Precursor Frequency and Competition Dictate the HLA-A2–Restricted CD8+ T Cell Responses to Influenza A Infection and Vaccination in HLA-A2.1 Transgenic Mice

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Precursor Frequency and Competition Dictate the HLA-A2–Restricted CD8+ T Cell Responses to Influenza A Infection and Vaccination in HLA-A2.1 Transgenic Mice

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The human HLA-A2–restricted CD8+ T cell response to influenza A virus (IAV) is largely directed against the matrix protein–derived M158–66 epitope and represents an archetypal example of CD8+ T cell immunodominance. In this study, we examined the CD8+ T cell hierarchy to M158–66 and two subdominant IAV-specific epitopes: NS1 122–130 and PA 46–55 in HLA-A2+ human subjects and HLA-A2.1 transgenic (HHD) mice. Using epitope-based lipopeptides, we show that the CD8+ T cell hierarchy induced by IAV infection could also be induced by lipopeptide vaccination in a context outside of viral infection when the Ag load is equalized. In the HHD HLA-A2.1 mouse model, we show that the naive T cell precursor frequencies, and competition at the Ag presentation level, can predict the IAV-specific CD8+ T cell hierarchy. Immunization of mice with subdominant epitopes alone was unable to overcome the dominance of the M1 58–66–specific response in the face of IAV challenge; however, a multiepitope vaccination strategy was most effective at generating a broad and multispecific response to infection. The Journal of Immunology, 2011, 187: 1895–1902.

The CD8+ T cell response plays a central role in the control and clearance of virus after acute infection. Although the viral genome potentially encodes a multitude of target epitopes, the CD8+ T cell response postinfection is often focused on one or a few epitopes in a phenomenon known as immunodominance (1, 2). Numerous processes contributing to the generation of CD8+ T cells are implicated in the expression of immunodominance hierarchies, which can be broadly divided into those factors concerning Ag presentation such as protein abundance, epitope processing and MHC binding (3–5), T cell factor TCR avidity (6, 7), and naive T cell precursor frequency (8–11). Competition between CD8+ T cells, where the presence of a dominant T cell population hinders the development or activation of subdominant populations (12, 13), is known as immunodominance. Immunodominance is thought to be a result of CD8+ T cell competition for access to the limited resources of peptide–MHC complexes, cell-bound costimulatory molecules present on APCs, and CD8+ T cell competition for soluble factors including cytokines such as IL-12 (14–18).

CD8+ T cell immunodominance hierarchies are maintained across inbred mouse populations and also in humans with similar or matching HLA phenotypes (2, 19, 20). An archetypal example of immunodominance is seen in the human HLA-A2–restricted response to influenza A virus (IAV) where CD8+ T cell responses to the virus are predominantly targeted to the M158–66 epitope derived from the matrix 1 protein (M1) (20–23). This epitope also constitutes the immunodominant response to IAV infection observed in HLA-A2.1 transgenic mice (24). In contrast, other HLA-A2–restricted epitopes such as NS1 122–130 (25) and PA 46–55 (21) have been identified, but the response to these epitopes is weaker and less frequently detected than are those to M1 58–66 (21, 26, 27). The abundance of the M1 and high binding affinity of the M1 58–66 epitope for HLA-A2 (21) are consistent with the observed immunodominance of this epitope, but correlation does not necessarily imply causation, and the factors governing the dominance of this M158–66–based immune response have yet to be determined. The naive precursor frequencies of Ag-specific CD8+ T cells are also major determinants of the response to viral infection (8, 9); however, detecting these naive populations in humans is difficult because of the extensive exposure of the human population to influenza A infection.

In this study, we have used HLA-A2.1 transgenic (HHD) mice that express a chimeric monochain of HLA-A2.1 to examine the hierarchy of the CD8+ T response to natural influenza A infection and also to epitope-based lipopeptide (LP) candidate vaccines. Using these mice, we hoped to illuminate some of those factors that dictate the CD8+ T cell hierarchy and immunodominance of the M158–67 response. We also examined the effect of epitope-based vaccination on the hierarchy of CD8+ T cell responses during IAV infection. By examining the Ag presentation requirements and naive T cell precursor frequencies, we present an explanation for the remarkable immunodominance of M1 58–67 in the CD8+ T cell response to IAV and, as we reveal, to vaccination.

