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CD4⁺ T Cell Priming Accelerates the Clearance of Sendai Virus in Mice, but Has a Negative Effect on CD8⁺ T Cell Memory¹

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Current vaccines designed to promote humoral immunity to respiratory virus infections also induce potent CD4⁺ T cell memory. However, little is known about the impact of primed CD4⁺ T cells on the immune response to heterologous viruses that are serologically distinct, but that share CD4⁺ T cell epitopes. In addition, the protective capacity of primed CD4⁺ T cells has not been fully evaluated. In the present study, we addressed these two issues using a murine Sendai virus model. Mice were primed with an HN₄₂₁₋₄₃₆ peptide that represents the dominant CD4⁺ T cell epitope on the hemagglutinin-neuraminidase (HN) of Sendai virus. This vaccination strategy induced strong CD4⁺ T cell memory to the peptide, but did not induce Abs specific for the Sendai virus virion. Subsequent Sendai virus infection of primed mice resulted in 1) a substantially accelerated virus-specific CD4⁺ T cell response in the pneumonic lung; 2) enhanced primary antiviral Ab-forming cell response in the mediastinal lymph nodes; and 3) accelerated viral clearance. Interestingly, the virus-specific CD8⁺ T cell response in the lung and the development of long-term memory CD8⁺ T cells in the spleen were significantly reduced. Taken together, our data demonstrate that primed CD4⁺ T cells, in the absence of pre-existing Ab, can have a significant effect on the subsequent immune responses to a respiratory virus infection. *The Journal of Immunology*, 2000, 164: 3274–3282.

Immune control of primary respiratory virus infections, such as influenza virus, is mediated primarily by CD8⁺ T cells (1). This protection is dependent on either Fas or perforin (2, 3) and is most likely due to cytolytic activity directed against virally infected lung epithelial cells (4, 5). Control of secondary viral infections is mediated primarily by Ab that is induced in a CD4⁺ T cell-dependent fashion during the latter stages of the primary infection. Although the Ab response is not thought to play a major role in the primary response, it is clear that Ab can control a primary infection when CD8⁺ T cells are absent. For example, primary challenge of CD8⁺ T cell-depleted mice with either influenza or Sendai virus results in impaired control of the virus (6–8) and, in the case of Sendai virus, increased mortality (7). This delayed control of the infection was dependent on both CD4⁺ T cells and B cells (8, 9), suggesting that it was mediated by CD4⁺-dependent generation of neutralizing Abs. In addition to promoting Ab production, there is accumulating evidence that CD4⁺ T cells can also act to control the infection in an Ab-independent manner. For example, adoptive transfer of influenza-specific CD4⁺ T cells into B cell-deficient mice led to partial protection against subsequent viral challenge (10). In addition, it has also been shown that primed, influenza-specific CD4⁺ T cells can result in limited control of an influenza virus infection in mice that lack both CD8⁺ T cells and

B cells (8, 11). This protective effect seems to depend on previously primed CD4⁺ T cells, inasmuch as unprimed CD4⁺ T cells appear to be ineffective under the same circumstances (9). However, it was not clear from any of these studies whether CD4⁺ T cells could have any significant impact on a viral infection in a fully immune competent mouse.

In addition to protective effects, primed CD4⁺ T cells have also been implicated in detrimental effects during viral infections. Pre-sensitization with the major surface glycoprotein (G protein) of respiratory syncytial virus (RSV)³ led to severe pulmonary eosinophilia upon challenge with RSV (12, 13). This enhanced disease has been found to be associated with a strongly Th2-biased pulmonary CD4⁺ response triggered by the G protein of RSV (14–16). Similar phenomena have also been observed in influenza virus model (17). Although adoptive transfer of the virus-specific Th1 clones conferred complete protection against viral challenge, transfer of virus-specific Th2 clones resulted in exacerbated lung pathology upon challenge with the virus (17). This indicates that the quality or nature of CD4⁺ T cell populations primed may have a significant impact on the nature of the subsequent responses to infection.

Current influenza vaccines are composed of Formalin-inactivated trivalent influenza viruses and are designed to induce protective humoral immunity (18). This vaccination regimen also induces potent CD4⁺ T cell memory (19, 20). However, little is known about the impact of these primed CD4⁺ T cells on the immune response to heterologous viruses that are serologically distinct, but that share CD4⁺ T cell epitopes. In the present study, we addressed the issue of CD4⁺ T cell-mediated antiviral immunity in immunologically intact mice, using Sendai virus as a model system. Our results demonstrate that CD4⁺ T cell priming had a

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³ Abbreviations used in this paper: RSV, respiratory syncytial virus; AFC, Ab-forming cell; BAL, bronchoalveolar lavage; BFA, brefeldin A; CLN, cervical lymph node; CTM, complete tumor medium; EID₅₀, egg infectious dose; ELISPOT, enzyme-linked immunospot; HN, hemagglutinin-neuraminidase; i.n., intranasal; MLN, mediastinal lymph node; NP, nucleoprotein; WBC, white blood cell.

significant influence on the profile of T and B cell-mediated responses to acute Sendai virus infection and also influenced the establishment of long-term CD8⁺ T cell memory. Most important, we show in this work that CD4⁺ T cell priming leads to accelerated viral clearance.

Materials and Methods

Mice

Female C57BL/6 mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and housed under specific pathogen-free conditions before infection with Sendai virus at 6–10 wk of age.

Priming of mice with peptide Ags

Sendai virus hemagglutinin-neuraminidase (HN) peptides (HN_{421–436}, HN_{559–574}, HN_{163–178}), nucleoprotein (NP) peptide (NP_{324–332}), and matrix protein (M) peptide (M_{149–164}) have been described previously (21, 22). The peptides were synthesized and purified with reverse-phase HPLC at St. Jude Children's Research Hospital. Mice were primed with 50 µg of either the HN_{421–436} or HN_{559–574} synthetic peptides emulsified in 100 µl of CFA by s.c. injection into the base of the tail. Control mice received an equal amount of CFA prepared with PBS. Seven to ten days later, the animals were boosted with 50 µg of the corresponding peptides in IFA or PBS in IFA as a negative control.

Virus infections and assays

The Enders strain of Sendai virus (23) was grown, titrated, and stored, as described previously (7). Mice that had been primed with HN peptides 10–30 days previously were infected intranasally (i.n.) under anesthesia with 500 egg infectious doses (EID₅₀) of Sendai virus. All infected mice were held under biosafety level 3 conditions. Virus titers of lung tissues were determined from lung homogenates by endpoint titration in embryonated hen eggs (logEID₅₀), as described previously (7).

CD4⁺ T cell proliferation assay

Mice were killed 10–30 days after immunization with Sendai virus HN peptides, and single-cell suspensions were prepared from the inguinal and paraortic lymph nodes. CD4⁺ T cells were enriched from pools of two to three spleens by negative selection with Dynabeads (Dyna, Oslo, Norway), as described previously (3). The enriched cell population usually comprised 82–87% CD4⁺ T cells, as determined by flow cytometry. Enriched CD4⁺ T cells (3–5 × 10⁵) were then cultured with T cell-depleted, X-irradiated (3000 rad) splenocytes from syngeneic naive mice (2.5 × 10⁵) in the presence of HN peptides (10 µg/ml) for 4 days in 200 µl of complete tumor medium (CTM) at 37°C with 10% CO₂. CTM is prepared by supplementing MEM (Life Technologies, Rockville, MD) with essential and nonessential amino acids, sodium pyruvate, L-glutamine, sodium bicarbonate, gentomycin, penicillin G, streptomycin sulfate, and 2-ME, as described previously (24). [³H]thymidine (1 µCi/well; DuPont, Boston, MA) was added for the final 18 h of culture. Cells were harvested using an automatic cell harvester (Tomtec, Vialac, Gaithersburg, MD), and the incorporated radioactivity was determined by an automatic beta scintillation counter system (LKB, Vialac). The results are expressed as cpm ± SD of triplicate cultures.

