

Acknowledgements

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Intraepithelial lymphocytes: their shared and divergent immunological behaviors in the small and large intestine

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Summary: At the front line of the body's immunological defense system, the gastrointestinal tract faces a large number of food-derived antigens, allergens, and nutrients, as well as commensal and pathogenic microorganisms. To maintain intestinal homeostasis, the gut immune system regulates two opposite immunological reactions: immune activation and quiescence. With their versatile immunological features, intraepithelial lymphocytes (IELs) play an important role in this regulation. IELs are mainly composed of T cells, but these T cells are immunologically distinct from peripheral T cells. Not only do IELs differ immunologically from peripheral T cells but they are also comprised of heterogeneous populations showing different phenotypes and immunological functions, as well as trafficking and developmental pathways. Though IELs in the small and large intestine share common features, they have also developed differences as they adjust to the two different environments. This review seeks to shed light on the immunological diversity of small and large intestinal IELs.

Keywords: intraepithelial T lymphocyte (IEL), small and large intestines, development trafficking, classical and non-classical MHC

Introduction

Mucosal surfaces of the gastrointestinal and respiratory tracts directly interact with the mucosal lumen, the harshest environment in our body and one that is constantly exposed to many foreign antigens, including food nutrients, allergens, and commensal and pathogenic microorganisms. To protect mucosal sites from these foreign materials and to maintain mucosal homeostasis, the aerodigestive tract is equipped with multiple physical, biological, and immunological barriers.

The acquired-type immunological barrier at the mucosal surface is initiated by the induction of antigen-specific immune responses through mucosa-associated lymphoid tissues (MALT) including the Peyer's patches (PPs), isolated lymphoid follicles (ILFs), and the nasopharynx-associated lymphoid tissues (1, 2). MALT are covered with a follicle-associated epithelium, which

contains antigen-sampling M (microfold) cells that allow for selective transport of antigens from the lumen to underlying antigen-presenting cells such as dendritic cells (DCs) and macrophages (3). These cells present the antigen to T and B cells in MALT, rendering them antigen-primed T and immunoglobulin A (IgA)-committed B cells, respectively. These T and B cells then migrate to effector tissues (e.g. intestinal epithelium, *lamina propria*, and nasal passages) via an immunological intranet known as the common mucosal immune system (CMIS). Because these anatomical and functional characteristics enable MALT to act as inductive tissues for the priming of antigen-specific T- and B-cell responses, they have often been made the target for vaccine delivery (4).

Several physical and biological barriers associated with the innate immune system also protect these sites from microbial invasion and help to maintain their mucosal homeostasis. Closely knit to one another via tight-junction proteins like occludins, claudins, and zonula occludens (5, 6) and characterized by brush-border microvilli as well as a dense mucin layer at the apical site, epithelial cells (ECs) physically bar the entry of pathogenic microorganisms by inhibiting attachment and penetration (7). In addition, they produce antimicrobial peptides such as a β -defensin (7). In addition to these antimicrobial peptides, secretory IgA (S-IgA), the predominant isotype at mucosal sites, is secreted and plays an important role in preventing pathogen invasion (4, 8). S-IgA forms a J-chain-mediated polymeric structure that interacts with the polymeric Ig receptor expressed on mucosal ECs, an interaction that is required for their transport into the lumen (9, 10). S-IgA contributes to both acquired and innate immunity. Acquired immunity, principally mediated by B2 B cells, and innate immunity, mediated by B1 B cells, both play equally important roles in S-IgA production in the murine intestinal *lamina propria* region (11). The B2 B-cell-mediated S-IgA is mainly derived from the CMIS and thus plays a key role in the recognition of T-dependent antigens, while B1 B cells produce antibodies to T-independent antigen, such as phosphorylcholine, a hapten-like antigen associated with many pathogenic bacteria (1, 2, 12). Based on the indiscriminating reactivity of B1 B-cell-derived S-IgA against commensal and pathogenic microorganisms (1, 2, 12), it has been generally considered that B1 B-cell-derived S-IgA plays a pivotal role in the prevention of attachment of both commensal and pathogenic microorganisms.

Intraepithelial lymphocytes (IELs) play an important role in the maintenance of mucosal homeostasis by actively or negatively regulating mucosal innate and acquired immunity. Residing as single cells among ECs, they monitor for stressed

or damaged ECs and express $\alpha\beta$ T-cell receptors (TCRs) or $\gamma\delta$ TCRs, which recognize antigenic peptides presented by conventional major histocompatibility complex (MHC) molecules (13) or by non-classical MHC molecules, respectively (14). In addition to the uniqueness of TCR expression, the developmental pathway and immunological functions of the IELs render them distinctive. Although most previous studies focused on IELs mainly in the small intestine, several lines of evidence have demonstrated that immunological and biological differences between the small and large intestine lead to differences in composition of small and large intestinal IELs as well. This review describes and discusses those features that IELs from the two environments share and those that make them distinct.

IEL subsets in the small and large intestines

Type a and type b IELs in the small and large intestine IELs are interspersed among ECs in both the small and large intestine, but their frequencies vary, with one IEL for every 4–10 ECs in the small intestine and for every 30–50 ECs in the large intestine (15). In both the small and large intestine, IELs mainly consist of T cells. Two unique characteristics of the IELs allow them to be divided into subsets. First, IELs contain cells expressing the homodimeric form of CD8 α (CD8 $\alpha\alpha$), which is only barely detectable in the systemic immune compartments. Second, IELs contain more cells expressing $\gamma\delta$ TCRs than do peripheral T cells, which almost exclusively express $\alpha\beta$ TCRs. These unique features allow us to divide IELs into two groups: 'type a', which is also detectable in the blood, lymph, and secondary lymphoid organs including PPs, and 'type b', which is far more prevalent in the mucosal epithelium. Type a mucosal T cells express $\alpha\beta$ TCRs with CD4 or CD8 α , while type b IELs express $\alpha\beta$ TCRs or $\gamma\delta$ TCRs with a unique coreceptor, CD8 $\alpha\alpha$, or lack CD8 and CD4 altogether [double negative (DN)]. In addition to the uniqueness of CD8 $\alpha\alpha$ and $\alpha\beta/\gamma\delta$ TCR expression, type b IELs can be distinguished from type a IELs because they lack some markers, such as CD2 (16), CD28 (17), cytotoxic T-lymphocyte antigen-4 (18), and Thy-1 (19). Although small and large intestines contain both type a and type b IELs, the ratio between type a and type b IELs differs markedly (Table 1). The small intestine is rich in CD8 $\alpha\alpha$ IELs, while the large intestine contains very few. For example, 65–75% of the IELs in the small intestine of BALB/c mice are type b IELs (60% are CD8 $\alpha\alpha$, 10% are DN) (Table 1). Because about 60–70% of small intestinal type b IELs were $\gamma\delta$ TCR⁺, 40% of the total IELs in the small intestine can be assumed to be $\gamma\delta$ TCR⁺ (Table 1). In contrast, CD8 $\alpha\alpha$ IELs represent only a minor

Table 1. Different composition of IELs in the small and large intestine*

T cell subsets	Small intestine (total cell number $5.4 \pm 1.4 \times 10^6$ cells), %		Large intestine (total cell number $4.3 \pm 1.8 \times 10^5$ cells), %	
	Among total IELs	Among the subset	Among total IELs	Among the subset
CD8 $\alpha\alpha$	62.7 \pm 2.5		4.7 \pm 0.6	
$\alpha\beta$ TCR		35.7 \pm 3.1		67.3 \pm 2.5
$\gamma\delta$ TCR		64.0 \pm 6.5		32.7 \pm 2.5
No TCR		N.D.		N.D.
CD8 $\alpha\beta$	15.6 \pm 2.0		7.3 \pm 1.2	
$\alpha\beta$ TCR		84.6 \pm 3.1		95.6 \pm 0.6
$\gamma\delta$ TCR		N.D.		N.D.
No TCR		15.3 \pm 3.0		4.3 \pm 0.6
CD4	9.0 \pm 1.7		31.0 \pm 5.6	
$\alpha\beta$ TCR		87.3 \pm 2.1		98.6 \pm 0.6
$\gamma\delta$ TCR		N.D.		N.D.
No TCR		12.7 \pm 2.1		1.7 \pm 0.6
DP	7.3 \pm 0.6		<0.1%	
$\alpha\beta$ TCR		52.0 \pm 6.1		N/A
$\gamma\delta$ TCR		2.0 \pm 1.0		N/A
No TCR		46.1 \pm 5.3		N/A
DN	5.4 \pm 1.2		57.3 \pm 5.2	
$\alpha\beta$ TCR		2.7 \pm 0.6		5.3 \pm 0.6
$\gamma\delta$ TCR		20.7 \pm 3.1		4.3 \pm 0.6
No TCR		76.6 \pm 3.2		90.4 \pm 1.0

N.D., not detectable; N/A, not applicable; type b IEL in italics.

*The data were obtained from female BALB/C mice (7–10 weeks) and represent means \pm SD ($n = 5$).

population in the large intestine (Table 1). Alternatively, the percentage of DN IELs in the large intestine is higher than in the small intestine (60% in the large intestine, 15% in the small intestine) (Table 1).