Materials and Methods

Peptides and LPs

Peptides and LPs were synthesized using conventional solid-phase and Fmoc chemistry with an automated synthesizer (Symphony/Multiplex, Protein Technologies, AZ), as described previously (28). LPs consisted of OT2 (ISQAVHAAHAEINEAG), a helper CD4+ T cell epitope (29) linked to a CD8+ T cell epitope through an intervening lysine residue. From the ε-amino group of the lysine, two serine residues separated a
pamP2Cys lipid moiety from the peptide chain comprising the Th and CD8+ T cell epitopes. The peptides used were GILGFVFTL (M13-26), AIMDKNIIIL (NS1122-130), and FMYSDFHFI (PA46-55). The peptides and their corresponding LPs, M1-LP, acid polymerase (PA)-LP, and non-structural 1 protein (NS1)-LP were synthesized and purified by reverse phase HPLC using a Vydac pre-packed C4 column (4.6 × 250 mm) installed in a Waters HPLC system. Mass spectrometry was performed to verify fidelity of the products using an Agilent 1100 Series ion trap mass spectrometer (Agilent Technologies, Santa Clara, CA).

**Human blood samples**

Human peripheral blood mononuclear cells (PBMCs) were obtained from buffy coat preparations (Australian Red Cross Blood Service, Parkville, Melbourne, VIC, Australia), and blood samples were obtained from healthy human volunteers from the Department of Microbiology and Immunology, University of Melbourne. Written informed consent was obtained from all donors before blood collection. Subjects were identified as HLA-A*27 by molecular typing (Rotary Bone Marrow Research Centre of the Australian Red Cross Blood Service) or by serological typing using FITC-labeled anti-human HLA-A2 Abs (BD Pharmingen).

The recruitment of donors and all experimental procedures using human blood-derived products were approved by the University of Melbourne Human Research Ethics Committee.

**Preparation of single-cell suspensions**

Spleens were collected from blood using Ficoll-Paque (15 ml; Amersham Pharmacia, Sweden) as described previously (30). PBMCs were resuspended in FCS (CSL, Parkville, VIC, Australia) containing 10% DMSO and frozen in liquid nitrogen until required.

**IFN-γ-detecting ELISPOT assay**

Cryopreserved PBMCs were thawed and counted by trypan blue dye exclusion and then 2–3 × 10^6 viable cells were incubated with 10 μg/ml CTL peptide epitope on membrane-based 96-well plates (MAIPS4510-Millipore, North Ryde, NSW, Australia), which were coated with anti-human IFN-γ capture Ab (1-D1K; Mabtech, Mosman, NSW, Australia) for 18 h at 37°C in 5% CO2. Cells secreting IFN-γ were detected using biotinylated mouse anti-IFN-γ detection Ab (clone 7-B6-1; Mabtech) as described elsewhere (31). Four wells that did not contain peptide epitope were included as negative controls. Spots formed by the deposition of enzyme substrate were counted using an ELISPOT plate reader (AID Autoimmune Diagnostics, Strasbourg, France) and analyzed using the AID software. The results expressed as spot-forming units (SFU) were calculated by subtracting the sum of the background value plus two SD. Responses were considered positive when the net SFU value was >20 SFU/10^6 PBMCs.

**Enrichment of naive CD8+ T cells**

Female HHD mice aged between 6 and 12 wk were used. HHD mice express HLA-A2.1 transgenic mice and were kindly supplied by Professor Andreas Suhrbier (Queensland Institute of Medical Research, Herston, QLD, Australia). Mice were bred and maintained in the Animal House Facility, Department of Microbiology, University of Melbourne. All procedures performed conformed to guidelines issued by the National Health and Medical Research Council of Australia and were approved by the University of Melbourne Animal Experimentation Ethics Committee.

