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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.

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-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 splenocytes were cultured with 30×10^6 irra-

diated (3000 rad) peptide pulsed (2 µg/ml specific peptide) syngeneic splenocytes for 5 days. Blast cells were enriched by Ficoll and then fused with BWZ.36 cells [19, 20]. The resulting clones were tested for specificity using peptide-pulsed L cells transfected with the I-A^b MHC genes.

2.6. Antigen presentation assays

Antigen presentation assays were performed as previously described [20–24]. Briefly, hybridomas (1×10^5) were cultured with virus-infected or peptide-pulsed cells in flat-bottomed microtiter plates. The plates were incubated overnight, washed with PBS and fixed with β-galactosidase fixative (2% formaldehyde/0.2% glutaraldehyde). Cells were washed again with PBS followed by the addition of 50 µl of a 1 mg/ml X-gal solution (5 mM potassium ferrocyanide, 5 mM potassium ferricyanide, and 2 mM magnesium chloride). After 4 h, the hybridomas were examined under a light microscope for the presence of blue cells.

2.7. Bone marrow derived dendritic cells and vaccination

Bone marrow was flushed from the femurs of C57BL/6 mice, depleted of erythrocytes, and 2×10^6 mononuclear cells were placed into a bacteriological Petri dish with media supplemented with 20 ng/ml recombinant murine granulocyte/macrophage colony-stimulating factor (rmGM-CSF; Peprotech, Rocky Hill, NJ) and incubated at 37 °C with 10% CO_2 [25]. On day 3, an additional 10 ml of CTM containing 20 ng/ml rmGM-CSF was added. On day 6, half of the cells were removed, centrifuged, and added back to the same plate in 10 ml of fresh media containing 20 ng/ml rmGM-CSF. On day 8 the cells were removed, centrifuged, and 2×10^6 cells were placed into new bacteriological Petri dishes in 10 ml of media supplemented with 20 ng/ml rmGM-CSF. On day 10 of the culture, the cells were resuspended at 5×10^6/ml and incubated at 37 °C for 3 h with peptide at a concentration of 50 µg/ml [18]. Following peptide pulsing, the dendritic cells were washed and 100 µl of cells in PBS were injected i.v. into mice at a final concentration of 1×10^6 cells per mouse [18]. Three weeks post dendritic cell vaccination, the mice were injected subcutaneously with 100 µg of peptide emulsified in incomplete Freud's adjuvant [26].

2.8. Viral titers

Homogenized lungs were serially diluted and injected into three 10 day old embryonated hen eggs per sample. After incubation at 35 °C for 48 h, allantoic fluid from each egg was sampled and assayed for hemagglutinating activity using chicken red blood cells as previously described [27]. Samples were scored as positive when two of the three eggs contained hemagglutinating activity [27].

3. Results

3.1. Primary screen for T cell epitopes within influenza virus proteins

To identify MHC class II-restricted epitopes from influenza virus, we synthesized a panel of 15-mer peptides (overlapping by 10 amino acids) from each of the published x31 influenza virus protein sequences and from the PR8 influenza virus hemagglutinin (HA) and neuraminidase (NA) protein sequences (Table 1). Altogether, a total of 1085 peptides were synthesized and are listed in Supplemental Tables 1 and 2. These peptides were then used to screen splenocytes from mice that had recovered from a prior intranasal x31 infection (21 days post-infection) in two independent ELISpot assays. As shown in Figure 1 and Figure 2, many of the peptides elicited ELISpot responses that were above three standard deviations of the background (i.e. more than 10 spots). The majority of these positive responses were elicited by peptides derived from the HA, nucleoprotein (NP), acidic polymerase (PA) and basic polymerase (PB1 and PB2) proteins of x31 (Figure 1). Relatively few positive responses were elicited by peptides derived from the NA, nonstructural (NS1 and

Table 1. Influenza virus proteins

Protein	Accession number (PubMed)	Size[a]	Number of peptides
Hemagglutinin x31 (HA)	PO 3437	566	112
Hemagglutinin PR8 (HA)	AAM 75158	565	112
Neuramidase x31 (NA)	BAC 77663	469	93
Neuramidase PR8 (NA)	NP 775534	454	90
Nucleoprotein (NP)	BAA 99400	498	99
Acidic polymerase (PA)	BAA 99401	716	142
PB1 polymerase (PB1)	BAA 99402	759	151
PB2 polymerase (PB2)	BAA 99403	757	151
Non-structural 1 (NS1)	BAA 99396	230	45
Non-structural 2 (NS2)	BAA 99396	121	23
Matrix protein 1 (M1)	BAA 99399	252	49
Matrix protein 2 (M2)	BAA 99398	97	18

[a] Amino acids.

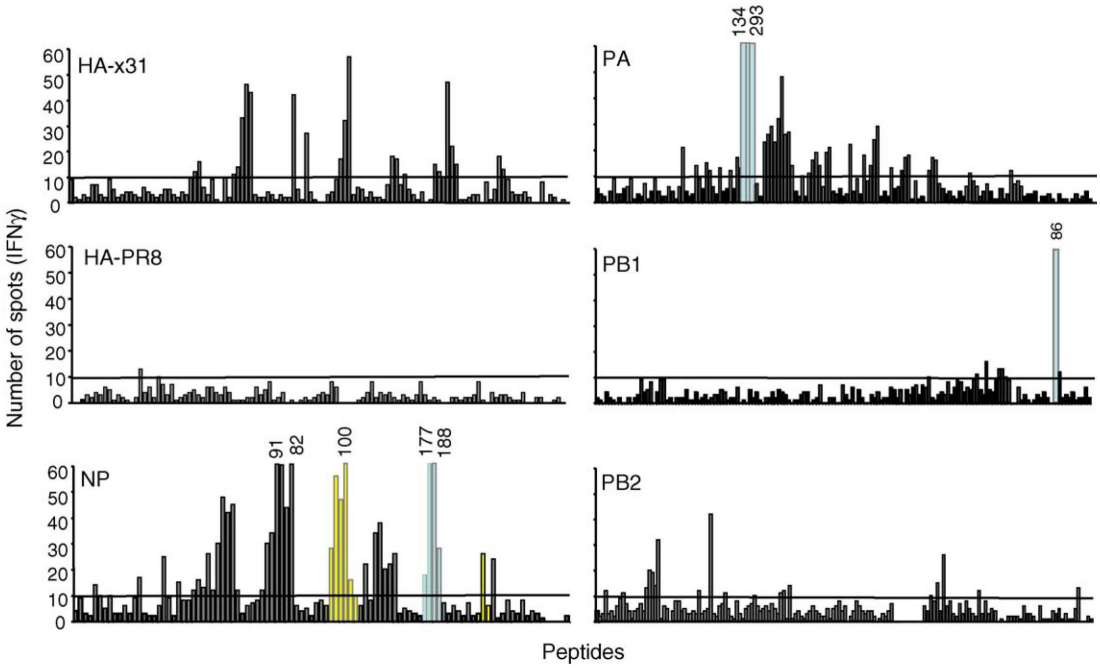

Figure 1. Infection induces T cells specific for peptides within the hemagglutinin, nucleoprotein protein, acidic polymerase, basic polymerase 1, and basic polymerase 2 of the x31 influenza virus. C57BL/6 mice were intranasally infected with 300 EID_{50} x31 influenza virus. On day 21 post infection the spleens were removed, enriched for lymphocytes, and incubated for 48 h with irradiated (3000 rad) syngeneic normal spleen cells, peptide, and IL-2 in a standard IFNγ specific ELISpot assay. Shown are the number of IFNγ positive cells from one of two representative experiments following incubation with peptides from the following proteins: HA derived from x31, HA derived from PR8, NP, PA, PB1, and PB2 (all derived from x31). Blue bars indicate the regions containing the known CD8 epitopes and yellow bars indicate the region containing the known CD4 epitope.

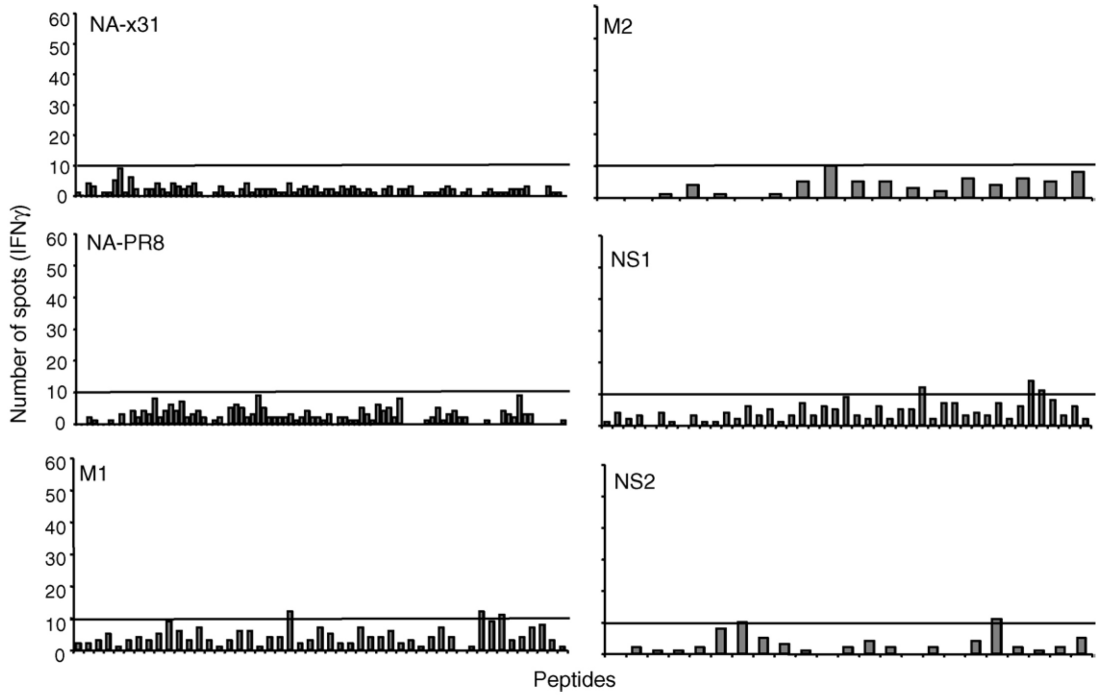

Figure 2. Infection does not induce T cells specific for peptides within the neuramidase, maxtrix 1, matrix 2, nonstructural 1, and nonstructural 2 proteins of the x31 influenza virus. C57BL/6 mice were intranasally infected with 300 EID_{50} x31 influenza virus. On day 21 post infection the spleens were removed, enriched for lymphocytes, incubated for 48 h with irradiated (3000 rad) syngeneic normal spleen cells, peptide, and IL-2 in a standard ELISpot assay. Shown are the number of IFNγ positive cells from one of two representative experiments following incubation with peptides from the following proteins: NA derived from x31, NA derived from PR8, M1, M2, NS1, and NS2 (all derived from x31).

NS2) and matrix (M1 and M2) proteins of x31 (Figure 2). In addition, peptides from the HA and NA proteins of PR8 virus did not elicit positive responses from the x31-primed spleen cells (Figure 1 and Figure 2), consistent with the relatively limited sequence homology between these proteins in the x31 and PR8 viruses. As expected, the screen clearly identified the regions of NP that had previously been reported by Gao et al. to contain an I-A^b epitope ($NP_{261–290}$ and $NP_{411–435}$) [9, 10]. These regions are indicated by the yellow bars in Figure 1.

Since the splenocytes used for the ELISpot assay included both CD4 and CD8 cells, the data did not discriminate between MHC class II- and class I-restricted responses. Although class I peptides are typically shorter than the 15-mer peptides synthesized for this study, it is known that 15 mer peptides can be presented to $CD8^+$ T cells under these conditions, albeit with reduced efficiency [28, 29]. Therefore, we scanned the ELISpot data for known immunodominant class I epitopes, $NP_{366–374}$, $PA_{224–233}$, and $PB1_{703–711}$. Responses to all three of these epitopes were detected in the ELISpot screen as indicated by the blue bars in Figure 1. Taken together, these results suggested that our screen likely identified a combination of class I and class II-restricted epitopes.

3.2. Contributions of selected epitopes to T cell responses to primary x31 infection

Since we had identified a reasonable number of epitopes that were not known class I-restricted epitopes, we assumed that most of these epitopes were likely to be class II-restricted CD4 T cell epitopes. To determine whether the RANKPEP algorithm could be used to accurately predict the CD4 epitopes identified in our screen, we screened the x31 viral proteins using the RANKPEP algorithm and, using this program, identified a large number of peptides that were predicted to bind I-A^b with a range of efficiencies (Table 2 and data not shown). However, there was only a partial correlation with the ELISpot data (Table 2 and data not shown), and many epitopes identified in our functional screen were not identified by the RANKPEP program. Interestingly, the algorithm did predict two potentially strong I-A^b binding peptides in the $NP_{261–290}$ region defined by Gao et al.; $_{266}$LRGSVAHKS$_{274}$ and $_{277}$PACVYGPAVA$_{286}$ (Table 2), suggesting that a subset of CD4 epitopes can be identified by this method.

Next, to further investigate the specificity of the T cell response to influenza virus infection, we selected 36 peptides for additional characterization (Table 2). These peptides were selected on the basis that (i) they gave greater than 20 ELISpots in two independent screening assays, and/or (ii) they had a RANKPEP score of greater than 32% optimal (this score was selected as it typically identified the top 10% of predicted I-A^b binders in any given protein). We also excluded all peptides that contained known class I epitopes and peptides from the regions previously described by Gao et al. [9, 10]. Table 2 shows the list of 36 peptides (plus the Gao et al. peptides) with the corresponding ELISpot results and RANKPEP scores. Even though these peptides were the top candidates for I-A^b-restricted epitopes, there was not a direct correlation between the number of ELISpots elicited by these peptides and their respective RANKPEP scores. For example, $NP_{311–325}$ elicited strong ELISpot responses but was only 20% optimal by RANKPEP whereas $PA_{456–470}$ elicited weak ELISpot responses but was 38% optimal by RANKPEP (Table 2). Of 36 peptides in Table 2 that were positive by ELISpot, only six received a RANKPEP score of greater than 32%.

