Development of a Vaccine to Prevent Group A Streptococci (GAS) Infection by Using a Recombinant Protein.

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Rheumatic fever (RF) is a post-infectious autoimmune disease that affects over 20 million children worldwide. RF affects 3% to 4% of untreated susceptible individuals infected by Group A *Streptococcus pyogenes* (GAS). The most severe manifestation of RF, carditis leads to severe and permanent valve lesions, which result in chronic rheumatic heart disease (RHD).

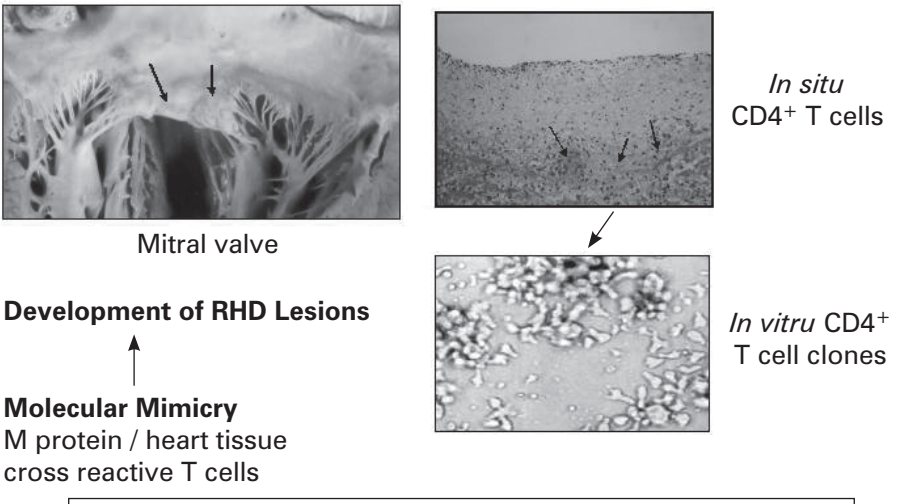
RHD continues to be a major public health problem in developing countries, leading to 233,000 deaths/year. Worldwide, the incidence of RHD is at least 15.6 million cases [1]. In Brazil, although the incidence of acute RF has decreased by 75% in the last 10 years, it is still high, reaching 5,000 new cases/year (data from the Brazilian Health Ministry).

The GAS cell wall consists of repeating units of N-acetyl beta-D-glucosamine carbohydrates linked to a polymeric rhamnose backbone. In addition, GAS contain M, T and R surface proteins and lipoteichoic acid (LTA), all of which are involved in bacterial adherence to throat epithelial cells. The complex structure of the cell surface accounts for many of the GAS virulence determinants, especially the determinants related to colonization of host cells, evasion of phagocytosis and the host immune response. The M protein is particularly important as a virulence factor and is the main molecule, being an attachment factor that provides the bacterium with an adherence advantage. The M protein extends from the bacterial cell wall and is composed of two polypeptide chains with approximately 450 amino acid residues in an alpha-helical coiled-coil configuration. The N-terminal portion contains the A repeat region with the antigenic variations that define the different serotypes, of which 130 have been identified to date. The B repeat region also presents antigenic variations among the streptococcal serotypes. The C-terminal half is preserved, and contains multiple repeat regions [2].

RF and RHD are due to a pathological autoimmune response triggered by a defensive response against GAS. Defined as the sharing of epitopes between antigens of the host and GAS, molecular mimicry is the mechanism that mediates these autoimmune reactions. By generating T-cell clones obtained from surgical fragments of heart lesions of RHD patients, we demonstrated for the first time the ability of $CD4^+$ T cells to simultaneously recognize M protein peptides and heart tissue proteins. Three M5 regions (residues 1-25, 81-103 and 163-177) cross-

reacted with several heart tissue protein fractions, mainly from valvular tissue with molecular masses of 95-150, 43-65 and 30-43kDa (3) (**Figure 1**).

