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Development, Homeostasis and Functions of Intestinal Intraepithelial Lymphocytes

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Abstract

The intestine is continuously exposed to commensal microorganisms, food and environmental agents, and also serves as a major portal of entry for many pathogens. A critical defense mechanism against microbial invasion in the intestine is the single layer of epithelial cells that separates the gut lumen from the underlying tissues. The barrier function of the intestinal epithelium is supported by cells and soluble factors of the intestinal immune system. Chiefly among them are intestinal intraepithelial lymphocytes (iIELs), which are embedded in the intestinal epithelium, and represent one of the single largest populations of lymphocytes in the body. Compared with lymphocytes in other parts of the body, iIELs exhibit unique phenotypic, developmental and functional properties that reflect their key roles in maintaining the intestinal epithelial barrier. Here, we review the biology of iIELs in supporting normal health and how their dysregulation can contribute to disease.

Introduction

Our bodies are continuously exposed to microbial organisms present in the environment, including commensal microbiota as well as many infectious agents. These encounters with microbial organisms primarily occur at external or internal body surfaces, including the skin and the mucosal membranes of the gastrointestinal, respiratory and genitourinary tracts. To deal with this onslaught of microbes, the immune system at mucosal surfaces has evolved specialized features to balance immune responsiveness against invaders with tolerance against commensal microorganisms and other environmental agents, such as food particles (1). These dynamic interactions between the host and microorganisms are particularly apparent in the intestine, which contains more than 1,000 distinct bacterial species and numerous viruses, fungi, and protozoa (2). It has been suggested that many features of the adaptive immune system, such as organized lymphoid structures, may have evolved initially in the gut of vertebrates in response to interactions with microbiota (3). Particularly intriguing is that the thymus, which is critical for the maturation of the majority of T lymphocytes, is derived from the embryonic intestine in vertebrates (4). The gut has been implicated in the development of a subset of γδT cells (5, 6), suggesting similarities in the

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functions and evolutionary origin of the thymus and gut-associated lymphoid tissues. Based on these considerations it has been postulated that the thymus may have evolved from gut-associated lymphoid tissues in the gills of early vertebrates (7).

The intestinal immune system consists of multiple levels to defend against microbial invaders (8). First is immunity provided by the gut microbiota, which compete with pathogens for nutrients and ecological niches, produce bacteriocins and proteinaceous toxins that can inhibit the growth of related bacterial species, and play a critical role in shaping host immunity (9). The second level of defense is provided by the single layer of intestinal epithelial cells (IECs) that separates the luminal contents from the gut tissue (10). IECs are securely joined together via distinct types of cell junctions, generating a barrier against harmful insults such as pathogenic microbes. Moreover, the epithelium contains specialized IECs such as mucus-producing goblet cells, anti-microbial – producing Paneth cells, and microfold (M) cells that sample antigens from the lumen for delivery to underlying lymphoid structures. The epithelium is protected by the mucus layer, which prevents microorganisms and toxic substances from reaching the surface of the epithelium (11). IECs can also respond to microbial products to produce a variety of mediators, including proinflammatory cytokines (e.g., IL-1β and IL-18) and factors that promote cell survival and repair (e.g., EGFR ligands), barrier function (e.g., mucins and anti-microbial products), and immunoregulatory responses (e.g., IL-25, TGF-β, TSLP and retinoic acid). Furthermore, IECs actively transport IgA antibodies, secreted by plasma cells in the mucosa, into the lumen. The third level of immune defense consists of innate and adaptive immunity. Dendritic cells (DCs) and macrophages are found throughout the intestinal lamina propria, the layer of connective tissue underlying the intestinal epithelium. Interestingly, mononuclear phagocytes found underneath the epithelium can sample the luminal contents by extending dendrites through the epithelial layer into the lumen (12, 13). The gut mucosa contains organized lymphoid structures such as the mesenteric lymph nodes and the Peyer's patches, and diffuse lymphoid follicles (14). Many innate and adaptive lymphocytes are also scattered throughout the lamina propria and other parts of the intestinal mucosa. Additionally, abundant lymphocytes, called intestinal intraepithelial lymphocytes (iIELs), are interspersed between IECs at the basolateral side of the epithelium (Figure 1) (15–18). The epithelium of the small intestine contains approximately 1 iIEL for every 10 IECs (19–21), making it one of the largest immune compartments of the body. While iIELs serve critical roles in supporting the barrier function of the intestinal epithelium, they sometimes also contribute to gastrointestinal inflammation and disease. Here, we review the unique features, developmental requirements and functions of iIELs.

General properties of iIELs

Recruitment of iIELs to the intestinal epithelium is mediated in part by the chemokine CCL25, which is produced by IECs and is recognized by the chemokine receptor CCR9 expressed by all iIELs (22, 23). Entry and retention of iIELs into the epithelium is further facilitated by interaction of E-cadherin on enterocytes with the integrin $\alpha_E\beta_7$ (β_7 is also called CD103) on iIELs (24, 25). Unlike lymphocytes in many other tissues, iIELs do not recirculate (26–28). Most iIELs exhibit an effector or memory phenotype, which endows these cells with the capacity to rapidly respond to activating signals (29–31). Many of them

