inflammation and local cytokine release, would induce class II expression by surrounding parenchymal (nonlymphoid) cells. Potentially autoreactive T cells that first encounter the combination of self tissue-specific antigen plus self class II on these cells rather than on Mϕ/DC would be paralysed, thus preventing the initiation of a 'bystander' autoimmune reaction. This might serve as an important fail-safe to complement the primary tolerance inducing function of the thymus. In some cases, other factors may tip the balance towards T-cell activation, and autoimmune responses would occur despite the expression of class II on parenchymal tissues.

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Note added in proof: Gaspari *et al.*[23] have recently reported similar *in vitro* results using class II$^+$ keratinocytes.

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Intestinal intraepithelial lymphocytes are a distinct set of γδ T cells

Marc Bonneville, Charles A. Janeway Jr*, Kouichi Ito, Wayne Haser, Isao Ishida, Nobuki Nakanishi† & Susumu Tonegawa

Howard Hughes Medical Institute, and Center for Cancer Research, and Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

Lymphocytes are most reliably subdivided on the basis of their receptors for antigen at the cell surface. Three subtypes of lymphocytes are well defined: B cells that bear surface immunoglobulin and make antibody, CD4$^+$T cells with CD3 αβ receptors specific for antigen associated with class II major histocompatibility complex molecules, and CD8$^+$T cells with CD3 αβ receptors specific for antigen associated with class I MHC molecules. These T cells are responsible for known forms of cell-mediated immunity. The discovery of a third rearranging T-cell specific gene called γ (refs 1 and 2) has revealed the presence of a new class of T cells bearing a new receptor type, CD3 γδ (refs 3–7). To date, neither the function nor the specificity of cells bearing this receptor has been determined. Because γδ T cells are the main lymphocyte of epidermis[8,9], it was proposed that such cells could be important in surveillance of all epithelia[10]. We have isolated intraepithelial lymphocytes from murine small intestine[11–13], and shown that they predominantly or exclusively express CD3 γδ receptors. Unlike the epidermal lymphocytes, these cells also express CD8, and they use a different V_γ gene to form their receptor. This strongly suggests that γδ T cells home in a very specific manner to epithelia, where they presumably mediate their function.

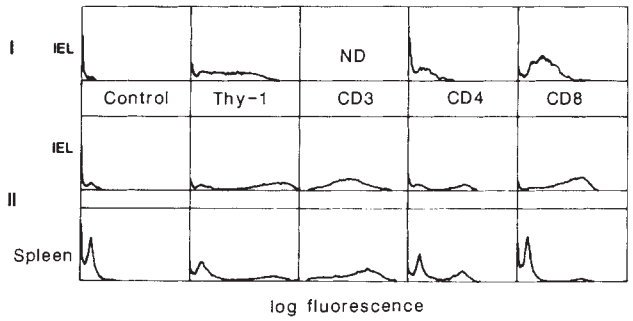

Fig. 1 FACS analysis of IELs and spleen cells. Two identical isolations were performed. IEL were prepared essentially as described[12]. Cells were stained with biotinylated monoclonal antibodies to the structures shown, counterstained with FITC-avidin, and analysed on an EPICS flow cytometer. The FACS profiles show that most IELs are T cells expressing CD8.

T cells with αβ receptors recognize fragments of foreign antigens associated with MHC molecules[14], and can be subdivided on the basis of expression of CD4 for class II MHC recognition and CD8 for class I MHC recognition[15]. T cells with γδ receptors have no known ligand and, in mouse, have been found to express neither CD4 nor CD8, being referred to as 'double negative' (DN) cells[4–7]. When cells expressing the murine pan T-cell marker Thy-1 were isolated and cloned from epidermis[16,17], these cells were shown to be DN CD3 γδ T cells. It has recently been shown that such cells almost exclusively express the products of the $V_{\gamma 5}$ gene (see refs 6 and 26 for the nomenclature of mouse V_γ gene segments) associated with $V_{\delta 1}$ (ref. 9; W. L. Havran and J. P. Allison, and J. Levis, P. Tucker, P. Bergstresser and R. Tigelaar, personal communication). Such cells are also the earliest T cells to appear in the thymus at about day 15 of gestation, and they are not found in the thymus after birth[18]. The homogeneity of these dendritic epidermal cells (DECs) suggests a very specific homing mechanism.

