Role of autoimmunity in rheumatic fever

Luiza Guilherme & Jorge Kalil

Rheumatic fever is the prototype of postinfectious autoimmune disease and is caused by untreated Streptococcus pyogenes throat infection in susceptible children. This review presents the major immune-mediated events leading to carditis, valvulitis and Sydenham’s chorea (SC). The role of genetic factors in susceptibility is also discussed. In the last 10–15 years, the pathogenesis of rheumatic autoimmune reactions has been clarified. A brief review on humoral immune response, focusing on crossreactive antibodies against laminin, vimentin and cardiac myosin, and the recent data on specific crossreactive antibodies against streptococcal antigens and glycosaminoglycans leads to the autoimmune reactions in SC is presented. T lymphocytes are the major effectors of crossreactivity between streptococcal and human proteins leading to rheumatic heart disease. Heart tissue T-cell infiltration is facilitated by adhesion molecules, myosin and laminin antibodies. A cytokine balance favoring Th helper 1 inflammatory cytokines and few IL-4-producing cells in heart valves leads to the progression and maintenance of valvular lesions.

Rheumatic fever (RF) is considered an autoimmune disease that affects mainly children and teenagers (aged 3–19 years) who have a genetic predisposition. The disease is caused by untreated throat infection by the Gram-positive bacteria Streptococcus pyogenes. Other environmental factors, such as poor quality of life and limited access to medical care, increase the risk of developing RF. The incidence of acute RF in some developing countries exceeds 50 per 100,000 children [1]. The main clinical features of acute RF were described by Jones in 1944, modified and revised twice; the latest revision remains useful to date [2]. Based on these criteria, the disease manifests as polyarthritis, carditis, chorea, erythema marginatum and/or subcutaneous nodules. Arthritis is the earliest feature of the disease, present in 60–80% of patients. Carditis, the most serious complication, occurs a few weeks after the infection in 30–45% of RF patients, and usually presents as pancarditis. Endocarditis frequently leads to chronic rheumatic heart disease (RHD). Valvular lesions and mitral and aortic regurgitation are the most common events caused by repeated valvulitis. RHD remains a major public-health problem in developing countries, leading to 233,000 deaths/year [3]. The worldwide incidence of RHD is at least 15.6 million cases and the highest documented incidence of the disease among children from developing countries is 5.7 per 1000 in sub-Saharan Africa [1,3].

Autoimmune mechanisms
The ability of S. pyogenes to provide the innate immune system with the correct stimuli for inducing the expansion and differentiation of the pathologic crossreactive, self-specific T-cell repertoire could be crucial for the maintenance of autoimmunity in RF. Both humoral and cellular immune responses are involved in the development of autoimmune reactions leading to RF/RHD in individuals with a genetic predisposition.

Susceptibility factors
The innate and adaptive immune response triggered against S. pyogenes leads to a cascade of autoimmune reactions in those individuals that have genetic predispositions to the disease. The dominant contributors to the autoimmune reactions in RF and RHD are the susceptibility MHC class II alleles (DR and DQ). Several HLA-DR and -DQ alleles have been reported to be associated with RF, RHD and Sydenham’s chorea [4]. Among the class II susceptibility alleles, HLA-DR7 was found to be associated with the disease in patients from four ethnically diverse countries (Brazil, Egypt, Latvia and Turkey) and the association of this allele with some DQ alleles seems to be linked to the development of valvular lesions in RHD [4]. The TNF-α gene is also located on chromosome 6, between MHC class I and class II, and presents an inflammatory function. The association of TNF-α alleles (-308A and -238A) with RF and RHD was recently described [5,6]. It is possible that this association could be due to linkage disequilibrium with MHC class II molecules and/or responsible for an exacerbation of inflammatory response with high levels of the TNF-α cytokine.
**Molecular mimicry**

The role of HLA molecules is to present antigens to the T-cell receptor, triggering the activation of the adaptive immune response. HLA class II molecules are expressed on the surface of antigen-presenting cells such as macrophages, dendritic cells and B lymphocytes. Although the molecular mechanism by which MHC class II molecules confer susceptibility to autoimmune diseases is not clear, the presentation of pathogen epitopes with structural or sequential similarity to self epitopes might activate autoreactive T lymphocytes that have escaped immune tolerance by the molecular mimicry mechanism. These autoreactive T cells can also activate B cells that produce antigen-specific antibodies.