also express surface receptors, including both activating and inhibitory receptors, that are characteristically expressed by natural killer (NK) cells (15, 17, 32). iIELs predominantly consist of T lineage cells, and compared with most other tissues, are enriched for T cells expressing γδT cell receptors (TCRs) (33). While TCRγδ$^+$ iIELs exhibit a strongly restricted TCR repertoire since birth, TCRαβ$^+$ iIELs exhibit a polyclonal TCR repertoire in newborn mice and humans (34). However, in adults, the repertoire of TCRαβ$^+$ iIELs becomes largely oligoclonal, in a manner that is driven by microbial colonization (34–37), suggesting that these cells recognize intestinal commensal antigens under physiological conditions. Another intriguing feature of iIELs is that many of them express a homodimer of CD8α (CD8αα), either in the presence, but more commonly in the absence of CD8αβ expression (15, 17). While CD8αβ functions as a T cell co-receptor to enhance interactions between the TCR and MHC class I-peptide complexes, the function of CD8αα on TCR$^+$ iIELs appears to be distinct (38, 39). CD8αβ and CD8αα exhibit similar affinity for multiple classical MHC class I molecules, yet CD8αα but not CD8αβ binds with high affinity to the thymus leukemia (TL) antigen (40, 41), a nonclassical MHC class I protein (42). TL, which is selectively expressed in the intestinal epithelium (43, 44), is not known to bind antigen of any kind (45), and therefore does not function as a traditional antigen-presenting molecule. Instead, TL engagement redirects CD8αα and its associated lck tyrosine kinase away from the TCR, leading to negative signaling in iIELs (46–48). In this manner, interactions between CD8αα and TL may assist in dampening uncontrolled immune responses in the intestinal epithelium (38, 39). Of note, CD8αα is also expressed by a subset of TCR$^-$ iIELs (49).

iIEL subsets

Approximately 90% of all iIELs are TCR$^+$ (Figure 1). These cells can be further divided into induced and natural TCR$^+$ iIELs (also called conventional or type a, and unconventional or type b iIELs, respectively) (15, 17). Induced TCR$^+$ iIELs are derived from conventional, antigen-specific T cells that were activated in the periphery and subsequently entered the epithelium. This group of iIELs includes both CD4$^+$ and CD8αβ$^+$ subsets. Natural TCR$^+$ iIELs include TCRαβ$^+$ and TCRγδ$^+$ subsets, which immediately enter the iIEL compartment following their generation. The prevalence of distinct TCR$^+$ iIEL subsets differs substantially between mice and humans (15, 32). The subset distribution of TCR$^+$ iIELs in mice consists of 10–15% CD4$^+$TCRαβ$^+$ and 20–30% CD8αβ$^+$TCRαβ$^+$ induced iIELs, and 20–50% CD8αβ$^-$CD8αα$^+$TCRαβ$^+$ and 40–70% CD4$^-$CD8αβ$^-$CD8αα$^{+/-}$TCRγδ$^+$ natural iIELs (50, 51). Humans contain 10–15% CD4$^+$TCRαβ$^+$ and 70–80% CD8αβ$^+$TCRαβ$^+$ induced iIELs, and <1% CD8αβ$^-$CD8αα$^+$TCRαβ$^+$ and 5–20% CD4$^-$CD8αβ$^-$CD8αα$^{+/-}$TCRγδ$^+$ natural iIELs (52, 53).

Although TCR$^-$ iIELs were described two decades ago (31, 54, 55), these cells have been characterized only in recent years (Figure 1) (17). TCR$^-$ iIELs include subsets resembling innate lymphoid cells (ILCs) found outside the intestinal epithelium. Cells resembling peripheral ILC1 cells have been identified in both mice and humans (56–59). Mice contain subsets of ILC1-like iIELs with or without expression of the natural cytotoxicity receptor (NCR) NKp46 (NCR1/CD335) (56, 59), and humans contain ILC1-like iIELs expressing NKp44 (NCR2/CD336) (56–58). A subset of iIELs expressing NKp44 and resembling

peripheral ILC3 cells has also been identified in humans (59). Another subset of TCR^- iIELs, present in both mice and humans, expresses intracellular CD3 (iCD3) chains, and is called $iCD3^+TCR^-$ iIELs (60). One subset of TCR^- iIELs, identified in both mice and humans, expresses iCD3 chains together with surface CD8αα, and is referred to as innate $CD8\alpha^+$ (iCD8α) cells (49, 60, 61).

iIEL development and maintenance

Induced TCR^+ iIELs

These are derived from conventional antigen-experienced T cells that enter the intestinal epithelium (Figure 2). Their ontogeny follows the conventional intrathymic development pathway, which will not be discussed here.

A fraction of $CD4^+$ and $CD8\alpha\beta^+$ T cells that migrate into the intestinal epithelium upregulate expression of CD8αα (15, 39). Recent studies have provided insight into the acquisition of CD8αα by $CD4^+$ iIELs. Traditionally, it was thought that the $CD4^+$ and $CD8^+$ T lineages were fixed, without converting from one to the other. However, some $CD4^+$ T cells that migrate into the intestine gain features of the $CD8^+$ T cell lineage, such as cytotoxicity, thus becoming $CD4^+CD8\alpha\alpha^+TCR\alpha\beta^+$ iIELs (62). This transition is governed primarily by the interplay between two transcription factors: ThPOK (encoded by *Zbtb7b*), which promotes the $CD4^+$ T cell lineage while suppressing the $CD8^+$ T cell lineage, and Runx3, which acts inversely to ThPOK (63–65). For peripheral $CD4^+$ T cells to gain expression of CD8αα, they must downregulate ThPOK and increase expression of Runx3 (and other factors, such as T-bet) (62, 66, 67). The microenvironment of the intestinal epithelium provides signals in the form of TGF-β, retinoic acid, IFN-γ and IL-27 to induce CD8αα expression in $CD4^+$ T cells entering the epithelium (66–69).