It is well known that many epithelia contain lymphoid populations interdigitating between the basolateral faces of epithelial cells. The best characterized of these are the intestinal intraepithelial lymphocytes (IELs), which have been isolated in several species. These cells are Thy-1$^+$, CD4$^-$, and mainly CD8$^+$ (refs 11-13). They have been shown to have cytolytic activity in a number of non-specific cytolytic assays[11,12]. The nature of their receptor or their ligand specificity is not known. If such cells were shown to be mainly or exclusively γδ T cells, this would extend the evidence that γδ T cells home to epithelia, and would imply that such γδ lymphocytes could be involved in surveillance of epithelial cell integrity; they could thus represent a first line of defence against infection and transformation of epithelial cells.

To determine the nature of the receptor on IELs, we isolated such cells from murine small intestines[13,19]. As previously shown[11-13], these cells were mainly Thy-1$^+$, CD8$^+$, CD4$^-$ (Fig. 1, preparation I); in some preparations, a significant number of CD4$^+$T cells were found (Fig. 1, preparation II). These probably represent contaminants from lamina propria or from Peyer's patch, but the techniques used in these experiments cannot

* Permanent address: Howard Hughes Medical Institute, Department of Pathology and Section of Immunobiology, Yale University School of Medicine, New Haven, Connecticut 06510, USA.
† Present address: Howard Hughes Medical Institute, Columbia University, New York, New York 10032, USA.

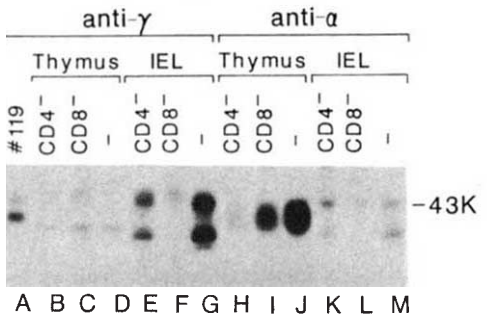

Fig. 2 Immunoprecipitated receptors on IEL and thymocytes. IEL and thymocytes were untreated, or treated with anti-CD4 or anti-CD8 and complement, the surviving cells labelled with ^{125}I, and lysates precipitated with anti-γ (lanes *A–G*) or subsequently with anti-α (lanes H–M), and resolved on SDS-PAGE minigels. Material in lanes *B–D*, *H–J* is derived from thymus; lanes *E–G*, *K–M*, from IEL; lane *A*, from hybridoma 119 expressing $V_{\gamma 1}$. Material in lanes *B*, *E*, *H* and *K* has been treated with anti-CD4; lanes *C*, *F*, *I* and *L*, treated with anti-CD8 and complement. IELs express large amounts of $\gamma\delta$ receptor, depleted by anti-CD8 and complement, whereas thymus has less $\gamma\delta$ receptor not depleted by anti-CD8. IELs lack $\alpha\beta$ receptors; bands precipitated represent residual $\gamma\delta$ receptor from the first immunoprecipitation. Note depletion of $\alpha\beta$ receptors in thymocytes by anti-CD4 and anti-CD8.

Methods. IEL and thymocytes were isolated from adult (nine weeks old) C57BL/6 mice, divided into aliquots of 2×10^6 IELs or 2×10^7 thymocytes, treated for 60 min at 37 °C with anti-CD4 (RL 172.4) or anti-CD8 (3.155.D14) plus rabbit complement (Lowtox, Cedarlane); live cells were recovered by centrifugation over Ficoll-hypaque, the six pools of cells were labelled with ^{125}I and lysed with NP40 in buffer[6]. Lysates were precipitated with anti-γ monoclonal antibody, immune complexes and beads were centrifuged out, and the remaining supernatant was subjected to a second immunoprecipitation with beads coated with purified anti-α antiserum. Anti-α antiserum was prepared in rabbits by immunization with a fusion protein of β-galactosidase constructed by cloning a 655-base pair fragment of the α-chain gene of pHSD58 isolated by digestion with RSA1 and *Hin*cII into λgt11. The clone was expressed in *Eschericia coli* and the fusion protein was purified by affinity chromatography on a column of anti-β-galactosidase. Rabbits were hyper-immunized with this protein, and the immunoglobulin component purified by protein A Sepharose chromatography. Anti-γ monoclonal antibody KN365 was raised by immunization of DBA/2 mice with a bacterial recombinant-derived γ-chain fragment consisting of 20 amino acids of $V_{\gamma 2}$, all of $J_{\gamma 2}$, and 123 amino acids of $C_{\gamma 2}$. KN365 reacts with an epitope on $C_{\gamma 1}$ and $C_{\gamma 2}$ regions after denaturation of the protein with SDS (ref. 23).

