

Rheumatic Heart Disease: Genes, Inflammation and Autoimmunity

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Abstract

Rheumatic heart disease is a sequel of rheumatic fever that follows an untreated group A streptococcal infection in young susceptible individuals. The disease is mediated by autoimmune reactions. Several genes related to both the innate and adaptive immune response are involved. Several HLA class II alleles have been associated with the disease. In the present review, we focus on punctual genetic polymorphisms associated with RF/RHD development, most of which are related innate immunity. The role of inflammatory cytokines as mediators of rheumatic heart lesions and a discussion of the major autoantigens recognized due to their molecular mimicry with streptococcal antigens are also presented. A vaccine against *S. pyogenes* is being developed, and an increase in the knowledge of the underlying mechanisms of the disease will certainly facilitate the development of an effective and safe vaccine.

Keywords: *S. pyogenes*; Autoimmunity; Molecular mimicry; Rheumatic fever; T cell receptor; Cytokines; Vaccine; Genetic susceptibility

Abbreviations: APC: Antigen-Presenting Cell; ARF: Acute Rheumatic Fever; ASO: Antistreptolysin O Titer; CD80: Cluster of Differentiation 80; CD86: Cluster of Differentiation 86; CTLA4: Cytotoxic T-Lymphocyte Antigen 4; DC: Dendritic Cell; FCγRIIA: Receptor for the Fc Fragments of Immunoglobulin G; FCN2: Ficolin 2; GAS: Group A *Streptococcus*; GlcNAC: N-acetylglucosamine; HCM: Human Cardiac Myosin; HLA: Human Leukocyte Antigen; HSPA5: Heat Shock 70 kDa Protein 5; IFNγ: Interferon gamma; IgG2: Immunoglobulin 2; IL: interleukin; IL1RA: Interleukin-1 Receptor Antagonist; LMM: Light Meromyosin; MBL: Mannose-Binding Lectin; MVL: Multivalvular Lesion; PDIA3: Protein Disulfide Isomerase family A, member 3; PRR: Pattern Recognition Receptors; RF: Rheumatic Fever; RHD: Rheumatic Heart Disease; SNP: Single Nucleotide Polymorphism; TCR: T Cell Receptor; TGFβ: Tumor Growing Factor-beta; Th: T helper lymphocyte subsets 1 and 2 and 17; TLR2: Toll Like Receptor 2; TNFα: Tumor Necrosis Factor-Alpha; Tr1: Type 1 (IL-10-producing) T regulatory cell; Treg: Regulatory T cells; VCAM1: Vascular Cell Adhesion Molecule 1; VNTR: Variable Number Tandem Repeat

Introduction

Rheumatic Fever (RF) is an autoimmune disease that is mediated by both the humoral and cellular immune responses that follow an untreated pharyngeal *Streptococcus pyogenes* infection. The disease is characterized by tissue inflammation that contributes to typical clinical characteristics, such as arthritis, chorea and carditis/valvulitis, which were first described by Jones in 1944 and modified and revised later [1]. The most serious complication is rheumatic heart disease (RHD), which occurs in 30 to 45% of RF patients and leads to chronic valvular lesions [2].

RF and RHD are complex diseases and depend on genetic and environmental factors. The autoimmune reactions are the hallmark of the pathogenesis of the disease. Molecular mimicry, the sharing of epitopes between antigens of the host and *S. pyogenes*, has been proposed to be the triggering factor leading to the disease. Both cross-reactive antibodies and T cells play a role in the cross-recognition between streptococcal antigens and human proteins leading to inflammation and autoimmunity [3].

In the present review, we focus on the genetic factors, the bacterial

and human protein cross-reactions and the inflammatory processes that lead to the heart tissue lesions in RHD patients.

The Role of Genes in the Pathogenesis of Rheumatic Fever and Rheumatic Heart Disease

Protection against pathogens relies on complex interactions between the genetically controlled innate and adaptive immune responses.

