Review

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Rheumatic Fever: From Sore Throat to Autoimmune Heart Lesions

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Key Words
Autoimmunity · Cytokines · Heart tissue proteins · M protein · Rheumatic heart disease · T cell receptor

Abstract
Molecular mimicry between streptococci and heart components has been proposed as the triggering factor leading to autoimmunity in rheumatic heart disease (RHD). In this review, we present data from cellular autoimmune responses, focusing on the interactions between HLA class II molecules, streptococcal peptides and heart tissue proteins and T-cell receptor (TCR) usage. HLA-DR7DR53 associated with DQ molecules seem to be related with the development of valvular lesions in severe RHD patients. DR7DR53 molecules were also involved in the recognition of an immunodominant M5 peptide in these patients. T cells infiltrating RHD hearts displayed several oligoclonal expansions. Intralesional T-cell clones presenting identical TCR-BVBJ AVAJ and -CDR3 sequences were able to recognize several antigens with little or low homology, showing an intramolecular degenerate pattern of antigen recognition. Peripheral blood mononuclear cells of rheumatic fever (RF) patients produced proinflammatory cytokines, and intralesional mononuclear cells from severe RHD patients produced predominantly Th1-type cytokines. These results illustrate the complex mechanisms leading to heart tissue damage in RF/RHD patients.

Introduction

Rheumatic fever (RF) is an inflammatory disease mediated by humoral and cellular autoimmune responses that occurs as a delayed sequelae of *Streptococcus pyogenes* infection in 3–4% of susceptible and untreated children and adolescents (aged 5–18 years). Carditis affects 30–45% of RF patients and is the most serious manifestation of the disease, leading to valvular lesions mainly in the mitral and aortic valves. It causes chronic rheumatic heart disease (RHD) that still remains a major public health problem in developing countries.

The pathogenic mechanisms involved in the development of RF/RHD are not fully understood. It is believed that the molecular mimicry mechanism is responsible for the cross-reactions between streptococcal antigens and human tissue proteins, mainly heart tissue proteins in susceptible individuals. It is now clear that the

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disease is mediated by both humoral and cellular immune responses and that the cellular branch of the immune response is more involved in the development of RHD.

In this review, we will focus on the cellular branch of autoimmune responses leading to heart lesions in RHD patients. The three main components involved in the T-cell responses: HLA class II molecules, antigenic peptides and T-cell receptors (TCR; trimolecular complex) will be discussed.

Trimolecular Complex

Early studies in animal models by Benacerraf [1] showed that immunization with synthetic antigens were able to induce the production of antibodies and that this immune response was determined by certain MHC haplotypes. Later experiments mapped the control of immune responses to MHC class II genes [2]. Although several HLA class II molecules have been associated with the development of humoral or cellular-mediated diseases and are considered as genetic markers of these diseases, now we know that, in fact, the major role of HLA class II molecules is to present antigens to Th (CD4+) cells. Antigen-presenting cells (APC), e.g. macrophages, dendritic cells and B lymphocytes, constitutively express HLA class II molecules. When activated by specific cytokines such as IFNγ, these APCs express large amounts of HLA class II molecules.

During throat infection by *S. pyogenes*, several streptococcal peptides are generated by APC. These peptides, mainly from the M protein, are associated with HLA class II molecules and presented to Th cells triggering an inflammatory or humoral response. In untreated individuals with genetic predisposition to develop RF/RHD, T-cell populations selected by some immunodominant streptococcal peptides combined with HLA class II molecules will be able to trigger an autoimmune reaction (discussed below – Molecular Mimicry).

In the case of acute rheumatic carditis, Aschoff's bodies, the pathognomonic sign of the disease, develop in the myocardium and/or endocardium [3]. The Aschoff body is constituted by an agglomeration of several cells, e.g. monocytes/macrophages and B lymphocytes that are probably acting as APCs. Furthermore, mononuclear cells that also include APC (macrophages and B lymphocytes) and T lymphocytes infiltrate the heart in patients with both acute and chronic RHD.

