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CD8αα-Mediated Intraepithelial Lymphocyte Snatching of Thymic Leukemia MHC Class Ib Molecules In Vitro and In Vivo

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Thymic leukemia (TL) is a MHC class Ib molecule that interacts with CD8αα homodimers. CD8αα is abundantly expressed by intraepithelial T lymphocytes (IELs) located in close proximity to TL-expressing intestinal epithelial cells. In this study, we show that CD8αα+ IELs “snatch” TL from the plasma membrane of TL-expressing cells and express TL in its proper orientation on their own cell surface. TL snatching is enhanced by cross-linking of IEL TCRs in a phosphatidylinositol kinase-dependent manner, and results in overall alterations to the IEL cell surface detected by enhanced binding of peanut agglutinin lectin. Induction of bowel inflammation results in the presence of TL on IELs, probably via in vivo snatching, providing the initial evidence for the interaction of CD8αα IELs with intestinal cells. The Journal of Immunology, 2006, 177: 1590–1598.

M
ouse thymus leukemia (TL)3 Ag is a MHC class Ib molecule expressed primarily in small intestine epithelial cells, immature thymocytes in TL+ mouse strains, and activated mature T cells (1–3). The crystal structure of TL revealed that its putative ligand-binding groove is too narrow to accommodate peptides or other ligands (4). This corroborates with the stable folding of TL in the absence of added ligands (5–8). TL binds to CD8αα homodimers with unusually high affinity relative to the interaction of CD8αα or CD8αβ molecules with classical MHC class I molecules (5, 6). Based on this interaction, TL tetramers can be used to identify CD8αα-expressing cells by flow cytometry (7).

Incubation of intraepithelial T lymphocytes (IELs) with TL-expressing cells has been reported to 1) enhance IEL secretion of IFN-γ and 2) inhibit IEL cytotoxic activity and proliferation induced by treating IELs with Abs that cross-link the TCR complex (5), but the physiological relevance of these findings made ex vivo uncertain (6). The transient expression of CD8αα by activated T cells has been linked to the generation of memory CD8+ T cells, and based on the simultaneous appearance of expression of TL on APCs it was proposed that the CD8αα-TL interaction is responsible for the generation of memory cells (9). This cannot be an absolute requirement, however, because memory T CD8+ can develop normally in mice with targeted deletion of β2-microglobulin (β2m−/−), which is absolutely required for cell surface expression of functional TL (10).

Despite this recent progress in understanding TL function, its physiological importance remains enigmatic. Given the high levels of TL on the basolateral surface of small intestinal epithelial cells (2, 6) and their close proximity to CD8αα IELs, it seems likely that the interaction between IEL CD8αα and epithelial cell TL plays a key role in regulating the activity of IEL, whose functions remain equally enigmatic. In this study, we show that the in vitro interaction of CD8αα-expressing cells with TL-expressing cells results in the unidirectional acquisition of TL. We use this insight to obtain the initial evidence for the physiological importance of TL-CD8αα interaction between IELs and gut epithelial cells.

Materials and Methods

Mice

B6, BALB/c, β2m−/−, and IL-10−/− animals were purchased from Taconic Farms. CD8α−/− and CD8β−/− mice were provided by Dr. A. Singer (National Cancer Institute (NCI), National Institutes of Health, Bethesda, MD). Five- to 6-wk-old IL-10−/− animals were orally fed with the non-steroidal anti-inflammatory drug piroxicam mixed with powdered rodent chow (200 ppm; Sigma-Aldrich) for at least 4–5 wk.

