A Critical Role for CD40–CD40 Ligand Interactions in Amplification of the Mucosal CD8 T Cell Response

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Summary

The role of CD40 ligand (CD40L) in CD8 T cell activation was assessed by tracking antigen-specific T cells in vivo using both adoptive transfer of T cell receptor transgenic T cells and major histocompatibility complex (MHC) class I tetramers. Soluble antigen immunization induced entry of CD8 cells into the intestinal mucosa and cytotoxic T lymphocyte (CTL) differentiation, whereas CD8 cells in secondary lymphoid tissue proliferated but were not cytolytic. Immunization concurrent with CD40L blockade or in the absence of CD40 demonstrated that accumulation of CD8 T cells in the mucosa was CD40L dependent. Furthermore, activation was mediated through CD40L expressed by the CD8 cells, since inhibition by anti-CD40L monoclonal antibodies occurred after adoptive transfer to CD40L-deficient mice. However, mucosal CD8 T cells in normal and CD40<sup>-/-</sup> mice were equivalent killers, indicating that CD40L was not required for CTL differentiation. Appearance of virus-specific mucosal, but not splenic, CD8 cells also relied heavily on CD40–CD40L interactions. The mucosal CTL response of transferred CD8 T cells was MHC class II and interleukin 12 independent. The results established a novel pathway of direct CD40L-mediated CD8 T cell activation.

Key words: CD8 • costimulation • CD40 • mucosa • virus

Cell activation is dependent on multiple signaling events that occur through engagement of cell-surface receptors. Prime among these is triggering the TCR via an antigenic peptide–MHC complex, which can result in T cell activation (1, 2). However, this signal alone generally results in a transient, nonproductive immune response (3, 4). Costimulatory signals are required in addition to TCR engagement to drive the response to fruition (5). Inflammatory mediators generated during microbial infections or by adjuvants can induce the required costimulation (6-8). For example, activation of CD4 T cells with antigen in the absence of costimulatory signals results in a transient, nonproductive immune response (3, 4). However, in the presence of costimulatory signals, T cell responses are enhanced (3, 4). The effect of adjuvants can be at the level of the antigen-presenting cell (APC) by up-regulation of CD28 costimulatory ligands such as B7, and by induction of proinflammatory cytokines (6, 7). Another important costimulatory receptor–ligand interaction is CD40–CD40L (9, 10). CD40L (CD154) is upregulated on activated CD4 T cells and interacts with CD40 expressed by APCs and by B cells (11-13). Blockade or absence of CD40L or CD40 impairs T cell help for B cell activation (14, 15). Furthermore, activation of APCs via inflammation can be mimicked by triggering of CD40 with agonist ligands or antibodies (16-18).

Although the importance of CD40L-mediated CD4 T cell interactions with B cells is recognized, it has been less clear what role CD40 plays in CD8 T cell activation (9). Recent reports indicate that CD40L–CD40-mediated activation of dendritic cells (DCs) by antigen-specific CD4 T cells is essential for subsequent priming of CD8 T cells (19-21). That is, in those cases where T cell help is required to generate a productive CD8 T cell response, triggering of the DCs by CD4 T cells is necessary before DC encounter with an antigen-specific CD8 T cell. The properties engendered to the DC that are involved in CD8 T cell activation are not known, but possibilities are the up-regulation of B7 costimulators and/or cytokines such as IL-12 (22). Although activated CD8 T cells can themselves express CD40L (23-25), there is little evidence for a direct functional interaction between CD8 T cell CD40L and CD40 expressed by APCs or other cell types (9).

