Innate (hard-wired) and Adaptive (programmable) Immune Responses: Lessons from Listeriosis and Other Infections

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1998: The centennial of the earliest description of innate immunity ("resisting power")?

"...scattered about...were the Martians - dead! - slain by the ... disease bacteria against which their systems were unprepared;... by the humblest things that God, in his wisdom, has put upon this earth....These germs of disease have taken toll of humanity since the beginning of things - taken toll of our prehuman ancestors since life began here. But by virtue of this natural selection of our kind we have developed *resisting power*; to no germs do we succumb without a struggle, ...By the toll of a billion deaths man has bought his birthright of the earth, and it is his against all comers;..."

— H. G. Wells. The War of the Worlds. 1898.

A growing body of data indicates that host defense against infections consists of innate and adaptive immune responses. In this grand rounds, I hope to accomplish the following:

1) Introduce the concepts of innate and adaptive immunity;

2) Discuss listeriosis as a model infection with an emphasis on clinical aspects; and

3) Compare and contrast the roles of innate and adaptive immunity in the host response against listeria. Our major focus will be on recent advances in our understanding of innate immunity.

Innate and adaptive immunity: an introduction.

After infection, each of us is protected by an ancient immune system that is an inheritance of our evolutionary experience as a species. This is called "innate" or "natural" immunity (1,2). We are also protected by "adaptive" or "acquired" immunity. This is the immunity generally discussed in textbooks. It is antigen-specific and mediated by antibodies and T-cell receptors. In contrast to the exquisitive specificity of the antibodies and T-cell receptors involved in adaptive immunity, innate immunity recognizes general patterns of pathogenic molecules (3) and tissue injury caused by pathogens (4). The pattern-recognizing receptors of the innate immune response are shared by all individuals in a species. In contrast, the specific antibodies and T cell receptors of the adaptive immune response are the product of an individual's experience. Since each of us has a different live-experience, each of us have different antibodies and T cell receptors. Innate immunity is our first line of defense. It controls infection during the days to weeks necessary for adaptive immunity to develop. It determines if adaptive immunity occurs and also determines the nature of the adaptive immune response. Although innate immunity is potent, it cannot completely sterilize infected tissue. That is a function of adaptive immunity. Furthermore, innate immunity has no memory; a second encounter with the same infection will not provoke a more efficient response. For a given individual, innate immunity is "hard wired." In contrast, adaptive immunity is "adaptive," and a second encounter with a pathogen will elicit a "memory" response. For a given individual, adaptive immunity is "programmable." Perhaps the most widely known component of innate

immunity is the alternative pathway of complement. The counterpart in adaptive immunity is the classical pathway of complement that has a requirement for antigen-specific antibodies. The essential features of innate and adaptive immunity are given in Figure 1.

	Innate Immunity	Adaptive Immunity
Onset	Rapid response (seconds to days)	Days to weeks
Recognition	Pattern recognition of pathogens Recognition of tissue injury	Specific antigens via classical antibodies and classical alpha/beta T cell receptors
	Reflects evolutionary experience of species	Reflects individual experience
Functions	First line of host defense	Sterilization
	Determines if adaptive immunity will occur	Immunologic memory
	Determines what type of adaptive immunity will occur	

Figure 1

Adaptive immunity is the ultimate evolutionary accomplishment of vertebrate immunity. Within a single lifetime, from a limited amount of genomic material, and using elegant processes of recombining segments of genes, randomly adding or deleting nucleotides at the recombination sites, adaptive immunity produces trillions of clones of B and T cells, each with a unique antigen receptor. The very diversity of these receptors ensures that most microbes will have some antigen which will be immunologically recognized. The generation of these diverse immunologic receptors occurs by stochastic genetic events and encounters with microbes within the lifetime of a single individual. Adaptive immunity is thus "programmed" during a single lifetime, and is uncoupled from the "wisdom" gained by the species during encounters with pathogens during evolution. "With no information from the past, these receptors [of adaptive immunity] can evaluate only antigenic structure and not its biological correlates" (5).

Innate immunity, on the other hand, is "hard-wired" by genes which exist only in their germline configuration. There are no recombinations, deletions, additions, or mutations within an individual's lifetime. The gene products of innate immunity are determined by evolution - the

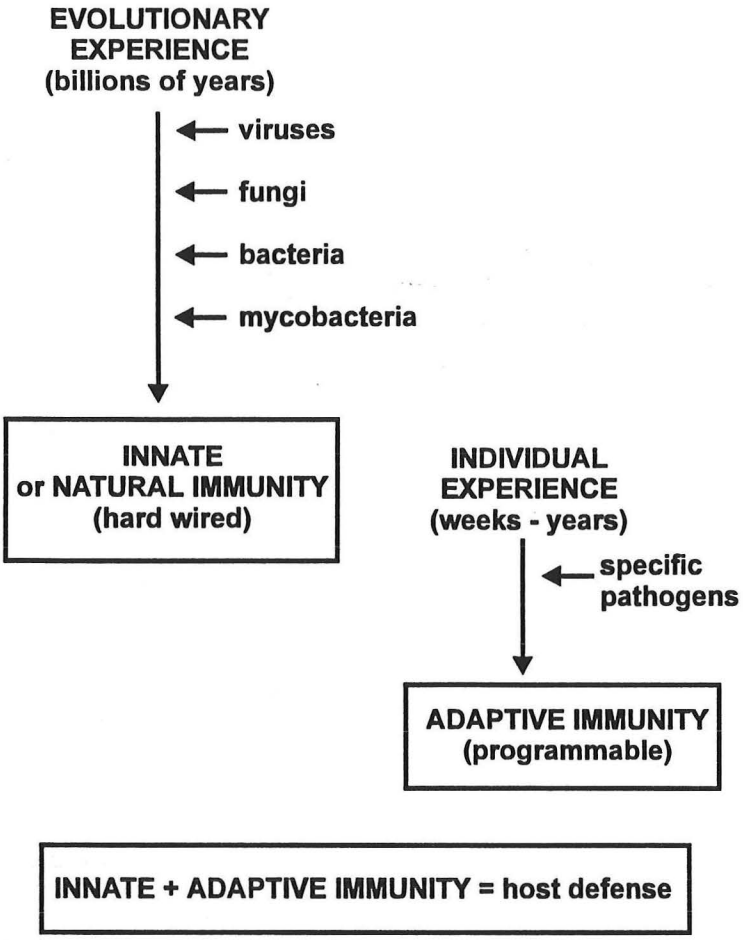

Figure 2

previous encounters of our species with viruses, fungi, and bacteria. These gene products recognize the patterns common to a class of infectious pathogen (1,2). For example, the endotoxin receptor recognizes the basic lipid A structure of endotoxin and thus all gram negative bacteria. In addition, innate immunity recognizes tissue injury which occurs during the course of an infection (4,6). See Figure 2. Innate immunity gives biological meaning to the antigens recognized by antibodies and T-cells of adaptive immunity.

I believe the first description of innate immunity was in 1898 by H.G. Wells in his book, War of the Worlds. His description is quoted at the beginning of this paper. In that science fiction book, Martians landed on earth and conquered man. But the Martians had not evolved on earth and had no innate immunity against our indigenous bacteria. They died, while humans, with our innate immunity against these bacteria, survived.

I will focus our analysis of innate and adaptive immunity by studying their function during listeriosis. We will begin by reviewing clinical listeriosis, then we will discuss the biology of the bacteria and its virulence genes, and finally we will analyze the innate and adaptive responses to listeria.

Clinical listeriosis:

I will discuss this area briefly so I can concentrate on the immunology of listeriosis. My main clinical focus will be on new data on the importance of food in transmitting listeria. There is an excellent clinical review by Dr. Justin Radolf in these Grand Rounds in 1992. In addition, there are number of recent published clinical reviews (7-11).

Listeriosis is caused by listeria monocytogenes; the other species of listeria are not pathogenic for humans. Listeriosis is an uncommon disease with a case rate of 4.4 per million.

However, it is important because it is fatal in 30% of patients. Early recognition and institution of antibiotic therapy is critical. In addition, listeriosis often results from contaminated food and early recognition, and destruction of the food will prevent or control an epidemic.

Listeria monocytogenes is widely distributed in the environment. It is associated with farm animals such as sheep, cattle, chickens, and turkeys. Fecal carriage is common. The bacteria contaminates manure, feed, and silage. It causes septic abortion of sheep and cattle, and also causes basilar meningoencephalitis ("circling disease") in these animals. Listeria is also found in soil, dust, water, and sewage. It may contaminate food and may thus cause human epidemics. Such foods include milk, soft-cheeses, deli-meats, and seafood. Listeria grows at refrigerator temperatures. The bacteria is a temporary component of the stool flora in 5% of normal people. Invasive listeriosis is uncommon and depends on virulence and the size of the innoculum, and the host immune status.

