

TRANSGENIC MICE DEMONSTRATE THAT EPITHELIAL  
HOMING OF  $\gamma/\delta$  T CELLS IS DETERMINED BY CELL  
LINEAGES INDEPENDENT OF T CELL  
RECEPTOR SPECIFICITY

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The search for the genes encoding the TCR- $\alpha/\beta$  led to the identification of the third rearranging gene, TCR- $\gamma$  (1-3), which in turn led to the realization of the occurrence of second TCR, TCR- $\gamma/\delta$  (4-6). One of the most intriguing features of  $\gamma/\delta$  T cells is the compartmentalization of their subsets with different TCR repertoires to various epithelia. Thus, most T cells associated with epidermis, which are referred to as dendritic epidermal cells (DEC)<sup>1</sup> (7, 8), bear TCRs encoded by a particular pair of  $\gamma$  and  $\delta$  genes containing V<sub>5</sub> $\gamma$  and V<sub>1</sub> $\delta$  gene segments (9). Likewise, most of the long-studied intraepithelial lymphocytes (IEL) of the gut (for a review see reference 10) turned out to be composed of  $\gamma/\delta$  T cells bearing TCRs whose  $\gamma$  subunits are preferentially encoded by V<sub>7</sub>-containing  $\gamma$  genes (11, 12). Yet, another subset of  $\gamma/\delta$  T cells with TCRs encoded by a particular V<sub>6</sub>  $\gamma$  gene and a particular V<sub>1</sub>  $\delta$  gene is associated with the mucosal epithelia of uterus, vagina, and tongue (13).

The compartmentalization of these  $\gamma/\delta$  T cell subsets with different TCR repertoires raised the possibility that their homing specificity is determined by a compartmentalization of TCR- $\gamma/\delta$  ligands in the various epithelia. We, therefore, examined

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<sup>1</sup> Abbreviations used in this paper: DEC, dendritic epidermal cell; GAH Ig-FITC, goat anti-hamster Ig antisera coupled to FITC; IEL, intestinal intraepithelial lymphocyte; TG, transgenic.

the homing properties of  $\gamma/\delta$  T cells with predetermined TCRs using the transgenic mouse technique. The results suggest that the homing specificities of these T cells do not depend on their TCR repertoires, but are determined by differentiation programs that may link the rearrangement of particular  $\gamma$  and  $\delta$  gene segments with the expression of particular homing receptors. In addition, our analysis of the IEL- $\gamma/\delta$  in normal, nude, and transgenic mice using an anti-TCR- $\gamma/\delta$  mAb (14) suggests that Thy-1 is a differentiation marker, while CD8 is an activation marker of this T cell subset.

### Materials and Methods

**Animals.** C57BL/6 mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Athymic nude *nu/nu* BALB/c mice and control *nu/+* BALB/c mice were obtained from Dr. M. Katsuki (Kawasaki, Japan).  $\gamma/\delta$  transgenic (TG) animals carrying the functionally rearranged  $V_4J_1C_1\gamma/V_5D_2D_1J_1C\delta$  (15; Ishida, I., S. Verbeek, M. Bonneville, A. Berns, and S. Tonegawa, manuscript submitted for publication) or  $V_5J_1C_1\gamma/V_1D_2J_2C\delta$  (16) chain genes were constructed after microinjection of DNA from hybridoma KN6 (derived from adult thymocytes) (15, 17) and hybridoma KI129 (derived from day 15.5 fetal thymocytes) (18), respectively, into the pronucleus of fertilized eggs, as described elsewhere (Ishida et al., manuscript submitted for publication).

**Antibodies.** CD8, CD4, and Thy-1.2 mAbs were purchased from Becton Dickinson & Co. (Mountain View, CA). The anti-CD3  $\epsilon$  mAb 2C11 (19) and the anti-pan TCR- $\alpha/\beta$  mAb H57-597 (20) were kindly provided by J. A. Bluestone and R. Kubo, respectively. F536 mAb directed against the  $V_5J_1C_1\gamma$  chain was a kind gift from W. Havran and J. Allison (21). 3A10, 8D6, and 5C10 mAbs are directed against a constant region of TCR  $\delta$  chain,  $V_{\gamma 4}/V_{\delta 5}$ -encoded TCR, and a unique epitope of the hybridoma KN6 TCR, respectively (14).

**Cells.** IEL were isolated according to the technique described by Petit et al. (22). Briefly, small intestine was cut into small pieces and incubated in HBSS without  $MgCl_2$ ,  $MgSO_4$ , and  $CaCl_2$ , supplemented with 0.1 mM EDTA. After washes in Hanks + 5% FCS and passage through a nylon wool column, cells were loaded on top of a 30–70% Percoll gradient, and lymphocytes from the 70% gradient were recovered and washed. DEC were prepared according to the technique described by Nixon-Fulton et al. (23). Mice were shaved, dorsal and abdominal skin was isolated, and, after elimination of subcutaneous tissue, cut into 5-mm slices. Skin slices were then incubated (dermal side down) on a 0.3% Trypsin solution in glucose (0.17%), NaCl (0.88%), and KCl (0.04%) at 4°C overnight. Epidermis was gently scraped and incubated 30 min at 37°C in a 0.3% Trypsin, 0.1 mg/ml DNase I solution. Epidermal cells were then washed twice, loaded on a Ficoll solution (Lymphoprep), spun 15 min at 2,000 rpm, and cells from the interface were recovered and analyzed.

**Immunofluorescence and FACS Analysis.** Cells were stained according to standard procedures using mAbs as hybridoma supernatant (F536, H57-597), purified FITC- (Thy-1.2, CD8, CD3, H57-597, 8D6, 5C10, 3A10), or Phycoerythrin- (CD4) conjugated or biotinylated (CD8, Thy-1.2, 8D6, 5C10, 3A10) antibodies. In the case of noncoupled mAbs, the secondary reagent used was a mouse-absorbed goat anti-hamster Ig antiserum coupled to FITC (GAH Ig-FITC) (Caltag, San Francisco, CA). In the case of biotinylated antibodies, the secondary reagent was streptavidin coupled to phycoerythrin (Becton Dickinson & Co.). After staining, cells were resuspended in PBS-propidium iodide (0.5  $\mu$ g/ml) solution and subjected to flow cytometric analysis using a FACSCAN apparatus. In all cases, dead cells, mostly composed of residual epithelial cells, were gated out on the FL3 channel.

