Predominant T-Cell Receptor V β Usage of Intraepithelial Lymphocytes during the Immune Response to Enteric Reovirus Infection

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Previous studies have found that intraepithelial lymphocytes (IEL) contain virus-specific cytotoxic T lymphocytes (CTL) that increase dramatically during the course of virus infection. In the present study, the T-cell receptor (TCR) V β pattern used by IEL against reovirus enteric infection was investigated both in conventional and in germfree mice. IEL were isolated by a modified rapid method, and their expression of 13 TCR V β s was examined by flow cytometric analysis. The virus-specific CTL activity of each TCR V β subset was assessed by subtraction with coated Dyna beads by a nonradioactive assay. There was a preferential perturbation of TCR V β s following virus challenge, including increases in cells expressing V β 7, -12, -14, and -17 in conventional mice and V β 2, -12, and -17 in germfree mice. In conventionally reared mice, IEL maintained and restimulated in culture had a preferential use of TCR V β 9, -12, and -17. TCR V β 12 and -17 subfamilies were found amplified in all conditions. Furthermore, TCR V β 12 and -17 accounted for 37 and 77% of the virus-specific CTL activity, respectively, after *in vitro* restimulation. This study provides evidence that virus-specific CTL activity may be due to the oligoclonal expansion of TCR V β subfamilies in IEL. Our findings suggest that *in vivo* infection selectively presents few T-cell epitopes and that the correct identification of these T-cell epitopes would increase the likelihood of success when designing subunit vaccines.

Intraepithelial lymphocytes (IEL) are interspersed among the epithelial cells of intestine and provide a first-line host defense against gut pathogens. The population of IEL consists predominantly of large granular cells that are phenotypically similar to either natural killer (NK)-like cells or CD8⁺ T cells (45). Previous studies showed that IEL function in the immune surveillance of the epithelial layer and respond to foreign antigens (1). Following intestinal infection with virus, cytotoxic T-lymphocyte (CTL) activity was present in the IEL (6, 28). In addition, IEL produce cytokines such as gamma interferon and interleukin-2, which may augment local immunity, and IEL also have NK cell activity (1, 30).

There has been considerable interest in examining the functional potential of intraepithelial T cells since the discovery that a relatively high percentage of these T cells bear the γ/δ T-cell receptor (TCR) (18). While the function of CD8⁺ cells bearing the γ/δ TCR may be to respond to altered host cells or pathogens expressing heat shock proteins (20), other roles for these cells in modulating the immune response are still uncertain (15). There are few studies that examine the repertoire of IEL bearing α/β TCRs in mice (36, 45). In humans, IEL bearing α/β TCRs are found and the majority of TCR variable genes can be utilized by IEL (4). Even though T cells bearing the γ/δ TCR comprise a high proportion of the IEL, intraepithelial T cells bearing the α/β TCR are responsible for the specificity of CTL for intracellular pathogens such as viruses (6, 28, 32). A role for specific subsets of T cells bearing V α and V β TCRs has been postulated due to preferential interactions with the major histocompatibility complex (MHC) (9, 11), and

these interactions may account for the preferential use of TCR variable genes in some virus-induced diseases (12, 34, 39).

Gastrointestinal infection with reovirus in mice has been a model for the study of gut mucosal immunity to viruses. Reovirus type 1 binds avidly to microfold (M) cells overlying Peyer's patches, which may contribute to its ability to activate the local humoral and cellular virus-specific immune responses (27, 34, 41, 47). Following enteric infection with reovirus, virus-specific precursor CTLs (pCTLs) have been isolated from the IEL compartment. These pCTLs bear the α/β TCR and can lyse virus-infected cells in an MHC-restricted manner following secondary *in vitro* restimulation. It was found that the average frequency of the pCTLs was approximately 200 per 10⁶ CD8⁺ IEL 1 week after peroral infection (6, 28). Little is known about IEL utilization of TCRs in viral gastrointestinal infection and whether any restrictions exist in the repertoire of responses. The objective of this study is to define whether specific restrictions occur in the IEL CTL repertoire responding to reovirus infection.

