Autoimmunity, February 2006; 39(1): 31–39

Taylor & Francis
Taylor & Francis Group

Molecular mimicry in the autoimmune pathogenesis of rheumatic heart disease

LUIZA GUILHERME[1], JORGE KALIL[1,2], & MADELEINE CUNNINGHAM[3]

[1]*Heart Institute—InCor, University of São Paulo, School of Medicine, São Paulo, Brazil,* [2]*Clinical Immunology and Allergy, Department of Clinical Medicine University of São Paulo, School of Medicine, São Paulo, Brazil, and* [3]*Department of Microbiology and Immunology, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA*

Abstract
Molecular mimicry is a hallmark of the pathogenesis of rheumatic fever where the streptococcal group A carbohydrate epitope, N-acetyl glucosamine, and the α-helical coiled-coil streptococcal M protein structurally mimic cardiac myosin in the human disease, rheumatic carditis, and in animal models immunized with streptococcal M protein and cardiac myosin. Recent studies have unraveled the potential pathogenic mechanisms by which the immune response against the group A streptococcus attacks the rheumatic valve leading to chronic rheumatic heart disease. Both B- and T-cell responses are involved in the process, and evidence for the hypotheses of molecular mimicry and epitope spreading are reviewed.

Keywords: *Streptococci, myosin, autoimmunity, rheumatic fever*

Introduction

Rheumatic fever, a sequela of group A streptococcal infection, is characterized by inflammation of the joints (arthritis), heart (carditis), central nervous system (chorea), skin (erythema marginatum) and/or subcutaneous nodules [1]. Any of these five major manifestations may be seen in rheumatic fever and are established as the Jones criteria as revised by the American Heart Association [2–4]. Rheumatic fever is autoimmune in nature and results from production of autoreactive antibodies and T-cells crossreactive with components of the group A streptococcus and host tissues. The medical importance of rheumatic fever is due to serious cardiac manifestations leading to death or valve replacement [1,4]. In 1900s, rheumatic fever was considered the most common cause of acquired heart disease in school-aged children in the US [5,6], and it is a major cause of acquired heart disease in children worldwide occurring most frequently in developing countries [6]. The incidence of rheumatic heart disease worldwide ranges from 0.55 to 11 per thousand [7]. A recent epidemiological survey in rural North India cited 210 cases of rheumatic heart disease per 100,000 school children ages 5 to 15 [7]. Epidemiological data leaves little doubt that rheumatic fever is a world health problem.

Advances have been made toward understanding the pathogenesis of rheumatic fever and its manifestations as a postinfectious autoimmune sequela. Risk factors in disease include the major histocompatibility complex (MHC) antigens, immune responses against host and streptococcal antigens as well as socio-economic conditions and ethnicity [8]. It is evident that T lymphocytes play an important role in the pathogenesis of rheumatic carditis, and data support the hypothesis that antibodies play an important role in the initiation of the disease at the valve endothelium [9]. Pathogenic epitopes of streptococcal and host antigens which cause autoimmune disease in animal models have been defined. Our review will summarize the current evidence for the role of crossreactive antibodies and T-cells in the pathogenesis of rheumatic carditis.

Correspondence: M.W. Cunningham, Department of Microbiology and Immunology, University of Oklahoma, HSC, Oklahoma, OK, USA. Tel: 1 405 271 3128. Fax: 1 405 271 2217. E-mail: madeleine-cunningham@ouhsc.edu

ISSN 0891-6934 print/ISSN 1607-842X online © 2006 Taylor & Francis
DOI: 10.1080/08916930500484674

Genetic susceptibility in rheumatic fever/rheumatic heart disease

The individual as well as familial predisposition of rheumatic fever has been postulated for more than a century (extracted from Taranta) [10]. The search for genetic markers revealed that human leukocyte-associated antigen (HLA) class II genes or other genes in linkage disequilibrium were potentially involved with the development of rheumatic fever/rheumatic heart disease. HLA class II genes are located in human chromosome 6 and are responsible for the control of immune responses. HLA class II molecules play an important role in antigen presentation to the T-cell receptor (TCR) and consequently in the triggering of cellular and humoral immune responses. Association with different HLA class II antigens has been found in several populations [11]. Among the HLA class II alleles described, HLA-DR7 was the allele most consistently associated with rheumatic heart disease [12–17]. The association of DR7 with different DQ-B or DQ-A alleles seems to be associated with the development of multiple valvular lesions (MVL) or mitral valve regurgitation (MVR) in rheumatic heart disease patients [15,17]. HLA-DR53, another HLA class II molecule, is in linkage disequilibrium with HLA-DR4, DR7 and DR9. This molecule was found to be in strong association with rheumatic fever/rheumatic heart disease in two studies with mulatto Brazilian patients [12,14] but not in Brazilian Caucasian patients [16]. Although this molecule has not been described in previous studies, HLA-DR4 and DR9 were found to be associated with rheumatic fever in American Caucasian and Arabian patients [18,19], whereas in Egyptian and Latvian patients, HLA-DR7 was associated with the disease [15,19]. In Japanese rheumatic heart disease patients, susceptibility to mitral stenosis seems to be in part controlled by the *HLA-DQA* gene or by genes in close disequilibrium linkage with *HLA-DQA** 0104 and DQB1*05031 [20]. *HLA-DQA**0501 DQB*0301 associated with DRB1*1601 (DR2) was associated with rheumatic heart disease in a Mexican Mestizo population [21]. In the Mexican rheumatic heart disease patients, increased frequencies for some tumor necrosis factor (TNF)-α alleles was also observed [22]. The *TNF-α* gene is located on chromosome six between HLA-B and HLA-DR suggesting linkage disequilibrium between these genes.

Molecular mimicry, degeneracy and epitope spreading

Molecular mimicry is defined as the sharing of epitopes between antigens of the host and streptococcal bacteria. For antibodies, three types of mimicry have been defined between antigens, including the sharing of: (1) identical amino acid sequences, (2) homologous but non-identical amino acid sequences and (3) epitopes on dissimilar molecules such as peptides and carbohydrates [23–26] or between DNA and peptides [27,28] or carbohydrates and gangiosides [29]. Although mimicry necessitates that the antibody recognize more than one antigen and results in lower affinity, the affinities of crossreactive antibodies differ and can be of high enough affinity to cause cytotoxity in the presence of complement or induce antibody mediated cell signaling of a cell surface receptor [9,29].