**Immunohistology Procedures.** The techniques used for light immunohistochemistry have been described in detail (24).

### Results

**Surface Phenotype Analysis of IEL.** Previous analyses of TCR- $\gamma/\delta$  expression by IEL were carried out by cell surface labeling of purified IEL, followed by immunoprecip-

itation (11, 12). It is difficult to obtain an accurate measurement of the proportion of TCR- $\alpha/\beta$ - or TCR- $\gamma/\delta$ -bearing IEL by these procedures. The availability of mAbs that recognize all  $\alpha/\beta$  T cells (H57-597) (20) or all  $\gamma/\delta$  T cells (3A10) (14) enabled us to address this issue directly and to study the expression of CD8 and Thy-1 antigens on the  $\alpha/\beta$  and  $\gamma/\delta$  IEL subsets. Intestinal tissue sections were stained with mAbs H57-597 and with 3A10.  $\gamma/\delta$  T cells were detected between columnar epithelial cells much more than in the lamina propria (Fig. 1 *A*). An opposite pattern of distribution was observed for  $\alpha/\beta$  T cells (Fig. 1 *B*). To determine the relative abundance of the two types of T cells in the epithelium, the IEL were isolated, stained with various antibodies, and analyzed by flow cytometry. About 50% of the cells expressed TCR- $\gamma/\delta$ , ~30% expressed TCR- $\alpha/\beta$ , and the remaining cells were CD3<sup>-</sup> and expressed neither TCR (Fig. 2). The presence of  $\alpha/\beta$  IEL in this abundance was unexpected because the  $\alpha/\beta$  heterodimers could not be detected in the previous immunoprecipitation studies (11, 12). This apparent discrepancy may be explained by the lower TCR density on  $\alpha/\beta$  (CD3 mean fluorescence intensity, 495) than on  $\gamma/\delta$  T cells (CD3 mean fluorescence intensity, 566) (Fig. 2).

Virtually all  $\gamma/\delta$  and  $\alpha/\beta$  IEL expressed CD8, while Thy-1 was expressed by about two thirds of the  $\gamma/\delta$  and  $\alpha/\beta$  IEL (Fig. 3).

*Homing of  $\gamma/\delta$  T Cells to Intestinal Epithelia in Nude Mice.* Previous studies have shown that Thy-1<sup>-</sup> CD8<sup>+</sup> IEL are present in the intestines of nude mice (23). We investigated the type of TCR expressed on these IEL using H57-597 and 3A10 mAbs. As shown in Fig. 4 *A*, almost all IEL from young (6–8 wk old) *nu/nu* BALB/c mice carry TCR- $\gamma/\delta$  on their surface, while those from *nu/+* littermates express TCR-

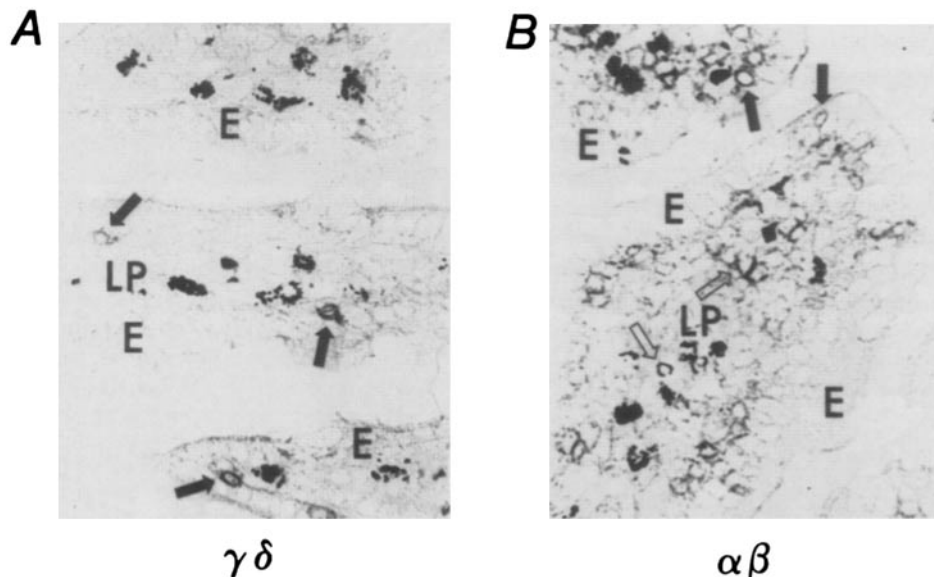


FIGURE 1.  $\alpha/\beta$  and  $\gamma/\delta$  T cell distribution in the gut mucosa. Staining of jejunum tissue sections using 3A10 (anti- $\gamma/\delta$ ) mAb (*A*) or H57-597 (anti- $\alpha/\beta$ ) mAb (*B*) was performed as described in Materials and Methods. Indicated are the areas corresponding to the epithelial layer (*E*) and the lamina propria (*LP*) of intestinal villi, as well as some intraepithelial (*closed arrows*) and intralamina propria (*open arrows*) stained lymphocytes.

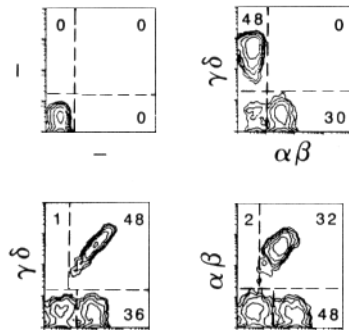


FIGURE 2. TCR- $\alpha/\beta$  and - $\gamma/\delta$  expression by IEL. IEL from 10-wk-old C57BL/6 mice isolated according to procedures described in Materials and Methods were incubated first with H57-597 (anti- $\alpha/\beta$ ) mAb (biotinylated or FITC conjugated), 2C11 (anti-CD3) mAb (FITC conjugated), or 3A10 (anti- $\gamma/\delta$ ) mAb (biotinylated), followed by an incubation with streptavidin-phycoerythrin. Indicated is the percent of cells in each quadrant.