Using an adoptive transfer system in which ovalbumin (OVA)-specific CD8 T cells (OT-I cells) are transferred to normal mice, we have shown that immunization with solu-
ble (s)OVA in the absence of adjuvant results in proliferation and phenotypic differentiation of OT-I cells residing in secondary lymphoid tissues (26). However, only low levels of CTL activity are induced in these OT-I cells. In the same animals, OT-I cells that had migrated to the intestinal mucosa after activation and were present in the lamina propria (LP) and intraepithelial lymphocyte (IEL) compartment became potent antigen-specific CTLs (26). The basis for this dichotomy in the outcome of primary peripheral versus mucosal CD8 T cell responses is unknown. One possibility is that the inflammatory nature of the intestinal mucosa generates signals that induce mucosal APCs to become competent to drive CTL responses even when presenting soluble antigen. This theory was supported by our finding that costimulation via B7-1 played a larger role in the mucosal than in the peripheral OT-I response to sOVA (26). With regard to CD8 cells in secondary lymphoid tissues, this system also allowed the separation in vivo of the induction of proliferation from the induction of CTL activity. Using this model, as well as MHC tetramers to identify endogenous virus antigen-specific CD8 T cells, we have now analyzed the role of CD40–CD40L interactions in primary mucosal and peripheral CTL induction. The results provide evidence for a critical role for CD40 in the optimal generation of a mucosal CD8 T cell response.

Materials and Methods

Mice. C57BL/6J (Ly5.1), C57BL/6 (B6)–IL-12 (B6-IL-12tm1Imx) (27), and B6-Tnfds55tm1mix (CD40–/–, reference 28) mice were purchased from The Jackson Laboratory. C57BL/6TacfBr-KO (K6) mice (29) were purchased from Taconic Farms Inc. B6-Ly5.2 mice were obtained from Charles River Laboratories through the National Cancer Institute animal program. The OT-I mouse line was provided by W.R. Heath (The Walter and Eliza Hall Institute of Medical Research, Parkville, Australia) and F. Carbone (Monash Medical School, Prahran, Victoria, Australia) (30) and was maintained as a B6-Ly5.2 or B6-Ly5.1 line on a recombinant-activating-gene (RAG)–/– background. B6-Cd40–/– mice (13) were provided by Dr. Hitoshi Kikutani (Osaka University, Osaka, Japan) via Dr. Nancy Philips (University of Massachusetts Medical Center, Worcester, MA).

A adoptive transfer. This method was adopted from Kearney et al. (3). 2.5–4 × 106 pooled CD8 LN cells from OT-I–RAG–/– (Ly5.1 or Ly5.2) mice were injected intravenously into B6 (Ly5.1 or Ly5.2) mice. 2 d later 5 mg of OVA (Grade V1; Sigma Chemical Co.) was administered by intraperitoneal injection. Lymphocytes were isolated at the indicated times and analyzed for the presence of transferred cells by flow cytometric detection of Ly5 differences. Antibody treatments were performed by intraperitoneal injection of 200 μg of MR1 (anti-CD40L) or hamster Ig as control (11). MR1 was provided by Dr. R. N. Oelke, Dartmouth Medical School, Hanover, NH. Injections were given daily starting 1 d before immunization. Cells were labeled with 5- (and -6)-carboxyfluorescein diacetate succinimidy ester (CFSE; Molecular Probes, Inc.) at a concentration of 5 μm for 10 min at 37°C (31, 32). Each experiment was performed a minimum of three times.

Detection of Vesicular Stomatitis Virus-specific CD8 T Cells with MHC Tetramers. Mice were infected by intravenous injection of 1 × 106 PFU of vesicular stomatitis virus (VSV), Indiana serotype. 6 d later lymphocytes were isolated, and VSV nucleoprotein (N)-specific CD8 T cells were detected using H-2Kb tetramers containing the N protein–derived peptide R G Y V Y Q G L, or as a control the OVA-derived peptide SIINFEKL (Research Genetics) (33). MHC tetramers were produced essentially as described previously (34, 35). Briefly, H-2Kb containing the BirA–dependent biotinylation substrate sequence was folded in the presence of human β2-microglobulin and the N peptide. Biotinylation was performed with biotin–protein ligase (AviDity). Tetramers were then produced from biotinylated HPLC–purified monomers by addition of streptavidin–allophycocyanin (Molecular Probes, Inc.).