There are several clinical syndromes of listeriosis. 30% of cases are pregnant women who suffer a self-limited "flu-like" illness. However, 21% of the pregnancies result in fetal loss. In some cases a severely ill neonate is born. 2-50% of these neonates will die despite appropriate antibiotics and supportive treatment. There are two syndromes of neonatal listeriosis. One is an early syndrome of overwhelming sepsis, "granulomatosis infantiseptica", characterized by papular cutaneous lesions and mucosal nodules. The other is a syndrome of meningitis and sepsis which is similar to the adult syndrome discussed below. This late syndrome occurs approximately one week after birth and may result from passage of the neonate through a contaminated birth canal. Adult listeriosis occurs in the elderly or immunosuppressed and is characterized by CNS infections. The immunosuppressed adults include patients with AIDS (12,13) where the case rate is 96 - 280 x normal population, but comparatively less than other AIDS associated infections such as PCP, etc. One explanation for the low incidence in AIDS patients may be the sensitivity of listeria to Bactrim and the use of Bactrim prophylaxis.

Listeria meningitis has features which distinguish it from other bacterial meningitis. Although the presentation is usually acute, it may be subacute and may mimic tuberculous meningitis. Nuchal rigidity is not present in 15%-20% of adults. Movement disorders such as ataxia, tremors, and myoclonus are more common (15%-20% of patients); seizures are more common (at least 25% of patients). Fluctuating mental status is common; blood cultures are more likely to be positive (75%) of cases). CSF findings include: a negative gram stain in most cases (organisms are seen in 40%); the glucose level is not low in most cases (levels are normal in 60% of cases); mononuclear cells predominate in about one-third of cases (10).

More than other bacteria, Listeria tends to infect the brain parenchyma in addition to the meninges. A unique presentation is Listeria rhombencephalitis which occurs in 5-10% of listerial CNS infections, and typically has two phases: A prodrome of headache, fever, nausea is followed by a bulbar phase which consists of cranial nerve abnormalities including ptosis, diplopia, dysarthria, dysphagia. In addition the patients may develop hemiparesis or quadraparesis. (9)

Listeria is a foodborne pathogen. See Figure 3. The epidemic of 1981 in Nova Scotia is of particular importance because it was the first well-documented epidemic with a foodborne

source (14). Thirty-four 34 cases of listeriosis were seen in the Maritime provinces between March and September of 1981. Twenty-seven of the 34 cases were pregnant women. They had an acute febrile illness followed by spontaneous abortion (5 cases), stillbirth (4 cases), birth of a seriously ill premature or term infant (23 cases), or birth of a well infant (2 cases). Despite aggressive antibiotics and supportive care, 27% of the infants who were born alive later died. Six of the non-pregnant patients had meningitis, and 33% of these patients died. One of the non-pregnant patients had had a previous CVA and developed aspiration pneumonia and sepsis. This patient was the key to solving the cause of the epidemic. Listeria as a cause of aspiration pneumonia is unusual and immediately attracted the attention of the CDC epidemiologists. Listeria was found in coleslaw in this patient's refrigerator and had been aspirated by the patient. All patients in the epidemic had eaten coleslaw from the same distributor. Cabbage for the coleslaw was grown on a farm where sheep from the farmer's flock had died from listeriosis one and two years prior to the human epidemic. The farmer had used manure from these sheep to fertilize the cabbage used for making the coleslaw. This is how listeria came to be ingested by the patients.

MAJOR FOODBORNE OUTBREAKS OF LISTERIOSIS IN THE U.S.

Date	Place	Cases (deaths)	Implicated vehicle	Reference
1981	Nova Scotia, Canada	41 (18)	Coleslaw	NEJM 308:203 `83
1983	Massachusetts	49 (14)	Pasteurized milk	NEJM 312:404 `85
1985	Los Angeles	142 (48)	Soft Mexican-style cheese	NEJM 319:823 `88
1994	Illinois	11 (0)	Chocolate milk	NEJM 336:100 `97

Figure 3

After this report, other well-documented foodborne epidemics were also described (15,16). See Figure 3. These prompted federal regulations for preventing and detecting listeria contamination; these were instituted in the late 1980's. Although these measures have decreased the incidence of listeriosis, the disease continues to occur. Unpasteurized dairy products are still sold in California, and unpasteurized juices and other foods are sought by those wishing to consume "natural" or "organic" foods (7). An epidemic of listeria gastroenteritis due to

contaminated chocolate milk occurred in 1994 (17), and 5 cases occurred at Parkland during the last 18 months.

Patients who have AIDS, receive immunosuppressive drugs, are elderly or are pregnant should follow the dietary suggestions of the CDC and FDA (10,18). "For all persons: Thoroughly cook raw food from animal sources, thoroughly wash raw vegetables before eating, keep uncooked meats separate from vegetables, cooked foods, and ready-to-eat foods, avoid consumption of raw (unpasteurized) milk or foods made from raw milk, wash hands, knives, and cutting boards after handling uncooked foods. Additional recommendations for persons at high risk for listeriosis (those immunocompromised by illness or medications, pregnant women, and the elderly): Avoid soft chesses including Mexican-style, feta, Brie, Camembert, and blue-veined cheeses (there is no need to avoid hard cheeses, cream cheese, cottage cheese, or yogurt), reheat leftover foods or ready-to-eat foods (eg: hot dogs) until steaming hot before eating, and although the risk for listeriosis associated with foods from delicatessen counters is relatively low, pregnant women and immunosuppressed persons may choose to avoid these foods or to thoroughly reheat cold cuts before eating."

To summarize, these are the clinical settings in which listeriosis should be considered: "Neonatal sepsis or meningitis; meningitis or parenchymal brain infection in patients with hematological malignancies, AIDS, organ transplants, or corticosteroid immunosuppression; meningitis or parenchymal brain infection in adults over 50 years of age; simultaneous infection of the meninges and brain parenchyma; subcortical brain abscess; fever during pregnancy, particularly in the third trimester; presence of "diptheroids" on gram stain or culture of blood, CSF, or other normally sterile specimens; foodborne outbreak of febrile gastroenteritis in which routine cultures fail to yield a pathogen." (10).

The therapy of listeriosis is ampicillin and gentamycin. Penicillin-allergic individuals may benefit from co-trimoxazole. Current cephalosporins should not be used. A prolonged course of therapy may be necessary, particularly in patients with CNS infections. These issues are discussed in a recent comprehensive review of anti-microbial therapy for listeriosis (19). Despite the sensitivity of listeria to these and other antibiotics in vitro, there is a high failure rate of anti-microbial therapy. This indicates that antibiotics are unable to overcome the deficits of innate and adaptive immunity against listeria. We will now discuss these responses in more detail, but first a review of the genes responsible for the virulence of listeria. We will see that the products of these genes have a major impact on immunity against listeria.

Virulence genes of listeria monocytogenes.

Two genes are responsible for the entry of Listeria into host epithelial cells, endothelial cells, and fibroblasts. These are Internalins A and B (20). The former binds to E-cadherin on

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[1]Note that phagocytosis by macrophages and polymorphonuclear cells does not require these specialized bacterial structures, but is mediated by receptors on the phagocyte cell surface for bacterial carbohydrates; anti-listerial antibodies; and collectins, pentactins, and complement components deposited on the bacteria (1).

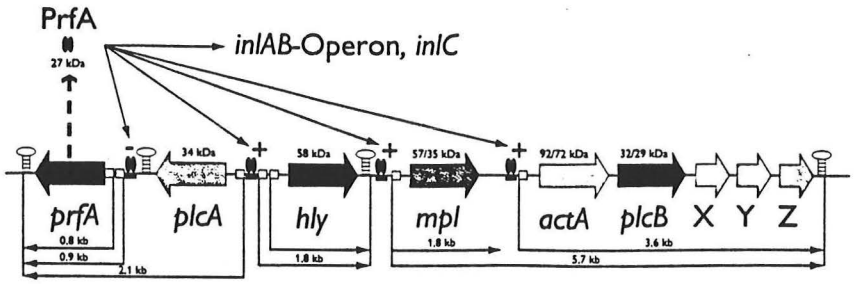

Figure 4

surface of epithelial cells and promotes endocytosis. E-cadherin is on the basolateral surface of epithelial cell. Therefore initial interaction of listeria is probably via M cell. Internalin B is necessary for entry of listeria into hepatocytes. The activation of the internalin genes is regulated by prfA (20-23).[1]