The third component of the trimolecular complex is the T lymphocyte. The majority of T cells (90%) present an antigen receptor (TCR) composed of α- and β-chains. The TCR α- and β-chain are produced by the assembly of variable (V), joining (J) and constant (C) gene segments and also diverse (D) gene segments for β-chain. Combinations of these genes generate around 10^{18} TCRs. Both chains have three regions designated as complementarity-determining region (CDR1, CDR2, CDR3). CDR1 and CDR2 of the TCR interact with MHC molecules. The peptide side chains of the MHC-peptide complex on the surface of APC interact most closely with the hypervariable region of the TCR (CDR3) encoded by the V-D-J region on the TCR-β-chain. The combinations between HLA class II molecules and antigens will drive the T-cell repertoire.

Genetic Susceptibility

Several genetic markers of susceptibility to RF/RHD have been studied [4], and associations with different HLA class II antigens have been observed in several populations, e.g. DR4/DR9 in American Caucasians and DR2 in American blacks [5, 6], DR4 in Arabians [7], DR3 in Indians [8], DR1 and DR6 in Africans [9] and DR5 in Turks [10]. Interestingly, DR7 was found to be associated with the disease in different countries [11–15]. Associations of HLA-DR7 with some HLA-DQ antigens seem to be related to the presence of multivalvular lesions in RHD patients [14–15]. HLA-DR53 is another class II molecule always associated with DR4, DR7 and DR9 molecules. In our studies, DR53 was also associated with the disease [11, 12]. The fact that several HLA class II antigens are associated with the development of RF/RHD in different countries is consistent with the possibility that different strains of group A streptococci could be implicated in the development of RF/RHD in different countries. The variable association may also be due to the important role that HLA class II antigens play in antigen presentation to the TCR.

In order to analyze the role of HLA-DR7 molecules in T-cell immune responses against the N-terminal region of the M5 protein, we studied the T-cell reactivity in the peripheral blood of RHD patients. Amongst the M5 peptides tested, it was possible to map the immunodominant regions recognized by severe and mild RHD patients. Mild RHD patients recognized preferentially the region of amino acid residues 1–25 of M5 protein by several HLA class II molecules. However, severe RHD patients

Table 1. HLA-DR7 and RF/RHD

Country	Population	HLA	Clinical picture of the disease	Functional study	References
Brazil	Mulatto	DR7, DR53 allogenotope[a] TaqI DRβ 13.81 kb	RF/RHD	M5(81–96) peptide preferentially recognized by DR7+ severe RHD patients; capacity of binding with the HLA-DR53 molecule	11, 12, 16
Brazil	Caucasian	DR7	RF/RHD		13
Egypt	Egyptian	DR7 DQ A1 02 01	RHD-MVL		14
Latvia	Latvian	DR7 DQ B1 03 02 DR7 DQ B1 04 01	RF/RHD-MVL RF/MVR Sydeham's chorea		15

[a] Defined by restriction fragment length polymorphism, 13.81-kb fragment corresponding to the HLA-DR53 antigen [12].

MVL = Multivalvular lesions; MVR = mitral valve regurgitation.

recognized the region of amino acid residues 81–96, defined as an immunodominant M5 epitope. Interestingly, 70% of severe RHD patients that recognized this M5 peptide were HLA-DR7+ DR53+, suggesting that M5(81–96) peptide was preferentially presented to the T cells in the context of DR7 and DR53 molecules. The analysis of the capacity to bind streptococcal M5 peptides showed that the M5(81–96) peptide was also able to bind to the DR53 molecule [16]. These results are presented in table 1.

Molecular Mimicry

Antigenic mimicry between streptococcal antigens, mainly M-protein epitopes and heart components, has been proposed as the triggering factor leading to autoimmunity in RF. M protein is the major antigen of *S. pyogenes* and extends from the cell wall. It is composed of approximately 450 amino acid residues showing antigenic variations but high homology on the amino-terminal (N-terminal) region, except for the 11 first amino acid residues that define the different serotypes, nowadays approximately 100 strains. The carboxyterminal (C-terminal) region contains multiple repeat regions and is conserved [17].