We next analyzed the capacity of the 36 selected peptides to stimulate IFNγ production during an acute response to influenza virus infection using an intracellular cytokine assay. This approach allowed us to: (i) confirm specific epitopes in an independent assay; (ii) identify those epitopes that made significant contributions to the acute response to infection and (iii) specifically distinguish class I and class II responses. C57BL/6 mice were intranasally infected with influenza virus and 10 days later cells were recovered from the lung airways. These cells were then assessed for their ability to produce IFNγ in an intracellular cytokine assay following stimulation with each of the 36 peptides (the known class I epitopes, $NP_{366–374}$ and $PA_{224–233}$, and the Gao peptide, $NP_{276–290}$, were included as controls). As shown in Figure 3 almost all of the peptides tested induced IFNγ in these effector T cells and over half of the peptides induced IFNγ production in greater than 1% of either the CD4 or CD8 T cell populations. Ten of these peptides stimulated CD4 T cells, eight stimulated CD8 T cells, while two peptides stimulated both CD4 and CD8 T cells to produce IFNγ. Representative data from six of these peptides are shown on the right hand panel of Figure 3.

3.3. T cells specific for the $HA_{211–225}$ and $NP_{311–325}$ epitopes make substantial contributions to the primary CD4 T cell response to influenza

We next took the two peptides that elicited the strongest IFNγ production in the intracellular cytokine staining assay ($HA_{211–225}$ and $NP_{311–325}$) and re-synthesized them as purified peptides to confirm that they corresponded to *bona fide* epitopes. Intracellular cytokine staining analysis of these re-synthesized peptides confirmed that they were both class II-restricted epitopes and also demonstrated that the $NP_{311–325}$ peptide did not stimulate CD8 T cells (data not shown). It is unclear why the crude $NP_{311–325}$ peptide gave an initial response as a mixed CD4 and CD8 epitope. However, we noted that the peptide was originally synthesized in a well adjacent to the known $NP_{366–374}/D^b$ epitope and we now speculate that there may have been some cross contamination either during the production or subsequent handling of the peptides. We next analyzed the kinetics of the primary T cell response to the purified $HA_{211–225}$ and $NP_{311–325}$ epitopes. As shown in Figure 4A, both epitopes stimulated strong $CD4^+$ T cell

Table 2. Amino acid sequence, ELISpot results, and RANKPEP results from influenza virus peptides selected for further investigation

Peptide name	Sequence	ELISpot results[a]	RANKPEP (Rank)[b]	RANKPEP (% Optimal)[c]
HA 201–215	PSTNQEQTSLYVQAS	33/14		
HA 206–220	EQTSLYVQASGRVTV	46/28	3	33.61
HA 211–225	YVQASGRVTVSTRRS	43/35	3	33.61
HA 261–275	INSNGNLIAPRGYFK	42/20		
HA 276–290	MRTGKSSIMRSDAPI	27/19	23	17.53
HA 321–335	CPKYVKQNTLKLATG	32/37	34	14.04
HA 326–340	KQNTLKLATGMRNVP	57/30	30	15.75
HA 441–455	AELLVALENQHTIDL	47/70		
HA 446–460	ALENQHTIDLTDSEM	22/42		
NP 91–105	KTGGPIYRRVNGKWM	25/17	2	38.05
NP 106–120	RELILYDKEEIRRIW	15/26		
NP 136–150	MMIWHSNLNDATYQR	26/14	22	17.72
NP 146–160	ATYQRTRALVRTGMD	30/17	5	27.89
NP 151–165	TRALVRTGMDPRMCS	48/22		
NP 161–175	PRMCSLMQGSTLPRR	45/22	47	8.96
NP 196–210	MIKRGINDRNFWRGE	30/21		
NP 201–215	INDRNFWRGENGRKT	34/39		
NP 206–220	FWRGENGRKTRIAYE	91/82		
NP 211–225	NGRKTRIAYERMCNI	82/61		
NP 216–230	RIAYERMCNILKGKF	44/43	49	8.53
NP 311–325	QVYSLIRPNENPAHK	38/41	14	19.98
NP 316–330	IRPNENPAHKSQLVW	20/23		
NP 326–340	SQLVWMACHSAAFED	26/17	41	10.71
PA 251–265	KEVNARIEPFLKTTP	26/14		
PA 256–270	RIEPKLKTTPRPLRL	29/17	22	18.00
PA 276–290	CSQRSKFLLMDALKL	26/27	30	15.83
PA 311–325	MRTFFGWKEPNVVKP	11/14	2	37.96
PA 316–330	GWKEPNVVKPHEKGI	16/16	2	37.96
PA 411–425	FNKACELTDSSWIEL	29/12	24	17.21
PA 456–470	RATEYIMKGVYINTA	18/13	1	38.11
NS2 91–105	ENSFEQITFMQALHL	11/61	1	21.32
PB2 91–105	VSPLAVTWWNRNGPM	20/20	33	14.86
PB2 106–120	TNTVHYPKIYKTYFE	32/17	21	19.35
PB2 196–210	CKISPLMVAYMLERE	42/30		
M1 201–215	EAMEVASQARQMVQA	12/32		
M1 211–225	QMVQAMITIGTHPSS	11/29	5	23.73
Region previously identified by Gao *et al.* [9, 10]				
NP 261–275	RSALILRGSVAHKSC	28/13	12	22.37
NP 266–280	LRGSVAHKSCLPACV	56/30	12	22.37
NP 271–285	AHKSCLPACVYGPAV	47/23	6	27.67
NP 276–290	LPACVYGPAVASGYD	100/69	3	32.96

Brackets indicate overlapping peptides.

[a] Number of ELISpots from 10^5 spleen cells, determined in two independent experiments (experiment 1/experiment 2).

[b] Relative rank of the predicted peptide within the input protein.

[c] Percentile score of the predicted peptide relative to that of the sequence that yields the maximum score for the selected profile ($I\text{-}A^b$).

responses that can be first detected on day 8 post infection in the lung airways. The frequency of $HA_{211–225}$ and $NP_{311–325}$ specific cells peaked at 10% and 11%, respectively, of the total CD4 cells. Since 67% of the total CD4 T cells in the lung airways rapidly respond to activation with PMA and ionomycin treatment, we conclude that approximately 31% of the total influenza-specific CD4 T cells in the lung airways are specific for the $HA_{211–225}$ and $NP_{311–325}$ epitopes (Figure 4B). Together, these data confirm that $HA_{211–225}$ and $NP_{311–325}$ are *bona fide* class II-restricted epitopes and further demonstrate that they are relatively dominant epitopes in the class II-restricted $CD4^+$ T cell response following influenza virus infection of C57BL/6 mice.

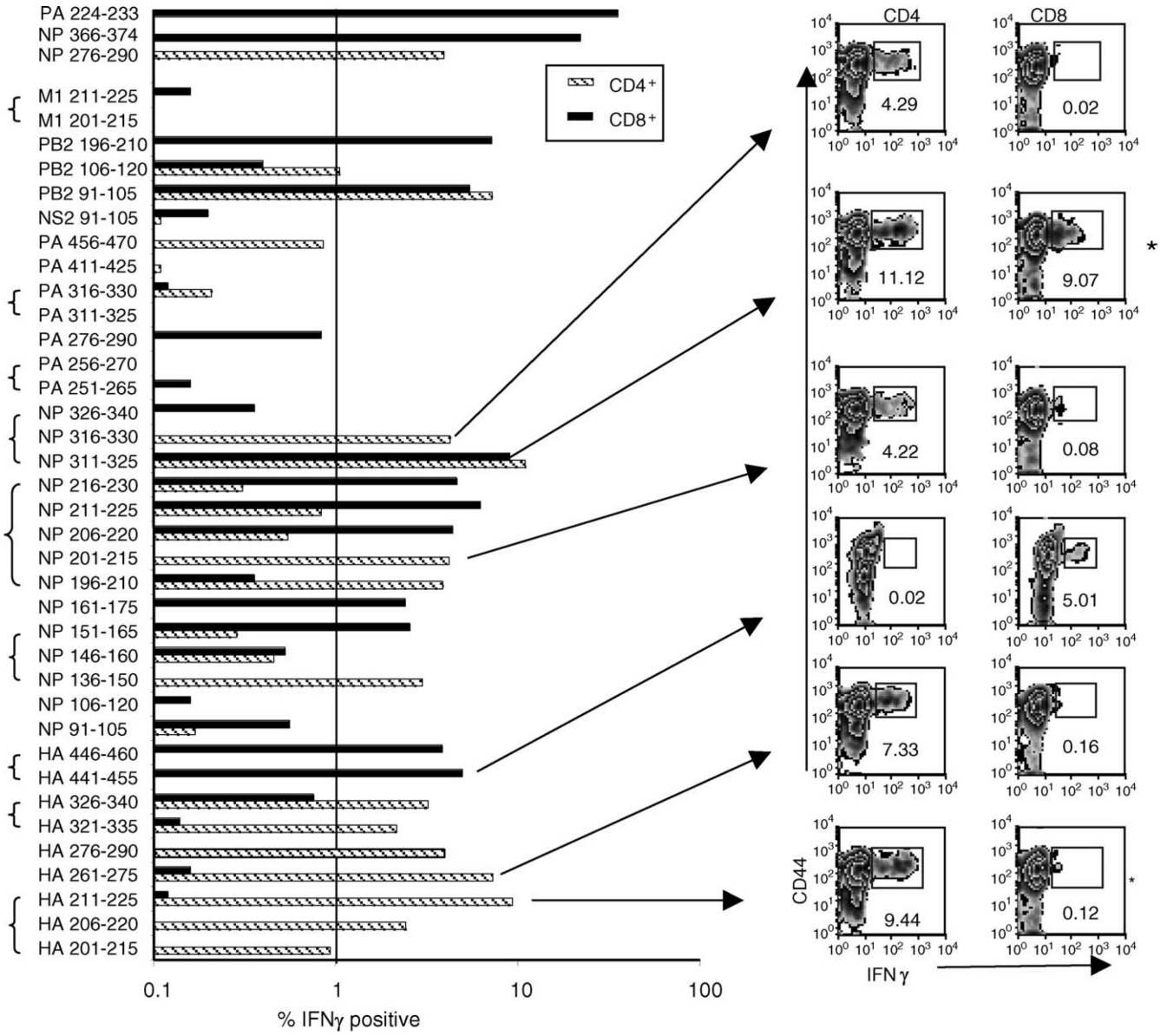

Figure 3. IFNγ production by antigen-specific CD4$^+$ and CD8$^+$ T cells on day 10 post infection. C57BL/6 mice were intranasally infected with 300 EID_{50} x31 influenza virus. On day 10 post infection, lung cells were collected and incubated for 5 h in the presence of the indicated peptide and Brefeldin A. Following stimulation, the cells were stained with anti-CD4 FITC, anti-CD8 PerCP, anti-IFNγ PE, and anti-CD44 allophycocyanin. Shown are the percentages of CD4 or CD8 cells producing IFNγ following stimulation with the indicated peptide from one of two representative experiments. Also shown are representative FACs plots. Brackets indicate overlapping peptides.

3.4. The $HA_{211-225}$ epitope is presented in the lungs and local draining lymph nodes during influenza virus infection

To further investigate the characteristics of the $HA_{211-225}/A^b$ epitope, we generated a panel of T cell hybridomas. Spleen cells from mice that had recovered from a prior x31 infection were stimulated in vitro with the $HA_{211-225}$ peptide and subsequently fused to a lacZ expressing variant of BW5147 [19]. The resulting hybridomas were screened for reactivity to the $HA_{211-225}$ peptide and one hybridoma, H-#-SM101.3, was selected for further study. This hybridoma was re-cloned and was shown to specifically respond to the $HA_{211-225}$ peptide pulsed L cells transfected with the I-A^b molecule (Figure 5A). The hybridoma also responded to syngeneic spleen cells infected with various doses of x31 influenza virus (Figure 5B). We next used the H-#-SM101.3 hybridoma to investigate the presentation of the $HA_{211-225}/A^b$ epitope ex vivo by antigen presenting cells isolated from infected mice. Cells were isolated from the lung airways, lung parenchyma, MLN, and spleen on days 4 (Figure 5C) and 6 (Figure 5D) post influenza virus infection. Increasing numbers of cells from infected mice were then cultured with the H-#-SM101.3 hybrid and the number of hybridoma cells responding to

the $HA_{211-225}$ peptide was determined one day later. As shown in Figure 5 panels C and D, cells presenting the $HA_{211-225}/A^b$ epitope were clearly detected at days 4 and 6 in the lung airways, lung parenchyma, and MLN, but not in the spleen. Extrapolation of the titration curves in Figure 4 indicates that at least 1 in 1000 cells in the lungs and MLN are presenting the $HA_{211-225}/A^b$ epitope at both time points. Together, these data indicate that the $HA_{211-225}/A^b$ epitope is widely expressed during the course of an influenza virus infection.

3.5. Partial control of viral infection in $HA_{211-225}$ vaccinated mice

To investigate the capacity of T cells specific for the $HA_{211-225}/A^b$ epitope to mediate antiviral control, we vaccinated mice with dendritic cells that had been pulsed with the $HA_{211-225}$ peptide (control mice were left unvaccinated). Three weeks post vaccination, the mice were boosted with peptide emulsified in incomplete Freud's adjuvant and then infected with x31 influenza virus 2 weeks later. The absolute numbers of antigen-specific T cells in the secondary lymphoid organs and the viral loads in the lungs were determined on various days post-infection. Vaccination with dendritic cells pulsed with the $HA_{211-225}$ peptide did not significantly alter the number of $CD8^+$ T cells migrating to the lungs following infection (data not shown). However, $HA_{211-225}/A^b$-specific cells were detected earlier in the lymphoid organs of vaccinated mice as compared to unvaccinated mice (day 6 versus day 8) (Figure 6A). In addition, substantially greater numbers of antigen-specific T cells were detected in vaccinated mice on day 8 post-infection (Figure 6A). Analysis of viral loads indicated that there was a significant reduction in viral titers on days 4 and 6 in the vaccinated mice (Figure 6B). However, viral clearance was not accelerated, with both vaccinated and unvaccinated mice clearing virus on day 12 (Figure 6B). Together these data indicate that vaccination with the $HA_{211-225}$ peptide resulted in a significantly enhanced T cell response to viral challenge and a corresponding decrease viral titers in the lungs. However, it did not result in the accelerated clearance of virus from the lungs.

