Brief Definitive Report

The Vβ Repertoire of Mouse Gut Homodimeric α CD8+ Intraepithelial T Cell Receptor α/β+ Lymphocytes Reveals a Major Extrathymic Pathway of T Cell Differentiation

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Summary

Gut intraepithelial lymphocytes (IEL) contain two independent T cell receptor α/β+ T cell populations, with different Vβ repertoires. In DBA/2 mice (Mls^a, IE+), the CD4+ and heterodimeric α/β CD8+ thymodependent T cell pool shows the same deletion of Vβ6, 8.1, and 11+ cells as found in peripheral lymphoid organs. In contrast, such deletions are not observed in the pool of IEL bearing homodimeric α CD8+ chains, in which these Vβ families are frequently observed in high amounts. The size of this gut homodimeric α CD8+ IEL pool and its different Vβ repertoire selection demonstrate the existence of a major extrathymic pathway of T cell differentiation with a gut-restricted localization. The large amount of the thymo-independent, homodimeric α CD8+ IEL found in the small bowel may contribute to a first line of defense against exogenous superantigens.

In mice bearing the Mls-1^a allele of the minor lymphocyte stimulating (Mls) locus or the product of the MHC IE^k or IE^d, T lymphocytes bearing, respectively, the TCR β chains variable segments Vβ6, Vβ8.1, or Vβ11 are eliminated during differentiation in the thymus (1–3). Gut intraepithelial lymphocytes (IEL) contain two major T cell populations (4). One is thymodependent and Thy-1+ TCR-α/β+, containing a majority of CD8+ cells (bearing heterodimeric CD8 α and β chains, as all peripheral CD8+ T lymphocytes) and a small minority of CD4+ cells. This population is the progeny of blasts arising in Peyer's patches after antigenic stimulation (4, 5). The second population is Thy-1+ or Thy-1−, expresses CD8 molecules containing only α chains (homodimeric α CD8+), and bears TCR made of γ/δ or α/β chains. This last population is thymoindependent; we have presented evidence that it derives from circulating bone marrow precursors attracted by the gut environment, which may also induce its local differentiation (i.e., expression of CD8 α chains and TCR gene rearrangement) (4). In the present work, we studied in DBA/2 mice (Mls-1^{a+} and IE^{d+}) the Vβ expression of TCR-α/β-bearing cells among these gut IEL populations. The results show that the heterodimeric α/β CD8+ and the CD4+ IEL are markedly depleted in Vβ6+, 8.1+, and 11+ cells, as are peripheral T cells; in contrast, cells bearing these Vβ chains are present in high percentages on the thymoindependent gut homodimeric α CD8+ IEL bearing TCR-α/β.

Materials and Methods

Animals. DBA/2 and C57Bl/6 mice were raised in conventional conditions in the INSERM U132 animal house. Pools of four to six mice were used in each experiment.

Cell Suspension and Immunofluorescence Analysis. Peripheral LN cell suspensions and IEL lymphocytes were prepared as described (5). Thy-1− cells were isolated by complement-mediated cytotoxicity and magnetic sorting, using the anti-Thy-1 mAb AT83 (a gift from F. Fitch, University of Chicago) and anti-Ig-coated Dynabeads (Dynal, Oslo, Norway). The following mAbs, coupled to FITC or biotin by standard methods (revealed with streptavidin-PE; Becton Dickinson & Co., Sunnyvale, CA) were used: GK1.5 (anti-CD4) (6); HO22 (anti-CD8 α chain) (7); H35-17-2 (anti-CD8 α/β chains) (4, 7); 30H-12 (anti-Thy-1) (8); H57-597 (anti- β TCR) (9); KJ16 (anti-Vβ8.1 and 8.2) (2); F23.2 (anti-Vβ8.2) (2); 44-22-1 (anti-Vβ6) (1), RR3.15 (anti-Vβ11) (3). Detection of surface antigens and fluorescence analysis was performed as described (8) using a FACScan® (Becton Dickinson & Co.), and the data were processed in a Hewlett Packard computer. The percentage of Vβ8.1+ cells was obtained by comparison between Vβ8.1+ + 8.2+ cells and Vβ8.2+ only cells.

Evaluation of the Intraepithelial and Peripheral Pool of T Lymphocytes. Seven longitudinal (1.5 cm in length) and transversal pieces of gut, taken at intervals of 7 cm along the small bowel, were processed for histological study. The numbers of villi per centimeter of length and per cross-section were determined on each section; we also evaluated the number of epithelial cells per villus, and the ratio of IEL per 100 epithelial cells. This permitted us to calculate the number of IEL per centimeter. The total IEL present in the

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small bowel was evaluated from the length of the small bowel (41 cm). The total number of CD3$^+$ cells was estimated in the spleen and the axillar, inguinal, and mesenteric lymph nodes, and the amount of lymphocytes in the peripheral pool was calculated as described (10).

