Rheumatic Heart Disease: Key Points on Valve Lesions Development

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Abstract

Rheumatic heart disease lesions are mediated by inflammatory and autoimmune reactions. Several genes related to both innate and adaptive immune responses are involved in the development of the disease. Adhesion molecules and chemokines facilitate heart tissue-T cell infiltration. T lymphocytes are the major effectors of cross-reactivity between streptococcal and human proteins leading to RHD. In vitro analysis of heart-tissue infiltrating T cells showed their ability to recognize streptococcal-M protein peptides as well as self-antigens by molecular mimicry mechanism. A cytokine balance favoring Th1 polarization and production of inflammatory cytokines and few IL-4 producing cells in the valves lead to the progression and maintenance of valvular lesions.

Keywords: S. pyogenes; Rheumatic heart disease; Genetic susceptibility; Inflammatory molecules; Autoimmune reactions

Introduction

Rheumatic Heart Disease (RHD) is the most serious complication of Rheumatic Fever (RF) and depends on several host factors that mediate an inflammatory and heart-tissue driven autoimmune response triggered by a protective immune response against S. pyogenes.

RF and RHD are the most convincing examples of molecular mimicry in human pathological autoimmunity, in light of the cross reactions between streptococcal antigens and human tissue proteins, mainly heart tissue proteins, that follow throat infection by S. pyogenes in susceptible individuals.

Molecular mimicry is the term used for more than 50 years [1] to define the mechanism by which self-antigens are recognized after an infection by cross reactivity. Four different mechanisms allowed the recognition of pathogen and self-antigens by molecular mimicry, as follows: i. identical amino acid sequences, ii. homologous but non-identical sequences, iii. Common or similar amino acid sequences of different molecules (proteins, carbohydrates) and iv. Structural similarities between the microbe and environmental agent and its host [2].

Genetic predisposition is one of the leading factors contributing to the development of RHD-autoimmune reactions. Several genes have been described to be associated with the development of the disease, in particular genes related with infection control. A recent review and meta-analysis of twin studies of concordance of development of rheumatic fever analyzed data from 435 twin pairs from six independent studies [3]. Although the limitations of the study the analysis indicate, that RF is a disease with a high heritability. Below we described the known genes associated with the susceptibility of the disease.

Genetic Features

The susceptibility to develop RF and RHD was first described in the beginning of the 80’s as being in association with HLA alleles (human leukocytes antigens) class II genes (DRB1, DQB and DQA), which are located on human chromosome 6. Several HLA class II alleles were described in association with the disease [4,5] and the most frequent association was with the HLA-DR7 allele, found in Brazilian, Turkish, Egyptian and Latvian patients. In addition, the association of DR7 with some DQ-B or DQA-A alleles may be connected to the development of multiple valvular lesions in RHD patients in Egypt and Latvia [6,7].

Beside these genes, molecular biology tools, bringing new insights about the mechanisms leading to the disease, described other genes more than 20 years later.

Genes that control the first steps of host defense against S. pyogenes

Innate immune response: TLR-2 [Toll like receptor 2] [8] and the Ficolin 2 [FCN2] genes play an important role in triggering the innate immune response either by binding collectin cellular receptors or activating the complement lectin pathway. In Brazilian chronic RHD patients, who show prolonged time of infection or repeated streptococcal infections together with low expression levels of ficolin 2 protein, the haplotype G/G/A (-986/-602/-4) was found to be more frequent than in controls [9].

The complement system consists of many proteins that are involved in the elimination of invading pathogens. Mannose Binding Lectin (MBL) is an acute phase inflammatory protein and functions as a soluble pathogen recognition receptor. Several components of the bacterial cell surface combine with Pattern Recognition Receptors (PRRs) such as MBL or the ficolin family of proteins. N-acetylgalactosamine, a sugar that is present in the cell wall of S. pyogenes, is a high affinity ligand for MBL. Different alleles of the MBL2 gene are associated with RF/RHD; these alleles code for large [10] or low [11] amounts of soluble MBL, which is related to the development of valvar stenosis or regurgitation. Of note, it is interesting that increased levels of complement activate the complement cascade leading to the deposition of immune complexes in the valvular tissue of RHD and consequently valvar stenosis. On the other hand, low amounts probably lead to delayed time of infection, tissue inflammation and necrosis, resulting in the induction of dendritic cell maturation and consequently T cell activation (adaptive
immune response). Figure 1 illustrates the role of MBL in the clearance of bacteria and/or tissue lesions.