4. Discussion

A major limitation in our understanding of the $CD4^+$ T cell response to influenza virus infection has been the lack of information on the numbers, source proteins, and relative contributions of class II epitopes that drive the response. To begin to address this problem, we undertook a peptide screen of all the major influenza virus proteins to identify specific epitopes in C57BL/6 mice. This approach identified a large number of T cell epitopes that were derived predominantly from a relatively limited number of proteins. We had initially speculated that the number of epitopes would reflect the length of the protein and its relative abundance in infected cells. Consistent with this, the nucleoprotein and hemagglutinin proteins contained large numbers of epitopes. However, the neuraminidase protein, which is also relatively abundant, did not contain any I-A^b-restricted epitopes at all and the acidic polymerase protein, which has a relatively low abundance, appeared to contain multiple epitopes. It should be noted that although no epitopes were detected in the NA protein, this did not appear to reflect a general feature of this protein as multiple epitopes were identified in x31 NA protein in a parallel screen with BALB/c (H-2^d) mice (data not shown). As expected, there was no cross-reactivity between the HA and NA proteins derived from x31 and PR8 viruses in C57BL/6 mice. This is consistent with reports that these proteins do not elicit cross-reactive antibodies or T cell responses [30, 31].

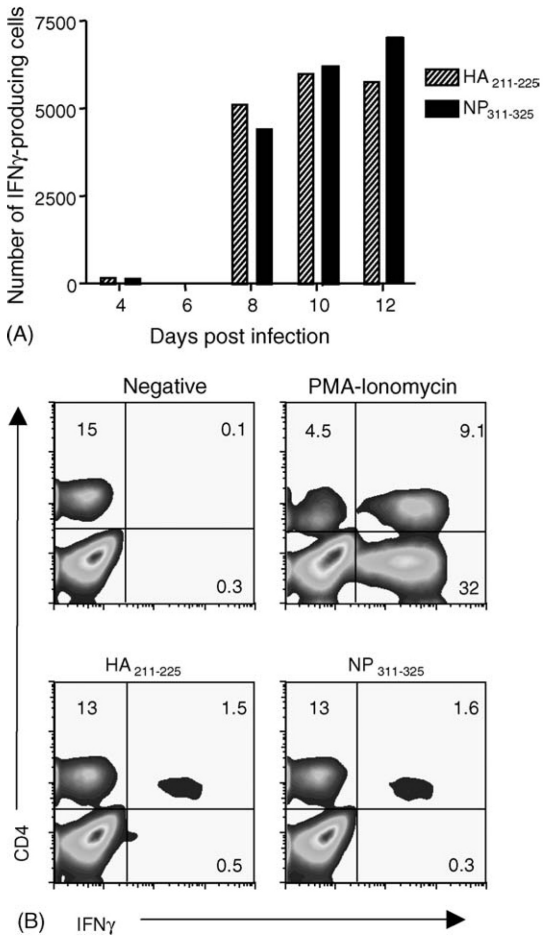

Figure 4. Kinetics of the antigen-specific $CD4^+$ T cell response following influenza virus infection. C57BL/6 mice were intranasally infected with 300 EID_{50} x31 influenza virus. Panel A: On days 4, 6, 8, 10, and 12 post infection, cells from the lung airways were collected and incubated for 5 h in the presence of the $HA_{211-225}$ or $NP_{311-325}$ peptide and Brefeldin A. Following stimulation, the cells were stained with anti-CD4 FITC, anti-CD8 PerCP, anti-IFNγ PE, and anti-CD44 allophycocyanin. Shown is the number of epitope-specific $CD4^+$ T cells on the different days post infection. Panel B shows the percentage of lung airway cells producing IFNγ following stimulation on day 10 post infection.

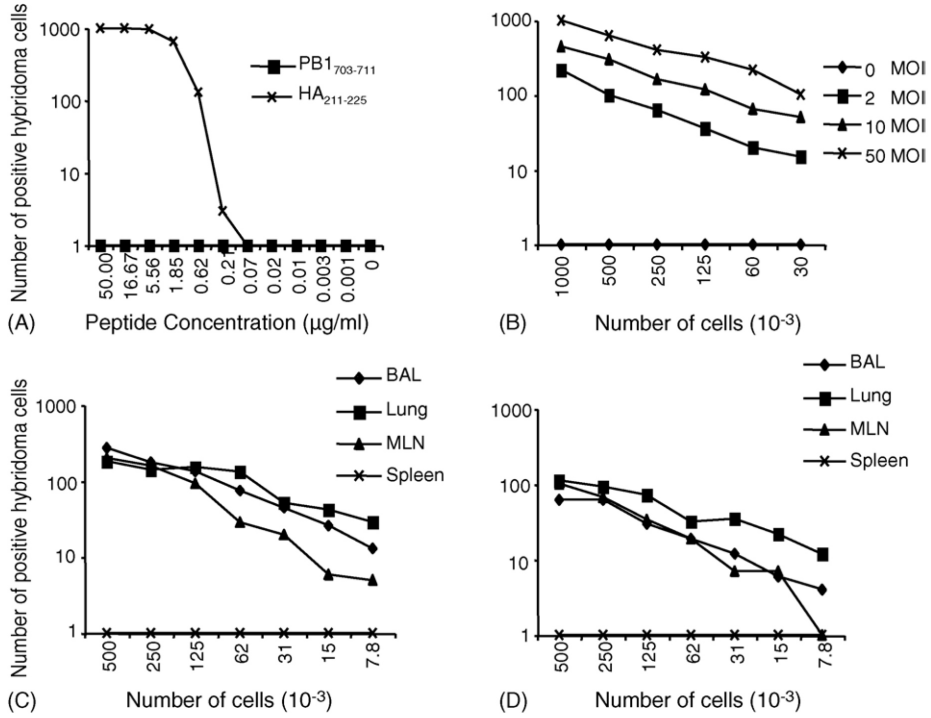

Figure 5. Antigen presentation of the $HA_{211-225}/A^b$ epitope following infection with influenza virus. Panel A: clone H-#-SM101.3 was screened using I-A^b transfected L cells incubated overnight with either the $PB1_{703-711}$ or $HA_{211-225}$ peptides at the indicated concentrations. The graphs show the number of positive Lac Z hybridomas per well (no more than 2000 spots were counted per well). Panel B: splenocytes were collected from uninfected mice and then infected in vitro with influenza virus at a multiplicity of infection of 0, 2, 10, or 50 for 4 h. The infected cells were irradiated and then plated in increasing numbers in a standard antigen presentation assay with the H-#-SM101.3 hybridoma. Panels C and D: mice were infected intranasally with x31 and cells from the lung airways, lung parenchyma, MLN, and spleens were collected on day 4 (panel C) or day 6 (panel D) post infection. Two-fold serial dilutions of cells were made in flat-bottom, 96-well plates starting at 10^5 cells/well and a standard antigen presentation assay was performed using the I-A^b $HA_{211-225}$ restricted H-#-SM101.3 Lac Z-inducible hybridoma.

An important question raised by these studies is what is the absolute number of class II-restricted epitopes involved in the response to infection? Any analysis of the ELISpot data must take into account that: (i) a single epitope can reside on up to three neighboring peptides; (ii) there is potential inclusion of class I-restricted epitopes and (iii) the data include some false positives. The issue of adjacent peptides and known class I epitopes is easily addressed, and taking these issues into account, we estimate that there are approximately 60 putative epitopes in H-2^b mice (approximately 10 each in HA and PB2 and 20 each in NP and PA). The issue of false positives is more difficult to address. However, we can estimate the frequency of real class II epitopes among positive responses based on the analysis of a selected set of peptides in Figure 3. In this case, we analyzed 36 peptides that corresponded to 21 putative epitopes (taking into account overlapping peptides and excluding peptides that were only positive in the RANKPEP analysis). Ten of them (48%) appeared to be real class II-restricted epitopes that elicited a significant T cell response in an independent intracellular cytokine assay (greater than 1% of $CD4^+$ T cells in the lung airways during an acute response to infection). Thus, we speculate that about 48% of the 60 putative epitopes (about 29 epitopes) identified in the entire screen are likely to be real epitopes. This is in the same range as the number of class I-restricted epitopes that have been identified in this MHC haplotype (16 K^b and D^b-restricted epitopes) [8, 32]. Moreover, two of the epitopes, $HA_{211-225}/A^b$ and $NP_{311-325}/A^b$, were shown dominate the $CD4^+$ T cell response to infection, accounting for 31% of the $CD4^+$ T cells present in the lung airways at the peak of the response. This is very similar to the pattern of immunodominance in the $CD8^+$ T cell response where a limited number of epitopes dominate the entire response [8, 32]. However, it should be noted that C57BL/6 mice only express a single MHC class II molecule (I-A^b) due to the deletion of the I-Eα gene. Thus, our estimate of the breadth of the $CD4^+$ T cell repertoire in C57BL/6 mice may underestimate the breadth of the repertoire in mice that express multiple MHC class II molecules. Further estimation of the repertoire will come from more detailed follow up analysis of the many putative epitopes identified in this study and a study of responses in mice expressing different MHC haplotypes.

In addition to analyzing T cell responses using an ELISpot assay, we also analyzed the proteins using the RANKPEP algorithm. There was generally only a modest correlation between the two approaches in terms of their ability to identify epitopes (Table 2). For example,

the $HA_{211-225}/A^b$ epitope, which was found to contribute substantially to the $CD4^+$ T cell response, was predicted by RANKPEP to be an immunodominant epitope with a 33.6% optimal binding score and a rank of 3. However, another epitope, which also contributed to the $CD4^+$ T cell response ($NP_{311-325}/A^b$), was not predicted as an immunodominant epitope by RANKPEP with only a 20% optimal binding score and a rank of 14. In contrast, the $PA_{456-470}$ peptide elicited weak ELISpot responses but was predicted by RANKPEP to be an optimal binder (38% optimal binding and number 1 rank). Therefore, while the RANKPEP algorithms can be useful in predicting immunodominant epitopes, this methodology can result in both "false positives" (such as $PA_{456-470}$) and "false negatives" (such as $NP_{311-325}$).

A critical question regarding the inflammatory $CD4^+$ T cell response has been its capacity to mediate protective immunity. Several studies have indicated that $CD4^+$ T cells are generally not required for protection [33, 34]. However, evidence from a parainfluenza virus system suggests that $CD4^+$ T cells can mediate substantial viral control against secondary challenge [16, 26] and studies using B cell deficient mice have suggested that CD4 T cells play an important role in enhancing protective antibody responses [35]. To address these issues in the influenza virus system, we vaccinated mice with the $HA_{211-225}$ peptide and demonstrated that this resulted in significant reductions in viral titers following a subsequent viral challenge. Interestingly, although the numbers of antigen-specific $CD4^+$ T cells was significantly enhanced in the vaccinated mice and persisted through day 12, this did not result in accelerated viral clearance. It is possible that this may reflect a general feature of inflammatory $CD4^+$ T cell responses to influenza virus or the need for antibodies to mediate late viral clearance. However, we have found that transgenic $CD4^+$ effector T cells are able to mediate viral clearance and protection in an adoptive transfer model, suggesting that the effect may be epitope-dependent and reflect differences in effector functions, such as levels of TNFα secretion [36]. In this regard, we have shown that different MHC class I-restricted influenza virus epitopes differ in their capacity to elicit protective immune responses. We are currently addressing these differences experimentally.

In summary, the data presented here indicate that the numbers of influenza virus MHC class II and class I-restricted epitopes in C57BL/6 mice are not significantly different and the T cell response to these epitopes tend to be dominated by only a limited number of epitopes. The data presented here will also provide a basis for further detailed studies of $CD4^+$ T cell responses to influenza virus.

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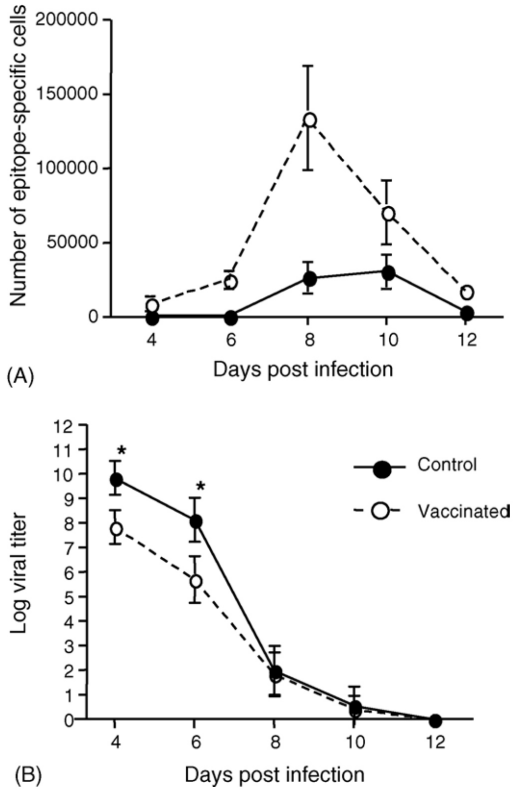

Figure 6. Vaccination with the $HA_{211-225}$ peptide results in increased epitope-specific T cells and decreased viral load on days 4 and 6. Mice were injected i.v. with 0.5×10^6 dendritic cells pulsed with the $HA_{211-225}$ peptide of influenza. Three weeks post vaccination, the mice were boosted by a subcutaneous injection of the $HA_{211-225}$ peptide in incomplete Feud's adjuvant. Two weeks following boosting, the mice were infected with 600 EID_{50} x31. Panel A: on days 4, 6, 8, 10, and 12 post infection, cells from the spleen were collected, restimulated with the $HA_{211-225}$ peptide, and stained anti-CD4 FITC, anti-CD8 PerCP, anti-IFNγ PE, and anti-CD44 allophycocyanin. Shown is the number (average ± S.D.) of epitope-specific $CD4^+$ T cells on the different days post infection. Panel B shows the viral titer (average ± S.D.) in the lungs on the different days post infection. The data shown are the average and standard deviation from three independent experiments with each experiment containing six mice per time point (three control and three vaccinated).

