Different Use of T Cell Receptor Transducing Modules in Two Populations of Gut Intraepithelial Lymphocytes Are Related to Distinct Pathways of T Cell Differentiation

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Summary

Most gut intraepithelial cells (IEL) of the mouse are T cells that bear CD8 molecules, present either as α-β chain heterodimers (CD8β^+) or as α chain homodimers (CD8β^-). All CD8β^+ IEL bear α/β T cell receptors (TCR); CD8β^- IEL bear either α/β or γ/δ TCR and are considered to be a thymus-independent (TI) population, probably arising locally from a small fraction of CD3$^-$ IEL containing the recombinant activating gene RAG proteins. Here we report that TI CD8β^- IEL, whether bearing α/β or γ/δ TCR, contain, in normal mice, mRNAs for both ζ and FcϵRI γ chains. These chains are present in their CD3-TCR complexes as homo- or heterodimers. In contrast, only ζ chain mRNA and homodimers are found in gut CD8α/β^+ IEL and in peripheral T lymphocytes. Intestinal CD3$^-$ precursor cells contain only γ chain, and CD3$^-$ IL-2R$^+$ thymocyte precursors only ζ chain mRNAs. Only very primitive thymocyte precursors contain detectable γ chain mRNA, and it thus appears that FcϵRI γ chain use is switched off at a very early stage during thymocyte differentiation. Thus, T cell differentiation in the gut epithelium differs from that occurring in the thymus, from which CD8β^+ IEL appear to derive. Use of different TCR transducing modules and CD8 accessory molecules between the TI and the thymus-derived T cell populations provides an explanation for their difference in reactivity to antigenic stimulations and thus in selection of repertoires.

In the mouse, we have identified two main populations of gut intraepithelial lymphocytes (IEL)[1]. One, which bears TCRα/β or γ/δ, is present in significant amounts only in this peculiar localization, and is characterized by the fact that it does not express CD4 and CD8α/β coreceptors, but CD8α homodimers (CD4CD8β^- IEL) (1). The other, in contrast, bears, as does the bulk of peripheral lymphocytes, TCRα/β and CD4 or CD8α/β molecules (CD4CD8β^+ IEL). IEL from both populations are differentiated and functional: they contain granules rich in granzymes and perforin and are cytotoxic (1-3). Since the CD4CD8β^- subset develops in the absence of a thymus (1), it is thymus independent (TI), and appears to differentiate locally from CD3$^-$ IEL which contain mRNA for the recombinase activating gene RAG-1 (1, 4). We have presented evidence (1, 5, 6), that, in contrast, the CD4CD8β^+ IEL are thymus dependent (TD).

Mice mutated in the gene coding for the ζ/η chain of the TCR-CD3 complex have severe defects in thymocyte maturation and in peripheral T cells and gut TD-IEL. In contrast, gut TI-IEL are little affected in these mutant mice, probably because they use FcϵRIγ chain dimers (7) instead of ζ chain dimers (8, 9) as a signal transmitter associated to their TCR-CD3 complex (10-13).

This study analyzes the signal transduction modules present, in normal mice, in the CD3 complexes of the various populations of IEL, peripheral lymphocytes and thymocytes, and explores the presence of mRNAs for ζ and FcϵRIγ chains (referred to below as γ chains) in the precursors of IEL and thymocytes. The resulting observations lead to the conclusion that T cell differentiation in the gut epithelium differs from that occurring in the thymus, and help to delineate two main ontogenic pathways among lymphocytes.

Materials and Methods

Animals. Conventional C3H DBA/2 F$_1$ mice, C57BL/6 mice transgenic for the α and β chains of a TCR specific for the HY

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[1] *Abbreviations used in this paper:* DN, double negative; IEL, intraepithelial lymphocyte; RAG, recombinant activating gene; TD, thymus dependent; TI, thymus independent; TN, triple negative.

antigen (14) were raised in our animal house. RAG 1$^{-/-}$ (15) and TCR$\beta^{-/-}$ (16) mutant mice, a kind gift of P. Mombaerts and S. Tonegawa (Howard Hughes Medical Institute, Massachusetts Institute of Technology, Cambridge, MA 02139), were raised at Centre National de la Recherche Scientifique (Orleans, France), as were RAG-2$^{-/-}$ (17) mutant mice, also used. Swiss nude mice were from Iffa Credo (L'Abresle, France).