Molecular mimicry for T-cells is different from that described for antibody and requires an understanding of mimicry and degeneracy and their relationship. Although the recognition of multiple antigens may be a degenerate property of some T-cells, it is not a property of all of them. Degeneracy may result in the recognition of amino acid sequences without any sequence homology between the two or many peptides. Mimicry in pathogenesis means that sequences are recognized by T-cells that are specific for the molecules related to the disease, as in the case of rheumatic carditis [30]. However, mimicry is a type of T-cell degeneracy. The ability of T-cells to recognize a wider range of antigens is important for the T-cell repertoire since TCRs do not undergo somatic mutation as do antibody *V* genes. Mimicry may begin the disease process in a target organ and lead to chronic autoimmune disease through epitope spreading [31]. In epitope spreading, T-cells at the site of disease may no longer recognize the original mimicking epitope but recognize epitopes in other proteins of the target organ which continue to perpetuate the disease long after the initiating antigen or infection has been eliminated.

Anti-streptococcal autoantibodies against the heart: Development of monoclonal antibodies

Anti-cardiac antibodies were associated with acute rheumatic fever in 1945 by Cavelti [32] and in 1964 by Kaplan [33] and in 1970 by Zabriskie and colleagues [34]. Antibody and complement were found deposited in hearts of patients with acute rheumatic heart disease [33]. Anti-heart antibodies persisted in patients with rheumatic recurrences, but declined by five years after the initial rheumatic episode. Zabriskie suggested that repeated episodes of streptococcal infections were important in development of acute rheumatic fever [34]. These previous data supported the hypothesis that acute rheumatic fever has an autoimmune origin.

Historically, anti-heart antibodies could be absorbed from human sera by group A streptococci, their cell walls or membranes [35–37], and sera from rheumatic fever patients or rabbit anti-group A streptococcal sera reacted with heart or skeletal muscle [38–40]. The streptococcal crossreactive antigens were found in streptococcal walls and

membranes and were associated with the streptococcal M protein and the group A carbohydrate [36,37,41]. It was evident from many studies that the group A streptococci were associated with autoantibody responses against heart and other tissues, however, crossreactivity was not understood due to the large number of antibodies present in human and animal sera. Since this time, monoclonal antibodies (mAbs) have been used to characterize the crossreactivity between streptococci and heart and have been important in understanding the pathogenesis of rheumatic heart disease.

Human and mouse anti-streptococcal mAbs [28,42,43] reacted with myocardium in heart tissue sections similar to that demonstrated for human and animal sera studied previously. Crossreactive anti-streptococcal mAbs identified myosin as the dominant autoantigen in heart [44,45] and have been shown to recognize streptococcal M protein as well as the group A carbohydrate [23,24,46]. mAbs validated the hypothesis of crossreactivity between group A streptococci and heart tissue. The specificities of mouse crossreactive mAbs were found to recognize either M protein or the group A carbohydrate epitope N-acetyl-β-D-glucosamine and cardiac myosin, and several α-helical proteins found in heart or valve including tropomyosin and vimentin, respectively [46,47]. The human crossreactive anti-streptococcal mAbs derived from rheumatic carditis patients reacted with cardiac myosin and with N-acetyl-glucosamine, the immunodominant epitope of the group A carbohydrate [9,23]. Reactivity with N-acetyl-glucosamine was an important feature of the human mAbs because elevated and persistent levels of anti-group A carbohydrate antibodies indicated a poor prognosis in cases of chronic rheumatic valvulitis [48]. Anti-DNA and anti-nuclear reactivity was observed among a few mouse mAbs which was not seen in the human mAbs [45]. Anti-nuclear or anti-DNA antibodies are not a feature of acute rheumatic fever. In the case of anti-N-acetyl-glucosamine antibodies, Shikhman et al. [24] demonstrated that some of the anti-streptococcal/anti-myosin mouse and human mAbs recognized cytoskeletal proteins and the epitope of the group A carbohydrate, N-acetyl-glucosamine. Antibodies which recognized N-acetyl-glucosamine also recognized peptides which could bind lectins and induced an immune response against N-acetyl-glucosamine [23–25]. These data illustrate that the N-acetyl-glucosamine antibody repertoire is closely linked with immune responses against myosin and other α-helical proteins in the heart as well as other target organs in rheumatic fever.

Anti-streptococcal crossreactive mAbs represented three major groups: (A) mAbs reactive with α-helical coiled-coil molecules such as myosin, tropomyosin and keratin, (B) mAbs reactive with myosin and DNA, and (C) mAbs reactive with myosin and N-acetyl-glucosamine. Affinity purified human anti-myosin antibodies from acute rheumatic fever sera demonstrated the reactivities observed in the mAb groups A and C, and identified a crossreactive epitope near the pepsin cleavage site in M5 and M6 proteins [43]. The amino acid sequence of the epitope was identified as Gln–Lys–Ser–Lys–Gln. Proteolytic fragments and synthetic peptides of human cardiac myosin were used to identify sites of crossreactivity in the myosin molecule. mAbs reacted with the heavy chain of myosin within the α-helical rod region. Reactivity with sites in cardiac myosin is particularly important since it has been shown to induce myocarditis when administered to susceptible animals whereas skeletal myosin does not [49,50].

The streptococcal M protein antigen also reacted with mAbs derived from mice and humans [46,51]. The M protein was identified by Manjula, Fischetti and colleagues as an α-helical heptad repeating structure which resembled α-helical proteins such as tropomyosin and the desmin–keratin family of molecules [52–54]. Anti-streptococcal crossreactive mAbs identified mimicry with α-helical coiled-coil proteins myosin, tropomyosin, vimentin, laminin and keratin [9,23,25,47,55–57]. In streptococcal M protein, the myosin crossreactive sequence (Gln–Lys–Ser–Lys–Gln) near the pepsin cleavage site in M5 and M6 proteins [43], while other myosin crossreactive sites were identified by Dale and Beachey in M5 [58] and M19 [59]. Using overlapping synthetic peptides of M5 protein, myosin crossreactive B-cell epitopes were identified in peptides from the A, B and C repeat regions of M5 protein [60]. Investigation of mAb 10B6, an anti-M protein mAb which recognized the class I epitope of M proteins, revealed that it reacted with M5 peptides containing the class I epitope and with cardiac and skeletal myosins [61]. The class I epitope shared homology with both skeletal and cardiac myosins, but peptides containing the class I epitope did not cause any tissue inflammation in animal models as described below.

