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# Cytotoxic and interferon γ-producing activities of γδ T cells in the mouse intestinal epithelium are strain dependent

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**ABSTRACT** **We have analyzed the cytolytic activity of freshly isolated intraepithelial T cells (i-IEL) from the intestines of several different mouse strains in an anti-T-cell receptor monoclonal antibody-mediated redirected lysis assay. The cytolytic activity of γδ i-IEL but not that of αβ i-IEL was strain dependent. Mouse strains could be divided into high (H), marginal (M), and null (N) strains. The anti-γδ T-cell receptor monoclonal antibody-induced interferon γ production showed the same strain-dependent variability, but the proliferative responses to γδ T-cell receptor crosslinking did not show this variability. The N phenotype of γδ i-IEL was found to be dominant in (H × N)$F_1$ mice. In radiation bone-marrow chimeras the H/N phenotype was determined by the genotype of the reconstituting bone-marrow-derived cells but was not determined by the genotype of the radioresistant host cells. Analysis of (H × N)$F_1$ backcross animals indicated that at least two genes are involved in determination of the H/N phenotype. One of these genes is major-histocompatibility-complex linked. No difference in the use of the variable region segment of the γ-chain or δ-chain was seen between the γδ i-IEL from H and N strains. Various models that might explain the strain-dependent γδ i-IEL phenotypes are discussed.**

The function and specificity of T cells bearing γδ T-cell receptor (TCR) are poorly understood (1, 2). One major γδ T-cell subset is associated with the intestinal epithelia of mice (3, 4) and other species, such as human (5), chicken (6), and sheep (7). The γδ intestinal intraepithelial T lymphocytes (i-IEL) differ from other γδ T cells in that they are thymus independent (8, 9) and in that many of these cells express γ-chain variable region ($V_\gamma$) 7 (10) and CD8αα homodimer (9). Freshly isolated γδ i-IEL as well as αβ i-IEL kill Fc receptor-positive target cells in the presence of anti-TCR monoclonal antibody (mAb) (11). It has been proposed that the γδ i-IEL play an important role in the first-line defense against microorganisms by destroying infected enterocytes (1, 12). However, at present, no evidence supports this hypothesis, and the biological role of γδ i-IEL remains unknown. To illuminate this issue we have analyzed the anti-TCR mAb-mediated lysis of Fc receptor-positive target cells by γδ i-IEL from several different mouse strains. The results described here show that, in contrast to αβ i-IEL, γδ i-IEL from different mouse strains vary in their anti-TCR-mediated cytolytic activity. In contrast, no such strain dependency was observed in the proliferative response to γδ TCR crosslinking. Similar strain dependency was also seen in interferon γ (IFN-γ) production upon polyclonal stimulation of γδ i-IEL. At least two genes that are expressed in hemopoietic cells control the strain-dependent cytotoxicity. One of these genes is major-histocompatibility-complex (MHC) linked.

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## MATERIALS AND METHODS

**Mice.** AKR/J, A/J, C57BL/6 (B6), B10, B10.A, B10.BR, B10.D2, BALB/c, C3H/HeJ (C3H), C3H.SW, and DBA/2 mice were purchased from The Jackson Laboratory. C3H/HeN mice were obtained from National Institutes of Health (Bethesda, MD). BALB.B mice were from our breeding colony at Massachusetts Institute of Technology. By crossing appropriate inbred mouse strains, we raised several different $F_1$ hybrid mice and backcrossed animals. For IFN-γ assays, C3H and (B6 × C3H)$F_1$ (B6C3F1) mice were purchased from Bomholtgard Breeding and Research Center Ltd. (Denmark).

**Isolation of Mouse i-IEL.** Unless otherwise stated, we used an improved method for the isolation of mouse i-IEL. Small intestine free of the lumen content was turned inside-out with the aid of polyethylene tubing. The inverted intestine was fastened to the tubing with a string and then cut into three or four segments. Up to 10 segments of intestines were transferred to a Tupperware box containing 250 ml of RPMI 1640 medium/2% fetal calf serum/25 mM Hepes/penicillin at 100 units/ml/streptomycin at 100 μg/ml, and the box was shaken at 37°C for 45 min (Oribital shaker, 150 rpm). Cell suspensions were collected in 250-ml tubes and further processed for the purification of i-IEL, according to the standard technique. In brief, the cell suspension was first passed through a glass-wool column to deplete cell debris and sticky cells and was then subjected to Percoll discontinuous-gradient centrifugation. More than 90% of i-IEL was recovered at the interphase of 44% and 70% Percoll solutions. The advantage of this method is that the contamination by lymphocytes from either Peyer's patches or lamina propria is minimal. For IFN-γ assays, we used the following method for i-IEL preparation: mesenteric tissues and Peyer's patches were removed from intestines that were then cut open longitudinally, washed with phosphate-buffered saline, and cut into smaller pieces. These pieces were incubated in 2% fetal calf serum/RPMI 1640 medium for 30 min at 37°C with vigorous shaking. This process was repeated twice with fresh medium. Subsequent procedures were as described above.