Cell Preparations and Separation of Cell Subsets. All subpopulations were sorted with a FACStar® flow cytometer (Becton Dickinson & Co., Mountain View, CA) after double labeling with appropriate antibodies. Biotinylated anti-TCR β (H57.597) or δ (GL3) mAbs (revealed with streptavidin-coupled PE), FITC-labeled anti-CD4 (GK1.5) plus CD8β (H35.17.2) mAbs (1), FITC-labeled PC61 5.3 mAb (CD25) (American Type Culture Collection, Rockville, MD), and biotinylated anti-HY TCRα transgene mAb (18), were used. IEL were isolated as described (19). Peripheral T cells were depleted of B cells by two successive pannings on plates coated with an anti–mouse Ig goat antibody. Double negative (DN) TCR$^+$ and $^-$ thymocytes were obtained by sorting of cells previously depleted of CD4$^+$ CD8$^+$ thymocytes by antibody plus complement treatment. In vivo activated T cells were collected from the thoracic duct lymph of F$_1$ irradiated mice undergoing a graft-vs.-host reaction 5 d after the injection of parental peripheral lymphocytes (20). In vitro activated T cells were obtained by 2-d stimulation of T cells from the thoracic duct lymph with 10 μg/ml Con A.

Northern Blot Analyses of ζ and FcϵRI γ Chain mRNAs. RNAs were prepared as described (1). The cmRNA riboprobes were obtained from full-length cDNAs (21, 22) inserted in pGEM-3 plasmids, and Northern blots were performed in conditions of stringency described previously (1), with hybridization at 62°C and washing at 70°C.

Western Blot Analyses of Anti-CD3 Immunoprecipitates from Cell Lysates. 2–3 × 10^6 sorted IEL or LN T cells were solubilized in 1 ml digitonin lysis buffer (1% digitonin, 0.02% N$_3$Na, 5 mM iodoacetamide, 10 μg/ml aprotinin, and 100 mM PMSF) for 30 min on ice. Supernatants were precipitated in the digitonin lysis buffer using 5 μl of protein A–purified hamster anti-CD3ϵ mAb (2C11) and 50 μl of a 50% solution of protein A–Sepharose beads (Pharmacia, Uppsala, Sweden). After centrifugation, the beads were incubated in sample buffer (2% SDS, 10% glycerol, 0.08 M Tris-HCl, pH 6.8, 0.02% bromophenol blue), and the supernatant electrophoresed in nonreducing conditions on 12.5% SDS polyacrylamide gels, followed by transfer to nitrocellulose. Coprecipitated chains were revealed with either a rabbit anti-FcϵRI γ antiserum (23) or a rabbit antiserum recognizing a ζ/η peptide (24), followed by incubation with horseradish peroxidase mouse anti–rabbit Ig (The Jackson Laboratory, Bar Harbor, ME) and use of the enhanced chemiluminescence protocol (Amersham International, Amersham, Bucks, UK). Note that for TCRγ/δ^+ IEL, lysates from 3 to 4 × 10^6 cells were sufficient to clearly observe the protein bands, whereas for TCRα/β^+ TI-IEL, lysates from 10 to 12 × 10^6 cells, requiring pooling of several cell sorting procedures, were necessary. This difference is probably related to the lower expression of CD3 molecules on TCRα/β^+ than on TCRγ/δ^+ IEL (data not shown).

Results

Gut IEL Subpopulations Differ in their Use of ζ and γ Chains. Northern blots of mRNA were hybridized with radioactive probes for mRNAs of ζ and γ chains simultaneously, in order to allow a direct comparison of the respective amounts of these mRNAs, identified by their difference in

size. Both mRNAs were found in RNAs from total gut IEL (data not shown). Gut IEL were then fractionated into three populations: CD4CD8β^+ IEL and TCRα/β^+ or γ/δ^+ CD8β^- IEL, referred to as TI-IEL (Fig. 1). Whereas ζ chain mRNA was found in all IEL preparations, γ chain mRNA was also present in extracts of the two TI-IEL populations and in higher amounts than ζ chain mRNA. In contrast, no γ chain mRNA was detected in CD4CD8β^+ IEL (Fig. 2). Since a peculiarly small subset of CD4$^+$ IEL coexpresses CD8α chains (probably due to the induction of this last molecule on all subsets of IEL by the gut microenvironment (6, 25), CD4$^+$ and CD8β^+ IEL were sorted separately. Both were found to contain only ζ chain transcripts (data not shown). Since TI-IEL can be Thy-1$^+$ or $^-$, these subsets were also separately studied. Both contained ζ as well as γ chain mRNAs. TCR$^+$ IEL obtained from nude mice (the majority TCRγ/δ^+) were also found to contain ζ and γ chain transcripts (Fig. 2). Finally, both mRNAs were also found in the TCRα/β^+, CD4CD8β^- TI-IEL sorted from male mice bearing an anti-HY TCR transgene (Fig. 2) (18).