T-cell immune responses in rheumatic fever/rheumatic heart disease

The first evidence of $CD4^+$T-cell involvement in rheumatic heart disease lesions was described by Raizada et al. [62] and led us to investigate their role in the development of heart-tissue lesions. Molecular mimicry between β hemolytic streptococci and heart tissue proteins was demonstrated through an analysis of the heart-tissue infiltrating T-cell repertoire leading to local tissue damage in rheumatic heart disease. By generating T-cell clones from heart lesions of four severe rheumatic heart disease patients we demonstrated for the first time the ability of 7.5% of these cells to simultaneously recognize M protein peptides and heart tissue-derived proteins. Three M5 regions

		M5 Immunodominant Region			
		M5 peptides	**81-96**	**83-103**	**91-103**
T cell clones	**Intralesional**	**Positive/163**	9	10	3
		(%)	**5.5**	**6.1**	**1.8**
	PBMC	**Positive/23**	2	1	0
		(%)	**8.6**	**4.3**	

Figure 1. Reactivity of PBMC and intralesional T cell clones against the M5(81–103) immunodominant region. Intralesional T cell clones were obtained from surgical fragments of rheumatic heart disease patients as previously described [63]. M5 peptides were tested by proliferation assays and considered positive when SI $\geq$ 3.0. Peptide sequences were based on sequences published by Manjula et al. [67]. M5 (81–96) DKLKQQRDTLSTQKET; M5(83–103) LKQQRDTLSTQKETLEREVQN; M5(91–103) STQKETLEREVQN.

(residues 1–25, 81–103 and 163–177) cross-reacted with several heart protein fractions, mainly those derived from valvular tissue with molecular masses of 95–150, 43–65 and 30–43 kDa were described [63]. An extension of this work defined the M5 (81–103) as an immunodominant region recognized by both peripheral blood mononuclear cells (PBMC) and intralesional T-cell clones (Figure 1) [64]. In murine models, in which mice were immunized with intact cardiac myosin, lymph-node T-cells cross reacted with overlapping M5 peptides NT5/6 [60] that aligned with the M5 (81–103) region recognized by human intralesional T-cells (Table I) reinforcing the dominance of this region. The sequence and α-helical nature of the streptococcal M protein has been previously reported [65–67].

Yoshinaga et al. [68] also isolated T-cells from rheumatic heart disease heart valves and compared the reactivity of phytohemagglutinin and streptococcal antigen-stimulated T-cell lines derived from heart valve specimens and PBMCs of rheumatic fever patients, and showed that, albeit these cells recognized cell wall and membrane streptococcal antigens, they failed to react with the M protein, myosin or other mammalian cytoskeletal proteins. These results are in contrast with those published [30,63,64] which could be due to the possibility that they may have tested only established T-cell lines not isolated T-cell clones or that the T-cells isolated from valves were reactive with different streptococcal or heart proteins. It is possible that T-cells in the valve may respond to antigens other than M protein or myosin following a streptococcal infection. In addition, we would expect that any activated T-cell could enter through the activated valve endothelium [69] and not all T-cells entering the valve would cause disease. Only T-cells expanded by valvular or crossreactive antigens would survive and cause disease.

In studies of peripheral blood T-cells in rheumatic heart disease, Ellis et al. [30] reported that peripheral human T-cell clones responsive to group A streptococcal recombinant M6 (rM6) protein were derived from rheumatic carditis and selected for dual recognition of rM6 and human cardiac myosin. Crossreactive T-cell clones recognized human cardiac myosin, tropomyosin and laminin, a valve protein. Ten $CD4^+$ and three $CD8^+$ rM6/human cardiac myosin crossreactive T-cell clones showed 100-fold greater avidity for rM6 than human cardiac myosin, and tenfold greater avidity for human cardiac myosin than laminin or tropomyosin [30]. The T-cell clones had a heterogeneous *Vβ*-gene and complementarity determining region (CDR)3 usage. The crossreactive response was MHC II restricted by DR or DQ, or HLA I restricted for $CD8^+$ clones. The human T-cell clones specifically proliferated to epitopes in the B-repeat region of streptococcal M protein and epitopes in the S2 and LMM regions of human cardiac myosin confirming molecular mimicry for T-cells from peripheral blood in rheumatic heart disease [30]. T-cell clones produced interferon (IFN)-γ in response to peptide antigen supporting a TH1 response. The study of peripheral human T-cell clones from rheumatic heart disease demonstrates mimicry at

Table I. M protein homologous regions recognized by human and murine T-cells.

Amino acid residues	Human heart T cell clones	Murine lymph node T-cells
*NT5 (59–76) KKEHEAEN**DKLKQQRDTL**		⋆
*NT6 (72–89) **QRDTL**STQKETLEREVQN		⋆
†M5 (81–96) **DKLKQQRDTL**STQKET	⋆	
†M5 (83–103) **LKQQRDTL**STQKETLEREVQN	⋆	
†M5 (91–103) STQKETLEREVQN	⋆	

⋆ Peptides were based on sequences published by Miller et al. [65] and presented myosin cross reactivity [60].
† sequences from Manjula et al. [67] and presented cross reactivity with human valvular tissue proteins [63]. Bold typed and underlined regions correspond to the identical residues among the different peptides.

the T-cell level between streptococcal M protein and human cardiac myosin epitopes and supports the work reported for T-cell mimicry in the valve.

In favor of the putative pathogenic role of the M5(81–96) peptide as a trigger of cross reactivity with heart tissue proteins is the fact that this peptide induces *in vitro* the production of IFN-γ, an inflammatory cytokine, by heart tissue infiltrating T-cell lines [70]. This data supports the observation that mononuclear cells infiltrating both myocardium and valvular tissue preferentially produced inflammatory cytokines (IFN-γ and TNF-α) confirming that rheumatic heart disease is mediated by an inflammatory immune response. An important finding was the fact that few mononuclear cells infiltrating the valves were able to produce interleukin (IL)-4, a regulatory cytokine. In contrast, in the myocardium we found large number of T-cells expressing IL-4. These results demonstrated that the significantly lower IL-4 expression in the valvular tissue might contribute to the progression of rheumatic heart disease leading to permanent valvular damage [70].