The innate immune response provides the first line of defense against *S. pyogenes* infections, in the case of RF, with complement cascade activation. During the innate response, the adaptive response is initiated through antigen processing and presentation to T cells and by cytokine secretion [4] (Figure 1).

Several polymorphisms in genes that code for molecules involved in mounting the effector innate and adaptive immune response contribute to RF and RHD susceptibility.

Within the early innate immune response, associations have been found between polymorphisms in genes that code for mannose-binding lectin (MBL2), ficolin-2 (FCN2), receptor for the Fc fragments of immunoglobulin G (FCγRIIA) and toll like receptor 2 (TLR2). In addition, polymorphisms in genes that play a role in both innate and adaptive immunity, such as tumor necrosis factor-alpha (TNFα), interleukin-1 receptor antagonist (IL1RA), tumor growth factor-beta (TGFβ), and cytotoxic T cell lymphocyte antigen 4 (CTLA4) (Table 1), can contribute to the pathogenesis of RF and RHD.

The complement system is part of the innate immune system and consists of many proteins that are involved in a cascade of proteolysis and protein complex assembly that culminates in the elimination of

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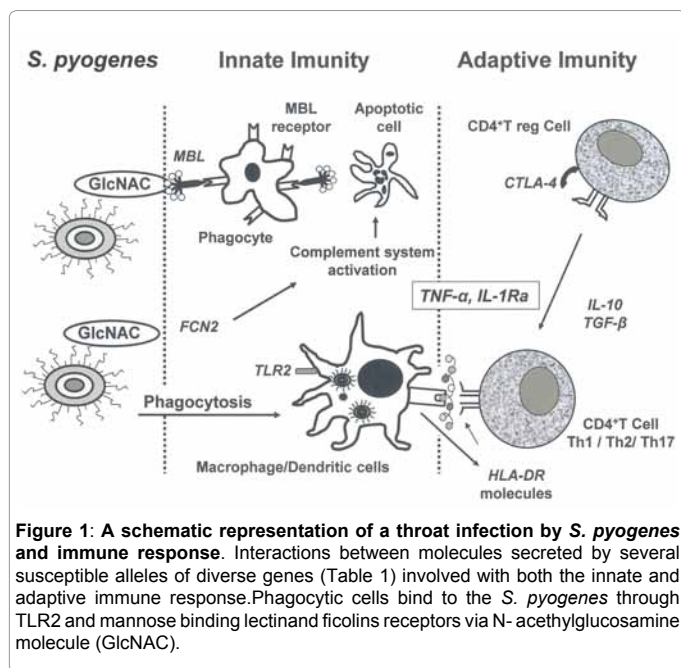


Figure 1: A schematic representation of a throat infection by *S. pyogenes* and immune response. Interactions between molecules secreted by several susceptible alleles of diverse genes (Table 1) involved with both the innate and adaptive immune response. Phagocytic cells bind to the *S. pyogenes* through TLR2 and mannose binding lectin and ficolins receptors via N-acetylglucosamine molecule (GlcNAC).

invading pathogens [5]. Several components of the bacterial cell surface combine with pattern recognition receptors (PRRs) such as MBL or the ficolin family of proteins. MBL is an acute phase inflammatory protein and functions as a soluble pathogen recognition receptor. It binds to a wide variety of sugars on the surface of pathogens and plays a major role in innate immunity due to its ability to opsonize pathogens, enhancing their phagocytosis and activating the complement cascade via the lectin pathway [6]. N-acetylglucosamine (GlcNAC), present in the cell wall of *S. pyogenes*, is a strong ligand for MBL.

Different variants of the promoter and exon 1 regions of the MBL2 gene are correlated with deficient MBL in the plasma and have been shown to be associated with recurrent infections in children and several infectious diseases [6]. Moreover, different alleles are associated with different clinical representations of RF and RHD (Table 1) [7,8] and probably play a role in the development of valvular lesions.