Molecular mimicry was first demonstrated in studies on humoral immune responses. Anti-streptococcal antibodies cross-reacted with several human tissues, including the heart, skin, brain, glomerular basement membrane, and striated and smooth muscles [18, 19].

One intersection point between humoral and cellular immune responses in RHD patients could be the fact that cross-reactive antibodies in the heart tissue may bind to the valvular endothelium leading to inflammation, cellular infiltration and valve scarring [20]. Once activated, the valvular endothelium expressed increased amounts of the adhesion molecule VCAM-1, that facilitates the binding/adhesion of T cells and consequently extravasation into the valves, leading to the cycle of scarring, neovascularization and infiltration of lymphocytes [21].

The presence of CD4+ T cells at lesion sites in the heart of RHD patients has been demonstrated, suggesting a direct role of these cells in the pathogenesis of RHD [22, 23].

We showed the significance of molecular mimicry between β-hemolytic streptococci and the heart tissue, analyzing the T-cell repertoire leading to local tissue damage in RHD. We demonstrated the capacity of infiltrating T cell clones from heart lesions of severe RHD patients in recognizing M protein peptides and heart tissue-derived proteins. Our results pointed out three M5 immunodominant regions (residues 1–25, 81–103 and 163–177) that cross-reacted to several heart protein fractions, mainly those derived from valvular tissue with a molecular mass of 95–150, 43–63 and 30–43 kD [24]. Peripheral T lym-

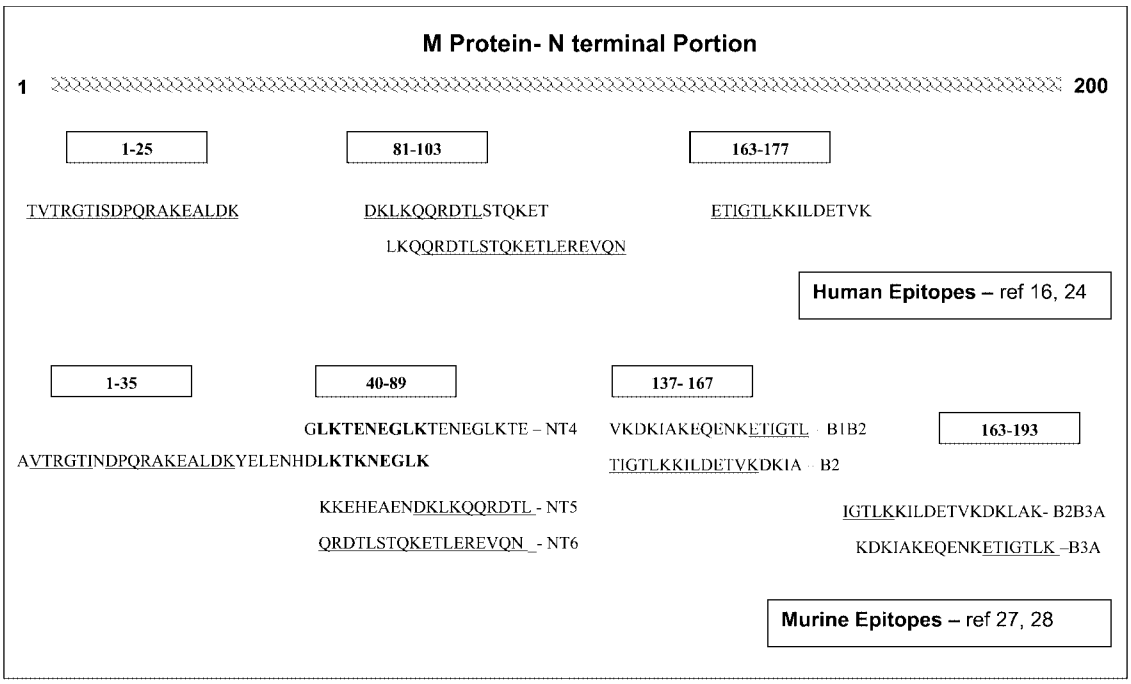

Fig. 1. Shared sequences of human and murine M5 epitopes recognized by T cells. Several T-cell immunodominant M5 protein peptides were described and here we presented only peptides with shared sequences. The sequences of M5 peptides [M5(1–25), M5(81–96), M5(83–103) and M5(163–177)] from references 16 and 24 were based on the sequence of the M5 protein published by Manjula et al. [29]. Sequences used on murine studies from reference 27 (peptides NT4, NT5, NT6, B1B2, B2, B2B3A and B3A) were from Miller et al. [30] and reference 28 [M5(1–35) peptide] refers to a mutant M5 protein published by the authors. Human and murine amino acid residues of overlapping peptides are underlined; murine amino acid residues of overlapping peptides are in bold type.

