DEFINING SOCIAL NETWORK STRUCTURE THROUGH TEXT SIMILARITY ANALYSIS: A MODEL FOR PROMOTING COLLABORATION AND EXAMINING CONDITIONS IMPACTING THE SUCCESS OF COLLABORATIVE ENDEAVORS WITHIN A RESEARCH COMMUNITY

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DEFINING SOCIAL NETWORK STRUCTURE THROUGH TEXT SIMILARITY ANALYSIS: A MODEL FOR PROMOTING COLLABORATION AND EXAMINING CONDITIONS IMPACTING THE SUCCESS OF COLLABORATIVE ENDEAVORS WITHIN A RESEARCH COMMUNITY

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Given the breadth and sheer volume of accumulated scientific knowledge, individual researchers often lack the requisite knowledge and resources to adequately address increasingly complex problems; therefore, many researchers are realizing the advantages afforded by collaborative research practices. The application of text data mining technologies to social networking strategies provides a novel approach to identifying opportunities for scientific collaboration through text similarity analysis, provided by the computer program eTSNAP. The data set submitted to eTSNAP comprised 137 research abstracts representing individual scientists affiliated with the Regional Centers of Excellence in Biodefense and Emerging Infectious

Diseases. Examination of the data in the form of tables, matrices, and interactive similarity network maps revealed the presence of eight discrete clusters of individuals, linked by the similarity of their abstracts. Further analysis of structural and functional characteristics of each cluster permitted the selection of a single cluster with the highest probability of collaborative success to serve as the pilot cluster. Members of this pilot cluster, renamed the "anthrax cluster" in reference to the common theme of research, received an introductory packet of information explaining the design of the project and soliciting participation in a preliminary survey, developed with intentions of assessing collaborative readiness and garnering practical information to assist in the preparation of a future teleconference. When multiple requests failed to elicit an adequate response, further attempts at establishing collaborative relationships between these researchers merely represented an exercise in futility. Evaluation of this project ultimately consisted of a secondary telephone interview with cluster members along with an in-depth literature review; both components of the final evaluation endeavored to isolate and examine factors that facilitate or inhibit collaboration within a research environment. Results suggest that similar interests alone cannot sustain successful collaboration; rather, complex interactions between a multitude of interconnected variables essentially determine collaborative outcomes.

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Abstract (scientific) – a brief summary describing the goals, methodology, significant results, and conclusions of a particular research endeavor.

Anthrax – an acute infectious disease caused by the pathogen *Bacillus anthracis* that exhibits high mortality in cases of pulmonary exposure; has demonstrated potential for use as a bioweapon on multiple occasions, including the September 2001 attacks on the United States.

Bibliometric study – a study that applies statistical methods to the analysis of a body of literature in order to examine development of subject fields along with patterns of authorship and publication.

Biodefense – the summation of defensive strategies taken to prevent the development and use of biological agents as weapons and mitigate the impact of a biological attack, should one occur.

Bioterrorism – the deliberate use of biological agents, such as pathogenic microorganisms or toxins produced by living organisms, to induce death or disease.

Cluster – in the context of this project, a subset of individuals within a social network distinguished and isolated by ties of similarity.

Collaboration – the process by which a group of individuals works cooperatively to complete a designated task.

Collaboratory – a form of scientific collaboration that relies almost completely upon electronic communications infrastructure to support communications between distant researchers.

Electronic bulletin board – an online resource that permits asynchronous communication between multiple individuals by establishing a virtual location for the collection and display of messages.

Electronic Text Similarity Network Analysis Package (eTSNAP) – a text data mining application that uses natural language processing algorithms to quantitatively evaluate the degree of similarity between pairs of text fields within a data set.

Hyperlink – a electronic link within a document, usually represented by highlighted words or images, that, when activated, directs a user to the document or portion of the document referenced by the link.

Hypertext Markup Language (HTML) – a computer coding syntax used for electronic publishing

Internet-based chat application – an online resource that permits synchronous communication between multiple users through text messages.

Java – an object-oriented programming language developed by Sun Microsystems; commonly used to enhance the functionality and interactivity of web pages.

Likert scale – a multi-point rating scale, frequently incorporated into surveys, by which respondents indicate their level of agreement with statements expressing either a favorable or unfavorable attitude toward a particular concept.

Matrix – in the context of this project, a method of presenting numerical data whereby values at the intersection of a row and column corresponds to a calculation between those particular elements.

National Institute of Allergy and Infectious Diseases (NIAID) – one of 27 institutes comprising the NIH, the NIAID manages basic and applied research related to infectious, immunologic, and allergic diseases.

National Institutes of Health (NIH) – a division of the United States Department of Health and Human Services and the primary agency of the United States government responsible for biomedical research.

Node – in the context of this project, a single locus on a social network map, representative of a particular scientist defined through his or her abstract.

Plain text – a software-independent text format, commonly used to exchange information between computer systems, that contains only text elements without additional formatting or structural information, such as font size, font, or layout information.

Portable Document Format (PDF) – a universal file format that preserves all formatting elements of a particular document and enables viewing consistency regardless of computer platform or settings.

Principle investigator (PI) – the lead investigator of a NIH-sponsored research endeavor.

PubMed – an online database maintained by the National Library of Medicine that provides access to over 15 million abstracts from MEDLINE and additional life sciences journals.

Regional Centers of Excellence in Biodefense and Emerging Infectious Diseases (RCEs) – a national network established by the NIAID comprising ten regional centers, each supporting research focused on countering threats from bioterror agents and emerging infectious diseases.

Social network – an association of individuals linked through common interests.

Stopwords – trivial words, such as 'and,' 'or,' 'with,' 'but,' 'not,' filtered from text data prior to or following data processing.

Tag-delimited formatting – a system of organizing data into specified fields to facilitate computational analysis.

Teleconference – a method of communication involving synchronous exchange of audio information between two or more remote locations through a telecommunications system, most often an established telephone line.

Text data mining – the automated extraction of latent and potentially useful information from large quantities of data existing in text format.

Threshold value – in the context of this project, a user-defined numerical value limited to the range of (0.0-1.0) which alters the sensitivity of similarity analysis; lower threshold values correspond to decreased sensitivity, while higher threshold values correspond to increased sensitivity and fewer instances of similarity.

Uniform Resource Locator (URL) – specifies the location of a particular file on the internet.

Videoconference – a method of communication employing a set of interactive telecommunication technologies that allows synchronous communication between two or more remote locations via two-way video and audio transmissions.

Word count vectors – a list of words and corresponding frequencies of occurrence within a defined text field used in text similarity analysis that permit statistical comparisons between natural language text fields.

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CHAPTER ONE
Introduction

Rapid expansion and specialization of the present scientific knowledge base reduce the chance of a single researcher possessing all the requisite knowledge, skill, and resources to address the complexities of modern scientific investigation. Consequently, many scientists have modified their perspective to research by embracing a more collaborative approach. Augmentation of communications infrastructure and development of new technologies, such as videoconferencing, chat rooms, and web portals, have supported this trend toward collaboration by reducing the impact of geographical distance on effective communication. Although documentation of scientific collaboration dates back to as early as 1665[1], only recently has the concept of collaboration reached such a level of widespread importance so as to encourage research into the practice of collaboration itself. Nevertheless, encouraged by prospects of increased effectiveness and efficiency of research, many policy-makers, private research companies, and governmental agencies have adopted collaborative research policies despite lacking a thorough understanding of the collaborative process and the factors affecting its success.

Background information

The National Institutes of Health (NIH), a division of the United States Department of Health and Human Services, serves as the primary federal agency for conducting and supporting biomedical research and maintains responsibility for 28 percent (approximately $28 billion) of national expenditures in biomedical research each year[2]. The National Institute of Allergy and Infectious Diseases (NIAID), one of the twenty-seven institutes of the NIH, manages basic and applied research within the realm of infectious, immunologic, and allergic diseases. The intentional dispersal of anthrax toxin through the United States Postal Service one week following the terrorist attacks of September eleventh brought latent concerns regarding bioterrorism to the forefront of national security issues. Reflecting the immediate necessity for development of

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[1] Luukkonen, Terttu, Olle Persson, and Gunnar Sivertsen. "Understanding Patterns of International Scientific Collaboration." *Science, Technology, and Human Values* 17.1 (1992): 101.
[2] Osterweil, Neil. "Medical Research Spending Doubled Over Past Decade." *Medpage Today* 20 Sept. 2005: n. pag. Online. Internet. 18 Apr. 2007. Available: http://www.medpagetoday.com/PublicHealthPolicy/HealthPolicy/tb/1767.

bioterrorism defense strategies, the NIAID's funding increased by $1.5 billion in 2003, while the Project BioShield Act of 2004 provided an additional $5.6 billion over the next ten years[3]. In 2005, the NIAID launched a national network of ten Regional Centers of Excellence in Biodefense and Emerging Infectious Diseases (RCEs) with the ultimate goal of producing a series of vaccines and therapeutics for a multitude of infectious agents, many of which demonstrate potential as weapons of biowarfare[4]. The gravity of the threat of bioterrorism demands the delivery of conclusive research in an efficient manner; therefore, the NIH strongly encouraged the RCEs, at their inception, to adopt collaborative research strategies. Through independent investigator funding mechanisms, each RCE supports various researchers at universities and complementary research institutions within a defined geographical area. Unfortunately, such funding practices, in addition to unavoidable circumstances of geographic isolation, have nurtured scientists' natural tendencies toward independence and autonomy, thus hindering collaborative efforts. In response, the NIH allocated roughly $500,000 to the initial development of videoconferencing capabilities and corresponding communications infrastructure in addition to establishing a $100,000 annual expenditure for equipment maintenance and enhancement; nevertheless, substandard levels of communication within and between regions continue to fragment the RCEs[5].

Thesis question

Initial conversations with committee member and Western RCE communications core director Dr. Garner illuminated the less than satisfactory condition of RCE communications and led to further discussion surrounding potential methods of ameliorating such conditions. Effective scientific collaboration requires first and foremost that researchers develop and maintain an awareness of current research occurring within their respective fields; therefore, developing a method of identifying scientists working on related projects would potentially yield increased levels of collaboration. A text data mining software application developed by members of Dr. Garner's laboratory at the University of Texas Southwestern Medical Center at Dallas (UT Southwestern) entitled eTSNAP (electronic text similarity network analysis package) provides quantitative measures of similarity between multiple text fields along with a visual representation of the connections between these text fields. Dr. Garner and I discussed the possibility of using a subset

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[3] Wright, Susan. "Taking Biodefense Too Far." *Bulletin of the Atomic Scientists* 60.6 (2004): 61.
[4] NIAID. "Biodefense" *Selected Areas of Scientific Research* (2005): 65. Online. Internet. 18 Apr. 2007. Available: http://www3.niaid.nih.gov/about/overview/profile/fy2005/pdf/research_biodefense.pdf.
[5] Garner, Harold. E-mail to the author. 12 Feb. 2007.

of scientific abstracts as inputted text fields to allow for the construction of a theoretical social network amongst authors of related abstracts and the subsequent identification of potential collaboration opportunities. This speculation led to the formation of the following question: Can an application of text data mining software form the basis of an effective model for the identification and construction of successful collaborative networks within a defined scientific community?

Goals, Objectives, and Limitations

Addressing my thesis question required a defined goal with clear objectives and parameters. The development of a model that identifies paths to successful scientific collaboration through the use of text data mining technology represented the ultimate goal of this project. Several objectives supported the pursuit of this goal and included:

1. developing a strategy for the identification of 'clusters' of authors with similar research through eTSNAP
2. selecting a single cluster for participation in communications activities aimed at fostering collaborative relationships
3. drafting an introductory letter and pre-collaborative survey for inclusion in an informative packet that will establish initial contact with the members of the cluster
4. along with committee member Dr. Garner, organizing and mediating a preliminary discussion via video- or teleconferencing session with members of the cluster
5. providing support for additional communications ventures, such as bulletin boards and chat rooms, as deemed necessary by cluster members, and
6. evaluating the advancement of collaborative relationships between members of the cluster.

The scope of this thesis limits the data mining software to eTSNAP and the analysis and the search for research clusters to the well-defined community of RCE-affiliated scientists who had submitted abstracts prior to January first of 2006. Subjects' access to resources, primarily communications equipment, and motivation to participate also impose potential limitations on this study.

Significance

The significance of this study lies in its potential to demonstrate the utility of text data mining in social networking applications and, consequently, to define a novel method of discovering opportunities for successful scientific collaboration. Increased levels of collaboration often translate to more efficient and higher quality research, saving time and consuming fewer raw materials and financial resources. Biodefense research in particular maintains a critical necessity for reliable and efficient research; however, other fields of scientific research could also expect to benefit from increased efficiency and quality through collaboration.

CHAPTER TWO
Review of the Literature

PRIMARY LITERATURE REVIEW: SOCIAL NETWORKING APPLICATIONS

As social creatures, human beings rely heavily on their interactions with others to explore and navigate the world around them. Each interaction establishes a relationship between two individuals through a common factor such as a shared interest or membership within a particular organization. An individual's unique collection of social interactions forms the basis of his or her social network and provides access to the overlapping networks of others through mutual acquaintances within a defined community. Social psychologist Stanley Milgram's early empirical research revealed the expansive nature of these networks and supported the concept of the small world phenomenon: the hypothesis that just a small chain of intermediary acquaintances connects any two randomly selected individuals within a given population[6]. The results of Milgram's experiments, specifically, suggested an average number of 6 intermediary contacts exist between any two randomly selected persons residing within the United States, giving rise to the phrase "six degrees of separation." Several variations on this theme, such as the Erdos number[7], which uses co-authorship data to calculate of the degrees of separation between any mathematician and the prolific Hungarian mathematician, Paul Erdos, and the Hollywood trivia game "six degrees of Kevin Bacon[8]," which attempts to connect the actor Kevin Bacon to any other actor via co-actor networks maintained by the internet movie database (IMDB), demonstrate the popularity of social networks. The utility of social networking extends far beyond that of entertainment, however, to benefit a diverse array of professional communities. The scientific community, in particular, maintains a plethora of well-developed and integrative relationships through collaborative activity and thus provides an optimal environment for the exploration of social networks. Bibliometric studies of co-authorship and citation networks comprise much of the early research into scientific

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[6] Newman, M.E.J. "The Structure of Scientific Collaboration Networks." *Proceedings of the National Academy of Sciences of the United States of America* 98 (2001): 404.
[7] Barabási, A.L. et al. "Evolution of the Social Network of Scientific Collaborations." *Physica A* 311 (2002) 591.
[8] University of Virginia Department of Computer Science. "The Oracle of Bacon." 1996: n. pag. Online. Internet. 16 Apr. 2007. Available: http://oracleofbacon.org.

social network structure[9], while advances in computer science along with increased accessibility and usability of the internet have resulted in the development of numerous computer- and web-based social networking applications.

Bibliometric Studies

Although technologically less sophisticated, co-authorship and citation network analyses continue to provide valuable information regarding the structure of scientific social networks. This method of analysis assumes the existence of a working relationship between co-authors of a particular manuscript or, in the case of citation networks, a shared interest at minimum. Bibliometric studies of co-authorship offer a more accurate means of documenting instances of collaboration than citation networks and therefore account for the majority of such collaboration-oriented scientific social network research. Although possible to construct a visual social network of ties between individual scientists via co-authorship data, the true strength of bibliometric analysis lies in its ability to expose structure and trends within extremely large sets of data[10]. The standardized approach of co-authorship bibliometric analysis decreases ambiguity by defining a collaborative relationship by joint publication; however, such a simple methodology has drawbacks and limitations. For example, large-scale collaborative studies may require the expertise of several researchers who ultimately report their findings to a lead investigator and do not, in fact, establish a working relationship amongst themselves. In addition, the occasional listing of 'courtesy authors' along with inconsistencies in name format and authors with identical names produces inaccuracies within the analysis; however, the considerable scale of these studies often renders such inaccuracies statistically insignificant[11]. Another drawback inherent to bibliometric analysis involves the retrospective nature of the research: historical publication data merely defines past relationships rather than revealing opportunities for future collaboration. Execution of a bibliometric analysis also presents further difficulty, for without additional tools to automate data collection or display results, such analyses require an exceptional amount of labor. Despite the value of bibliometric analysis in certain applications, the relatively small scale of this project, combined with a need for a more focused examination of individual researchers and their collaborative ties precluded the use of such analysis for this project.

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[9] Newman, M.E.J. "Coauthorship Networks and Patterns of Scientific Collaboration." *Proceedings of the National Academy of Sciences of the United States of America* 101 (2004): suppl.1 5200.

[10] Barabási, 591.

[11] Barabási, 594.

Referral Web

Similar in concept to bibliometric studies, the web-based social networking application Referral Web takes advantage of the internet's limitless quantity of information to generate a visual social network map through a process called automated referral chaining[12]. The term referral chaining describes the process of connecting one individual to another through a certain number of intermediary acquaintances. Referral Web automates this process by integrating the use of a standard internet search engine, in this case, AltaVista, with simple mathematical analysis[13]. Immediately following a user's registration with Referral Web, AltaVista initiates a search of the entire internet for any and all documents containing the user's name. From these resulting documents, Referral Web extracts additional names (typically several hundred) occurring in close proximity to the user's name. Subsequent analysis including the calculation of a Jaccard coefficient and application of a threshold value further refine the search results to generate an individual's social network map. By integrating the maps of multiple users, Referral Web allows an individual to explore his or her extended network within a user-specified field of study. The concept for Referral Web arose in response to the fact that the value of privately held information often exceeds that of information available within public domains such as the internet; therefore, access to quality information frequently depends upon social connections with its proprietor[14]. Through visual representation of personalized referral chains, Referral Web offers users a means to accessing this privately-held information through existing social ties. With a user-friendly visual display and a database that encompasses the entire internet, Referral web provides considerable advantages over bibliometric social network construction; however, consistency of individuals' names and identical names for different individuals remain notable weaknesses. Moreover, the lack of publication standards on the internet brings the reliability of Referral Web's information base into question. The utility of Referral Web increases substantially with each registered user due to network overlap; however, the process of manually submitting names for analysis hinders the construction of a sufficiently large and organized system of networks, especially considering the generation of initial networks can take up to 24 hours[15]. In addition to these limitations, as with bibliometric network construction, Referral Web's retrospective

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[12] Kautz, Henry, Bart Selman, and Mehul Shah. "The Hidden Web." *American Association for Artificial Intelligence*. Summer 1997: 29.
[13] Kautz, 29.
[14] Kautz, 28.
[15] Kautz, 32.

approach to composing social networks runs counter to this project's goal of exploring opportunities for establishing new relationships. Ultimately, however, the unavailability of Referral Web at the present time clearly precluded its consideration in this project.

Locus: Experimental Social Interface

The limitations inherent to bibliometric-related studies of social networks prompted the exploration of alternate means to network construction with particular emphasis on computer applications driven by text similarity analysis; Locus: ESI (experimental social interface), or simply Locus, provides an example of such an application. Marketed as a social experiment, instant messenger, and art piece, Locus assembles visually engaging social network maps from textual data obtained from incoming and outgoing instant messages[16]. Written in Python, a computer programming language, the internet chat protocol Jabber serves as the foundation of Locus, permitting the transmission and collection of instant messages between users. Locus implements Bayesian algorithms, such as those present in successful electronic mail spam filters, in its analysis of incoming and outgoing messages. Bayesian analysis permits the classification of incoming messages by user based on previous knowledge acquired through program training. Subsequent analysis of the textual information contained in the compilation of messages assigned to each user involves the removal of "stopwords," such as a, the, and, or, etc. and the extraction of various keywords. Similarity analysis between keywords of each and every user provides the final component of Locus' analysis and enables the construction of an interactive visual display of a user's social network. Although text similarity analysis and interactive visual elements of Locus provide distinct advantages over bibliometric-related applications, Locus' instant messaging foundation involves several limitations. To begin, individuals wishing to explore his or her social network through Locus must download and install the instant messaging software, which operates solely on a PC or Linux platform[17]. Although a user's contacts may continue to use their preferred proprietary instant messaging systems, such as AOL, Yahoo, and ICQ, for example[18], Locus restricts social networks to individuals within a user's instant messaging contact list; therefore, social networks only incorporate individuals with whom the user has previously established

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[16] "About Locus." Online. Internet. 16 Apr. 2007. Available: http://locus.e-mu.org/index.php?option=com_content&task=view&id=13&Itemid=44

[17] "Downloads." Online. Internet. 16 Apr. 2007. Available: http://locus.e-mu.org/index.php?option=com_docman&task=view_category&Itemid=50&subcat=1&catid=75&limitstart=0&limit=5

[18] "About Locus."

contact through instant messaging. Additionally, instant messaging lacks the formality of other methods of communication, with its users commonly resorting to the use of slang and abbreviations within the text of messages, thereby producing a substandard data set for computer-based text analysis. In a scientific community, the informality of instant messaging could also conceivably dissuade researchers from considering such forms of communication for important discussions pertaining to research. Despite the potential value of text similarity analysis, applying the instant messaging application Locus to a research community presents significant obstacles that would certainly hinder any attempts at social network construction.

Electronic Text Similarity Networking Analysis Package (eTSNAP)

eTSNAP, the application ultimately chosen for the social network analysis component of this project, provides an example of a computer-based application employing text similarity analysis in the generation of social networks. Developed by Garner Labs at the University of Texas Southwestern Medical Center at Dallas, eTSNAP functions as a text data-mining program that calculates similarity scores between pairs of text fields belonging to a specified data set. Improving upon simple keyword analysis, eTSNAP's algorithm incorporates two complementary methods of similarity analysis[19]. The first component, bearing resemblance to Locus' analysis, involves the removal of stopwords and the computation of word-count vectors which take into consideration the frequency of identified keywords. The second component then evaluates similarity through a sentence alignment mechanism which preserves word order information, assessing likeness through matrices. The summation of eTSNAP's multiple layers of analysis yields a single similarity score between each pair in a data set. Ultimately, eTSNAP presents the results of its analysis in one of three user-selected formats: matrix, tabular, or Java-based interactive map. Each display format offers distinct advantages to the user, provided he or she understands basic concepts of data presentation; novice users may experience difficulty in navigating the various options and fail to recognize the benefits afforded by integration of two or more data formats. Additionally, preparing the text document for submission to eTSNAP demands user competency with the fundamentals of computer "tag" language and may entail a considerable amount of effort, depending on the amount of data and user proficiency. Despite these drawbacks, the unparalleled sophistication of similarity analysis along with a multitude of

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[19] Lewis, James, et al. "Text Similarity: An Alternative Way to Search Medline." *Bioinformatics* 22 (2006): 2299.

display options which include an interactive visual social network map supply the tools necessary for advanced social network analysis; moreover, eTSNAP's network generation operates independently of subject interaction, thereby eliminating the prerequisite of subject participation. Such advantages render eTSNAP an ideal application for incorporation into this communications model.

The recent popularity of social networking has prompted the development of a vast number of applications that examine, depict, and facilitate the creation of social networks. This review focused exclusively on applications suitable for use within a scientific community; therefore, applications such as MySpace, a popular social networking tool among younger generations, did not receive attention in this survey. Since ties of similarity establish the foundation of social networks, applications capable of exposing similarity within data sets demonstrate great potential for incorporation into social networking utilities. Processing data for similarity presents significant difficulties due to the predominantly textual versus numerical nature of the raw data. Text similarity algorithms, such as those that power the analysis component of Locus and eTSNAP, aspire to accurately read and interpret unfiltered real text data for purposes of comparison. The unequaled precision of eTSNAP's similarity algorithm, along with several additional features unique to the eTSNAP application, comprise an ideal system for the social network analysis component of this project.

SECONDARY LITERATURE REVIEW: SCIENTIFIC COLLABORATION

In response to the increasing complexity of scientific problems and mounting concerns regarding the nation's preparedness for potential health crises, the NIH, in September of 2003, unveiled a new strategy to address these concerns through modification of fundamental scientific practice[20]. Developed in response to past difficulties encountered during large-scale multidisciplinary projects, The NIH Roadmap articulates a new collaborative philosophy and provides funding for infrastructure in support of this philosophy. For example, large scale production and analysis of membrane-bound proteins within the structural biology program and the formation of libraries and databases of small molecules with potential pharmaceutical applications both advance the concept

[20] Check, Erika. "NIH 'Roadmap' Charts Course to Tackle Big Research Issues." *Nature* 425 (2003): 438.

of 'big science.' Science on such a massive scale requires the cooperation of multiple individuals from diverse backgrounds; consequently, a large portion of the Roadmap initiative will focus on improving multidisciplinary collaborative relationships. At an initial cost of $125 million, rising to $2 billion by 2009[21], the NIH Roadmap venture represents a significant expense; such an investment clearly attests to the NIH's confidence in the potential merits of collaborative research practices.

Advantages of Collaboration

Lacking the validation of large-scale comprehensive studies, collaborative research has nevertheless received much praise, primarily on account of auspicious findings garnered from anecdotal and case study reports. Complementarity in research consistently emerges as a major impetus for collaboration, with authors citing rapidly changing technology, increasing complexity of research problems, and exponential growth of scientific knowledge with specialization of research as circumstances demanding complementary skills[22]. Collaboration allows researchers the opportunity to pool knowledge and physical resources in order to enhance productivity and address larger, more complicated problems[23]; moreover, successful collaborative research tends to produce higher quality results, due to increased detection of errors and formulation of innovative solutions resulting from integration of differing perspectives[24]. While many scientists collaborate in response to pressures of the changing research industry, some also choose to collaborate for more personal reasons such as to obtain prestige, learn new techniques, or simply for pleasure[25]. For students and recent graduates, especially, collaboration represents a certain rite of passage and may provide a means to gaining acceptance within the scientific community[26]. Whether to adapt to the changing research industry or for personal advancement or enjoyment, collaborative research has the potential to benefit both individual scientists and the research community as a whole.

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[21] Check, 438.

[22] Hara, Noriko, and Paul Solomon. "An Emerging View of Scientific Collaboration: Scientists' Perspectives on Collaboration and Factors that Impact Collaboration." *Journal of the American Society for Information Science and Technology* 54 (2003): 952, 962.

[23] Bozeman, Barry, and Elizabeth Corley. "Scientists' Collaboration Strategies: Implications for Scientific and Technical Human Capital." *Research Policy* 33 (2004): 601.

[24] Beaver, Donald deB. "Reflections on Scientific Collaboration (and its Study): Past, Present, and Future." *Scientometrics* 52 (2001): 370.

[25] Beaver, 373.

[26] Hara, 957.

Disadvantages of Collaboration

In comparison to the abundance of literature extolling its benefits, few papers address possible disadvantages of collaboration within scientific communities. Collaborative research environments have a tendency to anonymize scientists, often failing to recognize the efforts of individuals while focusing instead upon deliverables[27]. Additionally, collaborative approaches to research frequently involve a shift in laboratory dynamics. Principle investigators generally assume a more distant supervisory role, thereby losing touch with direct research, while lab scientists concern themselves with specialized prescribed tasks and experience fewer opportunities for creative thought. Although such specialization and division of labor can increase both productivity and quality of research, individual scientists may nevertheless lack a fundamental understanding of the research problem as well as an adequately diversified skill set[28]. Bearing additional administrative and organizational responsibilities that divert attention from research-oriented activities, principle investigators also may suffer the degradation of research skills[29]. Despite these concerns, however, scientific industry is embracing the concept of collaboration, though to many researchers, the transition may prove quite unsettling.

Origins of Collaboration

Formal examination of collaborative events reveals characteristics of the origination and course of collaborative activity. Most instances of collaboration emerge either informally within small groups of acquaintances or formally as part of a managed collaborative initiative. Small informal collaborations often stem from conversations among colleagues and usually consist of three to five individuals[30]. Scientists experiencing important life transitions or blocked advancement, in particular, have a tendency to seek out collaborative opportunities; moreover, research environments characterized by prolonged socialization, high levels of peer interaction, slow and uncertain mobility, limited access to mentors, and uniformity in employee status tend to stimulate collaborative activity[31]. The advent of widely accessible electronic communications media transformed the practice of science by reducing the impact of geographical distance on the flow of

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[27] Beaver, 370.
[28] Beaver, 370.
[29] Beaver, 370.
[30] Levine, John M. and Richard L. Moreland. "Collaboration: The Social Context of Theory Development." *Personality and Social Psychology Review* 8.2 (2004): 165.
[31] Levine, 165.

information[32]. Many of the aforementioned formal collaborative initiatives span multiple institutions throughout the nation and therefore rely to some degree upon electronic communications applications for rapid transmission of vital information. The modern "collaboratory," for example, operates almost exclusively through established communications infrastructure linking remote locations[33]. The former Great Lakes Regional Center for AIDS Research and the New York University Oral Cancer Research for Adolescent and Adult Health Promotion Center, both funded by the NIH, provide examples of prominent collaboratories. Whether a spontaneous event stemming from informal conversations among colleagues or a large-scale meticulously planned operation, collaborative endeavors exhibit a certain fragility, easily disrupted by various forces.

Factors Influencing Collaborative Outcomes

Geographical Distribution

Despite the wealth of communications resources permitting communication across great distances, geographic isolation remains a significant barrier to successful collaboration as indicated by results of bibliometric studies which reveal an exponential decrease in co-authorship as the distance between authors increases[34]. Communications technology plays an important role in facilitating collaboration, whether within a formal collaboratory or a small group of colleagues. Videoconferencing, internet-supported chat rooms and portal systems, and real-time whiteboarding sessions represent a few of the standard technology-based communication applications suitable to scientific collaboration. Despite its potential to enhance interactions within a research community, electronic communications technology does not always satisfy the needs of individual researchers. For example, a survey conducted within a research community found that although videoconferencing successfully cultivates an awareness of collaborative opportunities, scientists prefer to use other communications mechanisms, such as email, telephone, or in-person meetings, when engaging in actual research activity[35]. In present times, rapid expansion of the technology industry frequently obscures the value of simple face-to-face

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[32] Kling, Rob and Geoffrey McKim. "Not Just a Matter of Time: Field Differences and the Shaping of Electronic Media in Supporting Scientific Communication." *Journal of the American Society for Information Science* 51 (2000): 1306.

[33] Schleyer, Titus KL, Stephanie Teasley, and Rishi Bhatnagar. "Comparative Case Study of Two Biomedical Research Collaboratories." *Journal of Medical Internet Research* 7.5 (2005): n. pag. Online. Internet. 4 Apr. 2007. Available: http://www.jmir.org/2005/5/e53.

[34] Bozeman, 602.

[35] Hara, 963.

communication. Establishing opportunities for personal contact, such as conferences and retreats, builds trust amongst colleagues and has thus proven beneficial in sustaining collaborative relationships[36]. Though the availability of new communications technologies has diminished the adverse effects of geographical distance on successful collaboration, spatial proximity clearly remains an important variable in facilitating the formation and maintenance of collaborative initiatives.

Organizational and Leadership Structure

In addition to geography, certain features relating to the structure and organization of sizable collaborative research endeavors play a critical role in the success or failure of that endeavor. Articulating well-defined attainable goals early in the planning process provides direction and focus to collaborative efforts, as does the creation of an incentive program[37]. Through funding mechanisms, for example, incentives have proven useful in mitigating scientists' concerns regarding participation in collaborative research[38]. Composing a successful collaborative research group requires attention to several demographic factors. Small (less than twenty) or medium (twenty to fifty) groups of individuals with shared institutional or departmental identities tend to exhibit greater collaborative strength than larger, more diverse groups[39]. In cases of transdisciplinary collaborations, research groups that encompass a narrow versus broad scope of disciplines display a greater tendency toward success[40]. Regarding administrative structure, strong consistent leadership that has a simple versus complex hierarchical configuration enhances group operations and, consequently, facilitates effective collaboration[41]. In addition to social considerations, designing an environment conducive to collaboration also necessitates careful assessment of technological requirements. Computing platform incompatibilities, network infrastructure complexity, lack of technical support, and low computer and software literacy represent several technological issues that threaten the integrity of collaborative research activities[42]. Clearly, organizing a successful collaborative venture requires the expenditure of a

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[36] Stokols, Daniel, et al. "In Vivo Studies of Transdisciplinary Scientific Collaboration." *American Journal of Preventive Medicine 28* (2005): 212.
[37] Hara, 962.
[38] Schleyer, n. pag.
[39] Stokols, "In Vivo Studies," 206.
[40] Stokols, "In Vivo Studies," 206.
[41] Stokols, "In Vivo Studies," 206.
[42] Schleyer, n. pag.

considerable amount of time and energy. For example, administrators affiliated with the NIH's Transdisciplinary Tobacco Use Research Centers (TTURCs), a collaborative network of scientists and public health researchers, spent nearly two years resolving differences in scientific terminology, researching strategies and negotiating collaborative agreements[43]. Investing time and energy in pre-collaborative needs assessment and organizational planning does not represent time misspent; rather, identifying and addressing potential deficiencies in social and technical infrastructure prior to the start of collaborative activity increases the likelihood of success.

Personal characteristics and values

Collaborative research, by definition, entails a substantial amount of social interaction; therefore, individual character traits and personal values have the potential to significantly impact productivity. Similarity in work habits and writing style, along with shared scientific values and priorities typically enhance the collaborative experience, while factors breeding interpersonal tensions threaten to compromise collaborative integrity[44]. Individuals have a natural tendency to seek out others who embrace similar values and perspectives for purposes of validation, and while such activity creates a supportive environment, it can also suppress innovation through "groupthink," thus negating advantages afforded by collaboration[45]. Despite a penchant for similarity, scientists also demonstrate a certain appreciation for diversity in skill and knowledge, frequently citing complementary expertise as a reason for engaging in collaborative research[46]. Observable diversity, however, such as age, race, and sex, initially tends to impede group integration[47]. Personal compatibility engenders trust amongst researchers and thus plays a crucial role in the success or failure of collaborative relationships. To illustrate, a recent survey exploring scientists' perspectives on collaborative research reported that 78% of scientists consider personal relationships with potential collaborators important[48]. In a culture that cultivates unremitting competition, conflict often arises over ownership of ideas. With countless scientists vying for funds concentrated in the hands of a few, competition within the scientific community exhibits a

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[43] Stokols, "In Vivo Studies," 206.
[44] Stokols, "In Vivo Studies," 208.
[45] Stokols, "In Vivo Studies," 211.
[46] Hara, 962.
[47] Levine, 167.
[48] Qin, Jian, F. W. Lancaster, and Bryce Allen. "Types and Levels of Collaboration in Interdisciplinary Research in the Sciences." *Journal of the American Society for Information Science* 48 (1997): 907.

particular intensity found in few other industries[49]. Although collaborative research espouses cooperation, a traditional competitive ideology continues to pervade scientific research with many scientists experiencing collaboration as alternating intervals of cooperation and competition[50]. Typically, shifts from collaborative to competitive strategies occur later in the research process, as scientists converge upon a solution[51]. A comprehensive case study of research practices in the genetics field reveals that 87% of scientists reported that they consistently honored requests for unpublished findings or data, while 59% reported greater secrecy amongst their colleagues[52]. The same study also reports that in biotechnology fields, more than any other factor, potential financial loss, either in the form of patents of grant funding, prevented scientists from divulging significant findings, thereby crippling any prospects of collaboration. As a practice rooted in social interaction, successful collaboration demands compatibility within a group of individuals, each with his or her own unique attributes and values; given the extraordinary variability in personal habits and characteristics as well as competitive tendencies, predicting collaborative success amounts to a fairly monumental task.

A radical departure from traditional methods of scientific research, collaboration is transforming the scientific industry to such a degree that one particular journal article described the collaborative movement as the new industrial revolution in the 1980s[53]. Such a profound alteration in research philosophy will involve a lengthy period of acclimation, which David Korn of the Association of American Medical Colleges referred to as "a very unsettling change[54]." Collaborative research certainly has the capacity to elicit more efficient research of a higher quality, as evidenced by the results of numerous anecdotal case reports; however, collaborative arrangements also have significant drawbacks that require further study. Complex interactions of innumerable variables complicate the study of collaboration, especially considering that definitive

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[49] Atkinson, Paul, Claire Batchelor, and Evelyn Parsons. "Trajectories of Collaboration and Competition in a Medical Discovery." *Science, Technology, and Human Values* 23 (1998): 269.
[50] Atkinson, 260.
[51] Atkinson, 269.
[52] McCain, Katherine W. "Communication, Competition, and Secrecy: The production and Dissemination of Research-Related Information in Genetics." *Science, Technology and Human Values* 16 (1991): 494.
[53] McCain, 492.
[54] Check, 438.

results may not materialize until years or decades later[55]. Considering the significance of the collaborative trend in research, developing a systematic approach to the study of collaboration could ultimately, through comparative study, facilitate identification of factors influencing the success or failure of collaborative endeavors[56].

[55] Stokols, "Toward a Science," 68.

[56] Greene, Sarah M., Gene Hart, and Edward H. Wagner. "Measuring and Improving Performance in Multicenter Research Consortia." *Journal of the national Cancer Institute Monographs* 35 (2005): 31.

CHAPTER THREE
Methodology

Data Selection

With the purpose of developing a process through which to identify and subsequently facilitate collaborative opportunities within the RCEs, I began devising a strategy that would enable me to maximize the utility of eTSNAP's text similarity analysis. First, I considered available sources of text data. Gathering the data for and producing a formal manuscript can require several years of effort, and, therefore, may not reflect a researcher's precise interests upon publication; moreover, nascent researchers may have yet to publish a paper. Limited access to certain journals and the laborious process of searching for and obtaining publications severely hinder the collection of data, while journal-specific disparity in formatting and required content produce additional variance within the data set. Due to multiple difficulties inherent to the use of journal articles as input data, I elected to use NIH research abstracts as the source of data. The NIH requires every RCE principle investigator to submit an annual grant application containing an abstract that synopsizes the objectives, procedures, and significant findings of research conducted within the past year, thus ensuring a complete data set with standardized composition. Additionally, working with Dr. Garner established a connection to the RCE network and therefore facilitated the acquisition of these abstracts.

Each RCE supports a variety of initiatives, distributed among four categories: research, career development, and developmental projects, along with an administrative and facilities core. Research projects focus on existing concerns within the biodefense domain and account for the majority of projects funded by the RCE. Career development projects provide qualified individuals with opportunities to develop or refocus their careers in biodefense through basic research and clinical and/or translational ventures, while developmental projects explore developing technologies and new opportunities. Finally, administrative and facilities cores support the functions of all projects and ensure access to vital resources. Considering the intent of this project, I elected to include exclusively the abstracts of research projects in the final analysis on account of the experience level of the investigators and thoroughly defined fields of research.

Orientation to eTSNAP

Acquiring an analysis from eTSNAP requires composition of a single plain text (.txt) document in tag-delimited format (figure 3-1).

```
<TITLE>: title
<AUTHORS>: authors
<JOURNAL>: journal
<DATE>: date
<CITATION>: citation
<TYPE>: type
<ADDRESS>: address
<EMAIL>: email
<PHONE>: phone
<KEYWORDS>: keywords
<TEXT>: text
<END TAGS>
```

Figure 3-1: Tag-delimited formatting

A set of tags may comprise any number of tag names, or fields, with the first tag providing an identity to the associated entries. At the minimum, eTSNAP requires the designation of title and text fields for identification and analysis, respectively. Examples of supplemental tag names include authors, institutions and contact information. Following submission of the saved text file to the web interface, prior to any revisions, eTSNAP required the user to select a threshold value between 0.0 and 1.0 prior to commencing analysis. The similarity scores calculated by eTSNAP between each pair of text fields all fall within the range of 0.0 and 1.0, with values approaching 0.0 reflecting greater variance and those approaching 1.0 indicating increased uniformity among text fields. The threshold value designates a cut off value below which eTSNAP fails to indicate a connection between two nodes within a social network map. For example, with a calculated similarity score of 0.28 between a pair of text fields, a threshold value of 0.27 will yield a visible connection between these two nodes. If, however, the threshold value rises to 0.29, that connection disappears. Optimal threshold values display great variability, depending on the extent of similarity or difference within a data set, and selecting an appropriate value can therefore pose significant difficulties in the absence of any awareness regarding the distribution of similarity scores. As a result, subsequent revisions to the eTSNAP interface eliminated the selection of a

threshold value as a prerequisite to analysis. Currently, a post-analysis table lists predetermined threshold values in increments of 0.05 along with correlating information regarding average cluster size and number of clusters for each given threshold value. Examining the information contained within the table permits a user to make an educated decision regarding a suitable threshold value; hyperlinked threshold values then direct the user to the corresponding map.

Analysis, comprising the computation of similarity scores and the preparation of output displays, requires little time, approximately one to two minutes, with variation attributed to the number of text fields and quantity of text within each field. At the conclusion of analysis, eTSNAP provides links to results in three different formats: matrix, tabular and interactive map. Figure 3-2 presents each the different data presentation formats obtained from a sample analysis of nine pieces of poetry: *Sonnets I*, *II*, and *III* by William Shakespeare; *Messy Room*, *Where the Sidewalk Ends*, and *One Inch Tall* by Shel Silverstein; and *Alone*, *Annabel Lee*, and *Lenore* by Edgar Allen Poe.

Similarity Matrix

	1	2	3	4	5	6	7	8	9
1	1.00000	0.36905	0.37639	0.10227	0.02806	0.01919	0.01070	0.02451	0.11212
2	0.36905	1.00000	0.33473	0.08721	0.02699	0.00907	0.07669	0.08379	0.16647
3	0.37639	0.33473	1.00000	0.05032	0.00926	0.00944	0.01071	0.03839	0.15815
4	0.10227	0.08721	0.05032	1.00000	0.04831	0.01962	0.00000	0.00000	0.01254
5	0.02806	0.02699	0.00926	0.04831	1.00000	0.02943	0.01028	0.03540	0.04619
6	0.01919	0.00907	0.00944	0.01962	0.02943	1.00000	0.01048	0.03164	0.03777
7	0.01070	0.07669	0.01071	0.00000	0.01028	0.01048	1.00000	0.17331	0.05160
8	0.02451	0.08379	0.03839	0.00000	0.03540	0.03164	0.17331	1.00000	0.20844
9	0.11212	0.16647	0.15815	0.01254	0.04619	0.03777	0.05160	0.20844	1.00000

Key
1: Sonnet I
2: Sonnet II
3: Sonnet III
4: Messy Room
5: Where the Sidewalk Ends
6: One Inch Tall
7: Alone
8: Annabel Lee
9: Lenore

Figure 3-2a: Matrix display format

Topic	Most Similar Topics	Score
Sonnet I	Sonnet III	0.37639
	Sonnet II	0.36905
	Lenore	0.11212
Sonnet II	Sonnet I	0.36905
	Sonnet III	0.33473
	Lenore	0.16647
Sonnet III	Sonnet I	0.37639
	Sonnet II	0.33473
	Lenore	0.15815
Messy Room	Sonnet I	0.10227
	Sonnet II	0.08721
	Sonnet III	0.05032
Where the Sidewalk Ends	Messy Room	0.04831
	Lenore	0.04619
	Annabel Lee	0.03540
One Inch Tall	Lenore	0.03777
	Annabel Lee	0.03164
	Where the Sidewalk Ends	0.02943
Alone	Annabel Lee	0.17331
	Sonnet II	0.07669
	Lenore	0.05160
Annabel Lee	Lenore	0.20844
	Alone	0.17331
	Sonnet II	0.08379
Lenore	Annabel Lee	0.20844
	Sonnet II	0.16647
	Sonnet III	0.15815

Figure 3-2b: Tabular display format

The structure of the matrix essentially follows a grid format with each text field, in series, forming the headers for both horizontal and vertical fields. Where a column intersects a row, the matrix displays the similarity score for the pair. Matrix format permits display of the numerical values of every similarity score within a data set, unlike tabular format. Tabular format lists each text field along with the three most similar text fields and corresponding similarity scores. The Java-based interactive map presents results in pictorial format with a lesser emphasis on numerical values. Lines illustrate similarity between nodes, representative of the various text fields, and, as mentioned previously, respond to changes in the threshold value. Operated via slidebar, the zoom, rotate, and locality tools allow manipulation of this map and add functionality. Zoom and rotate functions facilitate proper viewing of results by magnification and alteration of orientation, respectively, while locality influences the degree of branching from a central node. Ultimately, the accurate interpretation of results depends on an understanding of the advantages provided by each method of data presentation.

