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Physiological Numbers of CD4+ T Cells Generate Weak Recall Responses Following Influenza Virus Challenge

Paul G. Thomas,* Scott A. Brown,* Melissa Y. Morris,* Wen Yue,*† Jenny So,* Cory Reynolds,* Richard J. Webb,† and Peter C. Doherty*‡

Naive and recall CD4+ T cell responses were probed with recombinant influenza A viruses incorporating the OVA OT-II peptide. The extent of OT-II-specific CD4+ T cell expansion was greater following primary exposure, with secondary challenge achieving no significant increase in numbers, despite higher precursor frequencies. Adoptive transfer experiments with OT-II TCR-transgenic T cells established that the predominant memory set is CD62Lhi, whereas the CD62Llo precursors make little contribution to the recall response. Unlike the situation described by other investigators, in which the transfer of very large numbers of in vitro-activated CD4+ cells can modify the disease process, providing CD62Llo OT-II–specific T cells at physiological levels neither enhanced virus clearance nor altered clinical progression. Some confounding effects of the transgenic model were observed, with decreasing primary expansion efficiency correlating with greater numbers of transferred cells. This was associated with increased levels of mRNA for the proapoptotic molecule Bim in cells recovered following high-dose transfer. However, even with very low numbers of transferred cells, memory T cells did not expand significantly following secondary challenge. A similar result was recorded in mice primed and boosted to respond to an endogenous IAα-restricted epitope derived from the influenza virus hemagglutinin glycoprotein. Depletion of CD8+ T cells during secondary challenge generated an increased accumulation of OT-II–specific T cells but only at the site of infection. Taken together, significant expansion was not a feature of these secondary influenza-specific CD4+ T cell responses and the recall of memory did not enhance recovery.

Antigen-specific CD4+ T cells are the central regulators of adaptive immunity, providing help via cell surface receptor contact and the secretion of cytokines to activate dendritic cells, promote Ab maturation and class-switching, and provide key factors for the survival and expansion of CD8+ T cells (1, 2). The part played by CD4+ T cells in resolving infection has been analyzed using CD4+ T cell-depleted or MHC class II (MHC II)−/− animals. In a variety of infections, clearance of the pathogen is delayed, the extent of viral pathology is increased, and memory B cell and CD8+ T cell responses are impaired (3–5). For example, MHC II−/− mice mount relatively normal CD8+ CTL responses following primary lymphocytic choriomeningitis virus (LCMV) infection. However, secondary challenge is characterized by greatly diminished CD8+ T cell expansion, an effect attributed to the aberrant expression of TRAIL on CD8+ memory CTLs generated without CD4+ T help (6). Other studies with Listeria monocytogenes indicated that immune CD4+ T cells secrete an unidentified survival factor that promotes CD8+ T cell memory (7, 8). The end result was failure of the CD8+ recall response in both sets of experiments. The situation for the influenza A viruses is less dramatic: although there is evidence of a partial defect in the absence of a concurrent CD4+ T cell immunity, the CD8+ CTLs still expand and retain the immunodominance profiles that are characteristic of wild-type (wt) mice (9, 10).

Individual CD4+ T cell expansions from the naive repertoire generally are smaller than the concurrent CD8+ CTL responses, with at least some of the effect being attributed to the greater diversity of MHC II-restricted epitopes (11–14). This, combined with the lack of widely available staining (tetramer or dimer) reagents, has resulted in the quantitative analysis of CD4+ T cell responses being relatively underaddressed. In one study following primary infection with a recombinant influenza A virus containing the OVA323–339 peptide (ISQAVHAAHAINEAGR peptide [OT-Ip]), adoptively transferred TCR-transgenic (Tg) cells replicated and trafficked to the lung and airways (11). The response was generally lower in magnitude than that characteristic of CD8+ CTLs, but it was comparable to the endogenous CD4+ T cell response.