phocytes also recognized these immunodominant M5 peptides and valve proteins. The M5(81–96) mentioned above is included in the M5(81–103) region peptide and was preferentially recognized by HLA-DR7+DR53+ in severe RHD patients (table 1) [16]. By using a proteomic approach, we were able to characterize some mitral-valve proteins identified by molecular weight and isoelectric point (pI). Several valve-derived proteins were recognized by peripheral blood and intralesional T-cell clones from severe RHD patients. Amongst them, we identified vimentin (molecular weight 53 kD/pI 5.12, recognized mainly by peripheral T cells) and other cytoskeleton proteins (recognized by both peripheral and intralesional T cells) [manuscript in preparation]. In line with these results, previous work showed the recognition of a 50- to 54-kD myocardial-derived protein by peripheral T lymphocytes from RHD patients [25].

We have also analyzed the intralesional T-cell responses against synthetic peptides from human cardiac myosin β-chain, and we found that 29% of these intralesional T-cell clones derived from both myocardium and valves were reactive. Taken together, our results indicate that several autoantigens were recognized, and vimentin could be the initial target of RF lesions resulting from polyarthritis reactions, while myosin could also be an immunodominant target during carditis episodes. The phenomena of epitope spreading described by Sercaz et al. [26] that lead to a broad diversity of recognition and triggers an amplification and diversification of immune responses could explain these results.

Myosin/M5 protein cross-reactive T-cell epitopes were also investigated in mice immunized with intact cardiac myosin [27]. Lymph node T cells were tested against overlapping M5 peptides named NT5/6/7 and B1B2/B2 and B2B3A/B3A aligned with the M5 regions identified by us, the M5(81–96) and M5(163–177), respectively. Robinson et al. [28] obtained lymph node T-cell clones from mice immunized with recombinant M5 protein that were able to recognize M5 epitopes. Amongst the M5 epitopes recognized by the mouse T-cell clones, only the M5(1–35)

Table 2. TCR analysis of cross-reactive intralesional T cell clones

T cell clone identification	TCR	BV BJ CDR3 (N-D-N) sequences	Antigens recognized mitral-valve-derived protein, LMM or M5 peptides
Lu 3.1.3		SGRQGRYEQY-10aa	35 kD/pI 8.84
Lu 3.1.8	BV13 BJ2S7 AV2 AV3	SGRQGRYEQY-10aa	35 kD/pI 8.84, LMM 28 (1647–1664) LMM 28B (1660–1677), LMM 32 (1699–1716)
Lu 3.1.29		SGRQGRYEQY-10aa	56–53 kD/pI 6.76
Lu 3. 2. 12.9	BV13 BJ2S7 AV2 AV7	SGRQGRYEQY-10aa	56–53 kD/pI 6.76
Lu 3.1.85	BV3 BJ2S1 AV5	SFTGRLDNEQF-11aa	79 kD/pI 5.12 M5 (1–20), M5 (11–25), M5 (81–96) M5(111–130), M5(121–140) M5(163–177), M5(183–201)

LMM = Light meromyosin peptides; LMM28 = SLQSLLKDTQIQLDDAVR; LMM28B = DDAVRANDDLKENIAIVE; LMM32 = RSRKLAEQELIETSERVQ; M5 peptides: 1–20 = VTRGTISDPQRAKEALDKY; 11–25 = QRAKEALDKYELENH; 81–96 = DKLKQQRDTLSTQKET; 83–103 = QQRDTLSTQKETLEREVQN; 111–130 = TRQELANKQQESKENEKALN; 121–140 = ESKENEKALNELLEKTVKDK; 163–177 = ETIGTLKKILDETVK; 183–201 = LDETVKDKLAKEQKSJQNI; NT = not tested. Shared sequences are underlined (adapted from Faé et al. [43]).

region aligns with the M5(1–25) region recognized by the human infiltrating T-cell clones. Figure 1 summarizes both human and murine reactivity against M protein, the N-terminal portion [29, 30].