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Appendix B. Supplementary data

Supplemental Table 1. *x31 Influenza virus peptides*

	Peptide Sequence	Elispot[a]	
HA 1-15	MKTIIALSYIFCLAL	9	14
HA 6-20	ALSYIFCLALGQDLP	2	6
HA 11-25	FCLALGQDLPGNDNS	1	5
HA 16-30	GQDLPGNDNSTATLC	3	4
HA 21-35	GNDNSTATLCLGHHA	2	3
HA 26-40	TATLCLGHHAVPNGT	7	2
HA 31-45	LGHHAVPNGTLVKTI	7	2
HA 36-50	VPNGTLVKTITDDQI	3	6
HA 41-55	LVKTITDDQIEVTNA	2	4
HA 46-60	TDDQIEVTNATELVQ	9	7
HA 51-65	EVTNATELVQSSSTG	5	5
HA 56-70	TELVQSSSTGKICNN	2	3
HA 61-75	SSSTGKICNNPHRIL	3	5
HA 66-80	KICNNPHRILDGIDC	4	4
HA 71-85	PHRILDGIDCTLIDA	4	7
HA 76-90	DGIDCTLIDALLGDP	3	5
HA 81-95	TLIDALLGDPHCDVF	2	5
HA 86-100	LLGDPHCDVFQNETW	6	8
HA 91-105	HCDVFQNETWDLFVE	4	8
HA 96-110	QNETWDLFVERSKAF	3	10
HA 101-115	DLFVERSKAFSNCYP	2	7
HA 106-120	RSKAFSNCYPYDVPD	3	6
HA 111-125	SNCYPYDVPDYASLR	5	5
HA 116-130	YDVPDYASLRSLVAS	5	4
HA 121-135	YASLRSLVASSGTLE	3	6
HA 126-140	SLVASSGTLEFITEG	2	4
HA 131-145	SGTLEFITEGFTWTG	4	4
HA 136-150	FITEGFTWTGVTQNG	3	2
HA 141-155	FTWTGVTQNGGSNAC	10	10
HA 146-160	VTQNGGSNACKRGPG	12	10
HA 151-165	GSNACKRGPGSGFFS	16	14
HA 156-170	KRGPGSGFFSRLNWL	6	5
HA 161-175	SGFFSRLNWLTKSGS	3	11
HA 166-180	RLNWLTKSGSTYPVL	9	4
HA 171-185	TKSGSTYPVLNVTMP	1	7
HA 176-190	TYPVLNVTMPNNDNF	0	6
HA 181-195	NVTMPNNDNFDKLYI	10	10
HA 186-200	NNDNFDKLYIWGIHH	2	4
HA 191-205	DKLYIWGIHHPSTNQ	11	4
HA 196-210	WGIHHPSTNQEQTSL	14	16
HA 201-215	**PSTNQEQTSLYVQAS**	**33**	**14**
HA 206-220	**EQTSLYVQASGRVTV**	**46**	**28**
HA 211-225	**YVQASGRVTVSTRRS**	**43**	**35**
HA 216-230	GRVTVSTRRSQQTII	3	5
HA 221-235	STRRSQQTIIPNIGS	2	8
HA 226-240	QQTIIPNIGSRPWVR	2	4
HA 231-245	PNIGSRPWVRGLSSR	4	8
HA 236-250	RPWVRGLSSRISIYW	3	6
HA 241-255	GLSSRISIYWTIVKP	1	4
HA 246-260	ISIYWTIVKPGDVLV	3	5
HA 251-265	TIVKPGDVLVINSNG	2	5
HA 256-270	GDVLVINSNGNLIAP	2	9
HA 261-275	**INSNGNLIAPRGYFK**	**42**	**20**
HA 266-280	NLIAPRGYFKMRTGK	5	13
HA 271-285	RGYFKMRTGKSSIMR	1	10
HA 276-290	**MRTGKSSIMRSDAPI**	**27**	**19**
HA 281-295	SSIMRSDAPIDTCIS	4	5
HA 286-300	SDAPIDTCISECITP	1	2
HA 291-305	DTCISECITPNGSIP	0	4
HA 296-310	ECITPNGSIPNDKPF	0	6
HA 301-315	NGSIPNDKPFQNVNK	3	2
HA 306-320	NDKPFQNVNKITYGA	4	4
HA 311-325	QNVNKITYGACPKYV	9	12
HA 316-330	ITYGACPKYVKQNTL	17	14
HA 321-335	**CPKYVKQNTLKLATG**	**32**	**37**
HA 326-340	**KQNTLKLATGMRNVP**	**57**	**30**
HA 331-345	KLATGMRNVPEKQTR	3	3
HA 336-350	MRNVPEKQTRGLFGA	6	6
HA 341-355	EKQTRGLFGAIAGFI	5	8
HA 346-360	GLFGAIAGFIENGWE	2	4
HA 351-365	IAGFIENGWEGMIDG	4	2
HA 356-370	ENGWEGMIDGWYGFR	2	4
HA 361-375	GMIDGWYGFRHQNSE	2	4
HA 366-380	WYGFRHQNSEGTGQA	1	2
HA 371-385	HQNSEGTGQAADLKS	7	10
HA 376-390	GTGQAADLKSTQAAI	18	20
HA 381-395	ADLKSTQAAIDQING	17	14
HA 386-400	TQAAIDQINGKLNRV	7	16
HA 391-405	DQINGKLNRVIEKTN	11	12
HA 396-410	KLNRVIEKTNEKFHQ	5	2
HA 401-415	IEKTNEKFHQIEKEF	3	2
HA 406-420	EKFHQIEKEFSEVEG	1	3
HA 411-425	IEKEFSEVEGRIQDL	4	1
HA 416-430	SEVEGRIQDLEKYVE	0	3
HA 421-435	RIQDLEKYVEDTKID	1	4
HA 426-440	EKYVEDTKIDLWSYN	15	10
HA 431-445	DTKIDLWSYNAELLV	12	6
HA 436-450	LWSYNAELLVALENQ	10	16
HA 441-455	**AELLVALENQHTIDL**	**47**	**70**
HA 446-460	**ALENQHTIDLTDSEM**	**22**	**42**
HA 451-465	HTIDLTDSEMNKLFE	15	14
HA 456-470	TDSEMNKLFEKTRRQ	1	3
HA 461-475	NKLFEKTRRQLRENA	1	2
HA 466-480	KTRRQLRENAEEMGN	2	3
HA 471-485	LRENAEEMGNGCFKI	3	3
HA 476-490	EEMGNGCFKIYHKCD	8	4
HA 481-495	GCFKIYHKCDNACIE	1	6
HA 486-500	YHKCDNACIESIRNG	5	5

	Peptide Sequence	Elispot[a]	
HA 491-505	NACIESIRNGTYDHD	18	14
HA 496-510	SIRNGTYDHDVYRDE	13	11
HA 501-515	TYDHDVYRDEALNNR	9	3
HA 506-520	VYRDEALNNRFQIKG	4	13
HA 511-525	ALNNRFQIKGVELKS	3	8
HA 516-530	FQIKGVELKSGYKDW	3	5
HA 521-535	VELKSGYKDWILWIS	4	12
HA 526-540	GYKDWILWISFAISC	2	4
HA 531-545	ILWISFAISCFLLCV	0	7
HA 536-550	FAISCFLLCVVLLGF	0	7
HA 541-555	FLLCVVLLGFIMWAC	8	3
HA 546-560	VLLGFIMWACQRGNI	1	6
HA 551-565	IMWACQRGNIRCNI	3	4
HA 556-566	QRGNIRCNICI	2	4
NA 1-15	MNPNQKIITIGSVSL	1	2
NA 6-20	KIITIGSVSLTIATV	0	5
NA 11-25	GSVSLTIATVCFLMQ	4	6
NA 16-30	TIATVCFLMQIAILV	3	5
NA 21-35	CFLMQIAILVTTVTL	0	6
NA 26-40	IAILVTTVTLHFKQY	1	4
NA 31-45	TTVTLHFKQYECDSP	1	5
NA 36-50	HFKQYECDSPASNQV	5	3
NA 41-55	ECDSPASNQVMPCEP	9	5
NA 46-60	ASNQVMPCEPIIIER	1	8
NA 51-65	MPCEPIIIERNITEI	6	8
NA 56-70	IIIERNITEIVYLNN	2	3
NA 61-75	NITEIVYLNNTTIEK	0	2
NA 66-80	VYLNNTTIEKEICPK	2	3
NA 71-85	TTIEKEICPKVVEYR	2	3
NA 76-90	EICPKVVEYRNWSKP	4	7
NA 81-95	VVEYRNWSKPQCQIT	2	5
NA 86-100	NWSKPQCQITGFAPF	1	6
NA 91-105	QCQITGFAPFSKDNS	4	5
NA 96-110	GFAPFSKDNSIRLSA	3	6
NA 101-115	SKDNSIRLSAGGDIW	2	8
NA 106-120	IRLSAGGDIWVTREP	3	5
NA 111-125	GGDIWVTREPYVSCD	4	10
NA 116-130	VTREPYVSCDHGKCY	1	1
NA 121-135	YVSCDHGKCYQFALG	0	4
NA 126-140	HGKCYQFALGQGTTL	0	6
NA 131-145	QFALGQGTTLDNKHS	1	7
NA 136-150	QGTTLDNKHSNDTIH	3	6
NA 141-155	DNKHSNDTIHDRIPH	1	4
NA 146-160	NDTIHDRIPHRTLLM	1	2
NA 151-165	DRIPHRTLLMNELGV	0	3
NA 156-170	RTLLMNELGVPFHLG	2	3
NA 161-175	NELGVPFHLGTRQVC	4	5
NA 166-180	PFHLGTRQVCIAWSS	1	4
NA 171-185	TRQVCIAWSSSSCHD	2	6
NA 176-190	IAWSSSSCHDGKAWL	2	7

	Peptide Sequence	Elispot[a]	
NA 181-195	SSCHDGKAWLHVCIT	2	5
NA 186-200	GKAWLHVCITGDDKN	2	6
NA 191-205	HVCITGDDKNATASF	1	3
NA 196-210	GDDKNATASFIYDGR	1	6
NA 201-215	ATASFIYDGRLVDSI	4	12
NA 206-220	IYDGRLVDSIGSWSQ	1	6
NA 211-225	LVDSIGSWSQNILRT	2	5
NA 216-230	GSWSQNILRTQESEC	3	6
NA 221-235	NILRTQESECVCING	2	5
NA 226-240	QESECVCINGTCTVV	3	4
NA 231-245	VCINGTCTVVMTDGS	1	6
NA 236-250	TCTVVMTDGSASGRA	2	3
NA 241-255	MTDGSASGRADTRIL	2	4
NA 246-260	ASGRADTRILFIEEG	1	5
NA 251-265	DTRILFIEEGKIVHI	3	4
NA 256-270	FIEEGKIVHISPLSG	2	5
NA 261-275	KIVHISPLSGSAQHV	3	3
NA 266-280	SPLSGSAQHVEECSC	2	6
NA 271-285	SAQHVEECSCYPRYP	1	4
NA 276-290	EECSCYPRYPGVRCI	2	14
NA 281-295	YPRYPGVRCICRDNW	1	4
NA 286-300	GVRCICRDNWKGSNR	0	7
NA 291-305	CRDNWKGSNRPVVDI	2	7
NA 296-310	KGSNRPVVDINMEDY	3	4
NA 301-315	PVVDINMEDYSIDSS	0	3
NA 306-320	NMEDYSIDSSYVCSG	2	2
NA 311-325	SIDSSYVCSGLVGDT	2	4
NA 316-330	YVCSGLVGDTPRNDD	3	13
NA 321-335	LVGDTPRNDDRSSNS	0	6
NA 326-340	PRNDDRSSNSNCRNP	0	3
NA 331-345	RSSNSNCRNPNNERG	1	2
NA 336-350	NCRNPNNERGNQGVK	1	4
NA 341-355	NNERGNQGVKGWAFD	1	4
NA 346-360	NQGVKGWAFDNGDDV	2	6
NA 351-365	GWAFDNGDDVWMGRT	3	4
NA 356-370	NGDDVWMGRTISKDL	2	5
NA 361-375	WMGRTISKDLRSGYE	0	4
NA 366-380	ISKDLRSGYETFKVI	1	2
NA 371-385	RSGYETFKVIGGWST	2	7
NA 376-390	TFKVIGGWSTPNSKS	0	5
NA 381-395	GGWSTPNSKSQINRQ	0	7
NA 386-400	PNSKSQINRQVIVDS	1	3
NA 391-405	QINRQVIVDSDNRSG	2	3
NA 396-410	VIVDSDNRSGYSGIF	1	3
NA 401-415	DNRSGYSGIFSVEGK	1	10
NA 406-420	YSGIFSVEGKSCINR	1	4
NA 411-425	SVEGKSCINRCFYVE	2	10
NA 416-430	SCINRCFYVELIRGR	2	12
NA 421-435	CFYVELIRGRKQETR	2	4
NA 424-438	VELIRGRKQETRVWW	3	7

