INTRAEPITHELIAL LYMPHOCYTES

Anatomical Site, Not T Cell Receptor Form, Dictates Phenotype and Function

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Peripheral T lymphocytes express plasma membrane receptors specific for foreign antigen that are composed of two disulfide-linked proteins containing constant and variable regions (1–3). This dimer is associated with a complex of several invariant proteins collectively termed CD3 (4, 5). Based on biochemical and genetic data, two forms of TCRs have been identified: those containing α and β proteins which are found on the majority of peripheral T cells (1–3), and those made up of γ and δ chain heterodimers (6–8). The latter TCRs are present on some murine, avian, and human $CD4^-CD8^-$ thymocytes and peripheral T cells (7, 9–11) and are found on murine Thy-1^+ dendritic epidermal cells in the skin (12–14). Recently we (15), and subsequently others (16), demonstrated that a major population of murine $CD8^+$ T cells found in the small intestine, termed intraepithelial lymphocytes (IEL),[1] express the γ/δ type of TCRs.

Recently, several laboratories have demonstrated that some γ, δ T cells can react with mycobacterial antigens (17–19) or with tetanus toxoid (20). In the case of tetanus toxid, human class I-restricted $CD8^+$ γ, δ T cells were shown to be reactive. In other cases the response to mycobacterial antigens by $CD4^-8^-$ T cells was either not MHC restricted (17, 18) or was restricted to as yet undefined restricting elements (19). In the mouse, IEL are unusual among T cells in that a large proportion of $CD3^+8^+$ cells are Thy-1^- (21, 22). In addition, some IEL express the leukocyte common antigen (T200)-associated carbohydrate differentiation antigen, CT1, an antigen not present on other peripheral T cells (23, 24). IEL are also functionally distinct from other peripheral T cell populations, in that freshly isolated IEL are cytolytic, although their specificity is unknown (15). However, externally derived stimuli, perhaps bacterial in nature, are required for induction of lytic activity in IEL since IEL from germ-free mice are not cytolytic (25). Lytic activity resides in the Thy-1^+ IEL subset and this population is absent in IEL from germ-free mice. Thus, IEL represent a distinct lineage of T cells based on phenotypic and functional characteristics, not the least of which is the predominant expression of TCR-γ/δ.

To further characterize IEL we developed mAbs specific for TCR-γ/δ. Fluorescence analysis and biochemical studies using this panel of mAbs demonstrated marked heterogeneity in the TCRs of IEL. Moreover, the constitutive lytic activity and the Thy-$1^\pm$ phenotype of IEL was not restricted to TCR-γ/δ^+ IEL, but TCR-α/β^+ IEL

[1] *Abbreviations used in this paper:* IEL, intraepithelial lymphocytes; LN, lymph nodes.

also exhibited these characteristics. Thus, the distinct attributes of IEL appear to be regulated by the intestinal milieu and their anatomical location rather than a priori expression of a particular subclass of TCRs.

Materials and Methods

Purification of Murine IEL. IEL were isolated essentially as previously described (15). Briefly, the small intestines from 4–10 mice were cut into 5-mm pieces that were then stirred at 37°C in HBSS with the addition of 1 mM dithioerythritol. The resulting supernatants containing a mixture of lymphocytes and epithelial cells were then centrifuged through a 44/67.5% Percoll gradient. Cells at the interface were panned on petri dishes coated with an anti-CD8 mAb. The final populations were 88–95% $CD8^+$.

Animals. Adult Armenian hamsters were purchased from Cytogen, Cambridge, MA. C57BL/6J and BALB/cJ mice were obtained from The Jackson Laboratory, Bar Harbor, ME.

T Cell Hybrids. T cell hybrids used in this study were DN2.9, DN7.1.13, DN7.3, DN12.1 (26), generously provided by Ada Kruisbeek (National Institutes of Health, Bethesda, MD); FT45.1 and PDN3.1 (27) were provided by Jeff Bluestone (University of Chicago, Chicago, IL); and 33BTE.67.1 was provided by Willi Born (National Jewish Hospital, Denver, CO).

Monoclonal Antibodies. mAbs used for these studies were: 3.168 (anti-CD8; reference 28) I3/2.3 (anti-T200; 29), CT1 (30), T24 (anti-Thy-1; 31), 13.4 (anti-Thy-1.2; 32) 145-2C11 (a hamster mAb specific for the CD3 ϵ-chain; 33), H57.597 (a hamster mAb that recognizes all TCR-α/β; generously provided by Dr. Ralph Kubo, National Jewish Hospital; 34) and RL172 (anti-CD4; 35).