The recognition of M5 protein peptides by peripheral blood T-cells also demonstrated evolution of carditis in rheumatic heart disease patients. The M5 (81–96) peptide elicited a cellular immune response in 46% of patients with severe carditis. Several heart tissue-derived proteins were also recognized by peripheral T-cells. In addition, 70% of severe rheumatic heart disease patients that recognized the M5 (81–96) peptide express the HLA-DR7 molecule [64] that is associated with the development of MVL [15,17]. In contrast, the M5 (11–25) peptide was preferentially recognized by mild rheumatic heart disease patients [64].

The activation of the cellular immune response involved the MHC class I and II molecules, peptides and the TCR. T-cell repertoire is defined by the assembly of the 24 V_β, 13 J_β, 31 V_α and 61 J_α families. The analysis of these regions may detect antigen-driven T-cell expansions in autoimmune diseases. We analyzed the T-cell repertoire in peripheral and intralesional T cell lines derived from rheumatic heart disease patients, and we found polyclonal expansions in the peripheral blood and several oligoclonal expansions in both myocardium and mitral valve tissue [71]. Intralesional T-cell clones crossreactive with streptococcal and heart tissue proteins frequently used the same TCR-$V_\beta J_\beta$ $V_\alpha J_\alpha$ and CDR3 sequences to recognize different antigens showing a degenerate pattern of recognition [11,72].

Superantigens are proteins that polyclonally activate T-cells through an MHC class II dependent, but MHC haplotype-unrestricted mechanism. Proliferative responses to superantigens are limited to T-cells expressing a particular TCR-V_β gene but are independent of antigen specificity. A superantigenic effect of streptococcal M5 protein is described by some groups [73,74] but in fact the exotoxin secreted by group A streptococci is responsible for the potent superantigen effect observed [75,76].

Pathogenic mechanisms in rheumatic carditis

The pathogenesis of rheumatic carditis in the initiating stages is hypothesized to be a two stage process whereby antibodies damage and inflame the endothelium of the valve making it susceptible to infiltration and attack by T-cells. Although the role of the crossreactive or polyspecific antibodies in the pathogenesis of rheumatic fever has been controversial, it has been shown that the antibodies that recognize cardiac myosin in the myocardium also recognize the valve endothelium and laminin [9] (Figure 2). Human monoclonal antibody from rheumatic carditis identified epitopes in laminin and cardiac myosin and was cytotoxic for the endothelium in the presence of complement. Although further studies are needed to demonstrate this principle in animal models, the evidence suggests that the antibody may be required to target the valve for T-cell infiltration and attack.

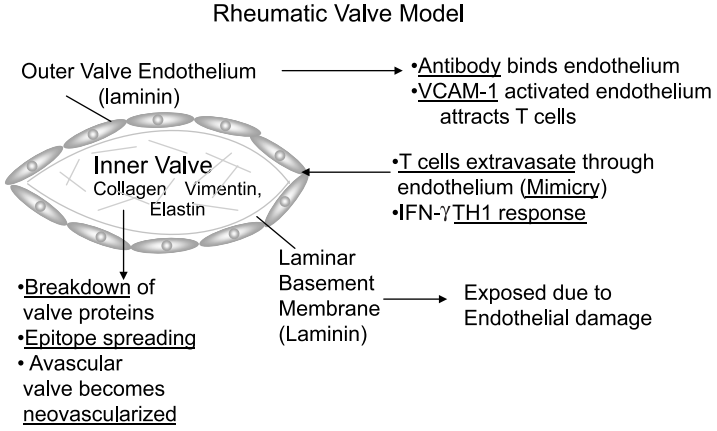

Figure 2. Diagram illustrating the pathogenic mechanisms in initiation and development of rheumatic heart disease.

There is strong evidence for inflammation at the valvular endothelium in acute rheumatic carditis. Vascular cell adhesion molecule-1 (VCAM-1) is upregulated on the endothelium of the valve in disease in children requiring valve replacements. The upregulation of VCAM-1 would promote T cell adhesion and infiltration into the avascular valve, which is the second stage of disease in the valve. The valve becomes infiltrated by M protein specific T-cells which produce primarily γ-IFN and result in the scarring in the valvular tissue [11,63,64,70,71]. Further discussion of these hypotheses and data can be found in a recent review by Cunningham [77]. The scarring leads to deformity and malfunctioning of the valve and to the neovascularization of scar tissue which upon reinfection with group A streptococci would promote further disease in the valve due to cellular infiltration through the blood vessels in the neovascularized valve tissue as well as at the surface endothelium of the valve. For this reason, penicillin prophylaxis is important to prevent further group A streptococcal infection and exacerbation of disease.

Finally, once disease has become chronic in the valve, other proteins in the valve such as laminin, vimentin, collagen and others may be presented to the immune system and epitope spreading would be predicted to occur (Figure 2). Immune responses would continue to promote a TH1 granulomatous response in the valve [70] with additional scarring. It is possible that responses against collagen, laminin and vimentin as well as myosin may occur during a chronic epitope spreading stage where antibodies and T cells against new epitopes and antigens would appear but may not have the specificity of the original mimicking antigen and by definition constitute epitope spreading.

Animal models of carditis

Animal models to study rheumatic fever are limited because man is the host and reservoir of group A streptococci. Animals are not easily infected with group A streptococci, and once infected, animal models do not maintain an infection for a lengthy period of time. Most animal models of rheumatic fever have relied on immunization of rabbits, mice, rats and monkeys [78–82].

The model that has been most useful in comparison to the human rheumatic fever histopathology in the heart has been the definition of a model in the Lewis rat immunized with recombinant group A streptococcal M protein serotype 6 [49,82]. In this model, the M protein induced valvular infiltration at the endothelium of the valve in approximately 50% of the rats immunized with the M protein. Lesions included verrucae and Aschoff-like lesions in the rat valves [82]. T cell lines were isolated from rats with disease. The T-cell lines crossreacted with streptococcal M protein and cardiac myosin [82]. In addition, immunization with peptides of streptococcal M protein as well as the pepsin fragment of M5 protein also induced valvular lesions (Cunningham, unpublished observations). These studies have been performed in the anticipation of identifying an animal model which through immunization procedures could be used to study rheumatic fever or rheumatic heart disease.