**Antibodies.** The following mAbs were used: anti-pan γδ TCR mAbs, 3A10 (13) and GL-3 (11); an anti-$V_\delta$4 mAb, GL-2 (11); an anti-pan αβ TCR mAb, H57-597 (14); an anti-CD3/TCR mAb, 145-2C11 (15); an anti-pan B-cell mAb, B220 (16); an anti-H-2K$^b$ mAb; and an anti-H-2K$^k$ mAb.

**Redirected Cytolysis Assay.** Redirected cytotoxic activity (4, 11, 17) was measured in a standard $^{51}$Cr-release assay. The medium was RPMI 1640 (GIBCO)/10% fetal calf serum/25 mM Hepes/50 μM 2-mercaptoethanol/4 mM glutamine/penicillin at 100 units/ml/streptomycin at 100 μg/ml (complete

Abbreviations: i-IEL, intestinal intraepithelial T lymphocytes; mAb, monoclonal antibody; TCR, T-cell receptor; MHC, major histocompatibility complex; FITC, fluorescein isothiocyanate; V, variable region; $V_\gamma$, γ-chain V; $V_\delta$, δ-chain V; IFN-γ, interferon γ.

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medium). Percent specific killing was calculated as [(experimental release − spontaneous release)/(total incorporated − spontaneous release)] × 100. Spontaneous release was always <10% of total $^{51}Cr$ incorporated into the target cells.

**Proliferation Assay.** For the proliferation assay, γδ i-IEL preparations of >98% purity were obtained by FACStar separation of i-IEL suspensions that had been stained with the fluorescein isothiocyanate (FITC)-conjugated anti-αβ TCR mAb H57-597 and anti-B-cell mAb B220.

**IFN-γ Assay.** IFN-γ activities in the culture supernatants were determined by ELISA, according to a described procedure (18).

**Flow Cytometric Analysis and Cell Sorting.** Immunofluorescence staining procedures have been described (13). Flow cytometric analyses were done by using a FACScan with FACSCAN software (Becton Dickinson). Positive and negative sortings of particular i-IEL subsets out of whole i-IEL populations stained with FITC-conjugated mAbs were done with a FACStar cytometer.

**PCR and Southern Blotting.** Total DNA was extracted from the sorted γδ i-IEL suspensions and recovered by ethanol precipitation with sonicated salmon sperm DNA as a carrier. PCR primers used for the reaction have been described (10). The Southern blots of $V_\delta 4$- and $V_\delta 7$-PCR products were hybridized with an oligonucleotide probe for δ-chain joining region as described (10).

## RESULTS

**γδ i-IEL Show Strain-Dependent Variation in Cytolytic Activity.** i-IEL isolated from various mouse strains (8- to 20-week old) showed similar cytolytic activities when the anti-αβ TCR mAb was used (data not shown). In contrast, strain-dependent differences were observed when the anti-γδ TCR mAb was used (Fig. 1). The mouse strains could be divided into three groups on the basis of their γδ i-IEL-mediated cytolytic activity: high (H)—C57BL/6; marginal (M)—B10.BR, BALB/c, and C3H.SW; and null (N)—AKR/J, C3H/HeJ(C3H), C3H/HeN, and DBA/2. B10 was typed as H strain, and A/J, B10.A, B10.D2, and BALB.B were typed as M strains (data not shown). The ratio of αβ to γδ TCR-positive i-IEL was ≈1:1 in H, M, and N strains (Fig. 2 and data not shown). We also examined the cytolytic activity of highly purified γδ i-IEL (>98%). The cells from B6 mice were cytolytic when activated by the anti-CD3/TCR or anti-γδ TCR mAb but were not cytolytic when activated by the anti-αβ TCR mAb. Cells from C3H mice were not cytolytic with any of the three mAbs.