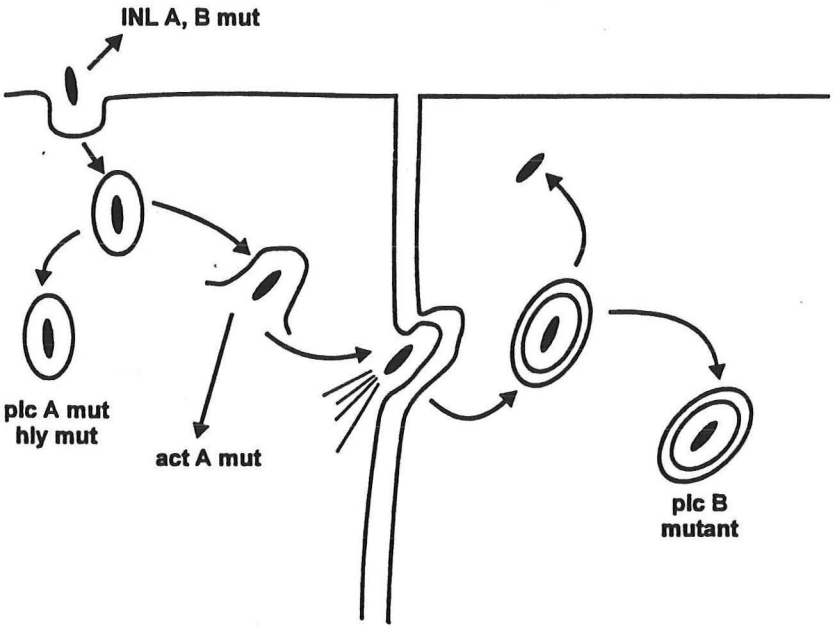

Figure 5

As shown in Figure 4 and Figure 5, other genes are responsible for the virulence of Listeria after the bacteria have been ingested (20,24). These genes are regulated by the transcription factor prfA. PrfA is produced when the bacteria finds itself in the stressful environment of the phagolysosome, and activates the genes hly, plcA, actA, and plcB. The gene products for hly and plcA are listeriolysin O and a phosphotidyl-inositol-specific phospholipase C. Together these enzymes lyse the phagolysosome and free the listeria to enter the cytoplasm (25). The listeria then produce the actA gene product (26) that reorganizes the host cells actin cytoskeleton such that the listeria is propelled to the cell surface and pushed into a neighboring cell. The listeria then finds itself within a double-membrane vacuole. These membranes are lysed by the plcB gene product, a non-specific phospholipase C (25). Transposon mutagenesis of each of the above specific genes has identified their role in bacterial virulence.

Innate and adaptive immunity to listeria monocytogenes.

As shown in Figure 6, the host is ordinarily protected from listeria by the innate and adaptive immune responses. The innate response is activated both by the stress induced by the activities of virulent listeria within infected cells and by pattern recognition of listerial products. Such activation controls infection until adaptive immunity can be activated.

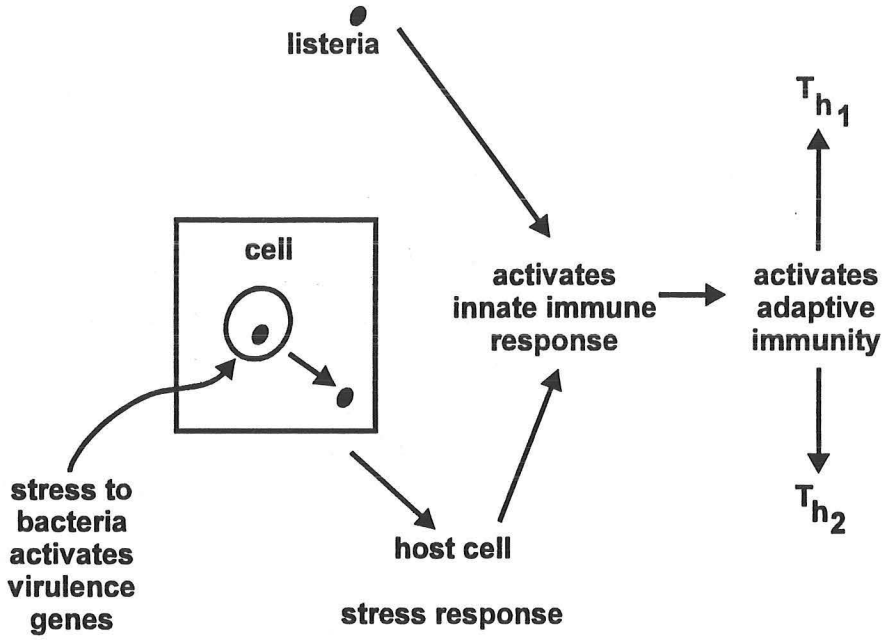

Figure 6

Activation of innate immunity by injury occurring during infection.

An important unexpected finding occurred when the ability of virulent versus avirulent to induce protective immunity was compared (27). Listeria with a mutated, nonfunctional hly gene were not virulent, and also did not induce protective immunity. I believe that this occurs because the innate immune response recognizes tissue injury and is stimulated only after infection by virulent listeria. See Figure 7.

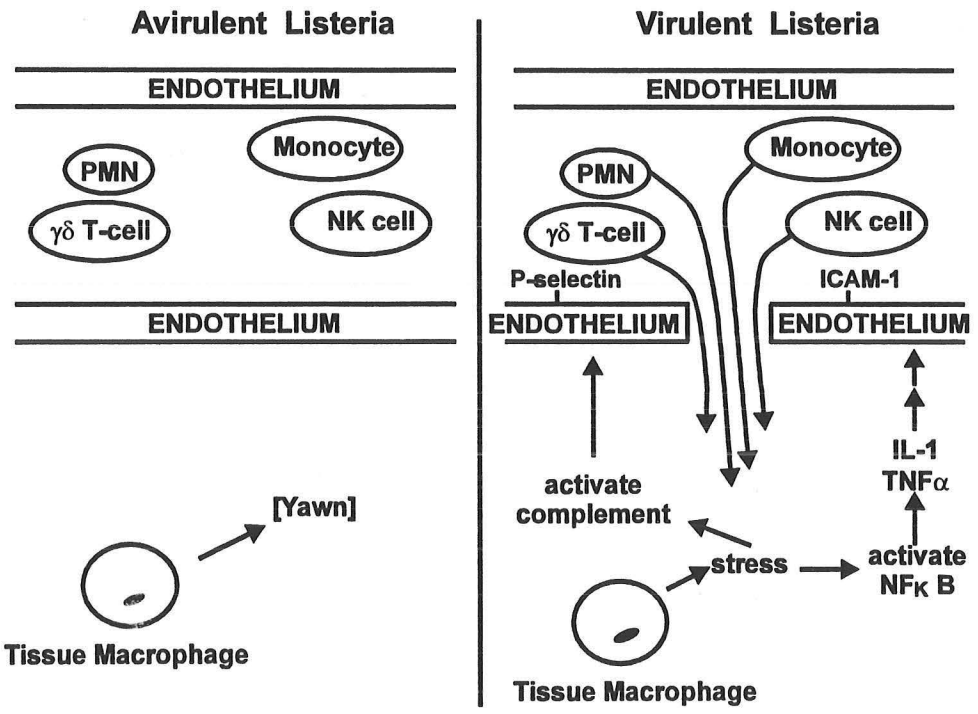

Figure 7

The presence of listeria in the cytoplasm of macrophages injures these cells (28). It induces the production of the stress (or heat-shock genes) hsp70 and hsp90 (29). Infection also activates the

transcription factor NFκ B (30). This is an extremely important event because this transcription factor participates in the activation of proinflammatory genes including interleukin 1, and TNFα (31-34). These transcription factors would, in turn, activate endothelium and cause the immigration of inflammatory cells into the site of infection and thus facilitate activation of innate immunity (35,36). Only virulent strains of listeria cause prolonged activation of NFκ B, only such strains injure the host cell, and only such strains cause the infected cells to release pro-inflammatory cytokines (28,37). The importance of NFκ B is confirmed by the increased susceptibility of mice with transgenic knockout of the p50 (NFκ B1) component of the NFκ B heterodimer (38).

The importance of injury is further demonstrated by the inability of avirulent listeria to induce the production of pro-inflammatory cytokines in the spleen and liver (39,40).

Included in the genes activated by virulent listeria are GM CSF and G CSF (41). The importance of these genes is the increased susceptibility of mice with transgenic knockout of these genes to listeriosis (42). These cytokines illustrate the communication between the site of infection and the bone marrow. GM CSF and G CSF produced at the site of infection are released into the blood, cause the generation of monocytes and neutrophils by the bone marrow. This causes the monocytosis and neutrophilia seen with infection, and allows the infiltration of large numbers of leukocytes into the site of infection.

In addition, cellular stress will also activate the alternative pathway of complement. This is best described after myocardial ischemia, but is also likely to occur in cells injured by any process, including intracellular infections. Ordinarily, there is slow activation of the alternative pathway via "C3 tickover". C3 has an unstable thioester such that a small amount of C3 is continuously activated to C3b. This C3b binds to repetitive hydroxyl and amine groups on cell surfaces. The cell-surface C3b forms a C3 convertase by binding factors B, D, and properdin (P). This C3 convertase activates more C3 in a positive amplification loop, activates C5 to form C5a anapylatoxin, which recruits and activates inflammatory cells, and initiates construction of the membrane attack complex (C5-9) which lyses cells. C3 tickover results in C3b deposition on the surfaces of both host cells and bacteria. Damage to host cells via C3 tickover and the alternative pathway is ordinarily prevented by inhibitory proteins on cell surfaces. These inhibitors include CD46 (membrane cofactor protein - MCP, CD55 (decay accelerating factor DAF), and CD59 (membrane inhibitor of reactive lysis - MIRL) (43). However, injury increases intracellular calcium which activates a phosphosphatidylinositol-specific phospholipase C. This enzyme cleaves the cell-surface complement inhibitory proteins; as a result the uninhibited alternative pathway produces C3a, and C5a that activates endothelia and recruit an inflammatory infiltrate. The C5-9 membrane attack complex is also produced, and this stimulates other cells to release interleukin 8 and platelet activating factor (PAF) which are chemotactic and activate endothelia (44).