Abs and flow cytometry

PCP-conjugated anti-CD8α, FITC-conjugated anti-CD4 and anti-Kd, PE-conjugated anti-CD8β, anti-TCRγ, and anti-CD3e, APC-conjugated anti-TCRβ, purified anti-CD3e, anti-CD8α, anti-CD8β, and anti-CD4 were purchased from BD Pharmingen-BD Biosciences. FITC-PNA (lectin peanut agglutinin) was purchased from Vector Laboratories. Alexa-647-conjugated anti-GFP was purchased from Molecular Probes. Anti-FcγII/III receptor CD16/CD32 mAb was produced from the supernatant of 2-4G2 hybridoma. Before all stainings, cells were treated with 2-4G2 Ab to block FcγR. Anti-TL mAb (HD168) was produced by Taconic Farms and labeled with Alexa Fluor 647 according to the manufacturer’s protocol (Molecular Probes). Intracellular staining was performed...
on surface-stained cells after fixation with 1% paraformaldehyde and permeabilization with 0.1% saponin. The cells were analyzed with a FACS Calibur (BD Biosciences).

Cell transfection

The full-length TL protein construct is described in Ref. 6. A mutant form of the full-length TL protein was constructed using directed mutagenesis by introducing a lysine at position AA253 instead of the original aspartic acid. We used two couples of oligomers: 5'-GGGCTACCTGGATGA CACTCAG-3' containing a unique NdeI site and 5'-TCTCCACAAGCTCT GTCTTTGG-3', which contained the mutation (underlined), and 5'- CGTGCTTTG-3' and 5'-CAGAAGACGGAGCTTGTGGAGA-3', which contained the mutation (underlined). The NdeI/BamHI fragment was synthesized and cloned back into the TL full-length DNA construct and was called TLm.

The full-length TL as well as the TLm DNA were subcloned in the pGFP-N1 vector (BD Clontech, BD Biosciences) so that the TL and TLm proteins were fused in frame with GFP, and were subsequently called TL-GFP and TLm-GFP, respectively. The murine CD8α-1 FLAG construct (tagged at the COOH terminus of the molecule) was a gift from R. Bosselut (NCI, National Institutes of Health, Bethesda, MD). The murine CCR5 (tagged at the COOH terminus of the molecule) was a gift from R. Bosselut.

Cell isolation

IELs and intestinal epithelial cells from the mice small bowel were isolated as described previously (11, 12). Lymph node (LN) lymphocytes were prepared from pooled inguinal, brachial, and axillary LNs.

FIGURE 1. Expression of TL before and after coculture with TL-transfected P815 cells. A. Freshly isolated IELs from a βm−/− (top row) or a B6 (bottom row) animal were stained with anti-CD8α and anti-TL Ab. Cells were gated on CD8α+ cell population. Ex vivo IELs: the cells were stained immediately after isolation. IELs+P815-TL: IELs were cocultured o/n with TL-transfected P815 cells in the presence or absence of anti-CD3 Ab before staining. B. B6 IELs were cocultured o/n with TL-transfected P815 in the presence of anti-CD3 Ab. Cells were stained with anti-TL Ab. Fluorescence geometric mean, 56.26 ± 4.89. C. Same as in B. Cells were stained with anti-K and anti-TL Ab. D. LN lymphocytes from a B6 animal were cocultured o/n with TL-transfected P815 cells in the presence of anti-CD3 Ab. Staining and gating were the same as in A. E. TL-GFP-transfected P815 cells were cocultured o/n with CD8α-transfected P815 cells. Cells were stained with anti-CD8α. The percentage of positive or negative cells is indicated. These data are representative of three (B6 IELs) and two (βm−/−) independent experiments.

Snatching assay

When indicated, 96-well plates (Corning) were treated with anti-CD3 mAb in PBS (overnight (o/n), 4°C, 1 μg/ml). Freshly isolated IELs were cocultured with P815 transfected with either TL, TLm, TL-GFP, TLm-GFP, CD8α-FLAG, CCR5-GFP (irrelevant) DNA constructs (ratio 1:1) or mock transfected for 4–16 h at 37°C (or for the indicated times in kinetics experiments) in RPMI 1640 containing 10% FCS in wells treated or not with anti-CD3 mAb. The cells were then stained with various mAbs.