Other studies developed mouse models immunized with peptides of the streptococcal M5 protein which elicited rheumatic-like lesions in mice. The first of these studies identified a streptococcal M5 protein amino acid sequence, GLKTENEGLKTENEGLKTE (NT4 peptide), which shared similarities with cardiac myosin and produced myocarditis in BALB/c and MRL/++ mice [60,83]. Myocarditis was also observed in BALB/c mice when immunized with peptides of M5 protein from the A and B repeat regions which included streptococcal M5 protein peptides NT4, NT5, NT6, B1A and B3A which elicited cellular infiltrates in the myocardium as previously described [60]. The A and B repeat regions of M proteins contain sequence homology with cardiac myosin, a known autoantigen in myocarditis. Peptides from the C repeat region shared homology with both skeletal and cardiac myosins and did not elicit an inflammatory reaction in the myocardium of mice [60].

The data are consistent with the hypothesis that only cardiac myosins and not skeletal myosin induce inflammatory heart disease [50]. The hypothesis in rheumatic heart disease is that unique sequences in M proteins break immune tolerance to pathogenic epitopes in human cardiac myosin and lead to an autoimmune mediated pathogenesis in rheumatic fever and rheumatic carditis. Although animal models support this hypothesis, there are multiple factors, which must be considered in an animal model of a human disease.

Most of the studies on T-cell epitopes in rheumatic fever and in animal models focus on the streptococcal M5 protein molecule, because M5 has been a serotype associated for many years with acute rheumatic fever outbreaks [84,85]. T- and B-cell epitopes of the M5 protein were defined in previous studies by Robinson and colleagues [86,87], by Good and Pruksakorn [88–90], and in our own laboratory [60]. T cell epitopes crossreactive with cardiac myosin and defined in animal models have been summarized previously by Cunningham [60]. The T cell crossreactive epitopes were found primarily in the A and B repeat regions of the M protein in mice and rats [60] (Cunningham, unpublished observations).

In studies of the B- and T-cell epitopes of streptococcal M5 protein which crossreacted with myosin were mapped using 23 overlapping synthetic peptides (18-mers) of the A, B and C repeat regions of the M5 protein [60]. Six dominant myosin

crossreactive sites in the streptococcal M5 molecule consistently stimulated T-cells from mice sensitized to human cardiac myosin. Dominant myosin cross-reactive T-cell epitopes of M5 protein in BALB/c mice were located in the same region as the streptococcal M5 sequences recognized by T cell clones from rheumatic heart valves [63]. M5 peptides were also reported by Pruksakorn, Good and colleagues to stimulate peripheral human T-cells from normal individuals and rheumatic fever which were crossreactive with myosin peptides [88]. The collective evidence suggest that amino acid sequences in M5 protein which share homology with cardiac myosin may break tolerance and promote T cell mediated inflammatory heart disease in animals and man [11,30,60,63,64,71,83].

Conclusion

Evidence presented in our review suggest that molecular mimicry and epitope spreading are likely pathogenic mechanisms of T cell responses in rheumatic valves after attack of the valve endothelium by anti-streptococcal antibody at the valve surface (Figure 2). Once the valve endothelium is activated, the valve is infiltrated by T-cells which recognize streptococcal M protein and cardiac myosin as well as other valve related proteins. Studies in animal models as well as in human rheumatic heart disease have provided evidence of antibody mimicry between the group A carbohydrate epitope and cardiac myosin and laminin and of T cell mimicry between streptococcal M protein and human cardiac myosin epitopes and other α-helical proteins present in the valve such as laminin or vimentin. The repeated stimulation of the T-cells in the valve leads to scarring and IFN-γ production with neovascularization and increased infiltration by T-cells with potentially an epitope spreading mechanism that perpetuates the disease.

References

[1] Stollerman GH. Rheumatic and heritable connective tissue diseases of the cardiovascular system. In: Braunmald E, editor. Heart disease: A textbook of cardiovascular medicine. Vol. 11 Philadelphia: W.B. Saunders; 1988. p 1706–1734.

[2] Guidelines for the diagnosis of rheumatic fever: Jones Criteria Special writing group of the committee on rheumatic fever, endocarditis, and kawasaki disease of the council on cardiovascular disease in the young of the American Heart Ass. JAMA 1992;268:2069–2073, update.

[3] Jones TD. The diagnosis of rheumatic fever. JAMA 1944;126:481–485.

[4] Veasy LG. Rheumatic fever-T. Duckett jones and the rest of the story. Cardiol Young 1995;5:293–301.

[5] Massell BF. Rheumatic fever and streptococcal infection: Unraveling the mysteries of a dread disease. Boston, MA: Harvard University Press for the Francis A. Countway library of medicine. 1997.

[6] Stollerman GH. Rheumatic fever. Lancet 1997;349:935–942.

[7] Kaur S, Kumar D, Grover A, Khanduja KL, Kaplan EL, Gray ED, Ganguly NK. Ethnic differences in expression of susceptibility marker(s) in rheumatic fever/rheumatic heart disease patients. Int J Cardiol 1998;64:9–14.

[8] Kurahara D, Tokuda A, Grandinetti A, Najita J, Ho C, Yamamoto K, Reddy DV, Macpherson K, Iwamuro M, Yamaga K. Ethnic differences in risk for pediatric rheumatic illness in a culturally diverse population. J Rheumatol 2002;29:379–383.

[9] Galvin JE, Hemric ME, Ward K, Cunningham MW. Cytotoxic mAB from rheumatic carditis recognizes heart valves and laminin. J Clin Invest 2000;106:217–224.

[10] Taranta A. A history of rheumatic fever. In: Narula J, Virmani R, Reddy KS, Tandon R, editors. Rheumatic Fever–1999. Washington, DC: American Registry of Pathology; 1999. p 1–40.

[11] Guilherme L, Kalil J. Rheumatic fever: How streptococcal throat infection triggers an autoimmune disease. In: Shoenfeld Y, Rose NR, editors. Infection and autoimmunity. Amsterdam: Elsevier; 2004. p 321–330.