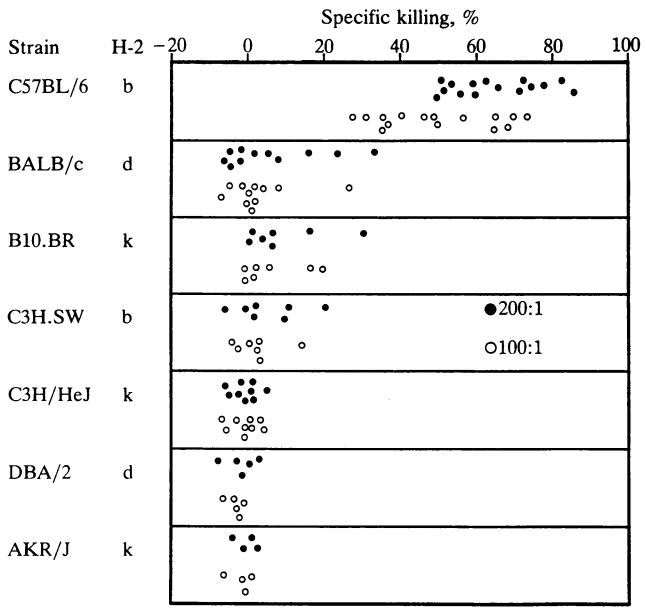


FIG. 1. Cytotoxic activity of γδ i-IEL from various mouse strains. i-IEL were assayed for lytic activity against $^{51}Cr$-labeled P815 target cells in the presence of 3A10 mAb (13) specific for a constant region of TCR δ chain (1 μg/ml). Serial dilutions of i-IEL were incubated in 96-well flat-bottom microliter plates with $2 \times 10^3$ target cells and incubated at 37°C for 5 hr. Virtually no difference in lytic activity was observed whether the anti-pan γδ TCR mAb GL3 (2 μg/ml) or 3A10 mAb (0.1, 1.0, or 10 μg/ml) was used. Lytic activities elicited only at effector-to-target ratios of 200:1 (●) and 100:1 (○) are presented, and each symbol represents an independent determination.

**The N Phenotype Is Dominant in (H × N)$F_1$ Mice.** We prepared three different (H × N)$F_1$ hybrid mice—namely, (B6 × C3H)$F_1$ (B6C3F1), (B6 × AKR/J)$F_1$ (B6AKR/JF1), and B6 × DBA/2)$F_1$ (B6D2F1) and examined the cytolytic activities of their γδ i-IEL. No cytolytic activity was seen with γδ i-IEL from any of these strains. A representative result is shown in Fig. 2. A maternal effect was excluded because the same results were obtained when parent-strain combinations were reversed.

**The H/N Phenotype Is Determined by Bone-Marrow-Derived Cells.** Five months after lethal irradiation and reconstitution by bone-marrow cells, almost all i-IEL (92–96%) were shown to be donor derived by H-2 typing. The ratio of αβ to γδ T cells was ≈1:1, as in normal mice (data not shown). In the (H × N)$F_1$ into H chimeras (B6C3F1 into B6), only the αβ i-IEL, but not the γδ i-IEL, were found to express cytolytic activity. In the H into (H × N)$F_1$ chimeras, both αβ and γδ i-IEL expressed cytolytic activity. These results indicate that the H/N phenotype is determined by genes expressed in radiosensitive hemopoietic cells.

**IFN-γ Production by γδ i-IEL Is Also Strain Dependent.** We examined whether IFN-γ production by γδ i-IEL exhibits a similar strain dependency to the cytolytic activity. Table 1 shows that in the presence of the anti-CD3/TCR mAb or anti-αβ TCR mAb, IFN-γ production was induced in i-IEL from all strains of mice examined. In contrast, stimulation with the anti-γδ TCR mAb induced IFN-γ production in i-IEL from B6 mice but did not induce it in i-IEL from C3H or B6C3F1 mice.

**The *in Vitro* Proliferative Response to γδ TCR-Crosslinking Is Not Strain Dependent.** We examined the *in vitro* proliferative responses of highly purified γδ i-IEL (>98%) from B6 and C3H mice to TCR crosslinking in the presence or absence of recombinant interleukin 2. Both B6 and C3H γδ i-IEL proliferated equally well, irrespective of whether their TCRs were crosslinked by the anti-CD3/TCR mAb or anti-γδ TCR mAb (Table 2 and data not shown).

