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Immunology

Generation of mucosal cytotoxic T cells against soluble protein by tissue-specific environmental and costimulatory signals

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ABSTRACT **We compared peripheral and mucosal primary CD8 T cell responses to inflammatory and noninflammatory forms of antigen in a T cell-adoptive transfer system. Immunization with the soluble antigen, ovalbumin (ova), administered i.p. or orally without adjuvant, activated nonmucosal CD8 T cells but did not induce cytotoxic activity. However, after activation, the transferred cells entered the intestinal mucosa and became potent antigen-specific killers. Thus, exogenous intact soluble protein entered the major histocompatibility complex class I antigen presentation pathway and induced mucosal cytotoxic T lymphocytes. Moreover, distinct costimulatory requirements for activation of peripheral versus mucosal T cells were noted in that the CD28 ligand, B7-1, was critical for activated mucosal T cell generation but not for activation of peripheral CD8 T cells. The costimulator, B7-2, was required for optimum activation of both populations. Infection with a new recombinant vesicular stomatitis virus encoding ovalbumin induced lytic activity in mucosal as well as peripheral sites, demonstrating an adjuvant effect of inflammatory mediators produced during virus infection. Generation of antiviral cytotoxic T lymphocytes was also costimulation-dependent. The results indicated that induction of peripheral tolerance via antigen administration may not extend to mucosal sites because of distinct costimulatory and inflammatory signals in the mucosa.**

Induction of immune tolerance is a potential therapeutic avenue for regulating immune responses and autoimmunity (1–3). However, the consequences of tolerance induction *in vivo* need to be defined to make such treatment feasible. The intestinal mucosa is a site of chronic but controlled inflammation (4), and the effect of tolerance induction on the mucosal immune system is unclear. Many CD8 T cell receptor-$\alpha\beta$ (TCR$\alpha\beta$) and TCR$\gamma\delta$ T cells with constitutive lytic activity are present in the epithelium and lamina propria of the small intestine of mice and humans (5–7). However, the antigen specificity of such cells is difficult to measure because of the heterogeneity of the cell populations. Moreover, the precise origin of these cells remains unclear. Recent data indicate that TCR$\gamma\delta$ intraepithelial lymphocytes (IEL) mature extrathymically while the production of most TCR$\alpha\beta$ IEL requires the thymus (8). Thus, although TCR$\gamma\delta$ IEL may be activated in the intestinal epithelium, it is possible that thymus-derived TCR$\alpha\beta$ IEL are activated outside of the epithelium and subsequently traffic to the intestinal mucosa.

Using a TCR transgenic mouse model in which ovalbumin-specific major histocompatibility complex class I restricted CD8 T cells can be analyzed, we demonstrated recently that activation of CD8 T cells outside of the intestinal mucosa, probably in peripheral and mesenteric lymph nodes, was required for entry of these cells into the mucosa (9). Antigen-specific cytotoxic T lymphocytes (CTL) were detected in the mucosa after immunization with soluble ova in the absence of adjuvant. However, it was unclear whether the induction of lytic activity was specific to the mucosa or whether CTL differentiation could be driven by soluble antigen in the absence of inflammatory signals. This is an important issue considering the high level of constitutive lytic activity detectable in the IEL compartment and in the lamina propria (LP). If presumably tolerogenic forms of antigen have differential effects on peripheral versus mucosal immunity, there will be critical implications for the form and use of tolerizing as well as immunizing antigen regimens. In addition, identification of the factors involved in the generation and control of intestinal immunity versus tolerance will be important in devising ways to protect against pathogens entering via mucosa, such as the HIV. The role of costimulation in generating peripheral and mucosal CTL *in vivo* also has not been addressed adequately. Thus, while some studies suggest that costimulation is involved in activation of CD8 T cells and CTL induction (10–12), LCMV-specific CTL are induced efficiently in mice lacking CD28 (13). Therefore, we have now examined the factors involved in generation of CTL in peripheral versus mucosal lymphoid tissue with regard to the form of immunogen and the costimulatory requirements.

MATERIALS AND METHODS

Mice. C57BL/6J (Ly5.1) mice were obtained from The Jackson Laboratory. C57BL/6-Ly5.2 mice were obtained from Charles River Breeding Laboratories through the National Cancer Institute animal program. The OT-I mouse line (14) was maintained as a C57BL/6-Ly5.2 line or on a C57BL/6-RAG-$1^{-/-}$ background (The Jackson Laboratory).