$\alpha/\beta$  as well. Both  $nu/+$  and  $nu/nu$  TCR- $\gamma/\delta^+$  IEL are CD8<sup>+</sup>, but, as reported previously (25), nude IEL are exclusively Thy-1<sup>-</sup>, while some  $nu/+$  IEL express Thy-1 (Fig. 4 B). Similarly, the rare  $\alpha/\beta^+$  IEL present in gut epithelium of nude mice are CD8<sup>+</sup>Thy-1<sup>-</sup> (data not shown). Taken together, these observations demonstrate that Thy-1<sup>-</sup>CD8<sup>+</sup>  $\gamma/\delta^+$  IEL selectively differentiate in the absence of a normal thymic environment and strongly suggest that Thy-1 expression depends on intrathymic differentiation.

*The TCR- $\gamma/\delta$  Does not Determine the Homing Specificity.* To test the hypothesis that TCR- $\gamma/\delta$  determines the homing specificity to a peripheral tissue, we made use of two kinds of  $\gamma/\delta$  double-transgenic mice.  $V_{\gamma 4}V_{\delta 5}$  transgenic mice were produced using productively rearranged  $V_4J_1C_1$   $\gamma$  and  $V_5D_1D_2J_1C$   $\delta$  genes isolated from a T hybridoma derived from an adult thymocyte (Ishida et al., manuscript submitted for publication).  $V_{\gamma 4}V_{\delta 5}$ -encoded TCR are the most frequently used by adult  $\gamma/\delta$  thymocytes (14, 17, 26). We also constructed a second set of transgenic mice (16) using productively rearranged  $V_5J_1C_1$   $\gamma$  and  $V_1D_2J_2C$   $\delta$  genes that encode the TCR expressed on most DEC (9). These mice will be referred hereafter to as KN6 ( $V_{\gamma 4}V_{\delta 5}$ ) and DEC ( $V_{\gamma 5}V_{\delta 1}$ ) transgenic mice.

Transgene expression on the surface of  $\gamma/\delta$  T cells was investigated by using mAbs that are specifically directed against the transgenic TCR. In the case of KN6 transgenic mice, we used either the 8D6 mAb that recognizes  $\gamma/\delta$  T cells carrying any  $V_{\gamma 4}V_{\delta 5}$ -containing TCR, or the 5C10 mAb that specifically reacts with the KN6 clonotype (14). To assess the expression of the transgene-coded TCR in DEC trans-

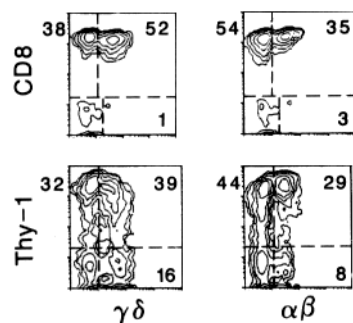
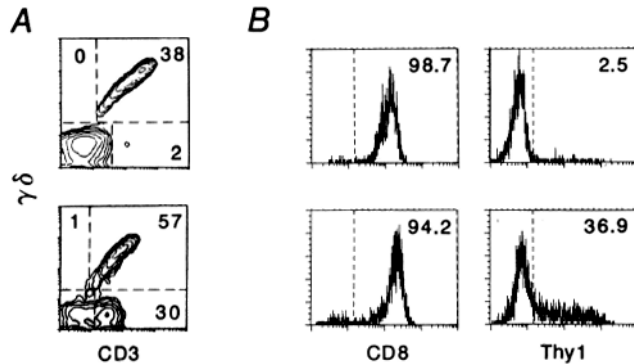


FIGURE 3. Thy-1 and CD8 expression by  $\alpha/\beta$  and  $\gamma/\delta$  IEL. IEL from 10-wk-old C57BL/6 mice were stained using CD8 or Thy-1.2 mAbs (biotinylated, streptavidin-phycoerythrin) and H57/597 (anti- $\alpha/\beta$ ) or 3A10 (anti- $\gamma/\delta$ ) FITC-conjugated mAbs. Indicated is the percent of cells in each quadrant.



by *nu/nu* (top) and *nu/+* (bottom)  $\gamma\delta$  + IEL. IEL were stained with 3A10 mAb (biotinylated, streptavidin-phycoerythrin) and either Thy-1.2 or CD8 (FITC-conjugated) mAbs. For the Thy-1 and CD8 fluorescence histograms, only 3A10<sup>+</sup> cells are shown.

FIGURE 4. TCR, CD8, and Thy-1 expression by IEL from athymic nude mice and euthymic *nu/+* control mice. IEL from 6-wk-old BALB/c *nu/nu* and *nu/+* mice were isolated and stained as described in Figs. 2 and 3. (A) A two-color analysis of *nu/nu* (top) and *nu/+* (bottom) IEL using 2C11 (anti-CD3) mAb (FITC conjugated) and 3A10 (anti- $\gamma\delta$ ) mAb (biotinylated, streptavidin-phycoerythrin). Indicated is the percent of cells in each quadrant. (B) CD8 and Thy-1 expression

