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Intestinal Intraepithelial Lymphocytes: Sentinels of the Mucosal Barrier

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Abstract

Intestinal intraepithelial lymphocytes (IEL) are a large and diverse population of lymphoid cells that reside between the intestinal epithelial cells (IEC) that form the intestinal mucosal barrier. Although IEL biology has traditionally focused on T cells, recent studies have identified several subsets of TCR-negative IEL with intriguing properties. New insight into the development, homeostasis and functions of distinct IEL subsets has been provided. Additional studies have revealed intricate interactions between different IEL subsets, reciprocal interactions between IEL and IEC, and communication of IEL with immune cells that reside outside of the intestinal epithelium. Here, we review sentinel functions of IEL in the maintenance of the mucosal barrier integrity, but also how dysregulated IEL responses can contribute to pathology.

Keywords

intraepithelial lymphocytes; mucosal immunity; intestinal immunity; lymphocyte development; intestinal inflammation

The Intestinal Intraepithelial Lymphocyte Compartment

The mucosal immune system contains the largest reservoir of immune cells in the body [1]. It is in close contact with the outside world, including microbiota, pathogens and environmental antigens, which requires fine-tuning of the immune response to enable efficient responses against pathogens while maintaining tolerance to innocuous stimuli. This is particularly the case for the gastrointestinal immune system, which contains organized structures such as mesenteric lymph nodes and **Peyer's patches** (see Glossary), diffuse lymphoid structures such as the **cryptopatches** of the **lamina propria** in the gut wall, and immune cells that are embedded within the intestinal epithelium [2]. In humans, the intestinal epithelium spans between 200-400 m^2 and consists of a single layer of intestinal epithelial cells (IEC) that are critically important for nutrient uptake and provide a barrier against harmful substances. The vast majority of immune cells within the intestinal epithelium are lymphocytes and are referred to as intestinal intraepithelial lymphocytes

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(IEL) [3]. The small intestine contains approximately 1 IEL per 10 IEC, and this ratio is lower in the colon. IEL are resident to the intestinal epithelium and do not recirculate [4–6]. They express a number of characteristic surface receptors (Box 1) such as the chemokine receptor CCR9, which interacts with CCL25 produced by IEC and thus assists in recruiting IEL to the gut mucosa. Intestinal IEL also express the integrin $\alpha_E\beta_7$ (α_E is also known as CD103), which interacts with E-cadherin on enterocytes, to facilitate entry and retention in the intestinal epithelium. The majority of IEL also express a homodimer of CD8α (CD8αα), which does not function as a co-receptor on these cells, but instead influences their functional characteristics (Box 1). Approximately 90% of all IEL express T cell receptors (TCR), and these cells have been the main focus of studies on IEL biology. However, recent work in this field has identified several subsets of TCR^- IEL with fascinating properties and functions. Here, we will review recent progress on the development, homeostasis and functions of distinct subsets of TCR^+ and TCR^- IEL in the intestine.

Classification of Intestinal IEL

Intestinal IEL can be classified into TCR^+ and TCR^- subsets (Figure 1). TCR^+ IEL can be further divided into *induced* (also called type a or conventional) and *natural* (also called type b or unconventional) IEL. The former cells are derived from antigen-experienced, conventional T cells that home to the intestinal epithelium, whereas the latter comprise cells that home immediately to the intestinal epithelium after their development [3]. All TCR^+ IEL express activation markers such as CD44 and CD69, and many express natural killer (NK) cell markers such as CD16, CD122 and CD161. Although TCR^- IEL were identified nearly two decades ago [7–9], they have only been characterized in recent years. The TCR^- IEL population includes subsets resembling **innate lymphoid cells (ILC)** (Box 2) [10], and cells expressing intracellular CD3 (iCD3) chains [11]. The TCR^-iCD3^+ IEL population includes a subset expressing CD8α homodimers, called innate $CD8\alpha\alpha^+$ (iCD8α) cells [12]. We will first discuss induced TCR^+ IEL, then natural TCR^+ IEL, and finally subsets of TCR^- IEL.

Induced TCR^+ IEL

Induced TCR^+ IEL are derived from antigen-experienced conventional $TCR\alpha\beta^+$ T cells [3]. Similar to conventional T cells, but unlike natural TCR^+ IEL, they express CD2, CD5, CD28, LFA-1 and Thy1. The induced TCR^+ IEL population includes subsets expressing CD4 or CD8αβ, usually together with CD8αα, which is induced upon entry into the intestinal epithelium [13] (Figure 1 and Table 1).

