Antigen-Specific CD8+ T Cells Persist in the Upper Respiratory Tract Following Influenza Virus Infection

James A. Wiley, Robert J. Hogan, David L. Woodland and Allen G. Harmsen

J Immunol 2001; 167:3293-3299; doi: 10.4049/jimmunol.167.6.3293
http://www.jimmunol.org/content/167/6/3293

References
This article cites 20 articles, 11 of which you can access for free at: http://www.jimmunol.org/content/167/6/3293.full#ref-list-1

Subscription
Information about subscribing to The Journal of Immunology is online at: http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Antigen-Specific CD8+ T Cells Persist in the Upper Respiratory Tract Following Influenza Virus Infection

James A. Wiley, Robert J. Hogan, David L. Woodland, and Allen G. Harmsen

Because little is known about lymphocyte responses in the nasal mucosa, lymphocyte accumulation in the nasal mucosa, nasal-associated lymphoid tissue (NALT), and cervical lymph nodes (CLN) were determined after primary and heterosubtypic intranasal influenza challenge of mice. T cell accumulation peaked in the nasal mucosa on day 7, but peaked slightly earlier in the CLN (day 5) and later (day 10) in the NALT. Tetrameric staining of nasal mucosal cells revealed a peak accumulation of CD8 T cells specific for either the H-2Dβ influenza nucleoprotein epitope 366–374 (DβNP366) or the H-2Dβ polymerase 2 protein epitope 224–233 (DβPA224) at 7 days. By day 13, DβPA224-specific CD8 T cells were undetectable in the mucosa, whereas DβNP366-specific CD8 T cells persisted for at least 35 days in the mucosa and spleen. After heterosubtypic virus challenge, the accumulation of CD8 T cells in the nasal mucosa was quicker, more intense, and predominately DβNP366-specific relative to the primary inoculation. The kinetics and specificity of the CD8 T cell response were similar to those in the CLN, but the responses in the NALT and spleen were again slower and more protracted. These results indicate that similar to what was reported in the lung, DβNP366-specific CD8 T cells persist in the nasal mucosa after primary influenza infection and predominate in an intensified nasal mucosal response to heterosubtypic challenge. In addition, differences in the kinetics of the CD8 T cell responses in the CLN, NALT, and spleen suggest different roles of these lymphoid tissues in the mucosal response.

The functional relationship of these induction and effector sites parallels that of the Peyer’s patch and lamina propria, respectively, of gut-associated lymphoid tissue (GALT). In the absence of Ag, the NALT is predominantly a naive immunological site, lacking germinial center formation and consisting of unswitched IgM+ and IgD+ B cells (4) and naive Th0 T cells (2). Following infection, the generation of an Ag-specific mucosal IgG and IgA isotype-specific humoral response in the NALT and nasal mucosa suggest that localized regulatory mechanisms involving CD4 T cells and B cells at these induction and effector sites are continually active (4). In contrast, the generation and regulation of an Ag-specific cell-mediated immune response at these sites in the upper airways following infection are far less understood.

The CTL responses directed against respiratory viruses are involved in the clearance of viruses from the lung (5, 6). Fas- and perforin-mediated mechanisms are the primary means by which viral-specific CTL attack and destroy infected target cells (7). To date, all the H-2Dβ-restricted influenza-specific CD8 T cells recognize specific peptide sequences derived from the internal proteins of the virus (8). The H-2Dβ nucleoprotein epitope (DβNP366) is recognized by ~12% of the CD8 T cells recovered from the bronchoalveolar lavage during the response to primary influenza virus infection (9, 10). The frequency of these DβNP366-specific CD8 T cells increases to ~60% in secondary responses (9). A second H-2Dβ-restricted virus-specific epitope derived from the polymerase 2 protein (DβPA224) has recently been shown to account for ~12–15% of the CD8 T cells in the lungs following a primary influenza infection (8). The frequency of the DβPA224-specific CD8 T cells is diminished relative to the DβNP366-specific CD8 T cells in a secondary response within the lungs (8).