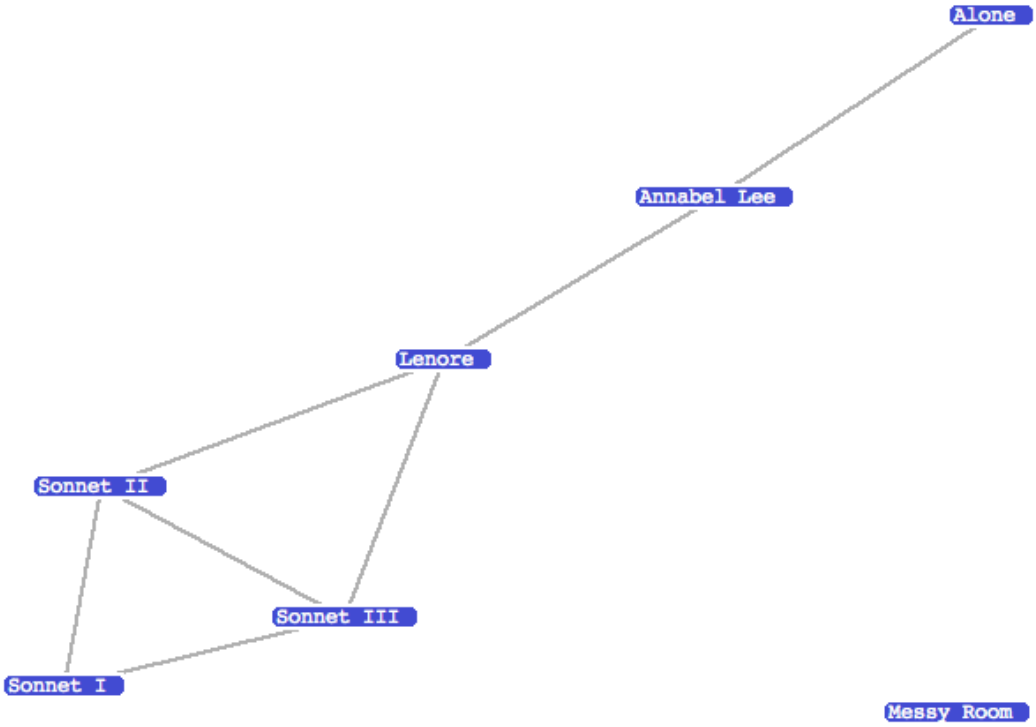

Figure 3-2c: Still image of interactive map display format

To better acquaint myself with the details of data preparation and analysis, I conducted a series of trials using the July 2005 abstracts from the Western Regional Center of Excellence as input data. Managing a smaller data set of 37 abstracts simplified the exploration of various methods of data interpretation and allowed me to concentrate more easily on individual relationships between abstracts. Interpreting results through integration of tabular and interactive map formats revealed two distinct clusters of individuals, one of which had already demonstrated a significant degree of collaborative activity, as determined through co-authorship literature searches within PubMed.

Data Collection and Processing

Having substantiated the efficacy of eTSNAP, I then proceeded to collect the required data, in the form of research abstracts, from eight of ten RCE regions. Due to variations in submission deadlines, abstracts from the Rocky Mountain Regional Center of Excellence and the Pacific Southwest Regional Center of Excellence did not constitute part of the final data set. Once I had gathered the requisite data, I composed the input document using a template that designated the following fields, or tag names: title, authors, journal, date, citation, type, address, email, phone,

keywords, and text. (see appendix A for a copy of the input document) The fragmentary nature of data supplementary to the abstract text, such as contact information of the PIs, made completion of all fields for a given abstract impossible; without affecting the outcome of the final analysis, I thus left several fields blank. To facilitate identification of abstracts as belonging to particular RCE, I preceded the abstract title with the acronym of the RCE to which the abstract belongs. After uploading the completed document to the eTSNAP web page, I then needed to select a threshold value; through trial and error, I deemed a threshold value of 0.25 appropriate to this particular analysis.

Cluster Identification and Analysis

With an objective of identifying clusters of abstracts with consistently strong similarity scores, I commenced my interpretation of the output with a survey of the results in tabular format (figure 3-3), searching for consistently high similarity scores among the top results for a particular abstract.

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the dimensions of this figure; please refer to figure3_3.pdf,
located in the supplemental images folder.

Figure 3-3: Results of analysis, tabular format

This procedure enabled a quick but thorough quantitative analysis of the relationships between abstracts, thus narrowing my investigation to a reasonable number of highly correlative abstracts, which I documented in a spreadsheet (table 3-1).

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the dimensions of this table; please refer to table3_1.pdf,
located in the supplemental images folder.

Table 3-1: Highly correlative abstracts

Having isolated several abstracts of interest, I began to examine these abstracts within the map environment (figure 3-4), considering in particular associations with other nodes.

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the dimensions of this figure; please refer to figure3_4.pdf,
located in the supplemental images folder.

Figure 3-4: Results of analysis, map format

The congregation of several interconnected nodes, typically 5 to 15, constitutes a cluster and warrants further investigation to determine the collaborative potential of that cluster. Ultimately, I distinguished eight clusters whose structural and functional information I recorded in a spreadsheet (table 3-2).

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the dimensions of this table; please refer to table3_2.pdf,
located in the supplemental images folder.

Table 3-2: Clusters identified through analysis

Note: The results as presented in matrix format did not provide additional benefit in this particular application of eTSNAP; nevertheless, I have included these results in appendix B for the purpose of completeness.

In addition to listing the RCE region, title, similarity score (relative to the first-listed abstract), principle investigator (PI) and institution for each abstract affiliated with a specific cluster, I included data indicating each PI's videoconferencing capabilities and prior intra-cluster collaborations, if any. Examination of RCE videoconferencing equipment distribution lists allowed me to determine capabilities of individual PIs, while ascertaining the presence of any prior collaborations required extensive use of PubMed, an internet-based search application providing access to a database containing over 16 million citations and abstracts of biomedical research articles. Performing an author search for every possible combination of two PIs within a single cluster yielded several instances of joint publication, indicating a history of collaboration. The final component of cluster analysis involved a qualitative review of abstract content to ensure a sufficient level of similarity among cluster members.

Selection and Modification of the Pilot Cluster

Through comparative analysis of structural and functional features of the eight potentially viable clusters, I selected the cluster perceived as having the greatest probability of success to serve as a pilot cluster. Cluster_8 (figure 3-5) entitled the "anthrax cluster," displayed several characteristics I regarded as favorable, including a moderately large group size with diverse RCE representation. Despite an absence of prior collaborative activity, consistently high similarity scores and a larger than average percentage of members with established access to videoconferencing equipment also conveyed a sense of collaborative readiness. Finally, with two exceptions, in reviewing the content of each abstract within a cluster, I detected a distinctive balance between heterogeneity and uniformity of research objectives among abstracts of the anthrax cluster. While each PI held vaccine development as his or her ultimate goal, techniques and strategies varied considerably within the cluster, with PIs investigating the structure and function of the anthrax spore and/or toxins and how each respective portion of the anthrax pathogen might present a target for future vaccines. The two exceptions, *Development and evaluation of human brucellosis vaccines* and *NBC Abstract #10 Theme 3: Optimizing VSV-based vaccine vectors* did not relate to the study of anthrax, but managed to secure a position within the anthrax cluster due to the similarity of such keywords as 'bacterial,' 'virulent,' 'human,' 'model,' 'pulmonary,' 'infection,' 'generate,' 'response,' 'pathogen,' 'disease,' 'candidate,' 'antigen,' and other non-specific terms; therefore, the authors of these two abstracts did not comprise part of the final cluster.

Having clearly defined the anthrax cluster identity, I elected to perform a keyword search within the entire set of RCE abstracts to identify any potential additions to the anthrax cluster. Standards of formal scientific literature dictate the use of binomial nomenclature when referring to biological organisms; therefore, I selected the keyword "anthracis" from *Bacillus anthracis*, the scientific name for the causative agent of anthrax disease. The keyword-directed search exposed four additional abstracts, two of which demonstrated relevancy to the research of the existing anthrax cluster: *Role of tissue-specific cell killing in anthrax-mediated cell killing* and *Therapeutic inhibition of* B. anthracis *pathogenesis*. Conversely, of the remaining two abstracts, *Pathogenic mechanisms of anthrax toxins* explores the merit of various therapeutic interventions in the medical management of anthrax, while *Salmonella type III secretion for bio-defense vaccines* focuses primarily on the use of avirulent strains of salmonella as antigen delivery vehicles for an assortment of pathogens, including *B. anthracis*. Lacking the fine discrimination of other search

methods, a simple keyword search nevertheless yielded beneficial results as evidenced by the identification of two additional cluster members. One final modification to the anthrax cluster involved the addition of Rick Lyons, primary researcher of the WRCE small animal core, on the basis of his established relationship with Dr. Garner and intended collaboration with Li Xu, another cluster member, in relation to murine pulmonary anthrax models. The finalized anthrax cluster, along with pertinent descriptive elements, appears in table 3-3.

(Figure 3-5 appears on the following page)

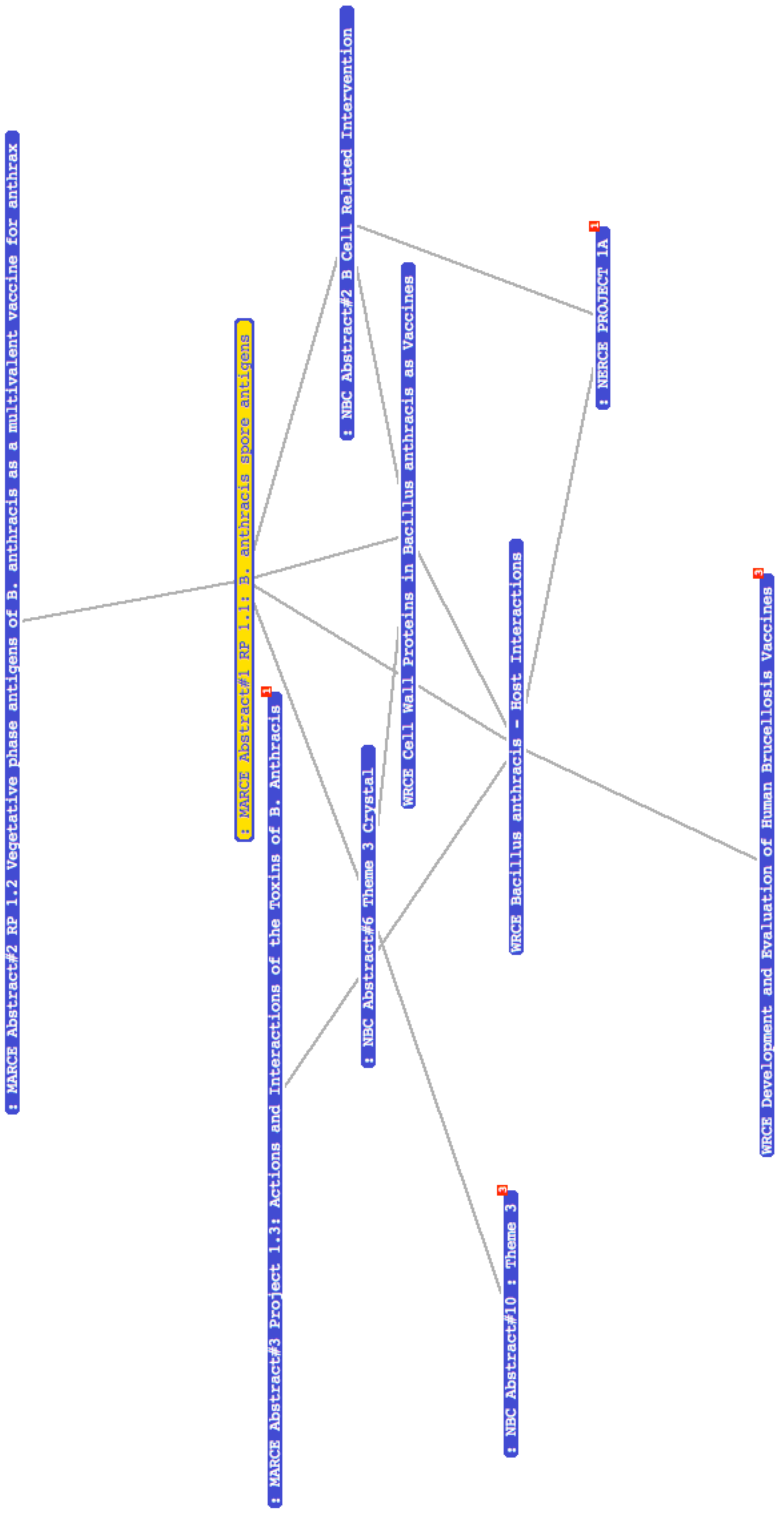

Figure 3-5: Map of anthrax cluster

Principle Investigator	Project Title	RCE Region	Institution	Email Address	Phone Number	V-con?
O'Brien, Alison D.	Abstract #1 RP1.1 B. anthracis spore antigens	MARCE	Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc.	aobrien@usuhs.mil		yes
Cross, Alan S.	Abstract #2 RP1.2 Vegetative phase antigens of B. anthracis as a multivalent vaccine for anthrax	MARCE	Center for Vaccine Development University of Maryland, Baltimore	across@medicine.umaryland.edu		yes
Xu, Yi	Cell wall proteins in bacillus anthracis as vaccines	WRCE	Texas A&M University			
Crystal, Ronald	Abstract #6 Theme 3 Crystal: Anti-B. anthracis vaccination and passive protection	NBC	Weill Medical College of Cornell University			
Koehler, Theresa	Bacillus anthracis - host interactions	WRCE	University of Texas Health Science Center at Houston	theresa.m.koehler@uth.tmc.edu		yes
Casadevall, Arturo	Abstract #2 B cell related intervention	NBC	Albert Einstein College of Medicine	casadeva@aecom.yu.edu		yes
Hewlett, Erik L.	Abstract #3 Project 1.3: Actions and interactions of the toxins of B. anthracis	MARCE	University of Virginia	eh2v@virginia.edu		yes
Collier, John R.	Project 1A: Direct inhibition of anthrax toxin	NERCE	Harvard Medical School			
Lyons, C. Rick	(mentioned in Xu abstract)	WRCE	University of New Mexico Health Science Center	clyons@wrce		yes
Brojatsch, Jurgen	Abstract #17: Role of tissue-specific cell killing in anthrax toxin-mediated cell killing	NBC	Albert Einstein College of Medicine			
Joachimiak, Andrzej	Therepeutic inhibition of B. anthracis pathogenesis	GLRCE	University of Chicago			

Table 3-3: Finalized anthrax cluster

Establishing Contact

Having finalized the composition of the anthrax cluster, I began designing a packet of information for distribution to each of the eleven cluster members. This packet ultimately consisted of the following elements:

1. An introductory contact letter explaining the purpose and goals of the project
2. A still image of the anthrax network generated by eTSNAP
3. A spreadsheet containing each cluster member's name, project title, RCE and institution affiliations, available contact information, and videoconferencing capabilities along with a brief statement identifying the unifying aspects of research within the cluster.
4. A compilation of the research abstracts obtained from each cluster member.
5. A brief survey designed to assess preexisting attitudes regarding collaboration, current trends in communications practices within and between RCEs, and individual familiarity with common tools of communication.

I merged items 1 through 4 into a single Word (.doc) document, importing the still image as a TIFF (.tif) file and the spreadsheet as an Excel (.xls) file. Finally, to preserve document formatting, I elected to export the word document as a PDF (.pdf) file (see appendix C for a copy of this document).

To lend credibility to the project, Linda Gunn, project coordinator for the Garner labs, assumed the role of intermediary in my correspondence with the cluster members, managing the distribution, via email, of the introductory information packet along with subsequent communications. Several weeks following the distribution of the introductory packet, one completed survey embodied the entire response to the project. When multiple ensuing requests failed to generate more than two additional responses, I conceded defeat in my attempts to instigate collaborative activity within the identified anthrax cluster.

These unforeseen circumstances prompted further exploration into factors that influence the successful implementation of collaborative research practices. In addition to an extensive secondary literature review, I developed a secondary survey with the intent of eliciting various reasons for nonparticipation as well as opinions regarding collaboration in general.

CHAPTER FOUR
Results

PRELIMINARY SURVEY

Development

The design of the preliminary survey (figure 4-1) considered three primary areas of focus: prevailing attitudes toward collaboration, availability of and familiarity with various communications resources, and collaborative history of the participants.

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the dimensions of this figure; please refer to figure4_1.pdf,
located in the supplemental images folder.

Figure 4-1: preliminary survey

The assessment of current sentiments toward collaboration along with the acquisition of focused histories regarding previous collaborative practices would provide an estimation of collaborative readiness and identify habits of prior collaborations, while an inventory of preferred and available communications tools would permit customization of communications strategies. Adequately collecting the necessary information required the use of several question formats: yes / no, checkboxes, free response, and multiple choice, with psychometric responses graded on a forced-choice six-point Likert scale. Within the initial information packet distributed via e-mail to each member of the anthrax cluster, a hyperlink directed participants to the anonymous HTML-based survey, temporarily located at the following URL: http://www.rcebiodefense.org/quest/index.html. The creation of a back-end database automated the collection of responses, with the final results presented in spreadsheet format. As mentioned previously, multiple requests for participation elicited only three responses.

Results

Despite the disappointing number of responses, the results of the preliminary survey nevertheless provided a substantial amount of constructive information. In general, responses reflected a favorable opinion of collaboration, despite a perceived reluctance, on the part of the respondents themselves and the respondents' colleagues, to engage in collaborative activities. All respondents reported having access to a wealth of communications resources including teleconferencing, internet, and e-mail technologies, with two respondents additionally indicating they had access to videoconferencing equipment. Nevertheless, responses revealed a preference for less technologically-advanced methods of communication, such as e-mail, telephone, and meetings; respondents expressed greater discomfort with videoconferencing, bulletin boards, and internet chat applications. Finally, all researchers who completed the survey reported personal involvement in some aspect of collaborative research, and while all respondents considered the collaborative experience worthwhile, two of the respondents realized tangible benefits of collaboration in the form of a grant application and manuscripts. (see table 4-1 for a summary of results)

(table 4-1 appears on next page)

Question:	Researcher 1	Researcher 2[1]	Researcher 3
1. I think that research collaboration is important to research productivity.	5	2	5
2. I am receptive to the idea of collaboration.	did not answer	2	4
3. I have found other RCE researchers to be receptive to the idea of collaboration.	5	2	4
4. Do you have access to the following forms of communication? (check all that apply)	Videoconferencing Teleconferencing Internet E-mail	Teleconferencing Internet E-mail	Videoconferencing Teleconferencing Internet E-mail
5. I am very comfortable using videoconferencing technology.	4	2	3
6. I am very comfortable using teleconferencing technology.	6	5	5
7. I am very comfortable using internet message board technology.	3	2	3
8. I am very comfortable using internet chat room technology.	3	5	3
9. I am very comfortable using email messaging technology.	6	1	6
10. If you have initiated contact with another RCE researcher, how did you discover him / her and his / her contact information?	Telephone	By national group meetings - RCE / PUBMED	Scientific meetings publications
11. Please briefly describe the resources available to help you locate a fellow researcher interested in a collaborative effort. (I.e. database, directory, meeting abstracts, RCE meetings, etc.)	RCE meetings and directories	PUBMED and RCE websites	all of those listed
12. Have you tried to contact other researchers working on projects like yours?	yes	yes	yes
13. How often do you attempt to contact researchers who might be working on similar projects?	once	occasionally	occasionally
14. Are the other researchers whom you have contacted members of your region?	yes	yes	no
15. Have you contacted members of other RCE regions?	yes	yes	yes
16. Was a collaboration formed after this contact?	yes	yes	yes
17. Was the experience worth the effort?	yes	yes	yes
18. Was something produced from the collaboration?	grant application, manuscript	not yet	manuscript
19. How did you contact this / these individual(s)? (check all that apply)	telephone internet	email	internet email scientific meeting
20. Which method was most valuable?	internet	email	scientific meeting
21. How often do you contact [members of the anthrax cluster]?	not applicable	regularly	regularly
22. How do you most often contact [members of the anthrax cluster]? (check all that apply)	not applicable	teleconference e-mail phone call	e-mail
23. What do you see as the most valuable thing you would get from a collaborative effort and why? (i.e. sharing reagents, sharing technology / cores, joint publications)	did not answer	sharing reagents joint publications	all of those listed[2]

Note: questions 1-3 and 5-9 follow a six-point Likert scale: 1 indicates strong disagreement and 6 indicates strong agreement with the given statement.

[1] Researcher 2 appeared to have reversed the Likert scale, given the consistent and polar disagreement with other researchers and the contradictory responses to certain questions, such as #9 and #20.

[2] Researcher 3 continued: "please note that with the exception of 2 investigators listed in your table, I already know these investigators and feel free to communicate with them whenever I see a need."

Table 4-1: Summary of preliminary survey responses

SECONDARY SURVEY

Development

In consideration of the poor response to the preliminary survey, the design of the secondary survey assumed an entirely different format from that of the former. Developed with the intent of gaining detailed insight into researchers' perspectives on collaboration as well as motivations for non-participation in this specific project, the secondary survey consisted of seven questions, primarily free response, which served as a guide for an informal telephone conversation. Conducting the survey via telephone minimized the effort required for participation and created an informal atmosphere conducive to the collection of more detailed and significant information. On account of his status and familiarity with the conventions of scientific research, Dr. Garner conducted the phone interviews via speakerphone while I recorded the responses. A summary of all four completed interviews appears below (see appendix B for completed surveys in their entirety). The initial series of telephone calls yielded two responses, while subsequent efforts procured an additional two surveys. A summary of the responses to the secondary survey appear below.

Results

Question 1: Do you recall receiving any correspondence regarding collaboration opportunities within the RCE on the subject of anthrax

Question 2: Did you take the time to read the cover letter? Look over the map of the cluster? Read the abstracts of the cluster members? Look over and/or complete the survey?

Responses to preliminary questions ascertained that all respondents did indeed remember receiving correspondence regarding collaboration opportunities within the RCE. Researchers A and B reported having reviewed all components, while Researcher C read only the cover letter, and Researcher D took no more than a cursory glance through the packet.

Question 3: Of the components that you did review, did anything catch your interest of compel you to keep reading? If so, what?

The responses of Researchers A and B, the only researchers to review all sections of the introductory information packet, demonstrated considerable variability. Researcher A reported

that he found the concept of the project intriguing, mentioning expressly the computer-generated social networking model. Researcher B, however, did not specifically address the question, but stated on numerous occasions that the project fundamentally failed to impress her.

Question 4: Did you glean any useful information from this initial packet of information sent to you? If so, what?

Once again, this question applied exclusively to Researchers A and B on account of their having reviewed the packet. Researcher A admitted that although he had a solid understanding of the current topics and major researchers in the field, he still considered the compilation of abstracts useful in keeping him apprised of current progress in the field of anthrax research. Researcher B, in contrast, felt that her twenty years in the field provided her with sufficient knowledge regarding the state of anthrax research, and she therefore did not garner any useful information from any element of the initial information packet.

Question 5: For what reason(s) did you choose not to participate in this project?

Researchers A and C both cited a lack of time as the principle reason for non-participation; researcher A, specifically, mentioned approaching deadlines for grant applications along with his recent attendance at a week-long conference as primary contributors to his lack of time. Researcher C also elaborated on his response, stating that the demands of research-related operations preclude many scientists from engaging in subsidiary activities, and that any such activities would have to demonstrate substantial benefits before scientists agree to divert time away from their research. Researcher B, again, felt that her experience and status already supplied her with ample knowledge of key researchers and significant developments in the field of anthrax research, and therefore chose not to participate. Additionally, researcher B revealed that she regularly collaborates with individuals within her own RCE and does not foresee any value to potential collaborations beyond those in which she already participates; moreover, should she deem additional collaboration necessary, she feels comfortable initiating these partnerships on her own. Researcher D did not answer the question.

Question 6: Do you feel collaborative efforts have potential value for your research efforts?

Researchers A, C, and D all responded favorably, with researcher C specifying two situations in which he felt collaboration had particular value: the emergence of new information or the need of a particular researcher to acquire resources outside the realm of his or her primary research. Researcher D expressed a strong desire to collaborate, mentioning that his anthrax vaccine outperformed competitors in murine models and that he would like to pool resources with colleagues in order to proceed with trials involving non-human primates. Her response deviating from those of the three other researchers, Researcher B declared that she did not see any value to collaboration as proposed by this project, though stated further that she believed this exercise may prove worthwhile in providing new researchers with a channel through which to identify major researchers and relationships within a particular field of research.

Question 7: Is there anything you feel would encourage or entice collaborative effort from you or your colleagues?

Although none of the researchers interviewed recommended specific strategies to elicit participation in collaborative activity, most alluded to the presence of certain obstacles which they felt discouraged collaboration. Researcher A asserted that, even within his own RCE, competition between PIs and a resulting reluctance to discuss works in progress severely hinder the success of collaborative endeavors. Similarly, researcher B indicated that effectual collaborations must cultivate an environment built upon mutual trust, for scientists naturally have a tendency to protect unpublished work; this penchant for secrecy, as researcher B pointed out, directly conflicts the NIH's goal of collaborative research. Additionally, Researcher D described a perceived need for a more nationally-coordinated and goal-driven approach to vaccine development. Researcher C did not answer the question.

CHAPTER FIVE
Conclusions and Recommendations

Project Summary

Developing a model that successfully identifies opportunities for scientific collaboration through the use of text data mining technology initially defined the goal of this project; however, unforeseen circumstances forced a re-assessment of original intentions, with the identification of variables predicting collaborative outcomes thus emerging as a secondary goal. The project commenced with the requisite procedures of data selection and preparation; NIH grant application abstracts of research projects from eight RCE regions comprised the data set, assuming the form of a single tag-delimited plain text document. Submission of this document to eTSNAP, along with the selection of a threshold value, enabled the computation of similarity scores and subsequent presentation of results in matrix, tabular, and interactive map formats. Through integration of tabular and interactive map displays, analysis revealed the presence of eight clusters of abstracts, each demarcated by bonds of similarity. Subsequent analysis of the structural and functional attributes of each cluster permitted the selection of a single cluster with the greatest perceived likelihood of success. This cluster, labeled the "anthrax cluster," then underwent further analysis and modification to ensure cluster integrity. An introductory information packet established initial contact with members of the finalized anthrax cluster and solicited participation in a preliminary survey. The unsatisfactory response to multiple requests for participation prompted the development of a secondary survey and the execution of an extensive literature review to elucidate the reasons for this failure.

Conclusions

In conclusion, the results of both surveys, in conjunction with information garnered from the literature review, indicate that a multitude of intertwined variables ultimately determines the outcome of a collaborative endeavor. Similar research interests alone cannot support collaborative relationships, and thus the effectiveness of eTSNAP in discovering opportunities for collaboration remains largely undefined. Most scientists acknowledge certain benefits of collaborative research yet, in practice, have difficulty surmounting the prevailing competitive tendencies of scientific industry. Particularly within the field of biomedical research, rapid expansion and specialization of scientific intelligence have instigated a trend toward collaborative research along with external

encouragement from institutions such as the NIH and private research establishments. Given the potential benefits of collaboration, research groups who collaborate effectively set a precedent for quality of research that makes it difficult for others to compete, thus reinforcing the tendency toward collaborative research practices.

Recommendations for Further Research

Given the significance of this trend toward scientific collaboration, efforts directed toward the development of a standardized method of describing and evaluating collaborative environments would allow comparative analysis of these environments and subsequently facilitate the detection of the various effects of certain conditions. Similarly, controlled experiments exploring factors believed to influence collaborative outcomes would also promote a functional understanding of the collaborative process and the variables impacting its success. Finally, exploring the utility of eTSNAP in additional contexts could determine the most appropriate application of this tool, given its current limitations;. Future modifications to eTSNAP's functionality that acknowledged variables other than strictly text similarity would redefine its purpose as an application committed to discovering opportunities for collaboration.

APPENDIX A

eTSNAP Input Document

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<TITLE>: NERCE National Small Molecule Screening
Laboratory
<AUTHORS>: Stephen Lory, Caroline Shamu, Su Chiang
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>: Harvard Medical School
<EMAIL>: Email
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<TEXT>: The National Small Molecule Screening
Laboratory for the RCEs in Biodefense and Emerging
Infectious Disease (NSRB) was established in 2004 with
New Opportunities funds to provide access to high-
throughput screening (HTS) capability for all U.S.
investigators conducting research on NIAID Priority
Pathogens.  The NSRB is a fully equipped screening
facility, staffed by personnel with expertise in
microbiology and HTS, and a medicinal chemistry group
to aid in advancing screening positives towards
pharmaceutical development. The NSRB supports multiple
screening platforms and interacts closely with other
screening programs at HMS.  The NSRB has recruited
multiple projects and is currently supporting 35
projects at various stages, over half of which are
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from outside the New England region. The NSRB Advisory Committee has fully endorsed this report and is pleased with the progress made over the current funding period.
<END TEXT>

<TITLE>: NERCE Project 1C
<AUTHORS>:
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
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<ADDRESS>: CBR Institute for Biomedical Research, Inc.
<EMAIL>: Email
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<TEXT>: Because vesicle trafficking has never been a major target for pharmaceutical development, few chemical agents are available to perturb specific aspects of membrane traffic. Those that do exist have provided considerable insights; for example, the importance of cholera toxins traffic from the Golgi and endosomal network to the endoplasmic reticulum (ER) was discovered using Brefeldin A. We recently developed an imaging-based medium-throughput screen with intact cells to identify chemicals that affect different pathways of membrane traffic. From two different screens and using over 19,000 compounds, we identified two chemicals, exo2 and secramine, with unique and specific effects on the function of cholera toxin and edema factor of anthrax. To expand on these results we propose studies whose goals are to determine the molecular basis for the biological activity of secramine and to elucidate its molecular target.
<END TEXT>

<TITLE>: NERCE PROJECT 1D
<AUTHORS>: John R. Murphy, Ajit Bharti
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation

<TYPE>: Type
<ADDRESS>: Boston Med Ctr
<EMAIL>: Email
<PHONE>: Phone
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<TEXT>: The catalytic domains of diphtheria toxin, anthrax lethal factor, anthrax edema factor, and all serotypes of botulinum neurotoxin are delivered into their respective target cell cytosols from acidified early endosomes. Using the diphtheria toxin-related fusion protein DAB389IL-2 as a model system, we have shown in vitro that the delivery of the diphtheria toxin catalytic domain to the external milieu requires the active participation of a cytosolic translocation factor (CTF) complex. While the full composition of this complex is not fully known, we have demonstrated that the heat shock protein Hsp 90 and thioredoxin reductase are essential for this process. In addition, we have recently identified a ten amino acid motif in transmembrane helix 1 of diphtheria toxin that is essential for membrane translocation of the catalytic domain. Furthermore, using the fusion protein GST-DT140-271 in pull down experiments, we have shown that that amino acid sequences that at least overlap the translocation motif specifically interact with -COP. Since anti--COP inhibits in vitro translocation of the catalytic domain, we now conclude that -COP is also an essential component of the CTF complex. We are currently examining by dose response analysis the protective antigen dependent entry of anthrax lethal factor into the cell using the fusion protein LFn-DTA. Using Hut102/6TG and diphtheria toxin resistant Hut102/6TG-T1 cells, we now find that the expression of the T1 motif in Hut102/6TG-T1 cells confers resistance to LFn-DTA intoxication. We are currently examining the role of the translocation motif in the translocation of anthrax LF by site directed mutagenesis. In addition, current work is underway to determine the precise site of interaction between the T1 motif and -COP. Since expression of the T1 peptide in target cell confers toxin resistance, we are working toward the identification of this binding site as the first step in the

development of an assay to screen for specific competitive inhibitors that will block toxin catalytic domain entry into the cytosol.
<END TEXT>

<TITLE>: NERCE PROJECT 2A
<AUTHORS>: Jon D. Goguen, Ph.D.,Egil Lien, Ph.D.,Shan Lu, M.D.,Hardy Kornfeld, M.D.

<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>: University of Massachusetts Medical School,Worcester, MA 01655
<EMAIL>: Email
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<TEXT>: Yersinia pestis, the causative agent of plague, is a major concern with respect to misuse in the context of bioterrorism or biological warfare. It is the only bacterial agent among the highest priority pathogens that is transmissible from person to person. The transmissible form of disease, pneumonic plague, may be initiated by aerosol infection. Pneumonic plague requires rapid treatment (within 18 hours of the onset of symptoms), and in the absence of such treatment is virtually uniformly fatal. Unfortunately, in the early stages, this disease resembles flu, making timely diagnosis of index cases difficult. There is currently no effective vaccine for prophylaxis against pneumonic plague. Moreover, antibiotic-resistant strains occur in nature and have also been intentionally produced for use as weapons. This project has two major goals: identification of novel antigens for use in improved subunit vaccines, and the development of small-molecule inhibitors of Type III secretion. This secretion function is essential for the virulence of Y. pestis, making such inhibitors potential therapeutics distinct from traditional antibiotics. Our strategy for the discovery of new antigens is based on screening

proteome arrays containing nearly all proteins produced by Y. pestis with antisera produced against a specially engineered highly immunogenic Y. pestis derivative. Discovery of compounds inhibiting Type III secretion is based on a novel high-throughput screening assay, which we have already used to screen in excess of 70,000 candidate compounds.
The products of this project are intended to promote public health and safety by providing an effective plague vaccine and new treatment options.
<END TEXT>

<TITLE>: NERCE PROJECT 3
<AUTHORS>: John Mekalanos, Darren Higgins, David Knipe
<JOURNAL>: Journal
<DATE>: Date
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<ADDRESS>: Harvard Medical School
<EMAIL>: Email
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<TEXT>: The objective of this project is to develop vector systems that facilitate immunization with antigens of biodefense (BD) relevance, with emphasis on allowing rapid construction of vaccine prototypes and evaluation of immune responses against expressed antigens. The vector systems under development include live vaccines based on Vibrio cholerae, killed vaccines based on E. coli, and live viral vectors based on replication-incompetent herpes simplex virus (HSV). Each system will be tested for ability to promote immune responses to anthrax protective antigen (PA), listeriolysin O (LLO), and West Nile envelope protein (WNE). Progress has been made with all three systems, and we anticipate assessing several prototype vaccines in animals during the next funding period.
<END TEXT>

<TITLE>: NERCE PROJECT 4
<AUTHORS>: Dennis L. Kasper

<JOURNAL>: Journal
<DATE>: Date
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<ADDRESS>: Harvard Medical School
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<TEXT>: Francisella tularensis is a highly infectious bacterium that poses a serious threat as an agent of bioterrorism. A live whole cell vaccine is currently available for at-risk populations, but it is associated with incomplete immunity and side-effects. Studies of humans and mice vaccinated with this preparation indicate that humoral and cell-mediated immune responses are required for complete protection in the infected host. Currently, little is known concerning the virulence factors associated with F. tularensis that contribute to its ability to cause lethal disease. In addition, few immunogenic molecules from this organism have identified. We hypothesize that the development of a safe and effective vaccine for the prevention of tularemia will require generation of conjugate vaccines that comprises both the O-antigen and immunogenic proteins expressed by this organism. To address this question, we propose to: 1) Determine the humoral and cell-mediated immune responses to the proteins of F. tularensis; and 2) Develop a conjugate vaccine for F. tularensis infections that comprises the O-antigen and the identified immunogenic proteins. These studies will employ a proteomics-based approach to identify new immunogenic proteins from F. tularensis that can be used as carriers in the development of novel acellular glycoconjugate vaccines. It is anticipated that these vaccines will activate both humoral and cell-mediated immune responses and elicit complete protection against tularemia. Glycoconjugate vaccines have been among the most effective biologics ever developed for the prevention of bacterial infections. It is expected that this approach can be applied successfully to the development of a vaccine that can

ultimately be tested in clinical trials for the prevention of tularemia.
<END TEXT>

<TITLE>: NERCE PROJECT 5
<AUTHORS>: Stephen C. Harrison
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>: Harvard Medical School
<EMAIL>: Email
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<TEXT>: Enveloped viruses initiate infection by fusing their membranes with a membrane of the host cell. Compounds that inhibit this step can be useful anti-viral drugs, as demonstrated by work on HIV-1. Structural studies of flavivirus envelope proteins show that the conformational changes that lead to fusion by these class 2 viral fusion proteins resemble in certain critical respects those seen in class 1 fusion proteins, such as the envelope protein of HIV. In particular, the two stages at which proven HIV entry inhibitors operate (by targeting a pocket in the prefusion structure and by inhibiting the zipping up of the postfusion structure, respectively) have parallels in flavivirus fusion. We therefore seek inhibitors that block one or the other of these two steps. We have developed an assay to screen small-molecule libraries for inhibitors of stem-peptide binding to the trimeric core of dengue virus E in its postfusion conformation. We will pursue this screen, using the high-throughput facilities of the NERCE screening core. We expect such molecules to retard or inhibit fusion, by preventing the stem from zipping up during the final stages of the fusion process. We will also synthesize compounds that bind in the kl-pocket of the dimeric, prefusion conformation, and use suitable competition assays to screen libraries for more drug-like molecules that may inhibit entry by occupying this site. Structural studies will

accompany both of these efforts, and assays for inhibition of viral entry will be carried out by a collaborator.
<END TEXT>

<TITLE>: NERCE PROJECT 7
<AUTHORS>: Edward Goldberg
<JOURNAL>: Journal
<DATE>: Date
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<TYPE>: Type
<ADDRESS>: Harvard Medical School
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<TEXT>: Our long-term goal is to develop a hand-held diagtnostic device that will use a versatile and robust non-destructive detection method to distinguish a variety of cellular, viral, and molecular targets in patient samples. Data for multiple diagnostic targets in a single sample will be analyzed, reported on the screen, and transmitted digitally, effectively in real time. Our immediate goal in this proposal is to develop the core sensing technology for this device. To provide an initial test system for assay of anthrax infection, we will use a high-affinity peptide specific to Bacillus anthracis protective antigen (PA) protein. PA is the central soluable component of the tripartite anthrax toxin and is most often used as an index of toxin production.
SPECIFIC AIMS:
1. To demonstrate the feasibility of the proposed approach we will construct a monodisperse composite sensor and test its ability to indicate the presence of a specific target.
2. To demonstrate the feasibilty of multiplex operation we will construct sensors of different lengths targeting different molecules and quantify their differential responses.
3. To demonstrate feasibility for diagnosis of clinically relevant targets we will test complete nanosensors with a ligands specific to PA. When mixed

with the target in solution, the sensor will bind the target and report changes in signal frequency and amplitude to an external analyzer.
Relevance to Public Health: Our system will be designed to use patient samples with little or no sample preparation. Groups of sensors can be designed for the identification of a variety of disease states and for differential diagnosis. The proposed device could be used by many types of first-responders with minimal training in hospitals, clinics, and other settings. Real-time, non-invasive monitoring of blood and other samples for health hazards by non-experts enhances the possibility of remote pre-symptomatic diagnosis by health care providers as well as the effectiveness of bedside treatment by direct monitoring of target proteins appropriate to the condition. Diagnostic devices of this type will be increasingly important to biodefense and public health as our database of molecular markers for pathogens continues to expand.
<END TEXT>

<TITLE>: NERCE PROJECT 1A
<AUTHORS>: R. John Collier
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>: Harvard Medical School
<EMAIL>: Email
<PHONE>: Phone
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<TEXT>: Besides exposing our general vulnerability to bioterrorism, the anthrax attacks of the fall of 2001 alerted the nation to the need to develop therapies and vaccines against Bacillus anthracis. B. anthracis produces two major virulence factors an antiphagocytic poly-D-glutamic acid capsule and a tripartite toxin. Injection of the purified toxin causes rapid death of sensitive rodents and non-human primates, and immunization against the toxin protects against infection. Thus it is generally believed that

death from anthrax infections results from the effects of the toxin. As a step towards developing new therapeutic interventions for anthrax, we have used high-throughput screens to identify low molecular mass compounds that inhibit the action of anthrax toxin at the cellular level. We have identified a number of inhibitors of anthrax toxin action. Building on the experience of the Collier laboratory in studying anthrax toxin, we will characterize these promising inhibitors to determine the specific step in toxin action that each inhibits. Lead compounds with low toxicity will be considered for testing in animal models of toxin action.
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<TITLE>: SERCEB Abstract#8 Project 1.1
<AUTHORS>: Garber
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<TEXT>: Concerns about the potential use of variola as an agent of bioterrorism have recently resulted in decisions to re-initiate smallpox vaccination programs. However, the currently available vaccinia-based smallpox vaccines (eg, Dryvax) are associated with high rates of adverse reactions and are not safe for use in immunodeficient individuals or those with a variety of common medical conditions. As such, development of new smallpox vaccines that are substantially safer, but of equivalent or better immunogenic potency than the current VV vaccine preparations is imperative. While certain attenuated strains of vaccinia, especially modified vaccinia Ankara (MVA), have highly desirable safety features and impressive immunogenicity when used to express heterologous antigens, a variety of data suggest that MVA may be an insufficiently immunogenic vaccine to

reliably engender protective responses against variola or other highly pathogenic orthopoxviruses. The overall goal of the proposal is to genetically modify the currently available strain of MVA, so that novel vaccine variants are derived with preserved safety profiles, but with substantially enhanced abilities to raise durable, high level humoral and cellular antiviral immune responses that are cross-reactive with major pathogenic orthopoxviruses. To accomplish this goal, we propose to (1) delete from the MVA genome, residual viral immune evasion genes; (2) express within recombinant MVAs specific cytokines and chemokines that promote DC recruitment and activation; and (3) express specific late viral structural gene products from early vaccinia promoters in recombinant MVAs to facilitate induction of increased host protective immune responses against key orthopoxvirus antigens.
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<TITLE>: SERCEB Abstract#9 Cross Protection Against Multiple Poxviruses
<AUTHORS>: Johnston
<JOURNAL>: Journal
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<TEXT>: Vaccination with the current poxvirus vaccine, vaccinia virus (Dryvax), effectively cross protects against multiple orthopoxviruses, but can result in serious complications. Since cessation of routine vaccination, the population for which the vaccine is contraindicated has grown, making vaccination more dangerous for a larger proportion of the population as a whole. As smallpox has been eradicated, the risks of mass vaccination with Dryvax may not outweigh the benefits of protection against a possible bioterrorist attack. The primary goal of our project is to use VEE

Replicon Particles (VRP) expressing immunodominant, protective poxvirus proteins as a vaccine that will be safe and cross protective against multiple orthopoxviruses. The VRP system directly targets dendritic cells allowing efficient antigen expression, processing, and presentation to the immune system resulting in long lasting systemic and mucosal immunity. Vector-based immunity does not appear to interfere with the response to VRP transgenes and only a small fraction of the worlds population has ever been exposed to VEE, thus reducing the importance of pre-existing anti-vector antibodies. VRP expressing HIV gag are currently in Phase I clinical trials. The cowpox virus structural proteins A33R, L1R, B5R, A27L, A17L, and A10L have been cloned and packaged into VRP. Balb/C mice primed and boosted with with B5R-VRP retain a high anti-B5R serum IgG titer through 50 weeks post-prime. The B5R reciprocal endpoint serum IgG titer from mice vaccinated with B5R-VRP is approximately ten fold higher than mice vaccinated with purified B5R protein. Furthermore, Balb/C mice vaccinated with a cocktail of B5R, A33R, L1R, and A27L-VRP had an anti-B5R serum IgG titer equivalent to mice vaccinated with B5R-VRP alone, indicating that a cocktail of several different VRP may be used for vaccination studies without obvious immunodominance or masking. Mice vaccinated with A27L-VRP, B5R-VRP, A33R-VRP individually or with a cocktail of A27L, B5R, A33R,
and L1R-VRP and challenged intranasally with 106 PFU the Brighton red strain of cowpox virus lost markedly less weight and exhibited less severe signs of clinical illness than mice vaccinated with the irrelevant HA-VRP vaccine control. Mice vaccinated with L1R-VRP were not protected against weight loss or clinical signs of illness after cowpox virus challenge. These initial protection studies are highly encouraging and suggest that engineered VRP viral vector vaccines may effectively protect against orthopoxviral diseases.
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<TITLE>: SERCEB Abstract#10 - Project 1.3:Anti-Orthopox Immunomodulatory Molecule Vaccine
<AUTHORS>: David Pickup
<JOURNAL>: Journal
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<TEXT>: The orthopoxviruses encode a large number of accessory proteins that suppress host immune responses to infection. Major targets of these viral accessory proteins include complement, IL-1, IL-18, TNF, CC-chemokines, type I IFN, type II IFN, and CD30L. These accessory proteins often contribute to the virulence of these viruses by inhibiting or delaying innate and adaptive responses to the infection.

The objectives of this project are to determine if protection against poxviral infection and disease will be maximized by vaccines designed to target not only structural proteins, but also the poxviral accessory proteins that interfere with host immune defenses. We hypothesize that the inactivation of these viral accessory proteins in the vaccine virus will both reduce the reactogenicity of the vaccine and increase the immunogenicity of the vaccine by abrogation of the immunosuppressive actions of these proteins. Although inactivation of these proteins might be achieved by deletion of the genes encoding these proteins, humoral and cellular immune responses against the viral accessory proteins encoded by the vaccine virus are also predicted to play an important role in protection against subsequent infection by viruses such as variola virus that encode similar accessory proteins. Therefore, to achieve maximum protective capacity we are constructing vaccine viruses that express inactive but immunogenic versions of these accessory proteins.

