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T-cell molecular mimicry in Chagas disease: identification and partial structural analysis of multiple cross-reactive epitopes between *Trypanosoma cruzi* B13 and cardiac myosin heavy chain

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Abstract

Chagas disease cardiomyopathy (CCC) is one of the few examples of post-infectious autoimmunity, where infectious episodes with an established pathogen, the protozoan parasite *Trypanosoma cruzi*, clearly triggers molecular mimicry-related target organ immune damage. $CD4^+$ T-cell clones infiltrating hearts from CCC patients cross-reactively recognize human cardiac myosin, the major heart protein, and the immunodominant B13 protein from *T. cruzi*. Moreover, in vitro priming with B13 leads to the recovery of cardiac myosin cross-reactive T-cell clones. In order to identify cross-reactive epitopes between B13 protein and human cardiac myosin, we used B13 peptide S15.4, preferentially recognized by CCC patients, to establish a T-cell clone from an HLA-DQ7 individual. The B13 S15.4 peptide-specific $CD4^+$ T-cell clone 3E5 was tested in proliferation assays against 15 Lys/His-substituted S15.4-derived peptides for TCR/HLA contact analysis. Together with previous HLA-binding data and molecular modeling of the HLA-DQ7-peptide S15.4 complex, Lys/His scanning analysis showed eight TCR/HLA contact positions. Clone 3E5 was also tested against 45 15-mer peptides from human β-cardiac myosin heavy chain bearing the central HLA-DQ7 binding motif. Clone 3E5 recognized 13 peptides from cardiac myosin. The alignment of cross-reactive peptides in cardiac myosin showed very limited sharing of residues or side chains with similar chemical/structural features at aligned positions, indicative of a very degenerate TCR recognition pattern. The existence of degenerate intramolecular recognition, with multiple low-homology, cross-reactive epitopes in a single autoantigenic protein may have implications in increasing the magnitude of the autoimmune response in CCC and other autoimmune diseases.

Keywords: B13 protein; Chagas disease cardiomyopathy; Human cardiac myosin; T-cell molecular mimicry; *Trypanosoma cruzi*

1. Introduction

Chagas disease cardiomyopathy (CCC) is one of the few examples of post-infectious autoimmunity, where infectious episodes with an established pathogen, the protozoan parasite *Trypanosoma cruzi*, clearly triggers

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molecular mimicry-related target organ immune damage. CCC is an inflammatory dilated cardiomyopathy that develops in 25–30% of the estimated 9 million individuals infected by the protozoan parasite in Latin America, 5–30 years after infection. Most of the remaining infected individuals remain asymptomatic ("indeterminate" patients, ASY). CCC heart lesions show histopathological findings consistent with inflammation: T cell/macrophage-rich myocarditis, fibrosis and heart fiber damage [1], in the presence of very scarce *T. cruzi* forms. Increased local expression of the cytokines IFN-γ, TNF-α, IL-6 [2,3] and IL-4 [2], as well as HLA class I/class II molecules and adhesion molecules were reported [3]. In murine models, local production of TNF-α, IFN-γ and IFN-γ-induced chemokines MIG, RANTES, IP-10, MCP-1, and MIP-1α was reported [4] and up-regulated production of IFN-γ in *T. cruzi*-infected IL-4 −/− mice enhanced late-phase myocarditis [5] suggesting a pathogenic role for IFN-γ. Together, these observations suggest that infiltrating inflammatory cells are the ultimate effectors of heart tissue damage, and chronic myocardial inflammation could contribute to CCC pathogenesis.