Other signals involved in the selection of $CD4^+CD8\alpha\alpha^+TCR\alpha\beta^+$ iIELs are provided by the microbiome. $CD4^+CD8\alpha\alpha^+TCR\alpha\beta^+$ iIELs are reduced in number in germ-free mice as compared with mice maintained under specific pathogen-free conditions (34, 70). In particular, *Lactobacillus reuteri* has been shown to promote the development of these cells, as mice deficient in this microorganism have significantly reduced numbers of $CD4^+CD8\alpha\alpha^+TCR\alpha\beta^+$ iIELs (71). Mechanistic studies showed that *L. reuteri* generates indol derivatives of tryptophan that activate the aryl hydrocarbon receptor (AhR) in $CD4^+$ T cells, allowing these cells to differentiate into $CD4^+CD8\alpha\alpha^+TCR\alpha\beta^+$ iIELs.

Natural TCR^+ iIELs

$CD8\alpha\alpha^+TCR\alpha\beta^+$ iIELs are of particular interest because they are exclusively found in the iIEL compartment. Although their antigen-specificity remains unclear, reactivity of these cells is restricted by classical or non-classical MHC class I molecules (72–76). Since their discovery, the origin and development of $CD8\alpha\alpha^+TCR\alpha\beta^+$ iIEL has been debated (77, 78). This controversy stems from the observation that athymic nude mice harbor considerable numbers of these cells (79, 80). Cryptopatches, small aggregates of lymphoid cells in the lamina propria, were suggested as the relevant site for their extrathymic development (26, 81, 82). However, athymic mice transplanted with a normal thymus reconstitute CD8αα

$^{+}$TCRαβ $^{+}$ cells in the iIEL compartment (83, 84), and neither iIELs nor cryptopatches express RAG proteins (85), providing strong evidence for thymus-dependent development (Figure 2). This conclusion was supported by fate mapping studies (86) and by analyzing the iIEL compartment of mice expressing TCR transgenes (87–89). The precursors for CD8αα $^{+}$TCRαβ^{+} iIELs are comprised of CD4^{-}CD8^{-}TCRβ^{+} thymocytes (87, 89, 90). These precursors undergo agonist positive selection (91), which is responsible for the tendency of mature CD8αα^{+}TCRαβ^{+} iIELs to exhibit self-reactivity (92). CD8αα^{+}TCRαβ^{+} iIEL precursors include two subpopulations: PD-1^{+}T-bet^{-} and PD-1^{-}T-bet^{+} cells (90). PD-1^{+}T-bet $^{-}$ precursors are restricted by classical MHC class I molecules and are enriched in self-reactive thymocytes, whereas the PD-1^{-}T-bet^{+} population includes cells restricted by non-classical MHC class I molecules (90). Thymic emigrants derived from CD4^{-}CD8^{-}TCRβ^{+} precursors migrate into the intestinal epithelium where they receive the appropriate cues for their final differentiation, such as expression of CD8αα (Figure 2) (87, 89, 90). This final differentiation step involves TGF-β, as revealed by defective CD8αα^{+}TCRαβ^{+} iIEL development in TGF-β- and TGF-β signaling-deficient mice, and enrichment of these cells in TGF-β transgenic mice (68).

The maintenance of CD8αα^{+}TCRαβ^{+} iIEL is influenced by the intestinal microbiota, as shown by their reduced prevalence in germ-free animals (34, 70). Mice deficient in the NOD2 pattern recognition receptor also harbor reduced numbers of CD8αα^{+}TCRαβ^{+} iIEL (93). Mechanistic studies showed that microbiota activate NOD2 in DCs and IECs, triggering IL-15 secretion, which promotes the survival and maintenance of CD8αα $^{+}$TCRαβ^{+} iIELs. IEC-derived IL-15 induces T-bet expression in CD8αα^{+}TCRαβ^{+} iIELs, which is critical for expression of CD8αα (94).

Although it is now generally accepted that the vast majority of natural TCRαβ^{+} iIELs are selected in the thymus, accumulating evidence indicates that natural TCRγδ^{+} iIELs predominantly develop extrathymically (Figure 2) (5, 6, 26, 33, 95). While a contribution for the thymus in generating TCRγδ^{+} iIEL progenitors cannot be excluded, a critical role for cryptopatches has been proposed (82, 96). Consistent with their lack of MHC-restriction, the development of murine TCRγδ^{+} iIEL is largely unaffected by MHC antigens that shape αβ T cell repertoires (97).

