AUTOIMMUNITY AND MOLECULAR MIMICRY IN THE PATHOGENESIS OF POST-STREPTOCOCCAL HEART DISEASE

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1. ABSTRACT

Molecular mimicry between pathogen and host has been proposed as a mechanism for the development of autoimmune diseases. Evidence suggests that microorganisms contain proteins which are similar enough to host proteins that they can stimulate existing B and T cells to respond to self proteins. The loss of immune regulation during responses against microbial antigens may explain development of pathogenic B and T cell responses in autoimmune diseases associated with infections. The study of B and T cell responses against the group A streptococcal antigens, N-acetyl-glucosamine, M protein and the autoantigen cardiac myosin has led to a better understanding of how molecular mimicry may play a role in disease. Studies of human monoclonal antibodies, T cell responses and animal models in comparison with the immunopathology in the human disease has provided information about the steps leading to inflammatory heart disease in autoimmune post-streptococcal rheumatic carditis. The new data indicate that the steps in pathogenesis of rheumatic heart disease following group A streptococcal infection include the following events. First, the development of crossreactive autoantibodies against the group A streptococcal carbohydrate antigen N-acetyl-glucosamine and cardiac myosin. Second, these antibodies react with valvular endothelium which becomes inflamed with expression of vascular cell adhesion molecule-1 (VCAM-1). After this event, T cells, CD4+ and CD8+, infiltrate through the endothelium/endocardium into the valve which is an avascular structure. Aschoff bodies or granulomatous lesions may form containing macrophages and T cells underneath the endocardium. The T cells are responsive to streptococcal M protein antigen sequences. The valve becomes scarred with eventual neovascularization and progressive, chronic disease in the valve. In the host, the mimicking antigens cardiac myosin and laminin have been involved in the myocardium and valve, respectively. As in other autoimmune diseases, both environmental and genetic factors are involved in the development of rheumatic carditis and inflammatory heart disease, a result of mimicry between the group A streptococcus and heart.

2. INTRODUCTION

Molecular mimicry between pathogen and host has been proposed as a mechanism for the development of autoimmune diseases(1-3). Evidence suggests that microorganisms contain proteins which are similar enough to host proteins that they can stimulate existing B and T cells to respond to self proteins. The loss of immune regulation during responses against microbial antigens may explain development of pathogenic B and T cell responses in autoimmune diseases associated with infections. The pathogenic response by a susceptible host most likely involves induction of crossreactive autoantibodies and the recognition of microbial epitopes by the major histocompatibility complex (MHC) proteins which present these epitopes to inflammatory (Th1, CD4+) and cytotoxic(CD8+) T cell subsets.

Production of large quantities of crossreactive antibodies by B lymphocytes may be important in antibody and complement deposition in tissues which may initiate inflammation in the target tissue. In some cases, the disease may solely be produced by antibody deposition, usually as a result of binding to the surface of the target cell in tissues and inducing changes in or death of the target cell. This may be through mechanisms such as complement mediated destruction of the target cell, cell signaling at the surface of the target cell, alteration of the nerve impulse transmission

or some other cell activity, cytokine induction by the target cell or upregulation of the cell adhesion molecules which attract T cells into the target tissue. The avidity and quantity of antibody may be important in the overall clinical manifestations of diseases produced by autoantibodies generated from infections(4).

