[0060] FIG.24 depicts binding of hybrid insulin peptides to DRB1*04:01 and two edited alleles of the present disclosure with pocket 1 mutations G86L and G86M, according to embodiments of the disclosure. Numbers in upper left corner of the boxes are binding ratios compared to negative controls.

[0061] FIG.25 depicts binding of neuroautoimmune peptides binding to DRB1*04:01 and edited alleles of the present disclosure with pocket 1 mutations G86L and G86M, according to embodiments of the disclosure. Numbers in upper left corner of boxes are binding ratios compared to negative controls.

[0062] FIG.26 depicts binding of arthritogenic peptides to DRB1*04:01 and engineered alleles of the present disclosure with pocket 1 mutations, according to embodiments of the disclosure. Numbers in upper left corner of boxes are binding ratios of citrullinated peptides compared to native peptides.

[0063] FIG.27 depicts binding of native and citrullinated arthritogenic peptides to DRB1*04:01 and engineered alleles of the present disclosure with pocket 1 mutations, according to embodiments of the disclosure. Numbers in upper left corner of boxes are binding ratios compared to the negative controls.

[0064] FIG.28 is a list of representative HLA alleles, amino acid positions, and mutations according to embodiments of the present disclosure.

[0064a] FIG. 29 lists mature protein sequences of various HLA alleles.

[0064b] FIG. 30 lists cDNA sequences of various embodiments of susceptible and engineered HLA alleles.

DETAILED DESCRIPTION

[0065] Disclosed herein are various methods and compositions useful in treating, reducing, or eliminating autoimmune disease in a subject suffering from or at risk of developing the same. Such autoimmune diseases include, without limitation, rheumatoid arthritis (RA), celiac disease, diabetes mellitus type 1, systemic lupus erythematosus (SLE), multiple sclerosis (MS), myelin oligodendrocyte glycoprotein antibody disorders (MOGAD), myasthenic syndromes and neuromyelitis optica (NMO), ankylosing spondylitis, Behçet’s syndrome, Birdshot uveitis, narcolepsy, narcolepsy type 1 (NT1; previously termed narcolepsy with cataplexy), Kawasaki disease, Crohn’s disease, psoriasis, dermatomyositis (DM), Addison's disease, irritable-bowl syndrome (IBS), Graves' disease, Henoch-Schönlein purpura (HSP), sarcoidosis, Sjögren’s syndrome, eosinophilic granulomatosis with polyangiitis, Hashimoto's disease, idiopathic thrombocytopenic purpura, polymyositis (PM), paraneoplastic neurological syndromes

(PNS), autoimmune encephalitis, lupus nephritis (LN), myasthenia gravis (MG), psoriatic arthritis, graft rejection, graft-versus-host disease (GVHD), an unwanted delayed-type hypersensitivity reaction, T-cell mediated pulmonary disease, neuritis, vitiligo,

2011). For the HLA-DRB1 and -DRB3 alleles, the retrovirus in the supernatant was used to transduce 1 x 10^5 T2 cells expressing DRA1*01:01 and sorted for high expression of HLA-DR+/GFP+ seven days post transduction (Anti-DR-APC (LN3) Invitrogen Cat#17-9956-42). For the -DQ alleles, the retrovirus of the HLA-DQB1 alleles was used to transduce 1 x 105 HLA Class II negative T2 cells and sorted for high GFP+ expression seven days post transduction. Then, the retrovirus for the corresponding HLA-DQA1 allele for the cis and trans dimer was used to transduce 1 x 10^5 DQB1+ T2 cells and sorted for high HLA-DQ+/GFP+ expression seven days post transduction (Anti-Human HLA-DP/DQ/DR Starbright Blue (WR18) 700 BioRad Cat# MCA477SBB700). Post-sort, RNA was isolated from each cell line to verify the HLA sequences for both cis and trans HLA alleles by Sanger sequencing (Quintara Biosciences). All cell lines were grown in IMDM-GlutaMAX (Life Technologies) supplemented with sodium pyruvate, thio-penicillin/streptomycin, and 10% fetal bovine serum (FBS).