Production of mAbs. Armenian hamsters were immunized in the rear footpads four times over a 2-mo period with 5–10 × 10^6 C57BL/6J IEL per footpad. For injection, the IEL were resuspended in normal saline and were either viable or lethally irradiated. 3 d after the last injection, popliteal lymph node cells were fused with the murine myeloma SP2/0 at a ratio of 4:1 (lymph node/myeloma) in 1 ml of 50% polyethelene glycol (PEG-1450; J. T. Baker Chemical Co., Phillipsburg, NJ) as previously described (36). The cells were then distributed in 96-well microtiter plates with HAT medium.

Screening of Fusions. Culture supernatants from growth-positive wells were tested for their ability to redirect lysis of the Fc $receptor^+$ DBA/2 mastocytoma P815 by IEL (37). We have previously shown that freshly isolated murine $CD8^+$ IEL are constitutively lytic. 100 μl of culture supernatant was added to 2.5 × 10^3 $Na^{51}Cr$-labeled P815 cells. Freshly isolated IEL were added to each well at a concentration of 10^5 cells per well followed by a 5-h incubation at 37°C. Percent specific lysis was calculated as 100 × [(cpm released with effector cells − cpm released alone)/(cpm released by detergent − cpm released alone)]. Cells from wells that resulted in >10% lysis were cloned twice by limiting dilution at a concentration of 0.5 cells/well in 96-well microtiter plates with 10^6 BALB/cJ irradiated spleen cells per well.

Immunofluorescent Staining. IEL and murine spleen cells were resuspended in PBS/0.2% BSA/0.1% NaN_3 followed by incubation at 4°C for 30 min with 200 μl of culture supernatant from hybridomas that redirected lysis. In other experiments, cells were incubated with appropriate dilutions of purified antibodies or antibodies coupled to biotin or fluorescein isothiocyanate. Cells were washed twice and incubated for 30 min with a FITC-coupled goat anti-hamster Ig (Cappel Laboratories, Malvern, PA) or avidin-phycoerythrin. Cells were then washed three times and resuspended at 10^6 cells/ml for cytofluorimetric analysis. Relative fluorescent intensities of individual cells were measured with a cytofluorograph (no. 50H; Ortho Diagnostic Systems Inc., Westwood, MA). Forward angle light scatter was used to exclude dead and aggregated cells. The results are presented as fluorescence histograms with the relative number of cells on a linear scale plotted vs. the relative fluorescence intensity on a logarithmic scale, both in arbitrary units.

Radioimmunoprecipitation. Cell surface proteins of IEL were radioiodinated by the Iodogen method (38). IELs were washed and resuspended in lysis buffer (PBS, 0.5% NP-40, 2 mM PMSF, 20 IU/ml aprotinin, 10 mg/ml BSA) and incubated on ice for 15 min. Lysates were centrifuged at 13,000 *g* for 20 min and precleared with Pansorbin (Calbiochem-Behring Corp., La Jolla, CA) followed by incubation with purified GL1, 2, 3, 4, or 5 coupled directly to

Sepharose 4B and 4°C overnight. The resulting precipitates were analyzed by SDS-PAGE using a 12.5% acrylamide gel in the absence or presence of 5% 2-ME. Endoglycosidose F treatments were carried out on immunoprecipitates according to the manufacturer's specifications (*N*-glycanase; Genzyme, Cambridge, MA). After drying, the gels were subjected to autoradiography at −70°C with Kodak XAR-5 x-ray film and Dupont Cronex intensifier screens.

Results

To obtain mAbs that would react with native cell surface TCRs, we immunized hamsters with intact purified IEL and generated hybridomas by fusing immune lymph node cells with the murine myeloma SP2/0. Since IEL are constitutively cytolytic, a redirected lysis assay was used for detection of mAbs specific for molecules capable of inducing lysis. This assay uses an Ig Fc receptor–expressing target cell and relies on crosslinking by the mAb of relevant cell surface proteins of IEL to the Fc receptor to trigger lysis. In this way several mAbs were identified that were able to redirect lysis of IEL and were specific for TCR-γ/δ. These mAbs were further screened by fluorescence analysis of binding to lymph node T cells and to IEL. The IEL in all experiments unless otherwise stated were 85–95% $CD3^+8^+$ with <5% of the cells being either surface Ig^+ or $CD4^+$ and 10% or fewer being nonlymphoid epithelial elements. The binding of five of the mAbs, termed GL1 through GL5, to IEL is depicted in Fig. 1, *A* and *B*. IEL from C57Bl/6J mice (B6) were 40–50% $GL1^+$ and 15–20% $GL2^+$ (Fig. 1 *A*). GL3 and GL4 reacted with 70–80% of IEL and 40–50% were $GL5^+$. The intensity of GL4 staining was consistently less than that observed using GL3. Purified lymph node (LN) T cells were also tested with the GL mAb series and <3% of LN T cells were positive using any of the mAbs (as an example, GL3 binding is shown in Fig. 1 *C*). The hamster mAb H57, which reacts with all TCR-α/β, stained nearly all LN T cells, but only 20–25% of B6 IEL (Fig. 1 *C*). This result was consistent with our previous demonstration that 7–10% of B6 IEL

