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Uneven distribution of MHC class II epitopes within the influenza virus

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Abstract

The identification of T cell epitopes is crucial for the understanding of the host immune response during infection. While much is known about the MHC class I-restricted response following influenza virus infection of C57BL/6 mice, with over 16 CD8 epitopes identified to date, less is known about the MHC class II-restricted response. Currently, only a few I-A^b-restricted T helper epitopes have been identified. Therefore, several important questions remain about how many class II epitopes exist in this system and whether these epitopes are evenly distributed within the most abundant viral proteins. In order to address these questions, we analyzed the repertoire of epitopes that drive the $CD4^+$ T cell response to influenza virus infection in C57BL/6 (H-2^b) mice. Using a panel of overlapping peptides from each of the viral proteins we show that approximately 20–30 epitopes drive the CD4 T cell response and that the majority of these peptides are derived from the NP and HA proteins. We were also able to demonstrate that vaccination with one of the newly identified epitopes, $HA_{211–225}/A^b$, resulted in increased epitope-specific T cell numbers and a significant reduction in viral titers following influenza virus challenge.

Keywords: T cells; MHC II; Influenza; Vaccination

1. Introduction

The T cell response to influenza virus infection is directed at processed viral peptides that are presented on the surface of APCs in the context of MHC class I and class II molecules [1,2]. While substantial progress has been made in understanding the mechanisms involved in the acquisition and processing of viral proteins into peptides, less is known about the number of epitopes involved in a T cell response. The best understood system is the class I-restricted $CD8^+$ T cell response to influenza virus infection in inbred mice. For example, 16 H-$2K^b$ and H-$2D^b$-restricted epitopes have been identified in C57BL/6 mice following influenza virus infection and these epitopes have provided insight into the breadth of the T cell response and patterns of immunodominance [3–8]. In addition, these epitopes have provided a basis for mechanistic and vaccine studies, and reagent development, including MHC-peptide tetramers. In stark contrast, much less is known about the class II-restricted $CD4^+$ T cell response in C57BL/6 mice, with only two putative I-A^b-restricted epitopes identified in the influenza x31 virus [9,10]. The comparative lack of information on the $CD4^+$ T cell response partially reflects the difficulties in identifying potential class II-restricted epitopes. In general, peptide binding to MHC class II molecules is less stringent than for peptide binding to MHC class I molecules. Thus, it has been correspondingly difficult to develop algorithms to predict potential class II-restricted epitopes [11,12]. One of the more recently developed algorithms, RANKPEP, allows for the input of protein sequences and then determines the rank and percentile optimal binding of the predicted class II epitope [11,12]. However, it is still unknown whether this algorithm can accurately predict class II epitopes in most proteins.

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Because of the lack of detailed information on class II-restricted epitopes in the murine influenza virus model, many questions remain unanswered regarding the specificity and diversity of the $CD4^+$ T cell response. For example, it is believed that the $CD4^+$ T cell response is much more diverse than the $CD8^+$ T cell response in terms of the number of epitopes recognized; however, there is no direct evidence for this supposition. In addition, it is not known whether $CD4^+$ T cell epitopes are evenly distributed within specific viral proteins, although there is some evidence that they may be enriched in regions of proteins that are recognized by antibodies [13,14]. There is also little information on the capacity of $CD4^+$ T cell epitopes to mediate effective antiviral immunity in the context of peptide-based vaccines. Clearly, there is a need to develop a better understanding of the numbers and distribution of MHC class II-restricted epitopes in the influenza virus.

Here we analyzed the breadth of the $CD4^+$ T cell response to influenza virus in C57BL/6 mice using a panel of peptides derived from all the major proteins of the x31 strain of influenza virus and compared the epitopes identified by functional studies with those predicted by the RANKPEP algorithm. We demonstrate that $CD4^+$ T cell epitopes are unevenly distributed in a limited number of proteins and estimate the total number of distinct epitopes to be in the range of 20–30, most of which were not predicted by the RANKPEP program. Two of these epitopes appear to be immunodominant inasmuch as they drive a major fraction of the $CD4^+$ T cell response to acute influenza virus infection. Additionally, we found that vaccination with one of these CD4 epitopes resulted in an enhanced $CD4^+$ T cell response and a significant decrease in viral loads following a subsequent influenza virus challenge.