The kinetics of the influenza-specific CTL response in the nose remains to be determined, despite the fact that the first susceptible tissue that this pathogen comes into contact with is the nasal mucosa. In this study, we present a kinetic analysis of the virus-specific CTL response at the mucosal effector sites and in the associated lymphoid induction sites of the nasal mucosa following both a primary influenza virus infection and a secondary infection...
by a heterosubtypic influenza virus strain. Development of influenza-specific cell-mediated resistance and establishment of the corresponding memory response in the mucosal effector sites and associated lymphoid induction sites of the upper respiratory tract (URT) were examined in detail for the first time using viral epitope-specific MHC class I tetramers. The data in this study show that the recruitment of D\(^{\text{NP}}\)_366-specific and D\(^{\text{PA}}\)_224-specific CD8 T cells into the nasal mucosa following a primary intranasal infection was analogous to the recruitment of these same effector cells into the lung following pulmonary influenza infection (10). In addition, these D\(^{\text{NP}}\)_366-specific CD8 T cells persisted in the nasal mucosa and were the dominant subset of virus-specific CD8 T cells responding to the secondary heterosubtypic influenza challenge. In both the primary and secondary infections, CD8 T cells were found to localize in the lamina propria and the intraepithelial lymphocyte compartment of the respiratory epithelium in the nasal mucosa. The results of this study encourage the continual evaluation of the ability of the CD8 T cell-mediated response in the nasal mucosa to limit the transmission of an influenza infection of the URT into the lungs.

**Materials and Methods**

**Mice**

Male and female C57BL/6 mice, 6–8 wk of age, were used for all experiments in this study. These animals were obtained from the Trudeau Institute animal breeding facility (Saranac Lake, NY).

**Viruses**

Influenza viruses A/PR/8/34 (PR8; H1N1) and A/HKx31 (X31; H3N2) were grown in the allantoic fluid of 10-day-old embryonated chicken eggs. The infected eggs were incubated for 48 h at 35°C. The allantoic fluid was then harvested and stored at −70°C until use.

**Immunization and challenge of mice**

Infectious influenza virus was used for immunization and challenge doses. Primary responses were induced by inoculating the mice with 5 \(\mu\)l nostril of allantoic fluid containing 5 \(\times\) 10^6 PFU X31 influenza virus. The mice were lightly anesthetized with halothane and allowed to inhale the inoculum directly into each nostril. For challenge experiments, the mice were given the same primary immunization and then challenged 35 days later. The mice were challenged with 5 \(\mu\)l nostril of allantoic fluid containing 2 \(\times\) 10^6 PFU PR8 influenza virus. The PR8 (H1N1) and X31 (H3N2) influenza strains share the same internal proteins, whereas their surface influenza hemagglutinin and neuraminidase proteins are different serotypes (9).

**Tissue sampling**

Following primary immunization or secondary challenge, five mice were taken for each time point. These mice were placed under deep halothane anesthetization and exsanguinated by perforation of their abdominal aorta. The spleen, left and right superficial cervical lymph nodes (CLN), NALT tissue, and the nasal mucosa region were taken for FACS analysis. The superficial CLN are located beneath the skin on the surface of the superficial portion of the masseter muscle (11). These lymph nodes have also been referred to as the mandibular lymph nodes (12). To reach the NALT and nasal mucosa, the head was removed from the body, and the skin was peeled off the head. The lower jaw and associated muscle and connective tissue were cut off to reveal the soft palate of the upper jaw. The NALT was obtained by peeling off the soft palate from the upper jaw. The bilateral strips of NALT tissue lay on the nasal or posterior surface of the soft palate. Separation of the rostrum from the head posterior to the eyes and removal of the front incisors were performed to isolate the nasal mucosa region. Each tissue was passed through a mesh screen to obtain a single-cell suspension. The cells were counted, washed, and resuspended at 10^7/mL. A 100-\(\mu\)l aliquot was used for FACS analysis of each tissue.