[12] Guilherme L, Weidebach W, Kiss MH, Snitcowsky R, Kalil J. Association of human leukocyte class II antigens with rheumatic fever or rheumatic heart disease in a Brazilian population. Circulation 1991;83:1995–1998.

[13] Ozkan M, Carin M, Sonmez G, Senocak M, Ozdemir M, Yakut C. HLA antigens in Turkish race with rheumatic heart disease. Circulation 1993;87:1974–1978.

[14] Weidebach W, Goldberg A, Chiarella J, Guilherme L, Snitcowsky R, Pileggi F, Kalil J. HLA class II antigens in rheumatic fever: Analysis of the DR locus by restriction fragment-length polymorphism and oligotyping. Hum Immunol 1994;40:253–258.

[15] Guedez Y, Kotby A, El-Demellawy M, Galal A, Thomson G, Zaher S, Kassem S, Kotb M. HLA class II associations with rheumatic heart disease are more evident and consistent among clinically homogeneous patients. Circulation 1999;99:2784–2790.

[16] Visentainer JE, Pereira FC, Dalalio MM, Tsuneto LT, Donadio PR, Moliterno RA. Association of HLA-DR7 with rheumatic fever in the Brazilian population. J Rheumatol 2002;27:1518–1520.

[17] Ayoub EM, Barrett DJ, Maclaren NK, Krischer JP. Association of class II human histocompatibility leukocyte antigens with rheumatic fever. J Clin Invest 1986;77: 2019–2026.

[18] Rajapakse CNA, Halim K, Al-Orainey L, Al-Nozha M, Al-Aska AK. A genetic marker for rheumatic heart disease. Br Heart J 1987;58:659–662.

[19] Stanevicha V, Eglite J, Sochnevs A, Gardovska D, Zavadska D, Shantere R. HLA class II associations with rheumatic heart disease among clinically homogeneous patients in children in Latvia. Arthritis Res Ther 2003;5:R340–R346.

[20] Koyanagi T, Koga Y, Nishi H, Toshima H, Sasazuki T, Imaizumi T, Kimura A. DNA typing of HLA class II genes in Japanese patients with rheumatic heart disease. J Mol Cell Cardiol 1996;28:1349–1353.

[21] Hernandez-Pacheco G, Aguilar-Garcia J, Flores-Dominguez C, Rodriguez-Perez JM, Perez-Hernandez N, Alvarez-Leon E, Reyes PA, Vargas-Alarcon G. MHC class II alleles in Mexican patients with rheumatic heart disease. Int J Cardiol 2003;92:49–54.

[22] Hernandez-Pacheco G, Flores-Dominguez C, Rodriguez-Perez JM, Perez-Hernandez N, Fragoso JM, Saul A, Granados J, Reyes PA, Vargas-Alarcon G. Tumor necrosis factor-α promoter polymorphisms in Mexican patients with rheumatic heart disease. J Autoimmun 2003;21:59–63.

[23] Shikhman AR, Cunningham MW. Immunological mimicry between N-acetyl-beta-D-glucosamine and cytokeratin peptides.