We are using Venezuelan Equine Encephalitis (VEE) replicon vectors expressing inactivated versions of

the cowpox virus accessory proteins, or purified accessory proteins, as immunogens to identify the most important accessory proteins for protective responses assayed in a cowpox virus (CPXV)-mouse challenge model. The specific aims of this project are:
1. Construct VEE replicons expressing mutationally inactivated CPXV accessory proteins.
2. Assemble and test prototype VEE replicon particle (VRP) vaccines for CPXV accessory proteins.
3. Evaluate the efficacy of prophylactic and therapeutic VRP-CPXV-accessory protein/purified protein vaccines in the mouse model.
4. Construct and test the efficacies of vaccinia virus MVA vaccines expressing inactive CPXV accessory proteins that affect immune responses to infection.
This strategy will enable us to develop novel vaccines and therapeutics for use against virulent poxviruses. In addition, it will help us to improve the safety and efficacy of current poxvirus vaccines, including vaccinia.
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<TITLE>: SERCEB Abstract#11 2.1
<AUTHORS>: Richard Moyer
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<TEXT>: The emergence of drug resistant viruses is an important concern in the development of new antiviral compounds. This project addresses that issue at several levels. First we propose to isolate orthopoxviruses that are resistant to various antiviral compounds and then map the location of the drug resistance in the genome. This will provide data concerning the frequency of drug resistance and the mapping will offer insights into the mechanisms of drug action. The second part of the project will be

to analyze what effects the drug resistance has on the pathogenesis of the virus. Cidofovir is a lead compound in the search for antivirals against orthopoxviruses. Cidofovir is a nucleoside analog approved for use against HSV. The mode of action for CDV has been hypothesized to be through the viral DNA polymerase but this has not been demonstrated for poxviruses. To date, we have isolated and mapped cidofovir resistant vaccinia viruses. All of the resistance mutants that we have isolated map to the DNA polymerase gene. The effects of CDV resistance on pathogenesis are being examined in collaboration with Dr. Earl Kern, University of Alabama. The technologies that we have developed are applicable to other antiviral drugs.
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<TITLE>: SERCEB Abstract#12 Development of Antiviral Therapeutics against Orthopox viruses
<AUTHORS>: Whitley
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<TEXT>: The primary aim of this project is to develop and evaluate potentially active compounds for treatment of smallpox and other orthopox viruses. The effort requires evaluation of new potential drug targets, high throughput screening of potential antiviral compounds, and design of new compounds based on the structure of selected target proteins. During the past year, the project teams have made great progress in identifying a number of potential soluble targets and prioritizing these targets in order to begin development of screening assays. Using in vitro assays, researchers have evaluated a total of 545 compounds for future testing of efficacy in animal models. Significant progress has been made also in

the synthesis of potential orally active prodrugs related to PMEA and PMEG. To date, 76 agents have been synthesized, and several of the most promising compounds have been submitted for evaluation against smallpox virus. Future plans for this program include continued development of therapeutic agents with improved selectivity and novel mechanisms of action against orthopox. In addition, the team will continue to synthesize new DAP and CPDAP phosphonate prodrugs and will pursue promising leads emerging from both the animal model screening of compounds in the Kern lab and testing of a number of prototypic antiviral drugs done in collaboration with other program researchers
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<KEYWORDS>: Keywords
<TEXT>: The Poxviridae family members Vaccinia and variola (a Class A pthogen) enter mammalian cells, replicate extranuclearly, and produce virions that travel to the cell surface along microtubules, fuse with the plasma membrane, and egress away from infected cells towards apposing cells on actin-filled membranous protrusions. We show that cell-associated enveloped virions (CEV) utilize Abl- and Src-family tyrosine kinases for actin motility, and that these kinases act in a redundant fashion, perhaps permitting motility in a greater range of cell types. Additionally, release of CEV from the cell requires Abl- but not Src-family tyrosine kinases, and is blocked by STI-571 (Gleevec), an Abl-family kinase inhibitor used to treat chronic myelogenous leukemia in humans. Finally, we demonstrate that STI-571

reduces viral dissemination by five orders of magnitude and promotes survival in infected mice, suggesting a possible utility of this drug in treating smallpox or complications associated with vaccination. Aim 1 will determine the mechanisms of recruitment of tyrosine kinases to the virion, and the targets of kinases associated withmotility and release of infectious virions. Aim 2 will determine whether Gleevec is effective against variola monkeypox and ectromelia infection in vitro. Aim 3 will determine the efficacy of Gleevec and PD, and other Abl inhibitors now in clinical trials as cancer therapeutics, in treating disease in mice infected with Vaccinia and ectromelia, and Aim 4 will determine whether these drugs affect the capacity of the immune system to facilitate clearance of the virus. This therapeutic approach may prove generally efficacious in treating microbial infections that rely on host tyrosine kinases, and because the drug targets host but not viral molecules, this strategy is much less likely to engender resistance compared to conventional anti-microbial therapies.
<END TEXT>

<TITLE>: SERCEB Abstract#14 3.1
<AUTHORS>: Parkos
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
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<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: The objective of the research proposed in Program 3 is to elucidate molecular mechanisms of orthopoxvirus attachment to host cells. Virus binding to specific host molecules on the cell surface is the initial interaction between virus and host. As such, virus-receptor engagement is a critical determinant of disease outcome and an attractive target for antiviral therapy. A potentially effective method to treat

orthopoxvirus infection would be to interfere with productive virus-receptor interaction using small-molecule inhibitors. To develop this approach, cellular receptors for orthopoxviruses and the appropriate viral attachment proteins must be identified. The goal of Program 3 is to begin this process by identification of host-cell receptors for vaccinia virus (VV).
<END TEXT>

<TITLE>: SERCEB Abstract#15 3.2
<AUTHORS>: Richard Moyer
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
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<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: Work on project 3.2 in the Moyer laboratory at the University of Florida has centered on the identification of virus proteins that are required for vaccinia virus to bind to and enter host cells. Proteins known to be involved in virus entry include A28, H2, A21, and L5. A temperature-sensitive mutant mapping to the A28 gene has been sequenced and found to truncate the A28 protein from 146 to 134 amino acids. Virions of VV A28 ts produced at 40 C can bind to cells normally, but are defective in entry, and cannot mediate cell-cell fusion ("fusion from without"). Entry of intracellular mature virus (IMV) into cells takes place by a fusion mechanism, and our evidence suggests that there is a hemifusion intermediate before virus cores are able to enter the cytoplasm. Interactions between entry proteins A28 and H2, and involving pairs of H2, A21, and L5, have been shown by coimmunoprecipitation. In addition we have studied proteins L1, F9, J5 and G6 by adding either FLAG or HA tags, and assessing interactions with known entry proteins. To date we have found interactions between A21 and L1, between L5

and L1, and between L5 and F9. The novel protein G6 is associated with both F9 and J5. We plan to construct conditional vaccinia mutants in which expression of a candidate entry protein (F9, J5, or G6) is inducible by IPTG, so that we can isolate virions lacking a specific protein and study their phenotype.
Virion membrane proteins likely to be involved in binding are being expressed in collaboration with Dr. Ming Luo at UAB. Purified F9, J5, and D8 proteins lacking the transmembrane domain ("ectodomains") are being tested for binding to susceptible cells, and for the ability to reduce binding of vaccinia IMV to cells. The D8 protein is a known binding protein and serves as a positive control.
We are testing mAbs against surface proteins on HeLa cells (produced by Charles Parkos at Emory) for ability to interfere with binding and entry of vaccinia virus. The mAbs were screened for interference with vaccinia growth by Terry Dermody and Bryan Youree at Vanderbilt, and then passed on to us for characterization in our assays for virus binding and entry into HeLa cells. We intend to use these mAbs to identify the cellular proteins needed for vaccinia virus binding and entry, in conjunction with other approaches based on vaccinia virus ligands that bind to attachment proteins and entry receptors.
<END TEXT>

<TITLE>: SERCEB Abstract#16 4.1
<AUTHORS>: Straley
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>:
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: : New Vaccines against pneumonic plague This project proposes to identify large (> 50 kDa) surface proteins that are expressed at 37oC by the plague

bacterium Yersinia pestis. These are being prioritized by conditions for expression, relative mRNA abundance, recognition by plague convalescent serum, indications from homology with the databases of potential virulence function, and protective efficacy against a non-encapsulated (F1-) strain against which vaccines currently in development will not protect adequately. 11 proteins are being evaluated. 10 were chosen based on homology to surface proteins in other pathogens. One of these has been used to vaccinate mice subcutaneously and was found to provide partial protection against 5 LD50 doses of an F1- pgm strain given intravenously. The 11th protein was an abundant protein recognized by a rabbit serum against an extract of surface proteins from Y. pestis grown at 37oC. Mice are being vaccinated by a combination of DNA and protein immunization with 8 of these proteins (plus vector alone and LcrV as a positive control). It is hoped that one or more of these will prove to be protective. Studies are being initiated in BSL3 with F1+ and F1- virulent Y. pestis to evaluate pathogenesis and expression of these proteins during lung infection of mice. Our findings will enhance our arsenal of means to protect against potential bioterrorism use of Yersinia pestis and also will improve our understanding of the pathogenesis of pneumonic plague. Our aims are: Aim 1. Identification of large surface proteins expressed in the mouse lung after aerogenic challenge. Aim 2. Characterization and sorting of surface proteins. Aim 3. Immunization studies.
<END TEXT>

<TITLE>: SERCEB Abstract#17 Innate Immune Mediators and Select Agents
<AUTHORS>: Jenny Ting
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>: Uiversity of North Carolina at Chapel Hill Lineberger Comprehensive Cancer Center

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Chapel Hill, NC 27599
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<KEYWORDS>: Keywords
<TEXT>: The discovery of Toll-like receptors (TLRs) in mammalians cells has revolutionized the understanding of innate immunity. These molecules constitute pattern recognition membrane molecules that recognize pathogenic products, including LPS, flagellin, proteoglycan, lipoteichoic acid, mycobacterium LAM, double stranded RNA, and bacterial CpG. The range of products derived from gram positive and negative bacteria, mycobacterium, fungi and viral products all activate the TLR pathways. As a consequence of TLR activation, cytokines that can activate adaptive immunity are also produced, thus they serve as a bridge between innate and adaptive immunity. Despite the pivotal role of these receptors, their involvement in defense against select agent is less well defined. In this proposal, we plan to directly examine the roles of TLRs and its downstream regulators in defense against a select agent, Yersinia pestis. We will focus on known TLRs and their downstream regulators as well as a new downstream regulator that we recently identified, the Monarch-1 protein. Monarch-1 downregulates TLR responses, including NF-/AP-1 activation, and TNF-a response. Thus it constitutes a novel suppressor of the TLR response that is likely to affect immunity against natural infection and vaccination. Accordingly, the Aims are (1) To produce cell lines lacking known and new molecules in the TLR pathway by the use of interference RNA (RNAi technology). We plan to generate two sets of monocytic THP-1 cells that are defective in Monarch-1 as well as selected TLRs and their downstream signals including TRAF2, IRAKs, MyD88, and Md-2. (2) To determine if the removal of the genes targeted in Aim 1 causes alterations in responses to Y. pestis, both in infective and cytokine production. (3) To understand how the removal of these genes change responses to Dengue virus.
<END TEXT>

<TITLE>: SERCEB Abstract#18 T-Regulatory Cell Modulation of Vaccine Repsonses
<AUTHORS>: Gregory Sempowski
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>: RP1-Room 113
Research Drive
Durham, NC 27710
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<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: Safely amplifying immune responses to vaccines is an important goal in development of emerging infection vaccines. One potential mechanism to enhance vaccine responses would be transient removal of regulatory cells of the adaptive immune system which are responsible for maintaining an endogenous level of immune suppression. Regulatory CD4+ T cells constitutively expresses CD25 and are ~10% of the peripheral T cell pool. T regulatory cells are thymus-derived endogenous T cells that actively suppress T and B cell immune responses and have been implicated in controlling autoreactive immune responses. We hypothesize that transient removal of regulatory T cells will enhance responses to weak or suboptimal immunogens. To test this hypothesis we have developed a model of transient CD4+/CD25+ T-reg cell depletion with monoclonal antibody in Balb/C mice. The overall goal of this project is to determine if transient T regulatory cell depletion or CD7/CD7L signaling will be beneficial as immune stimulant
or immune modulatory regimen to improve immune responses to weak or suboptimal immunogens.
<END TEXT>

<TITLE>: MARCE Abstract#1 RP 1.1: B. anthracis spore antigens

<AUTHORS>: Alison D. OBrien, Ph.D.
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
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<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: One way to increase the likelihood that individuals who are exposed to aerosolized Bacillus anthracis spores remain disease free is to prevent germination of the infectious dormant spores into active vegetative cells that can produce potentially lethal toxins. Our recent finding that rabbit antiserum raised against mixtures of B. anthracis spores decreases the level of both Ames and Sterne strain spore germination in vitro, taken with the fact that formaldehyde-inactivated spores can serve as a protective vaccine against anthrax in guinea pigs, led us to the following hypothesis. Vaccine candidates that contain spore-surface-expressed antigens, i.e. exosporium antigens, will evoke antibodies that block or reduce spore germination in vivo or render spores more susceptible to phagocytosis and ultimately killing by macrophages. Based on this theory, our goal is to identify B. anthracis exosporium antigens as potential targets for incorporation into a second-generation Protective Antigen (PA)-based vaccine. The specific aims are to: 1. clone, express, purify, and characterize as potential protective immunogens (with and without PA) exosporium-associated antigens recognized by the anti- B. anthracis spore serum that inhibits spore germination in vitro; 2. prepare polyclonal and monoclonal antibodies against selected, purified, exosporium proteins and test these antibodies for spore-binding and anti-germinating activities in vitro, and, if such in vitro activities are evident, for protective capacity when administered to mice prior to and after spore challenge; 3. construct single and, if appropriate, double mutants of B. anthracis Sterne strain in which exosporium genes are deleted and compare the mutants with wild-

type for virulence in the A/J mouse subcutaneous and inhalational challenge models; 4. develop a mouse model of inhalational anthrax in which separate lux operon fusions of B. anthracis Sterne strain are constructed so as to visualize by bioluminescence: i.) in vivo germination of spores; ii.) distribution and replication of vegetative cells and use this model to evaluate the efficacy of various active and passive immunization strategies to prevent anthrax. We envision that a vaccine that incorporates spore antigen(s) and PA could be used in both civilian and military populations at risk for exposure to anthrax spores.
<END TEXT>

<TITLE>: MARCE Abstract#2 RP 1.2 Vegetative phase antigens of B. anthracis as a multivalent vaccine for anthrax
<AUTHORS>: Cross, Alan S
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
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<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: B. anthracis (BA), the causative agent of anthrax infection, has been used in bioterrorist events. Consequently, the availability of preventive measures, such as vaccines, is highly desirable. The vegetative form of BA expresses a tri-partite toxin composed of protective antigen (PA), edema factor (EF) and lethal factor (LF) which combine to form two toxins, lethal toxin (PA+LF) and edema toxin (PA+EF). Current and next generation vaccines target PA; however, it would be desirable to prevent toxin formation by targeting the spore form of BA which initially infects the host, thereby preventing anthrax infection, We therefore are examining the role of vaccine- and naturally-acquired antibodies in mediating the killing of BA by macrophages, are

defining the mechanisms by which antibodies promote the killing of BA and are seeking new targets on BA for future generation vaccines. While subjects immunized with the UK vaccine make antibodies to PA, LF and EF, subjects immunized with the US vaccine make antibody to the PA only. Patients recovering from naturally-acquired cutaneous anthrax had stronger responses to LF than to PA. The anti-LF antibody had toxin-neutralizing activity in the absence of PA. Thus, LF make be a useful addition to US anthrax vaccines. Using a strain of BA that is unable to germinate, we found that macrophages are unable to kill the spore form of BA but do kill the vegetative form emerging within macrophages. Macrophages require the induction of nitric oxide (NO) to kill the BA. Enzymes present on the exosporium structure which covers the spore enable BA to escape macrophage killing, in part by inhibiting NO formation. Thus, vaccine-induced antibodies to these enzymes might neutralize these BA virulence factors. Antibodies, including purified anti-PA IgG, from the serum of an immunized subject enhanced the killing of BA by binding to the exoporium (since antibody-mediated killing is not observed in the absence of the exosporium). Addition of complement markedly increases the antibody-mediated killing. Since we hypothesize that proteins expressed during growth of BA in human blood may identify additional antigens for inclusion in next generation vaccines, we are comparing the expression of protein antigens in BA grown in laboratory media and in human blood. Any proteins expressed uniquely during growth in blood will then be evaluated as a vaccine candidate.
<END TEXT>

<TITLE>: MARCE Abstract#3 Project 1.3: Actions and Interactions of the Toxins of B. Anthracis
<AUTHORS>: Erik L. Hewlett
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
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<EMAIL>: Email
<PHONE>: Phone
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<TEXT>: One strategy for treatment or prevention of anthrax is to understand better the pathogenesis at the cellular and molecular levels. Sub-project 1.3 was initiated for the purpose of investigating the actions of edema toxin (ET) and lethal toxin (LT) on target cells in vitro and in animals in vivo, with the objective of identification of potential interventions. The fact that inhalational anthrax has a high mortality, in spite of appropriate antibiotics, suggests that B. anthracis is capable of evading or subverting the host response. Although it has been postulated that infection with B. anthracis leads to host death by eliciting cytokine release, it is clearly a more complex process and it appears that there is failure of the host innate immune response to control infection during the early stage. Both ET and LT have been implicated in the causation of this outcome.
We continue to evaluate the mechanisms and pathways by which B. anthracis is recognized by the host and the cellular and molecular effects of the toxins, ET and LT, on those responses. Specifically, we are studying the effects of LT and ET on murine macrophages, non-human primate macrophages, and primary human monocyte-derived macrophages in vitro. In addition, we are characterizing host innate immune recognition and response to B. anthracis in vitro and in vivo. For the latter, we are testing the role of Toll-like receptor (TLR) signaling in mice deficient in select TLR-signaling components in the response to aerosolized spore challenge.
Similarly, the pathogenesis of ET-induced edema formation and the role of ET-induced cAMP accumulation are completely unknown. We are addressing those questions using cultured cells and rabbits, into which we inject ET intradermally to produce edema. To date, our data indicate that edema does not result from a direct action of ET on the vascular endothelium or on mast cells, but rather some other type of cell that

initiates a biphasic cascade of events resulting in inflammation and edema. Inhibitors of local edema and inflammation are being tested for their abilities to influence the course of the ET-elicited edema.
In order to relate our data from human macrophages in vitro and rabbit data in vivo to what occurs during infection, we are testing effects of the toxins in vivo. In Sub-Project 1.3 Supplement, we soon will be performing a toxin challenge experiment in which the effects of intravenous LT or LT plus ET or PA alone will be compared. The cytokines and other indicators of the host response to the organism, as well as measurements of standard physiologic parameters, will be analyzed. It is our plan, depending on the results, to follow this toxin study with one in which we study the course and outcome of infection with virulent B. anthracis.
<END TEXT>

<TITLE>: MARCE Abstract#4 RP 1.4 Salmonella Typhi-Based Anthrax Vaccine Compatible with Prime-Boost Strategy
<AUTHORS>: Myron Levine, M.D., M.T.P.H.
<JOURNAL>: Journal
<DATE>: Date

<CITATION>: Citation
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<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: We hypothesize that by appropriately engineering of attenuated Salmonella Typhi live vectors, it is possible to construct a multivalent oral vaccine that will successfully prime the immune system to generate enhanced and accelerated systemic and mucosal immune responses against several antigens of a single Category A bioterrorism agent (or antigens of several different Class A agents) when those antigens are subsequently presented via an heterologous vaccine formulation. Following this

approach, we report here further progress toward development of a live vector-based anthrax vaccine. Initially, attenuated S. Typhi CVD 908-htrA was modified to express the eukaryotic cell-binding domain (D4) of anthrax toxin protective antigen (PA83), both as a secreted fusion protein (fused to ClyA) and as an unfused cytoplasmic heterologous antigen. Intranasal priming of mice with live vector delivering D4, followed by boosting with purified Protective Antigen, elicited neutralizing antitoxin responses. To enhance neutralizing antitoxin responses, we are investigating two further approaches: 1] expression of full-length PA83 and PA63, and 2] co-expression within our live vectors of the novel immunomodulator CTA1-DD, which combines the enzymatic activity of the A1 subunit of cholera toxin (CTA1) with two tandem B cell targeting moieties (DD) derived from an Ig-binding domain of Staphylococcus aureus protein A. We observed impressive expression levels of both PA83 and PA63 when exported as ClyA fusion proteins. Preliminary results in mice indicate all fusion proteins are immunogenic, with PA63 fusions generating not only the highest PA-specific titers but also neutralizing antibody. Additional preliminary results indicate that CTA1-DD further enhances immune responses when co-expressed in CVD 908-htrA with a given PA fusion protein. Our results continue to support the feasibility of using attenuated S. Typhi live vectors to enhance the immunoprophylaxis of anthrax using an heterologous prime-boosting strategy of immunization.
<END TEXT>

<TITLE>: MARCE Abstract#5 RP 2.1 Henipavirus and Bunyaviruses
<AUTHORS>: Christopher C. Broder, Ph.D.
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>:
<EMAIL>: Email
<PHONE>: Phone

<KEYWORDS>: Keywords
<TEXT>: Hendra virus (HeV) and Nipah virus (NiV) are closely related emerging viruses comprising the Henipavirus genus of the sub-family Paramyxovirinae and are distinguished by their ability to cause fatal disease in both animal and human hosts. These emerging viruses have been classified as agents of biodefense concern because they possess characteristics making them suitable for weaponization. Futher, the high mortality and person to person transmission associated with the most recent NiV outbreaks, as well as the very recent re-emergence of HeV, has confirmed the importance and necessity of developing effective therapeutic interventions. As a potential gap-filling approach for the rapid development of safe and efficacious antiviral therapies for these agents, we propose to identify, isolate and characterize neutralizing human monoclonal antibodies (nhMAbs) reactive to the native forms of the envelope glycoproteins (Envs) of these viruses. The development of these nhMAbs would provide a valuable battery of post-exposure or post-infection therapeutics to combat disease caused by these agents. Antibodies are being obtained by screening human phage display libraries against the viral Envs. Our focus will be the development of recombinant-based assays for measuring virus entry along with the production of native viral Envs as antigens for phage panning procedures. The overall objectives of the research project will be to develop a battery of potent nhMAbs capable of being used as passive immunotherapy against henipaviruses. The overall specific aims will be: 1) Develop high-throughput recombinant assays for virus entry and develop recombinant viral Envs; 2) identify and isolate Fabs capable of blocking virus entry by using recombinant viral Envs and/or functional pseudotyped or virus-like particles as antigens for phage panning; and 3) produce and test these Fabs as whole IgG nhMAbs against live virus and in vivo in an animal model of henipavirus infection. These research goals are priorities as immediate and
long-term objectives for biodefense research on these important emerging viruses.

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<TITLE>: MARCE Abstract#6 RP 2.4: Alphavirus Replicons
<AUTHORS>: Quinnan, Gerald V.
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
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<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: The two specific aims approved for this project were: (1) Study immunogenicity of viral envelope proteins using the established VEE replicon system; and (2) Construct a new alphavirus-based replicon system using non-structural protein coding sequences from two alternative alphavirus donors: (Fort Morgan viruses and the Southern elephant seal virus). Substantial progress has been made on each of the specific aims. Immunogenicity of alphavirus replicons expressing West Nile Virus prM and E proteins was demonstrated in mice. High potency neutralizing activity was induced consistently. Hendra and Nipah virus F and G proteins were each introduced into alphavirus replicons, protein expression verified, replicon particle preparations made, and mouse immunogenicity studies initiated. Development of alternate, more potent alphavirus replicons has been pursued by modification of the existing VEE replicon and cloning of the Fort Morgan Virus non-structural protein coding sequence. Prolonged expression replicons have been constructed, Hendra and Nipah Virus G proteins have been cloned into them, and immunogenicity in comparison to standard replicons is being evaluated. Fort Morgan virus replicon expressing green fluorescent protein has been constructed. After evaluation, Hendra and Nipah virus F and G protein genes will be introduced and studied for comparative immunogenicity. Analyses of Fort Morgan Virus NSP sequences demonstrate that it is a new subgroup of New World alphavirus

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<TITLE>: MARCE Abstract#7 Poxvirus Subproject #1: Subunit Vaccines
<AUTHORS>: Gary H. Cohen
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
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<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: Eradication of smallpox (variola) by vaccination with live vaccinia virus (VV, a related poxvirus) led to discontinuation of routine vaccination in the U.S. in 1972. Thus, the entire population is potentially susceptible. In response to a smallpox attack by bioterrorists, mass immunization is limited by the fact that the live-virus vaccine might produce severe complications, especially in immunocompromised people, pregnant women and infants. Our goal is to develop a protein subunit vaccine using vaccinia virus proteins that are targets of the protective response. Such a vaccine would be safely administered to individuals that cannot be vaccinated with the live virus vaccine. In addition, we will clone and express the variola virus homologues of these proteins in the baculovirus system in order to compare them with their VV homologues. These proteins may contain epitopes that could be more effective in a vaccine intended to protect against variola virus infection. Note that we will confine the cloning of the variola homologues to placement in an insect virus vector, not into VV. We will prepare our live VV stocks in a different laboratory than that used for any variola proteins. We plan to incorporate both variola and vaccinia proteins in the mixture. Variola proteins may differ from their vaccinia virus counterparts, hence affording a more protective response. Third, we will incorporate proteins that may overcome strategies devised by the virus to thwart the

hosts immune response. We propose three specific aims: 1. To clone, express and purify and characterize vaccinia glycoproteins in a baculovirus expression system. 2. To clone, express and purify the variola homologues of vaccinia virus proteins in order to compare them with vaccinia virus homologues. 3. To test the efficacy of vaccinia and variola proteins in animal models of vaccinia virus infection

Bioterrorism with variola virus is of immense concern because (a) virtually the entire world population is susceptible since routine vaccination was discontinued; (b) there are no treatments; (c) the virus in aerosol form is stable; (d) the virus is transmissible person-to-person; and (e) infection results in high morbidity and mortality. Vaccination with vaccinia virus was a key factor in eradicating smallpox. The necessity to vaccinate an at-risk population with vaccinia virus is central to preparing for the potential threat of smallpox bioterrorism. However recognized complications of vaccinia vaccination, especially in immunocompromised hosts, pregnant women, and infants impose serious limitations of this strategy. Consequently, there is a critical need to develop a safer vaccine.

As part of the MARCE Poxvirus Research Program, Subproject 2 is attempting to discover new targets of neutralizing antibodies. These newly identified protein targets will then be studied as part of a subunit vaccine strategy. Since the formation of the MARCE, our work has provided evidence that the major target of neutralizing antibody against the extracellular enveloped virus (EEV) is the B5 protein. Given this finding, we are now evaluating hybridomas developed from mice vaccinated with a recombinant vaccinia virus lacking the majority of the B5 ectodomain. Also, in collaboration with MARCE Poxvirus Subproject 1, we are testing passive and active immunization strategies in mice to better understand how these strategies alter poxvirus pathogenesis. The specific aims of this subproject are:

1.Construct a broad panel of monoclonal antibodies developed in mice immunized with VV and define the

specific proteins and epitopes responsible for VV neutralization
2.Test in vivo the ability of active and passive immunization strategies to modify vaccinia virus pathogenesis
<END TEXT>

<TITLE>: MARCE Abstract#8 Poxvirus Subproject #3: Targets of VIG
<AUTHORS>: Lambris, John D.
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
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<EMAIL>: Email
<PHONE>: Phone
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<TEXT>: In past smallpox vaccination efforts, individuals with complications arising from the use of live VV in
the U.S. were treated with human vaccinia immune globulin (VIG) obtained from VV immunized
people. This procedure of therapeutic passive immunization usually required administration of large volumes of immune globulin. Although this material had efficacy, little is known about which
components of the immune globulin were effective. Thus, it is imperative to develop antibodies that can act therapeutically in the event that mass vaccination is required. Our goal is to develop a cocktail of defined and high affinity antibodies to VV proteins that will replace the use of traditional VIG. Our hypothesis is that by identifying proteins recognized by VIG we can develop human
antibodies to proteins that will provoke a protective response. A cocktail of such antibodies will
provide a uniform and secure source of a VV immune therapeutic reagent. In addition, this
approach may identify targets that will be important to include in a subunit vaccine. During the third year we made significant progress and we have:

1. Expressed, properly folded, five key vaccinia proteins in E. Coli (A33R, A56R, A28, L1R, and D8L) which in addition to facilitating our studies will provide large amounts, in low cost, proteins for vaccine development and structural studies.
2. We have identified the first 3 human antibodies to A33R and, by the end of this funding period, will complete the screening of antibodies to stalk region of B5R. Recent studies have shown that mouse antibodies to this region neutralize the virus. In the event that we obtain such antibodies, it will dramatically expedite the process of developing these antibodies as therapeutics as they are already human antibodies and not mouse antibodies that have to be humanized.
3. We have identified A33R reactive peptides which if they inhibit A33Rs functions, might lead to the development of small molecule inhibitors which will be easier and less expensive to manufacture.
During the 4th year we will screen human antibody phage library for antibodies to B5R, A56R, A28, L1R, and D8L, biochemically characterize them and assesses their ability to neutralize VV.
<END TEXT>

<TITLE>: MARCE Abstract#9 Project 3.4 Ectomelia Pathogenesis
<AUTHORS>: Thomas J. Braciale, M.D., Ph.D.
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>:
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: The emerging threat of smallpox-variola virus (VA) as a terrorist weapon has multiple implications and ramifications for the citizens of the US and the world at large. First and foremost, because of the

world-wide eradication of VA more than 30 years ago, the human population is vulnerable to lethal infection. Second, since the likely delivery method of weaponized VA is via airborne dispersal (i.e. through the respiratory tract), understanding the initial events in the interaction of pox viruses with cells and mediators within the respiratory tract will be crucial in elucidating the pathogenesis of lethal pox virus infection, virus dissemination through the body, and the induction of the innate/adaptive immune responses to infection. Third, vaccination strategies to prevent lethal VA infection, as well as, therapeutic interventions to treat VA infection prior to systemic dissemination will require detailed information on the early events in the pox virus/host interaction within the respiratory tract. The elucidation of both the early events in pox virus infection and the host response requires an animal model of natural pox virus infection in the respiratory tract which is amenable to experimental manipulation. We plan to use virulent mouse pox (ectromelia-EV) infection in the murine model to examine the early events in pox virus/host interaction within the respiratory tract leading to the establishment of infection, virus dissemination from the respiratory tract, and the induction of the host adaptive response. We further plan to exploit the technologies and reagents (e.g. antibodies , viral subunit vaccines, etc.) to determine the efficacy of reagents generated to the related pox virus vaccinia (VV) in the prevention and treatment of respiratory EV infection. The specific aims of the project are: 1. To characterize the early innate response of dendritic cells to EV infection in the respiratory tract, and the impact of respiratory tract infection on the induction of the adaptive immune response; 2. To evaluate the efficacy of antibody reagents and vaccination strategies for the prevention/treatment of EV infection.
<END TEXT>

<TITLE>: MARCE Abstract#10 Multi-valent F. tularensis capsule/protein conjugate vaccines

<AUTHORS>: Thomas J Inzana
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
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<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: Our long term objective is to identify and characterize the putative capsule of F. tularensis for conjugation to a protein, such as CRM197, and to evaluate the immunoprotective capacity of such a conjugate for tularemia. Our specific aims are to 1) purify and chemically characterize the F. tularensis capsule; 2) conjugate the purified capsule to well-defined protein carriers; 3) determine the immunogenicity and protective efficacy of capsule and capsule-protein conjugates in mice. Once these aims are achieved, we will need to optimize the protective immune response through variation of A) the type of coupling chemistry; B0 the specific protein carrier; C) the type of adjuvant included.
F. tularensis is the etiologic agent of tularemia and is classified as a category A bioterrorism agent. A licensed vaccine is not available to prevent tularemia, and safe, effective vaccines are needed. A capsule around F. tularensis has been described based on electron microscopic analysis. In order to characterize this capsule and determine its role in bacterial virulence and immunoprotection, we must first purify the capsule and determine its chemical structure. Standard biochemical procedures using aqueous phenol/water or trichloroacetic acid, enzyme digestion, and ultracentrifugation will be used to isolate and purify the capsule from either the aqueous or organic phases. The composition and structure of the capsule will be carried out in collaboration with the University of Georgia Complex Carbohydrate Research Center. The capsule will be conjugated to an established carrier protein, such as CRM197 (derived from diphtheria toxoid). The immunogenicity and protective efficacy of the conjugate vaccine will be

tested in mice. The capsule may later be conjugated to meningococcal outer membrane protein, marine haemocyanin, or a protein from F.tularensis itself to enhance immunogenicity. Mice will also be immunized with the conjugate in various adjuvants designed to stimulate a Th1 response, such as CpG, IL-12, or saponin. The antibody responses and T cell responses (cytokines) will be measured. Immunization will be followed by intradermal and respiratory challenge with F. tularensis type A and LVS. To aid in identification of the capsule, the genes for its biosynthesis and export will be identified by Suppression Subtractive Hybridization, and nonencapsulated mutants will be generated by transposon or allelic exchange mutagenesis. Mutants will be evaluated for virulence and immunoprotection in mice. These experiments are expected to lead to a subunit vaccine that will provide adequate protection or supplement early protection against tularemia. Such a vaccine would also be safer nd more readily accepted for public use than a live attenuated vaccine.
<END TEXT>

<TITLE>: MARCE Abstract#11 Subproject 4.2: Innate and adaptive immune responses to Francisella tularensis
<AUTHORS>: Stefanie N. Vogel, Ph.D.
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>:
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: Francisella tularensis (Ft), a Gram negative intracellular bacterium, is the etiologic agent of tularemia. Ft has been classified by the Center for Disease Control as a Category A bioterrorism agent due to its ability to spread via the airborne route, its extremely low infectious dose, and its capacity to cause severe disease and death. While attenuated for humans, infection of mice with <10 Ft Live Vaccine

Strain (LVS) organisms intraperitoneally (i.p.) causes lethal infection that resembles human tularemia, while the LD50 for an intradermal (i.d.) infection is >106 organisms. To examine the immunological consequences of Ft LVS infection on the innate immune response, the inflammatory responses of mice infected i.p. or i.d. were compared. Mice infected i.p. displayed greater bacterial burden and markedly increased expression of pro-inflammatory genes, particularly in the liver, where numerous granulomas were detected within 48 hours of infection. In contrast to most lipopolysaccharides (LPS),
highly purified Ft LVS LPS (10 g/ml) was found to be only minimally stimulatory in primary murine macrophages and in HEK293T cells transiently transfected with TLR4/MD2/CD14 or any other TLR. In contrast, live Ft LVS bacteria were highly stimulatory for macrophages and elicited strong NF-B reporter activity in TLR2-expressing HEK293T cells. Despite the poor stimulatory activity of Ft LVS LPS in vitro, administration of 100 ng Ft LVS LPS 2 days prior to Ft LVS challenge severely limited both bacterial burden and cytokine mRNA and protein expression in the absence of detectable antibody at the time of bacterial challenge; yet, these mice developed, within two days of infection, a robust IgM antibody response directed predominantly against the Ft LPS moiety of the organism, and survived. These data suggest that prior administration of Ft LVS LPS protects the host by limiting bacterial burden and blunting an otherwise overwhelming innate inflammatory response to infection, while priming the adaptive immune response for development of a strong antibody response. This finding is important because it suggests that Ft LPS serves as a adjuvant to augment the protective antibody-mediated immune response to Ft. The immunologic basis for the protective effect conferred by Ft LPS will continue to be explored by analyzing the specificity of this response, the capacity of newly developed, non-replicating vaccine candidates to elicit inflammation, and the role of TLR2 signaling in the response to Ft LVS.
<END TEXT>

<TITLE>: MARCE Abstract#12 RP 4.3: Design of a Rationally-Attenuated, Mucosally-Administered F. tularensis Vaccine Strain
<AUTHORS>: Myron Levine, M.D., M.T.P.H.
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>:
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: Francisella tularensis biogroup tularensis (type A) is the most virulent etiological agent of tularemia in North America; the prototype member of this group is the strain Schu S4. Because this pathogen is capable of causing fatal disease, with doses that contain as few as 10 colony-forming units and because it can be delivered in an aerosol form, it is considered a high priority for biodefense. The live attenuated vaccine strain (LVS) is an attenuated strain derived from Francisella tularensis biogroup holartica (type B). This vaccine has been used to vaccinate over a thousand individuals and has proven protective against disease caused by F. tularensis. However, the basis for attenuation of this strain is unknown and its precise derivation does not make it suitable for widespread use in the population. Our goal is to create live, attenuated F. tularensis strains based on the SchuS4 prototype containing precise deletions in biosynthetic or virulence genes for use as vaccines. Genes targeted for deletion include the guaB or guaA genes, which result in guanine autotrophy and which have proven to be attenuating in enteric organisms. Genes considered for deletion as secondary mutations include mglA(macrophage growth locus), pdpA (pathogenicity determinant protein) and iglC (intracellular growth locus) which have been identified as important for macrophage survival. A genetic system for allelic exchange in tularensis has been developed based on

oriE1 plasmids which do not replicate in Francisella and which have been engineered to contain marker genes with Francisella-specific promoters. This system has been used to delete the guaB and guaA genes from the LVS strain. The resultant derivatives are auxotrophic for guanine, and attenuated for growth in macrophages in culture. The LVS-guaB mutant was attenuated for virulence in a mouse model where 100-1000 times more bacteria were required to cause disease in mice compared to the parental strain. Secondary mutations in the iglC and mglA genes have
been introduced into LVS and are currently being evaluated for attenuating effects. Our finding support the generation of attenuated vaccines with defined deletions in guaA and or guaB as well the standardization of molecular procedures applicable in the generation of attenuated Francisella tularensis Schu-S4 strains
<END TEXT>

<TITLE>: MARCE Abstract#13 Pathogenesis of F. tularensis
<AUTHORS>: Qureshi, Nilofer
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>:
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: Francisella tularensis, the causative agent of tularemia in man is a Gram negative bacterium that infects a wide variety of hosts. This bacterium has been classified by CD as a "Class A" bioterrorism agent that is likely to pose a national security risk. Lipopolysaccharide (LPS), an integral outer membrane component of Gram negative bacteria usually plays a pivotal role in the innate immune responses to infection, however, not much is known about the LPS of F. tularensis. The goal of this research program is to purify and establish the structure of LPS/lipid A from

this bacterium, and to use the purified LPS/lipid A for biological and signal transduction studies. The central hypothesis is that various phase variants (during infectious and chronic states) attenuated, and wild type strains of F. tularensis have different LPS with different lipid A structures which may activate macrophages differently. In addition, we will study the LPS/lipid A structures from and relate the structure of LPS to biological activities in macrophages. The following Specific aims are proposed: 1. To purify the LPS/lipid A from different strains of F. tularensis. Techniques developed in our laboratory for purification and structural determination of LPS/lipid A will be employed. The purified lipid A fractions will be analyzed by thin-layer chromatography, and by reverse-phase high pressure liquid chromatography. HPLC-purified methylated lipid A samples will be analyzed for phosphorus and fatty acids. The purified lipid A will also be analyzed by matrix assisted laser desorption/ionization/time of flight mass spectrometry (MALDI/TOF) and liquid secondary ion mass spectrometry (LSIMS). We will determine the molecular weight and the structure of lipid A using a combination of mass spectrometry and chemical analysis. If need arises, we will also use nuclear magnetic resonance to determine the complete structure of the lipid A. 2. To determine the biological properties of this purified LPS/lipid A. Most of this Specific aim 1 will be carried in Dr. Nilofer Qureshi's laboratory and most of the Specific aim 2 will be carried out in Dr. Stefanie Vogel's laboratory. These studies will lead to greater understanding of the role of LPS in pathogenicity and to the development of novel therapeutic intervention strategies for treatment of tularemia.
<END TEXT>

<TITLE>: MARCE Abstract#15 RP 4.31 Therapies for Tularemia
<AUTHORS>: Barbara J. Mann
<JOURNAL>: Journal
<DATE>: Date

<CITATION>: Citation
<TYPE>: Type
<ADDRESS>:
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: Francisella tularensis sp. tularensis has been designated a category A select agent because it has a low infectious dose (<10 organisms), can cause a severe illness, and be spread by aersolization. The live vaccine strain (LVS) has been used a vaccine against tularemia for many years but in the United States it has never been approved for widespread use. As a vaccine LVS has several drawbacks; it is a member of the less virulent subspecies F. tularensis holarctica, the nature of its attenuation is unknown, and although it appears to be quite effective in preventing infection to intradermal challenges it is less effective against aerosol challenge with the more virulent F. tularensis tularensis subspecies. The goal of this subproject is to create a defined attenuated strain in the F. tularensis tularensis background that could be used as a protective vaccine. The hypothesis is that an attenuated F. tularensis tularensis strain will have better efficacy against aerosol challenge than LVS. Specifically attenuated strains will be identified using a mutagenesis technique called signature-tagged mutagenesis. Attenuated strains identified by this technique will be characterized and tested for the ability to protect against both intradermal and aerosol challenge.
<END TEXT>

<TITLE>: MARCE Abstract#16 Project 5.1A, Cryptosporidium genomics, pathogenesis and vaccinology
<AUTHORS>: Gregory A. Buck
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>:
<EMAIL>: Email

<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: The focus of the research in the Project V1: Crytosporidium genomics, pathogenesis and vaccinology, is on the application of genome analysis to identify potential vaccine candidates from the complete genome sequence of C. hominis. Substantial progress has been made on our aims and our milestones to identify and express candidate C. hominis peptides of interest, prepare and test polyclonal and monoclonal antibodies, and develop in vitro and in vivo models of cryptosporidial infection. We have developed new qPCR methods to quantify infection in vitro and in vivo and have launched new collaborative microarray studies and studies of the innate immune inducer, profilin. We are now exploring new potential chemotherapeutic targets in this parasite. Our initial approach to finding immunoprophylactics and therapeutic targets, termed reverse vaccinology, involves the in silico analysis of the genome sequence to provide new potential vaccine antigens and therapeutic targets. This is complemented by additional methods of genome analysis including microarray and proteomic technologies. In addition, new MARCE collaborations with colleagues at USUHS and UMd include the development of the human intestinal organoid model and studies of ZOT-inhibitor and MBL, and we are exploring new studies of alanylglutamine as potential novel therapeutic deliverables. Hence our aims are:
1. Identify and express C. hominis candidate virulence, vaccine and chemotherapeutic targets, using bioinformatics and the whole genome sequence:
a. Scan the genome for candidate virulence, vaccine, and chemotherapeutic targets;
b. Express candidate sequences in bacteria/yeast expression vectors for functional and immunologic assays, and for structural analysis for identification of potential chemotherapeutic inhibitors;
c. Express, crystallize and define the role of C. hominis profilin in the host immune response.