The presence of ζ and γ chains in the CD3–TCR complexes of TI-IEL was then explored by Western blot analysis of the anti-CD3 precipitates obtained from lysates of TCRα/β^+ or γ/δ^+ TI-IEL, revealed with anti-ζ/η or -γ chain antibodies. Precipitates from lysates of LN cells, used as controls, contained only ζ chain homodimers. In contrast, those from the two subpopulations of TI-IEL, TCRα/β^+ or γ/δ^+, and those of TCR$^+$ IEL from nude mice, contained ζ and γ chain homodimers and ζ/γ heterodimers (Fig. 3). The relative proportions of these various dimers is difficult to evaluate with precision, since it varied somewhat between samples and experiments.

In Various Peripheral T Cell Populations of Euthymic Mice, only ζ Chain mRNA Is Detectable, in Contrast with What Is Observed in Peripheral T Cells of Nude Mice. In euthymic mice, the bulk of lymphocytes from peripheral LN or from the thoracic duct lymph, which are TCRα/β^+, CD4$^+$, or CD8β^+, contain only ζ chain mRNA (Fig. 4). Peripheral T cells activated in vivo (thoracic duct lymphocytes from mice undergoing an acute graft-vs.-host reaction [20]) or in vitro (2 d of culture) (Fig. 4), which were studied to explore whether
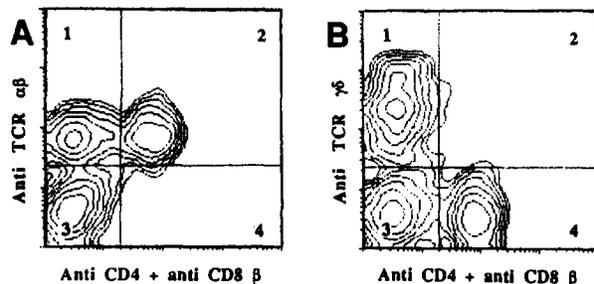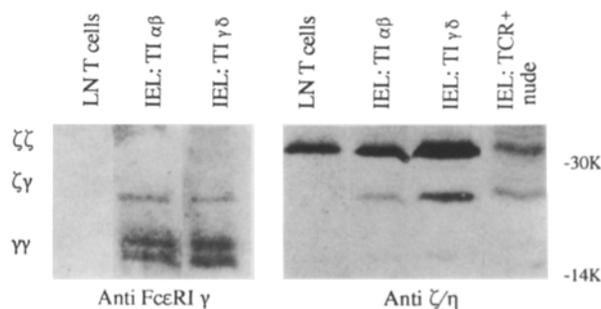