Results and Discussion

The CD4$^+$ and CD8$^+$ lymph node (LN) T lymphocytes of DBA/2 (Mls-1^a) and C57Bl/6 (Mls^b) mice were explored by double immunofluorescence flow cytometry analysis to determine the percentage of cells bearing the Vβ6 and Vβ8.1 TCR β chain phenotypes. From the results shown in Table 1, it can be calculated that there was a depletion in Vβ8.1$^+$ cells of 93% and 85%, and in Vβ6 of 85% and 67% among, respectively, the CD4$^+$ and CD8$^+$ LN cells of the DBA/2 mice, as compared with these populations in B6 mice. These results are in agreement with those reported by others (1, 2). Gut CD4$^+$ and heterodimeric α/β CD8$^+$ IEL of DBA/2 mice (i.e., cells bearing CD4 and α/β CD8 phenotype identical to that of peripheral T cells) were similarly analyzed. The percentage of Vβ8.1$^+$ and Vβ6$^+$ cells among these two subpopulations of gut IEL was low and comparable with that found in LN cells (Table 1). It can be calculated from the results shown in Table 1 that the CD4$^+$ and heterodimeric α/β CD8$^+$ LN or gut IEL of DBA/2 mice also had a 30–70% depletion in their Vβ11$^+$ cells, as compared with the CD4$^+$ and CD8$^+$ LN cells of B6 mice. This difference is comparable with that observed by others with LN T cells of DBA/2 mice (3, 11).

When gut IEL were depleted in Thy-1$^+$ cells, a procedure that leaves a large Thy-1$^-$ population consisting almost exclusively of homodimeric α CD8$^+$ cells bearing TCR-α/β or -γ/δ (4), analysis of Vβ expression showed a totally different pattern, since the percentage of Vβ8.1$^+$, Vβ6$^+$, or Vβ11$^+$ cells among TCR-α/β^+ IEL (pan β^+ cells) was comparable with that of LN T cells from B6 mice (Table 1).

In these experiments using the gut IEL Thy-1$^-$ popula-

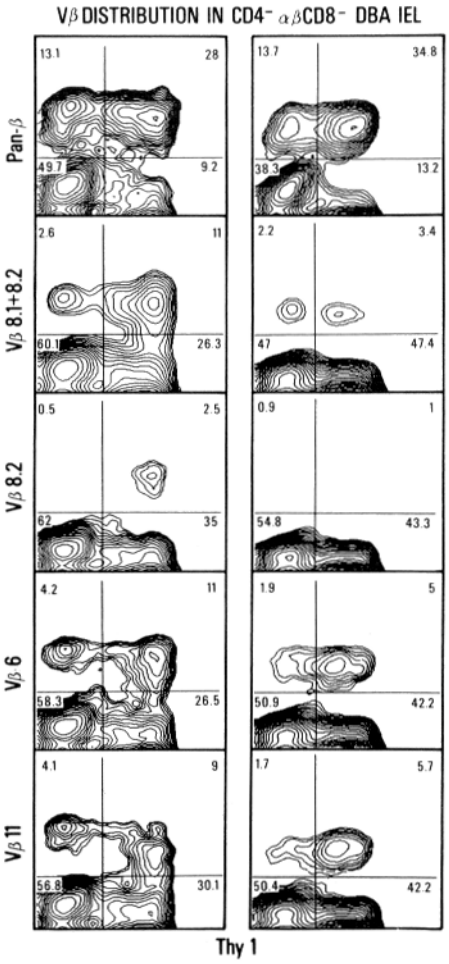

Figure 1. Flow cytometry analysis of the Vβ chains and TCR-α/β expression among the Thy-1$^+$ or Thy-1$^-$ α/β CD8$^-$, CD4$^-$ IEL from two DBA/2 mice (*left and right columns*) after sorting with a FACStar® (see Materials and Methods).

Table 1. *Vβ Gene Segment Expression among TCR-α/β^+ Gut IEL and LN Lymphoctyes*

		Vβ8.1	Vβ6	Vβ11
B6	CD4$^+$ LN	10 ± 3.9	14 ± 1.5	6 ± 1.4
DBA	CD4$^+$ LN	0.7 ± 0.5*	2.1 ± 0.8‡	1.8 ± 0.4‡
DBA	CD4$^+$ IEL	1.9 ± 0.6*	2.9 ± 1.7‡	3.3 ± 1.2*
B6	α/β CD8$^+$ LN	7.8 ± 2.4	14 ± 2.3	8 ± 0.6
DBA	α/β CD8$^+$ LN	1.2 ± 1.9*	4.6 ± 0.8‡	4.3 ± 0.4‡
DBA	α/β CD8$^+$ IEL	0.6 ± 0.5‡	5 ± 0.6‡	5.3 ± 1.4‡
DBA	Thy-1$^-$ IEL	10.3 ± 2.2	14.8 ± 5.6	12.1 ± 5.6

Results represent the percentage of cell bearing the various Vβ chains explored among the cells of the origin and phenotype indicated in the left column and the mean ± SD of six independent experiments. The percentage of Vβ bearing cells is based on that of the total TCR-α/β, detected with a pan-β TCR mAb, within each cell population. The significance of the differences in Vβ distribution in B6 and DBA/2 cell populations was analyzed using the Student *t* test.
* $p < 0.05$.
‡ $p < 0.01$.