In this study, we have been able to characterize the usage of TCR V β s in the IEL population during the immune response to enteric reovirus infection in conventional and germfree mice. We found that the CD8⁺ T-cell population stimulated by reovirus is represented by specific subsets of TCR V β s in the IEL. Moreover, CTL responses toward L-cell-infected targets demonstrate that the expanded CD8⁺ cells expressing TCR V β 12 and -17 account for virtually all of the activity. However, not all of the CD8⁺ T cells bearing V β s that are expanded respond as CTLs, thereby suggesting the potential for other roles for these cells.

MATERIALS AND METHODS

Animals. C3HeB/FeJ and BALB/cByJ mice were purchased from Harlan Industries (Indianapolis, Ind.) and housed in the Nashville Veterans Affairs

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TABLE 1. Effect of reovirus infection on TCR V β usage in C3H murine small bowel IEL population^a

Wk after infection	% of T cells bearing V β :												
	-2	-3	-5	-6	-7	-8	-9	-10	-11	-12	-13	-14	-17
Control	8.02	2.02	1.71	6.69	2.44	12.25	4.39	1.72	2.33	0.47	2.14	3.13	0.37
1	10.62	3.97	1.15	4.62	4.16	12.11	1.53	2.14	2.05	1.61	4.75	5.12	1.44
2	9.34	3.12	2.27	10.28	14.30	12.69	2.67	3.64	1.45	1.35	6.15	3.67	1.62
3	13.08	2.96	2.25	7.76	4.40	12.33	1.93	2.23	2.15	1.79	3.12	3.67	1.34
4	10.02	3.13	1.63	10.22	5.70	10.96	1.91	1.66	1.56	0.84	2.45	9.68	0.71
5	2.04	1.65	0.40	5.58	3.92	14.86	0.21	1.17	0.31	0.33	2.63	3.06	0.26

^a The percentage of T cells bearing various V β s in the IEL compartment of infected and noninfected mice was determined weekly following reovirus infection. Noninfected mice (control) were obtained randomly from within the mouse colony. Mice orally infected with reovirus type 1 were killed at weekly intervals. V β TCR usage was determined by FACS.

Medical Center animal facility. Germfree BALB/cByJ mice were bred in the gnotobiotic facility of the Department of Biology at the University of Pennsylvania (Philadelphia). These mice were housed in isolator bubbles with filtered air and fed autoclaved water and mouse chow. Virus-infected mice were housed in isolators in separate rooms from stock mice. Germfree mice were examined for bacterial colonization following reovirus infection, and they remained bacterium free for the length of the experiments. Mice were used at 6 to 12 weeks of age.

Virus and immunization. Reovirus serotype 1 was originally obtained from Bernard N. Fields. Viral stocks were prepared from infected L cells (37), and third-passage stocks of virus purified by CsCl centrifugation were used in all experiments. Conventionally reared and germfree mice were orally inoculated by passage of a tube (PE-50) attached to a 23-gauge needle on a tuberculin syringe into their stomachs. Reovirus type 1, 3×10^7 PFU suspended in 100 μ l of borate-buffered saline (pH 7.4) containing 0.2% gelatin (gel saline), was used for all virus inoculations (7, 37). The controls consisted of mice inoculated with gel saline alone (sham).

Isolation of IEL. IEL were isolated by a rapid method (8, 29) with slight modifications. Briefly, the small intestine was removed from four mice and flushed with complete culture medium consisting of RPMI 1640 (Irvine Scientific, Santa Ana, Calif.) containing 10% fetal bovine serum, 2 mM glutamine, 25 mM HEPES (pH 7.4), 100 U of penicillin per ml, 100 μ g of streptomycin per ml, and 0.25 μ g of fungizone per ml. After removal of the mesentery and Peyer's patches, the intestine was split longitudinally and cut into 0.5- to 1-cm pieces. Pieces of intestine were washed in 50 ml of phosphate-buffered saline with and without Ca^{2+} and Mg^{2+} twice, respectively. The pieces were then stirred for 1 h with a magnetic stirrer in 80 ml of Ca^{2+} - and Mg^{2+} -free Hank's balanced solution (Sigma Chemical Company, St. Louis, Mo.) containing 1 mM EDTA and 1 mM dithiothreitol, and the supernatant with extracted cells was harvested. Eluted cells were passed through a nylon wool column and then subjected to two-step discontinuous gradient centrifugation at $600 \times g$ for 20 min with 70 and 45% Percoll. IEL were collected from the interface between the Percoll layers.