Other adoptive transfer studies used large numbers of in vitro-expanded TCR-Tg CD4+ T cells specific for an A/Puerto Rico/34 (PR8) influenza A virus hemagglutinin (HA) epitope (15–19). These experiments indicated that such effectors operate to promote the direct clearance of virus via cytolytic mechanisms. Analysis with this model also showed that memory CD4+ T cells are generated from effector precursors as early as 3 d following initial stimulation (18), a finding consistent with experiments from our group characterizing the in vivo induction of CD8+ T cell memory (20). Similar studies using Tg or polyclonal in vitro-activated CD4+ cells...
CD8+ T cells. By all of the criteria analyzed, these two arms of investigators (2, 20) addressed in great detail for virus-specific rather than Ab-mediated, protective immunity that we and other extents of clonal expansion, and the capacity to mediate cellular, effector memory phenotype (based on CD62L and CD44), the (23). The analysis focuses on questions relating to central and epitope \([H3ova]\) in their respective HA (H1 and H3) proteins and H3N2 influenza A virus engineered to express the OT-II influenza A virus engineered to express the OT-II epitope \([H1ova]\) and H3N2 influenza A virus engineered to express the OT-II epitope \([H3ova]\) in their respective HA (H1 and H3) proteins (23). The analysis focuses on questions relating to central and effector memory phenotype (based on CD62L and CD44), the extent of clonal expansion, and the capacity to mediate cellular, rather than Ab-mediated, protective immunity that we and other investigators (2, 20) addressed in great detail for virus-specific CD8+ T cells. By all of the criteria analyzed, these two arms of cell-mediated immunity seem to be different. Secondary expansion of the OT-IIT cells seems to be severely limited, reflecting, perhaps, that the establishment and persistence of memory is in some way compromised by aspects of the TCR-Tg model (24, 25). Indeed, we found that high-dose transfers resulted in the upregulation of message for the proapoptotic molecule Bim. Because transferring excess TCR-Tg cells can be counterproductive, we tested low-number transfers that are more typical of physiological conditions. However, this fails to generate a memory base for substantial secondary expansion. Overall, the findings using this TCR-Tg model are consistent with what is seen for endogenous responses to influenza A, suggesting that memory CD4+ T cells play little part in secondary effector responses in this model.

### Materials and Methods

#### Viruses, mice, and sampling

Reverse genetics protocols were used to insert the OVA_{323-339} sequence (ISQAHHAAHAEINAGRR) after the glycines at residue 173 of the H1 (PR8, H1N1) and 174 of the H3 (the A/Avic HA of HKx31) HA glycoproteins (23). No residues were removed. The resultant H1ova and H3ova viruses were rescued in 10-d-old embryonated chicken eggs after the engineered plasmids were transfected into cocultures of 293 T cells and MDCK cells. A known epitope (26) in the H3 HA (A/Avic HA) QASSGRVTVSTRR protein of x31 (SLY1) was inserted into the H1 HA by the same method (H1dyl). Female C57BL/6J (B6) mice were purchased from The Jackson Laboratory (Bar Harbor, ME), whereas the OT-II/Thy1.1+ TCR-Tg mice were bred at St. Jude Children’s Research Hospital. All mice were held under specific pathogen-free conditions. Priming with the H1ova or H1sly viruses was by i.p. injection with 10^5 egg 50% infective dose (EID_{50}) of the H1 viruses. Intrapitoneal priming results in aborted replication, allowing very high doses to be given, generating robust and consistent cellular memory. Further, this protocol avoids the accumulation of cells in the lungs, allowing the examination of only recruited memory responses following challenge and not cells remaining from a primary infection (innate or adaptive). Intranasal (i.n.) challenge with the H3 viruses was performed following anesthesia by i.p. injection of 2,2,2-trichloroethanol (Avertin), i.e., delivery of 10^6 EID_{50} of the wild-type H3N2 (HKx31) virus (H3wt) or H3ova virus, as described in the figure legends. The mice were euthanized again at the time of sampling and exsanguinated by sectioning the axillary artery. Inflammatory cell populations were recovered from the infected respiratory tract by bronchoalveolar lavage (BAL), followed by removal of the mediastinal lymph nodes (MLNs) and spleen to prepare single-cell suspensions for lymphocyte analysis.

#### Identifying OT-IIT CD4+ T cells

The OT-II–specific CD4+ T cell response was analyzed by flow cytometry using a Becton Dickinson FACSCalibur (BD Biosciences, Franklin Lakes, NJ) and Flojo software (Tree Star, Ashland, OR) or a MoFlo sorter (Beckman Coulter, Brea, CA) for cell separation. Spleen and BAL lymphocytes were stained with Thy1.1 PE (OX-7), CD4 PE-Cy5 (L3T4), and, in some cases, Vv2 FFTC (B20.1). In some experiments, cells were also stained for CD44 and CD62L. Cells were negatively gated for CD8 and MHC II. The TCR-Tg populations used in cell transfer studies were first labeled with CFSE.