Determination of virus-specific serum Ab titers by ELISA

Sendai virus-specific serum Ab titers were determined by ELISA, as described previously (25). Briefly, serial dilutions of test serum samples were incubated on plates coated with purified Sendai virus Ag preparation. Alkaline phosphatase-labeled goat anti-mouse secondary Abs (Southern Biotechnology Associates, Birmingham, AL) were added, followed by development of the color with the peroxidase substrate (*p*-nitrophenyl phosphate; Sigma, St. Louis, MO). In all assays, a pooled normal mouse serum collected from naive C57BL/6 mice was used to determine the endpoint titer of test serum samples. The endpoint was defined as the final dilution of a test sample whose OD value is greater than the mean plus 3 SD of data obtained with normal mouse serum.

Differential WBC counting of the bronchoalveolar lavage (BAL)

Cells from the BAL were pooled from three to four infected mice, washed once with CTM, and resuspended in 80 µl of CTM. The concentrations and percentages of pulmonary inflammatory cells, including total WBC, neutrophils, eosinophils, basophils, monocytes, macrophages, and lymphocytes, were counted and calculated by an automatic cell counter (Hemavet 3500; CDC Technologies, Oxford, CT). The criteria used for the differen-

tial cell count by the instrument are based on the cell size, number, and morphology of different types of murine WBC. The results are expressed as absolute numbers of each inflammatory cell type per lung.

Flow cytometry

Staining with Sendai virus MHC class I-peptide tetramers was done as described previously (26). Briefly, B cells were first depleted from spleen samples by panning on anti-mouse IgG-coated flasks. Nonspecific staining was blocked in purified anti-mouse CD16/CD32 (FcγIII/II receptor; PharMingen, San Diego, CA). The cells were then stained with Sendai virus NP_{324–332}/K^b-PE or NP_{324–332}/D^b-PE tetramer, followed by staining with tricolor-conjugated anti-CD8 (Caltag, Burlingame, CA) and FITC-conjugated anti-CD44 or anti-CD62L (PharMingen). Two-color staining of BAL cells utilized FITC-conjugated anti-CD4 and PE-conjugated anti-CD8 mAbs (PharMingen). Stained samples were acquired on a Becton Dickinson FACScan flow cytometer, and the data were analyzed using CellQuest software (Becton Dickinson Immunocytometry System, San Jose, CA). For BAL samples, the results are expressed as the absolute cell numbers of CD4⁺, CD8⁺, or NP_{324–332}/K^b-tetramer⁺ cells in each lung, which were calculated based on the percentage of tetramer⁺/CD4⁺ or CD8⁺ cells among total lymphocytes, and the absolute numbers of lymphocytes were determined from the total WBC count of each lung. For spleen and MLN samples, the results are expressed as the percentage of NP_{324–332}/K^b-tetramer⁺ cells among total CD8⁺ T cells.

Intracellular IFN-γ staining following peptide stimulation

Cells from the BAL were pooled from 10 to 14 primed and infected mice of each group and absorbed onto plastic dishes to remove macrophages. The nonadherent cell populations were then cultured for 6 h in the presence or absence of 1 µg/ml of Sendai virus NP_{324–332} peptide or 10 µg/ml HN peptides in 250 µl of CTM containing 10 µg/ml brefeldin A (BFA). After culture, intracellular IFN-γ staining was performed, as described previously (27). Briefly, the responder cells were washed twice with PBS/BFA, blocked in mAb to FcR3/II receptor, and stained with rat anti-mouse CD8⁺ or anti-CD4⁺ FITC conjugates (PharMingen). They were then washed in PBS/BFA, fixed in 1% formaldehyde, and permeated in PBS/0.5% Saponin (Sigma). The cells were stained with rat anti-mouse IFN-γ PE or rat IgG1 PE isotype control. FACS analysis was done as described above. The results are expressed as the percentage of CD4⁺ IFN-γ⁺ or CD8⁺ IFN-γ⁺ double-positive populations among total CD4⁺ or CD8⁺ cells after subtraction of background obtained with an irrelevant Sendai virus peptide, M_{149–164}.

⁵¹Cr release assay

The cytotoxic activity of cells from the BAL of primed and infected mice was determined as described previously (28). Briefly, target cells (L-K^b and L-D^b transfectants) were labeled with ⁵¹Cr (Na⁵¹CrO₄; Amersham Life Science, Arlington Heights, IL) and then either pulsed with Sendai virus NP_{324–332} peptide or infected with Sendai virus. Unpulsed target cells were used as negative controls. Various numbers of BAL cells were incubated with 2 × 10⁵ targets for 5 h. The percentage of specific ⁵¹Cr release was calculated by the formula: percent specific release = (experimental – spontaneous)/(maximal – spontaneous). Spontaneous release was typically <10% of the total release induced by 0.5% Triton X-100.

Single cell ELISPOT assay for Sendai virus-specific Ab-forming cell (AFC)

The ELISPOT assay for virus-specific AFC was done as described previously (29, 30) using nitrocellulose Multiscreen HA filtration plates (Millipore, Bedford, MA) coated with purified Sendai virion Ags. Virus-specific AFC-producing IgM, IgG1, IgG2a, IgG2b, IgG3, and IgA were enumerated in single cell suspensions prepared from cervical lymph node (CLN) and MLN, and the results are expressed as mean ± SD.

Results

In vivo priming with Sendai virus HN peptides

Previous studies have shown that the CD4⁺ T cell response to Sendai virus infection in C57BL/6 mice is directed against dominant (HN_{421–436}/A^b) and subdominant (HN_{559–574}/A^b) epitopes derived from the viral HN protein (22). The goal of the current study was to investigate the effect of priming CD4⁺ T cells specific for these epitopes on various aspects of the immune response to a subsequent virus infection. Thus, we primed C57BL/6 mice in

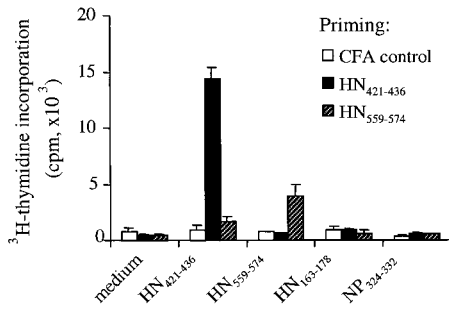


FIGURE 1. In vitro proliferative responses of CD4⁺ T cells to synthetic peptides of Sendai virus HN glycoprotein. CD4⁺ T cells were enriched from pooled spleens of C57BL/6 mice primed 15 days earlier with either HN₄₂₁₋₄₃₆ or HN₅₅₉₋₅₇₄ peptide. The enriched cells were then restimulated for 4 days in vitro with Sendai virus HN peptides or medium alone in the presence of T cell-depleted, irradiated, syngeneic splenocytes from naive mice. The results are expressed as mean \pm SD of triplicate wells. The data are representative of three independent experiments.

the base of the tail with either HN₄₂₁₋₄₃₆ or HN₅₅₉₋₅₇₄ peptides in CFA and boosted them 7–10 days later with the same peptides in IFA. To confirm priming of the mice, splenic CD4⁺ T cells were restimulated in vitro with either the HN₄₂₁₋₄₃₆ or HN₅₅₉₋₅₇₄ peptides, or a K^b-restricted Sendai virus nucleoprotein peptide (NP₃₂₄₋₃₃₂) as a negative control. As shown in Fig. 1, mice primed with the HN₄₂₁₋₄₃₆ peptide proliferated strongly in response to the HN₄₂₁₋₄₃₆ peptide in vitro, but not to HN₅₅₉₋₅₇₄ and NP₃₂₄₋₃₃₂ peptides. In contrast, CD4⁺ T cells from mice primed with the HN₅₅₉₋₅₇₄ peptide proliferated exclusively to the HN₅₅₉₋₅₇₄ peptide, and not the other peptides tested. CD4⁺ T cells taken from control mice that had been primed with PBS/CFA responded to none of the peptides tested. These results confirmed that vaccination with either the HN₄₂₁₋₄₃₆ or HN₅₅₉₋₅₇₅ peptides in CFA primed CD4⁺ T cells specific for the corresponding priming peptide.