T-Cell Repertoire

In the 90s, some researchers described a superantigenic effect of streptococcal M5 protein preparations (pepsin cleaved fragment – pepM5) for human T cells expressing TCR-BV2, BV4, and BV8 [31–35]. Superantigens are proteins that polyclonally activate T cells by an MHC class II-dependent, but haplotype-unrestricted mechanism. Proliferative responses to superantigens are limited to T cells expressing a particular TCR-BV gene but independent of antigen specificity. M protein has an important role in the host anti-streptococcal immune response, and for this reason it has been ascribed superantigenic properties. However, the superantigenic effect was later dismissed by some studies showing that the superantigenicity of pepM1 and pepM5 were due to contamination with pyrogenic exotoxins that had themselves a potent superantigen effect on BV2-bearing human T cells [36–39].

In a recent work, we compared the TCR-BV usage in peripheral blood and heart-infiltrating T cell lines (HIL) from severe RHD patients, looking for oligoclonal β-chain expansions in line with antigen-driven immune responses. T-cell receptor β-chain family (TCR-BV) usage and the degree of clonality were assessed by the analysis of the length of the β-chain CDR3. Our results showed expansion of several BV families with oligoclonal profiles, mainly in heart-infiltrating T-cell lines, and favor no superantigenicity of M proteins in RHD patients. Few oligoclonal BV expansions were shared by mitral valve- and left atrium-derived T-cell lines in the same individual. However, in this study, we described a case of 1 patient that presented a BV5 expansion with the same BJ2S3 segment in both valve and myocardium tissues. However, these T cells presented different amino acid CDR3 sequences, suggesting that different antigenic peptides could be predominantly recognized by T cells that infiltrate mitral valve and myocardium tissues [40]. The high frequency and the persistence of T-cell oligoclonal expansions in the damaged heart valves seem to be associated with the progression of the disease [41], probably due to the T-cell recognition of several heart tissue proteins exposed by local lesions. In agreement with these data, it has been described that it is possible to detect T-cell

expansions in damaged heart valves even 20 years after the acute RF episode [42].

Recently, we described intralesional T-cell clones with a degenerate pattern of reactivity [43]. Five heart tissue-derived T-cell clones (three from the mitral valve and one from the myocardium) obtained from a patient with severe RHD presented the same TCR-BV13 BJ2S7 with identical CDR3 sequences. They expressed two α-chains at the RNA level and recognized M5 epitopes or human cardiac β-chain synthetic myosin peptides or mitral valve-derived proteins. Interestingly, a mitral valve-derived protein (53–56 kD/pI7.76) was recognized by two intralesional T-cell clones, one from mitral valve tissue and the other from myocardial tissue. These T-cell clones expressed only one different α-chain. We also found other T-cell clones that recognized several different antigens bearing the same TCR-BV3 BJ2S1. These results are summarized in table 2. Our data are in agreement with those done by Mason [44, 45], in which the flexibility of T-cell antigen recognition was evaluated by the analysis of the immune response pattern against pathogens in experimental models. Using a mathematical approach, he estimated that T cells can react with a very large number of peptides. The high frequency of cross-reactivity was postulated as essential for keeping the T-cell repertoire active against the large number of foreign antigens that an individual can encounter and respond to in his/her life [44, 45]. Thus, it appears that the major role of degeneracy of T cells is to maintain the physiological immunity. However, our report is the first to identify intramolecularly degenerate pattern of recognition.

It is known that among autoreactive T cells it is possible to differentiate T-cell subsets triggering pathological compared to what could be called 'physiological autoimmunity' [46–48]. However, the mechanism used by degenerate T cells to recognize selected peptides without pathological potential and, on the other hand, the mechanism of degenerate T-cell reactivity leading to pathological autoimmunity in individuals with genetic susceptibility remain unknown.