Table 1. IFN-γ production by i-IEL from different mouse strains

| | IFN-γ, units/ml | | | |
|---|---|---|---|---|
| | P815 | i-IEL + P815 | | |
| Stimulation | alone | C57BL/6 | B6C3F1 | C3H/HeJ |
| Anti-CD3 mAb | <1.5 | 146.1 | 168.0 | 172.0 |
| Anti-αβ mAb | <1.5 | 60.8 | 85.0 | 100.0 |
| Anti-γδ mAb | <1.5 | 49.4 | 1.5 | <1.5 |
| Hamster IgG | <1.5 | <1.5 | <1.5 | <1.5 |
| Medium | <1.5 | <1.5 | <1.5 | <1.5 |

i-IEL ($2 \times 10^5$) were cultured with $1 \times 10^5$ irradiated (10 Gy) P815 cells in 225 μl of RPMI 1640 complete medium with anti-CD3/TCR mAb 145-2C11 at 2 μg/ml (15), anti-αβ TCR mAb H57-597 at 2 μg/ml (14), anti-γδ TCR mAb GL-3 at 2 μg/ml (11), hamster IgG at 2 μg/ml, or medium alone. Twenty-four hours later, supernatants were collected and assayed for IFN-γ by ELISA, as described by Singh *et al.* (18).

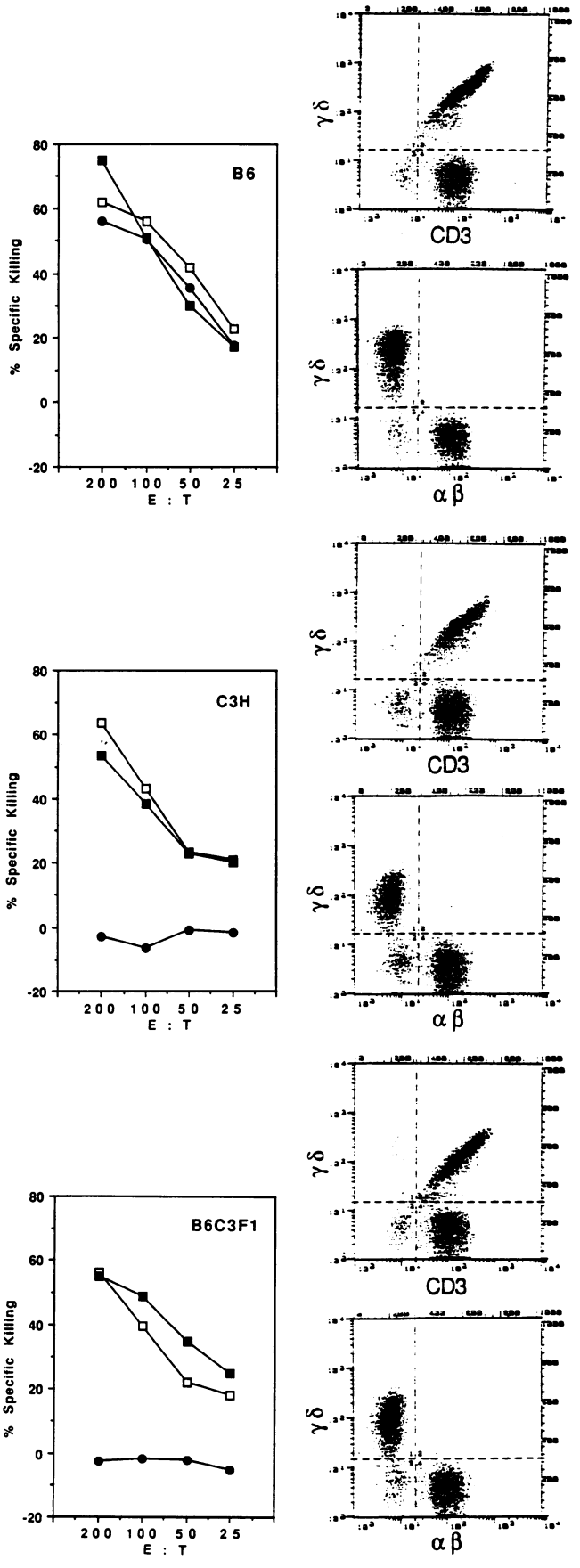


FIG. 2. Cytotoxic activity and two-color flow cytometric analysis of i-IEL from B6, C3H, and (B6 × C3H)$F_1$ (B6C3F1) mice. (*Left*) The redirected lysis assay was done in the presence of H57-597 mAb (0.2 μg/ml; ■), 3A10 mAb (1 μg/ml; ●) or 145-2C11 mAb (0.2 mg/ml; □). (*Right*) For flow cytometry, i-IEL were incubated first with the anti-γδ TCR mAb 3A10 (biotinylated) and anti-CD3/TCR mAb 2C11 (FITC conjugated) or with the anti-γδ TCR mAb 3A10 (biotinylated) and anti-αβ TCR mAb H57-597 (FITC conjugated), followed by an incubation with streptavidin–phycoerythrin. E:T, effector/target ratio.

