Qualitative and Quantitative Characteristics of Rotavirus-Specific CD8 T Cells Vary Depending on the Route of Infection

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CD8 T-cell response provides an important defense against rotavirus, which infects a variety of systemic locations in addition to the gut. Here we investigated the distribution, phenotype, and function of rotavirus-specific CD8 T cells in multiple organs after rotavirus infection initiated via the intranasal, oral, or intramuscular route. The highest level of virus-specific CD8 T cells was observed in the Peyer’s patches of orally infected mice and in the lungs of intranasally infected animals. Very low levels of virus-specific CD8 T cells were detected in peripheral blood or spleen irrespective of the route of infection. Rotavirus-specific CD8 T cells from Peyer’s patches of orally infected mice expressed high levels of CCR9, while CXCR6 and LFA-1 expression was associated with virus-specific CD8 T cells in lungs of intranasally infected mice. Oral infection induced the highest proportion of gamma interferon+ CD107a/b+ CD8 T cells in Peyer’s patches. When equal numbers of rotavirus-specific CD8 T cells were transferred into Rag-1 knockout mice chronically infected with rotavirus, the donor cells derived from Peyer’s patches of orally infected mice were more efficient than those derived from lungs of intranasally infected animals in clearing intestinal infection. These results suggest that different routes of infection induce virus-specific CD8 T cells with distinct homing phenotypes and effector functions as well as variable abilities to clear infection.

Rotavirus is recognized to be the most common cause of severe diarrhea in young children throughout the world. Resolution of rotavirus infection involves both CD8 cytotoxic T-lymphocyte (CTL) and antibody responses (13, 14, 32). Comprehensive studies using animal models have shown that CD8 T cells are needed for timely virus clearance while antibody is critical for protection from reinfection (15). Mice depleted of CD8 T cells by administration of an anti-CD8 monoclonal antibody (MAb) had a modest delay in clearance of primary viral infection (15). In contrast, mice depleted of CD4 T cells cleared viral infection just as well as their undepleted counterparts, despite a markedly reduced level of virus-specific intestinal immunoglobulin A (32). Moreover, depletion of CD8 T cells from previously infected B-cell-deficient JHD mice resulted in higher levels of virus shedding during rotavirus reinfection, indicating that partial but incomplete protection from reinfection was mediated by CD8 T cells (13). Taken together, these data indicate that protection against rotavirus infection is at least partially mediated by virus-specific CD8 T cells. The antiviral function of CD8 T cells is mediated by their cytokine production and direct killing of infected cells.

Previous studies have shown that the routes of viral immunization can affect the characteristics of induced T-cell responses (3, 4, 20). For example, intrarectal immunization of mice with human immunodeficiency virus type 1 (HIV-1) peptides induced a CTL response in the Peyer’s patches (PP), the spleen, and lamina propria of the intestine, and these responses were able to reduce the viral titer in the ovaries and the colorectal tissue upon intrarectal HIV-vaccinia virus challenge (3). In contrast, subcutaneous immunization with the same peptide antigen induced CTLs in the spleen but not in the PP or lamina propria of the intestinal tract and had no effect on the viral titers upon intrarectal challenge. However, other studies (4) using monkeys suggested that, even in the absence of priming at mucosal sites, systemic immunization could lead to control of mucosal infection with simian immunodeficiency virus. In studies of rotavirus immunization, a variety of experiments have demonstrated that intrarectal immunization with nonreplicating double-layered virus-like particles can induce protective immunity in the upper gastrointestinal tract that is not based on neutralizing antibodies (1, 38). These studies did not examine CD8 T-cell responses, however. On the other hand, intranasal administration of 2/6 virus-like particles to gnotobiotic piglets did not induce protective immunity to enteric challenge (45). In other studies, intramuscular (i.m.) immunization with replication-competent simian rotavirus was shown to elicit the movement of virus-specific memory B cells to the gut-associated lymphoid tissue that was associated with protection from a murine rotavirus challenge (7, 8). These studies did not examine the induction of CD8 T-cell immunity, nor did they specifically employ strategies to limit possible trafficking of systemically administered virus to the gut. There have been a number of studies that have examined the role of CD8 T cells induced after oral infection with live rotavirus, but little has been presented concerning the generation or function of such cells after infection at other mucosal or systemic sites (2). Studies of the related reovirus have demonstrated that enteric priming can induce effector CTLs that could clear the lower respiratory tract of infection in chronically infected SCID mice, but the reciprocal passive-transfer studies were not presented (46).
Rotavirus infection was originally thought to be limited to the gastrointestinal tract and to cause viral gastroenteritis. Later evidence (16, 36, 41), as well as a recent study from our group (12), indicates that rotavirus infection in mice and several other animal species including humans spreads beyond the intestine. In orally inoculated mice, virus and viral antigen (Ag) have been recovered from the blood, liver, spleen, kidneys, and lungs (16, 36, 41). In children, viral Ag and viral RNA have been detected in the central nervous system, liver, kidney, and, most commonly, in blood (36). However, neither the nature of the B-cell response nor the characteristics and the role of rotavirus-specific CD8 T cells that are generated during infection of anatomical sites other than the intestinal tract have been studied systematically.