### Real-time RT-PCR analysis

The primers for Bim, Nor1, and GAPDH were described previously (27). RNA from OT-IIT cells sorted at intervals postinfection was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA) and amplified by a separate reverse transcription step, followed by amplification using ABI SYBR Green MasterMix (Applied Biosystems, Foster City, CA), according to the manufacturer’s instructions. Every sample was analyzed in triplicate for each product on an ABI 7500 instrument (Applied Biosystems) with the ddCT Relative Quantitation protocol.

#### Cell transfers

Cell transfers were done in 0.2 ml PBS via i.v. injection in the lateral tail vein. The numbers of cells transferred for each experiment are given in the figure legends.

### CD4+ T cell ELISPOT

An established ELISPOT assay was used to quantify OT-II– or SLY1–specific IFN-γ–producing CD4+ T cells in spleen after stimulation with SLY1_{150-257}, OVA_{223-237} peptide, or no peptide, and the number of IFN-γ producers was measured as spots per 10^6 cells after 48 h at 37°C.

### Virus titration

Lung homogenates were titered by plaque assay on MDCK cells. Near confluent 25-cm² monolayers of MDCK cells were infected with 1-ml aliquots of diluted lung homogenate for 1 h at 37°C. Cells were washed with PBS and overlaid with 3 ml MEM containing 1 mg/ml L-1-tosylamido-2-phenylethyl chloromethyl ketone-treated trypsin (Worthington Biochemical, Lakewood, NJ) and 0.9% agarose. The cultures were incubated at 37°C with 5% CO₂ for 72 h. Plaques were visualized with crystal violet.

### Statistical analysis

Statistical analyses were performed using InStat (GraphPad, La Jolla, CA) software. In most cases, ANOVA (Kruskal-Wallis) was used to compare results.

### Results

#### Primary and secondary OT-IIT expansion

Questions relating to naive and memory CD4+ T cell precursor frequencies are readily probed using adoptively transferred, Thy1–different OT-IIT populations. The extent of proliferation following primary challenge was analyzed by giving 6 x 10⁵ naive OT-IIT cells to wt (Thy1.2°) B6 mice, which were then infected i.n. with variant H3N2 influenza A viruses on the following day. The spleen and BAL populations were harvested, and the extent of expansion was assessed for the Thy1.1° OT-IIT cells by comparing the values resulting from exposure to the H3wt or H3ova virus. Consistent with findings for similar TCR-Tg models, stimulation with the OT-II epitope greatly increased the number of OT-II T cells, representing a 16-fold expansion over the value found following infection with the wt virus (Fig. 1A). In the BAL population, the OT-IIT cells dominated the CD4+ T cell component, constituting >50% of those in the airways (primary, Fig. 1B).

To test for the establishment of memory, naive B6 mice were given 6 x 10⁶ naive OT-IIT cells and then were infected i.p. with a high dose of the H1ova or wild-type H1N1 (PR8) (H1wt) virus. After 50 d, the mice primed with the H1wt virus had few detectable OT-IIT cells, whereas individual spleens from the H1ova group contained substantial numbers (day 30, Fig. 1C). Groups of these H1ova or H1wt immune mice were then challenged i.n. with the homologous H1ova or H3wt virus, and spleen and BAL populations were harvested 8 d later (day 8, Fig. 1C). Although there was some indication (not statistically significant) that OT-IIT memory precursors had...
increased ~3-fold (to 3 x 10^5 cells/spleen) in number, this was much lower than the expansions that normally occur for CD8+ T cells (see Ref. 10 and PA [DPA224 CD8+ T cell responses] and PBI [KBPB1703 CD8+ T cell responses] in Fig. 5). Furthermore, the OT-IIT set constituted only ~10% of CD4+ T cell numbers in the secondary BAL compartment, a considerable decrease in the relative prevalence from that found following primary challenge (Fig. 1D).