Altogether the release of cytokines by cells infected by listeria and the activation of complement cause all four of the steps necessary for infiltration of leukocytes from the capillaries into the interstitial site of infection to occur (35,36,45). First, adhesion molecules appear locally on endothelium which has been activated by signals from nearby injured interstitial cells. Second, leukocytes adhere by weak, reversible interactions to P and E selectins,

VCAM-1, and hyaluronate on the surfaces of activated endothelium. Third, during this weak adherence, the leukocytes themselves receive activation signals, including chemokines, which change the conformation of their cell-surface beta-2 integrins so that these bind their counterligands on the endothelium. The beta-2 integrins on leukocyte cell surfaces are LFA-1, Mac-1, and VLA-4; they bind to the following counterligands on the endothelial surface: ICAM-1 and -2, and VCAM-1; the signals activating the leukocytes may include chemokines such as IL 8 and MCP-1. Fourth, the leukocyte moves across the endothelium (diapedesis).

Activation of innate immunity by pattern recognition of pathogenic molecules produced by infectious agents.

The recognition structures used by innate immunity evolved under the selective pressures imposed by infectious pathogens. On the one hand, infectious pathogens have an enormous variability and a high mutation rate. On the other hand, prior to the evolution of the somatic mechanisms used to generate the diversity of antibodies and T cell receptors, there was an upper limit on the number of receptor molecules which could be code by the germline genes of innate immunity. These problems were solved by receptors which recognized invariant structures common to large groups of pathogens. These are the so-called pathogen-associated molecular patterns. These receptors recognize molecules shared by large groups of pathogens. These molecules are essential for the survival or pathogenicity of the pathogens. Examples include lipid A which is a vital component of all gram negative bacteria, double stranded RNA which is a component of many RNA viruses, mycolic acid and lipoarabinomannas which are components of mycobacteria, and mannans which are components of fungi. An additional characteristic of these molecules is that they are not components of mammals, and therefore are distinct from self antigens (1,3). We will now discuss several of the recognition systems used by innate immunity to recognize these pathogen-associated molecular patterns.

Activation of complement, a component of the innate response, by listeria.

Complement activation, in the absence of listeria-specific antibody, is an important component of innate immunity. Not only is complement activated by injury caused by virulent listeria, the alternative pathway is also activated directly by the bacteria. As noted above C3, is constantly activated by C3 tickover, and this activation does not damage host cells because they have cell-surface inhibitory proteins. Since bacteria do not have these inhibitory proteins, they cannot prevent the activation of complement by the alternate pathway and are destroyed; in addition complement activation recruits an inflammatory infiltrate (46,47).

Listeria also causes complement to be activated by acute phase reactants. This is an effect which indirectly requires interleukin 6. At the site of infection, Listeria causes macrophages and other cells to produce interleukin 6. The importance of this cytokine is emphasized by the increased susceptibility of mice with transgenic knockout of this gene (48). Interleukin 6 has multiple effects during the host defense against listeria (49). One important effect of interleukin 6 is its ability to cause the liver to produce acute phase reactants. Two of these, C reactive protein and mannose-binding protein, are important for innate immunity. C reactive protein is a membrane of the pentraxin family; mannose-binding protein is a member of the collectin family.

INTRACELLULAR MOLECULES AND INNATE IMMUNITY

Antigen	Monomorphic MHC	Lymphocyte
mycolic acid lipoarabinomannan [M. tb and M. Leprae]	CD1	γδ T-cell
phosphorylated non-peptide [Mycobacteria]	?	γδ T-cell
bacterial hemolysins	?	γδ T-cell
listerial molecules	?	NK CD4 αB T-cell
f-met-leu-phe (found in procaryotes)	H 2 M 3	CD8 T-cell
bacterial hemolysin	Qa-1 (HLA-E)	CD8 T-cell
lysterial molecules	?	Natural killer cells

Figure 8

These proteins bind to bacteria. They are opsons. In addition they activate complement via C1q (1,50).

Listeria activates cellular components of innate immunity: natural killer (NK) cells and unconventional T cells (NK CD4 T cells with αß receptors and γδ T cells).

Lymphocytes of innate immunity recognize molecular patterns produced by intracellular pathogens such as listeria and M. tuberculosis. Mechanisms are required to shuttle these molecules from within the infected cell to the cell surface where they might be recognized by these lymphocytes. See Figure 8. We will first discuss these cells and the regulatory cytokines they produce. In the next section, we will discuss the mechanisms which shuttle the pathogenic molecules to the cell surface.

As shown in Figure 9, NK cells, and γδ T cells play a critical role in inducing an early defense against listeria. The role of NK cells has been best defined in mice with severe combined immunodeficiency, where these cells may be studied in the absence of T cells, and B cells (51). After ingesting listeria, macrophages produce interleukin 12, TNFα, and interleukin 1 beta. These activate NK cells which produce interferon gamma. This activates macrophages so that they can kill listeria and increase their expression of Class II MHC. The latter allow increased presentation of listerial antigen to CD4+ T cells. The importance of NK cells in conventional mice was demonstrated by the increased early mortality in animals receiving anti-sialo-GM-1, an antibody which eliminates NK cells (52). The molecules recognized by NK cells during listeria

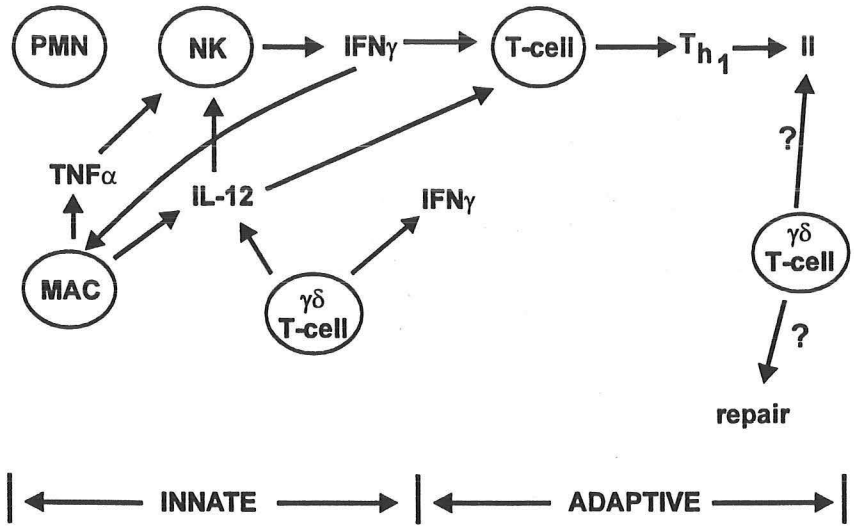

Figure 9

infections is unknown. The importance of interferon gamma, TNFα, interleukin 1, and interleukin 12 in the innate response to listeria is supported by the increased mortality in mice where these cytokines have been inactivated by transgenic knockout or injection of monoclonal antibodies (53-55).

Early in the course of listeriosis, there are unconventional lymphocytes with cell surface markers common to both NK cells and CD4 cells. These lymphocytes have αß T cell receptors. The molecules recognized by these lymphocytes is unknown. However, the cells are important in facilitating the production of chemokines which attract macrophages to the sites of infection (56).

Gamma delta T cells are also important during the innate response against Listeria. Deletion of γδ T cells using monoclonal antibodies or transgenic knockout results in increased susceptibility of mice to listeria (57-59). The γδ T cells secrete interferon gamma and other cytokines which activate macrophages, NK cells, and conventional T cells (60). Increased numbers of γδ T cells are seen in the peripheral blood of patients with listeriosis (61-63). Other γδ T cells act late during infection. These may inhibit the immune response or repair tissues damaged by the infection and the resultant immune response (64).

Gamma delta T cells have antigen receptors which are similar in structure to conventional T cells with αß receptors. The genes for these receptors are formed from the germline sequences by recombination of the various V genes to J, D, and C regions. Some classes of γδ T cells are striking for the homogeneity of the V,J,D, and C regions used and the absence of junctional diversity through the addition or subtraction of nucleotides at the joining regions (65). In other words, these are invariant receptors which might recognize a pattern of pathogenic molecules, much like other receptors in the innate immune response. In humans, γδ T cells with invariant Vγ2 Vδ2 chains recognize phosphorylated nonpeptide molecules produced by mycobacteria (66). Gamma delta T cells with invariant receptors also occur after human listeriosis and may recognize common regions of bacterial hemolysins (63).

Non-classical MHC present invariant pathogenic molecules to unconventional T cells.