The discrepancy between the scarcity of tissue parasites and the abundant infiltrating T cells suggested that tissue-damaging T cells were of autoimmune nature, possibly elicited by cross-reactive immune responses with *T. cruzi* parasite. For that matter, cross-reactive autoantibodies against several distinct host-*T. cruzi* antigenic pairs were described along the last decades (reviewed in [6,7]). In murine models of *T. cruzi* infection, cardiac myosin is an important target heart self-antigen. Cardiac myosin is recognized by $CD4^+$ T cells from mice chronically or acutely infected by *T. cruzi* [8,9]. Immunization with *T. cruzi* lysate generates cardiac myosin autoimmunity and vice versa, implying cross-reactivity [10]. Moreover, tolerance induction with cardiac myosin ameliorates *T. cruzi*-induced chronic cardiac inflammation and fibrosis [11]. Previous results from our group identified IgG autoantibodies cross-reactively recognizing cardiac myosin heavy chain, the major heart protein, and the tandemly repetitive *T. cruzi* membrane protein B13 in sera from CCC patients [12]. Significantly, heart-infiltrating $CD4^+$ T-cell clones from CCC patients also cross-reactively recognized human cardiac myosin heavy chain and *T. cruzi* B13 protein [13]. In vitro stimulation of peripheral blood mononuclear cells (PBMC) with *T. cruzi* B13 protein generated T-cell clones that cross-reactively recognized both cardiac myosin heavy chain and *T. cruzi* B13 protein [14].

T. cruzi B13 protein encompasses 19 slightly degenerate tandemly repeated 12 amino acid motifs. Ninety percent of patients displaying PBMC responses to B13 protein carry one of the HLA molecules capable of binding to B13 peptides, HLA-DQ7, DR1 and DR2 [15,16]. The minimal DQ7-restricted B13 T-cell epitope was the 10-mer FGQAAAGDKP, and the variant B13 peptide S15.4 was preferentially recognized by PBMC from HLA-DQ7 CCC patients ([16]; Abel LC, et al., submitted). Residues F1, G2 and A4 were identified as important HLA-DQ7 contact residues in binding assays ([16], Abel LC, submitted). While epitope identification was easier in B13 protein due to its tandemly repetitive nature, the large size of cardiac myosin heavy chain (>2000 residues) makes epitope mapping a difficult task. In this work, we established a T-cell clone against the B13 peptide S15.4 in an HLA-DQ7-positive individual, identifying the cross-reactive cardiac myosin heavy chain epitopes among sequences bearing HLA-DQ7 binding motifs. Furthermore, we studied the molecular aspects of the peptide contact sites of the peptide with the HLA and TCR molecules.

2. Materials and methods

2.1. Synthetic peptides

We selected 45 15-mer peptides from human β-cardiac myosin heavy chain (GenBank accession number NM-000257) bearing a central small residue (i, i+2) HLA-DQ7 binding motif [17] according to the "all box-no charge" algorithm (Table 1). Peptides were synthesized with Fmoc solid-phase strategy in an Advanced ChemTech (model 396, Louisville, KY, USA) multiple peptide synthesizer. Lysine/histidine substituted peptides derived from the S15.4 parent sequence (GenBank accession number AY-325808) were purchased from Mimotopes Inc. (San Diego, CA, USA) (Table 2). Peptides were analyzed by MALDI-TOF mass spectrometry (Tof-Spec E, Micromass, Manchester, UK) and by analytical reverse-phase HPLC (Shimadzu Inc., Tokyo, Japan) and were routinely >80% pure.

2.2. T-cell clone establishment

The T-cell clone was obtained from peripheral blood mononuclear cells (PBMC) of a healthy HLA-DQ7 individual. PBMC were initially isolated from peripheral blood by Ficoll–Hypaque density gradient centrifugation (d=1.077), washed and incubated with 25 μM of the S15.4 peptide in Dulbecco's modified Eagle's medium (DMEM; Gibco, Grand Island, NY, USA) supplemented with 2 mM L-glutamine, 1 mM sodium pyruvate, 50 μg/ml gentamicin, 10 mM HEPES buffer and 10% normal human serum (complete medium). After 6 days, the medium was supplemented with 20 U/ml of recombinant human interleukin-2 (hIL-2). After 10–15 days, wells with lymphoblasts were expanded for an additional 15–20 days with irradiated (5000 rad) allergenic antigen presenting cells (APC) (10^6/well in a 24-well plate) in complete medium with 2,5 μg/ml of