Since Sendai virus HN is a surface-exposed glycoprotein, it was possible that vaccination with the HN₄₂₁₋₄₃₆ and HN₅₅₉₋₅₇₄ peptides might induce virus-specific Ab responses that would interfere

Table I. Sendai virus-specific serum Ab titers after priming of C57BL/6 mice with HN peptides

Infection/Priming	Ab Titer ^a
Sendai virus	1/64,000
CFA control	< 1/25
HN ₄₂₁₋₄₃₆	< 1/25
HN ₅₅₉₋₅₇₅	< 1/25

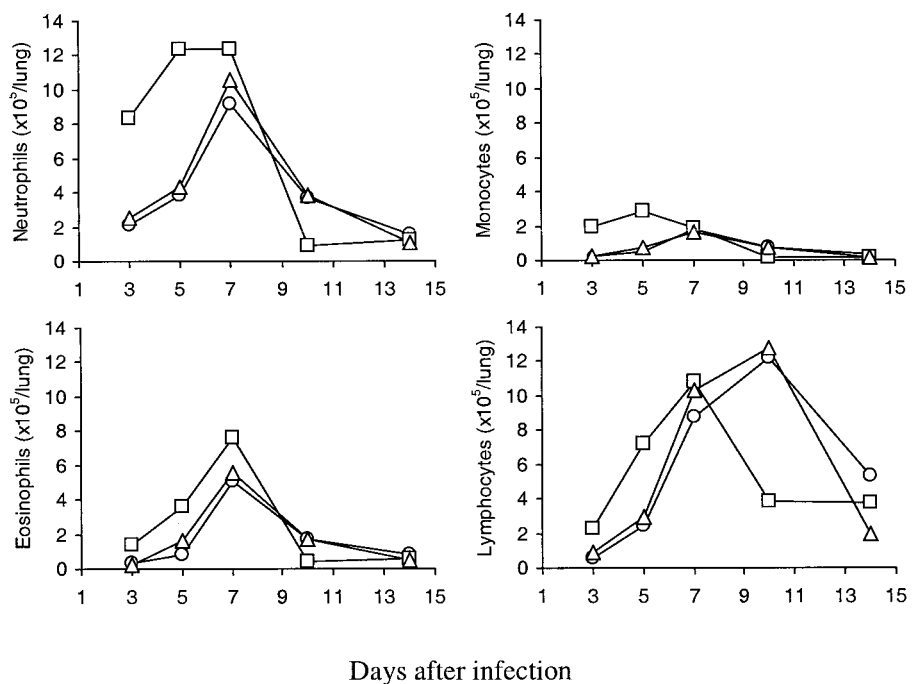
^a Pooled serum samples were obtained from either Sendai virus-infected (30 days after i.n. inoculation with 500 EID₅₀) or Sendai virus HN peptide-primed B6 mice (14 days after the boost). Serum Ab titers were determined by ELISA assay using a purified Sendai virus antigen preparation. The final titer of a serum is based on the final dilution of the sample whose OD value is greater than that of mean + 3 SD of negative control sera.

with a subsequent Sendai virus infection. To test this, sera were collected from peptide-primed or control mice, and virus-specific total IgG Abs were determined using an ELISA (31). As shown in Table I, a positive control serum obtained from Sendai virus-infected mice contained a high titer of virus-specific Abs, whereas none of three serum samples from CFA-, HN₄₂₁₋₄₃₆-, or HN₅₅₉₋₅₇₄-primed mice contained any detectable Abs specific for Sendai virus. This demonstrates that vaccination with either HN₄₂₁₋₄₃₆ or HN₅₅₉₋₅₇₄ peptides did not induce virus-specific Ab responses.

Accelerated recovery from pulmonary neutrophilia

To investigate the impact of CD4⁺ T cell priming on the subsequent pulmonary responses to Sendai virus, we first characterized a normal inflammatory process in the BAL of unvaccinated mice after a primary infection with Sendai virus. Inflammatory infiltrates consisted of a variety of inflammatory cells, including neutrophils, eosinophils, basophils, monocytes, and lymphocytes (Fig. 2). Neutrophils were the predominant inflammatory cell type during the first 7 days after infection. They peaked on day 7 postinfection, accounting for 67% of the inflammatory infiltrates, and dropped rapidly to background levels at about day 14 postinfection. Significant numbers of lymphocytes were first detected on day 7, peaked on day 10 postinfection (65%), and decreased quickly to background level within the next 3–5 days.

FIGURE 2. Enhanced cellular infiltration in the lungs of HN₄₂₁₋₄₃₆ peptide-immune mice after challenge with Sendai virus. Cells from the BAL were pooled from three mice of each group at indicated time points after infection. The types and percentages of inflammatory cells in the BAL of CFA-primed control mice (○), HN₄₂₁₋₄₃₆-immune mice (□), and HN₅₅₉₋₅₇₄-immune mice (△) were determined using an automatic cell counter. The data are representative of two independent experiments.



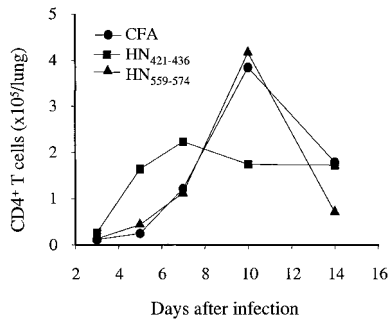


FIGURE 3. Enhanced numbers of CD4⁺ T cells in the lungs of HN₄₂₁₋₄₃₆ peptide-immune mice after challenge with Sendai virus. C57BL/6 mice primed with either HN₄₂₁₋₄₃₆ or HN₅₅₉₋₅₇₄ peptide 20 days before were inoculated i.n. with 500 EID₅₀ of Sendai virus. Cells from the BAL were pooled from three to four mice of each group at different time points after infection and analyzed for total numbers of WBC and percentages of CD4⁺ T cell subset, as described in *Materials and Methods*. The results are expressed as absolute numbers of total CD4⁺ T cells per lung. The data are representative of two independent time course experiments with the same results.

The inflammatory response to Sendai virus infection in HN₄₂₁₋₄₃₆ peptide-primed animals was significantly different and characterized by the accelerated appearance of enhanced numbers of inflammatory cells in the BAL. For example, the numbers of neutrophils present in the BAL on day 5 postinfection were 4-fold higher in mice primed with peptide than primed with adjuvant alone (the means of two independent experiments were 8.71×10^5 /lung in HN₄₂₁₋₄₃₆-immune mice vs 2.29×10^5 /lung in CFA control). Similarly, HN₄₂₁₋₄₃₆-primed mice showed a marginally accelerated appearance of lymphocytes, eosinophils, and monocytes. No significant differences were observed between the subdominant HN₅₅₉₋₅₇₄ peptide-primed and adjuvant alone-primed mice with respect to the magnitude and kinetics of pulmonary inflammatory cell recruitment.

Accelerated recruitment of HN-specific CD4⁺ T cells to the lung

We next asked whether CD4⁺ T cells primed by this vaccination protocol were able to traffic to the site of an acute infection and participate in the subsequent immune responses. Thus, we compared the kinetics of CD4⁺ T cell recruitment in HN₄₂₁₋₄₃₆-immune and unprimed mice that had been i.n. infected with Sendai virus. As shown in Fig. 3, the recruitment of CD4⁺ T cells was significantly accelerated in the BAL of HN₄₂₁₋₄₃₆-immune mice. The accumulation of CD4⁺ cells into the BAL peaked at day 7 postinfection (2.3×10^5 /lung), and remained relatively high for the rest of the 6-day period of observation, whereas significant numbers of CD4⁺ T cells (1×10^5 /lung) in the BAL of unprimed animals were only detected after 7 days and peaked on day 10 postinfection. Interestingly, the peak number of CD4⁺ T cells in unprimed mice was nearly twice as high as that of HN₄₂₁₋₄₃₆-primed mice ($\sim 4 \times 10^5$ /lung). Priming with the subdominant HN₅₅₉₋₅₇₄ peptide had no effect on total CD4⁺ T cell recruitment to the lung.