Induced $TCR\alpha\beta^+CD4^+$ IEL

$TCR\alpha\beta^+CD4^+$ T cells are the largest T cell subset in peripheral lymphoid organs and are also found in the IEL compartment. Following activation, especially in organs associated with the intestinal mucosa, some peripheral $TCR\alpha\beta^+CD4^+$ T cells migrate into the intestinal epithelium where they can respond to subsequent antigenic challenges as bona fide effector or tissue-resident memory T cells [14]. In mice, $TCR\alpha\beta^+CD4^+$ T cells are more abundant in intestinal regions closer to the cecum such as the ileum and proximal colon, and their proportion decreases in the proximal small intestine (duodenum) and distal colon. While

traditional dogma held that $CD4^+$ T cells only express CD8 chains during their thymic development, over 20 years ago, several research groups identified a fraction of murine $TCR\alpha\beta^+CD4^+$ IEL (~25 to 50% of all $TCR\alpha\beta^+CD4^+$ IEL, depending on the housing environment) expressing $CD8\alpha\alpha$ [15–17]. $TCR\alpha\beta^+CD4^+CD8\alpha\alpha^+$ IEL are also present in the human intestinal epithelium [18].

How $TCR\alpha\beta^+CD4^+$ IEL acquire expression of $CD8\alpha$ homodimers has been a topic of substantial debate, as it was traditionally thought that the $CD4^+$ and $CD8^+$ T cell lineages are fixed, without the possibility of converting from one to the other. T cell lineage commitment is governed by the action and counteraction of transcription factors, primarily **ThPOK** (encoded by *Zbtb7b*), which induces the $CD4^+$ T cell fate while suppressing $CD8^+$ T cell differentiation, and **Runx3**, which acts inversely to ThPOK [19–21]. In order for $CD4^+$ T cells to gain expression of $CD8\alpha\alpha$, they must activate aspects of the $CD8^+$ T cell lineage by diminishing expression of ThPOK, while gaining expression of Runx3, as well as **T-bet** [22–24]. $CD4^+$ T cells receive such cues for re-differentiation from external stimuli within the intestinal epithelium, such as TGF-β, **retinoic acid**, IFN-γ and IL-27 [23–26]. Nevertheless, $TCR\alpha\beta^+CD4^+CD8\alpha\alpha^+$ IEL do not gain all $CD8^+$ T cell qualities, as exemplified by their lack of CD8β expression.

Recent findings have identified the intestinal microbiome as another important factor that regulates the differentiation of antigen-activated $CD4^+$ T cells into $TCR\alpha\beta^+CD4^+CD8\alpha\alpha^+$ IEL [27]. *Lactobacillus reuteri* is a commensal microorganism that metabolizes tryptophan into indole derivatives, which in turn can activate the aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor that regulates intestinal immunity and inflammation. Interestingly, mice housed in the absence of *L. reuteri* contain significantly reduced numbers of $TCR\alpha\beta^+CD4^+CD8\alpha\alpha^+$ IEL as compared with mice where this bacterium is part of the microbiome [27]. This observation not only suggests that *L. reuteriy* is responsible for the development of $TCR\alpha\beta^+CD4^+CD8\alpha\alpha^+$ IEL, but also serves as a cautionary tale, as the microbiota of mice from different commercial vendors differ in the presence of this organism and, thus the prevalence of $TCR\alpha\beta^+CD4^+CD8\alpha\alpha^+$ IEL can be variable as well. Interestingly, additional studies have shown that reconstitution of *L. reuteri* in mice lacking this organism is sufficient to induce the differentiation of $TCR\alpha\beta^+CD4^+CD8\alpha\alpha^+$ IEL when these animals are also provided with a tryptophan-rich diet [27].

Although $TCR\alpha\beta^+CD4^+CD8\alpha\alpha^+$ IEL express large quantities of granzymes and exhibit cytolytic activities [22,28,29], the functional significance of these findings remains unclear. A potential regulatory function of $TCR\alpha\beta^+CD4^+CD8\alpha\alpha^+$ IEL was suggested by decreased frequencies of these cells in patients suffering from chronic intestinal inflammation [30,31]. This possibility was further explored by transferring $CD4^+$ T cells cultured ex vivo under T helper 2 (Th2)-differentiation conditions into **RAG**-deficient mice [13]. After their entry into the IEL compartment, a fraction of the transferred cells gained $CD8\alpha\alpha$ expression. Secondary transfer of these Th2 cell-derived $TCR\alpha\beta^+CD4^+CD8\alpha\alpha^+$ IEL into RAG-deficient recipients protected the animals against colitis induced by co-transferred pathogenic Th1 cells. Colitis suppression in this experimental system correlated with IL-10 production by the transferred $TCR\alpha\beta^+CD4^+CD8\alpha\alpha^+$ IEL. Recent experiments using intra-vital multi-photon microscopy and fate mapping showed that regulatory $\mathbf{Foxp3^+}$ T cells in

the lamina propria can migrate into the IEL compartment, where they acquire a TCRαβ^{+}CD4^{+}CD8αα^{+}Foxp3^{-} phenotype [32]. Importantly, these investigators further showed that TCRαβ^{+}CD4^{+}CD8αα^{+}Foxp3^{-} IEL possess regulatory properties that can compensate for the absence of CD4^{+}Foxp3^{+} T cells in the lamina propria to protect mice against intestinal inflammation. Thus, mucosal immune responses can be regulated at distinct, albeit adjacent, intestinal anatomical sites. These findings also highlight the concept that the intestinal epithelium constitutes a unique immunological niche.

