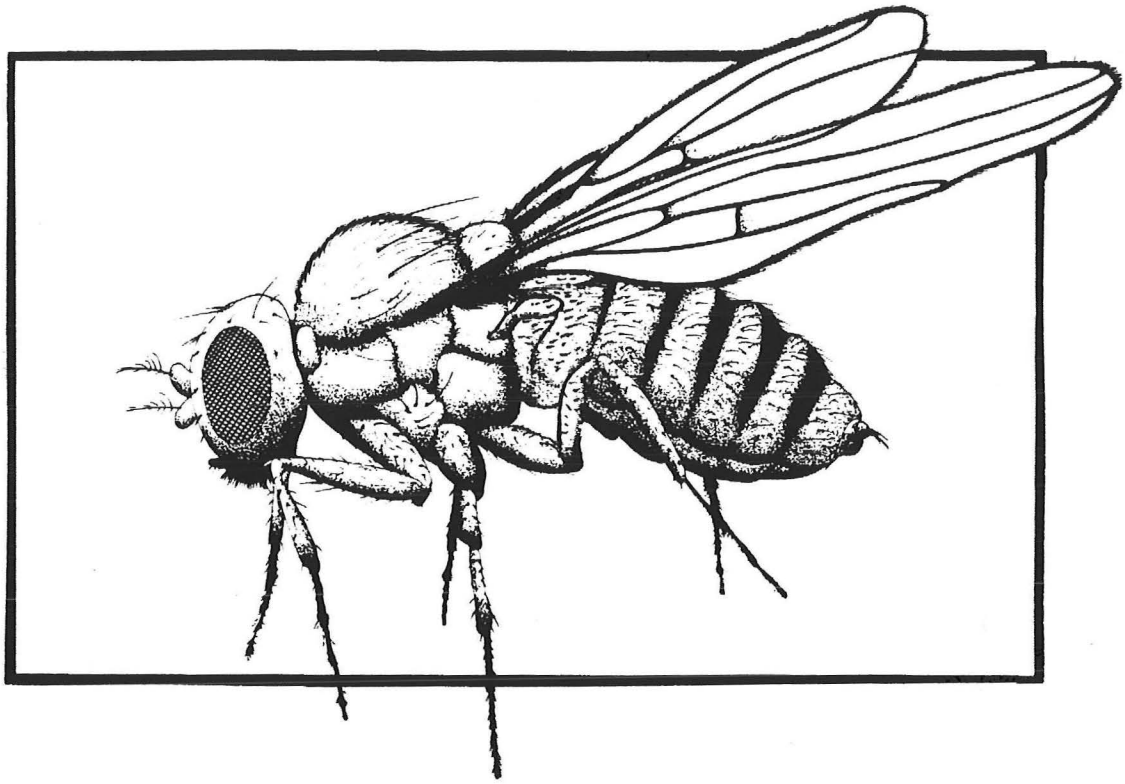

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ROLE OF INFECTIONS IN THE RHEUMATIC DISEASES: MOLECULAR MIMICRY BETWEEN BACTERIAL AND HUMAN STRESS PROTEINS?

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ROLE OF INFECTIONS IN RHEUMATIC DISEASES: MOLECULAR MIMICRY BETWEEN BACTERIAL AND HUMAN STRESS PROTEINS?

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COVER: Drosophila from cover of Drosophila: A Practical Approach. Oxford, IRL Press, 1986, 295 pp (1).

INTRODUCTION

The purpose of this presentation is to review the ways in which infections can cause or flare the rheumatic diseases. In particular, the immune cross-reactivity between bacterial stress (heat shock) proteins and normal tissue constituents, leading to autoimmunity, adds a new dimension to this discussion. Then, as an infection-induced model for other rheumatic diseases, rheumatic fever (RF) with its well-established link to prior group A streptococcal infection will be revisited, and other rheumatic diseases with direct links to infection will be compared to RF. And finally, the lessons learned from these diseases will be applied to ankylosing spondylitis, rheumatoid arthritis, Sjögren's syndrome and polymyositis in which a mounting body of circumstantial evidence suggests a probable infectious cause. The interplay of genetic susceptibility and infection with particular organisms will be discussed. The implications of this new information on present and future therapy of these rheumatic diseases will also be presented.

Infections Cause and Flare Rheumatic Diseases in Several Ways.

The different mechanisms by which infection can cause or exacerbate rheumatic diseases are outlined in Table 1.

Table 1. Role of Infections in Rheumatic Diseases

Molecular mimicry with autoantigens
Immune complex-induced inflammation
Activation of specific cytotoxic T-cells
Recruitment of non-specific NK-cell injury
Induction of stress (heat-shock) proteins

The concept of molecular mimicry makes it is easy to understand the induction of autoimmunity if the bacterial antigen shares reactive antigenic determinants (epitopes) with normal tissue components. In RF, several well-documented examples of this type of reaction have been observed. The N-acetyl-glucosamine of the carbohydrate of streptococcal cell wall cross-reacts with the N-acetyl-glucosamine in human heart valves, generating both circulating autoantibodies, and cytotoxic T-cell autoreactivity (2), the latter sometimes persisting for years, even when the patient has been on penicillin prophylaxis (3). Selected streptococcal M-proteins cross-react with heart muscle membranes (4). Patients with Sydenham's chorea have antibodies against neuronal antigens in the subthalamic and caudate nucleus region of the brain and these antibodies are absorbed out by treating the serum with group A streptococcal cell walls (5). The level of the anti-neuronal antibodies correlates with the severity of the chorea, and are present in increased concentrations in cerebrospinal fluid (2). Similar relationships between portions of the HLA-B27 histocompatibility antigen and selected bacteria have been shown (6), and mimicry of Staphylococcus aureus Protein A with the IgG-Fc fragment have led to bacterial explanations for generation of autoantibodies against IgG (rheumatoid factors) (7).

Immune complexes (IC's) are believed to play a dominant role in the induction of polyarthritis in the rheumatic diseases, and the same can be said about early presentation of skin rash, such as erythema marginatum in RF (8), or the skin rash seen in conjunction with polyarthritis in the emerging immunity in occasional patients with Hepatitis B (9). During flares of rheumatoid arthritis (RA), as with RF and the arthritis of Hepatitis B, high levels of circulating IC's can be shown (10,11). Not only do IC's activate the complement system, but when deposited in cartilage surface (as in RA) (12), or in blood vessel walls (as in polyarteritis nodosa or glomerulonephritis) chronic inflammation and

extensive tissue injury can follow.