**LP inoculation and IAV challenge**

Mice were anesthetized by inhalation of Penthrane or isoflurane before intranasal (i.n.) administration of either 25 μm M1-LP, PA-LP, or NS1-LP PBMCs, or 75 μm of a mixture of the three LPs in equimolar amounts (3LP). LPs were dissolved in saline and administered as 50 μl to anesthetized mice. Challenge with IAV was carried out by i.n. administration of 10^5 PFU Mem virus (a genetic reassortant of A/Memphis/1/71 [H3N2] and A/Queensland/27/1996 H1N1 influenza virus strains) in 50 μl PBS to cervical or brachial lymph nodes of individual mice.

**Preparation of bone marrow-derived dendritic cells**

Bone marrow was isolated from the femurs and tibias of HHD mice, and cells were cultured with DMEM (DMEM [Life Technologies] containing 10% FCS and supplements). Media were further supplemented with 10% and 5% heat-inactivated GM-CSF (35). On days 6, 8, and 10, half-media changes with Ag86 supernatant and DMEM sup media were made. On day 11, the nonadhesive cell population (representing the dendritic cells [DCs]) was harvested and then plated at a concentration of 3 × 10^6 cells in 3 ml DMEM media supplemented with Ag86SN in a six-well plate. DCs were then pulsed with LP (final concentration of 5 mmol LP/ml) for 24 h and then washed thoroughly. DCs (1 × 10^6) were resuspended in 150 μl PBS and administered by i.v. route to three different treatment groups of HHD mice. The DC1LP group received DCs pulsed with 3LP. The DCmus group received DCs pulsed with each of the LPs individually and then pooled together; DC1LP represents three groups of mice that received a single population of DCs pulsed with a single LP. Spleen cells were harvested from recipient mice 10 d after transfer and analyzed by ICS for epitope-specific CD8+ T cells. The epitope-specific response is representative of the total measured response to normalize the responses between the different treatment groups. This was calculated using the following formulae: for DCmus and DC1LP groups, (number of epitope-specific cells/sum of M1-, PA-, and NS1-specific cells) × 100; and
for the baseline DC,LP group, (number of epitope-specific cells/average number of M1-, PA-, and NS1-specific cells) × 100.

Statistical analysis

Statistical analysis was carried out using GraphPad Prism software. Differences were considered significant when p values were <0.05 with a 95% confidence level.

Results

The IAV-specific response in HLA-A2+ human donors

PBMCs obtained from 12 healthy HLA-A2+ donors were incubated with individual peptides representing the M138-66 (M1), PA46-55 (PA), or NS1122-130 (NS1) epitopes, and the responses to each epitope were determined by IFN-γ ELISPOT assay. In agreement with its reported immunodominance (20, 21), the M1 epitope was recognized by each of the 12 donors, whereas only half of the subjects responded to PA or NS1 (Fig. 1). This pattern was reflected at the individual level, with 11 of the donors exhibiting higher M1-specific responses, confirming the dominance of the M1 response with respect to two other IAV-specific epitopes.

These results indicate a distinct hierarchy of Ag-experienced influenza A–specific T cells but do not address the issues of how and at what stage in CD8+ T cell activation such immunodominance hierarchies emerge. We therefore analyzed the IAV-specific response in HLA-A2.1 transgenic mice to identify the processes that contribute to the development of immunodominance.

The IAV-specific response in HLA-A2.1 transgenic mice

HHD mice were challenged with a sublethal dose of H3N1 Mem virus, and CD8+ T cell responses to M1, PA, and NS1 epitopes were examined by measuring IFN-γ production by an ICS assay. The IAV-specific CD8+ T cell response in HHD mice was found to be reminiscent of the response in human donors where M1 was immunodominant and the responses to PA and NS1 were substantially and significantly lower (p < 0.0001; Fig. 2). This is, to our knowledge, the first time that such a hierarchy of responses to these three epitopes has been demonstrated in HHD mice. When expressed as a percentage of the IAV-specific response, the M1 response comprised 95% of the response. A similar CD8+ T cell hierarchy was also reflected in the lungs of infected mice (data not shown).

