StreptInCor: a model of anti-*Streptococcus pyogenes* vaccine reviewed

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Abstract *Streptococcus pyogenes* infections remain a health problem in multiple countries because of post-streptococcal sequelae, such as rheumatic fever and rheumatic heart disease. The epidemiological growth of streptococcal diseases in undeveloped and developing countries has encouraged many groups to study vaccine candidates for preventing group A streptococcus infections. We developed a vaccine epitope (StreptInCor) composed of 55 amino acid residues of the C-terminal portion of the M protein that encompasses both T and B cell protective epitopes. Using human blood samples, we showed that the StreptInCor epitope is recognized by individuals bearing different HLA class II molecules and could be considered a universal vaccine epitope. In addition, the StreptInCor molecular structure was solved by nuclear magnetic resonance spectroscopy, and a series of structural stability experiments was performed to elucidate its folding/unfolding mechanism. Using BALB-c and HLA class II transgenic mice, we evaluated the immune response over an extended period and found that StreptInCor was able to induce a robust immune response in both models. No cross-reaction was observed against cardiac proteins. The safety of the vaccine epitope was evaluated by analyzing histopathology, and no autoimmune or pathological reactions were observed in the heart or other organs. Vaccinated BALB/c mice challenged with a virulent strain of *S. pyogenes* had 100 % survival over 30 days. Taking all results into account, StreptInCor could be a safe and effective vaccine against streptococcus-induced disease.

Keywords *Streptococcus pyogenes* · M protein · Vaccine · Rheumatic fever · Rheumatic heart disease · T and B cells · HLA class II transgenic mice

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Introduction

Rheumatic fever (RF) is an autoimmune disease resulting from untreated throat infections of the gram-positive bacteria *Streptococcus pyogenes* in susceptible children. Rheumatic heart disease (RHD), the most serious complication, occurs in 30 %–45 % of RF patients and leads to chronic valvular lesions.

Suppurative streptococcal infections of the throat and skin generate stimuli that lead to RF in 1–5 % of susceptible children and teenagers (3–19 years of age). The disease initially manifests as polyarthritis, carditis/valvulitis, Sydenham's chorea, erythema marginatum, and/or subcutaneous nodules. Chronic renal disease can also occur. Rheumatic carditis usually presents as pancarditis affecting the endocardium, myocardium, and pericardium. Recurrent acute cardiac lesions frequently evolve into chronic RHD, of which valvular deformities are the most important

sequelae. These deformities lead to mitral and aortic regurgitation and/or stenosis. Valve replacement surgery is usually the only treatment for chronic RHD patients and incurs high costs for both public and private health systems [1].

The incidence of acute rheumatic fever (ARF) in some developing countries exceeds 50 per 100,000 children. The worldwide incidence of RHD is at least 15.6 million cases and is the cause of $\sim$233,000 deaths/year. However, as these estimates are based on conservative assumptions, the actual disease burden is most likely substantially higher. The incidence of ARF can vary from 0.7 to 508 per 100,000 children per year in different populations [2]. According to the WHO epidemiological model and data from IBGE (Brazilian Institute of Geography and Statistics), the number of *Streptococcal pharyngitis* infections in Brazil is around 10 million cases, which could lead to 30,000 new cases of RF and around 15,000 cardiac lesions leading to RHD [3].

Rheumatic fever and RHD development depends on several host factors that mediate a heart tissue-damaging autoimmune response that is triggered by a defensive immune response against *S. pyogenes*. Genetic predisposition is one of the leading factors contributing to the development of autoimmunity.

The first genetic associations described in the 1980s focused on the HLA class II alleles coded by the HLA-DRB1 and DQB1 genes, and studies with diverse genetic backgrounds led to multiple HLA class II alleles being associated with the disease. More recently, modern molecular biology tools made it possible to identify several more genes involved with the activation of both innate and adaptive immune responses in which new single nucleotide polymorphisms (SNPs) were associated with the development of RF/RHD.

The SNPs associated with RF/RHD are located near genes coding for proinflammatory molecules, including (1) mannose-binding lectin (MBL) initiating the complement lectin pathway; (2) Toll-like receptors (TLRs), sensors of foreign microbial products that initiate host defense responses in multicellular organisms; (3) Ficolins, proteins that trigger the innate immune response by either binding collectin cellular receptors or initiating the complement lectin pathway; and (4) FcγRIIA, the immunoglobulin receptor that plays a role in the clearance of immune complexes by macrophages, neutrophils, and platelets. In addition, several SNPs near inflammatory and regulatory cytokine genes (e.g., IL1Ra, TNF alpha, TGFB1, and IL-10) were also associated with the development of heart lesions [4, 5].