Several streptococcal and human protein cross-reactive antibodies found in the sera of RF patients and immunized rabbits and mice have been described and reviewed [7,8]. Antibodies against N-acetyl β-D-glucosamine, a polysaccharide present in both streptococcal cell-wall and heart valve tissue, displayed crossreactivity against laminin, an extracellular matrix α-helical coiled-coil protein, surrounding heart cells and also present in the valves [7,8]. Among human proteins, cardiac myosin and vimentin seem to be the major target antigens. By using affinity-purified antomyosin antibodies, Cunningham's group identified a five-amino-acid residue (Gln-Lys-Ser-Lys-Gln) epitope of the N-terminal M 5 and M 6 proteins as crossreactive with cardiac myosin [9].

Sydenham's chorea (SC), another important manifestation of RF, is mediated by antibodies able to bind to neuronal cells. Recently, it was demonstrated that lysoganglioside GM 1 from neuronal cells crossreacted with N-acetyl β-D-glucosamine, an antigen that is present in the cell wall of S. pyogenes, by molecular mimicry. These antibodies mediate signal transduction by calcium/calmodulin-dependent protein kinase II, triggering dopamine release from neuronal cells [10]. By using a monoclonal antibody derived from cells of an SC patient, the same group identified tubulin, an intracellular α-helical protein, as a putative autoantigen target of autoimmune reactivity in patients with SC [11].

The interplay of humoral and cellular immune responses in RHD was only recently demonstrated by two elegant studies by Cunningham's group in which they showed that in rheumatic carditis, streptococcal and human protein crossreactive antibodies upregulate the adhesion molecule VCAM-1 after binding to the endothelial surface [12], leading to inflammation, cellular infiltration and valve scarring [13]. These data definitively establish the role of heart tissue crossreactive antibodies (cardiac myosin and laminin) in the early stages of inflammation and T-cell infiltration in RHD lesions.

Studies performed in the last 25 years showed that CD 4+ cells are the major effectors of autoimmune reactions in the heart tissue in RHD patients [14–16]. Yoshinaga et al. reported that T-cell lines derived from heart-valve specimens and peripheral blood mononuclear cells from RF and RHD patients react with streptococcal cell-wall and -membrane antigens. However, these lymphocytes did not crossreact with M protein or mammalian cytoskeletal proteins [17]. The role of T cells in the pathogenesis of RF and RHD was demonstrated through the analysis of heart-tissue-infiltrating T-cell clones. Immunodominant peptides of the M 5 protein (residues 81–96 and 83–103) displayed crossreactivity with valvular proteins and cardiac myosin peptides by molecular mimicry [17,18]. These M 5 epitopes were also preferentially recognized by peripheral T lymphocytes from RHD patients when compared with normal individuals, mainly in the context of HLA-DR7 [19].

Table 1 summarizes these results. Another study showed that peripheral T cells from RHD patients stimulated in vitro were able to recognize a 50–54-kDa myocardial protein fraction, indicating autoreactivity to heart antigens probably caused by streptococcal infection [20].

Cardiac myosin is the well-known autoantigen target of the immune response in several inflammatory heart diseases [21]. Antibodies and T cells from RHD patients and cells from experimental myocarditis and/or valvulitis induced by myosin or streptococcal antigens were able to recognize several cardiac myosin epitopes [21]. Recently, two works demonstrated the mimicry between cardiac myosin and streptococcal M protein and pointed out different patterns of T-cell antigen crossrecognition. One of these studies focused on peripheral T-cell clones from a patient with RHD that recognized different α-helical coiled-coil proteins such as streptococcal M protein, myosin, laminin and tropomyosin. In addition, an in-depth analysis of the N-terminal portion of both streptococcal M 5 and cardiac myosin identifies three M 5 protein epitopes and three cardiac myosin (S2 and light meromyosin [LM M ] regions) epitopes by molecular mimicry [22]. Another study, performed by our group, focused on the reactivity of intraleisonal T-cell clones derived from myocardium and...
valvular tissue of six RHD patients against the LMM region of cardiac myosin, M5 streptococcal protein and valve-derived proteins. A high frequency of reactive T-cell clones was found (63%). These T cells displayed three patterns of crossreactivity, as follows:

- Cardiac myosin (LMM region) and valve-derived proteins;
- Cardiac myosin and M5 streptococcal peptides;
- Cardiac myosin, M5 streptococcal peptides and valve-derived proteins [19].