Figure 1. An example of immunofluorescent analysis of the gut IEL used for subpopulation sortings. Only TCR α/β^+ IEL bear CD4 or CD8β coreceptors. The three IEL subpopulations used are easily identified: CD4$^+$ or CD8β^+ TCR α/β^+ (TD-IEL) (*A*, quadrant *2* or *B*, quadrant *4*); TI-IEL (CD4$^-$ CD8β^-) TCR α/β^+ (*A*, quadrant *1*); and TI-IEL (CD4$^-$CD8β^-) TCR γ/δ^+ (*B*, quadrant *1*).
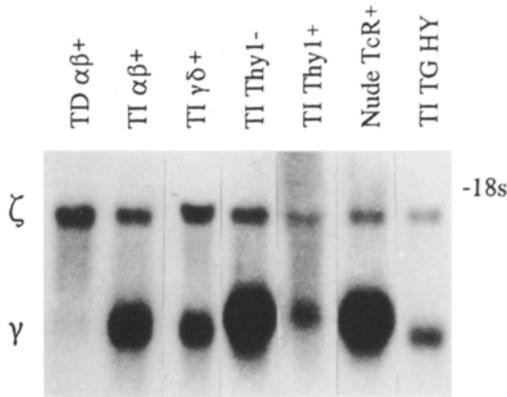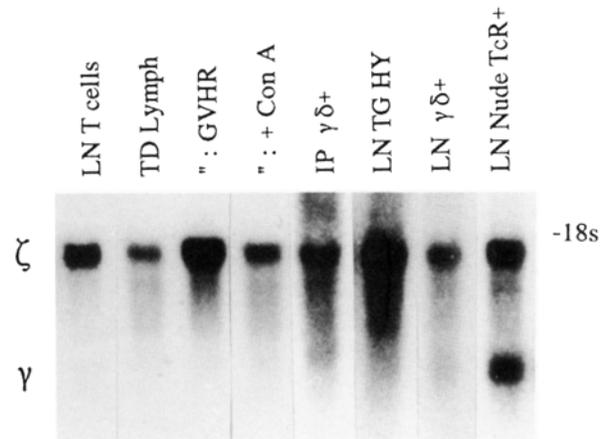
Figure 2. Northern blot analyses of ζ and FcεRI γ chains mRNA in various IEL populations. All Northern blots were hybridized simultaneously with both probes, in order to obtain a quantitative comparison of the presence of the two mRNAs. IEL populations were selected by cell sorting according to their CD4 CD8β and TCRα/β or γ/δ phenotype as shown in Fig. 1 or: as CD4CD8β⁻ Thy1⁻ or ⁺ (*TI Thy1⁻* and ⁺), as nude mice TCR⁺ IEL (α/β⁺ and γ/δ⁺) (*Nude TcR⁺*), and as CD8α/α⁺ and ⁻ IEL of transgenic male mice bearing the anti-HY TCR transgene (*TI TG HY*) (18).

Figure 4. Northern blot analyses of ζ and FcεRI γ chain mRNAs in various peripheral T cell populations. (*TD Lymph*) Lymphocytes from the thoracic duct lymph; (*GVHR*) graft-vs.-host reaction; (*Con A*) after 2 d in culture with Con A; (*IP γ/δ⁺*) intraperitoneal TCRγ/δ⁺ lymphocytes; (*LN TG HY*) lymphocytes from LN of transgenic male mice bearing an anti-HY TCR transgene (18) sorted as DN or CD8α/β; (*LN γ/δ⁺*) TCRγ/δ⁺ cells obtained from the LN of TCRβ⁻/⁻ mutant mice (16); (*LN Nude TcR⁺*) CD3⁺ cells obtained from the LN of nude mice.

short-term T cell activation may induce the expression of γ chains, contained only ζ mRNA. TCRγ/δ⁺ lymphocytes were obtained by cell sorting of peritoneal cells 3 d after LPS injection (as described [26]): they contained only ζ transcripts (Fig. 4). To study the DN CD4CD8β⁻ lymphocytes, which are too scarce in LN from normal mice to be isolated in sufficient number, we selected by cell sorting the DN and CD8β^low lymphocytes that are present in the peripheral LN of male mice bearing an anti-HY TCR transgene, and the TCRγ/δ⁺ lymphocytes that represent the only T cell population in TCRβ⁻/⁻ mutant mice (16). These two populations expressed only ζ chain transcripts (Fig. 4). In contrast, the TCR-CD3⁺ lymphocytes present in the peripheral LN of aged nude mice were found, after sorting, to contain both transcripts (Fig. 4).
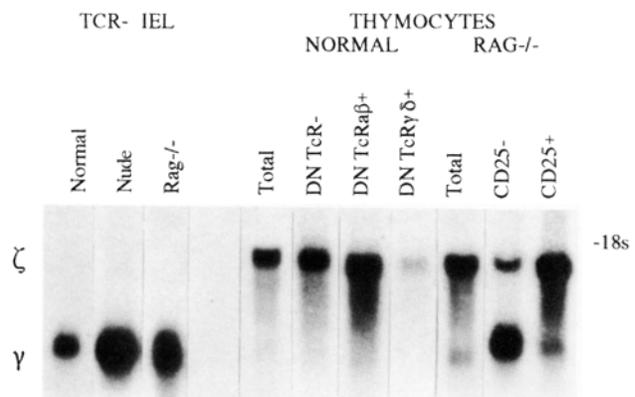
Precursors of Thymocytes and of Gut TI-IEL Express γ Homodimers, but in the Thymocyte Differentiation Pathway, Transcription of the γ Chain Is Switched Off Very Early. Precursors of TI-IEL are probably contained in the CD3⁻ IEL population (see Discussion). These cells, sorted from the IEL of normal young mice (since they are in increased percentage compared to adult mice) or of nude mice, contain only γ mRNA (Fig. 5). This is also the case with IEL of RAG⁻/⁻ mutant mice, which have a block in differentiation at the