In the current study, we used rhesus rotavirus (RRV), a heterologous (nonmurine) rotavirus strain that has the ability to replicate systemically in mice under specific circumstances, to study the effects of the route of infection on the distribution, trafficking phenotype, and effector function of the rotavirus-specific CD8 T-cell response during primary rotavirus infection.

MATERIALS AND METHODS

Animals. C57BL/6 female mice (Jackson Laboratory), 6 to 8 weeks old, and Rag-1-deficient (Rag-1) mice (Jackson Laboratory) were propagated and maintained at the Palo Alto Veterans Affairs Health Care System, Veteran Medicinal Unit (Palo Alto, CA). Mouse colonies were maintained on a 12-h light-dark cycle. All animal experiments were approved by the Stanford Institutional Animal Care Committee.

Virus preparation and animal infection. Tissue culture-adapted heterologous RRV was prepared as previously described, and the titer was determined as PFU cycle. All animal experiments were approved by the Stanford Institutional Animal Care Committee.

Isolation of lymphocytes. Mice were sacrificed at various time points postinfection. Heparinized peripheral blood samples were obtained from mice before euthanasia. Mononuclear cells were isolated by Ficoll-Paque gradient separation. Lymphocytes from PP and spleen were isolated by mechanical disruption through a cell strainer, and then washed with PBS. To isolate pulmonary intraparenchymal lymphocytes, PBS was injected into lungs to flush the pulmonary vascular bed. The lungs were then isolated using a 33% to 75% (vol/vol) Percoll (GE Healthcare) isolation kit (Stem Cell Technologies, Vancouver, BC, Canada). FlowJo software (Tree Star, Inc., Ashland, OR) was used for analysis of fluorescence-activated cell sorter data.

Detection of rotavirus antigen in stool. A sandwich enzyme-linked immunosorbent assay (ELISA) was used to detect rotavirus Ag in mouse stool. In brief, polyvinyl chloride plates were coated with rabbit hyperimmune antirotavirus virus antisera for 3 h at 37°C and then blocked with 5% milk at 37°C for 2 h before being incubated with 10% stool suspension overnight at 4°C. After a washing, virus Ags were detected by adding a guinea pig hyperimmune sera against rotavirus to each well and incubating wells at 37°C for 1 h, followed by addition of peroxidase-conjugated anti-guinea pig antibodies (Kirkgaard & Perry Laboratories, Gaithersburg, MD). Methylbenzidine was used as the enzyme substrate, and the reaction was terminated with 1% HCl. Optical density was measured at 450 nm. Naive mouse stool and wells without a feces sample served as negative controls.