2. Define roles of Cryptosporidium genes in pathogenesis and immunity:

a. Develop and explore protection in tissue culture and animal models, examining blockade (using products and antibody) of attachment and/or invasion in vitro, and using microarray technology to identify parasite and host genes expressed during infection, also examining candidate products for expression and immunologic recognition in human infection using western blots;
b. Prepare and test polyclonal and monoclonal antibodies;
c. Move toward production of candidate therapeutic/vaccine deliverables (by year 5).
3. Examine genetic determinants of human susceptibility to cryptosporidia.
NOTE: this document contains a combined writeup from UVA (Guerrant) and VCU (Buck)
<END TEXT>

<TITLE>: MARCE Abstract#17 RP 5.2 EHEC and Shigella dysenteriae: New Vaccines and Therapies
<AUTHORS>: Eileen Barry, Ph.D.
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
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<ADDRESS>:
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: The main objective of this project is to develop a safe and effective vaccine for Shigella dysenteriae (S. dysenteriae) type 1, which has been classified by NIAID as a Category B priority pathogen and is considered a potential biological weapon. Currently, there is no available vaccine for Shigella and there are only limited treatment options for infections with multiple antibiotic resistant strains. S. dysenteriae type 1 is the serotype that poses the greatest threat. This serotype is capable of pandemic spread with strains that harbor multiple antibiotic resistance and induce high attack rates and mortality in all age groups. Given the shortcomings of

available public health measures to successfully control this infection, and bioterrorism potential, development of a safe and effective S. dysenteriae type 1 vaccine would be an important advance.
The first phase of this project encompasses the development of a challenge model with wild type Shiga toxin-positive S. dysenteriae 1 in cynomolgus macaques, as well as studies on the immunological mechanisms that might be involved in protection from S. dysenteriae 1 infection. The second phase of this project involves the intragastric vaccination of cynomolgus monkeys at days 0 and 14 with 1010 cfu of Shigella vaccine strain constructs engineered at the CVD or at Walter Reed or placebo vaccine vehicle. Seventy-five days post immunization, the animals will be challenged with wild type S. dysenteriae 1. Immune responses to be evaluated include measurements of antibody responses in serum and stool specimens and antibody secreting cells in peripheral blood and colonic mucosa as well as cell-mediated immune responses in cells isolated from blood, colonic mucosa and mesenteric lymph nodes draining the colon. These techniques have been successfully used in studies involving non-human primates. We will also investigate whether challenge with shiga toxin positive wild type Shigella strains elicits specific B and T cells in circulation and in mucosal tissues characterized by the expression of homing molecules, such as L-selectin, integrin a4b7 and chemokine receptors, that direct effector T cells and antigen secreting cells to distinct immune effector sites. These studies will provide valuable insights into the development of attenuated vaccine strains for S. dysenteriae.
<END TEXT>

<TITLE>: MARCE Abstract#18 Genetic Determinants of Immunity to Cryptosporidiosis
<AUTHORS>: Kirkpatrick, Beth Diane
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation

<TYPE>: Type
<ADDRESS>:
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: This work examines the genetic susceptibility of humans to infection with Cryptosporidium species. The results of this work are hoped to lead to a rapid diagnostic test which would identify individuals at risk of infection and therapuetic agents or prophlyactic agents against disease. We have screened over 9000 samples to identify children with known Cryptosporidium infection, and have used the DNA from these children for evaluation of genetic polymorphims in genes involved in the immune response to infection. The genetic data is strengthened by our ability to evaluate it with extensive clinical and epidemiologic data from the corresponding database of 220 children, followed for approximately five years.
To data we have analyzed the HLA (MHC Class I (A, B, C) and II) polymorphisms for all these children, as well as the single nucleotide polymorhpims in the IL-8 promoter and in TNF-. In addition we have evaluated mutations in the mannose binding lectin gene, a component of the innate immune response. Results have demonstrated that children with the HLA II allele DQB1 0300 have a 2.5x increased risk of disease, but no MHC class I polymorphims are clearly associated with infection. In addition, it appears that decreased mannose binding serum levels are associated with an increase of cryptosporidiosis, and this corresponds to MBL genotype. Ongoing work is evaluating this data in light of issues of malnutrition, recurrent infection and severity of illness.
<END TEXT>

<TITLE>: MARCE Abstract#19 Project V.1: Cryptosporidium genomics, pathogenesis, and vaccinology
<AUTHORS>: Richard L. Guerrant
<JOURNAL>: Journal
<DATE>: Date

<CITATION>: Citation
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<ADDRESS>:
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: The focus of the research in the Project V1: Crytosporidium genomics, pathogenesis and vaccinology, is on the application of genome analysis to identify potential vaccine candidates from the complete genome sequence of C. hominis. Substantial progress has been made on our aims and our milestones to identify and express candidate C. hominis peptides of interest, prepare and test polyclonal and monoclonal antibodies, and develop in vitro and in vivo models of cryptosporidial infection. We have developed new qPCR methods to quantify infection in vitro and in vivo and have launched new collaborative microarray studies and studies of the innate immune inducer, profilin. We are now exploring new potential chemotherapeutic targets in this parasite.
Our initial approach to finding immunoprophylactics and therapeutic targets, termed reverse vaccinology, involves the in silico analysis of the genome sequence to provide new potential vaccine antigens and therapeutic targets. This is complemented by additional methods of genome analysis including microarray and proteomic technologies. In addition, new MARCE collaborations with colleagues at USUHS and UMd include the development of the human intestinal organoid model and studies of ZOT-inhibitor and MBL, and we are exploring new studies of alanylglutamine as potential novel therapeutic deliverables. Hence our aims are:
1. Identify and express C. hominis candidate virulence, vaccine and chemotherapeutic targets, using bioinformatics and the whole genome sequence:
a. Scan the genome for candidate virulence, vaccine, and chemotherapeutic targets;
b. Express candidate sequences in bacteria/yeast expression vectors for functional and immunologic assays, and for structural analysis for identification of potential chemotherapeutic inhibitors;

c. Express, crystallize and define the role of C. hominis profilin in the host immune response.
2. Define roles of Cryptosporidium genes in pathogenesis and immunity:
a. Develop and explore protection in tissue culture and animal models, examining blockade (using products and antibody) of attachment and/or invasion in vitro, and using microarray technology to identify parasite and host genes expressed during infection, also examining candidate products for expression and immunologic recognition in human infection using western blots;
b. Prepare and test polyclonal and monoclonal antibodies;
c. Move toward production of candidate therapeutic/vaccine deliverables (by year 5).
3. Examine genetic determinants of human susceptibility to cryptosporidia
<END TEXT>

<TITLE>: MARCE Abstract#20 New Therapeutics for Shiga Toxin E. Coli Disease
<AUTHORS>: Obrig, Tom G
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
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<ADDRESS>:
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: This project is designed to identify and test new therapeutic agents for enterohemorrhagic E. coli diseases.
No effective agents are currently available for Shiga toxin producing E. coli (STEC) disease. This first involves identifying new therapeutic targets in a murine model of Shiga toxin-dependent disease. Secondly,
therapeutic agents are to be identified for the targets. Thirdly, the agents are to be prepared and tested in

well-established cell culture and whole animal models of STEC-related disease. Our long term goal is to test these new therapeutic agents in humans for safety and efficacy.
<END TEXT>

<TITLE>: MARCE Abstract#21 RP 5.21: EHEC and Shigella dysenteriae type 1
<AUTHORS>: Alison D. OBrien, Ph.D.
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
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<ADDRESS>:
<EMAIL>: Email
<PHONE>: Phone
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<TEXT>: Efforts to identify and evaluate interventions for the prevention and treatment of Shiga toxin (Stx)-mediated disease in humans hinge on the availability of a representative small animal model of infection with Shiga toxin-producing Escherichia coli (STEC). In humans, the most frequent outcome of infection with STEC is hemorrhagic colitis. However, in five to 10% of those infected, particularly children and the elderly, the life-threatening toxin-mediated sequela, the hemolytic uremic syndrome occurs. HUS is characterized by hemolytic anemia, thrombic thrombocytopenia, kidney failure, and those who survive HUS may have persistent central nervous system or kidney damage. There are currently no approved interventions for HUS, and paradoxically, antibiotics are contraindicated for treatment of STEC infections because they accelerate the induction of lysogenic toxin-converting bacteriophages, which in turn, increases Shiga toxin-production and development of adverse toxin-mediated symptoms.
In mice pretreated with streptomycin to reduce normal intestinal flora, we have established gastrointestinal STEC infections and Shiga toxin-mediated disease. However, in that model, hemorrhagic colitis is not seen, and Stx kidney pathology in mice is tubular

necrosis rather than the glomerular necrosis seen in human HUS. Nonetheless, we have shown in that model that humanized chimeric monoclonal antibodies (C) directed against Stx1 and Stx2 are protective against toxin challenge. C-Stx2 also protects mice from lethal infection with O91:H21 E.coli that produce the Stx2d-activatable variant of Stx2. Others have demonstrated in the mouse model that Stx toxicity is exacerbated by the production of pro-inflammatory cytokines.

In this work we have focused on ferrets as a more representative model for STEC infection because, unlike mice, ferrets develop diarrhea and glomerular kidney damage. As in humans, these more serious manifestations are seen in only10 to 20% of infected ferrets. One objective has been to optimize conditions for STEC infection in ferrets such that symptoms of HUS occur more consistently. The improved ferret model will then be used to test the efficacy of the C-Stx1 and -Stx2 antibodies in amelioration of the symptoms and complications of Stx-mediated disease. Similarly, the improved ferret STEC-infection model will be used to test the effect of anti-inflammatory drugs in alleviation of cytokine-accelerated Stx effects.

Thus far we have been unsuccessful in increasing the HUS-rate in ferrets. Next we plan to test O91:H21, the highly virulent strain for mice, in the ferrets to increase the likelihood of HUS symptoms.

Additionally, we plan to intoxicate the ferrets by injection of toxin rather than the more natural oral infection route. Although this latter strategy does not reflect the manner in which humans acquire disease, it should provide a more uniform response to toxin such that the interventions with antibodies and anti-inflammatory drugs can be effectively tested.

<END TEXT>

<TITLE>: MARCE Abstract#22 RCE 6.1 McKenzie Component Project
<AUTHORS>: Robin McKenzie, M.D.
<JOURNAL>: Journal
<DATE>: Date

<CITATION>: Citation
<TYPE>: Type
<ADDRESS>:
<EMAIL>: Email
<PHONE>: Phone
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<TEXT>: The long-term objective of the 6.1 project was to evaluate alternate methods of delivering vaccines for biodefense and emerging infectious diseases, especially needle-less methods. The funding for the 6.1 component will be discontinued, but we will submit a new proposal as mentioned below in the progress report summary

<END TEXT>

<TITLE>: MARCE Abstract#23 6.1a Needle Free Technologies for Biodefense
<AUTHORS>: James Campbell, MD
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
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<EMAIL>: Email
<PHONE>: Phone
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<TEXT>: This project involves the use of a new method of injection for mass campaigns which is applicable to vaccines directed against bioterrror, emerging infectious pathogens, pandemics, and other pathogens of global significance. The method is a new form of jet injector, specifically designed for mass campaigns. Single use cartridges are filled with vaccine and attached to the injector. Up to 600 immunizations can be given in an hour and no needles are used, adding to the safety for the vaccinee, the vaccinator, and the public who may be exposed to the biomedical waste.
Although the jet injector will eventually be studied in trials using vaccines against agents of bioterror and biowarfare, there are currently no new vaccines of

this type far enough along in clinical development to test a new method of administration. That is, the new biodefense vaccines, such as recombinant Protective Antigen (anthrax vaccine) or plague vaccines do not yet have final formulations, doses, and schedules using needle and syringe. In order to test the jet injector prior to availability of the biodefense vaccines, we have decided to use a newly licensed vaccine, meningococcal conjugate vaccine (MCV4 or Menactra), that is now a recommended vaccine in the US and could be used to protect against emerging epidemic disease elsewhere.
The specific aims of this project are to test the safety, tolerabilty, and immunogenicity of the quadrivalent meningococcal conjugate vaccine when administered by a jet injector designed for mass campaigns (LectraJetHS).
<END TEXT>

<TITLE>: MARCE Abstract#24 RP 6.2 Immunogenetics of Human Responses to Biodefense
<AUTHORS>: Donald Burke, MD
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
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<ADDRESS>:
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: Sub-Project 6.2. Public health Response Research: Immunogenetics of human responses to infectious diseases and vacccines
The broad objective of this research is to utilize novel technologies to gain an in-depth understanding of human immune response in peripheral blood cells to Category A-C pathogens and related vaccines.
Specific Aims
1.Define the changes in the RNA transcript levels in peripheral blood mononuclear cells in human subjects with well-defined acute febrile infectious diseases

including bacterial diseases (shigella), parasitic diseases (malaria), and viral diseases (dengue).
2.Define vaccinia virus-induced changes in the RNA transcript levels in peripheral blood mononuclear cells in vaccinated human subjects, and compare these to changes observed in other infections.
These aims will be evaluated by collecting peripheral blood cells from human volunteers under the forementioned conditions and preserving RNA. The RNA will be subjected to standard preprocessing and labeling for microarray hybridization to Affymetrix microarray chips. The chips will subsequently be scanned and images will be analyzed.
These studies are relevant to the long-term objectives of the RCEs. The overalll goals of the RCE program as stated on the main website are to develop and maintain a strong infrastructure and to support multifaceted research and development activities that will provide the scientific information leading to the next generation of therapeutics, vaccine and diagnostics agains NIAID Category A, B and C priority pathogens. This projects focus on the human immune response to infection with and vaccination for those pathogens is an important part of working toward these goals.
<END TEXT>

<TITLE>: MARCE Abstract#25 6.3 Rothman Universal Diagnostic Platforms for Bioterrorist Events
<AUTHORS>: Richard E. Rothman
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
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<ADDRESS>:
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: The objective of this project is to accelerate the development, clinical evaluation and deployment of genomic and proteomic based diagnostic technologies that may be crucial for an effective public health response to a bioterrorist or emerging infectious

disease event. Progress has continued in both laboratory and clinical components of the study, with one cutting edge technology leading to new patent filing and industry collaboration. Our multiprobe PCR which is capable of universal bacterial identification and species identification has been optimized in preclinical validation studies and demonstrated to have high sensitivity and specificity for detection of common causes of meningitis and bacteremia; limits of detection surpass traditional culture-based methodologies. The assay also has been demonstrated to detect five category A/B BT agents. Several new patents are being filed and existing patents are in negotiation for licensing. With regard to clinical sample collection, over 120 cerebrospinal fluid, 140 blood and 25 nasopharyngeal aspirates have been collected. To optimize the sample set for evaluation, a new protocol has been devised and is currently under IRB review, allowing for identity-unlinked waste sample collection from the hospital laboratory. The DiversiLab Microbial characterization system, which is a semi-automated platform that uses repetitive sequence-based PCR and microfluidics chip technology, is being validated for clinical use because it has the potential to rapidly discern whether bacterial pathogens recovered from a group of patients have the same genetic profile (important in a suspected BT event). These clinical trials will be used towards FDA approval of the platform. In the first phase of the project we demonstrated Diversilab to have superior accuracy and discriminatory capability for strain typing Staphylococcus aureus and Clostridium difficile, relative to existing standards (PFGE). Recently, the kit failed to accurately distinguish Burkholderia complex from other non-fermenters, versus four other methods of identification. This finding led the company to further optimize its database; studies with Bacillus strain characterization are now underway. Progress with our universal viral detection assay (DOP-PCR) has included optimization of the non-specific amplification procedure and use of artificial CSF to optimize virus genome extraction procedures. Similar optimization experiments with a respiratory

matrix are underway, as are clinical CSF and human respiratory sample testing. Progress with the APL breath detection platform (mass spectrophometric measures of host response) has included (1) IRB approval of human subject testing of healthy human controls for purposes of device optimization and selection; (2) delineation of procedures, enrollment of subjects and collection of clinical data; and (3) amendment of human subjects protocol for evaluation of ED patients. A separate abstract and report is attached for the New Opportunities Award which was funded this past year.
<END TEXT>

<TITLE>: MARCE Abstract#26 Early Detection and Classification of Flu Like Illness
<AUTHORS>: Maria Salvato, Ph.D.
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>:
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: The Salvato project is to make a diagnostic tool for detection of flu-like infections based on human blood cell mRNA profiles. In the first 2 years, human blood and nasal wash specimens were collected as part of a Flu surveillance effort to provide RNA samples for eventual validation of the diagnostic. In the first, second and third years human blood cell cultures were exposed to flu-like viruses (Influenza, parainfluenza, RSV, rhinovirus, and arenaviruses) for various timepoints and their RNA was used to make cDNA for hybridizaton to human genomic microarrays. This resulted in mRNA profiles, and those gene expression changes greater than background were analyzed for their potential to uniquely describe the virus-specific disease. The validation of these profiles has not been completed (using real-time quantitative PCR to confirm differential expression of all mRNAs).

For each virus, about 50 genes were identified as belonging to virus-specific responses. We do not yet have data from 3
replicates of this process for each virus. It is clear from the data that we have obtained already that the viruses most closely related (the arenavirus group and the RSV/parainfluenza group) have closely related gene-expression profiles, yet can be distinguished from each other by their gene expression profiles. After PCR validation, a small number of virus-specific genes will be used to form a Select array that will be validated by hybridization to human samples from the patients with flu-like symptoms. We are also exploring the use of monkey samples for validating some of the microarray results since the human samples we collected represent a late disease stage and the cell culture samples represent an early disease stage, samples from an animal model could bridge the temporal gap. We are looking for an industrial partner willing to produce a small array that can use optical readout for quick diagnosis, and make such a diagnostic feasible for clinical use.
<END TEXT>

<TITLE>: MARCE Abstract#35 Flaviviruses: West Nile Virus
<AUTHORS>: Robert W. Doms
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>:
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: Since the initiation of this project, we have developed genetic systems that make it possible to screen inhibitors of West Nile virus in a quantitative, high-throughput format, developed a rigorous neutralization assay that can be performed under BSL2 conditions, identified a West Nile virus receptor, and have made progress in characterizing other WNV receptors and attachment factors. Our

Management and Oversight Committee (MOC) feels that West Nile virus is no longer as germane to the goals of the RCE as it once was. West Nile is now endemic throughout the United States, and good progress is being made in vaccine development. As a result, the MOC has asked that I shift the focus of my RCE-funded research to Rift Valley Fever Virus, a Category A agent. Thus, for the coming year, my WNV will be completed, while my work on RVFV will be accelerated. Importantly, our RVFV work is being done in collaboration with Dr. Mark Heise of the Southeast RCE at the University of North Carolina. Thus, the Specific Aims for the coming year are:

1. Define WNV neutralization epitopes in vivo. While neutralizing antibodies to WNV have been produced in mice, the epitopes to which in vivo neutralizing antibodies are directed are not known. Using an extensive panel of seras from horses either naturally infected with WNV or immunized with a WNV vaccine, we will characterize the in vivo neutralizing antibody response.
2. Determine the role that carbohydrate plays in WNV tropism. We have found that the presence or absence of the carbohydrate addition site in the WNV E protein can significiantly affect WNV tropism, though the mechanism for this is not clear.
3. Develop and optimize a rapid, quantitative RVFV neutralization assay. We have developed a quantitative, single-cycle infection system for studying RVFV entry that allows the quantification of neutralizing antibodies in BSL2 facilities. This assay will be utilized to evaluate the efficacy of the alphavirus-based vaccine developed by our colleagues at the University of North Carolina in Chapel Hill.

<END TEXT>

<TITLE>: MRCE Abstract #1
<AUTHORS>: Stanley, Samuel L., Craig T. Morita
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type

<ADDRESS>: University of Iowa Roy J. and Lucille A. Carver College of Medicine, Department of Internal Medicine Division of Rheumatology Eckstein Medical Research Building 340F Iowa City, IA 52242
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: Francisella tularensis infects at local sites before being disseminated throughout the body via bacteremia and/or infected macrophages. The first few days of infection are often critical in determining whether or not the host will survive. Positioned at the interface between innate and adaptive immunity, NK cells and T cells are present in large numbers and respond rapidly to infection by a variety of viruses, bacteria, and parasites. These "rapid response" lymphocytes kill infected cells and secrete large amounts of IFN- and TNF- that stimulate infected macrophages to kill intracellular bacteria. Human V2V2 T cells expand rapidly during tularemia infections to very high levels (22-50% of circulating T cells) supporting their important role in the immune response to Francisella tularensis. We have shown that identical expansions of monkey V2V2 T cells have characteristics of memory T cell responses and that these expansions coincide with the resolution of mycobacterial infections. We find that V2V2 T cells use their TCRs to recognize bacterial and protozoal infections by responding to a common microbial metabolic intermediate, (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMBPP). In support of memory responses by T cells, we find that adult V2V2 T cells are almost exclusively memory cells that are divided into 3 subsets, central memory, effector memory, and effector CD45RA+ memory. We hypothesize that the response of human memory V2V2 T cells to phosphoantigens on the surface of F. tularensis infected cells provides vital 'bridge immunity" in tularemia before T cell and B cell responses.Despite the importance of V2V2 T cells in human tularemia there are no vaccines that stimulate this T cell subset. Priming or boosting V2V2 T cell memory should help provide early, partial immunity and

will also have an adjuvant effects on pathogen-specific T cells for tularemia and for other infections with pathogens that produce HMBPP including anthrax and plague. Here we propose to (1) test V2V2 T cell recognition of F. tularensis infected cells, (2) determine the contribution of V2V2 T cell to the survival of human PBMC-SCID-beige mice infected with F. tularensis, (3) derive live bacterial vaccines for V2V2 T cells that overproduce HMBPP from attenuated Salmonella, and (4) test HMBPP/adjuvant vaccines and live bacterial vaccines for their ability to stimulate V2V2 T cell immunity in monkeys. These studies will elucidate the critical role of V2V2 T cells in tularemia and will develop vaccines that target V2V2 T cells.
<END TEXT>

<TITLE>: MRCE Abstract #2: Project 5 Fremont
<AUTHORS>: Fremont, Daved
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>: Washington University
660 South Euclid Avenue
St. Louis, MO 63110
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: Poxviruses are a family of large DNA viruses that encode up to 200 distinct open reading frames. The large size of the poxvirus genome is an important feature that has allowed them to acquire multiple immunomodulatory genes and thereby evolve unique strategies for evasion from host anti-viral responses. Ectromelia virus (EV) is a member of the orthopoxvirus family and is a highly virulent rodent pathogen that causes the disease mousepox. EV is similar to variola virus, the causative agent of human smallpox. Our primary hypothesis is that secreted and cell membrane associated proteins encoded by EV likely serve important roles in viral evasion of host mediated

innate and adaptive immune responses. Using a bioinformatics approach coupled to the established literature, we have selected target proteins from the EV Moscow strain genome that will be investigated by a combination of biochemical, functional, and crystallographic tools. Our primary targets of investigation include the soluble decoy receptors encoded by the virus that are specific for IFN-alpha, TNF, and CC-chemokines. We are also targeting three proteins with sequence similarity to natural killer receptors of the C-type lectin family. We have the following specific aims for the exploration of these potential agents of immune subterfuge: (1) Establish baculovirus and bacterial oxidative refolding expression systems for targeted EV encoded proteins to be used in functional and structural studies; (2) Identify and characterize the interactions between EV proteins and their host ligands and receptors; (3) Determine the structural basis of EV protein function by x-ray crystallography and structure-based mutagenesis.
<END TEXT>

<TITLE>: MRCE Abstract #3: Project1 - Yokoyama
<AUTHORS>:
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
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<ADDRESS>: Washington University
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St. Louis, MO 63110
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<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: Although natural killer (NK) cells are best known for their capacity to kill tumor cells in a perforin-dependent manner, recent studies have also indicated an important role for NK cells in innate immunity to pathogens, especially viruses. In prior work, the applicants laboratory has elucidated the basis for genetic resistance of certain strains of

mice to murine cytomegalovirus (MCMV). This is due to a genetic locus in the NK gene complex (NKC) for an NK cell activation receptor that recognizes an MCMV encoded ligand. In vitro and in vivo studies have revealed two phases of NK cell responses during MCMV infection, a non-specific early phase followed by selective proliferation of NK cells bearing the relevant activation receptor. Available data from the literature and the applicants laboratory strongly suggest that mouse NK cell responses to poxviruses (ectromelia virus, vaccinia virus) are highly related to their responses to MCMV, and involve both the non-specific early phase and a specific phase involving another NK cell activation receptor encoded in the NKC. Therefore, the applicant proposes the following specific aims to study: 1). The basic characteristics of the NK cell response to poxviruses; 2) Specific NK cell receptor triggering; 3) The role of NKG2D in poxvirus infections; and 4) Innate immune evasion by poxviruses. These studies will provide unique insight into the innate NK cell immune response to poxviruses.
<END TEXT>

<TITLE>: MRCE Abstract #4: Project4
<AUTHORS>: Diamond, Michael S.
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>: Washington University
660 South Euclid Avenue
St. Louis, MO 63110
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: West Nile Virus (WNV) is an emerging mosquito-borne human and animal pathogen that has caused outbreaks of fatal encephalitis in Europe, Asia, the Middle East, and most recently, the United States. Much remains to be learned about the pathogenesis of WNV infection and the immune system response that

prevents extension into the central nervous system (CNS) in most hosts. Recently, using a mouse model of WNV encephalitis, we have demonstrated that a deficiency of antibody or complement proteins leads to a disseminated, fatal infection. Based on these observations, the proposed research plans to directly determine how B cells, antibody and complement orchestrate a protective immune response against WNV. In the initial progress reports, we reported on the role of IgM in controlling infection, and on experiments that elucidated how antibodies protect against WNV infection. Since then, the interaction between antibody and complement in preventing dissemination, and the role of the alternative, classical, and lectin pathways of complement activation in triggering the adaptive immune response against WNV has been defined. This information has been instrumental in informing our studies that have generated a humanized neutralizing monoclonal antibody against WNV envelope protein with therapeutic potential. As part of this grant, we will (a) continue to characterize the mechanism(s) by which antibodies inhibit WNV infection in vivo, (b) assess the mechanisms by which the different complement activation pathways inhibit infection and prime adaptive immunity against WNV, and (c) investigate the mechanism(s) by which complement receptors induce a protective immune response against WNV. The identification of specific mechanisms for controlling early dissemination of WNV infection and triggering long-term protection will enhance our understanding of viral pathogenesis and the epidemiology of WNV-induced disease, and possibly provide a rationale for therapeutic intervention.
<END TEXT>

<TITLE>: MRCE Abstract #5: Project 6 - Virgin
<AUTHORS>: Virgin, Herbert W.
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type

<ADDRESS>: Washington University
St. Louis, MO
<EMAIL>: Email

<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: Interferons (IFNs) play a critical role in innate defense against infection with RNA and DNA viruses. A detailed understanding of how these critical cytokines inhibit viral infection might lead to pharmacologic approaches to induce resistance to multiple potentially dangerous agents without the necessity of inducing prior antigen-specific immunity. Given the large number of potential biological weapons for which no vaccine exists, this is an important goal. We seek to identify novel but general mechanisms to inhibit virus infection of proven relevance in vivo. This approach should allow us to identify approaches to combating agents that are functional across viruses and therefore are particularly well suited to dealing with new emerging or engineered biological threats. IFNs work via induction or repression of proteins that interfere with viral replication or alter the function of immune cells. In this project we proposed originally to identify new antiviral molecules. We have succeeded in identifying one novel antiviral, ISG15. ISG15 has antiviral roles against herpesviruses, alphaviruses such as Sindbis virus, and influenza. Studies are ongoing to identify the mechanisms of ISG15 action. We have continued to look for novel antivirals. The novel aspect of our approach is that we identify candidates in vivo, thus allowing us to detect important activities that are not observed in tissue culture. Using these approaches we have identified an additional antiviral candidate, VPS34, a component of the PI3Kinase thought to regulate the antiviral process of autophagy. We have germ line transmission of a mutation in the VPS34 gene in mice, and are thus well on our way to determining if we have identified a second physiologically relevant antiviral molecule. The Aims remain: Aim 1. Determine the molecular mechanism(s) of the antiviral effect of ISG15.Aim 2.

Identify candidate novel antiviral proteins and prove their physiologic relevance.
<END TEXT>

<TITLE>: MRCE Abstract #6: Project 7- Storch, G
<AUTHORS>: Storch, Gregory
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>: Washington University School of Medicine, St. Louis, Missouri
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: The original broad objective of this project was to use conventional and molecular techniques to define the virologic events following smallpox vaccination in vaccinia-nave and vaccinia-experienced individuals. This project was put on hold following the decision not to administer Dryvax vaccine to human subjects. Before that time, a quantitative PCR assay for vaccina DNA was developed and validated, and will be used for additional studies of smallpox vaccination currently in the planning stage. Last year, the project was redirected to study the role of viruses in severe acute respiratory illness, based on the idea that a bioterrorist event would likely involve use of a respiratory agent, and improved respiratory viral diagnostics would be helpful in recognizing and responding to such an event. The Specific Aims of the new project are: 1) To develop nucleic acid amplification assays for all of the common respiratory viruses, including influenza A and B, human metapneumovirus, coronavirus, picornaviruses (including rhinoviruses and enteroviruses), respiratory syncytial virus, parainfluenza viruses 1-4, and adenovirus; and 2) to apply the assays developed in Specific Aim 1 along with a pan-viral microarray technique developed by Dr. David Wang to analyze specimens from selected groups of patients with illness that appears to be caused by an

infectious agent but are negative by conventional testing. Very substantial progress has been made on Specific Aim 1, with development of RT-PCR assays for influenza A and B, human coronaviruses 229E, OC43, NL43/NH, and SARS, human rhinovirus, and the newly discovered bocavirus. A study called the Severe Acute Respiratory Illness (SARI) study has been initiated, which will enroll patients hospitalized in the intensive care units at Barnes-Jewish and St. Louis Childrens Hospitals with severe acute respiratory illnesses. A retrospective collection of brochoalveolar lavage samples is available for testing, and a prospective study has been initiated to collect specimen material and patient data. Both the retrospective and prospective studies have been approved by the Washington University IRB. The assays to be developed and the collaborative relationships with intensive care clinicians that have been established will provide an infrastructure that will be helpful in detecting and responding to a bioterrorist attack on the United States.
<END TEXT>

<TITLE>: MRCE Abstract #7: Project 9- Stanley
<AUTHORS>: Stanley, Samuel L.
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>: Washington University
660 South Euclid Avenue
St. Louis, MO 63110
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: The major goal of this project is to identify genes that are involved in susceptibility and resistance to human vaccinia infection and influenza infection, and to identify loci that may predispose individuals to adverse events or non-response to smallpox and influenza vaccines, and more severe disease after natural infection. In specific aim 1,

we are using a cohort of previously vaccinated individuals to identify single nucleotide polymorphisms that predispose individuals to develop fever and other adverse events after live attenuated vaccinia (Dryvax) immunization. In specific aim 2, we are performing a prospective study on individuals that obtain modified vaccinia Ankara (MVA) and looking at innate immune responses through analysis of transcriptional changes in peripheral blood mononuclear cell populations, and any correlation between particular loci and either adverse events or more profound immune responses. In the third specific aim, we are collaborating with the Colonna laboratory to look
at the innate antiviral response in specific antigen presenting cell types infected with either vaccinia or influenza virus. These studies are designed to identify why certain virally-infected cells produce large quantities of interferons, while other APC do not. Understanding these pathways may aid in the development of agents that can naturally stimulate host cells to produce higher levels of interferons. Taken as a whole, these studies represent a novel approach to understanding the human innate immune response to live or attenuated viruses at the cellular level, and may lead to new insights into innate immunity, and the nature of protective immune responses, to two critically important biologic agents.
<END TEXT>

<TITLE>: MRCE Abstract #8: Project 10 - Virgin
<AUTHORS>: Virgin, Herbert
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>: Washington University
St. Louis, MO
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords

<TEXT>: Effective responses to biological threats are inextricably related to effective responses to emerging pathogens [Countering Bioterrorism: the Role of Science and Technology, NAS report 2002]. The etiologies of many human diseases are unknown, but may be infectious. New viruses can enter the human population with devastating effect, and the intentional release of new or engineered agents is a danger. When new technologies are applied to the problem of detecting emerging agents or agents associated with diseases of unknown etiology, new pathogens have been discovered, resulting in significant advances in human health and in the development of new and useful diagnostics. These diagnostics, particularly if they are relatively unbiased in what they can detect can play a role in detecting emerging pathogens or biological threats. Thus, discovery of new agents walks hand in hand with effective responses to bioterrorism. In light of these considerations, we propose to identify new agents of human
disease by integrating the efforrts of three principal investigators in a Project designed to: (i) bring the most advanced technologies to the problem of discovering new pathogens, (ii) apply these technologies and approaches in a serious and high throughput way to clinical samples carefully selected to contain unknown or emerging pathogens, and (iii) through this effort bring new diagnostic and discovery tools on line for identifying epidemic agents in clinical specimens. Potential deliverables from this Project include new pathogens associated with human disease, the infrastructure and trained professionals required to identify pathogens during an outbreak, and diagnostic tests and technologies. If this project is successful we envision being able to consider proposing a National Core as part of the MRCE renewal. We propose to accoplish these goals via three Aims: Aim 1) Identify clinical materials appropriate for pathogen discovery experiments. Aim 2) Identify novel pathogens by applying:
Aim 2a) Pan-viral microarray technology. Aim 2b) Representational Difference Analysis (RDA). Aim 2c)

Passage of agents in severely immunocompromised mice.
Aim 3) Determine if novel pathogens are present in clinical samples.
<END TEXT>

<TITLE>: MRCE Abstract #9: Project 11
<AUTHORS>: Belshe, Robert B.
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>: Saint Louis University
Division of Infectious Diseases
3635 Vista Avenue, FD 8-N
St. Louis, MO
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: Four projects are underway in the clinical core. The first one is a design project in which a new protocol to evaluate the biology of modified vaccinia (Ankara) (MVA) and to develop its potential to induce rapid protection against poxviruses. We propose to determine the correlates of immune protection to poxvirus infection using Dryvax vaccination as a surrogate challenge virus. We will also explore the potential for MVA to be used in post exposure prophylaxis. Detailed laboratory studies are proposed. The immunogenetics of common adverse reactions to vaccinia has been studied. Four genes were found to be significantly associated with acute vaccinia syndrome. These genes were IL18, IL1A, IL1B and IL1R1.
Cell mediated immunity studies of poxvirus infections are underway. Vaccinia specific gamma delta T cell responses using a newly developed flow cytometric assay for studying memory effector T cell responses is proposed. The kinetics of specific vaccinia antibodies has also been assessed. We have determined the baseline and 30 day post vaccination specific antibody titers were 79 nave for previously vaccinated smallpox vaccine recipients versus membrane proteins

B5 or A33 or A27L and L1R and the results were presented at the 8th Annual Conference on Vaccine Research In May, 2005.
<END TEXT>

<TITLE>: MRCE Abstract #10
<AUTHORS>: Craig T. Morita
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>: University of Iowa Roy J. and Lucille A. Carver College of Medicine
Department of Internal Medicine
Division of Rheumatology
Eckstein Medical Research Building 340F
Iowa City, IA 52242
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: Francisella tularensis infects at local sites before being disseminated throughout the body via bacteremia and/or infected macrophages. The first few days of infection are often critical in determining whether or not the host will survive. Positioned at the interface between innate and adaptive immunity, NK cells and T cells are present in large numbers and respond rapidly to infection by a variety of viruses, bacteria, and parasites. These "rapid response" lymphocytes kill infected cells and secrete large amounts of IFN- and TNF- that stimulate infected macrophages to kill intracellular bacteria. Human V2V2 T cells expand rapidly during tularemia infections to very high levels (22-50% of circulating T cells) supporting their important role in the immune response to Francisella tularensis. We have shown that identical expansions of monkey V2V2 T cells have characteristics of memory T cell responses and that these expansions coincide with the resolution of mycobacterial infections. We
find that V2V2 T cells use their TCRs to recognize bacterial and protozoal infections by responding to a

common microbial metabolic intermediate, (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMBPP). In support of memory responses by T cells, we find that adult V2V2 T cells are almost exclusively memory cells that are divided into 3 subsets, central memory, effector memory, and effector CD45RA+ memory. We hypothesize that the response of human memory V2V2 T cells to phosphoantigens on the surface of F. tularensis infected cells provides vital 'bridge immunity" in tularemia before T cell and B cell responses.
Despite the importance of V2V2 T cells in human tularemia there are no vaccines that stimulate this T cell subset. Priming or boosting V2V2 T cell memory should help provide early, partial immunity and will also have an adjuvant effects on pathogen-specific T cells for tularemia and for other infections with pathogens that produce HMBPP including anthrax and plague. Here we propose to (1) test V2V2 T cell recognition of F. tularensis infected cells, (2) determine the contribution of V2V2 T cell to the survival of human PBMC-SCID-beige mice infected with F. tularensis, (3) derive live bacterial vaccines for V2V2 T cells that overproduce HMBPP from attenuated Salmonella, and (4) test HMBPP/adjuvant vaccines and live bacterial vaccines for their ability to stimulate V2V2 T cell immunity in monkeys. These studies will elucidate the critical role of V2V2 T cells in tularemia and will develop vaccines that target V2V2 T cells.
<END TEXT>

<TITLE>: MRCE Abstract #11: Project 11- Hultgren, Scott J
<AUTHORS>: Hultgren, Scott
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>: Washington University in St. Louis
<EMAIL>: Email
<PHONE>: Phone

<KEYWORDS>: Keywords
<TEXT>: Pathogenic gram-negative bacteria such as Salmonella, Escherichia, and Yersinia pestis the causative agent of the bubonic plague, assemble adhesive fibers on their surfaces via the chaperone/usher pathway. Presently, there are over 100 different chaperone/usher pathways, which can be divided into two distinct homologous families, the FGS family that assembles pilus type structures and the FGL family, which assembles a variety of structures including the protective capsule-like structures found on Y. pestis. Both FGS and FGL chaperones are made up of two immunoglobulin domains that are oriented in the shape of a boomerang. These chaperones catalyze subunit folding by forming hydrogen-bonding interactions with the C-terminal carboxyl group of the pilin subunit to the invariant Arg and Lys found in the active site cleft of the chaperone. Based on computer modeling using the crystal structure of PapD as a template, we have and continue to design small molecules inhibitors that mimic the specific interactions that the chaperone makes with pilus subunits and thus inhibits pilus or capsule assembly and ultimately disrupts the infection process. One class of inhibitors that is currently being developed is the 2-pyridone carboxylic acids. These inhibitors called pilicides were designed to bind to the active site of chaperone and have been shown to reduce adherence, biofilm formation, and mannose sensitive hemagglutination in type 1 pili by approximately 90%. Since the targeted chaperone active site is invariant in all FGS and FGL chaperones, we believe that these compounds should have a broad spectrum of activity by blocking chaperone function and have the potential to block capsule formation in Y. pestis and disrupt its infection. We will test the ability of these compounds to bind to Caf1M, which is the chaperone responsible for capsule assembly in Y. pestis and inhibit capsule assembly.
A chemical platform with broad applications and flexibility was rationally designed to interrupt complicated molecular machines important in microbial pathogenesis. This work represents a blueprint of how

to blend computer-aided design, synthetic organic chemistry and molecular biology with genetics to investigate and disrupt complex biological systems important in disease.
<END TEXT>

<TITLE>: MRCE Abstract #12: Project 12-Goldman
<AUTHORS>: Goldman, William
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>: Washington University, St. Louis, MO
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: Abstract: Pneumonic plague, caused by the same bacterial strains responsible for bubonic plague, is the deadliest manifestation of disease caused by Yersinia pestis. However, there is remarkably little information on the cellular and molecular mechanisms responsible for Y. pestis-triggered pathology in the lung. This project will focus on the pathogenesis of pneumonic plague by following two specific aims: (i) to characterize the interaction between Y. pestis and pulmonary cells using model systems of infection; and (ii) to identify Y. pestis genes that are selectively upregulated as a consequence of bacterial interaction with the respiratory tract.
Lay description: Pneumonic plague is the deadliest manifestation of disease caused by the bacterium Yersinia pestis. Though rare compared to the bubonic form, which is acquired by skin penetration, the possible combination of widespread aerosol dissemination and rapid disease progression are of particular concern for defense against bioterrorism. This project will use molecular biology strategies to understand how the bacteria interact with the lung during the course of pneumonic plague.
<END TEXT>

<TITLE>: MRCE Abstract #13: Project 14-Majerus, Philip
<AUTHORS>: Majerus, Philip M.
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>: Washington University, St. Louis, MO
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: We have discovered two human genes by their homology to the Burkholderia pseudomallei virulence factor BopB. The homology was restricted to the putative phosphatase active site motif CX5RI. We cloned and expressed cDNAs encoding both of these genes. They both catalyzed the hydrolysis of phosphatidylinositol 4,5-bisphosphate forming phosphatidylinositol 5-P as the product. Phosphatidylinositol 5-P is a recently discovered signalling molecule that is important in cell cycle control and these newly discovered enzymes provide a route for its synthesis. These two 4-phosphatasses are widely expressed and are located on the outer surface of lysosomes.
<END TEXT>

<TITLE>: MRCE Abstract #14: Project 16-LeVine, Steven
<AUTHORS>: LeVine, Steven
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>: University of Kansas Medical Center, Kansas City, Kansas
University of Kansas, Lawrence, Kansas
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: Therapeutic interventions for the medical management of anthrax (Bacillus anthracis infection) are inadequate. Key to the pathogenesis of B. anthracis are the actions of protective antigen (PA)

and lethal factor (LF) which act together to yield a lethal toxin (LeTx). We have shown that LeTx leads to disseminating intravascular coagulation (DIC) and the development of hemorrhagic/hypovolemic shock in mice. Studies were undertaken to attempt to improve the outcome following LeTx exposure in mice. Compounds that have been shown to ameliorate shock and/or DIC by other research groups were tested alone and/or in combination with one another in LeTx mice. The compounds inclued activated protein C, aprotinin, diluted plasma and/or heparin, dimaprit, N-acetylcysteine, or Ilomastat (GM6001). N,N'-diacetyl-L-cystine (a disulfide dimer of N-acetylcysteine) was also tested. The disease course in experimental LeTx mice was compared to that in LeTx mice given vehicle. No single compound or tested combination of compounds significantly improved the survival of LeTx mice compared to the control groups, although some compounds may have had a mild effect on delaying the time to death/moribund state following LeTx injection. Since treatments targeting late pathophysiological events such as DIC and hemorrhagic/hypovolemic shock did not substantially alter the disease course, an earlier pathogenic step was targeted. The binding of protective antigen to its receptors is pivotal to subsequent pathogenesis induced by lethal factor or edema factor. Since the crystal structure of protective antigen binding to one of its cellular receptors, CMG2, had been published (Santelli et al., Nature 2004; 430:905-908), we mapped the region of protective antigen that binds CMG2 and then performed a virtual search of the ChemNavigator Database that contains >5 million compounds. Six compounds were identified that had strong binding affinities, < -23 kcal/mole, and one of the six had a binding affinity of -35 kcal/mole. In addition, we have performed studies to try to investigate the role of heat shock proteins in vessel injury following LeTx exposure. Also, we and other researchers have found that CAST/Ei mice given LeTx have a shorter survival time compared to C57BL/6 mice given LeTx. Studies are currently ongoing to help determine whether or not LeTx CAST/Ei mice have a faster development of DIC and/or

hemorrhagic/hypovolemic shock compared to that for LeTx C57BL/6 mice.
<END TEXT>

<TITLE>: MRCE Abstract #15: Project 17-Colonna, Marco
<AUTHORS>: Colonna, Marco
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>: Washington University
St. Louis, MO
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: Small pox epidemics secondary to a bioterror release of variola virus represent a potentially public health threat. Thus, it is critical to establish anti-variola agents that can provide specific therapy in the case of a viral outbreak. Variola, vaccinia and other poxviruses encode homologues of human CD47 defined here as vCD47. CD47 is a cell surface molecule that inhibits the immune system by interacting with the signal regulatory protein (SIRP). In addition, CD47 interacts with integrins and the extracellular matrix protein thrombospondin, regulating migration and adhesion of leukocytes. The original goal of this proposal was to determine the role of vCD47 in subverting host immune responses and to determine whether vCD47 can be suitable targets for the generation of anti-small pox therapeutics. In our completed Specific Aim 1, we have succeeded in producing the first mAb against vCD47, which we have used to demonstrate that vCD47 is expressed on vaccinia virus-infected cells. Thus, our anti-vCD47 mAb provides a diagnostic tool to detect vaccinia and small pox infections. Moreover, we found that soluble recombinant proteins encoding SIRP or other SIRP receptors did not bind cells expressing vCD47. Therefore we conclude that vCD47 most likely interacts with other endogenous CD47 ligands. In Specific Aim 2 we are currently analyzing the impact

of vCD47 on host immune responses in vivo by infecting mice with wild-type and recombinant EGFP-vaccinia viruses in which vCD47 has been deleted. If vCD47 subverts immune responses, our anti-vCD47 mAb may provide an important therapeutic tool to combat smallpox.
<END TEXT>

<TITLE>: MRCE Abstract #16: Project 18-M. Cho
<AUTHORS>: Cho, Michael
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>: Case Western Reserve University, Cleveland, Ohio
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: The etiological agent of severe acute respiratory syndrome (SARS) has been identified as a novel coronavirus (SARS-CoV). During the 2002-2003 outbreak, close to 8,100 people have been infected worldwide with an overall mortality rate of over 9%. Based on worldwide reactions and events that have occurred during this outbreak, larger epidemics in the future could have a devastating global impact. Consequently, SARS-CoV is a potential agent of bioterrorism with severe health and socioeconomic consequences. It has been shown that SARS-CoV uses angiotensin-converting enzyme 2 (ACE2) as a receptor. However, very little is known about the pathogenic mechanism. To prepare for future epidemics, a vaccine and/or antiviral agents need to be developed. Additionally, better understanding of pathogenic properties of the virus is needed. Towards this goal, we have been evaluating antigenic properties of SARS S glycoprotein and screening for peptides that have antiviral activity using SARS pseudovirus neutralization assay. In this study, we propose to develop a small animal model for SARS, which would complement ongoing studies. The specific aims are:

(1) to construct and characterize a plasmid that encodes human ACE2 under the control of inducible mouse metallothionine promoter; and (2) to generate transgenic mice expressing human ACE2. Developing a mouse model for SARS infection would facilitate understanding of viral pathogenesis as well as vaccine development and evaluation of antiviral agents.
<END TEXT>