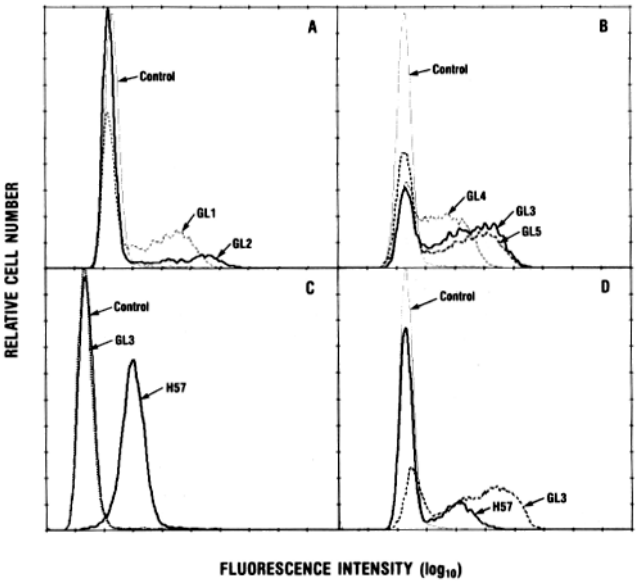

FIGURE 1. Reactivity of anti-TCR mAb with IEL. Purified IEL (*A*, *B*, *D*) or purified lymph node T cells (*C*) were reacted with the indicated mAb followed by staining with an FITC-conjugated goat anti-hamster Ig reagent. Individual cells were then analyzed by fluorescence flow cytometry.

are stained by the Vβ8-reactive mAb F23.1 (23), which reacts with ~20% of peripheral T cells from this strain. Together, mAbs GL3 and H57 were able to identify all $CD3^+$ IEL (see below).

Since some of the mAbs reacted with IEL subpopulations we wished to examine their ability to trigger TCR-mediated lytic activity of the respective subsets. Purified GL1, GL2, and anti-CD3 were compared in the redirected lysis assay (Fig. 2). All of the mAbs could trigger lysis by IEL, with anti-CD3 being the most efficient. The levels of lytic activity obtained using GL1 and GL2 also correlated with the number of cells expressing each of these determinants, with GL2, which reacts with 15–20% of B6 IEL, triggering low levels of activity. GL3-GL5 also efficiently triggered lysis. Thus, all of the subpopulations identified by the GL mAb were cytolytic.

To assess the reactivity of the GL mAb with TCR proteins IEL were radioiodinated and lysates were subjected to immunoprecipitation. Several interesting features of IEL TCR proteins were evident (Fig. 3). When the precipitates were analyzed under nonreducing conditions two protein species of apparent M_r 78,000 and 73,000 were present in precipitates from GL1, 3, 4, and 5. In contrast, GL2 precipitated only the 78,000 M_r dimer (Fig. 3 *A*, lane *2*). Analysis under reducing conditions revealed that GL1, 3, 4, and GL5 precipitated 46,000 and 42,000 δ M_r proteins and 35,000 and 34,000 γ M_r proteins. These results were identical to those obtained by precipitation with an anti-Cγ sera (15) or with anti-CD3. In contrast, GL2 reacted primarily with TCRs containing the higher M_r δ chain of 46,000, consistent with the precipitation of only the 78,000 M_r dimer by this mAb. However, it became clear that a third δ protein of 43,000 M_r was also precipitated by GL2 (Fig. 3, lane *2*, *arrow*). The presence of two distinct TCR heterodimers on IEL was further examined by two-dimensional nonreducing/reducing electrophoresis (Fig. 4). While the anti-CD3 and GL1 mAb precipitated dimers that contained two δ chains that were easily separated, the GL2 mAb reacted only with dimers containing

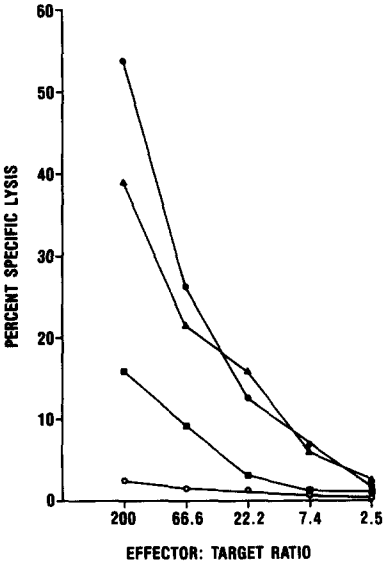

FIGURE 2. IEL subpopulations are constitutively cytolytic. Serial dilutions of IEL were incubated for 5 h at 37°C with 2.5×10^3 ^{51}Cr- sodium chromate-labeled P815 target cells that had been previously incubated with 2 μg/ml of anti-CD3 (●), GL1 (▲), GL2 (■), or medium alone (○). Spontaneous release was <10%.