Adaptive transfer of CD8 T cells into chronically infected Rag-1 mice. Rice-1 knockout (KO) mice (<3 weeks old) were orally inoculated with 5 × 10⁵ 50% diarrhoea doses of murine rotavirus strain EC after feeding 100 μl of 1.33% sodium bicarbonate to neutralize stomach acid. Stools were collected 2 to 3 weeks after viral inoculation to confirm the establishment of chronic infection. Donor C57 BL/6 mice were infected with RRV by p.o. or i.n. inoculation. At day 7 after infection, the donor mice were sacrificed to prepare lymphocytes from either PP or lungs. After the red blood cells were lysed, CD8 T cells were first obtained by using a mouse CD8 T-cell enrichment kit, further purified by staining with MBs specific for CD3 and CD8, and then sorted for the CD3+ CD8+ population on a FACSAria cell sorter (BD Bioscience). The purity of sorted CD8 T cells was >99%. Cells were resuspended in PBS and transferred by intravenous (i.v.) injection into chronically infected Rag-1 mice. Each Rag-1 mouse received 5 × 10⁶ CD8 T cells from PP or lungs infected donor mice, a corresponding number of CD8 T cells containing the same numbers of Vp6 and Vp7 tetramer+ cells from the lungs of infected donor mice, or the same numbers of total CD8 T cells from PP or lungs of uninfected mice.

Statistical analysis. Values were expressed as means ± standard errors of the means (SEM). Analysis of variance (ANOVA) was used to determine statistical significance for tetramer+ CD8 T-cell distribution and homing marker expression data. The unpaired t test was used for intracellular IFN-γ, CD107a/b, and viral load data. A P value of <0.05 was considered statistically significant.

RESULTS

Kinetics of rotavirus-specific CD8 T-cell response after infection. First we examined the rotavirus-specific CD8 T-cell response following oral infection of mice with RRV. To examine CD8 T-cell responses in infected mice, lymphocytes were isolated from either PP or lungs at days 3, 5, 7, and 15 after oral or i.n. infection and stained with selected Vp6 and Vp7 tetramers based on prior epitope mapping studies of rotavirus-
specific CD8 T cells (19). In addition, PP lymphocytes collected on days 3, 5, 7, and 9 were stimulated ex vivo with a mixture of Vp6 and Vp7 peptides and stained for intracellular IFN-γ production to correlate tetramer findings with prior studies of the intracellular cytokine response. The percentages of both Vp6- and Vp7-specific tetramer CD8 T cells peaked on day 7 in both PP after oral infection and in lungs after i.n. inoculation. These peaks corresponded temporally with peaks of IFN-γ+ CD8 T cells, as detected by IFN-γ flow cytometric assay (data not shown). Therefore, day 7 postinfection was chosen for all subsequent studies of rotavirus-specific CD8 T cells.

Distribution of virus-specific CD8 T cells in mice infected with RRV through different routes. To investigate the effect of infection route on the level of virus-specific CD8 T cells at different locations, mice were inoculated with RRV by the p.o., i.n., or i.m. route. On day 7 postinfection, lymphocytes isolated from peripheral blood, PP, spleen, liver, and lung were stained with Vp6 and Vp7 tetramers to determine the frequency of tetramer+ CD8 T cells in each organ. p.o. infection induced the highest levels of tetramer+ CD8 T cells in PP compared to other routes of infection (Fig. 1). The i.n. route repeatedly induced more rotavirus-specific CD8 T cells in PP than the i.m. route, although within each experiment these numbers were not statistically different and both were significantly less than those seen following p.o. infection. Following p.o. infection, PP were found to have a significantly higher percentage of rotavirus-specific CD8 T cells than blood, spleens, livers, and lungs, which all had comparably low levels of the specific CD8 T cells. In contrast, in mice infected via the i.n. route, the frequency of tetramer+ CD8 T cells was significantly higher in lungs than in other organs. After i.m. inoculation, no significant difference among the percentages of rotavirus-specific CD8 T cells in any of the sites examined was detected. In general very low levels of Ag-specific CD8 T cells were detected in peripheral blood and the spleen irrespective of the route of infection. Taken together, these results suggest that, after p.o. or i.n. infection, rotavirus-specific CD8 T cells localize predominantly to PP or lungs, respectively. In contrast, i.m. immunization resulted in only modest levels of rotavirus-specific T cells that were relatively evenly distributed in a variety of systemic and mucosal sites at day 7 after inoculation, when a specific organ-preferred distribution of rotavirus-specific T cells was evident for both p.o. and i.n. infection (Fig. 1).