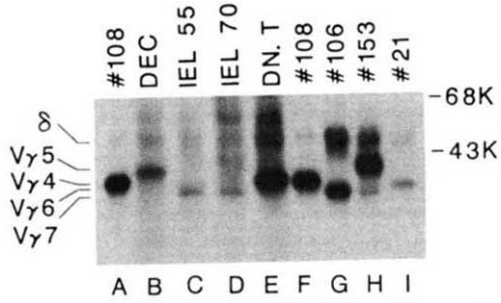

Fig. 3 Immunoprecipitation of the $\gamma\delta$ protein complex from DECs, IELs, DN thymocytes and T-hybridomas. DECs, IELs, DN thymocytes and selected T-cell hybrids were surface iodinated, and $\gamma\delta$ proteins were precipitated with anti-γ monoclonal antibody and resolved on one-dimensional gel electrophoresis. Lane *B*, DECs; lane *C*, IEL from 55% Percoll gradient; lane *D*, IEL from 70% Percoll gradient; lane *D*, DN thymocytes; lanes *A*, *F*, *G*, *H* and *J*, $\gamma\delta$-expressing hybridomas (number 108: $V_{\gamma 4}V_{\delta 1}$; number 106: $V_{\gamma 7}V_{\delta 5}$; number 153: $V_{\gamma 5}V_{\delta 1}$ and number 21: $V_{\gamma 6}V_{\delta 1}$). The reference mobilities of γ-chains from hybridomas using $V_{\gamma 4}$, $V_{\gamma 5}$, $V_{\gamma 6}$ and $V_{\gamma 7}$ are indicated on the left. Note the presence of three species immunoprecipitated from $V_{\gamma 5}$-expressing hybridoma number 153. The lowest species corresponds to a deglycosylated $V_{\gamma 5}J_{\gamma 1}C_{\gamma 1}$ product[9].

Methods. DEC were prepared from trypsinized epidermal sheets as described [24] and incubated overnight in culture medium at 37 °C before immunoprecipitation to allow recovery of their surface receptors. IELs were isolated according to the technique described by Petit *et al.*[19] and lymphocytes from 55% and 70% Percoll gradients were analysed separately. DN thymocytes were obtained after complement-dependent lysis of $CD4^+$ and $CD8^+$ cells, as described in Fig. 2. T-cell hybrids were prepared by fusing DN thymocytes from mice at different ages with BW5147 thymoma, selected in HAT medium, and secreened, cloned, and characterized as descibed elsewhere[18,20]. Cells were iodinated and lysates immunoprecipitated with anti-γ monoclonal antibody KN365 as described previously[5,6].

determine this conclusively. These isolated cells were then labelled with ^{125}I, and the receptors immunoprecipitated using either anti-γ monoclonal antibody or a purified anti-α antiserum. As can be seen in Fig. 2, there are abundant $\gamma\delta$ heterodimers on IELs (Fig. 2, lane *G*), but little $\gamma\delta$ detectable on adult thymocytes from the same donors (Fig. 2, lane *D*). Thymocytes show abundant $\alpha\beta$ receptors, whereas IELs show no detectable $\alpha\beta$ receptor (Fig. 2, lanes *J* and *M* respectively).

As virtually all the IELs isolated express CD8, and as most or all of the receptor protein precipitated is $\gamma\delta$, it seems likely that the T cells expressing $\gamma\delta$ receptors are mainly $CD8^+$. We and others have previously shown that anti-CD8 plus complement treatment of thymocytes kills most of the cells, but does not deplete cells expressing $\gamma\delta$ receptors[4–7]; we have confirmed this result, see Fig. 2, lanes *C* and *D*. Using IELs, however, anti-CD8 plus complement almost totally eliminates T cells bearing $\gamma\delta$ receptors (Fig. 2, lanes *F* and *G*). Thus, most (or all) of the $CD8^+$T cells isolated from the gut express $\gamma\delta$ T-cell receptors. As a control, anti-CD4 plus complement was shown not to deplete $\gamma\delta$ T cells from either thymocytes (Fig. 2, lane *B*) or IEL (Fig. 2, lane *E*), as expected. Finally, sequential precipitation of the same lysates with anti-α antibody precipitates no $\alpha\beta$ receptor proteins from IELs and demonstrates that anti-CD8 plus complement, and especially anti-CD4 plus complement, greatly deplete $\alpha\beta$ receptor in the thymocyte population (Fig. 2, lanes *H–J*).