**Phenotypes of precursor and expanded CD4+ memory T cells and lack of protection**

The results presented in Fig. 1 indicate that OT-II–specific CD4+ memory T cells show less capacity for further expansion than the comparable virus or OT-I–specific CD8+ sets (20). Does this difference correlate in any way with the relative prominence of effector (CD62Llo) versus central (CD62Lhi) memory precursors (28, 29)? Following adoptive transfer, influenza virus-specific CD44hiCD8+CD62Llo and CD62Lhi memory T cells show evidence of a substantial capacity for further expansion and effector function (20). Also, is there evidence that physiological numbers of effector or central CD4+ memory T cells can protect against viral challenge?

Thus, the phenotypic and functional characteristics of immune OT-IIT cells were analyzed using the experimental plan illustrated at the top of Fig. 2. Naive (6 x 10^5) OT-IIT cells were transferred to B6 mice, which were then infected i.n with the H3ova virus and “rested” for 30 d. Spleen populations were then sorted to isolate the Thy1.1+CD44hi, CD62Lhi, or CD62lo subsets, and 1 x 10^5 OT-IIT CD44hiCD62Llo or CD44hiCD62Lhi memory T cells were transferred into individual recipients (Fig. 2B). Along with controls that were injected with diluent (PBS) or given an equivalent number of naive OT-IIT cells, all mice were infected i.n with a potentially lethal (high path, Fig. 2C, 2D) or readily survivable (low path, Fig. 2E, 2F) dose of the H1ova or H3ova virus, respectively.

Following high- (Fig. 2C) or low- (Fig. 2E) path virus challenge, the extent of OT-IIT cell expansion on day 8 was no greater than expected.
for CD44hiCD62Lhi memory T cells than for an equivalent number of naive precursors, whereas the smallest counts were found in those given the primed CD44hiCD62Llo set (∼one fourth of the naive and CD62Lhi sets). In several experiments, there was no significant difference between the number of cells recovered from the BAL in the CD44hiCD62Lhi- or CD44hiCD62Llo-recipient animals (data not shown). The numbers recovered for all subsets were lower than from the spleen, and the trend (two of three experiments) was toward higher recovery from the CD44hiCD62Lhi-recipient animals, despite the tissue tropism expected of CD44hiCD62Llo cells. Furthermore, neither the immune CD44hiCD62Lhi nor the CD44hiCD62Llo memory T cells conferred any greater protection against virus-induced weight loss (Fig. 2D,2F), virus growth, or mortality (Table I) than naive or no OT-IIT cells. In addition, all of the transferred OT-II T cell subsets gave rise to CD44hiCD62Llo and CD44hiCD62Lhi OT-IIT progeny on day 8 (data not shown), with those from the memory CD44hiCD62Llo precursors being least likely to express their starting phenotype after further Ag challenge. Again, although the evidence for greater proliferative capacity of the CD44hiCD62Lhi versus CD44hiCD62Llo T cells is what might be expected from the broader literature in this field, the findings are generally different from the situation found previously for influenza A virus-specific CD8+ T cells (30).

Cell number effects on recall efficiency

Transferring too many TCR-Tg cells can be counterproductive, because giving lower cell doses can result in greater proliferation and total cell accumulation (27, 31). Similarly, resting CD4+ T cell numbers can decline when large numbers of clonotypic T cells are transferred, whereas more heterogeneous populations may be maintained (25). To test whether we would see more efficient expansion at smaller cell doses, we gave recipient mice 10³, 10⁴, or 10⁵ naive OT-IIT cells. Mice were infected i.p. with the H1ova virus. Some were sampled 30 d later to determine the numbers of OT-IIT memory T cells, whereas others were challenged i.n. with H3ova on day 30, and OT-IIT cells were counted for BAL, MLN, and spleen samples taken 8 d later. None of the mice showed any evidence of substantial CD4+ T cell expansion from the memory compartment, irrespective of the initial, naive TCR-Tg numbers (Fig. 3A). The results were no different from those found for the high-dose transfers analyzed in Fig. 1. We repeated the analysis of the 10³ transfer at additional time points to determine whether increased expansion might occur at earlier or later time points than