Differential expression of lymphocyte trafficking molecules on rotavirus-specific CD8 T cells in mice infected via different routes. We examined the expression of lymphocyte trafficking molecules on virus-specific CD8 T cells isolated from different organs of mice infected through the p.o., i.n., or i.m. route. On day 7 after infection, lymphocytes isolated from different organs were stained with antibodies against CCR9, LFA-1, and CXCR6 to determine the frequency of tetramer+ CD8 T cells expressing each of these markers. The percentage of total tetramer+ cells expressing each marker was calculated based on results with Vp6 and Vp7 tetramers. (A) CCR9 expression on tetramer+ CD8 T cells. (B) LFA-1 expression on tetramer+ CD8 T cells. (C) CXCR6 expression on tetramer+ CD8 T cells. Standard errors and the ANOVA test were based on five mice in each group. Displayed are means and SEM for five mice in each group. The experiment was repeated four times with similar results. *, $P < 0.05$ (ANOVA).
in other organs including peripheral blood, spleens, livers, and lungs, regardless of the route of infection; however, the highest level of CCR9 expression on PP tetramer+ CD8 T cells was seen in the p.o. infected mice (Fig. 2A). Interestingly, the highest level of CCR9 expression on Ag-specific CD8 T cells in lungs was detected in mice receiving i.n. infection.

In mice infected by the i.m. route, the average level of expression of LFA-1 on rotavirus-specific CD8 T cells was significantly higher (P < 0.05) in livers than in other organs (Fig. 2B), while in i.n. infected mice, LFA expression was highest in the lungs. The p.o. inoculation route was not efficient at inducing LFA expression (<40% in each of the organs studied) although the levels of expression were higher in the livers and lungs than in the other organs (Fig. 2B).

Finally, the average level of expression of CXCR6 on virus-specific CD8 T cells in i.n. infected mice was significantly higher (P = 0.05) in lungs than in any other organs of the same mouse or in any organ of mice infected via other routes (Fig. 2C).

Taken together, these findings suggest that CCR9 expression is associated with rotavirus-specific CD8 T cells in the intestine, irrespective of the route of infection. Our data also suggest that LFA-1 expression is associated with these cells in the liver after i.m. infection.

Expression of functional markers in RRV-specific CD8 T cells induced after infection via different routes. To investigate potential effector functions of rotavirus-specific CD8 T cells in the intestine after immunization via various routes, lymphocytes were isolated from PP of mice on day 7 after infection. These cells were then cultured with or without Vp6 and Vp7 peptides for 6 h and then stained for intracellular IFN-γ and CD107a/b, a marker for cytotoxicity, to determine the percentages of rotavirus-specific CD8 T cells expressing IFN-γ, CD107a/b, or both. Figure 3A shows representative fluorescence-activated cell sorters plots of the expression of IFN-γ and CD107a/b in RRV peptide-specific CD8 T cells isolated from PP of mice infected via the three different routes, while the results for all mice are summarized in Fig. 3B and C. In agreement with results of tetramer staining (Fig. 1), the total percentage of RRV peptide-specific PP CD8 T cells expressing IFN-γ and/or CD107a/b was significantly higher in p.o. infected mice than in mice infected i.n. or i.m. (Fig. 3B). Interestingly, the percentage of RRV-specific cytotoxic CD8 T cells that did not secrete IFN-γ (CD107a/b- IFN-γ-) was significantly higher in the PP of mice infected by the p.o. route than in mice infected i.m. or i.n. (P < 0.05) (Fig. 3C).

Virus-specific CD8 T cells resolved rotavirus infection when transferred into chronically infected Rag-1-deficient mice. We isolated CD8 T cells from PP or lungs of p.o. or i.n. infected mice, respectively, and transferred them by i.v. injection into Rag-1 KO mice chronically infected with murine rotavirus to determine the efficacy of rotavirus immune CD8 T cells derived from different organs in clearing intestinal infection. The numbers of rotavirus Vp6- and Vp7-specific CD8 T cells were rendered equivalent in the transfers from lungs and PP. PP or lung CD8 T cells from uninfected mice served as controls. The recipient mice were tested for fecal rotavirus antigen shedding to monitor clearance of intestinal infection after adoptive transfer. Chronically infected Rag-1 KO mice receiving CD8 T cells from PP of p.o. infected mice resolved infection between days 8 and 10 posttransfer, while those receiving CD8 T cells from lungs of i.n. infected mice cleared infection between days 12 and 14 (Fig. 4). The difference in the mean numbers of viral shedding days between these two groups was statistically significant (P < 0.01, Student’s t test). Adoptive transfer of CD8 T cells from PP or lungs of uninfected mice had no effect on stool viral shedding.