Confocal microscopy

Freshly isolated IELs from B6 animals and TL-GFP-transfected P815 cells were layered separately on 3 ml of lymphocyte separation medium (Cambrex BioScience) to eliminate dead cells and debris. After centrifugation, the cell layer was harvested and washed. IELs and transfected cells were cocultured at 37°C as described above for 2 h. They were then put onto glass coverslips at the bottom of plastic chambers. After the cells had settled, they were observed by confocal fluorescence microscopy on a Leica SP2 (Leica). Final composites were constructed in Adobe Photoshop (Adobe).

Immunoelectron microscopy

Cells were fixed in 4% paraformaldehyde/0.05% glutaraldehyde (EMS) in 0.1 M phosphate buffer, washed in 0.1 M phosphate buffer, and then incubated at 37°C in 10% gelatin. A pellet was formed by centrifugation. The sample was placed in ice to solidify. The pellet was cut at 4°C into small cubes infiltrated with 2.3 M sucrose in 0.1 M phosphate buffer and frozen on pins in liquid nitrogen. The pins were stored in liquid nitrogen. Ultracryosections were cut on a Leica Ultracut FCS microtome and picked up in a solution of 2.3 M sucrose and 2% methyl cellulose (50:50) on a loop and dropped on formvar/carbon-coated grids then placed on 2% gelatin on ice. After melting the gelatin, the sections...
Cells were stained with anti-TL Ab. The data in A were then added, and the cells were stained with anti-CD8 Ab after 4-h incubation with P815 cells (Fig. 1B). Incubation of IELs with nontransfected P815 cells or P815 cells transfected with a control gene failed to result in TL-stained IELs (data not shown), suggesting that TL was obtained from TL-P815 cells. To test this idea, we incubated TL-P815 cells with IEL from βm<sup>-/-</sup> mice. This revealed that TL expression by IELs does not require IEL β<sub>m</sub> synthesis, which indicates that IEL must obtain TL from P815-TL cells (Fig 1, A and B).

Multicolor flow cytometry indicated that virtually all TL<sup>+</sup> IEL are CD8α<sup>+</sup> and express either αβ or γδ TCRs (data not shown). Activation of IELs by coculturing with TL-P815 cells in wells coated with anti-CD3 mAb increased the fraction of TL-expressing IELs by ~1.7-fold, without increasing the amount of TL expressed per cell (Fig. 1B). Anti-CD3-mediated activation of IELs resulted in a similar increase in the number of viable IELs recovered following overnight culture, suggesting a relationship between TCR-mediated signals resulting in TL-transfer and increased IEL survival.

To examine the specificity of TL transfer to IELs from P815 cells, we stained cocultured anti-CD3-activated CD8<sup>+</sup> IELs for the classical MHC class I molecule K<sub>4</sub>, which is expressed by P815 cells (Fig. 1C). Although K<sub>4</sub> is expressed in greater amounts than TL on TL-P815 cells (in terms of staining intensity were quenched in 0.02 M glycine in 0.1 M phosphate buffer, blocked in 0.1% fish skin gelatin in 0.01 M phosphate buffer (Sigma-Aldrich), and incubated with anti-GFP rabbit polyclonal Ab (Abcam). The sections were then washed in 0.1% fish skin gelatin in 0.01 M phosphate buffer and final washes were in deionized, filtered water. The sections were stained in 9:1 2% methyl cellulose (Sigma-Aldrich) to 2% uranyl acetate (EMS) and picked up by loops to air dry. Sections were imaged using a FEI CM100 transmission electron microscope.

**P13K inhibition**

Freshly prepared IELs from B6 animals were pretreated for 45 min with 1 μM wortmannin (Calbiochem) at 37°C. TL-GFP-transfected P815 cells were then added, and the cells were stained with anti-CD8α Ab after 4-h coculture.