In separate experiments, we have identified the $\gamma\delta$ T-cell receptor proteins of a series of T-cell hybrids derived from fetal and adult DN thymocytes fused with the T-cell lymphoma Bw5147 (refs 18 and 20). By analysing DNA and RNA, we could determine which γ- and δ-genes encode the $\gamma\delta$ heterodimers expressed on each of these hybridomas[18–20]. This analysis revealed that γ-chains encoded by different V_γ gene segments migrate with distinct mobilities in SDS-PAGE, as shown in Fig. 3, lanes *A*, *F*, *G*, *H* and *I*. On the basis of this criterion the IEL γ-chain is clearly distinct from those encoded by $V_{\gamma 4}$ and $V_{\gamma 5}$ which are the chief γ-chain types expressed on adult thymocytes (ref. 20 and Fig. 3, lane *E*) and DEC (ref. 9; lane *B*) respectively. Instead, IEL γ chains seem to comigrate, either with $V_{\gamma 7}$- or $V_{\gamma 6}$-encoded γ-chains (Fig. 3, lanes *C*, *D*, *G* and *I*).

In a further attempt to assign the IEL γ-chains to a specific V_γ gene, we examined RNA present in IEL using V_γ-specific hybridization probes. As shown in Fig. 4, $V_{\gamma 7}$ probe detected a moderate level of γ RNA in IELs (Fig. 4*a*, lanes *F* and *G*), whereas RNA was absent or present at a barely detectable level when $V_{\gamma 4}$ (Fig. 4*b*, lanes *F–G*), $V_{\gamma 6}$ (Fig. 4*c*, lanes *F–G*) or $V_{\gamma 5}$ (data not shown) probes were used. This contrasted with the results from DN thymocytes, where we found large amounts of $V_{\gamma 4}$ transcripts (Fig. 4*b*, lane *E*) and very low or zero levels of $V_{\gamma 7}$, $V_{\gamma 6}$, or $V_{\gamma 5}$ mRNAs (Fig. 4*a* and *c*, lane *E*, and data not shown). These results corroborate those for the proteins and indicate that IEL γ chain could be primarily encoded by the $V_{\gamma 7}$ gene segment.