Unique features of type a IELs

Although type a IELs exclusively express $\alpha\beta$ TCRs and the coreceptors CD4 or CD8 $\alpha\beta$, traits typical of peripheral T cells, they also differ from conventional T cells in a number of ways. For instance, the ratio of CD8 $\alpha\beta$ to CD4 in the small intestinal IELs is much higher than in the spleen, while the ratio in the large intestine is similar to that in the spleen. Additionally, in the small but not the large intestine, CD8 $\alpha\alpha$ expression can be coexpressed by CD8 $\alpha\beta$ (20) and CD4 (21) conventional mucosal T cells as well as by single-positive IELs.

Natural-killer-like cells in the intestinal epithelium

An additional unique feature of IELs is the ability of some, the so-called natural killer T (NKT) IELs (22–24), to express NK receptors. The ligand for one of these NK receptors, NKG2D, is the human non-classical MHC molecule MICA/B (MHC class I-chain-related gene A/B), which is predominantly expressed on damaged or transformed ECs (25). Interestingly, $\gamma\delta$ TCR recognizes the same MICA/B molecules (26, 27), implying that IELs can use both $\gamma\delta$ TCR and NKG2D to recognize damaged or stressed ECs through MICA.

Also present in the IEL population are TCR⁻ NK cells whose characteristics differ from those of splenic NK cells (22, 23, 28). Our group previously demonstrated that the cytotoxic effects of NK IELs were enhanced by interleukin-15 (IL-15) (28). IL-15 has also been reported to regulate NKG2D (29) as well as MICA expression (30). Furthermore, it has recently been reported that IL-15 induces CD94 expression and interferon- γ (IFN- γ) and IL-10 production from IELs, allowing them to show Fas-ligand-mediated killing activity (31). Although little has been reported about NK or NKT IELs in the large intestine (24), it is likely that these NK-like IELs directly and/or indirectly interact with ECs for the maintenance of intestinal homeostasis.

Recent thymic emigrants are a novel subclass of IELs. Staton et al. (32) have reported that a naive population in IELs is made up of CD8⁺ recent thymic emigrants (RTEs). In general, naive T cells migrate into the intestine after activation in the gut-associated lymphoid tissue (GALT) [e.g. PPs and mesenteric lymph nodes (MLNs)], but RTEs are distinguished by the ability to migrate into the small intestine without activation (32) (Fig. 1). Unlike naive T cells in the periphery, RTEs exclusively express $\alpha 4\beta 7$ integrin, αE integrin, and CCR9, making them gut-tropic T cells (33) (Fig. 1). After migrating directly into the intestinal epithelium from the thymus, they begin to proliferate in response to antigen exclusively present in the gut and to show a phenotype similar to that of resident IELs (32). Based on the

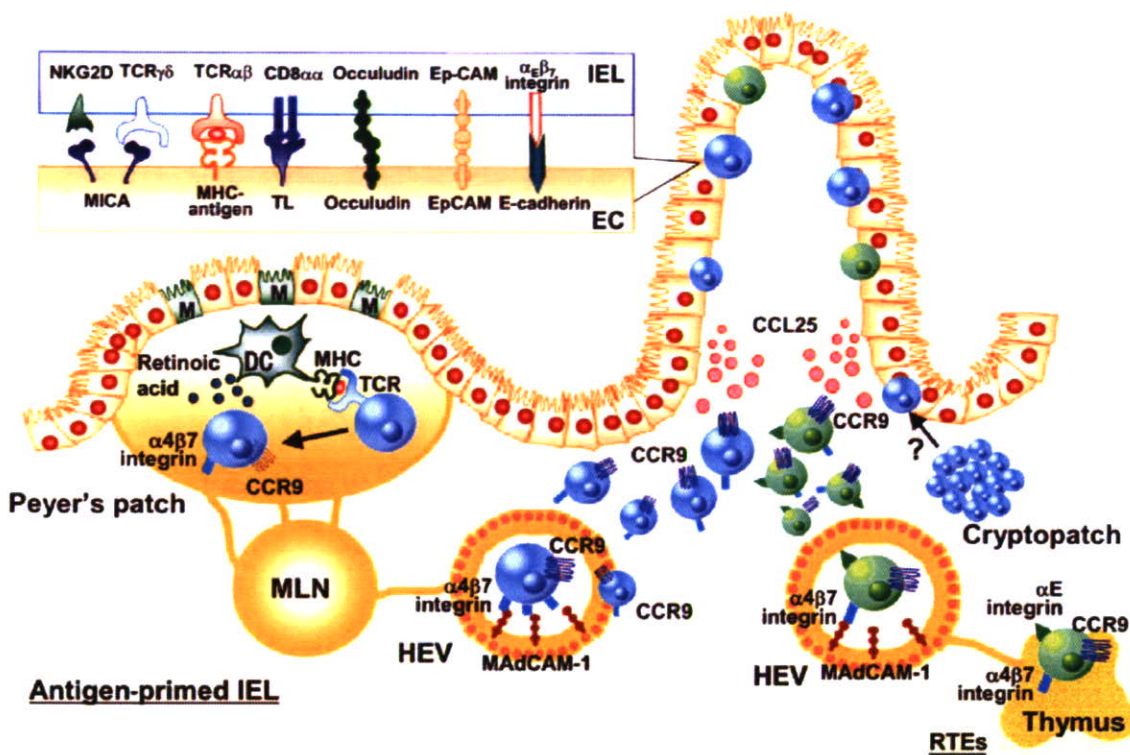


Fig. 1. Unique trafficking pathways for IELs in the intestinal compartments. In the antigen-primed IEL trafficking pathway, DCs in PPs take up antigens transported through M cells and present them to T cells in an MHC-dependent manner. Simultaneously, DCs produce retinoic acid, which causes antigen-primed T cells to express $\alpha 4\beta 7$ integrin and CCR9. $\alpha 4\beta 7$ integrin interacts with intestinal high endothelial venules (HEV) expressing MAdCAM-1, while CCR9 allows specific migration to the CCL25, a chemokine produced by intestinal ECs.

In the other pathway, RTEs migrate into the intestinal epithelium without antigen priming at PPs and MLNs. Alternatively, RTEs have already expressed $\alpha 4\beta 7$ integrin, CCR9, and αE integrin in the thymus. The other population of IELs may originate from the CPs, but their trafficking pathway has not been fully elucidated. Upon migration into the intestinal epithelium, IELs express several unique molecules capable of tethering ECs (e.g. αE integrin, Ep-CAM, and occludin) or of recognizing stressed or infected ECs (e.g. $\alpha \beta$ TCR, $\gamma \delta$ TCR, NKG2D, and CD8).

results obtained by the Study (32), one can surmise that almost half of the naive IELs in the small intestine are derived from RTEs, while PPs and MLNs contain a naive population of RTEs with much less frequency (less than 3%). With their diverse TCR repertoires, RTEs seem likely to play an important role in maintaining TCR diversity in the intestine. At present, however, it remains unknown whether or not RTEs are present in the large intestinal epithelium.

Differing compositions of naive and activated/memory IELs in the small and large intestine

Not surprisingly given the different environmental circumstances faced in the small and large intestine, IELs in the two environments differ with regard to the ratio between naive and activated/memory IELs. It is well established that small intestinal IELs are functionally characterized as antigen-experienced activated/memory cells expressing CD69 and that they contain few naive cells (less than 5%) (34–36). In contrast, the large intestine contains large numbers of naive-type cells

expressing CD62L^{high} and CD44^{int} (more than 40%) but a far less abundant supply of activated/memory cells (34–36).

A correlation may exist between the abundant presence of activated/memory IELs and of CD8 $\alpha\alpha$ IELs in the small intestine. As mentioned above, the small intestine contains a large number of type b IELs expressing CD8 $\alpha\alpha$ as well as type a IELs expressing CD8 $\alpha\alpha$ together with CD4 or CD8 $\alpha\beta$ (Table 1). CD8 $\alpha\alpha$, which can be expressed on activated conventional T cells of the lymph nodes and spleen, plays a key role in mediating the survival of those effector cells that further differentiate into mature memory T cells (37, 38). These surviving conventional T cells acquire the ability to migrate to and reside long-term in non-lymphoid tissues, including the gut, and they readily reinduce CD8 $\alpha\alpha$ expression after secondary restimulation. These observations indicate that the expression of CD8 $\alpha\alpha$ by the various subsets of mucosal T cells is not a marker to identify their origin or the specificity of their TCRs, but rather this expression reflects their effector/memory phenotype and plays a unique

role in maintaining immune homeostasis within the intestine.