Recombinant Vesicular Stomatitis Virus (VSV) Production and Infection. VSV-ova was produced by ligation of a *Xho*I-*Xba*I cDNA fragment containing the entire ovalbumin coding sequence into the pVSV-XN2 vector restricted by *Xho*I and *Nhe*I (15, 16). The ova gene-containing vector was transfected along with helper plasmids into BHK cells, and recombinant VSV was recovered as described previously (15, 16). Ova production was assessed by Western blot analysis of detergent lysates and culture supernatants of infected BHK cells. West-

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Abbreviations: CTL, cytotoxic T lymphocyte; IEL, intraepithelial lymphocyte; TCR, T cell receptor; ova, ovalbumin; LN, lymph node; LP, lamina propria; VSV, vesicular stomatitis virus; APC, antigen-presenting cell.

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ern blots were performed after separation of proteins by SDS/PAGE on 15% acrylamide gels and transfer to nitrocellulose. Rabbit anti-ova sera (Cappel) was used as primary antibody and was detected by using the ECL kit (Amersham) according to the manufacturer's instructions. For VSV infection of mice, 1×10^6 pfu of wild-type Indiana serotype VSV or VSV-ova were injected i.v. Five days later cells were isolated and analyzed for the presence of donor cells and cytotoxicity.

Adoptive Transfer. This method was adopted from Kearney *et al.* (17). Pooled lymph node (LN) cells (1×10^7) from OT-I (C57BL/6-Ly5.2) or OT-I-RAG$^{-/-}$ (Ly5.1$^+$) mice were injected i.v. into either C57BL/6J (Ly5.1) mice or C57BL/6-Ly5.2 mice, respectively. Two days later 5 mg of ovalbumin (Grade VI, Sigma) was administered by i.p. injection or 10 mg was given orally. Three to 10 days later lymphocytes were isolated and analyzed for the presence of transferred cells by flow cytometric detection of Ly5.1$^+$ or Ly5.2$^+$ cells. Antibody treatments were performed by i.p. injection of 100 μg of purified anti-B7-1 [clone 16-10A1 (18)] and/or 100 μg anti-B7-2 [clone GL1(19)], or 100 μg of hamster Ig as control. Similarly, 100 μg of CTLA4-Ig or the CTLA4-Ig mutant 104 as control (20) was injected i.p. Mutant 104 does not bind to B7-1 or B7-2 but retains Fc receptor binding. Injections were given daily starting on the day of immunization.

Isolation of Lymphocyte Populations. IEL and LP cells were isolated as described previously (21, 22). For cytotoxicity assays, panning of Percoll-fractionated IEL on anti-CD8 mAb-coated plates was performed to remove contaminating epithelial cells. Inguinal, brachial, and cervical lymph nodes and spleens were removed, and single-cell suspensions were prepared by using a tissue homogenizer. The resulting preparation was filtered through Nytex, and the filtrate was centrifuged to pellet the cells.

Immunofluorescence Analysis. Lymphocytes were resuspended in PBS/0.2% BSA/0.1% NaN_3 (PBS/BSA/NaN_3) at a concentration of 1×10^6 to 1×10^7 cells/ml followed by incubation at 4°C for 30 min with 100 μl of properly diluted mAb. The mAbs were either labeled directly with fluorescein isothiocyanate, phycoerythrin, or cychrome, or were biotinylated. For the latter, avidin-Red 670 (Av-R670; Life Technologies, Gaithersburg, MD) was used as a secondary reagent for detection. After staining, the cells were washed twice with PBS/BSA/NaN_3 and fixed in 3% paraformaldehyde buffer. Relative fluorescence intensities then were measured with a FACSCalibur (Becton Dickinson). Data were analyzed by using LYSYS II or WINMDI software.

Measurement of Cytolytic Activity. Cytolytic activity was measured by using [^{51}Cr] sodium chromate-labeled EL4 cells (an H-2^b 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-bottom microtiter plates with 2.5×10^3 target cells for 6 hr at 37°C. Percentage of specific lysis was calculated as: $100 \times$ [(cpm released with effectors) $-$ (cpm released alone)]/[(cpm released by detergent) $-$ (cpm released alone)].