<TITLE>: MRCE Abstract #17: Project 19-Fan
<AUTHORS>: Fan, Xiaofeng, M.D., Ph.D.
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>: Saint Louis University School of Medicine
Department of Internal Medicine
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St. Louis, MO 63104
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: Dengue viruses are mosquito-borne flaviviruses that most commonly cause dengue fever (DF), and less frequently cause the life-threatening illness, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). There is no effective vaccine available in spite of extensive exploration using several approaches, including live-attenuated vaccines, subunit vaccines, DNA vaccines and chimeric virus vaccines. Major problems for dengue vaccine development include the low immunogenicity and the existence of a distinct phenomenon, antibody-dependent enhancement (ADE), which requires that broad and strong neutralizing antibodies against all four dengue virus serotypes must be elicited upon vaccination. We hypothesize that these problems can be overcome by using recombinant adenoviruses expressing ancestral dengue virus envelope genes. To test this hypothesis, the following specific aims are planned. Specific Aim 1: Inference and assembly of the ancestral dengue virus envelope gene. Specific Aim 2:

Investigate the immunogenicity of recombinant adenoviruses expressing ancestral dengue envelope gene. Specific aim 1 has been almost achieved in the first year (see progress report) and if successfully renewed, we will accomplish specific aim 2 in the second year.
<END TEXT>

<TITLE>: MRCE Abstract #18: Project 21-Olivo, P.
<AUTHORS>: Olivo, Paul D.
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>: Apath, LLC
893 N. Warson Rd.
St. Louis, MO 63141
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: The goal of this proposal is to identify an inhibitor of Ebola virus (EBOV) that targets the viral protein 30 (VP30) a transcriptional transactivator. This proposal is part of our ongoing efforts to discover novel antiviral agents against the filoviruses Ebola virus and Marburg virus (MBGV). Filoviruses are category A bioterrorism agents that cause fatal hemorrhagic fever syndromes. Neither a vaccine and nor reliable therapeutic options are available for EBOV. We have developed an antiviral screening method based on an infection-independent minigenome expression system. The EBOV minigenome-expression system depends on VP30 which has been shown to promote EBOV transcription. The transcriptional activation activity of VP30 depends on homo-oligomerization which has been mapped to a domain on the polypeptide chain. A 23-mer peptide derived from this domain has been shown to inhibit oligomerization, transcriptional activation, and viral replication (Hartlieb et al. JBC 2003). VP30 therefore represents a possible target for an anti-EBOV therapy. The work of this proposal is to confirm and extend

this observation using our minigenome-based system. This will significantly promote efforts to develop a small molecule inhibitor of VP30 to toward the overall goal of developing a therapeutic agent for Ebola virus infection.
<END TEXT>

<TITLE>: MRCE Abstract #19: Project 22-Reilly
<AUTHORS>: Reilly, Thomas J.; Johnson, Gayle
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>: University of Missouri-Columbia (UMC)
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: Tularemia, a bacterial zoonosis is caused by the intracellular bacterial pathogen Francisella tularensis. The organism can be acquired from numerous sources, readily grown in broth culture, aerosolized and is one of the most infectious pathogenic organisms known to man requiring inoculation or inhalation of as few as ten organisms to cause disease. As such F. tularensis has been designated a Category A Priority Pathogen and is considered a potent and sought after weapon of bioterrorism. While antimicrobial agents and vaccines are available, little is known about how effective these agents are against genetically manipulated strains of the pathogen. It is therefore imperative that we stay ahead of the bioterrorist threat posed by F. tularensis. Staying ahead of the threat requires better understanding of F. tularensis pathogenesis mechanisms, development of agents specifically targetet to abrogate or attenuate these mechanisms, as well as discovery or design of agents which prevent infection. The acid phosphatase of F. tularensis (AcpA) is one molecule with which we may achieve these goals concomitantly. Preliminary biochemical data obtained with highly purified preparations of the acid phosphatase suggests AcpA is important to the survival

of F. tularensis within a host. Purified preparations inhibit the respiratory burst, a potent antimicrobial defense mechanism of professional phagocytes, in a dose-dependent manner. Our long-term objectives are to discern AcpA's properties with respect to intracellular colonization and survival, develop agents specifically targeted to abrogate AcpA's unique catalytic activity, and to utilized recombinant AcpA (rAcpA) in an improved vaccine. This application is a request for funds to address the latter of these objectives. Milligram quantities of rAcpA, expressed at high levels from an overexpression vector are to be produced in E. coli, purified, characterized, and used as an immunogen in the mouse strain C3H/HeN. This strain is known to be highly susceptible with an LD50 of ~1 CFU of the live vaccine strain when injected intraperitoneally Protection afforded by immunity to purified recombinant moleclue will be assessed by clinical signs, statistically significant increase in LD50 in the vaccinated group, reduction of bacterial load in affected organs, and by reduction in pathologic lesions.
<END TEXT>

<TITLE>: MRCE Abstract #20: Project 23-Rowland
<AUTHORS>: Rowland, Raymond R. R.
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>: Kansas State University
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: Severe acute respiratory syndrome (SARS) is an emerging disease caused by the SARS coronavirus (SARS-CoV), a newly discovered member of the coronavirus family. The nucleocapsid (N) protein is the most abundant viral protein and during infection of humans, anti-N antibodies are produced to high levels. Therefore, N represents an ideal target for novel drug strategies and diagnosis of infection. Very little is

known about the individual functional domains within the SARS-N protein, including the role of individual amino acid residues involved in its various functions. One area of active interest is to understand the functions of nuclear transport signal (NLS) sequences. In other coronavirus N proteins, NLS sequences play an import role in locating the N protein to the nucleus. Progress towards the understanding of the function of several nuclear transport domains within the SARS N protein failed to identify N localization to the nucleus of infected and transfected cells. Retention of N in the cytoplasm is the result of a single amino acid located at position 372 of the protein. During the course of this project, several anti-N monoclonal antibodies were developed. These reagents are being tested for their utility in the detection of N protein in cells and tissues. The eventual goal is to incorporate these reagents into SARS diagnostic tests.
<END TEXT>

<TITLE>: MRCE Abstract #21: Project 24-Bann, James
<AUTHORS>: Bann, James G.
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>: Wichita State University
Wichita, Kansas
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: The ongoing threat of the use of anthrax as a bioterrorism agent necessitates the development of therapies to block the action of anthrax toxin at any stage of infection. Anthrax infection is well recognized to be pH dependent as a consequence of membrane bound pore formation by multimers of the anthrax B toxin protective antigen. Upon lowering of the pH of the endocytic vesicle from ~8 to ~6, extensive conformational changes occur in which the 2-3 strands of domain 2 of PA form a pore in the membrane. This pH dependent conformational change also

occurs in vitro in the absence of the receptor, but the change in pH required for pore formation is less (8 to ~7.5-7), suggesting that the receptor plays a direct role in restricting the pH dependent conformational change. The physical-chemical basis of the pH dependent conformational changes that occur in these toxins or the receptor is not understood. Understanding the physical basis of these pH dependent conformational changes would potentially guide the design of therapeutics aimed at preventing anthrax toxin entry. The present objective is to understand how pH governs the large conformational change in the B toxin protective antigen to form a pore. The proposed research will capitalize upon the biosynthetic incorporation of 2-fluorohistidine (2-F-His), which has a dramatically lower pKa, to probe the effect of histidine on pH dependent conformational changes. Our central hypothesis is that the formation of a membrane spanning pore in the presence of the receptor is dependent on the protonation of histidine residues. The hypothesis is based on preliminary experiments that show that labeling of PA63 with 2-F-His, which has a pKa of 1, prevents the pH dependent conformational change from occurring in the presence of CMG2. In the absence of the CMG2 receptor, pore formation occurs at pH values coincident with the unlabeled protein. 19F-NMR experiments on the 2-F-His labeled PA indicate an environmental change from a pH of 8 to 7, with no further changes observed from a pH of 7 to 5, suggesting other titratable residues are involved in the pH dependent conversion. Future experiments will focus on characterizing the functionality of the 2-F-His PA, and include studies in collaboration with Dr. R. John Collier (Harvard) on the ability to insert into planar lipid bilayers and to translocate LFn-DTA into the cytosol of CHO-K1 cells. 2-F-His PA may represent a novel therapeutic that can inhibit anthrax toxin action by blocking pore formation.
<END TEXT>

<TITLE>: MRCE Abstract #22: Project 25-
Carayannopoulos, Leonidas
<AUTHORS>: Carayannopoulos, Leonidas
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>: Washington University
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<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: Innate lymphocytes include natural killer (NK-) NKT-, and T cells. They survey the surfaces of surrounding cells for expression of numerous constitutive and inducible markers many of which possess an MHC class I-like protein fold. If the profile of such markers deviates from normal by virtue of cellular stress caused by viral infection, the cell will be recognized as infected by the innate lymphocyte. This results in killing of the infected cell and secretion of important regulatory cytokines by the effector cell. While the cytokine environment bathing the lymphocyte sets the range of normal, it is the marker profile itself that triggers the recognition event. Because of the importance of these markers and their cognate receptors to antiviral immunity, viruses have evolved countermeasures against these molecules functions. Of particular interest to biodefense are the countermeasures employed by zoonotic orthopoxviruses. These agents are amenable to bioengineering, have animal
reservoirs beyond the reach of vaccination, and can occasionally display surprising propensity for interhuman spread (e.g. monkeypox Zaire).
This proposal relates to a novel MHC Class I-like protein (OMCP) discovered through the use of structurally constrained hidden Markov models. It is conserved in all sequenced cowpox and monkeypox viruses and hypothesized to serve as a countermeasure to innate immunity. We have recently found this molecule to fold as a dimer and bind to an undefined receptor on NK cells and B cells. Ongoing work is

aimed at: cloning the host receptor for OMCP, producing better immunoreagents for the Brighton Red cowpox variant, and characterizing the effects of OMCP on NK cells.
Achievement of these objectives will lay the groundwork for a later RO1-level proposal.
<END TEXT>

<TITLE>: MRCE Abstract #23: Project 26-Haslam, David B.
<AUTHORS>: Haslam, David B.
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>: Washington University
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<KEYWORDS>: Keywords
<TEXT>: Shiga, cholera, ricin, and several other toxins follow a retrograde pathway in order to reach the host cell cytoplasm. This pathway is an attractive target for therapeutic intervention and considerable effort has been placed in identifying the host molecules involved in toxin trafficking. Yet, directed examination of known vesicle trafficking molecules have thus far failed to identify conserved components of toxin trafficking pathways. We propose to take an alternate, unbiased approach. Rather than focus on known vesicular trafficking proteins, we will employ a small molecule screen for inhibitors of shiga toxin action. We will then determine the intracellular sites of toxin inhibition. Over the long term, the host targets of inhibitory molecules will be identified, and the effects of these inhibitors on susceptibility to other toxins will be determined.
In order to carry out a screen for small molecule inhibitors of toxin trafficking, we have developed a highly sensitive and reproducible assay for shiga toxin (Stx) activity, based on the toxins ability to

inhibit protein synthesis. Vero cells are transduced with an adenoviral vector encoding luciferase (modified such that the protein has a short intracellular half-life). Upon exposure to Stx, luciferase synthesis is inhibited, existing luciferase is degraded, and cells have diminished light output upon exposure to luciferase substrate. We have adapted this assay to a multiwell format, such than thousands of wells may be exposed to toxin plus small molecule compounds. This screen will be carried out at the Institute for Chemistry and Cell Biology (ICCB) at Harvard University. Molecules found to have inhibitory activity will be examined in detail by determining the stage at which toxin trafficking or activity is inhibited. Finally, we hope to identify the intracellular target of inhibitory molecules using a proteomics approach. These studies will have important implications for the study of vesicular trafficking and cell biology. More importantly, we hope that some of these compounds may be developed for therapies against disease caused by Stx, cholera, ricin, and other bacterial toxins.
<END TEXT>

<TITLE>: MRCE Abstract #24: Project 27-Pontow, Suzanne
<AUTHORS>: Pontow, Suzanne
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>: Washington University
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: The potential misuse of poxvirus vectors as agents of bioterrorism necessitates rapid development of
antiviral therapies to combat infection in non-immunized people. Recent studies from our laboratory and others indicate that inhibition of signal transduction pathways can effectively limit infection or spread of viruses. A complex signaling network

centered on the Rho-family GTPases Cdc42, Rac1, RhoA and RhoG, is a common target of diverse microbes, including all of the Category A pathogens, that exploit cellular membrane systems and cytoskeletal remodeling or transport mechanisms as requisite features of their infectious cycles. Vaccinia virus (VV), a close relative of Variola major and Category A Priority Pathogen, provides an excellent system for studying opportunistic use of cellular membranes and cytoskeletal plasticity. Recombinant VVs encoding dominant-negative Rac1, Cdc42 or RhoA or constitutive-active Rac1 or Cdc42 point mutants were characterized utilizing a series of assays designed to evaluate individual milestones in VV maturation and egress. Our results indicate that Rho-family GTPases are an integral part of the host cell signaling apparatus utilized during the processes of VV assembly, transport and spread. The expression of Rho-family activity mutants disrupted VV morphogenesis at distinct stages, mimicking morphogenetic blocks described for specific VV deletion mutants and antiviral drugs. A model of VV morphogenesis and spread was developed, based on evidence supporting the participation of Rac1 and specific regulatory partners in the processes of VV transport, envelopment, externalization, actin tail formation, and release, with a roles for Cdc42 in actin recruitment and tail formation. Future work will be driven by the relationships between VV proteins and host cell signaling molecules and cytoskeletal elements that are suggested by the model.
<END TEXT>

<TITLE>: MRCE Abstract #25: Project 28-Tanner, John
<AUTHORS>: Tanner, John J.;Reilly, Thomas J.;Felts, Richard;Ou, Zhonghui

<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>: University of Missouri-Columbia (UMC)

<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: Francisella tularensis is the highly infectious bacterium that causes tularemia, a potentially fatal plague-like disease. The CDC has classified F. tularensis as a category A agent because of its potential for use as a bioterrorism agent. The long-term goal of this project one that we are seeking NIH funding for - is to determine high resolution crystal structures of F. tularensis virulence proteins as a prelude to structure-based inhibitor and vaccine development. The goal of this 1-2 year MRCE project is to obtain preliminary crystallographic data for several F. tularensis virulence proteins, which we feel, is needed for a competitive NIH application. Involvement of the target proteins in virulence has been established by us and others using a variety of proteomics, molecular genetics and cell biological techniques. However, the precise functions of these proteins are not well understood. The crystal structures determined in this project will provide clues about the functions of these proteins and further our understanding of their roles in the life cycle of F. tularensis and in virulence. This project is timely because there are currently no crystal structures of any F. tularensis protein. Thus, structural biology of the F. tularensis proteome is a newly emerging research area that we have the potential to lead.
This proposal has two aims. (1) Determine the crystal structure of Francisella tularensis AcpA, a respiratory burst-inhibiting acid phosphatase. (2) Obtain preliminary crystallographic data for several other putative F. tularensis virulence proteins. Aim 1 was completed with the 1.75 resolution structure of AcpA bound to the competitive inhibitor vanadate. This work resulted in two publications and 1 deposition to the protein data bank. Aim 2 was completed beyond expectation. Although the goal was to obtain preliminary crystallographic data, we, in fact, determined three structures of a F. tularensis periplasmic histidine acid phosphatase, grown crystals

of a F. tularensis surface-localized (class C) phosphatase and determined two structures of the class C phosphatase homologue from Bacillus anthracis. Moreover, we have recently obtained high level expression of soluble MglA and IglC expressed as fusion proteins with maltose-binding protein. Protein purification and crystallization trials of these proteins are in progress.
<END TEXT>

<TITLE>: MRCE Abstract #26: Project 13-P Biswas
<AUTHORS>: Biswas, Pratim; Angenent, Largus
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>: Washington University
St. Louis, MO 63110
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: The long term objectives of this project is to continue developing and testing the SXC for capture and inactivation. One current study that is underway is to test the performance of the unit in BSL 3 type facilities, instead of testing with only simulated agents.
The other focus is to identify a commercial partner to adopt this technology and have a specific application wherein it is used.
Relevance of the project to public health is significant as it could be used in ventilation systems that will prevent the spread of viruses and bacteria that cause disease. Very specific is the airborne transmission and spread can be completely eliminated in indoor environments. For example, airborne transmission of corona virus, SARS virus, and other flu viruses could be eliminated in closed environments.
<END TEXT>

<TITLE>: MRCE Abstract #27: Project 2-Atkinson
<AUTHORS>: Atkinson, John P.
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>: Washington University St. Louis, MO
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: The overall goal for our grant is to characterize the complement regulatory activity of the poxviral inhibitors of complement enzymes. We cloned and expressed the vaccinia complement regulatory protein, VCP. Next we modified (changed 11 amino acids) in this construct to create the complement regulator of variola called SPICE. Additionally, we cloned the ectromelia (mousepox) inhibitor (and termed it EMICE) and are collaborating with Mark Buller to establish an in vivo system to investigate the role of complement in ectromelia infection. Because of the recent outbreak of monkeypox infection in the U.S., we also cloned and are evaluating its complement regulator (and termed it MOPICE). Of much interest, through a collaboration with Mark Bullers group at Saint Louis University, it was determined that the more virulent Central African strains of monkeypox express this regulator while the less virulent West African strains have deleted it. Indeed, the major difference between the two strains appears to be the presence or absence of MOPICE, suggesting its importance as a virulence factor in monkeypox infections.
We demonstrated that complement regulatory activities parallel severity of human infection. We also characterized MOPICE and the functional significance of homodimers of poxviral complement regulators. These data imply that 1) cofactor activity is critical to block complement regulation in the host since MOPICE lacks convertase disassociating activity (DAA); 2) DAA for the classical and/or lectin pathway is key for viral pathogenesis; 3) dimers are more efficacious inhibitors than monomers; and, 4) heparin binding

sites on secreted poxviral proteins represent a creative way to regulate complement activation in that one protein can serve in both fluid-phase and on host membranes. Mouse studies demonstrated increased mortality in the C3-/- mice infected with ectromelia indicating complement is required for either direct viral inactivation or induction of the immune response or possibly both. The death of antibody-deficient mice at or before the generation of specific IgM or IgG suggests that natural antibody
is also important for controlling poxviruses at early time points. The activation of complement by natural antibody could be a key aspect of host defense during the first week of infection. Activation of complement by specific IgM that arises in the second week following inoculation could be critical to resolving the infection.
<END TEXT>

<TITLE>: MRCE Abstract #28: Project 20-Molina, Hector
<AUTHORS>: Molina, Hector
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>: Washington University School of Medicine, St. Louis, MO
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: There are concerns for safety and risks related to orthopoxvirus (OPV) outbreaks. It is imperative to find methods to enhance the natural immune defenses of the host and provide protection against infection. One mechanism to achieve this goal is to study the role of the complement system. It is a natural adjuvant that orchestrates the activation of multiple immunological effector pathways directed against pathogenic invasion. That complement is important in OPV infection is underscored by the expression of complement regulatory proteins by these viruses and by the acquisition of host complement

regulatory molecules by the viral envelope. Understanding the immunobiology of complement during OPV infection will help us generate better vaccines and immune-based strategies to control OPV infections. In collaboration with Dr. Mark Buller, we have infected mice deficient in the third component of complement (C3) with Ectromelia virus as a representative OPV The effect on clinical outcome was measured.
Survival curves were recorded. In general, no difference was noted in morbidity and mortality comparing WT mice to C3-deficient mice. The number of mice per group, however, was small making it difficult to determine any real effect from the C3-deficiency and making it difficult to assess any statistical analysis.
<END TEXT>

<TITLE>: MRCE Abstract #29: Post-DVM Training- Riley
<AUTHORS>: Riley, Lela
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>: University of Missouri-Columbia
Columbia, MO
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: The University of Missouri will continue its efforts to train veterinarians in research related to biodefense and emerging pathogens. There remains a critical need for veterinarians with expertise in agents of bioterrorism recognizing that 80% of the organisms on the Select Pathogen list are zoonotic pathogens. This training program is designed to address the shortage of veterinarians trained in this field. To date, the MRCE program has successfully trained two students and two additional post-DVM trainees were recently recruited into the program. Trainees funded by the award are conducting research of importance to biodefense and emerging pathogens.

In addition, individuals recruited into the program receive training in conducting animal experimentation with BSL3 level pathogens and participate in development of protocols for operation of a Regional Biocontainment Laboratory that will be constructed at the University of Missouri. Trainees completing this program will be eligible to conduct research with highly pathogenic agents and emerging pathogens, assist investigators in performance of research using animals models of infection, and oversee animal facilities in which highly pathogenic agents are studied.
Additionally, seminars and lectures presented by MRCE faculty at the University of Missouri on a variety of topics related to research in biodefense and emerging pathogens have been provided to trainees funded by this award as well as to a numerous other graduate students, postdoctoral fellows and faculty on the University of Missouri campus and the Washington University. Thus, the funding provided by the MRCE is being exceptionally well utilized to benefit the scientific community.
<END TEXT>

<TITLE>: MRCE Abstract #30: Project 8-Kazura
<AUTHORS>: Kazura, James
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>: Case Western Reserve University
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: The present threat of an intentional release of smallpox in a bioterrorism attack requires the consideration of implementing mass vaccination. However, Dryvax - the only vaccine known to protect against smallpox - has been shown to cause serious complications, especially in individuals with a compromised immune system. Therefore, there has been a desire to develop a safer vaccine to protect against smallpox. In order to understand the immunological

features of long-term memory elicited by Dryvax, we have studied the phenotype of IFN- producing vaccinia specific CD8+ memory T cells of persons given Dryvax from 1 to more than 30 years previously. A population of CD8+ TEMRA+ effector memory T cells produce IFN- and are present in individuals last vaccinated over 30 years ago. The long-term maintenance of this CD8+ T cell memory population suggests that it may play a role in conferring protection in concert with neutralizing antibodies. These data can be used for comparisons following smallpox
immunization using newer generations of vaccines such as modified Vaccinia Ankara strain and cloned vaccinia virus preparations.
<END TEXT>

<TITLE>: NBC Abstract#1 Theme 1 - Briese
<AUTHORS>:
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>:
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: Post exposure prophylaxis and treatment are critical challenges in the management of emerging viral diseases. Vaccines may be helpful in post exposure prophylaxis of neurologic diseases such as rabies where agents replicate slowly or have long incubation periods. However, in most instances, vaccines are not useful for post-exposure because active immunity does not occur in a time frame wherein disease can be prevented or ameliorated. Passive immunotherapy has an established track record in management of many acute viral diseases. The protective effects of passive immunotherapy are specific, rapid, and long lasting. Indeed, passive antibody theapy is currently the only available strategy for providing immediate immunity to susceptible populations. Although the efficacy and safety of antivirals will undoubtedly improve, passive

immunotherapy, an old yet effective tool in our armamentarium, will continue to be a significant primary or complementary line of defense in emerging infectious diseases. In the three decades since Khler and Milstein first reported derivation of murine hybridomas, methods have been established for creating MAbs through phage display and for humanizing murine MAbs. Advantages of fully human monoclonal antibodies (fhMAbs) include improved circulation half-life, reduced immunogenicity, and potential for improved performance through complement-mediated cytotoxicity and antibody-dependent cytotoxicity. Technical advances have enabled economical GMP production of highly active MAbs for passive immunotherapy of human disease in virtually inexhaustible quantities.
The recent global spread of H5N1 influenza virus has precipitated investment in vaccines and antivirals. Less attention has been focused on the potential utility of antiviral MAbs as primary or complementary therapeutics. This program will build on unique expertise and resources at four NBC institutions to rapidly develop and implement therapeutic MAbs for H5N1 influenza virus through pursuit of four specific aims: Aim 1, Clone cDNA encoding HA of H5N1 influenza viruses isolated from humans and birds in 1997 to 2005; Aim 2, Create fhMAb and mouse MAb to H5N1 influenza virus using recombinant HA; Aim 3, Establish fhMAbs to H5N1 influenza virus using PBMC from survivors of H5N1 infection; Aim 4, Characterize and select for future development the fhMAbs obtained in Aims 2 and 3.
<END TEXT>

<TITLE>: NBC Abstract#2 B Cell Related Intervention
<AUTHORS>: Arturo Casadevall
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>:
<EMAIL>: Email
<PHONE>: Phone

<KEYWORDS>: Keywords
<TEXT>: We have continued our studies to identify the molecular determinants of antibody efficacy against B. anthracis toxins. Specifically we have identified a novel neutralizing epitope on the PA20 fragment of B. anthracis protective antigen (PA). The mechanism of antibody-mediated protection appears to be inhibition of the rate of proteolysis by cell proteases. In addition we have taken on several new research directions. First, we have generated a large panel of antibodies to the B. anthracis capsule, and some prolong survival in mice infected with anthrax spores. Second, we have developed a new way of radiolabeling anthrax toxins that promises to significantly enhance studies of toxin action, cellular localization, etc. The method of radiolabeling was used to study the fate of anthrax toxin components in vivo (mice) and provided evidence that they localized to liver and spleen. Third, we have made the first mAbs to B. anthracis anthrolysin. In the Staphylococcus enterotoxin B project Dr. Fries has succeeded in making a large set of mAbs that will be characterized for protective efficacy.
<END TEXT>

<TITLE>: NBC Abstract#3 Theme 1 - Lipkin
<AUTHORS>:
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>:
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: Passive antibody administration is the only strategy available for conferring immediate immunity to individuals exposed to biological weapons. Furthermore, toxin-specific antibodies are toxin neutralizing agents par excellence and currently constitute the only means of neutralizing toxins in the human host. The efficacy of antitoxin sera in

human therapy is known since the 1890s when Behring and Kitasato developed antisera to tetanus and diptheria toxins and demonstrated their prophylactic and therapeutic properties. However, despite a century of immunological study we know relatively little about what antibody properties are important for antitoxin efficacy and the mechanisms involved in toxin neutralization are largely conjecture. This application is focused on the generation of antibodies to four toxins: B anthracis toxins (protective antigen, lethal factor, and edema factor protein subunits), ricin, and Staphylococcus aureus and enterotoxin with the goal of generating therapeutic antibodies to protect against biological attack with these agents. In addition we will generate neutralizing antibodies to West Nile Virus (WNV). The United States is currently in the midst of an unfolding WNV epidemic and there has been some concern that this agent was deliberately introduced into this country in an act of bioterrorism. While this is unlikely, the morbidity and mortality asociated with WNV remain a significant public health concern.
<END TEXT>

<TITLE>: NBC Abstract#4 RCE in Biodefense and Emerging Disease
<AUTHORS>: Ralph M. Steinman
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>:
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: We will continue to develop a new approach to vaccination against antigens that are pertinent to biodefense, beginning with Yersinia pestis LcrV protein. The approach aims to harness the principles of generating T cell dependent immune responses of the Th1 type. Currently, the vaccine is designed to target LcrV protein within monoclonal antibodies to

maturing dendritic cells within peripheral lymphoid organs. Dendritic cells are antigen presenting cells that control the generation of immunity, including memory for T and B cell responses, and this takes place within lymphoid tissues. The first monoclonal antibody to be tested is to DEC-205/CD205, an endocytic receptor on a subset of dendritic cells. LcrV protein has been successfully engineered in frame into the antibody. The fusion monoclonal antibody delivers V protein efficiently to dendritic cells in mice. When an adjuvant is simultaneously administered with the fusion antibody, to insure that the dendritic cells differentiate or mature, strong Th1 type CD4+ T cell immunity develops, and this is associated with a secondary response that includes high antibody titers spanning several IgG isotypes. To further develop this strategy, we will conduct protection experiments in mice exposed to lethal Y. pestis via the intranasal route, in order to set standards for assessing the quality of our immunization readouts. Our experiments are directed to 1) optimizing the adjuvant used to mature the antigen-capturing dendritic cells, 2) establishing the quality and duration of immune memory, 3) assessing an additional receptor using an antibody that targets a subset of dendritic cells distinct from CD205 positive cells, 4) developing human DEC-205 transgenic mice, and 5) identifying anti-dendritic cell antibodies that mediate antigen delivery and processing in humans and monkeys. We are aiming to identify optimal targeting antibodies for delivery of vaccine antigens to dendritic cells, particularly reagents that might be used in several different mammals to help develop this approach broadly in biodefense.
<END TEXT>

<TITLE>: NBC Abstract#5 Antibodies to the Yop Translocon and Immunity to Plague
<AUTHORS>: James B. Bliska
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation

<TYPE>: Type
<ADDRESS>:
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: Yersinia pestis, the agent of bubonic and pneumonic plague, is classified as a category A pathogen. This microbe is highly infectious, and can spread from human to human, by the aerosol route. There is no vaccine currently available in the United States to prevent plague in humans. Current treatment strategies rely on the use of antibiotics. The generation of antibiotic resistant strains through natural selection or by human intervention poses a significant health threat to the population. Therefore, new vaccines and novel therapeutic strategies are needed to counteract the intentional use of plague as a biological weapon. An antiphagocytic protein capsule that is produced by Y. pestis has been shown to function as a protective antigen. However, this capsule is not required for virulence, and capsule-deficient strains may therefore pose an additional challenge to current experimental vaccination strategies. In addition to producing a capsule, Y. pestis secretes a set of antiphagocytic protein toxins known as Yops directly into human cells. These toxins are delivered across the host cell membrane by a bacterial surface structure referred to as the Yop translocon. The translocon is thought to be composed of at least three proteins: LcrV, YopD and YopB. LcrV, YopD and YopB are thought to form a complex that inserts a channel in the host cell membrane through which the Yops are delivered. LcrV is a protective antigen, and together with the capsular protein, has been developed into an experimental two-subunit vaccine. A previous study indicates that YopD is partially protective against non-encapsulated Y. pestis. It is not known if YopB can function as a protective antigen. We will determine if YopD and YopB can function as protective antigens against non-encapsulated Y. pestis in mice. Mice will be vaccinated with YopD and YopB either alone or in combination with LcrV with the aim of

developing a multi-subunit vaccine that is highly efficacious against non-encapsulated Y. pestis (Aim 1). We will also determine if protective antibodies to YopD and YopB block Yop delivery, as has been reported for antibodies to LcrV (Aim 2). In addition, we will isolate monoclonal antibodies to YopD and YopB that could be used to passively immunize humans against plague (Aim 3).
<END TEXT>

<TITLE>: NBC Abstract#6 Theme 3 Crystal
<AUTHORS>:
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>: Weill Medical College of Cornell University (Weill Cornell)
New York, NY
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: Anthrax is caused by infection with B. anthracis, a category A bioterrorism agent. Any strategy to protect against B. anthracis must recognize that the pneumonic form of the disease develops quickly, and thus anti-B. anthracis protection has to be functional within days following an attack. This proposal presents a strategy for both rapid and long lasting protection against B. anthracis based on our experience in using adenovirus (Ad) gene transfer vectors to develop anti-bacterial vaccines, and the ability of Ad vectors to code for single chain antibodies against specific antigens. The underlying concept is that Ad-based gene transfer vectors can be used to evoke systemic, robust acquired immunity, as well as rapid passive immunity against B. anthracis antigens, and that both forms of protection can be achieved with a single administration of a single vector. The proposal uses an in vivo gene transfer-based strategy with a single Ad vector to simultaneously evoke rapid humoral immunity against B.

anthracis (via an anti-B. anthracis protective antigen (PA) single chain antibody coded by the vector), while also functioning as a vaccine to evoke endogenous host responses against B. anthracis PA (via B. anthracis PA coded by the vector). The 3 specific aims outline studies to achieve these goals by developing the vaccine and single chain antibody strategies independently, and then combined. Aim 1. To evaluate the hypothesis that a vaccine based on a replication deficient Ad vector encoding the B. anthracis PA or fragments of PA will evoke robust systemic humoral immunity against PA and protect against challenge with B. anthracis. Aim 2. To assess the hypothesis that an Ad vector encoding a single chain antibody against an epitope of B. anthracis PA will provide robust, rapid humoral immunity against PA and protect against challenge with B. anthracis. Aim 3. To examine the hypothesis that a combined passive and active anti-B. anthracis protection can be achieved with a single administration of a single Ad vector expressing anti-B. anthracis single chain antibody and B. anthracis PA antigen.
<END TEXT>

<TITLE>: NBC Abstract#7 Salmonella type III secretion for bio-defense vaccines
<AUTHORS>: Jorge E. Galn
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>: Yale University School of Medicine
Section of Microbial Pathogenesis
295 Congress Avenue, BCMM 354 New Haven, CT 06536
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: Avirulent strains of Salmonella typhimurium endowed with the ability to express cloned genes from other pathogens are being widely considered as platforms for the construction of polyvalent vaccines. We have recently developed a system that significantly

improves the utility of Salmonella as an antigen delivery vehicle. This system is based on the use of a specialized protein secretion apparatus (termed type III) that is normally utilized by Salmonella to deliver effector bacterial proteins into the extracellular medium as well as into the cytosol of infected cells. We have adapted this system to deliver heterologous proteins into class I- and class-II antigen presenting compartments and found that antigens delivered by this system stimulate strong immune responses both in-vivo and in-vitro. It is the objective of our proposed research project to develop the type III secretion-based delivery system into a versatile platform capable of delivering antigens from pathogens that could be used in a bioterrorist attack to different compartments of the antigen-presenting cellular machinery. More specifically we propose: 1) To construct avirulent strains of S. typhimurium capable of delivering through both of its type III secretion systems, protective antigens from Bacillus anthracis, Yersinia pestis and Burkolderia mallei; 2) To evaluate the ability of these vaccine strains to induce protective immune responses.
<END TEXT>

<TITLE>: NBC Abstract#8 Jacobs Theme 3
<AUTHORS>:
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>:
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: BCG represents an attractive platform for the development of multivalent vaccines since it: 1) is the worlds most widely used vaccine (over 3 billion doses administered since 1928) having a low incidence of serious complications, 2) can be administered at or any time after birth and is unaffected by maternal antibodies, 3) can be given as a single does and

sensitizes to tuberculoproteins for 5 to 50 years, 6) inexpensive to produce, and 7) can be readily manipulated genetically with a diverse set of vectors and tools. Moreover, numerous studies have demonstrated that immu7nization of mice with BCG expressing foreign antigens elicit antibody and T-cell immune responses to viral, bacterial, and parasitic antigens. In recent studies, we have generated novel attenuated M. tuberculosis (AMTB) and demonstrated that they are safer than BCG in immunocompromised mouse models and yet offer protection comparable to BCG. These new vectors represent attractive new platforms for vaccine candidates. We hypothesize that rBCG and rAMTB can be developed as novel protective vaccines for diseases beyond tuberculosis. As proof of principle, we intend to clone and express genes encoding protective antigens from West Nile Virus and influenza virus in rBCG and rAMTB vectors and demonstrate that mice immunized with these strains can be protected against viral challenges. These studies will demonstrate proof of principle to move forward to clone protective antigens from Bacillus anthracis and Small Pox virus for the generation of effective vaccines against these bioterrorist agents.
<END TEXT>

<TITLE>: NBC Abstract#9 Theme 3

<AUTHORS>: Peter Palese
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>:
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: This proposal aims at developing Newcastle disease virus (NDV) as a vaccine vector platform for use in animals and humans. Work was initially focused on optimizing the backbone of the recombinant NDV: (1) The F protein cleavage site was altered to allow

better spread in mammalian cells; (2) A cassette for the easy insertion of the foreign immunogen was developed which contained the cytoplasmic tail and the transmembrane region of the NDV F protein; (3) A murine Il-2 gene was inserted into the backbone to enhance immunogenicity. The early work was done using the anthrax PA antigen as the foreign immunogen. We will now switch to the virulence antigen of Yersinia pestis (LcrV) as the foreign antigen because it will allow direct comparison of the viral and bacterial vectors developed by the five laboratories of the vaccine platform group. It will also allow establishment of optimal prime-boost protocols in the Yersinia pestis mouse model system. We have also started to focus on the development of a chimeric NDV/influenza H5 vaccine. This vaccine should make it possible to simultaneously vaccinate chickens against NDV and the H5 hemagglutinin of the bird flu. It is proposed that such a vaccine would effectively protect against NDV and the presently circulating virulent H5N1 influenza viruses, and prevent the spread of both diseases in poultry.
<END TEXT>

<TITLE>: NBC Abstract#10 : Theme 3

<AUTHORS>: John K. Rose
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>:
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: Recombinant vesicular stomatitis viruses (VSVs) expressing appropriate foreign antigens are highly effective vaccines that protect against infection and disease in numerous viral challenge models. VSV vectors can be grown easily to very high titers in cell lines approved for vaccine production, and could be prepared quickly for use in emergencies.

One goal of this project was to determine if the VSV platform was effective against the SARS coronavirus, a virus that has now been controlled, but could re-emerge. Protection was achieved after only a single vaccination of animals with a VSV recombinant expressing the SARS glycoprotein. Another goal of this project is development of optimized VSV-based vectors expressing antigens from bacterial agents such as Yersinia pestis that cause fatal human disease and could potentially be used as biological warfare agents. Although VSV vectors had not been used previously to express bacterial antigens, it was hypothesized that they would be an excellent vaccine platform for generating protective responses. Indeed, protection against challenge with Y. pestis has now been demonstrated in an animal model using a prime-boost combination of VSV-based recombinants expressing a single protein of Y. pestis. Future studies will be directed at further optimization of the VSV-based vector so that complete protection can be achieved with a single vaccination.
<END TEXT>

<TITLE>: NBC Abstract#11 Identification and characterization of IFN-antagonists: critical virulence factors
<AUTHORS>: Basler
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>:
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: Numerous viruses encode gene products that counteract host interferon (IFN) responses. In several model systems, the viral IFN-antagonists are essential for full virulence. Thus, we anticipate that IFN-antagonists encoded by NIAID category A, B and C viruses will function as virulence factors. This project seeks to identify and characterize these IFN-

antagonists, viewing these as potential antiviral targets. Also, viruses with mutated IFN-antagonists may be useful as live, attenuated vaccines. Therefore, this project continues to use biological screening methods in conjunction with other established assays to identify and characterize IFN-antagonists from the filoviruses(NIAID priority category A), the arenaviruses Lassa virus (category A), the bunyavirus LaCrosse virus (LaCV) (category B) and the henipaviruses (category C). These studies build upon our previous studies on IFN-antagonists encoded by Ebola virus (the VP35 protein) and Nipah virus (the P, V and W proteins). Additional experiments will address the mechanisms by which these IFN-antagonists. Specifically, we have cloned, expressed and tested for IFN-antagonist activity proteins from different species of Ebola virus and Marburg virus, from Nipah virus, from Lacrosse virus and from several arenaviruses. We are in the process of defining the mechanisms by which the Ebola virus VP24 protein inhibits IFN-signaling and by which the Lassa virus NP protein inhibits IFN alpha/beta production. Finally, methods are being developed to screen for inhibitors of the Ebola virus VP35 and VP24 IFN-antagonists.
<END TEXT>

<TITLE>: NBC Abstract#12 Role of Dengue virus non-structural proteins in inhibiting innate immunity
<AUTHORS>: GARCA-SASTRE, Adolfo
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>:
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: The interferon system operates as a defense mechanism in infected cells, resulting in inhibition of viral replication. Many viruses display countermeasures against the interferon response that

allow them to replicate. Positive-stranded RNA viruses like hepatitis C virus and poliovirus posses such mechanisms and so do many negative-stranded RNA and DNA viruses. This proposal outlines a series of experiments designed to investigate the mechanisms employed by dengue virus to antagonize the antiviral effects of interferon. Reporter systems have been developed and applied to identify dengue virus proteins with the ability to block interferon signaling, or to enhance viral replication in the presence of interferon. Functional assays and mutational analysis will delineate the involvement of the dengue interferon antagonists identified. I plan to engineer dengue viruses expressing a defective interferon antagonist, and we expect that their capacity to replicate will be hindered in interferon-competent hosts. Thus, our findings may have therapeutic implications. The identification of dengue virus-encoded interferon antagonists will probably point to homologues in other insect-borne flaviviruses of medical or bioterrorism importance. Therefore, our studies may uncover mechanisms of pathogenesis present in several important viruses.
<END TEXT>

<TITLE>: NBC Abstract#13 Age-related endocrine and immune factors in viral encephalitis
<AUTHORS>: Mady Hornig
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>: Columbia University
722 W. 168th St. room 1801
New York, NY 10032
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: Age has important influences on morbidity and mortality associated with viral infection. In some instances, neonates are particularly vulnerable (e.g., Sindbis virus); in others, advanced age creates risk

(e.g., influenza virus, West Nile/Japanese encephalitis viruses). Changes in immune (Th1 vs Th2 cytokines, CD4/CD8 T cell ratios, humoral responses) and endocrine (melatonin, dihydroepiandrostenedione, cortisol) status are potential correlates of age-sensitive differential susceptibility. Pharmacologic manipulation of immune and endocrine parameters may improve clinical outcome independent of antiviral or passive immunotherapy, or may boost the effect of more specific therapies. Alterations in cellular and humoral immune function will be assessed in young adult and aged C3H/HeJ mice infected subcutaneously with high or low dose WNV. Serum DHEA sulfate, melatonin sulfate, cortisone, and individual cytokine levels will be quantitated at baseline and at regular intervals following infection. Mortality, viral burden in blood and brain, IgM and IgG antibody status, motor function, and neuropathology will also be assessed. These same parameters will be measured in mice treated with DHEA sulfate or melatonin sulfate (reversal of age-related endocrine changes); cortisone or aminogluthethimide (enhancement vs inhibition of peripheral corticosteroids); and IL-2 and IL-4 (enhancement of Th1 vs Th2 responses).
<END TEXT>

<TITLE>: NBC Abstract#14
<AUTHORS>: David E Levy PhD
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>: Department of Pathology
New York University School of Medicine
550 First Ave MSB548
New York NY 10016
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: The underlying motivation of this project is to develop a more comprehensive understanding of the signaling mechanisms activating interferon (IFN) genes

and inducing IFN stimulated gene expression in response to infection with viruses important in biodefense. Our ultimate goal is to exploit this information in order to develop novel antiviral strategies for prophylactic and/or therapeutic modalities for combating relevant pathogens. This strategy is based on the hypothesis that augmenting the normal IFN response and/or preventing viral antagonism of the IFN response represents a novel and potentially effective approach to combating viral disease. The specific aims of our research are (1) characterize the mechanism of transcriptional induction of IFN-stimulated genes, in particular the role of histone deacetylase (HDAC) activity in this process; and (2) define mechanisms of IFN gene induction during viral infection, specifically at sites of primary infection, such as in epithelial cells and
fibroblasts. This information will be used to design therapeutic and prophylactic approaches to antiviral drug discovery based on augmenting IFN responses or thwarting viral mechanisms of IFN antagonism
<END TEXT>

<TITLE>: NBC Abstract#15
<AUTHORS>: Erich Mackow
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>:
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: Hantaviruses cause two diseases, hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS, 45% mortality rate), and have the potential to be used as bioterrorism agents. We have defined potential determinants of hantavirus pathogenesis within HPS hantavirus proteins; however, there is currently no means for genetically altering hantaviruses and studying the role of encoded elements

in viral pathogenesis or viral attenuation. Reverse genetics, the ability to generate RNA viruses from cloned cDNA, has been used to genetically modify other negative stranded, segmented, RNA viruses like influenza virus. The success of reverse genetics for influenza viruses suggests that an established approach can be used to develop reverse genetics for hantaviruses. Hantavirus reverse genetics have not been established for several reasons. Hantaviruses grow poorly and to low titers in tissue culture and hantavirus surface glycoproteins (G1, G2) are expressed at low levels during infection. We have recently defined elements within the HPS hantavirus G1 protein that direct its ubiquitination and degradation as well as elements that bind key kinases that direct immune and endothelial cell receptor signaling responses. We have determined that mutating G1 elements or adding proteasomal inhibitors to cells blocks G1 ubiquitination and degradation and dramatically enhances protein expression. We have recently defined hantavirus gene and protein functions that regulate cellular interferon (IFN) responses and modifying these elements should attenuate hantaviruses once a reverse genetics system is devised for modifying hantaviviruses using recombinant approaches. Objective: We propose to develop hantavirus reverse genetics using approaches recently developed for bunyaviruses. These findings provide the basis for the genetic manipulation of hantaviruses and thereby a means to define determinants of hantavirus pathogenesis and to generate hantavirus vaccines. Specific Aims: Aim 1) Genetic Reporter of Recombinant Hantavirus Gene Transcription We will define hantavirus sequences required for transcription of luciferase reporters. Aim 2) Helper Virus Approach to Reverse Genetics NY-1V M and S segments will be provided in trans in order to rescue PHV replication. Aim 3) Plasmid-Based Reverse Genetics for Hantaviruses Plasmids used for bunyavirus virus reverse genetics have been generated and modified to accommodate hantaviruses. Novel vectors permit the precise transcription of hantavirus RNAs required for reverse

genetics. Clones of Andes Virus, which replicates to high titers will be used to develop reverse genetics.
<END TEXT>

