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Toxoplasma gondii HLA-B*0702-restricted $GRA7_{20-28}$ peptide with adjuvants and a universal helper T cell epitope elicits $CD8^+$ T cells producing interferon-γ and reduces parasite burden in HLA-B*0702 mice

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ABSTRACT

The ability of $CD8^+$ T cells to act as cytolytic effectors and produce interferon-γ (IFN-γ) was demonstrated to mediate resistance to *Toxoplasma gondii* in murine models because of the recognition of peptides restricted by murine major histocompatibility complex (MHC) class I molecules. However, no *T gondii*–specific HLA-B07-restricted peptides were proven protective against *T gondii*. Recently, 2 *T gondii*–specific HLA-B*0702-restricted T cell epitopes, $GRA7_{20-28}$ (LPQFATAAT) and $GRA3_{27-35}$ (VPFVVFLVA), displayed high-affinity binding to HLA-B*0702 and elicited IFN-γ from peripheral blood mononuclear cells of seropositive HLA-B*07 persons. Herein, these peptides were evaluated to determine whether they could elicit IFN-γ in splenocytes of HLA-B*0702 transgenic mice when administered with adjuvants and protect against subsequent challenge. Peptide-specific IFN-γ-producing T cells were identified by enzyme-linked immunosorbent spot and proliferation assays utilizing splenic T lymphocytes from human lymphocyte antigen (HLA) transgenic mice. When HLA-B*0702 mice were immunized with one of the identified epitopes, $GRA7_{20-28}$ in conjunction with a universal $CD4^+$ T cell epitope (PADRE) and adjuvants ($CD4^+$ T cell adjuvant, GLA-SE, and TLR2 stimulatory Pam_2Cys for $CD8^+$ T cells), this immunization induced $CD8^+$ T cells to produce IFN-γ and protected mice against high parasite burden when challenged with *T gondii*. This work demonstrates the feasibility of bioinformatics followed by an empiric approach based on HLA binding to test this biologic activity for identifying protective HLA-B*0702-restricted *T gondii* peptides and adjuvants that elicit protective immune responses in HLA-B*0702 mice.

1. Introduction

Toxoplasma gondii is an intracellular parasite that infects a broad range of mammalian hosts and can cause severe disease in immune-compromised or immunologically immature persons. The infection can cause severe ocular, neurologic, and systemic disease in these settings [1–3]. Because no medicines eradicate all life cycle stages of this parasite [4], development of a vaccine against *T gondii* would be extremely useful, especially if a vaccine could prevent acquisition of the parasite completely.

The ideal vaccine to protect against toxoplasmosis in humans would include epitopes that elicit a protective T helper (Th)-1 biased-immune response, characterized by the generation of long-lived interferon-γ (IFN-γ)-producing $CD8^+$ T cells [5]. $CD4^+$ and $CD8^+$ T cells have a critical role in protective immunity to *T gondii* in murine models and humans [6,7]. $CD8^+$ T cells and IFN-γ are significant effectors mediating resistance to acute and chronic *T gondii* infection [8]. Peptide-based vaccines derived from $CD8^+$ T cell epitopes are a promising strategy to mobilize the immune system against *T gondii* in humans. However, until now no *T gondii*–specific

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HLA-B07-restricted $CD8^+$ T cell epitopes that were proven to be protective had been identified.

Previously, HF10 (HPGSVNEFDF), a single decapeptide, derived from the dense granule protein 6 (GRA6) was identified as the protective, immunodominant L^d-restricted epitope in H–2^d mice infected with type II *T gondii* parasite [9]. Our earlier work had definitively proven that L^d was essential for the restriction of cyst number and brain pathology in L^d mice. Our recent study also demonstrated that immunization of H-$2L^d$ mice with the lipopeptide constructed by HF10 and PADRE or HF10 with monophosphoryl lipid A (GLA-SE, No. 2573) protected Balb/c mice. However, HF10 administered to HLA-B07 transgenic mice in a similar manner was not immunogenic despite a common L^d and B7 peptide-binding motif [10]. Therefore, to find effective peptides for *T gondii*–derived HLA-$B07^+$-restricted $CD8^+$ T cells, we utilized bioinformatic algorithms to identify novel, *T gondii*–derived, $CD8^+$ T cell epitopes restricted by the HLA-B07 supertypes, which will collectively provide coverage for 30% of the human population worldwide [11,12]. We screened the amino acid sequences from 9 surface and secreted proteins (SAG1, SUSA1, GRA2, GRA3, GRA6, GRA7, ROP2, ROP16, and ROP18) of the type II *T gondii* strain, ME49, for HLA-B*0702-specific peptide-binding scores using bioinformatic analysis. Peripheral blood mononuclear cells (PBMCs) from seropositive and seronegative individuals were tested for response to these peptides using an IFN-γ enzyme-linked immunosorbent SPOT (ELISPOT) assay. Strikingly, 2 *T gondii*–specific HLA-B*0702-restricted T cell epitopes, 1 derived from $GRA7_{20-28}$ (LPQFATAAT) and the other derived from $GRA3_{27-35}$ (VPFVVFLVA), both displayed high-affinity binding to HLA-B*0702 and stimulated IFN-γ production by peripheral blood cells from HLA-B*0702 individuals infected with *T gondii*, but not seronegative controls [10].