Evidence for a microbially driven anti-keratin antibody response. J Immunol 1994;152:4375–4387.
[24] Shikhman AR, Greenspan NS, Cunningham MW. A subset of mouse monoclonal antibodies cross-reactive with cytoskeletal proteins and group A streptococcal M proteins recognizes N-acetyl-beta-D-glucosamine. J Immunol 1993;151:3902–3913.
[25] Shikhman AR, Greenspan NS, Cunningham MW. Cytokeratin peptide SFGSGFGGGY mimics N-acetyl-beta-D-glucosamine in reaction with antibodies and lectins, and induces in vivo anti-carbohydrate antibody response. J Immunol 1994;153:5593–5606.
[26] Barnett LA, Fujinami RS. Molecular mimicry: A mechanism for autoimmune injury. FASEB J 1992;6:840–844.
[27] Putterman C, Diamond B. Immunization with a peptide surrogate for double stranded DNA (dsDNA) induces autoantibody production and renal immunoglobulin deposition. J Exp Med 1998;188:29–38.
[28] Cunningham MW, Hall NK, Krisher KK, Spanier AM. A study of anti-group A streptococcal monoclonal antibodies cross-reactive with myosin. J Immunol 1986;136:293–298.
[29] Kirvan CA, Swedo SE, Heuser JS, Cunningham MW. Mimicry and autoantibody-mediated neuronal cell signaling in Sydenham chorea. Nat Med 2003;9:914–920.
[30] Ellis NMJ, Li Y, Hildebrand W, Fischetti VA, Cunningham MW. T cell mimicry and epitope specificity of cross-reactive T cell clones from rheumatic heart disease. J Immunol 2005;175:5448–5456.
[31] McMahon EJ, Bailey SL, Castenada CV, Waldner H, Miller SD. Epitope spreading initiates in the CNS in two mouse models of multiple sclerosis. Nat Med 2005;11:335–339.
[32] Cavelti PA. Autoantibodies in rheumatic fever. Proc Soc Exp Biol Med 1945;60:379–381.
[33] Kaplan MH, Bolande R, Rakita L, Blair J. Presence of bound immunoglobulins and complement in the myocardium in acute rheumatic fever. Association with cardiac failure. N Engl J Med 1964;271:637–645.
[34] Zabriskie JB, Hsu KC, Seegal BC. Heart-reactive antibody associated with rheumatic fever: Characterization and diagnostic significance. Clin Exp Immunol 1970;7:147–159.
[35] Zabriskie JB. Mimetic relationships between group A streptococci and mammalian tissues. Adv Immunol 1967;7:147–188.
[36] Zabriskie JB, Freimer EH. An immunological relationship between the group A streptococcus and mammalian muscle. J Exp Med 1966;124:661–678.
[37] Kaplan MH. Immunologic relation of streptococcal and tissue antigens. I. Properties of an antigen in certain strains of group A streptococci exhibiting an immunologic cross reaction with human heart tissue. J Immunol 1963;90:595–606.
[38] Kaplan MH, Meyeserian M. An immunological cross-reaction between group—A streptococcal cells and human heart tissue. Lancet 1962;1:706–710.
[39] Kaplan MH, Suchy ML. Immunologic relation of streptococcal and tissue antigens. II. Cross reactions of antisera to mammalian heart tissue with a cell wall constituent of certain strains of group A streptococci. J Exp Med 1964;119:643–650.
[40] Kaplan MH, Svec KH. Immunologic relation of streptococcal and tissue antigens. III. Presence in human sera of streptococcal antibody cross reactive with heart tissue. Association with streptococcal infection, rheumatic fever, and glomerulonephritis. J Exp Med 1964;119:651–666.
[41] Goldstein I, Halpern B, Robert L. Immunological relationship between streptococcus A polysaccharide and the structural glycoproteins of heart valve. Nature 1967;213:44–47.
[42] Cunningham MW, McCormack JM, Talaber LR, Harley JB, Ayoub EM, Muneer RS, Chun LT, Reddy DV. Human monoclonal antibodies reactive with antigens of the group A streptococcus and human heart. J Immunol 1988;141:2760–2766.
[43] Cunningham MW, McCormack JM, Fenderson PG, Ho MK, Beachey EH, Dale JB. Human and murine antibodies cross-reactive with streptococcal M protein and myosin recognize the sequence GLN-LYS-SER-LYS-GLN in M protein. J Immunol 1989;143:2677–2683.
[44] Cunningham MW, Hall NK, Krisher KK, Spanier AM. A study of anti-group A streptococcal monoclonal antibodies cross-reactive with myosin. J Immunol 1985;136:293–298.
[45] Cunningham MW, Swerlick RA. Polyspecificity of antistreptococcal murine monoclonal antibodies and their implications in autoimmunity. J Exp Med 1986;164:998–1012.
[46] Fenderson PG, Fischetti VA, Cunningham MW. Tropomyosin shares immunologic epitopes with group A streptococcal M proteins. J Immunol 1989;142:2475–2481.
[47] Gulizia JM, Cunningham MW, McManus BM. Anti-streptococcal monoclonal antibodies recognize multiple epitopes in human heart valves: Cardiac myosin, vimentin and elastin as potential valvular autoantigens. In: Orefici G, editor. New perspectives on streptococci and streptococcal infections. Proceedings of the XI lancefield international symposium (Zentralbl. Bakteriol. Suppl. 22). New York: Gustav-Fischer-Verlag; 1992. p 267–269.
[48] Dudding BA, Ayoub EM. Persistence of streptococcal group A antibody in patients with rheumatic valvular disease. J Exp Med 1968;128:1081–1098.
[49] Galvin JE, Hemric ME, Kosanke SD, Factor SM, Quinn A, Cunningham MW. Induction of myocarditis and valvulitis in Lewis rats by different epitopes of cardiac myosin and its implications in rheumatic carditis. Am J Pathol 2002;160:297–306.
[50] Neu N, Rose NR, Beisel KW, Herskowitz A, Gurri-Glass G, Craig SW. Cardiac myosin induces myocarditis in genetically predisposed mice. J Immunol 1987;139:3630–3636.
[51] Quinn A, Adderson EE, Shackelford PG, Carroll WL, Cunningham MW. Autoantibody germ-line gene segment encodes VH and VL regions of a human anti-streptococcal monoclonal antibody recognizing streptococcal M protein and human cardiac myosin epitopes. J Immunol 1995;154:4203–4212.
[52] Fischetti VA. Streptococcal M protein: Molecular design and biological behavior. Clin Microbiol Rev 1989;2:285–314.
[53] Manjula BN, Fischetti VA. Sequence homology of group A streptococcal pep M5 protein with other coiled-coil proteins. Biochem Biophys Res Commun 1986;140:684–690.
[54] Manjula BN, Trus BL, Fischetti VA. Presence of two distinct regions in the coiled-coil structure of the streptococcal pep M5 protein: Relationship to mammalian coiled-coil proteins and implications to its biological properties. Proc Natl Acad Sci USA 1985;82:1064–1068.
[55] Krisher K, Cunningham MW. Myosin: A link between streptococci and heart. Science 1985;227:413–415.
[56] Kraus W, Seyer JM, Beachey EH. Vimentin-cross-reactive epitope of type 12 streptococcal M protein. Infect Immun 1989;57:2457–2461.
[57] Swerlick RA, Cunningham MW, Hall NK. Monoclonal antibodies cross-reactive with group A streptococci and normal and psoriatic human skin. J Invest Dermatol 1986;87:367–371.
[58] Dale JB, Beachey EH. Multiple, heart-cross-reactive epitopes of streptococcal M proteins. J Exp Med 1985;161:113–122.
[59] Bronze MS, Beachey EH, Dale JB. Protective and heart-crossreactive epitopes located within the NH2 terminus of type 19 streptococcal M protein. J Exp Med 1988;167:1849–1859.
[60] Cunningham MW, Antone SM, Smart M, Liu R, Kosanke S. Molecular analysis of human cardiac myosin-cross-reactive B- and T-cell epitopes of the group A streptococcal M5 protein. Infect Immun 1997;65:3913–3923.

[61] Quinn A, Ward K, Fischetti V, Hemric M, Cunningham MW. Immunological relationship between the class I epitope of streptococcal M protein and myosin. Infect Immun 1998;66:4418–4424.

[62] Raizada V, Williams Jr, RC, Chopra P, Gopinath N, Prakash K, Sharma KB, Cherian KM, Panday S, Arora R, Nigam M, Zabriskie JB, Husby G. Tissue distribution of lymphocytes in rheumatic heart valves as defined by monoclonal anti-T cell antibodies. Am J Med 1983;74:90–96.

[63] Guilherme L, Cunha-Neto E, Coelho V, Snitcowsky R, Pomerantzeff PMA, Assis RV, Pedra F, Neumann J, Goldberg A, Patarroyo ME, Pileggi F, Kalil J. Human heart-filtrating T cell clones from rheumatic heart disease patients recognize both streptococcal and cardiac proteins. Circulation 1995;92:415–420.

[64] Guilherme L, Oshiro SE, Fae KC, Cunha-Neto E, Renesto G, Goldberg AC, Tanaka AC, Pomerantzeff PM, Kiss MH, Silva C, Guzman F, Patarroyo ME, Southwood S, Sette A, Kalil J. T-cell reactivity against streptococcal antigens in the periphery mirrors reactivity of heart-infiltrating T lymphocytes in rheumatic heart disease patients. Infect Immun 2001;69:5345–5351.