Induced TCRαβ^{+}CD8αβ^{+} IEL

Conventional CD8^{+} T cells express the CD8αβ heterodimer, which interacts with classical MHC class I molecules and functions as a TCR co-receptor to enhance the overall functional avidity of T cells for antigen. CD8^{+} T cells play a critical role in antiviral and tumor immunity. TCRαβ^{+}CD8αβ^{+} cells are also present in the IEL compartment, comprising around 10–15% of the total IEL population in mice [3], and approximately 70–80% in humans [18]. Most of the TCRαβ^{+}CD8αβ^{+} IEL represent effector or memory cells derived from peripherally activated CD8^{+} T cells that subsequently migrated into the intestinal epithelium [33]. This conclusion is based on infection studies with vesicular stomatitis virus or *Listeria monocytogenes*, which promotes the migration of pathogen-specific TCRαβ^{+}CD8αβ^{+} cells into the IEL compartment as early as 5 days post infection, remaining within that compartment well over 250 days [33]. Following their migration into the IEL compartment, antigen-experienced CD8^{+} T cells do not require antigen for their retention, indicating that TCRαβ^{+}CD8αβ^{+} IEL represent effector or memory cells [33]. However, the phenotype of antigen-experienced TCRαβ^{+}CD8αβ^{+} IEL is distinct from antigen-experienced CD8^{+} T cells that reside in peripheral lymphoid organs such as the spleen [34]. Unlike splenic antigen-experienced CD8^{+} T cells, TCRαβ^{+}CD8αβ^{+} IEL constitutively express granzyme B, CD69, CD103 and β7 integrin. Moreover, as compared with their splenic counterparts, TCRαβ^{+}CD8αβ^{+} IEL produce lower amounts of TNF-α and IFN-γ. Another key difference is that some TCRαβ^{+}CD8αβ^{+} IEL express CD8αα [35], which appears to increase their activation threshold [36–38] (Box 1). In humans, but not in mice, TCRαβ^{+}CD8αβ^{+} IEL also express inhibitory and co-activating NK cell receptors that control their activation status [39]. While the precise contribution of TCRαβ^{+}CD8αβ^{+} IEL to mucosal immune responses remains unclear, in humans these cells have been implicated in the pathogenesis of **celiac disease** [40,41]. During the development of celiac disease, IEC express high levels of IL-15 together with ligands for NKG2D. IL-15 leads to NKG2D upregulation on TCRαβ^{+}CD8αβ^{+} IEL, allowing them to kill IEC. Although the TCRs expressed by these IEL are critical for acquisition of killer activity and NK cell receptor expression, the potential contribution of their antigen-specificity in this IEL-mediated pathology remains to be identified.

Natural TCR^{+} IEL

Natural TCR^{+} IEL include TCRαβ^{+} or TCRγδ^{+} T cells (Figure 1) with a rapid-response phenotype, and these cells home to the intestinal epithelium immediately following their development [3]. In contrast with induced TCR^{+} IEL, natural TCR^{+} IEL lack expression of CD2, CD5, CD28, LFA-1 and Thy1. Most of these cells express CD8αα, exhibit an

activated phenotype with expression of cytotoxic mediators such as granzyme B, and display a variety of NK cell receptors at their surface (Table 1). In mice, natural TCR^+ IEL express the NK cell receptors Ly49, CD94/NKG2A and 2B4 (CD244), and in both mice and humans they also express NKG2D, an activating NK cell receptor that interacts with stress-induced self-proteins [40].

Natural TCRαβ+ IEL

One of the most intriguing populations of IEL is the subset of $TCR\alpha\beta^+CD4^-CD8\alpha\beta^-CD8\alpha\alpha^+$ cells (hereafter called $TCR\alpha\beta^+CD8\alpha\alpha^+$ IEL) [3]. In mice, $TCR\alpha\beta^+CD8\alpha\alpha^+$ IEL are present at birth and, together with $TCR\gamma\delta^+$ cells, are the most predominant IEL population in the intestines. Natural $TCR\alpha\beta^+CD8\alpha\alpha^+$ IEL represent approximately one third of the total $TCR\alpha\beta^+$ IEL population. However, their prevalence decreases as mice age, primarily due to the expansion of induced $TCR\alpha\beta^+CD4^+$ and $TCR\alpha\beta^+CD8\alpha\beta^+$ IEL [42]. In humans, natural $TCR\alpha\beta^+CD8\alpha\alpha^+$ IEL are present during gestation but are rare in adults [43].