To determine whether vaccination affected the numbers of Ag-specific CD4⁺ T cells that accumulated in the lung, we used intracellular IFN- γ staining to determine the frequency of HN₄₂₁₋₄₃₆/A^b-specific IFN- γ ⁺ T cells in the BAL on days 7 and 10 postinfection. These time points were selected because maximal numbers of CD4⁺ T cells into the lung were observed at these times in HN₄₂₁₋₄₃₆-primed and control mice (Fig. 3). As shown in Fig. 4, more than 14% of the CD4⁺ cells present in the BAL of HN₄₂₁₋₄₃₆-immune mice were specific for the dominant HN₄₂₁₋₄₃₆/A^b epitope on day 7 after infection, compared with only about 4% of the CD4⁺ cells in the BAL of control mice. CD4⁺ T cells specific for the subdominant HN₅₅₉₋₅₇₄/A^b epitope were only a minimal component of the inflammatory infiltrates of lungs in all the three groups at this time point. Similar results were obtained at day 10. Priming with the subdominant HN₅₅₉₋₅₇₄ peptide did not lead to an enhanced recruitment of HN₅₅₉₋₅₇₄/A^b-specific CD4⁺ T cells in the lung. CD4⁺ T cells producing IL-4, IL-5, and IL-10 were not detectable in intracellular cytokine-staining assays (data not shown). Taken together, the data demonstrate that the priming of

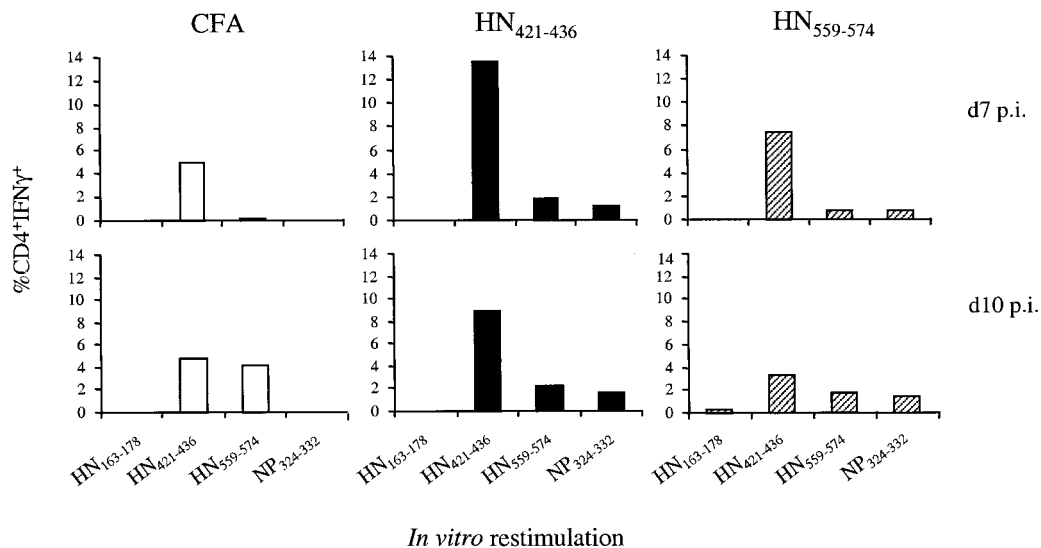


FIGURE 4. Frequencies of Sendai virus HN-specific CD4⁺ T cells in the lungs of HN peptide-immune mice after challenge with Sendai virus. C57BL/6 mice previously primed with either the HN₄₂₁₋₄₃₆ or HN₅₅₉₋₅₇₄ peptides were infected i.n. with 500 EID₅₀ of Sendai virus. Cells from the BAL were pooled from 14–16 mice of each group at days 7 and 10 after infection and stimulated for 6 h with HN peptides in the presence of BFA. The cells were stained for CD4⁺ and intracellular IFN- γ , and the results are expressed as percentages of CD4⁺ IFN- γ ⁺ populations among total CD4⁺ T cell populations in the lymphocyte/lymphoblast gate. The data are representative of two separate experiments with similar results.

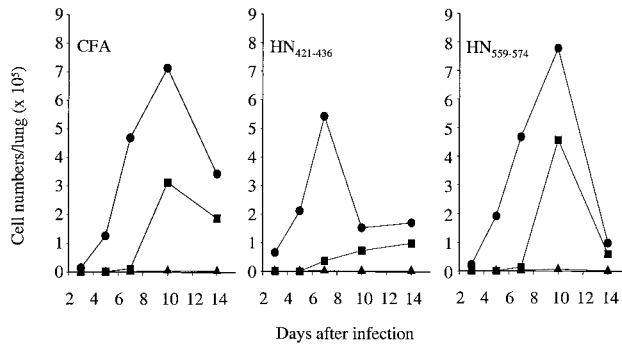


FIGURE 5. Decreased numbers of NP₃₂₄₋₃₃₂/K^b-specific T cells in the lungs of HN₄₂₁₋₄₃₆ peptide-immune mice after challenge with Sendai virus. C57BL/6 mice previously primed with either the HN₄₂₁₋₄₃₆ or HN₅₅₉₋₅₇₄ peptides were infected i.n. with 500 EID₅₀ of Sendai virus. Cells from the BAL were pooled from three to four mice of each group at indicated time points after infection. The absolute numbers of total CD8⁺ T cells per lung (●) were calculated based on the total WBC counting and CD8⁺ FACS staining of pooled cells from the BAL, as described in *Materials and Methods*. The frequencies of CD8⁺ T cells in the BAL specific for the dominant (■) and subdominant (▲) CD8⁺ epitopes of Sendai virus NP molecule were determined by NP₃₂₄₋₃₃₂/K^b or NP₃₂₄₋₃₃₂/D^b tetramer staining.

CD4⁺ T cells specific for the HN₄₂₁₋₄₃₆/A^b epitope peptide resulted in an accelerated CD4⁺ T cell response to a primary Sendai virus infection.

Priming with the HN₄₂₁₋₄₃₆ CD4⁺ T cell epitope alters the CD8⁺ CTL response to Sendai virus infection

Previous studies have established that NP₃₂₄₋₃₃₂/K^b-specific CD8⁺ effector T cells dominate the inflammatory infiltrates of the lung at about day 10 after Sendai virus infection and play a major role in the clearance of the virus from respiratory tract (7). Therefore, we investigated whether CD4⁺ T cell priming to the HN₄₂₁₋₄₃₆ peptide influenced the subsequent CD8⁺ T cell response to Sendai virus infection. The kinetics of NP₃₂₄₋₃₃₂/K^b-specific CD8⁺ T cell recruitment into the lung after infection with Sendai virus was determined using tetramer reagents that detect CD8⁺ T cells specific for either the dominant (NP₃₂₄₋₃₃₂/K^b) or subdominant (NP₃₂₄₋₃₃₂/D^b) class I-restricted epitopes (26, 32). Consistent with previous observations (7), maximal CD8⁺ T cell recruitment was observed in control mice on day 10 postinfection (Fig. 5). T cells specific for the dominant NP₃₂₄₋₃₃₂/K^b epitope were recruited to the lung with similar kinetics and represented ~50% of the total CD8⁺ T cells in the lung at day 10 postinfection. In contrast, the kinetics of CD8⁺ T cell recruitment to the lungs of HN₄₂₁₋₄₃₆-primed mice was significantly different. Peak numbers of CD8⁺ T cells were detected at day 7 and fell rapidly by day 10. Interestingly, the numbers of T cells specific for the NP₃₂₄₋₃₃₂/K^b epitope stayed relatively low through day 14 postinfection. CD8⁺ T cells specific for the subdominant NP₃₂₄₋₃₃₂/D^b epitope were not detectable in the pulmonary infiltrates of all three groups of mice tested during the entire 14-day period of observation, consistent with previous studies (26). The accumulation of total and virus-specific CD8⁺ T cells to the respiratory tract of the subdominant HN₅₅₉₋₅₇₄-primed and virus-infected mice was similar to that seen in control mice with regard to their magnitude and kinetics.