Microbial components can induce production of large quantities of influential cytokines that will upregulate expression of MHC class I and II molecules in tissues and will determine the T cell subsets involved in the immune response in the susceptible host. In the case of an inflammatory and cell mediated immune response, activated T cells migrate into target tissues by infiltration through activated vascular endothelium(5). Activation of autoreactive T cells may be affected by microbial superantigens and other mitogens produced by the infection (6-9). Activated lymphocytes which enter and affect target tissues in inflammatory conditions are usually the CD4+ Th1 subset and the CD8+ cytotoxic subset, although CD4+ Th2 responses can be manifested in an eosinophilic infiltrate in tissues(10, 11). The inflammatory CD4+ T cells (Th1) produce tissue inflammation and scarring while the cytotoxic CD8+ T cell subsets are important in destruction of target tissues. CD8+ T cell subsets may also be involved in regulatory functions (12, 13). The inflammatory CD4+ Th1 lymphocytes produce cytokines such as gamma-interferon which may influence the CD8+ T cells to become more effective cytotoxic lymphocytes. Eventually, the CD4+ Th1 response leads to scarring of the target tissue(11).
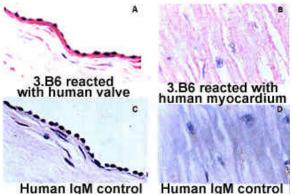
In studies to understand the mechanisms and microbial and host antigens of autoimmune and post-infectious sequelae, production of both monoclonal antibodies and T cell clones have been utilized(14-18). Animal models of autoimmune and infectious disease are important in understanding the mechanisms of disease as well as defining pathogenic epitopes of the autoantigens and microbial antigens involved. The use of animal models can lead to a better understanding of the human disease in some instances. However, animal models are not a substitute for work in humans and should be a guide only to investigation of the human disease. This review explores how the pathogenesis of rheumatic heart disease is related to mimicry between the group A streptococcus and cardiac myosin in the heart and to both antibody and T cell responses against the group A streptococcus.

3. RHEUMATIC FEVER AND RHEUMATIC HEART DISEASE

Group A streptococci are important human pathogens responsible for a number of suppurative infections in man, including pharyngitis, which can lead to acute rheumatic fever (ARF) and rheumatic heart disease (RHD) in a susceptible host (19). ARF is a leading cause of heart disease in children world-wide (19), and since 1985 a resurgence of ARF has been observed in the United States (20-22). The pathogenesis of ARF is complex and may depend on a number of streptococcal and host factors, particularly autoimmune and inflammatory responses in

host tissues. Immune crossreactivity or molecular mimicry between group A streptococcal M protein, N-acetyl-glucosamine, the dominant group A carbohydrate epitope, and cardiac myosin may in part lead to tissue destruction in ARF (23, 24). Host susceptibility is also an important factor in the development of ARF (25, 26). Expression of certain class II MHC molecules (27-29) and/or the D8/17 alloantigen (26, 30) are associated with increased risk for development of ARF. Both hereditary and environmental factors influence susceptibility to ARF (28, 30-32). Extensive reviews have recently been written on the pathogenesis of group A streptococcal infections and their sequelae (1, 33).

Autoimmune or rheumatic diseases may be associated with particular major histocompatibility antigen phenotypes. Presentation of streptococcal epitopes to T cells is controlled by the MHC antigens of the host. In previous studies, Ayoub reported a higher incidence of ARF in DR4 caucasian populations and in DR2 African-American populations (28). Interestingly, these associations have not been confirmed using HLA typing by molecular techniques (34). In South African populations with rheumatic fever, HLA DR1 and DRw6 were frequently associated with the disease (35). Different HLA class I and class II phenotypes were associated with ARF and with different ethnic groups. The reason for this confusing situation may be due in part to the fact that the studies were performed by serological methods and not by the more current molecular methods for detection of the MHC class II type. However, different ethnic groups may have different haplotypes which are important risk factors for disease. Guedez and Kotb (29) have grouped rheumatic heart disease patients into a more defined group with mitral valve disease such as mitral regurgitation and mitral stenosis. The frequency of DRB1*0701 and DQA1*0201 alleles and the DRB1*0701-DQA1*0201 and DRB1*13-DQA1*0501-3-DQB1*0301 haplotypes were found more often in the rheumatic heart disease patients than in ethnic controls. The data suggested that a stronger association with MHC class II type appeared more consistent when analyzed in patients with more homogeneous clinical manifestations. The results from the study (29) suggested that DRB1*0701, DR6, and DQB1*0201 confer susceptibility to rheumatic fever and are in agreement with those reported for Turkish (36), Mexican (37), South African (35), and Japanese rheumatic fever patients (38) where >50% of the cases evaluated were from mitral valve disease.