A question of continued interest is the MHC restriction of $TCR\alpha\beta^+CD8\alpha\alpha^+$ IEL. The development of these cells is strongly impaired in **β_2-microglobulin**-deficient mice [44,45], suggesting predominant reactivity with MHC class I molecules. Nevertheless, their development is only modestly affected in mice deficient in the classical MHC class I molecules H2-K and H2-D, and in mice singly deficient in **non-classical MHC class I molecules**, such as CD1d, Qa2 or TL (thymus leukemia antigen) [37,44–46]. These observations suggest that $TCR\alpha\beta^+CD8\alpha\alpha^+$ IEL represent a heterogeneous population of cells with diverse MHC class I restriction. Indeed, different $TCR\alpha\beta^+CD8\alpha\alpha^+$ IEL clones appear to require distinct MHC class I restriction, by either polymorphic or non-polymorphic elements, which is characteristic of classical or non-classical MHC proteins, respectively [47].

The origin and development of $TCR\alpha\beta^+CD8\alpha\alpha^+$ IEL has been controversial. Because substantial numbers of these cells are found in athymic nude mice, it was originally suggested that $TCR\alpha\beta^+CD8\alpha\alpha^+$ IEL develop outside of the thymus, possibly within cryptopatches [4,48]. However, the cells that are found in athymic mice appear to represent a vestigial pathway of intestinal lymphopoiesis. Further experiments showed that transplantation of a normal thymus reconstitutes the $TCR\alpha\beta^+CD8\alpha\alpha^+$ IEL compartment in athymic mice [49,50], indicating a thymic origin for these cells. This conclusion was further supported by studies with TCR transgenic systems [51]. Subsequent studies showed that $TCR\alpha\beta^+CD8\alpha\alpha^+$ IEL are derived from thymic $TCR\beta^+CD5^+CD122^+H\text{-}2K^{b+}CD4^-CD8^-$ precursors [51–53]. for mRNA expression of factorsesd signlingallyWithin this population two subsets were identified: the first subset expresses **programmed death-1 (PD-1)**, whereas the second lacks PD-1 expression but is $T\text{-}bet^+$. The former represents enriched, self-reactive thymocytes, whereas the latter includes thymocytes restricted by non-classical MHC class I molecules. Whether these two precursor subsets give rise to distinct populations of mature $TCR\alpha\beta^+CD8\alpha\alpha^+$ IEL remains unclear. Nevertheless, a key property of the precursors for natural $TCR\alpha\beta^+CD8\alpha\alpha^+$ IEL is that they undergo agonist positive selection [54], resulting in a tendency for self-reactivity [55].

The thymic emigrants derived from the TCRαβ$^+$CD8αα$^+$ IEL precursors do not yet possess the CD8αα$^+$ phenotype, which is only acquired upon entry into the intestinal epithelium. Similar to induced TCRαβ$^+$CD8αα$^+$ IEL, natural TCRαβ$^+$CD8αα$^+$ IEL receive cues for their final differentiation in the intestinal epithelium. TGF-β plays a critical role in this process, as shown by defective natural TCRαβ$^+$CD8αα$^+$ IEL generation in mice lacking TGF-β or its canonical signaling molecule Smad3, and enrichment of these cells in TGF-β transgenic mice [25]. Consistent with this conclusion, *in vitro* culture of TCRαβ$^+$CD8αα$^+$ IEL precursors with TGF-β induces CD8αα expression in association with diminished ThPOK expression [25]. The transcription factor T-bet, which may mediate signals from IEC-derived IL-15, is also required for CD8αα upregulation [56].

The homeostasis of TCRαβ$^+$CD8αα$^+$ IEL is influenced by the normal gut microbiota, as shown with mice lacking the NOD2 pattern recognition receptor, which contain reduced numbers of TCRαβ$^+$CD8αα$^+$ IEL [57]. NOD2 is expressed by antigen-presenting cells and IEC, and its activation in these cells triggers production and secretion of IL-15, promoting the survival and maintenance of TCRαβ$^+$CD8αα$^+$ IEL. Vitamin D, a micronutrient recognized by the vitamin D receptor (VDR) on a variety of immune cells, also influences TCRαβ$^+$CD8αα$^+$ IEL homeostasis. VDR-deficient mice contain reduced numbers of natural TCRαβ$^+$CD8αα$^+$ IEL [58].

The functional role of TCRαβ$^+$CD8αα$^+$ IEL in mucosal immunity remains incompletely understood. Nevertheless, gene expression profiling studies have suggested a regulatory role for these cells [59]. TCRαβ$^+$CD8αα$^+$ IEL express NK cell receptors and their signaling components, such as members of the Ly49 family, DNAX activating protein of 12kD (DAP-12), 2B4, CD94, and others. Moreover, compared with induced TCRαβ$^+$CD8αβ$^+$ IEL, these cells are enriched for factors associated with immune regulation, including TGF-β3, lymphocyte activating 3 (LAG-3), which is involved in immune suppression by regulatory T cells, and fibrinogen-like protein 2 (Fgl-2), which suppresses dendritic cell maturation [59]. Consistent with this expression profile, TCRαβ$^+$CD8αα$^+$ IEL protect against colitis when co-transferred with naïve (CD4$^+$CD45RB^{hi}) T cells into T cell-deficient animals [60]. These effects on colitis require IL-10 production by the donor-derived TCRαβ$^+$CD8αα$^+$ IEL.

