Characterizing the CD4+ T Cell Response to an Epstein-Barr Virus gp350 Vaccine

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Background

Epstein-Barr virus (EBV) infects at least 90% of the global adult population.[1] In addition to causing infectious mononucleosis, EBV is associated with many types of cancers, including Burkitt lymphoma, nasopharyngeal carcinoma, and Hodgkin lymphoma.[2] Preventing EBV infection would therefore have an enormous impact on public health, but despite several preclinical trials, a vaccine against the virus has not yet been licensed for use in humans. EBV vaccine research thus far has focused on the resulting neutralizing antibody response because its clinical and protective benefits are well-established.[3] However, the vaccine's ability to induce a memory T cell response has not been reported on in detail. Investigation in this area is needed to better understand the efficacy and safety of an EBV-gp350 vaccine. The findings of this ongoing project will help our laboratory make progress towards developing a vaccine that is suitable for human use.

Hypothesis

The aim of this study was to establish a profile of the anti-gp350 CD4+ T cell response in mice receiving an EBV-gp350 + GLA/SE vaccine. We expected to observe an increase in the number and activity of gp350-specific CD4+ memory cells in vaccinated mice.

Methods

Mice
6- to 8-week old C57BL/6 mice were housed under specific pathogen-free conditions at the University of Minnesota Cancer and Cardiovascular Research Building, and all experiments were conducted according to federal and institutional guidelines under approved Institutional Animal Care and Use Committee protocols.

Immunization
5 mice were injected with 5 ug gp350 + 5 ug GLA/SE adjuvant, and 5 mice were injected with 5 ug GLA/SE only. Booster injections were administered after one month.

gp350 tetramer
Phycoerythrin (PE)- and allophycocyanin (APC)-conjugated pMHCII tetramers containing the gp350 peptide EIPEFPFYPTCNVCT were produced and provided by Dr Marc Jenkins of the University of Minnesota's Department of Microbiology and Immunology.

Data Collection
Ten days after receiving the booster, immunized mice were injected with 200 ug of gp350 peptide. Two hours after the exposure, the spleen and inguinal lymph nodes were harvested.[4] Samples were then stained with the tetramer in addition to fluorescence-conjugated antibodies specific for CD44, a memory CD4+ T cell marker, and IFN-y, a marker for their activity.[4]

Fluorescence-Activated Cell Sorting (FACS)
Samples were run on an LSR II flow cytometer with 14 fluorescent parameter capability. Irrelevant tetramer and isotype control antibodies were used to measure non-specific background staining.

Data Analysis
Data was analyzed using FlowJo software. The number of anti-gp350 CD4+ T cells was determined by gating on live CD4+ CD44+ cells that also stained positively for the gp350:MHCII tetramer.

Preliminary Results

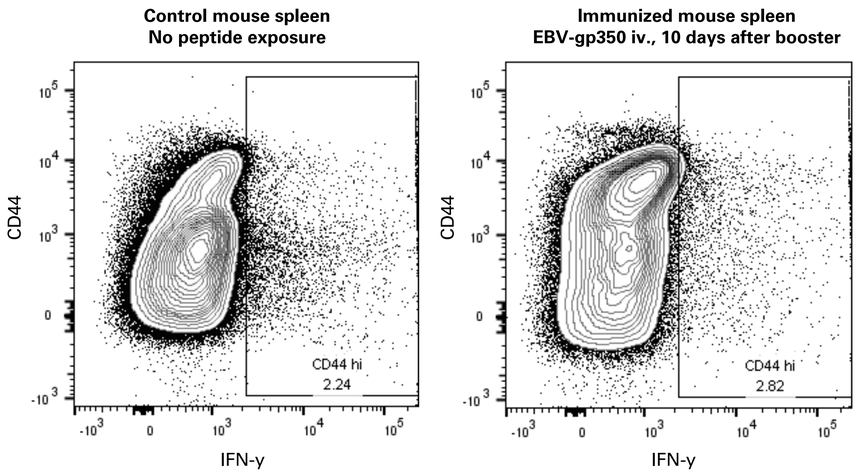

Figure 1. Flow cytometry plots of the indicated molecules for live CD4+ T cells from spleen samples from mice that were or were not injected with gp350 peptide two hours before collection and analysis.