To confirm the tetramer studies, we also investigated the lytic activity of CD8⁺ T cells in the lungs of infected mice using NP₃₂₄₋₃₃₂-pulsed L-K^b target cells. As shown in Fig. 6, strong NP₃₂₄₋₃₃₂/K^b-specific CTL activity could be detected in the BAL of both HN₅₅₉₋₅₇₄-primed and adjuvant alone-primed mice on day

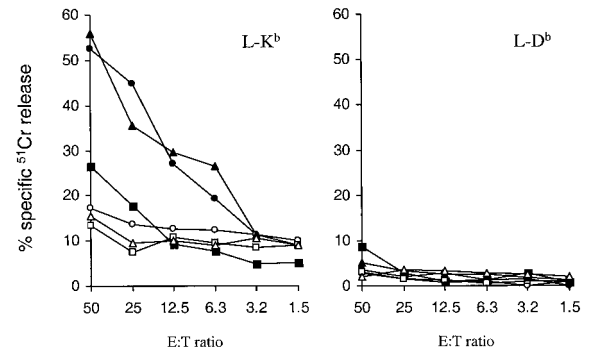


FIGURE 6. Decreased cytotoxic activity in the BAL populations of HN₄₂₁₋₄₃₆ peptide-immune mice after challenge with Sendai virus. Cells were isolated from the BAL on day 10 after infection and analyzed for the cytotoxic activity against NP₃₂₄₋₃₃₂-pulsed (closed symbols) or unpulsed (open symbols) L-K^b or L-D^b targets in a standard ⁵¹Cr release assay. The effector cells were from CFA-primed control mice (●,○), HN₄₂₁₋₄₃₆-immune mice (■,□), and HN₅₅₉₋₅₇₄-immune mice (▲,△).

10 after infection. However, as predicted by the NP₃₂₄₋₃₃₂/K^b tetramer staining (Fig. 5), NP₃₂₄₋₃₃₂/K^b-specific CTL activity was significantly reduced in the BAL of mice primed previously with HN₄₂₁₋₄₃₆ peptide. Consistent with NP₃₂₄₋₃₃₂/D^b tetramer staining (Fig. 5), no CTL activity against the subdominant NP₃₂₄₋₃₃₂/D^b epitope was detected in all the three groups of mice tested (Fig. 6).

Previous studies have shown that virus-specific, MHC class II-restricted CD4⁺ CTL activity has been detected in mice deficient in CD8⁺ T cell function (7, 33). Therefore, we also asked whether the CD4⁺ T cells elicited by Sendai virus in HN₄₂₁₋₄₃₆ peptide-vaccinated mice expressed *in vitro* cytotoxic activity. BAL from all three vaccinated groups were tested for their ability to lyse Sendai virus-infected L-I-A^b target on day 10 after infection (data not shown). No cytolytic activity was detected, consistent with the finding that MHC class II-restricted CD4⁺ CTL activity is usually not detectable in mice with an intact immune system (7, 33).

Significantly decreased frequencies of virus-specific long-term memory CD8⁺ T cells in peripheral lymphoid organs

Recent studies have shown that high frequencies of long-term CD8⁺ T cell memory to both the dominant NP₃₂₄₋₃₃₂/K^b and the subdominant NP₃₂₄₋₃₃₂/D^b epitope could be detected in the spleen after a primary infection (26). Given that CD4⁺ T cell priming with the HN₄₂₁₋₄₃₆ peptide resulted in a dramatic decrease in the numbers of NP₃₂₄₋₃₃₂/K^b-specific CTL effectors in pneumonic lung, we next asked whether this influenced the establishment of memory CD8⁺ T cells. Thus, we analyzed the frequencies and phenotypes of virus-specific memory CD8⁺ T cells by staining spleen cells with the NP₃₂₄₋₃₃₂/K^b tetramer at different time points after infection with Sendai virus. As shown in Table II, high frequencies of CD8⁺ NP₃₂₄₋₃₃₂/K^b-specific T cells were detected in both infected control mice and HN₄₂₁₋₄₃₆-primed mice (ranging from 4.0–4.9) during acute infection (day 12 postinfection). All of the CD8⁺ NP₃₂₄₋₃₃₂/K^b-specific T cells expressed a CD44^{high}/CD62L^{low} phenotype at this time (data not shown). This indicates that priming with the HN₄₂₁₋₄₃₆ peptide does not affect the numbers of NP₃₂₄₋₃₃₂/K^b-specific T cells in the peripheral lymphoid tissues during the acute infection. However, analysis of T cell frequencies at day 31 after infection, when immunological memory status is considered to have been established, revealed a significant impact of HN₄₂₁₋₄₃₆ peptide vaccination on the frequencies of Ag-specific memory CD8⁺ T cells. Whereas the frequency of

Table II. Effect of CD4⁺ T cell priming on establishment of Sendai virus-specific CD8⁺ T cell memory in the spleen^a

Priming	Infection	% CD8 ⁺ NP ₃₂₄₋₃₃₂ /K ^{b+}		% CD8 ⁺ NP ₃₂₄₋₃₃₂ /D ^{b+}	
		d12	d31	d12	d31
None	None	0.3	0.1	0.3	0.2
CFA	Sendai	4.9	2.4	0.4	0.3
HN ₄₂₁₋₄₃₆	Sendai	4.0	0.4	0.3	0.2
HN ₅₅₉₋₅₇₅	Sendai	3.1	1.9	0.3	0.1

^a C57BL/6 mice primed with either HN₄₂₁₋₄₃₆ or HN₅₅₉₋₅₇₄ peptide 20 days before were inoculated i.n. with 500 EID₅₀ of Sendai virus. Spleen cells were pooled from three to four primed mice of each group at different time points after infection. Spleen cells from naive B6 mice were used as controls. The frequencies of CD8⁺ T cells specific for Sendai virus dominant (NP₃₂₄₋₃₃₂/K^b) and subdominant (NP₃₂₄₋₃₃₂/D^b) CD8⁺ epitope were determined by NP₃₂₄₋₃₃₂/K^b or NP₃₂₄₋₃₃₂/D^b tetramer staining. The results are presented as percentage of CD8⁺NP₃₂₄₋₃₃₂/K^{b+} population among total CD8⁺ cells in lymphocyte/lymphoblast gates. The data are representative of two independent experiments.

CD8⁺ NP₃₂₄₋₃₃₂/K^b-specific T cells in spleen of unprimed animals was 2.4%, frequencies of CD8⁺ NP₃₂₄₋₃₃₂/K^b-specific T cells were only 0.4% in spleen of HN₄₂₁₋₄₃₆ peptide-primed memory mice. Similar results were obtained when analyzed on day 60 after infection (data not shown). These results demonstrate that vaccination based on priming CD4⁺ T cells not only has a significant influence on the effector phase of virus-specific CD8⁺ CTL responses, but also on the absolute numbers of CD8⁺ memory T cells that are established.

Accelerated virus clearance in the lung

The data to date demonstrate that priming with the HN₄₂₁₋₄₃₆ peptide resulted in significant effects on the immune responses to Sendai virus infection. Virus-specific CD4⁺ responses become dominant and cytotoxic CD8⁺ T cell responses were reduced. To investigate the biological consequence of these changes on virus clearance, we assessed virus titers in the lung at various times after infection. As shown in Fig. 7A, high titers of Sendai virus were detected in the lung homogenates of CFA alone-primed control mice on days 3 and 5. From day 7 on, virus titers dropped rapidly and no virus was detected in all six animals tested on day 10. In contrast, virus clearance was significantly increased in HN₄₂₁₋₄₃₆-primed animals. As shown in Fig. 7B, virus was completely eliminated from the lung tissues of three mice on day 8, a 2-day advantage over control mice. Interestingly, slightly accelerated viral clearance was also observed following priming with the subdominant HN₅₅₉₋₅₇₄ peptide. As shown in Fig. 7C, while none of the three control mice cleared virus on days 8 and 9, virus was only detected in two of three mice on day 8, and only one of three mice on day 9 from the lung homogenates.