Peptide Design and Synthesis for Peptide Binding Assays

[0178] Hybrid Insulin Peptides HIP1- WE14 (GQVELGGWSKMDQLA), HIP6- IAPP2 (GQVELGGGNAVEVLK), HIP8- NPY (GQVELGGGSSPETLI), and HIP11- C peptide (SLQPLALEAEDLQV) were synthesized with a biotinylated PEG3 linker on the N-terminus to >98% purity with Trifluoroacetic acid (TFA) removal by Genscript (Piscataway, NJ) (Delong 2016, Baker2019). The HIPs used in this study were selected because of their capability to stimulate available T cell clones (Table 2). Biotinylated GAD65265-281 (AMMIARFKMFPEVKEKG), Insulin Mimotope (HLVEELYLVAGEEG), and Influenza A (PKYVKQNTLKLAT) peptides were also synthesized as controls for HLA-DR and DQ binding [S. Dai, at doi.org/10.1073/pnas.1716527115]. All peptides, except HIP6, were reconstituted in Dimethyl Sulfoxide (DMSO), then equal parts water, and finally Dulbecco's phosphate buffered saline (DPBS) (Life Technologies) to 400 µM concentration and kept frozen at -20ºC until use in peptide binding and T cell studies. HIP6 was reconstituted in 3% ammonia water, then equal parts water, 75uL of 1M HCL to restore a neutral pH, and finally DPBS to 400 µM.

Patient Specific HLA-Class II Expressing T2 Cells Peptide Binding

[0179] T2 cell lines expressing the HLA-Class -DR and -DQ genotype of Pt3977 were harvested, resuspended, plated with 100µM HIP1, and cultured overnight as mentioned above. Plates were washed twice with DPBS to remove unbound peptide, then resuspended in 100 µL 1:1000 diluted eBioscience™ Fixable Viability Dye eFluor™ 780 for 30min at 4ºC. Then, the cells were processed and stained as before. Data were acquired on the Canto II flow cytometer (BD Biosciences) and analyzed by FlowJo Version X (Tree Star). The average binding ratio (MFI of HLA

which also binds the susceptible allele, but not the resistant allele. These results suggest that the peptide binding profile of DRB1*03:01 may be affected by mutating positions in the peptide binding groove, to prevent or lessen binding of AQP4 peptides, and thus prevent autoimmunity. Candidate positions (e.g. as disclosed elsewhere target amino acid positions 9, 11, 13, 26, 28, 30, 32, 33, 37, 38, 40, 47, 57, 58, 67, 71, 74, 78, 85, and 86) for such a mutation can be identified from the sequence alignment of DRB1*03:01 and DRB1*07:01 (FIG. 8; note positions 38, 40, and 85 are not polymorphic indicate 03:01 and 07:01; note also that aligned sequence positions are numbered based on mature protein sequence).

Example 6A - HLA alleles associated with RA

[0194] The HLA-DRB1 allele *04:05 also shows susceptibility to RA. In particular, this allele shows a strong association with RA in Japanese population. For these studies, position 71 in DRB1*04:05 was mutated from R to E (see FIG. 10).

[0195] These studies indicate that DRB1 allele *04:05 does not show a strong preference for binding the immunodominant collagen peptide (FIG. 11). However, when position 71 is mutated to glutamic acid (R71E), the low level of binding is further reduced. In the case of vimentin and α-enolase, the *04:05 allele preferentially binds the citrullinated versions over the native versions. As with to the K71E mutation, this preference is reduced by changing arginine at position 71 in DRB1*04:05 to glutamic acid (R71E). MFI Binding Ratio averages for 2 experiments.

[0196] As disclosed herein, other DR4 alleles susceptible to RA, for example DRB1*04:03, *04:04, and *04:08, may confer resistance (i.e. resistant alleles) when position 71 is changed from arginine (R) to glutamic acid (E).