Though both show a memory phenotype, type a and type b IELs represent distinct types of memory cells. Type a IELs resemble conventional memory T cells found in the periphery and lamina propria regions (39), and given that TCR β clonotypes were comparable for type a IELs and lamina propria T cells (39), it is possible that they differentiate via the same pathway. In contrast, CD8 $\alpha\alpha$ ($\alpha\beta$ IELs) are thought to be natural memory T cells. CD8 $\alpha\alpha$ $\alpha\beta$ TCR IELs show oligoclonal TCR repertoires that are different from those of type a IELs (40), indicating that CD8 $\alpha\alpha$ $\alpha\beta$ IELs differentiate via a distinct pathway. The accumulation of autoreactive TCRs in CD8 $\alpha\alpha$ $\alpha\beta$ IELs (41), along with their early appearance in neonatal mice (42) and human fetal intestine (43), when exposure to exogenous antigen is minimal, provides evidence for the self-specificity of these T cells. In addition, most human fetal IELs already express an effector/memory cell phenotype (43), indicating that a differentiation pathway other than exposure to peripheral exogenous antigen resulted in the acquisition by these cells of an activated memory-like phenotype. However, unlike the CD8 $\alpha\alpha$ $\gamma\delta$ TCR IELs ($\gamma\delta$ IELs), the $\alpha\beta$ IELs do not appear in the small intestine of germ-free mice lacking gut flora, although bacterial colonization of these animals restores the population of CD8 $\alpha\alpha$ $\alpha\beta$ IELs (44). These observations indicate that local stimulation from the luminal microflora is required for generation and maintenance of the CD8 $\alpha\alpha$ $\alpha\beta$ IELs.

Antigen recognition by IELs

Type a IELs are reactive to classical MHC-restricted antigen presentation

Similar to peripheral T cells, type a IELs express $\alpha\beta$ TCRs together with the coreceptor CD8 $\alpha\beta$, which recognizes MHC class I, or the coreceptor CD4, which recognizes MHC class II. In general, MHC class I presents peptide generated from cytoplasmic protein (endogenous antigen) (13), while antigens proteolytically processed after endocytosis/phagocytosis are presented by MHC class II molecules (45). Although both MHC class I and class II molecules express self- and non-self-antigen, type a IELs are specific for non-self-antigen because of the positive and negative selection in the thymus.

Antigen recognition by type b CD8 $\alpha\alpha$ $\alpha\beta$ IELs

The MHC restriction of type b CD8 $\alpha\alpha$ $\alpha\beta$ IELs is still enigmatic. CD8 $\alpha\alpha$ $\alpha\beta$ IELs are drastically reduced in number in β 2-microglobulin-deficient mice (46–49), but they are present in mice deficient either in the transporter associated with antigen

processing (TAP) (49) or in the classical class I molecules (K^b -/- D^b -/-) (50, 51). This finding suggests that type b IELs require a TAP-independent non-classical MHC class Ib molecule.

It has been reported that mice deficient in Qa-2, a non-classical MHC class I molecule that is TAP dependent (52), possessed fewer CD8 $\alpha\alpha$ $\alpha\beta$ IELs, suggesting that some CD8 $\alpha\alpha$ $\alpha\beta$ IELs are reactive to Qa-2 (53). However, when they compared BALB/c mice with the Qa-2-null Bailey substrain of BALB/c mice, Cheroutre's group (54) were unable to confirm these findings. Furthermore, Qa-2 is broadly expressed by the IELs themselves (18), and its expression is mostly TAP dependent. Two other non-classical class I molecules, the thymic leukemia (TL) antigen (55) and CD1d (56), have been reported to be expressed by mouse intestinal ECs, although this is controversial in the case of mouse CD1d (57). Both TL and CD1d are TAP independent, but they require β 2-microglobulin expression (58–60). However, the TCRs of this IEL subset in mice have never been shown to recognize either of these Ib molecules. Recent structural data indicate that the TL antigen has a very narrow and closed groove that makes interacting with $\alpha\beta$ TCRs unlikely (61).

Researchers using several MHC class-II-restricted TCR transgenic systems (62, 63) showed that CD8 $\alpha\alpha$ IELs were generated when agonist self-peptides were present, just as in the MHC class-I-restricted systems. Despite this finding, peripheral T cells in these same MHC class-II-restricted TCR transgenic mice were CD4⁺. These observations indicate that CD8 $\alpha\alpha$ does not function as a TCR coreceptor and that expression of CD8 $\alpha\alpha$ on the $\alpha\beta$ IELs does not necessarily imply MHC class I restriction. From these results, it can be surmised that CD8 $\alpha\alpha$ $\alpha\beta$ IELs, unlike type a IELs, are self-reactive but are not selected for self-reactivity to a single antigen and that they may include cells reactive to classical class I molecules, non-classical class I molecules, and MHC class II molecules.

Specificity of $\gamma\delta$ TCR IELs ($\gamma\delta$ IELs)

It is still uncertain to what extent the $\gamma\delta$ TCRs require a selection event for their development, especially in mice. Murine $\gamma\delta$ IELs make predominant use of the V γ 5 (also known as V γ 7) gene segment together with several V δ genes (64). Their CDR3 regions are more diverse than the invariant $\gamma\delta$ TCRs expressed in the skin and reproductive tracts (65). Although the great majority of these cells express CD8 $\alpha\alpha$, their numbers are not reduced in β 2-microglobulin-deficient mice (47, 48). In humans, the $\gamma\delta$ TCRs expressed by IELs predominantly use V δ 1 (66), and they have an oligoclonal CDR3 repertoire that can be sustained over a period of many months (67). These TCRs recognize MICA and

MICB, class-I-like molecules that are induced by heat shock or stress on ECs (26, 68, 69). Like TL antigen in mice, MIC molecules are not capable of presenting peptides or other ligands (61). As mentioned above, MICA is also recognized by NKG2D. Although mice do not have a functional MIC gene ortholog, they do have NKG2D receptors that recognize class-I-like molecules such as H60 (70) and members of the RAE class-I-like family (71). There are no reports, however, indicating that significant populations of $\gamma\delta$ IELs or other mouse $\gamma\delta$ T cells recognize these class-I-like molecules.

TL antigen and MICA for CD8 $\alpha\alpha$ IELs

Given the MHC class I or class II specificity of the TCRs of CD8 $\alpha\alpha$ $\alpha\beta$ IELs together with their requirement for β 2-microglobulin, an MHC class I-molecule-mediated function other than peptide presentation must be required for CD8 $\alpha\alpha$ $\alpha\beta$ IEL development and/or maintenance. In this regard, Leishman et al. (20) have shown that the mouse TAP-independent, β 2-microglobulin-dependent, non-classical MHC class I molecule TL is a unique ligand for CD8 $\alpha\alpha$. TL is constitutively and abundantly expressed by the ECs of the small intestine and so is in close proximity to the CD8 $\alpha\alpha$ IELs. The structural analysis of the molecule indicates the absence of an obvious antigen-binding groove (61). The

specific interaction of TL with CD8 $\alpha\alpha$ on activated CD8 $\alpha\alpha$ $\alpha\beta$ IELs modifies the TCR-activation signals to allow for regulated immune responses that are compatible with the gut environment (20). Unlike CD8 $\alpha\beta$, CD8 $\alpha\alpha$ is not internalized with the TCR from the surface of activated T cells (72), and associated p56^{lck} is inefficiently provided to the TCR complex, in part because of the inability of CD8 $\alpha\alpha$ to locate effectively into lipid rafts (73). It is, therefore, possible that the interaction of TL with CD8 $\alpha\alpha$ might sequester CD8-associated p56^{lck} away from the TCR complex, consequently reducing activation signals while promoting survival. In contrast to the abundant expression of TL in the small intestinal epithelium, TL was only weakly expressed in the large intestinal epithelium (74), which might be consistent with the small numbers of CD8 $\alpha\alpha$ IELs in the large intestine.

Our previous study constructed a transgenic mouse with gut-specific MICA expression driven by a T3b promoter, and we surprisingly observed a clonal expansion of CD4⁺ T cells also possessing CD8 $\alpha\alpha$ in the small intestine (75), suggesting that MICA positively regulates the expression of CD8 $\alpha\alpha$ (Fig. 2). Furthermore, studies in the inflammatory bowel disease model showed that transgenic MICA was able to attenuate the acute colitis induced by dextran sodium sulfate administration