2. Materials and methods

2.1. Generation of influenza peptides and epitope prediction

Amino acid sequences were obtained from PubMed for the proteins of the A/HK-x31 (x31, H3N2) and A/PR8/34 (PR8, H1N1) strains of the influenza virus. Lyophilized non-amidated peptides, 15 mers overlapping by 10, were generated and purchased from New England Peptide (Gardner, MA) and solubilized with a 50:50 acetonitrile/H_2O solution. To avoid multiple freeze/thaw cycles, the peptides were then diluted to a concentration of 0.5 μg/ml with Hank's Balance Salt Solution and aliquoted into round bottom 96 well plates. Potential T cell epitopes (MHC II—$I\text{-}A^b$) were predicted using the matrix-based algorithm RANKPEP [11,12].

2.2. Viruses, animals, and infections

The reassortant influenza virus strain A/HK-x31 (x31, H3N2 = A/Hong Kong/1/68 × A/Puerto Rico/8/34) was grown, stored and titrated as previously described [15]. Female C57BL/6 mice were purchased from Jackson Laboratories (Bar Harbor, ME). Mice (6–12 weeks) were anesthetized by i.p. injection with 2,2,2 tribromoethanol and infected intranasally with 300 or 600 50% egg infectious doses (EID_{50}) of influenza virus.

2.3. Enzyme-linked immunospot assay (ELISpot)

The numbers of IFNγ-secreting cells derived from spleens of infected mice were determined after stimulation with influenza peptides in a standard enzyme-linked immunospot (ELISpot) assay [16]. Briefly, 96 well Multiscreen mixed ester nitrocellulose plates (Millipore, Bedford, MA) were coated overnight at 4 °C with 100 μl well of rat anti-mouse IFNγ (B-D Pharmingen, San Jose, CA) at a concentration of 10 μg/ml. The plates were then washed and blocked before the addition of 10^5 responding cells, irradiated (3000 rad) syngeneic normal spleen cells, 10 μg/ml peptide, and 40 U/ml IL-2. Plates were then incubated 48 h at 37 °C and developed overnight with a biontinylated detection antibody, rat anti-mouse IFNγ (B-D Pharmingen, San Jose, CA). The plates were then incubated with streptavidin-alkaline phosphatase (DakoCytomation, Carpinteria, CA) for 1 h, washed, and incubated with BCIP/NBT alkaline phosphatase substrate (Sigma, St. Louis, MO) for 2 h at room temperature. Visible spots of IFNγ secreting cells were then enumerated using an Olympus SZH stereo zoom microscope system.

2.4. Intracellular cytokine staining

Lymphocytes were collected from the spleens or lung airways (*broncoalveloar lavage*) as previously described [17]. Following collection, the cells were washed and depleted of erythrocytes. Isolated cells (10^6cells/condition) were cultured at 37 °C for 5 h in the presence of 10 μg of the indicated peptide in 250 μl of complete tumor medium (CTM) containing 10 μg/ml Brefeldin A (BFA; Epicenter Technologies, Madison, WI) and 10 U/ml IL-2 (R&D Systems, Minneapolis, MN) [18]. After culture, the cells were blocked with monoclonal antibodies to FcRIII/II receptor (B-D Pharmingen, San Jose, CA) and stained with anti-CD4 conjugated to FITC anti-CD8 conjugated to PerCP, and anti-CD44 conjugated to allophycocyanin antibodies (B-D Pharmingen, San Jose, CA) in PBS/BFA. The cells were then fixed in 2% formaldehyde, permeabilized with buffer containing 0.5% saponin, and stained with anti-IFNγ conjugated to PE (B-D Pharmingen, San Jose, CA) monoclonal antibody. 200,000 events were collected on a Becton Dickinson FACSCalibur flow cytometer. Data was analyzed using FlowJo (TreeStar) software.

2.5. Generation of LacZ-inducible T cell hybridomas

Splenocytes were harvested from C57BL/6 mice 28 days after intranasal challenge with A/HKx31. 30×10^6 immune