The different sequential clinical features of RF can be segregated by the phase of the immune response involved. The early phase of polyarthritis and erythema marginatum involve primarily IC's containing streptococcal antigens, components of the classical complement pathway (C1q, C4b, C3b), and C-reactive protein (CRP) (10).

In RF, as the immune response matures, the role of IC's diminish and trapped antigen (or autoantigens) in peripheral blood vessels, heart valves or the brain become targets for a local immune response by antigen-specific T-lymphocytes (13). This type of response has been demonstrated in excised mitral valve leaflets examined many years after the first attack of RF with carditis (13). The fibroblasts in the heart valve show an unusual activation expressing DR (Ia) surface proteins suggesting that they can be antigen presenting cells for glycoprotein antigens in heart valve which drive a chronic rheumatic valvulitis even in the absence of new group A streptococcal exposure (14). This could explain the chronic valvular scarring and destruction present in some patients long after known attacks of RF.

A less specific, but nevertheless destructive role for NK cells has been proposed in RA where such cells have been demonstrated in large numbers in the synovial fluid in patients with chronic synovitis (15).

Induction of Stress (Heat-shock) Proteins. A new dimension has been added during the last four years to ways in which microorganisms can cause or flare rheumatic diseases. This has resulted from the discovery of a class of proteins in bacterial and mammalian cells known as "stress" or "heat-shock" proteins. In terms of biology, these stress proteins are not new. Ritossa (16) working in the Naples International Laboratory of Genetics and Biophysics in 1962 observed that the giant salivary gland chromosomes of the fly, Drosophila busckii, would form puffs on the chromosome at exactly the same location following exposure of the living fly to sub-lethal high temperatures (>30° C) (See Figure 1). He used radioactively labeled cytidine to demonstrate an intense local synthesis of RNA at the sites of these chromosomal puffs, and correctly concluded that heat exposure had induced selective synthesis of a protein or proteins which allowed repair of heat-induced injury. Ritossa named these proteins, "heat-shock" proteins. He also observed that certain drugs such as dinitrophenol and sodium salicylate would induce the same chromosomal puffing. These observations contributed a major advance in our knowledge of the induction of adaptive proteins to environmental changes in multicellular organisms, but until 10 years ago, their broader implications remained limited to studies of flys.

About 10 years ago, it was discovered that remarkably similar heat shock proteins were produced in virtually every cell from bacteria to man (17). Other stress factors such as anoxia or glucose deprivation as well as a number of noxious chemical substances could also sublethally "shock" or "stress" cells, and induce the same proteins. Terms like glucose-regulated proteins (GRP's) were added to heat-shock proteins (HSP's) and finally all were combined under the more general term of "stress proteins" (18).

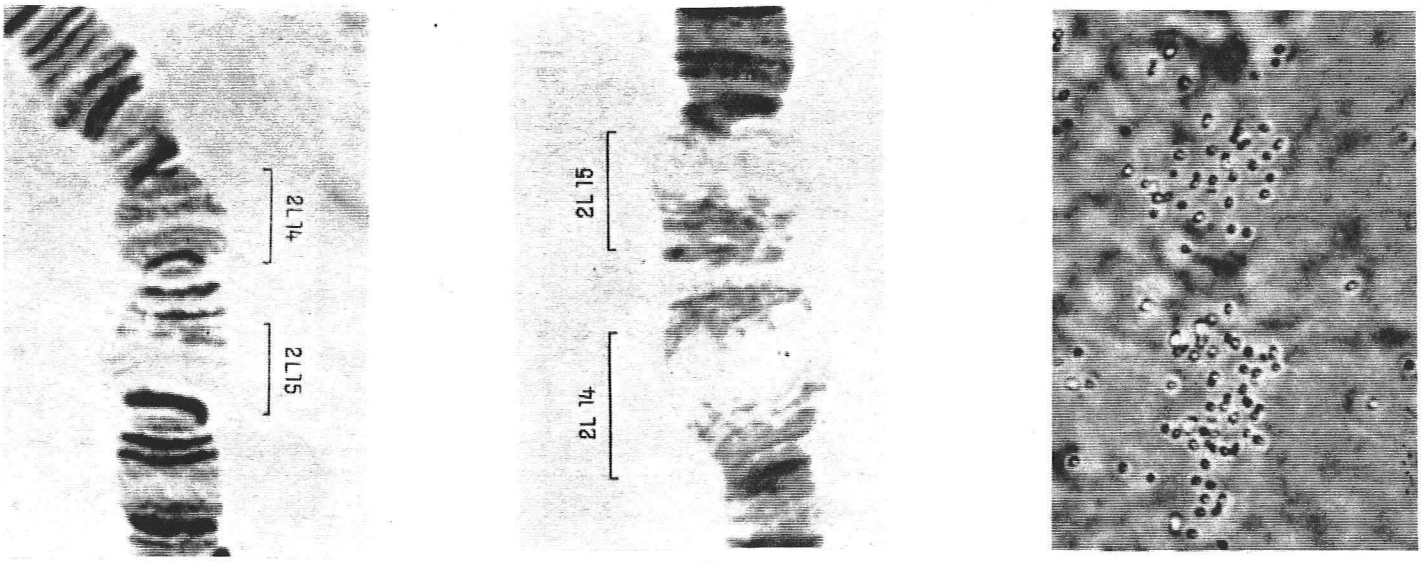

Figure 1. First demonstration of heat-shock effect was shown in Drosophila busckii. Panel A. Salivary gland chromosome from larva maintained at 25° C. Panel B Salivary gland chromosome from larva maintained for 30 min at 30° C. Panel C Radioautograph of chromosome shown in Panel B after incubation for 10 min with tritiated cytidine (s.a. 1 C/mM) showing active synthesis of nucleic acid over the "puff" areas. Developed 4 days on Kodak AR 10 film. (reference 16)