**Both MHC-Linked and MHC-Unlinked Genes Determine the γδ i-IEL Cytotoxic Activity.** The data of Fig. 1 suggest that both MHC-linked and MHC-unlinked genes are involved in the determination of the H/N phenotype of γδ i-IEL. To confirm this point, we backcrossed B6C3F1 mice to the B6 parent and determined the cytolytic activity and H-2 haplotype of individual mice. Fig. 3 shows that γδ i-IEL from homozygous H-$2^{b/b}$ mice showed significantly higher cytolytic activity than those from heterozygous H-$2^{k/b}$ mice ($P = 0.002$). In contrast, the anti-αβ TCR mAb elicited the same degree of cytotoxicity in i-IEL populations, regardless of their H-2 haplotype. We conducted a similar study using a smaller number of B6 × B6D2F1 backcross mice. Again, homozygous H-$2^{b/b}$ γδ i-IEL showed higher cytolytic activity than heterozygous H-$2^{b/d}$ γδ i-IEL ($P = 0.005$), whereas no such difference was seen in αβ i-IEL (data not shown). Taken together, these results indicate that an MHC-linked dominant gene(s) present in H-$2^k$(C3H) and H-$2^d$(DBA/2) haplotypes is involved in the determination of the N phenotype. In addition, the considerable variability in γδ i-IEL-mediated cytolytic activity among H-2-identical individuals (Fig. 3) suggests an involvement of an MHC-unlinked gene(s).

**The Vγ and the V Gene of the δ Chain ($V_\delta$) Segment Use by H and N Strains.** The H/N phenotype could be determined, at least in part, by specificity of the γδ TCR. Thus, a self-component(s) of N strains could either positively select (induce) the noncytolytic phenotype or delete the cytolytic phenotype in γδ i-IEL or their progenitors. In either case, one would expect that the γδ TCR repertoire of cytolytic γδ i-IEL differs from that of noncytolytic γδ i-IEL. In an attempt to identify such differences, we carried out two types of experiments. (*i*) We used the anti-$V_\delta 4$ mAb GL-2 (11) in redirected cytolysis assay. The results of Fig. 4*A* demonstrate that not only $V_\delta 4^+$ i-IEL but also $V_\delta 4^-$ i-IEL are cytolytic and that, based on the titration, the former activity comprises approximately a quarter of the total cytotoxicity.

(*ii*) We extracted genomic DNA from highly purified γδ i-IEL (99.9% pure) preparations and measured the relative amounts of V–joining region (J) γ or V–diversity region (D)–J δ rearrangements by PCR using appropriate V and J sequence primers. For analysis of the TCR γ genes, we examined $V_4$–$J_1$ γ and $V_7$–$J_1$ γ rearrangements because previous studies indicated that these are the two major γ genes expressed in i-IEL. No significant differences in the levels of these rearrangements in B6 (H), C3H (N), and B6C3F1 (N) mice were found. In contrast, a considerable strain-to-strain variation was observed in the levels of $V_4$–D–$J_1$ δ and $V_7$–D–$J_1$ δ rearrangements, as shown in Fig. 4 *B* and *C*, respectively. However, no obvious relationship between the level of either type of δ-gene rearrangement and the degree of cytolytic activity of the γδ i-IEL was discernable.

## DISCUSSION

We have described a genetic control for effector functions of γδ i-IEL: the constitutive cytotoxicity and IFN-γ-secreting activity of γδ i-IEL are strain dependent, whereas those of αβ i-IEL are not strain dependent. The mechanism underlying the genetic control of these constitutive effector functions of γδ i-IEL is unknown. Among many possible explanations, we regard two as more likely on the basis of our and others'

Table 2. Proliferative responses of γδ i-IEL

| γδ i-IEL responders | Mitomycin C-treated spleen cells | Anti-γδ mAb | rIL-2 | [³H]Thymidine incorporated, cpm | |
|---|---|---|---|---|---|
| | | | | Day 3 | Day 5 |
| B6 | B6 | − | − | 519 | 656 |
| B6 | B6 | + | − | 515 | 554 |
| B6 | B6 | − | + | 1,474 | 4,490 |
| B6 | B6 | + | + | 14,092 | 137,164 |
| B6 | | + | + | 1,701 | 4,297 |
| | B6 | + | + | 1,183 | 1,023 |
| C3H | C3H | − | − | 765 | 748 |
| C3H | C3H | + | − | 950 | 468 |
| C3H | C3H | − | + | 5,427 | 19,897 |
| C3H | C3H | + | + | 21,405 | 136,239 |
| C3H | | + | + | 3,431 | 6,251 |
| | C3H | + | + | 629 | 789 |