Cytokines

The cytokine pattern produced by Th cells in response to defined antigens is crucial to drive the humoral or cellular immune responses. In addition, the concept of pathological or physiological autoimmune reactions depends on the cytokine produced in response to the autoantigens that are being recognized by T cells. RF manifests different clinical pictures such as arthritis, chorea, carditis, erythema marginatum and/or subcutaneous nodules [49]. All these manifestations involve particular autoantigens as targets of pathological autoimmunity. Arthritis, chorea and mild RHD are in part due to a pathological autoimmune reaction probably mediated by Th2-type cytokines, leading to an exacerbated humoral response, as reported in several studies [19]. On the other hand, severe RHD is mediated mainly by T lymphocytes [16, 22–24]. The production of TNFα, IL-1 and IL-2 in the peripheral blood of acute RF and active RHD patients have been described [50, 51]. Other authors have confirmed these findings and have also noted increased plasma levels of TNFα in RF/RHD patients [52–54].

In heart lesions during the acute phase of RHD, the production of IL-1, TNFα and IL-2 was correlated with progression of the Aschoff nodule [55] localized mainly in the endocardium, subendocardium or perivascular regions of the myocardial interstitium.

Recently, we have shown that intralesional mononuclear cells from heart lesions predominantly secret IFNγ and TNFα in both acute RF and chronic RHD patients, with a scarce production of IL-4 [submitted]. When stimulated with streptococcal M5 antigens, mitral valve-derived intralesional T-cell lines produced IFNγ but not IL-4, while myocardial intralesional T-cell lines produced IFNγ, IL-10 and IL-4. The predominant Th1-type cytokine produced mainly by CD4+ T cells infiltrating valve tissue could mediate the severe RHD valve lesions, and the fact that myocardial-infiltrating cells were able to produce regulatory cytokines could have a role in the mildness of myocardial damage in RHD.

Conclusions

The development of RF/RHD involves a complex network of autoimmune reactions comprising the following major points. (1) Molecular mimicry between streptococcal antigens and human tissues, mainly heart tissue, leads to rheumatic heart lesions in RHD patients. (2) CD4+ T lymphocytes are the major effectors of heart lesions and display a degenerate pattern of antigen recognition. (3) Several streptococcal immunodominant peptides generate cross-recognition of vimentin, myosin and several mitral valve-derived proteins, possibly resulting from an epitope-spreading mechanism. (4) Several HLA class II molecules are associated with the disease, and HLA-DR7/DR53 combined with some HLA-DQ molecules seem to be associated with the development of multiple valvular

