Molecular Mimicry, Autoimmunity, and Infection: The Cross-Reactive Antigens of Group A Streptococci and their Sequelae

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ABSTRACT The group A streptococci are associated with a group of diseases affecting the heart, brain, and joints that are collectively referred to as acute rheumatic fever. The streptococcal immune-mediated sequelae, including acute rheumatic fever, are due to antibody and cellular immune responses that target antigens in the heart and brain as well as the group A streptococcal cross-reactive antigens as reviewed in this article. The pathogenesis of acute rheumatic fever, rheumatic heart disease, Sydenham chorea, and other autoimmune sequelae is related to autoantibodies that are characteristic of autoimmune diseases and result from the immune responses against group A streptococcal infection by the host. The sharing of host and streptococcal epitopes leads to molecular mimicry between the streptococcal and host antigens that are recognized by the autoantibodies during the host response. This article elaborates on the discoveries that led to a better understanding of the pathogenesis of disease and provides an overview of the history and the most current thought about the immune responses against the host and streptococcal cross-reactive antigens in group A streptococcal sequelae.

INTRODUCTION

Cross-reactive antigens are molecules on the group A streptococci that mimic host molecules and during infection or immunization induce an autoimmune response against host tissues, leading to the autoimmune group A streptococcal sequelae ([1]–[25]). "Molecular mimicry" is the term used to describe immunological cross-reactivity between the host and bacterial antigens. Immunological cross-reactions between streptococcal and host molecules have been identified involving antibodies or T cells that react with streptococcal components and tissue antigens ([6], [7], [22], [24], [25]). The advent of monoclonal antibodies (mAbs) and T cell clones/hybridomas has greatly advanced the identification of the host and streptococcal antigens responsible for immunological cross-reactions associated with immunization, infection, and autoimmune sequelae. The identification of cross-

reactive antigens in group A streptococci and important in our understanding of the pathogenesis of autoimmune sequelae, such as rheumatic fever including carditis ([8](#), [11](#)) and Sydenham chorea ([7](#)), which may follow group A streptococcal infection ([3](#)).

Molecular mimicry between host and bacterial antigens was first defined as identical amino acid sequences shared between different molecules present in tissues and the bacterium ([26](#)–[28](#)). The investigation of molecular mimicry through the use of mAbs has identified other types of molecular mimicry. The second type of mimicry involves antibody recognition of similar structures such as alpha-helical coiled-coil molecules such as streptococcal M protein, and the host proteins myosin, keratin, tropomyosin, vimentin, and laminin that share regions containing 40% identity or less and cross-reactive sites are not completely identical ([4](#), [29](#)–[43](#)). A third type of molecular mimicry is revealed in immunological cross-reactions between molecules as diverse as DNA and proteins ([36](#), [40](#), [44](#)) or carbohydrates and peptides ([45](#)–[47](#)). The three types of molecular mimicry are summarized in [Table 1](#). Studies of the cross-reactive antigens of the group A streptococci have contributed greatly to our knowledge about molecular mimicry, autoimmunity, and infection and the antibody molecules involved. The antibody molecules described as polyreactive or cross-reactive indicate recognition of multiple antigens. As described, the basis of the immunological cross-reactions may be identical or homologous amino acid sequences shared between two different proteins or may be epitopes shared between two entirely different chemical structures.

A theme in molecular mimicry suggests antibody recognition of an internal biomarker, often a structural protein, which cross-reacts with the streptococcal antigen and a cell surface antigen leading to cytotoxicity or to functional cell signaling ([6](#), [7](#)). Through these mechanisms, tissue is altered and T cells are recruited to the site of attack ([48](#)).

HISTORICAL PERSPECTIVE

Cross-reactive antigens and antibodies were first found to be associated with acute rheumatic fever (ARF) and group A streptococci when it was discovered that rheumatic fever sera or anti-group A streptococcal antisera reacted with human heart or skeletal muscle tissues ([15](#), [17](#), [19](#)). Antibodies against group A streptococci in the serum of rheumatic fever patients were shown to be absorbed from the sera with human heart extracts, and in contrast, the antiheart antibodies were shown to be absorbed with group A streptococci or streptococcal membranes ([49](#), [50](#)). Rabbit antisera produced against group A streptococcal cell walls reacted with human heart tissue, and antibodies produced against human heart tissue reacted with group A streptococcal antigens ([49](#), [51](#), [52](#)). In rheumatic fever, heart-reactive antibodies appeared to persist in patients with rheumatic recurrences, and there appeared to be a relationship between high titers of antiheart antibodies and recurrence of rheumatic fever ([15](#), [19](#)). Heart-reactive antibodies were reported to decline within 5 years of the initial rheumatic fever attack.

Subsequent research on this early work by Kaplan and Suchy ([49](#)) implicated the streptococcal cell wall containing the M protein as the cross-reactive antigen recognized by the antiheart antibodies, while studies by Zabriskie and Freimer ([15](#), [17](#)) implicated the streptococcal membrane as the site of the cross-reactive antigen. Beachey and Stollerman ([53](#)) and Widdowson and Maxted ([54](#)–[56](#)) reported an M-associated non-type-specific antigen and an M-associated protein, respectively, which were thought to be associated with immunological cross-reactions in ARF. In 1977, van de Rijn et al. demonstrated that highly purified peptides from group A streptococcal membranes reacted with the antiheart antibodies in rheumatic fever serum ([57](#)). Taken together, this evidence suggested that cross-reactive antigens were located in both the cell wall and membrane of the group A streptococci.

Evidence also supported the hypothesis that the group A polysaccharide was a cross-reactive antigen ([58](#)). Goldstein and colleagues showed that glycoproteins in heart valves contained the *N*-acetyl-glucosamine determinant that they proposed to be responsible for immune cross-reactivity of group A streptococci with heart tissues. Dudding and Ayoub demonstrated the persistence of anti-group A carbohydrate antibody in patients with rheumatic valvular disease ([59](#)). Lyampert in Russia also published studies suggesting that the group A streptococcal polysaccharide antigen induced responses against host tissues ([60](#)–[62](#)). McCarty suggested that the terminal O-linked *N*-acetyl-glucosamine might cross-react with antibodies against the group A carbohydrate and host tissues ([63](#)). Additional evidence suggested that the hyaluronic acid capsule of the group A streptococcus

**TABLE 1** Types of immunological mimicry (cross-reactivity)

Identical amino acid sequences in different proteins
Similar protein structures shared among different proteins
Diverse molecules such as DNA, carbohydrates, and proteins

also might induce responses against joint tissues ([64](), [65]()). Administration of a peptidoglycan-polysaccharide complex prepared from group A streptococci to rats induced carditis and arthritis ([66]()–[71]()). The studies in the 1960s and 1970s left little doubt that group A streptococci induced autoantibodies against the heart and other host tissues and that streptococcal components could induce inflammatory lesions resembling arthritis and carditis in animal tissues.

CROSS-REACTIVE mAbs RECOGNIZE CARDIAC MYOSIN AND ALPHA-HELICAL COILED-COIL MOLECULES IN HEART AND BRAIN

mAbs cross-reactive with group A streptococci and human heart tissues were produced from mice immunized with streptococcal cell wall and membrane components ([4](), [33](), [40](), [72]()) and from rheumatic carditis patients ([35]()). Figure 1 illustrates the cross-reactivity of an antistreptococcal mAb with myocardium. In 1985, Krisher and Cunningham identified myosin as a cross-reactive host tissue antigen that provided the link between streptococci and the heart ([4]()). In 1987, it was demonstrated that cardiac myosin could induce myocarditis in genetically susceptible mice ([73]()). Myosin is an alpha-helical coiled-coil molecule that was shown to be an important tissue target of the cross-reactive mAbs ([72]()). Other host tissue antigens recognized by antistreptococcal mouse and human mAbs included the alpha-helical coiled-coil molecules tropomyosin ([29](), [40]()), keratin ([29](), [45](), [74]()),

FIGURE 1 Reaction of mouse antistreptococcal mAb with a human tissue section of myocardium in a indirect immuno-fluorescence assay. Mouse IgM (20 µg/ml) was unreactive (not shown). mAbs were tested at 20 µg/ml. (Taken from reference [40]() with permission from the *Journal of Immunology*.)
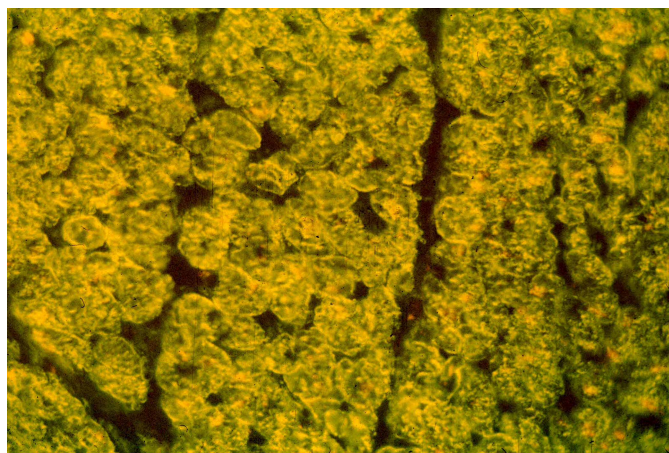
vimentin ([36]()), laminin ([6](), [30](), [75]()), DNA ([36]()), and *N*-acetyl-β-D-glucosamine ([45]()–[47]()), the immunodominant epitope of the group A polysaccharide. A summary of the cross-reactive antistreptococcal mAb host tissue targets is provided in Table 2. The tissue targets identified by the cross-reactive mAbs may be important in the manifestations of group A streptococcal rheumatic fever sequelae of arthritis, carditis, chorea, and erythema marginatum ([76](), [77]()). A summary of the cross-reactive human and mouse mAb specificities has been published in previous reviews ([78]()–[81]()).

The cross-reactive antibodies were divided into three major subsets based on their cross-reactivity with (i) myosin and other alpha-helical molecules, (ii) DNA, or (iii) *N*-acetyl-glucosamine. Figure 2 illustrates the subsets of cross-reactive antistreptococcal/antimyosin mAbs. All three subsets were identified among mAbs from mice immunized with group A streptococcal components, but in humans, the predominant subset reacted with the *N*-acetyl-glucosamine epitope and myosin and related molecules. This result is not surprising, since rheumatic fever patients do not develop antinuclear antibodies during the course of their disease. Elevated levels of poly-reactive antimyosin antibodies found in ARF sera ([35]()) and in animals immunized with streptococcal membranes or walls ([17](), [49](), [50](), [52]()) most likely account for the reactivity of these sera with myocardium and other tissues. Similar types of cross-reactive mouse and human antibodies have been investigated by Lange and colleagues ([82]()) and by Young and colleagues ([83]()), respectively.