RESULTS

To visualize activation of CD8 T cells in a defined system we employed a transgenic mouse line, OT-I, expressing a TCR specific for an ova peptide presented in the context of H-2K^b (9, 14). To test the consequences of activation of OT-I cells in a host with an intact immune system we adoptively transferred C57BL/6-Ly5.2 OT-I T cells into normal C57BL/6-Ly5.1 mice. Transferred cells can be detected by expression of Ly5.2 using flow cytometry. In the absence of immunization, 1.7% of LN cells expressed Ly5.2 and relatively low levels of CD11a (Fig. 1). However, 3 days after intraperitoneal immunization with soluble whole ova in the absence of adjuvant, donor T cells increased to 6.8% of total LN cells and expressed high levels

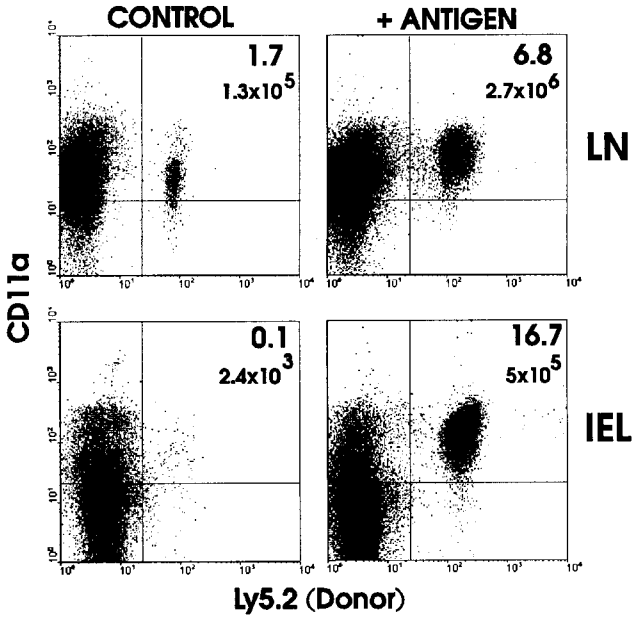

FIG. 1. Appearance of CD8 T cells in the intestinal mucosa after activation of transferred T cells. The adoptive transfer method was modified from Kearney *et al.* (17). C57BL/6-Ly5.2 OT-I T cells (1×10^7) were injected i.v. into C57BL/6-Ly5.1 mice. Two days later 5 mg of ovalbumin (+ antigen) was administered i.p. Three days later cells were isolated and analyzed for the presence of transferred cells (Ly5.2$^+$) and CD11a expression by fluorescence flow cytometry. Total donor cell numbers were calculated by multiplying the number of Ly5.2$^+$ cells by the total number of LN cells isolated. This experiment has been performed at least 10 times with similar results.

of CD11a. Overall, donor cells increased ≈21-fold after immunization since total lymph node cells increased ≈3-fold after immunization. Thus, intact ova was able to enter and be processed by the major histocompatibility complex class I antigen presentation pathway, resulting in T cell activation. In the intestinal epithelium, few transgenic T cells could be detected before immunization. Immunization resulted in a large increase in CD11a^{high} adoptively transferred cells in the IEL compartment. The increase in donor cells in the epithelium by day 3 was 208-fold in this experiment, indicating that the majority of these cells had migrated into the intestinal epithelium after immunization. Similar results were observed for LP lymphocytes (data not shown).

The functional consequences of soluble ova immunization were assessed by measurement of induction of cytolytic activity. A major advantage of the adoptive transfer system is the ability to calculate precisely the actual ratio of potential effectors to target cells in the cytotoxicity assay. A comparison of ova-specific lytic activity of mucosal versus peripheral OT-I T cells revealed an interesting dichotomy. Thus, although transferred OT-I LN cells were clearly activated by immunization, these cells exhibited only low levels of lytic activity (Fig. 2*A*). Similar results were obtained at earlier and later time points up to 10 days after immunization and when spleen cells were used as effectors (data not shown). Moreover, even in the intact transgenic mouse in which all T cells have the potential to be effectors, minimal lytic activity was induced by immunization with soluble ova (data not shown). In striking contrast, i.p. immunization resulted in the induction of high levels of ova-specific lytic activity in IEL that was attributable to the donor transgenic T cells (Fig. 2*A*). Based on actual effector-to-target ratios (as the values are plotted in Fig. 2), the lytic activity of IEL was 15- to 20-fold greater than that of LN or splenic T cells. Adoptively transferred OT-I-RAG$^{-/-}$ LN cells also did not produce lytic effectors in the LN but did generate