MHC molecules evolved to facilitate the immune response against intracellular pathogens such as Listeria. Class II MHC shuttle pathogenic molecules from the phagolysosome

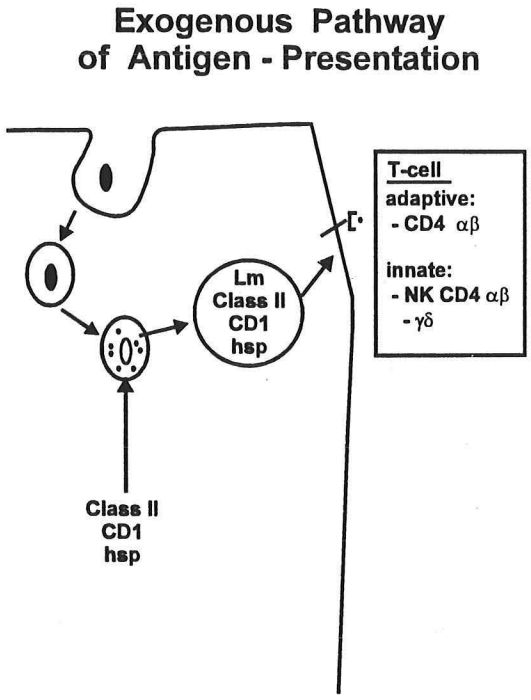

Figure 10

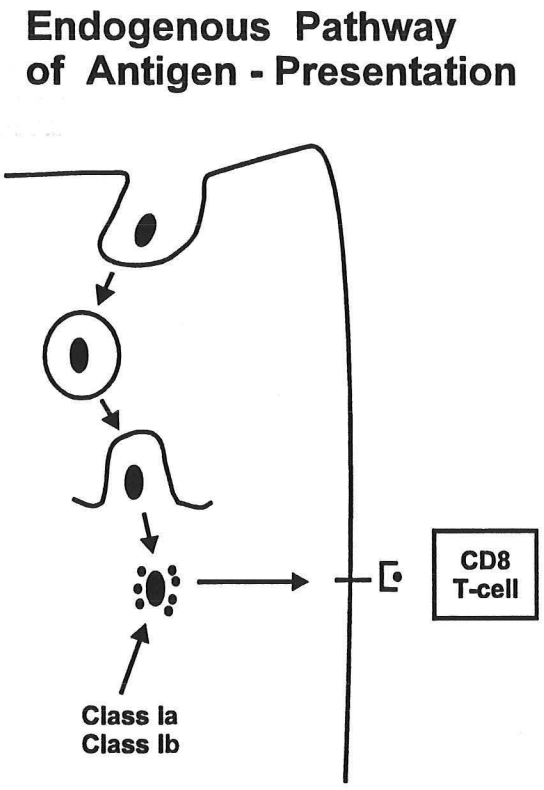

Figure 11

to the cell surface where they stimulate CD4 T cells. This is the "exogenous" pathway, Figure 10, which evolved to handle pathogens such as M. tuberculosis or Listeria which can reside in the cytoplasm. Class I MHC shuttle pathogenic molecules from the cytoplasm to the cell surface where they stimulate CD8 T cells. This is the "endogenous" pathway and evolved to defend the host against pathogens such as viruses or Listeria which reside in the cytoplasm, Figure 11. Conventional Class I and Class II MHC stimulate conventional CD8 and CD4 αß T cells, respectively. The pathogenic molecules are peptides. These systems are part of adaptive immunity.

Recent data indicates that innate immunity uses similar shuttle molecules. See Figure 12. Those that transport pathogenic molecules from the phagolysosome are unconventional Class II MHC. These are the MHC coded by the 5 gene loci of the CD1 complex. Those that transport pathogenic molecules from the cytoplasm are Class Ib MHC, as opposed to the conventional Class Ia MHC.

Consistent with the idea that innate immunity recognizes patterns of pathogenic molecules, these non-classical MHC present these molecules to unconventional T cells with invariant receptors. For example, CD1 is a non-classical MHC molecule which binds to mycolic acid and lipoarabinomannan. These are molecules made by Mycobacteria leprae or tuberculosis (67,68). CD1 transports these

	Exogenous pathway of antigen presentation		Endogenous pathway of antigen presentation	
Location of pathogen	Listeria residing within phagolysosome		Listeria in cytoplasm after lysing the phagolysosome membrane	
MHC molecule	Adaptive Class II	Innate CD1 ? hsp ? none	Adaptive Class Ia	Innate Class Ib H2M3 Qa1
Responding cell	CD4 T-cell	NK-CD4 T-cell γδ T-cell	CD8 T-cell	CD8 T-cell

Figure 12

molecules from phagolysosomes containing the mycobacteria to the cell surface in the same manner that classical Class II MHC shuttle peptides from the phagolysosome to the cell surface. However, CD1 stimulates γδ T cells whereas classical Class II MHC stimulate CD4 T cells with αß T cell receptors. Unlike classical Class II MHC, there are few alleles of CD1. For example, there are 122 known alleles at the human HLA DR Class II MHC locus (see Figure 13), but there is only one known allele at the human CD1b locus. This would be expected if CD1 evolved to

MHC Molecules Used by the Innate and Adaptive Immune Responses

Balanced polymorphism of Class Ia and classical Class II MHC compared with no or little polymorphism of Class Ib and non-classical Class II MHC.

	Innate (known alleles)	Adaptive (known alleles)
Class I	HLA-E (Qal) 1	HLA-B - 111
Class II	CD1 1	HLA-DR - 122

Figure 13

bind to a small number of critical molecules important for the host defense against a common pathogen such as M. tuberculosis.

Other non-classical MHC shuttle pathogenic molecules between the cytoplasm and the cell surface where they stimulate CD8+ cytotoxic T lymphocytes. An example is HLA-E which is the mouse analogue of Qa1. This is a Class Ib MHC molecule which recognizes listeriolysin and possibly other related bacterial hemolysins as well (69). Like other receptor-type molecules of innate immunity, there are very few alleles. This is in marked contrast to the 111 alleles of HLA B (70).

In the mouse, another non-classical MHC molecule is H2M3. This currently has no human counterpart. This Class Ib MHC molecule has few alleles and binds to formylated-methionine-leucine-phenolalanine. This is the leader sequence for procaryotic proteins. H2M3 may have evolved to shuttle bacterial peptides from the cytoplasm to the cell surface so that CD8 T cells could be stimulated. After listeriosis, H2M3 binds to an immunogenic formylated peptide which is tightly associated with a bacterial cardiolipin (71). This stimulates CD8 T cells.

Innate immunity regulates the subsequent adaptive immunity.

Innate immunity not only defends the host during the first several days of infection, but also activates and regulates adaptive immunity. Indeed, although the recognition of non-self by T cells via their antigen-receptors is one major component of immune responses, this by itself is not sufficient to resolve the following two fundamental questions discussed below (3,4,72-74).

Question 1) Should there be an immune response or not?

In other words, should the antigen be ignored, should the antigen be attacked with an active response, or should there be tolerance? For example, non-self antigen injected in the context of adjuvant induces an active response, while same antigen in soluble form may induce tolerance (75,76). An appropriate immune response is critical to survival after infection. This is illustrated by the infectious mortality and morbidity of immunosuppressed transplant patients and patients with AIDS. However, an inappropriate immune response, even against non-self antigens, is inherently dangerous. For example, the severe respiratory distress occurring after CMV pneumonitis results from the inappropriate activation of CMV-specific T cells after the virus has already been eliminated (77). Clearly the question whether or not there should be a response against a non-self-antigen is critical to survival of the host after an infection.

Innate immunity initiates adaptive immunity by causing inflammation as discussed in the previous sections. Among the leukocytes entering the site of infection are the CD4 and CD8 T cells of adaptive immunity.

In addition, TNFα and other cytokines produced by innate immunity facilitate activation of CD4 and CD8 αß T cells of adaptive immunity. These cytokines increase the expression of Class I and II MHC on antigen presenting macrophages and dendritic cells. In addition, these cytokines also increase the expression of costimulatory signals. Effective activation of CD4 and

CD8 T cells requires the above two classes of signals. One signal is delivered to the TcR via the MHC; the other is a costimulatory or "accessory" signal. If a T cell receives a signal only via its TcR, in the absence of the costimulatory signal, tolerance rather than an active response may occur. Although there are many costimulatory molecules, including HSA and ICAM-1 (78), the best understood are B7-1 and B7-2. These interact with CD28 on T cells. These costimulatory signals are increased by cytokines produced during innate immunity (3).

Question 2) If there should be an immune response, <u>what kind of immune response should occur?</u>

The immune system is not monolithic. Instead there are a variety of potential responses. For example, B cells may secreted - IgE, IgA, IgM, complement-fixing versus non-complement-fixing IgG antibodies. IgE is appropriate for some helminthic infections, IgA for infections involving the GI tract. There are also a large variety of potential T cell responses, and which occurs is determined by the nature of the innate immune response. We will discuss how these are regulated by innate immunity later in this paper. As we noted in the discussion of Figure 2, innate immunity reflects the evolutionary wisdom of the species. It gives biological meaning to antigen recognition by the antigen-specific receptors of the adaptive immune response; it determines which adaptive immune response is appropriate.