Table 2. Biologically Conserved Stress Proteins in Human Cells

Accepted Name	Cell Localization	Unusual Feature
60K-Hsp-Family (65K GroEL)	Mitochondria Cell Membranes	Autoantibodies to 60K-Hsp in RA but not in normals or SLE Pts
70K-Hsp-Family	Nucleolus, Nucleus Cell Surfaces	Binds IgG-Fc (induces RF?) Releases clathrin triskets
90K-Hsp-Family	Cytoplasm, cell membranes, nucleolus	Most abundant stress protein Elicits anti-lymphocyte Ab SLE
75K-Grp	Mitochondria	Function unknown
78K-Grp	Endoplasmic reticulum	A phosphoprotein, Structurally similar to 70K-hsp, Binds IgG-Fc
100K-Grp	Cytoplasm	Structurally similar to 90K-hsp
Histone H2B	Nucleus	Autoantibodies to Histone H2B in RA, Drug-induced lupus, SLE
Ubiquitin	Nucleus Binds H2A:H2B	Autoantibodies to ubiquitin in 80% of SLE patients
28K-hsp	Cytoplasm	4 forms, 3 are phosphorylated
32K-hsp	Cytoplasm	Induced by exposure to UV light, ? role in SLE
47K-hsp	Cell membrane	A glycoprotein which binds to collagen

The remarkable thing about these stress proteins was the high degree of similarity in structure, amino acid sequence and DNA homology from the lowest bacteria to the highest mammal. Between 50-90% homology had been conserved throughout biological development. No other proteins had maintained such similarity. This conservation suggested that these proteins perform very important functions, and that infrequent genetic mutations have been allowed because of the pressures of biological selection (19). Table 2 catalogues the best studied of the stress proteins known to exist in human cells.

Table 3 presents several common properties which most of these proteins share with one another.

Table 3. Common Properties of Stress Proteins

Most bind to hydrophobic portions of proteins and peptides.
Most possess ATPase activity which is used to provide the energy to fold, unfold and refold bound proteins.
These functions allow stress proteins to transport, stabilize, or chaperone proteins or peptides during assembly, move proteins from one cell compartment to another, restructure denatured proteins, and eliminate damaged proteins by "excretion" from cells. All these functions are necessary to repair damage induced by heat, other radiation, chemicals, glucose starvation, or anoxia. In macrophages and other antigen-presenting cells this peptide-binding function assists the immune response.

Unfortunately, the remarkable similarity of the stress proteins of microorganisms to the proteins of similar function in mammalian cells means that both viral and bacterial infections can induce stress proteins which cause autoimmunity in genetically susceptible patients. Some potential problems created by the molecular mimicry or immunological tolerance resulting from the extreme biological conservation of structure in the stress proteins across species barriers are outlined in Table 4.

Table 4. Problems Caused by Molecular Mimicry of, or Immune Tolerance to Microbial Stress Proteins

In spite of 50-90% structural homology with human stress proteins, bacterial stress proteins are superantigens for most normal people.
In the genetically susceptible host, bacterial stress proteins elicit autoantibodies and cytotoxic T-cell responses to epitopes on the body's own stress proteins. Autoantibodies of this type are found in SLE and RA. Cellular immune responses to human stress proteins are thought to play a major role in tuberculosis and in the tuberculoid form of leprosy.
In other genetically determined hosts, bacterial stress proteins are seen as self, and profound tolerance to these otherwise superantigens occurs. This form of anergy plays a role in lepromatous leprosy, miliary tuberculosis, mucocutaneous candiasis, and perhaps sarcoidosis.

Rheumatic Fever: Acute Model of Infection-induced Rheumatic Disease.

In the late 1800's several European physicians noted "post-scarletinal arthritis" after attacks of scarlet fever or pharyngitis. In 1900 Dr. Bela Schick suggested a link between streptococcal infection and RF (20). However, until 1929, no one had gone to the trouble to collect enough solid data to convince medical practitioners of this relationship. Then, Dr. Alvin Coburn, still a pediatric housestaff officer, collected the address of every child admitted to Babies Hospital in New York City with RF for the previous 10 years (21). He put a dot on the map of the City for each child. This distribution is shown in Figure 2. Most of the children with rheumatic fever lived in three areas, Hell's kitchen, Lower East Side, and Harlem. At that time, these three areas had the greatest poverty in New York City, and therefore, had homes and apartments with the greatest crowding together of family members.

Figure 2. Map of Manhattan Island showing clustering of RF cases in 3 major proverty areas: Hell's Kitchen, Lower East Side and Harlem. (From Coburn, AF: The Factor of Infection in the Rheumatic State (21).

After investigating this unexpected clustering of patients with RF, Dr. Coburn concluded that streptococcal infection, especially pharyngitis, caused RF, and set out to convince the Chief of the Department of Health of this association. "How could streptococcal infection cause rheumatic fever? Look at how many children have strep throats, and never get the disease. Impossible!" Dr. Coburn's "new" idea was ridiculed, he was labeled a crackpot and eventually forced out of his medical school position. Then in 1939 came sulfadiazine, the first real antibiotic, and with it, a precipitous fall in the incidence of streptococcal infections, followed immediately by a parallel plunge in the incidence of new cases of rheumatic fever. Overnight, the leaders of New York City medicine realized that Dr. Coburn had been right, and all jumped on the bandwagon, but not one acknowledged Dr. Coburn's role.

During World War II, it had been shown that the chance of infecting another soldier if you had a streptococcal sore throat was inversely proportional to the distance between the beds in the army barracks, thus

explaining the high incidence of RF under conditions of extreme crowding (22). Later, the military also showed that treating every new recruit with Bicillin (depo-Penicillin G) reduced the incidence of streptococcal pharyngitis and also of subsequent rheumatic fever more than 30-fold (23). Rising or falling anti-streptolysin O (ASO) titers provided solid proof of the role of antecedent group A (beta hemolytic) streptococcal infection 14 to 21 days prior to the onset of RF.

The last 50 years have added many new findings about RF which have allowed a much better understanding of its pathogenesis. Nevertheless, several aspects of RF remain unresolved. Table 5 lists several of the clinical and immunological peculiarities yet to be explained about rheumatic fever.

Table 5. Clinical Quirks of Rheumatic Fever

Antibiotic given in the first 9 days of pharyngitis blocks the first attack of RF.
Onset of RF lags an average of 18 days after symptoms of pharyngitis.
There is no shortening of the lag period with the second or third attacks of RF.
The highest titers of antibody are against extracellular streptococcal antigens.
Steroid treatment prolongs the duration of the RF attack.