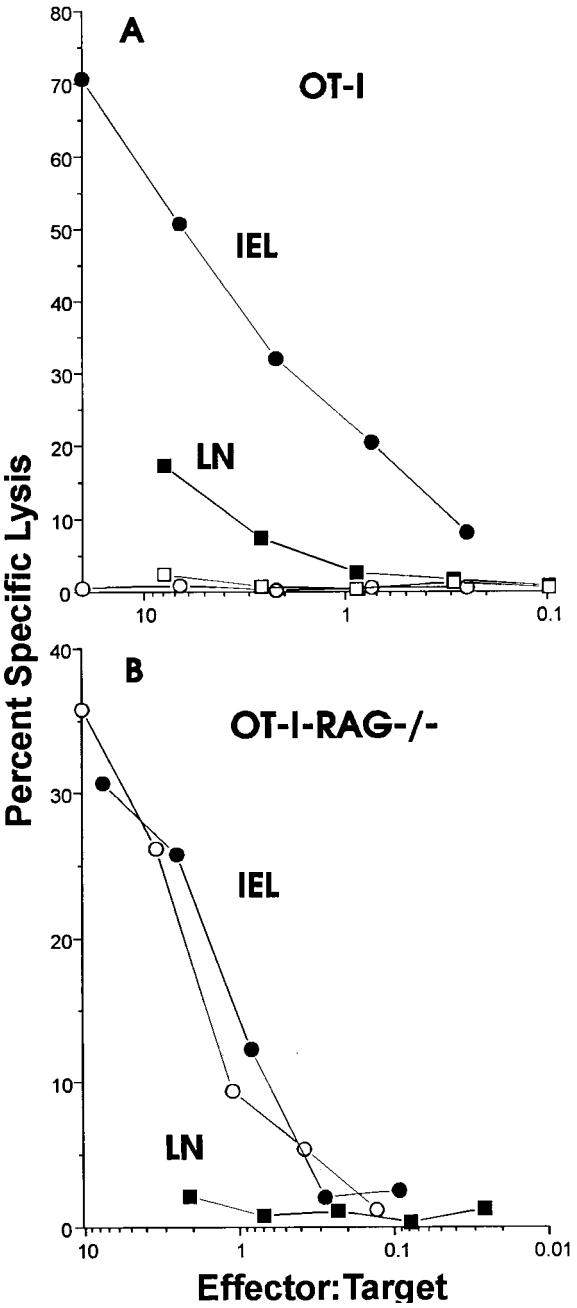

FIG. 2. Immunization with soluble antigen induces primary CTL in the intestinal mucosa but not in the periphery. OT-I or OT-I-RAG$^{-/-}$ T cells (1×10^7) were transferred to normal C57BL/6 mice and, 2 days later, were either injected i.p. with 5 mg ova or fed 10 mg ova by intubation. Four days later cells were isolated and cytolytic activity was measured by using [^{51}Cr] sodium chromate-labeled EL4 cells (an H-2^b 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-bottom microtiter plates with 2.5×10^3 target cells for 6 hr at 37°C. Percentage of specific lysis was calculated as: $100 \times$ [(cpm released with effectors) − (cpm released alone)]/[(cpm released by detergent) − (cpm released alone)]. (*A*) i.p. injection of ova. Open symbols, EL4 no peptide; closed symbols, EL4 with peptide. (*B*) Open circles, fed antigen; closed circles, i.p. injection; closed squares, LN cells from i.p. injected mouse; all symbols, EL4 with peptide as targets. Lytic activity without peptide added was <5%. Effector-to-target ratios were adjusted to actual donor cell percentages. This experiment has been performed eight times with similar results.

potent IEL effectors after i.p. immunization, indicating that activation via endogenous nontransgenic receptors was not responsible for CTL induction in the mucosa (Fig. 2*B*). Moreover, oral administration of antigen also induced mucosal antigen-specific CTL (Fig. 2*B*), indicating that oral tolerance did not extend to the intestinal mucosa for CD8 T cells, at least in the primary CTL response. IEL from normal mice (in the absence of transferred cells) did not exhibit lytic activity, and immunization was required for detection of ova-specific lytic activity. These results suggested that the intestinal mucosa is a highly efficient site of CTL differentiation for cells activated in the periphery presumably as part of the surveillance function of T cells in inflammatory sites.