Isolation of Lymphocyte Populations. IELs and LP cells were isolated as described previously (36, 37). For cytotoxicity assays, panning of Percoll-fractionated IELs on anti-CD8 mAb-coated plates was performed to remove contaminating epithelial cells. Without panning, lytic activity of IELs is less consistent between replicates, but overall activity is similar to that of panned cells. Lytic activity of LPLs is measured without prior panning and is similar to that of IELs, indicating that the anti-CD8 panning step does not affect lytic function (data not shown). LNs and spleens were removed, and single cell suspensions were prepared using a tissue homogenizer. Peripheral LN s included brachial, axillary, and superficial inguinal nodes. The preparation was filtered through Nitex, and the filtrate was centrifuged to pellet the cells.

Immunofluorescence Analysis. Lymphocytes were resuspended in PBS, 0.2% BSA, 0.1% NaN3 (PBS/BSA/NaN3) at a concentration of 105–106 cells ml followed by incubation at 4°C for 30 min with 100 μl of properly diluted mAb. The mAbs were either directly labeled with FITC, PE, Cy5, allophycocyanin, or were biotinylated. For the latter, avidin-PE-Cy7 (Caltag Laboratories) was used as a secondary reagent for detection. After staining, the cells were washed twice with PBS/BSA/Nan3 and fixed in 3% paraformaldehyde in PBS. Relative fluorescence intensities were then measured with a FACSCalibur™ (Becton Dickinson). Data were analyzed using WinMDI software (Joseph Trotter, Scripps Clinic, La Jolla, CA).

Material measurement. Cytolytic activity was measured using [3H]sodium chromate-labeled EL4 cells (HL-A2b thymoma), with or without the addition of 10 μg/ml of the OVA-derived peptide SIINFEKL. Serial dilutions of effector cells were incubated in 96-well round-bottomed microtiter plates with 2.5 × 104 target cells for 5 h at 37°C. Percent specific lysis was calculated as: 100[(cpm released with effectors) – (cpm released alone)]/(cpm released by detergent) – (cpm released alone).

Results

Blockade of CD40 L inhibits OT-I T-cell accumulation in mucosal tissues. Our previous results indicate that adoptively transferred OVA-specific T cells migrating into the intestinal mucosa become potent CTLs after immunization with OVA. To test whether CD40–CD40L interactions were involved in this activation, we treated mice during immunization with a blocking mAb specific for CD40L (11). Naive OT-I cells do not enter the intestinal mucosa (26, 38) but a small population of naive cells (~0.5%) can be detected in the peripheral LN s (PLN s) and mesenteric LN s (MLN s) by virtue of differences in Ly5.1/Ly5.2 expression between the
transferred and host lymphocytes (Fig. 1). 3 d after intraperitoneal immunization with sOVA, a large increase in OT-I cells in PLNs and MLNs was observed. Although the magnitude of this response is dependent on antigen dose, immunization with 0.5 mg sOVA resulted in detectable T cell activation and migration to the mucosa (data not shown). Responses in MLNs were partially inhibited (~30%), whereas PLN OT-I responses were not affected (Figs. 1, 2 A, and 3). Although the inhibition of the MLN response is not impressive, it was consistently observed, as was the lack of inhibition of the PLN response. However, treatment with anti-CD40L mAb resulted in ~70–80% inhibition of appearance of OT-I cells in the LP (Fig. 1) and LP (Fig. 2 B) compartments. This inhibition was not merely a kinetic delay in the response, as the effect of early anti-CD40L blockade was maintained throughout the response (8 d and longer after immunization; data not shown). Thus, there was an apparent requirement for CD40–CD40L interactions to generate an optimal mucosal CD8 T cell response.