Table 2 shows the amino acid sequence alignment of crossreactive M5 (81–103) peptide and cardiac myosin peptide (LMM10, residues 1413–1430) and illustrates the second pattern of crossreactivity. The homology observed reached 87%, based on the identities of amino acid residues and also on the conserved substitutions [19].

Table 2. Amino acid residue homology between cardiac myosin and streptococcal M protein peptides.

<table>
<thead>
<tr>
<th>Crossreactive epitopes recognized by intraleisonal T cells</th>
<th>Amino acid sequence alignment</th>
<th>Homology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac myosin - LMM10 (1413–1430)</td>
<td>C S S L E K T K H R L Q N E I E D L</td>
<td></td>
</tr>
<tr>
<td>Streptococcal – M5 (83–103)</td>
<td>L K Q Q R D T L S T Q K E T L E R E V Q N</td>
<td>87%</td>
</tr>
</tbody>
</table>

*Identical amino acids residues.
**Conserved substitutions.
§Semi-conserved substitutions.
Adapted from [19].
The BV13 family in the periphery was 4.3% and displayed a polyclonal profile, while in heart tissue the relative frequency was 8.5% in an oligoclonal expansion [24]. The BV13 family has the possibility to combine with 13 JB segments. Although we did not determine the frequencies of JB segments in the peripheral blood, we analyzed all the JB segments for those intralesional T cells bearing the TCR-BV13 that were in oligoclonal expansion, as mentioned above. The results showed that this family combined preferentially with the BJ2S7 segment (62%) [24]. Figure 1 shows the CDR3 patterns found for lymphocytes from a RHD patient bearing the TCR-BV13 for both peripheral and intralesional T-cell populations, the possible JB segment combinations in the periphery and the major populations found in the heart tissue as oligoclonal expansions (BV13–BJ2S7 and BV13–BJ1S5). Several intralesional T-cell clones bearing BV13–JB2S7 presented the same amino acid sequences in the CDR3 region of the TCR, which, as mentioned above, interacts with the antigenic peptide and recognizes several streptococcal M-protein peptides, heart-tissue proteins and cardiac myosin peptides derived from the LMM region, indicating degeneracy in antigen.

**Figure 1. T-cell populations from peripheral blood to rheumatic heart disease lesions.**

<table>
<thead>
<tr>
<th>CD</th>
<th>CDR3</th>
<th>Relative Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>BJ1S5</td>
<td>10aa</td>
<td>11%</td>
</tr>
<tr>
<td>BJ2S7</td>
<td>10aa</td>
<td>62%</td>
</tr>
</tbody>
</table>

TCR diversity is based on the combination of 22 VB and 13 JB families. The degree of clonality is determined by the β-chain CDR3 length. Here we showed the pattern found for lymphocytes from one RHD patient bearing the TCR-BV13 for both peripheral and intralesional T-cell populations and the possibilities of the 13 JB-segment combinations in the periphery and the major populations found in the heart tissue as oligoclonal expansions (BV13 BJ2S7 and BV13 BJ1S5). The relative frequency of T cells from a PBMC-bearing TCR-BV13 family was 4.2% as a polyclonal population. The same TCR-BV13 family was found in the mitral-valve tissue as an oligoclonal population representing 8.5% of infiltrating T cells, indicating that this population was preferentially expanded in the tissue. Most of this T-cell population in the mitral-valve tissue combines with the JB2S7 segment (62%) with a CDR3 region length of ten amino acid residues. Their reactivity is shown in Table 3. Another TCR-BV13 oligoclonal population combines with BJ1S5 with ten amino acid residues in the CDR3 region, with relative frequencies of 11%

aa: Amino acid; CDR: Complementarity–determining region; PBMC: Peripheral blood mononuclear cell; RHD: Rheumatic heart disease; TCR: T-cell antigen receptor.
Table 3. T-cell clones bearing the same T-cell receptor recognize several antigens.