genic mice, cells were stained with the F-536 mAb (21) specific to the DEC TCR. In both KN6 and DEC transgenic mice, most or all TCRs expressed  $\gamma\delta$  T cells encoded by the transgenes because the rearrangement of the endogenous  $\gamma$  and  $\delta$  genes are inhibited in these T cells (16; Ishida et al., manuscript submitted for publication). Thus, if the  $V_{\gamma 7}V_{\delta}$ -coded TCRs are required for the homing of  $\gamma\delta$  T cells to gut epithelia, one would expect no or a highly diminished level of  $\gamma\delta$  IEL in both the KN6 ( $V_{\gamma 4}V_{\delta 5}$ ) and DEC ( $V_{\gamma 5}V_{\delta 1}$ ) transgenic mice. Similarly, if  $V_{\gamma 5}V_{\delta 1}$ -coded TCR are the homing receptors for skin, no or little DEC will be observed in the KN6 ( $V_{\delta 4}V_{\delta 5}$ ) transgenic mice. However, we found that  $\gamma\delta$  T cells are present at normal levels in the IEL preparations from either the KN6 or DEC transgenic mice (data not shown), and that these T cells bear TCR- $\gamma\delta$  encoded exclusively by the respective transgenes (Fig. 5).  $\gamma\delta$  T cells are also present, albeit at a diminished level ( $\sim 5$ – $20\%$  of the normal level), in the DEC preparations of the KN6 transgenic mice, and the majority of these T cells bear the  $V_{\gamma 4}V_{\delta 5}$  transgene-coded TCR- $\gamma\delta$  (Fig. 6 A).

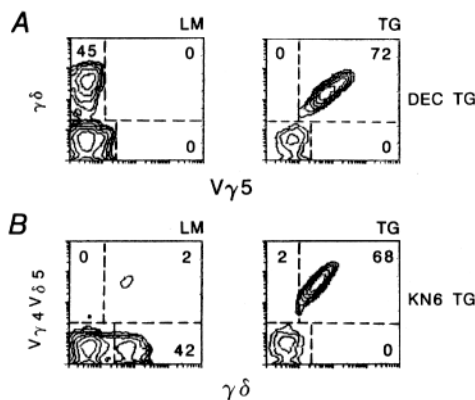


FIGURE 5. All the  $\gamma\delta$  IEL from young KN6 and DEC transgenic mice express transgene-encoded TCR. (A) IEL from an 8-wk-old  $V_{\gamma 5}V_{\delta 1}$  transgenic mouse (right) and a nontransgenic littermate (left) were stained using F536 (anti- $V_{\gamma 5}$ ) mAb (followed by an incubation with GAH Ig-FITC) and 3A10 (anti- $\gamma\delta$ ) mAb (biotinylated, streptavidin-phycoerythrin). (B) IEL from an 8-wk-old  $V_{\gamma 4}V_{\delta 5}$  transgenic mouse (right) and a nontransgenic littermate (left) were stained using 3A10 mAb (FITC-conjugated) and 8D6 (anti- $V_{\gamma 4}V_{\delta 5}$ ) mAb (biotinylated, streptavidin-phycoerythrin).

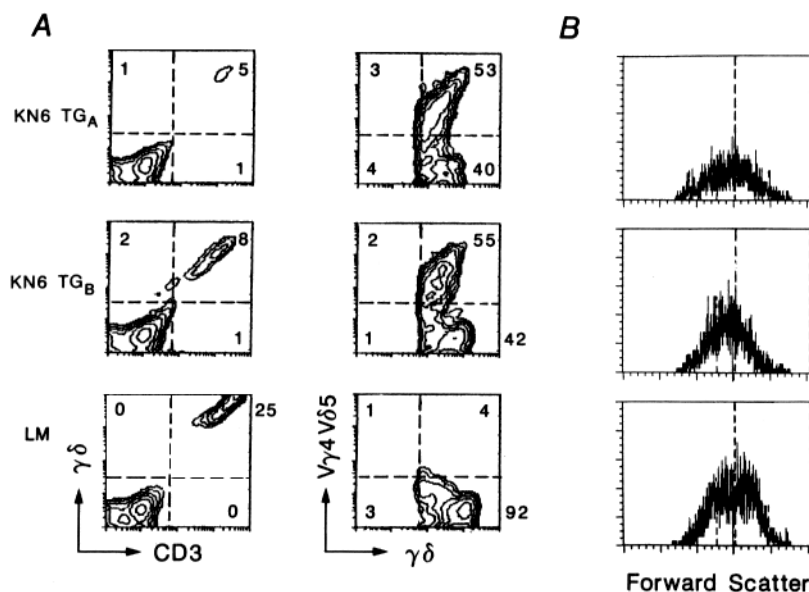


FIGURE 6. TCR phenotype and size of DEC cells from KN6 transgenic mice. (A) DEC cells from two  $V\gamma4V\delta5$  transgenic mice (*top* and *middle*) and from a nontransgenic littermate (*bottom*) were isolated according to procedures described in Materials and Methods and stained with 2C11 (anti-CD3) mAb (FITC conjugated) and 3A10 (anti- $\gamma\delta$ ) mAb (biotinylated, streptavidin-phycoerythrin) (*left*), or with 3A10 mAb (FITC conjugated) and 8D6 (anti- $V\gamma4V\delta5$ ) mAb (biotinylated, streptavidin-phycoerythrin) (*right*). Indicated is the percent of cells in each quadrant. The right panels were obtained after gating out the 3A10<sup>-</sup> cells. (B) Size of  $\gamma\delta$  (3A10)<sup>+</sup> DEC cells from two  $V\gamma4V\delta5$  transgenic mice (*top* and *middle*) and from a control littermate (*bottom*) was analyzed by forward-angle scatter analysis.

We investigated by immunohistology whether the  $\gamma/\delta$  T cells in the gut and skin of the transgenic mice are associated with epithelial cells, as are these T cells in normal mice. As shown in Fig. 7, the overall distribution of  $\gamma/\delta$  T cells in the gut of either KN6 or DEC transgenic mice was not different from that of nontransgenic control mice (Fig. 1). Indeed, unlike  $\alpha/\beta$  T cells, which are preferentially localized in the lamina propria,  $\gamma/\delta$  T cells are mostly observed within the epithelial cell layer. Similarly, the  $\gamma/\delta$  T cells in the skin of the KN6 transgenic mice are associated with epithelial cells (Fig. 8), although there is clearly a reduction of the T cell density in the epidermis of these mice in comparison with the normal or DEC transgenic mice, in agreement with the results of the immunostaining analysis of DEC populations (Fig. 6 A). In addition, these DEC are on average smaller than the control DEC (Fig. 6 B), which accounts at least in part for the reduced anti- $\gamma/\delta$  or anti-CD3 staining (Fig. 6 A).