**Virus quantification assay**

On days 0, 5, 7, 10, 13, and 31 after primary infection or secondary challenge (day 3) was included for the secondary infections), the amount of influenza virus present in the nasal mucosa and lungs of the infected animals was quantified by plaque assay on Madin–Darby canine kidney cells as previously described (13). Viral recovery was calculated as the total amount of virus recovered from the nasal mucosa or lungs. The limit of detection in this assay was 20 PFU.

**D\(^{\text{NP}}\)_366 and D\(^{\text{PA}}\)_224 tetramers and FACS analysis**

H-2D\(^{\text{c}}\)-restricted, virus-specific CD8 T cells that recognize the influenza nucleoprotein peptide, NP\(_{366-374}\) (ASSENEMETH), or the influenza polymerase-2 peptide, PA\(_{224-232}\) (SSLNFRAYV), were detected by FACS analysis using PE-conjugated tetrameric complexes. These tetrameric complexes were synthesized by the Molecular Core Unit at the Trudeau Institute according to previously described procedures (14). T lymphocytes were also stained with FITC-conjugated anti-mouse CD4 and CyChrome-conjugated anti-mouse CD8\(\alpha\) (BD Pharmingen, San Diego, CA). The data were acquired on a FACSscan and then analyzed using CellQuest software (BD Pharmingen).

**Immunohistochemical staining of nasal passage**

Nasal tissue sections were cut from the rostrum of infected and noninfected mice. The rostrum was cut from the head of the animal, fixed in periodate-lysine-parafomaldehyde for 24 h at 4°C, and then decalcified over a 10–12 day period at −5°C in an EDTA/glycerol (12/15%, w/v) solution as described by Mori et al. (15). Once the tissues were sufficiently decalcified, they were sequentially rinsed in PBS/glycerol solutions of 15, 10, 5, and 0% glycerol over 8 h. The rostroms were then impregnated with Tissue-Tek OCT embedding medium (Miles, Elkhart, IN) at increasing concentrations of 10, 25, 50, 70, and 100% under a vacuum. The tissues were then frozen in 100% OCT over liquid N\(_2\) and stored at −70°C. Tissue sections of 5 \(\mu\)m were cut from the frozen blocks, placed on silane (Sigma, St. Louis, MO)-coated slides, and air-dried. The sections were blocked with an avidin-biotin blocking kit (Vector Laboratories, Burlingame, CA), followed by rat serum. The sections were then incubated with biotinylated anti-mouse CD8\(\alpha\) (TIB210, Trudeau Institute). The location of the bound Ab was revealed with the avidin-biotin-peroxidase kit with 3,3'-diaminobenzidine as a substrate (Vector Laboratories). The sections were then counterstained with methyl-green.

**Results**

**Viral clearance**

We have previously observed in our intranasal infection models (data not shown) and those of others (16) that the use of a small volume of inoculum reduced the direct deposition of the inoculum into the lung and thereby more closely mimicked the descent of the infection into the lower respiratory tract. In our model, detectable levels of virus were recovered from the nasal mucosa of most mice up to 13 days after primary infection with the X31 influenza strain (Fig. 1). Although detectable levels of virus were found in the lungs of four of five mice at day 5 postinfection (p.i.), these levels were 1–2 log\(_{10}\) less than those recovered from their nasal mucosa. Only one animal had a detectable level of virus in its lung by day 7 p.i. Before the secondary PR8 influenza challenge, no virus was detected in either the lungs or nasal mucosa of any of the mice.

Clearance of the secondary PR8 infection from the nasal mucosa and lungs of the challenged mice (Fig. 1) was faster than clearance of the primary infection. Virus was recovered from the nasal mucosa of all mice 3 days postchallenge (p.c.), in four of five mice on day 5 p.c., and in only two of five mice by day 7 p.c. The amount of virus recovered from each of these mice was substantially lower than seen at the same time during the primary response. No virus was recovered from the nasal mucosa after day 7 p.c. Only one mouse on day 3 p.c. had detectable virus in the lung. This animal also had the greatest amount of virus recovered from the nasal mucosa on day 3 p.c. No virus was found in the lungs of any other mice that were challenged with the PR8 influenza strain.