<table>
<thead>
<tr>
<th>T-cell clone</th>
<th>Antigen recognized</th>
<th>TCR BV family</th>
<th>CDR3 region</th>
<th>CTR BJ family</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1.8</td>
<td>*Miv 35 kDa/pI 8.84 LMM 22 (1567–1585) LMM 24 (1594–1611) LMM 46 (1881–1898) LMM 49 (1920–1936)</td>
<td>BV13</td>
<td>SGRQGRYEQY (10aa)</td>
<td>BJ2S7</td>
</tr>
<tr>
<td>3.1.10</td>
<td>M5 (81–96) M5 (83–103)</td>
<td>BV13</td>
<td>SGRQGRYEQY (10aa)</td>
<td>BJ2S7</td>
</tr>
<tr>
<td>3.1.29</td>
<td>*Miv 56–53 kDa/pI 6.76</td>
<td>BV13</td>
<td>SGRQGRYEQY (10aa)</td>
<td>BJ2S7</td>
</tr>
</tbody>
</table>

- T-cell clones were derived from mitral valve tissue (3.1.3, 3.1.8, 3.1.10 and 3.1.29) and myocardium (3.2.12.9); *Mitral valve-derived protein identified by molecular weight (kDa) and isoelectrical point (pI). Cardiac myosin peptides (LMM) sequences:

Streptococcal M5(81–96) and M5(83–103) peptides sequences are shown in Table 1.

<table>
<thead>
<tr>
<th>T-cell clone</th>
<th>Antigen recognized</th>
<th>TCR BV family</th>
<th>CDR3 region</th>
<th>CTR BJ family</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1.8</td>
<td>*Miv 35 kDa/pI 8.84 LMM 22 (1567–1585) LMM 24 (1594–1611) LMM 46 (1881–1898) LMM 49 (1920–1936)</td>
<td>BV13</td>
<td>SGRQGRYEQY (10aa)</td>
<td>BJ2S7</td>
</tr>
<tr>
<td>3.1.10</td>
<td>M5 (81–96) M5 (83–103)</td>
<td>BV13</td>
<td>SGRQGRYEQY (10aa)</td>
<td>BJ2S7</td>
</tr>
<tr>
<td>3.1.29</td>
<td>*Miv 56–53 kDa/pI 6.76</td>
<td>BV13</td>
<td>SGRQGRYEQY (10aa)</td>
<td>BJ2S7</td>
</tr>
</tbody>
</table>

T-cell clones were derived from mitral valve tissue (3.1.3, 3.1.8, 3.1.10 and 3.1.29) and myocardium (3.2.12.9); *Mitral valve-derived protein identified by molecular weight (kDa) and isoelectrical point (pI). Cardiac myosin peptides (LMM) sequences:

Streptococcal M5(81–96) and M5(83–103) peptides sequences are shown in Table 1.

aa: Amino acid; LMM: Light meromyosin; Miv: Mitral valve; TCR: T-cell receptor.

recognition [18,23,25]. These data are summarized in Table 3. Different streptococcal and cardiac-myosin peptides (LMM and S2 regions) and laminin crossreactive T-cell clones from the periphery use a unique TCR [22]. Together, these data provide support for the idea that antigen-specific peripheral T-cell populations migrate to the heart tissue, expand locally and become capable of recognizing several antigens by an epitope-spreading mechanism [26] in which the reactivity during an ongoing autoimmune response is induced against self epitopes that are distinct from the pathogen-inducing epitope. In addition, the evolution of an autoimmune pathology may easily remove any evidence of the initial target antigens, such as streptococcal M protein, and crossreactive self antigens can consequently display a degenerate pattern of antigen recognition.

Cytokines

An effective immune response depends on cytokine production. CD4+ T-helper cells are crucial regulators of the adaptive immune response. Antigen-activated CD4+ T cells polarize to T-helper (Th)1 or Th2 based on the pattern of cytokines they secrete. Th1 cells are involved in the cellular immune response and produce IL-2, IFN-γ and TNF-α. Th2 cells mediate humoral and allergic immune responses and produce IL-4, IL-5 and IL-13. Although IL-17 was described more than 10 years ago, Th cells producing IL-17 were only recently classified as a new CD4 T-cell subset [27]. TGF-β, IL-6 and IL-23 are the differentiator factors of the Th17 lineage. In vitro studies indicate a proinflammatory function of IL-17, and its expression was found to be associated with some inflammatory and autoimmune diseases, reviewed in [27,28].