**Results**

**IELs acquire TL from TL-P815 cells via interaction with CD8αα**

In the course of studying the functional consequence of CD8αα-TL interaction (6), we observed that CD8αα IELs from B6 mice, which fail to express TL (Fig. 1A), became TL-positive after overnight incubation with P815 cells transfected with TL (TL-P815) (Fig. 1B).
with the mAbs used), we detected Kd on only a very small percentage of IEL, virtually none of which were TL+. This demonstrates that TL transfer is not accompanied by the wholesale transfer of P815 cell surface molecules. In the same experiment, we found that LN CD8+ T lymphocytes failed to acquire the TL molecules from P815 cells (Fig. 1D). Because LN T cells do not express CD8α, this suggests that TL transfer is based on its interaction with CD8α. Consistent with this idea, TL is transferred between P815 cells by simply incubating TL-P815 cells with P815 cells transfected with CD8α (Fig. 1E). Additional experiments revealed that TL can also be transferred to IELs from 293 or BMA cells expressing TL following transfection with the TL-expression plasmid (data not shown).

The role of CD8 in transfer of TL from P815 cells to IELs was further explored by using IELs from mice with targeted disruption in CD8α or CD8β gene. This revealed that TL transfer required expression of CD8α but not CD8β (Fig. 2A). Consistent with this finding, TL transfer to B6 IELs was blocked by addition of a CD8α-specific mAb but not CD8β- or CD4-specific mAbs (Fig. 2B). Finally, we incubated IELs with P815 cells transfected with a gene encoding TL with a mutation in the CD8 binding site that abrogates interaction with CD8α (TLm) (Fig. 2C). Despite being expressed at similar levels to wild-type TL on P815 cells (data not shown), TLm is not acquired by IELs.

Based on these findings, we conclude that IEL cell surface CD8α mediates the acquisition of TL from the surface of TL-expressing cells. In none of the experiments described above did we observe the acquisition of CD8α by TL-expressing cells as measured by flow cytometry using anti-CD8 mAb (data not shown). Thus, the transfer interaction of CD8α with TL results in TL snatching, i.e., the unidirectional transfer of TL to CD8α-bearing cells in the absence of indiscriminate transfer of other cell surface proteins, as indicated by the lack of Kd transfer.

Cell biology of TL snatching

To facilitate further study of TL snatching, we genetically conjoined the COOH terminus of TL to enhanced GFP and inserted the TL-GFP gene into an expression plasmid that we used to transfect P815 cells. After adding B6 or BALB/c IELs to TL-GFP-P815 cells, we quantitated transfer of TL-GFP to CD8α+ IELs by directly measuring GFP fluorescence via flow cytometry (Fig. 3A). This revealed that TL transfer can be detected within 30 min of cell mixing in a few percentage of IELs, increases nearly linearly for the next 4.5 h, and then less rapidly over the next 11 h. Anti-CD3 activation of cells increases the fraction of TL-snatching IELs.

Fig. 3B shows the mean fluorescence of TL+ IELs. This reveals that the number of TL molecules snatched by positive cells increases only slightly with time and is not greatly influenced by anti-CD3-mediated activation. These data suggest that snatching is largely a quantal event: IELs acquire TL-GFP from TL-GFP-P815 cells during a single interaction that results in the transfer of a substantial number of molecules. IEL activation increases the number of cells capable of snatching, without increasing the number of molecules snatched or the number of times that snatching can occur.

We next used anti-GFP Abs to examine the topology of TL-GFP snatched by IELs. Staining of TL-GFP-P815 cells with anti-GFP resulted in preferential cell permeabilization, consistent with the predicted cytosolic topology of GFP (data not shown). Importantly, anti-GFP staining of the vast majority of TL-GFP+ IELs also required permeabilization (Fig. 4, compare A and B). Because the TL extracellular domain is stained on live cells by TL-specific mAbs following snatching, these findings indicate that during the process of snatching, the orientation of TL in the plasma membrane is maintained.