HN₄₂₁₋₄₃₆ peptide priming considerably enhances antiviral Ab responses

Ab-mediated mechanisms play a key role in controlling secondary Sendai virus infections. In addition, it has been shown that Ab is able to control primary Sendai virus infections when CD8⁺ T cell responses are absent, albeit with slower kinetics (6–8). In light of the classic observation that preimmunization with carrier leads to accelerated and enhanced Ab response to hapten (34), we hypothesized that CD4⁺ T cell priming would result in accelerated and enhanced B cell responses to Sendai virus. To test this, we first used a single cell ELISPOT assay to determine the induction of virus-specific Ab-forming cells (AFC) in the MLN and CLN. These sites were chosen as they have been shown to contribute to

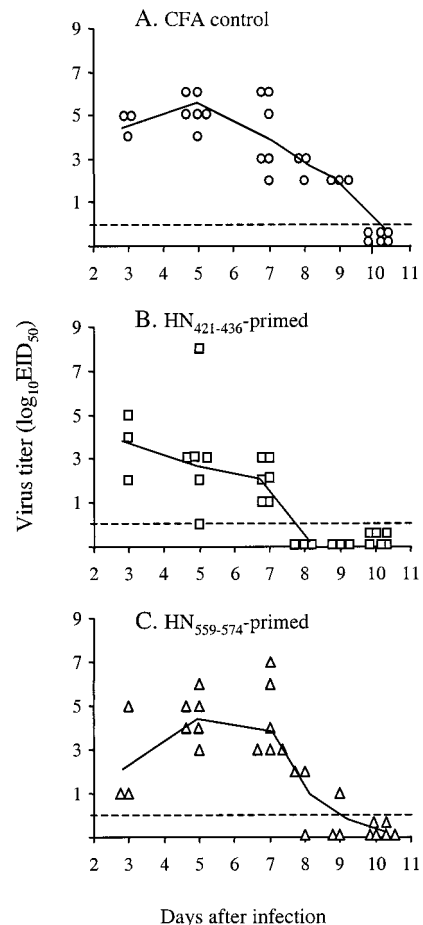


FIGURE 7. Enhanced viral clearance in the HN₄₂₁₋₄₃₆ peptide-immune mice after challenge with Sendai virus. C57BL/6 mice primed with either HN₄₂₁₋₄₃₆ or HN₅₅₉₋₅₇₄ peptide 15 days before were infected i.n. with 500 EID₅₀ of Sendai virus. The lungs were sampled individually at different time points after infection. The virus titers were determined by titrating lung homogenates in embryonated chicken eggs, followed by hemagglutination test. The results are expressed as log₁₀EID₅₀ and represent the combined data from two independent experiments. Virus titers lower than 10⁰ (undiluted) were considered as negative, as indicated by the dotted lines.

the generation of virus-specific Abs in respiratory secretions and serum during acute Sendai virus infection (25).

The overall antiviral AFC response in the MLN was both accelerated and enhanced in magnitude by previous immunization with HN₄₂₁₋₄₃₆ peptide. As shown in Fig. 8, the frequencies of AFC-producing virus-specific IgM, IgG1, IgG2a, IgG2b, and IgG3 isotypes in the MLN of HN₄₂₁₋₄₃₆ peptide-vaccinated mice were higher than those of control mice and could be detected 1 day earlier. Immunization with the subdominant HN₅₅₉₋₅₇₄ peptide was much less effective in promoting the antiviral AFC response in the MLN. Only slightly increased numbers of AFC-producing IgG1 and IgA were present in the CLN.

Next we determined the Sendai virus-specific serum Ab titers of the same mice used for the AFC assay. We found that only low titers of Sendai virus-specific serum IgM and IgG Abs could be detected in all of the three test groups on day 8 after viral challenge, when compared with a positive control serum. The Ab titers of the IgG1, IgG2a, IgG2b, and IgG3 isotypes were slightly higher (3-fold increase) in both HN₄₂₁₋₄₃₆- and HN₅₅₉₋₅₇₄-immune mice than those of CFA-primed control mice. In contrast, no significant differences in serum titers were found among the three groups on

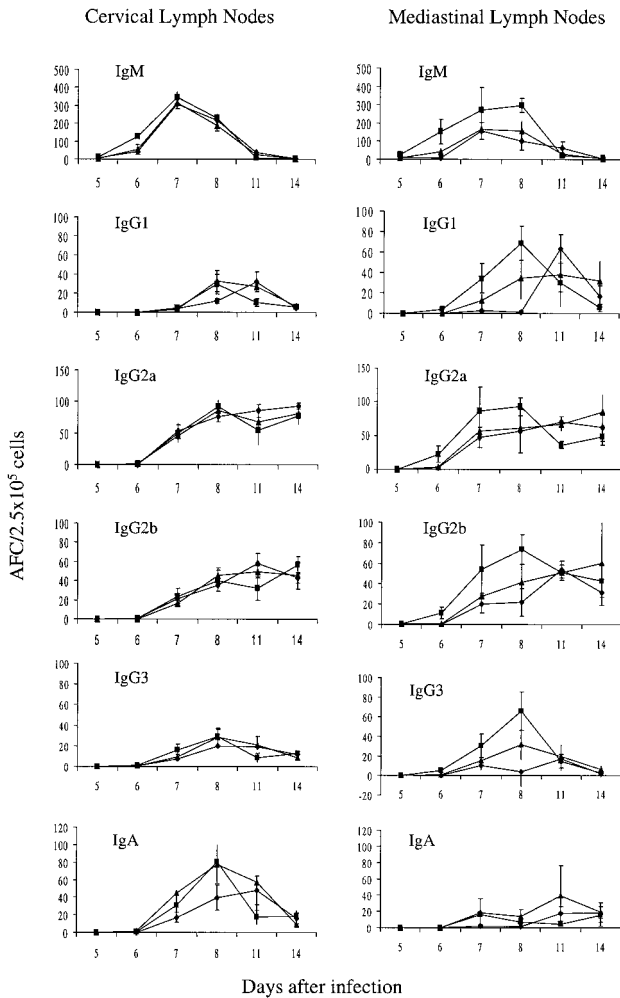


FIGURE 8. Sendai virus-specific AFC responses in the draining lymph nodes of HN peptide-immune mice after challenge with Sendai virus. C57BL/6 mice primed with either HN₄₂₁₋₄₃₆ (■) or HN₅₅₉₋₅₇₄ peptide (▲) or CFA in PBS (◆) 20 days before were inoculated i.n. with 500 EID₅₀ of Sendai virus. CLN and MLN were sampled from five individual mice of each group at indicated time points after infection. An ELISPOT assay was used to determine the number of cells producing IgM, IgG1, IgG2a, IgG2b, IgG3, and IgA specific for Sendai virus. The results are expressed as mean \pm SD of five individual mice in each group.

day 14 after infection (data not shown). The low serum Ab titers detected at day 8 suggest that the mechanism of viral clearance in this system is not mediated exclusively by an Ab response.

Discussion

Vaccines designed to promote humoral immunity, such as the current influenza vaccines, induce strong CD4⁺ T cell priming as a prerequisite of inducing strong Ab responses. These primed CD4⁺ T cells may have a tremendous impact (either positive or negative) on subsequent virus infections. This may be particularly important for influenza virus, in which reassorted viruses that are serologically distinct may nonetheless be cross-reactive at the T cell level (19, 20). Thus, there is need to better understand the impact that CD4⁺ T cells can have on the course of respiratory virus infections. The current study provides the first evidence that previous CD4⁺ T cell priming shapes the qualitative and quantitative nature of subsequent cellular and humoral responses to a parainfluenza virus infection. Sendai virus infection of mice that had been primed with a peptide representing the dominant CD4⁺ T cell

epitope exhibited a dramatically altered immune response to the virus. Whereas Ag-specific CD4⁺ T cells were recruited to the lung more rapidly, CTL responses in the lung were significantly reduced and there was also a reduction in the absolute numbers of CD8 memory cells induced. Despite these changes, virus was more rapidly cleared in primed animals.

The mechanism through which CD4⁺ T cell epitope vaccination resulted in significantly enhanced viral clearance is unclear. It was not due to an Ab response to the immunizing peptide since Sendai virus-specific Abs were not detected in the primed animals before infection. It is also unlikely that enhanced viral clearance was mediated by Ag-specific CD8⁺ CTL since these cells were significantly reduced in number. Similarly, the CD4⁺ T cells that were recruited rapidly to the lung were not themselves cytotoxic. One possibility is that the primed CD4⁺ T cells mediated an accelerated and enhanced antiviral Ab response, leading to an accelerated viral clearance. Indeed, the virus-specific AFC numbers were both accelerated and increased in magnitude in MLN in HN₄₂₁₋₄₃₆ peptide-primed mice following Sendai virus infection. However, slightly enhanced Sendai virus-specific serum Abs were detected on day 8 after viral challenge in both HN₄₂₁₋₄₃₆- and HN₅₅₉₋₅₇₄-primed mice. The fact that only HN₄₂₁₋₄₃₆-primed, but not HN₅₅₉₋₅₇₄-primed, mice cleared virus from the lung at this time point suggests that this level of Ab is not the primary factor in mediating accelerated viral clearance. Additional experiments are underway in our lab to formally rule out this possibility by testing the role of Ab in this system using B cell-deficient mice. Together, our data are consistent with the idea that primed CD4⁺ T cells are able to promote accelerated clearance of a Sendai virus infection in an Ab-independent manner.