Effect of Ag dose on immunodominance hierarchy

Despite numerous reports on the immunodominance of the M1 response, little is known about the factors that govern the preferential recognition of this epitope. Epitopes derived from the more abundant nucleoprotein and M1 of IAV have been found to contribute more frequently to immunodominant responses (36), indicating that perhaps protein abundance may underlie the immunodominance of this epitope.

To determine whether the immunodominance hierarchy is driven by differences in Ag dose, we examined the effect on the hierarchy of the CD8+ T cell response by equalizing epitope dose. To achieve this, we used Pam2Cys-based LPs, synthetic epitope-based immunogens that are capable of inducing specific CD8+ T cell responses against numerous infectious targets including IAV (30, 37, 38). We synthesized three LPs containing the M1, PA, or NS1 CD8+ T cell epitope (Fig. 3A) and admixed these in equal quantities to generate the immunogen, 3LP. We also administered each LP separately to determine whether CD8+ T cell priming is affected by the presence of other epitopes, and consequently whether the subdominance of the PA or NS1 responses is the result of immunodominance in the presence of the M1 epitope.

The results (Fig. 3B) demonstrate that in HHD mice inoculated with 3LP, the CD8+ T cell hierarchy was similar to that seen after natural IAV infection, indicating that Ag load alone is not responsible for the immunodominance hierarchy. When LPs were delivered individually, although the M1 response was still dominant, the PA- and NS1-specific responses were considerably and significantly (p = 0.0353 for PA, p = 0.009 for NS1) higher than when these epitopes were codelivered with M1 (Fig. 3B). These findings demonstrate that the PA and NS1 responses are subject to immunodominacy by the M1 response, and that immunodominance amplifies M1 dominance.

The frequency of naive CD8+ T cells predicts the immunodominance hierarchy

Having ruled out epitope load as a major contributor to the CD8+ T cell hierarchy, we considered the possibility that there could be differences in the naive CD8+ T cell precursor frequencies of the M1, PA, or NS1 populations. These populations were identified using a novel tetramer-based enrichment strategy (39) that has been modified to allow the direct enumeration of naive epitope-specific CD8+ T cells in mice (8, 34). This technique was used to enumerate the M1, PA, or NS1 Ag-specific CD8+ T cell populations. In naive HHD mice, we identified a significantly higher
number of precursors to the M1 epitope than to the PA (p < 0.0015) or NS1 (p < 0.0016) epitopes (Fig. 4). The M1-specific precursor frequency was, on average, 6.2 times more abundant than those of PA- or NS1-specific cells. Although the numbers of PA and NS1 naive CD8+ T cells often fell below the accepted limit of detection of five precursors per mouse, these results nonetheless highlight the significantly greater number of M1 precursors. This technique was applied to mice previously infected with IA V to identify distinct populations of M1, PA, or NS1-tetramer–positive cells. The frequency of B220+CD11b+CD11c+CD4+CD8+CD62L+ naive epitope-specific cells was determined in individual naive mice and expressed per 107 CD8+ T cells. Individual symbols indicate the cells detected in an individual mouse; p values indicate significant differences between the frequencies of epitope-specific population.

**FIGURE 3.** 3LP versus individual LP inoculation. LPs are branched peptide structures incorporating a CTL epitope, the OT2 Th epitope, and a pendant Pam2Cys lipid moiety (A). HHD mice were inoculated with 75 nmol 3LP or 25 nmol of the M1- LP, PA-LP, or NS1-LP via the i.n. route. On day 7 after inoculation, the spleens of mice were harvested and M1-, PA-, and NS1-specific IFN-γ-producing CD8+ T cells were detected ex vivo by ICS assay (B). The peptide-specific response as a percentage of the LP-induced response is indicated by the pie charts. Bars show the average response for each inoculation group (n = 3), and error bars indicate the SD. The p values indicate significant differences between the responses as indicated by connecting lines (two-tailed, Student t test). Results were obtained from a single experiment and are representative of three repeat experiments.