DISCUSSION

In this study, we investigated the effects of the route of rotavirus infection on the distribution, phenotype, and function of rotavirus-specific CD8 T cells in multiple organs of infected
sets of T cells contained 0.15

munogenic. Third, RRV infection induces protection against

routes was substantial (serum ELISA titer, 4,000 to 10,000),

rotavirus fecal shedding on subsequent challenge with murine

parably efficient at inducing high levels of protection against

i.n. administration, and i.m. administration of RRV were com-

(our unpublished data) demonstrated that p.o. administration,

for rotavirus Ag by ELISA. The graph is based on results of

an experiment with four mice in each group, which was repeated twice

with similar results.

mice. We used both tetramer staining and intracellular IFN-γ

staining to demonstrate that primary rotavirus infection by the

p.o. or i.n. route induced a virus-specific CD8 T-cell response

that peaked on day 7 postinfection, which corresponds to the

time when mice clear fecal viral shedding (14, 15). This finding

is compatible with prior findings that CD8 T cells are involved

with the timely resolution of primary infection (14). Note that

prior studies had indicated that peak CD8 T-cell responses in

PP and liver occurred on day 5 rather than day 7, as observed

in this study (19). The basis for this difference is not entirely

clear but does not appear to be entirely due to the current use

of a tetramer-based assay since time course experiments meas-

uring intracellular IFN-γ also showed responses peaking on
day 7. However, prior studies using peptide stimulation and

intracellular IFN-γ assays demonstrated that VP7-reactive CD8 T
cells did peak on day 7 while VP6-reactive cells peaked on
day 5 (19). It goes without saying that this analysis focused

in this study (19). The basis for this difference is not entirely

clear, but among them might be that i.m. induced

replication potential for RRV versus the murine EDIM strain

is responsible for these differences.

Rotavirus-reactive CD8 T cells in PP elicited after p.o. in-

fection expressed CCR9 more frequently than comparable

cells induced following i.m. or i.n. infection. i.m., but not i.n.,
infection was also relatively effective at eliciting rotavirus-spe-
cific CD8 T cells, but in this instance, the cells were primarily

localized in the lung. Expression of CXCR6 and LFA-1, but

not CCR9, was associated with virus-specific CD8 T cells in the

lungs of i.n. infected mice. Interestingly, i.m. administration,

while highly effective at inducing protective immunity and se-

rum antibodies (data not shown), was relatively inefficient at

inducing a cytotoxic T-cell response. The reasons for this find-

ing are not clear, but among them might be that i.m. induced

CD8 T cells do not localize to the sites sampled in this analysis

or that day 7 does not represent the peak timing for the

generation of CD8 T cells after i.m. administration.

While both i.n. and p.o. administration of RRV elicited

relatively robust CD8 T-cell responses, these responses tended

to be localized to the site of original viral administration and

not to spread to distant mucosal or systemic sites. Several

groups have suggested that one might take advantage of the

common mucosal immune system to immunize at one mucosal

location and induce protective immunity at another (18, 33). In

fact, in this murine rotavirus model system, all three routes of

immunization are sufficient to induce protection from reinfec-

tion. However, it is evident that the three routes are not

equally effective at inducing cellular immunity in the gut. It is

clear that, in the case of rotavirus infection, the site of viral

administration is the site of greatest CD8 T-cell response. That

said, it appears that i.n. administration was somewhat more

effective than i.m. infection at eliciting a CD8 T-cell response

in the gut and that, reciprocally, p.o. administration was some-
what more efficient than i.m. administration at eliciting CD8 T-cell responses in the lungs. In agreement with the findings of others (31, 34), our data indicated that mucosal inoculation induced the strongest immune response at mucosal sites, rather than systemic sites. It is interesting to note that none of the immunization routes elicited a substantial CD8 T-cell response in the circulation, and if this was the site of sampling for evaluation of responsiveness (as it generally is in people), one would incorrectly conclude that rotaviruses were very inefficient at stimulating cytotoxic CD8 T-cell immunity.