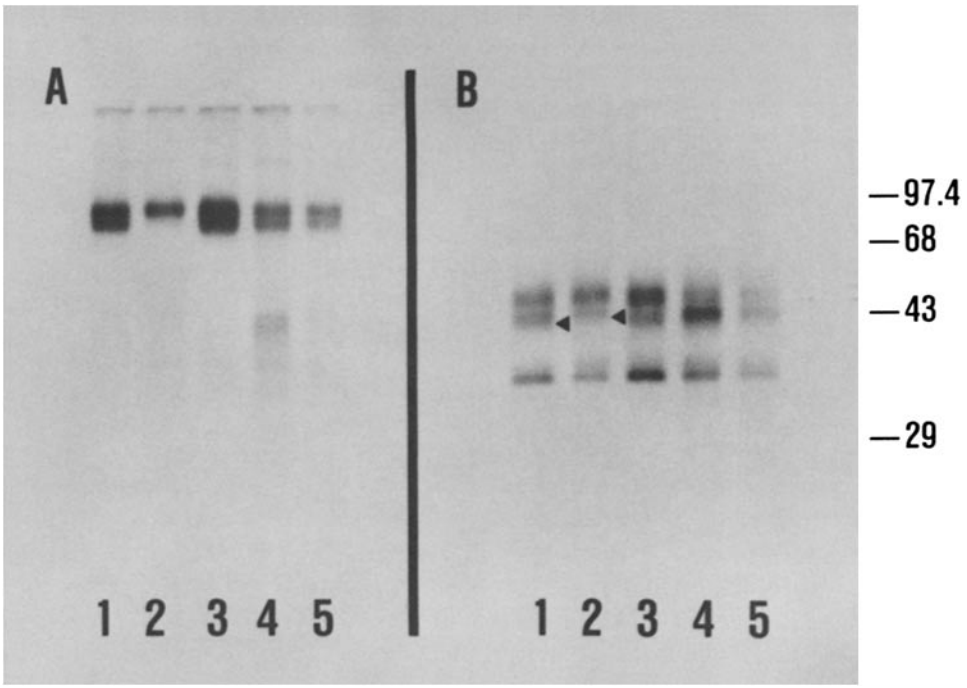

FIGURE 3. Immunoprecipitation of TCR-γ/δ by the GL mAb. IEL were surface labeled with ^{125}I and lysates were subjected to immunoprecipitation with GL1 (lane *1*); GL2 (lane *2*); GL3 (lane *3*); GL4 (lane *4*); or GL5 (lane *5*). The precipitates were analyzed on a 12.5% SDS-polyacrylamide gel under nonreducing (*A*) or reducing (*B*) conditions followed by autoradiography.

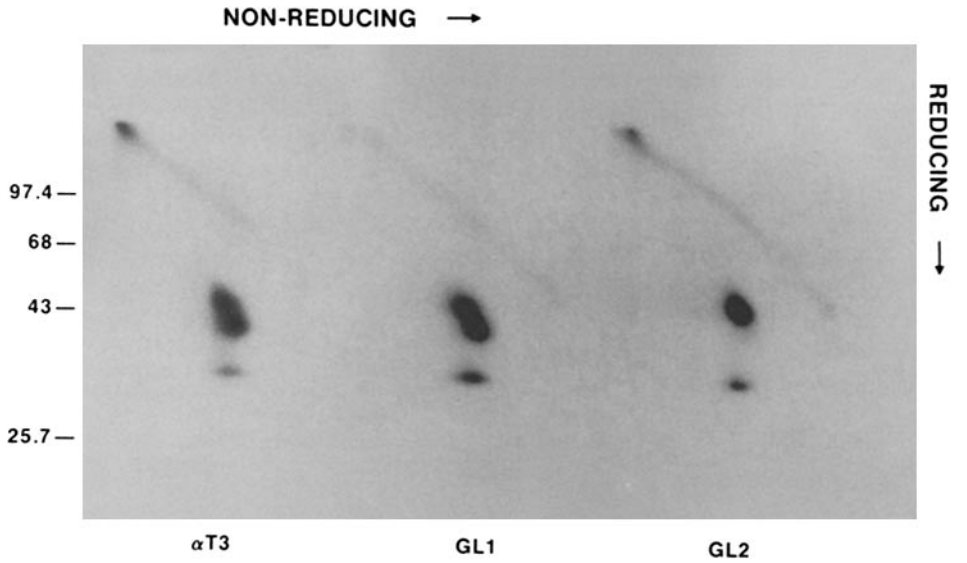

FIGURE 4. Two-dimensional electrophoretic analysis of TCR-γ/δ of IEL. IEL immunoprecipitates were separated under nonreducing conditions in a 12.5% SDS-polyacrylamide gel in 3-mm tubes followed by separation in the second dimension under reducing conditions in a 12.5% slab gel. Precipitations were carried out on digitonin lysates with the indicated mAb.