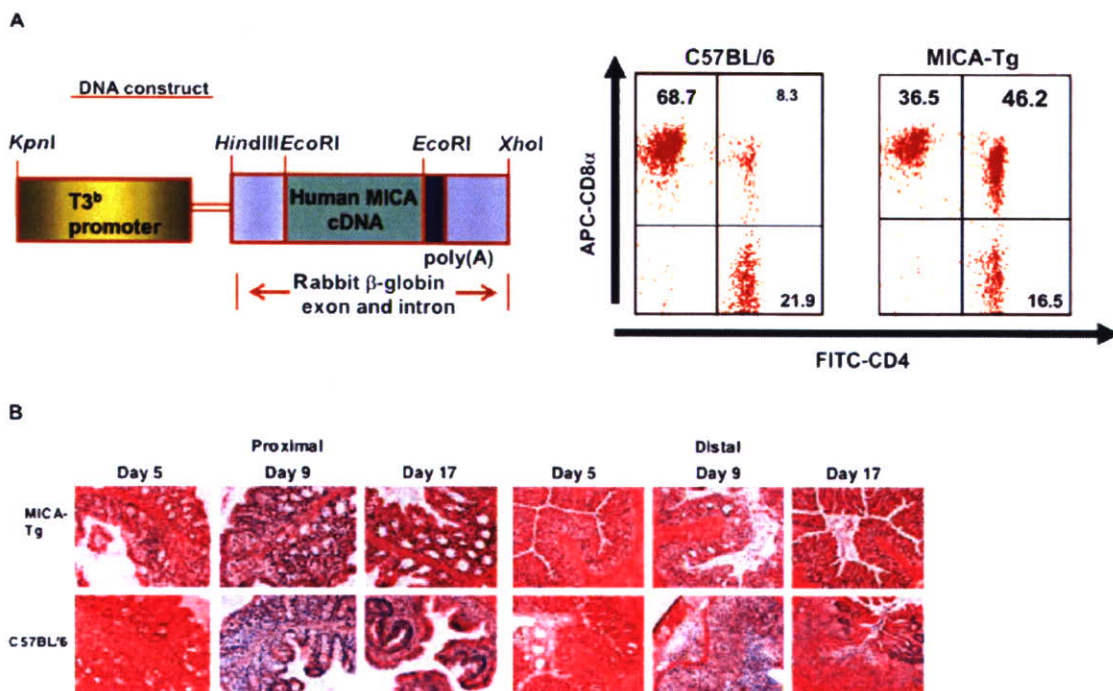


Fig. 2. CD8 $\alpha\alpha$ expression by mucosal T cells plays a unique role in maintaining immune homeostasis within the intestine. A transgenic mouse with gut-specific MICA expression driven by a T3b promoter was constructed. Surprisingly, a clonal expansion of CD4⁺ T cells also possessing CD8 $\alpha\alpha$ was observed in the small

intestine, suggesting that MICA positively regulates the expression of CD8 $\alpha\alpha$ (A). Furthermore, studies in the inflammatory bowel disease model show that transgenic MICA was able to attenuate the acute colitis induced by dextran sodium sulfate administration (B).

(Fig. 2). In this regard, it should be noted that MICA and TL, both of which belong to MHC class Ib molecules, are preferentially expressed in the intestinal ECs (55, 76). Thus, although MICA is both β 2-microglobulin and TAP independent, while TL is TAP independent but β 2-microglobulin dependent, it is likely that both molecules play a similar role in the development of IELs. Several years ago, Madakamutil et al. (38) demonstrated that TCR ligation and activation of CD8 α IELs by TL resulted in increased expression of the anti-apoptotic proteins Bcl-2 and Bcl-xL, suggesting that TL may abrogate antigen-induced cell death.

Unique signal transduction through TCR and Fc ϵ R1 γ in type b IELs

Type b IELs in both the small and large intestine exclusively express homodimers of Fc ϵ R1 γ or heterodimers of Fc ϵ R1 γ with CD3 ζ (77–79), while type a IELs and peripheral T cells predominantly express homodimers of CD3 ζ , which can form a complex with TCR that is important for signal transduction (77). Thus, most T cells from CD3 ζ -deficient mice showed abnormal development (78, 80). However, type b IELs could express TCR in the CD3 ζ -deficient mice because Fc ϵ R1 γ compensated for the CD3 ζ function in the TCR–CD3 complex (78–80). Because CD8 α molecules are not able to transfer signaling through TCR (73), selective expression of Fc ϵ R1 γ on type b IELs might act as an alternative pathway for type b IEL activation.

Uniqueness of IEL developmental pathways

Agonist selection in CD8 α IELs

Although controversy remains as to what extent the $\gamma\delta$ IELs require thymic selection (42, 81), much evidence exists in mice indicating that self-specific CD8 α $\alpha\beta$ IELs are actively and preferentially selected in the thymus under agonist selection conditions (63, 82). The outcome of thymic selection of conventional $\alpha\beta$ T cells is determined by the signal strength received through the TCR when interacting with MHC molecules presenting self-antigens. Intermediate TCR signal strength results in positive selection of conventional $\alpha\beta$ T cells, whereas strong TCR-mediated signals delete cells that express those TCR from the conventional T-cell repertoire. By contrast, CD8 α $\alpha\beta$ IELs preferentially accumulate under conditions that lead to the deletion of conventional $\alpha\beta$ T cells, and, as a consequence, autoreactive TCRs are enriched in the TCR repertoire of CD8 α $\alpha\beta$ IELs (41). The existence of an agonist-dependent selection of CD8 α $\alpha\beta$ IELs has been shown using mice that express a transgenic TCR and cognate antigen during thymic selection. The H-Y TCR is specific for a Y-chromosome-encoded peptide presented by H-2D^b, and H-Y TCR transgenic

mice provide a model for the self-based agonist selection of CD8 α $\alpha\beta$ IELs (63, 83). When the H-Y TCR transgenic mice were crossed onto a recombination-activating gene (Rag)^{-/-} background, CD8 $\alpha\beta$ thymocytes matured in the thymus and populated the peripheral tissues of H-Y antigen-negative female mice, whereas few CD8 $\alpha\beta$ IELs and no CD8 $\alpha\alpha$ IELs were detected in these female mice. By contrast, despite deletion of conventional CD8 $\alpha\beta$ H-Y TCR⁺ T cells in Rag^{-/-} male H-Y TCR transgenic mice, large numbers of H-Y TCR⁺ IELs largely expressing CD8 $\alpha\alpha$ developed (83).

Similar observations have been made in TCR transgenic mice that coexpress the endogenous cognate antigen, such as 2C TCR transgenic mice expressing the H-2L^d alloantigen recognized by the 2C TCR (84) and double-transgenic mice expressing both the TCR and the cognate antigen. Examples of the latter include OT-1 TCR transgenic/RIPmOVA mice [membrane-bound ovalbumin (OVA) antigen transgene under the control of the rat insulin promoter (RIP)] (63), influenza virus nucleoprotein (NP)-specific F5 TCR transgenic mice that express NP peptides (85), and P14 lymphocytic choriomeningitis virus (LCMV)-specific TCR transgenic mice that coexpress the cognate LCMV-specific epitope GP33-41 (86).

The selection of CD8 α $\alpha\beta$ IELs is most effective in the presence of high-affinity interactions between the TCR and the agonist self-antigens, as was shown using the clonotypic F5 TCR, which has a high affinity for the influenza virus epitope NP68. NP68/F5 TCR double-transgenic mice preferentially generated CD8 α $\alpha\beta$ IELs, whereas double-transgenic F5 TCR mice coexpressing the antagonist peptide NP34 did not (85). Similarly, CD8 α IELs expressing the 2C TCR were readily generated in mice expressing the H-2L^d high-affinity ligand, whereas CD8 $\alpha\beta$ T cells developed preferentially in the presence of the low-affinity ligand presented by H-2K^b (84). Similarly, H-Y TCR⁺ CD8 α IELs were more efficiently generated in H-2D^{b/b} homozygous male mice than in their H-2D^{b/d} heterozygous counterparts (87).

Similar to conventional $\alpha\beta$ T cells, CD8 α $\alpha\beta$ IELs require the expression of pre-T α during the initial steps of $\alpha\beta$ TCR rearrangements (88). The fact that the efficient agonist selection of CD8 α $\alpha\beta$ IELs also requires the expression of the TCR α chain connecting peptide domain (α -CPM) further suggests that the agonist and conventional selection processes have similar features and may have evolved simultaneously (63).

Agonistic selection in the thymus

The marked reduction in the numbers of CD8 α $\alpha\beta$ IELs in congenic athymic nude mice (89, 90) and in mice that had been neonatally thymectomized (91) indicates that the thymus is

required for the generation of agonist-selected CD8 $\alpha\alpha$ $\alpha\beta$ IELs. This requirement is further supported by the observation that thymus grafts from male H-Y TCR transgenic mice generated CD8 $\alpha\alpha$ H-Y TCR⁺ IELs when transplanted into female recipient mice (63), and wildtype thymus grafts efficiently generated CD8 $\alpha\alpha$ $\alpha\beta$ IELs when transplanted into recipient nude mice (42). In the latter case, it was shown that CD8 $\alpha\alpha$ IELs and conventional CD4 and CD8 $\alpha\beta$ IELs were generated with different kinetics. Fetal thymus grafts or grafts from mice up to weaning age mainly repopulated the intestine of the recipient mice with CD8 $\alpha\alpha$ $\alpha\beta$ IELs, whereas thymus grafts from older mice were more efficient in generating the conventional $\alpha\beta$ T-cell subsets (42). It should not be assumed, however, that the adult thymus had a reduced ability to generate CD8 $\alpha\alpha$ $\alpha\beta$ IELs as the adult thymus grafts from the male H-Y TCR transgenic *Rag*^{-/-} mice efficiently did so (63). It is possible that in the absence of conventional positive selection, as in the case of the male H-Y TCR transgenic mice on a *Rag*^{-/-} background, the generation of CD8 $\alpha\alpha$ $\alpha\beta$ IELs can continue in adult mice as long as a functional thymus is present.