Natural TCRγδ$^+$ IEL

In mice, TCRγδ$^+$ IEL comprise 50–60% of the IEL compartment in the small intestine [61], and in humans, only about 10–15% of all IEL are TCRγδ$^+$ cells [18,62]. The majority of mouse TCRγδ$^+$ IEL express Vγ7 and some express Vγ1 or Vγ4 [63], whereas most human TCRγδ$^+$ IEL express Vγ4 and are enriched for Vδ1-expressing cells [64]. Similar to most peripheral γδ T cells, the TCR specificity of TCRγδ$^+$ IEL remains uncertain, and there is no evidence for MHC-restricted antigen recognition.

While there is now strong evidence that the vast majority of natural TCRαβ$^+$ IEL are thymus-derived, recent studies have confirmed earlier reports that many TCRγδ$^+$ IEL have an extrathymic origin [65,66]. The development of these cells is independent of microbial and food antigens [65]. However, a recent study showed that the size of the TCRγδ$^+$ IEL compartment greatly depends on the expression of butyrophilin-like (BTNL) molecules [65],

a family of poorly understood gene products with structural similarity to B7 co-stimulatory molecules. Members of the BTNL protein family had already been implicated in modulating the response of human peripheral blood TCR$\gamma\delta^+$ T cells to phosphoantigens and to drive the maturation of murine TCR$\gamma\delta^+$ dendritic epidermal T cells [67]. The new study showed that expression of BTNL1 by enterocytes promotes the maturation and expansion of murine V$\gamma7^+$ IEL [65]. Additionally, BTNL1 and BTNL6 induce TCR-dependent responses by murine V$\gamma7^+$ IEL, and BTNL3 and BTNL8 similarly induce TCR-dependent responses of human colonic of V$\gamma4^+$ IEL [65]. These findings thus reveal that IEC can provide signals that instruct the development and function of the local TCR$\gamma\delta^+$ IEL compartment.

TCR$\gamma\delta^+$ IEL exhibit cytotoxic properties and produce cytokines such as IFN-γ, TNF-α, TGF-β, IL-10, and IL-13, prothymosin β4, keratinocyte growth factor (KGF), and antimicrobial proteins. While the traditional paradigm held that TCR$\gamma\delta^+$ IEL are stationary within the intestinal epithelium, recent studies have shown that these cells can migrate dynamically within the intestinal epithelium by contacting enterocytes via homotypic interactions mediated by the **tight-junction** protein occludin [68–70].

The main function of TCR$\gamma\delta^+$ IEL is to protect the intestinal epithelium against attack by pathogenic microorganisms and inflammatory insults. Recent studies have shown that TCR$\gamma\delta^+$ IEL are a critical component of the mucosal immune response against resident intestinal bacteria [71,72]. Within the first few hours after exposure to intestinal microbiota TCR$\gamma\delta^+$ IEL produce antimicrobial effectors such as the antibacterial lectin RegIIIγ. This is dependent on bacterial stimulation of **MyD88** signaling in IEC, indicating that cross-talk between IEC and TCR$\gamma\delta^+$ IEL promotes homeostasis with the resident microbiota. Such interactions might also be critical for immune responses against pathogenic microorganisms, as TCR$\gamma\delta^+$ IEL have been implicated in limiting bacterial invasion following mucosal infection with Salmonella typhimurium [72] and *Toxoplasma gondii* [73], and in initiating rapid expulsion of *Nippostrongylus brasiliensis* parasites [74]. Another function of TCR$\gamma\delta^+$ IEL during infection is to protect the epithelium against inflammatory damage. TCR$\gamma\delta^+$ IEL produce a variety of factors such as TGF-β, prothymosin β4, and KGF that promote healing and safeguard the integrity of the intestinal epithelium. These properties of TCR$\gamma\delta^+$ IEL are likely responsible for the increased tissue damage observed in TCR$\gamma\delta$-deficient mice following infection with Listeria *monocytogenes* [75]. TCR$\gamma\delta^+$ IEL can also suppress inflammatory responses by inhibiting the activation of other immune cells, as shown in a model of infection induced by the protozoan parasite *Eimeria vermiformis* [76]. Thus, TCR$\gamma\delta^+$ IEL have a dual role in microbial infection, inhibiting microbial invasion early, while limiting excessive inflammation and tissue damage late during infection.