Poly-specific or cross-reactive autoantibodies have emerged as a theme in autoimmunity and molecular mimicry ([36](), [84]()–[86]()). The V-D-J region genes of the human and mouse cross-reactive mAbs have been sequenced ([29](), [30](), [87]()), but there is no consensus sequence to explain the molecular basis of poly-specificity and cross-reactivity. It is worth noting that the three groups of reactivities in mice do not have specific antibody V gene families or VH and VL gene combinations that correlate with a specific reactivity. However, higher-avidity cross-reactive antimyosin mAb ([87]()) was associated with

TABLE 2 Cross-reactive monoclonal antibody host tissue targets

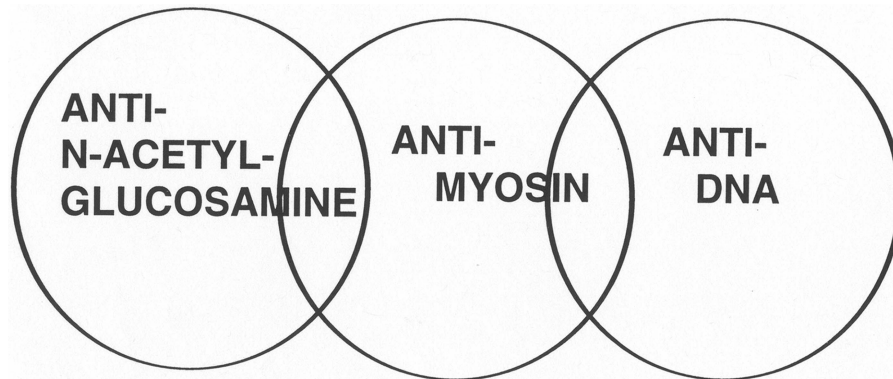| |
|---|
| Cardiac myosin |
| Skeletal myosin |
| Tropomyosin |
| Keratin |
| Vimentin |
| Laminin |

FIGURE 2 Human and murine antistreptococcal/antimyosin mAbs were divided into three subsets based on their reactivity with myosin and *N*-acetyl-glucosamine, the dominant group A carbohydrate epitope, with DNA and the cell nucleus, a property found among murine mAbs, and reactivity with myosin and a family of alpha-helical coiled-coil molecules. (Taken from reference 203 with permission from Indiana University School of Dentistry Press.)

reactivity with laminin and complement-mediated heart cell cytotoxicity (30, 75). The V genes of cross-reactive human mAbs from rheumatic fever were encoded by a heterogeneous group of VH3 family genes (VH-3, VH-8, VH-23, VH-30) and a VH4-59 gene segment (29). Wu et al. reported a similar group of V gene sequences for their human antistreptococcal/antimyosin antibodies produced from Epstein-Barr virus-transformed B cell lines (83). Many sequences were found to be either in germline configuration or were highly homologous with a previously sequenced germline V gene. It has been proposed that a germline antibody may be poly-reactive due to conformational rearrangement and configurational change, permitting binding of diverse molecules (88).

Cytotoxic mouse and human mAbs have identified the extracellular matrix protein laminin as a potential tissue target present in basement membrane surrounding myocardium and valve surface endothelium (6, 30, 75, 89, 90). Cross-reactive antibodies may become trapped in extracellular matrix that may act like a sieve to capture antibody and lead to inflammation in host tissues. The cross-reactive antibodies in rheumatic heart disease, even though they react with cardiac myosin, target valve surface endothelium and laminin (6) as shown in Fig. 3 and 4 and initiate the inflammation at the endocardium, where T cells then target the activated valve endothelium and infiltrate the valve (48) as shown in Fig. 5 and lead to eventual scarring and neovascularization of the normally avascular valve. Vascular cell adhesion molecule-1 (VCAM-1) expression has been found on the valve surface endothelium that attracts very late antigen-4 (VLA-4) on the activated T cell (48).

mAbs IDENTIFY STREPTOCOCCAL CROSS-REACTIVE ANTIGENS

Introduction

The discovery leading to the development of mAbs (91) was transformative for the investigation of auto-antibodies and their specificities, including the cross-

FIGURE 3 Diagram illustrating the potential mechanism of antibody in the pathogenesis of rheumatic heart disease. Cross-reactive antibody is shown binding directly to endo-thelium (top diagram) or binding to basement membrane of the valve (bottom diagram) exposed due to shear stress or damage by antibody and complement. (Reproduced with permission from ASM press).

Potential Mechanisms of Pathogenic Antibodies in Acute Rheumatic Fever
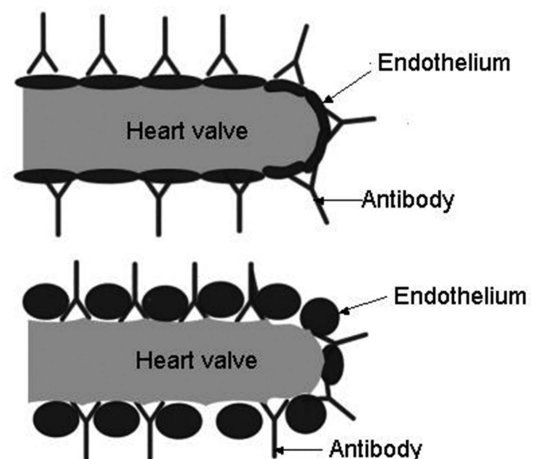
FIGURE 4 Reactivity of antistreptococcal/antimyosin mAb 3.B6 with normal human valve endothelium and myocardium. Formalin-fixed human mitral valve **(A)** and myocardium **(B)** were reacted with mAb 3.B6 at 10 μg/ml. mAb 3.B6 binding was detected using biotin-conjugated antihuman antibodies and alkaline phosphatase-labeled streptavidin followed by fast red substrate. Control sections did not react with human IgM at 10 μg/ml **(C,D)**. (Reproduced with permission from Lippincott Williams and Wilkins).

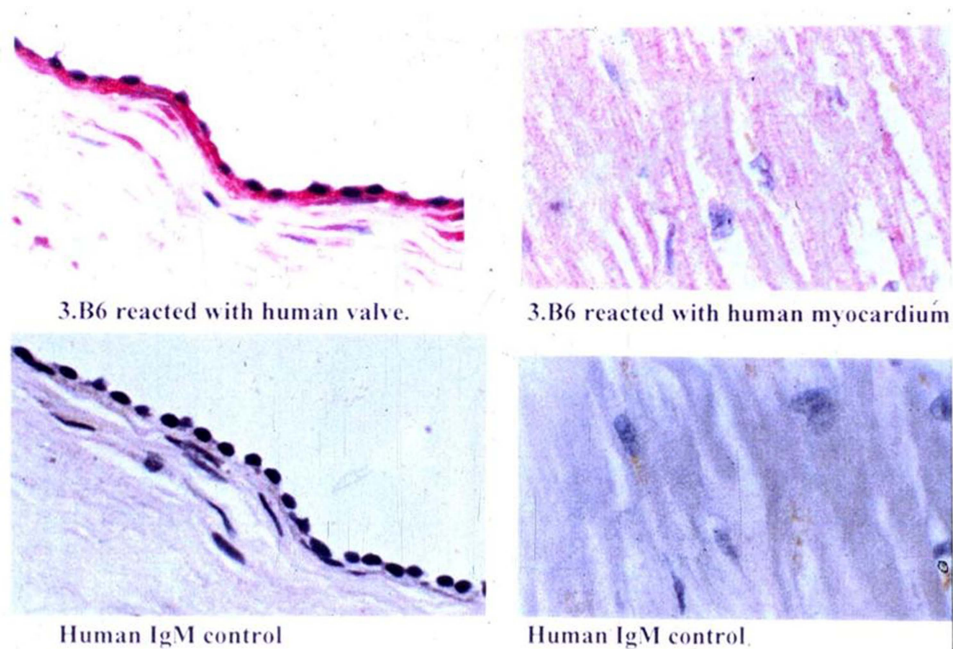reactivities of the antibodies of the group A streptococci (4). Because a single mAb can represent a minor specificity and not the antibodies represented in the disease, the sera from patients with the disease should be investigated to prove that the mAbs reflect the disease and potentially its pathogenesis. Antimyosin mAbs identified cross-reactive antigens in streptococcal membranes (36, 92) and walls (36). The streptococcal M protein was shown to react with the heart or myosin cross-reactive mAbs (40, 92). In addition, a 60-kDa protein present in the cell membrane (93, 94) was cross-reactive with myosin in the heart, and a 67-kDa protein that was immunologically similar to class II major histocompatibility molecules was also identified (95). The data suggested that cross-reactive antigens were present in both the wall and membrane of the group A streptococci. The data support previous evidence from both Kaplan (49–52, 96) and Zabriskie (15, 17, 19). Although these data were once thought to be conflicting, it is clear that the cross-reactive antibodies recognize more than one antigen in the streptococcal cell. The data previously reported by Goldstein (58) are also supported by the evidence that a subset of cross-reactive mAbs recognized *N*-acetyl-β-D-glucosamine, the immunodominant epitope

of the group A polysaccharide. *N*-acetyl-glucosamine is a major epitope of some of the cross-reactive mouse mAbs and virtually all of the human cross-reactive mAbs investigated (6, 45–47). The studies link together the cross-reactivity of the group A carbohydrate epitope, GlcNAc, human cardiac myosin, and streptococcal M protein. Antigenic redundancy due to cross-reactivity may be an important factor in triggering disease in a susceptible host. The cross-reactive antigens are now seen as separate entities recognized by mAbs that recognize more than one antigen molecule. Thus, the previous studies, which before seemed to be conflicting, were all correct. The use of mAbs has allowed dissection of the group A streptococcal cross-reactive antigens that would not have been possible using polyclonal sera. The following sections provide more evidence about the identification and analysis of the cross-reactive antigens of the group A streptococci. Table 2 summarizes the host tissue protein antigens targeted by antistreptococcal antibodies in disease and following immunization with group A streptococcal components. Table 3 summarizes the group A streptococcal cross-reactive antigens, of which the group A carbohydrate (6) and the M proteins (8, 97–101) are the most well investigated.
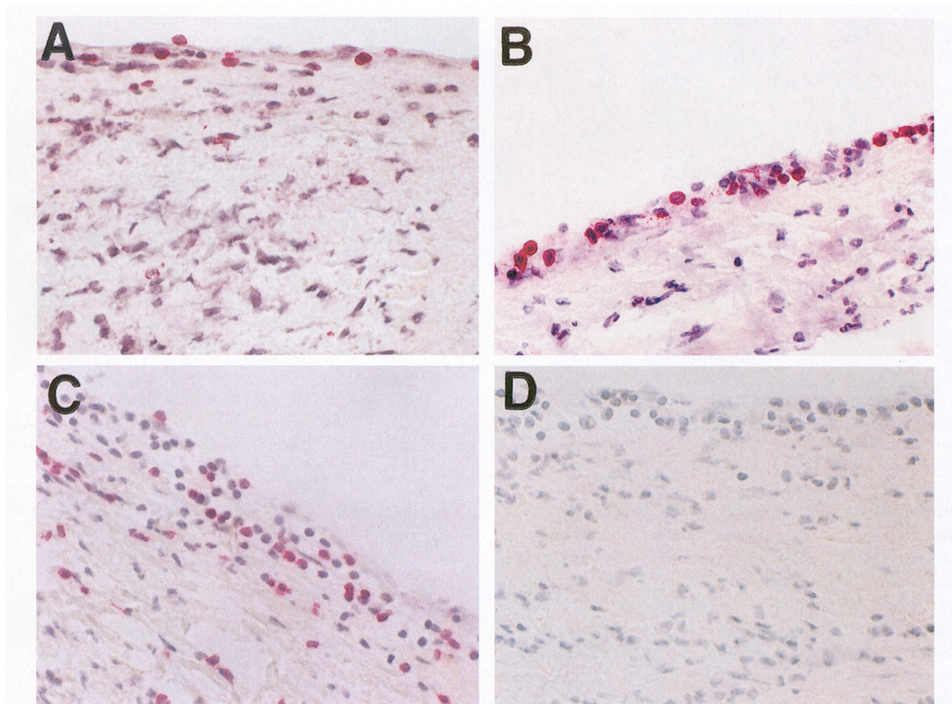
FIGURE 5 Adhesion and extravasation of T lymphocytes into an ARF valve in valvulitis. **(A,B)** Extravasation of CD4$^+$ lymphocytes (stained red; original magnification, 200× and 400×, respectively). **(C)** Extravasation of CD8$^+$ lymphocytes (stained red) into the valve through the valvular endothelium (magnification, 200×). An IgG$_1$ isotype control mAb (IgG$_1$) did not react with the same valve (not shown). (With permission from the *Journal of Infectious Diseases*, Chicago Press).