Since immunization with soluble protein resulted in functional differences in CTL induction in peripheral versus mucosal lymphoid tissue, it was possible that the costimulatory pathways involved in generating each population were distinct. Therefore, we tested the role of the costimulatory ligands of CD28, B7-1 and B7-2, in activation of adoptively transferred CD8 T cells. After transfer of T cells, mice were treated during immunization with antibodies specific for B7-1 and B7-2 (23, 24). Immunization resulted in large increases in Ly5.2$^+$ cells in the LN and in the intestinal epithelium (Fig. 3). These cells were activated as manifested by a substantial increase in cell size (data not shown) and up-regulation of CD44 (Fig. 3) and CD11a (Fig. 1). The level of CD44 expression of IEL routinely was found to be less than that observed for activated LN T cells. In the LN, a 21-fold increase in transgenic cells occurred by 3 days after immunization while a 542-fold increase in transferred cells occurred in IEL. Anti-B7-2 treatment resulted in a 9-fold decrease in activated donor LN cells and a 19-fold decrease in donor IEL, indicating that B7-2-mediated costimulation was critical for activation of OT-I T cells regardless of their location. In contrast, treatment with anti-B7-1 mAb had little effect on cell number increases in the LN. Similarly, a previous study showed that anti-B7-1 mAb treatment had minimal effect on CD4 T cell activation in the periphery (25). However, anti-B7-1 mAb treatment inhibited by 6-fold the appearance of Ly5.2$^+$ IEL. This result suggested that B7-1-dependent costimulation played a significant role in activation of mucosal CD8 T cells as compared with peripheral CD8 T cells. The combination of anti-B7-1,B7-2 antibody treatment nearly completely inhibited donor T cell expansion in both sites, and the remaining cells expressed intermediate levels of CD44 as compared with nonimmune and immune control cells. The level of inhibition of expansion correlated with the level of inhibition of CTL activity (data not shown). Treatment with CTLA4-Ig also completely inhibited expansion (data not shown). These results indicated that a lack of costimulation was not responsible for the lack of induction of CTL activity in peripheral lymphoid tissues. However, the quality or strength of costimulation (26) in periphery versus mucosa appeared distinct, with mucosal responses relying heavily on B7-1 involvement.

The split tolerance induced by soluble antigen, that is, induction of proliferation and phenotypic changes without induction of lytic activity in LN T cells, suggested that the environment in which CTL precursors undergo functional maturation is critical for optimal primary CTL development *in vivo*. Thus, the term split tolerance also could be applied based on anatomical site since CTL activity was generated in the mucosa but not in peripheral lymphoid tissues. In analogy, adjuvant in addition to soluble antigen is needed to induce sufficient CD4 T cell help to promote an antibody response (17). To determine whether a response to a soluble antigen in the context of an inflammatory response could drive CTL production, a recombinant VSV was engineered to encode ova (VSV-ova). Ovalbumin could be detected in lysates and in tissue culture supernatants from VSV-ova infected cells (Fig. 4). VSV is a lytic virus able to infect many cell types, and infection of mice with VSV efficiently induces primary CTL responses *in vivo* (27). In this system, VSV-ova would be expected to infect cells, resulting in production of secreted,