Herein, we evaluated the peptides identified previously using bioinformatics [13–17] and *in vitro* analyses as vaccine components administered in conjunction with pan DR epitope (PADRE), a universal helper T lymphocyte epitope in HLA-B*0702 transgenic mice [13,14]. Immunization of HLA-B*0702 transgenic mice with the peptide LPQFATAAT with the help of PADRE and GLA-SE and Pam_2Cys as adjuvants activated $CD8^+$ T cells to produce IFN-γ and protected against subsequent challenge with a high burden of a type II parasite.

2. Subjects and methods

2.1. Peptides and adjuvants

HLA-B*0702-restricted peptides—$GRA7_{20-28}$ (LPQFATAAT) and $GRA3_{27-35}$ (VPFVVFLVA), PADRE (AKFVAAWTLKAAA) and Pam_2Cys (Pam_2-KSS) [18]—were synthesized by Synthetic Biomolecules (San Diego, CA) at >90% purity in lyophilized form. The TLR4 agonist was a GLA-SE mimetic that was synthesized by the Infectious Diseases Research Institute (IDRI, Seattle, WA), used as previously described [19].

2.2. Mice

Female HLA-B*0702 transgenic mice produced at Pharmexa-Epimmune (San Diego, CA) and bred at the University of Chicago express a chimeric HLA-B07/H2-D^b major histocompatibility complex (MHC) class I molecule, are on a C57BL/6 × Balb/C background backcrossed through many generations, and have been previously described [11]. HLA-A*1101 mice were also utilized in an experiment. All studies were conducted with the approval of the Institutional Animal Care and Use Committee at the University of Chicago.

2.3. Analysis of peptide solubility

Peptides were dissolved in phosphate-buffered saline (PBS) followed by brief vortexing to yield a concentration of 5 mg/mL. Samples were centrifuged at 15,000 rpm at room temperature for 1 hour. Supernatant concentrations were determined by measuring absorbance at 280 nm and by high-performance liquid chromatography. The percentage solubility of the samples was calculated, although no detailed studies of optimal concentrations of peptides or adjuvant were performed herein.

2.4. Immunizations and challenge

HLA-B*0702 mice were inoculated subcutaneously at the base of the tail using a 30-gauge needle with single peptides or a mixture of 2 of the identified B7 $CD8^+$ T cell peptides (amino acid sequence LPQFATAAT [LT9] and amino acid sequence VPFVVFLVA [VA9]; 50 μg of each peptide per mouse), with or without 50 μg PADRE and 50 μg Pam_2Cys emulsified in 20 μg of GLA-SE (TLR4 agonist). Control mice were injected with PBS. Mice were boosted twice at 2-week intervals with a total of 50 μg of each peptide per mouse with each immunogen. For challenge studies, mice were challenged 14 days postimmunization with peptide emulsions via intraperitoneal inoculation with Prugniaud (Pru), a type II strain luciferase-expressing parasite [20]. In an experiment to compare treatment with PBS, GLA-SE, and PADRE and GLA-SE, PADRE, and LT9, a Pru parasite transfected with luciferase that produced more cysts was kindly provided by Laura Knoll (Madison, WI) and used for challenge.

2.5. ELISPOT assay

Mice were euthanized 10 to 14 days after the last immunization. Spleens were harvested, pressed through a 70-μm screen to form a single-cell suspension, and depleted of erythrocytes with AKC lysis buffer (160 mM NH_4Cl, 10 mM $KHCO_3$, 100 μM EDTA). Cultures were prepared separately, each with the spleen cells from an individual mouse. Cells were cultured for 24 hours as described previously [10]. Murine ELISPOT assays were performed using antimouse IFN-γ monoclonal antibody (mAb; AN18) and the biotinylated antimouse IFN-γ mAb (R4–6A2) and 5×10^5 splenocytes were plated per well. All antibodies and reagents used for the ELISPOT assay were obtained from Mabtech (Cincinnati, OH). Cells were plated in at least 3 replicate wells for each condition. Results were expressed as the number of spot-forming cells per 10^6 murine splenocytes. Murine splenocytes were incubated with the relevant blocking antibody for 1.5 to 2 hours at 37°C and 5% CO_2 before they were seeded at 5×10^5 cells per well. Anti–$CD4^+$ antibody (RM4–5, BioLegend, San Diego, CA), anti-CD8 monoclonal antibody (53–6.7, BD Biosciences), and their relevant isotype control (Rat IgG_{2a}, BD Biosciences) were added to each sample at a final concentration of 10 μg/mL. To assay for B7-restricted IFN-γ production, splenocytes were incubated with 0.5 μg of FcBlock (BD Biosciences) per 10^6 cells for 10 to 15 minutes at 4°C and washed before anti-HLA-B*0702 (BioLegend) mAb or its isotype control (mouse IgG_{2a}, BioLegend) was added at a final concentration of 20 μg/mL.

2.6. T cell proliferation assay

A splenocyte suspension containing 5×10^6 cells/mL from immunized mice was plated into 96-well U-bottom tissue culture plates (100 μL per well) along with 100 μL of each stimulant diluted to appropriate concentrations in complete RPMI 1640. The stimulant used was peptide at 10 μg/mL. Concanavalin A at 10 μg/mL was used as a positive control and cells cultured with medium alone were used as negative controls. The plates were incubated for 3 days in 5% CO_2 at 37°C and pulsed with 1 μCi [^{3}H]thymidine per well for the final 18 hours. The cells were then harvested onto a unifilter 96-well plate using a cell harvester (Packard Instrument, Meriden, CT). After cells were dried, 25 μL of Microscint was added to each well and the filter plate was sealed and counted in a TopCount (Packard Instruments) to determine radioactivity incorporated into DNA. Results are expressed as the stimulation index (SI), calculated