A naive CD4+ αß T cell is pluripotent, and during an immune response will differentiate into T cells which produce a few of the large number of possible lymphokines - interferon gamma, interleukins 2,3,4,5, etc. Which differentiation pathway occurs is determined by the initial interactions between T cell and antigen (79,80).

In several well-described infections, including listeriosis, CD4 T cells differentiate into Th1 or Th2 cells. The outcome of infection - death or cure - is determined by which response occurs. For example, the progressive and ultimately fatal lepromatous response to Mycobacteria leprae is associated with Th2 cells, but the tuberculoid response, which ultimately results in cure, is associated with a Th1 response (81). The signature lymphokine produced by Th1 cells is IFNγ. This activates macrophages to destroy Mycobacteria leprae. IFNγ also regulates B-cells to produce IgG antibody isotypes which bind to pathogens and then promote their destruction by activating complement and by inducing phagocytosis via binding to Fc receptors on these cells. As another example, a Th2 response protects against infection by helminths such as Nippostrongylus brasiliensis. The signature lymphokines of a Th2 response are IL 4 and IL 5. IL 5 activates eosinophils; IL 4 induces B cells to secrete IgE, IgM, and non-complementing activating IgG isotypes. IL 4 also changes gut physiology such that the helminth is expelled. Only those strains of mice which mount a Th2 response to infection are cured. Injections of Th1 lymphokines increase susceptibility to infection. See review (82). In addition to their signature lymphokines, both Th1 an Th2 cells produce many lymphokines in common; these include IL 3, and GM-CSF (79,80).

A major factor in determining which differentiation program T cells follow during the above infections are the cytokines present when the T-cell - antigen interaction takes place (Figure 14). These cytokines are produced by NK cells, and unconventional T cells, which are T-cells with both NK and CD4 T cell markers and γδ T cells. Immediately after infection, these

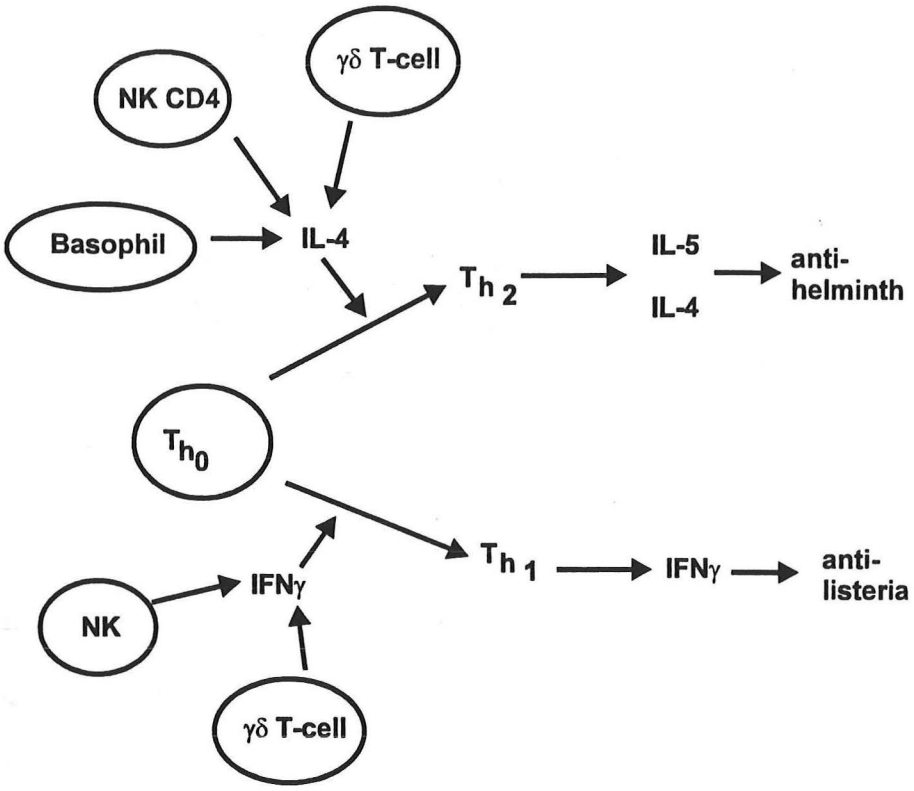

Figure 14

cells are activated by Listeria monocytogenes, Mycobacterium tuberculosis, or Mycobacteria leprae (51,68,83,84), and produce IFNγ, and macrophages which have ingested the bacteria produce IL 12. In the context of IFNγ and IL 12, naive CD4 T cells, which interact with bacterial antigens, differentiate down a Th1 pathway. These Th1 cells then secrete the lymphokines necessary for an effective response against these intracellular pathogens. In contrast, after helminthic infections, mast cells secrete interleukin 4 that causes naive T cells to differentiate down a Th2 pathway and produce the lymphokines necessary for an effective response against this parasite. Gamma delta T cells may direct differentiation down either Th1 or Th2 pathways depending upon whether they interact with Listeria monocytogenes or Nippostrongylus brasiliensis, respectively (85).

Adaptive immunity.

As discussed above, conventional αß CD4 and CD8 T cells with specific antigen receptors are activated during the course of innate immunity. These sterilize the tissues of listeria. The CD8 T cells recognize listeria infected cells and lyse them. This releases the listeria into the extracellular space. These are ingested by macrophages. These macrophages have been activated by interferon gamma produced by the CD4 T cells, and have acquired the ability to kill the listeria.

Figure 15 illustrates the functions of innate versus adaptive immunity in normal mice and in mice where one or the other immune systems has been inactivated by monoclonal antibodies or transgenic knockout. Both innate and adaptive immune responses are necessary for survival

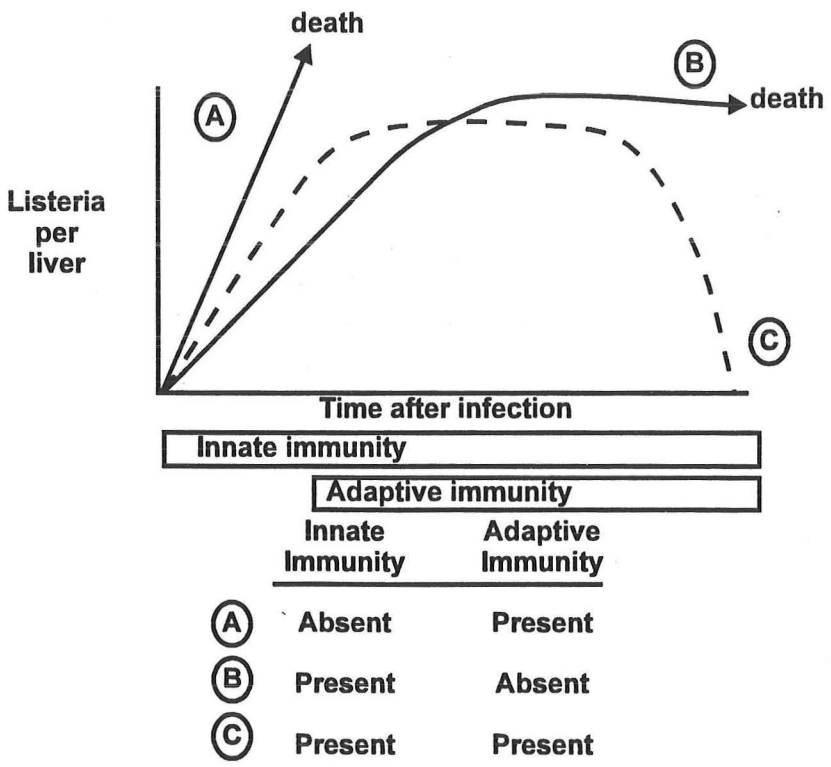

Figure 15

after listeriosis. Elimination of the innate response, for example by antibodies which eliminated neutrophils, or prevented immigration of neutrophils and macrophages into the site of infection (86) or by elimination of gamma-delta T cells (59,87,88), decreased survival after listeriosis (curve A in Figure). Elimination of the adaptive immune response by eliminating T cells resulted in control of listeria early during infection, but the animals eventually died because tissues could not be sterilized (51).

Summary.

Listeria monocytogenes is a foodborne pathogen. It causes septic abortions in pregnant women, and CNS infections in immunocompromised hosts and the elderly. Despite appropriate antibiotics and supportive therapy, the fatality rate remains at approximately 30%. The antimicrobial therapy cannot overcome the immunologic deficits in these patients.