Only about 70% of patients developing RF can recall having had a sore throat, although the remaining 30% have rising antibody titers to extracellular antigens of group A streptococci providing proof of recent infection (24). In those patients with clinical features of pharyngitis, if penicillin or another effective antibiotic is administered within 9 days of symptoms of a sore throat, RF does not develop. This is strange in view of the fact that antibody would be expected to form within three days of streptococcal antigen exposure. By 9 days, near-maximum titers would be present, yet killing the streptoccocci at that point prevents an attack. Cellular immune mechanisms would also be fully operative by the ninth day, yet no RF develops if treatment is given. An average of yet another 9 days follows before the earliest manifestations of RF (arthritis, fever, skin rash) appear. Most investigators feel that these earliest features of RF result from immune-complex induced disease (25), but the cause of the prolonged lag period is unknown. In addition, if the patient does not receive antibiotic prophylaxis, and acquires another streptococcal infection of an appropriate type, it again requires an average lag period of 18 days prior to the onset of clinical features of RF. This lack of shortening of the lag period with the second and third RF attacks makes an anamnestic response to the streptococcal antigens a less significant contributor to the pathogenesis of RF. Perhaps each new attack is generated by a different (?rheumatogenic) strain of streptococci with sufficiently different variations in bacterial antigens that a de novo immune response is required. This is quite possible as we will see when rheumatogenic and nephritogenic strains of group A streptococci are discussed later. The de novo immune response might then produce the same constant lag period.

Another unusual feature of RF is the exaggerated serum antibody responses to extracellular streptococcal antigens such as streptolysin O, streptococcal hyaluronidase, and streptococcal DNAase. Most of the cell wall and and cytoplasmic components of streptococci elicit a relatively weak antibody response. Perhaps the soluble extracellular antigens are more widely disseminated, thus stimulating a larger proportion of the immune system.

Finally another mystery regarding RF has been noted. If arthritis or other features of RF are abruptly terminated by the administration of steroids, then steroids are withdrawn at predetermined intervals when the average untreated RF-patient would be expected to have fully recovered, it is found that the steroid-treated patient will flare his suppressed RF (24). One possible explanation for this flare would be the interference of the administered steroids with the catabolism of immune complexes (IC's) causing them to pile up in tissues. When the steroids are stopped, the phagocytic cells which contribute so much to inflammation renew their attack on the IC's and a flare-up of disease activity occurs.

Twists and Turns in the Epidemiology of RF Since 1862. One of the earliest well-studied outbreaks of RF occurred in Denmark in 1862 with an incidence of 250/100,000 children observed (26). At that time, the streptococcal cause was unknown, but the full clinical description of the disease had been established. Beginning about 1900, the annual incidence of RF began to gradually decline. Since the genetics of the US and European populations would be expected to change little in a few generations, and since the decline in RF incidence began before the availability of antibiotics, other factors must have played a major role. Better housing, smaller families, separate rooms for children to sleep all probably played a major role in slowing the spread of infection. Better nutrition and greater cleanliness also played a substantial role in the drop in transmission of group A streptococcal infections. By 1950, the incidence of RF was down to 17/100,000, and by 1980, the incidence of new cases of rheumatic fever had decreased to 0.23/100,000 (27). This last precipitous drop was partly related to widespread treatment of streptococcal pharyngitis with penicillin. However, antibiotics alone cannot be credited with the enormous drop in RF incidence between 1950 and 1980. Only 70% of children with strep throat realize they had any disease. Even with optimally available medical care, only about two-thirds of patients would show symptoms which would indicate a need for antibiotic treatment (8). Some other factor caused this last drop in RF.

In the 1970's and 1980's Third World countries continued to have a high incidence of new cases of RF. Even in the United States, exceptions to the overall decline in incidence of RF existed. Somoan children in Hawaii had an incidence of RF of 206/100,000 hospitalized children in 1987 (28). Genetic susceptibility factors may have played some role in these isolated outbreaks such as the Somoan children, and among certain other Polynesian, Hispanic, American Indian and Black populations where higher incidence and more severe RF were observed (29).

However, far more important than environmental and genetic factors, changes in the strain of group A streptococci causing epidemic outbreaks of RF radically altered the incidence of new cases. Three recent epidemic outbursts of RF in Salt Lake City, Pittsburgh, and Columbus, Ohio, dramatically make this point (30,31,32). The Salt Lake City outbreak resulted in a RF incidence of 18.1/100,000 children between 5 and 17 years if age, and represented an eight-fold jump over the average incidence for the preceding 15 years! This well-studied outbreak showed no differences in incidence rates for white and ethnic minority children even though substantial differences in access to medical care separated these two groups. The difference in these three recent US outbreaks of RF was the group A streptococcal strain causing the epidemic.

Streptococci with M-protein type 18, an M-type rarely detected during the decline in RF incidence in the US (33), was shown to be responsible for the Utah and Ohio outbreak. Both isolation from the involved population during the outbreak, and the specific antibody responses in RF patients linked M-18 type group A streptococci to the Utah outbreak (30). The last time M-18 type group A streptococci had been found in an epidemic of RF had been in the Rocky Mountain region in the 1960's in military recruits (33). In addition, overcrowding may still have played some role in the Utah epidemic where the RF cases came from households twice as crowded as the state average (30). The clinical presentations of 99 of the Utah patients during the M-18 streptococcal outbreak in 1985-1986 are shown in Table 6.

Table 6. Acute Rheumatic Fever by Three Major Manifestations of Jones Criteria in the 1985-86 Utah Epidemic

Major Manifestations 99 patients	Percentage of Patients
Carditis and polyarthritis	44
Polyarthritis alone	14
Carditis alone	14
Carditis and chorea	14
Carditis, chorea and polyarthritis	6
Polyarthritis and chorea	4
Chorea alone	4

From MMWR 36:108, 1987 (34).

Rheumatogenic Versus Nephritogenic Strains of Group A Strep.