**Kinetics of the primary and secondary CD4 and CD8 T cell responses**

To determine the kinetics of the T cell response to primary and secondary intranasal influenza virus infection, tissues of the URT corresponding to induction and effector sites were assessed for the
The presence of CD4 and CD8 T cells. Following the primary X31 infection, a small peak in the accumulation of CD4 and CD8 T cells occurred in the nasal mucosa on day 7 p.i. (Fig. 2A, inset). At this time, the accumulation of CD8 T cells at this effector site was slightly greater than that of CD4 T cells. Before and after day 7 p.i., the level of CD8 T cell accumulation in the nasal mucosa was equal to or less than that of the CD4 T cells in this tissue. At the time of the PR8 challenge, the levels of CD4 and CD8 T cells in the nasal mucosa were equivalent and not substantially different from those during the later stages of the primary response. Five days after the secondary PR8 challenge the peak accumulation levels of CD8 and CD4 T cells were 10 and 20 times greater than their respective peak accumulation levels during the primary response. By day 7 p.c. and beyond, the accumulation of CD4 and CD8 T cells in the nasal mucosa diminished to and remained at levels equivalent to or only slightly greater than those at the time of the secondary PR8 challenge.

The accumulation of CD4 and CD8 T cells in the NALT following primary X31 infection spiked to its maximal level on day 10 p.i. (Fig. 2B). By day 13 p.i., this peak accumulation level had diminished by 75% and continued to gradually decrease for the remainder of the response. The accumulation of CD4 and CD8 T cells in the NALT during the secondary PR8 response differed from that during the primary response in that the T cells levels fluctuated within a maximal range between days 5 and 10 p.c., after which they returned to their prechallenge levels.

During the primary response to X31 influenza infection, the accumulation of CD4 and CD8 T cells in the CLN reached a maximum level on day 5 p.i. and then gradually diminished to a level slightly greater than that seen before the infection (Fig. 2C). Throughout the primary response, CD4 levels remained higher than CD8 levels. Following the secondary PR8 challenge, CD4 and CD8 T cell accumulation peaked at equivalent levels on day 5 p.c. The accumulation of both T cell subsets diminished rapidly to day 7 p.c. and then at a more gradual rate until it reached a level slightly greater than seen at the time of challenge.

**Kinetics of the primary and secondary Db NP 366- and Db PA 224-specific CD8 T cell responses**

The specificity of the anti-viral CD8 T cell response in the lung is known to evolve in favor of a Db NP 366-specific CD8 T cell response that subsequently dominates the memory pool of viral-specific effector CD8 T cells (9). To determine whether this also held true for the nasal mucosa, we evaluated the CD8 T cell population at this effector site by tetramer staining. In the nasal mucosa the recruitment rate of Db NP 366- and Db PA 224-specific CD8 T cells was almost identical before their maximum accumulation on day 7 p.i. following primary X31 influenza infection (Fig. 3A). At day 7 p.i., the level of Db NP 366-specific CD8 T cells was slightly greater than that of Db PA 224-specific CD8 T cells, and it remained this way for the duration of the primary response. The nasal mucosa was the only tissue in which a substantial increase in the accumulation of Db PA 224-specific CD8 T cells was observed during the secondary response to PR8 challenge. This increase occurred on day 5 p.c. and then returned to and remained at the prechallenge level for the remainder of the response. The accumulation of Db NP 366-specific CD8 T cells during the secondary response to the PR8 challenge spiked to a maximum at this same time. Two days later, the number of Db NP 366-specific CD8 T cells had rapidly diminished to a level slightly greater than that of Db PA 224-specific CD8 T cells and remained at this level for the duration of the secondary response.