**CD40L Delivers Proliferative Signals to CD8 T Cells.** The effect of anti-CD40L mAb on mucosal OT-I accumulation could have been due to inhibition of migration or of proliferation. In addition, inhibition of proliferation outside of the mucosa could result in fewer cells migrating to the mucosa. To determine the point at which CD40L interactions were involved in activation, we transferred CFSE-labeled OT-I cells and then immunized with OVA in the presence or absence of anti-CD40L mAb. 3 d after immunization, lymphoid tissues were analyzed for the presence of donor cells and their CFSE fluorescence was measured. CFSE is distributed equally between daughter cells upon division and can be detected over nine divisions (i.e., no fluorescence after nine divisions [31, 32]). In all sites, OT-I cells from control mice had divided at least nine times and thus retained no fluorescence above background (Fig. 2, A and B). Transferred cells in PLNs from MR1-treated mice also exhibited little fluorescence, indicating that the cells had divided at least nine times, and that CD40L blockade had not greatly affected the proliferation of cells in the PLNs. In MLNs, ~40% of the cells from anti-CD40L-treated mice had undergone eight divisions, at least one less than control OT-I cells. In the LP from MR1-treated mice, ~50% of the OT-I cells had divided less than nine times, with 28% having divided seven times, and 7% having undergone six divisions. In IELs from MR1-treated mice, ~60% of the cells had undergone less than nine divisions, and 22% had divided seven times. This inhibition of
proliferation correlated well with the decreased percentage and total cell number (data not shown) of OT-I cells in LP and IELs. These results suggested that CD40L-mediated proliferative signals, whether delivered within or outside of the mucosa, were important for generation of the mucosal CD8 response.

The results thus far demonstrated a role for CD40L in CD8 T cell activation, but did not indicate whether this effect was direct or indirect. To test this, we transferred OT-I–RAG2−/− cells into CD40L-deficient mice and attempted to block activation with MR1. In this experiment, only the transferred CD8 T cells were capable of expressing CD40L, so that any inhibitory effect must be mediated at the level of the OT-I cells. After OT-I cell transfer, immunization of CD40L−/− mice with sOVA resulted in substantial proliferation in the periphery and appearance of OT-I cells in the LP (Fig. 3). Treatment with anti-CD40L mAb resulted in partial inhibition of OT-I accumulation in MLNs and much greater inhibition of OT-I accumulation in the LP (Fig. 3). Little inhibition of OT-I expansion in PLNs was observed (data not shown). These results were similar to those obtained in CD40L-competent mice (Figs. 1 and 2), and indicated that CD40L expressed by CD8 T cells was responsible for activation of OT-I cells destined for the mucosa.

CD40 expression is required for mucosal CD8 T cell responses of transferred or endogenous CD8 T cells. Although anti-CD40L mAb treatments have been widely employed to test in vivo function, it was possible that mAb binding to CD40L directed untoward effects on the T cells. Therefore, we performed adoptive transfer and immunization in mice lacking CD40 (Fig. 4). 4 d after antigen administration, substantial populations of OT-I cells were detected in PLNs, MLNs, LP, and IELs of normal mice. However, in the absence of CD40, OT-I cells were reduced by ≈80% in IELs and LP, just as they were with MR1 treatment. Interestingly, the inhibition of OT-I cell expansion in both PLNs and MLNs was greater than that observed with MR1 treatment. However, a difference remained between PLNs (≈40% reduction) versus MLNs (≈75% reduction).