Ten thousand cells from purified γδ i-IEL preparations (>98%) were cultured in each well of a 96-well flat-bottom microplate, and 1 μCi of [³H]thymidine (1 Ci = 37 GBq) was added 12 hr before termination of culture. Mitomycin C-treated spleen cells ($2.5 \times 10^5$), anti-γδ TCR mAb 3A10 at 0.1 μg/ml (13), and recombinant interleukin 2 (rIL-2) at 100 units per ml were used. Means of triplicated determinations are presented.

findings. (*i*) Genetic control could be exerted at the activation step that converts cytotoxic γδ i-IEL precursors to the constitutively cytotoxic state. Whatever the mechanism for this activation step, we suggest that N strains carry genes that encode products which inhibit the activation process in a TCR-specificity-independent fashion. This hypothesis explains why the N phenotype is dominant over the H phenotype in $(H \times N)F_1$ mice and is consistent with the apparent lack of correlation between an H/N phenotype and V-gene segment use. However, this hypothesis demands that the postulated inhibitor genes would be active only in γδ i-IEL and would not be active in αβ i-IEL.

(*ii*) An alternative, more likely, hypothesis for the genetic control of the constitutive effector functions of γδ i-IEL is that it occurs during differentiation of immature T cells in the form of TCR-mediated cellular selection. Various versions of the selection hypothesis are conceivable; however, in light of the $(H \times N)F_1$ phenotype and the similarity to the previously known αβ T-cell system (19, 20), we favor the following version. In this version the N phenotype results from a negative selection of those immature γδ i-IEL-expressing TCRs specific for the products of genes carried by N strains but not specific for the products of genes carried by H strains. According to this hypothesis, the activation of the mature γδ i-IEL from the cytotoxic precursor state to the constitutively cytotoxic state occurs in H strains in a TCR-specific manner upon recognition of gut epithelium-associated antigens that mimic or cross react with the negatively selecting products of the N strain genes. γδ i-IEL from H strains are highly cytotoxic in the *in vitro* antibody-redirected assay because the activated cytotoxic cells are fully present, whereas γδ i-IEL from N strains are not cytotoxic in the same assay because these strains negatively select the cytotoxic precursors and, hence, lack constitutively cytotoxic T cells. Many other inbred strains and some individual mice produced by an $H \times (H \times N)F_1$ backcross lack some of the genes that encode products which act as negative selectors and, therefore, exhibit various intermediate levels of constitutive cytoxicity.

Our results indicate that both MHC-linked and MHC-unlinked genes are involved in the postulated negative selection of γδ i-IEL. Strain-dependent negative selection of a specific subset of αβ T cells by MHC-linked and MHC-unlinked gene products has been reported for endogenous superantigens (21). For instance, both MHC class II I-E genes and multiple non-MHC-linked superantigen genes have been shown to determine the depletion of $V_\beta 11$-bearing T cells (22, 23). In this system, as for γδ i-IEL, individual progeny derived from an $F_1 \times$ parent backcross exhibited various intermediate phenotypes.