Taken together, these results demonstrate that  $\gamma/\delta$  cells bearing TCRs encoded by "wrong"  $\gamma$  and  $\delta$  genes can home to gut or skin epithelia. This strongly suggests that TCR- $\gamma/\delta$  per se are not homing receptors for specific epithelia.

*CD8 and Thy-1 Expression on IEL of DEC and KN6 Transgenic Mice.* It was previously suggested that expression of Thy-1 and CD8 by at least some  $\gamma/\delta$  T cells depends on their activation via TCR engagement (27, 28). To obtain an insight into

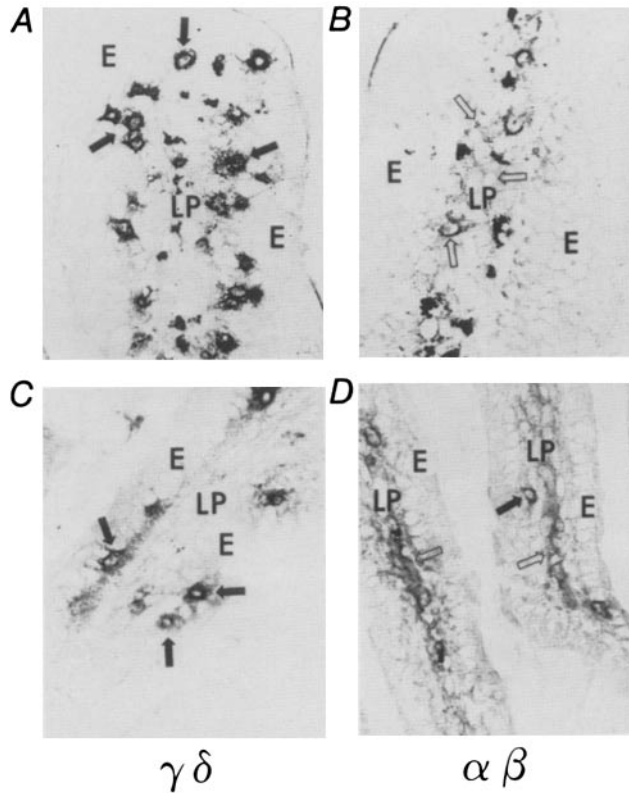


FIGURE 7.  $\alpha/\beta$  and  $\gamma/\delta$  T cell distribution in the gut mucosa of KN6 and DEC transgenic mice. Jejunum tissue sections from KN6 (A and B) and DEC (C and D) transgenic mice were stained with 3A10 (anti- $\gamma/\delta$ ) mAb or with H57-597 (anti- $\alpha/\beta$ ) mAb. Indicated are the areas corresponding to the epithelial layer (E) and the lamina propria (LP) of the villi. In addition, closed and open arrows indicate some intraepithelial and intra-lamina propria lymphocytes, respectively. Staining of DEC transgenic tissue sections with F536 ( $V\gamma\delta$ ) mAb gave results that are virtually identical to those obtained with 3A10 mAb (not shown).

these hypotheses, we investigated the effect of “wrong” TCRs on the expression of these proteins on the surface of  $\gamma/\delta$  IEL.

Most  $\gamma/\delta$  IEL in 8-wk-old DEC transgenic mice were Thy-1<sup>+</sup> (Fig. 9). TCRs of these IEL are almost exclusively encoded by the transgenes (Fig. 5). On the other hand, about half of the  $\gamma/\delta$  IEL of KN6 transgenic mice, like those of nontransgenic littermates, were Thy-1<sup>+</sup> (Fig. 9).

As to the CD8 expression, the proportion of CD8<sup>-</sup> cells among  $\gamma/\delta$  IEL was much higher than that in nontransgenic mice; ~90% of  $\gamma/\delta$  IEL from 8-wk-old nontransgenic mice are CD8<sup>+</sup>, while no more than 50% of these cells in the age-matched DEC and KN6 transgenic mice expressed CD8 molecules.

### Discussion

Previous studies suggested that  $\gamma/\delta$  T cells were specifically enriched in gut epithelium (11, 12). By using three mAbs that react with both  $\alpha/\beta$  and  $\gamma/\delta$  T cells or only with one of the two T cell subsets, we have now determined the proportion of  $\alpha/\beta$  and  $\gamma/\delta$  T cells among IEL; ~60% of CD3<sup>+</sup> cells are TCR- $\gamma/\delta$ <sup>+</sup>, and the rest are TCR- $\alpha/\beta$ <sup>+</sup> (Figs. 1 and 2). The vast majority of these T cells are CD8<sup>+</sup> while less than half of the cells in each T cell class are Thy-1<sup>+</sup>. IEL in the intestine of nude mice are almost all Thy-1<sup>-</sup>CD8<sup>+</sup>  $\gamma/\delta$  T cells; only very few Thy-1<sup>-</sup>CD8<sup>+</sup>