HLA class II molecules are expressed on the surface of antigen-presenting cells (APCs), e.g., macrophages, dendritic cells, and B lymphocytes and play a major role in

triggering immune system activation. In the case of RF and RHD, autoimmune reactions are triggered by T cell populations activated by self-antigen stimulation, and these reactions produce multiple inflammatory cytokines that damage heart tissue [6]. These observations are corroborated by the fact that during the acute phase of disease, Aschoff bodies, a granulomatous lesion containing macrophages, multinucleated Anitschkow cells, and polymorphonuclear leukocytes develop in the myocardium and/or endocardium of RHD patients. Inflammatory cytokines such as IL-1, TNF-alpha, and IL-2 have been found during specific Aschoff body developmental phases [7] and, as mentioned above, most likely initiate the inflammatory process leading to heart-tissue rheumatic lesions.

The inflammatory process that follows a *S. pyogenes* throat infection in individuals with genetic predispositions leads to intense cytokine production by monocytes and macrophages and triggers the activation of B and T lymphocytes. These cells are the major effectors of the heart tissue-damaging autoimmune reactions created in RHD as a result of similarities between human and streptococcal proteins.

To ameliorate the heart valve damage caused by *S. pyogenes* infections in susceptible young individuals, many studies have attempted to develop a vaccine against *S. pyogenes* to prevent infection and complications. There are four anti-group A streptococci (GAS) vaccine candidates that target the M protein and eight other candidates targeting alternative streptococcal antigens, including group A CHO, C5a peptidase (SCPA), cysteine protease (Spe B), binding proteins similar to fibronectin, opacity factor, lipoproteins, Spes (super antigens), and streptococcal pili [8, 9].

The M protein has been the target of multiple models utilizing different immunization approaches. Initially, vaccines based on the multivalent N-terminus were developed based on six serotypes that are more prevalent in the USA. More recently, a 26-valent vaccine comprised of short sequences from 26 different strains entered phase I clinical trials with promising results [10].

The C-terminal region is highly conserved among *S. pyogenes* and is the focus of several potential vaccine models. Interestingly, an Australian group has identified a C-terminal minimal B cell epitope that induces protective antibodies in animal models [8].

The model we developed is composed of 55 amino acid residues from the C-terminal portion of the M5 protein and encompasses both T and B cell protective epitopes [11]. This vaccine model is named "StreptInCor" (medical identity). The StreptInCor molecular structure was probed by nuclear magnetic resonance (NMR) spectroscopy. The structural analysis revealed a unique molecular folding composed of two disordered micro domains corresponding to the T and B epitopes separated by an 18-amino-acid α-helix (Fig. 1). In addition, StreptInCor structural stability

experiments monitored by circular dichroism in the presence and absence of chemical denaturing reagents and temperature variation showed that its folding/unfolding behavior proceeds as a two-state reversible process [12]. These characteristics result in generous requirements for excipients, pH, processing temperature, transport and storage conditions, which are all desirable qualities in vaccines.

StreptInCor is a universal vaccine candidate epitope

The analysis of a large panel of peripheral blood mononuclear cells bearing HLA class II molecules showed the
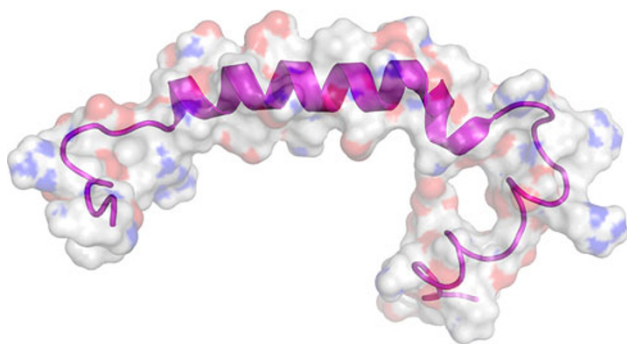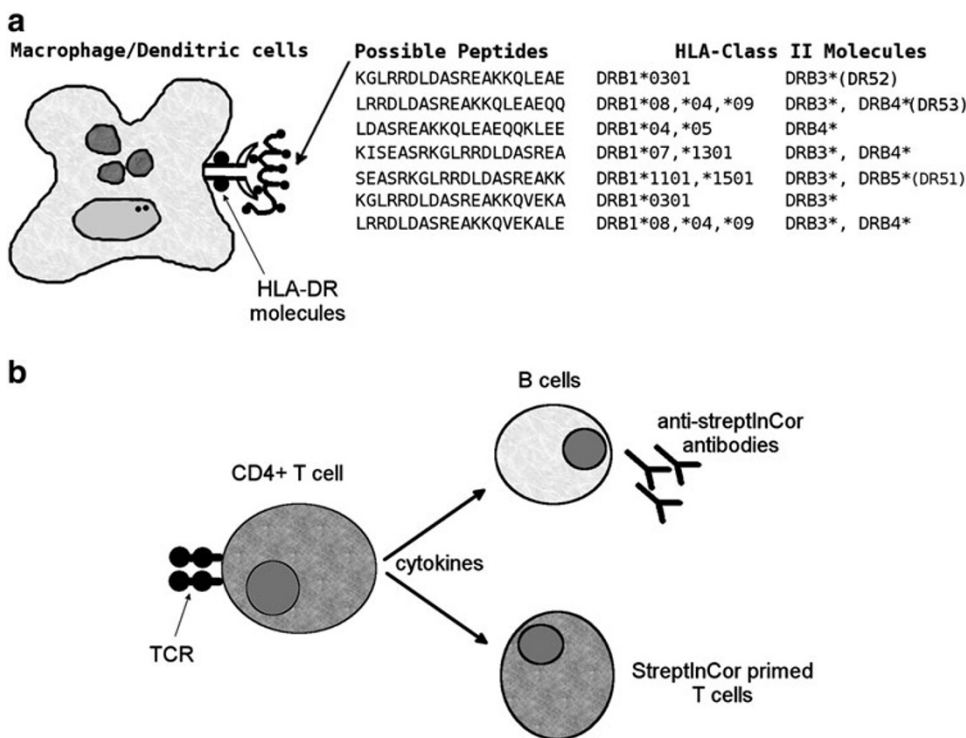