[65] Miller LC, Gray ED, Beachey EH, Kehoe MA. Antigenic variation among group A streptococcal M proteins: Nucleotide sequence of the serotype 5 M protein gene and its' relationship with genes encoding types 6 and 24 M proteins. J Biol Chem 1988;263:5668–5673.

[66] Manjula BN, Fischetti VA. Tropomyosin-like seven residue periodicity in three immunologically distinct streptococal M proteins and its implications for the antiphagocytic property of the molecule. J Exp Med 1980;151:695–708.

[67] Manjula BN, Acharya AS, Mische SM, Fairwell T, Fischetti VA. The complete amino acid sequence of a biologically active 197-residue fragment of M protein isolated from type 5 group A streptococci. J Biol Chem 1984;259:3686–3693.

[68] Yoshinaga M, Figueroa F, Wahid F, Marcus RH, Suh E, Zabriske JB. Antigenic specificity of lymphocytes isolated from valvular specimens of rheumatic fever patients. J Autoimmun 1995;8:601–613.

[69] Roberts S, Kosanke S, Terrence Dunn S, Jankelow D, Duran CM, Cunningham MW. Immune mechanisms in rheumatic carditis: Focus on valvular endothelium. J Infect Dis 2001;183:507–511.

[70] Guilherme L, Cury P, Demarchi LM, Coelho V, Abel L, Lopez AP, Oshiro SE, Aliotti S, Cunha-Neto E, Pomerantzeff PM, Tanaka AC, Kalil J. Rheumatic heart disease: Pro-inflammatory cytokines play a role in the progression and maintenance of valvular lesions. Am J Pathol 2004;165:1583–1591.

[71] Guilherme L, Dulphy N, Douay C, Coelho V, Cunha-Neto E, Oshiro SE, Assis RV, Tanaka AC, Pomerantzeff PM, Charron D, Toubert A, Kalil J. Molecular evidence for antigen-driven immune responses in cardiac lesions of rheumatic heart disease patients. Int Immunol 2000;12:1063–1074.

[72] Fae K, Kalil J, Toubert A, Guilherme L. Heart infiltrating T cell clones from a rheumatic heart disease patient display a common TCR usage and a degenerate antigen recognition pattern. Mol Immunol 2004;40:1129–1135.

[73] Tomai M, Kotb M, Majumdar G, Beachey EH. Superantigenicity of streptococcal M protein. J Exp Med 1990;172:359–362.

[74] Kotb M, Majumdar G, Tomai M, Beachey EH. Accessory cell-independent stimulation of human T-cells by streptococcal M protein superantigen. J Immunol 1990;145:1332–1336.

[75] Degnan B, Taylor J, Hawkes C, O'Shea U, Smith J, Robinson JH, Kehoe MA, Boylston A, Goodacre JA. Streptococcus pyogenes type 5 M protein is an antigen, not a superantigen for human T-cells. Hum Immunol 1997;53:206–215.

[76] Li PLL, Tiedemann RE, Moffat SL, Fraser JD. The superantigen streptococcal pyrogenic exotoxin C (SPE-C) exhibits a novel mode of action. J Exp Med 1997;186:375–383.

[77] Cunningham MW. Autoimmunity and molecular mimicry in the pathogenesis of post-streptococcal heart disease. Front Biosci 2003;8:s533–s543.

[78] Massell BF, Honikman LH, Amezcua J. Rheumatic fever following streptococcal vaccination. JAMA 1969; 207:1115–1119.

[79] Murphy GE, Swift HF. Induction of cardiac lesions, closely resembling those of rheumatic fever, in rabbits following repeated skin infections with group A streptococci. J Exp Med 1949;89:687–698.

[80] Murphy GE, Swift HF. The induction of rheumatic-like cardiac lesions in rabbits by repeated focal injections with group A streptococci. Comparison with the cardiac lesions of serum disease. J Exp Med 1950;91:485–498.

[81] Cromartie WJ, Craddock JG. Rheumatic like cardiac lesions in mice. Science 1966;154:285–287.

[82] Quinn A, Kosanke S, Fischetti VA, Factor SM, Cunningham MW. Induction of autoimmune valvular heart disease by recombinant streptococcal M protein. Infect Immun 2001;69(6):4072–4078.

[83] Huber SA, Cunningham MW. Streptococcal M protein peptide with similarity to myosin induces $CD4^{+}$T cell-dependent myocarditis in MRL/++ mice and induces partial tolerance against coxsackieviral myocarditis. J Immunol 1996;156:3528–3534.

[84] Bisno AL. Non-suppurative poststreptococcal sequelae: Rheumatic fever and glomerulonephritis. In: Mandell GL, Bennett JE, Dolin R, editors. Principles and practice of infectious diseases. Vol.2 New York: Churchill Livingstone; 1995. p 1799–1810.

[85] Bisno AL. The concept of rheumatogenic and non-rheumatogenic group A streptococci. In: Read SE, Zabriskie JB, editors. Streptococcal diseases and the immune response. New York: Academic Press Inc; 1980. p 789–803.

[86] Robinson JH, Atherton MC, Goodacre JA, Pinkney M, Weightman H, Kehoe MA. Mapping T-cell epitopes in group A streptococcal type 5 M protein. Infect Immun 1991;59:4324–4331.

[87] Robinson JH, Case MC, Kehoe MA. Characterization of a conserved helper T-cell epitope from group A streptococcal M proteins. Infect Immun 1993;61:1062–1068.

[88] Pruksakorn S, Currie B, Brandt E, Phornphutkul C, Hunsakunachai S, Manmontri A, Robinson JH, Kehoe MA, Galbraith A, Good MF. Identification of T cell autoepitopes that cross-react with the C-terminal segment of the M protein of group A streptococci. Intl Immunol 1994;6:1235–1244.

[89] Pruksakorn S, Currie B, Brandt E, Martin D, Galbraith A, Phornphutkul C, Hunsakunachai S, Manmontri A, Good MF. Towards a vaccine for rheumatic fever: Identification of a conserved target epitope on M protein of group A streptococci. Lancet 1994;344:639–642.

[90] Pruksakorn S, Galbraith A, Houghten RA, Good MF. Conserved T and B cell epitopes on the M protein of group A streptococci. Induction of bactericidal antibodies. J Immunol 1992;149:2729–2735.