In vitro culture of IEL. IEL were cultured for 3 to 6 days in 12-well plates at the density of 10^6 cells/ml of complete culture medium in 1 ml/well. Thioglycolate-elicited murine peritoneal macrophages, pulsed with reovirus serotype 1 for 4 h, were used as a source of antigen-presenting cells (APC). A total of 2×10^5 APC were added to each well containing IEL. One day after initiation, cultures were supplemented with the supernatant from concanavalin A-stimulated mouse spleen cells at a final dilution of 10%. Following in vitro culture, the cells were harvested and centrifuged on Percoll gradients as described above (6) with yields of IEL being approximately 10 to 30% of preculture cell counts.

Flow cytometry study. Isolated cells were stained with the following monoclonal antibodies: phycoerythrin-conjugated anti-CD8, fluorescein isothiocyanate (FITC)-conjugated anti-CD4, anti-V β 2 (B20.6) (40), anti-V β 3 (KJ25) (35), anti-V β 5.1/5.2 (V β 5) (MR9-4) (2), anti-V β 6 (RR4-7) (23), anti-V β 7 (TR310) (33), anti-V β 8.1/8.2 (V β 8) (MR5-2) (22), anti-V β 9 (MR10-2) (43), anti-V β 10 (B21.5) (31), anti-V β 11 (RR3-15) (17), anti-V β 12 (MR11-1) (3), anti-V β 13 (MR12-3) (48), anti-V β 14 (MR14-2) (25), and anti-V β 17 (KJ-23) (24) (Pharmingen, San Diego, Calif.). The analysis was performed on a FACScan (Becton Dickinson, Mountain View, Calif.). The proportion of T cells bearing the CD4 and CD8 antigens in the IEL population was determined by CD4 and CD8 two-color staining. The CD4/CD8 ratio was less than 0.4 in all cases.

Cytotoxicity assay. Target cells were prepared by infecting L cells with reovirus type 1 for 1 h and culturing the infected cells for 18 h at 37°C in T-25 tissue culture flasks (26). Two weeks following enteric infection, IEL from conventional mice were stimulated in vitro as described above for 4 days and then used as the effector cells. In some experiments, cells expressing particular TCR V β s were removed by adsorption with anti-V β specific rat or mouse monoclonal antibodies followed by goat anti-rat or sheep anti-mouse antibodies linked to magnetic beads (DYNAL Inc., Great Neck, N.Y.) (44). Cytotoxicity was assessed by a nonradioactive assay that measured lactate dehydrogenase (LDH) release from cell lysis (Promega, Madison, Wis.) (10).

All assays were performed in triplicate. Percent specific cytotoxicity was calculated by the following formula: percent specific cytotoxicity = $[(E - S -$

$TS)/(TM - TS)] \times 100$, where E represents the LDH released in experimental wells, S represents the spontaneous release rate of targets and effectors, TM represents the counts of target maximal release, and TS represents the spontaneous release rate of targets.

RESULTS

TCR V β usage in vivo. To characterize the TCR repertoire of cells bearing V β s found in the IEL compartment, lymphocytes were isolated from control and infected groups of conventionally reared C3H mice weekly following infection. Freshly isolated IEL were stained with TCR V β -specific antibodies and analyzed by flow cytometry (fluorescence-activated cell sorting [FACS]). TCR V β utilization in noninfected C3H mouse IEL revealed that TCR V β 2, -6, and -8 were overrepresented (8.20, 6.69, and 12.25%, respectively). The TCR bearing V β 17 was least represented (0.37%). One to four weeks following reovirus enteric infection, the percentage of T cells expressing TCR V β 7, -12, -14, and -17 increased at least threefold compared to noninfected controls. In contrast, the percentage of lymphocytes expressing TCR V β 9 and -11 decreased following the infection (Table 1).