M Proteins and Rheumatic Fever

Investigation of the M proteins has provided important information about the sequence and primary structure of the molecule. The hypothesis that M proteins and myosin have immunological similarities was supported by the structural studies of Fischetti and colleagues ([102](#)–[105](#)) that demonstrated the seven-amino-acid-residue periodicity that is common among group A streptococcal M proteins and shared with proteins such as tropomyosin, myosin, desmin, vimentin, and keratin. [Figure 6](#) illustrates the seven-residue periodicity and the homology characteristic of alpha-helical coiled-coil proteins such as tropomyosin and myosin. The cross-reactivity between group A streptococci and myosin was first identified by Krisher and Cunningham using mouse mAbs from mice immunized with group A streptococcal anti-

TABLE 3 Cross-reactive antigens of group A streptococci

M proteins
Hyaluronic acid capsule
N-acetyl-glucosamine/group A polysaccharide
60-kDa wall-membrane antigen
67-kDa antigen

gens ([4](#)). Subsequent studies by Dale, Beachey, Bronze, and colleagues using polyclonal sera and affinity purified antibodies further demonstrated immunological cross-reactivity between M proteins and myosin ([37](#)–[39](#), [106](#)). Studies using the cross-reactive mAbs also identified immunological cross-reactivity between streptococcal M proteins (both PepM [the N-terminal half of the molecule] and recombinant molecules) and cardiac and skeletal myosins ([31](#), [36](#), [40](#)).