Ficolins also trigger the innate immune response by either binding to collectin cellular receptors or initiating the complement lectin pathway. Polymorphisms within the promoter region of the *FCN2* gene are associated with plasma levels of this protein in chronic RHD patients [9] and probably prolong the time of infection or repeated streptococcal infections (Table 1).

Toll like receptors are capable of recognizing a wide spectrum of organisms, including viruses, bacteria and other parasites. TLR activation leads to the production of proinflammatory cytokines that enable macrophages and dendritic cells (DC) to eliminate invading pathogens. TLR2 is reported to interact with different bacterial structures, including lipoproteins, peptidoglycan and lipoteichoic acid [10]. Some of these structures are present on the surface of *S. pyogenes* [11]. A single nucleotide polymorphism (SNP) in exon 3 (Table 1) is associated with a high or low susceptibility to acute rheumatic fever (ARF). This association is correlated with ASO levels in the sera [10]. A recent study reported that *in vitro* human cardiac myosin (HCM) binds to TLR2 and TLR8 thus activating human monocytes to release pro-inflammatory cytokines that will trigger the activation of the adaptive immune response. Based on these observations, the authors suggested that pathogenic T cell epitopes from human cardiac myosin may link

the innate and adaptive responses to promote chronic inflammation in the myocardium [12].

FCγRIIA protects the host against foreign antigens by removing antigen-antibody complexes from the circulation [13]. SNPs in the FCγRIIA gene are expressed in a codominant manner and cause changes in the ability of receptor to bind to human IgG2. The genotypes of the SNPs are associated with different levels of risk of developing RF [14].

Polymorphisms in the cytokine genes also seem to be involved with the disease. The interleukin 1 (IL-1) gene cluster located on chromosome 2 includes the genes expressing the proinflammatory cytokines IL-1a and IL-1b and their inhibitor IL-1 receptor antagonist (IL-1RA). The ratio of IL-1RA to IL-1 is important in determining the duration and intensity of the inflammatory response [15]. The absence or misrepresentation of two alleles of VNTR from the IL-1RA gene results in a strong inflammatory response. RHD patients with severe carditis had low frequencies of one of these alleles, suggesting the absence of inflammatory control (Table 1) [16,17].

The TNFA gene codes for the inflammatory cytokine TNFα. An association of TNFα polymorphisms with RF and RHD (Table 1) has been shown in three independent studies. Thus, the variants of TNFα may be one of the predisposing risk factors for RF and RHD contributing to the development of valve lesions; TNFα is likely to act in synergy with other factors, both genetic and environmental, in the development of the disease [18-21].

IL-10, together with TGFβ and IL-35, is one of the most important anti-inflammatory cytokines. It is produced by activated immune cells, especially monocytes/macrophages and T cell subsets including regulatory T cells (Tr1 and T reg) and Th1 cells [22]. A large number of polymorphisms have been identified in the IL-10 gene promoter. Polymorphisms in this region are overrepresented in RHD patients and are associated with both the development of multiple valvular lesions (MVL) and the severity of RHD (Table 1) [16]. Similarly, some studies showed that alleles of the TGFβ1 gene were risk factors for the development of valvular RHD lesions (Table 1) [23,24].

In summary, functional polymorphisms of innate immune response genes involved with inflammatory reactions and host defenses against pathogens that are associated with the disease probably contribute to the development of valvular lesions and can determine the type of rheumatic valvular lesions (stenosis, regurgitation, or both) that occur in RHD patients (Table 1). More recently, a polymorphism in gene that codes for CTLA4, a negative regulator of T cell proliferation that plays a role in the adaptive immune response, has also been associated with RHD because the polymorphism affects the inhibitory function of the protein coded by this gene (Table 1) [25].

HLA class II alleles were described more than 30 years ago and are coded by several genes located on human chromosome 6. These alleles code for the HLA molecules that are expressed on the surface of antigen-presenting cells (APCs) and are crucial for triggering the adaptive immune response via the T cell receptor. Several HLA class II alleles have been described to be associated with RF and RHD [26,27]. The HLA-DR7 allele seems to be most frequently associated with the disease [27].