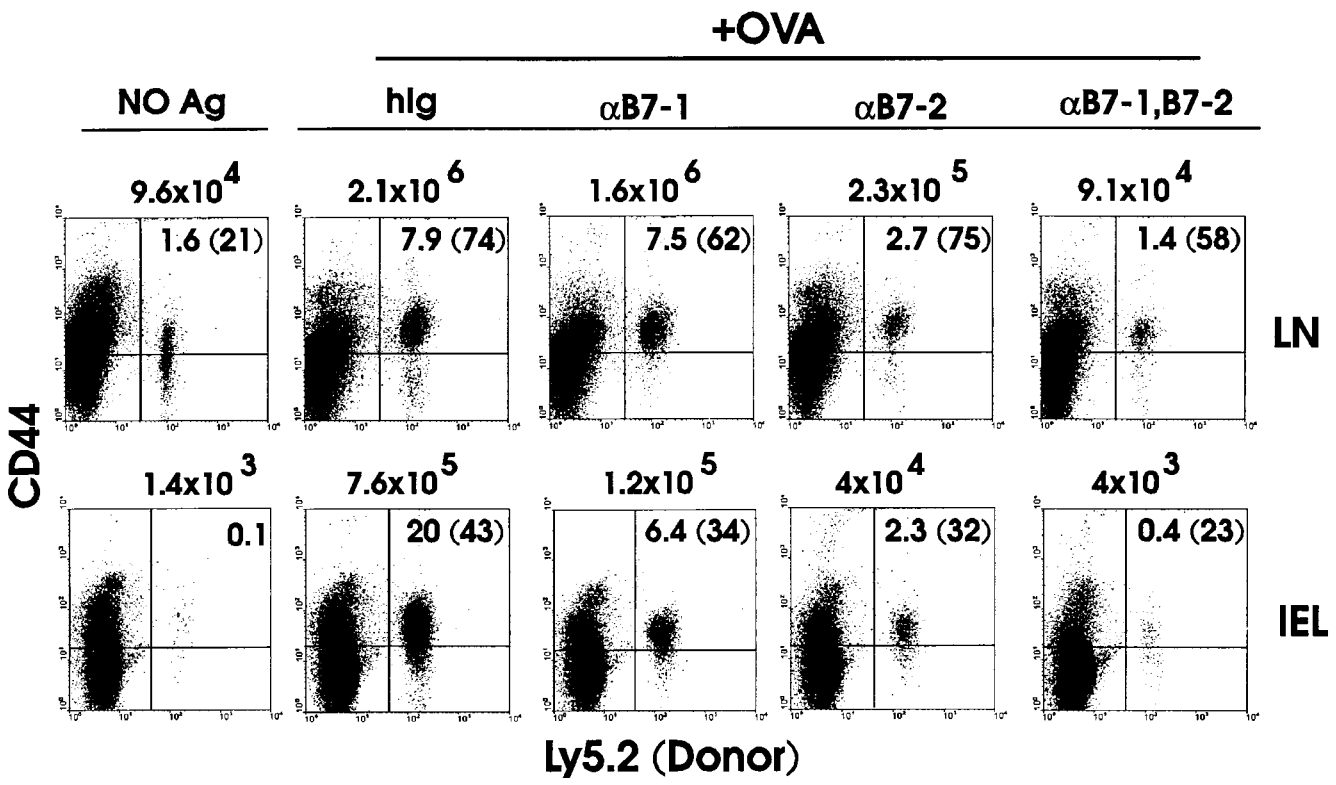

FIG. 3. Costimulatory requirements for activation of adoptively transferred CD8 T cells. C57BL/6-Ly5.2 OT-I T cells (1×10^7) were injected i.v. into C57BL/6-Ly5.1 mice. Two days later 5 mg of ovalbumin was administered i.p. One hundred micrograms of the indicated antibody was injected i.p. daily beginning on the day of immunization. Three days after immunization cells were isolated and analyzed for Ly5.2 and CD44 expression by fluorescence flow cytometry. Numbers in parentheses indicate the mean fluorescence intensity of CD44 expression for the donor cells. Numbers above the plots indicate the total number of donor cells. This experiment has been performed three times with similar results.

soluble ovalbumin. After adoptive transfer of ova-specific CD8 transgenic T cells, mice were infected with VSV-ova and the status of donor cells was assessed by flow cytometry (Fig. 5). Fewer OT-I cells were transferred (2.5×10^6) for the virus-infection experiments because of the greater expansion of cells induced by VSV-ova as compared with soluble ova. Five days after infection transferred cells had expanded 116-fold in the LN. No naive CD8 T cells were detectable in the intestinal epithelium before infection (Fig. 5). However, after infection with VSV-ova there was a major influx of activated CD8 T cells into the intestinal epithelium (at least 700-fold but this value is only an estimate since donor cells were essentially not detectable before immunization). These cells expressed high, up-regulated levels of CD11a (Fig. 5) and CD44 (data not shown). Thus, secretion of ova by infected cells and/or processing of ova by infected antigen-presenting cell (APCs) was able to effectively trigger CD8 T cell activation. This response was costimulation-dependent since treatment of VSV-ova-infected mice with CTLA4-Ig resulted in a 33-fold decline in OT-I cell expansion in both LN and IEL. These results indicate that infection with VSV-ova resulted in processing of whole

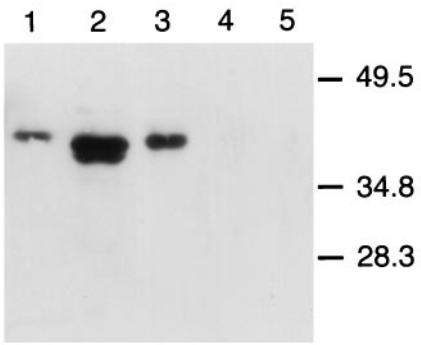

FIG. 4. Recombinant VSV containing the ovalbumin gene directs secretion of ovalbumin in infected cells. Western blot analysis of ova expression with purified ova (80 ng) (lane 1), VSV-ova-infected BHK cell lysates (lane 2), supernatant from VSV-ova-infected BHK cells (lane 3), wild-type VSV-infected BHK cell lysates (lane 4), or supernatant (lane 5).

