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Flexible peptide recognition by HLA-DR triggers specific autoimmune T-cell responses in autoimmune thyroiditis and diabetes

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ABSTRACT

Autoimmune polyglandular syndrome 3 variant (APS3v) refers to the co-occurrence of autoimmune thyroiditis (AITD) and type 1 diabetes (T1D) within the same individual. HLA class II confers the strongest susceptibility to APS3v. We previously identified a unique amino acid signature of the HLA-DR pocket (designated APS3v HLA-DR pocket) that predisposes to APS3v. We hypothesized that both thyroid and islet peptides can be presented by the unique APS3v HLA-DR pocket, triggering AITD + T1D together. To test this hypothesis we screened islet and thyroid peptides for their ability to bind to the APS3v HLA-DR pocket. Virtual screen of all possible thyroglobulin (Tg), thyroid-stimulating hormone receptor (TSHR), thyroid peroxidase (TPO), insulin (Ins), and glutamic acid decarboxylase 65 (GAD65) peptides identified 36 peptides that bound to this unique pocket. *In vitro* binding assays using baculovirus-produced recombinant APS3v HLA-DR identified 11 thyroid/islet peptides (of the 36 predicted binders) that bound with high affinity. By immunizing humanized HLA-DR3 mice carrying the APS3v HLA-DR pocket we identified 4 peptides (Tg.1571, GAD.492, TPO.758, TPO.338) that were presented by antigen presenting cells and elicited T-cell response. We conclude that both thyroid and islet peptides can bind to this flexible APS3v HLA-DR pocket and induce thyroid and islet specific T-cell responses. These findings set the stage to developing specific inhibitors of the APS3v HLA-DR pocket as a precision medicine approach to treating or preventing APS3v in patients that carry this genetic HLA-DR pocket variant.

1. Introduction

Autoimmune endocrine disorders frequently occur in the same individual. This association is referred to as autoimmune polyglandular syndrome (APS) [1]. The most common type of APS consists of the co-occurrence of autoimmune thyroid disease (AITD), in which the target endocrine gland is the thyroid, and type 1 diabetes (T1D), in which the target endocrine gland is the pancreatic islets beta cells [2–5]. The clinical phenotype of AITD + T1D within the same individual is referred to as a variant of APS type 3 (APS3v). Indeed, AITD and T1D share common etiology and immunological abnormalities including infiltration of the target glands by lymphocytes reactive to self-antigens and production of antibodies directed at the target organs [1,6]. While the prevalence of APS3v has not been calculated directly it can be estimated based on the prevalence of T1D in the US population (1.93 per 1000) [7] and the frequency of AITD in T1D patients (20–30%) [8]; based on these data the prevalence of APS3v in the US population is approximately 0.4–0.6 per 1000.

Family and population studies provide consistent evidence for the strong association of AITD and T1D. Whole genome linkage and Genome Wide Association Studies (GWAS) support a genetic etiology for this association [2] and identified five gene-loci that confer susceptibility to APS3v. They include the major histocompatibility (MHC) class II locus, cytotoxic T-lymphocyte antigen 4 (CTLA-4), protein tyrosine phosphatase non-receptor type 22 (PTPN22), G-protein-coupled receptor 103 (GPR103) and Forkhead box P3 (FOXP3) [9–12]. Among them, the MHC class II locus showed the strongest linkage and association with APS3v, and Human Leukocyte Antigen-DR3 (HLA-DR3) conferred most of the

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shared risk [8]. Our group has previously identified a pocket amino acid signature, DRβ-Tyr26, DRβ-Leu67, DRβ-Gln70, DRβ-Lys71 and DRβ-Arg74 that is strongly associated with APS3v [9]. We hypothesized that this unique pocket predisposes to both AITD and T1D (APS3v) by enabling the presentation of thyroid and islet peptides within the same pocket structure.

To test this hypothesis we screened all potential peptides originating from the major AITD and T1D associated self-antigens (thyroglobulin [Tg], thyroid-stimulating hormone receptor [TSHR], thyroid peroxidase [TPO], insulin [Ins], and glutamic acid decarboxylase 65 [GAD65]) for binding to the APS3v HLA-DR pocket. We then tested whether peptides that showed strong binding to the APS3v-pocket induced T-cell responses in a humanized mouse model carrying the core APS3v pocket signature. Our data support the hypothesis that the APS3v HLA-DR pocket we identified enables the presentation of pathogenic thyroid and islet peptides to T-cells, thereby triggering both thyroid and islet T-cell responses and autoimmunity.

2. Materials and methods

2.1. Production of recombinant APS3v HLA-DR

APS3v HLA-DR protein was produced using the baculovirus system using a similar strategy we described previously [13]. We designed 2 separate constructs for the α and β chains of the APS3v HLA-DR. The β chain construct contained the extracellular portion of the APS3v β chain fused to the coiled coil region of the basic Leucine zipper domain of JunB, and the α chain construct contained the extracellular portion of the α chain fused to the coiled coil region of the basic Leucine zipper domain of Fos. A tobacco etch virus (TEV) protease cutting site was introduced in each chain to allow removal of the dimerization motif. The JunB and Fos dimerization motifs allowed the protein to dimerize and form the final APS3v HLA-DR protein. The α chain has a His_6 tag while the β chain has a V5 epitope tag for purification purposes (Fig. 1). These constructs were used to produce the APS3v HLA-DR protein in a Baculovirus system using the Life Technologies Baculovirus protein production custom services (Carlsbad, CA, USA).