Aberrant immune responses in ARF patients could predispose them to the disease. Cunningham and colleagues have searched for unique antibody idiotypes in ARF. Purified anti-myosin antibodies from ARF were used to immunize rabbits and develop an anti-idiotypic serum which recognized antibodies present in ARF. The idiotype associated with ARF was named My1 and was found highly elevated in sera from ARF, acute glomerulonephritis (AGN), systemic lupus erythematosus, and Sjogren's syndrome (39). Normal levels of idiotype were found in sera from group A streptococcal pharyngitis, Chagas disease, myocarditis, IgA nephropathy., and rheumatoid

Figure 1. Reactivity of anti-streptococcal/anti-myosin mAb 3.B6 with normal human valve endothelium and myocardium. Formalin fixed human mitral valve (**A**) and myocardium (**B**) were reacted with mAb 3.B6 at 10 ug/ml. mAb 3.B6 binding was detected using biotin conjugated anti-human antibodies and alkaline phosphatase labeled streptavidin followed by fast red substrate. Control sections did not react with human IgM at 10 ug/ml (panels **C and D**) (With permission from Lippincott Williams and Wilkins)

arthritis. The data suggest that the My1 idiotype was associated with streptococcal sequelae ARF and AGN, but not with uncomplicated pharyngitis (39).

The major clinical manifestations of ARF include carditis/valvulitis, chorea, polyarthritis, erythema marginatum and subcutaneous nodules (40). Polyarthritis occurs as the most common manifestation of the disease, while carditis is the most serious(41, 42). The clinical signs of carditis include development of organic heart murmurs, cardiac enlargement, pericardial friction rubs, signs of effusion, and congestive heart failure. Pathologic signs of rheumatic carditis which include Anitshkow myocytes (43) and Aschoff's nodules may develop in heart tissues of ARF patients (42). In valvular injury, verrucae or nodules form on the mitral valve with edema and cellular infiltration of the leaflets(44) . Valves are damaged resulting in valvular stenosis or regurgitation.

ARF is an important model to study autoimmune disease following a confirmed bacterial infection, and is an excellent example of molecular mimicry between host and pathogen (23, 45-49). Sera of patients with ARF contain heart-reactive or myosin-reactive antibodies, frequently in high titers (15, 16, 46). Previous studies have demonstrated the presence of both CD4+ and CD8+ T-cell subsets within ARF valves (50-53), and MHC class II antigen expression on vessel endothelium and valvular fibroblasts (52, 54).

3.1. Antibodies against streptococcal carbohydrate n-acetyl-glucosamine and cardiac myosin recognize valvular endothelium and laminin
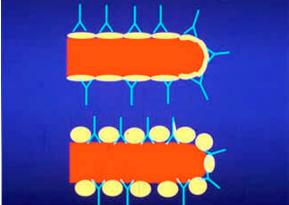
The first important event following streptococcal infection is the production of the crossreactive autoantibodies against myocardium and valve tissues. Studies suggest that immune responses against group A streptococci and the autoantigen cardiac myosin may play a

significant role in pathogenesis of ARF (15, 55-57). The importance of cardiac myosin surpasses all other alpha-helical coiled-coil antigens due to its prime role in producing inflammatory heart disease(58). Since the early work of Kaplan, antibodies and complement have been observed deposited in the myocardium and valves of ARF patients (59). Studies of anti-streptococcal/anti-heart monoclonal antibodies (mAbs) from rheumatic carditis (60-62) have revealed that cardiac myosin , and N-acetyl-glucosamine, the immunodominant epitope of the group A carbohydrate antigen, are the crossreactive antigens involved in antibody deposition on the valve (63). Furthermore, in a reverse experiment, anti-myosin mAbs have been shown to react with N-acetyl-glucosamine and to passively transfer inflammatory heart disease in mice(64).The reactivity of the human crossreactive mAbs with N-acetyl-glucosamine, the immunodominant epitope of the group A streptococcal carbohydrate, is an important feature because previous work by Dudding and Ayoub demonstrated the persistence of increased levels of anti-group A carbohydrate antibody in rheumatic valvular heart disease and their association with a poor prognosis (65).