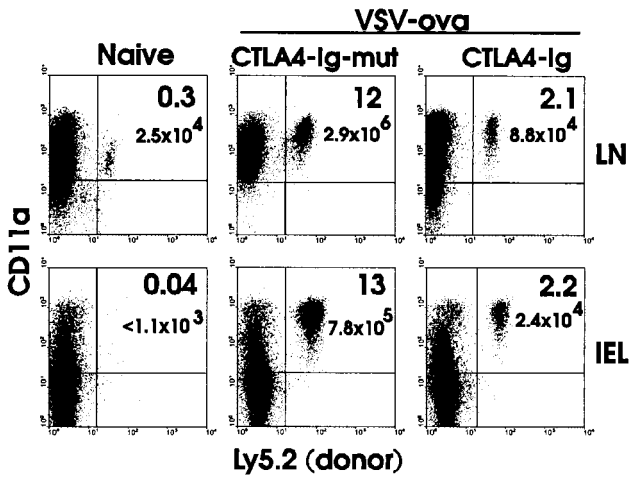

FIG. 5. Infection with VSV-ova activates ova-specific T cells and induces CD8 T cell migration to the intestinal mucosa. C57BL/6-Ly5.2 OT-I T cells (2.5×10^6) were transferred to C57BL/6 mice, and 2 days later mice were infected by i.v. injection with 1×10^6 pfu of VSV-ova. One hundred micrograms of the indicated reagent was injected i.p. daily beginning on the day of immunization. Five days later cells were isolated and analyzed for expression of Ly5.2 and CD11a expression by fluorescence flow cytometry.

ova and presentation of ova-derived peptide by APCs to OT-I T cells in a costimulation-dependent reaction.

Since immunization with soluble ova did not induce peripheral CTL it was important to test whether CTL could be induced when ova was presented in the context of an infection. Therefore, after infection with VSV-ova the lytic activity of spleen and LN cells and of IEL was measured (Fig. 6). In contrast to the results obtained with soluble ova, substantial ova-specific lytic activity was detected in spleen (data not shown) and LN cells (Fig. 6) from VSV-ova-infected mice. Antigen-specific lytic activity also was detected in IEL (Fig. 6). Interestingly, the lytic activity of IEL was ≈3-fold greater than that of peripheral CTL, again suggesting that factors in the intestinal mucosa were up-regulating CTL activity. Treatment with CTLA4-Ig greatly inhibited CTL induction in both peripheral and mucosal tissues (Fig. 6), demonstrating that CD28-mediated costimulation was essential for the anti-VSV-ova CD8 CTL response. CTAL4-Ig was less effective at inhibition of IEL CTL activity as compared with cellular expansion, again reiterating the higher lytic activity of IEL on a per cell basis as compared with LN T cells. Infection with wild-type VSV did not result in anti-ova CTL generation in this system (data not shown), indicating that the response was not because of nonspecific bystander activation (28). These findings indicated that the quality of the response to a soluble antigen was modulated by a concomitant viral infection and resulted in a productive immune response.

DISCUSSION

Our results emphasize the distinct nature of mucosal immune responses while demonstrating an integration of the peripheral and mucosal immune systems. That is, CD8 T cells activated via interaction with injected or fed soluble antigen in a costimulation-dependent reaction trafficked to the intestine, where they underwent productive differentiation to CTL. The findings presented have significant implications for the use of immune tolerance-inducing regimens for the treatment of autoimmune diseases, particularly those associated with mucosae. Thus, although injected or fed soluble antigen may induce peripheral tolerance as assessed by *in vitro* analyses, a productive organ-specific primary immune response may occur as a result. Indeed, fed ova can prime for a secondary CTL response in normal mice (29). It will be important to determine whether our findings apply to the CD4 arm of the immune system.