<table>
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<th>Table I.</th>
<th>Lung titers and mortality in mice receiving CD4 memory or naive populations following lethal H1ova influenza challenge</th>
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<tr>
<td></td>
<td>PBS (No. Cells)</td>
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<tr>
<td>Day 8 titer (log₁₀ PFU) (mean ± SEM)</td>
<td>4.71 ± 0.048</td>
</tr>
<tr>
<td>Mortality by day 8 or 30% weight loss (N [%])</td>
<td>5/15 (33)</td>
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Mice were infected with a lethal dose of H1ova (10⁴ EID₅₀). Lung titers were determined by MDCK plaque assay. Titers are representative of at least two independent experiments, with three to five mice per group. Mortality was determined across three independent experiments. Mice were humanely euthanized after 30% weight loss. No statistical differences were found among the groups by Cox Proportional Hazards test.
and a memory response on day 30. No difference was observed in induction of these factors between a primary response on day 5 and a secondary response on day 30. Previous reports (27) suggested that key regulators of apoptosis (Bim and Nor1) might be selectively modifying memory T cell homeostasis for the higher-dose transfers. Using real-time RT-PCR, we compared the level of expression of these factors between naive and memory CD4+ T cells from high-dose transfer OT-IIT cells compared with splenic cells isolated on day 5 following primary infection, with data normalized to internal controls. Data represent averages of at least three mice per group and are representative of two independent experiments. *p < 0.05 by ANOVA (Kruskal-Wallis, Tukey posttest).

Endogenous CD4+ T cell responses

To determine whether the poor secondary expansions observed in our TCR-Tg model could be replicated for an endogenous epitope, we tested this hypothesis by comparing the magnitude of the recall, endogenous, H2IA6+OT-IIt epitope (OT-Ii6e)-specific CD4+ T cell response with that for two subdominant, secondary CD8+ sets (Fig. 5A). The CD8+KbPB1703 T cell populations were essentially comparable in size following priming and boosting with the H1wt and H3wt or H1ova and H3ova viruses; however, as might be expected, only the latter combination led to the expansion of an OT-Ii6e-specific set. However, the numbers of Ag-specific CD4+ T cells were significantly lower than the counts for the two CD8+ T cell responses.

An endogenous MHC II-restricted epitope, SLY1, derived from the x31 HA, was described previously (26). We engineered this sequence into the same site on the H1 HA and measured the magnitude of the response (Fig. 5B) in mice primed with H1wt PA224 and CD8+KbPB1703 T cell populations were essentially comparable in size following priming and boosting with the H1wt and H3wt or H1ova and H3ova viruses; however, as might be expected, only the latter combination led to the expansion of an OT-Ii6e-specific set. However, the numbers of Ag-specific CD4+ T cells were significantly lower than the counts for the two CD8+ T cell responses.

FIGURE 4. CD8 depletion enhances OT-IIT memory. Secondary anti-influenza responses are characterized by effective viral control by cross-reactive, memory CD8+ T cells. Is this efficient cell-mediated clearance limiting the activation and expansion of memory CD4+ T cells? We tested this hypothesis by transferring 103 or 105 OT-IIT cells into naive animals, priming with H1ova, and resting the animals for 30 d to generate OT-IIT memory cells. We then depleted CD8+ T cells from these animals by i.p. administration of an anti-CD8 mAb (2.43), followed by infection with H3ova. The Ab was administered twice prior to infection and, subsequently, on alternating days until day 8, when the mice were euthanized, and BAL, spleen, and MLNs were analyzed for OT-IIT expansion (Fig. 4). There was a 10-fold increase in the number of OT-IIT cells recovered from the BAL of the 105 transfer group (Fig. 4A), whereas there was an 8-fold increase in the 103 transfer group (Fig. 4A). Higher numbers of OT-IIT cells were found in all organs examined, but these differences were only statistically significant for BAL.

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and challenged with H3wt (a primary response to SLY1) or mice primed with H1sly and challenged with H3wt (a secondary response to SLY1). The magnitude of this and OT-Ile-specific endogenous responses were comparable, regardless of the priming regimen. There was no statistically significant increase in response magnitude resulting from earlier exposure to this epitope.