TCR$\gamma\delta^+$ IEL also appear to play divergent roles during different phases of colitis. Several studies have shown a pathogenic role of these cells in colitis induction [77–80]. However, during the later stages of colitis, TCR$\gamma\delta^+$ IEL protect the epithelium against inflammation-induced damage [81–83].

TCR$\gamma\delta^+$ IEL are significantly increased in the small intestine of patients with celiac disease [40,84] but their relevance to disease remains unclear. Based on the critical role of IL-15 in driving TCR$\gamma\delta^+$ IEL expansion, the observed increase in TCR$\gamma\delta^+$ IEL in these patients

might be related to upregulated IL-15 production by IEC. The potential role of TCR$\gamma\delta^+$ IEL in controlling immune responses to food products such as gluten is supported by earlier studies providing evidence that these cells can regulate the induction and maintenance of oral tolerance in mice [85,86].

TCR$^-$ IEL

Recent studies have identified multiple subsets of TCR$^-$ IEL (Figure 1 and Table 1). This includes subsets of cells with phenotypic, developmental and functional similarities to subsets of ILC found outside the intestinal epithelium (Box 2). Additionally, the intestinal epithelium contains TCR$^-$ lymphocytes that are developmentally distinct from ILC and express iCD3 chains (TCR$^-$iCD3$^+$ IEL), including a subset that expresses CD8$\alpha\alpha$ (iCD8α cells).

ILC-like IEL

A subset of IEL with similarities to ILC1 cells (see Box 2) that colonize the intestinal epithelium and express NKp44 in humans [10,87,88], or NKp46 and NK1.1 in mice [10,89], has been identified. While the development of these cells is not affected in mice with defective IL-15 receptor signaling, their numbers are considerably reduced in mice deficient for the transcription factors **Nfil3** and T-bet [10]. These two transcription factors are also critical for the development of conventional NK cells [90], suggesting a lineage relationship between murine intraepithelial NKp46$^+$ ILC1-like cells and conventional NK cells. Human intraepithelial NKp44$^+$ ILC1-like cells have a memory-activated phenotype, produce IFN-γ in response to IL-12 and IL-15, and are amplified in patients with **Crohn's disease** [10], and in patients that received intestinal allografts [88]. In mice, intraepithelial NKp46$^+$ ILC1-like cells play a pathogenic role in the development of colitis induced by anti-CD40 antibodies [10].

In addition to murine intraepithelial NKp46$^+$ ILC1-like cells, a subset of NKp46$^-$ cells that express Ly49E and harbor a group 1 ILC transcriptional profile has been identified in the intestinal epithelium of mice [89]. Like intraepithelial NKp46$^+$ ILC1-like cells, intraepithelial NKp46$^-$Ly49E$^+$ cells require T-bet expression for their development. However, in sharp contrast with intraepithelial NKp46$^+$ ILC1-like cells, intraepithelial NKp46$^-$Ly49E$^+$ cells require IL-15 signaling for their development or survival. The latter cells are also capable of producing IFN-γ in response to IL-12, IL-15, or IL-18 activation in vitro, and in response to *T. gondii* infection *in vivo*.

A recent study has identified a human TCR$^-$ IEL subset expressing NKp44, **RORγt**, IL-23R and IL-22 in the intestinal epithelial immune compartment, which is expanded in patients that received intestinal allografts [88]. The phenotype of these cells is most similar to human NKp44-expressing group 3 ILC (see Box 2). Cells with this phenotype remain to be identified in mice.

iCD8α Cells and other TCR$^-$iCD3$^+$ IEL

Expression of CD8$\alpha\alpha$ is not limited to intestinal TCR$^+$ IEL. iCD8α cells are innate lymphocytes characterized by surface expression of CD8$\alpha\alpha$ [12], intracellular expression of

CD3ε and CD3γ (iCD3), and TCR rearrangements [11], which develop independently of a functional thymus [11]. The development and/or maintenance of these cells requires IL-15, the transcription factor **Notch1**, the Cd8α enhancer $E8_I$, and the thymus leukemia (TL) antigen, a non-classical MHC class I molecule expressed by IEC that serves as a high-affinity ligand for CD8 (Box 1). T-bet and other transcription factors such as **Id2**, Ahr and RORγt are dispensable for their development [12]. These results indicate that iCD8α cells share some developmental features with $TCR^+CD8\alpha\alpha^+$ IEL (i.e., IL-15-dependence) but differ in others (i.e., T-bet-dependence for $TCR^+CD8\alpha\alpha^+$ IEL but not iCD8α cells). iCD8α cells have also been identified in humans, where they are expanded in newborns with necrotizing enterocolitis [12]. These cells have functional properties related to innate immune cells, such as production of the cytokines monocyte chemotactic protein-1 (MCP-1), IFN-γ and osteopontin, and cytotoxic and phagocytic activities. Remarkably, iCD8α cells express MHC class II molecules and can present peptide antigens to MHC class II-restricted $CD4^+$ T cells. Functional studies have shown that these cells can protect mice against *Citrobacter rodentium* infection, and exacerbate experimental colitis induced by anti-CD40 antibodies [12,91].