The quantal nature of snatching noted in the kinetic experiment was most consistent with the direct interaction of IELs and TL-P815 cells. In agreement with this idea, snatching did not occur when IELs were separated from TL-P815 by a 0.4-μm filter (data not shown). To visualize snatching, we performed real-time confocal microscopy of TL-GFP-P815 cells incubated with CD8α+ IELs (Figs. 5 and 6).4 TL-GFP is predominantly located at the plasma membrane of P815 cells (Fig. 5A). Attachment of an IEL to a TL-GFP-P815 cell resulted in the concentration of a “plate” of TL-GFP at the site of attachment in some (L in panels A and B) but not all encounters (L* in panel B). We routinely observed lymphocytes no longer in contact with P815 cells that displayed a plate of TL-GFP similar in dimensions to that observed at the point of contact between IELs and TL-GFP-P815 (Fig. 5C), indicating that TL-GFP remains in an organized membrane domain following transfer. In

4 The online version of this article contains supplemental material.
that inhibits the function of phosphatidylinositol kinases, most induced by TL snatching. Wortmannin is a fungal metabolite to the induction of TL snatching and the alterations in IEL function.

We next examined the biochemical signals in IEL that contribute shown). A rotable three-dimensional reconstruction of the P815 cell is shown. 4 right cell (P) in contact with two IELs showed that IEL on the left (L) had higher intensity of GFP at the contact area. However, contact area with the right IEL (L*) showed normal intensity. Time-lapse data of this area demonstrated that L, but not L*, made a stable interaction with P (data not shown). A rotatable three-dimensional reconstruction of the P815 cell is shown. 4 C, An IEL with highly localized plasma membrane GFP.

FIGURE 5. Visualization of TL-GFP in cocultures of B6 IELs and TL-GFP-transfected P815 cells by confocal microscopy. A, Purified IELs were incubated with purified TL-GFP-transfected P815 cells for 2 h at a ratio of 3:1 before visualization of GFP via confocal microscopy. Panels show GFP autofluorescence overlaid on the transmitted light image collected with differential interference contrast optics. In a typical TL-GFP-transfected P815 cell (P), GFP is largely concentrated at the plasma membrane, as demonstrated by its centripetal distribution. GFP was also present in filopodia and/or pseudopodia (data not shown). IEL on the right (L) showed no fluorescence, except at the interface with P815 cells. (Note: IELs are normally smaller than P815 cells and easily distinguished by their morphology and motility from nontransfected P815 cells.) The contact area between the P and L demonstrated higher intensity of GFP expression compared with the noncontact area of transfected-P815 cell. B, A TL-GFP-transfected P815 cell (P) in contact with two IELs showed that IEL on the left (L) had higher intensity of GFP at the contact area. However, contact area with the right IEL (L*) showed normal intensity. Time-lapse data of this area demonstrated that L, but not L*, made a stable interaction with P (data not shown). A rotatable three-dimensional reconstruction of the P815 cell is shown. 4 C, An IEL with highly localized plasma membrane GFP.

one instance, we could visualize the transfer of TL-GFP, which occurred as the IEL retracted from the TL-GFP-P815 cell over a 20-min period (Fig. 6.

To characterize snatched TL-GFP, we performed cryoimmunoelectron microscopy on IELs isolated by cell sorting following their incubation with TL-GFP-P815 cells. TL-GFP was localized by indirect staining with rabbit anti-GFP Abs followed by colloidal gold conjugated to anti-rabbit IgG Abs. Specific intense localized staining of a low percentage of cells was clearly demarcated from the nonspecific scattered gold particles observed for all cells (Fig. 7). These positive cells demonstrated one to three densely stained regions of the plasma membrane of similar dimension to the fluorescent-snatched patches observed on IELs by confocal microscopy (~1 μ). The plasma membrane containing the gold particles appeared normal (see inset in Fig. 7C for high magnification view). The staining observed was not due to contamination with TL-GFP-P815 cells, which displayed uniform gold staining along the plasma membrane (data not shown).

These results eliminate the possibility that TL is transferred via vesicular structures (e.g., exosomes) released from P815 cells that bind in an intact form to IELs. Rather, in conjunction with the real-time confocal microscopy, the ultrastructural analysis supports the conclusion that TL molecules are physically removed from the transfected cell surface as a relatively large intact membrane domain that is integrated into the IEL plasma membrane in a manner that resists diffusion and dispersion of TL molecules. This resembles the previously described process of trogocytosis (13) in which T cells and NK cells acquire plasma membrane fragments from the region of their interaction with APCs or target cells.