The size of the TCRγδ^{+} subset of natural iIELs in mice does not appear to be influenced by the intestinal microbiota (6, 98, 99), but these cells require AhR signaling for their maintenance in the epithelium (100). TCRγδ^{+} iIELs have a highly restricted TCR repertoire, with a dominant Vγ7 subset in mice and a dominant Vγ4 subset in humans. How these TCRγδ^{+} subsets are selected and retained within the intestinal epithelium has remained unclear until recently. Two studies showed that G protein-coupled receptors (GPRs) expressed by TCRγδ^{+} iIELs play a critical role in their recruitment and migration into the intestinal epithelium (101, 102). The orphan receptor GPR18 augmented accumulation of TCRγδ^{+} cells in the epithelium (101), whereas GPR55, which mediates migration inhibition in response to lysophosphatidylinositol, counteracted this accumulation (102), suggesting tight control over the entry of these cells into the iIEL compartment. Another recent study reported that IECs selectively express certain members of a family of receptors, called butyrophilin-like (BTNL) proteins, that are structurally related to CD80 co-stimulatory and

PD-L1 inhibitory molecules (6). These investigators showed that BTNL1 and BTNL6 heterodimers expressed by murine IECs are critically important for the selection and function of V$\gamma7^+$ iIELs in mice, and that human gut epithelial cells expressing BTNL3 and BTNL8 heterodimers induce responses of human colonic V$\gamma4^+$ iIELs. Surprisingly, these effects of BTNL products on TCR$\gamma\delta^+$ iIEL selection and function are TCR-dependent, suggesting TCR-specificity for individual (or pairs of) BTNL family members (6, 103). Additionally, intestinal inflammation and colon cancer alters expression of BTN and BTNL genes (104), which might contribute to some of the alterations in TCR$\gamma\delta^+$ iIEL numbers and activity observed in these conditions. These findings also raise the possibility that unique BTNL products expressed in different epithelia may play a role in the selection and function of specific γδT cell subsets in these tissues. The latter hypothesis is supported by studies showing that the murine BTNL protein SKINT1 is selectively expressed by thymic epithelial cells and keratinocytes, and mediates selection of V$\gamma5^+$ dendritic epidermal T cells in the skin of mice (33, 103, 105, 106). Whether selection of specific γδT cell subsets in the epithelium of the respiratory and reproductive systems similarly involves expression of tissue-specific BTNL proteins remains to be determined.

TCR$^-$ iIELs

Consistent with their lack of antigen-specific receptors, TCR$^-$ iIELs develop extrathymically (Figure 2) (17). The development of murine NKp46$^+$ ILC1-like iIELs is dependent on the transcription factors Nfil3 and Id2 (56), which are also required for the development of all peripheral ILC subsets. The development of these cells also requires T-bet expression (56), as would be expected from its role in ILC1 development. Development of murine NKp46$^-$ ILC1-like iIELs similarly depends on T-bet expression (59). Both iCD3$^+$TCR$^-$ iIELs and iCD8αα cells differentiate in the absence of Id2 and require Notch1 signals for their development (49, 60).

The NKp46$^-$ subset, but not the NKp46$^+$ subset, of murine ILC1-like cells requires IL-15 for its survival in the intestinal epithelium (56, 59). Similarly, both iCD3$^+$TCR$^-$ iIELs and iCD8αα cells require IL-15 signaling for their homeostasis or survival (49, 60). iCD8αα cells also require interactions between CD8αα and TL for selection or survival in the epithelium, as shown in mice lacking TL expression or defective for the CD8α enhancer E8$_I$ (49).

iIEL functions

Induced TCR$^+$ iIELs

Induced iIELs are comprised of conventional, MHC-restricted CD4$^+$ and CD8αβ$^+$ T cells that, after activation in the mesenteric lymph nodes or the lamina propria, migrate into the intestinal epithelium. Once in the epithelium, these cells remain as sentinels to protect the mucosal barrier, either as *bona fide* effector cells or tissue-resident memory T cells (Table 1) (29). Many of these cells also induce expression of CD8αα upon entry into the epithelium, which not only increases their activation threshold, but may also modulate their functional properties (38, 39).

The frequencies of $CD4^+CD8\alpha\alpha^+TCR\alpha\beta^+$ iIELs are reduced in individuals with chronic intestinal inflammation (107, 108), suggesting an anti-inflammatory role for these cells. This possibility was tested in an animal model following adoptive transfer of *in vitro* polarized Th2 cells into RAG-deficient mice (109). The transferred cells were able to enter the intestinal epithelium of the recipient animals and also acquired CD8αα expression. Secondary transfer of these $CD4^+CD8\alpha\alpha^+TCR\alpha\beta^+$ iIELs protected recipient RAG-deficient mice against Th1 cell-driven inflammation in a manner that required IL-10. These findings suggested that $CD4^+$ T cells activated and polarized anywhere outside the epithelium may yield $CD4^+CD8\alpha\alpha^+TCR\alpha\beta^+$ iIELs. A recent study provided further support for these findings by showing that regulatory $Foxp3^+$ T cells from the lamina propria can migrate into the intestinal epithelium, where they gain CD8αα expression but lose Foxp3 expression, yet retain regulatory properties and can suppress inflammation (Table 1) (110). The regulatory properties of these iIELs complemented the immunosuppressive functions of $CD4^+Foxp3^+$ T cells resident to the lamina propria.