The Inflammatory Process Leading to Rheumatic Heart Disease Lesions

A *S. pyogenes* throat infection triggers an inflammatory reaction that involves the production of several inflammatory cytokines by

Gene	Chromosome localization	Polymorphism	Position in gene	Allele/Genotype/Haplotype associated with disease	Clinical picture	Functional alterations	Population studied	Ref.
MBL2	10q11.2-q21	-221 X,Y A (52C, 54G, 57G), O (52T, 54A, 57A) A (52C, 54G, 57G), O (52T, 54A, 57A)	Promoter and exon 1	YA/YA, YA/XA	RHD - MS	High production of MBL	Brazilian	7
				O, O/O	RHD - AR	Low level of MBL in sera	Brazilian	8
FCN2	9q34	-986G/A, -602G/A, -4G/A	promoter	G/G/A	RHD	Low levels of ficolin 2	Brazilian	9
TLR2	4q32	2258A/G (753 Arg/ Gln)	exon 3	753Gln, Arg753Gln	ARF	Low level of pathogen recognition	Turkish	10
FCγRIIA	1q21-q23	494A/G (131H/R)	exon 4	131R, R/R (high risk), R/H (intermediate risk)	ARF	Low ability of binding human IgG2	Turkish	14
IL1RA	2q14.2	A1,A2,A3,A4	Intron 2	A1/A1	RHD	Low level of receptor expression	Egyptian	16
				A1, A1/A1	RHD		Brazilian	17
TNFα	6p21.3	-308G/A	promoter	A	RHD	Th1/Th2 imbalance	Mexican	20
				A/A	RHD-MVL		Egyptian	16
				A/A, G/G	RHD-MVD		Egyptian	16
				A	ARF/RHD		Brazilian	18
				A	ARF/RHD	High production of TNF alpha	Turkish	19
TGFβ	19q13.1	-509C/T	promoter	G, G/G	RHD		Mexican	18
				A	ARF/RHD		Brazilian	19
				T	RHD	Th1/Th2 imbalance	Egyptian	24
IL-10	1q31-q32	869T/C -1082G/A	Exon 1 promoter	C/C	HD x RHD	Protection to RHD	Chinese	23
				T, T/T	RHD		Egyptian	24
CTLA4		+49A/G	Exon 1	G/G	RHD-MVD	Th1/Th2 imbalance	Egyptian	16
				A/A	RHD-MVL		Egyptian	16
				G/G	RHD	CTLA-4 affected inhibitory function	Turkish	25

TNFα: Tumor Necrosis Factor alpha; **TGFβ:** Transforming Growth Factor beta; **IL-1RA:** IL-1 Receptor Antagonist; **MBL:** Mannan Binding Lectin; **TLR2:** Toll Like Receptor 2; **FCN2:** Ficolin 2; **FCγRIIA:** IgG Fc receptor; **CTLA4:** Cytotoxic T cell Lymphocyte Antigen 4; **ARF:** Acute Rheumatic Fever; **RHD:** Rheumatic Heart Disease; **AR:** Aortic Regurgitation; **MS:** Mitral Stenosis; **MVD:** Mitral Valve Disease; **MVL:** Multivalvular Lesions; **HD:** Healthy Donors

Table 1: Punctual genetic polymorphism associated with RF/RHD development.

peripheral mononuclear cells aimed at controlling the infection. In individuals with a genetic predisposition, an exacerbated inflammatory reaction occurs leading to intense cytokine production by monocytes and macrophages that triggers the activation of B and T lymphocytes. Specific antibodies activate the heart tissue valvular endothelial cells increasing the expression of some adhesion molecules such as VCAM1, which facilitates cellular infiltration by neutrophils, monocytes, B and T cells [28].