**Discussion**

Analyzing the characteristics of CD4+ T cell expansion, phenotype, and possible effector function following primary or secondary challenge with engineered influenza A viruses has shown intriguing differences from the more familiar CD8+ CTL response profiles (32, 33). Although CD4+ OT-IIT cells respond robustly on primary infection with OT-Ipt+ influenza A viruses (expanding 16-fold), the extent of proliferation following secondary challenge is much less (~3-fold and not significant). This is very different from the established profile for most influenza virus-specific CD8+ T cell responses (10, 34). Perhaps although the OT-II system has been widely used (20), OT-Ile is a very poor epitope, or the OT-IIT TCR is suboptimal. However, this would also need to be true for the native H3SLY1 response. As a general point, there is still a great deal to learn about what constitutes the best fit for peptide+MHC complexes and TCRs and how that, in turn, translates into response profiles.

The possibility of intraclonal competition limits the wide applicability of TCR-Tg models and emphasizes the need to transfer physiological numbers of cells (25, 31). Phenotypic differences can be induced by varying the cell dose, suggesting that a compromised response profile (including functional impairment) can be a consequence of transferring too many TCR-Tg cells (24). In this influenza virus model, transferring decreasing numbers of TCR-Tg cells increased the efficiency of memory formation, although not to the dramatic extent described for the LCMV system, in which a 100-fold lower cell dose can expand to higher absolute counts (31). The moderate increase in expansion efficiency observed in our transfer experiments may be partially explained by enhanced message levels for the proapoptotic molecule Bim found in memory cells generated following high-dose transfer. Combined with previous reports using LCMV, this suggests a universal apoptosis enhancement mechanism for minimizing clonal dominance in CD4+ T cell populations. Studies showing a role for self-MHC II complexes in homeostatic proliferation and survival suggest one mechanism by which high doses of TCR-Tg T cells may compete for a scarce resource (specific self-MHC II complexes), resulting in apoptosis (35, 36). Even so, despite this effect, secondary expansion was severely compromised for even low-dose OT-IIT transfers, a finding replicated with the endogenous SLY1 epitope.

Within the OT-IIT memory CD4+ population, the central memory CD62L+CD44hi subset showed a substantially greater capacity for further expansion than the CD62L+CD44+ effector memory subset. In fact, the sorted CD62L+ OT-IIT cells behaved much like the more potent naive set following challenge with a lethal virus. Furthermore, the CD44/CD62L partitioning of CD4+ OT-IIT cell memory contrasts with comparable experiments using endogenous influenza A virus-specific CD8+ memory CTLs, which showed no difference in transfer capacity for the central and effector populations defined by the CD62L marker (20). In that study, the optimal CD8+ memory T cells localized preferentially to the draining MLN, which contained more potent CD62Llo and CD62Lhi memory CTL precursors compared with the spleen at all time points tested. However, when we tested CD4+ OT-IIT memory populations from the MLN and the spleen, there did not seem to be a significant advantage for the lymph node precursors (data not shown).

Direct intratracheal transfer of effector CD4+ T cell populations from Sendai- or influenza-infected lung showed some efficacy in reducing viral titers following challenge. This correlated with a rapid production of IFN-γ and was based on the transfer of 5 × 107 polyclonal effectors (37). It may be that the limited memory numbers generated in our system did not provide enough cytokine support to generate a protective effect. Overall, the numbers of CD4+ OT-Ile–specific T cell memory generated in this system provided no protection against virus-induced pathology (as measured by weight loss and mortality) and caused no increase in the magnitude of concurrent CD8+ T cell responses (data not shown). This contrasts with findings from a Sendai virus pneumonia model that shows robust CD4+ T cell recall responses following in vivo virus challenge and with an influenza-based experimental system in which very large numbers of in vitro-stimulated, TCR-Tg or polyclonal CD4+ memory T cells are transferred (15, 21, 38). In these instances, CD4+ effectors seemed to contribute some measure of protection and even mediate virus clearance. However, other experiments (32) that did not use this in vitro culture step showed no evidence that influenza-specific CD4+ T cells can control virus in the absence of Ab.

Compared with other viral systems, might the relative CD4+ T help independence of influenza-specific CD8+ T cell responses be a function of a relatively weak CD4+ T cell response? The robust CD8+ T cell secondary response is capable of controlling infection; in its absence, we observed increased numbers of OT-IIT cells in the BAL. However, the numbers in other organs were only slightly elevated, and this was not statistically significant. It suggests some mechanism for the competitive exclusion of OT-IIT cells being exerted by CD8+ T cells, possibly by the removal of Ag.