**FIGURE 4.** Frequencies of M1-, PA-, and NS1-specific CD8+ T cell precursors in naive HHD mice. Epitope-specific CD8+ T cells were isolated from the spleen and all major lymph nodes of naive or IA V-infected HHD mice using magnetic enrichment of M1, PA, or NS1-tetramer–positive cells. The frequency of B220+CD11b+CD11c+CD4-CD8+CD62L+ naive epitope-specific cells was determined in individual naive mice and expressed per 107 CD8+ T cells. Individual symbols indicate the cells detected in an individual mouse; p values indicate significant differences between the frequencies of epitope-specific population.

different combinations of LP to study the effect of differential Ag presentation on CD8+ T cell priming. We examined three different scenarios of APC to CD8+ T cell encounters. First, DCs were pulsed with a single LP and then administered to individual groups of mice. This scenario establishes the baseline CD8+ T cell response that is elicited by a single epitope in the absence of cellular competition and is collectively referred to as DC_LP. Second, three populations of DCs were individually pulsed with either the M1-LP, PA-LP, or NS1-LP; then the pulsed cells were mixed together (DC_mix) to represent a situation where competition might occur directly between CD8+ T cells for access to non-APC–bound soluble factors, but not for surface-bound peptide–MHC complexes or cell-bound costimulatory factors bound on the APC surface. Third, DCs were pulsed simultaneously with an equimolar mixture of the 3LP to produce a population of Ag-loaded DCs (DC_3LP) where competition could occur at all levels of Ag presentation including peptide loading onto MHC molecules and naive CD8+ T cell access to APCs.

The various preparations of LP-pulsed DCs were transferred i.v. into separate groups of mice, and the CD8+ T cell responses were examined ex vivo on day 10 after cell transfer. No responses were detected in mice injected with supernatant obtained from the final wash of the DCs pulsed with 3LP, confirming that LP had been effectively removed during the washing procedure (data not shown).

The results (Fig. 5) show that in DC_LP baseline group that received a single LP, the M1 response was, on average, 6- and 4-fold greater than PA and NS1, respectively; a result that could be predicted based on the number of naive CD8+ T cell precursors and in the absence of external competitive pressures. When compared with this baseline group, the DC_mix group displayed comparable magnitudes and differences between the M1 and PA or NS1 responses, suggesting that negligible competition occurs directly between the CD8+ T cells, and that the subdominant responses can be generated maximally provided that the different epitopes have an adequate opportunity to be presented by DCs. In contrast, when M1, PA, and NS1 LPs were simultaneously exposed to DC, the subdominant responses were substantially suppressed and the M1 response in the DC_3LP group was, on average, 18- and 35-fold greater than the PA and NS1 responses, respectively. Therefore, it is when all epitopes are simultaneously exposed to the APC that the subdominant population is suppressed, indicating that competition is occurring at the Ag pre-
apparent that prevaccination with PA-LP and NS1-LP increased (Fig. 6). was a distinct M1 dominance in the response to viral challenge population. In groups that received either 3LP or M1-LP, there outcome of vaccination with these epitopes. It is, expression in the human population also suggests that a high level associated with pandemic infection. The prevalence of HLA-A2 reactivity of M1- and NS1-specific CD8+ T cell responses to PA, and NS1-based vaccines might be effective against multiple avian or swine origin viruses in vitro (27, 36, 43) suggest that M1, repertoire of the CD8+ T cell responses to a subsequent IA V inoculation on the populations, we examined the impact of LP inoculation on the early and group that received PBS was included as a representative of the LP, or 3LP, and challenged with Mem virus 30 d later. A control challenge. HHD mice were inoculated with M1-LP, PA-LP, NS1- specific IFN-γ-producing CD8+ T cell responses were examined in each treatment group. The bars indicate the epitope-specific response as a percentage of the total measured response (mean ± SD). The relative differences between the M1 and the PA or NS1 responses in each group are displayed above each bar. The p values indicate significant differences between each group for the given epitope specificity (one-way ANOVA analysis and post hoc Dunnett’s multiple-comparison test). The results shown were obtained from a single experiment (n = 3) and are representative of two separate experiments.