NALT CD8 T cells were stained for their viral specificity to determine whether this tissue might be involved in the establishment of an Ag-specific cell-mediated response in the nasal mucosa. The Db NP 366- and Db PA 224-specific CD8 T cell levels in the NALT did not significantly change relative to their preinfection levels, nor did their accumulation levels differ significantly from one another throughout the primary response to the X31 infection (Fig. 3B). Unlike the nasal mucosa, the accumulation and diminution of Db NP 366-specific CD8 T cells in the NALT during the secondary response to PR8 occurred over a longer time course and
reached its maximal level on day 7 p.c., 2 days after these cells reached their maximum levels in nasal mucosa. Early in the primary response to X31 infection, the accumulation of Db NP 366- and Db PA 224-specific CD8 T cells in the CLN were identical (Fig. 3C). After day 5 p.i., the accumulation of Db NP 366-specific CD8 T cells increased to a small peak on day 7 p.i. whereas the accumulation of Db PA 224-specific CD8 T cells diminished. For the remainder of the response, the accumulation of Db NP 366-specific CD8 T cells was slightly greater than that of Db PA 224-specific CD8 T cells. Following the secondary PR8 challenge, only Db NP 366-specific CD8 T cells showed a significant level of accumulation in the CLN. The accumulation of these cells reached a maximum level on day 5 p.c. and continually decreased thereafter. In the spleen, the accumulation of Db NP 366- and Db PA 224-specific CD8 T cells was equivalent up to 7 days after the primary infection with X31 (Fig. 3D). Db PA 224-specific CD8 T cells reached their maximum accumulation level at this time and then gradually decreased to their preinfection levels at the time of PR8 challenge. The Db NP 366-specific CD8 T cells continued to accumulate and reached a peak level by day 13 p.i., after which the accumulation of these cells decreased to slightly greater the pre-infection level. Following the secondary PR8 challenge, the rate of accumulation of Db NP 366-specific CD8 T cells in the spleen was similar to that in the CLN, except that greater cell numbers were involved in the spleen. Following the day-5 p.c. maximum, the Db NP 366-specific CD8 T cells in the spleen stabilized at the level found on day 7 p.c.

CD8 T cell localization within the nasal passages

CD8 T cells recruited into the infected nasal mucosa localize at the level of the respiratory epithelium and lamina propria in the nasal passages (Fig. 4, B–D). In addition, CD8 T cells were detected in the respiratory epithelium of the Vulmer’s organ, associated nasal sinuses, and NALT following infection (not shown). The presence of CD8 T cells in the nasal mucosa of uninfected animals was negligible (Fig. 4A). The CD8 T cell recruitment at the peak of the primary response (Fig. 4B) was significantly less than that observed at the peak of the secondary response (Fig. 4C). No CD8 T cells were detected in the transition area into and throughout the sensory epithelium of the nasal passages and Vulmer’s organ (data not shown). Within the respiratory epithelium, CD8 T cells were...
detected in the intraepithelial lymphocyte compartment (Fig. 4D, arrows) and in the lamina propria (Fig. 4D, arrowhead).

Discussion
The first susceptible tissue with which airborne influenza virus particles can make contact is the respiratory epithelium of the nasal mucosa. Thus, it follows that the nasal mucosa is also the site at which effective resistance to influenza infection needs to be rapidly established to limit the proliferation of progeny virus and their subsequent transmission into the lung. To limit the proliferation of progeny virus, infected epithelial cells are targeted for destruction by the CD8 T cell-mediated response (7). MHC class 1 tetramers recognizing Db PA 224 and Db NP 366 epitope-specific CD8 T cells have been used extensively to study the recruitment of virus-specific CD8 T cells into the lung following influenza virus infection (8, 9, 17). The experiments presented in our study are unique, in that they make use of these MHC class 1 tetramers to address the establishment and persistence of the primary and secondary viral-specific CD8 T cell-mediated responses to influenza virus infection of the nasal mucosa.