Since streptococcal M proteins have been investigated for potential epitopes that were recognized by the cross-reactive mAbs, affinity purified antimyosin antibodies from ARF sera were reacted with peptides of streptococcal M5 protein. Affinity purified antimyosin antibodies from ARF reacted with an M5 amino acid sequence (residues 184 to 188) near the pepsin cleavage site (near the center of the molecule) in M5 and M6 proteins ([40](#)). The epitope, located in the B repeat region of M5 and M6 proteins, appeared to be a B cell epitope for antibody-mediated cross-reactivity with myosin ([32](#), [40](#)). An M5 peptide, containing the B repeat region epitope Gln-Lys-Ser-Lys-Gln (QKSKQ) was shown to inhibit antimyosin antibodies in ARF ([40](#)). Furthermore,

```
            bc    g     e        ef    c  e    bc
            ||    |     |        ||    |  |    ||
                310         320          330         340
            abcdefgabcdefgabcdefgabcdefgabcdefgabcdefg
M6.1        LEEANSKLAALEKLNKELEESKKLTEKEKAELQAKLEAEAKA
            ::...::. .:.   .   ::. .:.:  . :::...:.. ...
Tropomyosin YEEVARKLVIIESDLERAEERAELSEGKCAELEEELKTVTNN
                170         180          190         200
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FIGURE 6 Sequence alignment of streptococcal M6 protein and human cardiac tropomyosin in a region exhibiting significant homology. Lowercase letters a to g directly above the sequence designate the position of these amino acids within the seven-residue periodicity in both segments. Lowercase letters at the top of the figure designate identities at external locations in the heptad repeat. Double dots indicate identities, and single dots indicate conservative substitutions. Within this segment of the streptococcal M6 molecule, 31% homology is observed with tropomyosin. Since both molecules are alpha-helical coiled-coil proteins, they contain the seven-residue repeat pattern where positions a and d are usually hydrophobic. Similar homologies are seen between M proteins and myosin heavy chains and any of the three laminin chains. (Reproduced from reference 40 with permission from the *Journal of Immunology*.)

an M5 peptide that contained the QKSKQ sequence induced antibodies in BALB/c mice against cardiac or skeletal myosins and the light meromyosin (LMM) fragment of myosin. This evidence further supported the previous findings indicating that the QKSKQ sequence is important in the antibody-mediated cross-reactions with myosin in ARF (40). M5 residues 164 to 197 were demonstrated to induce antibodies against sarcolemmal membrane of heart tissue (107), and M5 residues 84 to 116 induced heart-reactive antibody and reacted with antimyosin antibody purified from ARF sera (40, 107). Studies by Kraus and colleagues (42) demonstrated a vimentin cross-reactive epitope present in the M12 protein, and M protein peptides containing brain cross-reactive epitopes were localized to the M5 protein sequence 134 to 184 and the M19 sequence 1 to 24 (98, 99). A summary of the currently known myosin cross-reactive B cell epitopes in M5 protein is shown in Fig. 7, which illustrates the A, B, and C repeat regions of M protein.

Immunization of BALB/c mice with each of 23 overlapping synthetic peptides of M5 protein has revealed that the M5 peptides NT3-NT7, B2B3B, and C1A-C3 induce anti-human cardiac myosin antibodies as shown

in Fig. 8. Similar overlapping synthetic peptides spanning the M5 protein molecule have been reported elsewhere (32). Titers of the anti-M5 peptide sera were 10 times greater against cardiac myosin than against skeletal myosin, tropomyosin, vimentin, and laminin

FIGURE 7 Antimyosin antibody cross-reactive sites in M5 protein A, B, and C repeat regions of the molecule. Arrows point to sites determined to induce human cardiac myosin cross-reactive antibody (32). The asterisk (*) marks the location of peptide NT4 containing several repeats of an epitope in cardiac myosins that causes myocarditis in MRL/++ and BALB/c mouse strains (32, 116). Site QKSKQ is the epitope determined to react with antimyosin antibody in ARF sera (40). (Reproduced from *Effects of Microbes on the Immune System* with permission from Lippincott Williams and Wilkins.)
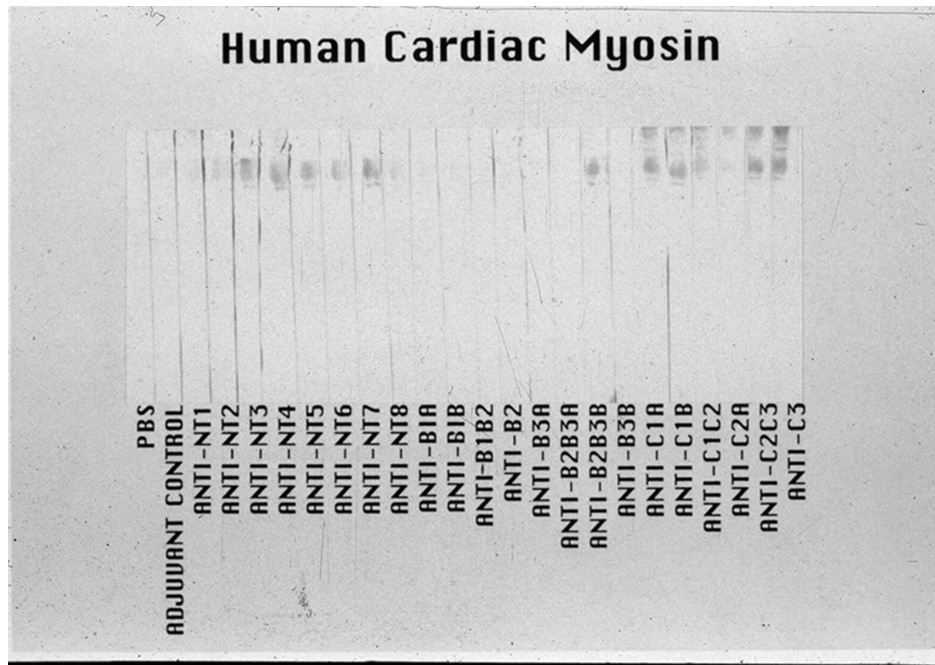
FIGURE 8 Reactivity of the antistreptococcal M5 peptide sera in a Western immunoblot of human cardiac myosin. The 200-kDa protein band of the purified human cardiac myosin, shown in the stained portion of the Western blot, reacted most strongly with antipeptide sera from mice immunized with the M5 peptides NT3-7, B2B3B, C1A, C1B, C2C3, and C3. Sera were tested at a 1:1000 dilution. A control antimyosin mAb CCM-52 (a gift from William Clark, Cardiovascular Research Institute, Michael Reese Hospital and Medical Center, Chicago, IL) reacted with the 200-kDa band present in our purified preparation of human cardiac myosin heavy chain. Purification of the human cardiac myosin heavy chain to homogeneity was previously described by Dell et al. ([204]). The Western blot confirms the data seen in the enzyme-linked immunosorbent assay with human cardiac myosin. (Reproduced from reference [32] with permission from ASM Press.)

([32]). Certain M5 peptides appeared to induce a cardiac myosin-directed response in BALB/c mice. Furthermore, BALB/c mice developed mild myocarditis when immunized with M5 peptides NT4, NT5, NT6, B1A, and B3B ([32]). Peptides from the C-repeat region of M5 protein did not produce myocardial lesions when administered to BALB/c mice.

The C-repeat region of class I M proteins contains the class I epitope, which is identified by reactivity with anti-M protein mAb 10B6 ([108]–[111]). Class I M protein serotypes were streptococcal strains associated with pharyngitis and rheumatic fever. Responses against the class I epitope were stronger in patients with rheumatic fever than in those with uncomplicated disease ([112], [113]). mAb 10B6, which recognizes the C-repeat epitope, also reacted with skeletal and cardiac myosins and the heavy meromyosin subfragment of myosin ([112]). The C-repeat class I epitope in M5 contains the amino acid sequence KGLRRDLDASREAK, which shares homology with RRDL, a conserved amino acid sequence found in cardiac and skeletal myosins. The myosin sequence RRDL is located in the heavy meromyosin subfragment of the cardiac and skeletal myosin heavy chain ([112]).

It was reported that reactivity of IgG from ARF sera was greater to the class I peptide sequence than IgG from uncomplicated pharyngitis ([112]). This was confirmed in a study by Mori and colleagues ([114]). However, the lower immune response to the class I epitope in uncomplicated pharyngitis most likely occurred because the patients with uncomplicated pharyngitis had been treated with penicillin ([115]). Antibodies against the class I epitope were affinity purified from ARF sera and shown to react with myosin ([112]).

Studies by Huber and Cunningham demonstrated that streptococcal M5 peptide NT4 containing the amino acid sequence GLKTENEGLKTENEGLKTE could produce myocarditis in MRL/++ mice, an autoimmune-prone strain ([116]). The studies demonstrated that the myocarditis was mediated by CD4$^+$ T cells and class II major histocompatibility complex molecules. Antibodies against the IA^k major histocompatibility molecule or antibody against CD4$^+$ T cells abrogated the myocarditis ([116]). Amino acid sequence homology between the NT4 peptide of M5 and human cardiac myosin demonstrates 80% identity. [Figure 9] illustrates the homology between the M5 peptide NT4 sequence and the cardiac myosin

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Streptococcal M Protein M5 Peptide NT4        GLKTENEG
                                              : :::
                        cardiac myosin        KLQTENGE
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FIGURE 9 Sequence homology between human cardiac myosin and peptide NT4 that causes myocarditis (32, 119). The homologous sequence repeats four times in the streptococcal M5 protein and in NT4 and once in cardiac myosin. Repeated sequences in M proteins that mimic cardiac myosin may be important in inducing inflammatory heart disease. (Adapted from reference 119 with permission from the *Journal of Immunology*.)

sequence. The mimicking sequence in NT4 is repeated four times in the M5 protein and is present in myosin only once. Repeated regions of the M protein that mimic cardiac myosin may be important in breaking tolerance in the susceptible host and producing autoimmune disease. The data support the hypothesis that epitopes in streptococcal M5 protein that mimic cardiac myosin may be important in breaking tolerance to this potent auto-antigen. Table 4 summarizes the M protein amino acid sequences observed to induce inflammatory myocardial lesions in mice.

Streptococcal M proteins not only mimic epitopes in cytoskeletal proteins, but they mimic epitopes in other strong bacterial and viral antigens, namely, heat shock protein (Hsp-65) (117) and coxsackie viral capsid proteins (31, 116, 118, 119). These immunological cross-reactions may be vital to the survival of the host and suggest that antibody molecules may recognize and neutralize more than a single infectious agent. Such antibodies may be an important first line of defense and would be highly advantageous for the host. Multiple pieces of evidence from experiments using synthetic peptides have shown that sites in the M protein mimic a site(s) in the VP1 capsid protein of coxsackie virus (31, 120). Further evidence demonstrated that some of the antistreptococcal/antimyosin mAbs neutralized enteroviruses and were cytotoxic for heart cells (31). This was extremely interesting because coxsackie viruses cause autoimmune myocarditis in susceptible hosts (121–

123). The Hsp-65 antigen has been shown to play a role in the development of arthritis and diabetes (124, 125). Cross-reactive epitopes shared between streptococcal M proteins and Hsp-65 play a role in arthritis sequelae. Antibodies against heat shock protein 60 have been implicated in cytotoxicity against endothelium (126). Shared epitopes among pathogens may be important in molecular mimicry, may break tolerance to cryptic host molecules, and may influence the development of autoimmune diseases. Table 5 summarizes bacterial and viral proteins that have immunological similarities with M protein.