To further examine the role of CD40 in mucosal CD8 T cell responses, we visualized the response of endogenous antiviral CD8 T cells using MHC tetramers. B6 or CD40−/− mice were infected with VSV, and 6 d later spleen cells, LPLs, and IELs were isolated. N-specific CD8 T cells were identified using H-2Kb–N peptide MHC tetramers. In normal mice, N-specific cells consistently comprised ≈10% of the splenic CD8 T cells, whereas in the CD40−/− mouse shown the response was partially inhibited, with 45% of the CD8 cells reactive with N-peptide (Fig. 5). However, the difference observed in the splenic response between control (10.4 ± 1.8, n = 6) versus CD40−/− (7.6 ± 2; n = 6) mice was not statistically significant. In contrast, the anti-VSV CD8 response in LP was greatly inhibited in the ab-
The presence of CD40. In the experiment shown, 21% of LP CD8 cells from control mice were N-specific, while only 2.5% were N-specific in CD40<sup>−/−</sup> mice (Fig. 5). The difference between the response in control mice (19.7 ± 0.8, n = 6) versus CD40<sup>−/−</sup> mice (4 ± 0.6; n = 6) was highly significant (P = 0.001) and was independent of total CD8 cell numbers (data not shown). Although we do not yet know whether CD40L expressed by antiviral CD8 cells is involved in activation, the results nevertheless demonstrated the importance of CD40–CD40L interactions in the mucosal antiviral CD8 response. Generation of Mucosal OT-I CTLs Is CD40 Independent. The adoptive transfer system allows a comparison of lytic activity on a per cell basis, and so whether CD40L triggering results in enhanced lytic activity was tested. We analyzed CTL activity of transferred OT-I cells from IELs and spleen from immunized, normal, or CD40<sup>−/−</sup> mice (Fig. 6). sOVA immunization induced poor lytic activity in OT-I cells in spleen despite their activated state, as determined by phenotype and proliferation (data not shown, and reference 26). In contrast, sOVA immunization resulted in appearance of potent OT-I IEL effectors (Fig. 6). In the absence of CD40, OT-I proliferation was severely inhibited in the IEL compartment, as shown in Fig. 4. Nevertheless, when the lytic activity of OT-I effectors was compared on a per cell basis, OT-I IELs in CD40<sup>−/−</sup> mice were equally effective at inducing antigen-specific lysis as IELs from control mice. This result demonstrated that CD40–CD40L interactions were essential for optimal induction of proliferation of CD8 T cells, but that differentiation to CTLs in the intestinal mucosa was CD40 and CD40L independent. MHC Class II–selected CD4 T Cells or IL-12 Are Not Required for Induction of Peripheral or Mucosal CTLs. The MR1 blocking studies predicted that CD4 cells were not likely to be involved in the sOVA activation of mucosal OT-I cells. To test this directly, the lytic activity of OT-I IELs was measured after adoptive transfer and immunization of MHC class II–deficient mice. The absence of MHC class II had no effect on generation of CTLs in the mucosa (Fig. 7). Proliferation of OT-I cells in MHC class II–deficient mice was also no different from that observed in normal mice.

Figure 5. Amplification of the mucosal antiviral CD8 T cell response requires CD40. B6 or CD40<sup>−/−</sup> mice were infected by intravenous injection of 1 × 10<sup>6</sup> PFU of VSV. 6 d later, spleen and LP cells were isolated and stained for three-color flow cytometry with allophtocyanin-labeled H-2K<sup>b</sup>-N peptide tetramers (N-tet-APC), anti-CD8-PE, and anti-CD11a-FITC. CD8<sup>+</sup> cells were positively gated and then analyzed for tetramer and CD11a staining. Negative control staining of cells from infected mice was performed with H-2K<sup>b</sup>-OVA peptide tetramers, and no staining was observed (data not shown).

Figure 6. Mucosa-specific induction of CD8 lytic activity does not require CD40. Naive OT-I–RAG<sup>−/−</sup> cells (Ly5.2) were transferred to C57BL/6J or B6-CD40<sup>−/−</sup> mice, and 2 d later the mice were immunized intraperitoneally with 5 mg OVA. 3 d later, lymphocytes were isolated and tested for cytolysis activity against SIINFEKL-coated (filled symbols) or untreated (open symbols) 51Cr-labeled EL4 target cells. The effector-to-target ratios shown indicate actual percentages of OT-I cells based on flow cytometric data. , B6 IELs; , CD40<sup>−/−</sup> IELs; , B6 spleen cells. Spontaneous 51Cr release was <10%.