the higher M_r δ proteins. This experiment also demonstrated that similar γ chains were able to associate with the high and low M_r δ proteins, although we do not know whether different V regions are being used by γ chains with similar M_r. Thus, not only do the GL mAb subdivide the IEL on the basis of cell surface binding but this heterogeneity was manifested in the chain composition of the TCRs recognized by the mAb.

To determine whether the distinct TCR proteins were due to differences in asparagine-linked glycosylation GL1 and GL3 immunoprecipitates were subjected to endoglycosidase F treatment (Fig. 5). The two major δ proteins of 46,000 and 42,000 M_r were each reduced in molecular weight by ~6,000 after Endo F digestion, while the major 34,000 M_r γ protein was reduced by 3,000. These results suggest that two *N*-linked oligosaccharide chains were present on the δ chains and that a single *N*-linked oligosaccharide was present on the 34,000 M_r γ chain, consistent with previous reports. Moreover, the presence of two major δ proteins was not due to heterogeneity of *N*-linked glycosylation.

Two-color fluorescence analysis was used to determine the interrelationships between the IEL subsets delineated by the GL mAb. Simultaneous staining with GL1 and GL2 showed that 95–98% of $GL2^+$ cells were contained within the $GL1^+$ population (Fig. 6 *B*). Thus, GL2 identified a subset of $GL1^+$ IEL that preferentially expressed the high M_r δ proteins as shown above. Sequential immunoprecipitations revealed that the 46,000 M_r δ protein contained within the $GL1^+$ population was entirely $GL2^+$ (see below). However, preclearance of lysates with GL2 followed by precipitation with GL3 indicated that other $GL2^-$ 46,000 M_r δ proteins were present (Lefrancois, L., and T. Goodman, unpublished results). Further fluorescence binding studies demonstrated that the $GL5^+$ subset overlapped completely with the $GL1^+$ population. The mAbs were also tested for reactivity with a panel of TCR-γ/δ^+ hybrids derived from fetal thymocytes or from $CD4^-8^-$ peripheral T cells (Table I). The GL1 and GL5 mAbs did not react with any of the hybrids shown. GL2 reacted with two hybrids with similar γ and δ chains (Vγ2Cγ1/Vδ4). However, GL2 did not react with other Vγ2Cγ1 hybrids with as yet uncharacterized δ chains, and IEL contain <5% $V\gamma2^+$ cells. Thus, we can tentatively assign GL2 to Vδ4 specificity. GL3 and GL4 reacted with all hybrids tested regardless of γ or δ chain expression.

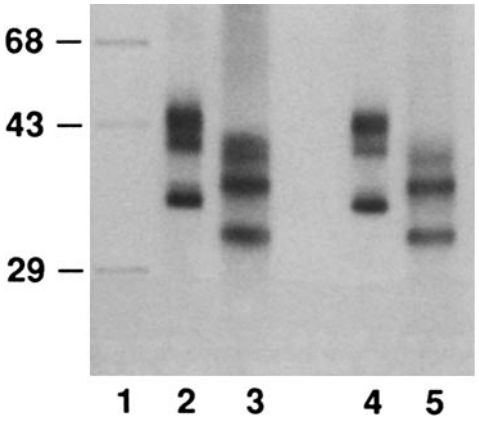

FIGURE 5. Analysis of *N*-linked glycans of IEL TCR proteins. Lysates from ^{125}I surface-labeled IEL were subjected to immunoprecipitation with GL1 or GL3 coupled to Sepharose 4B. The resulting precipitates were treated with *N*-glycanase (lanes *3*, *5*) or with reaction buffer without the enzyme added (lanes *2*, *4*) for 16 h followed by analysis on a 12.5% SDS-polyacrylamide gel. (Lane *1*) Molecular weight standards; (lanes *2*, *3*) GL1 precipitate; (lanes *4*, *5*) GL3 precipitates.