In order to prove that a given disease is caused by infection medical science has required that features of that disease satisfy Koch's postulates: that is, the putative organism has to be found in every patient with the disease; the organism has to be isolated and identified from the patient; the disease has to be produced by introducing the organism into an experimental subject; and the organism again has to be demonstrated in the experimentally-diseased subject. As you will see in our analysis of the association of group A Streptococcus as the causative agent in RF, there would remain room for considerable doubt for this association had satisfaction of Koch's postulates been necessary, primarily because we would have been making the false assumptions that all strains of group A Streptococci are alike, and that previously unimmunized human subjects would all respond in a predictable manner to infection. As it turns out, genetic variations in the host and strain variations in the organism interact in such a complicated manner that Koch's postulates in the broad sense would appear not to be satisfied.

Dr. Gene Stollerman and his colleagues (35) prospectively followed 300 Chicago school children with proven group A streptococcal pharyngitis in an effort to determine the fraction which would develop RF. None developed RF. This troublesome negative result flew in the face of the dictum worked out in previous streptococcal pharyngitis outbreaks that 3% of children having group A streptococcal pharyngitis would develop RF. Not only did it show that the 3% generalization was wrong, but the Chicago study suggested that most group A streptococci pose little threat of RF. Subsequent studies have shown that different M-proteins in group A streptococci are associated with both variable virulence and disease tropism which has been designated "rheumatogenic" when associated with later development of RF, and "nephritogenic" when later associated with acute post-streptococcal glomerulonephritis. Table 7 lists those strains best characterized as having these two different potentials.

Table 7. Disease Associations of Streptococci with Different Type M-Proteins

Disease Association	M-Protein Type
Pharyngitis and Rheumatic Fever "Rheumatogenic"	5,6,14,18,19,24,41
Impetigo and Glomerulonephritis "Nephritogenic"	1,2,4,12,49,55
Pharyngitis without Rheumatic Fever	Remaining 75+ M-types

Pope RM, Bull Rheum Dis 38:1-8(1989) (8).

The Role of Genetics in the Predisposition to RF. A slightly increased risk to develop RF exists in patients who are HLA-B5 because of what appears to be a hyperresponsiveness to streptococcal antigens (36). Similarly, there is a slightly increased risk to develop carditis if RF occurs in patients who are HLA-DR-2,3, or 4 (37,38). It should be stressed that these genetic types pose only a slightly increased risk for RF. However, Zabriskie and his colleagues at the Rockefeller Institute (2,39) have shown that a protein on the surface of human B-lymphocytes is found in nearly 100% of patients with RF, whether that patient lives in New York City or in Bogota, Columbia. This B-lymphocyte surface protein has been termed an "alloantigen", meaning that it shows genetic pleomorphism or variability. This protein has been designated B-cell alloantigen 883. It has been detected with mouse monoclonal antibodies produced in hybridomas after immunization of a mouse with B-cells from a patient with RF. This same alloantigen 883 has been shown to be present in only 15% of the general population of New York City and Bogota. If we put this information together with epidemiological studies from past epidemic outbursts of RF, we can conclude that up to 15% of patients with a "rheumatogenic" strain of group A streptococcal pharyngitis may be at risk to develop RF because of genetic susceptibility, and that intensity of infection, prior immunity, or mild undetected disease reduces this to the observed 3% incidence of clinically evident RF. This B-lymphocyte alloantigen 883 has been shown to be located adjacent to the B-lymphocyte receptor for group A streptococcal membrane antigens, and to co-cap with these antigens and presumably their surface gamma-globulin receptor following antigen exposure in vitro (2). This has been interpreted as indicating that this B-lymphocyte alloantigen 883 may be similar to an immune response gene (DR or Ia) product. Since it is uniformly present in RF patients, it may function to allow reaction to epitopes on streptococcal antigens shared by the patient's normal tissues. Reaction to these epitopes would presumably not occur in at least 85% of patients with group A streptococcal pharyngitis because of persistence of appropriate immune tolerance mechanisms.

The Necessary Linkage of the Wrong Genes and the Right Organism

The most impressive lesson learned about the pathogenesis of RF in the last few years centers on the virtually absolute requirement for a patient to have the wrong genetic susceptibility (B-lymphocyte alloantigen 883) and to be infected with a "rheumatogenic" strain of group A Streptococcus. Although RF follows a relatively acute clinical course, it can still be examined as a model for more chronic conditions, such as rheumatoid arthritis. To design appropriate studies of pathogenesis, the same interplay between genetic predisposition and exogenous factors such as infection can be assumed.

Table 8 below summarizes the known genetic associations that have been found to increase the risk of developing various rheumatic diseases.

Table 8. HLA (Class I,II,and III) and Other Genetic Pleomorphisms Increasing the Potential Risk to Develop Rheumatic Disease

HLA-Class I (HLA-A,B,C)	HLA Allele	Rel.Risk	%Pt/C
Ankylosing Spondylitis	B27	69.1	90/9
Reiter's Disease	B27	30.0	80/9
Psoriatic Arthritis (Spinal)	B27	10.8	47/9
	B38	10.3	23/3
	Bw16	7.8	13/7
Psoriatic Arthritis (Periph.)	B27	2.0	14/9
	B38	5.5	15/3
	B17	5.1	25/7
Behcet's (Oriental)	B5	7.2	69/23
(US or Western Europe)	B5	3.8	31/12
Inflam. Bowel Disease (Spinal)	B27	10.2	52/9
Whipple's Disease	B27	4.6	30/9
HLA-Class II (HLA-DR,DP,DQ) (Ia)			
Rheumatoid Arthritis	DR4	3.6	56/25
	DR1	1.4	20/17
	DR2	-2.2	13/25
	DR3	-1.4	16/21
	DR5	-3.1	7/19
	DRw6	-3.1	2/6
	DR7	-1.7	14/22
Systemic Lupus Erythematosus	DR2	2.3	44/25
	DR3	2.5	40/21
Polymyositis/Dermatomyositis	DR3	10.7	74/21
Sjogren's Syndrome	DR2	5.2	73/33
	DR3	3.6	56/26
Lyme Arthritis, chronic	DR4	4.1	58/25
HLA-Class III (C4A-complement)			
Systemic Lupus Erythematosus	C4A-null	3.7	30/10
Other (B-cell Alloantigen 883)			
Rheumatic Fever	Ag-883	561.0	99/15

(Adapted from references 2,40,41,42.)