Similar to other tissue-resident $CD8^+$ T cells, $CD8\alpha\beta^+TCR\alpha\beta^+$ iIELs represent effector or memory cells activated in immune organs associated with the intestines (Table 1) (111). For example, in mice infected with vesicular stomatitis virus or *Listeria monocytogenes*, $CD8\alpha\beta^+TCR\alpha\beta^+$ T cells migrate to the iIEL compartment as early as 5 days post infection, and are retained there over 250 days (111). Additional studies have provided evidence that $CD8\alpha\beta^+TCR\alpha\beta^+$ iIELs expressing CD8αα are enriched for high-affinity TCRs (112), suggesting that TL on IECs preferentially retains such high-affinity memory T cells in the epithelium. Once in the iIEL compartment, $CD8\alpha\beta^+TCR\alpha\beta^+$ iIELs do not require antigen to persist in the epithelium, which suggests that $CD8\alpha\beta^+TCR\alpha\beta^+$ iIELs exhibit effector-like qualities (111, 113). Despite this similarity, the phenotype of antigen-experienced iIELs is distinct from $CD8^+$ T cells in peripheral immune organs such as the spleen and lymph nodes (114). For example, unlike conventional $CD8^+$ T cells, $CD8\alpha\beta^+TCR\alpha\beta^+$ iIELs constitutively express granzyme B, CD69, CD103 and β7 integrin, and produce lower amounts of TNF-α and IFN-γ. In humans with celiac disease, $CD8\alpha\beta^+TCR\alpha\beta^+$ iIELs contribute to disease pathogenesis (Table 1) (32, 115). Gluten-derived products can induce IL-15 production by IECs, which in turn augments expression of activating NK cell receptors such as NKG2D on $CD8\alpha\beta^+TCR\alpha\beta^+$ iIELs. Engagement of NKG2D on the iIELs with its ligand MICA (MHC class I polypeptide-related sequence A), an MHC class I-related protein expressed by stressed IECs, subsequently leads to IEC lysis. However, there is currently no evidence that the TCRs expressed by these iIELs recognize specific antigens derived from the IECs.

Natural TCR^+ iIELs

The role of $CD8\alpha\alpha^+TCR\alpha\beta^+$ iIELs in the intestinal epithelium remains incompletely understood. However, these cells possess a transcriptional profile consistent with regulatory potential (116). For example, $CD8\alpha\alpha^+TCR\alpha\beta^+$ iIELs express NK cell receptors and their signaling components, such as Ly49 family members, DNAX activating protein of 12KD (DAP-12), 2B4, CD94 and others. These cells are also enriched in immunomodulatory factors, including TGF-β3, lymphocyte activating 3 (LAG-3, which is involved in immunosuppression by regulatory T cells), and fibrinogen-like protein 2 (Fg1–2, which suppresses DC maturation) (116). Consistent with this transcriptional profile, CD8αα

$^{+}$TCRαβ^{+} iIEL protect mice against colitis induced following adoptive transfer of naïve CD4^{+} T cells into immunodeficient animals, in a manner that requires IL-10 production (Table 1) (117, 118).

The TCRγδ^{+} subset of iIELs can produce a variety of pro-inflammatory cytokines (IFN-γ and TNF-α), anti-inflammatory cytokines (TGF-β and IL-10), factors associated with wound healing (TGF-β, prothymosin β4, and keratinocyte growth factor [KGF]), antimicrobial proteins (RegIIIγ), pro-fibrotic factors (IL-13), and granzymes (15). A number of recent studies have shown that TCRγδ^{+} iIELs are highly motile and rapidly migrate within the space between the epithelial layer and the basement membrane (119–121), while contacting enterocytes via homotypic interactions mediated by the tight-junction protein occludin (119). This motility of TCRγδ^{+} iIELs is driven by commensal bacteria, resulting in an efficient immune surveillance program. Consistent with these findings, it has been shown that TCRγδ^{+} iIELs play a critical role in mucosal immune responses against gut microbiota, in a manner that involves MyD88-mediated signaling in IECs and production of the antibacterial lectin RegIIIγ (122, 123). Following infection with microbial pathogens, TCRγδ^{+} iIELs quickly change their motility and pattern of movement within the epithelium. Interestingly, this response is associated with a metabolic switch toward glycolysis, indicating pathogen-induced alterations in energy utilization pathways (121). Such crosstalk between TCRγδ^{+} iIELs and IECs is critical for the antimicrobial properties of these cells (Table 1), which were previously implicated in limiting bacterial invasion of *Salmonella typhimurium* (123) and *Toxoplasma gondii* infection (124), and to clear *Nippostrongylus brasiliensis* parasites (125). In addition to promoting pathogen clearance, TCRγδ^{+} iIELs can also limit tissue damage after infection, as seen during infection with *Listeria monocytogenes* (126) and *Eimeria vermiformis* (127). The latter properties of TCRγδ^{+} iIELs are likely mediated by their capacity to produce factors such as TGF-β, prothymosin β4 and KGF that promote healing and fortify the mucosal barrier (Table 1).

In patients with inflammatory bowel disease, disease severity correlates with increased numbers of TCRγδ^{+} cells in the intestinal mucosa (128, 129). In several models of colitis, TCRγδ^{+} T cells promote inflammation (118, 130–133). However, in later stages of colitis, these cells can protect the epithelium against inflammation-induced damage (118, 134–136). Consistent with this role in strengthening the epithelial barrier, TCRγδ^{+} iIELs have been implicated in promoting the induction and maintenance of oral tolerance in mice (137, 138). The iIEL lymphocytosis observed in patients with celiac disease, which is due to loss of tolerance against gluten, includes a significant expansion of TCRγδ^{+} iIELs (32, 139, 140). However, whether TCRγδ^{+} iIELs contribute to disease pathogenesis or are involved in the healing process following tissue damage remains unclear.