Further studies by Shikhman and colleagues have shown that peptides from coiled-coil molecules actually mimic the N-acetyl-glucosamine molecule in reactivity with anti-GlcNAc antibodies and specific lectins(60-62). Immunization of animals with the peptide mimic resulted in production of anti-GlcNAc antibodies (60). These investigations shed light on the molecular basis of polyreactivity and suggest that some of the crossreactive antibodies may behave like lectins and bind peptide mimics and GlcNAc. The studies also link together the crossreactivity of the group A carbohydrate epitope, GlcNAc, human cardiac myosin, and streptococcal M protein. Antigenic redundancy due to crossreactivity among several molecules may also be important in triggering disease in a susceptible host.
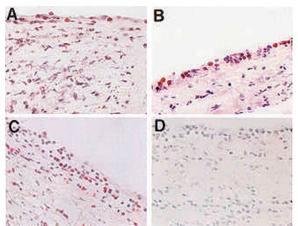
Most important in the disease process is the attachment of anti-streptococcal carbohydrate antibodies to valvular endothelium which may serve as an inflammatory signal to upregulate expression of adhesion molecules on valvular surface endothelium, an infiltration site for lymphocyte extravasation into the valve. Human anti-streptococcal/anti-myosin antibodies from rheumatic carditis which recognize group A carbohydrate and human valve endothelium and basement membrane support such a hypothesis (63). It was shown that human anti-streptococcal carbohydrate mAb 3B6 from rheumatic carditis recognized a site both on the valve surface endothelium as well as the myocardium. The study demonstrated that the mAb 3B6 recognized both cardiac myosin in the myocardium and laminin at the valve surface and within the basement membrane as shown in Figure 1.

Laminin is a large 900kD molecule composed of three chains, A, B1 or B2, which contain alpha-helical coiled-coil domains which are highly homologous with streptococcal M proteins and cardiac myosins. The laminin sequence HTQNT, shared between cardiac myosin and laminin, was located, synthesized and shown to inhibit the

Figure 2. Diagram illustrates the potential mechanism of antibody in the pathogenesis of rheumatic heart disease. Crossreactive antibody is shown binding directly to endothelium (top diagram) or binding to basement membrane of the valve (bottom diagram) exposed due to shear stress or damage by antibody and complement. *(With permission from ASM press)*

Figure 3. Adhesion and extravasation of T lymphocytes into ARF valve in valvulitis. **Figures A and B** (original magnification 200X and 400X, respectively) demonstrate extravasation of CD4+ lymphocytes (stained red) while **figure C** (magnification 200X) illustrates extravasation of CD8+ lymphocytes (stained red) into the valve through the valvular endothelium. An IgG_1 isotype control mAb (IgG_1) did not react with the same valve (not shown) (magnification 400X) (With permission from *the Journal of Infectious Diseases*, Chicago Press)

reactivity of the anti-streptococcal/anti-myosin antibody with the valve endothelium and basement membrane (63). The mechanism for antibody deposition on the valve would indicate that laminin or some other similar crossreactive protein exposed at the valve surface and within the basement membrane may trap antibody on the valve surface. Laminin or other crossreactive proteins on the valve surface and in the basement membrane would

contribute to the collection of antibody on the valve as well as enhance the upregulation of inflammatory signals by the endothelium. Figure 2 shows a diagram of the mechanism by which antibody binds to valve surface directly to endothelial cells or to valvular basement membrane exposed during valve stress.