	Peptide Sequence	Elispot[a]	
NA 429-443	GRKQETRVWWTSNSI	0	5
NA 434-448	TRVWWTSNSIVVFCG	0	4
NA 439-453	TSNSIVVFCGTSGTY	0	4
NA 444-458	VVFCGTSGTYGTGSW	3	8
NA 449-463	TSGTYGTGSWPDGAN	1	4
NA 454-468	GTGSWPDGANINFMP	1	4
NA 459-469	PDGANINFMPI	0	12
NP 1-15	MASQGTKRSYEQMET	4	5
NP 6-20	TKRSYEQMETDGERQ	9	6
NP 11-25	EQMETDGERQNATEI	3	10
NP 16-30	DGERQNATEIRASVG	2	15
NP 21-35	NATEIRASVGKMIGG	14	5
NP 26-40	RASVGKMIGGIGRFY	10	12
NP 31-45	KMIGGIGRFYIQMCT	5	6
NP 36-50	IGRFYIQMCTELKLS	10	10
NP 41-55	IQMCTELKLSDYEGR	3	3
NP 46-60	ELKLSDYEGRLIQNS	3	12
NP 51-65	DYEGRLIQNSLTIER	6	11
NP 56-70	LIQNSLTIERMVLSA	3	6
NP 61-75	LTIERMVLSAFDERR	9	12
NP 66-80	MVLSAFDERRNKYLE	17	5
NP 71-85	FDERRNKYLEEHPSA	3	3
NP 76-90	NKYLEEHPSAGKDPK	2	9
NP 81-95	EHPSAGKDPKKTGGP	2	8
NP 86-100	GKDPKKTGGPIYRRV	6	5
NP 91-105	**KTGGPIYRRVNGKWM**	**25**	**17**
NP 96-110	IYRRVNGKWMRELIL	9	13
NP 101-115	NGKWMRELILYDKEE	2	10
NP 106-120	**RELILYDKEEIRRIW**	**15**	**26**
NP 111-125	YDKEEIRRIWRQANN	8	2
NP 116-130	IRRIWRQANNGDDAT	8	12
NP 121-135	RQANNGDDATAGLTH	12	11
NP 126-140	GDDATAGLTHMMIWH	16	6
NP 131-145	AGLTHMMIWHSNLND	13	7
NP 136-150	**MMIWHSNLNDATYQR**	**26**	**14**
NP 141-155	SNLNDATYQRTRALV	12	16
NP 146-160	**ATYQRTRALVRTGMD**	**30**	**17**
NP 151-165	**TRALVRTGMDPRMCS**	**48**	**22**
NP 156-170	RTGMDPRMCSLMQGS	42	12
NP 161-175	**PRMCSLMQGSTLPRR**	**45**	**22**
NP 166-180	LMQGSTLPRRSGAAG	12	5
NP 171-185	TLPRRSGAAGAAVKG	3	5
NP 176-190	SGAAGAAVKGVGTMV	6	3
NP 181-195	AAVKGVGTMVMELVR	7	3
NP 186-200	VGTMVMELVRMIKRG	8	8
NP 191-205	MELVRMIKRGINDRN	12	16
NP 196-210	**MIKRGINDRNFWRGE**	**30**	**21**
NP 201-215	**INDRNFWRGENGRKT**	**34**	**39**
NP 206-220	**FWRGENGRKTRIAYE**	**91**	**82**
NP 211-225	**NGRKTRIAYERMCNI**	**82**	**61**

	Peptide Sequence	Elispot[a]	
NP 216-230	**RIAYERMCNILKGKF**	**44**	**43**
NP 221-235	RMCNILKGKFQTAAQ	64	8
NP 226-240	LKGKFQTAAQKAMMD	6	6
NP 231-245	QTAAQKAMMDQVRES	4	8
NP 236-250	KAMMDQVRESRNPGN	5	6
NP 241-255	QVRESRNPGNAEFED	2	0
NP 246-260	RNPGNAEFEDLTFLA	7	3
NP 251-265	AEFEDLTFLARSALI	8	5
NP 256-270	LTFLARSALILRGSV	6	4
NP 261-275	RSALILRGSVAHKSC	28	13
NP 266-280	LRGSVAHKSCLPACV	56	30
NP 271-285	AHKSCLPACVYGPAV	47	23
NP 276-290	LPACVYGPAVASGYD	100	69
NP 281-295	YGPAVASGYDFEREG	16	6
NP 286-300	ASGYDFEREGYSLVG	10	3
NP 291-305	FEREGYSLVGIDPFR	6	3
NP 296-310	YSLVGIDPFRLLQNS	22	2
NP 301-315	IDPFRLLQNSQVYSL	8	2
NP 306-320	LLQNSQVYSLIRPNE	34	8
NP 311-325	**QVYSLIRPNENPAHK**	**38**	**41**
NP 316-330	**IRPNENPAHKSQLVW**	**20**	**23**
NP 321-335	NPAHKSQLVWMACHS	22	7
NP 326-340	**SQLVWMACHSAAFED**	**26**	**17**
NP 331-345	MACHSAAFEDLRVLS	3	7
NP 336-350	AAFEDLRVLSFIKGT	5	15
NP 341-355	LRVLSFIKGTKVLPR	4	3
NP 346-360	FIKGTKVLPRGKLST	3	5
NP 351-365	KVLPRGKLSTRGVQI	2	10
NP 356-370	GKLSTRGVQIASNEN	18	14
NP 361-375	RGVQIASNENMETME	177	139
NP 366-380	ASNENMETMESSTLE	188	3
NP 371-385	METMESSTLELRSRY	28	13
NP 376-390	SSTLELRSRYWAIRT	7	2
NP 381-395	LRSRYWAIRTRSGGN	3	2
NP 386-400	WAIRTRSGGNTNQQR	6	4
NP 391-405	RSGGNTNQQRASAGQ	4	3
NP 396-410	TNQQRASAGQISIQP	2	4
NP 401-415	ASAGQISIQPTFSVQ	7	4
NP 406-420	ISIQPTFSVQRNLPF	2	3
NP 411-425	TFSVQRNLPFDRTTI	3	4
NP 416-430	RNLPFDRTTIMAAFN	26	8
NP 421-435	DRTTIMAAFNGNTEG	6	10
NP 423-437	TIMAAFNGNTEGRTS	24	11
NP 428-442	FNGNTEGRTSDMRTE	1	3
NP 433-447	EGRTSDMRTEIIRMM	3	2
NP 438-452	DMRTEIIRMMESARP	8	7
NP 443-457	IIRMMESARPEDVSF	4	14
NP 448-462	ESARPEDVSFQGRGV	3	3
NP 453-467	EDVSFQGRGVFELSD	8	4
NP 458-472	QGRGVFELSDEKAAS	2	3

	Peptide Sequence	Elispot[a]	
NP 463-477	FELSDEKAASPIVPS	4	7
NP 468-482	EKAASPIVPSFDMSN	3	3
NP 473-487	PIVPSFDMSNEGSYF	0	4
NP 478-493	FDMSNEGSYFFGDNA	0	2
NP 484-498	EGSYFFGDNAEEYDN	0	5
NP 489-498	FGDNAEEYDN	2	3
PA 1-15	MEDFVRQCFNPMIVE	5	2
PA 6-20	RQCFNPMIVELAEKT	3	8
PA 11-25	PMIVELAEKTMKEYG	2	6
PA 16-30	LAEKTMKEYGEDLKI	0	8
PA 21-35	MKEYGEDLKIETNKF	4	8
PA 26-40	EDLKIETNKFAAICT	9	4
PA 31-45	ETNKFAAICTHLEVC	3	12
PA 36-50	AAICTHLEVCFMYSD	3	8
PA 41-55	HLEVCFMYSDFHFIN	5	10
PA 46-60	FMYSDFHFINEQGES	6	10
PA 51-65	FHFINEQGESIIVEL	9	6
PA 56-70	EQGESIIVELGDPSA	1	7
PA 61-75	IIVELGDPSALLKHR	3	10
PA 66-80	GDPSALLKHRFEIIE	2	10
PA 71-85	LLKHRFEIIEGRDRT	7	7
PA 76-90	FEIIEGRDRTMAWTV	1	9
PA 81-95	GRDRTMAWTVVNSIC	4	10
PA 86-100	MAWTVVNSICNTTGA	9	12
PA 91-105	VNSICNTTGAEKPKF	4	14
PA 96-110	NTTGAEKPKFLPDLY	2	9
PA 101-115	EKPKFLPDLYDYKEN	2	6
PA 106-120	LPDLYDYKENRFIEI	6	8
PA 111-125	DYKENRFIEIGVTRR	4	4
PA 116-130	RFIEIGVTRREVHIY	1	4
PA 121-135	GVTRREVHIYYLEKA	6	1
PA 126-140	EVHIYYLEKANKIKS	21	4
PA 131-145	YLEKANKIKSEKTHI	4	7
PA 136-150	NKIKSEKTHIHIFSF	2	6
PA 141-155	EKTHIHIFSFTGEEM	3	5
PA 146-160	HIFSFTGEEMATKAD	14	5
PA 151-165	TGEEMATKADYTLDE	9	3
PA 156-170	ATKADYTLDEESRAR	5	4
PA 161-175	YTLDEESRARIKTRL	15	6
PA 166-180	ESRARIKTRLFTIRQ	12	4
PA 171-185	IKTRLFTIRQEMASR	6	3
PA 176-190	FTIRQEMASRGLWDS	3	3
PA 181-195	EMASRGLWDSFRQSE	13	3
PA 186-200	GLWDSFRQSERGEET	4	4
PA 191-205	FRQSERGEETIEERF	5	22
PA 196-210	RGEETIEERFEITGT	12	15
PA 201-215	IEERFEITGTMRKLA	5	8
PA 206-220	EITGTMRKLADQSLP	17	8
PA 211-225	MRKLADQSLPPNFSS	13	5
PA 216-230	DQSLPPNFSSLENFR	134	24

	Peptide Sequence	Elispot[a]	
PA 221-235	PNFSSLENFRAYVDG	293	53
PA 226-240	LENFRAYVDGFEPNG	74	8
PA 231-245	AYVDGFEPNGYIEGK	7	4
PA 236-250	FEPNGYIEGKLSQMS	0	3
PA 241-255	YIEGKLSQMSKEVNA	2	3
PA 246-260	LSQMSKEVNARIEPF	23	14
PA 251-265	**KEVNARIEPFLKTTP**	**26**	**14**
PA 256-270	**RIEPFLKTTPRPLRL**	**29**	**17**
PA 261-275	LKTTPRPLRLPNGPP	23	3
PA 266-280	RPLRLPNGPPCSQRS	32	6
PA 271-285	PNGPPCSQRSKFLLM	48	6
PA 276-290	**CSQRSKFLLMDALKL**	**26**	**27**
PA 281-295	KFLLMDALKLSIEDP	27	6
PA 286-300	DALKLSIEDPSHEGE	14	3
PA 291-305	SIEDPSHEGEGIPLY	4	5
PA 296-310	SHEGEGIPLYDAIKC	2	9
PA 301-315	GIPLYDAIKCMRTFF	10	7
PA 306-320	DAIKCMRTFFGWKEP	2	9
PA 311-325	**MRTFFGWKEPNVVKP**	**11**	**14**
PA 316-330	**GWKEPNVVKPHEKGI**	**16**	**16**
PA 321-335	NVVKPHEKGINPNYL	19	8
PA 326-340	HEKGINPNYLLSWKQ	14	12
PA 331-345	NPNYLLSWKQVLAEL	6	3
PA 336-350	LSWKQVLAELQDIEN	19	6
PA 341-355	VLAELQDIENEEKIP	21	4
PA 346-360	QDIENEEKIPKTKNM	4	4
PA 351-365	EEKIPKTKNMKKTSQ	3	6
PA 356-370	KTKNMKKTSQLKWAL	5	6
PA 361-375	KKTSQLKWALGENMA	2	4
PA 366-380	LKWALGENMAPEKVD	3	2
PA 371-385	GENMAPEKVDFDDCK	22	2
PA 376-390	PEKVDFDDCKDVGDL	4	6
PA 381-395	FDDCKDVGDLKQYDS	9	4
PA 386-400	DVGDLKQYDSDEPEL	3	5
PA 391-405	KQYDSDEPELRSLAS	18	3
PA 396-410	DEPELRSLASWIQNE	8	3
PA 401-415	RSLASWIQNEFNKAC	14	10
PA 406-420	WIQNEFNKACELTDS	24	10
PA 411-425	**FNKACELTDSSWIEL**	**29**	**12**
PA 416-430	ELTDSSWIELDEIGE	2	4
PA 421-435	SWIELDEIGEDVAPI	4	6
PA 436-440	ELDEIGEDVAPIEHI	2	8
PA 431-445	GEDVAPIEHIASMRR	13	8
PA 436-450	PIEHIASMRRNYFTS	5	3
PA 441-455	ASMRRNYFTSEVSHC	6	5
PA 446-460	NYFTSEVSHCRATEY	12	16
PA 451-465	EVSHCRATEYIMKGV	17	16
PA 456-470	**RATEYIMKGVYINTA**	**18**	**13**
PA 461-475	IMKGVYINTALLNAS	1	15
PA 466-480	YINTALLNASCAAMD	8	7

	Peptide Sequence	Elispot[a]	
PA 471-185	LLNASCAAMDDFQLI	1	6
PA 476-490	CAAMDDFQLIPMISK	12	10
PA 481-495	DFQLIPMISKCRTKE	17	12
PA 486-500	PMISKCRTKEGRRKT	16	4
PA 491-505	CRTKEGRRKTNLYGF	7	3
PA 496-510	GRRKTNLYGFIIKGR	5	2
PA 501-515	NLYGFIIKGRSHLRN	3	2
PA 506-520	IIKGRSHLRNDTDVV	4	7
PA 511-525	SHLRNDTDVVNFVSM	2	4
PA 516-530	DTDVVNFVSMEFSLT	1	7
PA 521-535	NFVSMEFSLTDPRLE	4	5
PA 526-540	EFSLTDPRLEPHKWE	2	4
PA 531-545	DPRLEPHKWEKYCVL	6	3
PA 536-550	PHKWEKYCVLEIGDM	11	2
PA 541-555	KYCVLEIGDMLIRSA	8	3
PA 546-560	EIGDMLIRSAIGQVS	6	6
PA 551-565	LIRSAIGQVSRPMFL	2	6
PA 556-570	IGQVSRPMFLYVRTN	4	2
PA 561-575	RPMFLYVRTNGTSKI	7	3
PA 566-580	YVRTNGTSKIKMKWG	4	4
PA 571-585	GTSKIKMKWGMEMRR	1	3
PA 576-590	KMKWGMEMRRCLLQS	1	4
PA 581-595	MEMRRCLLQSLQQIE	5	11
PA 586-500	CLLQSLQQIESMIEA	1	16
PA 591-605	LQQIESMIEAESSVK	3	16
PA 596-610	SMIEAESSVKEKDMT	12	17
PA 601-615	ESSVKEKDMTKEFFE	7	32
PA 606-620	EKDMTKEFFENKSET	8	24
PA 611-625	KEFFENKSETWPIGE	6	5
PA 616-630	NKSETWPIGESPKGV	2	5
PA 621-635	WPIGESPKGVEESSI	3	2
PA 626-640	SPKGVEESSIGKVCR	3	2
PA 631-645	EESSIGKVCRTLLAK	4	5
PA 636-650	GKVCRTLLAKSVFNS	2	7
PA 641-655	TLLAKSVFNSLYASP	2	4
PA 646-660	SVFNSLYASPQLEGF	3	3
PA 651-665	LYASPQLEGFSAESR	1	8
PA 656-670	QLEGFSAESRKLLLI	0	1
PA 661-675	SAESRKLLLIVQALR	3	3
PA 666-680	KLLLIVQALRDNLEP	1	1
PA 671-685	VQALRDNLEPGTFDL	1	2
PA 676-690	DNLEPGTFDLGGLYE	0	1
PA 681-695	GTFDLGGLYEAIEEC	1	2
PA 686-700	GGLYEAIEECLINDP	3	1
PA 691-705	AIEECLINDPWVLLN	1	3
PA 696-710	LINDPWVLLNASWFN	2	8
PA 701-715	WVLLNASWFNSFLTH	4	7
PA 706-716	ASWFNSFLTHALS	1	1
PB1 1-15	MDVNPTLLFLKVPAQ	2	4
PB1 6-20	TLLFLKVPAQNAIST	1	7