TCR^{-} iIELs

Both NKp46^{+} and NKp46^{-} ILC1-like iIELs in mice can produce IFN-γ in response to cytokine stimulation (56, 59). The NKp46^{+} subset has been shown to cause pathology in a model of innate cell-mediated colitis induced by anti-CD40 antibodies (Table 1) (56). In humans, NKp44^{+} ILC1-like iIELs exhibit a memory-activated phenotype and produce IFN-γ in response to IL-12 and IL-15 stimulation (56). Further, these cells are expanded in

patients with Crohn's disease (56) and in patients who received intestinal allografts (58). Human ILC3-like iIELs were shown to produce IL-22 and, like ILC1-like cells, were expanded in patients who received intestinal allografts (58).

iCD3$^+$TCR$^-$ iIELs produce granzyme B in response to IL-15 stimulation (60). In some patients with a refractory form of celiac disease, iCD3$^+$TCR$^-$ iIELs undergo massive expansion in response to IEC-derived IL-15 production and develop NK-like cytotoxicity against IECs (60, 141). Eventually, some of these cells may develop into clonal lymphomas (Table 1).

iCD8α cells produce a variety of innate cytokines such as MCP-1, IFN-γ and osteopontin, and these cells exhibit cytotoxic and phagocytic properties (49). They also express MHC class II proteins and are capable of presenting antigens to MHC class II-restricted T cells. In mice, these cells provide protection against infection with *Citrobacter rodentium* (49), and exacerbate colitis induced by anti-CD40 antibodies (17) (Table 1). In humans, the numbers of these cells are decreased in newborns with necrotizing enterocolitis (49).

Crosstalk between iIEL subsets

Due to their close proximity, it is likely that distinct iIEL subsets are engaged in extensive interactions with each other. Yet, few examples of such crosstalk are available. A human study has provided evidence that TCRγδ$^+$ iIELs control the number and activation of CD8αβ$^+$TCRαβ $^+$ iIELs, in part by reducing expression of the activating NK cell receptor NKG2D on these cells, in a process that may be relevant to the pathogenesis of celiac disease (142). Additionally, the finding that iCD8α cells have a functional MHC class II antigen presentation pathway (49) suggests that these cells might be able to present antigens to CD4$^+$TCRαβ $^+$TCRαβ$^+$ iIELs, a possibility that remains to be explored. Clearly, crosstalk between iIEL subsets is an important area for future research.

Crosstalk between iIELs and other immune cells

Many studies have shown that iIELs interact with immune cells outside the intestinal epithelium, especially the lamina propria. For example, lamina propria DCs constitutively produce retinoid acid and TGF-β, which induce gut-homing and differentiation of induced iIELs (143–146). DCs also are important for presenting antigens to induced TCRαβ$^+$ iIELs (147, 148). In human celiac disease, anti-gluten CD4$^+$ T cells in the lamina propria contribute to activation of induced CD8αβ$^+$TCRαβ$^+$ iIELs (149). *In vitro* co-culture studies have shown that TCRαβ$^+$ iIELs from mice primed with antigen by the oral route can function as helper cells to antigen-pulsed B cells to induce IgG and IgA antibodies (150), suggesting that these cells can influence antibody responses. In colitis models, IL-10-producing natural CD8αα$^+$TCRαβ$^+$ iIELs were able to prevent inflammation induced by CD4$^+$CD45RB^{hi} T cells (109, 117). Finally, the finding that TCRγδ$^+$ iIELs can promote oral tolerance (137, 138) suggests that these cells can influence CD4$^+$ T cell responses in the lamina propria.

Outstanding Questions

Although recent studies have provided new insight into the development, homeostasis and functions of distinct iIEL subsets, a variety of outstanding questions remain. First, it is likely that additional subsets of TCR^- iIEL are yet to be discovered. Whether all subsets of iIELs are conserved between mice and humans also requires further investigation. Second, the precise developmental relationships between the different subsets of iIELs and with other lymphoid cells residing outside the intestinal epithelium remain to be elucidated. Third, a key question regarding the population of natural $TCR\gamma\delta^+$ iIELs is the tissue location and pathway for their development, which appears to be largely thymus-independent. Fourth, the factors that are responsible for the induction and maintenance of $CD8\alpha\alpha$ expression by the majority of iIELs remain incompletely defined. The functional implications of this expression also require further attention. Fifth, while it is clear that the intestinal microbiota greatly impacts iIEL numbers and functions, a better understanding of the effects of individual microbial species on iIEL biology could be employed to devise methods to enhance or suppress iIEL functions. Finally, the precise interactions of distinct iIEL subsets with IECs, with each other, and with immune cells outside the intestinal epithelium remain incompletely understood. Answers to these questions should inform efforts to manipulate iIELs for the development of vaccines and immunotherapies.

Conclusions

The intestinal epithelium contains a wide variety of lymphoid cells with diverse developmental requirements and effector functions. The majority of iIELs are TCR^+, and may enter the epithelium after antigen encounter elsewhere (for induced TCR^+ iIELs), or immediately after their generation (for natural TCR^+ IELs). In recent years, several subsets of TCR^- iIELs have been identified, some of which resemble ILCs found in many other mucosal tissues, and cells expressing intracellular CD3 chains. The latter population includes a subset of iIELs that co-express iCD3 chains and surface $CD8\alpha$ homodimers ($iCD8\alpha$ cells). While their main function is to promote the integrity of the epithelial barrier, iIELs can also occasionally contribute to inflammation and disease. Further studies in this field should be instrumental in the development of iIEL-based vaccines and immunotherapies for infectious and inflammatory diseases.