Identification of the Molecular Basis of Mimicry in the Streptococcal M Protein

Studies were undertaken to crystallize a portion of the streptococcal M1 protein (127) to evaluate its virulence properties when portions of the amino acid sequence were mutated, but also to investigate regions and structures responsible for eliciting or reacting with cross-reactive antibodies that participate in molecular mimicry. The structural study revealed irregularities and instabilities in the coiled coil of the M1 fragment crystal. Similar structural instabilities and irregularities occur in myosin and tropomyosin that had previously been demonstrated to cross-react with the streptococcal M proteins (37–40). Stabilization of the irregularities of the M protein structure, by creating mutants of the M protein gene to alter specific amino acids, enhanced the stability of the coiled coil and diminished the virulence properties of the M1 protein, including decreased cross-reactivity with autoantibodies that cross-reacted with heart tissues, cardiac myosin, and group A streptococcal M proteins (127). Loss of the heart or tissue cross-reactivity of the streptococcal M protein improves its potential for use as a vaccine antigen.

TABLE 4 M5 protein sequences that produce myocarditis in mice[a]

Peptide	Amino acid sequence	Residues
A repeat region		
NT4	GLKTENEGLKTENEGLKTE	40-58
NT5	KKEHEAENDKLKQQRDTL	59-76
NT6	QRDTLSTQKETLEREVQN	72-89
B repeat region		
B1A	TRQELANKQQESKENEKAL	111-129
B3B	GALKQELAKKDEANKISD	202-219

[a]NT4 produced myocarditis in BALB/c (32) and MRL/++ (116) mice. The other peptides shown produced myocarditis in BALB/c mice. (Reproduced from *Effects of Microbes on the Immune System* with permission from Lippincott Williams and Wilkins.)

TABLE 5 Bacterial and viral antigens with immunological similarities with group A streptococcal M proteins

Heat shock protein 65
Coxsackievirus VP1, 2, 3 capsid proteins
Group A streptococcal carbohydrate

M Proteins and Cross-Reactive T Cells

M proteins have also been shown to stimulate human T lymphocyte responses (97, 128–135), including cytotoxic T lymphocytes from ARF patients (128, 136). PepM protein (pepsin-extracted M protein fragment) has been reported to be a superantigen that stimulated Vβ2, Vβ4, and Vβ8 bearing T cell subsets (133–135, 137). Other reports show that neither recombinant nor native forms of M proteins are superantigenic (138–140). A superantigenic site reported for the M5 protein has been localized to the amino acid sequence located in M5 residues 157 to 197 (KEQENKETIGTLKKILD ETVKDKLAKE QKSKQNIGALKQEL) in the B3 repeat region (137). The site has a significant amount of sequence homology with other superantigens. The role of M protein or other superantigens in ARF may be to activate large numbers of T cells, including some that are cross-reactive and may lead to ARF.

Studies of T cell epitopes in rheumatic fever and in animal models focus on the M5 protein molecule because serotype M5 has often been associated with ARF outbreaks (141). T and B cell epitopes of the M5 protein were defined in earlier studies by Robinson and colleagues (131, 132), by Good and colleagues (129, 130), and in my laboratory (32).

Guilherme et al. isolated cross-reactive T cells from mitral valves, papillary muscle, and left atrium of rheumatic fever patients previously infected with M5 group A streptococci (97). T cell lines were responsive to several peptides of the streptococcal serotype M5 protein and proteins from heart tissue extracts. Sequences from the A and B repeat regions of M5 protein that stimulated the valvular T cells are shown in Table 6. In BALB/c mice using synthetic peptides, myosin-cross-reactive T cell epitopes from the A, B, and C repeat regions of the M5 protein were identified (32). Six dominant myosin cross-reactive T cell epitopes were located in the M5 molecule (32). Table 6 summarizes (i) the dominant myosin cross-reactive T cell epitopes of M5 protein in BALB/c mice (32), (ii) the M5 sequences recognized by T cell clones from rheumatic heart valves (97), and (iii) the M5 peptides reported by Good and colleagues to stimulate human T cells from normal controls and rheumatic fever patients that were responsive to myosin peptides (129, 130). An important correlation seen in Table 6 indicates that M5 peptides NT4/NT5 (GLKTENEGLKTENEGL KTE and KKEHEAENDKLKQQRDTL) and B1B2/B2 (VKDKIAKEQENKETIGTL and TIGTLKKILDETVK DKIA), which were dominant cross-reactive T cell epitopes in the BALB/c mouse, contain sequences similar to those reported to be recognized by T cells from rheumatic valves (97). Peptides NT4 and NT5 produced inflammatory infiltrates in the myocardium of animals immunized with those peptides (32). The collective evidence on cross-reactive T cells suggests that amino acid sequences in M5 protein that share homology with cardiac myosin may break tolerance and promote T cell-mediated inflammatory heart disease in animals and humans (32).

T cells isolated and cloned from the peripheral blood or from human rheumatic mitral valves of human rheumatic heart disease patients recognized streptococcal M5 peptides and LMM peptides (21, 22, 25). The cross-reactive T cells taken from ARF and RHD rheumatic heart disease heart valves mainly recognize three

TABLE 6 Summary of myosin or heart cross-reactive T cell epitopes of streptococcal M5 protein[a]

Peptides	Sequence[a]	Origin of T cell clone or response[c]
1–25	TVTRGTISDPQRAKEALDKYELENH[b]	ARF/valve (142)
81–96	DKLKQQRDTLSTQKETLEREVQN[b]	ARF/valve (142)
163–177	ETIGTLKKILDETVK[b]	ARF/valve (142)
337–356	LRRDLDASREAKKQVEKAL	Normal/PBL (130)
347–366	AKKQVEKALEEANSKLAALE	Mice (129)/normal PBL (130)
397–416	LKEQLAKQAEELAKLRAGKA	ARF/PBL (130)
NT4 40–58	GLKTENEGLKTENEGLKTE	BALB/c/Lymph node[d]
NT5 59–76	KKEHEAENDKLKQQRDTL	
B1B2 137–154	VKDKIAKEQENKETIGTL	
B2 150–167	TIGTLKKILDETVKDKIA	
C2A 254–271	EASRKGLRRDLDASREAK	
C3 293–308	KGLRRDLDASREAKKQ	

[a]Taken from reference 32 with permission from ASM press.
[b]The amino terminal TVTRGTIS sequence was taken from the M5 amino acid sequence published by Manjula et al. (201) and deviates from the M5 sequence published by Miller et al. (202) at positions 1 and 8. Other marked sequences ([81–96] and [163–177]) were taken from the PepM5 sequence as reported by Manjula (201). These two sequences are found in the sequence as 67–89 and 174–188, respectively, as reported by Miller et al. (202). All other sequences shown above are from the M5 gene sequence reported by Miller et al. (202).
[c]PBL, peripheral blood lymphocytes, ARF, acute rheumatic fever.
[d]BALB/c mice immunized with purified human cardiac myosin and the recovered lymph node lymphocytes were stimulated with each of the peptides in tritiated thymidine uptake assays.

regions (residues 1 to 25, 81 to 103, and 163 to 177) of the amino-terminal region of M5 protein, several valve-derived proteins, and peptides of the β-chain of the human cardiac LMM region, which is the smaller of two subunits produced by tryptic digestion of human cardiac myosin ([21], [22], [25], [97]). Mimicry between M proteins and cardiac myosin stimulates cross-reactive T cells in the peripheral blood of the host ([25], [142], [143]). The T cells then travel to the valve once the endothelium of the valve becomes activated and inflamed ([48]). This extravasation event at the valve endocardium allows the cross-reactive T cells to enter the valve, where they recognize and proliferate to valvular proteins such as vimentin ([89]), which may include cardiac myosin from papillary muscle. T cells that remain in the valve survive if they continue to be stimulated by host alpha helical proteins within the valve. The activated cross-reactive T cells when continually stimulated within the valve become pathogenic for the host and produce the TH1 mechanism of pathogenesis with scarring in the valve. Figure 10 illustrates the events in rheumatic carditis that result from immune responses against the cross-reactive antigens of the group A streptococci ([144]).

The granulomatous Th1 cell response and the presence of interferon-γ (IFNγ) have been reported as dominant in rheumatic valves ([145]). Studies of rheumatic heart disease have suggested that Th17 cells in peripheral blood are elevated in rheumatic heart disease with concomitant downregulation of the Treg phenotype ([146]). In ARF and rheumatic heart disease, elevated numbers of Th17 cells, high interleukin-17A (IL-17A) levels, and decreased T regulatory cells were reported in peripheral blood ([146]). Although less is known about Th17 cell responses in rheumatic heart disease, Th17 cells are important in group A streptococcal infections and have been identified in nasopharyngeal and tonsillar lymphoid tissues in streptococcal infection animal models ([147]–[149]). Different routes of bacterial infection may induce long-lived Th1 memory cells and short-lived Th17 cell responses ([148]). IL-17 triggers effective immune responses against extracellular bacteria, such as promoting neutrophil responses ([150]–[153]).

The Lewis rat model of rheumatic heart disease also suggests the presence of IL-17A in the pathogenesis of experimental valvulitis induced by immunization with group A streptococcal antigens ([13], [101]). This finding does not preclude Th1, which is often found in tandem with Th17 responses in autoimmune disease models and in human autoimmune diseases ([148]). Although both Th1 and Th17 are proinflammatory, Th17 responses have been found to be more damaging in the chronic
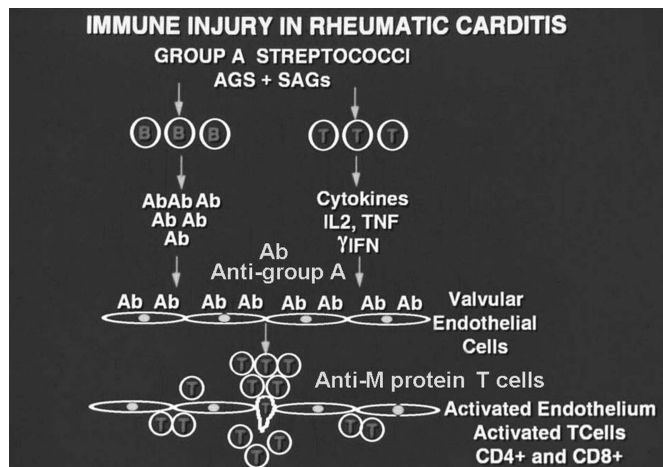