The presence of persistent D<sup>3</sup>PA<sub>224</sub>-specific and particularly D<sup>3</sup>NP<sub>366</sub>-specific CD8 T cells within the nasal mucosa may well be responsible for the accelerated clearance of the secondary PR8 challenge. D<sup>3</sup>NP<sub>366</sub>-specific CD8 T cells isolated from the lungs well after the clearance of influenza Ag acquire strong cytolytic activity only after re-exposure to Ag (17). This is in contrast to vesicular stomatitis virus-specific CD8 T cells, which were found to possess constitutive cytolytic activity upon isolation from the intestinal lamina propria (18) and other nonlymphoid sites (19) following clearance of a vesicular stomatitis virus infection. Nonetheless, in both models these in situ virus-specific CD8 T cells are poised to rapidly respond to the presence of virus in the tissue. The evidence in our study, in conjunction with the findings of others, suggest that the D<sup>3</sup>NP<sub>366</sub>-specific CD8 T cells found in the nasal mucosa could rapidly respond to the heterosubtypic challenge. These cells may be responsible for the level of protection observed in the nasal mucosa and, by extension, for the reduced transmission into the lungs of immunized mice. The possibility of non-neutralizing cross-reactive Abs contributing to heterosubtypic cross-protection has been demonstrated in a mucosal vaccine
model that uses an inactivated influenza virus (20). However, whether such Abs play a role in our model is not known.

The maximum T cell accumulation levels in the nasal mucosa were greater during the secondary response (day 5 p.c.) than at the peak of the primary response (day 7 p.i.) to influenza infection. This augmented accumulation of T cells at effector sites following secondary infections has been observed in the lung (21). However, in the NALT, the accumulation of T cells was greater during the primary response than during the secondary response to PR8 challenge. The maximum T cell accumulation levels in the NALT 10 days after primary infection were concomitant with a substantially reduced viral load and a diminished level of T cell accumulation in the nasal mucosa. Similarly, T cell accumulation did not peak in the spleen until after clearance of the primary infection from the nasal mucosa (data not shown). In a similar manner, the maximal accumulation of Db NP 366-specific CD8 T cells in the NALT following the PR8 challenge occurred as the accumulation of these cells in the nasal mucosa was diminishing and at a time when the viral burden was very low in the nasal mucosa. This suggests that the NALT may not act as an initial induction site for Ag-specific proliferation of T cells after influenza infection of the nose. It is possible that as the virus load is reduced in the nasal mucosa, the subsequent reduction of recruitment signals from the nasal mucosa could cause T cells recruited from the CLN to accumulate in the NALT. This recruitment pattern of T cells to the nasal mucosa via the NALT would support earlier ideas that the NALT is an important staging area for lymphocyte recirculation into the nasal mucosa (1). In contrast, the delayed accumulation of these T cells in the NALT may be associated with the reduced availability of viral Ag that would normally provide the necessary proliferative signals to induce clonal expansion of the viral-specific CD8 T cells. However, the CLNs, which are more distal from the nasal mucosa than the NALT, exhibited a CD8 T cell accumulation pattern similar in kinetics to that of the nasal mucosa. It would appear unlikely that the availability of viral Ag was responsible for the delayed accumulation of these viral-specific CD8 T cells in the NALT and did not cause a similar delay in a more distal lymphoid tissue.