3.2. Lymphocytes invade the valve through the valvular surface endothelium in rheumatic carditis

Since valvular injury is the most serious consequence of rheumatic carditis, the understanding of pathogenic mechanisms in valvular inflammation is crucial to understanding the basis of rheumatic heart disease. Studies of the valve surface endothelium in humans with ARF suggested that it becomes inflamed by some mechanism, such as by antibody and complement binding. T cell infiltration would take place through an activated valvular surface endothelium. Since the valve is originally an avascular tissue, the entrance of the T cells into the valve would either be through the surface endothelium or from adjacent myocardium. Studies in our laboratory suggest that the infiltration of T cells is through the inflamed endothelial cells at the surface of the valve, the endocardium. Once inflammation is initiated, the exposure of the valve surface may ensure further binding of crossreactive antibody to the valve. Endocarditis is a common feature in the pathology of rheumatic heart disease.
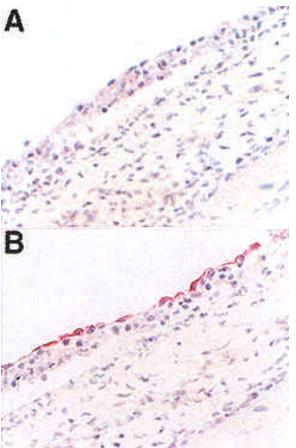
Evidence supports the hypothesis that the surface endothelium of the valve is the initial site of entry of lymphocytes in rheumatic heart disease (5). Since the valve is considered to be an avascular tissue, the most logical explanation or hypothesis is that the valve is traumatized and inflamed by antibody deposition and subsequent lymphocytic infiltration. The evidence in support of this hypothesis comes from studies of rheumatic valves from young children (5). Immunochemical staining of rheumatic valves demonstrated the attachment and infiltration of valve surface endothelium with CD4+ or CD8+ T cells as shown in Figure 3.

3.3. Vascular cell adhesion molecule-1 (VCAM-1) is upregulated on the surface valvular endothelium in rheumatic carditis

Further evidence shows that vascular cell adhesion molecule-1 was upregulated on the valve surface endothelium as shown in Figure 4. Upregulation of VCAM-1 promotes lymphocyte adhesion to the endothelium. The infiltration of the valve with lymphocytes may allow for the formation of the Aschoff nodule in the valve which is most likely a granulomatous lesion containing both lymphocytes and macrophages which are characteristic of chronic inflammatory lesions. Figure 5 shows the formation of the Aschoff lesion underneath activated valvular endothelium. CD4+ T cells are shown to be entering through the endothelium in waves.

3.4. T lymphocytes in rheumatic heart lesions react with streptococcal M protein sequences

Historically, T lymphocytes from ARF patients were found highly responsive to streptococcal cell wall or membrane antigens (66-68). T cells are important in the

Figure 4. Expression of VCAM-1 by ARF valvular endothelium (stained red) in valvulitis(**panel A**) Anti-VCAM-1 mAb reacted with rheumatic valvular endothelium but not with normal valve (not shown) An IgG$_1$ isotype control did not react with rheumatic valve (**panel B**) Original magnification 400X (With permission from *the Journal of Infectious Diseases*, Chicago Press)

development of valvular heart disease in ARF since they may lead to development of granulomatous lesions in the valve and subsequent scarring. Studies by Zabriskie and colleagues demonstrated CD4+ and CD8+ T cells present in heart valves from ARF (51, 52), and aberrant expression of HLA-DR antigen on valvular fibroblasts from patients with active rheumatic carditis were reported (51, 52). Aschoff nodules in valves have been shown to express cytokines IL-1 and TNF (69). The Th1 cytokine gamma-IFN may play a pivotal role in the inflammatory responses in heart valves.

The specificity of lymphocytes within the lesions has been investigated by Guilherme and colleagues (53) who determined that the valve-infiltrating lymphocytes were specific for M protein and heart antigens. The study suggested that the T lymphocytes which infiltrated the valve were reactive with streptococcal M protein. Studies in mice have suggested that streptococcal M protein sequences in the A and B repeat regions that are similar to sequences of the heart protein cardiac myosin are the most likely involved in the disease (23, 70). Although the class I

epitope of M proteins has been shown to crossreact with myosins, its sequence is more homologous to a sequence present in skeletal myosins which are not involved in production of inflammatory heart disease (58, 71, 72).