TL snatching requires signal transduction and results in cell surface alterations in CD8α+ IELs

We next examined the biochemical signals in IEL that contribute to the induction of TL snatching and the alterations in IEL function induced by TL snatching. Wortmannin is a fungal metabolite that inhibits the function of phosphatidylinositol kinases, most prominently PI3K, a ubiquitous lipid kinase involved in receptor signal transduction by tyrosine kinase receptors. We pretreated freshly isolated IELs with the wortmannin before 4-h coculture with TL-GFP P815 cells in the presence or absence of anti-CD3, and used flow cytometry to evaluate TL snatching by CD8α+ IELs (Fig. 8A). Wortmannin treatment resulted in a 50% decrease in the number of CD8α+ TL+ cells in cultures with or without anti-CD3 mAb. This effect could not be attributed to decreased IEL viability, which was not reduced by wortmannin treatment. These data suggest that TL snatching is regulated by a PI3K signaling mechanism that increases the snatching capacity of the CD8α+ IELs.

To examine the possible effects of TL snatching on IEL function, we screened a number of known activation markers, including the PNA, which binds to nonsialylated O-linked glycans on several cell surface proteins, including CD8 itself. We incubated CD8α+ IELs and TL-GFP-transfected- or mock-transfected P815 cells in the presence and absence of anti-CD3 for 4 h and measured PNA binding to CD8α+ TL+ and CD8α− TL+ IELs. This revealed that TL+ CD8α+ IELs bound 25% more PNA than TL− CD8α+ IELs (Fig. 8, B and C). PNA binding was not significantly affected by anti-CD3 activation.

We cannot account for increased PNA binding strictly by selection of the highest PNA binding cells for snatching. Rather, at least part of the increase must be due to the increased expression of a PNA-binding ligand, because there was an absolute increase in the PNA binding by the highest binding cells indicated by a shift in the leading edge of the histogram. Nor can we account for the increase in PNA binding by a simple increase in CD8α expression, which was nearly identical in TL+ vs TL− cells. Thus, the increase is likely to be due to enhanced expression of other PNA-binding glycoproteins. In any event, the increased binding of PNA demonstrates that even during this relatively short period (4 h) of the assay, TL snatching is associated with biochemical alterations of the IELs that obtain TL. Notably, increases in PNA binding has been previously linked to alterations in the activation status of T cells (14).
Gut inflammation induces TL snatching in vivo

Interestingly, though we do not detect TL on IELs prepared by the method that results in the greatest recovery and purity of IELs (e.g., see Fig. 1), we do find that 15–20% of IELs that contaminate small intestinal epithelial preparations do express cell surface TL (Fig. 9A). Given the clear ability of IELs to acquire TL by snatching, it is likely that this process contributes to TL expression by these IELs, and may completely account for its expression. Snatching may occur during or after the initiation of the isolation procedure, because this would be consistent with the absence of TL on IELs prepared by the protocol that separates them at an early stage from epithelial cells.

Indeed, the absence of TL on B6 and BALB/c IELs that have been separated rapidly from epithelial cells suggests that TL snatching does not occur with high frequency under conditions in which mice are maintained in a disease-free environment. To explore the effect of an acute gut inflammation on IEL snatching of TL, we used a model system in which B6 mice with a targeted disruption of the IL-10 gene (IL-10−/−) develop spontaneous chronic inflammatory bowel disease characterized by increased inflammatory cytokine production as well as IFN-γ from Th1 CD4+ T cells (15, 16). This process is hastened by treating mice with piroxicam, a nonsteroidal anti-inflammatory drug (17).

Histological sections of small intestine of IL-10−/− mice treated for 4 wk with piroxicam demonstrated the presence of cellular infiltrates and thickening of the epithelial lining (data not shown). No pathological alterations were detected in untreated animals (data not shown). IELs from untreated IL-10−/− animals contained a small percentage of CD8+TL+ cells (Fig. 9, −piroxicam). Treating mice with piroxicam tripled the number of TL+ IELs without altering the amount of TL present on each cell (Fig. 9B, +piroxicam).