Several streptococcal-primed T cells migrate from the periphery to the heart tissue (myocardium and valves) of patients with RHD. These antigen-driven oligoclonal T cell expansions probably cause the rheumatic heart lesions [29]. These cells are CD4+ and produce inflammatory cytokines (TNFα and IFNγ). The expression of regulatory cytokines IL-10 and IL-4 were also evaluated in the heart tissue. A similar numbers of IL-10 producing cells were found in both myocardium and valvular tissue, however a scarce numbers of IL-4+ cells (less than 10%) were found in the valve lesions of RHD patients (Figure 2). IL-4 is a Th2-type cytokine and plays a regulatory role in the inflammatory response mediated by Th1 cytokines. Our findings indicate that the low numbers of IL-4-producing cells in the valves probably induces progressive and permanent valve damage while the Th1/Th2 cytokine balance has a role in healing myocarditis [30] (Figure 2). Another lineage of CD4+ T cells (Th17) have been more recently

described and produce a complex set of cytokines initially identified as IL-17, TGFβ, IL-6 and IL-23. This set of cells plays a role in several autoimmune diseases [31].

Recently, using immunohistochemistry, we identified myocardium and valvular infiltrating cells that were positive for IL-17 and IL-23. The expression of these cytokines were also observed in the valvular endothelium [manuscript in preparation] showing that Th17 cells also play an important role in the inflammatory process in RHD heart lesions.

Triggering Autoimmunity by Molecular Mimicry

The molecules produced during the innate immune response act as signals to activate adaptive immunity. In RF and RHD, the adaptive immune responses play an important role in the maintenance and propagation of inflammation that leads to tissue lesions. Antigen presenting cells (APCs), such as DCs, are activated and express costimulatory (CD80 and CD86) and MHC molecules on their cell surface that enable these cells to present processed antigens to T cells through the T cell receptor (TCR). Cytokines, such as TNFα and IFNγ, act at the site of infection and can affect pathogen survival and control the immune response [4]. The down-regulation of the immune response is driven by T regulatory cells and is critical to avoid exacerbated reactions and to maintain tolerance to self-antigens.

Several heart tissue-derived proteins and cardiac myosin synthetic peptides were identified as target antigens during the autoimmune process leading to rheumatic heart lesions [32-34]. Briefly, these studies described the reactivity of both peripheral T cells and heart tissue intralesional T cell clones against rheumatic heart disease valve tissue-derived proteins such as laminin, PDIA3, HSPA5 and several synthetic myosin peptides derived from the light meromyosin region (LMM) that spans 316 amino acid residues. The mechanism that leads to the recognition of self-antigens is molecular mimicry. In RHD, after a non treated throat infection, self-proteins are recognized by the similarities of their amino acids residues with *S. pyogenes* proteins or conformational structures. The autoimmune reaction can be exacerbated by a mechanism called epitope spreading in which the reactivity against an immunodominant antigen triggers the recognition of several other self-proteins leading to a broad inflammatory immune response (Figure 3).

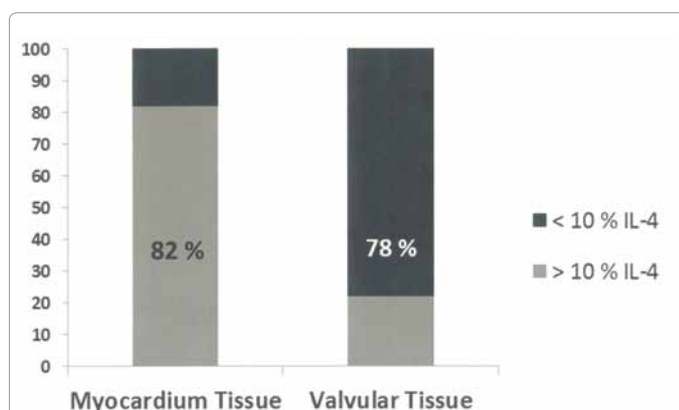


Figure 2: The regulatory cytokine IL-4 is misrepresented in the valves. Mononuclear cells producing IL-10 and IL-4 were evaluated by immunohistochemistry in myocardium and valvular tissue of RHD patients (n=11) [30]. Most of samples from the myocardium tissue (82%) had IL-4 positive cells, while 78% of valvular samples have low numbers of IL-4 positive cells.