4. ANIMAL MODEL OF RHEUMATIC/VALVULAR HEART DISEASE DEMONSTRATED IN LEWIS RATS IMMUNIZED WITH STREPTOCOCCAL M PROTEIN OR CARDIAC MYOSIN

Development of an animal model for rheumatic heart disease has been a challenge. No animal model has been accepted as the gold standard animal model of rheumatic fever. Futhermore, man is the primary host and reservoir for group A streptococci. One of the underlying complications of developing a model of ARF is that an infection model which displays all of the symptoms of the disease has not easily been attained due to the difficulty in infecting an animal model. Models have relied on immunization with adjuvants and streptococcal cell wall components in the past, and more recently purified recombinant streptococcal or host antigens have been utilized to substitute for disease in the animal. Animal models described in the 1950s and 1960s reported lesions using various crude streptococcal cell wall components or whole streptococci (43, 73-78). Unny and Middlebrook have reviewed many of the older studies which utilized crude streptococcal antigens to induce lesions in animals (79). In early studies crude streptococcal preparations and equivocal results made the experiments difficult to interpret and were discouraging for the field in general. Murphy and Swift immunized rabbits with whole streptococci and reported heart lesions in rabbits (73, 74). Schwab and colleagues have utilized group A streptococcal peptidoglycan-polysaccharide complexes in mice and rats to establish carditis in animals (75-78, 80, 81). The evidence suggested that the peptidoglycan-polysaccharide complexes persisted in the tissues and acted to continually stimulate tissue injury and immune complex deposition. The persistence of antigen may be an important feature in disease in man.

More recent models include the DBA/2 mouse model where anti-myosin antibody deposition led to development of inflammatory heart disease(64). It was postulated that the DBA/2 mice may have a target organ sensitivity since in other strains of mice the anti-myosin mAbs did not deposit or lead to disease. In another model, autoimmune prone MRL/++ mice immunized with the NT4 peptide of streptococcal M5 protein developed myocarditis (70). NT4 is a cardiac myosin mimicking peptide. In the MRL model, disease was shown to be caused by CD4+ T cells and MHC class II-IAd. In BALB/c mice which are also MHC class II-IAd, immunization with peptides of streptococcal M5 protein which mimic cardiac myosin produced inflammatory heart lesions (23). The data support the mimicry hypothesis that unique sequences in streptococcal M proteins which mimic epitopes in cardiac myosin may break tolerance and induce an immune attack on the heart. Although animal models support the molecular mimicry hypothesis, multiple factors including host susceptibility must be considered in any animal model of ARF.
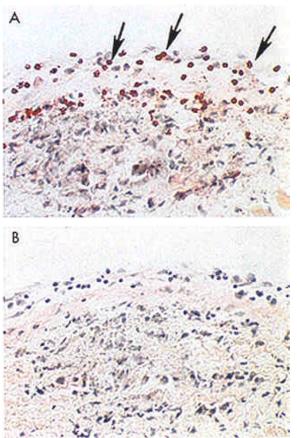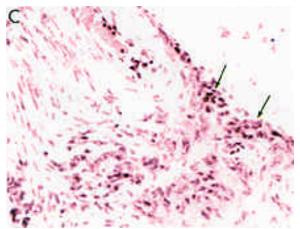
Figure 5. Extravasation of CD4+ lymphocytes (stained red) into valve above Aschoff's body in the subendocardium of the left atrial appendage (**panel A**) Similar results were seen with anti-CD8 mAb staining of the valve, but there were fewer CD8+ T-cells observed (not shown) Isotype control IgG1 mAb did not react with valve tissue (**panel B**) Original magnification 200X (With permission from the Journal of Infectious Diseases, Chicago Press)