The TNF-α gene [12-15] is responsible for the production of an inflammatory cytokine, which is active during the initial episodes of the S. pyogenes throat infection and also in later stages of the disease, during the development of myocarditis and valvulitis. It is interesting to note that both TNF-α gene and some components of the complement (C2, C4 and B factor) are also located in the human chromosome 6, between HLA class I and II genes that as mentioned above, are associated with the development of the disease.

Other genes that code for cytokines that play a role in the inflammatory reactions leading to RHD tissue lesions will be briefly described.

The IL-10 gene is involved in inflammation control. The IL-1Ra gene, for which the most frequent alleles are 1 and 2, encodes the IL-1α and IL-1β receptor antagonist, both of which are inflammatory cytokines. Two studies in Brazilian and Egyptian RHD patients with severe carditis showed low frequencies of allele 1, suggesting lack of inflammatory control [16,17].

The TGF-β1 gene controls cellular proliferation and is considered to be a possible risk factor for the development of valvular RHD lesions in Egyptian and Taiwan RHD patients [18,19].

Heart lesions

Adaptive Immune response: Rheumatic fever is characterized by inflammatory reactions and exudative modifications in joints, heart, kidney and subcutaneous tissues and nerves, being the heart lesions the most severe, leading to permanent disability. Rheumatic valvular lesions result from chronic inflammation and CD4+ and CD8+ T cells infiltration in the valves. Recurrent acute cardiac lesions frequently evolve into RHD, of which valvular deformities are the most important rheumatic chronic sequelae, and lead mainly to mitral and aortic regurgitation and/or stenosis.

a. Inflammatory process: Integrins, adhesion molecules and chemokines complicate the progression of rheumatic heart lesions encompassing molecules that play a role in the inflammatory process in the valves leading to progressive and permanent damage. Integrons, adhesion molecules and chemokines are involved in the recruitment of leucocytes into the tissues. Lymphocytic infiltration through the valve surface endothelium appears to be the initiating step for tissue damage and disease pathogenesis. Several molecules are associated with the recruitment of inflammatory and T cells in the heart tissue (Table 1). Vascular Cell Adhesion Molecule 1 [VCAM-1] is up regulated by anti-streptococcal antibodies, which are cross reactive with cardiac tissue proteins, and leads to the leucocyte infiltration into the rheumatic valves [20]. We observed that other molecules also involved in the recruitment of inflammatory cells to the heart tissue were the ICAM and P and E-selectins (unpublished data). Recently we showed that some chemokines and their receptors (Table 1) are associated with the recruitment of mainly CD4+ T cells in the heart tissue. Briefly, CCL3/MIP1α gene expression was up-regulated in the myocardium, while CCL1/1-I-309, CXCL9/Mig, CCR5 and CCR8 were highly expressed in the valves. Valve lesion-infiltinating T cells were CD4+CD45RO+ and migrated mainly toward CCL1/1-I-309 and CCL17/TARC and CXCL9/Mig gradients [21].

b. Cytokines

The effectiveness of the immune response depends on the production of cytokines, which are important secondary signals following an infection. These cytokines trigger effective immune responses in most individuals as well as probably deleterious responses in patients with autoimmune disease.

Three subsets of T helper cytokines are currently described. Antigen-activated CD4+ T cells polarize to the Th1, Th2 or Th17
subsets, depending on the secreted cytokine. Th1 is involved with the cellular immune response and produces IL-2, IFN-γ and TNF-α. Th2 cells mediate humoral and allergic immune responses and produce IL-4, IL-5 and IL-10. Th17 cells have been more recently described as a mediator of a proinflammatory response mediated by IL-17. The cytokines TGF-β, IL-6 and IL-23 are the factors that induce the Th17 lineage (or polarization).