Table 2. *Vβ Expression in Thy-1$^+$ and Thy-1$^-$ Thymus-independent IEL*

		Vβ8.1	Vβ6	Vβ11
Left	Thy$^+$	30	32	32
	Thy$^-$	16	39	31
Right	Thy$^+$	7	14	16
	Thy$^-$	9.5	14	12.5

Percentage of CD4$^-$, α/β CD8$^-$ IEL expressing individual Vβ TCR chains among the TCR-α/β^+ cells analyzed (as determined from the "pan β" plot), for each of the two mice (left and right).

tion, part of the Vβ repertoire of the gut homodimeric α CD8+ IEL was not explored, since, while all Thy-1− Vβ+ IEL bear homodimeric CD8 α chains, not all IEL of the homodimeric α CD8+ population are Thy-1− (4). To explore the Vβ repertoire and the Thy-1 expression of this entire population, CD4+ and heterodimeric α/β CD8+ IEL of DBA/2 mice were first eliminated by cell sorting using appropriate mAbs. Double immunofluorescence analysis was then performed on the remaining cells, using FITC-labeled anti-Thy-1 mAb and anti-β TCR mAbs (Fig. 1, Table 2). This led to the following conclusions: (*a*) ~40–50% of these cells were Thy-1+; (*b*) 40–50% of these cells, either Thy-1+ or Thy-1−, did not bear TCR-α/β (pan-β−; Fig. 1); they are known to bear TCR-γ/δ (12); (*c*) Vβ8.1+, Vβ6+, and Vβ11+ cells have a comparable distribution between Thy-1+ and Thy-1− cells. These results show that the thymus-directed deletion in the Vβ TCR repertoire is only present in the gut IEL bearing a phenotype identical to that of peripheral T cells. They also confirm that the two lineages of thymodependent and independent gut IEL (4) cannot be distinguished by the expression of the Thy-1 antigens but by that of the homodimeric CD8 molecules.

The homodimeric α CD8+ gut IEL population is not the only T cell population escaping deletion of Vβ+ cells that recognizes Mls and I-E antigens. A comparable observation has been made with the TCR-α/β+ cells found among two other T cell populations. One is that of the spleen cells of inbred nude mice (8, 13). The Vβ repertoire of these cells is pauciclonal as shown by the dominance of the expression of certain Vβ genes in some mice and the extensive variation of Vβ expression among individual mice (14, 15). The gut homodimeric α CD8+ cells share the same characteristics. A comparison of left and right columns in Fig. 1 shows large individual variations; in the left column, it can be seen that in this particular experiment, the Vβ8.1, Vβ6, and Vβ11 cells account for most of the α/β+ cells in the gut homodimeric α CD8+ IEL. Because of this high percentage, two-color staining with the appropriate mAbs was performed, and confirmed that a single TCR Vβ chain family was expressed by individual cells. In a total of 14 experiments, overexpression of Vβ11+ cells was found in 64%, and that of Vβ6+ cells in 43% of the homodimeric α CD8+ IEL (data not shown). Whatever the conditions leading to this nonrandom distribution of the Vβ repertoire in this population of gut IEL, these observations further emphasize the contrast between the Vβ expression in this population and in that of thymic-derived gut IEL or peripheral lymphocytes. The other T cell population that escapes deletion of Vβ+ cells in Mls-1a+, IE+ strains is the small population of TCR-α/β+, CD4− CD8− double-negative (DN) thymocytes; these cells, however, appear to display less variability in their Vβ repertoire than that found in the TCR-α/β+ spleen cells of nude mice (16). It is of interest to note that, after lectin stimulation, DN thymocytes and TCR-γ/δ+ peripheral lymphocytes also express the homodimeric α CD8 phenotype (17, 18). All these cells may belong to the same T cell lineage, expressing first γ/δ, and subsequently and in certain conditions only, TCR-α/β (8, 19).

Homodimeric α CD8+ lymphocytes are virtually absent among spleen and LN T lymphocytes, but represent ~60% of gut IEL. The size of this peculiar gut IEL pool has been calculated (see Materials and Methods) to be of ~3–4 × 10^7 T cells, i.e., to be as large as the pool of T lymphocytes in the spleen, its size thus amounting to ~40% of that of the peripheral T cell pool. Thus, our results demonstrate the existence of a major pathway of T cell differentiation that follows rules of selection different from those observed during maturation in the thymus. The antigenic specificity of the homodimeric α CD8+ TCR-α/β+ cells, as well as their localization restricted to the gut, may bear some relationship with their function in vivo. Deletion of thymocytes recognizing endogeneous "superantigens" such as Mls during the process of T cells differentiation within the thymus results in the elimination of cells expressing certain Vβ genes. However, cells expressing these Vβ genes may be required for the response to exogeneous bacterial antigens present in the gut, and even to exogeneous superantigens such as bacterial enterotoxins (20). The existence of an alternative pathway of T cell differentiation that maintains the expression of some Vβ genes but is restricted to the environment of the gut epithelium may thus be of major importance as a first line of immune defense against some antigens.

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