Stimulated TCR V β bias in vitro. To examine the contribution of the subpopulations of CD8⁺ cells expressing particular TCR V β s following reovirus stimulation, CD4⁺-expressing cells were removed from murine IEL with rat anti-CD4 antibodies and goat anti-rat immunoglobulin-coated magnetic beads. A portion of the CD8⁺ IEL were examined by flow cytometry for V β TCR expression, and the remainder of the cells were restimulated in vitro for 3 to 6 days with reovirus-pulsed APC. The TCR V β repertoire of the sham-infected IEL, depleted of CD4⁺ T cells, was similar to that present in non-CD4 T-cell-depleted IEL, with V β 2 (12.66%), V β 6 (10.58%), and V β 8 (19.36%) highly represented and V β 17 (0.77%) at the level of background for this assay.

There was a dramatic increase in the presence of CD8⁺ T cells expressing a limited number of TCR V β s among cells obtained from reovirus-infected mice after restimulation in vitro. After 3 days of in vitro culture, the CD8⁺ T-cell population had increased 4-fold for T cells bearing TCR V β 10⁺, 5-fold for cells bearing V β 13⁺, 6-fold for cells bearing V β 12⁺, and 10-fold for cells bearing V β 17⁺. In contrast to the results obtained for freshly isolated IEL, following in vitro restimulation, CD8⁺ T cells expressing TCR V β 2, -6, and -8 decreased. The remaining TCR V β subgroups had only minor changes in percentage (Table 2).

Germfree mouse V β repertoire following reovirus infection. There was a naturally dominant representation of IEL expressing some of the TCR V β s, such as V β 2, -6, and -8, in conventionally reared mice. The high background of cells expressing TCR V β s that were overrepresented in the noninfected pop-

TABLE 2. In vitro reovirus restimulation biases the TCR V β repertoire of cultured IEL^a

Day of culture	% of IEL with V β :												
	-2	-3	-5	-6	-7	-8	-9	-10	-11	-12	-13	-14	-17
Control	12.66	4.86	1.98	10.58	4.65	19.36	0.68	0.99	2.17	0.63	1.81	4.88	0.77
3	7.30	4.24	2.49	6.89	6.77	12.66	4.65	4.22	5.58	4.08	9.57	9.65	7.87
6	5.14	4.77	2.36	4.67	5.04	9.69	1.62	1.56	4.87	1.36	5.89	7.05	2.81

^a Mice were infected with reovirus type 1 orally, and CD4⁺-depleted IEL were placed in culture in the presence of reovirus-pulsed APC. The percentage of the IEL utilizing TCR V β genes was determined by FACS at 3 and 6 days in culture. Control IEL consisted of cells obtained from noninfected mice.

ulation may mask alterations in V β usage that occur during the course of infection. This may especially be the case if reovirus stimulates T cells that express one of the overrepresented V β s. Therefore, mice bred and housed in the germfree facility were used to assess changes in TCR usage. Germfree mouse IEL had consistently lower numbers of all groups of TCR V β s than those in noninfected conventionally reared mice. However, even in germfree mice, TCRs bearing V β 6 and -8 were slightly more represented (1.76 and 2.75%, respectively). The TCR V β 17-bearing cells were undetectable (0.07%). T cells bearing the TCR V β 2 subset, which were overrepresented in the IEL from conventionally reared mice, were nondetectable in the IEL from germfree mice (0.06%). Within 4 weeks following reovirus enteric challenge, the percentages of T cells rose in the IEL, with cells bearing TCR V β 2, -12, and -17 increasing more than 10-fold compared to those of sham-infected controls (Fig. 1 and 2). Thus, following reovirus infection, the percentages of cells bearing particular TCR V β s were found to

be similar in germfree BALB/cByJ mice and conventionally reared C3H mice.