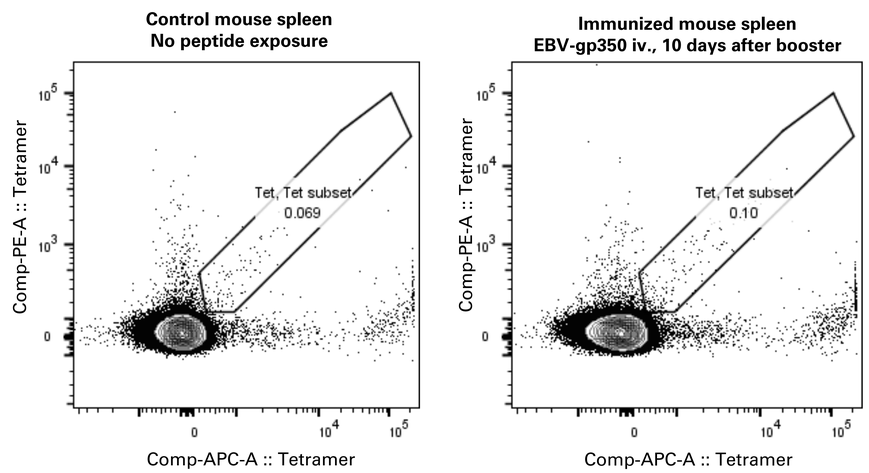

Figure 2. Flow cytometry plots live CD4+ CD44+ T cells from spleen samples from mice that were or were not injected with gp350 peptide two hours before collection and analysis.

Tetramer-Based Detection

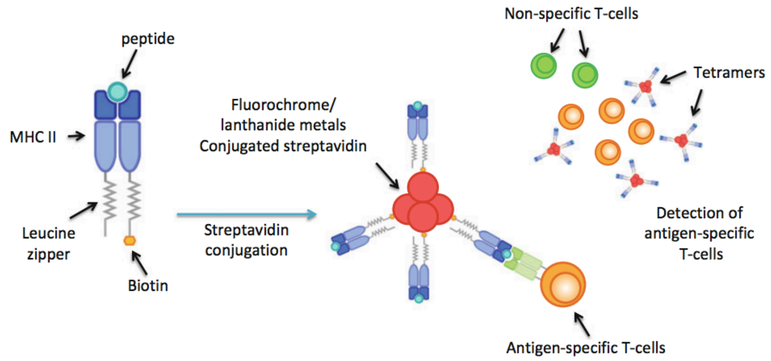

Figure 3. MHC class II tetramer technology as a method for detecting antigen-specific CD4+ T cells.

Conclusions & Future Directions

- We observed a high background signal from the IFN-y assay (Figure 1). To minimize this in future trials, we will reduce the duration of incubation with the anti-IFN-y antibody.
- Few of the CD44+ CD4+ T cells detected were bound to the PE- and APC-conjugated tetramers. This suggests that we need to optimize our tetramer staining and detection assay.
  - CD4 enrichment
  - Tetramer-based enrichment
  - Performing an experiment with a different peptide-tetramer pairing to demonstrate proof-of-concept
- We can try to maximize the memory CD4+ T cell number and activity detected by harvesting spleen and lymph node samples earlier than 10 days post-booster.
- While these challenges prevent us from making confident conclusions, the preliminary data suggest a higher specific memory CD4 T cell response in immunized mice.
- When our assay is optimized, we will further characterize phenotype and activity by looking at:
  - Surface molecules CXCR5, CXCR3, and CX3CR1
  - Intracellular molecules Bcl-6, T-bet, and RORγT

References

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3. Heeke, D.S.; Lin, R.; Rao, E.; Woo, J.C.; McCarthy, M.P.; Marshall, J.D. Identification of GLA/SE as an effective adjuvant for the induction of robust humoral and cell-mediated immune responses to EBV-gp350. *Vaccine.* 2016, 34: 2562-2569.
4. Krueger, P.D.; Goldberg, M.F.; Hong, S.; Osum, K.C.; Langlois, R.A.; Kotov, D.I.; Dileepan, T.; Jenkins, M.C. Two sequential activation modules control the differentiation of protective T helper-1 (Th1) cells. *Immunity.* 2021, 54: 687-701.

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