<TITLE>: NBC Abstract#16 Development of small-molecule dependent viruses
<AUTHORS>: Rice, Charles M.
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>:
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: We propose to develop and apply a technology platform for engineering viral and microbial pathogens to be stably dependent on a small-molecule drug. Such a conditional-live (CL) system would present numerous applications relevant to bio-defense, including vaccine development, bio-safety and containment, and basic research on highly pathogenic agents. The core technology to be applied is the conditional protein splicing (CPS) system developed by out collaborator Tom Muir. In the first year of the grant, we will focus on optimizing the CPS system, applying it in the context of three Category A-C pathogens which we have prior experience manipulating (Dengue virus, West Nile virus, and yellow fever virus), and evaluating the performance and stability of these CL viruses in cell culture. In future years, we will extend the project in two directions. We will continue to develop and test CL systems towards clinical and other in vivo applications, via the intermediate of a small animal model. The
project will also be extended via trans-center collaborations to include other high-priority pathogens, including additional viral agents and bacterial and other unicellular organisms.
<END TEXT>

<TITLE>: NBC Abstract#17 Role of Tissue-specific Cell Killing in Anthrax toxin-mediated Cell Killing
<AUTHORS>: Jurgen Brojatsch
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>:
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: The long-term objective of the proposed project is to understand anthrax lethal toxin-mediated pathogenesis, which is caused by the Gram-positive Bacillus anthracis. The bacterium releases lethal toxin (LT), which is sufficient to reproduce most anthrax associated pathology when injected into mice. LT kills select cell types in culture, and induces broad cytopathic effects in LT-injected mice. The aim of this study is to identify key target cells in anthrax pathogenesis. To determine the role of LT killing in this process, we analyzed cytopathic effects in murine strains that differ in their susceptibility to LT. Specifically, we used a murine strain (BALB/c) that is highly susceptible to the toxin, and a more resistant murine strain (C57BL/6). We found that LT triggers apoptosis in C57BL/6-derived antigen presenting cells, and necrotic death in BALB/c-derived murine cell types. We also found rapid depletion of antigen-presenting cells in LT-treated mice, which presumably leads to an impairment of their adaptive immunity. Activation of distinct cell death pathways by LT will be helpful in our attempt to identify primary LT targets in anthrax. Our findings will allow us to distinguish between cells directly targeted by the toxin, via induction of apoptosis, from cells killed indirectly, via hypoxia. Hypoxic cells will be identified using antibodies against hypoxic markers (Hif1) expressed on these cells. We are focusing our studies on the vasculature, as several physiological changes point to a major contribution of vascular damage in LT-mediated pathogenesis. Preliminary data of cross-sections of

LT-injected C57BL/6 mice revealed TUNEL-positive cells, presumably endothelial cells, lining arteries in the liver of these mice. Studies of LT-injected mice will be performed in conjunction with in vitro assays using primary cells isolated from BALB/c and C57BL/6 mice. We found that LT directly triggered significant permeability changes across primary murine endothelial monolayers.
The contribution of direct LT cell killing and permeability changes of the vasculature in LT-mediated disease progression will be analyzed. We also detected that LT induced necrotic cell death in BALB/c-derived cells leads to cytolysis and the release of specific cytokines, including IL-18 and IL-1. The effect of released cytokines on permeability changes of the endothelium and LT-mediated cytopathic effects will be analyzed.
<END TEXT>

<TITLE>: NBC Abstract#18 Prospective Comparative Trial of the Humoral and Cell Mediated Immune Responses to the Trivalent Subviron Influenza Vaccine in Pediatric Liver Transplant Recipients as an Indicator for Response of Immunocompromised Subjects to Vaccination against Agents of Bioterrorism
<AUTHORS>: Campbell
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>:
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: Background: Epidemic influenza remains a significant cause of morbidity and mortality worldwide. The majority of deaths occur in high risk populations. Recent events such as the influenza vaccine shortage of 2004, the harsh influenza A season of 2003 and the persistent avian influenza (H5N1) outbreak in Southeast Asia reaffirm the possibility of a rapidly spreading influenza pandemic the likes of

which has not been seen since 1918. Influenza vaccination of all pediatric solid organ transplant recipients; however, few studies have been done to assess the immunogenicity of vaccination in this high risk population. To date, no study has evaluated the cell-mediated immune response to the influenza vaccine in pediatric solid organ transplant recipients. Objective: To investigate the humoral and cell mediated immune response to the trivalent subviron influenza vaccine (TIV) in pediatric liver transplant recipients and to correlate the humoral and cell mediated immune response with parameters that may predict a subjects ability to respond to the vaccine, such as time from transplant, immunosuppressive agents, previous episodes of rejection or cytomegalovirus infection, T-cell numbers and T-cell responses to mitogens or antigens. Design/Methods: Prospective comparative trial of the cell-mediated and humoral immune response in 44 pediatric liver transplant recipients and 22 healthy siblings over two influenza seasons. All patients will be vaccinated according to the ACIP recommendations. T-cell numbers, interferon gamma production in response to each of the viral strains contained within the vaccine, to phytohemagglutinin and tetanus and hemagglutination inhibition titers will be evaluated on all study subjects at the time of vaccination and one month and three months following vaccination. Results: To date, 44 subjects (22 transplant recipient and 22 sibling controls) have been enrolled. Mean ages at enrollment are (8.8 years vs 10.5 years, p=.117) for transplant recipients and controls. At the
time of vaccination initiation there was no difference in the mean white blood cell count (6138 vs 6205, p=.799), absolute neutrophil count (2840 vs 3109, p=.124) absolute lymphocyte count (2413 vs 2360, p=.911), CD4+ T-cells (889 vs 804, p=.569) and CD8+ T-cells (498 vs 552, p=.481) between transplant recipients and sibling controls. Proportions of transplant recipients (n=5) and controls (n=9) experiencing a four fold rise in antibody titer are as follows: New Caledonia (60% vs 89%, p=.504), Wyoming

(60% vs 56%, p=1.0) and Shanghai (40% vs 67%, p=.58) following full immunization. One control subject experienced self-limited fever following immunization. No other adverse events were reported. No patients developed influenza during the 2004-2005 winter season. Conclusions: Prior to vaccination, transplant recipients and health controls were similar with respect to demographics, absolute white blood cell, neutrophil and lymphocyte counts. Antibody responses were similar between transplant recipients and
healthy controls to each of the viral strains contained within the vaccine. Very few adverse events occurred following immunization. Results of assays to determine T-cell function at the time of vaccination and immune response to vaccination are pending. Correlation of baseline immune function with immune response to vaccine will be aide in determining the most appropriate vaccine strategy for pediatric liver transplant recipients.
<END TEXT>

<TITLE>: NBC Abstract#19 Screening IFN antagonists for novel smallpox antivirals
<AUTHORS>: Isabelle Marie, PhD
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>: 550 1st Ave MSB556
New York University School of Medicine
New York NY 10016
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: Our proposed research will provide a three-pronged approach to the validation and development of inhibitors of viral IFN antagonists. We will take a genetic and proteomic approach to defining the biochemical mechanism underlying the inhibitory function of E3L by analyzing its inhibition of and/or interaction with signaling components known to be

involved in the activation of IRF proteins; we will screen by yeast two-hybrid for novel E3L interacting proteins; and we will establish a reporter system amenable to screening for lead compounds capable of reversing the inhibitory action of E3L. These three approaches are sufficiently independent to proceed simultaneously in parallel, and we predict that significant progress on each aim can be accomplished within the timeframe of the proposed research. Ultimately, knowledge of the mechanisms underlying the ability of E3L to disrupt the IFN induction cascade will be invaluable for predicting the kinds of small molecules that could inhibit its action and for deciphering the potential mechanisms of their action.
<END TEXT>

TITLE>: NBC Abstract#21
<AUTHORS>: Anne Moscona
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>:
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: Hendra virus (HeV) is a recently identified paramyxovirus, fatal in humans and a possible bioterrorism agent. For HeV, the receptor binding protein (G) is required in order for the fusion (F) protein to mediate fusion, and analysis of the triggering/activation of HeV F by G should lead to strategies for interfering with this key step in viral entry.
-Alterations in Gs cytoplasmic tail (CT) that modulate F-mediated fusion: We recently found that truncation of the CT of HeV G leads to activation of its fusion promotion activity. A severely truncated HeV G, in which 32 of the 46 CT residues are absent, is far more effective at promoting fusion by HeV F. We will determine how this truncation of the CT enhances fusion by the corresponding F protein, using assays that we recently developed for HeV fusion and for

triggering of HeV F by HeV G. We will determine whether CT truncation results in increased inherent F-triggering activity, or in enhancement of fusion pore formation. The results will help elucidate the mechanism of F-triggering by HeV G.
-HeV and HPIV3 peptides are effective at inhibiting HeV fusion: The ectodomain of F contains two conserved heptad repeat (HR) regions, designated HRA and HRB, located near the fusion peptide and the transmembrane domains. Peptides derived from the HRA and HRB regions of F are proposed to inhibit fusion by preventing F from forming the 6-helix bundle (6HB) structure that is required for fusion. We found that that an HPIV3 HRB F-peptide is more effective at inhibiting HeV fusion than the HeV-derived peptide. We will assess whether the effective inhibitory F-peptides bind to HeV F, as would be predicted if the peptides are preventing the formation of the 6HB believed to be required for fusion, and whether inhibitory activity of a panel of peptides correlates with affinity of binding to F sequences. Biacore analysis of the interaction between HRB inhibitory peptides and HRA peptides from HeV F or HPIV-3 F provides the binding affinity information. These analyses will provide candidate molecules for use in probing a phage display library for short peptides (10 aa) that have high likelihood of inhibiting HeV fusion and would be feasible as therapeutic agents.
<END TEXT>

<TITLE>: NBC Abstract#22 Immunosuppression in Filovirus Infections
<AUTHORS>: Palacios, Gustavo
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>: 722 West 168th Street, Room 1801 New York, NY 10032
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords

<TEXT>: The filoviruses Ebola and Marburg virus can cause outbreaks of hemorrhagic fever with up to 80% mortality. Since the 1976 Ebola outbreak in Zaire (now the Republic of Congo) there have been more than 1,900 cases of human filovirus infection, with the most recent outbreaks occurring in Gabon and the Republic of the Congo in 2003, and Angola, this year. Profound depression of innate and adaptive immunity are hallmarks of the disease and are posited to contribute to pathogenesis by facilitating rapid unchecked viral replication. Apoptosis and loss of NK cells are prominent findings, suggesting the importance of innate immunity in determining the fate of the host. CD4+ and CD8+ lymphocyte counts decrease 60-70 % during the first 4 days after infection. In contrast, the number of CD20+ B lymphocytes in the blood does not change significantly. Filovirus glycoproteins play an important role in cell tropism, the spread of infection and pathogenicity. During the course of building a viral
universal microarray, we discovered a region of strong secondary structure conservation between the c-terminal domain of the envelope glycoprotein of filoviruses and an immunosuppressive domain described in the retroviral envelope protein. In addition, this domain was extraordinarily conserved among the members of each genus. This domain is located in an exposed region on the GP2 glycoprotein. Here we report that the synthetic filovirus peptides representing the prototypic aminoacid sequence of the conserved domain in the Ebola and Marburg virus glycoprotein have immunosuppressive properties. Peripheral blood mononuclear cells (PBMCs) from healthy donors were stimulated with PHA, exposed to different doses of the filoviral peptide, and characterized after culture by multi-color flow cytometry. 80-90 % of the PBMCs were depleted when incubated with high doses of the peptide (60 mM). Cell depletion was also observed at lower doses (6 mM), mainly in the CD4+ lymphocyte population. In contrast, no effect was
observed on CD19+ B lymphocytes and CD14+ monocytes. T-lymphocyte subsets exposed to the peptide had reduced immune response to PHA activation. Apoptosis

was detected as early as 6 hours post-peptide exposure (60 mM), among the CD4+ and CD8+ subsets of T lymphocytes. Cytokine analysis in the supernatants from peptide treated PBMCs revealed a significant decrease in expression of cytokines involved in activation of T cells (IL-2, IFN-, IL12-p40). These data suggest that the conserved filoviral peptide causes immunosuppression by inhibiting activation of lymphocytes and eliminating those subsets that are required for mounting an effective response to the virus. This highly conserved immunosuppressive peptide is a candidate target for pharmacological intervention in filovirus infections.
<END TEXT>

<TITLE>: NBC Abstract#23 Broad-spectrum passive immunoprophylaxis for flavivirus infection
<AUTHORS>: Charles M. Rice, Ph.D.
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>: The Rockefeller University
1230 York Ave., Box 64
New York, NY 10021
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: Flaviviruses are major arthropod-borne human pathogens responsible for encephalitis, hepatitis and/or hemorrhagic fevers. These enveloped viruses contain a single-stranded, positive-sense RNA of approximately 11 kB that encodes 3 structural and 7 nonstructural proteins. The nonstructural glycoprotein NS1 is essential for flavivirus viability. During host-cell infection in vitro, NS1 is found associated to intracellular organelles, where it participates to the viral RNA replication process, or is alternatively transported to the cell surface. In addition, the protein is released into the extracellular fluid of dengue virus (DENV)-infected mammalian cells. NS1 secretion was further demonstrated during the acute

phase of the disease in DENV-infected patients. Interestingly, concentrations of NS1 in sera appeared higher in patients who developed the hemorrhagic form rather than the mild form of DEN, suggesting that the protein could contribute to viral pathogenesis. More recently, we have demonstrated
that NS1 in blood is also a hallmark of clinical West Nile virus (WNV) infections in horses and humans. WNV is widely distributed in Africa, the Middle East, Asia and Southern Europe and has strinkingly spread across North America since 1999, leading to hundreds of human meningoencephalitis that often proved fatal in the absence of any appropriate prophylaxis. In order to reduce the incidence of WNV-induced mortality, we are currently evaluating an immunotherapeutic approach through the use of NS1-specific monoclonal antibodies (mAbs). A panel of mAbs raised against WN NS1, or DEN NS1 and cross-reactive against WN NS1, are being purified and tested for their ability to protect adult mice against a lethal challenge with WNV. Post-exposure protective efficacy, as well as potential cross-protection against DENV and yellow fever virus infections, will be assessed for the most promising mAbs. If successful, this approach would constitute a novel strategy for broad-spectrum prevention and treatment of flavivirus
infections.
<END TEXT>

<TITLE>: NBC Abstract#24
<AUTHORS>: CR Roy
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type
<ADDRESS>:
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: Coxiella burnetii is an obligate intracellular pathogen that encodes a type IV secretion system related to the Legionella pneumophila Dot/Icm system.

It is predicted that the C. burnetii Dot/Icm system will modulate host cell functions and assist in the creation of a specialized vacuole that supports bacterial growth. Thus, identification and characterization of substrate proteins injected into host cells by the C. burnetii Dot/Icm system will reveal bacterial strategies of host infection. Additionally, these proteins should make excellent targets for antimicrobial therapy and vaccine development. Using Legionella as a surrogate host, we have shown that several C. burnetii Dot/Icm substrate proteins that were predicted to be bacterial effectors based on homology to eukaryotic proteins are indeed substrates of the Dot/Icm system. Having established a system to detect C. burnetii proteins that are Dot/Icm substrates, we performed an unbiased genetic screen in an attempt to identify new C. burnetii Dot/Icm substrates. This screen was conducted during the initial period of funding of this developmental project and was successful. We identified 10 new C. burnetii proteins translocated into host cells by the Dot/Icm system. Interestingly, the proteins were unique, meaning that they have no significant amino acid similarity to proteins in prokaryotic or eukaryotic organisms. In the next phase of this developmental project, we will test whether these newly identified C. burnetii proteins are required for bacterial replication in host cells. To accomplish this, we will generate antibodies to these proteins and test whether microinjection of these antibodies prevents establishment of a vacuole that supports C. burnetii growth. The goal of this work is to establish the importance of Dot/Icm substrates in the C. burnetii intracellular lifestyle.
<END TEXT>

<TITLE>: NBC Abstract#25
<AUTHORS>: CA Sariol
<JOURNAL>: Journal
<DATE>: Date
<CITATION>: Citation
<TYPE>: Type

<ADDRESS>:
<EMAIL>: Email
<PHONE>: Phone
<KEYWORDS>: Keywords
<TEXT>: 50 to 100 million cases of Dengue Fever (DF) and 500,000 cases of Dengue Hemorrhagic Fever (DHF) each year. Even though these animals do not show the clinical signs of infection observed in humans, non-human primates are the best animal model to test the effectiveness of drugs or vaccine formulations against dengue virus (DV). In addition, this model allows for the study of these responses during sequential infections with different DV serotypes. However, there are few or no reports on the specific cells that support viral replication on differential gene expression or on cytokine profiles induced after infection in rhesus macaques. The main goal of this study is to provide preliminary data on differential gene expression and on cytokine production after DV infection in either nave or DV-primed animals. In this protocol, we infected four rhesus macaques with DV 1, strain western pacific 74, and two animals were mock infected. We showed that in addition to macrophages, B cells support DV replication after in vivo infection. This had not been shown before for this animal model. We showed that after a primary infection with DV 1, both B cells and macrophages from rhesus macaques produce an impressive immune response by up regulating the IFN-stimulated response genes (ISGs). These genes include OASL, OAS1, RSAD2 (viperin), IF15, IF27, MX1, and MX2, among others. However, this immune response showed only a discrete increase in the gene transcriptions or cytokine production of IL-10, IL-8, and TNF. One significant contribution of this research protocol is that, for the first time, we have evidence that B cells are infected with dengue virus. However, in spite of infection, neither B cells nor macrophages express the early activation surface marker as CD69. In contraposition, in spite of infection, macrophages do not show this surface activation marker when tested five days after infection. The implication of these findings for the dengue pathogenesis in rhesus macaques will be evaluated in further research

protocols. In the next phase of this grant, four animals will be secondarily and two nave animals will be primarily infected with dengue 2 virus, New Guinea C strain.
Using the extra funding period of this grant, we plan to proceed with a third infection using a dengue 3 virus strain. In both infections described above, both cytokine expression profiles and production will be examined in B cells and macrophages.
<END TEXT>

<TITLE>: GLRCE A Putative Virulence Factor in Yersinia
<AUTHORS>: Karla Fullnew Satchell
<JOURNAL>: journal
<DATE>: Jan0106
<CITATION>: citation
<TYPE>: type
<ADDRESS>: Northwestern
<EMAIL>: email
<PHONE>: phone
<KEYWORDS>: keywords
<TEXT>: Yersinia pestis is a Gram-negative bacterium that causes both pneumonic and bubonic plague. The easy human-to-human transmission of pneumonic plague and the high rates of lethality raise fears that Y. pestis could re-emerge or be used as a bioterrorist agent. The recent publication of the genomic sequence of three Y. pestis strains facilitates identification of additional virulence factors that could be problematic for creation of safe, attenuated vaccines strains if the factors were not identified. This grant will examine a new putative virulence factor related to the RTX family of toxins that is present on the genome of all three sequenced strains and is also present on the genome of the closely related species Y. pseudotuberculosis.
The gene encodes a putative multifunctional cytotoxin. The deduced protein product from Y. pestis is 3110 aa in length and is 100% identical in the sequence among the Y. pestis strains while the Y. pseudotuberculosis deduced protein is 3103 aa in length and shares 96% identity with the Y. pestis protein. In all strains, the putative virulence factor is a divergent member of

the RTX toxin family that likely arose from an ancestral pore-forming RTX toxin similar to one encoded by Y. enterocolitica that subsequently acquired three cytotoxic activities from the Vibrio RTX toxins included cell lysis, kinase, and GAP activities.
This grant will investigate three aspects of this putative virulence factor. In specific aim 1, more focused approaches including slot blot or RT-PCR analysis of transcripts and Western blotting for protein under a variety of growth conditions will be performed to confirm reports from microarray studies that this gene is expressed and is not a pseudogene. In specific aim 2, the putative activity domains will be expressed in mammalian cells as GFP fusion proteins and affects on cell physiology will be assessed by microscopy and by 2-D gel analysis of phospho-proteins. To determine if this factor is virulence-associated in specific aim 3, mutants with insertions in the gene on the chromosome of both Y. pseudotuberculosis and Y. pestis will be created and the mutant strains will be assessed in relevant mouse models of disease. Taken together, these experiments will determine if this is an expressed factor that carries cytotoxic activities important for pathogenesis of Y. pestis and Y. pseudotuberculosis. If it is found that this putative virulence factor is a cytotoxin that contributes to Yersinia pathogenesis, the gene encoding this factor would need to be removed from attenuated vaccine strains to assure safety of these vaccines that could be used to protect people in the event of a worldwide return of the plague.
<END TEXT>

<TITLE>: GLRCE Prevention and Control of Ebola Virus Infection
<AUTHORS>: Yoshihiro Kawaoka
<JOURNAL>: journal
<DATE>: Jan0106
<CITATION>: citation
<TYPE>: Research Project
<ADDRESS>: University of Wisconsin
<EMAIL>: email

<PHONE>: phone
<KEYWORDS>: keywords
<TEXT>: Filoviruses continue to cause outbreaks of fatal hemorrhagic fever with mortality rates as high as 90%; yet, neither protective vaccines nor therapeutic antiviral treatments are currently available. We therefore proposed to generate replication-incompetent virus-like particles (VLPs) for use as Ebola virus vaccines (Aim 1) and for establishing large-scale screening systems to identify potentially effective antiviral compounds (Aim 2). More specifically, for Aim 1, we proposed to establish replication-incompetent Ebola VLPs that lack the VP40 and VP24 genes and can only be maintained in cell lines expressing these two proteins. While we were able to establish cell lines expressing VP40, the establishment of cell lines expressing VP24 has proven more challenging, likely because of the cytotocixity of VP24. Therefore, we are now pursuing a second approach that uses influenza virus for the expression of an Ebola virus protein. To this end, we have generated a replication-incompetent influenza virus that expresses the Ebola virus GP1 protein and have demonstrated that this virus induces antibodies to both Ebola and influenza virus in mice. In yet another approach, we assessed the potential of live attenuated Ebola vaccines by generating an Ebola virus that expresses the GFP reporter protein. The resulting virus was attenuated and induced sterile immunity in nonhuman primates, demonstrating the potential of attenuated virus strains for protection against Ebola virus infections. For Aim 2, we have established a system to screen for drugs that interfere with Ebola virus binding or internalization, and a system to screen for drugs that interfere with Ebola virus replication. Both systems have been approved for screening at the NSRB at Harvard University, Boston. A first round of screening for antivirals that interfere with Ebola virus binding or internalization yielded more than one compound with potential antiviral activity.
<END TEXT>

<TITLE>: GLRCE Construction of F. tularensis Regulatory Mutants
<AUTHORS>: Thomas C. Zahrt
<JOURNAL>: journal
<DATE>: Jan0106
<CITATION>: citation
<TYPE>: No designation
<ADDRESS>: Medical College of Wisconsin
<EMAIL>: email
<PHONE>: phone

<KEYWORDS>: keywords
<TEXT>: Francisella tularensis is a Gram-negative facultative intracellular pathogen with an exceedingly low infectious dose of between 10-50 viable organisms. It causes severe infections in animals and humans and can enter through broken skin, mucosal epithelium of the eye, the throat, or the lungs. Currently there is no vaccine to protect against F. tularensis infections even though this microorganism has been designated a Select Agent by the Centers for Disease Control and Prevention. Genetic studies in F. tularensis have been limited by the inability to efficiently introduce DNA, the lack of stably replicating vectors, and absence of useful genetic tools and markers. Work from our laboratories that was funded under a previous GLRCE Developmental Project (DP5) supported the development of several novel Francisella shuttle vectors (pFNLTP-based) that: (i) can efficiently be transformed into a variety of Francisella spp., (ii) is stably maintained in the host during cultivation on laboratory medium or in macrophage cell lines in the absence of selection, (iii) possess useful selectable markers applicable to studies in type A strains of F. tularensis, (iv) can be conditionally maintained at the permissive temperature but lost following shift to the non-permissive temperature, and (v) express useful reporter or counterselectable genetic determinants. In this application, pFNLTP-based vectors and other suicide plasmids will be used to construct, complement, and characterize a set of regulatory mutants that are expected to contribute to intracellular proliferation of F. tularensis in the

macrophage. Virulence of constructed mutants will be investigated by in vitro and in vivo model systems of infection, and downstream regulated genes identified by microarray-based approaches. Overall, this study should enhance our understanding of the mechanisms utilized by F. tularensis for proliferation in host phagocytic cells. Importantly, results from this study may also lead to development of new attenuated vaccine candidates.
<END TEXT>

<TITLE>: GLRCE Therapeutic Inhibition of B. anthracis Pathogenesis
<AUTHORS>: Andrzej Joachimiak, Philip Hanna, Brian Kay, Dominique Missiakas, Olaf Schneewind
<JOURNAL>: journal
<DATE>: Jan0106
<CITATION>: citation
<TYPE>: Research Project
<ADDRESS>: address
<EMAIL>: email
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<KEYWORDS>: keywords
<TEXT>: GLRCE Research Project 1 scientists aim to develop anti-infective therapies that can be used for therapy of anthrax infections. To achieve this goal, the genetic determinants of B. anthracis that are required for the pathogenesis of anthrax disease are being established. Such determinants are then examined for their physiological properties and biochemical activities. If such activity can be determined and measured in vitro, the corresponding virulence genes can be considered targets of anti-infective therapy. Penultimate goal of this research is the identification of small-molecule inhibitors of virulence targets and exploration of the therapeutic properties of these compounds in animal models of anthrax disease. Thus, virulence targets will be pursued in this research program by screening NSRB small molecule libraries for inhibitory properties. These targets include (1) B. anthracis factors required for the secretion of protective antigen or

other virulence factors (PrsC & EssC), (2) B. anthracis factors required for anchoring of poly-D-glutamic acid capsule to the cell wall envelope (CapD), (3) B. anthracis sortases, i.e. factors required for anchoring of surface proteins to the bacterial envelope (SrtA, SrtB & SrtC), (4) superoxide dismutases (SOD) and (5) factors involved in D-alanylation of lipoteichoic acid (Dlt). For example, high-throughput screening of NRSB compound libraries for inhibitors of sortases led to the identification of sortizins. X-ray crystallography structure determination of virulence targets with their inhibitors is used to achieve mechanistic appreciation of inhibitor function. Small molecule inhibitors are explored for their therapeutic values in animal models of anthrax disease.
<END TEXT>

<TITLE>: GLRCE Diagnostic Protein Arrays for Category A Agents
<AUTHORS>: Darrell Chandler
<JOURNAL>: journal
<DATE>: Jan0106
<CITATION>: citation
<TYPE>: Research Project
<ADDRESS>: Argonne National Laboratory
<EMAIL>: email
<PHONE>: phone
<KEYWORDS>: keywords
<TEXT>: The objective of this project is to develop a diagnostic protein array for clinical surveillance and surge-capacity testing for all Category A agents, a technology platform that is and will be extensible to Category B and C agents. In so doing, we directly address and meet the Great Lakes Region V RCE emphasis and requirements for platform products and rapid implementation of basic science discoveries into useful diagnostic products and systems. In organizing the project and proposing a specific protein array in this application, we maintain a "dual-use" view of technology by recognizing that bioterrorism is a low-probability event and that a "platform product" should

therefore be equally useful and efficacious in a more conventional public health and regulatory setting. The project is organized into two phases, wherein the Phase 1 objective is to develop a semi-quantitative antibody array and analysis method for all Category A agents, and test the prototype array on agents amended into (mouse) nasal lavage or blood. Successful completion of Phase 1 will warrant a Phase 2 proposal to translate the science of multiplexed agent detection from Phase 1 into a fluidic test cartridge and diagnostic instrument that is currently under development by Argonne National Laboratory (ANL) and its commercialization partners (Akonni Biosystems, Inc., Aurora Photonics, Inc., and Micronics, Inc.). The concept of operations for the Category A agent antibody array (in both Phase 1 and 2) is to provide a rapid (~30 min), point-of-use clinical test that also meets bioterrorism surveillance requirements for use immediately after the onset or discovery of a bioterrorism incident or suspicious event. The decision logic and results from the protein array will enable public health officials to identify infected persons and intelligently administer costly and/or limited quantities of antibiotics, anti-toxins or anti-viral therapies either in the clinic or in the field. We will meet the Phase 1 objective by taking advantage of ANL's extensive intellectual property portfolio and long-standing work with the U.S. Department of Defense (DoD) for the manufacture and use of three-dimensional gel element microarrays, field-portable microarray imagers and analysis software for biodefense applications, U. Chicago BSL-3 facilities and animal models, and related RCE fundamental science aimed at identifying novel membrane or soluble protein targets (i.e. drug development candidates) that can then be translated and incorporated into the base diagnostics platform developed herein. Finally, this project fills a fundamental technology gap between traditional clinical or disease diagnostics research and the stated NIH and Great Lakes Regional Center of Excellence (GLRCE) commitment to biodefense

diagnostics and platform products
(http://www.glrce.org/product_dev.php).
<END TEXT>

<TITLE>: GLRCE Immunity to Yersinia pestis Infections
<AUTHORS>: Robert Brubaker, Bana Jabri, Olaf Schneewind, John Xu
<JOURNAL>: journal
<DATE>: Jan0106
<CITATION>: citation
<TYPE>: Research Project
<ADDRESS>: address
<EMAIL>: email
<PHONE>: phone
<KEYWORDS>: keywords
<TEXT>: Research Project 1 scientists aim to develop a safe and effective plague vaccine from purified recombinant Yersinia pestis antigens. Y. pestis secretes LcrV during infection, thereby triggering release of interleukin 10 (IL-10) by host immune cells and suppression of pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-_) and interferon-gamma (IFN-() as well as innate defense mechanisms required to combat the pathogenesis of plague. Further, LcrV plays an important role in the type III secretion machinery-dependent delivery of effector Yops into host cells as a bacterial means to combat innate immune responses. Although immunization of animals with LcrV elicits protective immunity, the associated suppression of host defense mechanisms precludes the use of full length LcrV as a human plague vaccine. An LcrV variant lacking amino acid residues 271-300 (rV10) elicits immune responses that protected mice against a lethal challenge with fully virulent Y. pestis strain CO92. When compared to full-length LcrV, rV10 immunization affords greater levels of vaccine protection in a murine model of pneumonic plague. In contrast to full length LcrV, rV10 displayed reduced ability to release IL-10 from mouse and human macrophages. Further, LPS-stimulated release of pro-inflammatory cytokines by human or mouse macrophages is inhibited by full length LcrV but not

by the rV10 variant. GLRCE RP1 scientists are pursuing the development of rV10 as a safe vaccine to generate protective immunity against plague in humans. Other research work examines surface protein antigens of Y. pestis for their protective antigen properties and for their molecular mechanisms of pathogenesis during plague.
<END TEXT>

<TITLE>: GLRCE A Screening System for Anti-Virals
<AUTHORS>: Yoshihiro Kawaoka
<JOURNAL>: journal
<DATE>: Jan0106
<CITATION>: citation
<TYPE>: No Designation
<ADDRESS>: University of Wisconsin, Madison
<EMAIL>: email
<PHONE>: phone
<KEYWORDS>: keywords
<TEXT>: The long term goal of this research is to develop effective therapeutics to important human pathogens such as SARS coronavirus (SARS Co-V), Nipah virus, and La Crosse virus. SARS Co-V causes severe acute respiratory syndrome and the outbreak in 2003 had major medical and worldwide social and economical consequences. Nipah virus infections result in symptoms ranging from mild headaches to severe acute encephalitis with high mortality rates, while La Crosse virus is the causative agent of pediatric viral encephalitis in certain areas of the United States and Canada. The latter two viruses are classified in the NIAID Biodefense and Emerging Infectious Diseases Research Opportunities program as 'priority pathogens' that can potentially be used as bioweapons, while SARS Co-V is currently not listed as a 'priority pathogen'. For these viruses, neither effective antiviral drugs nor protective vaccines are available. Here, we propose to generate replication-incompetent vesicular stomatitis virus-like particles (VSV-VLPs) complemented with the viral glycoprotein(s) of SARS Co-V, Nipah virus, or La Crosse virus. These particles do not produce infectious progeny viruses and can

therefore be handled safely in biosafety level 2 facilities. We propose to use these VLPs for large-scale screening of the compound library available at the NSRB (National Screening Laboratory for the Regional Centers of Excellence in Biodefense and Emerging Infectious Disease, Boston, MA). Likewise, but not part of this application, we are planning to perform screening experiments for VSV-VLPs complemented with Ebola virus glycoprotein. Combining our expertise in the generation of recombinant VLPs with screening resources made available through the Regional Centers of Excellence (RCE) provides a powerful approach for the identification of potential therapeutics for the above-described virus infections.
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<TITLE>: GLRCE Designed Antibiotic Peptides and Mimetics
<AUTHORS>: Kevin Mayo
<JOURNAL>: journal
<DATE>: Jan0106
<CITATION>: citation
<TYPE>: Research Project
<ADDRESS>: University of Minnesota, Minneapolis
<EMAIL>: email
<PHONE>: phone
<KEYWORDS>: keywords
<TEXT>: Bacterial genetic mutations and natural selection can lead to attenuated antibiotic potential and even full drug resistance of specific protein-and enzyme-targeted antibiotics. Bacterial membrane disintegrating peptides offer promising alternatives as antibiotics of the future, primarily because they show broad spectrum antibacterial activity and disrupt integrity of the entire bacterial cell membrane, thereby reducing risk of drug resistance. Such compounds have considerable potential as therapeutic agents in the biodefense initiative. Most bacterial membrane-disintegrating agents are proteins and peptides that possess amphipathic character imparted by a hydrophobic face and a hydrophilic, cationic face. Although most are greater than about 15 amino

acid residues, we have identified a bactericidal peptide 12mer (SC4) and discovered non-peptidic, calixarene-based mimetics of SC4. Via specific bacterial membrane disintegration, peptide SC4 kills bacteria in the nanomolar to sub-micromolar ranges, whereas the first round of calixarene-based SC4 mimetics has produced "small molecule" agents effective in the micromolar range. There is no doubt that lead molecules currently in hand can be improved. The overall goal of this research project, therefore, is to develop such bacterial membrane disintegrating peptides and their "small molecule" mimetics for use against infectious disease. The significant body of data already in hand provides support and momentum for the planned studies, which have the following aims:
AIM 1: Optimize bactericidal activity of lead peptide 12mer (SC4).
AIM 2: Design and synthesize additional, more potent, calixarene-based SC4 mimetics.
AIM 3: Assess biological efficacy of bactericidal agents in vitro and in vivo.
This application presents a comprehensive and integrated plan that will enable us to capitalize on the exciting discovery of the antibacterial properties of membrane-disintegrating peptides.
<END TEXT>

<TITLE>: GLRCE Animal Models and Correlates of Immunity for Plague
<AUTHORS>: Debra Anderson, Kristin DeBord
<JOURNAL>: journal
<DATE>: Jan0106
<CITATION>: citation
<TYPE>: No Designation
<ADDRESS>: University of Chicago
<EMAIL>: email
<PHONE>: phone
<KEYWORDS>: keywords
<TEXT>: Yersinia pestis, the causative agent of plague, is a feared bio-threat agent due to its high mortality and its ability to be spread person to person via respiratory droplets. There is currently no licensed vaccine to prevent plague. Most strains of

plague are sensitive to antibiotic treatment however recent outbreaks of plague in Madagascar were caused by a multiply antibiotic resistant strain. Thus, Americans are extremely vulnerable to an attack with Y. pestis and there is an urgent need for the development of safe vaccines to prevent plague. Current attempts to develop a plague vaccine focus on two protein antigens: Caf1 and LcrV. LcrV is essential for the pathogenesis of Y. pestis infections, but vaccination with LcrV antigen, while providing high level protection against bubonic and pneumonic plague, induces a concomitant induction of IL-10 release from immune cells. As IL-10 is known to play a role in suppressing the immune response, this side effect raises serious issues of safety, particularly for the immuno-compromised population. Research Project 4 of the GLRCE has generated LcrV variants that appear to retain their protective property but have lost the ability to stimulate IL-10 release suggesting that these variants may be appropriate for inclusion into a vaccine cocktail. In order to rapidly develop these variants into a vaccine that can be licensed for humans, we propose to develop animal models and correlates of protection to measure efficacy of LcrV variant vaccination. These experiments are expected to provide data to move forward with GLP testing of the variant vaccine in support of licensure with the U.S. Food and Drug Administration.
<END TEXT>

<TITLE>: GLRCE Development of Antiviral Strategies
<AUTHORS>: Richard J. Kuhn
<JOURNAL>: journal
<DATE>: Jan0106
<CITATION>: citation
<TYPE>: Research Project
<ADDRESS>: Purdue University
<EMAIL>: email
<PHONE>: phone
<KEYWORDS>: keywords
<TEXT>: Our laboratories have a long-term interest in examining the structure-function relationships of

proteins from several groups of enveloped viruses: the alphaviruses, the flaviviruses, and the filoviruses. Using this information, as well as data obtained in the first two years of this grant's funding, we have, and will continue to identify and target specific aspects of the virus replication cycle for the development of inhibitory compounds. The flaviviruses comprise a genus within the family Flaviviridae of plus-strand RNA viruses. Many of its members cause significant disease in humans. Several examples of flaviviruses include dengue, yellow fever, West Nile, and tick-borne encephalitis. We will also carry out studies with the alphaviruses, a genus of plus-strand RNA viruses found in the Togaviridae family. Several examples of alphaviruses are Sindbis, Semliki Forest, and Venezuelan equine encephalitis, a virus known to be weaponized. In addition, we will examine the major glycoprotein GP1 of Ebola virus using structural approaches and utilize this information to develop antivirals. All three groups of viruses contain members that have been suggested as possible bioterrorism agents. To date, there are few effective vaccines for these viruses and no efficacious antiviral agents.
Our laboratories have built a wealth of information on virus structure and assembly using crystallography, electron microscopy and molecular genetics. We exploited this information in the first two years of this project to identify new antiviral compounds. By targeting steps in the virus replication cycle that may be susceptible to antiviral compounds, compounds were identified using structure-based design and tested against flavivirus and alphavirus. Our initial results on the activity of these potential compounds are promising, and we aim to further develop these, and other compounds in the next years of this project.
<END TEXT>

<TITLE>: GLRCE Vaccines and Therapies Against Botulism
<AUTHORS>: Joseph Barbieri
<JOURNAL>: journal
<DATE>: Jan0106
<CITATION>: citation

<TYPE>: Research Project
<ADDRESS>: Medical College of Wisconsin
<EMAIL>: email
<PHONE>: phone
<KEYWORDS>: keywords
<TEXT>: Botulinum neurotoxins A-G (BoNT) are zinc-dependent proteases that enter neurons and cleave proteins that are essential for exocytosis of neurotransmitters, resulting in paralysis and death. The A-G serotypes are designated by the ability of anti-sera to neutralize a specific serotype of BoNT. BoNTs are the most toxic proteins toxin for humans and are category A reagent. Botulism occurs in three forms: ingested, inhalation and cutaneous. There are currently limited vaccines and therapies against botulism. This application is a consortium among investigators at the University of Wisconsin, the University of Illinois-Champaign Urbana, and the Medical College of Wisconsin. The specific aims of this application are to: 1) Generate a recombinant holotoxin vaccine against BoNT intoxication, 2) Analyze the protective immune response to BoNT, 3) Engineer single-chain antibody-BoNT/A chimeric protein as an alternative antitoxin strategy for intracellular neutralization of BoNT, 4) Identify irreversible inhibitors of botulinum toxin metalloproteases, and 5) Identify neuronal receptors of the BoNT. Completion of these aims will provide new and novel strategies for the development of vaccine and therapies against botulism.
<END TEXT>

<TITLE>: GLRCE Subunint Vaccines for Botulism
<AUTHORS>: Joseph T. Barbieri
<JOURNAL>: journal
<DATE>: Jan0106
<CITATION>: citation
<TYPE>: No Designation
<ADDRESS>: Medical College of Wisconsin
<EMAIL>: email
<PHONE>: phone
<KEYWORDS>: keywords

<TEXT>: The botulinum neurotoxins (BoNT) are the most potent protein toxins for humans and are on the Select Agent List. Botulism can be contracted upon ingestion of preformed BoNT, through cutaneous routes following infection of a wound by BoNT producing Clostridia, or where Clostridia are ingested, grow in the intestine, and produce BoNT. Militarization of BoNT presents a new form of intoxication via inhalation. While an effective immunogen, the current BoNT vaccine has several limitations. First, the product consists of a crude extract of clostridia proteins; second, the material is dangerous to produce and there is a high cost associated with preparing the vaccine; and third only five of the seven serotypes are presented in the formulation.

The receptor binding domain of BoNT/A (HCc/A) has been subcloned and expressed as a soluble protein in E. coli. Using a conventional purification ~ 10 mg of HCc/A can be purified to near homogeneity from a 1 liter shake culture of E. coli. Anti-sera prepared against HCc neutralized BoNT toxicity in a mouse model assay. These properties make E. coli expressed HCc a more viable vaccine and therapy against botulism than Yeast-derived HCc, the current expression system for the generation of recombinant HCc vaccine.

The aims of this translational proposal are to: 1. Generate recombinant HCc from serotypes B-G of BoNT in E. coli. The Johnson laboratory will amplify and confirm the sequence of the HC serotypes B-G. The Barbieri laboratory will sub-clone, express and purify recombinant HCc. 2. Analyze the protective immune response to BoNT. Anti-sera will be prepared against purified HCc and used to determine the ability to neutralize BoNT in a mouse model by the Johnson laboratory. Purified HCc will also be used as a therapy to block BoNT intoxication and as a vaccine to prevent BoNT intoxication in a mouse model.

Completion of these studies will provide a platform for development of an effective vaccine and therapy against botulism.