TCR V β element-related specific cytotoxicity. We determined whether the oligoclonally expanded TCR V β s from the IEL infected with reovirus in vivo play an important role in the virus-specific CTL activity. IEL from conventionally reared mice, 2 weeks following infection, were cocultured with reovirus-infected peritoneal macrophages in vitro for 4 days, and cells bearing particular TCR V β s were depleted with mouse monoclonal antibodies and sheep anti-mouse immunoglobulin linked to magnetic beads. The CTL activity of total IEL was 87% (control). With depletion of IEL bearing TCR V β 12, a 37% decrease in CTL activity was observed, whereas depletion of TCR V β 17 resulted in a 77% decrease in CTL activity. In contrast, depleting the V β 11 subset led to an increase in CTL activity of approximately 20% (from 87 to 106% of control). Removal of IEL expressing other TCR V β s did not affect CTL activity (Fig. 3). This data indicates that TCR V β 12- and -17-expressing cells in IEL contain the majority of reovirus-specific CTLs. These results are in concordance with the analysis of

FIG. 1. Representative FACS obtained from the IEL of germfree mice. Germfree BALB/cByJ mice were orally inoculated with 3×10^7 PFU of reovirus type 1. Two weeks following infection, IEL were isolated and cells were stained and analyzed by FACS with phycoerythrin-conjugated anti-CD8, FITC-conjugated anti-CD4, and TCR V β antibodies. (a and b) Two-color flow cytometric analysis shows IEL populations that are CD8⁺ CD4⁻ (70%), CD8⁻ CD4⁻ (20%), CD8⁻ CD4⁺ (5%), or CD8⁺ CD4⁺ (5%). (c and d) The cells bearing TCR V β 12 in control germfree IEL were 0.16%, and they increased by >10-fold (to 1.76%) 2 weeks following reovirus enteric infection.

FIG. 2. TCR V β expression in the IEL of germfree mice following reovirus enteric infection. Germfree BALB/cByJ mice were orally inoculated with 3×10^7 PFU of reovirus type 1. IEL were obtained weekly, stained with FITC-conjugated TCR V β -specific antibodies, and analyzed by FACS to determine the percentage of cells expressing TCR V β s. T cells bearing TCR V β 2, -12, and -17 increased by 10-fold, whereas subsets of T cells bearing other V β s did not change (represented by V β 9 and -11).

FIG. 3. TCR V β s associated with CTL activity in IEL populations. IEL were isolated 2 weeks following reovirus enteric infection of C3H/HeJ mice and cocultured with reovirus-infected macrophages for 4 days. Each TCR V β was removed from the IEL-cultured cells by immunomagnetic beads. Cytotoxicity was assessed by a nonradioactive assay that measured LDH released during cell lysis. Reovirus-infected L cells ($H2^k$) were used as the target cells, and IEL were present at an effector-to-target ratio of 40:1. Reovirus-specific CTL activity of nondepleted IEL was 87% (control). It decreased by 37% with depletion of the V β 12-bearing cells and 77% with depletion of the V β 17-bearing cells. Depletion of the V β 11 cells resulted in an increase in CTL activity (19% more than control). These results were repeated twice.

IEL TCR V β percentage changes observed during infection (Fig. 3).

DISCUSSION

The gastrointestinal tract serves as a major site for primary infection as well as entry of pathogens, and the immune response at the mucosal surface provides the first barrier for protection and defense during invasion. We have been particularly interested in the T-cell responses that are necessary for the containment and clearance of viral infection (6, 26–28, 41). In this study, we characterized the perturbation of the TCR V β repertoire of lymphocytes in the intraepithelial compartment by enteric infection with reovirus in conventionally reared and germfree mice. Our data indicate that conventionally reared mice, maintained in pathogen-free conditions, have a high percentage of IEL that are accounted for by a limited repertoire of CD8 $^+$ cells expressing TCR. TCR V β 2, -6, and -8 subgroups each accounted for greater than 10% of the CD8 $^+$ IEL. The percentage of IEL that utilize V β 6 and -8 are comparable to those previously reported by Rocha and colleagues (36). For the mice maintained in the specific-pathogen-free facility, the percentage of IEL utilizing different V β s was relatively stable, compared to data presented by Viney et al. (45). However, in one noninfected group of mice, TCR V β 2 was increased to approximately 70% of the IEL, indicating unsuspected exposure to a nominal antigen (possibly a superantigen) prior to evaluation (data not shown). The expanded families of TCR V β genes utilized in conventionally reared mice contrasted with results obtained from the germfree mice in which TCR V β expression in IEL was uniformly low. These data suggest that sporadic introduction of microorganisms into the syngeneic mouse colony may occur and result in biologic variability of T-cell responses under experimental conditions. Food antigens apparently result in minimal expansion of α/β TCR $^+$ T-cell subpopulations in the absence of commensal organisms, although we have not compared the IEL T cells in germfree versus antigen-free mice.