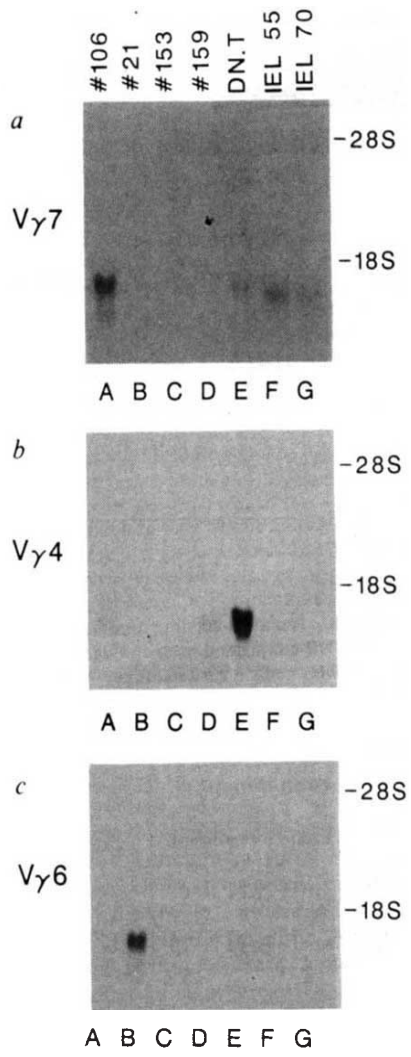

Fig. 4 Northern blot analysis of IELs and DN thymocytes. Total RNA from IELs, DN thymocytes and T-cell hybrids were electrophoresed in agarose gel, blotted to nylon membranes and hybridized to $V_{\gamma 7}$ (*a*), $V_{\gamma 4}$ (*b*) or $V_{\gamma 6}$ (*c*) probes. Lanes *A*-*D*: T-cell hybridomas (number 106: $V_{\gamma 7}$; number 21: $V_{\gamma 6}$; number 153: $V_{\gamma 5}$; number 159: $V_{\gamma 4}$); lane *E*: DN thymocytes; lane *F*: IEL from 55% Percoll gradient; lane *G*: IEL from 70% Percoll gradient. Hybridoma number 106 expresses both $V_{\gamma 4}$ and $V_{\gamma 7}$ mRNAs but analysis of cDNAs revealed that the $V_{\gamma 4}J_{\gamma 1}C_{\gamma 1}$ gene was joined out-of-frame[20]. **Methods.** IELs, DN thymocytes and T-cell hybrids were prepared as described in Fig. 3. Total RNA (5 μg) was electrophoresed, blotted on to nylon membranes, prehybridizded and hybridized with random primed probes[18,20]. Details of $V_{\gamma 4}$, $V_{\gamma 5}$, $V_{\gamma 6}$ and $V_{\gamma 7}$ probes are described elsewhere[18,20].

Our data have shown that another significant intraepithel lymphocyte population besides DECs, that of the small intestine, is made up of T cells with γδ receptors. It should be noted that our analysis applies to the cells isolated by two different procedures described in Fig. 1 and Fig. 3. Nevertheless, to establish whether these cells are representative of all IELs will require an *in situ* approach. Assuming the isolated IEL population is representative, then IELs differ from DECs in two important respects: IELs express CD8 and IELs use a different $V_\gamma V_\delta$ combination to form receptors. These data have several important consequences.

First, that both epidermis and intestinal epithelium are sites of predominant γδ T-cell populations suggests that such cells have some critical influence in epithelia. Indeed, the virtual absence of T cells expressing αβ receptors in these tissues, and the fact that the unusual $CD8^+$ $V_{\gamma 7}V_\delta$ phenotype of the IEL is seldom found in any somatic lymphoid organ[20], both suggest that such cells are specialized for homing to intestinal epithelium. That IELs and DECs differ in the V regions used in forming their receptors further points to a high degree of tissue specificity in this homing pattern. This, in turn, strongly suggests that at least these populations of γδ T cells are specialized for epithelial surveillance in particular sites, as discussed[21].

Although it has been difficult to demonstrate that DECs are present in species other than the mouse, it is clear that there are IELs in chickens and man, and that these cells also express γδ receptors and CD8 (R. P. Bucy *et al.*, personal communication). The restricted *V*-gene usage in these epithelial sites suggests that ligand recognition in these tissues is limited. Whether such receptors are truly autoreactive to MHC class[10], or whether they have some different function and specificity altogether, remains to be determined. Likewise, the mechanism of homing or selective retention of such cells in particular epithelial sites is not understood. Nevertheless, the finding of different γδ T cells in different epithelia strongly points to a highly specific biological function for such cells in these anatomical locations.

After this study was completed, we became aware that T. Goodman and L. Lefrancois[22] independently showed that $CD8^+$ T cells isolated from intestinal epithelium express γδ receptors, although they do not specify which γ-gene is used in this site.

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Killing of antigen-reactive B cells by class II-restricted, soluble antigen-specific $CD8^+$ cytolytic T lymphocytes

Nobukata Shinohara*†, Motoo Watanabe‡, David H. Sachs* & Nobumichi Hozumi‡

* Immunology Branch, National Cancer Institute, NIH Bethesda, Maryland 20892, USA
‡ Mount Sinai Hospital, Research Institute, Toronto, Ontario, Canada

Cytolytic T lymphocytes (CTLs) are generally thought to recognize cellular antigens presented by class I MHC molecules. A number of studies, however, have revealed responses of considerable magnitude involving both $CD8^+$ and $CD4^+$ CTLs with class II restriction[1-5], suggesting that class II-restricted CTLs recognizing exogeneous protein antigens may exist. As class II antigens are normally expressed on limited types of cells such as B cells and macrophages, such CTLs might be expected to exert a suppressive

† Present address: Laboratory of Cellular Immunology, Mitsubishi Kasei Institute of Life Sciences, Tokyo, Japan.