<END TEXT>

<TITLE>WRCE Bacillus anthracis - Host Interactions
<AUTHORS>Theresa M. Koehler
<JOURNAL>
<DATE>
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<TYPE>research project
<ADDRESS>university of texas health science center at houston
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<PHONE>
<KEYWORDS>
<TEXT>Anthrax disease results from a complex series of interactions between the invading bacterium, Bacillus anthracis, and the mammalian host. For inhalation anthrax, infection begins with entry of spores into the lung. Alveolar macrophages phagocytose the spores and transport them to lymph nodes of the mediastinum. Ultimately, the metabolically active form of the bacterium disseminates to the blood and other body tissues, reaching concentrations up to 108 colony-forming units per ml and secreting anthrax toxin proteins. In recent years, research emphases have focused on toxin protein structure and function. However, anthrax disease, whether acquired naturally or as the result of intentional dissemination of spores, results from infection with B. anthracis, not simply acquisition of toxin. Despite the importance of human infection with B. anthracis, there is almost a complete lack of knowledge of fundamental cellular and molecular mechanisms by which the bacterium interacts with its host. Results of studies proposed here will fill this critical gap in knowledge and reveal bacterial and host targets for generating new therapeutics for anthrax. We will use an in vitro macrophage model and in vivo murine model to identify pathogen and host targets important for multiple early steps in infection. The importance of pathogen and host factors during early infection will be assessed in both models by modulating expression of candidate B. anthracis and macrophage targets. In Aim 1, we will identify and characterize B. anthracis and macrophage molecular targets important for multiple steps of early infection. We will establish a detailed model of

B. anthracis-macrophage interactions. A major part of this work will be to characterize the modulation of both bacterial and macrophage gene expression as a result of B. anthracis-macrophage interactions, using transcriptional profiling and proteome analyses. In Aim 2, we will investigate B. anthracis development in a mouse nasal installation model for anthrax, focusing on the pulmonary response. We will test B. anthracis mutants for attenuation of pathogenesis in the model. B. anthracis germination, survival, and persistence in the lung will be correlated with lung histopathology and immune response. We will track development of B. anthracis in the whole animal using chemiluminescence-based in vivo imaging technology. Using these assays, we will establish the spatial and temporal development of a fully virulent B. anthracis strain and isogenic mutants deleted for genes encoding therapeutic candidates. Our long-term objective is to generate new therapeutics to block interactions of B. anthracis spores with alveolar macrophages. The most powerful strategy will probably employ a cocktail of inhibitors targeting multiple steps in the infectious process. Bacterial and macrophage targets shown experimentally to be important for B. anthracis-macrophage interactions will be immediately forwarded to RCE core facilities for recombinant expression and crystallization for high-resolution structural analysis. The structural data will be used for structural-based identification of lead-inhibitor templates.
<END TEXT>

<TITLE>WRCE Alphavirus Vaccines for Biodefense
<AUTHORS>Scott C. Weaver
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<KEYWORDS>
<TEXT>Alphaviruses, including Venezuelan (VEEV), eastern (EEEV) and western equine encephalitis viruses (WEEV), are highly developed agents of biological warfare and terrorism (BWT) and are important naturally emerging zoonotic viruses. Current biodefense measures against these viruses are inadequate, and widespread morbidity and mortality could be inflicted upon exposure of civilians or military personnel. Effective, licensed vaccines, critical first lines of defense and important tools for biodefense research, are badly needed. We will exploit recent advances in alphaviral genetics and vaccine design, as well as the unique alphavirology expertise within our regional group, to develop safe and effective vaccines suitable for licensure. We will generate a set of live-attenuated, chimeric Sindbis-based vaccine candidates against VEEV, EEEV and WEEV, including strains of both Sindbis and the encephalitic vaccine targets that vary in virulence. A major advantage of these chimeric alphavirus strains, which have proved successful for flaviviruses, is that none will have the potential to generate or retain the encephalitic alphavirus parent virus or its complete genome. We will also combine these vaccines with several different adjuvants expressed from replicon systems to optimize the immune response. These candidate alphavirus vaccines will be evaluated in rodent models for safety, immunogenicity and protection against challenge including aerosol and mosquito infection. The live-attenuated alphaviruses developed in this project will take advantage of the greater antigen presentation, cell-mediated immunity, and longer lasting antibody levels characteristic of exposure to replicating viruses. In addition to protection of civilian and military populations from an anticipated alphavirus BWT event and protection of laboratory personnel doing critical BWT and public health research, the methods we will develop can be exploited to rapidly and efficiently develop vaccines against newly recognized, emerging alphaviruses or an engineered alphavirus weapon. This research will also be useful for protecting populations at risk of

natural exposure to these zoonotic agents in many parts in the New World.
<END TEXT>

<TITLE>WRCE Novel Genetic Tools for Viral Biodefense
<AUTHORS>Peter Mason
<JOURNAL>
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<ADDRESS>university of texas medical branch at galveston
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<TEXT>Among pathogens considered likely agents of biological warfare and terrorism (BWT), RNA viruses comprise many of the most amenable for use as weapons. With modest cell culture capacity, large amounts of these viruses can be produced and lyophilized to generate stockpiles of aerosol-infectious material. Many of these viruses are readily available from natural sources, increasing the risk of acquisition by terrorists or governments seeking biological weapons of mass destruction. For these and other reasons, the viruses we propose to study are Category A-C agents, and many are also categorized as select agents by the Centers for Disease Control and Prevention. Current defense against most BWT RNA viruses is ineffective or non-existent. Ribavirin and interferon are effective against a few BWT RNA viruses but only if administered early during the course of disease, usually before severe disease is apparent; no effective therapy exists for later stages of infection when severe signs such as hemorrhage and encephalitis are first recognized. No licensed vaccines are available for any of the RNA viruses likely to be deployed for biological terrorism or warfare. Furthermore, diagnostic methods for detecting and characterizing human infection can be slow and imprecise. We therefore propose to exploit recent advances in RNA virus genetics, as well as the unique virology

expertise within our regional group, to 1) develop cross-cutting, low containment, rapid and high-throughput screening methods for identifying new antiviral drugs against BWT agents; 2) develop rapid, sensitive, and specific detection systems for identifying infectious BWT viruses and antibodies in infected persons; and 3) utilize state-of-the-art siRNA technology to identify host factors that could serve as the basis for development of new classes of antivirals with broader reactivity and less likelihood of genetic evasion by the viral pathogen. This research will meet critical, immediate needs for viral biodefense by accelerating the development of antivirals, diagnostics, and vaccine development and by moving much of this viral BWT research into the low-containment (BSL2) arena. Our project will also provide important new tools for basic research on BWT viruses, such as the identification of cellular receptors and mechanisms of pathogenesis, which will benefit both basic and applied virology research now and in the future.
<END TEXT>

<TITLE>WRCE Development and Evaluation of Human Brucellosis Vaccines
<AUTHORS>L. Garry Adams
<JOURNAL>
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<CITATION>
<TYPE>research project
<ADDRESS>texas a&m university
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<KEYWORDS>
<TEXT>Brucella melitensis is a Category B agent and an intracellular bacterial pathogen that subverts or avoids both innate and acquired immunity to cause a debilitating acute disease and to establish a chronic disease in man and animals alike. While moderately advanced diagnostics and vaccines exist for domestic livestock, antiquated diagnostics and no vaccines are licensed for human brucellosis. Currently available

vaccine strains are virulent in humans that, along with their lack of genetic definition, make them unsuitable for human use. The short-term aim of the proposed studies is to classify mutations in these genes according to their effect on survival in the mouse model (i.e., short- vs. long-term survival). The long-range goal of our research program is to expand the fundamental knowledge base for improved disease prevention through safer, more effective vaccines for human brucellosis. Identification of specific virulence genes will be used to derive attenuated candidate strains for use as live vaccines. The ultimate goal of the proposed experiments is to perform the same experiments using Macaca mulatta nonhuman primates based on the predictive capabilities of the mouse model, and to estimate the safety and efficacy of these strains for human use. Our specific aims are: (1) To identify B. melitensis genes necessary for survival and virulence using the TraSH system for generating and screening mutants. Mariner transposon mutagenesis will be used to generate a bank of mutants that will be screened using the mouse model of infection to identify mutants that exhibit reduced survival and chronic persistence in mice. Screening for survivability and persistence will enhance identification of genes that are important for persistence in humans. (2) To determine the safety and protection induced by B. melitensis vaccine candidates in the mouse model. Protection will be evaluated in this model to identify the optimal vaccine candidates for subsequent testing in nonhuman primates based on resistance to colonization of vaccinated animals to aerosol challenge with virulent B. melitensis. (3) To evaluate safety, protection and host gene expression in response to candidate live B. melitensis vaccines in nonhuman primates. The Macaca mulatta aerosol model for acute and chronic infection will be used to characterize pulmonary colonization, systemic dissemination and subsequent induced pathology. Protection will be evaluated based on resistance to pulmonary colonization, magnitude and burden of dissemination, and persistence of wild type after aerosol challenge with wild type B. melitensis

16M. Because of the threat posed by Brucella as a weapon, unavailability of vaccines for human use, and antibiotic therapies that may be unreliable, development of vaccines against human brucellosis is clearly the optimal approach for long-range protection of the public.
<END TEXT>

<TITLE>WRCE Rapid Diagnostic Tools for Q Fever
<AUTHORS>James E. Samuel
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<DATE>
<CITATION>
<TYPE>research project
<ADDRESS>texas a&m university
<EMAIL>
<PHONE>
<KEYWORDS>
<TEXT>The incidence of Q fever in the U.S. is likely underestimated because infections are seldom diagnosed with confirmatory tests and clinical presentation is similar to a variety of fevers of unknown origin. Infections are not verified by culturing of the organisms because of difficulties inherent to propagation of the organism, and the value of DNA identification of the organism during infection has not been established. Serodiagnostic testing currently relies upon reaction with whole organisms as antigen, and assays are not well standardized and not readily adaptable to large-scale screening. The objectives of this application are to create recombinant serodiagnostic reagents and improved PCR detection methods to adequately prepare for identifying infected individuals. The working hypothesis of the serodiagnostic component is that unique specific protein antigens are recognized early in infection during acute Q fever. We have identified and cloned a variety of immunodominant proteins from Coxiella burnetii. We propose to completely characterize the key antigens recognized during infection using proteomic and genomic approaches. The expressed recombinant antigens will be evaluated for their

utility as serodiagnostic reagents initially in an enzyme-linked immunosorbent assay (ELISA) format with recombinant His-tagged purified proteins. These reagents will then be compared with current assays for sensitivity and specificity using a large collection of human Q fever patient sera. The working hypothesis of the polymerase chain reaction (PCR) detection component is that organisms can be detected in blood samples early in infection, prior to the development of clinical symptoms and a specific immune response. To test this hypothesis, we will establish an aerosol-delivered acute Q fever model in guinea pigs to accurately represent human disease caused by inhalation, the natural route of infection and the likely route of infection resulting from a bioterrorist release. Previous studies suggest that organisms can be routinely detected by PCR in blood samples from patients serologically confirmed to have acute Q fever, but critical issues concerning when patients become bacteremic after infection and how long a bacteremic state exists have not been elucidated. PCR detection offers an attractive alternative approach to identify C. burnetii in blood since bacterial culturing is cumbersome, time-consuming, and requires BSL3 containment.
<END TEXT>

<TITLE>WRCE New Diagnostic Methods for Acute Rickettsial Infections
<AUTHORS>Juan P. Olano
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<DATE>
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<KEYWORDS>
<TEXT>The long-term goal of this research project is the development of novel, ultrasensitive, and rapid diagnostic methods for rickettsial infections during

the acute phase of the disease. Rickettsiae are obligately intracellular bacteria responsible for potentially lethal diseases such as epidemic and endemic typhus, scrub typhus, and Rocky Mountain spotted fever. In addition, Rickettsia prowazekii and R. rickettsii are part of the Centers for Disease Control and Prevention and NIH category B agents and the North Atlantic Treaty Organization select agent list for their potential use as bioterrorist/biowarfare agents. Bioterrorist attacks can occur in multiple settings, and it is widely accepted that most terrorist attacks are covert, and therefore the infectious agent will be unknown until the first person becomes acutely ill and seeks medical help. A fast and accurate diagnosis is most imperative at this stage so that proper identification of the agent is performed and adequate treatment is started immediately. Current diagnostic techniques are either insensitive during the acute stage of the disease, or the techniques needed to perform the tests are cumbersome and expensive. The specific aims designed to test the hypotheses that diagnosis of rickettsial infections during the acute phase of the disease is possible by using ultrasensitive antigen detection methods and by using host biosignature analysis are: 1) develop a highly sensitive and specific diagnostic test for rickettsial infections based on antigen detection in blood by using ultrasensitive detection methods (electrochemiluminescence and tyramide signal amplification coupled to enzyme-Iabeled fluorescence), and 2) develop reliable biosignature patterns in human hosts that allow proper identification of rickettsial infections during the acute stage based on analysis of several serum analytes (proteins, hormones, and others). The research design includes production of polyclonal, monoclonal and recombinant antibodies for the development of ruthenium and alkaline phosphatase-based sandwich immunoassays on magnetic beads to capture rickettsial organisms. The detection systems will use electrochemiluminescence and fluorescence analyzers. In addition, biosignature analysis will include 80 serum analytes and will be performed at the University of New Mexico Small Animal Core followed by

computerized analysis at the Pathogenesis Expression Core.
<END TEXT>

<TITLE>WRCE Discovery of Subunit Vaccines for Smallpox
<AUTHORS>Kathryn F. Sykes
<JOURNAL>
<DATE>
<CITATION>
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<PHONE>
<KEYWORDS>
<TEXT>Eradication of smallpox as a natural pathogen happened over 20 years ago. Eradication of smallpox as a biothreat is now our objective. New stockpiles of safe and efficacious smallpox vaccine are needed to protect both the civilian and military populations against deliberate release of the smallpox virus. Currently there is no commercially available vaccine, and the previously approved one is a live vaccinia inoculum associated with more adverse events than any other U.S. approved vaccine. Most recent work is focused on improving the manufacturing process of the original vaccinia vaccine strain and testing other live attenuated viruses. We propose to discover new vaccine candidates from viral components. A molecular-based design would be safer and more easily controlled during manufacture. Since we anticipate obtaining multiple protective components, these can be mixed and matched to effectively defend against wild type, natural variants, and bioengineered smallpox isolates. To identify antigens of smallpox that carry vaccine potential, the goal of our proposed project is to screen all the genes of the closely-related cowpox virus for their ability to protect against disease in its natural murine host. This genome-level approach is feasible because the viral genome databases are available and we have developed the platform technologies that make a comprehensive screen fast and efficient. We will establish the electronic and

molecular protocols to synthetically generate a thousand codon-optimized gene sequences. It will be used to produce a library of high quality synthetic cowpox open-reading-frames. An advanced library-screening method employing multiplex arrays will enable us to screen all the ORFs for protection in one challenge- protection experiment. Candidates selected by the screen will be individually validated to identify protective antigens. These will be tested as genes and protein subunits in two murine models of smallpox disease and immune characterized, in preparation for subsequent development as human variola vaccine candidates.
<END TEXT>

<TITLE>WRCE Discovery of Subunit Vaccine Candidates Against Glanders
<AUTHORS>D. Mark Estes
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<DATE>
<CITATION>
<TYPE>research project
<ADDRESS>university of texas medical branch at galveston
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<TEXT>Glanders is a severe disease that already has a history of use as an effective bioweapon. It is naturally an equine disease but zoonotically transmits to humans. Even if quickly diagnosed as Burkholderia mallei, antibiotic treatments have shown low efficacy and disease control requires long regimes with multiple drugs. Even if the patient survives the acute phase, chronic infection can be suffered for as long as 20 years. The most rational plan of defense is a vaccine, but to date none have been successfully developed. The immune evading tactics, including genomic fluidity, of this complex pathogen have rendered the performance of even live vaccines disappointing. New approaches are warranted. Therefore, we propose to identify a vaccine candidate

against glanders that is both more efficacious and safer than any previously developed. It will be designed from a collection of pathogen subunits that will include antigens that stimulate protective host immune responses yet exclude those that are immune-evading or superfluous. This conceptually solves the problem that whole pathogen vaccines are likely to provide too many components (dilution of useful ones by not useful ones) and the problem of subunit vaccines that is likely to provide too few components (limited antigenic coverage). We will accomplish this by engaging in a genomic-scale search of all B. mallei coding sequences for protective antigens by expression library immunization in a murine model of glanders. Our specific approach to identifying this condensed filtrate of protective antigens is to: 1) establish optimal molecular and animal model protocols for conducting protection assays on a large scale; 2) build all of the coding sequences of B. mallei into a library of expression constructs for genetic immunization; and 3) directly assay the protective capacity of every B. mallei coding sequence in vivo. At the conclusion of this project, we will have generated protective subunits that will be ready for delivery and modality optimization studies. Development of a B. mallei vaccine is anticipated to facilitate greatly the development of B. pseudomallei and B. cepacia vaccines against these ancient diseases.
<END TEXT>

<TITLE>WRCE Development of Novel Pseudoinfectious Flavivirus Vaccines
<AUTHORS>Peter Mason
<JOURNAL>
<DATE>
<CITATION>
<TYPE>research project
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<KEYWORDS>
<TEXT>There are numerous NIAID Category A, B, and C Priority Pathogens in the family Flaviviridae. These agents are arthropod-borne members of the Flavivirus genus, which can cause encephalitis or hemorrhagic fever. Current approved vaccines for these diseases include a live-attenuated vaccine (LAV) for yellow fever (YF) and inactivated virus vaccines (INV) for Japanese encephalitis (JE) and tick-borne encephalitis (TBE). The methods used to produce these vaccines have not yet yielded approved vaccines to prevent dengue fever/hemorrhagic fever or encephalitis caused by West Nile virus (WNV). Currently, LAVs for dengue are being developed based on the effective LAV for YF (17D strain). However, the 17D vaccine has recently been associated with several cases of disease in healthy adults, including the death of a 22-year-old, and this LAV is not acceptable for use in the immunocompromised (including pregnant women and infants). INV products, on the other hand, are expensive to produce, due to the large amounts of antigen needed to immunize vaccinees. Interestingly, the Japanese government has recently withdrawn its recommendation for universal vaccination with its JE vaccine due to concerns about residual mouse brain antigen present in this INV. To address the need for new and better vaccines for flavivirus diseases we have developed a method to produce genetically engineered, "pseudo-infectious" flaviviruses (PIFV) that offer great promise as vaccine candidates. These PIFVs consist of viruses that contain large deletions in the essential capsid (C) protein, and are thus non-infectious in animals, man, or traditional cell cultures. PIFVs can be propagated in cells expressing C; in cells lacking the C protein (e.g., cells in vaccinated hosts) PIFV undergo a limited replication cycle, producing large amounts of a highly immunogenic subviral immunogen. We have shown that a WNV PIFV does not cause disease in animals, and that animals inoculated with this PIFV produce high titers of WNV-neutralizing antibodies. Thus, PIFVs are ideal safe and effective vaccine candidates. The goal of this New Opportunities project is to take our proof-of principle studies on

PIFV generation to the next step, by developing methods for large-scale production of PIFV, and demonstrating that they can be used to safely immunize animals against challenge with flaviviruses. To achieve this goal we have developed the following specific aims: 1. Develop a replication-defective PIFV vaccine candidate for YF and WNE. 2. Develop methods to produce high yields of the YFV and WNV PIFV in certified cell lines.
<END TEXT>

<TITLE>WRCE Diagnostic DNA Microarray for Category A, B, &C Priority Viruses
<AUTHORS>Robert B. Tesh
<JOURNAL>
<DATE>
<CITATION>
<TYPE>new opportunity
<ADDRESS>university of texas medical branch at galveston
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<KEYWORDS>
<TEXT>The overall objective of this inter-regional New Opportunities project is to develop an integrated DNA microarray platform for detection of NIAID Category A, B, & C priority viruses in clinical samples. The entire diagnostic assay, which consists of nucleic acid isolation from clinical samples, random sequence-independent PCR amplification, fluorescent labeling, microarray hybridization, microarray scanning and automated interpretation of data, can be completed within 24 hours. The focus of this project is on the Category A, B, & C viruses associated with hemorrhagic fever and encephalitis; the agents in these two categories include most of the NIAID priority viruses. The project will involve scientists at two Regional Centers of Excellence for Biodefense and Emerging Infectious Diseases (RCE), the Western and the Midwestern RCEs. It represents a collaboration between researchers at the University of Texas Medical Branch in Galveston (WRCE) and at Washington University in

St. Louis (MRCE). Accordingly, the project and division of work will be divided into two parts. Dr. David Wang at Washington University will be responsible for the microarray design, hybridization experiments, and microarray data interpretation. His work will utilize the MRCE's Massively Parallel Viral Screening Core in St. Louis. Drs. Robert Tesh, Scott Weaver and Michael Holbrook at UTMB will be responisble for generating clinical samples and nucleic acids for each of the viruses of interest and for producing selected reassortant and recombinant viruses for testing by Dr. Wang; some of their work will be done in the Shope Laboratory, part of the WRCE BSL4 Core located in Galveston. The proposed research is highly innovative and complementary; it combines infectious disease research strengths, virologic expertise, and unique containment facilities at UTMB with the outstanding sequencing, genetic, and computational capabilities at Washinton University. The development of this diagnostic microarray would greatly advance our ability to rapidly and systematically diagnose the viral etiologies of hemorrhagic fever and encephalitis. Such an assay would be a benefit to clinical medicine and public health, as well as to biodefense.
<END TEXT>

<TITLE>WRCE Reassortant Vaccine for Lassa Fever: Advanced Proof-of-concept in Small Nonhuman Primates
<AUTHORS>Jean Patterson
<JOURNAL>
<DATE>
<CITATION>
<TYPE>new opportunity
<ADDRESS>southwest foundation for biomedical research
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<KEYWORDS>
<TEXT>Among the hemorrhagic fevers (HF), Lassa HF affects the second largest number of people (dengue HF is the largest). The at-risk seronegative population in West Africa may number as high as 59 million, with

an annual incidence of illness of 3 million, fatalities up to 67 thousand, and up to 3 million re-infections. The sizable disease burden and the possibility that Lassa virus (LASV) can be used as an agent of biological warfare make a strong case for vaccine development. A chimeric virus, clone ML29, was generated using the replication machinery of non-pathogenic Mopeia virus, a close genetic relative of LASV. ML29 encodes the major antigens that induce protective immunity against LASV. Proof-of-concept studies in guinea pigs proved that the ML29 vaccine is safe, immunogenic, and fully protective against homologous and distantly-related LASV strains. The LASV is a rodent-born pathogen and is treated differently by the immune system of rodents and nonhuman primates (NHPs). Recently a NHP model of human Lassa HF was developed in common marmosets at SFBR for the WRCE. This proposal was designed to naturally extend our proof-of-concept studies from the guinea pig model to NHPs to comply with the FDA's "Two Animal Rule" for pre-clinical development of biodefense vaccines (21 CFR 314 and 601). In this study, we will test the hypothesis that the ML29 vaccine is safe, immunogenic, and efficacious in common marmosets. Our specific goals are: 1. Efficacy: test the hypothesis that ML29 vaccine will protect primates against LASV challenge. 2. Safety: test the hypothesis that the ML29 induces a short, inapparent, self-limited infection failing to induce morphological or biochemical alterations in immunized monkeys. 3. Immunogenicity: test the hypothesis that ML29 vaccination will stimulate specific and cross-reactive cell-mediated immune responses.
This is an inter-regional RCE project. Specific Aim 1 will be conducted in BSL4 Facility at SFBR in San Antonio, TX (WRCE) and Specific Aims 2 and 3 will be performed by Dr. Igor Lukashevich in the Institute of Human Virology, UMBI, Baltimore, MD (MARCE).
<END TEXT>

<TITLE>WRCE Assays for the Detection and Discrimination of Burkholderia Species

<AUTHORS>Katherine A. Brown
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<DATE>
<CITATION>
<TYPE>new opportunity
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<TEXT>Burkholderia pseudomallei and Burkholderia mallei are the causative agents of the diseases melioidosis and glanders, respectively. Infections are particularly debilitating and can lead to abscesses in the lungs and other organs with accompanying septicemia. B. pseudomallei and B. mallei are recognized bioterrorism agents and are particularly dangerous as there are no vaccines currently available and treatment with antibiotics for melioidosis is prolonged and often ineffective (with fatality rates of about 40%). Improved, sensitive methods for whole organism detection may be of great value in protecting both military and civilian populations, particularly as infection would most likely be in the form of an aerosol. Furthermore, development of detection methods to identify Burkholderia relative to other gram-negative organisms and to distinguish between species would be of significant value in ensuring that correct countermeasures could be rapidly implemented. We propose to express surface proteins from both species of Burkholderia, to develop aptamers that can bind to these surface proteins, and to adapt Luminex dye-bead technology (which can be used in the military field conditions) to generate spectrally sensitive assays for the detection of these two Burkholderia species. In this context, the specific aims of the project are: Specific Aim 1. To identify, express, and purify antigen candidates from B. pseudomallei and B. mallei, selected based upon genome comparisons and proteomic data. Specific Aim 2. To generate high-affinity aptamers and antibodies against these antigens. Specific Aim 3. To develop robust assays for the detection of antigens and whole organisms.
<END TEXT>

<TITLE>WRCE Vaccine for Rocky Mountain Spotted Fver
<AUTHORS>Xuejie Yu
<JOURNAL>
<DATE>
<CITATION>
<TYPE>new opportunity
<ADDRESS>university of texas medical branch at galveston
<EMAIL>
<PHONE>
<KEYWORDS>
<TEXT>Rickettsia rickettsii is a select agent and a potential agent of biological warfare and terrorism. Rocky Mountain spotted fever caused by R. rickettsii is a serious human disease that is endemic in the United States, Canada, and Central and South America. Lethality in the pre-antibiotic era was high (23-80%) and even with the availability of effective antibiotic treatment, the mortality rate is around 3-4%, a consequence of late diagnosis and delay in starting appropriate therapy. Efforts to develop a vaccine for Rocky Mountain spotted fever began as early as the 1920s, but killed rickettsial vaccines derived from ticks, embryonated eggs, and cell culture failed to protect vaccinated volunteers challenged with virulent R. rickettsii. While subunit vaccines have shown some promise in animal studies, the development of an effective and safe vaccine for humans remains elusive. Currently there is no vaccine available for Rocky Mountain spotted fever despite the potential use of R. rickettsii as a biological weapon and the high case-fatality rates similar to or greater than Category A agents such as Francisella tularensis, Lassa virus, and Rift Valley fever virus. An attenuated strain of R. prowazekii was shown to be an effective vaccine providing excellent protection (94%) to human populations. Unfortunately, the strain could revert to a virulent form, an unacceptable property for a vaccine that may be given to millions of individuals. However, the success of this vaccine did demonstrate that a non-reversible attenuated strain would be a

safe and effective vaccine. This provides the basis for the proposed studies. We hypothesize that inactivating a gene that codes for a key virulence determinant, but is non-essential for rickettsial survival, will attenuate R. rickettsii and make it a safe and effective vaccine. The goal of this project is to develop an effective and safe vaccine for Rocky Mountain spotted fever. This will be accomplished through the following specific aims. Specific Aim 1: To genetically inactivate selected R. rickettsii virulence determinant genes, which are key virulence factors for rickettsiae, but are not essential for their survival. We will use homologous recombination techniques to insertionally inactivate the target genes. We will ensure that these resulting mutations are not reversible. Specific Aim 2: To determine the safety, immunogenicity, and protective immunity of the candidate attenuated vaccine strains generated in Specific Aim 1. To determine the efficacy and safety of the vaccine, guinea pigs and mice will be immunized with the gene knockout R. rickettsii strains and challenged with homologous virulent R. rickettsii or with heterologous R. typhi or R. conorii. The attenuated R. rickettsii vaccine generated in this project will have a significant impact on preventing Rocky Mountain spotted fever. This is an inter-regional RCE project. Specific Aim 1 will be conducted by David Wood, University of South Alabama, Mobile, AL (SERCEB) and Specific Aim 2 will be conducted by Xuejie Yu, UTMB, Galveston, TX (WRCE).
<END TEXT>

<TITLE>WRCE Rickettsial Vaccine Development by Microarray Analysis
<AUTHORS>Donald H. Bouyer
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<DATE>
<CITATION>
<TYPE>career development
<ADDRESS>university of texas medical branch at galveston
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<PHONE>
<KEYWORDS>
<TEXT>Rickettsia prowazekii is an obligately intracellular bacterium that is the causative agent of epidemic typhus. However, due to its stable extracellular form, low dose infectivity, high mortality rate, ability to be aerosolized easily and its previous history of being utilized as a bioweapon, R. prowazekii has been classified as a select agent by both NIH and CDC. Although the mechanisms of R. prowazekii pathogenicity were known in general, there was a dearth of information on specific genes involved in the disease process. Therefore, we utilized novel gene expression techniques to identify the rickettsial genes responsible for escape of R. prowazekii from the vacuole, which plays a critical role in the survival of the organism in the host. Our analysis identified potential membranolytic genes (tlyA, tlyC, pld and pat1) in the R. prowazekii genome that we suspected to be responsible for vacuolar escape. Reverse transcriptase-PCR analysis determined that tlyC and pld were transcribed during the escape process. The functionality of both genes was determined by complementation experiments using a strain of Salmonella that does not escape the vacuole. Experiments are ongoing to determine the role of tlyA and pat. Complementation analysis has also determined that each of the genes selected has homologs in many species of microbial pathogens. Our study on the roles of tlyC and pld in the escape of R. prowazekii from host cell endosomal vacuoles into the cytosol may also give a broader insight into the pathogenic mechanism of other microbes.
<END TEXT>

<TITLE>WRCE Transition Metal-mediated SPOS for Biodefense Discovery
<AUTHORS>Luis E. Martinez
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<PHONE>
<KEYWORDS>
<TEXT>The broad long-term goal of our program is to develop solid phase organic synthesis (SPOS) methods for the synthesis of small molecule compound libraries and to utilize these compounds for the identification of therapeutically relevant proteins that can serve as targets for future drug and biocide discovery efforts for biodefense and emerging infectious diseases. The particular focus of this research is twofold. The first focus is on the further development of transition metal-mediated solid phase organic synthesis techniques for the generation of differentially substituted aromatic and heteroaromatic compound libraries. The second focus is on the concurrent assessment of the biological activity of the resulting compounds as potential anti-infectives and biocides against CDC Category A-C Agents. We have developed transition metal-mediated benzannulation and trimerization of acetylenes under microwave conditions for the formation of multicomponent, differentially substituted compound libraries. We will now apply these compound libraries to identify therapeutically relevant proteins that can serve as targets for future efforts in biocide and therapeutic candidate discovery of CDC Category A-C Agents and elucidate information about the structure, function, and protein-ligand interactions of these proteins. Additionally, by interfacing this research with existing projects and cores of the RCE using short term, repetitive visits and long-distance mentoring/advising by two RCE investigators, it is anticipated that research in the areas of biodefense and emerging infectious diseases will be an important component of this investigator's future career development.
<END TEXT>

<TITLE>WRCE Cell Wall Proteins in Bacillus anthracis as Vaccines
<AUTHORS>Yi Xu

<JOURNAL>
<DATE>
<CITATION>
<TYPE>career development
<ADDRESS>texas a&m university system health science center
<EMAIL>
<PHONE>
<KEYWORDS>
<TEXT>Anthrax is an old disease with reborn relevance to both scientists and the general public. The current human anthrax vaccine, Anthrax Vaccine Absorbed, consists of protective antigen (PA) as the principal protective immunogen and other uncharacterized minor components. It requires a lengthy administration procedure with annual boosters and may have undesirable side effects. Several studies indicate that immunogens, in addition to PA, are required for full and long-lasting immunity to anthrax. Consequently, it is important to develop a vaccine that has defined components, affords long-term protection, and has reduced side effects. Gram-positive pathogenic bacteria possess cell wall anchored proteins (CWAPs) that are critical for virulence and are excellent vaccine candidates. By analogy, the CWAPs of B. anthracis are likely to show equal relevance and utility. Analysis of the B. anthracis genome revealed nine previously uncharacterized CWAPs. Studies from our lab indicate that two of these CWAPs are able to bind collagen and mediate adherence to immobilized collagen when expressed on the surface of a heterologous bacterial host. One of the CWAPs is involved in adherence to and invasion of human fibroblasts and epithelial cells. Together these findings support the hypothesis that CWAPs of B. anthracis are significant in the pathogenesis of anthrax and are potential vaccine candidates. The aims of this proposal are: 1) to determine the protective efficacy of the nine CWAPs in a murine pulmonary anthrax model against challenge from virulent B. anthracis, and in an A/J mouse model against challenge from B. anthracis Sterne strain; and 2) to determine if the CWAPs contribute to the

virulence of B. anthracis in the two mouse models. These studies will be performed in collaboration with Dr. Theresa Koehler at the University of Texas Medical School, and Dr. Rick Lyons at the University of New Mexico. I hypothesize that results from these studies will lead to the development of new human anthrax vaccines and provide an initial mechanistic understanding of the roles of these proteins in B. anthracis pathogenesis. This will set the foundation for future research with the long-term goal of developing improved human vaccines through a better understanding of the molecular interactions of B. anthracis and its host.
<END TEXT>

<TITLE>WRCE Revealing the Attenuating Mutations of F. tularensis LVS
<AUTHORS>Joseph Petrosino
<JOURNAL>
<DATE>
<CITATION>
<TYPE>career development
<ADDRESS>baylor college of medicine
<EMAIL>
<PHONE>
<KEYWORDS>
<TEXT>Francisella tularensis is one of the most infectious bacteria known and is a serious bioterrorism threat. A weakened strain (named LVS) has proven to be an effective vaccine for this organism in humans, but is not approved for use in the U.S., in part because its mechanism of attenuation is unknown. Based on genetic comparisons with a parental virulent strain, we have found relatively few genetic candidates for attenuation. Subsequent experiments in an infection model will test which genetic differences underlie the attenuating mechanism in LVS. This data may help lead to U.S. approval of the LVS vaccine and will help in the design of additional attenuated vaccine strains for Francisella and other pathogens.
<END TEXT>

<TITLE>WRCE Rational Design and Optimization of New Live-attenuated Vaccines for Alphaviral Encephalitides
<AUTHORS>Katherine Ryman
<JOURNAL>
<DATE>
<CITATION>
<TYPE>career development
<ADDRESS>louisiana state university health science center
<EMAIL>
<PHONE>
<KEYWORDS>
<TEXT>Eastern equine encephalitis virus (EEEV), an alphavirus in the family Togaviridae, is classified in Category B of the NIH Priority Pathogens List. It is also listed as a high consequence livestock pathogen by the USDA because it is highly lethal for humans and equines, and because effective vaccines and therapies are lacking for EEEV. The formalin-inactivated vaccine strain of EEEV is not suitable for wide-scale human use due to poor immunogenicity and possible residual virulence. Clearly, alternative strategies for vaccine production are required. Our long-term goal is to develop a live-attenuated virus vaccine with sufficient degree of attenuation to be safe for human populations. The objective of the proposed research is the rational design of attenuated strains via the selective deletion (or disabling) of innate immune evasion properties. This is based on the hypothesis that EEEV possesses mechanism(s) to antagonize the interferon alpha/beta (IFN-_/_) response elicited by infected dendritic cells (DCs) which can be disabled to attenuate the virus and enhance the immune response. Using the non-pathogenic alphavirus Sindbis (SB) as a "baseline" for IFN-_/_ sensitivity, we will determine which of the IFN-_/_-mediated responses that suppress SB replication do not do so for EEEV. We will infect cultures of primary myeloid bone marrow derived DCs (BMDCs). The use of these cells is particularly important as they are representative of the cells targeted by alphaviruses in vivo following subcutaneous inoculation. By infecting BMDCs with

chimeric replicon particles in which either the SB or the EEEV genome is encapsidated in SB structural proteins, we will for the first time be able to dissociate SB versus EEEV replication events from the role of the non-structural proteins (nsPs) from attachment/entry steps and thereby ensure equal delivery of RNA genomes to each cell. We have compelling evidence that the nsPs and/or cis-acting sequences in the viral RNA encode the IFN-_/_ resistance of the virulent alphaviruses. Therefore, in Aim 1 we will determine whether individual EEEV proteins or cis-acting elements alter replicative capability and IFN-_/_ sensitivity. In Aim 2, we will characterize the effects of targeted mutations in the EEEV genome on antagonism/evasion of the IFN-_/_-mediated response, focusing on the following pivotal steps in the IFN-_/_ antiviral pathway: i) the activity of antiviral effector mechanisms, particularly PKR; ii) IFN-_/_ induction; and iii) IFN-_/_ signaling. We anticipate that these studies will allow the identification and disablement of EEEV-encoded product(s) that antagonize/resist IFN-_/_ activity. Our long-term goal is the rational design of attenuated alphavirus strains with sufficient degree of attenuation to be safe for human populations via the selective inactivation of innate immune evasion properties.
<END TEXT>

<TITLE>WRCE Micro-NMR and Nanoparticle Amplification for Botulinum Toxin Diagnostics
<AUTHORS>Todd M. Alam
<JOURNAL>
<DATE>
<CITATION>
<TYPE>developmental project
<ADDRESS>sandia national laboratories
<EMAIL>
<PHONE>
<KEYWORDS>
<TEXT>Botulinum toxin is one of the most potent substances known. At present, laboratory diagnostic

testing for botulism in the United States is based on the mouse bioassay that can detect as little as 0.03 ng of botulinum toxin, but requires between 1 to 2 days for results. In this project, a portable micro-nuclear magnetic resonance (_-NMR) spectrometer platform utilizing nanoparticle amplification will be developed for the rapid diagnosis of botulinum toxicity. The sensitivity of this device relies on recent advances in micro-fabrication coupled with super-paramagnetic iron oxide nanoparticle (SPION) signal amplification for NMR detection. There have been recent advances in micro-fabrication techniques, drastically improving the sensitivity of _-NMR, thus reducing the detection volume of NMR to below one nanoliter. In this proposal, we will be combining _-NMR detection coils fabricated at Sandia National Laboratories with further miniaturization of permanent magnets to produce a portable _-NMR device that can be used in diagnostic laboratories. The increase in _-NMR sensitivity will be coupled to the relaxation-induced amplification afforded through the use of SPIONs, thereby allowing bioagent detection. The biospecificity of the SPION amplification is obtained through antibody conjugation. By utilizing different specific antibodies, it will be possible to provide rapid diagnostics of the seven distinct antigenic types of botulinum toxin. This diagnostic _-NMR platform will be able to identify and quantify all of the Clostridium botulinum toxin types (A through G) existing in blood, serum, and tissue samples in about five minutes. This generalized _-NMR amplification scheme can also be easily modified towards other NIAID agents, positively affecting future public health issues.
<END TEXT>

<TITLE>WRCE Recombinant Envelope Protein Domain III as a Candidate Subunit Dengue Vaccine
<AUTHORS>Alan Barrett
<JOURNAL>
<DATE>
<CITATION>

<TYPE>developmental project
<ADDRESS>university of texas medical branch at galveston
<EMAIL>
<PHONE>
<KEYWORDS>
<TEXT>Effective vaccines exist for only a few members of the genus Flavivirus, including yellow fever, tick-borne encephalitis, and Japanese encephalitis. There are no effective prophylactic treatments for the diseases caused by any of these viruses, including dengue (DEN). The disease DEN is caused by four mosquito-borne, serologically related flaviviruses known as DEN1 to DEN4, and is the most important arboviral disease of humans. An estimated 50-100 million cases of dengue fever and hundreds of thousands of cases of dengue hemorrhagic fever occur in the tropics each year. Thus there is a need for a tetravalent vaccine. Since DEN is an emerging disease that is a major public health problem throughout tropical regions of the world, developing a DEN vaccine fits into the strategic plan of the WRCE to develop vaccines against biothreat agents and emerging infectious diseases. To date no candidate DEN vaccines have gotten beyond Phase II clinical trials, demonstrating a need to take a novel approach to developing a DEN vaccine. Each DEN virus consists of multiple genetic groups, termed genotypes. In the last few years, there is evidence that Asian and American genotype viruses differ antigenically, and that Asian genotype viruses are not neutralized by sera prepared against American genotype viruses, at least for DEN2 virus. This latter point may become an important issue for vaccine development as all candidate vaccines to date are based on DEN strains from Thailand in Asia. Therefore, the long-term objective of these studies is the development of a candidate subunit vaccine that induces neutralizing antibodies, but not antibody-dependent enhancement antibodies, against all genotypes of all four DEN viruses. To achieve this goal, we are proposing preclinical studies on a subunit vaccine based on domain III of the envelope protein (ED3). We hypothesize that a monovalent ED3

immunogen will induce antibodies that will neutralize members of all genotypes of the particular DEN virus and that residue E-390 in ED3 is part of a critical neutralizing epitope on DEN2 virus. Furthermore, we hypothesize that a tetravalent ED3 DEN vaccine will induce a protective immune response against all four DEN viruses without the induction of cross-reactive antibodies that may enhance DEN infection and lead to DHF. For this developmental project we will focus on DEN2 virus to justify our hypothesis. The following specific aims are proposed: 1) express DEN2 virus domain III of the virus envelope protein (ED3) each from an Asian and American genotype virus and compare their immunogenicity in a mouse model; and 2) evaluate a tetravalent rED3 vaccine in the mouse model.
<END TEXT>

<TITLE>WRCE A Highly Sensitive, Low-labor Pathogen Detector Based on Retroreflector-linked Immunosorbent Assay
<AUTHORS>Paul Ruchhoeft
<JOURNAL>
<DATE>
<CITATION>
<TYPE>developmental project
<ADDRESS>university of houston
<EMAIL>
<PHONE>
<KEYWORDS>
<TEXT>We propose to fabricate a new and very sensitive biomolecule label system and diagnostic tool based upon the extremely bright optical signals readily obtained from retroreflectors. For this proposed project, corner-cube micro-retroreflectors will be used to establish a field-compatible diagnostic for the Norwalk virus, the most common cause of viral gastroenteritis and a Group B biodefense agent. These retroreflectors consist of a transparent cube with three orthogonal, mutually touching mirrored surfaces that return incident radiation directly back to its source, making them highly detectable. In fact, a set of cubical retroreflectors on the moon's surface is

routinely detected and ranged as part of ongoing experiments on lunar orbital dynamics. Retroreflectors are also used in road lane markers and bicycle reflectors (as an array of embossed cubes), but have not previously been applied to bioanalytical methods. In our approach, we will fabricate micro-retroreflectors from glass cubes, about 5_m on a side, and coat three sides with gold. Next, the gold surfaces will be decorated with Norwalk antibodies and the cubes will be suspended into the sample solution on a sensor chip. Select areas of the surface of the sensor chip will also be decorated with stripes of monoclonal Norwalk antibodies so that, in the presence of the virus, the cubes attach to these areas on the surface. After washing, a detection step using inexpensive and field compatible detectors will probe for the bright, distinctive retroreflectance of the cubes and determine, with the aid of reference and control signals, the presence and concentration of the Norwalk virus. As a final demonstration, we will implement and validate an assay for Norwalk virus using human clinical specimens. This work will create a low-cost diagnostic tool platform with broad applications in low-labor, inexpensive assays of very high sensitivity. With further development, the micro-retroreflector technology may potentially revolutionize diagnostics directed against NIAID Category A, B, and C agents. We expect to be able to detect the presence of single cubes, making the device extremely sensitive to even very low concentrations of pathogens.
<END TEXT>

<TITLE>WRCE Genetic Screens to Identify the Ebola Virus Receptor
<AUTHORS>Richard E. Sutton
<JOURNAL>
<DATE>
<CITATION>
<TYPE>developmental project
<ADDRESS>baylor college of medicine
<EMAIL>

<PHONE>
<KEYWORDS>
<TEXT>Ebola virus (EBO) is a member of the filoviridae and is the cause of fatal hemorrhagic fevers. It is currently categorized as both a BSL4 and class A bioterrorism agent. It is a filamentous, enveloped RNA virus, with a wide cellular tropism, both in terms of cell types and mammalian species. At present, no prophylactic vaccine yet exists for EBO and there are no effective therapies for infected patients. EBO encodes a precursor glycoprotein (GP) that is processed into GP1 and GP2. It is thought that GP1 mediates the initial binding of EBO to cell surfaces. Several years ago, folate receptor __(FR_) was identified as a putative cofactor for EBO entry. Viral entry was inhibited by both soluble folate and folate binding protein, but recent published data suggests that FR_ plays little if no role in EBO entry and it is likely there are other, as of yet uncharacterized, cellular factors that are involved in this process. This two-year WRCE developmental project seeks to characterize further EBO binding and entry into cells in two related but separate aims. The first specific aim is to utilize a short hairpin (sh) RNA retroviral vector library introduced into EBO-susceptible cells to identify one or more cell clones specifically resistant to EBO infection. The corresponding cDNA clone(s) will be recovered and introduced into non-permissive T cells to determine whether it confers susceptibility to EBO, both as pseudotyped particles but also as replication-competent virus, the latter being performed under BSL4 conditions at Southwest Foundation for Biomedical Research (San Antonio) in collaboration with Jean Patterson. cDNA(s) will also be tested for their ability to mediate spread of Marburg (MAR) virus. In the second aim (performed in parallel to the first) a genetic strategy using HIV-pseudotyped particles will be employed to identify genes that may mediate EBO binding and entry. A representational cDNA library will be introduced into an EBO non-permissive B cell line, and then the modified non-permissive cells subjected to infection with EBO GP pseudotyped viral particles. Candidate

cDNAs will be further characterized and tested as described under Aim 1. It is hoped that these studies will allow a better understanding of filovirus binding and entry into cells, perhaps resulting in insights into filovirus pathogenesis in man and leads in small molecule inhibitors of this deadly viral pathogen.
<END TEXT>

<TITLE>WRCE High-throughput Assay Development Against Cryptosporidium Glycolytic Enzymes
<AUTHORS>Guan Zhu
<JOURNAL>
<DATE>
<CITATION>
<TYPE>developmental project
<ADDRESS>texas a&m university
<EMAIL>
<PHONE>
<KEYWORDS>
<TEXT>Cryptosporidium parvum is a unicellular pathogen that can cause severe watery diarrhea in humans and animals. Because the infectious oocysts are highly resistant to chemical stresses including chlorine treatment applied to the community water supplies, C. parvum is a significant water- and food-borne pathogen, and is listed as one of the Category B priority pathogens in the NIH and CDC biodefense research programs. Currently, only a single drug (nitazoxanide) has been approved for treating cryptosporidiosis in Central and South America, or cryptosporidiosis in immunocompetent (but not immunocompromised) patients in the United States. Therefore, there is an urgent need to develop new anti-Cryptosporidium drugs. Drug development against cryptosporidiosis has been a slow process, which is primarily due to the poor understanding of the basic metabolic pathways in the parasite. Many well-defined or promising drug targets found in other apicomplexans are either absent or highly divergent in C. parvum. However, on the positive side, recent advancements in genome sequencing and biochemical studies have revealed many unique proteins that may serve as drug

targets in this parasite, which include bacterial-type lactate dehydrogenase (CpLDH1) and two distinct alcohol dehydrogenases (CpADH-E and CpADH2). Our long-term goal is to develop drugs that target the C. parvum CpLDH1, CpADH-E and CpADH2 enzymes. Because C. parvum relies solely on glycolysis for its energy, we hypothesize that the glycolytic enzymes may serve as rational drug targets in this parasite. As a first step to reach the long-term goal and to test the hypothesis, we plan to perform experiments to achieve the following two specific aims: Aim 1: To characterize the molecular features of CpLDH1, CpADH-E, and CpADH2, which includes validating whether these enzymes could serve as drug targets, analyzing expression patterns of C. parvum LDH and MDH genes, and detecting these enzymes in the parasite's complex life cycle. Aim 2: To develop assays for high-throughput screening (HTS) of compounds inhibiting these glycolytic enzymes, which include expressing C. parvum LDH and ADH enzymes as recombinant proteins, determining their enzymatic and inhibitory kinetics, developing assays in HTS format, and screening inhibitors from compound libraries. Completion of these aims will not only deepen our understanding on the energy metabolism of C. parvum, but also has a great potential for the development of new drugs against this medically important parasite.
<END TEXT>

<TITLE>WRCE Model for Oral Ingestion of Ricin Toxin
<AUTHORS>Seth Pincus
<JOURNAL>
<DATE>
<CITATION>
<TYPE>developmental project
<ADDRESS>children's hospital of new orleans
<EMAIL>
<PHONE>
<KEYWORDS>
<TEXT>Ricin toxin represents one of the most likely agents of bioterrorism because of its easy availability. Oral ingestion is the most probable

route of exposure of civilian populations. Early recognition of such intoxication is critical for identifying that an event has occurred and for instituting specific therapies. Yet there is virtually no knowledge regarding the clinical syndrome resulting from ingestion of the toxin, and it is unclear whether emergency room personal would be able to distinguish oral ricin intoxication from more common forms of gastroenteritis that occurs in clusters (e.g., due to staphylococcal toxins). Virtually all human experience is with the ingestion of the plant material, castor beans, which must first be digested before the toxin is released in the intestine. Animal studies have used ricin toxin as an aerosol, fed as an oil emulsion, or administered by gavage; none have involved the direct feeding of the toxin in an aqueous form or adsorbed to food. It is the goal of the proposed studies to study the clinical syndrome in mice resulting from ingestion of ricin toxin. To accomplish this goal, we propose the following specific aims: To feed ricin to mice and observe the clinical, pathological, and clinical laboratory findings associated with oral ricin toxicosis. To develop a sensitive ricin antigen-capture assay and utilize it to study ricin levels in saliva, gastrointestinal washings, stool, plasma, and tissue. These studies will allow the development of a set of clinical and laboratory criteria that will allow for the early recognition of ricin intoxications. The model may also be used for testing the utility of post-exposure treatments.
<END TEXT>

APPENDIX B
Results of analysis, matrix format

Formatting constraints of the thesis document cannot accommodate
the dimensions of this figure; please refer to figureb_1.pdf,
located in the supplemental images folder.