After infection, lymphocytes are recruited to infection sites. This lymphocyte trafficking process is mediated by unique combinations of adhesion molecules on their surfaces, which recognize chemokines produced by different tissues (27, 28). In this work we have examined several surface molecules as potential homing markers for various locations. The chemokine receptor CCR9 is expressed by the majority of human and murine small intestinal T cells. Its ligand CCL25 is constitutively expressed in the small intestine by the small intestinal epithelial cells but not in the colon (21). Unlike T cells deficient in another gut homing marker, α4β7, which is not absolutely required for T-cell migration to the intestinal mucosa (26), CCR9-deficient CD8 T cells were heavily disadvantaged in their trafficking to intestinal sites (22). LFA-1 is a promigratory receptor on T cells. Its principal ligand is ICAM-1 (42). CXCR6 is another chemokine receptor expressed on T cells and natural killer T cells, with CXCL16 as its ligand (35). Both LFA-1 and CXCR6 have been related to T-cell homing to liver and lung (35, 42). It has been suggested that expression of chemokine receptors and integrins is determined by the microenvironment of the local tissue where the activated antigen-specific T cells differentiate into effector memory T cells (27, 28). O’flit et al. (37) demonstrated that the frequency of rotavirus-specific CTL precursors in PP was 25- to 30-fold greater after oral inoculation than after footpad inoculation. However, the lymphocyte trafficking molecules associated with this enrichment of specific T cells was not defined. A recent study (6) has shown that, when mice were injected with tumor cells subcutaneously and intraperitoneally (i.p.) at the same time, tumor-specific CD8 T cells expressed either the skin- or gut-homing phenotype but not both. The authors postulated that CD8 T cells specific for the i.p. implanted tumors were imprinted with the gut-homing phenotype, suggesting that the site of antigen entry, rather than the identity of lymph nodes where T cells are activated, is responsible for their homing phenotype. Our previous study (26) demonstrated that expression of α4β7 facilitated normal intestinal B-cell immune trafficking to the gut but was not required for effective CD8 T-cell immunity against rotavirus. In the current study, we demonstrate that p.o. infection induced PP enrichment of virus-specific CD8 T cells that predominantly expressed CCR9, while i.n. infection induced lung enrichment of virus-specific CD8 T cells that predominantly expressed LFA-1. Our study suggested that virus entry sites affect the distribution of rotavirus-specific CD8 T cells by imprinting them with organ-specific homing markers. Apparently, none of the three markers that we elected to study are selectively elicited on CD8 T cells originating from i.m. administered RRV.

Our results suggest that the route of infection, in addition to affecting the distribution of virus-specific CD8 T cells, also affects the their effector functions. The immunization route proved to be an important factor in determining whether an efficient primed CD8 T-cell response is mounted (10, 11). Estcourt et al. (11) demonstrated that the route of vaccination affected the localization of priming events and efficiency of Ag-specific T cells. This finding was supported by Depierreux et al. (10) using the pseudorabies virus model. They showed that, although vaccination via the i.p., i.v., or footpad route induced a strong CTL response, i.p. vaccination was the most capable of eliciting a cytotoxic response at a low dose. No CTL activity was detected after subcutaneous immunization. Both studies demonstrated that the route of infection affected the localization and function of effector T cells.