FIGURE 10 Diagram representing the proposed immuno-pathogenesis of poststreptococcal rheumatic heart disease. Initially, B and T cells are activated by specific streptococcal antigens and superantigens, leading to strong immune responses against streptococcal and host antigens. The development of pathogenic clones of B and T lymphocytes is important in the development of the disease. Initially, antibodies develop against the group A carbohydrate, which are cross-reactive with the valve surface, and glycoproteins such as laminin and bind to the valve surface endothelium (endocardium), leading to inflammation and 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 ([48]). The formation of scar tissue in the valve followed by neovascularization allows the disease to continue in the valve. The specificity of the T cells in blood ([25]) and T cells entering the valve have been shown to react to M protein ([22], [97], [144]). T cell subsets include Th1 (IFNγ) ([145]) in the pathogenesis of proinflammatory responses and the development of the scarred and fibrotic valve. IL-17A has also been associated with rheumatic heart disease in humans and animal models, suggesting that Th17 cells are involved in disease ([101], [146], [149]).

stages of disease leading to fibrosis, and IL-17A was dispensable in the earlier proinflammatory stages of myocarditis in mice ([154]) but promoted fibrosis in later stages and was associated with heart failure in humans ([155]).

A New Model of Valvular Heart Disease Induced by Group A or Group G Streptococcal M Proteins in the Lewis Rat

A new model of experimental autoimmune valvulitis and rheumatic heart disease was developed by Quinn et al. by immunization of the Lewis rat model with the recombinant M6 protein ([23]). The lesions that developed demonstrated characteristics of rheumatic heart disease in humans with verrucae (Fig. 11C) ([23]),
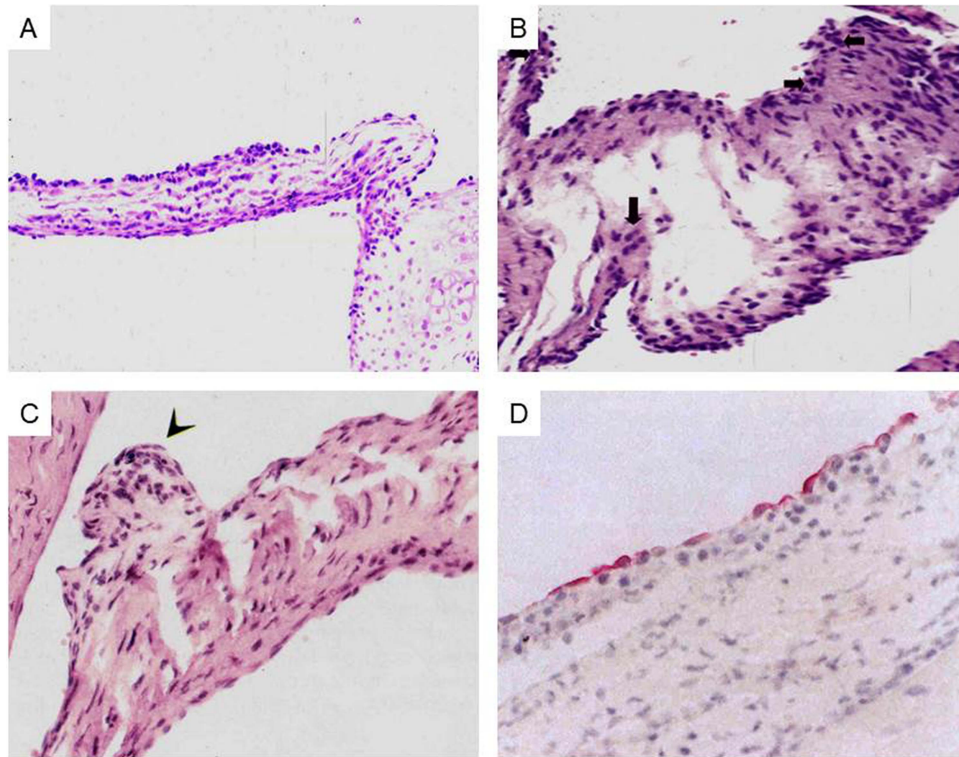
FIGURE 11 **(A)** Induction of valvulitis and cellular infiltration in hematoxylin- and eosin-stained heart valves from Lewis rats immunized with group A streptococcal M5 peptides NT1 to NT4/5 (AVTRGTINDPQRAKEALD amino acid [aa] residues 1 to 18; NT-2 KEALDKYELENHDLKTKN aa residues 14 to 31; NT-3 LKTKNEGLKTENEGLKTE aa residues 27 to 44; NT-4 GLKTENEGLKTENEGLKTE aa residues 40 to 58; NT-4/5 GLKTEKKEHE AENDKLK aa residues 54 to 70). (Taken from Kirvan et al. [8] with permission from the *Journal of Translational Cardiology*.) **(B)** Induction of valvulitis, edema, and cellular infiltration in hematoxylin- and eosin-stained heart valves from Lewis rats immunized with group A streptococcal M5 peptides NT1 to NT4/5 (AVTRGTINDPQRAKEALD aa residues 1 to 18; NT-2 KEALDKYELENHDLKTKN aa residues 14 to 31; NT-3 LKTKNEGLKTENEGLKTE aa residues 27 to 44; NT-4 GLKTENEGLKTENEGLKTE aa residues 40 to 58; NT-4/5 GLKTEKKEHEAENDKLK aa residues 54 to 70). (Taken from Kirvan et al. [8] with permission from the *Journal of Translational Cardiology*.) **(C)** Verrucous nodule observed on Lewis rat valve after immunization with group A streptococcal rM6 protein. (Taken from Quinn et al. [23] with permission from *Infection and Immunity*.) **(D)** VCAM-1 expressed on rheumatic valve. (Taken from Roberts et al. [48] with permission from the *Journal of Infectious Diseases*.)

Anitschkow cells, and edema with cellular infiltration into the endocardium of the valve (Fig. 11A and B) (8, 23). T cell lines from the model proliferated to both the rM6 protein and cardiac myosin. The model appeared to be very similar in appearance to the VCAM-1 activation (Fig. 11D) in humans and infiltration of CD4+ T cells into the VCAM-1 positive endothelium/endocardium (not shown) of the valve in rheumatic heart disease (48) (Fig. 12). More recent studies identified A and B region peptide epitopes of M5 protein that induced valvulitis in the Lewis rat (8). Repeat region peptides NT4, NT5/6, and NT7 (see Table 6) induced valvulitis similar to the

intact pepsin fragment of M5 protein, PepM5. T cell lines from rats with valvulitis proliferated to M5 peptides NT5/6 and NT6. Passive transfer of an NT5/6-specific T cell line into naive rats produced valvulitis characterized by infiltration of CD4+ cells and upregulation of VCAM-1 in valvular endothelium, while another NT6-specific T cell line did not target the valve (8). Thus, the infiltrating T cell line appeared to be important in the induction of inflammation in the valve.

Recent studies have used the Lewis rat model to demonstrate the induction of valvular heart disease by the C repeat region peptides of group A streptococcal
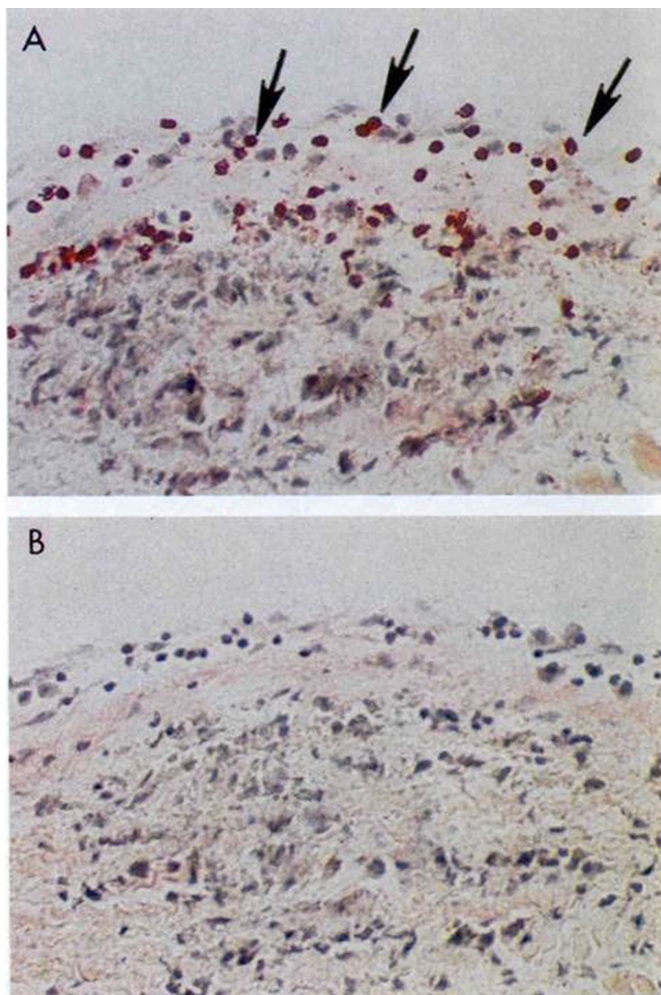
FIGURE 12 Extravasation of CD4$^+$ lymphocytes into valve above Aschoff's body in the subendocardium of the left atrial appendage. Original magnification, 200×. **(A)** Stained with anti-CD4 Mab; **(B)** stained with antibody isotype control. (Taken from Roberts et al. [48]).

M5 protein (12, 13). T cells from the model proliferated to M protein and cardiac myosin. Further studies by Ketheesan and colleagues demonstrated that exacerbation and increasing severity of valvulitis followed repeated group A streptococcal immunizations with group A streptococcal M protein (156). Lewis rats were immunized with group A streptococcal recombinant M5 protein and boosted once, twice, or three times. Anti-cardiac myosin antibodies and T cell responses increased with the booster immunizations. More severe lesions were observed in the repetitively immunized Lewis rats (156). Thus, the model demonstrates increased cardiac damage as seen in humans after repeated streptococcal infections. Immunization of the Lewis rat model with M protein from group G streptococci also led to valvular lesions (101). In the experimental autoimmune valvulitis

model, both group A and group G M proteins and cardiac myosin induced proliferation of the T cells. IL-17A and IFNγ were found to be upregulated in the model (101). Thus, roles for IL17A in ARF and rheumatic heart disease are supported by the studies of patients with carditis.
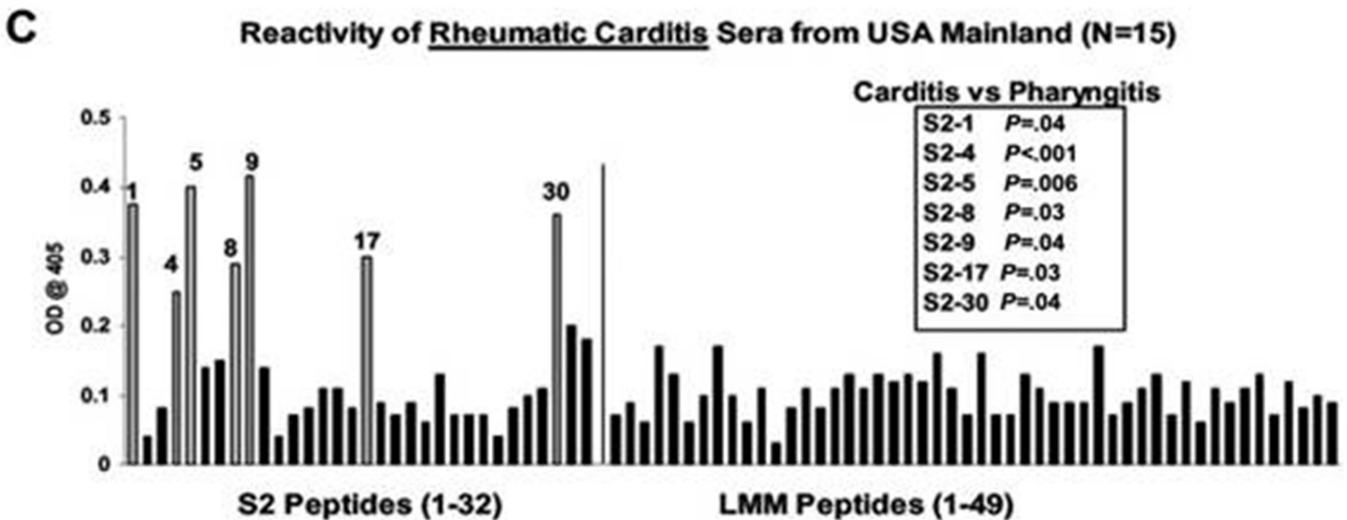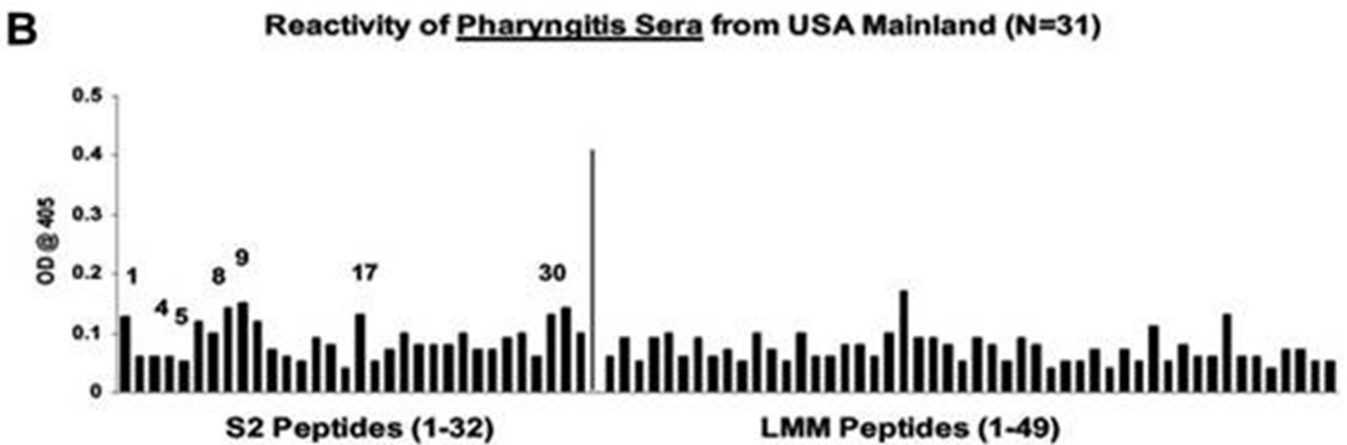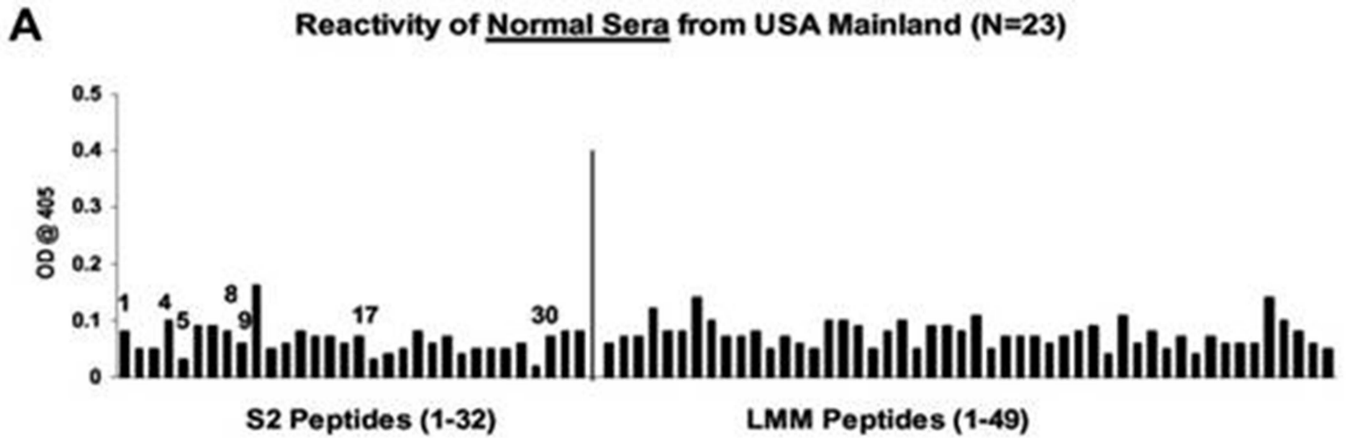
Human Cardiac Myosin Peptide Epitopes in Rheumatic Heart Disease in Human Populations and Their Use in Monitoring Progression of Disease

Studies of humans with rheumatic fever and rheumatic heart disease have identified disease-specific epitopes of human cardiac myosin in the development of rheumatic carditis in humans (24). Immune responses to cardiac myosin were similar in rheumatic carditis among a small sample of worldwide populations, in which serum IgG targeted disease-specific human cardiac myosin epitopes in the S2 subfragment hinge region within S2 peptides containing amino acid residues 842 to 992 and 1164 to 1272 (Fig. 13) (24). Analysis of the anti-cardiac myosin autoantibodies in rheumatic carditis in a Pacific Islander family further confirmed the specific responses against cardiac myosin in rheumatic heart disease and confirmed the presence of potential rheumatogenic epitopes in the S2 region of human cardiac myosin (24). The study suggested that cardiac myosin epitopes targeted in rheumatic carditis were in the S2 hinge region of cardiac myosin and that the anti-cardiac myosin responses were similar among populations with rheumatic carditis worldwide regardless of the infecting group A streptococcal M protein serotype.

Further study of cardiac myosin peptide epitopes revealed that Australian patients with active rheumatic heart disease reacted significantly more to peptides from the S2 or LMM regions of human cardiac myosin than did healthy controls (11). Comparison of serum from active rheumatic heart disease patients to serum from patients treated for over a year with antibiotics revealed that the treatment led to a reduction in the anti-cardiac myosin peptide autoantibodies (11). Thus, identifying the immune response against the cardiac myosin peptides may monitor progression of rheumatic heart disease and may also determine effective treatment with a concomitant decline of the autoantibodies to cardiac myosin.

M Proteins and Poststreptococcal Acute Glomerulonephritis

Kefalides and colleagues showed that sera from patients with acute poststreptococcal glomerulonephritis contained antibodies against laminin, collagen, and other

A Reactivity of <u>Normal Sera</u> from USA Mainland (N=23)

S2 Peptides (1-32)   LMM Peptides (1-49)

B Reactivity of <u>Pharyngitis Sera</u> from USA Mainland (N=31)

S2 Peptides (1-32)   LMM Peptides (1-49)

C Reactivity of <u>Rheumatic Carditis</u> Sera from USA Mainland (N=15)

Carditis vs Pharyngitis

S2-1	$P=.04$
S2-4	$P<.001$
S2-5	$P=.006$
S2-8	$P=.03$
S2-9	$P=.04$
S2-17	$P=.03$
S2-30	$P=.04$

S2 Peptides (1-32)   LMM Peptides (1-49)

S2   LMM

S1

Diagram of Myosin Fragments (S1, S2, and LMM)

macromolecules found in the glomerular basement membrane (157). The epitope recognized in collagen was identified and shown to be located in the 7-S domain of type IV collagen (158). Streptococcal antigens, immunoglobulins, and complement were detected in the kidney glomeruli in acute poststreptococcal glomerulonephritis (159). Studies by Lange and Markowitz suggested that the glomerular basement membrane shared antigens with streptococcal M12 protein (160) and/or between the group A streptococcus and glomeruli (41, 161–163). Kraus and Beachey discovered a renal autoimmune epitope (Ile-Arg-Leu-Arg) in M protein (43). Evidence has suggested that certain M protein serotypes are associated with nephritis and that molecular mimicry or immunological cross-reactivity between glomeruli and M protein could be one mechanism by which antiglomerular antibodies are produced during infection. In animal models of nephritis induced by nephritogenic streptococci (M type 12), antiglomerular antibodies eluted from kidney glomeruli reacted with the type 12 streptococcal M protein (164). Furthermore, an antiglomerular mAb reacted with the M12 protein (163). These studies support immune-mediated mechanisms in poststreptococcal acute glomerulonephritis (AGN). Streptococcal and renal antigens may be an important source of mimicking antigen present in the kidney and play a role in binding immunoglobulin and complement with production of nephritis.

Novel Cross-Reactive Protein Antigens in Group A Streptococci