Is it true that the type b IELs are developed in the thymus? Given that CD8 $\alpha\alpha$ $\alpha\beta$ IELs continued to be efficiently generated under conditions that caused deletion of CD8 $\alpha\beta$ T cells in the

thymus, some speculated that the precursors of these cells had not been submitted to thymic negative selection (41, 83). Taken together with observations that, in the absence of a thymus, these type b IELs nevertheless develop in lymphopenic mice grafted with normal bone marrow, fetal liver, or intestine (92, 93), this finding suggests that CD8 $\alpha\alpha$ $\alpha\beta$ IELs are likely the progeny of extrathymic progenitor cells localized within the intestine itself.

Kanamori et al. (94) demonstrated that approximately 1500 cryptopatch aggregates (CPs) are dispersed throughout the wall of the small and large intestine in mice (Fig. 3). Cells within the CP are composed of mostly lymphoid progenitors expressing stem cell factor receptor, or c-kit, and IL-7R α , but lacking the lineage markers (CD3, B220, Mac-1, Gr-1, and TER-119). They possess transcripts for germline TCR genes, messenger RNA (mRNA) for CD3 ϵ as well as proteins (i.e. RAG-2 and pre-T α) involved in TCR gene rearrangement (95), and are able to generate TCR⁺ IELs, type b CD8 $\alpha\alpha$ $\alpha\beta$ IELs, as well as $\gamma\delta$ IELs, albeit with a strong bias toward the generation of $\gamma\delta$ T cells, in irradiated severe combined immune-deficient mice. These findings demonstrated that c-kit⁺ CP cells are committed to the T-cell lineage and are competent for the generation of IELs (93, 96). Both IL-7R α ^{-/-} mice (94) and common cytokine receptor γ -chain (γ_c)-deficient mice (95) lack CP structure, but local

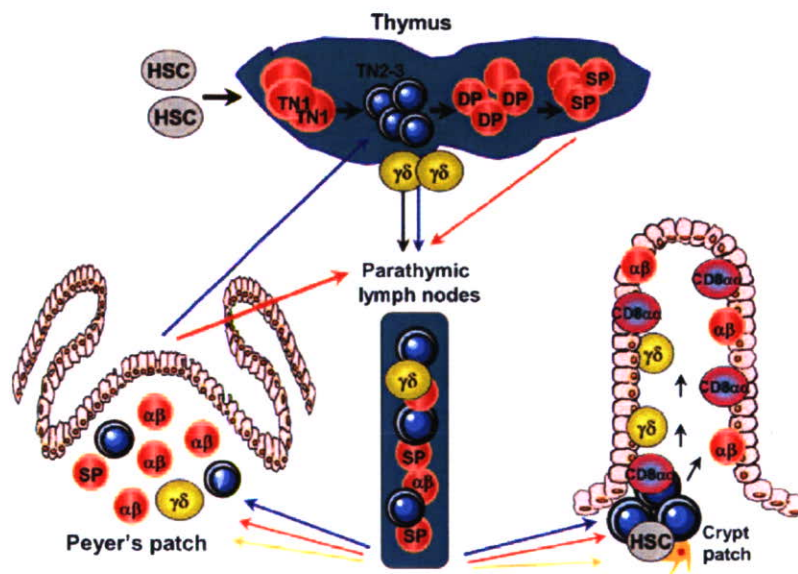


Fig. 3. Thymus-dependent versus thymus-independent IEL development. Bone marrow-derived or fetal-liver-derived hematopoietic stem cells (HSCs) enter the thymus as T-committed cells. Signals from the stroma and from other thymocytes (CD4⁺ CD8⁺ DP thymocytes?) induce differentiation toward type b unconventional T cells. Other immature CD4⁺ CD8⁺ CD3⁻ (TN) cells gradually switch off unconventional T-cell genes and differentiate toward conventional CD4⁺ or CD8⁺

single-positive (SP) $\alpha\beta$ T cells. Both immature and mature cells exit from the thymus. Unconventional T cells and their progenitors (TN2-3 cells) more readily populate the gut epithelium, possibly via CPs, where the progenitors may complete their differentiation into CD8 $\alpha\alpha$ IELs. Some TN2-3 progenitors may circulate back to the thymus. The gut epithelium could also be populated by bone marrow-derived HSCs that differentiate into type b $\gamma\delta$ T cells in CPs.

(fatty-acid-binding protein promoter) transgene expression of IL-7 by mature enterocytes of IL-7^{-/-} mice restored the development of CPs and $\gamma\delta$ IELs, whereas $\gamma\delta$ T cells remained absent from all other tissues (97), suggesting an important role for IL-7R signaling in their generation. These observations indicate a correlation between the presence of CP cells and lymphopoiesis in the gut, especially of $\gamma\delta$ IELs.

Lambolez et al. (88) reported difficulty identifying specific T-lineage-committed precursors among the CP cells. Using multiple lineage markers, they performed a detailed characterization of the progenitor cells isolated from CPs. They also transferred bone marrow from nude mice to irradiated thymectomized γ_c and RAG-2 double-deficient recipient mice to establish the sequence of appearance of these precursors during lymphoid development in the gut. This analysis provided little evidence that CP lineage (Lin)⁻ cells are T-cell committed, a finding consistent with data showing that several gene mutations that affect T-cell differentiation, including pre-T α , TCR α , and CD3 ϵ , had no effect on the appearance or ratio of CP precursor cells (88). However, CP Lin⁻ cells did express GATA-2, a transcription factor found in pluripotent hematopoietic progenitor cells, indicating that these CP cells might represent precursors of other hematopoietic cells. There is relatively little cell turnover in CPs, and very few CP cells express RAG-2 mRNA (88). In contrast, potential precursor cells isolated from the epithelium were more committed to the T-cell pathway, and TCR α mutation dramatically altered subtype ratios within the immature Lin⁻ cells of the epithelium (88).

In seeking to investigate which immune cells synthesize RAG-2, subsequent studies (98) have shown that RAG-2 production is detected exclusively in the thymus in normal mice with an intact thymus (euthymic), but RAG-2 is found in the MLNs of the gut and minimally in the PPs of nude or other athymic mice. Their findings suggest that extrathymic T-cell development is totally repressed in normal euthymic mice and becomes active only in cases of severe T-cell depletion. Whether in normal and athymic mice, neither CP cells nor IELs showed fluorescence-tagged RAG-2. To evaluate these findings, Nonaka et al. (99) generated athymic nude mice that lacked all lymph nodes and PPs by administering lymphotoxin- β receptor Ig and tumor necrosis factor (TNF)-R55-Ig fusion proteins into pregnant nu/+ mice and nu/nu aly/aly (alymphoplasia) mice that lacked all lymph nodes, PPs, as well as intestinal ILFs. These two kinds of mice, which harbored numerous DN as well as CD8 $\alpha\alpha$ $\gamma\delta$ IELs possessing TCR V γ 1, V γ 4, and V γ 7 segments in the small intestines, were found to retain CPs. These findings provide compelling evidence that in the lamina propria of the

intestine, at least some of the DN and CD8 $\alpha\alpha$ $\gamma\delta$ IELs developed within the tiny clusters, that is, CPs, that are filled with c-kit⁺IL-7R⁺Lin⁻ lymphopoietic cells.

Podd et al. (100) explored whether CD3 ϵ ⁺ CP cells are linearly related to $\gamma\delta$ IELs using laser capture microdissection analysis. They observed that in-frame TCR V γ 5 rearrangements were detected in the CD3 ϵ ⁺ CP cells that are shared with a subset of $\gamma\delta$ IELs. However, they did not detect mRNA for RAG-1 gene expression in the CP aggregates. Thus, their data lend support to the hypothesis that CPs are perhaps reservoirs for some antigen-experienced $\gamma\delta$ IELs in mice.

Eberl and Littman (101) performed interesting experiments in mice bearing two transgenes, a Cre recombinase transgene under the control of the retinoid-related orphan receptor γ 1 (ROR γ 1) promoter and a green fluorescent protein (GFP) transgene controlled by a ubiquitous promoter that is only transcribed when a lox-flanked stop sequence is removed by Cre. ROR γ 1, an orphan nuclear hormone receptor, was detected in immature DP thymocytes, fetal lymphoid-tissue-inducer cells, and c-kit⁺Lin⁻ cells in CPs. In these transgenic mice, all progeny of a Cre-expressing cell become constitutively GFP⁺ because the stop sequence of the GFP transgene has been deleted. These experiments, also performed with a CD4-Cre transgene, showed that GFP was expressed by all DP thymocytes and also by all single-positive T cells and $\alpha\beta$ IELs, including CD8 $\alpha\alpha$ $\alpha\beta$ IELs, but not by any other immune cells such as $\gamma\delta$ IELs, B cells, or NK cells. This finding elegantly demonstrates that all $\alpha\beta$ IELs are of thymic origin and must have been selected by the DP pathway. However, these findings did not exclude an extrathymic origin for $\gamma\delta$ and other IELs. They did suggest, however, that the contribution of intestinal precursors including c-kit⁺Lin⁻ cells is minimal in healthy mice but could become more substantial in lymphopenic mice or in mice in which large numbers of T cells have been mobilized in response to intestinal inflammation (101). However, Eberl and Littman (102) mentioned elsewhere that the principal function of the CP cells is to induce the formation of lymphoid follicles in the intestinal lamina propria in a manner similar to the induction of lymph nodes and PPs by the ROR γ 1-expressing fetal lymphoid tissue inducer cells.