Alternatively, other investigators concluded that long-lived CD4+ T cell memory is not a general feature of immunity, especially if CD4+ T help is relatively dispensable for potent effector recall responses (39, 40). These studies came to similar conclusions as our own, showing that memory CD4+ T cells proliferate poorly compared with naive cells in an immunization-based model or in response to systemic LCMV infection. Thus, increased CD4+ T cell secondary responses are only a function of higher precursor frequency in the memory compartment, although LCMV infection during challenge drove stronger memory CD4+ T cell proliferation than immunization. Our experiments have extended these findings to a localized infectious model system in which, in contrast to LCMV, we showed relatively robust CD8+ T cell proliferation following secondary challenge, which correlated with a rapid production of IFN-γ and was based on the transfer of 5 × 107 polyclonal effectors (37). It may be that the limited memory numbers generated in our system did not provide enough cytokine support to generate a protective effect. Overall, the numbers of CD4+ OT-Ile–specific T cell memory generated in this system provided no protection against virus-induced pathology (as measured by weight loss and mortality) and caused no increase in the magnitude of concurrent CD8+ T cell responses (data not shown). This contrasts with findings from a Sendai virus pneumonia model that shows robust CD4+ T cell recall responses following in vivo virus challenge and with an influenza-based experimental system in which very large numbers of in vitro-stimulated, TCR-Tg or polyclonal CD4+ memory T cells are transferred (15, 21, 38). In these instances, CD4+ effectors seemed to contribute some measure of protection and even mediate virus clearance. However, other experiments (32) that did not use this in vitro culture step showed no evidence that influenza-specific CD4+ T cells can control virus in the absence of Ab.

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Alternatively, other investigators concluded that long-lived CD4+ T cell memory is not a general feature of immunity, especially if CD4+ T help is relatively dispensable for potent effector recall responses (39, 40). These studies came to similar conclusions as our own, showing that memory CD4+ T cells proliferate poorly compared with naive cells in an immunization-based model or in response to systemic LCMV infection. Thus, increased CD4+ T cell secondary responses are only a function of higher precursor frequency in the memory compartment, although LCMV infection during challenge drove stronger memory CD4+ T cell proliferation than immunization. Our experiments have extended these findings to a localized infectious model system in which, in contrast to LCMV, we showed relatively robust CD8+ T cell proliferation following secondary challenge, which correlated with a rapid production of IFN-γ and was based on the transfer of 5 × 107 polyclonal effectors (37). It may be that the limited memory numbers generated in our system did not provide enough cytokine support to generate a protective effect. Overall, the numbers of CD4+ OT-Ile–specific T cell memory generated in this system provided no protection against virus-induced pathology (as measured by weight loss and mortality) and caused no increase in the magnitude of concurrent CD8+ T cell responses (data not shown). This contrasts with findings from a Sendai virus pneumonia model that shows robust CD4+ T cell recall responses following in vivo virus challenge and with an influenza-based experimental system in which very large numbers of in vitro-stimulated, TCR-Tg or polyclonal CD4+ memory T cells are transferred (15, 21, 38). In these instances, CD4+ effectors seemed to contribute some measure of protection and even mediate virus clearance. However, other experiments (32) that did not use this in vitro culture step showed no evidence that influenza-specific CD4+ T cells can control virus in the absence of Ab.

Compared with other viral systems, might the relative CD4+ T help independence of influenza-specific CD8+ T cell responses be a function of a relatively weak CD4+ T cell response? The robust CD8+ T cell secondary response is capable of controlling infection; in its absence, we observed increased numbers of OT-IIT cells in the BAL. However, the numbers in other organs were only slightly elevated, and this was not statistically significant. It suggests some mechanism for the competitive exclusion of OT-IIT cells being exerted by CD8+ T cells, possibly by the removal of Ag.
cell recall responses in CD4−deficient animals (although the memory compartment itself seems partially compromised) (9, 10, 41). It is possible that the strong priming environment provided by the inflammatory milieu in influenza virus infection allows comparatively poor CD4+ T cell memory and functionally independent CD8+ T cell responses. This hypothesis warrants further investigation.

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Disclosures

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