After Ag stimulation, CD8 T cells develop a variety of effector functions, including cytotoxicity-related degranulation, cytokine production, and proliferation. IFN-γ is one of the cytokines released by CD8 T cells which exhibits antiviral properties (40). CD107a/b is a glycoprotein located on a lipid bilayer of membrane-bound secretory lysosomes, which contain both perforin and granzymes (39). Normally CD107a/b is not found on the surfaces of T cells. Acquisition of cell surface CD107a/b on a CD8 T cell is associated with loss of intracellular perforin and is an indication of degranulation (5). Following the conjunction of a CD8 T cell with a target cell, lytic granules are transported and the granule contents are released into the immune synapse. A granule membrane containing glycoproteins CD107a/b becomes transiently expressed on the T-cell surface upon granule exocytosis (43). Measurement of CD107a/b expression has been shown to be a sensitive assay to detect Ag-specific CD8 T-cell functions (5, 24, 25). In this study, we showed that, by different routes of infection, Ag-specific CD8 T cells at different locations expressed various levels of IFN-γ and/or CD107a/b. Although virus-specific CD8 T cells were detected in PP of mice infected through p.o., i.n., and i.m. routes, a significantly higher proportion of virus-specific cytotoxic (CD107a/b+) CD8 T cells that are incapable of producing IFN-γ were present in PP of mice infected orally compared to the other routes. There are currently insufficient data to understand whether CD8 T cells with different phenotypes, such as CD107a/b+ IFN-γ− versus CD107a/b− IFN-γ+, actually function differently in vivo during resolution of a viral infection, but this seems likely. IFN-γ production has been reported to be correlated with CD107 expression in virus-specific CD8 T cells in some reports (9, 23, 25) but not others (29, 30). McElroy et al. (30) showed that, in HIV-specific CD8 T cells derived from patients coinfected with HIV-1 and Schistosoma mansoni, the expression of CD107 was reduced and the expression of interleukin 10 was increased, while expression of IFN-γ was similar to that in HIV-monoinfected patients. They suggested that S. mansoni coinfection resulted in increased expression of the Th2 cytokine interleukin 10, which subsequently suppressed the cytotoxicity of HIV-specific CD8 T cells without affecting their IFN-γ production. It seems reasonable to propose that, in the case of rotavirus infection, cytotoxic activity of virus-specific CD8 T cells is more important than IFN-γ production for clearing rotavirus from the intestine. It remains unclear whether the PP-localized virus-specific CD8 T cells generated following immunization at differing locations are equally effective in mediating an antiviral effect in vivo.
whether virus-specific CD8 T cells derived from different mucosal sites function differently in clearing intestinal infection. The lung and PP were ideal for the adoptive-transfer study because the percentages of virus-specific CD8 T cells were similar in these two organs. Therefore, after normalization for the number of virus-specific CD8 T cells, each recipient got relatively similar total numbers of donor cells from lungs or PP. Our study indicated that PP of orally infected mice are more efficient in clearing chronic rotavirus infection than virus-specific CD8 T cells derived from the lungs of i.n. infected mice. When equal numbers of virus-specific cells were transferred i.v., the PP-derived cells cleared the chronic infection more rapidly than the cells derived from the lung. These two cell populations differed in at least two important ways. First, the PP-derived cells cleared the chronic infection more rapidly than the cells derived from the lung. These two cell populations differed in at least two important ways. First, the PP-derived cells expressed higher levels of CCR9 than the lung-derived cells, and this might have enhanced their trafficking to the gut. We have previously demonstrated (26) that β7 expression on CD8 T cells did not influence rotavirus clearance in the chronic-infection model, but the role of CCR9 has not yet been studied directly in this model. Second, the PP-derived cells had a higher proportion of CD107a/b+ IFN-γ+ cells, which could be more efficient in mediating clearance. These potential mechanisms should be investigated in future studies. We also demonstrated, for the first time, that the CD8 T-cell-dependent ability to resolve rotavirus infection is not strictly homologous since T cells generated in response to the simian rotavirus strain remained effective in resolving a chronic rotavirus infection. This finding is compatible with a wide array of other studies that have indicated that rotavirus immunity is not strictly homotopic.

In summary, we demonstrated that the route of infection not only affects the distribution of rotavirus-specific CD8 T cells but also the trafficking features of these cells as well as their relative expression of two important effector markers, CD107a/b and IFN-γ. We also demonstrated that the phenotypic and functional characteristics of rotavirus-specific CD8 T cells are associated with their ability to clear chronic viral infection in the gut. These findings have significant implications for the design and evaluation of preventive and therapeutic vaccines for organ-specific viral infection.

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