Antimyosin antibodies from ARF were used to identify group A streptococcal cross-reactive antigens other than M proteins. The cross-reactive antigens were detected in a *Streptococcus pyogenes* strain that had spontaneously lost its *emm* gene. The affinity purified antimyosin antibodies were used to screen gene libraries and the antigen preparations from this M protein-deficient streptococcus. A 60-kDa streptococcal actin-like protein (165), a 60-kDa wall-membrane protein (93, 94), and a 67-kDa protein from a cloned gene product (95) were identified. All of the antigens were unique and interesting. The 60-kDa actin-like molecule behaved biochemically like an actin and produced actin-like filaments observed by electron microscopy (165). The 67-kDa cloned gene product recognized by antimyosin antibody from ARF sera was novel in that it shared enough sequence homology with major histocompatibility molecules that it reacted with sera against mouse major histocompatibility complex class II molecules and exhibited a hydrophilicity plot almost identical to that of major histocompatibility complex class II molecules (95). The gene for the 67-kDa protein was present only in pathogenic streptococcal groups A, C, and G (95). This gene was not found in other streptococcal groups, *Escherichia coli*, or staphylococci when they were screened for hybridization with the gene probe. The role of the 67-kDa protein as a potential virulence factor in the pathogenesis of streptococcal infections or sequelae is not yet known.

IMMUNE RESPONSES TO *N*-ACETYL- β-ᴅ-GLUCOSAMINE, DOMINANT EPITOPE OF GROUP A POLYSACCHARIDE, IN THE PATHOGENESIS OF RHEUMATIC HEART DISEASE AND SYDENHAM'S CHOREA IN ARF

Immunological Cross-Reactivity Between Carbohydrates and Peptides

The structure of the group A polysaccharide is a polymer of rhamnose with *N*-acetyl-β-ᴅ-glucosamine linked

FIGURE 13 Reactivity of serum samples originating from the United States with human cardiac myosin peptides from the S2 and LMM regions in the enzyme-linked immunosorbent assay (1:100 serum dilution). **(A)** Mean reactivity of normal serum samples from control donors with no evidence of streptococcal infection or heart disease on the U.S. mainland against S2 and LMM peptides. These samples rarely reacted with any S2 or LMM peptide at an optical density of 0.2. **(B)** Mean reactivity of serum samples from patients with streptococcal pharyngitis in the United States against S2 and LMM peptides. These samples rarely reacted with any S2 or LMM peptide at an optical density of 0.2. **(C)** Serum immunoglobulin G from patients with rheumatic carditis in the United States. Serum samples from patients with rheumatic carditis from the United States reacted predominantly with peptides S2-1, S2-4, S2-5, S2-8, S2-9, S2-17, and S2-30, compared with the reactivity of serum samples from patients with pharyngitis in the United States against those same peptides (B). **(C)** Unadjusted Mann-Whitney *P* values for the comparison between carditis and pharyngitis on the United States mainland. The comparison for S2-4 is statistically significant on the basis of a two-sided alpha level adjusted to preserve the false-discovery rate at 5%. (Taken from Ellis et al. [24] with permission from the *Journal of Infectious Diseases*).

to the rhamnose backbone. The *N*-acetyl-glucosamine is the immunodominant epitope of the group A carbohydrate that distinguishes *S. pyogenes* from other streptococcal species. The *N*-acetyl glucosamine and the group A polysaccharide have been identified in cross-reactions between streptococci and heart tissues (58, 61, 62). Recently, Shikhman, Adderson, and others reported that a subset of cross-reactive antimyosin/antistreptococcal mAbs from immunized mice and virtually all human mAbs from rheumatic carditis patients reacted strongly with an *N*-acetyl-glucosamine epitope of the group A streptococcal carbohydrate (29, 45–47). Furthermore, synthetic peptides from keratin (45, 47), coxsackie virus (46), and human cardiac myosin (29) reacted with mAbs against *N*-acetyl-glucosamine and myosin as previously described. In addition, a synthetic cytokeratin peptide, SFGSGFGGGY, mimicked *N*-acetyl-glucosamine in its reaction with antibodies to *N*-acetyl-glucosamine and lectins. Mimicry between the group A carbohydrate epitope and peptide molecules is a form of mimicry between chemically diverse molecules. Based on results with peptides altered at a single amino acid residue, it was deduced that aromatic and hydrophobic interactions were important in cross-reactive anticarbohydrate antibody binding to the peptide. Our data as well as that of others (166, 167) clearly link anticardiac myosin and anti-*N*-acetyl-glucosamine responses with antiheart cross-reactivity.

Rheumatic Heart Disease

Dudding and Ayoub described the elevation and persistence of anti-group A polysaccharide antibody in rheumatic valvulitis (59). Studies of the human antistreptococcal/antimyosin mAbs that reacted strongly with *N*-acetyl-glucosamine have revealed that one of the mAbs was cytotoxic for human endothelium and reacted with valvular endothelium in tissue sections of a human valve (6). The cytotoxic mAb recognized the extracellular matrix protein laminin that is part of the basement membrane underlying the valvular endothelium. These data suggest that human antibody cross-reactivity with valve tissues is established through antimyosin/anti-*N*-acetyl-glucosamine/antilaminin reactivity. Antibodies that react with valve endothelium may lead to inflammation at the valve surface and promote T cell infiltration of the valve in rheumatic heart disease. Based on our current knowledge as illustrated in Fig. 10 (143, 144), the proposed events in rheumatic valvulitis/carditis involve both antibodies and T cells. Cross-reactive antibodies target the valve and are believed to act against the group A carbohydrate and valve endothelium based on several lines of evidence described herein (6, 58, 59, 89, 96). T cells are cross-reactive with streptococcal M proteins and homologous alpha helical protein antigens such as myosin, laminin, tropomyosin, or vimentin and become activated and extravasate through activated endothelium into the valve, where they differentiate into CD4⁺ TH1 cells producing IFNγ and valvular scarring (21, 48, 97, 142–144, 168). VCAM-1 was upregulated on valve endothelium (endocardium) in rheumatic carditis, indicating that the endothelium of the valve in rheumatic carditis was activated (48). Evidence strongly supports the cross-reactive T cell component of the disease, where T cells are shown to penetrate the valve endothelium into an originally avascular valve (21, 32, 48, 97, 136, 142, 143, 168–174). T cells in peripheral blood of patients (24) and the valve (21, 22, 97, 175) have been shown to be cross-reactive with M protein and cardiac proteins, including cardiac myosin epitopes, and they are both CD4⁺ and CD8⁺ phenotypes, but the CD4⁺ phenotype and Th1 cells dominate. The development of scarring and fibrosis in the valve is part of the pathogenesis caused by IFNγ (3, 145, 175) production and IL-17A (101, 146), since the scarring promotes neovascularization and development of a blood supply into normally avascular valve tissue. T cells can subsequently enter the valve through blood vessels developed in the scar. The valve becomes predisposed to cellular infiltration through both the activated valve endocardial surface and the neovascularized scar tissue. Although antibodies develop against collagen in rheumatic heart disease (176, 177), they have not been found to be cross-reactive but may contribute to the pathogenesis of disease (178) once mimicry damages the valve and collagen is exposed to the immune system.

Recent reviews on rheumatic heart disease describe the collective studies and proposed pathogenesis of molecular mimicry in the disease (1–3, 179–184). Initial damage at the chordae tendineae would be sufficient to begin the process of damage to the endothelium of the valve (25) where T cells infiltrate and congregate as they recognize laminin and other valvular proteins that are part of the basement membrane (185). These mechanisms lead to the upregulation of inflammatory molecules such as VCAM-1 (48), which allows the valve to be infiltrated by the T cells, and then the continual damage of the valve with every repetitive streptococcal infection. Treatment with antibiotic prophylaxis as part of the guidelines from the American Heart Association for treatment of rheumatic fever is an important deterrent to the development of rheumatic fever and heart disease in children.

Sydenham Chorea and Pediatric Autoimmune Neuropsychiatric Disorder Associated with Streptococcal Infection (PANDAS)

Sydenham chorea is the major neurological manifestation and movement disorder of rheumatic fever. Human mAbs produced from disease were compared with sera from Sydenham chorea and used to identify the potential antibody targets as well as provide a better understanding of pathogenesis. Human Sydenham chorea-derived mAbs and serum IgG demonstrated specificity for mammalian lysoganglioside and the group A streptococcal carbohydrate epitope N-acetyl-β-D-glucosamine ([7](#)). Chorea-derived mAbs and acute serum antibodies, as well as cerebrospinal fluid from Sydenham chorea, reacted with lysoganglioside and targeted the surface of human neuronal cells. These antibodies also induced elevated calcium/calmodulin-dependent protein kinase II activity (CaM kinase II) ([7](#)). Although the mAbs were IgM, the serum antibodies that triggered the cell signaling in human neuronal cells were IgG. IgG can penetrate the blood-brain barrier when breached by bacterial components or other molecules such as epinephrine ([186](#)–[188](#)), and the IgG can reach brain tissues, where it can target specific cells in the brain tissue and produce disease.

Thus, the human chorea mAb 24.3.1 IgG was shown to penetrate the brain when the genetic information for the chorea antibody V genes were expressed in a transgenic mouse model ([189](#)). The antibody was expressed by B cells in the transgenic model and was produced by B cells and specific IgG accumulated in mouse serum. Once the blood-brain barrier was broken, the antibody that was tagged with a specific mouse allotype was observed in the basal ganglia, where the allotype-tagged chorea antibody IgG penetrated dopaminergic neurons in the ventral tegumental area or the substantia nigra as shown in [Fig. 14](#) ([189](#)). The chorea antibodies were then shown in tritiated thymidine experiments to lead to production of excess dopamine by neuronal cells that could lead to the symptoms of chorea. Other animal models demonstrated that antibodies induced by streptococcal immunization led to deposition in the basal ganglia and the development of behaviors characteristic of Sydenham chorea, such as holding a food pellet or holding on to a beam or demonstrating obsessive compulsive grooming after water spray to fur ([190](#), [191](#)). Passive transfer of the autoantibodies to animals also led to behaviors characteristic of neuropsychiatric symptoms and obsessive compulsive disorders ([192](#)–[194](#)).