Resolution of the thymus-dependent versus thymus-independent controversy

Just when researchers seemed to hit an impasse regarding thymus involvement in IEL production, a truly incendiary report by Lambolez et al. (103) was published. In this paper, they proposed a previously unknown pathway for T-cell

development that has implications for the thymus every bit as great as for the gut. Reexamining assumptions germane to the basic biology of T-cell development in the thymus, they noticed that neonatal thymus grafts leak specific subsets of early T-committed progenitor cells that will repopulate both the host thymus, in which they rapidly complete normal T-cell development, and the host gut, where they survive for many weeks and give rise via defined intermediates to type b IELs (103). This finding conflicted with previous assumptions that the thymus is a source solely of mature T cells and that it does not have the potential for long-term reconstitution. Furthermore, this report emphasizes that the leaked cells are early CD4⁻CD8⁻CD3⁻ triple-negative stage 2 (TN2) and TN3 progenitors that are T-cell committed but that have not yet rearranged their TCR genes. However, CD8 α IELs could not be reconstituted even after adoptive transfer of other more mature DP or single-positive thymocyte populations (Fig. 3).

Using an indirect assay, the authors (103) also showed that the leaked TN cells are much more efficient at completing T-cell differentiation in the gut than are cells derived directly from the bone marrow (Fig. 3). These results strongly suggest that stroma cells in the thymus and/or other thymocytes could help 'persuade' progenitors to differentiate into unconventional CD8 α IELs. The thymic imprinting might include gene products that favor survival and/or are biased toward unconventional IEL development.

It should be underlined that recent studies using H-Y TCR transgenic mice seem to exclude the possibility that DP thymocytes could repopulate the intestine (104) because H-Y TCR β and TCR α transgenes in this model are expressed abnormally in DN thymocytes and the mice have no TN2–TN4 cells. Those phenotypes were shown to be artifacts of the transgenic systems. In conditions in which the TCR α chain is physiologically expressed in DP thymocytes of the H-Y mice, the male mice have many DP thymocytes expressing the self-reactive $\alpha\beta$ T cells, but no increase in *Smyc* peptide-specific CD8 α IELs is found in the gut. Thus, the accumulation of self-reactive TCR transgenic cells in the gut of conventional H-Y transgenic mice seems to be because of the anomalous expression of the TCR α transgene in early TN thymocytes.

It is not clear whether leaked TN2–TN3 cells home exclusively to the gut or to several mucosal sites. CPs may be special features of the gut that attract immature cells. In this regard, Onai et al. (105) reported that CD11c⁺ DCs in CP aggregates express CCL25 and that the signaling via the chemokine receptor CCR9 that is present on c-kit⁺ CP cells might play a central role in CP and CD8 α IEL development. When the expression of CCR9 on the surface of c-kit⁺ bone

marrow cells was blocked by a viral construct expressing an intracellular form of CCL25, bone-marrow-recipient mice largely failed to develop CP aggregates and CD8 α IELs. By contrast, the cellularity and composition of the thymus and spleen of these mice were well developed. These findings led Onai et al. (105) to hypothesize that CCR9–CCL25 interaction is required for CP maturation in the generation of TCR⁻ and TCR⁺ IEL subsets.

The gut itself then seems to retain immature thymic emigrants and to nurture their development. As shown before, this nurturing includes local production of IL-7, which promotes local TCR gene rearrangement (97). A capacity for gene rearrangement might seem unexpected, given a report that a reporter gene expressed from the *Rag-2* promoter is not detected outside the thymus (98). Nonetheless, it has been shown that *Rag-1* is expressed in the gut, albeit in low numbers, from an alternative 5' exon that may be conserved in the mouse (106). The reporter construct that has been used previously (95) might not register expression by an unorthodox *Rag* exon (98). In summary, the gut is a viable site for T-cell differentiation because it expresses *Rag*, locally produces IL-7, and can accommodate immature T-cell-committed progenitors.

Cytokine requirements for IEL development

IELs require cytokines for their development. As mentioned above, γ_c -deficient mice show impaired development of IELs expressing either $\alpha\beta$ TCRs or $\gamma\delta$ TCRs (107, 108). Several cytokines (IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21) share γ_c as a part of their receptors. Consistent with impaired CP development, IL-7- or IL-7 receptor (IL-7R)-deficient mice did not have $\gamma\delta$ IELs but possessed substantial numbers of $\alpha\beta$ IELs (109, 110). Local transgene expression of IL-7 by the ECs of IL-7^{-/-} mice restored the development of $\gamma\delta$ IELs associated with CP development (97). Although both $\alpha\beta$ TCR- and $\gamma\delta$ TCR-positive CD8 α IELs belong to type b IELs, the developmental pathway of each is distinct, at least in terms of IL-7 requirements. $\gamma\delta$ IELs could be preferentially impaired at least in part because IL-7-mediated signaling is essential for the rearrangement of TCR γ genes (111). Additionally, the presence of substantial numbers of $\alpha\beta$ IELs in IL-7- or IL-7R-deficient mice implies that other cytokines using γ_c play a pivotal role in the regulation of IEL development, particularly of $\alpha\beta$ IELs.

Much attention is currently focused on IL-15, a cytokine that is produced by several kinds of cells including DCs and ECs and that includes an IL-2 receptor β chain (IL-2R β) as a part of its receptor. As one might expect from the elimination of $\alpha\beta$ and $\gamma\delta$ IELs from IL-2R β -deficient mice (112), disruption of the IL-15- or IL-15R α -encoding gene also resulted in a significant

reduction in IELs expressing either $\alpha\beta$ TCRs or $\gamma\delta$ TCRs (113, 114). Reciprocally, IL-15 stimulates IEL proliferation and restricts the TCR repertoire of IELs by selectively regulating local V γ gene chromatin modification (115, 116). In regard to IL-15-induced IEL development, a recent finding has indicated that MyD88 (myeloid differentiation factor 88), an adapter molecule associated with Toll-like receptors, mediates IL-15 production by ECs. As expected, the number of IELs significantly decreased in MyD88-deficient mice (117), suggesting that a MyD88 regulation pathway may help maintain the intestinal homeostasis against commensal microorganisms via the IEL and EC cross-talk system.

IEL trafficking pathway

Determination of IEL tropism by chemokine

It is generally accepted that chemokines play an important role in the regulation of lymphocyte trafficking (118, 119). For the regulation of IEL trafficking into the small intestine, CCR9 has been considered to be a key chemokine (120, 121) (Fig. 1). Its ligand, thymus-expressed chemokine (CCL25), is constitutively expressed on the ECs in the small intestine (122, 123) (Fig. 1). CCL25 has been shown to mediate the localization of type a IELs, especially CD103⁺-naive cells and, more recently, to activate CD69⁺ cells in the small intestine (124). CCL25 also regulates precursor differentiation during the generation of type b IELs, but it is less involved in steps that involve IEL retention (125). Mice with defective responses against CCL25 exhibited a significant reduction in CPs and IELs in the small intestine. In contrast to these implications, however, disruption of the CCR9 gene resulted in only a modest decrease in IELs, indicating that other chemokines might be involved in the IEL migration into the small intestine (126, 127). Furthermore, although there are CCR9⁺ cells in the large intestine, the CCR9/CCL25-mediated pathway is not involved in IEL recruitment into the large intestine because its ligand (CCL25) is poorly expressed on large intestinal ECs (120, 122, 123). It would stand to reason then that chemokines other than CCR9⁺ are involved in the regulation of IEL trafficking in the large intestine. Indeed, CCL28, which acts as a ligand for CCR10⁺-IgA-secreting cells (128, 129), is the predominant chemokine expressed on ECs in the large but not the small intestine (130). Although memory-type T cells in the intestine did not express CCR10 (128), it is possible that a CCL28/CCR10-mediated pathway might contribute to the regulation of large intestinal IEL trafficking, especially naive-type IELs preferentially residing in the large intestine. In addition, the various chemokine receptors expressed on IELs, such as CCR3, CCR4, CCR5, and

CXCR3 (131–133), may play a pivotal role in the alternative pathway of IEL trafficking. Although CCR6 is not expressed on mature IELs, CCR6-deficient mice showed impaired development of CD4 CD8 $\alpha\alpha$ $\alpha\beta$ IELs and CD8 $\alpha\alpha$ $\alpha\beta$ IELs but not CD8 $\alpha\alpha$ $\gamma\delta$ IELs because of the dysregulated development of CPs (134). These findings suggest that CD8 $\alpha\alpha$ $\alpha\beta$ IEL and CD8 $\alpha\alpha$ $\gamma\delta$ IEL developments have different chemokine requirements.