Fig. 1 Charged surface representation of the StreptInCor molecular structure elucidated by NMR spectroscopy. Both epitope B, on the *left*, and epitope T, on the *right*, are linked by an 18-aa α-helix. The Cα representation of the molecule can be observed inside the surface. This figure was drawn with PyMol (http://www.pymol.org)

capacity of StreptInCor to be processed by APC and presented to T cells via HLA class II molecules and T cell receptors (TCR). This presentation induces a strong immune response in humans [12]. Figure 2 illustrates the ability of APCs to generate diverse peptides for T cell recognition. This recognition leads to the activation of both T and B cells and the production of strong and specific antibodies and T and B memory cells.

Pre-clinical assays

BALB/c mice

We first evaluated the immune response against StreptInCor (10 μg) adsorbed onto Freund adjuvant subcutaneously injected in BALB/c mice. Our results showed that the vaccine peptide induced high titers of IgG1 and IgG2a antibodies [13]. To study whether StreptInCor peptide could maintain the production of specific antibodies without inducing any deleterious reactions, we immunized BALB/c mice with a higher dose (10 times) and observed them until 9 months after immunization. These animals maintained a huge specific humoral response, and no deleterious or autoimmune reactions were observed in the heart, liver, spleen, kidneys, or lymph nodes [13]. In another set of experiments, using an aluminum hydroxide (ALUM) adjuvant, we observed high humoral (titer > 1:12,800) and strong cellular (SI ≥ 5.0)
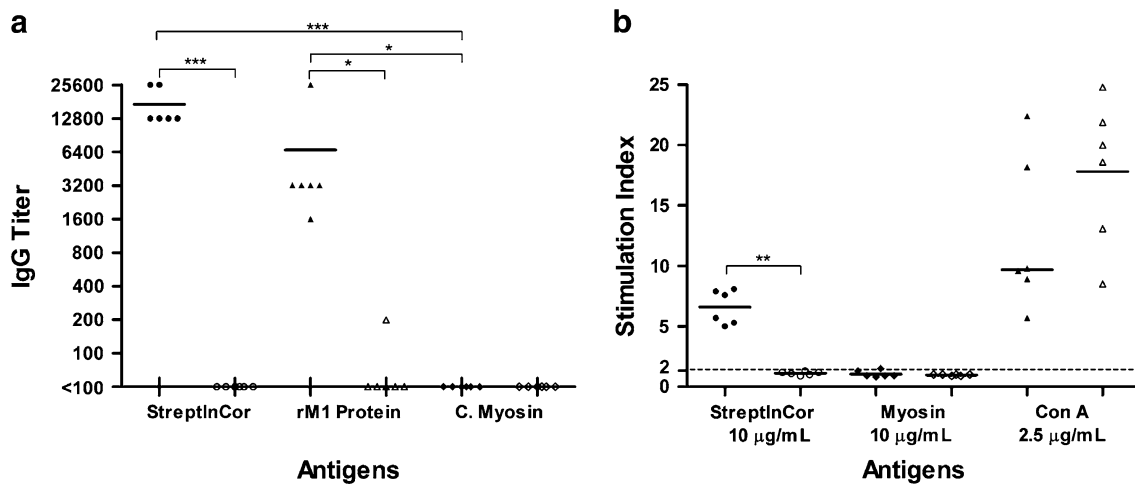
Fig. 2 Theoretical possibilities of StreptInCor processing and antigen presentation to the T cell receptor. StreptInCor is a peptide 55 amino acids long. **a** Several peptides (13 or 15 amino acids long) generated by antigen processing cells (APC); **b** diverse peptides in combination with HLA class II molecules will be presented to the T cell receptor (TCR). This will trigger the activation of both T and B cell immune responses and produce StreptInCor specific antibodies and T cells