The host defense against listeria consists of innate and adaptive immunity. Innate immunity results from the evolutionary experience of our species with pathogens. It is "hard-wired" and cannot be changed during an individual lifetime. Innate immunity recognizes the tissue injury caused by infection and pathogen-associated molecular patterns. One such pattern is the common chemical structure of endotoxin. Innate immunity protects the host during the days necessary for adaptive immunity to develop. Adaptive immunity recognizes specific non-self antigens via antibodies and T cell receptors. Within one lifetime, a wide diversity of these receptors is generated by somatic mechanisms - recombinatorial joining of various genes

and additions and deletions at the joints. Adaptive immunity is adaptive to the environment. It reflects an individual's experience and is "programmable." However, the adaptive immune system cannot give biological meaning to the antigens it recognizes. This biological meaning is defined by evolution and is interpreted by innate immunity. Innate immunity determines if an adaptive immune response should occur against an antigen, and determines what type of response occurs.

Reference List

1. Fearon DT and Locksley RM: The instructive role of innate immunity in the acquired immune response, *Science* 272:50-54, 1996

2. Medzhitov R and Janeway CA: Innate immunity - the virtues of a nonclonal system of recognition, *Cell* 91:295-298, 1997

3. Medzhitov R, Janeway CA, Jr.: Innate immunity: impact on the adaptive immune response. [Review] [40 refs]. *Curr Opin Immunol* 9:4-9, 1997

4. Matzinger P: Tolerance, danger, and the extended family, *Annu Rev Immunol* 12:991-1045, 1994

5. Fearon DT: Seeking wisdom in innate immunity, *Nature* 388:323-324, 1997

6. Janeway CA, Jr., Goodnow CC, Medzhitov R: Danger -pathogen on the premises: Immunological tolerance. [Review] [20 refs]. *Curr Biol* 6:519-522, 1996

7. Schlech WF: Listeria gastroenteritis—old syndrome, new pathogen [editorial; comment]. *N Engl J Med* 336:130-132, 1997

8. Southwick FS and Purich DL: Intracellular pathogenesis of listeriosis, *N Engl J Med* 334:770-776, 1996

9. Mielke MEA, Held TK, Unger M: Listeriosis, in *Pathology of Infectious Diseases*, edited by Connor DH, Chandler FW, Manz HJ, Schwartz DA, Lack EE, Baird JK, Utz JP, Stamford, Appleton and Lange, 1997, p. 621-633

10. Lorber B: Listeriosis. [Review] [125 refs]. *Clin Infect Dis* 24:1-9, 1997

11. Broome CV, Pinner RW, Schuchat A: Listeria monocytogenes, in *Infectious Diseases*, edited by Gorbach SL, Bartlett JG, Blacklow NR, Philadelphia, W.B.Saunders, 1998, p. 1750-1755

12. Jurado RL, Farley MM, Pereira E, Harvey RC, Schuchat A, Wenger JD, Stephens DS: Increased risk of meningitis and bacteremia due to Listeria monocytogenes in patients with human immunodeficiency virus infection. *Clin Infect Dis* 17:224-227, 1993

13. Ewert DP, Lieb L, Hayes PS, Reeves MW, Mascola L: Listeria monocytogenes infection and serotype distribution among HIV-infected persons in Los Angeles County, 1985-1992. *J Acquir Immune Defic Syndr Hum Retrovirol* 8:461-465, 1995

14. Schlech WF, Lavigne PM, Bortolussi RA, Allaen AC, Haldane EV, Wort AJ, Hightower AW, Johnson SE, King SH, Nicholls ES, Broome CV: Epidemic Listeriosis - evidence for transmission by food. *N Engl J Med* 308:302-206, 1983

15. Linnan M J, Mascola L., Lou X D, Goulet V., others., Broome C V: Epidemic listeriosis associated with Mexican-style cheese. *N Engl J Med* 319:823-828, 1988

16. Fleming DW, Cochi SL, MacDonald KL, Brondum J, Hayes PS, Plikaytis BD, Holmes MB, Audurier A, Broome CV, Reingold AL: Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. *N Engl J Med* 312:404-407, 1985

17. Dalton CB, Austin CC, Sobel J, Hayes PS, Bibb WF, Graves LM, Swaminathan B, Proctor ME, Griffin PM: An outbreak of gastroenteritis and fever due to Listeria monocytogenes in milk [see comments]. *N Engl J Med* 336:100-105, 1997

18. Food and Drug Administration: Efforts to prevent foodborne listeriosis, *J Am Med Assoc* 268:180 1992

19. Hof H, Nichterlein T, Kretschmar M: Management of listeriosis. [Review] [144 refs]. *Clin Microbiol Rev* 10:345-357, 1997

20. Ireton K, Cossart P: Host-pathogen interactions during entry and actin-based movement of Listeria monocytogenes. [Review] [136 refs]. *Annu Rev Genet* 31:113-38:113-138, 1997

21. Mengaud J, Ohayon H, Gounon P, Mege RM, and Cossart P: E-cadherin is the receptor for internalin, a surface protein required for entry of L. monocytogenes into epithelial cells, *Cell* 84:923-932, 1996

22. Dramsi S, Biswas I, Maguin E, Braun L, Mastroeni P, Cossart P: Entry of Listeria monocytogenes into hepatocytes requires expression of inlB, a surface protein of the internalin multigene family. *Mole Microbiol* 16:251-261, 1995

23. Gaillard JL, Berche P, Frehel C, Gouin E, Cossart P: Entry of L. monocytogenes into cells is mediated by internalin, a repeat protein reminiscent of surface antigens from gram-positive cocci. *Cell* 65:1127-1142, 1991

24. Portnoy DA, Chakraborty T, Goebel W, Cossart P: Molecular determinants of Listeria monocytogenes pathogenesis. *Infect Immun* 60:1263-1267, 1992

25. Songer JG: Bacterial phospholipases and their role in virulence [see comments]. [Review] [65 refs]. *Trends Microbiol* 5:156-161, 1997

26. Kocks C, Gouin Ej, Tabouret M, Berche P, Ohayon H, Cossart P: L. monocytogenes-induced actin assembly requires the actA gene product, a surface protein. *Cell* 68:521-531, 1992

27. Berche P, Gaillard JL, Sansonetti PJ: Intracellular growth of Listeria monocytogenes as a prerequisite for in vivo induction of T cell-mediated immunity. *J Immunol* 138:2266-2271, 1987

28. Kuhn M, Goebel W: Responses by murine macrophages infected with Listeria monocytogenes crucial for the development of immunity to this pathogen. *Immunol Rev* 158:57-67, 1997

29. Schwan WR, Goebel W: Host cell responses to Listeria monocytogenes infection include differential transcription of host stress genes involved in signal transduction. *Proc Natl Acad Sci U S A* 91:6428-6432, 1994

30. Hauf N, Goebel W, Serfling E, and Kuhn M: Listeria monocytogenes infection enhances transcription factor NF-kappa B in P388D1 macrophage-like cells, *Infect Immun* 62:2740-2747, 1994

31. Barnes PF and Karin M: Nuclear factor - kappa B - a pivotal transcription factor in chronic inflammatory diseases, *N Engl J Med* 336:1066-1071, 1997

32. Kopp EB and Ghosh S: NF kappa B and rel proteins in innate immunity, *Adv Immunol* 58:1-27, 1995

33. Baeuerle PA and Henkel T: Function and activation of NF-kappa B in the immune system, *Annu Rev Immunol* 12:141-179, 1994

34. Sienbenlist U, Franzoso G, Brown K: Structure, regulation and function of NF-kappa B. *Annu Rev Cell Biol* 10:405-455, 1994

35. Butcher EC and Picker LJ: Lymphocyte homing and homeostasis, *Science* 272:60-66, 1996

36. Springer TA: Traffic signals for lymphocyte recirculation and leukocyte emigration, *Cell* 76:301 1994

37. Vazquez MA, Sicher SC, Wright WJ, Proctor ML, Schmalzried SR, Stallworth KR, Crowley JC, Lu CY: Differential regulation of TNF alpha production by listeriolysin-producing versus non-producing strains of Listeria monocytogenes. *J Leuko Biol* 58:556-562, 1995

38. Sha WC, Liou HC, Tuomanen EI, Baltimore D: Targeted disruption of the p50 subunit of NF-kappa B leads to multifocal defects in immune responses. *Cell* 80:321-330, 1995

39. Poston RM, Kurlander RJ: Cytokine expression in vivo during murine listeriosis. *J Immunol* 149:3040-3044, 1992

40. Ehlers S, Mielke MEA, Blankenstein T, Hahn H: Kinetic analysis of cytokine gene expression in the livers of naive and immune mice infection with listeria monocytogenes. *J Immunol* 149:3016-3022, 1992

41. Wagner RD, Czuprynski CJ: Cytokine mRNA expression in livers of mice infected with Listeria monocytogenes. *J Leuko Biol* 53:525-531, 1993

42. Zhan Y, lieschke GJ, Grail D, Dunn AR, Cheers C: Essential roles for granulocyte-macrophage colony-stimulating factor (GM-CSF) and G-CSF in the sustained hematopoietic response of Listeria monocytogenes-infected mice. *Blood* 91:863-869, 1998

43. Frank MM, Fries LF: Complement, in *Fundamental Immunology*, edited by Paul WE, New York, Raven Press, Ltd., 1989, p. 679-701