There is evidence to support the notion that virus infection selectively induces monoclonal or oligoclonal expansion of certain TCR subpopulations, such as V β 8.3 T cells in influenza (12), V β 6, -5, -8, and -9 T cells in virus-induced thymic lymphomas (39), and V β 10, -8, and -13 T cells in acute myocarditis caused by coxsackievirus (38). However, the TCR utilization of IEL during the mucosal immune response to virus invading the gastrointestinal mucosa has not been studied previously. In this study, we found that there was a preferential expansion of CD8 $^+$ cells bearing TCR V β s of IEL after peroral infection with reovirus. In conventionally reared C3H mice, there was a marked increase in TCR V β 7-, -12-, -14-, and -17-bearing IEL subpopulations, with each increasing threefold within 4 weeks following reovirus infection *in vivo*. These results were similar to those found in germfree BALB/cByJ mouse-derived IEL, in which a 10-fold increase in V β 2, -12, and -17 was observed after enteric infection. In both syngeneic mouse strains, marked enrichment of V β 12- and -17-expressing cells of IEL was induced by reovirus. Therefore, the oligoclonal expansion of these TCR V β -bearing T-cell subpopulations in IEL suggests a role for them in gut mucosal immunity against reovirus. The MHC haplotypes of BALB/c ($H2^d$) and C3H ($H2^k$) mice are different, yet in both strains there was an increase in T cells expressing V β 12 and -17. It is possible that plasticity in the capacity of MHC class I to present antigen allows the same epitopes to be presented in mice bearing the $H2^k$ and $H2^d$ MHC haplotypes. Whether the increase in TCR V β 2 seen in BALB/cByJ germfree mice or V β 7 and -14 observed in C3H conventionally reared mice is due directly to reovirus stimulation or indirectly to genetic or environmental effects is at present being investigated.

The C3H/HeJ mouse does not express the V β 17 TCR protein due to a stop codon, TAG, near the 3' end of the open reading frame of the gene (V β 17b) (46). Using a commercially available anti-V β 17 monoclonal antibody initially isolated by Kappler and colleagues (24), we found that cells bearing the V β 17 TCR were a major T-cell subset accounting for immune response to reovirus. Therefore, it seems likely that these C3H mice are able to make a functional V β 17 protein. Consistent with this finding has been the recovery of a variant V β 17 transcript by standard reverse transcriptase production of cDNA and amplification by PCR with primer sets 5' from the V β 17a gene and the 3' primer from the C β gene. Our data show that IEL from noninfected C3H mice express mRNA containing a stop codon as previously described, whereas IEL from infected mice express mRNA coding for a variant V β 17 (data not shown). Therefore, amplification of cells expressing a variant gene occurs following reovirus infection, which accounts for V β 17 expression. Whether this reflects a second gene closely related to V β 17a or hypermutation of the V β 17b gene is at present under investigation.

Further evidence indicating that reovirus enteric infection results in the expansion of a selected population of TCR V β -bearing IEL was obtained by examining TCR V β expression following *in vitro* culture of IEL with reovirus-infected APC. We found that IEL expressing TCR V β 9, -12, -13, and -17 increased at least sixfold by 3 days in culture. At 6 days in culture, all TCR V β subsets decreased, indicating low cell viability of CD8 $^+$ cells bearing α/β TCR. However, marked increases in TCR V β families were still observed, and these differences were maintained for approximately 1 week in culture. IEL stimulated *in vitro* differed from freshly isolated IEL in the percentage of CD8 cells bearing TCR V β 2, -6, and -8. Freshly isolated IEL had minimal increases in these TCR V β groups, whereas in *in vitro*-maintained cells CD8 $^+$ cells bearing TCR V β 2, -6, and -8 decreased.