Figure B-1: Results of analysis, matrix format

APPENDIX C
Introductory Information Packet

Note: Due to formatting constraints of the thesis document, the format of the letter as it appears below varies slightly from the original PDF document. Content remains unchanged.

Greetings RCE scientist:

My name is Courtney Moser, and I am a graduate student in the Biomedical Communications program at the University of Texas Southwestern Medical Center at Dallas. I also currently work for Dr. Skip Garner within the Trans-RCE communications core. The communications core supports video conferencing initiatives and maintains the RCE web site along with its customized web-based portals for all RCE researchers. We are presently seeking new ways to enhance the productivity of the RCEs through enhanced communication and collaboration. In pursuit of this goal, we have been exploring the use of text data mining to identify individual RCE researchers with similar interests who may benefit from collaboration. The ultimate goal of the project is to encourage and enhance communication among researchers within and between the ten RCE regions.

The first part of this project involved the use of eTSNAP (electronic text similarity network analysis package), a text data mining software program developed here at UT Southwestern, to identify clusters of individuals working on similar research topics based upon abstracts submitted to each RCE; it is from this analysis that you were identified as belonging to a cluster with a common interest in anthrax. More specifically, your cluster is concerned with the structure and function of the anthrax spore and/or toxins and how each respective portion of the anthrax pathogen might be used as a target for future vaccines.

The next part of the project will comprise the greater part of the effort and involves initiating and facilitating collaboration within each identified cluster of individuals if the group agrees that this is a worthwhile effort. The initial group meeting will take place via teleconference or videoconference during which Skip and I will act as facilitators and moderators for the discussion. This meeting will allow time for each member of the cluster to introduce him/herself and his/her research and to identify any potential for collaboration. If warranted by the group, subsequent contacts may be made through a variety of telecommunications resources, such as video conferencing and live chat rooms, based upon feedback we receive regarding the effectiveness and convenience of such resources. Our role in this endeavor is to stimulate conversation and ensure access to the desired modes of contact for the purpose of collaboration.

The last part of the project involves a final evaluation in which we will consider the value of this method of social networking and the success of the various communications tools at facilitating collaboration within the scientific community. Our hope is that, through the use of eTSNAP, we can identify individuals who might benefit from collaboration, and that by initiating contact between such individuals and providing them with the necessary communications infrastructure, they will gain knowledge and insight that will help them in their research pursuits.

We would like to have our initial meeting sometime within the second week of October (the 9th through the 13th), depending on the availability of each member. Would you please let me know your availability during that week?

Accompanying this letter is a quick survey I ask that you complete before the initial contact. This survey will help me to understand the current state of communications between researchers and to

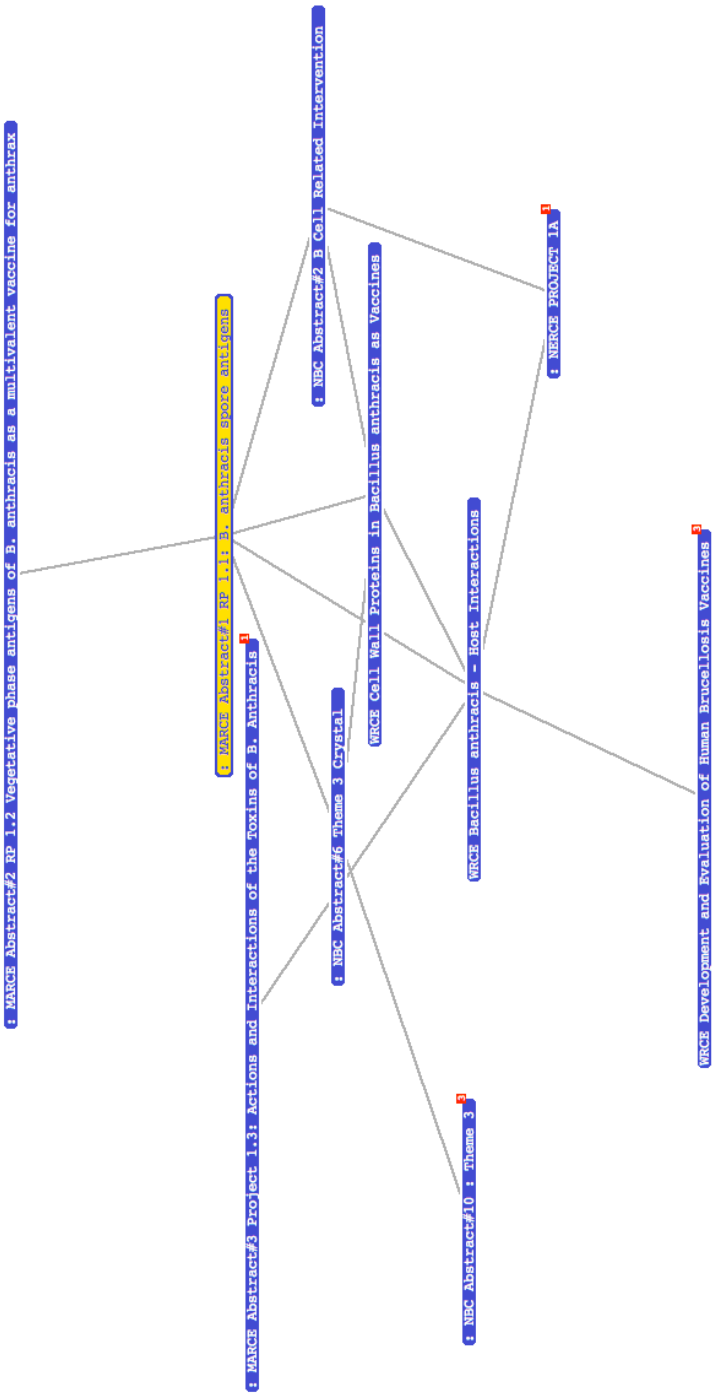
assess attitudes regarding collaborative efforts and communication within the RCE. I have also attached a copy of the cluster diagram generated by eTSNAP* and the abstracts of each member of the cluster. Please take some time prior to our initial meeting to review these two documents to ensure that you have a basic understanding of the research objectives of each cluster member and how these objectives might relate to your own work.

I appreciate your participation and look forward to working with you shortly.

Sincerely,
Courtney Moser

* Slight modifications to the cluster were made following a review of the abstracts and a keyword search through all RCE abstracts for the term "anthracis." Rick Lyons (mentioned in Xu abstract), Jurgen Brojatsch (NBC Abstract #17: *Role of tissue-specific cell killing in anthrax toxin-mediated cell killing)* and Andrzej Joachimiak (GLRCE: *Therapeutic inhibition of B. anthracis pathogenesis*) were added to the cluster; John Rose (NBC Abstract #10 Theme 3: *Optimizing VSV-based vaccine vectors*) and Gary Adams (WRCE: *Development and evaluation of human brucellosis vaccines)* were removed from the cluster.

Attachment 1: eTSNAP-generated social network for anthrax research.



Attachment 2: Members of the Anthrax Cluster

Principle Investigator	Project Title	RCE Region	Institution	Email Address	Phone Number	V-con?
O'Brien, Alison D.	Abstract #1 RP1.1 B. anthracis spore antigens	MARCE	Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc.	aobrien@usuhs.mil		yes
Cross, Alan S.	Abstract #2 RP1.2 Vegetative phase antigens of B. anthracis as a multivalent vaccine for anthrax	MARCE	Center for Vaccine Development University of Maryland, Baltimore	across@medicine.umaryland.edu		yes
Xu, Yi	Cell wall proteins in bacillus anthracis as vaccines	WRCE	Texas A&M University			
Crystal, Ronald	Abstract #6 Theme 3 Crystal: Anti-B. anthracis vaccination and passive protection	NBC	Weill Medical College of Cornell University			
Koehler, Theresa	Bacillus anthracis - host interactions	WRCE	University of Texas Health Science Center at Houston	theresa.m.koehler@uth.tmc.edu		yes
Casadevall, Arturo	Abstract #2 B cell related intervention	NBC	Albert Einstein College of Medicine	casadeva@aecom.yu.edu		yes
Hewlett, Erik L.	Abstract #3 Project 1.3: Actions and interactions of the toxins of B. anthracis	MARCE	University of Virginia	eh2v@virginia.edu		yes
Collier, John R.	Project 1A: Direct inhibition of anthrax toxin	NERCE	Harvard Medical School			
Lyons, C. Rick	(mentioned in Xu abstract)	WRCE	University of New Mexico Health Science Center	clyons@wrce		yes
Brojatsch, Jurgen	Abstract #17: Role of tissue-specific cell killing in anthrax toxin-mediated cell killing	NBC	Albert Einstein College of Medicine			
Joachimiak, Andrzej	Therepeutic inhibition of B. anthracis pathogenesis	GLRCE	University of Chicago			

Attachment 3: Abstracts of anthrax cluster members

The following 8 individuals (O'Brien, Cross, Xu, Crystal, Koehler, Casadevall, Hewlett, and Collier) were identified as being within the original cluster as defined by eTSNAP and are still considered to be part of the cluster following a review of their respective abstracts.

O'Brien, Alison D. MARCE
Abstract #1 RP1.1: *B. anthracis spore antigens*

One way to increase the likelihood that individuals who are exposed to aerosolized Bacillus anthracis spores remain disease free is to prevent germination of the infectious dormant spores into active vegetative cells that can produce potentially lethal toxins. Our recent finding that rabbit antiserum raised against mixtures of B. anthracis spores decreases the level of both Ames and Sterne strain spore germination in vitro, taken with the fact that formaldehyde-inactivated spores can serve as a protective vaccine against anthrax in guinea pigs, led us to the following hypothesis. Vaccine candidates that contain spore-surface-expressed antigens, i.e. exosporium antigens, will evoke antibodies that block or reduce spore germination in vivo or render spores more susceptible to phagocytosis and ultimately killing by macrophages. Based on this theory, our goal is to identify B. anthracis exosporium antigens as potential targets for incorporation into a second-generation Protective Antigen (PA)-based vaccine. The specific aims are to: 1. clone, express, purify, and characterize as potential protective immunogens (with and without PA) exosporium-associated antigens recognized by the anti- B. anthracis spore serum that inhibits spore germination in vitro; 2. prepare polyclonal and monoclonal antibodies against selected, purified, exosporium proteins and test these antibodies for spore-binding and anti-germinating activities in vitro, and, if such in vitro activities are evident, for protective capacity when administered to mice prior to and after spore challenge; 3. construct single and, if appropriate, double mutants of B. anthracis Sterne strain in which exosporium genes are deleted and compare the mutants with wild-type for virulence in the A/J mouse subcutaneous and inhalational challenge models; 4. develop a mouse model of inhalational anthrax in which separate lux operon fusions of B. anthracis Sterne strain are constructed so as to visualize by bioluminescence: i.) in vivo germination of spores; ii.) distribution and replication of vegetative cells and use this model to evaluate the efficacy of various active and passive immunization strategies to prevent anthrax. We envision that a vaccine that incorporates spore antigen(s) and PA could be used in both civilian and military populations at risk for exposure to anthrax spores.

Cross, Alan S. MARCE
Abstract #2 RP1.2: *Vegetative phase antigens of B. anthracis as a multivalent vaccine for anthrax*

B. anthracis (BA), the causative agent of anthrax infection, has been used in bioterrorist events. Consequently, the availability of preventive measures, such as vaccines, is highly desirable. The vegetative form of BA expresses a tri-partite toxin composed of protective antigen (PA), edema factor (EF) and lethal factor (LF) which combine to form two toxins, lethal toxin (PA+LF) and edema toxin (PA+EF). Current and next generation vaccines target PA; however, it would be desirable to prevent toxin formation by targeting the spore form of BA which initially infects the host, thereby preventing anthrax infection, We therefore are examining the role of vaccine- and naturally-acquired antibodies in mediating the killing of BA by macrophages, are defining the mechanisms by which antibodies promote the killing of BA and are seeking new targets on BA for future generation vaccines. While subjects immunized with the UK vaccine make antibodies to

PA, LF and EF, subjects immunized with the US vaccine make antibody to the PA only. Patients recovering from naturally-acquired cutaneous anthrax had stronger responses to LF than to PA. The anti-LF antibody had toxin-neutralizing activity in the absence of PA. Thus, LF make be a useful addition to US anthrax vaccines. Using a strain of BA that is unable to germinate, we found that macrophages are unable to kill the spore form of BA but do kill the vegetative form emerging within macrophages. Macrophages require the induction of nitric oxide (NO) to kill the BA. Enzymes present on the exosporium structure which covers the spore enable BA to escape macrophage killing, in part by inhibiting NO formation. Thus, vaccine-induced antibodies to these enzymes might neutralize these BA virulence factors. Antibodies, including purified anti-PA IgG, from the serum of an immunized subject enhanced the killing of BA by binding to the exosporium (since antibody-mediated killing is not observed in the absence of the exosporium). Addition of complement markedly increases the antibody-mediated killing. Since we hypothesize that proteins expressed during growth of BA in human blood may identify additional antigens for inclusion in next generation vaccines, we are comparing the expression of protein antigens in BA grown in laboratory media and in human blood. Any proteins expressed uniquely during growth in blood will then be evaluated as a vaccine candidate.

Xu, Yi WRCE
Cell wall proteins in bacillus anthracis as vaccines

Anthrax is an old disease with reborn relevance to both scientists and the general public. The current human anthrax vaccine, Anthrax Vaccine Absorbed, consists of protective antigen (PA) as the principal protective immunogen and other uncharacterized minor components. It requires a lengthy administration procedure with annual boosters and may have undesirable side effects. Several studies indicate that immunogens, in addition to PA, are required for full and long-lasting immunity to anthrax. Consequently, it is important to develop a vaccine that has defined components, affords long-term protection, and has reduced side effects. Gram-positive pathogenic bacteria possess cell wall anchored proteins (CWAPs) that are critical for virulence and are excellent vaccine candidates. By analogy, the CWAPs of B. anthracis are likely to show equal relevance and utility. Analysis of the B. anthracis genome revealed nine previously uncharacterized CWAPs. Studies from our lab indicate that two of these CWAPs are able to bind collagen and mediate adherence to immobilized collagen when expressed on the surface of a heterologous bacterial host. One of the CWAPs is involved in adherence to and invasion of human fibroblasts and epithelial cells. Together these findings support the hypothesis that CWAPs of B. anthracis are significant in the pathogenesis of anthrax and are potential vaccine candidates. The aims of this proposal are: 1) to determine the protective efficacy of the nine CWAPs in a murine pulmonary anthrax model against challenge from virulent B. anthracis, and in an A/J mouse model against challenge from B. anthracis Sterne strain; and 2) to determine if the CWAPs contribute to the virulence of B. anthracis in the two mouse models. These studies will be performed in collaboration with Dr. Theresa Koehler at the University of Texas Medical School, and Dr. Rick Lyons at the University of New Mexico. I hypothesize that results from these studies will lead to the development of new human anthrax vaccines and provide an initial mechanistic understanding of the roles of these proteins in B. anthracis pathogenesis. This will set the foundation for future research with the long-term goal of developing improved human vaccines through a better understanding of the molecular interactions of B. anthracis and its host.

Crystal, Ronald NBC
Abstract #6 Theme 3: *Anti-B. anthracis vaccination and passive protection*

Anthrax is caused by infection with B. anthracis, a category A bioterrorism agent. Any strategy to protect against B. anthracis must recognize that the pneumonic form of the disease develops quickly, and thus anti-B. anthracis protection has to be functional within days following an attack. This proposal presents a strategy for both rapid and long lasting protection against B. anthracis based on our experience in using adenovirus (Ad) gene transfer vectors to develop anti-bacterial vaccines, and the ability of Ad vectors to code for single chain antibodies against specific antigens. The underlying concept is that Ad-based gene transfer vectors can be used to evoke systemic, robust acquired immunity, as well as rapid passive immunity against B. anthracis antigens, and that both forms of protection can be achieved with a single administration of a single vector. The proposal uses an in vivo gene transfer-based strategy with a single Ad vector to simultaneously evoke rapid humoral immunity against B. anthracis (via an anti-B. anthracis protective antigen (PA) single chain antibody coded by the vector), while also functioning as a vaccine to evoke endogenous host responses against B. anthracis PA (via B. anthracis PA coded by the vector). The 3 specific aims outline studies to achieve these goals by developing the vaccine and single chain antibody strategies independently, and then combined. Aim 1. To evaluate the hypothesis that a vaccine based on a replication deficient Ad vector encoding the B. anthracis PA or fragments of PA will evoke robust systemic humoral immunity against PA and protect against challenge with B. anthracis. Aim 2. To assess the hypothesis that an Ad vector encoding a single chain antibody against an epitope of B. anthracis PA will provide robust, rapid humoral immunity against PA and protect against challenge with B. anthracis. Aim 3. To examine the hypothesis that a combined passive and active anti-B. anthracis protection can be achieved with a single administration of a single Ad vector expressing anti-B. anthracis single chain antibody and B. anthracis PA antigen.

Koehler, Theresa WRCE
Bacillus anthracis – host interactions

Anthrax disease results from a complex series of interactions between the invading bacterium, Bacillus anthracis, and the mammalian host. For inhalation anthrax, infection begins with entry of spores into the lung. Alveolar macrophages phagocytose the spores and transport them to lymph nodes of the mediastinum. Ultimately, the metabolically active form of the bacterium disseminates to the blood and other body tissues, reaching concentrations up to 108 colony-forming units per ml and secreting anthrax toxin proteins. In recent years, research emphases have focused on toxin protein structure and function. However, anthrax disease, whether acquired naturally or as the result of intentional dissemination of spores, results from infection with B. anthracis, not simply acquisition of toxin. Despite the importance of human infection with B. anthracis, there is almost a complete lack of knowledge of fundamental cellular and molecular mechanisms by which the bacterium interacts with its host. Results of studies proposed here will fill this critical gap in knowledge and reveal bacterial and host targets for generating new therapeutics for anthrax. We will use an in vitro macrophage model and in vivo murine model to identify pathogen and host targets important for multiple early steps in infection. The importance of pathogen and host factors during early infection will be assessed in both models by modulating expression of candidate B. anthracis and macrophage targets. In Aim 1, we will identify and characterize B. anthracis and macrophage molecular targets important for multiple steps of early infection. We will establish a detailed model of B. anthracis-macrophage interactions. A major part of this work will be to characterize the modulation of both bacterial and macrophage gene expression as a result of B.

anthracis-macrophage interactions, using transcriptional profiling and proteome analyses. In Aim 2, we will investigate B. anthracis development in a mouse nasal installation model for anthrax, focusing on the pulmonary response. We will test B. anthracis mutants for attenuation of pathogenesis in the model. B. anthracis germination, survival, and persistence in the lung will be correlated with lung histopathology and immune response. We will track development of B. anthracis in the whole animal using chemiluminescence-based in vivo imaging technology. Using these assays, we will establish the spatial and temporal development of a fully virulent B. anthracis strain and isogenic mutants deleted for genes encoding therapeutic candidates. Our long-term objective is to generate new therapeutics to block interactions of B. anthracis spores with alveolar macrophages. The most powerful strategy will probably employ a cocktail of inhibitors targeting multiple steps in the infectious process. Bacterial and macrophage targets shown experimentally to be important for B. anthracis-macrophage interactions will be immediately forwarded to RCE core facilities for recombinant expression and crystallization for high-resolution structural analysis. The structural data will be used for structural-based identification of lead-inhibitor templates.

Casadevall, Arturo NBC
Abstract #2: *B cell related intervention*

We have continued our studies to identify the molecular determinants of antibody efficacy against B. anthracis toxins. Specifically we have identified a novel neutralizing epitope on the PA20 fragment of B. anthracis protective antigen (PA). The mechanism of antibody-mediated protection appears to be inhibition of the rate of proteolysis by cell proteases. In addition we have taken on several new research directions. First, we have generated a large panel of antibodies to the B. anthracis capsule, and some prolong survival in mice infected with anthrax spores. Second, we have developed a new way of radiolabeling anthrax toxins that promises to significantly enhance studies of toxin action, cellular localization, etc. The method of radiolabeling was used to study the fate of anthrax toxin components in vivo (mice) and provided evidence that they localized to liver and spleen. Third, we have made the first mAbs to B. anthracis anthrolysin. In the Staphylococcus enterotoxin B project Dr. Fries has succeeded in making a large set of mAbs that will be characterized for protective efficacy.

Hewlett, Erik L. MARCE
Abstract #3, Project 1.3: *Actions and interactions of the toxins of B. anthracis*

One strategy for treatment or prevention of anthrax is to understand better the pathogenesis at the cellular and molecular levels. Sub-project 1.3 was initiated for the purpose of investigating the actions of edema toxin (ET) and lethal toxin (LT) on target cells in vitro and in animals in vivo, with the objective of identification of potential interventions. The fact that inhalational anthrax has a high mortality, in spite of appropriate antibiotics, suggests that B. anthracis is capable of evading or subverting the host response. Although it has been postulated that infection with B. anthracis leads to host death by eliciting cytokine release, it is clearly a more complex process and it appears that there is failure of the host innate immune response to control infection during the early stage. Both ET and LT have been implicated in the causation of this outcome. We continue to evaluate the mechanisms and pathways by which B. anthracis is recognized by the host and the cellular and molecular effects of the toxins, ET and LT, on those responses. Specifically, we are studying the effects of LT and ET on murine macrophages, non-human primate macrophages, and primary human monocyte-derived macrophages in vitro. In addition, we are characterizing host innate

immune recognition and response to B. anthracis in vitro and in vivo. For the latter, we are testing the role of Toll-like receptor (TLR) signaling in mice deficient in select TLR-signaling components in the response to aerosolized spore challenge. Similarly, the pathogenesis of ET-induced edema formation and the role of ET-induced cAMP accumulation are completely unknown. We are addressing those questions using cultured cells and rabbits, into which we inject ET intradermally to produce edema. To date, our data indicate that edema does not result from a direct action of ET on the vascular endothelium or on mast cells, but rather some other type of cell that initiates a biphasic cascade of events resulting in inflammation and edema. Inhibitors of local edema and inflammation are being tested for their abilities to influence the course of the ET-elicited edema. In order to relate our data from human macrophages in vitro and rabbit data in vivo to what occurs during infection, we are testing effects of the toxins in vivo. In Sub-Project 1.3 Supplement, we soon will be performing a toxin challenge experiment in which the effects of intravenous LT or LT plus ET or PA alone will be compared. The cytokines and other indicators of the host response to the organism, as well as measurements of standard physiologic parameters, will be analyzed. It is our plan, depending on the results, to follow this toxin study with one in which we study the course and outcome of infection with virulent B. anthracis.

Collier, John R. NERCE
Project 1A: *Direct inhibition of anthrax toxin*

Besides exposing our general vulnerability to bioterrorism, the anthrax attacks of the fall of 2001 alerted the nation to the need to develop therapies and vaccines against Bacillus anthracis. B. anthracis produces two major virulence factors an antiphagocytic poly-D-glutamic acid capsule and a tripartite toxin. Injection of the purified toxin causes rapid death of sensitive rodents and non-human primates, and immunization against the toxin protects against infection. Thus it is generally believed that death from anthrax infections results from the effects of the toxin. As a step towards developing new therapeutic interventions for anthrax, we have used high-throughput screens to identify low molecular mass compounds that inhibit the action of anthrax toxin at the cellular level. We have identified a number of inhibitors of anthrax toxin action. Building on the experience of the Collier laboratory in studying anthrax toxin, we will characterize these promising inhibitors to determine the specific step in toxin action that each inhibits. Lead compounds with low toxicity will be considered for testing in animal models of toxin action.

The following three individuals (Lyons*, Brojatsch, and Jurgen) were not identified by eTSNAP as belonging to the original cluster, but were identified through a keyword search for “anthracis” within the collection of abstracts from projects of all RCE regions:
*Lyons identified by name in Xu abstract, included in cluster due to ties to UTSouthwestern

Lyons, C. Rick WRCE
(no abstract - name mentioned in Xu abstract)

Brojatsch, Jurgen NBC
Abstract #17: *Role of tissue-specific cell killing in anthrax toxin-mediated cell killing*

The long-term objective of the proposed project is to understand anthrax lethal toxin-mediated pathogenesis, which is caused by the Gram-positive Bacillus anthracis. The bacterium releases

lethal toxin (LT), which is sufficient to reproduce most anthrax associated pathology when injected into mice. LT kills select cell types in culture, and induces broad cytopathic effects in LT-injected mice. The aim of this study is to identify key target cells in anthrax pathogenesis. To determine the role of LT killing in this process, we analyzed cytopathic effects in murine strains that differ in their susceptibility to LT. Specifically, we used a murine strain (BALB/c) that is highly susceptible to the toxin, and a more resistant murine strain (C57BL/6). We found that LT triggers apoptosis in C57BL/6-derived antigen presenting cells, and necrotic death in BALB/c-derived murine cell types. We also found rapid depletion of antigen-presenting cells in LT-treated mice, which presumably leads to an impairment of their adaptive immunity. Activation of distinct cell death pathways by LT will be helpful in our attempt to identify primary LT targets in anthrax. Our findings will allow us to distinguish between cells directly targeted by the toxin, via induction of apoptosis, from cells killed indirectly, via hypoxia. Hypoxic cells will be identified using antibodies against hypoxic markers (Hif1) expressed on these cells. We are focusing our studies on the vasculature, as several physiological changes point to a major contribution of vascular damage in LT-mediated pathogenesis. Preliminary data of cross-sections of LT-injected C57BL/6 mice revealed TUNEL-positive cells, presumably endothelial cells, lining arteries in the liver of these mice. Studies of LT-injected mice will be performed in conjunction with in vitro assays using primary cells isolated from BALB/c and C57BL/6 mice. We found that LT directly triggered significant permeability changes across primary murine endothelial monolayers. The contribution of direct LT cell killing and permeability changes of the vasculature in LT-mediated disease progression will be analyzed. We also detected that LT induced necrotic cell death in BALB/c-derived cells leads to cytolysis and the release of specific cytokines, including IL-18 and IL-1. The effect of released cytokines on permeability changes of the endothelium and LT-mediated cytopathic effects will be analyzed.

Joachimiak, Andrzej GLRCE
Therapeutic inhibition of B. anthracis pathogenesis

GLRCE Research Project 1 scientists aim to develop anti-infective therapies that can be used for therapy of anthrax infections. To achieve this goal, the genetic determinants of B. anthracis that are required for the pathogenesis of anthrax disease are being established. Such determinants are then examined for their physiological properties and biochemical activities. If such activity can be determined and measured in vitro, the corresponding virulence genes can be considered targets of anti-infective therapy. Penultimate goal of this research is the identification of small-molecule inhibitors of virulence targets and exploration of the therapeutic properties of these compounds in animal models of anthrax disease. Thus, virulence targets will be pursued in this research program by screening NSRB small molecule libraries for inhibitory properties. These targets include (1) B. anthracis factors required for the secretion of protective antigen or other virulence factors (PrsC & EssC), (2) B. anthracis factors required for anchoring of poly-D-glutamic acid capsule to the cell wall envelope (CapD), (3) B. anthracis sortases, i.e. factors required for anchoring of surface proteins to the bacterial envelope (SrtA, SrtB & SrtC), (4) superoxide dismutases (SOD) and (5) factors involved in D-alanylation of lipoteichoic acid (Dlt). For example, high-throughput screening of NRSB compound libraries for inhibitors of sortases led to the identification of sortizins. X-ray crystallography structure determination of virulence targets with their inhibitors is used to achieve mechanistic appreciation of inhibitor function. Small molecule inhibitors are explored for their therapeutic values in animal models of anthrax disease.

The following two individuals (LeVine and Galn) were also identified via “anthracis” keyword search, but did not seem to relate as well to the theme of the overall cluster.

LeVine, Steven MRCE
Abstract #14, Project 16: *Pathogenic mechanisms of anthrax toxins*

Therapeutic interventions for the medical management of anthrax (Bacillus anthracis infection) are inadequate. Key to the pathogenesis of B. anthracis are the actions of protective antigen (PA) and lethal factor (LF) which act together to yield a lethal toxin (LeTx). We have shown that LeTx leads to disseminating intravascular coagulation (DIC) and the development of hemorrhagic/hypovolemic shock in mice. Studies were undertaken to attempt to improve the outcome following LeTx exposure in mice. Compounds that have been shown to ameliorate shock and/or DIC by other research groups were tested alone and/or in combination with one another in LeTx mice. The compounds inclued activated protein C, aprotinin, diluted plasma and/or heparin, dimaprit, N-acetylcysteine, or Ilomastat (GM6001). N,N'-diacetyl-L-cystine (a disulfide dimer of N-acetylcysteine) was also tested. The disease course in experimental LeTx mice was compared to that in LeTx mice given vehicle. No single compound or tested combination of compounds significantly improved the survival of LeTx mice compared to the control groups, although some compounds may have had a mild effect on delaying the time to death/moribund state following LeTx injection. Since treatments targeting late pathophysiological events such as DIC and hemorrhagic/hypovolemic shock did not substantially alter the disease course, an earlier pathogenic step was targeted. The binding of protective antigen to its receptors is pivotal to subsequent pathogenesis induced by lethal factor or edema factor. Since the crystal structure of protective antigen binding to one of its cellular receptors, CMG2, had been published (Santelli et al., Nature 2004; 430:905-908), we mapped the region of protective antigen that binds CMG2 and then performed a virtual search of the ChemNavigator Database that contains >5 million compounds. Six compounds were identified that had strong binding affinities, < -23 kcal/mole, and one of the six had a binding affinity of -35 kcal/mole. In addition, we have performed studies to try to investigate the role of heat shock proteins in vessel injury following LeTx exposure. Also, we and other researchers have found that CAST/Ei mice given LeTx have a shorter survival time compared to C57BL/6 mice given LeTx. Studies are currently ongoing to help determine whether or not LeTx CAST/Ei mice have a faster development of DIC and/or hemorrhagic/hypovolemic shock compared to that for LeTx C57BL/6 mice.

Galn, Jorge E. NBC
Abstract #7: *Salmonella type III secretion for bio-defense vaccines*

Avirulent strains of Salmonella typhimurium endowed with the ability to express cloned genes from other pathogens are being widely considered as platforms for the construction of polyvalent vaccines. We have recently developed a system that significantly improves the utility of Salmonella as an antigen delivery vehicle. This system is based on the use of a specialized protein secretion apparatus (termed type III) that is normally utilized by Salmonella to deliver effector bacterial proteins into the extracellular medium as well as into the cytosol of infected cells. We have adapted this system to deliver heterologous proteins into class I- and class-II antigen presenting compartments and found that antigens delivered by this system stimulate strong immune responses both in-vivo and in-vitro. It is the objective of our proposed research project to develop the type III secretion-based delivery system into a versatile platform capable of delivering antigens from pathogens that could be used in a bioterrorist attack to different compartments of the

antigen-presenting cellular machinery. More specifically we propose: 1) To construct avirulent strains of S. typhimurium capable of delivering through both of its type III secretion systems, protective antigens from Bacillus anthracis, Yersinia pestis and Burkolderia mallei; 2) To evaluate the ability of these vaccine strains to induce protective immune responses.

The following two individuals (Rose, Adams) were identified by eTSNAP as outlying members of the initial cluster but were removed due to a lack of correlation with the overall cluster theme.

Rose, John K. NBC
Abstract #10 Theme 3: *Optimizing VSV-based vaccine vectors*

Recombinant vesicular stomatitis viruses (VSVs) expressing appropriate foreign antigens are highly effective vaccines that protect against infection and disease in numerous viral challenge models. VSV vectors can be grown easily to very high titers in cell lines approved for vaccine production, and could be prepared quickly for use in emergencies. One goal of this project was to determine if the VSV platform was effective against the SARS coronavirus, a virus that has now been controlled, but could re-emerge. Protection was achieved after only a single vaccination of animals with a VSV recombinant expressing the SARS glycoprotein. Another goal of this project is development of optimized VSV-based vectors expressing antigens from bacterial agents such as Yersinia pestis that cause fatal human disease and could potentially be used as biological warfare agents. Although VSV vectors had not been used previously to express bacterial antigens, it was hypothesized that they would be an excellent vaccine platform for generating protective responses. Indeed, protection against challenge with Y. pestis has now been demonstrated in an animal model using a prime-boost combination of VSV-based recombinants expressing a single protein of Y. pestis. Future studies will be directed at further optimization of the VSV-based vector so that complete protection can be achieved with a single vaccination.

Adams, L. Gary WRCE
Development and evaluation of human brucellosis vaccines

Brucella melitensis is a Category B agent and an intracellular bacterial pathogen that subverts or avoids both innate and acquired immunity to cause a debilitating acute disease and to establish a chronic disease in man and animals alike. While moderately advanced diagnostics and vaccines exist for domestic livestock, antiquated diagnostics and no vaccines are licensed for human brucellosis. Currently available vaccine strains are virulent in humans that, along with their lack of genetic definition, make them unsuitable for human use. The short-term aim of the proposed studies is to classify mutations in these genes according to their effect on survival in the mouse model (i.e., short- vs. long-term survival). The long-range goal of our research program is to expand the fundamental knowledge base for improved disease prevention through safer, more effective vaccines for human brucellosis. Identification of specific virulence genes will be used to derive attenuated candidate strains for use as live vaccines. The ultimate goal of the proposed experiments is to perform the same experiments using Macaca mulatta nonhuman primates based on the predictive capabilities of the mouse model, and to estimate the safety and efficacy of these strains for human use. Our specific aims are: (1) To identify B. melitensis genes necessary for survival and virulence using the TraSH system for generating and screening mutants. Mariner transposon mutagenesis will be used to generate a bank of mutants that will be screened using the mouse model of infection to identify mutants that exhibit reduced survival and chronic persistence

in mice. Screening for survivability and persistence will enhance identification of genes that are important for persistence in humans. (2) To determine the safety and protection induced by B. melitensis vaccine candidates in the mouse model. Protection will be evaluated in this model to identify the optimal vaccine candidates for subsequent testing in nonhuman primates based on resistance to colonization of vaccinated animals to aerosol challenge with virulent B. melitensis. (3) To evaluate safety, protection and host gene expression in response to candidate live B. melitensis vaccines in nonhuman primates. The Macaca mulatta aerosol model for acute and chronic infection will be used to characterize pulmonary colonization, systemic dissemination and subsequent induced pathology. Protection will be evaluated based on resistance to pulmonary colonization, magnitude and burden of dissemination, and persistence of wild type after aerosol challenge with wild type B. melitensis 16M. Because of the threat posed by Brucella as a weapon, unavailability of vaccines for human use, and antibiotic therapies that may be unreliable, development of vaccines against human brucellosis is clearly the optimal approach for long-range protection of the public.

APPENDIX D
Results of Secondary Surveys

Note: The template for the secondary survey appears below, followed by the four completed interviews of Researchers A-D.

Secondary Survey for RCE Collaboration Project

Date:
Time:
Interviewee:

1. Do you *recall receiving any correspondence* regarding collaboration opportunities within the RCE on the subject of anthrax?

Yes No

2. Did you *take the time to read* the cover letter? Look over the map of the cluster? Read the abstracts of the other cluster members? Look over and/or complete the survey?

Cover Letter Map Abstracts Survey

3. Of the components that you did review, did anything *catch your interest* or compel you to keep reading? If so, what?

4. Did you *glean any useful information* from this initial packet of information sent to you? If so, what?

5. For what reason(s) did you *choose not to participate* in this project?

6. Do you feel *collaborative efforts have potential value* for your research efforts?

7. Is there anything you feel would *encourage or entice collaborative effort* from yourself or your colleagues?

Secondary Survey for RCE Collaboration Project

Date: November 13, 2006
Time: 10:00
Interviewee: Researcher A

1. Do you *recall receiving any correspondence* regarding collaboration opportunities within the RCE on the subject of anthrax?

Yes

2. Did you *take the time to read* the cover letter? Look over the map of the cluster? Read the abstracts of the other cluster members? Look over and/or complete the survey?

Yes, all.

3. Of the components that you did review, did anything *catch your interest* or compel you to keep reading? If so, what?

Researcher A found the concept intriguing and mentioned specifically that the computer models caught his attention.

4. Did you *glean any useful information* from this initial packet of information sent to you? If so, what?

Researcher A stated that although he knew quite a bit about the current topics and major researchers in the field of anthrax from conferences and presentations he had been to, he did mention that this compilation of abstracts was useful in keeping him informed of the research of individuals whose presentations he had not had a chance to attend or whose work he had not had a chance to otherwise review.

5. For what reason(s) did you *choose not to participate* in this project?

Researcher A mentioned that he had a five-day conference he was coming back from and had a lot to catch up on. He also states that he had "a bunch of grants due on the first" that took up most of his time. Researcher A thus indicated that his lack of participation was mostly a time issue and offered to respond to the initial inquiry at this time.

6. Do you feel *collaborative efforts have potential value* for your research efforts?

Yes.

7. Is there anything you feel would *encourage or entice collaborative effort* from yourself or your colleagues?

Researcher A cites competition between PIs and resulting reluctance to discuss works in progress as major barriers to open communication and collaboration. Even within his own RCE, Researcher A states these factors are present and severely limit the amount of collaboration that takes place. No suggestions for ways to encourage collaboration.

Secondary Survey for RCE Collaboration Project

Date: November 13, 2006
Time: 10:00
Interviewee: Researcher B

1. Do you *recall receiving any correspondence* regarding collaboration opportunities within the RCE on the subject of anthrax?

Yes

2. Did you *take the time to read* the cover letter? Look over the map of the cluster? Read the abstracts of the other cluster members? Look over and/or complete the survey?

Yes

3. Of the components that you did review, did anything *catch your interest* or compel you to keep reading? If so, what?

Although this question was not directly asked or answered, Researcher B did say that she was "not very impressed" and really didn't see any value to this project.

4. Did you *glean any useful information* from this initial packet of information sent to you? If so, what?

Researcher B stated that she has been "doing this for 20 years" and therefore knows pretty much everything that is going on at the moment with regards to anthrax research. She did not find any new or useful information and also thought that there were some individuals who she thought should have been included in this collaboration attempt.

5. For what reason(s) did you *choose not to participate* in this project?

Again, with her background, Researcher B feels that she already has a very good knowledge of current anthrax research and the individuals involved in this research. She also states that she already meets regularly with certain individuals within her own RCE to discuss anthrax research and that she does not see any need for collaboration beyond that which she currently participates in. Should she desire to collaborate with other individuals, she feels comfortable seeking out these individuals on her own and does not see the value of eTSNAP in seeking our potential collaborators (again, due to her status in the field of anthrax research).

6. Do you feel *collaborative efforts have potential value* for your research efforts?

Researcher B does not see any value of collaborative efforts such as was proposed in this project. She already collaborates to some degree with her own group of anthrax researchers and therefore must see some value to collaboration in general. She does state that our efforts may prove worthwhile for researchers new to a field of research and would help these newcomers to orient themselves to the other major researchers in their field.

(Continued)

7. Is there anything you feel would *encourage or entice collaborative effort* from yourself or your colleagues?

With regard to this question, Researcher B states that collaboration in general, irregardless of the medium through which it is conducted, requires a great deal of trust on the part of the participants, and that it is natural for scientists to want to protect their unpublished work, rather than share it with a large group of scientists whom they might not know very well. The NIH goal of collaborative enterprise conflicts severely with the competitive nature of research.

Secondary Survey for RCE Collaboration Project

Date: Nov. 17, 2006
Time: 11:20
Interviewee: Researcher C

1. Do you *recall receiving any correspondence* regarding collaboration opportunities within the RCE on the subject of anthrax?

Yes

2. Did you *take the time to read* the cover letter? Look over the map of the cluster? Read the abstracts of the other cluster members? Look over and/or complete the survey?

Cover Letter

3. Of the components that you did review, did anything *catch your interest* or compel you to keep reading? If so, what?

(did not answer)

4. Did you *glean any useful information* from this initial packet of information sent to you? If so, what?

(did not answer)

5. For what reason(s) did you *choose not to participate* in this project?

Too busy. Everybody is very busy, and so anything requiring his time will have to be very good and worthwhile. Knows many in the field. Knows all in his regions, but not really familiar with all RCE investigators outside his region.

6. Do you feel *collaborative efforts have potential value* for your research efforts?

Yes, definitely. It is most valuable in two situations, when there is a side issue with regard to their primary research focus or when something new that comes up to the field. In both cases these involve needing to find out about "resources" that can be both stuff for the lab or a person who has specialized knowledge or capabilities.

7. Is there anything you feel would *encourage or entice collaborative effort* from yourself or your colleagues?

(did not answer)

Secondary Survey for RCE Collaboration Project

Date: Nov. 21, 2006
Time: 8:13
Interviewee: Researcher D

1. Do you *recall receiving any correspondence* regarding collaboration opportunities within the RCE on the subject of anthrax?

Yes

2. Did you *take the time to read* the cover letter? Look over the map of the cluster? Read the abstracts of the other cluster members? Look over and/or complete the survey?

Did not really read it.

3. Of the components that you did review, did anything *catch your interest* or compel you to keep reading? If so, what?

He repeatedly mentioned that he would be happy to collaborate on a variety of topics, especially vaccine development for Anthrax or Yersenia Pestis (Plague).

4. Did you *glean any useful information* from this initial packet of information sent to you? If so, what?

(did not answer)

5. For what reason(s) did you *choose not to participate* in this project?

(did not answer)

6. Do you feel *collaborative efforts have potential value* for your research efforts?

Yes, and very much would like to do so. For example, he has a vaccine for anthrax that in his region in mouse trials outcompeted the rest, and would like to move that research forward with tests in non-human primates. Any help, including collaborations, that would help that experiment would be welcome. Indicated that more than collaboration tools will be needed, including altering how cores are supported and prioritization of experiments to move good leads towards an effective vaccine. This will probably require coordination between PIs of regions and the NIH towards a more goal driven project from the more independent research organization now.

7. Is there anything you feel would *encourage or entice collaborative effort* from yourself or your colleagues?

Has no specific suggestions, but feels that there needs to be more over-reaching nationally coordinated focused efforts to translate results to effective clinical vaccines.

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VITAE

Courtney Joy Moser, daughter of Rev. Candace Moser and Dr. Charles Moser, earned her high school diploma in 1999 from Mounds Park Academy, located in her hometown of Saint Paul, Minnesota. Abandoning her Midwest roots, she relocated to Houston, Texas where she attended Rice University, graduating Summa Cum Laude in 2003 with a B.A. in kinesiology and studio art, concentrating in exercise physiology and sculpture, respectively. Concurrent with her enrollment at Rice, she spent a year at San Jacinto Community College, completing requirements for paramedic licensure, which she acquired in August of 2002. In 2004, having spent a year after graduation working as a paramedic, she entered the Biomedical Communications graduate program at the University of Texas Southwestern Medical Center at Dallas. She received her degree of Master of Arts in Biomedical Communications in May of 2007 and is currently preparing to attend veterinary school.

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