In addition to $TCR^-iCD3^+CD8\alpha\alpha^+$ IEL (i.e., iCD8α cells), $TCR^-iCD3^+CD8\alpha\alpha^-$ IEL have been identified [11]. Like iCD8α cells, $TCR^-iCD3^+CD8\alpha\alpha^-$ IEL differentiate in the absence of Id2 and require Notch1 and IL-15 signals for their development. Considering the similarities between the $CD8\alpha\alpha^+$ and $CD8\alpha\alpha^-$ subsets of TCR^-iCD3^+ IEL, and the finding that CD8αα expression is often acquired by IEL following entry into the intestinal epithelium, it is tempting to speculate that $TCR^-iCD3^+CD8\alpha\alpha^-$ IEL might be precursors to $TCR^-iCD3^+CD8\alpha\alpha^+$ IEL. Nevertheless, it is equally possible that the $CD8\alpha\alpha^+$ and $CD8\alpha\alpha^-$ subsets are part of a larger population of TCR^- IEL expressing iCD3 chains.

Patients with a refractory form of celiac disease are characterized by an expanded population of innate IEL that selectively respond to IL-15, develop NK-like cytotoxicity against epithelial cells, and may develop into lymphomas [92]. These malignant cells express iCD3 chains, contain TCR rearrangements, and harbor gain-of-function mutations in Janus kinase 1 (Jak1) or Signal transducer and activator of transcription 3 (Stat3) that enhance their response to IL-15. Thus, these findings provide strong evidence that malignant IEL in this refractory form of celiac disease arise from TCR^-iCD3^+ IEL.

Conclusions and Future Perspectives

IEL represent a population of cells that reside in an environment characterized by constant and diverse antigenic encounters. Because of the vast antigenic load within the gastrointestinal tract, the IEL compartment has evolved a variety of cell types with distinct effector functions, including cells with cytotoxic, helper and regulatory properties. Thus, a key aspect of the IEL compartment is its diversity of cell types. This diversity also implies an intricate network of interactions of individual IEL subsets with other IEL subsets, with IEC, and with immune cells outside the intestinal epithelium. Collectively, these cellular interactions strengthen the mucosal barrier during homeostatic conditions and following exogenous insults. Considering the complexity of IEL biology, it is remarkable that these cells only rarely cause disease. Many questions regarding the development, homeostasis and

functions of distinct subsets of IEL remain (see Outstanding Questions Box). The information gleaned from the answers to these questions should provide novel avenues to harness the immune cells that reside at the mucosal frontline to fight infectious and inflammatory diseases. In particular, better reagents and tools are needed to identify individual IEL subsets, which will be critically important for studying their interactions, and to target these cells for therapeutic purposes. Potential avenues to this end include global transcriptional analyses to identify unique markers, which would facilitate the generation of antibodies and other reagents to selectively deplete or activate specific IEL subsets.

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Glossary

β_2-microglobulin:
A small, non-polymorphic protein that binds non-covalently with MHC class I heavy chains. It is critically important for surface expression of all classical MHC class I molecules and many non-classical MHC class I molecules (e.g., CD1d, Qa-2 and TL)

Celiac disease:
An inflammatory disorder of the gut due to an immune reaction against gluten in food products in genetically predisposed individuals

Crohn's disease:
A type of inflammatory bowel disease that may affect any part of the gastrointestinal tract, caused by a combination of environmental, microbial and immune factors in genetically susceptible individuals

Cryptopatches:
Small aggregates of lymphoid cells found in the intestinal lamina propria

Foxp3:
The forkhead box P3 transcription factor functions as a master regulator for the development and function of regulatory T cells

Id2:
A member of the inhibitor of DNA binding (ID) family of transcription factors that is critically important for the development of all subsets of innate lymphoid cells

Innate lymphoid cells (ILC):
A family of innate lymphocytes that require the transcription factors Id2 and Nfil3 for their development and exhibit functions analogous to distinct effector T cell subsets

Lamina propria:
A layer of connective tissue beneath the epithelium of mucosal membranes

MyD88:

The myeloid differentiation gene 88 product is an adaptor protein critical for signaling through most toll-like receptors

Nfil3:
Nuclear factor, interleukin-3 regulated (also called E4BP4) is a bZIP transcription factor required for the development of all subsets of innate lymphoid cells

Non-classical MHC molecule:
Glycoproteins related to the classical MHC molecules that exhibit limited polymorphism, expression patterns, and presented antigens

Notch1:
A receptor of the conserved notch signaling pathway critical for T cell lineage commitment

Nude mice:
These mice exhibit a spontaneous mutation in the Foxn1 transcription factor that causes abnormal hair growth and defective development of the thymic epithelium, resulting in the absence of T cells