Inoculation with epitopes eases but does not overcome M1 dominance

The high level (>90–100%) of conservation in the PA and M1 epitopes across IAV subtypes (21, 42) and the observed cross-reactivity of M1- and NS1-specific CD8+ T cell responses to avian or swine origin viruses in vitro (27, 36, 43) suggest that M1, PA, and NS1-based vaccines might be effective against multiple subtypes of virus and against newly emerging viruses that are associated with pandemic infection. The prevalence of HLA-A2 expression in the human population also suggests that a high level of population coverage could be afforded by such vaccines. It is, therefore, of interest to study the effects of competition on the outcome of vaccination with these epitopes.

To determine whether we could alter the immunodominance hierarchy by expanding the subdominant PA and NS1 CD8+ T cell populations, we examined the impact of LP inoculation on the repertoire of the CD8+ T cell responses to a subsequent IAV challenge. HHD mice were inoculated with M1-LP, PA-LP, NS1-LP, or 3LP, and challenged with Mem virus 30 d later. A control group that received PBS was included as a representative of the natural response in “unvaccinated” mice. To track the early and late responses in IAV infection, we examined the CD8+ T cell response at days 5 and 10 postinfection.

On day 5 after viral challenge, it was necessary to perform in vitro restimulation of the spleen cells to detect the subdominant responses that were present (in particular, in the PBS groups) only at very low frequencies. As a result, the epitope-specific CD8+ T cell responses are expressed as a percentage of the CD8+ T cell population. In groups that received either 3LP or M1-LP, there was a distinct M1 dominance in the response to viral challenge (Fig. 6A). In mice inoculated with PA-LP and NS1-LP, it was apparent that prevaccination with PA-LP and NS1-LP increased the size of the PA- and NS1-specific responses, respectively, and at this time point, PA or NS1 constituted the dominant population. The responses seen at day 5 indicate that expansion of subdominant CD8+ T cell populations before viral challenge enables their early emergence in the face of infection, though these populations do not expand to the same extent as M1-specific cells in M1-LP vaccinated mice.

At day 10 postinfection, responses were similar in both spleen and lung, and for simplicity only, the responses obtained from the lungs are shown. At this later stage of infection, there was no significant difference in the magnitude of the total measured CD8+ T cell response, and M1 dominance was apparent in all treatment groups (Fig. 6B). In the PA-LP and NS1-LP groups, though there was an increased relative contribution to PA and NS1, respectively (Fig. 6C), these populations did not expand sufficiently to shift M1 dominance late in infection. These findings indicate that the M1-dominated hierarchy is eventually established during infection and cannot be offset or overcome by prior vaccination. The observation that there was a generally broader response to all three epitopes in the 3LP group compared with the single epitope vaccination groups suggests that, despite competition at the time of priming, a multispecific response is best established by multiepitope vaccination.

![FIGURE 5](image)

**FIGURE 5.** CD8+ T cell responses generated under potentially competitive conditions. HHD mice were injected with bone marrow-derived DCs i.v. pulsed with a single LP (DCLP, the baseline group), an admixture of DCs that had been pulsed with each of the LPs individually then pooled together (DCmix), or DCs pulsed with the three LPs simultaneously (DC3LP). Ten days after transfer of the DCs, the M1-, PA-, and NS1-specific IFN-γ-producing CD8+ T cell responses were examined in each treatment group. The bars indicate the epitope-specific response as a percentage of the total measured response (mean ± SD). The relative differences between the M1 and the PA or NS1 responses in each group are displayed above each bar. The p values indicate significant differences between each group for the given epitope specificity (one-way ANOVA analysis and post hoc Dunnett’s multiple-comparison test). The results shown were obtained from a single experiment (n = 3) and are representative of two separate experiments.