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Abbreviations

AhR	aryl hydrocarbon receptor
BTNL	butyrophilin-like
iCD3	intracellular CD3
iCD8α	innate $CD8\alpha\alpha^+$

IEC	intestinal epithelial cell
iIEL	intestinal intraepithelial lymphocyte
ILC	innate lymphoid cell
NCR	natural cytotoxicity receptor
TL	thymus leukemia

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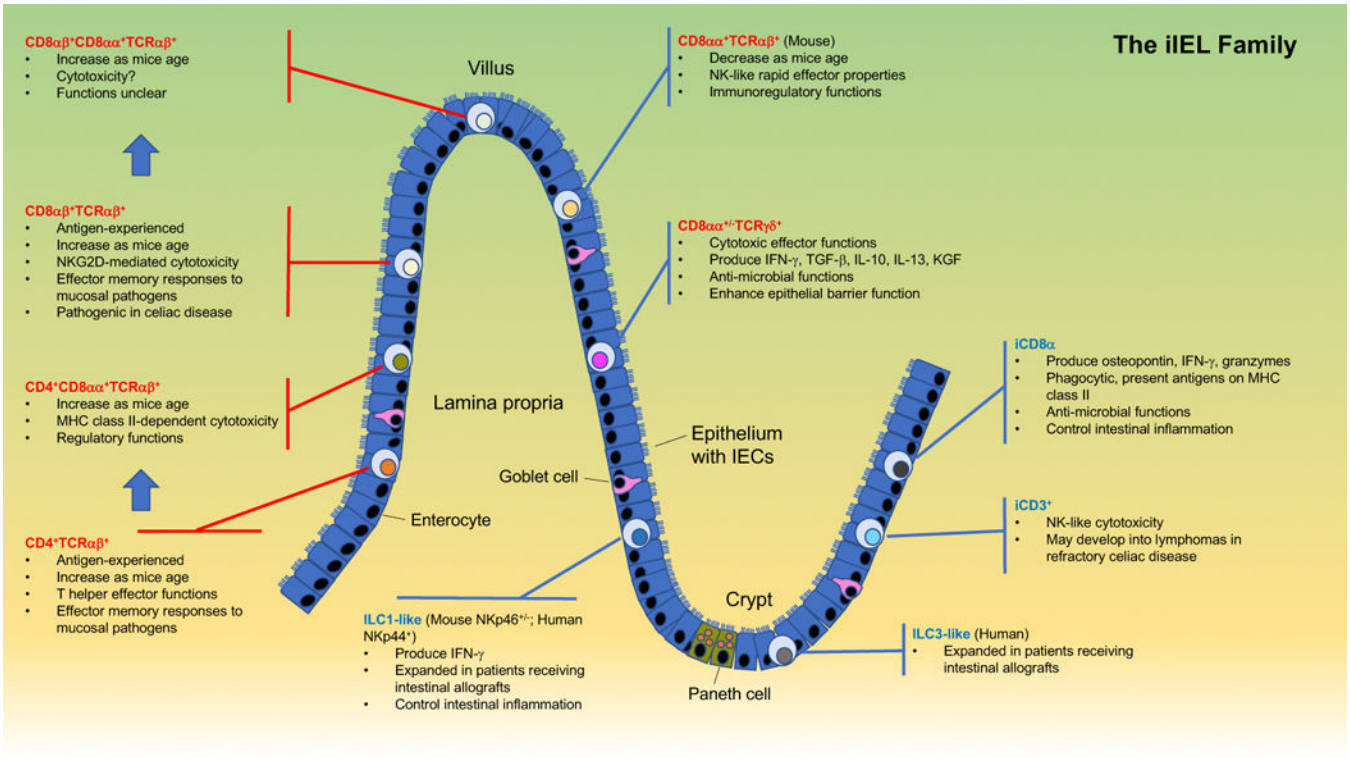

FIGURE 1.

Subsets and salient features of iIELs. iIELs include TCR^+ (red font) and TCR^- (blue font) populations, with subsets that are derived from conventional, antigen-experienced T cells (called induced iIELs, red brackets) and subsets that home to the intestinal epithelium immediately after their generation (called natural iIELs, blue brackets). Upon entry into the epithelium induced TCR^+ iIELs often initiate expression of CD8αα (indicated by blue arrows). The TCR^- iIEL population includes subsets resembling ILCs found in barrier tissues outside the intestinal epithelium (i.e., ILC1- and ILC3-like iIELs), cells expressing iCD3 chains ($iCD3^+TCR^-$ iIELs), and cells co-expressing iCD3 and surface CD8αα (iCD8α cells). A few differences between mouse and human iIEL subsets are highlighted. Key features of distinct iIEL subsets, mostly derived from studies with mice, are listed. Localization of iIEL subsets within the figure does not represent their normal distribution among villi and crypts.