Inflammatory reactions are triggered by S. pyogenes infection. The sera of patients with RF/RHD and peripheral mononuclear cells stimulated by streptococcal antigens produced increased amounts of proinflammatory cytokines (IL-1 and IL-6) and the inflammatory cytokines TNF-α and IFN-γ [8]. In the heart tissue (myocardium and valves) of acute and chronic RHD patients, we identified a large number of mononuclear cells able to secrete inflammatory cytokines (TNF-α and IFN-γ) and the regulatory cytokine IL-10 by immunohistochemistry. While a significant number of IL-4+ cells were found in the myocardium, these cells were very scarce in valve lesions of RHD patients [29]. These observations demonstrated the role of Th1/Th2 cytokine balance in healing myocarditis and in the induction of progressive and permanent valve damage [29]. Th17 cytokines have not yet been studied in RHD and it is possible that they also play an important role in the development of heart lesions. In support of this idea, some autoimmune diseases, such as multiple sclerosis, rheumatoid arthritis, psoriasis and Crohn's disease, traditionally viewed as prototypic Th1 CD4+ cell diseases, are now being defined as Th17-driven autoimmune inflammatory diseases [30].
Future perspective

The identification of the protein targets of autoimmune reactions in RHD is now being evaluated using proteomics. Their identification could be useful for the development of an RF/RHD clinical diagnostic test. The study of new genes involved in susceptibility to the disease is also important. The elucidation of the Th17 cytokines and chemokines involved with the recruitment of Th1/Th2 and Th17 cell subsets will complete the picture of the heart tissue inflammatory process in RHD. Several candidate vaccines aimed at the prevention of RF/RHD are in development, and a safe and effective vaccine may be available in the next 10 years.

Acknowledgements

We acknowledge Dr Edecio Cunha-Neto from the Heart Institute and anonymous peer reviewers for their suggestions.

Financial & competing interests disclosure

This work was supported by grants from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Executive summary

- In rheumatic carditis, streptococcal and human protein crossreactive antibodies induce the augmentation of adhesion molecules, leading to inflammation and the facilitation of cellular infiltration. Heart-tissue and streptococcal antigen crossreactive CD4+ T cells are the major effectors of heart lesions.

- In Sydenham's chorea, crossreactive antibodies against lysoganglioside and N-acetyl-β-D-glucosamine interfere with dopamine release in neuronal cells and are probably involved in the development of the disease.

- Both epitope spreading and T-cell receptor degeneracy mechanisms increase the possibility of crossreactivity between the infectious agent and self antigen.

- Large numbers of mononuclear cells infiltrating rheumatic heart lesions produce inflammatory cytokines (TNF-α and IFN-γ), and the imbalance of Th2 anti-inflammatory cytokine IL-4-producing cells in the valve tissue might contribute to the progression and maintenance of rheumatic valvular lesions.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.


D demonstrates the augmentation of the adhesion molecule VCAM-1 on valve specimens from RHD patients that facilitates the inflammatory process in rheumatic valve endocardium.


** Reactivity of RHD heart lesion-derived T-cell clones against cardiac myosin peptides highlights three patterns of autoimmune crossreactivity.


** D escribes the reactivity of peripheral T-cell clones derived from one RF patient against streptococcal antigens and human cardiac myosin and laminin.


** Review of the relevance of polyspecificity in lymphocyte development, activation and disease process.


** Several heart-tissue infiltrating T-cell clones expressing the same T-cell receptor BV family were able to recognize multiple antigens.


** Cytokine profile was analyzed in 20 heart-tissue fragments of 14 RHD patients. Heart-tissue infiltrating T-cell lines were also studied for the production of Th1/Th2 cytokines upon M-protein stimulation.


** D cribes the role of Th17 cell subset in human autoimmune diseases and possible new treatments.

Affiliations
- Luiza Guilherme
  Heart Institute (InCor), School of Medicine, University of São Paulo and Institute for Immunology Investigation, Millenium Institute, Av. Dr. Eneas de Carvalho Aguiar, 44 05403-903 São Paulo, SP, Brazil
  Tel.: + 55 113 069 5901
  Fax: + 55 113 069 5953
  luizaguil@usp.br

- Jorge Kaliil
  Heart Institute (InCor) and Clinical Immunology and Allergy Division, School of Medicine, University of São Paulo and Institute for Immunology Investigation, Millenium Institute, Av. Dr. Eneas de Carvalho Aguiar, 44 05403-903 São Paulo, SP, Brazil
  Tel.: + 55 113 069 5901
  Fax: + 55 113 069 5953
  jkaliil@usp.br