	Peptide Sequence	Elispot[a]	
PB1 11-25	KVPAQNAISTTFPYT	0	2
PB1 16-30	NAISTTFPYTGDPPY	4	12
PB1 21-35	TFPYTGDPPYSHGTG	2	4
PB1 26-40	GDPPYSHGTGTGYTM	1	6
PB1 31-45	SHGTGTGYTMDTVNR	0	1
PB1 36-50	TGYTMDTVNRTHQYS	2	4
PB1 41-55	DTVNRTHQYSEKGRW	0	10
PB1 46-60	THQYSEKGRWTTNTE	0	10
PB1 51-65	EKGRWTTNTETGAPQ	2	5
PB1 56-70	TTNTETGAPQLNPID	0	6
PB1 61-75	TGAPQLNPIDGPLPE	2	5
PB1 66-80	LNPIDGPLPEDNEPS	3	40
PB1 71-85	GPLPEDNEPSGYAQT	9	6
PB1 76-90	DNEPSGYAQTDCVLE	4	12
PB1 81-95	GYAQTDCVLEVMAFL	5	7
PB1 86-100	DCVLEVMAFLEESHP	8	13
PB1 91-105	VMAFLEESHPGIFEN	0	11
PB1 96-110	EESHPGIFENSCIET	4	9
PB1 101-115	GIFENSCIETMEVVQ	9	6
PB1 106-120	SCIETMEVVQQTRVD	9	11
PB1 111-125	MEVVQQTRVDKLTQG	1	4
PB1 116-130	QTRVDKLTQGRQTYD	2	12
PB1 121-135	KLTQGRQTYDWTLNR	0	6
PB1 126-140	RQTYDWTLNRNQPAA	2	11
PB1 131-145	WTLNRNQPAATALAN	2	3
PB1 136-150	NQPAATALANTIEVF	2	6
PB1 141-155	TALANTIEVFRSNGL	5	4
PB1 146-160	TIEVFRSNGLTANES	1	12
PB1 151-165	RSNGLTANESGRLID	1	9
PB1 156-170	TANESGRLIDFLKDV	4	18
PB1 161-175	GRLIDFLKDVMESMN	1	7
PB1 166-180	FLKDVMESMNKEEMG	2	5
PB1 171-185	MESMNKEEMGITTHF	3	4
PB1 176-190	KEEMGITTHFQRKRR	1	6
PB1 181-195	ITTHFQRKRRVRDNM	2	5
PB1 186-200	QRKRRVRDNMTKKMI	3	3
PB1 191-205	VRDNMTKKMITQRTM	3	9
PB1 196-210	TKKMITQRTMGKKKQ	5	9
PB1 201-215	TQRTMGKKKQRLNKR	4	4
PB1 206-220	GKKKQRLNKRSYLIR	1	5
PB1 211-225	RLNKRSYLIRALTLN	2	6
PB1 216-230	SYLIRALTLNTMTKD	2	14
PB1 221-235	ALTLNTMTKDAERGK	6	3
PB1 226-240	TMTKDAERGKLKRRA	0	6
PB1 231-245	AERGKLKRRAIATPG	1	7
PB1 236-250	LKRRAIATPGMQIRG	0	8
PB1 241-255	IATPGMQIRGFVYFV	5	3
PB1 246-260	MQIRGFVYFVETLAR	2	11
PB1 251-265	FVYFVETLARSICEK	4	4
PB1 256-270	ETLARSICEKLEQSG	1	3

	Peptide Sequence	Elispot[a]	
PB1 261-275	SICEKLEQSGLPVGG	0	4
PB1 266-280	LEQSGLPVGGNEKKA	2	0
PB1 271-285	LPVGGNEKKAKLANV	2	4
PB1 276-290	NEKKAKLANVVRKMM	4	4
PB1 281-295	KLANVVRKMMTNSQD	2	32
PB1 286-300	VRKMMTNSQDTELSF	5	12
PB1 291-305	TNSQDTELSFTITGD	4	7
PB1 296-310	TELSFTITGDNTKWN	3	5
PB1 301-315	TITGDNTKWNENQNP	1	7
PB1 306-320	NTKWNENQNPRMFLA	0	16
PB1 311-325	ENQNPRMFLAMITYM	1	10
PB1 316-330	RMFLAMITYMTRNQP	1	9
PB1 321-335	MITYMTRNQPEWFRN	3	10
PB1 326-340	TRNQPEWFRNVLSIA	4	17
PB1 331-345	EWFRNVLSIAPIMFS	4	16
PB1 336-350	VLSIAPIMFSNKMAR	0	9
PB1 341-355	PIMFSNKMARLGKGY	1	7
PB1 346-360	NKMARLGKGYMFESK	2	6
PB1 351-365	LGKGYMFESKSMKLR	7	3
PB1 356-370	MFESKSMKLRTQIPA	0	6
PB1 361-375	SMKLRTQIPAEMLAS	0	7
PB1 366-380	TQIPAEMLASIDLKY	2	6
PB1 371-385	EMLASIDLKYFNDST	2	5
PB1 376-390	IDLKYFNDSTRKKIE	0	7
PB1 381-395	FNDSTRKKIEKIRPL	0	6
PB1 386-400	RKKIEKIRPLLIEGT	7	2
PB1 391-405	KIRPLLIEGTASLSP	3	2
PB1 396-410	LIEGTASLSPGMMMG	1	1
PB1 401-415	ASLSPGMMMGMFNML	6	5
PB1 406-420	GMMMGMFNMLSTVLG	0	15
PB1 411-425	MFNMLSTVLGVSILN	5	7
PB1 416-430	STVLGVSILNLGQKR	2	2
PB1 421-435	VSILNLGQKRYTKTT	0	3
PB1 426-440	LNLGQKRYTKTTYWW	0	4
PB1 431-445	KRYTKTTYWWDGLQS	3	8
PB1 436-450	TTYWWDGLQSSDDFA	2	7
PB1 441-455	DGLQSSDDFALIVNA	4	3
PB1 446-460	SDDFALIVNAPNHEG	1	5
PB1 451-465	LIVNAPNHEGIQAGV	2	7
PB1 456-470	PNHEGIQAGVDRFYR	2	11
PB1 461-475	IQAGVDRFYRTCKLL	6	4
PB1 466-480	DRFYRTCKLLGINMS	5	18
PB1 471-485	TCKLLGINMSKKKSY	2	6
PB1 476-490	GINMSKKKSYINRTG	5	4
PB1 481-495	KKKSYINRTGTFEFT	3	1
PB1 486-500	INRTGTFEFTSFFYR	6	1
PB1 491-505	TFEFTSFFYRYGFVA	7	19
PB1 496-510	SFFYRYGFVANFSME	3	16
PB1 501-515	YGFVANFSMELPSFG	7	22
PB1 506-520	NFSMELPSFGVSGIN	6	8

	Peptide Sequence	Elispot[a]	
PB1 511-525	LPSFGVSGINESADM	10	7
PB1 516-530	VSGINESADMSIGVT	2	2
PB1 521-535	ESADMSIGVTVIKNN	1	4
PB1 526-540	SIGVTVIKNNMINND	3	2
PB1 531-545	VIKNNMINNDLGPAT	4	3
PB1 536-550	MINNDLGPATAQMAL	3	1
PB1 541-555	LGPATAQMALQLFIK	8	1
PB1 546-560	AQMALQLFIKDYRYT	3	2
PB1 551-565	QLFIKDYRYTYRCHR	6	2
PB1 556-570	DYRYTYRCHRGDTQI	9	4
PB1 561-575	YRCHRGDTQIQTRRS	8	8
PB1 566-580	GDTQIQTRRSFEIKK	4	6
PB1 571-585	QTRRSFEIKKLWEQT	6	5
PB1 576-590	FEIKKLWEQTRSKAG	5	6
PB1 581-595	LWEQTRSKAGLLVSD	9	3
PB1 586-600	RSKAGLLVSDGGPNL	11	0
PB1 591-605	LLVSDGGPNLYNIRN	5	0
PB1 596-610	GGPNLYNIRNLHIPE	3	3
PB1 601-615	YNIRNLHIPEVCLKW	16	7
PB1 606-620	LHIPEVCLKWELMDE	5	4
PB1 611-625	VCLKWELMDEDYQGR	4	8
PB1 616-630	ELMDEDYQGRLCNPL	9	9
PB1 621-635	DYQGRLCNPLNPFVS	13	9
PB1 626-640	LCNPLNPFVSHKEIE	13	6
PB1 631-645	NPFVSHKEIESMNNA	10	5
PB1 636-650	HKEIESMNNAVMMPA	9	6
PB1 641-655	SMNNAVMMPAHGPAK	0	2
PB1 646-660	VMMPAHGPAKNMEYD	0	1
PB1 651-665	HGPAKNMEYDAVATT	6	2
PB1 656-670	NMEYDAVATTHSWIP	1	5
PB1 661-675	AVATTHSWIPKRNRS	3	2
PB1 666-680	HSWIPKRNRSILNTS	1	1
PB1 671-685	KRNRSILNTSQRGVL	0	2
PB1 676-690	ILNTSQRGVLGDEQM	1	4
PB1 681-695	QRGVLGDEQMYQRCC	3	2
PB1 686-700	GDEQMYQRCCNLFEK	2	1
PB1 691-705	YQRCCNLFEKFFPSS	4	3
PB1 696-710	NLFEKFFPSSSYRRP	2	0
PB1 701-715	FFPSSSYRRPVGISS	86	36
PB1 706-720	SYRRPVGISSMVEAM	12	8
PB1 711-725	VGISSMVEAMVSRAR	2	3
PB1 716-730	MVEAMVSRARIDARI	3	3
PB1 721-735	VSRARIDARIDFESG	2	5
PB1 726-740	IDARIDFESGRIKKE	4	3
PB1 731-745	DFESGRIKKEEFTEI	1	3
PB1 736-750	RIKKEEFTEIMKICS	2	4
PB1 741-755	EFTEIMKICSTIEEL	2	7
PB1 746-759	MKICSTIEELRRQK	6	4
PB1 751-759	TIEELRRQK	2	4
PB2 1-15	MERIKELRNLMSQSR	4	3

	Peptide Sequence	Elispot[a]	
PB2 6-20	ELRNLMSQSRTREIL	2	1
PB2 11-25	MSQSRTREILTKTTV	3	3
PB2 16-30	TREILTKTTVDHMAI	12	11
PB2 21-35	TKTTVDHMAIIKKFT	3	5
PB2 26-40	DHMAIIKKFTSGRQE	4	6
PB2 31-45	IKKFTSGRQEKNPAL	2	7
PB2 36-50	SGRQEKNPALRMKWM	6	8
PB2 41-55	KNPALRMKWMMAMKY	9	6
PB2 46-60	RMKWMMAMKYPITAD	11	5
PB2 51-65	MAMKYPITADKRITE	7	8
PB2 56-70	PITADKRITEMIPER	4	4
PB2 61-75	KRITEMIPERNEQGQ	1	6
PB2 66-80	MIPERNEQGQTLWSK	4	7
PB2 71-85	NEQGQTLWSKMNDAG	5	15
PB2 76-90	TLWSKMNDAGSDRVM	7	7
PB2 81-95	MNDAGSDRVMVSPLA	4	6
PB2 86-100	SDRVMVSPLAVTWWN	13	10
PB2 91-105	**VSPLAVTWWNRNGPM**	**20**	**20**
PB2 96-110	VTWWNRNGPMTNTVH	19	22
PB2 101-115	RNGPMTNTVHYPKIY	14	12
PB2 106-120	**TNTVHYPKIYKTYFE**	**32**	**17**
PB2 111-125	YPKIYKTYFERVERL	1	4
PB2 116-130	KTYFERVERLKHGTF	6	6
PB2 121-135	RVERLKHGTFGPVHF	5	2
PB2 126-140	KHGTFGPVHFRNQVK	0	4
PB2 131-145	GPVHFRNQVKIRRRV	4	14
PB2 136-150	RNQVKIRRRVDINPG	8	4
PB2 141-155	IRRRVDINPGHADLS	8	13
PB2 146-160	DINPGHADLSAKEAQ	4	10
PB2 151-165	HADLSAKEAQDVIME	5	12
PB2 156-170	AKEAQDVIMEVVFPN	3	6
PB2 161-175	DVIMEVVFPNEVGAR	3	12
PB2 166-180	VVFPNEVGARILTSE	4	7
PB2 171-185	EVGARILTSESQLTI	3	8
PB2 176-190	ILTSESQLTITKEKK	12	7
PB2 181-195	SQLTITKEKKEELQD	3	6
PB2 186-200	TKEKKEELQDCKISP	4	9
PB2 191-205	EELQDCKISPLMVAY	5	11
PB2 196-210	**CKISPLMVAYMLERE**	**42**	**30**
PB2 201-215	LMVAYMLERELVRKT	3	4
PB2 206-220	MLERELVRKTRFLPV	2	5
PB2 211-225	LVRKTRFLPVAGGTS	6	3
PB2 216-230	RFLPVAGGTSSVYIE	7	6
PB2 221-235	AGGTSSVYIEVLHLT	11	10
PB2 226-240	SVYIEVLHLTQGTCW	5	5
PB2 231-245	VLHLTQGTCWEQMYT	2	7
PB2 236-250	QGTCWEQMYTPGGEV	1	5
PB2 241-255	EQMYTPGGEVRNDDV	8	2
PB2 246-260	PGGEVRNDDVDQSLI	3	3
PB2 251-265	RNDDVDQSLIIAARN	1	7