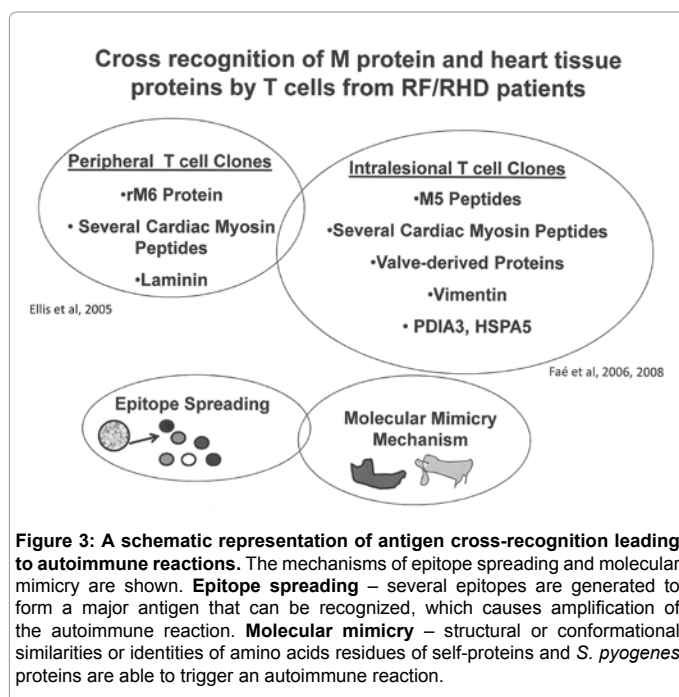


Figure 3: A schematic representation of antigen cross-recognition leading to autoimmune reactions. The mechanisms of epitope spreading and molecular mimicry are shown. **Epitope spreading** – several epitopes are generated to form a major antigen that can be recognized, which causes amplification of the autoimmune reaction. **Molecular mimicry** – structural or conformational similarities or identities of amino acids residues of self-proteins and *S. pyogenes* proteins are able to trigger an autoimmune reaction.

Altogether, the data on the mechanisms leading to RF and RHD suggest a complex network of inflammatory and immune reactions controlled by several genes that can drive the rheumatic heart lesions.

Development of a Vaccine against Streptococcus Pyogenes

Seeking the prevention of *S. pyogenes* infections and its complications, many studies have been performed to develop a vaccine against the bacteria reviewed by Steer and Carapetis [35]. The greatest challenge in the development of a GAS vaccine is to promote immunity without cross-reactivity to human tissue. Increased knowledge about the mechanisms leading to the autoimmune reactions mentioned above should favor the development of a vaccine against *S. pyogenes* without triggering autoimmune reactions.

Thus, we constructed a vaccine epitope (StreptInCor) composed of 55 amino acids residues of the C-terminal portion of the M protein that encompasses both T and B cell protective epitopes, which are defined by a large panel of both peripheral T cells and antibodies [36,37]. The structural, chemical and biological properties of this peptide were evaluated and showed that StreptInCor is a very stable molecule. Using human blood samples, we showed that the StreptInCor epitope is able to bind to different HLA class II molecules and that it could be considered a universal vaccine epitope [38]. An experimental model of HLA class II transgenic mice showed that the immune response against aluminum hydroxide-absorbed StreptInCor after a period of one year was robust and safe without deleterious reactions in several organs [39].

Conclusion

Rheumatic fever and rheumatic heart disease lesions result from a complex network of several genes that control both the innate and adaptive immune responses after a *S. pyogenes* throat infection. An inflammatory process permeates the development of heart lesions with high production of inflammatory cytokines (IFN γ , TNF α , IL-17 and IL-23) and low numbers of cells producing IL-4, a regulatory cytokine of inflammation. Autoreactive CD4+ T cells infiltrate the heart tissue and trigger autoimmune reactions through molecular mimicry.

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