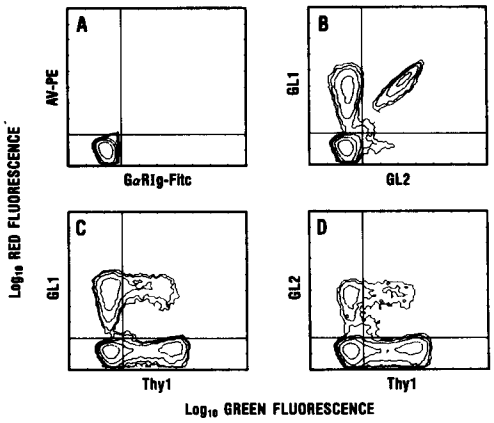

FIGURE 6. Two-color fluorescence analysis of TCR-γ/δ and Thy-1 expression of IEL. Purified IEL were reacted with FITC-conjugated anti-Thy-1 mAb (*C*, *D*) or GL2 (*B*) followed by reaction with biotinylated GL1 (*B*, *C*) or GL2 (*D*). Biotinylated mAbs were detected with avidin-phycoerythrin (PE). Control cells were reacted with avidin-PE and a FITC-coupled goat anti-rat Ig reagent.

To further delineate the fine specificity of the mAb, competitive binding studies were performed (Table II). Interestingly, binding of GL2 was effectively competed for by GL3 and GL4. GL3 and GL4 also crossblocked the binding of each other. Since GL2 appears to be specific for a Vδ4 determinant, GL3 and GL4 can be tentatively assigned to Cδ specificity. None of the mAbs react with TCR proteins after reduction and alkylation of the heterodimer, so we have not as yet been able to assign chain specificities by biochemical means.

We also examined the relationship between Thy-1 expression and the restricted TCR epitopes. In the experiment shown in Fig. 6 the IEL were 46% Thy-1$^+$, 41% GL1$^+$ and 18% GL2$^+$. 17% of B6 IEL were Thy-1$^+$ GL1$^+$ (*C*) and 10% were Thy-1$^+$ GL2$^+$ (*D*). The remaining GL mAbs gave similar results: both Thy-1$^+$ and Thy-1$^-$ IEL were stained. Thus, although IEL that express Thy-1 are cytolytic, while Thy-1$^-$ IEL are not, there did not appear to be a partitioning of a particular TCR subtype into either class of IEL.

The absence of Thy-1 and the presence of the CT1 carbohydrate antigen on some IEL serve to distinguish these cells from other peripheral T cells. No other mature T lymphocytes yet described have these characteristics. Moreover, few murine TCR-γ/δ$^+$ T cells other than IEL express CD8. Our original observations (15) led us to suggest that the expression of these receptors was linked to the distinct phenotype of IEL. To address this possibility we analyzed the expression of Thy-1 and CT1

TABLE I

Reactivity of the GL mAb with TCR-γ/δ$^+$ T Cell Hybrids

	Hybrids and TCR proteins expressed						
mAb	DN2.9 Vγ1Cγ2	DN12.1 Vγ1Cγ4	DN7.1.13 Vγ4Cγ1	DN7.3 Vγ2Cγ1	FT45.1 Vγ3Cγ1	PDN3.1 Vγ2Cγ1/Vδ4	33BTE.67.1 Vγ2Cγ1/Vδ4
GL1	–	–	–	–	–	–	–
GL2	–	–	–	–	–	+	+
GL3	+	+	+	+	+	+	+
GL4	+	+	+	+	+	+	+
GL5	–	–	–	–	–	–	–

The hybrids were reacted with FITC-coupled GL mAb and analyzed by fluorescence flow cytometry.

TABLE II

Competitive Binding Analysis of the GL mAb

	FITC-labeled mAb				
Unlabeled mAb	GL1	GL2	GL3	GL4	GL5
GL1	+	–	–	–	–
GL2	–	+	+	+	–
GL3	–	+	+	+	–
GL4	–	+	+	+	–
GL5	–	–	–	–	+

Saturating levels of unlabeled mAb were incubated with IEL followed by washing and incubation with FITC-coupled mAb. The cells were then analyzed by fluorescence flow cytometry.

on TCR-α/β^+ IEL with the H57 mAb that is reactive with all TCR-α/β. We first compared the populations stained by this mAb with those stained by GL3, since the latter appears to react with all TCR-γ/δ. In the experiment shown the IEL were 67% GL3$^+$, 25% H57$^+$, 46% CT1$^+$, 26% Thy-1$^+$, and 8% CD3$^-$. GL3 and H57 stained mutually exclusive IEL subpopulations and together reacted with all CD3$^+$ IEL. Simultaneous staining with GL3 and anti-Thy-1 or with the CT1 mAb indicated that 50% of IEL were Thy-1$^-$ GL3$^+$ (74% of GL3$^+$ IEL, Fig. 7 *C*), while 32% were CT1$^+$ GL3$^+$ (48% of GL3$^+$ cells; Fig. 7 *D*). Similar reactions using H57 rather than GL3 revealed that a substantial portion of TCR-α/β^+ IEL were Thy-1$^-$. In this experiment, 18% of IEL, representing 72% of TCR-α/β^+ cells were Thy-1$^-$ H57$^+$ (Fig. 7 *E*). Moreover, 12% of IEL were CT1$^+$ H57$^+$, or 48% of TCR-α/β-expressing IEL (Fig. 7 *F*). These results indicate that the unusual Thy-1$^-$ CT1$^+$ phenotype was not restricted to TCR-γ/δ^+ IEL but that similar TCR-α/β^+ IEL were also present. Thus, IEL were of a distinct phenotype that was likely to be due to