Notice in Table 8 that different studies assign different frequencies among controls to some alleles, emphasizing the possibility of selection errors when small numbers of patients and controls are studied, perhaps based on clustering of racial or family groups in less than random fashion when compared to a more general population frequency.

In addition to the above Major Histocompatibility Complex (MHC) variations and B-lymphocyte alloantigenic variations which give genetic susceptibility to selected rheumatic diseases, the V-genes (variable sequence) for the four possible chains of the T-lymphocyte antigen receptor also show genetic variations which may alter immune response (or create non-response). V-gene variability is of particular relevance when considering the response to the bacterial heat shock proteins discussed above and the potential for generation of autoimmunity by cross-reaction with stress proteins of great similarity in human cells. It has been shown that the gamma/delta chains common in fetal thymus, but otherwise seldom found on peripheral blood T-cells, form the T-cell antigen receptors for response to bacterial heat shock (stress) protein antigens (43,44,45). Since much less is known about the gamma/delta chains of T-cell receptors than the more common alpha/beta chains of the T-cell antigen receptor, further study for the potential of genetic variations in gamma/delta to generate autoimmunity in the rheumatic diseases is needed.

In summary, the genetic diversity of the immune system helps to guarantee that no unusual microorganism will appear causing an epidemic which will wipe out the human race. At the same time, the great genetic variation in the immune system has a paradoxical disadvantage, because it makes some individuals respond to antigens which cross-react to their own tissues, and an autoimmune disease results. Not every person with the genetic susceptibility gets disease because not every person of that genetic type is exposed to just the right combination of exogenous factors. Perhaps the most impressive experiment of Nature emphasizing this last point is the impact of acquired immunodeficiency (AIDS) on existing rheumatic diseases. Patients with the spondyloarthropathies (Reiter's disease, psoriatic arthritis) show great exacerbation of their disease manifestations when full-blown AIDS appears (46, 47). This suggests a dominant role for CD8-cytotoxic T cells in their disease or the release of a previously partially-suppressed infection by AIDS. On the other hand, patients with rheumatoid arthritis or systemic lupus erythematosus go into complete remission, possibly emphasizing the need for CD4-helper T-lymphocytes in the perpetuation of RA and SLE (48). At this point creation of a partial immunodeficiency state using methotrexate, azathiaprine, or cyclophosphamide has been shown to be a highly effective form of palliative therapy for some patients with severe RA or SLE, but the lack of selective immunosuppression also creates substantial risk for these patients for serious infectious complications of treatment.

Table 9. Rheumatic Diseases Known to Be Caused by Infection

Organisms invading tissues:	Septic Arthritis Viral Arthritides Lyme Disease? Whipple's Disease?
Products of Infection-IC's:	Hepatitis B Arthritis Serum Sick. Arthritis Polyarteritis Nodosa Rheumatic Fever
Cross-reactivity Bacterial Antigens with Autoantigens:	Rheumatic Fever Reiter's Disease

Rheumatic Diseases Probably Induced by Infection

The lessons learned above for RF and arthritis induced by known infectious agents such as *Borrelia bergdorferi* (Lyme Disease) need to be applied to what is known (and not known) about the more common rheumatic diseases such as rheumatoid arthritis. A mounting body of circumstantial evidence suggests a probable infectious cause for anklylosing spondylitis, rheumatoid arthritis, Sjogren's syndrome and polymyositis/dermatomyositis. The data suggesting infectious cause will be presented for each disease.

Table 10 presents the links of ankylosing spondylitis (AS) to prior or co-existing infection. "The probability that AS is due to a peculiar immunologic relationship between the patient and his enteric organisms, and that this is determined by his HLA-B27 gene products seems great (48)."

Table 10. Does Ankylosing Spondylitis Have an Infectious Cause?

Association with Reiter's disease due to proven prior infection with *Salmonella sp.* , *Shigella flexneri*, *Campylobacter jejuni*, *Yersinia sp.* (48)
High frequency of coexistent chronic prostatitis (49)
Increased IgG-bearing B-cells in wall of rectum (50)
Elevated titers of serum IgA to *Klebsiella pn.* (51)
Shared amino acid sequence homology between *K. pneum.* nitrogenase and genetic variants of HLA-B27 (52)
Mystery of cross-reaction of rabbit anti-Klebsiella antibody with B27+ cells *only* from pts with AS (53)
Elevated 3',5'-oligoadenylate in serum in AS (54)

The puzzling observation that rabbit antiserum prepared by immunization with *Klebsiella pneumoniae* will lyse HLA-B27+ cells derived from patients with AS, but not HLA-B27+ cells from non-AS subjects suggests that some other factor besides the HLA-B27 gene generates antigenic determinants on the cell surface in AS. HLA-B27 is a MHC Class I cell surface molecule which is not usually involved in antigen presentation. However, there is an exception to this rule. When a cell is infected by a virus, viral antigen is presented on the cell surface along with Class I molecules to form a target for attack by cytotoxic (CD8+) T-cells, thus lysing infected cells. A suggested hypothetical scheme has been generated to explain this puzzling observation, and is shown in Figure 3.