In addition to chemokine-mediated pathways, we recently found that sphingosine-1-phosphate (S1P) plays a role in IEL trafficking, especially in the large intestine (Jun Kunisawa, unpublished data). S1P, a lipid mediator originating from sphingomyelin, has been linked to the regulation of lymphocyte emigration from the secondary lymphoid organs and thymus by an accumulating body of evidence (118). Our new finding suggests that some type a IELs also use S1P in their exit from the GALT (Jun Kunisawa, unpublished data). Because previous studies proposed a mutual interaction between S1P- and chemokine-mediated pathways in lymphocyte trafficking (135, 136), the cooperative pathway mediated by both S1P and chemokines may determine the selective trafficking of IELs into the small and large intestine.

Integrin-mediated IEL trafficking and distribution

In addition to chemokines, adhesion molecules also contribute to the regulation of IEL trafficking (Fig. 1). Among them, β 7 integrin has been considered to be an important molecule in determining the localization of intestinal IELs (Fig. 1). Thus, β 7-integrin-deficient mice showed critically reduced numbers of IELs in the intestine (137, 138). IEL numbers were equally reduced in the small and large intestine, indicating that a chemokine-mediated rather than an adhesion-molecule-mediated pathway determines whether IELs migrate to the small or large intestine.

Several lines of evidence have demonstrated that DCs derived from the GALT (PPs and MLNs) but not those derived from the spleen are capable of determining the gut tropism of intestinal T cells by the induction of high levels of α 4 β 7 integrin and CCR9 expression (139–141). A recent study showed that retinoic acid, which induces the α 4 β 7 integrin and CCR9 expression on T cells (142), is dominantly expressed by GALT DCs (Fig. 1). Thus, mucosal T cells activated by orally administered antigens presented by GALT DCs tend to migrate into distant intestinal effector sites, including the intestinal epithelium, by obtaining mucosal trafficking molecules (e.g. α 4 β 7 integrin and CCR9). Regarding the integrated integrin- and chemokine-mediated induction pathway, CCL25, a ligand for CCR9, is expressed in the crypt region of the small intestine, which is close to the mucosal addressin cell adhesion molecule 1 (MAdCAM-1)⁺ vessels (123, 127). These findings further indicate that α 4 β 7

integrin–MAdCAM-1 and CCL25–CCR9 interactions synergistically recruit gut-tropic IELs into the small intestine.

The unique migration pathway of RTE into the small intestine

Because the migration of RTE into the small intestinal epithelium was detected in the lymphotoxin- α -deficient mice lacking organized secondary lymphoid organs such as PPs and MLNs, their migration must have been independent of the GALT structure (32). In contrast, the migration of RTEs was abolished in CCR9-deficient mice or mice treated with anti-CCL25 antibody or anti- $\alpha 4\beta 7$ integrin antibody, suggesting that the migration was dependent on a CCL25/CCR9-mediated and $\alpha 4\beta 7$ -integrin-mediated pathway (32) (Fig. 1). As one might expect given the preferential expression of CCR9 on RTEs, RTEs homed to the small intestinal epithelium more efficiently than did non-RTE-naive T cells (32). Although RTEs share with type a IELs a similar dependency on CCL25/CCR9 and $\alpha 4\beta 7$ integrin, RTEs do not require antigen-mediated activation. Thus, much unlike type a IELs, RTEs isolated from OT-1⁺Rag1^{-/-} mice can migrate into the small intestinal epithelium without antigen stimulation (32, 143).

Retention molecules on IELs

The intimate integrin-mediated interaction between ECs and IELs plays a pivotal role not just in the infiltration of the IELs into the intestine but also in the retention of IELs at the epithelium. Many attempts have been made at identifying the molecules that tether IELs and ECs together in the small intestine. CD103 (αE integrin), which interacts with the E-cadherin expressed on ECs, is expressed on IELs (144) (Fig. 1). It was previously reported that transforming growth factor- β (TGF- β) induced downregulation of $\alpha 4\beta 7$ integrin and simultaneously upregulated CD103 (145). Thus, $\alpha 4\beta 7$ integrin expression was reduced following IEL entry into the small intestinal epithelium, a reduction that coincided with an increase in CD103 expression (146). In addition to the TGF- β -mediated pathway, a recent study (146) proposed that CCR9-mediated signaling promoted the induction of CD103. In CCR9-deficient CD8⁺ T cells, upregulation of CD103 after migration into the small intestinal epithelium was markedly delayed (146). Additionally, the interaction between CD103 and E-cadherin was involved in the destruction of the intestinal epithelium during graft-versus-host disease (147), indicating that CD103 is a pivotal molecule regulating lymphocyte trafficking not only under natural conditions but also during pathological situations. Further, although CD103 was not required for the entry into the intestine (138), CD103-deficient mice exhibited

a decreased number of IELs (148). However, the reduction was modest, suggesting that other molecules contribute to the retention of IELs at the epithelium. In this regard, several lines of evidence have demonstrated that IEL retention may be mediated by their expression of some integrins (e.g. $\alpha 1\beta 1$ integrin, $\alpha 4\beta 1$ integrin, and $\beta 2$ integrin) (149–151). As in CD103-deficient mice, no or only a partial reduction in IELs was seen following the disruption of each of these integrins, suggesting that other integrins and/or molecules may compensate for their lack (151, 152). Additionally, we previously reported that IELs expressed the EC adhesion molecule (Ep-CAM) (153) (Fig. 1). Because Ep-CAM is also expressed on ECs and mediates homophilic cell-to-cell adhesion (154), interaction between IELs and ECs via Ep-CAM may play a pivotal role in IEL retention. Further, a recent study (155) indicates that IELs express tight-junction molecules such as occludins and zonula occludens-1 (Fig. 1). Taken together, the intimate biological interactions between ECs and IELs provide physiological and immunological barriers that act as a first line of defense at the intestine.

Physiological and biological functions of IELs

CD8 $\alpha\alpha$ $\alpha\beta$ IELs in immune protection and mucosal homeostasis

Despite their high frequency, potentially autoreactive type b CD8 $\alpha\alpha$ $\alpha\beta$ IELs are not self-destructive under normal conditions. Rather, their presence in the gut epithelium correlates with immune regulation and immune quiescence. Because endogenous, natural, specific self-antigens for these CD8 $\alpha\alpha$ $\alpha\beta$ IELs have not yet been identified, studies analyzing their functional ability are limited to the use of TCR transgenic mice, which express a cognate antigen for this TCR either naturally or through expression of a second transgene.

These transgenic mice, which have few conventional $\alpha\beta$ T cells expressing the transgenic TCR in the thymus, spleen, and lymph nodes, generate enhanced numbers of transgenic CD8 $\alpha\alpha$ $\alpha\beta$ IELs specific for the self-antigen (63) that are not anergic (83, 86, 156). Upon a systemic LCMV infection in double-transgenic mice expressing both an LCMV epitope (GP33-41)-specific TCR transgene and the cognate antigen transgene (GP33-41) driven by an MHC class I promoter, CD8 $\alpha\alpha$ $\alpha\beta$ IELs showed signs of virus-induced activation (86). This activation was antigen specific as the CD8 $\alpha\alpha$ $\alpha\beta$ IELs from double-transgenic mice expressing an OVA-peptide-specific TCR and the OVA antigen did not show activation following LCMV infection. Unlike conventional (GP33-41-specific) CD8 $\alpha\beta$ T cells, which lose self-tolerance upon LCMV infection, the activation of these self-specific CD8 $\alpha\alpha$ $\alpha\beta$ IELs did not result in

a loss of tolerance. The activated CD8 $\alpha\alpha$ $\alpha\beta$ IELs did not show antigen-specific cytotoxicity nor did they promote an inflammatory response. Instead, the responding CD8 $\alpha\alpha$ $\alpha\beta$ IELs secreted enhanced levels of TGF- β . The constitutive expression of measurable levels of mRNA for immunoregulatory cytokines, including IFN- γ , TGF- β , and IL-10, even without specific antigenic stimulation, further underscores that these type b IELs might exert regulatory functions rather than affording immune protection against specific pathogens.

The regulatory ability of these self-reactive T cells was first showed by analysis of gene-targeted animals, including IL-2/IL-2R-deficient mice, which have specific defects in this mucosal T-cell subset. These mice, in which the CD8 $\alpha\alpha$ $\alpha\beta$ IEL population is reduced, are highly susceptible to gut inflammatory diseases (157, 158).

Direct evidence that CD8 $\alpha\alpha$ $\alpha\beta$ IELs could regulate immune responses by conventional mucosal T cells was provided by sophisticated adoptive transfer experiments of CD8 $\alpha\alpha$ $\alpha\beta$ IELs into immune-deficient Rag^{-/-} recipient mice that also received pathogenic CD4⁺CD45RB^{high} splenocytes (156). In the absence of CD8 $\alpha\alpha$ $\alpha\beta$ IELs, transferred pathogenic CD4⁺ T cells migrate to the intestine and induce an uncontrolled inflammatory immune response, whereas mice that had previously received CD8 $\alpha\alpha$ $\alpha\beta$ IELs were protected against this CD4⁺ T-cell-mediated pathology. Surprisingly, CD4⁺CD45RB^{high} T-cell-induced colitis was prevented by transfer of CD8 $\alpha\alpha$ $\alpha\beta$ IELs isolated from H-Y TCR transgenic male donor mice (156). This protection, however, was observed only in H-2D^b male recipient mice and not in female mice, indicating that TCR must recognize self-antigen if it is to mediate regulatory function.