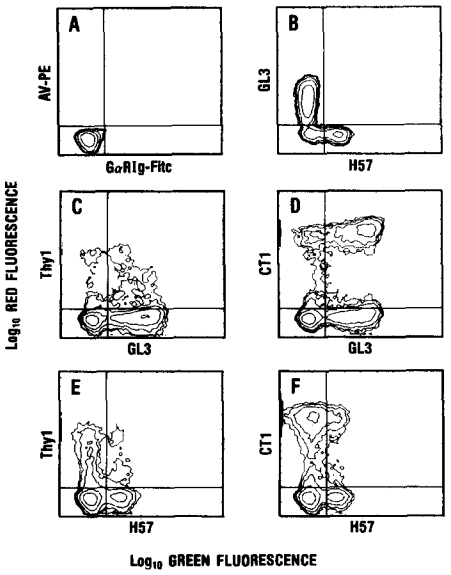

FIGURE 7. Thy-1 and CT1 expression are not linked to IEL TCR expression. IEL were analyzed by fluorescence flow cytometry after reaction with FITC-conjugated H57(anti-TCR-α/β) or GL3 followed by incubation with biotinylated GL3, anti-Thy-1, or CT1 as indicated.

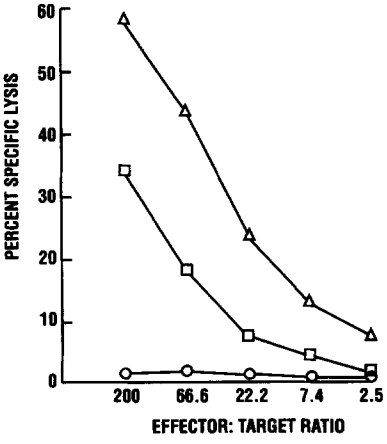

FIGURE 8. TCR-α/β^+ IEL are constitutively cytolytic. The redirected lysis assay (described in the legend to Fig. 2) was used to assay lytic activity of TCR-γ/δ^+ versus TCR-α/β^+ IEL. P815 target cells were preincubated with 2 μg/ml of GL3 (Δ), H57 (□), or with medium alone (○). Assay time was 5 h.

their anatomical site and specialized environment, rather than the type of TCR that was being used.

Since TCR-γ/δ^+ IEL were constitutively cytolytic upon isolation, we wished to determine if TCR-α/β^+ IEL also exhibited this characteristic. IEL were isolated and titrated into the redirected lysis assay with the addition of either GL3 or H57. As can be seen in Fig. 8, relatively high levels of lytic activity were detectable using GL3. Furthermore, H57 was also capable of redirecting lysis by IEL albeit at a lower level than GL3. This result is consistent with only 20% of IEL being $H57^+$ in this experiment. Similar results were obtained using several IEL preparations. It should be noted that $CD8^+$ peripheral T cells isolated by anti-CD8 panning are not lytic in this assay. These results suggest that the functional programming of IEL in vivo appears to be independent of TCR utilization.

Discussion

The results presented demonstrated marked heterogeneity in TCR-γ/δ of IEL. The TCR-γ/δ-specific mAbs that were generated divided IEL into at least three subpopulations: $GL1^+2^+$, $GL1^+2^-$ and $GL1^-2^-3^+$. We believe that the GL3 and GL4 mAbs react with all TCR-γ/δ and may be specific for a determinant present on Cδ (Table I). GL2 precipitates only TCRs containing the higher M_r δ chains, as well as both observed γ chains, and appears to be specific for a Vδ4 determinant (Table I, Figs. 3 and 4). The predominant γ chain mRNA present in IEL has been shown to contain the V_5 variable region sequence (Asarnow, D., L. Lefrancois and J. P. Allison, manuscript submitted for publication; and reference 16), although other Vγ protein mRNAs are also detected in IEL. We have also analyzed IEL with a Vγ3-specific mAb (39) and found that virtually no IEL expressed this receptor. In addition, Vγ2 expression was tested using a V2-specific mAb and <5% of B6 IEL were $V2^+$ (data not shown). Thus, the bulk of IEL appear to use V5-containing receptors.