Figure 1: T-cell mediated rheumatic heart lesions.



$CD4^+$ T-cells infiltrate mitral valve lesions. T-cell clones isolated from the mitral valve lesions were able to recognize M5 peptides and heart tissue proteins by molecular mimicry mechanism and probably trigger valvular lesions in RHD [3].

Considering that RF/RHD are the prototypes for post-infectious autoimmune disease, the greatest challenge is to develop a vaccine to prevent GAS infection and the diseases it causes without inducing autoimmune reactions.

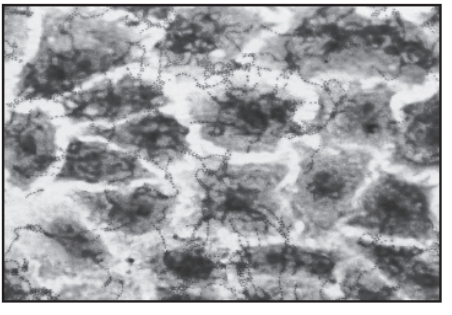
The anti-streptococcal vaccine model that we propose is based on protective T- and B-cell epitopes that do not trigger autoimmune reactions. We studied the T lymphocytes reactivity and sera against a panel of 620 human blood samples, testing against 80 overlapping peptides that cover an area of 100 amino acid residues of the C-terminal portion of the streptococcal M protein, differing by only one amino acid residue. This strategy has allowed us to define T- and B-cell epitopes composed by 22 and 25 amino acid residues, respectively [4]. The identified peptide sequences were deposited at the Brazilian Patent office, INPI020050020064.

A pool of sera from healthy individuals reactive to the B epitope was able to inhibit the adhesion and invasion of several streptococci strains (**Figure 2**). By cloning the streptococcal emm gene we obtained a recombinant protein that contains both T and B epitopes. As an autoimmunity control, we are using heart-tissue infiltrating T cell lines obtained from rheumatic heart disease patients to test their ability to recognize the selected peptides (both T and B epitopes) by proliferation assay and cytokine production. Our preliminary results showed no cross reactivity with

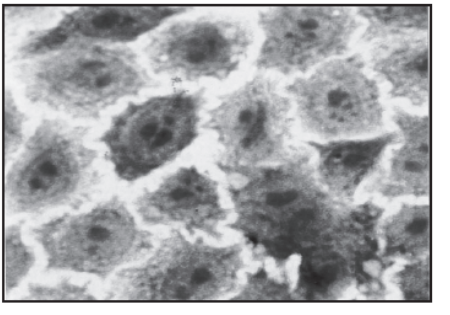
heart-tissue proteins, indicating that the selected epitopes could be a good vaccine candidate. The efficacy of the vaccine epitope for inducing both humoral and cellular responses will be tested in experimental animal models as a second step in the validation of the selected epitope as a vaccine candidate.

Figure 2: Human sera reactive against the vaccine candidate epitope inhibited the adhesion and invasion of group A streptococci strains to the HEp cells in vitro.

Adhesion - *S. pyogenes*



Adhesion/ Invasion Inhibition, human sera



S. pyogenes strain	Adhesion / Invasion Inhibition %
M5	99
M6	99
M44/61	98
M87	97
M1	90
M71 and M22	70

UFC without sera > 200.000

Normal individuals sera, epitope B reactive

References

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3. Guilherme L, Cunha-Neto E, Coelho V, Snitcowsky R, Pillegi F and Kalil J. Human heart-infiltrating T cell clones from rheumatic heart disease patients recognize both streptococcal and cardiac proteins. Circulation. 1995, 92:415-420.
4. Guilherme L, Fae KC, Higa F, Chaves L, Oshiro SE, Freschi de Barros S, Puschel C, Juliano MA, Tanaka AC, Spina G, Kalil J. Towards a vaccine against rheumatic fever. Clin. Immunol. Dev. (Taylor & Francis Journal), 2006. In Press.