Figure 3. Western blot analyses of anti-CD3 immunoprecipitates from cell lysates. In lysates of TCRα/β⁺ and TCRγ/δ⁺ CD4CD8β⁻ (TI) IEL, ζ homodimers (migrating at 32 Kd), ζ/γ heterodimers (at 24 Kd), and γ homodimers (at 16–18 Kd) are seen, whereas only ζ homodimers are found in lysates of peripheral T lymphocytes. The appearance of γ homodimers as a double band is a common observation (42).

Figure 5. Northern blot analyses of IEL precursors and of DN thymocytes. The DN TCR⁺ thymocytes from normal mice, as the DN TCR⁻ cells, contain only ζ chain mRNA, but cannot be subdivided in TCRα/β⁺ and γ/δ⁺ cells, since their number is too low. Thus, DN TCRα/β⁺ thymocytes were obtained by cell sorting from the thymus of male transgenic mice bearing an anti-HY TCR transgene (87% of the thymocytes were DN) and TCRγ/δ⁺ thymocytes were obtained from β⁻/⁻ mutant mice (20% of thymocytes), in smaller number, however, explaining the fainter ζ chain mRNA band.

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precursor level (15, 17) and whose IEL are thus all CD3$^-$ (Fig. 5).

Since only ζ mRNA is detectable in total thymocytes, subpopulations of thymocytes were studied. CD4$^-$CD8$^-$ DN thymocytes, sorted in TCR$^-$ and TCR$^+$ DN cells, contain only ζ chain mRNA (Fig. 5 and data not shown). This last result was confirmed for the two populations of DN TCR$^+$ thymocytes, by studying transgenic and mutant TCR$\beta^{-/-}$ mice (see above) in which DN TCRα/β^+ or γ/δ^+ thymocytes can be isolated in sufficient number (Fig. 5). In RAG$^{-/-}$ mutant mice, total thymocytes are present in very reduced number and are all DN, and ζ chain mRNA is abundant but γ chain mRNA is also occasionally detectable in trace amounts. Thus, these thymocytes were sorted into CD25$^-$ and CD25$^+$ (IL-2Rα^+) subpopulations, since, in the ontogenic pathway of thymocytes, CD25$^-$ lymphocytes are considered the earliest precursors, not yet fully committed to the thymocyte pathway (27). It is striking that whereas little or any γ chain mRNA is detectable in the CD25$^+$ thymocytes, both γ and ζ chain mRNAs are found in CD25$^-$ thymocytes (Fig. 5).

Discussion

These observations show that the two main populations of gut IEL, in normal adult mice, have different CD3-associated transmission molecules. The first population, which bears TCRα/β and CD4 or CD8α/β molecules, uses only ζ chains. In this respect, CD4$^+$ or CD8β^+ IEL are identical to the CD4$^+$ or CD8β^+ TCRα/β^+ postthymic cells of peripheral lymphoid organs. This observation is in keeping with the demonstration that all these cells are thymus derived (6). The second population, CD4 CD8β^-, TCRα/β^+ or γ/δ^+, which is TI since its presence does not need that of a thymus, uses, in addition to ζ chains, FcϵRI γ chains, and contain, linked to the CD3 complex, homo- or heterodimeric molecules made of these chains.

To properly evaluate the possible functional and ontogenic meanings of these observations, the various uses or presence of γ and/or ζ chains in T cells in different conditions or at different stages of their ontogenic pathways have to be considered.