	Peptide Sequence	Elispot[a]	
PB2 256-270	DQSLIIAARNIVRRA	5	5
PB2 261-275	IAARNIVRRAAVSAD	6	3
PB2 266-280	IVRRAAVSADPLASL	7	10
PB2 271-285	AVSADPLASLLEMCH	10	5
PB2 276-290	PLASLLEMCHSTQIG	6	3
PB2 281-295	LEMCHSTQIGGIRMV	4	10
PB2 286-300	STQIGGIRMVDILRQ	5	6
PB2 291-305	GIRMVDILRQNPTEE	6	8
PB2 296-310	DILRQNPTEEQAVDI	4	3
PB2 301-315	NPTEEQAVDICKAAM	7	5
PB2 306-320	QAVDICKAAMGLRIS	5	4
PB2 311-325	CKAAMGLRISSSFSF	3	6
PB2 316-330	GLRISSSFSFGGFTF	11	12
PB2 321-335	SSFSFGGFTFKRTSG	12	12
PB2 326-340	GGFTFKRTSGSSVKR	1	10
PB2 331-345	KRTSGSSVKREEEVL	14	7
PB2 336-350	SSVKREEEVLTGNLQ	1	5
PB2 341-355	EEEVLTGNLQTLKIR	3	10
PB2 346-360	TGNLQTLKIRVHEGY	4	4
PB2 351-365	TLKIRVHEGYEEFTM	2	9
PB2 356-370	VHEGYEEFTMVGRRA	1	10
PB2 361-375	EEFTMVGRRATAILR	3	6
PB2 366-380	VGRRATAILRKATRR	4	7
PB2 371-385	TAILRKATRRLIQLI	6	13
PB2 376-390	KATRRLIQLIVSGRD	4	8
PB2 381-395	LIQLIVSGRDEQSIA	3	7
PB2 386-400	VSGRDEQSIAEAIIV	2	7
PB2 391-405	EQSIAEAIIVAMVFS	2	4
PB2 396-410	EAIIVAMVFSQEDCM	5	3
PB2 401-415	AMVFSQEDCMIKAVR	1	7
PB2 406-420	QEDCMIKAVRGDLNF	5	4
PB2 411-425	IKAVRGDLNFVNRAN	6	11
PB2 416-430	GDLNFVNRANQRLNP	8	14
PB2 421-435	VNRANQRLNPMHQLL	4	4
PB2 426-440	ANQRLNPMHQLLRHF	6	4
PB2 431-445	NPMHQLLRHFQKDAK	0	1
PB2 436-450	LLRHFQKDAKVLFQN	2	4
PB2 441-455	QKDAKVLFQNWGVEP	4	6
PB2 446-460	VLFQNWGVEPIDNVM	8	6
PB2 451-465	WGVEPIDNVMGMIGI	6	6
PB2 456-470	IDNVMGMIGILPDMT	4	5
PB2 461-475	GMIGILPDMTPSIEM	3	9
PB2 466-480	LPDMTPSIEMSMRGV	4	1
PB2 471-485	PSIEMSMRGVRISKM	4	6
PB2 476-490	SMRGVRISKMGVDEY	1	2
PB2 481-495	RISKMGVDEYSSTER	3	2
PB2 486-500	GVDEYSSTERVVVSI	2	4
PB2 491-505	SSTERVVVSIDRFLR	4	3
PB2 496-510	VVVSIDRFLRIRDQR	7	4
PB2 501-515	DRFLRIRDQRGNVLL	6	3

	Peptide Sequence	Elispot[a]	
PB2 506-520	IRDQRGNVLLSPEEV	4	4
PB2 511-525	GNVLLSPEEVSETQG	10	7
PB2 516-530	SPEEVSETQGTEKLT	8	4
PB2 521-535	SETQGTEKLTITYSS	15	9
PB2 526-540	TEKLTITYSSSMMWE	4	2
PB2 531-545	ITYSSSMMWEINGPE	26	2
PB2 536-550	SMMWEINGPESVLVN	6	3
PB2 541-555	INGPESVLVNTYQWI	8	4
PB2 546-560	SVLVNTYQWIIRNWE	3	6
PB2 551-565	TYQWIIRNWETVKIQ	2	2
PB2 556-570	IRNWETVKIQWSQNP	7	4
PB2 561-575	TVKIQWSQNPTMLYN	3	6
PB2 566-580	WSQNPTMLYNKMEFE	8	3
PB2 571-585	TMLYNKMEFEPFQSL	5	2
PB2 576-590	KMEFEPFQSLVPKAI	3	2
PB2 581-595	PFQSLVPKAIRGQYS	2	2
PB2 586-600	VPKAIRGQYSGFVRT	12	2
PB2 591-605	RGQYSGFVRTLFQQM	3	3
PB2 596-610	GFVRTLFQQMRDVLG	8	5
PB2 601-615	LFQQMRDVLGTFDTA	3	1
PB2 606-620	RDVLGTFDTAQIIKL	5	1
PB2 611-625	TFDTAQIIKLLPFAA	4	3
PB2 616-630	QIIKLLPFAAAPPKQ	0	2
PB2 621-635	LPFAAAPPKQSRMQF	1	4
PB2 626-640	APPKQSRMQFSSFTV	0	4
PB2 631-645	SRMQFSSFTVNVRGS	2	3
PB2 636-650	SSFTVNVRGSGMRIL	2	4
PB2 641-655	NVRGSGMRILVRGNS	2	6
PB2 646-660	GMRILVRGNSPVFNY	2	9
PB2 651-665	VRGNSPVFNYNKATK	1	4
PB2 656-670	PVFNYNKATKRLTVL	6	20
PB2 661-675	NKATKRLTVLGKDAG	3	5
PB2 666-680	RLTVLGKDAGTLTED	3	2
PB2 671-685	GKDAGTLTEDPDEGT	6	1
PB2 676-690	TLTEDPDEGTAGVES	1	2
PB2 681-695	PDEGTAGVESAVLRG	1	4
PB2 686-700	AGVESAVLRGFLILG	1	1
PB2 691-705	AVLRGFLILGKEDKR	4	4
PB2 696-710	FLILGKEDKRYGPAL	3	5
PB2 701-715	KEDKRYGPALSINEL	5	44
PB2 706-720	YGPALSINELSNLAK	1	4
PB2 711-725	SINELSNLAKGEKAN	3	5
PB2 716-730	SNLAKGEKANVLIGQ	1	6
PB2 721-735	GEKANVLIGQGDVVL	1	10
PB2 726-740	VLIGQGDVVLVMKRK	5	5
PB2 731-745	GDVVLVMKRKRDSSI	13	5
PB2 734-748	VMKRKRDSSILTDSQ	0	8
PB2 737-751	RDSSILTDSQTATKR	0	29
PB2 742-756	LTDSQTATKRIRMAI	2	1
PB2 747-757	TATKRIRMAIN	1	3

	Peptide Sequence	Elispot[a]	
NS1 1-15	MDPNTVSSFQVDCFL	1	5
NS1 6-20	VSSFQVDCFLWHVRK	4	3
NS1 11-25	VDCFLWHVRKRVADQ	2	2
NS1 16-30	WHVRKRVADQELGDA	3	7
NS1 21-35	RVADQELGDAPFLDR	0	8
NS1 26-40	ELGDAPFLDRLRRDQ	4	2
NS1 31-45	PFLDRLRRDQKSLRG	1	4
NS1 36-50	LRRDQKSLRGRGSTL	0	3
NS1 41-55	KSLRGRGSTLGLDIK	3	3
NS1 46-60	RGSTLGLDIKTATRA	1	5
NS1 51-65	GLDIKTATRAGKQIV	1	1
NS1 56-70	TATRAGKQIVERILK	4	3
NS1 61-75	GKQIVERILKEESDE	2	3
NS1 66-80	ERILKEESDEALKMT	6	4
NS1 71-85	EESDEALKMTMASVP	3	2
NS1 76-90	ALKMTMASVPASRYL	5	4
NS1 81-95	MASVPASRYLTDMTL	1	5
NS1 86-100	ASRYLTDMTLEEMSR	3	3
NS1 91-105	TDMTLEEMSRDWSML	7	3
NS1 96-110	EEMSRDWSMLIPKQK	3	2
NS1 101-115	DWSMLIPKQKVAGPL	6	12
NS1 106-120	IPKQKVAGPLCIRMD	5	23
NS1 111-125	VAGPLCIRMDQAIMD	9	5
NS1 116-130	CIRMDQAIMDKNIIL	3	3
NS1 121-135	QAIMDKNIILKANFS	2	5
NS1 126-140	KNIILKANFSVIFDR	6	1
NS1 131-145	KANFSVIFDRLETLI	2	4
NS1 136-150	VIFDRLETLILLRAF	5	8
NS1 141-155	LETLILLRAFTEEGA	5	5
NS1 146-160	LLRAFTEEGAIVGEI	12	2
NS1 151-165	TEEGAIVGEISPLPS	2	3
NS1 156-170	IVGEISPLPSLPGHT	7	6
NS1 161-175	SPLPSLPGHTAEDVK	7	2
NS1 166-180	LPGHTAEDVKNAVGV	3	2
NS1 171-185	AEDVKNAVGVLIGGL	4	6
NS1 176-190	NAVGVLIGGLEWNDN	3	4
NS1 181-195	LIGGLEWNDNTVRVS	7	3
NS1 186-200	EWNDNTVRVSETLQR	2	2
NS1 191-205	TVRVSETLQRFAWRS	6	1
NS1 196-210	ETLQRFAWRSSNENG	14	2
NS1 201-215	FAWRSSNENGRPPLT	11	3
NS1 206-220	SNENGRPPLTPKQKR	8	7
NS1 211-225	RPPLTPKQKREMAGT	3	11
NS1 216-230	PKQKREMAGTIRSEV	6	8
NS1 221-230	EMAGTIRSEV	2	10
NS2 1-15	MDPNTVSSFQDILLR	0	2
NS2 6-20	VSSFQDILLRMSKMQ	2	3
NS2 11-25	DILLRMSKMQLESSS	1	0
NS2 16-30	MSKMQLESSSEDLNG	1	1
NS2 21-35	LESSSEDLNGMITQF	2	0

	Peptide Sequence	Elispot[a]	
NS2 26-40	EDLNGMITQFESLKL	8	5
NS2 31-45	MITQFESLKLYRDSL	10	6
NS2 36-50	ESLKLYRDSLGEAVM	5	3
NS2 41-55	YRDSLGEAVMRMGDL	3	5
NS2 46-60	GEAVMRMGDLHSLQN	1	4
NS2 51-65	RMGDLHSLQNRNEKW	0	3
NS2 56-70	HSLQNRNEKWREQLG	2	6
NS2 61-75	RNEKWREQLGQKFEE	4	2
NS2 66-80	REQLGQKFEEIRWLI	2	6
NS2 71-85	QKFEEIRWLIEEVRH	0	6
NS2 76-90	IRWLIEEVRHKLKIT	2	10
NS2 81-95	EEVRHKLKITENSFE	0	3
NS2 86-100	KLKITENSFEQITFM	4	4
NS2 91-105	**ENSFEQITFMQALHL**	**11**	**61**
NS2 96-110	QITFMQALHLLLEVE	2	5
NS2 101-115	QALHLLLEVEQEIRT	1	8
NS2 106-120	LLEVEQEIRTFSFQL	2	4
NS2 111-121	QEIRTFSFQLI	5	5
M1 1-15	MSFLTEVETYVLSII	2	5
M1 6-20	EVETYVLSIIPSGPL	2	6
M1 11-25	VLSIIPSGPLKAEIA	3	7
M1 16-30	PSGPLKAEIAQRLED	5	5
M1 21-35	KAEIAQRLEDVFAGK	1	13
M1 26-40	QRLEDVFAGKNTDLE	3	3
M1 31-45	VFAGKNTDLEVLMEW	4	2
M1 36-50	NTDLEVLMEWLKTRP	3	2
M1 41-55	VLMEWLKTRPILSPL	5	6
M1 46-60	LKTRPILSPLTKGIL	9	3
M1 51-65	ILSPLTKGILGFVFT	6	2
M1 56-70	TKGILGFVFTLTVPS	3	16
M1 61-75	GFVFTLTVPSERGLQ	7	4
M1 66-80	LTVPSERGLQRRRFV	3	3
M1 71-85	ERGLQRRRFVQNALN	1	3
M1 76-90	RRRFVQNALNGNGDP	3	4
M1 81-95	QNALNGNGDPNNMDK	6	3
M1 86-100	GNGDPNNMDKAVKLY	6	2
M1 91-105	NNMDKAVKLYRKLKR	1	2
M1 96-110	AVKLYRKLKREITFH	4	14
M1 101-115	RKLKREITFHGAKEI	4	6
M1 106-120	EITFHGAKEISLSYS	12	4
M1 111-125	GAKEISLSYSAGALA	2	2
M1 116-130	SLSYSAGALASCMGL	3	6
M1 121-135	AGALASCMGLIYNRM	7	6
M1 126-140	SCMGLIYNRMGAVTT	5	4
M1 131-145	IYNRMGAVTTEVAFG	2	10
M1 136-150	GAVTTEVAFGLVCAT	2	7
M1 141-155	EVAFGLVCATCEQIA	7	7
M1 146-160	LVCATCEQIADSQHR	4	4
M1 151-165	CEQIADSQHRSHRQM	4	16
M1 156-170	DSQHRSHRQMVTTTN	6	3
M1 161-175	SHRQMVTTTNPLIRH	2	6
M1 166-180	VTTTNPLIRHENRMV	3	2
M1 171-185	PLIRHENRMVLASTT	1	2
M1 176-190	ENRMVLASTTAKAME	4	7
M1 181-195	LASTTAKAMEQMAGS	7	6
M1 186-200	AKAMEQMAGSSEQAA	4	2
M1 191-205	QMAGSSEQAAEAMEV	0	3
M1 196-210	SEQAAEAMEVASQAR	1	9
M1 201-215	**EAMEVASQARQMVQA**	**12**	**32**
M1 206-220	ASQARQMVQAMITIG	9	35
M1 211-225	**QMVQAMITIGTHPSS**	**11**	**29**
M1 216-230	MITIGTHPSSSAGLK	3	8
M1 221-230	THPSSSAGLKNDLLE	4	14
M1 226-240	SAGLKNDLLENLQAY	7	3
M1 231-245	NDLLENLQAYQKRMG	8	5
M1236-250	NLQAYQKRMGVQMQR	3	5
M1 241-252	QKRMGVQMQRFK	1	2
M2 1-15	MSFLTEVETPIRNEW	0	5
M2 6-20	EVETPIRNEWGCRCN	0	5
M2 11-25	IRNEWGCRCNGSSDP	1	4
M2 16-30	GCRCNGSSDPLTIAA	4	4
M2 21-35	GSSDPLTIAANIIGI	1	6
M2 26-40	LTIAANIIGILHLTL	0	4
M2 31-45	NIIGILHLTLWMLDR	1	5
M2 36-50	LHLTLWMLDRLFFKC	5	14
M2 41-55	WMLDRLFFKCIYRRF	10	5
M2 46-60	LFFKCIYRRFKYGLK	5	10
M2 51-65	IYRRFKYGLKGGPST	5	6
M2 56-70	KYGLKGGPSTEGVPK	3	3
M2 61-75	GGPSTEGVPKSMREE	2	3
M2 66-80	EGVPKSMREEYRKEQ	6	4
M2 71-85	SMREEYRKEQQSAVD	4	4
M2 76-90	YRKEQQSAVDTDDGH	6	8
M2 81-95	QSAVDTDDGHFVSIE	5	10
M2 86-97	TDDGHFVSIELE	8	10

[a] Number of Elispots from 10^5 spleen cells, determined in two independent experiments. Peptides were screened on mice that had recovered from an x31 infection. The regions containing the known Class I and Class II epitopes are indicated by shading and the peptides investigated further are in bold.