Figure 6. Illustration of valvulitis and cellular infiltration in hematoxylin and eosin stained mitral valve from Lewis rats immunized with streptococcal rM6 protein. The figure demonstrates valvulitis in the mitral valve and shows disruption of endocardial (endothelium) surface of the valve with infiltrating cells (arrows) Magnification 200X. *(With permission from Infection and Immunity, ASM Press)*
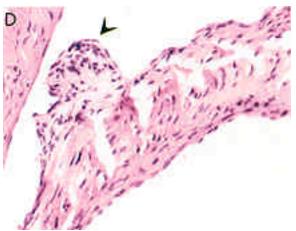
Recently, the Lewis rat has been used most extensively and successfully to develop a model with the characteristic lesions of rheumatic heart disease(58, 82). Rats are immunized with antigens recombinant streptococcal M protein or cardiac myosin or their peptides in adjuvants which affect the immune system by elevating inflammatory cytokines to levels expected in disease. The immunization scheme with the elevated cytokines presumably activates endothelium so that T cells can enter the target tissues in the susceptible rat strain. The streptococcal or host antigen breaks tolerance against the heart and induces specific T cells which infiltrate the target tissue through endothelium and play a role in the disease.

4.1. Streptococcal M protein and cardiac myosin induced rheumatic lesions in lewis rats
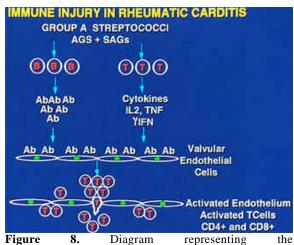
In studies in the Lewis rat, recombinant streptococcal M6 protein was administered with adjuvants and induced rheumatic heart disease in 50 percent of the animals tested (3 out of 6 rats developed valvular heart lesions) (82). The lesions demonstrated cellular infiltration through the valvular endothelium similar to that seen in the human disease. Figure 6 illustrates the cellular infiltration of the valve in the Lewis rat model of valvulitis after immunization with recombinant M protein. Other lesions suggestive of rheumatic heart disease were verrucae which were seen on the valve leaflet in the Lewis rat as shown in Figure 7. In this model, Anitschkow cells were also present. Lymphocytes from the recombinant M protein immunized rats proliferated in the presence of cardiac myosin and not skeletal myosin. Data from experiments in mice have suggested that the cardiac myosin-like sequences within the A and B sequence repeats of the streptococcal M protein are responsible for the development of heart-specific T cells that penetrate the valve(23). Similar experiments have been performed in the rat model which support this hypothesis. M protein regions which induce cardiac myosin reactive T cells are located in the A and B repeat regions of the M protein(23).

Most important are the data from studies of T cells collected from rheumatic hearts which indicate that the heart infiltrating T cells in rheumatic carditis responded to M protein peptides from the A and B repeat regions of the molecule and contained sequences more homologous to cardiac rather than skeletal myosins (23, 53). Cardiac myosin has been shown to induce both myocarditis and valvulitis in the Lewis rat model while skeletal myosin and other alpha helical molecules do not (58). Recombinant streptococcal M protein can also cause focal myocarditic lesions characteristic of those seen in rheumatic fever, but it does not produce a fulminating myocarditis that can be seen in rats immunized with cardiac myosins.

A rat T cell line specific for M protein and cardiac myosin suggested that crossreactive T cells were important in the production of disease (82). Most recently, we have produced highly specific M protein reactive T cell clones from humans with rheumatic heart disease which are crossreactive with sequences in streptococcal M protein and cardiac myosin (Mertens *et al,* manuscript in

Figure 7. The figure illustrates a verrucae-like nodule on the valve surface following immunization of the Lewis rat with group A streptococcal recombinant M6 protein. Anitschow cells were present in valves (not shown), and hematoxylin and eosin stained heart valve tissue sections from control rats immunized with PBS and adjuvants were normal with no verrucae or cellular infiltrates. (With permission from *Infection and Immunity*, ASM Press)
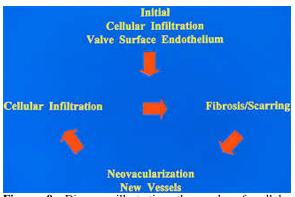