Although that the D^b NP<sub>366</sub> and D^b PA<sub>224</sub>-derived viral epitopes represent only two of a known number of H-2D<sup>b</sup> influenza virus-specific CD8 T cell epitopes, (10), they are useful for the purpose of studying the recruitment of virus-specific CD8 T cells within effector and induction sites. At the peak of the primary response in the nasal mucosa, the D<sup>b</sup>PA<sub>224</sub>- and D<sup>b</sup>NP<sub>366</sub>-specific CD8 T cells accounted for 30–40% of the CD8 T cells recovered from this tissue. This is similar to the recovery levels of D<sup>b</sup>PA<sub>224</sub>- and D<sup>b</sup>NP<sub>366</sub>-specific T cells from the airways of the lungs of mice with primary influenza pneumonia (8). Although the recruitment of D<sup>b</sup>PA<sub>224</sub>-specific CD8 T cells was equivalent or slightly greater at an early stage of the primary response in the nasal mucosa and CLN, the D<sup>b</sup>NP<sub>366</sub>-specific CD8 T cell response became increasingly dominant after day 5 p.i. and remained so for the duration of the primary response in our study. This aspect of the URT response differed somewhat from that found in the lung and mediastinal lymph node. Following primary infection of the lung, the D<sup>b</sup>PA<sub>224</sub>-specific CD8 T cells were more dominant relative to the
The Journal of Immunology

3299

D\(^{\text{b}}\)NP\(^{\text{366}}\)-specific CD8\(^{\text{c}}\) T cells over the course of the response (8) (R. J. Hogan, unpublished observations).

Although the dominance of the D\(^{\text{b}}\)PA\(^{\text{224}}\) CD8\(^{\text{c}}\) T cell-specific response seemed to differ between those of lung and nasal mucosa, the ability to establish a D\(^{\text{b}}\)PA\(^{\text{224}}\)-specific memory CD8\(^{\text{c}}\) T cell response in these two tissues did not. As the primary response in the nasal mucosa waned, the D\(^{\text{b}}\)NP\(^{\text{366}}\)-specific CD8\(^{\text{c}}\) T cell component accounted for an increasingly greater percentage of the virus-specific CD8\(^{\text{c}}\) T cells. This facilitated a more efficient establishment of a D\(^{\text{b}}\)NP\(^{\text{366}}\)-specific memory CD8\(^{\text{c}}\) T cell response in the URT. The preferential establishment of a D\(^{\text{b}}\)NP\(^{\text{366}}\)-specific memory CD8\(^{\text{c}}\) T cell pool has been attributed to a number of possibilities, including the avidity and/or affinity of each peptide for its MHC, the engagement by MHC:peptide complexes of a spectrum of TCR\(^{\text{a}}\) pairs ranging in their affinity and/or avidity for the complexes (8), the nature of the naive T cell repertoire, and the extent of the precursor CTL pool (9). The preponderance of the local D\(^{\text{b}}\)NP\(^{\text{366}}\)-specific epitopes within the virus and within the infected cell during the infection process may also influence the development of the virus-specific memory CD8\(^{\text{c}}\) pool.

The evidence in this study demonstrates that the dominance of the D\(^{\text{b}}\)NP\(^{\text{366}}\)-specific CD8\(^{\text{c}}\) T cell response extends beyond the lung to the tissues of the nasal mucosa. The similar kinetics of the T cell responses of the mucosa and CLN suggest that T cells produced in the CLN transit through the NALT on their way to the nasal mucosa. That the T cell accumulations of the NALT and spleen were more delayed and protracted suggest that these lymphoid tissues played a different role in the mucosal response than does the CLN.

The rapid clearance of the secondary heterosubtypic infection from the nasal mucosa was associated with a minimal level of infection to the tissues of the nasal mucosa. This suggested that the establishment and persistence of the local D\(^{\text{b}}\)NP\(^{\text{366}}\)-specific CD8\(^{\text{c}}\) T cell response in the nasal mucosa may be capable of providing a substantial level of protection against transmission of an URT influenza infection into the lungs. These results encourage the continual investigation of peptide-based aerosol vaccines as a means of eliciting protective CTL-mediated immunity against heterosubtypic influenza infections at the level of the nasal mucosa.

Acknowledgments

We thank Jean Brennan, Mike Tighe, Sharon Sadzy, and Tres Cookenham of the Trudeau Institute Molecular Core Facility for generation of the MHC class 1 tetramers.

References