![FIGURE 6](image)

**FIGURE 6.** Impact of LP inoculation on IAV-specific responses after challenge with live virus. HHD mice were inoculated i.n. with either 75 nmol 3LP, 25 nmol M1-LP, PA-LP, or NS1-LP, or PBS (n = 3/group), and 1 no later, mice were challenged i.n. with 10^4.5 PFU H3N1 Mem virus. A. On day 5 postinfection, the spleen cells were harvested and restimulated for 6 d by in vitro culture in the presence of each individual peptide. The M1-, PA-, and NS1-specific IFN-γ-producing CD8+ T cells were detected using ICS assay. On day 10 postinfection, the M1-, PA-, and NS1-specific IFN-γ-producing CD8+ T cells in the lung were detected ex vivo by ICS (B). C. Excerpt highlights the PA- and NS1-specific lung responses at day 10. The bars show the average response to each epitope for each inoculation group, and the error bars indicate the SD. The p values indicate significant differences in the total response to the PBS group. Asterisk indicates significant differences in the PA-specific response (PA-LP: *p < 0.05 versus all groups, one-way ANOVA and post hoc Dunnett’s multiple-comparison test). Results shown were obtained from a single experiment and are representative of two separate experiments.
Discussion

It has been widely observed that the HLA-A2–restricted T cell response to IAV in humans is characterized by an immunodominant M1\textsubscript{58–66}–specific response (20–22). The observation is not perhaps surprising given the abundance of the M1 protein and the high affinity of binding between HLA-A2 and the M1\textsubscript{58–66} epitope (21). The determinants for the dominance of the M1 response have, however, yet to be fully explained. In this study, we have demonstrated that the influenza A–specific CD8\(^+\) T cell hierarchy is similar in human HLA-A2\(^+\) subjects and HHD mice. Moreover, this hierarchy is also apparent outside of the viral context and when the epitope load is equalized, suggesting that the response is dictated by the host’s ability to respond to these epitopes. Although HLA peptide-binding affinity may be a strong correlate for immunogenicity (5) in some cases, we observed little association between the magnitude of the CD8\(^+\) T cell response and the reported HLA-A*0201 binding affinities of the epitopes that we used (IC\textsubscript{50} of 6.6 nM for M1, 3.5 nM for PA, and 260 nM for NS1) (21, 25). In this study, we demonstrate that precursor frequencies of naive M1-, PA-, and NS1-specific CD8\(^+\) T cells are the primary predictors for the hierarchy of the CD8\(^+\) T cell responses observed for influenza A infection and 3LP vaccination. This observation is in line with other studies that have demonstrated an association between precursor frequency and CD8\(^+\) T cell responses to lymphocytic choriomeningitis and vesicular stomatitis viral infections (8, 9).

We also observed that T cell competition contributes to subdominance of the PA and NS1 responses after vaccination, thereby exaggerating the CD8\(^+\) T cell hierarchy and consequently affecting the recall response to infection. Immunodominance of the subdominant CD8\(^+\) T cells populations occurred when epitopes were simultaneously exposed to DCs, suggesting that competition occurs at the level of Ag presentation favoring M1 priming over that of PA and NS1. Therefore, it is possible that competition between epitopes occurs during Ag cleavage and liberation of epitope by the proteosome and/or MHC class I loading of peptide by TAP and tapasin, processes that affect overall peptide–MHC levels, availability, and ultimately, T cell priming.

Several studies have established that although HLA binding affinity is important, it is not the sole determinant in immunodominance (14, 44). The NS1 response, for example, was severely impacted by the presence of M1, a finding that fits well with a lower MHC loading capacity of this low-affinity epitope (25). An explanation for the preferential MHC loading of M1 at the expense of PA and the consequent immunodomination when presented by the same APC could be explained by the fact that peptides have differing affinities for TAP transporters (45, 46), as well as for Tapasin (47, 48).

Although we have not measured the T cell avidity of M1 and PA responses, the competition observed when peptides are presented by the same APC may also be a consequence of characteristics of the responding T cell population. It has been shown that immunodominant CD8\(^+\) T cells can experience faster TCR–peptide–MHC on rates, and that this results in more rapid priming of T cells (49). If we assume that M1–specific CD8\(^+\) T cells exhibit a faster on rate, then such cells could more rapidly and preferentially engage peptide–MHC complexes on the APC surface, potentially obscuring access of PA–specific CD8\(^+\) T cells to the APC. This could result in PA domination when the peptide–MHC complex is present on the same APC.

Although speculative, these ideas do suggest a situation where subdominant and immunodominant responses are shaped by inherent features of the epitope itself, as well as cellular process-
Disclosures
The authors have no financial interests of interest.

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