Discussion

We have demonstrated that IELs acquire cell surface TL from donor cells based on the interaction of TL with IEL CD8+ cells. Expression of CD8α by P815 cells was sufficient to enable TL snatching by these cells, suggesting that specialized accessory molecules are not required for snatching. In contrast, P815 cells were less adept than IELs at snatching, indicating that IELs possess features that facilitate snatching. Real-time observation of IELs interacting with TL-P815 cells revealed that IELs could maintain prolonged intimate contact with TL-transfected cells and still fail to acquire TL. Thus, snatching requires more than simple contact between TL and CD8α on their respective cells. Indeed, only a subset of CD8α+ IELs is capable of snatching, suggesting that snatching is regulated by IELs.

Consistent with this possibility, we found that activation of IELs by TCR cross-linking increased the fraction of cells capable of snatching, and further that snatching could be partially inhibited by treating IELs with the PI3K inhibitor wortmannin, which also interfered with the enhancing effects of TCR cross-linking. Thus, specific phosphorylation events that both precede and result from TCR-based activation may play a key role in facilitating the snatching mechanism.

Our real-time observations of snatching show that the region of contact between IELs and TL-P815 cells resemble the immunological synapse (18), with TL clustering at the cell contact area (Fig. 5 and footnote 4). It will be of interest in future studies to examine the distribution of CD8α at cellular contact site, as well as the distribution of other molecules that might participate in the interaction, such as cellular adhesion molecules, cytoskeletal elements, and cytosolic signal transduction elements.

TL snatching bears a number of striking similarities to trogocytosis, the capture of APC molecules present at the immune synapse formed with T, B, and NK cells (13). In both processes, the
following occurs: 1) transfer of material from targets cells is uni-directional—immunocyte surface molecules at the immune synapse are not known to be transferred to the APC, and we failed to detect transfer of CD8αα or TCRs to TL-P815 cells; 2) transferred molecules maintain their normal topology following transfer; and 3) transfer is based on the interaction of receptor tyrosine kinases with ligands (TCR and activating NK receptors in trogocytosis, CD8αα in snatching) and is inhibited by wortmannin and other kinase inhibitors.

IELs are not known to be transferred to the APC, and we failed to detect transfer of P815 Kd molecules to IELs. This transfer of many species of molecules from target cells, whereas we failed to detect transfer of P815 Kd molecules to IELs. This difference may simply reflect an absence of Kd from the region of IEL-P815 cell contact. Further characterization of the P815 molecules present at the site of contact will enable a directed investigation of the specificity of the transfer process. Second, one of the most unusual features of TL snatching is the persistent presence of TL in highly restricted regions of the plasma membrane of IELs and no detectable internalization or redistribution. By contrast, MHC class I and II molecules acquired by trogocytosis are typically internalized rapidly into endosomal compartments where they are susceptible to proteolytic degradation. This difference may not, however, reflect basic differences between snatching and trogocytosis but rather special features of TL molecules or IELs. Unlike MHC class I or class II molecules, the TL cytoplasmic domain lacks obvious internalization sequences. The patchiness of snatched TL in the IEL membrane indicates that diffusion of transferred TL is highly limited. This may be due to direct interactions between transferred TL molecules. Alternatively, IELs may somehow sequester the acquired plasma membrane. To our knowledge, this mechanism would be unprecedented, and therefore would be of great interest and importance to characterize and understand. These possibilities can be discriminated by fluorescence recovery after photobleaching experiments comparing the properties of TL-GFP to other integral membrane proteins on the surface of IELs.