Studies collectively demonstrated that the IgG from Sydenham chorea targeted a group of cross-reactive antigens in the brain ([7](#)–[9](#), [195](#), [196](#)). These include lysoganglioside, as mentioned above, and they also target tubulin, an intracellular protein abundant in the brain. More importantly, the cross-reactive antibodies have been shown to react with and signal the dopamine receptors D1 and D2 that have led to the characterization of Sydenham chorea as a dopamine receptor encephalitis where the autoantibodies signal the D2 receptor ([189](#)). The autoantibodies have been found in both serum and CSF of patients with Sydenham chorea, and the IgG autoantibodies as well as human mAbs signal human neuronal cells and activate CaMKII, which is an important signaling molecule in the brain. These cross-reactive antistreptococcal chorea-derived antibodies lead to the production and release of excess dopamine by neuronal cells in the basal ganglia that ultimately leads to chorea.

Similar antineuronal autoantibodies and autoantibody-mediated neuronal cell signaling are elevated in serum and CSF from PANDAS with the choreiform movements. In both Sydenham chorea and PANDAS, the presence of abnormally elevated antidopamine receptor autoantibodies suggests a dopamine receptor encephalitis ([197](#), [198](#)). Studies of mice infected intranasally with group A streptococci illustrated the importance of infection and its role in opening the blood-brain barrier that must be broken to allow IgG to penetrate the brain. Studies by Agalliu and Cleary and colleagues show that intranasal group A streptococcal infection leads to activated Th17 cells that traverse the olfactory bulb and open the blood-brain barrier to proteins such as IgG ([199](#), [200](#)). Upon opening the blood-brain barrier, IgG antineuronal autoantibodies can penetrate the brain and potentially lead to Sydenham chorea and PANDAS.

The studies suggest (i) that the antibodies against streptococci and brain in Sydenham chorea and related diseases produce central nervous system dysfunction through a neuronal signal transduction and subsequent excess dopamine release mechanism and (ii) that the molecular targets of the chorea antibodies include lysoganglioside and the dopamine receptors in neuronal cell membranes. The antineuronal autoantibodies also target the group A streptococcal carbohydrate epitope N-acetyl-β-D-glucosamine present on the rhamnose backbone of the carbohydrate and present in the cell membrane and wall of the group A streptococci as well as the intracellular brain protein tubulin. The diagram in [Fig. 15](#) illustrates proposed events of how antineuronal autoantibodies against lysoganglioside and the dopamine receptors D1 and D2 may function in Sydenham chorea and PANDAS ([3](#)).

Tg Expressed Human Sydenham Chorea-Derived Ab 24.3.1 IgG1ᵃ Ab binds dopaminergic neurons *in vivo*

Co-localization of Anti-Mouse IgG1ᵃ (FITC-labeled) and Tyrosine Hydroxylase (TH) Ab (TRITC-Labeled) in Tg Mouse Brain

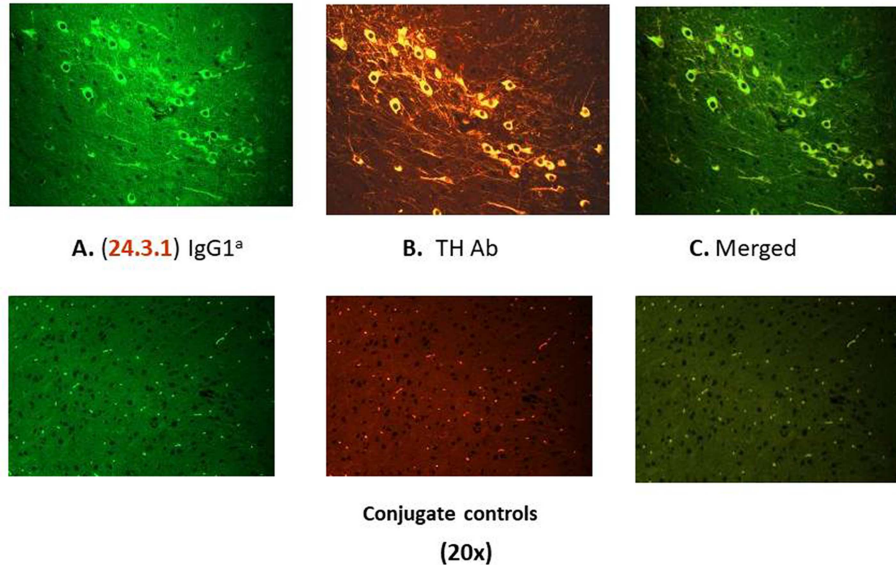**A.** (24.3.1) IgG1ᵃ   **B.** TH Ab   **C.** Merged

Conjugate controls

(20x)

FIGURE 14 The human Sydenham chorea MAb 24.3.1 V gene expressed as the human V gene-mouse IgG1a constant region in transgenic (Tg) mice targets dopaminergic neurons. Chimeric Tg24.3.1 VH IgG1a antibody expressed in Tg mouse sera penetrated dopaminergic neurons in Tg mouse brain *in vivo*. Colocalization of Tg 24.3.1 IgG1a (anti-IgG1a Ab; left panel) and tyrosine hydroxylase antibody (anti-TH Ab; middle panel). TH is a marker for dopaminergic neurons. The left-hand panel shows IgG1a (labeled with fluorescein isothiocyanate [FITC]), the center panel shows TH Ab (labeled with tetramethylrhodamine isothiocyanate [TRITC]), and the right-hand panel shows a merged image (FITC-TRITC). Brain sections (basal ganglia) of VH24.3.1 Tg mouse (original magnification, 320×), showing **(A)** FITC-labeled anti-mouse IgG1a, **(B)** TRITC-labeled anti-TH Ab, and **(C)** the merged image. Controls treated with secondary antibody are negative. (Figure taken with permission from Cox et al. [189].)

CONCLUSIONS

The theme in mimicry, as found in the above-described studies, suggests that cross-reactive antibodies target an intracellular biomarker such as myosin in the heart and tubulin in the brain, but for the antibodies to be pathogenic, they must target a cell surface antigen such as the extracellular matrix protein laminin in the valve endocardial basement membrane or dopamine receptors present at the surface of neuronal cells, leading to the initiation and manifestations of disease. In general, the autoantibodies present as a group of specificities in disease include the cross-reactive antigens recognized by the autoantibodies in disease sera. The cross-reactivity found in individual sera from the disease has been characterized using human mAbs that reflect the serum IgG in the disease.

Cross-reactive antibodies and cross-reactive T cells against group A streptococci are important in the pathogenesis of autoimmune sequelae that characterizes rheumatic fever following streptococcal infection. Cross-reactive antibodies have been defined as those that recognize host tissue alpha-helical coiled-coil antigens such as myosin, tropomyosin, keratin, vimentin, and laminin in the heart (79) and tubulin, lysoganglioside, and dopamine receptors in the brain (9). Cross-reactive antistreptococcal antibodies recognize peptide sequences in alpha-helical proteins such as the M protein virulence determinant of the group A streptococci as well as

Dopamine Receptors are the Targets of Autoantibodies in Sydenham chorea and Pediatric Autoimmune Neuropsychiatric Disorder Associated with Streptococcal Infections(PANDAS)
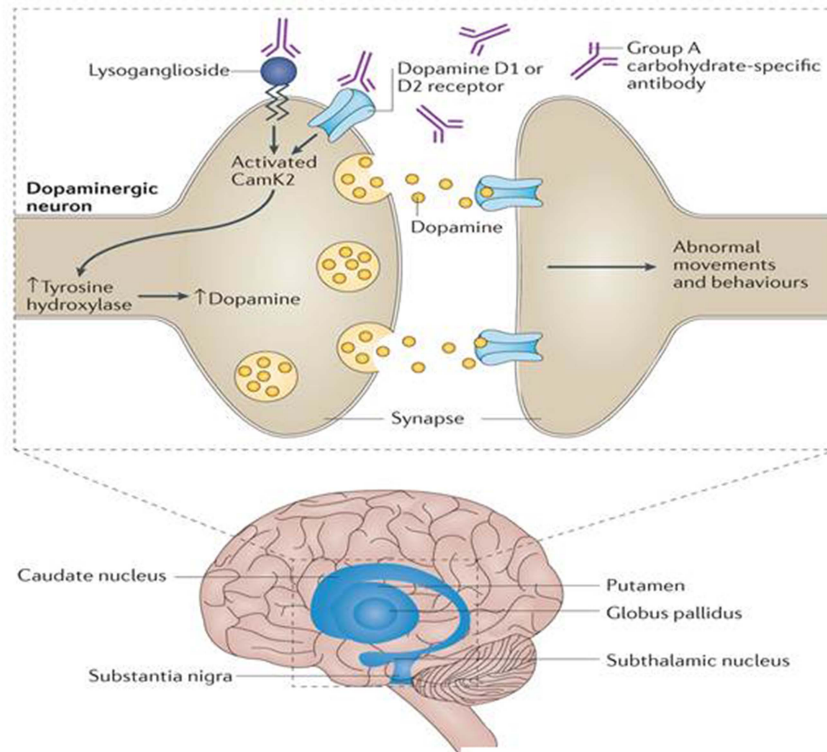
Taken with permission from Carapetis, J. R., Beaton, A., Cunningham, MW *et al.* (2016) Acute rheumatic fever and rheumatic heart disease. *Nature Reviews Disease Primers* doi:10.1038/nrdp.2015.84

FIGURE 15 Diagram of proposed events leading to the group A streptococcal sequelae Sydenham chorea and PANDAS. Autoantibodies against brain tissues in Sydenham chorea and PANDAS with piano-playing choreiform movements cross-react with the group A streptococcal carbohydrate, lysoganglioside, and dopamine receptors D1R and D2R. The antibodies in Sydenham chorea and PANDAS react with the surface of neuronal cells and trigger cell signaling events, leading to upregulation of calcium/calmodulindependent protein kinase II (CaMKII) and excess dopamine release that leads to the involuntary movements in Sydenham chorea or PANDAS with piano-playing choreiform movements. Both Sydenham chorea and PANDAS are likely to be a dopamine receptor encephalitis based on our data ([189](#), [205](#)) and those of Dale et al. ([197](#), [198](#)). (Taken from *Nature Reviews Disease Primers* with permission).

the *N*-acetyl-glucosamine molecule, the dominant epitope of the group A streptococcal carbohydrate ([45](#)–[47](#)). Glycosylated proteins are expected to be targets of the cross-reactive antistreptococcal antibodies. Historically, Goldstein et al. determined autoantibody reactivity with carbohydrates on heart valves ([58](#)), and Dudding and Ayoub demonstrated that the group A carbohydrate autoantibodies were an indicator of poor prognosis in valvular heart disease ([59](#)). This evidence supports our findings and confirms the importance of the autoantibodies against streptococcal antigens in the

development of cross-reactivity that is the basis of the initial disease processes in rheumatic fever and rheumatic heart disease.

In rheumatic heart disease, laminin, an extracellular matrix molecule present in the basement membrane of the valve, may trap cross-reactive anticarbohydrate autoantibodies at the endocardial cell surface and lead to damage or inflammation of the endothelium. Activated endothelium leads to subsequent extravasation of streptococcal M protein/myosin-cross-reactive T cells into the valve ([8](#), [48](#), [97](#), [142](#), [168](#)). Cross-reactive group A

streptococcal antigens with apparent roles in the pathogenesis of rheumatic heart disease include the M proteins and the group A polysaccharide. Amino acid sequences of streptococcal M5 protein that have been shown to be pathogenic in animals have also been reported to be recognized by T cells from rheumatic heart valves ([21], [97], [142], [143], [168]). The identification of human cardiac myosin cross-reactive B and T cell epitopes of M5 protein has been a step forward in understanding the cross-reactive epitopes that produce disease in animals and humans ([32]) and are hallmarks of molecular mimicry.

In Sydenham chorea, mimicry between the *N*-acetylglucosamine molecule of the group A carbohydrate and brain gangliosides potentially leads to antibodies that bind to the surface of neuronal cells and triggers induction of dopamine in the disease ([7]). In the pathogenesis of chorea, the development of IgG responses and higher-affinity cell surface reactive antibody that can bind strongly enough to alter host tissues may lead to disease. In carditis, T cells can enter the valve, but only those that are consistently stimulated with local antigens retain a strong immune response. The Th1 T cell subset and its proinflammatory IFNγ cytokine are dominant in valves with a potential role also for IL-17A and the Th17 subset of T cells in fibrosis and scarring.

Although the identity and pathogenic mechanisms of cross-reactive antigens and their cross-reactive antibodies and T cells is much clearer now than in the past, there will always be new lessons to be learned about the pathogenesis of the autoimmune sequelae of ARF and how cross-reactivity and mimicry of host tissues by the group A streptococci leads to autoimmune sequelae following streptococcal disease.

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