2.2. Construction of model structures and MD simulations

The structural models of the APS3v HLA-DR molecule were constructed based on the X-ray crystallographic structure of HLA-DR3 with a CLIP peptide complex (PDB ID: 1A6A) [14]. The amino acids at positions β37, β47 and β86 were mutated using the VMD facility [15] N37- > Y, F47- > Y and V86- > G. The structural models of the peptides in complex with APS3v were constructed by mutating the original sequence of the CLIP peptide using the VMD facility. The initial structures of the complexes of APS3v with the peptides were used to construct the system for simulations using the AMBER facility LEaP [16]. The system was placed in a truncated octahedron with walls 10 Å from the solute, which was filled with water molecules and ions to create an ionic strength of 0.15 M. The systems consisted of approximately 70,000 atoms. The systems were minimized, heated and equilibrated with the solute positionally restrained while gradually removing the restraints. The equilibrated system was simulated under constant temperature and pressure (NPT) for 100 ns. The trajectories were recorded every 2 ps. After a short period (~1 ns) the systems usually stabilized showing a root mean square deviation from the initial structure of the order of 3 Å. The first 10 ns of the trajectories were discarded and the remaining data were processed to remove the waters and ions. The resulting trajectories were used to calculate the binding energies with the MM-PBSA module in AMBER. Specifically, the residue contributions were calculated using the *decomp* option. The values discussed in the paper refer only to those associated with the peptide [17].

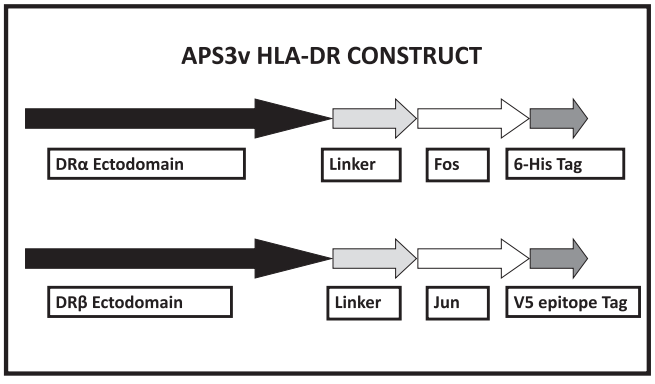

Fig. 1. Diagram depicting the APS3v HLA-DR protein construct we produced. The α chain construct contained the DRα chain fused to the coiled coil region of the basic Leucine zipper domain of Fos. The β chain construct contained the coiled coil region of the basic Leucine zipper domain of JunB. The Jun and Fos dimerization motifs allowed the protein to dimerize to form the APS3v HLA-DR protein. The α chain has a His_6 tag while the β chain has a V5 epitope tag for purification purposes.

2.3. Virtual screening of thyroidal and islet peptides

We used the web-based server NetMHCIIpan-2.0 to predict binding of peptides to HLA alleles. The method is based on Artificial Neural Network trained on available binding data of peptides to HLA alleles [18]. The sequences of the antigen proteins (Tg [D1KKB3], TPO [P35419], TSHR [P16473], GAD65 [Q9UGI5] and Ins [A6XGL2]; all accession numbers are from uniprot.org) were used to perform the virtual screen on a selected DRB subtype DRB1*0301. Top ranked peptides from the list were selected and redundancies (i.e., proximal sequences) were removed based on a predicted common binding core of 9 residues. This resulted in the 36 predicted peptide binders listed in Table 1.

2.4. Peptide synthesis

The peptides used in this study were synthesized by Genscript (Piscataway, NJ). Based on the results of the virtual screen the following peptides were tested *in vitro* for binding to the APS3v HLA-DR: 5 thyroglobulin (Tg) peptides (Tg.2098, Tg.726, Tg.1951, Tg.1571 and Tg.202), 2 thyroid-stimulating hormone receptor (TSHR) peptides (TSHR.132 and TSHR.197), 6 insulin (Ins) peptides (Ins. B9-23, Ins.44, Ins.25, Ins.76, Ins.89, Ins.98), 10 glutamic acid decarboxylase 65 (GAD65) peptides (GAD.378, GAD.492, GAD.116, GAD.247, GAD.260, GAD.274, GAD.379, GAD.555, GAD.192, GAD.456) and 13 thyroid peroxidase (TPO) peptides (TPO.763, TPO.489, TPO.122, TPO.28, TPO.423, TPO.758, TPO.405, TPO.338, TPO.513, TPO.589, TPO.161, TPO.344 and TPO.351). The apopeptide (APO), a peptide that was previously shown by us [13] and others [19] to be the best binder to this pocket, was used as a positive control, and a scrambled thyroglobulin peptide (scr2098) was used as a negative control. The sequences of the peptides are listed in Table 1.

2.5. In vitro screening of peptide binding to APS3v HLA-DR

The 36 peptides predicted by the virtual screen to bind the APS3v HLA-DR with high affinity were tested for binding to the APS3v HLA-DR pocket using a unique immunoassay we developed