The expanded V β 12 and -17 subfamilies in IEL were found to play a crucial role in the virus-specific cytotoxicity. This was most dramatic for CD8⁺ T cells bearing TCR V β 17 in which depletion of this subpopulation of T cells virtually eliminated CTL responsiveness. Depletion of CD8⁺ T cells bearing TCR V β 12 resulted in a 37% reduction in CTL responsiveness. These data suggest that there is a limited number of T-cell epitopes on reovirus polypeptides that result in reovirus-specific T-cell responses. This finding is consistent with the findings obtained in other viral systems. For influenza virus, the nucleoprotein is the major determinant for CTL responses (12). Under experimental conditions, the host may make additional responses, as is evident in the development of CTL responses to the influenza virus hemagglutinin (42). Therefore, selection of T-cell determinants as immunogens should be predicated by the host cell response during natural infection.

In reovirus gastrointestinal infection, the host response is restricted to a limited number of V β s. However, for other viral systems the relationship between oligoclonal expansion of certain TCR subpopulations and CTL responses has been confusing. Some studies have agreed with our findings that oligoclonal expansion of certain TCR subpopulations occurred following virus infection (12, 38, 39, 42). These include data from humans infected with human immunodeficiency virus, in which a patient had a profound increase in the percentage of CD8⁺ T cells bearing TCR V β 19, with some of these cells having CTL responses toward human immunodeficiency virus-infected cells. However, other infected individuals had increases in other TCR V β subtypes and less marked restrictions in responsiveness (21). For lymphocytic choriomeningitis virus, CTL clones derived from virus-primed lymphocytes had T cells bearing different TCR V β groups recognizing the same determinant. These data suggest that restrictions in repertoire were not related to TCR V β family (19). Alternatively, it is possible that the method of expansion of CTLs used in the study of lymphocytic choriomeningitis virus-specific responses in utilizing hybridomas provided a selective advantage toward T cells not common during natural infection.

Our work demonstrates that there is expansion of subsets of CD8⁺ IEL in both C3H and BALB/cByJ mice that appear to have no CTL responsiveness. For example, although V β 7 and -14 were obviously amplified in the mucosal immune response to the virus infection, depletion of either subpopulation did not decrease CTL activity. There are several possible explanations for this finding. Firstly, expansion of CD8⁺ cells without CTL activity may be regulating cells producing cytokines necessary for virus clearance (1, 16, 32). Secondly, these cells may regulate CTL responses themselves. For example, CTL responsiveness to reovirus is achieved by in vitro culture in the presence of infected APC prior to assay (13, 27). The lack of CTL responsiveness of freshly isolated immune IEL to infected targets may be due to suppression of responses induced by a second cell type. In support of this notion is the finding that there is only a 3-fold expansion of CD8⁺ IEL bearing TCR V β 17 cells in vitro compared to a 10-fold expansion of TCR V β 17-bearing T cells obtained a week after infection in vivo. Such an increase in cell numbers is easily achieved by increasing the effector-to-target ratio with freshly isolated cells. However, even at effector/target ratios of 100:1, CTL responses are not observed in freshly isolated IEL. Therefore, instead of expansion of a population of cells, in vitro culture conditions may decrease a regulatory (suppressor) cell (5, 13, 30).

Our study is consistent with the hypothesis that, during the gut mucosal immune response to reovirus enteric infection, a small number of TCR subfamilies of IEL are expanded selectively, based on recognition of viral epitopes, and that those

play a crucial role in the virus-specific cytotoxicity. Our data indicate that the development of protective immunity requires the oligoclonal expansion of particular virus-specific TCR V β subpopulations.

ACKNOWLEDGMENTS

We thank A. Chaney, W. F. Green, and M. C. Sutcliffe for assistance in the germfree animal facility, flow cytometric analysis, and virus purification, respectively. We thank E. Eisenberg, B. Michael, B. Graham, and T. Dermody for review of the manuscript.

This work was supported by NIH grant AI-23970 and a Merit Review grant from Veterans Affairs.

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