In the heart tissue (myocardium and valves) of acute and chronic RHD patients, we identified by immunohistochemistry a large number of mononuclear cells able to secrete inflammatory cytokines (TNF-α and IFN-γ). The regulatory cytokine IL-10 in both myocardium and valves, however only scarce numbers of IL-4+ cells (also a regulatory cytokine) were found in valve lesions of RHD patients, indicating the role of Th1/Th2 cytokine balance in healing myocarditis. However, the lack of cells producing IL-4, a regulatory cytokine, in the valves favors the progressive and permanent valve damage [22].

c. Heart-Tissue - T cell infiltration

We identified oligoclonal T cell populations in the heart-tissue of RHD patients by the analysis of their T cell Receptors (TCR) that takes into consideration the molecular structure of the TCR; this T cell receptor is composed by both alpha and beta chains, which are produced through the assembly of variable (V), joining (J), and constant gene segments. Combinations of these genes generate around 10^14 different TCRs.

We observed several expanded T cell populations with an oligoclonal profile in the heart tissue of chronic and acute RHD patients; these oligoclonal T cell populations are in contrast with the peripheral blood, which contains polyclonal populations (Table 2). A high number of T cell oligoclonal expansions were found in the valvular tissue, indicating that specific and cross-reactive T cells migrate to the valves [23], and upon specific cytokine stimulation, they expand at the site of the lesions.

d. Molecular mimicry and recognition of self proteins

Heart-tissue infiltrating T cells were mainly CD4+ and able to recognize heart-tissue derived proteins and streptococcal antigens in the absence of the bacteria by molecular mimicry [24].

Immunodominant M5 protein peptides (residues 81-96, 83-103) and valvular and myocardium-derived proteins were recognized by both myocardium and valve infiltrating T-cell clones by molecular mimicry [24]. These M5 epitopes were also preferentially recognized by polyclonal peripheral T lymphocytes from RHD patients. Most of these RHD patients presented the HLA-DR7 allele, one of the most frequent in association with the disease; this means that antigen presenting cells (monocytes/macrophages and dendritic cells) bear the HLA-DR7 molecule in their surface, and consequently antigen recognition occurred mainly in the context of this molecule [25] (Figure 2).

Cardiac myosin is one of the well-known autoantigen targets of the immune response in several inflammatory heart diseases [26]. Antibodies and T lymphocytes from rheumatic heart disease patients and cells from myosin or streptococcal antigens induced-experimental myocarditis and/or valvulitis were able to recognize several cardiac myosin epitopes [26]. Dr Cunningham’s group showed that peripheral T cell clones from one patient with RHD were able to recognize different alpha helical coiled coil proteins such as streptococcal M protein, Light Meromyosin (LMM) region of cardiac myosin, laminin and tropomyosin [27]. Another study performed in parallel by our group focused on the reactivity of intraletral T cell clones derived