Fig. 3 StreptInCor induces both humoral and cellular immune responses without cardiac myosin cross reactivity. Six BALB/c mice received 10 μg of StreptInCor adsorbed onto 60 μg of aluminum hydroxide (*black symbols*). **a** Humoral immune response: specific IgG anti-StreptInCor antibodies (*black circles*), anti-rM1 protein (*black triangles*); cardiac myosin (*black diamonds*). Controls received only 60 μg of aluminum hydroxide (*open symbols*). The titer obtained for each sample is represented as a data point. Samples not reactive at 1:100 dilutions were considered negatives (values = <100). **b** Cellular immune response: StreptInCor positive spleen cells (10 μg/mL, *black circles*) and Concanavalin A (2.5 μg/mL, *black triangles*). Control mouse spleen cells treated only with Concanavalin A (2.5 μg/mL, *open triangles*). Stimulation index ≥2.0 (*dotted line*) were considered positive. Each sample is represented as a data point plus a line at the mean. *P* values: *** <0.001; ** <0.010 and >0.001; * >0.01 and <0.05)

StreptInCor-specific immune responses without cross reactivity against cardiac myosin (Fig. 3a, b).

The capacity of StreptInCor to promote protection against the *S. pyogenes* M1 strain was tested in vaccinated BALB/c mice challenged by intraperitoneal injections of 1.5×10^7 CFU/100 μL of M1 strain. These animals were followed for 30 days and maintained 100 % survival. Non-vaccinated mice suffered 60 % mortality 2 days post-challenge. In another set of experiments, Swiss mice presented similar results.

HLA class II transgenic mice

Another model we examined was transgenic mice that has a complete deletion of murine H2 molecules [14] and human HLA class II genes (DRB1 and DQB1) acquired by gene transfection. After immunizing these mice with StreptInCor in the presence of aluminum hydroxide, we evaluated immune response and safety over a long period [15]. These HLA transgenic mice express the human HLA molecules DR2, DR4, DQ6, and DQ8 on the surface of APCs (monocytes, macrophages, B lymphocytes). The animals responded to immunization with high titers of specific antibodies for a period of 1 year. Interestingly, we observed no cross-reactive antibodies against human myocardium tissue and no tissue damage in the heart (myocardium, mitral, aortic, and tricuspid valves), joints, kidney, spleen, or liver [15]. Figure 4 is a picture of the heart chambers, focusing on the valves in which no morphological alterations or tissue damage were observed
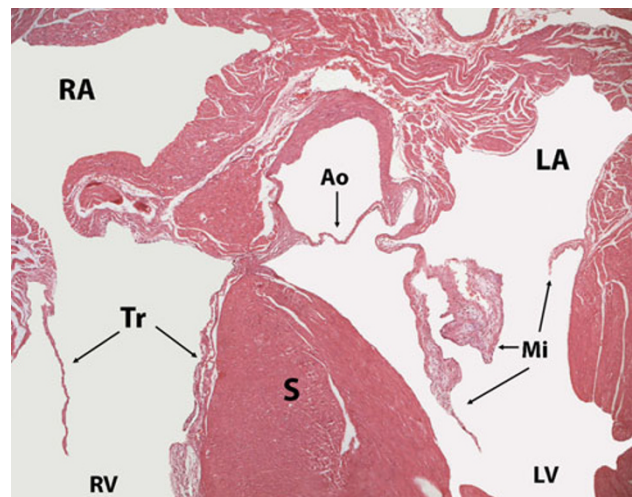

Fig. 4 Histological analysis of the heart tissue chambers with special focus on the valves Photomicrograph of a four-chamber histological section of the heart of an HLA class II transgenic mouse immunized with StreptInCor and followed for 9 months. No morphological alterations were observed in the right atrium (*RA*), tricuspid valve (*Tr*), right ventricle (*RV*), ventricular septum (*S*), left atrium (*LA*), mitral valve (*Mi*), left ventricle (*LV*) or aortic valve (*Ao*). Hematoxylin and eosin (H&E) staining 50×

1 year post-vaccination. Taken together, our data indicate that StreptInCor is a safe vaccine.

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