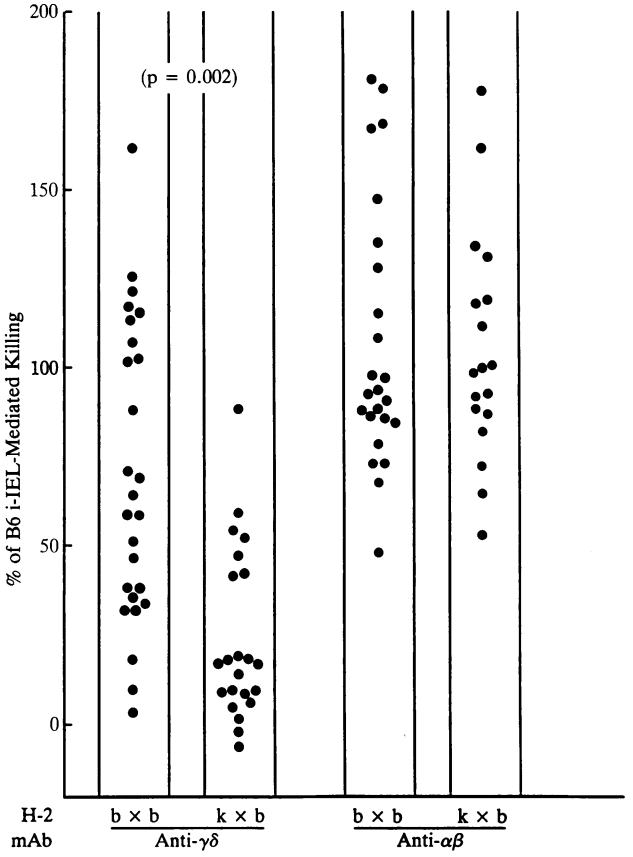


FIG. 3. Cytotoxic activity of γδ and αβ i-IEL from B6 × (B6 × C3H)$F_1$ backcross mice. i-IEL were isolated from the individual backcross mice, and their cytotoxic activities were evaluated in the presence of 3A10 or H57-597 mAb, as described in the legend for Fig. 2. The lytic activities at the effector-to-target ratio of 200:1 collected from five separate experiments are depicted as percentage of B6 γδ or αβ i-IEL-mediated killing (positive control). In each experiment, i-IEL isolated from two age-matched B6 mice were examined individually for their γδ and αβ i-IEL-mediated cytotoxic activity as the positive control. To determine the H-2 haplotype of the backcross mice, an aliquot of i-IEL suspension was incubated first with anti-H-$2K^k$ mAb (FITC-conjugated) and anti-H-$2K^b$ mAb (biotinylated), followed by an incubation with streptavidin–phycoerythrin.

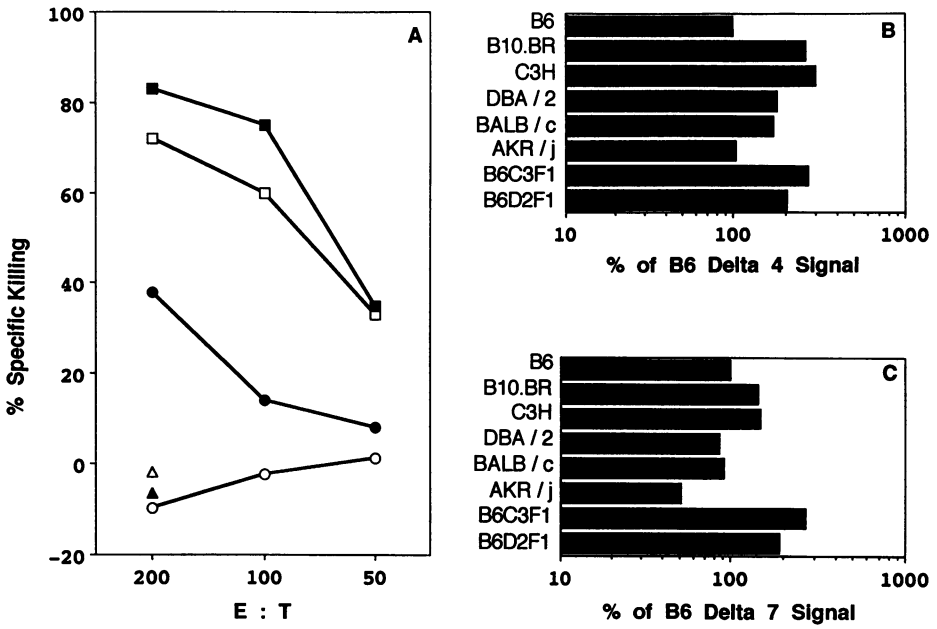


FIG. 4. Analysis of cytotoxic activity of B6 γδ i-IEL in relation to their $V_\delta$ gene usage. (*A*) B6 γδ i-IEL (■, ●, ▲) or B6 γδ i-IEL devoid of $V_\delta4^+$ TCR-expressing cells (□, ○, △) were sorted out by a FACStar from an i-IEL suspension stained with either the FITC-conjugated mAb H57-597 and mAb B220 or the FITC-conjugated mAb H57-597, mAb B220, and FITC-conjugated anti-$V_\delta4$ mAb GL 2 (11); their lytic activities were then assayed against P815 target cells in the presence of 3A10 mAb (1 μg/ml; ■, □), GL 2 mAb (2 μg/ml; ●, ○), or H57-597 mAb (0.2 μg/ml; ▲, △). (*B* and *C*) PCR-amplified $V_\delta4$ (*B*) and $V_\delta7$ (*C*) signals of purified γδ i-IEL DNA from various mouse strains were compared with the corresponding PCR-amplified $V_\delta4$ and $V_\delta7$ signals of purified B6 γδ i-IEL DNA. The strain-specific signals were normalized as the PCR-amplified $V_\delta4$ or $V_\delta7$ signal divided by the PCR-amplified actin signal. Three independent determinations produced similar data. Representative data are presented as percentages of the B6 $V_\delta4$ signal in *B* and as percentages of the B6 $V_\delta7$ signal in *C*.