Although the mechanism of CD4⁺ T cell-mediated control of Sendai virus infection is unknown, the most likely candidate is through the production of antiviral cytokines. For example, both Th1 (IFN- γ , IL-2, and TGF- β) and Th2 cytokines (IL-4, IL-5, and IL-10) have been identified during acute viral infections induced by respiratory tract viruses, including Sendai virus (35, 36). It was found that adoptive transfer of large numbers of activated Th1 clones specific for influenza A virus, but not Th2 clones, was able to confer protection against lethal viral challenge in vivo (17). In the present study, we show that following priming with the dominant Sendai virus HN epitope, the frequency of IFN- γ -producing CD4⁺ T cells was increased in the pneumonic lung, while CD4⁺ T cells producing IL-4, IL-5, and IL-10 were not detectable in intracellular cytokine-staining assays (data not shown). This indicates that CD4⁺ T cell priming to the HN₄₂₁₋₄₃₆ epitope using CFA as an adjuvant does not change the normal cytokine pattern of the CD4⁺ T cell response in the lung after Sendai virus infection. Rather, it strengthens a virus-specific Th1 response. It is possible that early or enhanced IFN- γ secretion could facilitate viral clearance, as has been demonstrated in other systems (37, 38). Other inflammatory cytokines, including IFN- $\alpha\beta$, might also contribute to an accelerated viral clearance in our system. In this regard, it should be noted that pulmonary recruitment of monocytes/macrophages, the major cell source of IFN- $\alpha\beta$, was obviously accelerated and enhanced in HN₄₂₁₋₄₃₆-primed animal. Taken together, the observed acceleration of viral clearance may reflect a combined effect of different antiviral cytokines in pulmonary infiltrates.

The requirement of CD4⁺ T cell help for generation of CD8⁺ T cell responses is controversial. Numerous experiments have shown that depletion of CD4⁺ T cell populations had little effect on the generation of CD8⁺ cytotoxic effectors against influenza A virus, Sendai virus, or lymphocytic choriomeningitis virus (7, 39–42).

On the other hand, many recent studies have documented the critical involvement of CD4⁺ T cell help in the generation and maintenance of effective CD8⁺ responses against some chronic viral infections. For example, it was found that virus-specific CD8⁺ T cell responses were completely lost in CD4⁺ T cell-deficient mice chronically infected with MHV-68 or lymphocytic choriomeningitis virus (43, 44). In this study, our data suggest a new aspect of CD4⁺/CD8⁺ interactions in the Sendai virus model. Virus-specific CD4⁺ T cells induced by peptide resulted in decreased virus-specific CD8⁺ effector function, and decreased frequency of CD8⁺ long-term memory. The reasons for this are unclear. One possibility is that HN₄₂₁₋₄₃₆-primed animals were able to clear virus more efficiently, resulting in a lower Ag load and reduced CD8⁺ T cell differentiation. Since memory T cells are derived, at least in part, from effector T cells (45), this would result in low frequency of CD8⁺ memory cells. This possibility is supported by a recent observation in an in vitro model system that differentiation of cytotoxic effectors was Ag dose dependent (46). However, an alternative possibility is that biased CD4⁺ expansion may negatively regulate the primary activation of CD8⁺ T cells, or the recruitment of activated T cells to the lung, possibly through the secretion of specific cytokines.

The negative effect of primed CD4⁺ T cells on the generation of virus-specific CD8⁺ memory may have significant implications for current vaccination strategies against respiratory virus infection. It has been shown that the CD8⁺ T cell arm is the major mediator of such a broad protection among serologically distinct influenza viruses (47). For example, infection of mice with an influenza A virus of one subtype led to induction of partial protection to infection with a virus of a different subtype (47–49). The results presented in this work imply that although primed, cross-reactive CD4⁺ T cells can enhance protection against challenge with a homologous virus, they could potentially block the development of cross-reactive CD8⁺ T cell memory and thereby reduce protection against some virus strains. These results argue that a vaccine approach that targets multiple arms of the immune system to develop broad immunity would be preferable.