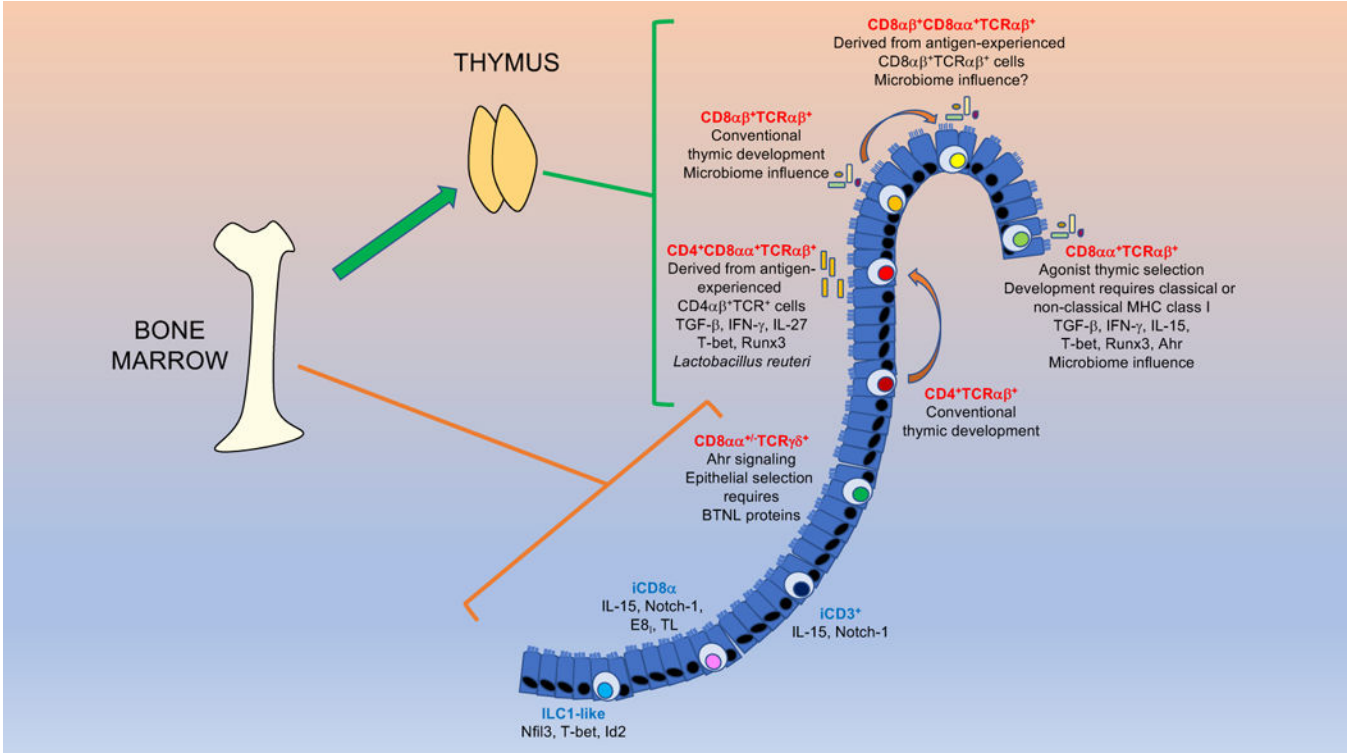

FIGURE 2.

Development and homeostasis of iIEL subsets. Except for TCRγδ$^+$ iIELs, all TCR$^+$ (red font) iIEL develop in the thymus. All TCR$^-$ (blue font) iIELs develop extrathymically. Induced TCR$^+$ iIEL follow a conventional thymic development and selection pathway, whereas natural CD8αα$^+$TCRαβ $^+$ iIELs undergo agonist selection. iIELs require a variety of transcription factors for their development and function. Many TCR$^+$ iIELs initiate CD8αα expression upon entry into the epithelium. The development, maintenance and homeostasis of iIELs requires a variety of factors, as indicated.

Table 1

Examples illustrating the diverse immune functions of iIELs in disease.

iIEL subset	Infection	Inflammation/colitis	Celiac disease	References
CD4$^+$TCRαβ$^+$	Effector memory Th1 and Th17 responses against mucosal pathogens	Pathogenic	Unknown	(29)
CD4$^+$CD8αα$^+$TCRαβ$^+$	Unknown	Possibly regulatory functions mediated by IL-10	Unknown	(107–110)
CD8αβ$^+$TCRαβ$^+$	Effector memory responses against mucosal pathogens, such as vesicular stomatitis virus and *L. monocytogenes*	Unknown	Lyse IECs via NKG2D-MICA interactions	(29, 32, 111, 115)
CD8αβ$^+$CD8αα$^+$TCRαβ$^+$	Unknown	Unknown	Unknown	
CD8αα$^+$TCRαβ$^+$	Unknown	Possibly regulatory functions mediated by TGF-β3, Lag-3, IL-10	Unknown	(116–118)
CD8αα$^{+/-}$TCRγδ$^+$	Anti-microbial activity against pathogens such as *S. typhimurium, T. gondii, N. brasiliensis*; RegIIIγ-mediated	Pro-inflammatory in early stages of murine colitis; promote healing and protect epithelial integrity in late stages of inflammation and colitis	Expansion during disease but function is not well understood	(15, 32, 118, 122–125, 128–136, 139, 140)
ILC1-like	Unknown	Cause IFN-γ-mediated pathology in anti-CD40 antibody-induced colitis	Unknown	(56, 58)
iCD8α	Promote bacterial clearance of organisms such as *C. rodentium*; phagocytic; capable of MHC class II-restricted antigen presentation	Decreased numbers in necrotizing enterocolitis; promote intestinal inflammation via granzymes	Unknown	(17, 49)
iCD3$^+$	Unknown	Unknown	May develop into lymphomas in some patients with refractory celiac disease	(60, 141)