It has been observed that activated T cells, in vivo or in culture, may use γ chains in association with, or in place of, ζ chains. The two main populations of IEL have several features of differentiated and activated T cells: they contain cytotoxic granules, display cytotoxicity in redirected lysis assays (2, 3), and bear CD69 (data not shown), a molecule present on activated T cells and on NK cells (28, 29). It does not seem likely that the presence of γ chains in gut TI-IEL merely reflects a recent activation of these cells, since both TD and TI-IEL appear activated whereas only TI-IEL express γ chains. Besides, peripheral T cells activated in vivo (thoracic duct lymphocytes from mice undergoing an acute graft vs. host reaction) or in vitro after 2 d of culture contain only ζ chain mRNA (Fig. 4). However, since at least a fraction of TI-IEL bear α/β TCR known to be potentially autoreactive (30), chronic stimulation of some autoreactive TD-IEL might lead

to the progressive appearance of γ chains within these cells. When a situation of this type is experimentally created, in male transgenic mice bearing an anti-HY TCR transgene, CD8α/α IEL, which all bear the autoreactive TCR transgene (18), contain γ chain mRNA, but the CD8$\alpha/\beta^{\text{low}}$ peripheral T cells bearing the same TCR transgene which are recovered from the LN (having escaped negative selection in the thymus) (31) contain only ζ chain mRNA (Figs. 2 and 4). This observation suggests that it is the site of differentiation of T cells rather than repeated stimulation by (auto)antigens which governs the use of γ chains as CD3-associated transmission molecules.

Whereas all CD4 CD8β^+ lymphocytes, whether as IEL or as peripheral lymphocytes, use only ζ chain homodimers, ζ and γ homo- and heterodimers linked to CD3 molecules are found in the IEL of nude mice as in the TI-IEL of euthymic mice. The rare DN TCRα/β^+ and γ/δ^+ in peripheral organs of euthymic mice contain only ζ chain mRNA, whereas the rare TCR CD3$^+$ LN lymphocytes of nude mice contain ζ as well as γ chain mRNA. Thus, the use of ζ chain homodimers is not restricted to lymphocytes of thymic origin. Rather, it appears that it is the use of CD3-transducing molecules with both ζ and γ chains as homo- or heterodimeric molecules which is a hallmark of extrathymic origin, whether in athymic mice or in the TI-IEL of euthymic mice, and whether bearing α/β or γ/δ TCRs[2]. Only studies at the clonal level will allow determination of whether each TI-IEL uses simultaneously these three varieties of transmission molecules or whether their use corresponds to various lymphocyte subpopulations, perhaps at different degrees of maturation.

Comparison of the presence of ζ and γ chain mRNAs in the putative precursors of the TD or TI lineages (the latter being mostly represented, in adult euthymic mice, by TI-IEL) leads to interesting conclusions. The earliest thymic lymphoid cells, which are observed in 14-d fetal thymus, contain γ and ζ chain mRNAs and bear CD16 molecules (35). It has been suggested that these cells represent the earliest thymocyte progenitors and may be common to both T and NK lineages (35, 36). In normal adult thymus, these cells, if present, make up a percentage too minute to be detected, and even after selecting the least differentiated thymocytes, CD4$^-$ CD8$^-$ CD3$^-$ (triple negative [TN]) cells, γ chain mRNA cannot be detected, whereas ζ chain mRNA is abun-

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[2] The absence of γ chain mRNA in TCRγ/δ^+ peripheral lymphocytes (Fig. 4) contrasts with the detection of ζ and γ chains in TCRγ/δ^+ cells obtained after culture of splenocytes (32). This may result from the culture conditions in IL-2, since we have also observed the presence of both ζ and γ chain mRNAs after prolonged cultures. Absence of γ chain mRNA in ex vivo conditions may suggest that these peripheral TCRγ/δ^+ lymphocytes are thymus derived. The precise nature of the transmission molecules used by the TCRγ/δ^+ T cells present in the epithelia of the skin and female reproductive organs, which derive from fetal waves of thymocytes (33), remains to be elucidated. The presence of these cells in $\zeta^{-/-}$ mutant mice has suggested that they might use γ chains (34). It is not known if fetal TCRγ/δ^+ thymocytes contain γ chain mRNA, in contrast to adult thymocytes which seem to contain only ζ chain mRNA as shown in the present observation.