Freshly isolated IEL are constitutively cytolytic. Yet, $CD8^+$ IEL do not possess natural killer or spontaneous cytotoxic abilities (15, 23). In this report we have shown that all of the subpopulations defined by our mAb were cytolytic, indicating that

a particular subset of IEL expressing certain TCRs were not solely responsible for the activity (Fig. 2). This point was further exemplified by the demonstration that IEL expressing TCR-α/β also displayed lytic ability, indicating that the intestinal milieu dictated the functional programming of IEL regardless of TCR utilization. Intriguing implications for the apparent dichotomy in TCR-α/β versus TCR-γ/δ expression were suggested by these results. Although murine IEL as a population were heavily skewed toward TCR-γ/δ^+-bearing cells (γ/δ:α/β was ~3:1 in B6 mice), common functional and phenotypic characteristics were observed for both populations. In particular, a large percentage of both γ/δ^+ and α/β^+ IEL were Thy-1$^-$.

What does this signify in terms of T cell differentiation? In the least, a distinct pathway of T cell trafficking into the IEL compartment would necessarily exist. It is also possible that some T cell differentiation occurs in situ in the gut mucosa. Since cytolytic IEL are Thy-1$^+$ but noncytolytic IEL are Thy-1$^-$ (25) it is tempting to speculate that Thy-1$^-$ IEL once activated differentiate into Thy-1$^+$ cells with cytolytic ability. Indeed, our preliminary results suggest that this phenomenon can occur in vitro in response to activation by anti-TCR mAb (Lefrancois, L., and T. Goodman, unpublished results). Whether the Thy-1$^-$ IEL arise from an extrathymic differentiation pathway or via the thymus as Thy-1$^+$ or Thy-1$^-$ precursors remains to be seen. Thy-1$^-$ TCR$^+$ thymocytes have not been described. It is also possible that seeding of the gut mucosa by T cells or their precursors occurs early in ontogeny and is not active in the normal, mature animal. In any case a mechanism for IEL precursors or their progeny to specifically home to the intestinal mucosa must exist. More precisely, IEL would require a mechanism for trafficking to the intestinal epithelium since other mucosal T cell populations such as lamina propria and Peyer's patch lymphocytes are not phenotypically similar to IEL (40). It is interesting to note that IEL do not express the MEL-14 lymphocyte homing receptor (23). It has also recently been demonstrated that a second type of homing receptor exists that is used by Peyer's patch lymphocytes, although it was not determined whether IEL expressed this receptor (41). Perhaps a third type of homing receptor is required for proper IEL trafficking. The question of IEL specificity remains to be answered. Although environmental stimuli can clearly influence the cytolytic activity and Thy-1 expression of IEL (25) the precise antigen(s) responsible for these effects are not known. However, in vivo IEL activation occurs over a relatively lengthy time period (2–4 wk), suggesting that colonization by intestinal bacterial flora may be responsible. Attempts at generating TCR-γ/δ^+ virus-specific IEL have thus far failed (data not shown). We are currently using flora-defined mice to assess the problem of IEL reactivity. The mAb described here should prove invaluable in further analyzing the ontogeny and function TCR-γ/δ^+ T cells.

Summary

The function and structure of the TCR proteins of intraepithelial lymphocytes (IEL) were examined using a panel of mAbs specific for TCR-γ/δ. Three subsets of TCR-γ/δ^+ IEL could be detected with five mAbs, termed GL1–GL5. The mAbs were able to trigger lysis via crosslinking of the IEL TCR and all of the subsets were constitutively cytolytic. Immunoprecipitation of IEL TCR proteins revealed that the GL2 mAb reacted only with γ, δ heterodimers containing high M_r δ chains, while the other mAbs precipitated all of the observed γ and δ proteins. Two-color

fluorescence analysis showed that the GL2$^+$ subset was contained within the larger GL1$^+$ subset. The GL3 and GL4 mAbs appear to be specific for all TCR-γ/δ while GL2 was Vδ4 specific. Analysis of IEL for TCR-α/β expression demonstrated that ~20% of B6 IEL were TCR-α/β^+. Interestingly, this population of IEL contained Thy-1$^-$ and CT1$^+$ cells, indicating that the unique phenotype of IEL was not restricted to TCR-γ/δ^+ cells. Moreover, the TCR-α/β^+ IEL were also constitutively cytolytic, suggesting that the intestinal milieu was controlling the functional programming of IEL regardless of TCR type. The mAbs reported here as well as the ability to exploit the distinct phenotype of IEL should prove useful in determining the function of IEL and the TCR-γ/δ.

We gratefully acknowledge the constant encouragement from and the enlightened discussions with Dr. Jeff Bluestone, University of Chicago.

Received for publication 2 May 1989 and in revised form 1 August 1989.

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