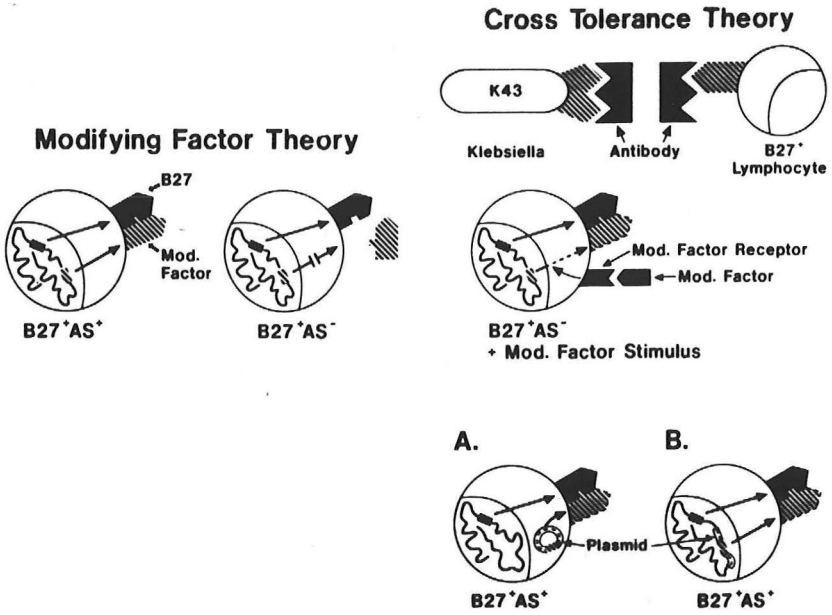

Figure 3. Infectious etiology in ankylosing spondylitis. The Modifying Factor Theory suggests that a genetically controlled "modifying factor" in the AS patient results in a closely spaced two-component surface antigen which can be recognized by the rabbit antiserum. Since this Factor is missing from the HLA-B27+ normal control, no recognition by the rabbit antiserum occurs. The Cross Tolerance Theory suggests that a bacterial plasmid acts like a human virus, and gains entry into the HLA-B27+ cell, much like a routine viral illness. Again, the normal person who is HLA-B27+ does not posess this viral-like antigen and no attachment by the rabbit antibody occurs. From Saag and Bennett (55).

In further support for the concept that AS may have a virus-like modifying factor would be the strong link to the class I HLA-B27 allotype. Class I surface proteins are usually associated with the presentation of viral-, not bacterialor auto-antigens, to CD8(+) cytotoxic T-cells. The recent report of elevated serum levels of 2',5'-oligoadenylate, an intracellular mediator of interferon in blocking viral replication, in the serum of AS patients also has been suggested as supporting some type of viral agent (?bacterial plasmid) in the infectious etiology of AS (56).

Rheumatoid Arthritis

For the last 50 years, many physicians have pondered the possibility that rheumatoid arthritis is caused by infection. After all, most patients wait until about age 35 before their arthritis begins. Then these patients often run low-grade fever, lose weight, develop a normocytic, normochromic anemia involving macrophage sequestration of bone marrow iron stores (the anemia of chronic infection), show hyper-gamma-globulinemia, leukocytosis, splenomegaly, lymphadenopathy and a flu-like illness in addition to polyarthritis. Within the joint, there is synovial tissue infiltration by T-lymphocytes, B-lymphocytes including plasma cells, and macrophages. The synovial infiltrate produces interleukin-1 and interleukin-2 (usually associated with active antigenic stimulation) and large amounts of immunoglobulins (principally IgG and IgA). The RA synovium makes more immunoglobulin than an equal weight of spleen or lymph node tissue (57). Complement (C1q,C4,C3) is actively fixed within the joint space, and immune complexes can be found in the joint fluid as well as near the surface of joint cartilage, tendons, joint capsule and adjacent collagen structures (12). The remarkable similarity of RA to the chronic arthritis found in some patients with Lyme disease has stimulated the medical community to reconsider some type of infection as the initiating factor in RA. Table 11 outlines the links between rheumatoid arthritis and prior or co-existing infection.

Table 11. Is Rheumatoid Arthritis Caused by Infection?

Treatment with sulfasalazine (Azulfidine) causes improvement or remission in 30% of pts (58,59).
Increased ab. to and increased culture of Clostridial species from stool in RA (70% vs. 45%) (60,61).
Elevated antibodies to a commonly shared bacterial cell wall peptidoglycan occur in RA (62).
Increased ab. to Proteus mirabilis in RA (63) Proteus surface antigens cross-react with DR4 (64).
RA synovial fluid lymphocytes proliferate after stimulation with bacterial HSP-65K (65).
RA sera contain elevated antibody titers to bacterial HSP-65K (66).
Post-rubella arthritis can become chronic and resemble RA, and live Rubella virus has been occasionally isolated from RA synovia (67,68).
Epstein-Barr virus antigens (EBV-gp110, EBNA-5) cross-react with DR-w4,DR-w14,DR-1, and type II collagen molecules (69,70).
Bacterial peptidoglycan, lipopolysaccharide, and staph. Protein A experimentally induce anti-IgG (rheum. factor) in vivo and/or in vitro (71,72,73).

Sjögren's Syndrome (SS)

Dry eyes, dry mouth and RA form the traditional triad referred to as Sjögren's (or sicca) syndrome (SS). However, about half of the patients do not have RA, but have either another rheumatic disease such as lupus or scleroderma (25%), or no identifiable rheumatic disease (about 25%). All forms share in common a dense infiltration of glandular structures throughout the body with a mixed population of T-cells, B-cells and macrophages very similar to the infiltrate of the RA synovium. As a late complication, this infiltrate can extend beyond the margin of the infiltrated gland (pseudolymphoma), or become monoclonal (B-cell lymphoma). Much recent evidence suggests that patients with SS are reacting to Epstein-Barr virus (EBV) (74).

Most adults have been infected with EBV by the time they are 21 years of age, most without having identifiable symptoms. Some show a variable illness, infectious mononucleosis, and rare children show nasopharyngeal carcinoma (Burkitt's lymphoma). In a small fraction of patients, live virus continues to reside in the salivary glands and provide infected saliva to propagate the infection to others. In occasional patients, SS follows a classical attack of infectious mononucleosis (74). EBV also infects B-lymphocytes, and can be detected in about one/million B-cells after usual EBV infection. However, patients with SS show a substantially larger number of infecteec cells, along with a polyclonal increase in Ig and an activation of B-cells making IgM-rheumatoid factor. SS patients also show increased antibody to the diffuse antigen (EA-D) of EBV (75), and the anti-SSB (La) autoantibody complexes with EBV-encoded RNA (76).

It has been suggested that the formation of this complex between La:EBV-RNA is responsible for the self + X-type of autoantibody production (76). Direct immunofluorescent staining of salivary epithelial cells was positive for EBV in 8 out of 14 patients with SS, but in none of 14 control patients. Salivary gland DNA examined in two patients with SS for EBV-related DNA by slot blot hybridization was positive in both patients. The saliva contained virus in 33 of 41 patients with SS, but in only of 6 of 26 controls (77,78). Although a high frequency of SS patients' peripheral blood lymphocytes would spontaneously culture out EBV-infected B-cells producing live virus, only rare patients with RA without SS provided peripheral blood lymphocytes which could produce EBV-infected B-cell lines, and none produced infectious EBV particles (78). The above data strongly associate EBV with SS, either as a causative agent, or as a superimposed secondary invader (Table 12).