It is noteworthy that TL expression by IELs examined immediately ex vivo from healthy mice depends on the method of IEL preparation. Although we did not detect TL on purified IELs and lamina propria lymphocytes (data not shown), TL is clearly present on IEL that contaminate epithelial cell preparations (Fig. 9A). It was previously reported that IELs express TL mRNA (2). We believe, however, that this is unlikely to be due to bona fide transcription of TL mRNA by IELs. Although we could confirm TL-encoding mRNA is present in “contaminating” TL-expressing lymphocyte preparations (after sorting IELs away from epithelial cells by flow cytometry), such preparations contain even higher amounts of mRNA-encoding E-cadherin, an epithelial cell marker gene product (J. Gibbs and N. Pardigon, unpublished observations). This suggests that TL mRNA present in IEL preparations is a contaminant derived from epithelial cells. We think that it is far more likely that TL-expressing IEL coisolated with IELs obtain TL via snatching due to their more intimate contact with epithelial cells, which may occur either in vivo or ex vivo during sample preparation.

Extending this conclusion, we believe that by inducing intestinal inflammation, IELs normally sequestered from epithelia cells can now interact intimately with these cells and acquire TL via snatching. Inflammation may also induce alterations in IEL (such as those we document following TCR ligation in vitro) and/or epithelial cell physiology that enhance TL snatching. In either event, our findings provide the initial evidence for the functional interaction of IELs with TL-expressing epithelia.

Presumably, TL snatching by IELs modifies their function in some useful way. We found that snatching is associated with increased binding of the lectin PNA, which binds to a highly limited set of glycoproteins (20). This is unlikely to be due to PNA binding to TL itself, because TL expression on P815 cells is not itself associated with increased PNA binding. The extent to which this represents remodeling of pre-existing PNA-binding oligosaccharides vs expression of new PNA-binding glycoproteins (or glycolipids) associated with the snatching process remains to be established. In either event, whatever is binding PNA may alter the function of IELs in some manner.

Indeed, snatched TL itself may alter IEL function by enabling IEL to interact with CD8αα-expressing cells, such as IEL themselves, activated T cells, or the CD8αα dendritic cells. A possible
role of TL on IELs might be to alert other immune cells of problems with the epithelium (e.g., pathogen breach or inflammation). Alternatively, TL on the IEL surface may modulate signals by interfering with CD8α/β coreceptor and/or TCR/CD3 complex signaling.

In conclusion, IEL snatching of TL provides the most direct evidence to date for the functional interaction of IELs with small intestinal epithelial cells. We believe that further analysis of TL snatching will provide critical insight into the functions of TL and IELs, enigmatic constituents of the immune system, which have surrendered their secrets with great reluctance.

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Disclosures
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FIGURE 8. Modulation of snatching and IEL function. A, TL snatching is partially inhibited by wortmannin. Freshly isolated IELs from B6 animals were pretreated for 1 h with 1 µM wortmannin before coculture with TL-transfected P815 cells for 4 h in the presence (+anti-CD3) or absence (−anti-CD3) of anti-CD3 Ab. The results are expressed in percentage of CD8α−TL− cells. B, CD8α−TL− IELs show increased binding to PNA. IELs from B6 animals were cocultured with TL-transfected or mock-transfected P815 cells for 4 h in the absence (top row, anti-CD3) or presence (bottom row, +anti-CD3) of anti-CD3 Ab. The samples were stained with PNA, anti-CD8α, and anti-TL Ab. Staining with anti-CD8α and PNA of TL− (black profile line) and TL+ (solid gray profile) cell subpopulations are presented. C, Same experiment as in B. Mean fluorescence level for PNA staining was determined for CD8α−TL− (□) and CD8α−TL+ (■) IEL subpopulations cocultured with TL-transfected P815 cells, and for CD8α+ (▲) IEL subpopulation cocultured with mock-transfected P815 cells. The results are representative of two independent experiments.

FIGURE 9. TL is present on IELs that contaminate epithelial preparations and on IELs from animals with chronic gut inflammation. A, IELs from epithelial preparations display TL on their surface. Freshly isolated epithelial cells from B6 animals were stained with anti-CD8α and anti-TL Abs. B, IELs from IL-10−/− animals treated with piroxicam have more TL molecules at their surface. Freshly isolated IELs from IL-10−/− animals treated (+piroxicam) or not (−piroxicam) with piroxicam were stained with anti-CD8α and anti-TL Ab. The percentage of each cell subpopulation is indicated. The data are representative of two independent experiments.

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