Example 6B - HLA alleles associated with Type I Diabetes – T1D

[0197] DQB1 alleles associated with susceptibility to Type I diabetes were tested for the ability to confer resistance with one or more mutations in the antigen binding groove. Specifically, several DQB alleles and corresponding A57D variants were cloned into T2 cell lines. Aspartic acid is found at position 57 in several resistant alleles.

[0198] Table 2. Selected Hybrid Insulin Peptide Information (See also FIG. 22)

Hybrid Insulin Peptides	Abbreviations	Sequences	References	SEQ ID NO
HIP1- WE14	HIP1	GQVELGG-WSKMDQLA	*Delong et al. 2016*	
HIP6- IAPP2	HIP6	GQVELGGG-NAVEVLK	*Delong et al. 2016*	
HIP8- NPY	HIP9	GQVELGGG-SSPETLI	*Delong et al. 2016*	
HIP11- C	HIP11	SLQPLAL-EAEDLQV	*Baker et al. 2019*	

[0199] Peptide Selection - Hybrid Insulin Peptides HIP1- WE14 (GQVELGGWSKMDQLA), HIP6- IAPP2 (GQVELGGGNAVEVLK), HIP8- NPY (GQVELGGGSSPETLI), and HIP11- C peptide (SLQPLALEAEDLQV) were synthesized with a biotinylated PEG3 linker on the N-terminus to >98% purity with Trifluoroacetic acid (TFA) removal by Genscript (Piscataway, NJ) (Delong 2016, Baker2019). The HIPs used in this study were selected because of their capability to stimulate and availability of T cell clones (Table 2). Biotinylated $GAD65^{265-281}$ (AMMIARFKMFPEVKEKG), Insulin Mimotope (HLVEELYLVAGEEG), and Influenza A (PKYVKQNTLKLAT) peptides were also synthesized as controls for HLA-DR and DQ binding [S. Dai, available at doi.org/10.1073/pnas.1716527115].

[0200] Hybrid insulin peptides were tested for their binding to these cell lines at various concentrations. Specifically, peptide binding of the native HLA DQB1 allele was compared with its A57D mutated form. Susceptible alleles were hypothesized to bind the hybrid insulin peptides.

[0201] These studies showed that the susceptible DQ2 and DQ8 alleles do not bind the HIP8-NPY peptide, but the DQ2 trans HLA molecule does (FIG. 12). When position 57 is changed from A to D, the binding of this hybrid insulin peptide is increased on DQ2 and DQ8 but is reduced on DQ2 trans. Similarly, DQ2 and DQ8 do not bind the HIP11-C peptide (FIG. 13), but the DQ2 trans molecule does. When A57D is introduced, DQ2 shows peptide binding. While DQ2 trans binds this peptide less, binding is not abolished. Binding of the insulin mimotope peptide follows a similar pattern as to the above peptides (FIG. 14). Specifically, DQ2 does not bind the mimotope peptide, but DQ2 trans and DQ8 bind the peptide. When the A57D mutation is introduced, DQ2 and DQ2 trans now bind the peptide. While the mutation reduces binding to the DQ8 molecule.

[0202] The effect on T cell stimulation by the A57D mutation was also tested. Specifically, E2 T-cells, restricted to DQ2 and specific for the HIP11-C peptide, were obtained. These T cells were stimulated in culture with T2 cells expressing DQ2 or the DQ2 trans molecule in the presence of different concentrations of the HIP11-C peptide overnight. Both molecules with the A57D mutations, were also tested. T cell stimulation was then measured by staining the cells for the IL-2R (CD25) on the surface of the cells.

[0203] These studies showed that the DQ2 T2 cell lines stimulate the E2 T cell clone much better than the parent EBV line (FIG. 15, top panel). Introducing the A57D mutation into these alleles results in less stimulation of the E2 T cell. As shown in the bottom panel of FIG. 15, the E2 T cell clone is stimulated by the DQ2 trans molecule but