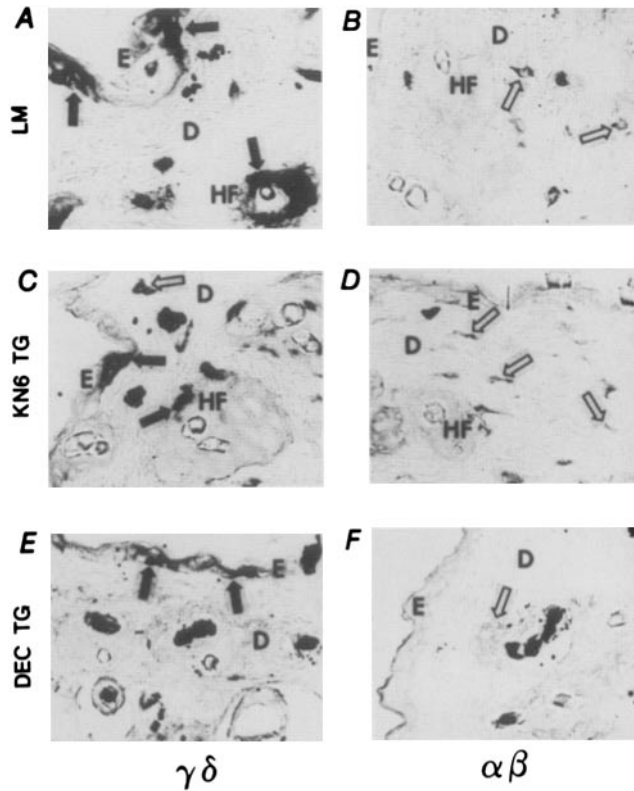


FIGURE 8.  $\alpha/\beta$  and  $\gamma/\delta$  T cell distribution in the skin of KN6 and DEC transgenic and nontransgenic mice. Skin tissue sections of nontransgenic (*A* and *B*), KN6 transgenic (*C* and *D*), and DEC transgenic (*E* and *F*) mice were stained with 3A10 or H57-597 mAb. Indicated are the areas corresponding to epidermis (*E*), hair follicles (*HF*), and dermis (*D*). Closed and open arrows indicate intraepithelial and intradermal lymphocytes, respectively.

$\alpha/\beta$  T cells are found. These observations suggest that the homing of  $\gamma/\delta$  T cells to intestinal epithelia is not dependent on thymic education nor on factors produced by intraepithelial  $\alpha/\beta$  T cells.

It was previously shown that  $\gamma/\delta$  T cells with different TCR repertoires are compartmentalized in different epithelia (9, 11, 13). This raised the possibility that the homing specificity of these  $\gamma/\delta$  T cells is determined by a compartmentalization of

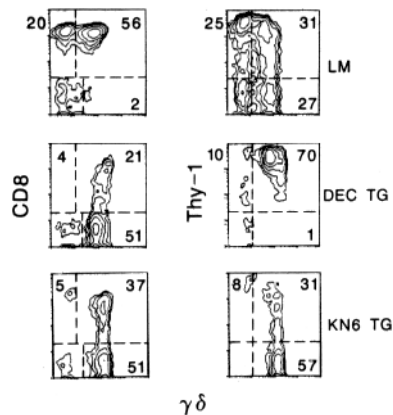


FIGURE 9. Comparison between CD8 and Thy-1 expression by IEL from DEC and KN6 transgenic mice and age-matched nontransgenic mouse. IEL from  $V\gamma_4V\delta_5$  transgenic (*bottom*),  $V\gamma_5V\delta_1$  transgenic (*middle*), and nontransgenic (*top*) 8-wk-old mice were stained with 3A10 (anti- $\gamma/\delta$ ) mAb (FITC conjugated) and either CD8 (*left*) or Thy-1.2 (*right*) mAbs (biotinylated, streptavidin-phycoerythrin). Indicated is the percent of cells in each quadrant.



TCR- $\gamma/\delta$  ligands in the various epithelia. The homing behavior of the transgenic  $\gamma/\delta$  T cells described here does not support this hypothesis; we found cells expressing "wrong" transgene-encoded TCRs in the intestinal epithelium as well as in the epidermis. This suggests that  $\gamma/\delta$  T cells in different epithelia belong to different  $\gamma/\delta$  T cell sublineages. Each sublineage has a particular program of differentiation that may link the rearrangements to particular  $\gamma$  and  $\delta$  gene segments of particular homing receptors. This model predicts that the rearrangement of different  $\gamma$  and  $\delta$  gene segments depends on different regulatory factors.

While our findings are consistent with this model, we cannot exclude the possibility that the compartmentalization of TCR- $\gamma/\delta$  ligands does play a role in the compartmentalization of  $\gamma/\delta$  T cells. In the case of  $\alpha/\beta$  T cells, interactions of the TCR- $\alpha/\beta$  with MHC-encoded proteins are not only required for the maturation of these cells in the thymus (29), but may also prevent a rapid decay of these cells in the periphery (30). Thus, the interaction between TCR- $\gamma/\delta$  and their ligands differentially expressed in various epithelia could serve a supplementary role in the compartmentalization of these T cells. Indeed, occurrence of such interactions is suggested by a few observations made in this study. First, normal DEC expressing the  $V_5\gamma V_1\delta$  TCR are larger than the DEC expressing the  $V_4\gamma V_5\delta$  transgene-encoded TCR (Fig. 6 B). Second, in DEC TG mice, the lack of CD8 expression by many intestinal IEL (Fig. 9) could reflect the failure of transgene-expressing  $\gamma/\delta$  T cells to interact with the TCR ligands in this epithelium (see below).

It is noteworthy that while the T cells expressing the KN6 transgenes do find their way to epidermis, their number is 10–40-fold lower than the number of DEC cells in normal mice. Furthermore,  $\gamma/\delta$  DEC cells expressing the endogenously rearranged (presumably  $V_5\gamma V_1\delta$ -encoded)  $\gamma$  and  $\delta$  genes are also detectable in the KN6 TG mice. These observations can be explained as follows.

As discussed elsewhere, the progenitors of DEC cells may proliferate in the fetal thymus as a result of their interaction with a TCR- $\gamma/\delta$  ligand (31, 32). Thus, the occurrence in KN6 TG mice may be explained by the expansion of a small number of thymocytes that escaped allelic exclusion. This expansion would not occur in the case of thymocytes expressing the TG-encoded TCR, hence the low number of DEC cells with this TCR in KN6 TG mice.

Interestingly, the proportion of CD8<sup>+</sup> cells among  $\gamma/\delta$  IEL is much lower in the transgenic mice than in normal mice (Fig. 9). One possible interpretation of this finding is that the introduction of the transgenes and their premature expression in developing T cells affect their differentiation and CD8 expression. An alternative explanation is suggested by the recent findings that TCR- $\gamma/\delta$  ligands can induce CD8 expression in vitro (27, and our unpublished results). Thus, TCR ligands in the intestine may induce CD8 in normal IEL but not in transgenic IEL.