Despite these similarities, the genetic control of γδ i-IEL may not be completely analogous to that of known superantigen-reactive αβ T cells. For instance, we have been unable to show a correlation between the absence of constitutive cytotoxicity and depletion of γδ i-IEL expressing particular $V_\gamma$ or $V_\delta$ gene segments. This lack of correlation may mean that the postulated interaction between TCR and the ligands that occurs during the negative selection is not as V segment-specific in γδ i-IEL as in superantigen-reactive αβ T cells (19–23). Alternatively for γδ T cells, negative selection may not result in cellular deletion but may result in functional inactivation (anergy). Indeed, we have previously observed that a clone of self-reactive γδ T cells undergoes inactivation of proliferating and lymphokine-producing activities, rather than cellular deletion in the thymus (24). Finally, if the negative selection hypothesis is correct, the identification and characterization of the controlling genes may shed light on the specificities of, at least some, γδ i-IEL.

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1. Hass, W., Pereira, P. & Tonegawa, S. (1992) *Annu. Rev. Immunol.* **11**, 637–685.
2. Bluestone, J. A., Cron, R. Q., Barrett, T. A., Houlden, B., Sperling, A. I., Dent, A., Hedrick, S., Rellahan, B. & Matis, L. A. (1991) *Immunol. Rev.* **120**, 5–33.
3. Bonneville, M., Janeway, C. A., Jr., Ito, K., Haser, W., Ishida, I., Nakanishi, N. & Tonegawa, S. (1988) *Nature (London)* **336**, 479–482.
4. Goodman, T. & Lefrancois, L. (1988) *Nature (London)* **333**, 855–858.
5. Groh, V., Porcelli, S., Fabbi, M., Lanier, L. L., Picker, L. J., Anderson, T., Warnke, R. A., Bhan, A. K., Strominger, J. L. & Brenner, M. B. (1989) *J. Exp. Med.* **169**, 1277–1294.
6. Bucy, R. P., Chen, C.-L. H., Clihak, J., Losch, U. & Cooper, M. D. (1988) *J. Immunol.* **141**, 2200–2205.
7. Hein, W. R. & Mackay, C. R. (1991) *Immunol. Today* **12**, 30–34.
8. Bandeira, A., Itohara, S., Bonneville, M., Burlen-Defrannoux, O., Mota-Santos, T., Coutinho, A. & Tonegawa, S. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 43–47.
9. Guy-Grand, D., Cerf-Bensussan, N., Malissen, B., Malassis-Seris, M., Briottet, C. & Vassalli, P. (1991) *J. Exp. Med.* **173**, 471–481.
10. Takagaki, Y., DeCloux, A., Bonneville, M. & Tonegawa, S. (1989) *Nature (London)* **339**, 712–714.
11. Goodman, T. & Lefrancois, L. (1989) *J. Exp. Med.* **170**, 1569–1581.
12. Janeway, C. A., Jr., Jones, B. & Hayday, A. (1988) *Immunol. Today* **9**, 73–76.
13. Itohara, S., Nakanishi, N., Kanagawa, O., Kubo, R. & Tonegawa, S. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 5094–5098.
14. Kubo, R. T., Born, W., Kappler, J. W., Marrack, P. & Pigeon, M. (1989) *J. Immunol.* **142**, 2736–2742.
15. Leo, D., Foo, M., Sachs, D., Samelson, L. E. & Bluestone, J. A. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 1374–1378.
16. Dessner, D. S. & Loken, M. R. (1981) *Eur. J. Immunol.* **11**, 282–285.
17. Lefrancois, L. & Goodman, T. (1989) *Science* **243**, 1716–1718.
18. Singh, I. G., Mukherjee, R., Talwar, G. P. & Kaufmann, S. H. E. (1992) *Infect. Immunol.* **60**, 257–263.
19. Kappler, J. W., Staerz, U., White, J. & Marrack, P. (1988) *Nature (London)* **332**, 35–40.
20. MacDonald, H. R., Schneider, R., Lees, R. K., Howe, R. C., Acha-Orbea, H., Festernstein, H., Zinkernagel, R. M. & Hengartner, H. (1988) *Nature (London)* **332**, 40–45.
21. Herman, A., Kappler, J. W., Marrack, P. & Pullen, A. M. (1991) *Annu. Rev. Immunol.* **9**, 745–772.
22. Vacchio, M. S., Ryan, J. J. & Hodes, R. J. (1990) *J. Exp. Med.* **172**, 807–813.
23. Dyson, P. J., Knight, A. M., Fairchild, S., Simpson, E. & Tomonari, K. (1991) *Nature (London)* **349**, 531–535.
24. Bonneville, M., Ishida, I., Itohara, S., Verbeek, S., Berns, A., Kanagawa, O., Hass, W. & Tonegawa, S. (1990) *Nature (London)* **344**, 163–165.