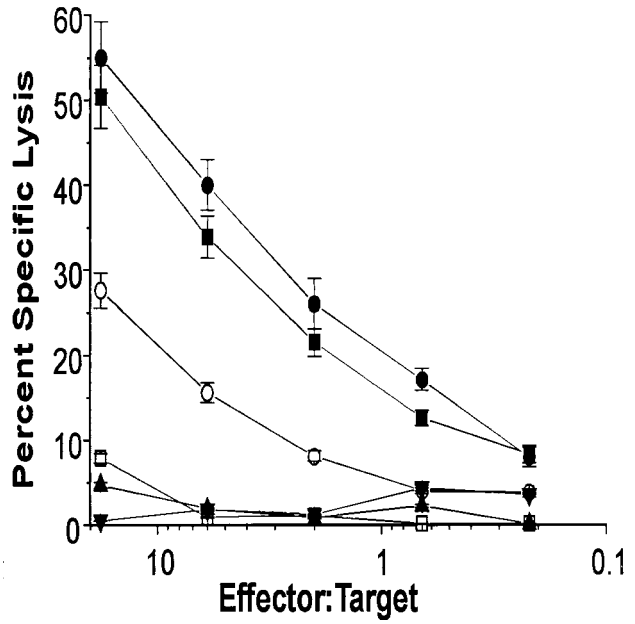

FIG. 6. Infection with recombinant VSV-ova induces anti-ova peripheral and mucosal primary CTL. VSV-ova induces primary anti-ova CTL in LN T cells and IEL. LN cells or IEL from CTLA4-Ig-mut-treated mice (■, LN; ●, IEL) or CTLA4-Ig treated mice (□, LN; ○, IEL) were tested for lytic activity against ova-peptide-coated EL4 cells. LN cells and IEL from CTLA4-Ig-mut-treated mice reacted with EL4 cells in the absence of peptide (▲, IEL; ▼, LN). Effector-to-target ratios were adjusted to actual donor cell percentages found in CTLA4-Ig-mut-treated mice. This experiment has been performed three times with similar results. SE bars are shown.

Our results also indicated that the requirements for mucosal vaccination may be distinct from those for peripheral vaccination. The induction of potent CTL in the intestinal mucosa by soluble antigen establishes that soluble factors and/or unique costimulatory interactions operating in the mucosa can substantially up-regulate an immune response. In regard to the latter, the generation of activated mucosal but not peripheral CD8 T cells specific for soluble antigen required B7-1 while B7-2 was important in both sites.

Since the mucosal transgenic T cells likely were activated outside of the intestine, this result suggests at least two nonmutually exclusive possibilities: (*i*) a low level of inhibition of peripheral responses by anti-B7-1 results in greater apparent inhibition in the mucosa because of the requirement for extensive amplification of the response before mucosal entry and/or (*ii*) upon entry into the mucosa CD28-B7-1 interactions occur resulting in expansion of the population and induction of CTL activity. In regard to the latter, the nature of mucosal APCs also could play a role in driving CTL differentiation. That is, because of the activated status of the LP, APCs in that site, such as dendritic cells, may express high levels of B7-1 and/or B7-2 as compared with peripheral APCs. Since B7-1 is only up-regulated after APC activation (19) and soluble ova would not be expected to activate APC, the preactivated APC of the intestinal mucosa may be preferentially suited to induce full CTL activation to noninflammatory antigens. These processes with regard to OT-I T cell activation appear to be CD4 T cell-independent since activation, expansion, and CTL differentiation in the mucosa occur in $RAG^{-/-}$ mice after adoptive transfer and activation of OT-I T cells (data not shown). In addition, immunization of OT-I-$RAG^{-/-}$ mice with ova results in induction of CTL activity in the IEL compartment (9).

Interestingly, costimulation also was important for CTL induction in a VSV-ova infection. This finding is in contrast to that obtained with LCMV infection in CD28-deficient mice in which a CTL response is induced (13) but is in keeping with the CD28 dependence of the primary CTL response to influenza virus (12) and to nonrecombinant VSV (30). The reasons for these differences in costimulation dependence are thought to be related to the duration of infection and, therefore, availability of antigen (30). Whereas LCMV infection results in chronic antigen exposure, VSV replicates only for a short period of time in mice (31). Thus, even when secreted antigen is produced by VSV-ova infected cells *in vivo*, which may be expected to provide a larger window of antigen exposure, the response remains costimulation-dependent. Although the intestinal mucosa generally has been thought of as an immunosuppressive environment, this is clearly not the case for CD8 T cell activation. The conversion by virus infection of a nonproductive peripheral CD8 T cell response to a productive response suggested a parallel between factors constitutively present in the intestinal mucosa and those produced during an antiviral response. Identification of the CTL-inducing factor(s) in our system could provide a means to regulate CTL responses *in vivo*. This result also demonstrated the utility of recombinant viruses as potential vaccines for induction of cytotoxic responses to poorly immunogenic soluble proteins. The system described here will allow a comparison at the cellular and molecular levels of an immune response to nonimmunogenic

and immunogenic forms of the same protein in the peripheral and mucosal immune systems.

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