CD8 $\alpha\alpha$ $\gamma\delta$ IELs and immune protection

The antigen specificity of CD8 $\alpha\alpha$ $\gamma\delta$ IELs is poorly defined, and cell-transfer studies have indicated that the CD8 $\alpha\alpha$ $\gamma\delta$ IELs have only minimal pathogen-specific activity (159, 160). Nevertheless, in the case of recipient mice infected with *Toxoplasma gondii*, it was shown that successful protection by CD8 $\alpha\alpha$ $\alpha\beta$ IELs was, in part, dependent on the presence of CD8 $\alpha\alpha$ $\gamma\delta$ IELs (160). Although these CD8 $\alpha\alpha$ $\gamma\delta$ IELs show only minimal pathogen-specific activity, their ability to respond rapidly to unprocessed self-antigens expressed by stressed or transformed ECs and to control the activity of the non-self-reactive CD8 $\alpha\alpha$ $\alpha\beta$ IELs has led to the idea that these cells might provide the first line of defense against invading pathogens (54).

Alternatively, because activated $\gamma\delta$ IELs can produce keratinocyte growth factor (KGF), which is important for epithelial growth and the repair of damaged tissue, some $\gamma\delta$ IELs could be involved in the repair of tissue damage elicited during

inflammatory immune responses (161). The synthesis of KGF by $\gamma\delta$ IELs has led to the hypothesis that these cells are important for the integrity and healing of the epithelium. Consistent with this hypothesis, the epithelium of the intestine in TCR δ -chain-deficient mice had reduced numbers of crypts (162). By contrast, the epithelium had a higher mitotic index in the presence of $\gamma\delta$ TCR transgene (163). In addition, TCR δ -chain-deficient mice show increased susceptibility to epithelial damage caused by 2,4,6-trinitrobenzene sulfonic-acid-induced colitis; indeed, the transfer of $\gamma\delta$ IELs to TCR δ -chain-deficient mice ameliorated the hapten-induced colitis, which correlated with decreased IFN- γ and TNF- α production and increased TGF- β production by IELs (164).

It remains to be determined if the effect of $\gamma\delta$ IELs on ECs is mediated solely by KGF or if other factors are involved. $\gamma\delta$ IELs synthesize mRNA for TGF- β 1 (18) and TGF- β 3 (165), and TGF- β has been reported to aid in the healing of epithelial damage (166). Furthermore, a lack of $\gamma\delta$ T cells often results in severe pathological conditions mediated by autoantibodies and/or in destructive cell-mediated immune responses by $\alpha\beta$ T cells (167). TCR δ -deficient mice that have been orally infected with *Eimeria vermiformis*, an enterocyte-specific parasite, can efficiently control the infection, but they also show severe bleeding in the small intestine because of uncontrolled immune responses by infiltrating CD4⁺ $\alpha\beta$ T cells (168). Transfer of normal IELs to these TCR δ -deficient mice could prevent mucosal injury. These results suggest that $\gamma\delta$ IELs exert significant control over the immune responses mediated by mucosal $\alpha\beta$ T cells.

Not all immune responses mediated by $\gamma\delta$ IELs are beneficial. For example, in the case of celiac disease, it was shown that dysregulated IL-15 secretion by wheat gliadin elicited overexpression of MICA/MICB on ECs and IELs, possibly including $\gamma\delta$ T cells. When isolated from patients with active celiac disease, $\gamma\delta$ T cells exhibited NKG2D-mediated cytotoxicity of the EC line HT29 possessing MICA (30).

$\gamma\delta$ IELs for mucosal IgA responses

$\gamma\delta$ IELs appear to be necessary for the induction and maintenance of humoral immune responses in the intestine. Interestingly, in TCR α -chain-deficient mice, an increase in the numbers and overall proportion of $\gamma\delta$ IELs is seen after environmental microbial challenge (169), which is accompanied by B-cell maturation and production of high levels of Igs (170–172). Collaboration between B and non- $\alpha\beta$ T cells, including $\gamma\delta$ T cells as well as $\beta\beta$ TCR⁺ T cells ($\beta\beta$ T cells), sustains the production of germinal centers, lymphoid follicles that ordinarily are the anatomical signature of $\alpha\beta$ T-cell and B-cell interactions.

Murine $\gamma\delta$ IELs, if administered in the periphery, can enhance antigen-specific IgA responses to orally ingested antigens and thus might be capable of abrogating oral tolerance (173, 174). However, because it is unlikely that $\gamma\delta$ IELs recirculate from the intestinal mucosa, their potential role in inducing or suppressing systemic oral tolerance is questionable. Although antibody responses against some T-cell-dependent antigens are impaired in mice lacking $\alpha\beta$ T cells, mucosal $\gamma\delta$ T cells may enhance antigen-specific as well as polyclonal S-IgA responses in the lamina propria of the small intestine (175).

Conclusions and future perspectives

In the harsh environment of the gut, the mucosal immune system must prevent the invasion of pathogenic micro-

organisms from the lumen while remaining quiescent against commensal microorganisms and food antigens, if disordered immune responses are to be prevented. Remarkably, IELs play a pivotal role in both immune surveillance and immune regulation, helping to maintain intestinal homeostasis by adjusting their immunological function to the different circumstances that prevail in the small and large intestines. However, the origin and development of these key players remain a subject of debate, and so it is crucial that we work to define their precise function and delineate their developmental pathway. Further investigation of IELs in both the small and large intestine will undoubtedly be key to the development of novel mucosal vaccines against infectious diseases and to immune therapy for inflammatory bowel diseases, food allergies, and celiac diseases.

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Enhanced TLR-mediated NF-IL6-dependent gene expression by Trib1 deficiency

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Toll-like receptors (TLRs) recognize a variety of microbial components and mediate downstream signal transduction pathways that culminate in the activation of nuclear factor κ B (NF- κ B) and mitogen-activated protein (MAP) kinases. Trib1 is reportedly involved in the regulation of NF- κ B and MAP kinases, as well as gene expression *in vitro*. To clarify the physiological function of Trib1 in TLR-mediated responses, we generated Trib1-deficient mice by gene targeting. Microarray analysis showed that Trib1-deficient macrophages exhibited a dysregulated expression pattern of lipopolysaccharide-inducible genes, whereas TLR-mediated activation of MAP kinases and NF- κ B was normal. Trib1 was found to associate with NF-IL6 (also known as CCAAT/enhancer-binding protein β). NF-IL6-deficient cells showed opposite phenotypes to those in Trib1-deficient cells in terms of TLR-mediated responses. Moreover, overexpression of Trib1 inhibited NF-IL6-dependent gene expression by down-regulating NF-IL6 protein expression. In contrast, Trib1-deficient cells exhibited augmented NF-IL6 DNA-binding activities with increased amounts of NF-IL6 proteins. These results demonstrate that Trib1 is a negative regulator of NF-IL6 protein expression and modulates NF-IL6-dependent gene expression in TLR-mediated signaling.

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Abbreviations used: 24p3, lipocalin-2; BLP, bacterial lipoprotein; C/EBP, CCAAT/enhancer-binding protein; Jnk, c-Jun N-terminal kinase; MALP-2, macrophage-activating lipopeptide-2; MAP, mitogen-activated protein; mPGES, prostaglandin E synthase; TLR, Toll-like receptor.

Innate immunity is promptly activated after the invasion of microbes through recognition of pathogen-associated molecular patterns by pattern-recognition receptors, including Toll-like receptors (TLRs) (1). The recognition of microbial components by TLRs effectively stimulates host immune responses such as proinflammatory cytokine production, cellular proliferation, and up-regulation of co-stimulatory molecules, accompanied by the activation of NF- κ B and mitogen-activated protein (MAP) kinases (2, 3). Although the inhibitory protein I κ B family members sequester NF- κ B in the cytoplasm of unstimulated cells, TLR-dependent I κ B phosphorylation by the I κ B kinase complex and degradation by the ubiquitin-proteasome pathway permit translocation of NF- κ B to the nucleus (4). MAP kinases such as c-Jun N-terminal kinase (Jnk) and p38 are also rapidly phosphorylated

and activated by upstream kinases in response to TLR stimulation (5). Moreover, TLR-mediated activity of NF- κ B and MAP kinases is shown to be regulated at multiple steps regarding the strength and the duration of the activation (6).

Recent extensive experiments have identified a variety of modulators that have positive and negative effects on the activation of NF- κ B and MAP kinases, including a family of serine/threonine kinase-like proteins called Trib (7). Trib consists of three family members: Trib1 (also known as c8fw, GIG2, or SKIP1), Trib2 (also known as c5fw), and Trib3 (also known as NIPK, SINK, or SKIP3) (7–12). Trib3 has been shown to interact with the p65 subunit of NF- κ B and to inhibit NF- κ B-dependent gene expression *in vitro* (11). In terms of MAP kinases, Trib1, Trib2, and Trib3 reportedly bind to Jnk and p38, and affect the activity of MAP kinases and IL-8 production in response to PMA or

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