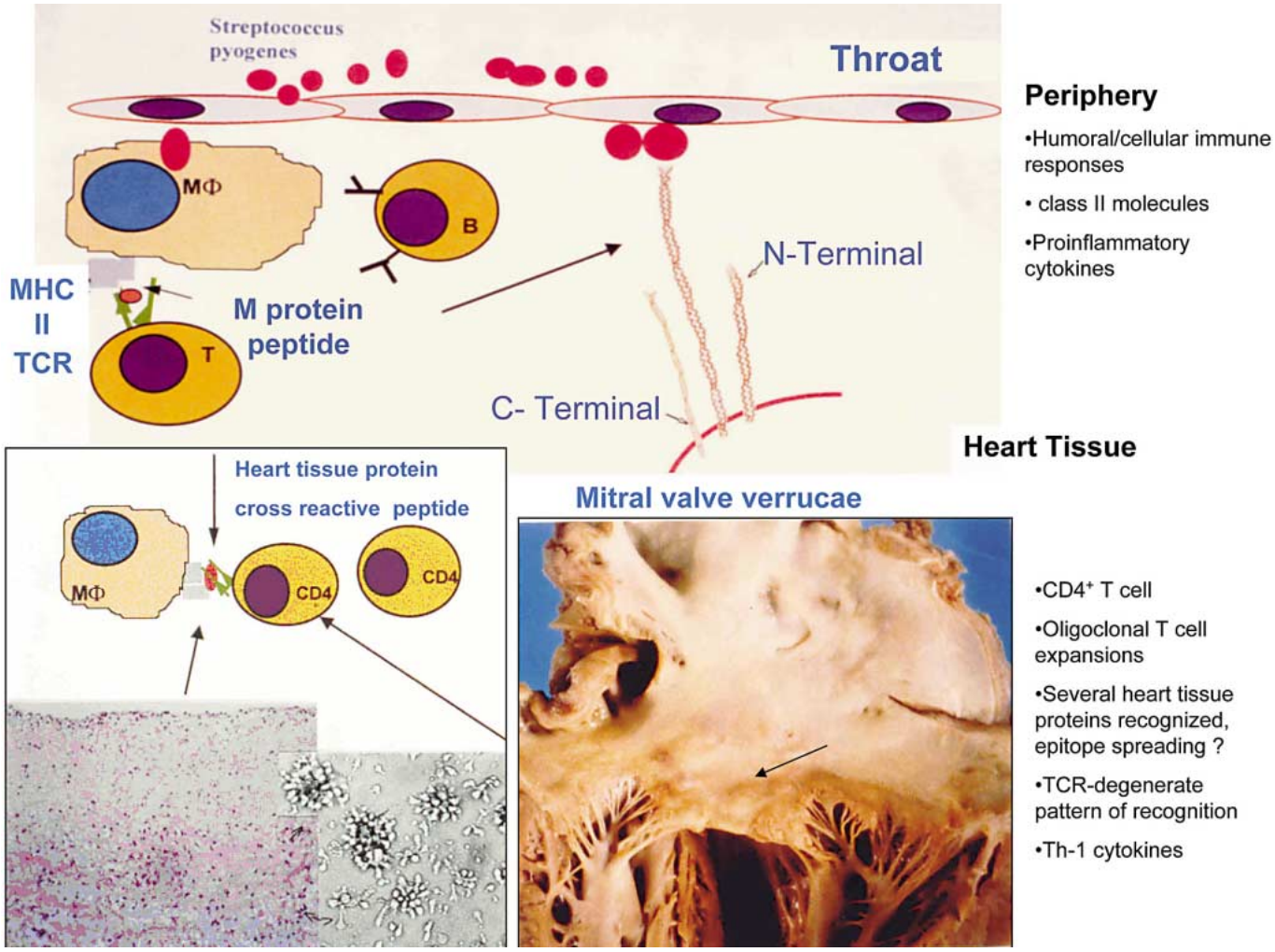

Fig. 2. Model of the development of RF/RHD. After group A streptococcal throat infection, untreated susceptible individuals (5–18 years old) developed RF/RHD. Humoral and cellular immune responses against *S. pyogenes* trigger an autoimmune attack to human tissues by molecular mimicry. The autoimmune reaction is initiated in the periphery where T cells recognize immunodominant M5 peptides presented by APC (macrophages/monocytes) in the context of HLA class II molecules. Proinflammatory cytokines were produced in the periphery. Activated T CD4+ cell clones expanded and migrate to the heart (myocardium and valvular tissues as shown in the picture), and several heart tissue proteins are recognized by molecular mimicry. Epitope spreading amplifies the autoimmune response. Several autoreactive T cell clones are generated and display degenerate TCR capable of recognizing several different antigens. Intralesional mononuclear cells also produced predominant Th1-type cytokines (IFNγ and TNFα). Mitral valve picture from 1 RHD patient shows verruca lesions as indicated by the arrow. On the lower left, a fragment of the mitral valve lesions (HE, ×20) shows infiltrating mononuclear cells in the endocardium. In vitro growing of T cells shows lymphoblasts as 'flowers'. ×100.

lesions in RHD patients. (5) T-cell recognition displays an intramolecular degenerate reactivity against streptococcal and human protein epitopes with low homology. (6) Th1-type cytokines seem to be predominant in heart lesions, especially valvular lesions.

All these points extend the knowledge on the development of RHD (summarized in fig. 2) and may open new possibilities of immunotherapy.

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