Figure 7. Mucosal induction of CD8 T cell lytic activity occurs independently of MHC class II and IL-12. Naive OT-I–RAG<sup>−/−</sup> cells (4 × 10<sup>6</sup>; Ly5.2) were transferred to C57BL/6J, or B6-AP<sub>b</sub>–IL-12<sup>−/−</sup>, or B6–IL-12<sup>−/−</sup> mice, and 2 d later the mice were immunized intraperitoneally with 5 mg OVA. 3 d later IELs were isolated and tested for cytolysis activity against SIINFEKL-coated 51Cr-labeled EL4 target cells. The effector-to-target ratios shown indicate actual percentages of OT-I cells based on flow cytometric data. Host mice were (A) B6; (B) MHC class II<sup>−/−</sup>; (B) IL-12<sup>−/−</sup>. Specific lysis in the absence of peptide was <10%. Spontaneous 51Cr release was <10%.
Discussion

The results presented here identified for the first time a critical role for CD40-CD40L interactions in mucosal CD8 T cell responses. Blockade or removal of CD40L interactions demonstrated a more stringent requirement for CD40-CD40L engagement to generate a CD8 response in mucosal effector sites (LP, IELs) compared with responses in peripheral secondary lymphoid tissue. This was true for the response of adoptively transferred OT-1 cells (Figs. 1-4), as well as for an endogenous antiviral CD8 T cell response (Fig. 5). Recently, it was reported that the splenic anti-VSV CTL response was CD40 independent as determined by measuring lytic activity (43). Our present results using MHC tetramer staining corroborate these findings (Fig. 5). Perhaps more importantly, our data showed that despite the fact that the splenic anti-VSV CD8 response was CD40 independent, the LP CD8 response had a stringent requirement for CD40. These results demonstrated a role for tissue-specific regulation of the CD8 immune response via CD40. The results also suggested, but did not prove, that CD8 cells migrating to the mucosa encountered APCs in the LP, which induced further proliferation and differentiation. There are few, if any, professional APCs in the intestinal epithelium, so the inhibition of the IEL response by CD40-CD40L blockade or absence was likely the result of a decrease in the LPL response and/or the peripheral response, thereby resulting in fewer cells available for migration to the epithelium. Indeed, the inhibition of the LPL response always paralleled the decrease in the IEL response.

If the mucosal response is in fact modulated by interaction of migrating CD8 cells with LP APCs, then the resulting effects could be linked to the status of APCs in the LP. That is, LP APCs may be at a heightened activated state compared with those in nonmucosal areas. This possibility was supported by the induction of CTL activity in mucosal effector sites but not in peripheral tissues. DCs from the gut migrate to MLNs (44, 45), and this could form the basis of the observed role for CD40L in that site. However, signals in addition to CD40L triggering must be involved in CTL induction, since activated OT-1 cells in MLNs exhibited poor lytic activity (data not shown). Overall, the results supported a generalized role for CD40-CD40L interactions in amplification of intestinal CD8 T cell responses, whether or not the relevant CD8 T cell-APC interaction occurred in the mucosa.

Our finding that CD40L expressed by activated OT-1 cells was interacting with CD40 expressed by APCs made sense, considering that MHC class II-restricted CD4 T cells were not required for the OT-1 response. The importance of CD40L expressed by OT-1 cells was unequivocally established by showing that inhibition of OT-1 expansion by CD40L blockade occurred when OT-1 cells were the only cells capable of expressing CD40L (Fig. 3). However, this finding does not preclude an indirect role for CD4 T cells, when they are present, in CTL expansion via an effect on APCs. Thus, CD4 cells could induce upregulation of CD40 on APCs, which would subsequently interact with CD40L expressed by CD8 T cells. Nevertheless, the recent demonstration that CD8 T cells can directly activate dendritic cells (43) supports the concept that some CD8 responses do not require CD4 T cells to activate APCs.