dant. Among the thymocytes of RAG$^{-/-}$ mutant mice, chosen to detect more easily early progenitors since thymocyte differentiation is blocked in these mice at an immature TN stage because of their inability to recombine TCR genes, only trace amounts of γ chain mRNA are detectable, whereas ζ chain mRNA is present in large amounts. These RAG$^{-/-}$ thymocytes were sorted into a CD25^{-} (the most undifferentiated) and a CD25^{+} subset (the differentiation stage where the first RAG gene transcripts and the first mRNAs for rearranged β, γ, and δ chains are found in normal mice [37]); both subsets contain ζ chain mRNA, but γ chain mRNA is conspicuously detectable only in CD25^{-} thymocytes. Its presence probably corresponds to a still smaller population of very early progenitors that are CD16^{+}, IL-2Rβ^{+} (data not shown) and which has been reported to be found in fetal thymocytes, as mentioned above (38). It thus appears that very early in their differentiation, thymocytes become restricted to ζ chain use, the expression of which is required for further differentiation and maturation of TD lymphocytes. Indeed, in ζ/η$^{-/-}$ mutant mice, thymocyte development is blocked at an early stage, DN thymocytes accumulate, few DP thymocytes are generated, and CD4 CD8β^{+} lymphocytes are scarce in peripheral lymphoid organs and among IEL, and do not express detectable levels of TCR chains (10–13). In conclusion, it appears that, whereas very early thymocyte progenitors contain γ chains, transcription of these chains is very rapidly switched off, so that the differentiation pathway within the thymus eventually rests exclusively on ζ chains, the only chains whose mRNA can be detected in all main maturing populations of thymocytes of normal adult mice, including the DN TCRα/β^{+} and γ/δ^{+} thymocytes.

This situation contrasts with that of the pathway of TI-IEL. Although the very early precursors of these cells probably come from the BM or the fetal liver, since lymphocyte lineages may derive from a common ancestor, the more immediate precursors of TI-IEL are probably present in the CD3^{-} IEL. These last cells, whose relative number among IEL is low in euthymic adult mice and higher in young mice before weaning or in nude mice (they are the only IEL found in RAG$^{-/-}$ mice), are characterized by a high proliferation rate and a high level of c-*kit* expression (data not shown), a growth factor receptor mostly expressed on incompletely differentiated hematopoietic cells and on early thymocytes (27). About 20% of them express RAG mRNA (4). All CD3^{-} IEL populations from whatever type of mice, contain γ but not ζ chain mRNA. The expression of ζ chains is not necessary for the development of the ζ and γ chain

containing TI-IEL, since these last cells, in complete contrast with the CD4 CD8β^{+} peripheral lymphocytes and IEL, are present in ζ/η$^{-/-}$ mutant mice, although bearing γ/δ or α/β TCR at lower levels than normal (10). In γ FcεRI$^{-/-}$ mutant mice, thymus development and peripheral T lymphocytes are normal (39). Preliminary studies (Guy-Grand, D., J. V. Ravetch, and P. Vassalli) on IEL have shown that CD4 CD8β^{+} IEL are, as expected, normal, and that TI-IEL are also observed, but bear decreased amounts of α/β or γ/δ TCR. This emphasizes the existence of two pathways of differentiation for T lymphocytes. First, the thymic pathway, using only ζ chain homodimers as CD3-dimeric transmission molecules, except at the earliest stage, does not require expression of γ chains but absolutely requires that of ζ chains. Second, the gut TI pathway, using both γ and ζ chains, is less altered by the lack of either chain. Whether the CD3 IEL of γ$^{-/-}$ mutant mice substitute ζ chains for the missing γ chains or have some capacity of differentiation without ζ and γ FcεRI chain dimers, since the transducing unit made of CD3 γ, δ, and ε chains is sufficient to transmit some signals (8), will be established by further study.

In conclusion, epithelial cells of the thymus and of the gut mucosa appear to exert on T lymphocyte precursors, microenvironmental influences inducing distinct ontogenic pathways of T cell differentiation. It is important to recognize that the use of different signal transducing systems by these two distinct pathways is also associated with the use of distinct T cell accessory molecules (CD8α/α instead of CD8α/β, and lack of CD28 [40]). Differences in signal receiving and in signal transducing systems may be equally essential. The thymic pathway, in contrast to the gut IEL pathway, requires the expression of CD8β chains, since CD8β gene$^{-/-}$ mutant mice have a severe defect in thymocytes and in peripheral T cell differentiation (41). It can be speculated that these differences in signal receiving and transducing machinery are related to essential functional differences in vivo in the TD and TI pathways of differentiation, in particular to contrasting mechanisms of selection in response to antigen during differentiation. Thymocytes undergo an extensive process of antigenic "negative" and "positive" selection, which shapes up the TCR repertoire of peripheral thymic selected T lymphocytes. In contrast, the CD8α/α IEL population does not appear to undergo negative selection (30), and whereas it probably results from a process of selection by antigen (18), this process is different from that of the positive selection seen in the thymus, which probably results from "lower affinity" interactions.

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