Supplemental Table 2 .*PR8 Influenza virus peptides*

	Peptide Sequence	Elispot[a]	
HA 1-15	MKANLLVLLSALAAA	0	0
HA 6-20	LVLLSALAAADADTI	0	0
HA 11-25	ALAAADADTICIGYH	1	1
HA 16-30	DADTICIGYHANNST	3	1
HA 21-35	CIGYHANNSTDTVDT	2	1
HA 26-40	ANNSTDTVDTVLEKN	4	2
HA 31-45	DTVDTVLEKNVTVTH	3	3
HA 36-50	VLEKNVTVTHSVNLL	6	4
HA 41-55	VTVTHSVNLLEDSHN	5	2
HA 46-60	SVNLLEDSHNGKLCR	1	1
HA 51-65	EDSHNGKLCRLKGIA	3	5
HA 56-70	GKLCRLKGIAPLQLG	2	1
HA 61-75	LKGIAPLQLGKCNIA	1	1
HA 66-80	PLQLGKCNIAGWLLG	0	0
HA 71-85	KCNIAGWLLGNPECD	2	2
HA 76-90	GWLLGNPECDPLLPV	13	6
HA 81-95	NPECDPLLPVRSWSY	4	1
HA 86-100	PLLPVRSWSYIVETP	6	3
HA 91-105	RSWSYIVETPNSENG	2	4
HA 96-110	IVETPNSENGICYPG	10	2
HA 101-115	NSENGICYPGDFIDY	7	1
HA 106-120	ICYPGDFIDYEELRE	3	3
HA 111-125	DFIDYEELREQLSSV	7	5
HA 116-130	EELREQLSSVSSFER	1	4
HA 121-135	QLSSVSSFERFEIFP	2	6
HA 126-140	SSFERFEIFPKESSW	3	7
HA 131-145	FEIFPKESSWPNHNT	4	1
HA 136-150	KESSWPNHNTNGVTA	5	2
HA 141-155	PNHNTNGVTAACSHE	3	4
HA 146-160	NGVTAACSHEGKSSF	2	1
HA 151-165	ACSHEGKSSFYRNLL	6	2
HA 156-170	GKSSFYRNLLWLTEK	6	3
HA 161-175	YRNLLWLTEKEGSYP	4	5
HA 166-180	WLTEKEGSYPKLKNS	3	4
HA 171-185	EGSYPKLKNSYVNKK	6	1
HA 176-190	KLKNSYVNKKGKEVL	4	0
HA 181-195	YVNKKGKEVLVLWGI	1	0
HA 186-200	GKEVLVLWGIHHPPN	1	1
HA 191-205	VLWGIHHPPNSKEQQ	1	1
HA 196-210	HHPPNSKEQQNIYQN	2	2
HA 201-215	SKEQQNIYQNENAYV	2	3
HA 206-220	NIYQNENAYVSVVTS	6	4
HA 211-225	ENAYVSVVTSNYNRR	3	5
HA 216-230	SVVTSNYNRRFTPEI	5	2
HA 221-235	NYNRRFTPEIAERPK	8	1
HA 226-240	FTPEIAERPKVRDQA	1	5
HA 231-245	AERPKVRDQAGRMNY	2	1
HA 236-250	VRDQAGRMNYYWTLL	4	3
HA 241-255	GRMNYYWTLLKPGDT	0	0
HA 246-260	YWTLLKPGDTIIFEA	1	0
HA 251-265	KPGDTIIFEANGNLI	0	0
HA 256-270	IIFEANGNLIAPMYA	1	5
HA 261-275	NGNLIAPMYAFALSR	2	4
HA 266-280	APMYAFALSRGFGSG	1	3
HA 271-285	FALSRGFGSGIITSN	2	1
HA 276-290	GFGSGIITSNASMHE	3	5
HA 281-295	IITSNASMHECNTKC	4	1
HA 286-300	ASMHECNTKCQTPLG	3	7
HA 291-305	CNTKCQTPLGAINSS	8	5
HA 296-310	QTPLGAINSSLPYQN	6	1
HA 301-315	AINSSLPYQNIHPVT	0	2
HA 306-320	LPYQNIHPVTIGECP	0	2
HA 311-325	IHPVTIGECPKYVRS	0	0
HA 316-330	IGECPKYVRSAKLRM	0	0
HA 321-335	KYVRSAKLRMVTGLR	1	0
HA 326-340	AKLRMVTGLRNTPSI	2	0
HA 331-345	VTGLRNTPSIQSRGL	4	1
HA 336-350	NTPSIQSRGLFGAIA	8	3
HA 341-355	QSRGLFGAIAGFIEG	2	2
HA 346-360	FGAIAGFIEGGWTGM	4	5
HA 351-365	GFIEGGWTGMIDGWY	3	1
HA 356-370	GWTGMIDGWYGYHHQ	4	5
HA 361-375	IDGWYGYHHQNEQGS	2	4
HA 366-380	GYHHQNEQGSGYAAD	1	7
HA 371-385	NEQGSGYAADQKSTQ	3	6
HA 376-390	GYAADQKSTQNAING	2	3
HA 381-395	QKSTQNAINGITNKV	1	1
HA 386-400	NAINGITNKVNTVIE	3	1
HA 391-405	ITNKVNTVIEKMNIQ	8	2
HA 396-410	NTVIEKMNIQFTAVG	3	2
HA 401-415	KMNIQFTAVGKEFNK	1	1
HA 406-420	FTAVGKEFNKLEKRM	2	2
HA 411-425	KEFNKLEKRMENLNK	1	1
HA 416-430	LEKRMENLNKKVDDG	0	2
HA 421-435	ENLNKKVDDGFLDIW	0	1
HA 426-440	KVDDGFLDIWTYNAE	2	5
HA 431-445	FLDIWTYNAELLVLL	2	3
HA 436-450	TYNAELLVLLENERT	1	0
HA 441-455	LLVLLENERTLDFHD	2	0
HA 446-460	ENERTLDFHDSNVKN	2	0
HA 451-465	LDFHDSNVKNLYEKV	3	3
HA 456-470	SNVKNLYEKVKSQLK	8	4
HA 461-475	LYEKVKSQLKNNAKE	1	2
HA 466-480	IGNGCFEFYHKCDNE	4	1
HA 471-485	FEFYHKCDNECMESV	2	3
HA 476-490	KCDNECMESVRNGTY	3	1
HA 481-495	CMESVRNGTYDYPKY	3	3
HA 486-500	RNGTYDYPKYSEESK	2	1
HA 491-505	DYPKYSEESKLNREK	1	3
HA 496-510	SEESKLNREKVDGVK	1	1
HA 501-515	LNREKVDGVKLESMG	4	2
HA 506-520	VDGVKLESMGIYQIL	0	1
HA 511-525	LESMGIYQILAIYST	0	0

	Peptide Sequence	Elispot[a]	
HA 516-530	IYQILAIYSTVASSL	2	1
HA 521-535	AIYSTVASSLVLLVS	2	4
HA 526-540	VASSLVLLVSLGAIS	0	0
HA 531-545	VLLVSLGAISFWMCS	0	0
HA 536-550	LGAISFWMCSNGSLQ	1	0
HA 541-554	FWMCSNGSLQCRIC	2	1
HA 546-555	NGSLQCRICI	0	3
NA 1-15	MNPNQKITTIGSICL	0	4
NA 6-20	KITTIGSICLVVGLI	0	0
NA 11-25	GSICLVVGLISLILQ	2	1
NA 16-30	VVGLISLILQIGNII	1	2
NA 21-35	SLILQIGNIISIWIS	8	4
NA 26-40	IGNIISIWISHSIQT	1	3
NA 31-45	SIWISHSIQTGSQNH	0	2
NA 36-50	HSIQTGSQNHTGICN	3	1
NA 41-55	GSQNHTGICNQNIIT	0	4
NA 46-60	TGICNQNIITYKNST	4	3
NA 51-65	QNIITYKNSTWVKDT	2	5
NA 56-70	YKNSTWVKDTTSVIL	4	4
NA 61-75	WVKDTTSVILTGNSS	3	1
NA 66-80	TSVILTGNSSLCPIR	8	3
NA 71-85	TGNSSLCPIRGWAIY	2	4
NA 76-90	LCPIRGWAIYSKDNS	6	1
NA 81-95	GWAIYSKDNSIRIGS	4	3
NA 86-100	IRIGSKGDVFVIREP	7	0
NA 91-105	KGDVFVIREPFISCS	2	0
NA 96-110	VIREPFISCSHLECR	3	0
NA 101-115	FISCSHLECRTFFLT	4	3
NA 106-120	HLECRTFFLTQGALL	2	1
NA 111-125	TFFLTQGALLNDKHS	0	4
NA 116-130	QGALLNDKHSNGTVK	1	1
NA 121-135	NDKHSNGTVKDRSPY	2	0
NA 126-140	NGTVKDRSPYRALMS	0	0
NA 131-145	DRSPYRALMSCPVGE	5	2
NA 136-150	RALMSCPVGEAPSPY	6	2
NA 141-155	CPVGEAPSPYNSRFE	5	1
NA 146-160	APSPYNSRFESVAWS	2	4
NA 151-165	NSRFESVAWSASACH	2	3
NA 156-170	SVAWSASACHDGMGW	3	2
NA 161-175	ASACHDGMGWLTIGI	9	5
NA 166-180	DGMGWLTIGISGPDN	5	4
NA 171-185	LTIGISGPDNGAVAV	2	1
NA 176-190	SGPDNGAVAVLKYNG	2	2
NA 181-195	GAVAVLKYNGIITET	2	3
NA 186-200	LKYNGIITETIKSWR	2	5
NA 191-205	IITETIKSWRKKILR	3	5
NA 196-210	IKSWRKKILRTQESE	1	3
NA 201-215	KKILRTQESECACVN	2	3
NA 206-220	TQESECACVNGSCFT	4	4
NA 211-225	CACVNGSCFTIMTDG	2	2
NA 216-230	GSCFTIMTDGPSDGL	2	3

	Peptide Sequence	Elispot[a]	
NA 221-235	IMTDGPSDGLASYKI	1	6
NA 226-240	PSDGLASYKIFKIEK	3	2
NA 231-245	ASYKIFKIEKGKVTK	0	0
NA 236-250	FKIEKGKVTKSIELN	2	0
NA 241-255	GKVTKSIELNAPNSH	2	1
NA 246-260	SIELNAPNSHYEECS	1	2
NA 251-265	APNSHYEECSCYPDT	1	5
NA 256-270	YEECSCYPDTGKVMC	5	0
NA 261-275	CYPDTGKVMCVCRDN	3	1
NA 266-280	GKVMCVCRDNWHGSN	1	2
NA 271-285	VCRDNWHGSNRPWVS	6	5
NA 276-290	WHGSNRPWVSFDQNL	4	3
NA 281-295	RPWVSFDQNLDYQIG	5	4
NA 286-300	FDQNLDYQIGYICSG	2	3
NA 291-305	DYQIGYICSGVFGDN	8	2
NA 296-310	YICSGVFGDNPRPED	0	2
NA 301-315	VFGDNPRPEDGTGSC	0	5
NA 306-320	PRPEDGTGSCGPVYV	0	3
NA 311-325	GTGSCGPVYVDGANG	0	3
NA 316-330	GPVYVDGANGVKGFS	1	1
NA 321-335	DGANGVKGFSYRYGN	2	2
NA 326-340	VKGFSYRYGNGVWIG	5	3
NA 331-345	YRYGNGVWIGRTKSH	1	4
NA 336-350	GVWIGRTKSHSSRHG	3	2
NA 341-355	RTKSHSSRHGFEMIW	4	2
NA 346-360	SSRHGFEMIWDPNGW	2	1
NA 351-365	FEMIWDPNGWTETDS	2	0
NA 356-370	DPNGWTETDSKFSVR	0	0
NA 361-375	TETDSKFSVRQDVVA	0	3
NA 366-380	KFSVRQDVVAMTDWS	0	2
NA 371-385	QDVVAMTDWSGYSGS	1	2
NA 376-390	MTDWSGYSGSFVQHP	0	1
NA 381-395	GYSGSFVQHPELTGL	0	2
NA 386-400	FVQHPELTGLDCMRP	4	1
NA 391-405	ELTGLDCMRPCFWVE	4	2
NA 396-410	DCMRPCFWVELIRGR	3	3
NA 401-415	CFWVELIRGRPKEKT	2	4
NA 406-420	LIRGRPKEKTIWTS	9	7
NA 411-425	PKEKT IWTSASSIS	3	2
NA 416-430	IWTSASSISFCGVN	3	1
NA 421-435	ASSISFCGVNSDTVD	0	0
NA 426-440	FCGVNSDTVDWSWPD	0	1
NA 431-444	SDTVDWSWPDGAELP	0	2
NA 436-450	WSWPDGAELPFSIDK	0	0
NA 441-450	GAELPFSIDK	1	0

[a] Number of Elispots from 10^5 spleen cells, determined in two independent experiments.

Peptides were screened on mice that had recovered from an x31 infection. The regions containing the known Class I and Class II epitopes are indicated by shading and peptides investigated further are in bold.