Figure 8. Diagram representing the immunopathogenesis of post streptococcal heart disease. Initially, B and T cells are activated by specific streptococcal antigens and superantigens leading to strong responses against streptococcal and host antigens. The development of pathogenic clones of B and T lymphocytes are important in development of the disease. The antibodies against the group A carbohydrate, which is crossreactive with the valve surface, bind to the valve surface endothelium (endocardium) and lead to damage and/or upregulation of cell adhesion molecules such as VCAM-1 on activated surface endothelium of the valve. M protein-reactive T cells enter the valve through the surface endothelium by binding to cell adhesion molecules such as VCAM-1 and extravasate into the valve. The formation of scar tissue in the valve followed by neovascularization allows for the disease to continue in the valve. The specificity of the T cells entering the valve has been shown to be M protein (38)

preparation). These data strongly support the hypothesis that the T cells which infiltrate the valve are reactive with the streptococcal M protein and its cardiac myosin like peptide sequences.

5. CONCLUSIONS AND PERSPECTIVES

The data support the hypothesis that mimicry between alpha-helical cardiac myosins and the group A carbohydrate and the alpha-helical streptococcal M protein are important in producing rheumatic heart disease. Elevated antibody titers against the group A carbohydrate have been implicated in the prognosis of valvular heart disease in the past (65) and the investigation of anti-streptococcal/anti-myosin monoclonal antibodies led to the discovery that the anti-group A carbohydrate epitope, N-acetyl-glucosamine, crossreacted immunologically with cardiac myosins and reacted with both myocardium and valve (61, 63). Antibody has been found deposited on both valve and myocardium in rheumatic fever (59). However, the reaction with the valve is now linked to the reactivity of the antibody with laminin on the surface of the valve(63). Further evidence supports the hypothesis that the disease begins by inflammation at the endothelium of the valve where VCAM-1 is upregulated in the disease and leads to infiltration of the valve by lymphocytes reactive with the streptococcal M protein (5, 53). Therefore, there is a role for both M protein and group A carbohydrate in the development of rheumatic heart disease. The anti-group A carbohydrate antibody would initiate the disease by reacting with the valvular endothelium, and the T cells specific for M protein and cardiac myosin shared sequences infiltrate the valve and lead to the scarring. A diagram in Figure 8 illustrates this hypothesis.

Although cardiac myosin is a profound autoantigen in animal models of myocarditis and valvulitis(58), this may not exclude other cardiac proteins which may yet be found to be involved in the disease. In addition, the endothelium of the valve may be activated by a variety of mechanisms which include immune complexes or cytokine influence on the valve in addition to anti-group A carbohydrate antibodies. T cells entered the valve through surface endothelium both in humans and in animal models. The data strongly suggest that the only entrance into the avascular valve tissue is through the surface endothelium which must become activated by antibody in order to allow cellular infiltration into the valve. Once the endothelium is activated, T cells infiltrate and inflammatory Th1 cytokines would be produced in the valve and scarring occurs. The scarred tissue eventually becomes neovascularized with vessels developing in the previously avascular valve tissue. The neovascularization within the scarring allows the disease to progress within the valve. Figure 9 illustrates the cycle in the valve in rheumatic heart disease. The valve is initially damaged at the surface endothelium allowing cellular infiltration which would subsequently continue after the initial insult at the valvular endothelial surface. Scarring and neovascularization in the valve tissues leads to endstage disease or irreversible deformation of the valve and its malfunction in rheumatic heart disease.

Figure 9. Diagram illustrating the cycle of cellular infiltration, scarring and neovascularization and continual cellular infiltration through the neovascularized valve.

6. ACKNOWLEDGEMENTS

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