Finally, we would like to discuss our finding that the proportion of Thy-1<sup>+</sup> cells among the transgenic  $\gamma/\delta$  IEL is at least as high as the proportion of Thy-1<sup>+</sup> among  $\gamma/\delta$  IEL of normal mice (Fig. 9). We believe that Thy-1 is not an activation marker of IEL. The opposite conclusion was recently drawn by LeFrancois and Goodmann (28) on the basis of their finding that IEL from germ-free mice are mostly Thy-1<sup>-</sup>, and that acclimation of these mice to nonsterile conditions resulted in the generation of Thy-1 IEL. However, the present study has shown that a substantial amount (40%) of CD3<sup>+</sup> IEL bear TCR- $\alpha/\beta$  (Fig. 2), and our own analysis of IEL in germ-

free and specific pathogen-free mice indicates that the difference in the expression of Thy-1 by IEL of these mice is attributable to the difference in the ratio of  $\gamma/\delta$  and  $\alpha/\beta$  T cells (A. Bandeira, C. Hausser, S. Itohara, S. Tonegawa, and A. Coutinho, unpublished observations). In addition, Thy-1 is not expressed by  $\gamma/\delta$  IEL of nude mice (Fig. 4 B). It is therefore likely that Thy-1 is a differentiation marker for  $\gamma/\delta$  IEL as it is for other T cells.

### Summary

$\gamma/\delta$  T cells with different TCR repertoires are compartmentalized in different epithelia. This raises the possibility that the TCR- $\gamma/\delta$  directs homing of T cells to these epithelia. Alternatively, the signals that induce TCR- $\gamma/\delta$  expression in developing T cells may also induce homing properties in such cells, presumably in the form of cell surface receptors. We have examined this issue by studying the homing of  $\gamma/\delta$  T cells in transgenic mice constructed with specific pairs of rearranged  $\gamma$  and  $\delta$  genes. In such mice, most  $\gamma/\delta$  T cells express the transgene-encoded TCR. We find that homing to both skin and gut epithelia is a property of T cells and is not determined by the type of  $\gamma$  and  $\delta$  genes used to encode their TCR. We also studied the effect of TCR replacement on the expression of Thy-1 and CD8 proteins on the  $\gamma/\delta$  T cells associated with gut epithelia. Our results show that the expression of the appropriate type of TCR- $\gamma/\delta$  is not required for the Thy-1 expression by these T cells, suggesting that Thy-1 is not an activation marker. In contrast, CD8 expression by gut  $\gamma/\delta$  T cells seems to depend on the expression of the appropriate type of TCR.

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### References

1. Saito, H., D. M. Kranz, Y. Takagaki, A. C. Hayday, N. H. Eisen, and S. Tonegawa. 1984. Complete primary structure of a heterodimeric T-cell receptor deduced from cDNA sequences. *Nature (Lond.)* 309:757.
2. Kranz, D. M., H. Saito, M. Heller, Y. Takagaki, W. Haas, N. N. Eisen, and S. Tonegawa. 1985. Limited diversity of the rearranged T cell gamma ( $\gamma$ ) gene. *Nature (Lond.)* 313:752.
3. Hayday, A. C., H. Saito, S. D. Gillies, D. M. Kranz, G. Tanigawa, H. N. Eisen, and S. Tonegawa. 1985. Structure organization and somatic rearrangement of T cell 'gamma genes'. *Cell* 40:259.
4. Brenner, M. B., J. McLean, D. P. Dialynas, J. L. Strominger, J. A. Smith, F. L. Owen, J. G. Seidman, S. Ip, F. Rosen, and M. S. Krangel. 1986. Identification of a putative second T-cell receptor. *Nature (Lond.)* 322:145.
5. Bank, I., R. A. dePinho, M. B. Brenner, J. Cassimeris, F. W. Alt, and L. Chess. 1986. A functional T3 molecule associated with a novel heterodimer on the surface of immature human thymocytes. *Nature (Lond.)* 322:179.
6. Weiss, A., M. Newton, and D. Crommie. 1986. Expression of T3 in association with