44. Homeister JW and Lucchesi BR: Complement activation and inhibition in mycocardial ischemia and reperfusion injury, *Annu Rev Pharmacol Toxicol* 34:17-40, 1994

45. Pober JS, Cotran RS: The role of endothelial cells in inflammation. *Transplantation* 50:537-544, 1990

46. Brown E, Atkinson JP, Fearon DT: Innate immunity: 50 ways to kill a microbe [editorial]. *Curr Opin Immunol* 6:73-74, 1994

47. Atkinson JP, Farries T: Separation of self from non-self in the complement system. *Immunology Today* 8:212-215, 1987

48. Kopf M, Baumann H, Freer G, Freudenberg M, Lamers M, Kishimoto T, Zinkernagel R, Bluethmann H, and Kohler G: Impaired immune and acute-phase responses in interleukin-6-deficient mice, *Nature* 368:339-342, 1994

49. Liu Z, Simpson RJ, Cheers C: Role of IL-6 in activation of T cells for acquired cellular resistance to Listeria monocytogenes. *J Immunol* 152:5375-5380, 1994

50. Turner MW: Mannose-binding lectin: the pluripotent molecule of the innate immune system. *Immunol Today* 17:532-540, 1996

51. Unanue ER: Studies in listeriosis show the strong symbiosis between the innate cellular system and the T-cell response, *Immunol Rev* 158:11-25, 1997

52. Dunn PL, North RJ: Early gamma interferon production by natural killer cells is important in defense against murine listeriosis. *Infection & Immunity* 59:2892-2900, 1991

53. Kaufmann SHE: Bacterial and protozoal infections in genetically disrupted mice. *Curr Opin Immunol* 6:518-525, 1994

54. Tripp CS, Gately MK, Hakimi J, Ling P, Unanue ER: Neutralization of IL-12 decreases resistance to Listeria in SCID and C.B-17 mice. *J Immunol* 152:1883, 1994

55. Rogers HW, Sheehan KCF, Brunt LM, Dower SK, Unanue ER, Schreiber RD: Interleukin 1 participates in the development of anti-Listeria responses in normal and SCID mice. *Proc Nat Acad Sci U S A* 89:1011-1015, 1992

56. Kaufmann SHE, Emoto M, Szalay G, Barsig J, and Flesch IEA: Interleukin 4 and listeriosis, *Immunol Rev* 158:95-105, 1997

57. Mombaerts P, Arnoldi J, Russ F, Tonegawa S, Kaufmann SHE: Different roles of alpha beta and gamma delta T cells in immunity against an intracellular bacterial pathogen. *Nature* 53:56, 1993

58. Hiromatsu K, Yoshikai Y, Matsuzaki G, Ohga S, Muramori K, Matsumoto K, Bluestone JA, Nomoto K: A protective role of gamma / delta T cells in primary infection with Listeria monocytogenes in mice. *J Exp Med* 175:49-56, 1992

59. Fu YX, Roark CE, Kelly K, Brevets D, Campbell P, O'Brien R, and Born W: Immune protection and control of inflammatory tissue necrosis by gamma delta T cells, *J Immunol* 153:3101-3115, 1994

60. Kaufmann SHE: Gamma / delta and other unconventional T lymphocytes -what do they see and what do they do, *Proc Natl Acad Sci U S A* 93:2272-2279, 1996

61. Bertotto A, Spinozzi F, Gerli R, Bassotti G, Forenza N, Vagliasindi C, Vaccaro R: Peripheral blood gamma delta T cells in human listeriosis. *Acta Paediatr* 84:1434-1435, 1995

62. Munk ME, Elser C, Kaufmann SH: Human gamma/delta T-cell response to Listeria monocytogenes protein components in vitro. *Immunol* 87:230-235, 1996

63. Guo Y, Ziegler HK, Safley SA, Niesel DW, Vaidya S, Klimpel GR: Human T-cell recognition of Listeria monocytogenes: recognition of listeriolysin O by TcR alpha beta + and TcR gamma delta + T cells. *Infect Immun* 63:2288-2294, 1995

64. Boismenu R and Havran WL: Short analytical review: gamma delta T cells in host defense and epithelial cell biology, *Clin Immunol Immunopath* 86:121-133, 1998

65. Haas W, Pereira P, Tonegawa S: Gamma / delta cells. *Annu Rev Immunol* 11:637-685, 1993

66. Tanaka Y, Brenner MB, Bloom BR, Morita CT: Recognition of nonpeptide antigens by T cells. [Review] [63 refs]. *J Mol Med* 74:223-231, 1996

67. Julien D, Stenger S, Ernst WA, and Molin.R.L.: CD1 presentation of microbial nonpeptide antigens to T cells, *J Clin Invest* 99:2071-2074, 1997

68. Bendelac A: CD1: presenting unusual antigens to unusual T lymphocytes, *Science* 269:185-186, 1995

69. Bouwer HGA, Barry RA, and Hinrichs DJ: Acquired immunity to an intracellular pathogen: immunologic recognition of L. Monocytogenes-infected cells, *Immunol Rev* 158:137-146, 1997

70. Parham P and Ohta T: Population biology of antigen presentation by MHC Class I molecules, *Science* 272:67-74, 1996

71. Kurlander R and Nataraj C: Characterization of the murine H2-M3 wt-restricted CD8 response against a hydrophobic, protease-resistant, phospholipid-associated antigen from Listeria monocytogenes, *Immunol Rev* 158:123-128, 1998

72. Bendelac A, Fearon DT: Innate pathways that control acquired immunity. *Curr Opin Immunol* 9:1-3, 1997

73. Cohen IR: The cognitive principle challenges clonal selection. *Immunology Today* 13(11):441-444, 1992

74. Ibrahim MAA, Chain BM, and Katz DR: The injured cell: the role of the dendritic cell system as a sentinel receptor pathway, *Immunol Today* 16:181-186, 1995

75. Perez VL, van Parijs L, Biuckians A, Zheng XX, Strom TB, Abbas AK: Induction of peripheral T cell tolerance in vivo requires CTLA-4 engagement. *Immunity* 6:411-417, 1997

76. Weigle WO and Romball CG: CD4+ T-cell subsets and cytokines involved in peripheral tolerance, *Immunol Today* 18:533-538, 1997

77. Tanaka K, Nakazawa H, Okada K, Umezawa K, Fukuyama N, and Koga Y: Nitric oxide mediates murine cytomegalovirus associated pneumonitis in lungs that are free of the virus, *J Clin Invest* 100:1822-1830, 1997

78. Quill H: Commentary: anergy as a mechanism of peripheral T cell tolerance, *J Immunol* 156:1325-1327, 1996

79. Abbas AK, Murphy KM, and Sher A: Functional diversity of helper T lymphocytes, *Nature* 383:787-793, 1996

80. Mosmann TR and Sad S: The expanding universe of T-cell subsets: Th1, Th2 and more, *Immunol Today* 17:138-139, 1996

81. Yamamura M, Uyemura K, Deans RJ, Weinberg K, Rea TH, Bloom BR, Modlin RL: Defining protective responses to pathogens: cytokine profiles in leprosy lesions. *Science* 254:277, 1991

82. Finkelman FD, Shea-Donohue T, Goldhill J, Sullivan CA, Morris SC, Madden KB, Gause WC, and Urban JFJr: Cytokine regulation of host defense against parasitic gastrointestinal nematodes: lessons from studies with rodent models, *Annu Rev Immunol* 15:505-533, 1997

83. Melian A, Beckman EM, Porcelli SA, Brenner MB: Antigen presentation by CD1 and MHC-encoded class I-like molecules. [Review] [41 refs]. *Curr Opin Immunol* 8:82-88, 1996

84. Morita CT, Beckman EM, Bukowski JF, Tanaka Y, Band H, Bloom BR, Golan DE, Brenner MB: Direct presentation of nonpeptide prenyl pyrophosphate antigens to human gamma delta T cells. *Immunity* 3:495-507, 1995

85. Ferrick DA, Schrenzel MD, Mulvania T, Hsieh B, Ferlin WG, and Lepper H: Differential production of interferon gamma and interleukin 4 in response to Th1 and Th2 - stimulating pathogens by gamma delta T cells in vitro, *Nature* 373:255-257, 1995

86. Czuprynski CJ and Haak-Frendscho M: Non-specific resistance mechanisms to listeriosis: implications for experimental and naturaly occurring infection, *Immunol Rev* 158:47-56, 1997

87. Ladel CH, Blum C, Kaufmann SH: Control of natural killer cell-mediated innate resistance against the intracellular pathogen Listeria monocytogenes by gamma/delta T lymphocytes. *Infect Immun* 64:1744-1749, 1996

88. Skeen MJ, Ziegler HK: Intercellular interactions and cytokine responsiveness of peritoneal alpha / beta and gamma / delta T cells from Listeria-infected mice: synergistic effects of interleukin 1 and 7 on gamma / delta T cells. *J Exp Med* 178:985-996, 1993