Table 12. Is Sjögren's Syndrome Caused by EB-Virus ?

SS occasionally begins following infectious mononucleosis (74).
The SSB(La) antigen complexes with EBV-encoded RNA. May explain autoantibody anti-La (76).
Slot blot DNA hybridization shows EBV-related DNA in salivary gland DNA from SS pts. (77).
Early EBV antigen (EA-D) stainable in salivary glands. 57% SS patients vs. 0% controls (77,78).
Live EBV in the saliva of 80% of SS patients vs. 23% of control patients (78).
Circulating lymphocytes produce EBV-B-cell lines frequently in SS, but rarely in RA pts. without SS. SS B-cell lines generate live virus (78).

Polymyositis/Dermatomyositis

Polymyositis is a chronic inflammation of proximal limb-girdle muscles and occasionally of cardiac muscle which may be also associated with a scaly, erythematous rash of the upper eyelids, upper chest and over the extensor surfaces of joints such as the knees, elbows and knuckles. Its pathogenesis has been traced to an autoimmune reaction of cytotoxic T-cells against fast-twitch skeletal muscle fibers (79). It may be associated with a neoplasm in 16% of patients, with an innocent by-stander destruction of muscle linked to the body's cellular immune response to the cancer (80). If untreated, 65% of patients die within one year of onset, but with suppression of T-cell activity by high doses of steroids, 60% of patients survive. Most of the patients with associated neoplasm do not respond to steroids, but occasional patients recover completely after surgical resection of their tumor (80). In steroid resistant polymyositis, about 15% respond to immunosuppression with methotrexate or chlorambucil. Since most patients have a self-limited disease running an average course of one to two years, this allows disease suppression with less long-term risk (81).

Another small group of patients with polymyositis develop the clinical features of the disease as a result of a drug reaction. For example 1% of Japanese patients and 0.5% of Caucasian patients taking D-penicillamine for RA or scleroderma develop polymyositis (82).

An increasing amount of data suggest that most patients with polymyositis/dermatomyositis without a neoplasm or drug reaction may have developed their disease in response to some type of infection. Table 13 summarizes the findings in support of this suggestion.

Table 13. Do Most Patients with Polymyositis (PM) Have Infection?

Toxoplasma gondii IgM-antibodies may be elevated (83).
Occasional pediatric and rare adult patients have live Coxsackie A9 virus cultured from muscle (84).
Early cases of childhood polymyositis show elevated levels of antibody to Coxsackie B virus (85).
Slot blot DNA hybridization with DNA obtained from muscle biopsies examined with Coxsackie B specific cDNA probe demonstrated evidence for viral genome in 55% of 9 PM patients, but not in the DNA from 10 controls (86).

One interpretation of the multiple causes of PM/DM listed above would be generation of cytotoxic T-cell autoimmunity against the normal antigens on fast-twitch skeletal or cardiac muscle by a self + X mechanism in which products of intracellular bacterial (Toxoplasma) or viral infection appear on the cell surface near normal muscle proteins. The immune system recognizes the normal muscle protein and the closely associated microbial antigen as a unit. In genetically susceptible patients (HLA-B8,DR3) autoimmunity against normal muscle results. The secretion of tumor necrosis factor beta (TNF-beta) (lymphotoxin) when PM peripheral blood lymphocytes were exposed to normal muscle cell cultures, and the lysis of those cultures by the tumor necrosis factor beta would support this mechanism (79). Muscle lysis becomes independent of viral, bacterial or drug-related antigen when muscle lysis is occurring in chronic polymyositis. This would allow explanation of polymyositis associated with a neoplasm. The intense stimulation of TNF-beta by the cancer causes innocent by-stander destruction of susceptible muscle fibers, often at distant sites from the cancer itself. This explanation would also help explain the generally good response to steroid therapy in classical polymyositis, and the relative resistance of neoplasm-related polymyositis where larger amounts of TNF-beta may be released and steroid suppression of this release may be less effective.

Implications for Current and Future Therapy of Rheumatic Disease

The evidence presented above for the joint role of genetic susceptibility and specific infections in causing or flaring rheumatic diseases offers several interesting possibilities for present and future treatment of these conditions. The much more focused manipulation of the immune system provided by monoclonal antibodies against selected cell surface proteins such as T-lymphocyte surface antigens is already underway in several medical centers, and offers the most immediate potential for the management of patients with severe, life-threatening illness. As mentioned above, the observations with AIDS patients who also have RA or SLE suggest that disease remission follows loss of CD4(+) T-cells. As with AIDs, the depletion of CD4(+) helper-T-cells poses a significant threat of serious infectious complications. The selective ablation of the CD8(+) cytotoxic-T-cell population might prove of similar benefit in patients with spondyloarthropathies such as Reiter's disease or psoriatic arthritis (46,47).

Use of cDNA probes, and the polymerase chain reaction technology to multiply available DNA from small samples of blood should allow rapid, much less expensive typing of patients with suspected rheumatic diseases. The burgeoning data on genetic influences on disease susceptibilty and on likelihood of response to treatment will provide future physicians in practice new diagnostic and treatment tools. The Human Genome project now underway to map all human genes is scheduled to be completed within 10 years at a cost of three billion dollars, and will provide the normal baseline data for comparison with the DNA of patients with rheumatic diseases and other illnesses. I am convinced that this information will be made readily accessible with computer technology for comparison with deviations seen in sick patients, and that the resulting data will revolutionize medical diagnosis and treatment.

Similar advances in molecular biology using cDNA probes will allow detection of latent and active viruses in cell nuclei using hybridization techniques (86). Several serious rheumatic diseases have already been studied by DNA hybridization methods as described above (41).

If a firm linkage can be established between bacteria or viruses, and a given rheumatic disease, then immunization with recombinant bacterial or viral antigens which pose no threat of infection or autoimmunity could provide lasting protection, especially for selected genetic subpopulations who may be at particular risk.

Finally, if chronic carrier status for a bacteria or viral agent could be shown to be perpetuating chronic illness, appropriate antibiotic therapy could be used. Unlike the essentially blind approach of our current immunosuppressive treatment of several of the rheumatic diseases, antibiotics could be sharply targeted, and a measurable microbiological or serological end-point for successful treatment should be available.

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