- a molecule distinct from the T-cell antigen receptor heterodimer. *Proc. Natl. Acad. Sci. USA.* 83:6998.
7. Stingl, G., K. C. Gunter, E. Tschachler, H. Yamada, R. I. Lechler, W. Yokoyama, G. Steiner, R. N. Germain, and E. M. Shevach. 1987. Thy-1<sup>+</sup> dendritic epidermal cells belong to the T-cell lineage. *Proc. Natl. Acad. Sci. USA.* 84:2430.
  8. Kuziel, W. A., A. Takashima, M. Bonyhadi, P. R. Bergstresser, J. P. Allison, R. E. Tigelaar, and P. W. Tucker. 1987. Regulation of T-cell receptor  $\gamma$ -chain RNA expression in murine Thy-1<sup>+</sup> dendritic epidermal cells. *Nature (Lond.)* 328:263.
  9. Asarnow, D. M., W. A. Kuziel, M. Bonyhadi, R. E. Tigelaar, P. W. Tucker, and J. P. Allison. 1988. Limited diversity of  $\gamma\delta$  antigen receptor genes of Thy-1<sup>+</sup> dendritic epidermal cells. *Cell.* 55:837.
  10. Guy-Grand, D., and P. Vassalli. 1988. Origin and traffic of gut mucosal lymphocytes and mast cells. In *Migration and Homing of Lymphoid Cells*. Alan Husband, editor. CRC Press, Inc., Boca Raton. 100-112.
  11. Bonneville, M., C. A. Janeway Jr., K. Ito, W. Haser, I. Ishida, N. Nakanishi, and S. Tonegawa. 1988. Intestinal intraepithelial lymphocytes are a distinct set of  $\gamma\delta$  T-cells. *Nature (Lond.)* 336:479.
  12. Goodman, T., and L. Lefrancois. 1988. Expression of the  $\gamma\delta$  T-cell receptor on intestinal CD8<sup>+</sup> intraepithelial lymphocytes. *Nature (Lond.)* 333:855.
  13. Itohara, S., A. G. Farr, J. J. Lafaille, M. Bonneville, Y. Takagaki, W. Haas, and S. Tonegawa. 1990. Homing of a  $\gamma\delta$  thymocyte subset with homogeneous T-cell receptors to mucosal epithelia. *Nature (Lond.)*. In press.
  14. Itohara, S., N. Nakanishi, O. Kanagawa, R. Kubo, and S. Tonegawa. 1989. Monoclonal antibodies specific to native murine T cell receptor  $\gamma\delta$ : analysis of  $\gamma\delta$  T cells in thymic ontogeny and peripheral lymphoid organs. *Proc. Natl. Acad. Sci. USA.* 86:5094.
  15. Bonneville, M., K. Ito, E. G. Krecko, S. Itohara, D. Kappes, I. Ishida, O. Kanagawa, C. A. Janeway Jr., D. B. Murphy, and S. Tonegawa. 1989. Recognition of a self MHC TL region product by  $\gamma\delta$  T cell receptors. *Proc. Natl. Acad. Sci. USA.* 86:5928.
  16. Bonneville, M., I. Ishida, P. Mombaerts, M. Katsuki, S. Berbeek, A. Berns, and S. Tonegawa. 1989. Blockade of  $\alpha\beta$  T cell development by  $\gamma\delta$  TCR transgenes. *Nature (Lond.)* 342:931.
  17. Takagaki, Y., N. Nakanishi, I. Ishida, O. Kanagawa, and S. Tonegawa. 1989. TCR  $\gamma$  and  $\delta$  gene preferentially utilized by adult thymocytes for the surface expression. *J. Immunol.* 142:2112.
  18. Ito, K., M. Bonneville, Y. Takagaki, N. Nakanishi, O. Kanagawa, E. G. Krecko, and S. Tonegawa. 1989. Different  $\gamma\delta$  T-cell receptors are expressed on thymocytes at different stages of development. *Proc. Natl. Acad. Sci. USA.* 86:631.
  19. Leo, D., M. Foo, D. Sachs, L. E. Samelson, and J. A. Bluestone. 1987. Identification of a monoclonal antibody specific for a murine T3 polypeptide. *Proc. Natl. Acad. Sci. USA.* 84:1374.
  20. Kubo, R. T., W. Born, J. W. Kappler, P. Marrack, and M. Pigeon. 1989. Characterization of a monoclonal antibody which detects all murine  $\alpha\beta$  T-cell receptors. *J. Immunol.* 142:2736.
  21. Havran, W. L., and J. P. Allison. 1988. Developmentally ordered appearance of thymocytes expressing different T-cell antigen receptors. *Nature (Lond.)* 335:443.
  22. Petit, A., P. B. Ernst, A. D. Befus, D. A. Clark, K. L. Rosenthal, T. Ishizaka, and J. Bienenstock. 1985. Murine intestinal intraepithelial lymphocytes. 1. Relationship of a novel Thy-1<sup>-</sup>, Lyt-1<sup>-</sup>, Lyt-2<sup>+</sup> granulated subpopulation to natural killer cells and mast cells. *Eur. J. Immunol.* 15:211.
  23. Nixon-Fulton, J. L., P. R. Bergstresser, and R. E. Tigelaar. 1986. Thy-1<sup>-</sup> epidermal cells proliferate in response to concanavalin A and interleukin 2. *J. Immunol.* 136:2776.

24. Farr, A. G., and P. K. Nakane. 1981. Immunohistochemistry with enzyme-labelled antibodies: a brief review. *J. Immunol. Methods.* 47:129.
25. Klein, J. R. 1986. Ontogeny of the Thy-1<sup>-</sup>, Lyl2<sup>+</sup> murine intestinal intraepithelial lymphocyte. Characterization of a unique population of thymus-independent cytotoxic effector cells in the intestinal mucosa. *J. Exp. Med.* 164:309.
26. Elliot, J. R., E. P. Rock, P. A. Patten, M. M. Davis, and Y-h. Chien. 1988. The adult T-cell receptor  $\delta$ -chain is diverse and distinct from that of fetal thymocytes. *Nature (Lond.)* 331:627.
27. Mingari, M. C., P. Varese, C. Bottino, G. Meliori, A. Moretta, and L. Moretta. 1988. Clonal analysis of CD4<sup>-</sup>CD8<sup>-</sup> human thymocytes expressing a T cell receptor  $\gamma\delta$  chain. Direct evidence for the de novo expression of CD8 surface antigen and of cytolytic activity against tumor targets. *Eur. J. Immunol.* 18:1831.
28. Lefrancois, L., and T. Goodman. 1989. *In vivo* modulation of cytolytic activity and Thy-1 expression of TCR- $\gamma\delta$ <sup>+</sup> intraepithelial lymphocytes. *Science (Wash. DC)* 243:1716.
29. Kieselow, P., H. Bluthmann, U. D. Staerz, M. Steinmetz, and H. von Boehmer. 1988. Tolerance in T-cell-receptor transgenic mice involves deletion of nonmature CD4<sup>+</sup>8<sup>+</sup> thymocytes. *Nature (Lond.)* 333:742.
30. Blackman, M. A., P. Marrack, and J. Kappler. 1989. Influence of the major histocompatibility complex on positive thymic selection of V $\beta$ 17A<sup>+</sup> T cells. *Science (Wash. DC)* 244:214.
31. Lafaille, J., A. DeCloux, M. Bonneville, Y. Takagaki, and S. Tonegawa. 1989. Junctional sequences of T cell receptor  $\gamma\delta$  genes: implications for  $\gamma\delta$  T cell lineages and for a novel intermediate of V-(D)-J joining. *Cell.* 59:859.
32. Lafaille, J., W. Haas, A. Coutinho, and S. Tonegawa. 1990. *Immunol. Today*. In press.