<table>
<thead>
<tr>
<th>Group</th>
<th>Molecule</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell adhesion molecules</td>
<td>VCAM-1</td>
<td>Vascular cell adhesion molecule that mediates the adhesion of lymphocytes to vascular endothelium.</td>
</tr>
<tr>
<td></td>
<td>ICAM-1</td>
<td>Intercellular adhesion molecule, expressed on endothelial cells, involved in leukocyte endothelial transmigration.</td>
</tr>
<tr>
<td></td>
<td>Selectin E</td>
<td>Cell adhesion molecule expressed on endothelial cells activated by cytokines. Recruits leukocytes to the site of injury.</td>
</tr>
<tr>
<td></td>
<td>Selectin P</td>
<td>Cell adhesion molecule expressed on endothelial cells. Recruits leukocytes to the site of injury.</td>
</tr>
<tr>
<td>Chemokines</td>
<td>CCL3/MIP1α</td>
<td>Inflammatory chemokine; acts in the recruitment and activation of leukocytes.</td>
</tr>
<tr>
<td></td>
<td>CCL1/I-309</td>
<td>Inflammatory chemokine that attracts monocytes, NK cells, B cells and DCs.</td>
</tr>
<tr>
<td></td>
<td>CXCL9/MIG</td>
<td>Monokine induced by IFN-γ that attracts T cells.</td>
</tr>
<tr>
<td></td>
<td>CCL17/TARC</td>
<td>Expressed constitutively in thymus and transiently in activated PBMC; T cell chemoattractant.</td>
</tr>
<tr>
<td>Chemokine</td>
<td>CCR5</td>
<td>Plays a role inflammatory response to infections; expressed mainly on T cells, macrophages, DCs. Binds CCL1, 3 and 5.</td>
</tr>
<tr>
<td>Receptors</td>
<td>CCR8</td>
<td>Chemoattract activated T cells injury areas and lymphoid organs. Binds CCL1.</td>
</tr>
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Table 1: Molecules involved in T cells migration to the heart tissue.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Site of oligoclonal T cell expansions</th>
<th>TCR-Vbfamilies</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLA</td>
<td>Periphery</td>
<td>7, 8, 23</td>
</tr>
<tr>
<td></td>
<td>Heart lesion - mitral valve</td>
<td>1, 2, 3, 5, 8, 9, 13, 17</td>
</tr>
<tr>
<td>WFA</td>
<td>Periphery</td>
<td>Nondetected</td>
</tr>
<tr>
<td></td>
<td>Heart lesion - mitral valve</td>
<td>4, 5, 8, 14, 15, 17, 20</td>
</tr>
<tr>
<td></td>
<td>Heart lesion - aortic valve</td>
<td>2, 5, 7, 13, 15</td>
</tr>
<tr>
<td>JSS</td>
<td>Periphery</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Heart lesion - mitral valve</td>
<td>1, 2, 3, 4, 5, 9, 11, 16, 17, 18, 20, 21, 23, 24</td>
</tr>
<tr>
<td></td>
<td>Heart lesion - aortic valve</td>
<td>1, 2, 13, 16, 18, 22, 23</td>
</tr>
</tbody>
</table>

Some expanded and oligoclonal T cell populations were found in both peripheral blood and the valves (bold), indicating that these expansions are antigen-driven thus auto reactive [23]

Table 2: Oligoclonal T cell expansions in peripheral blood and heart- tissueintralesional lineages.
from myocardium and valvular tissue of six RHD patients against the same region of cardiac myosin and M5 streptococcal protein and valve-derived proteins; in this study, a high frequency of reactive heart-tissue intralesional T cell clones was found (63%) indicating a broad recognition of self antigens [28]. In addition, we demonstrated by proteomic approach the recognition of valve-derived proteins such as vimentin and disulfide isomerase ER-60 precursor [PDI3A] and 78 kD glucose-regulated protein precursor [HSP5A] by T lymphocytes that infiltrate the valvular tissue of both acute and chronic RHD patients [29]. These proteins are probably exposed during the inflammatory process in the heart-tissue and were recognized by S. pyogenes-primed T cells that migrated from the peripheral blood to the cardiac tissue leading to valve damage.

Conclusions

Several genes contribute to the development of rheumatic heart lesions after a S. pyogenes infection. The HLA class II molecules are involved in the process of antigen presentation to T lymphocytes and activation of the immune response, and probably play a key role in the development of the autoimmune reactions that lead to the valve damage in RHD patients. Adhesion molecules and chemokines facilitate the cellular infiltration into the heart tissue. CD4+ T cells are the major effectors of the autoimmune reactions, in which valve proteins (vimentin, collagen, among others) are injured damaged as a result of autoimmune reactions. Inflammatory cytokines and the lack of regulatory IL-4 cytokine contribute to the progression of the valve damage. The knowledge of the mechanisms leading to the disease certainly will contribute to the design of new drugs and an efficient vaccine against S. pyogenes.

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