In all other in vivo responses examined thus far, CD40L expressed by CD4 T cells was critical for CD8 T cell activation (19-21, 46, 47). However, in contrast to the system in which CD40 triggering is required to induce CTLs to OVA-loaded spleen cells (20), induction of mucosal CTLs to soluble antigen was CD40 independent. That is, despite the fact that the increase in OT-1 cells in LP and IELs was greatly inhibited by CD40L blockade, the lytic activity of the remaining cells was not affected. Thus, although CD40 triggering in some situations can drive CTL differentiation (21), this is apparently not a requisite step for mucosal CTL induction. It should be noted that in previous studies it was not possible to determine the effect of CD40 on proliferation versus CTL differentiation, since antigen-specific T cells were not quantified. The importance of CD40/CD40L in the mucosal CD8 T cell response indicates that the nature of the CD40 expressed by intestinal APCs may be distinct from that of APCs in nonmucosal sites, perhaps in terms of level of expression as well as activation status of the cell. The significant numbers of activated B cells in the LP could participate in the response as APCs. Further, the density and/or anatomy of the APC network in the LP may regulate T cell interactions differently from those occurring in secondary lymphoid tissue.

Although we do not yet know the factors required for mucosal induction of CTLs, we showed here that IL-12 was not essential. Exogenous IL-12 augments alloreactive CTL activity in vitro (39) and in vivo (41). Yet, it is not clear from these studies whether IL-12 acts directly on CD8 T cells or affects CD4 T cells which then influence the CTL response. Moreover, the primary alloreactive CTL response in vivo was unaffected in IL-12-deficient mice (27). In another report, low doses of IL-12 increased the number of CD8 cells in lymphocytic choriomeningitis virus-infected mice, whereas high doses of IL-12 inhibited the CTL response (42). Thus, although splenic CD8 cells may respond to exogenous IL-12, it remains unclear whether IL-12 is involved in systemic CD8 T cell immunity. In the case of mucosal T cell responses, IL-12 appears to play a significant role. In a model of colitis in which IFN-γ-producing Th1 cells are involved, inhibition of IL-12 results in abrogation of the disease (48). Furthermore, inhibition of CD40L-CD40 interaction inhibited the induction of the disease by blocking priming of Th1 cells by IL-12 (49). IL-12 may also play a role in some mucosal CD8 responses. Mucosal immunization of mice with a multideterminant HIV peptide results in the appearance of...
antigen-specific CTLs in spleen, Peyer's patches (PPs), and LP. However, CTL priming was completely abrogated when IL-12 was blocked or IFN-γ was absent (50). In our system, primary mucosal CTL development was IL-12 independent. This finding may be related to the demonstration that this response was also CD4 T cell independent, while the colitis model and the response to the HIV peptide require CD4 T cells. Therefore, as discussed above, the requirement for IL-12 in CD8 responses may be indirect and related to the action of this cytokine on CD4 T cells.

Our findings support a model for mucosal CD8 T cell activation in which sequential encounters with APCs may occur. As shown here for sOVA immunization and VSV infection, CD40–CD40L interaction allowed a significant amplification of the mucosal CD8 response. This system could provide a powerful mechanism for potentiation of mucosal immune responses. The goal of the immune system is to send functional CTLs to sites of potential pathogen entry, the intestine being foremost on this list. When the intestinal mucosa is infected, antigens are most likely presented to T cells in the draining organized secondary lymphoid tissue such as PPs and M LN s, followed by migration of activated T cells to the LP. Our hypothesis suggests that upon entry into the LP, CD8 T cells may reencounter antigen presented by APCs and receive further proliferative and differentiative signals. This theory predicts a second level of regulation for CD8 T cells patrolling the mucosa: i.e., if antigen is not encountered in the mucosa, further activation of CD8 T cells does not occur, thereby conserving resources and decreasing the opportunity for destructive autoimmune reactions. Deciphering the specialized signals for T cell activation in the intestinal mucosa will provide tools for manipulating inflammatory bowel disease and for potentiation of mucosal vaccination and immune barrier function.

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