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References

- Doherty, P. C., D. J. Topham, R. A. Tripp, R. D. Cardin, J. W. Brooks, and P. G. Stevenson. 1997. Effector CD4⁺ and CD8⁺ T-cell mechanisms in the control of respiratory virus infections. *Immunol. Rev.* 159:105.
- Kagi, D., F. Vignaux, B. Ledermann, K. Burki, V. Depraetere, S. Nagata, H. Hengartner, and P. Golstein. 1994. Fas and perforin pathways as major mechanisms of T cell-mediated cytotoxicity. *Science* 265:528.
- Topham, D. J., R. A. Tripp, and P. C. Doherty. 1997. CD8⁺ T cells clear influenza virus by perforin or Fas-dependent processes. *J. Immunol.* 159:5197.
- Yap, K. L., G. L. Ada, and I. F. McKenzie. 1978. Transfer of specific cytotoxic T lymphocytes protects mice inoculated with influenza virus. *Nature* 273:238.
- Lukacher, A. E., V. L. Braciale, and T. J. Braciale. 1984. In vivo effector function of influenza virus-specific cytotoxic T lymphocyte clones is highly specific. *J. Exp. Med.* 160:814.
- Eichelberger, M., W. Allan, M. Zijlstra, R. Jaenisch, and P. C. Doherty. 1991. Clearance of influenza virus respiratory infection in mice lacking class I major histocompatibility complex-restricted CD8⁺ T cells. *J. Exp. Med.* 174:875.
- Hou, S., P. C. Doherty, M. Zijlstra, R. Jaenisch, and J. M. Katz. 1992. Delayed clearance of Sendai virus in mice lacking class I MHC-restricted CD8⁺ T cells. *J. Immunol.* 149:1319.
- Topham, D. J., and P. C. Doherty. 1998. Clearance of an influenza A virus by CD4⁺ T cells is inefficient in the absence of B cells. *J. Virol.* 72:882.
- Mozdzanowska, K., M. Furchner, K. Maiese, and W. Gerhard. 1997. CD4⁺ T cells are ineffective in clearing a pulmonary infection with influenza type A virus in the absence of B cells. *Virology* 239:217.
- Graham, M. B., and T. J. Braciale. 1997. Resistance to and recovery from lethal influenza virus infection in B lymphocyte-deficient mice. *J. Exp. Med.* 186:2063.
- Epstein, S. L., C.-Y. Lo, J. A. Misplon, and J. R. Bennink. 1998. Mechanism of protective immunity against influenza virus infection in mice without antibodies. *J. Immunol.* 160:322.
- Openshaw, P. J., S. L. Clarke, and F. M. Record. 1992. Pulmonary eosinophilic response to respiratory syncytial virus infection in mice sensitized to the major surface glycoprotein G. *Int. Immunol.* 4:493.
- Hancock, G. E., D. J. Speelman, K. Heers, E. Bortell, J. Smith, and C. Cosco. 1996. Generation of atypical inflammatory responses in BALB/c mice after immunization with the native attachment (G) glycoprotein of respiratory syncytial virus. *J. Virol.* 70:7783.
- Alwan, W. H., F. M. Record, and P. J. Openshaw. 1992. CD4⁺ T cells clear virus but augment disease in mice infected with respiratory syncytial virus: comparison with the effects of CD8⁺ T cells. *Clin. Exp. Immunol.* 88:527.
- Alwan, W. H., W. J. Kozłowska, and P. J. Openshaw. 1994. Distinct types of lung disease caused by functional subsets of antiviral T cells. *J. Exp. Med.* 179:81.
- Srikiathachorn, A., and T. J. Braciale. 1997. Virus-specific CD8⁺ T lymphocytes down-regulate T helper cell type 2 cytokine secretion and pulmonary eosinophilia during experimental murine respiratory syncytial virus infection. *J. Exp. Med.* 186:421.
- Graham, M. B., V. L. Braciale, and T. J. Braciale. 1994. Influenza virus-specific CD4⁺ T helper type 2 T lymphocytes do not promote recovery from experimental virus infection. *J. Exp. Med.* 180:1273.
- Subbarao, K. 1999. Influenza vaccines: present and future. *Adv. Virus Res.* 54:349.
- Effros, R. B., P. C. Doherty, W. Gerhard, and J. Bennink. 1977. Generation of both cross-reactive and virus-specific T-cell populations after immunization with serologically distinct influenza A viruses. *J. Exp. Med.* 145:557.
- Hurwitz, J. L., C. J. Hackett, E. C. McAndrew, and W. Gerhard. 1985. Murine TH response to influenza virus: recognition of hemagglutinin, neuraminidase, matrix, and nucleoproteins. *J. Immunol.* 134:1994.
- Kast, W. M., L. Roux, J. Curren, H. J. Blom, A. C. Voordouw, R. H. Meleno, D. Kolakofsky, and C. J. Melief. 1991. Protection against lethal Sendai virus infection by in vivo priming of virus-specific cytotoxic T lymphocytes with a free synthetic peptide. *Proc. Natl. Acad. Sci. USA* 88:2283.
- Cole, G. A., J. M. Katz, T. L. Hogg, K. W. Ryan, A. Portner, and D. L. Woodland. 1994. Analysis of the primary T-cell response to Sendai virus infection in C57BL/6 mice: CD4⁺ T-cell recognition is directed predominantly to the hemagglutinin-neuraminidase glycoprotein. *J. Virol.* 68:6863.
- Portner, A., P. A. Marx, and D. W. Kingsbury. 1974. Isolation and characterization of Sendai virus temperature-sensitive mutants. *J. Virol.* 13:298.
- Kappler, J. W., B. Skidmore, J. White, and P. Marrack. 1981. Antigen-inducible, H-2-restricted, interleukin-2-producing T cell hybridomas: lack of independent antigen and H-2 recognition. *J. Exp. Med.* 153:1198.
- Sangster, M., L. Hyland, R. Sealy, and C. Coleclough. 1995. Distinctive kinetics of the antibody-forming cell response to Sendai virus infection of mice in different anatomical compartments. *Virology* 207:287.
- Usherwood, E. J., R. J. Hogan, G. Crowther, S. L. Surman, T. L. Hogg, J. D. Altman, and D. L. Woodland. 1999. Functionally heterogeneous CD8⁺ T-cell memory is induced by Sendai virus infection of mice. *J. Virol.* 73:7278.
- Prussin, C., and D. D. Metcalfe. 1995. Detection on intracytoplasmic cytokine using flow cytometry and directly conjugated anti-cytokine antibodies. *J. Immunol. Methods* 188:117.
- Cole, G. A., T. L. Hogg, M. A. Coppola, and D. L. Woodland. 1997. Efficient priming of CD8⁺ memory T cells specific for a subdominant epitope following Sendai virus infection. *J. Immunol.* 158:4301.
- Sarawar, S. R., M. Sangster, R. L. Coffman, and P. C. Doherty. 1994. Administration of anti-IFN- γ antibody to β_2 -microglobulin-deficient mice delays influenza virus clearance but does not switch the response to a T helper cell 2 phenotype. *J. Immunol.* 153:1246.
- Sangster, M., F. S. Smith, C. Coleclough, and J. L. Hurwitz. 1995. Human parainfluenza virus type 1 immunization of infant mice protects from subsequent Sendai virus infection. *Virology* 212:13.
- Sangster, M. Y., X. Y. Mo, R. Sealy, and C. Coleclough. 1997. Matching antibody class with pathogen type and portal of entry: cognate mechanisms regulate local isotype expression patterns in lymph nodes draining the respiratory tract of mice inoculated with respiratory viruses, according to virus replication competence and site of inoculation. *J. Immunol.* 159:1893.
- Flynn, K. J., G. T. Belz, J. D. Altman, R. Ahmed, D. L. Woodland, and P. C. Doherty. 1998. Virus-specific CD8⁺ T cells in primary and secondary influenza pneumonia. *Immunity* 8:683.
- Muller, D., B. H. Koller, L. Whiton, K. E. LaPan, K. K. Brigman, and J. A. Frelinger. 1992. LCMV-specific, class II-restricted cytotoxic T cells in β_2 -microglobulin-deficient mice. *Science* 255:1576.
- Katz, D. H., W. E. Paul, E. A. Golgl, and B. Benacerraf. 1970. Carrier function in anti-hapten immune responses. I. Enhancement of primary and secondary anti-hapten antibody responses by carrier preimmunization. *J. Exp. Med.* 132:261.
- Sarawar, S. R., S. R. Carding, W. Allan, A. McMickle, K. Fujihashi, H. Kiyono, J. R. McGhee, and P. C. Doherty. 1993. Cytokine profiles of bronchoalveolar lavage cells from mice with influenza pneumonia: consequences of CD4⁺ and CD8⁺ T cell depletion. *Reg. Immunol.* 5:142.
- Mo, X. Y., S. R. Sarawar, and P. C. Doherty. 1995. Induction of cytokines in mice with parainfluenza pneumonia. *J. Virol.* 69:1288.
- Karupiah, G., R. V. Blanden, and I. A. Ramshaw. 1990. Interferon γ is involved in the recovery of athymic nude mice from recombinant vaccinia virus/interleukin 2 infection. *J. Exp. Med.* 172:1495.

38. Ramsay, A. J., J. Ruby, and I. A. Ramshaw. 1993. A case for cytokines as effector molecules in the resolution of virus infection. *Immunol. Today* 14:155.
39. Allan, W., Z. Tabi, A. Cleary, and P. C. Doherty. 1990. Cellular events in the lymph node and lung of mice with influenza: consequences of depleting CD4⁺ T cells. *J. Immunol.* 144:3980.
40. Hou, S., X. Y. Mo, L. Hyland, and P. C. Doherty. 1995. Host response to Sendai virus in mice lacking class II major histocompatibility complex glycoproteins. *J. Virol.* 69:1429.
41. Ahmed, R., L. D. Butler, and L. Bhatti. 1988. T4⁺ T helper cell function in vivo: differential requirement for induction of antiviral cytotoxic T-cell and antibody responses. *J. Virol.* 62:2102.
42. Rahemtulla, A., W. P. Fung-Leung, M. W. Schilham, T. M. Kundig, S. R. Sambhara, A. Narendran, A. Arabian, A. Wakeham, C. J. Paige, and R. M. Zinkernagel. 1991. Normal development and function of CD8⁺ cells but markedly decreased helper cell activity in mice lacking CD4. *Nature* 353:180.
43. Cardin, R. D., J. W. Brooks, S. R. Sarawar, and P. C. Doherty. 1996. Progressive loss of CD8⁺ T cell-mediated control of a γ -herpesvirus in the absence of CD4⁺ T cells. *J. Exp. Med.* 184:863.
44. von Herrath, M. G., M. Yokoyama, J. Dockter, M. B. Oldstone, and J. L. Whitton. 1996. CD4-deficient mice have reduced levels of memory cytotoxic T lymphocytes after immunization and show diminished resistance to subsequent virus challenge. *J. Virol.* 70:1072.
45. Dutton, R. W., L. M. Bradley, and S. L. Swain. 1998. T cell memory. *Annu. Rev. Immunol.* 16:201.
46. Opferman, J. T., B. T. Ober, and P. G. Ashton-Rickardt. 1999. Linear differentiation of cytotoxic effectors into memory T lymphocytes. *Science* 283:1745.
47. Liang, S., K. Mozdzanowska, G. Palladino, and W. Gerhard. 1994. Heterosubtypic immunity to influenza type A virus in mice: effector mechanisms and their longevity. *J. Immunol.* 152:1653.
48. Yetter, R. A., S. Lehrer, R. Ramphal, and P. A. Small Jr. 1980. Outcome of influenza infection: effect of initial infection and heterosubtypic immunity. *Infect. Immun.* 29:654.
49. Yetter, R. A., W. H. Barber, and P. A. Small Jr. 1980. Heterotypic immunity to influenza in ferrets. *Infect. Immun.* 29:650.