Peyer's patches:
Small lymphoid follicles throughout the ilium of the small intestine

Programmed death-1 (PD-1):
An inhibitory surface receptor expressed by chronically activated or regulatory T cells that plays a critical role in downregulating immune responses

RAG:
The recombination activating gene 1 and 2 products are critical for the rearrangement of B and T cell receptor genes

Retinoic acid:
A metabolite of vitamin A that binds with its nuclear receptor in lymphocytes to promote mucosal tolerance

RORγt:
The retinoic acid-related orphan receptor γt transcription factor is critical for the differentiation of T helper 17 cells

Runx3:
The runt-related transcription factor-3 acts as a key regulator for commitment of immature T cells to the CD8 lineage

T-bet:
A T-box transcription factor encoded by the Tbx21 gene and selectively expressed by T helper 1 cells to control expression of the IFN-γ gene

ThPOK:

The T helper-inducing POZ/Krüppel factor acts as a key regulator for commitment of immature T cells to the CD4 lineage

Tight junctions:
Closely associated areas between cells whose membranes join together to form a barrier

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Box 1:

Characteristic Surface Markers of Intestinal IEL

CCR9:

The CC-chemokine receptor 9 (CCR9) is primarily expressed by developing thymocytes and intestinal lymphocytes. It binds with its ligand, chemokine ligand 25 (CCL25), which is expressed at high levels in thymic dendritic cells and intestinal epithelial cells (IEC). This chemokine receptor/chemokine pair is critically important for recruitment of lymphocytes to the intestinal epithelium.

$\alpha_E\beta_7$:

The integrin $\alpha_E\beta_7$ (α_E is also known as CD103) is expressed by all intestinal epithelial lymphocytes (IEL) and interacts with E-cadherin on mucosal epithelial cells, thus mediating homing of lymphocytes to the intestinal epithelium.

CD8αα:

With the exception of some TCR^- IEL, one feature that distinguishes conventional peripheral lymphocytes from intestinal IEL is the expression of CD8α homodimers by the latter cells. Conventional $CD8^+$ T cells express the CD8αβ heterodimer, which is considered a co-receptor that enhances interactions between the TCR and MHC class I-peptide complexes to augment signal transduction. Although CD8αα has similar binding affinity to multiple classical MHC class I molecules, there is ample evidence that CD8αα possesses a distinct role during T cell activation [93], serving as a repressor of IEL activation via interaction with the thymus leukemia (TL) antigen, a non-classical MHC class I molecule with much higher affinity for CD8αα than classical MHC class I molecules. Evidence pointing towards CD8αα as a repressor of TCR signaling comes from several different experimental approaches. Overexpression of CD8αα in double-negative thymocytes results in weakened signal transduction events [94], the effects of CD8αα co-expression in CD8αβ T cells are proportional to the strength of TCR stimulation [95], and transgenic expression of high-affinity peptide agonists induces development of $CD8\alpha\alpha^+$ but not $CD8\alpha\beta^+$ T cells [96]. Therefore, the available evidence suggests that $TCR^+CD8\alpha\alpha^+$ IEL, which are considered to be in a “partially activated state” [14], express CD8αα to quench the response to high-affinity antigens. A subset of TCR^- IEL, called iCD8α cells, express CD8αα homodimers in the absence of a TCR, but its role in regulating the effector functions of these cells remains unclear. Instead, the available evidence suggests that the development and/or maintenance of iCD8α cells depends on interactions between CD8αα on these cells with TL on IEC [12].

Box 2:

Innate Lymphoid Cells (ILC)

Recent studies in immunology have identified several subsets of innate lymphocytes, referred to as innate lymphoid cells (ILC), that lack T cell receptor (TCR) expression but share effector functions with distinct subsets of effector T cells of the adaptive immune system [97]. These cells have been classified into three groups (group 1, 2 and 3 ILC) and all require expression of the transcription factors Id2 and Nfil3 (also called E4BP4) for their development. Group 1 ILC produce IFN-γ and express the transcription factor T-bet. This group includes natural killer (NK) cells and ILC1 cells, which share effector functions with $CD8^+$ cytotoxic T lymphocytes and T helper 1 (Th1) cells, respectively. Group 2 ILC consists of ILC2 cells (also called nuocytes, natural helper cells or innate helper 2 cells) that produce the cytokines IL-4, IL-5 and IL-13, and express the transcription factor GATA-3, which are characteristics shared with Th2 cells. Group 3 ILC produce the cytokines IL-17 and IL-22 and express the transcription factor RORγt. This group includes lymphoid tissue inducer (LTi) cells that play a critical role in the development of lymphoid tissues, and subsets of ILC3 cells that share properties and functions with Th17 cells. ILC are enriched in mucosal tissues, respond early in an immune response, and influence the quality of adaptive immune responses. In concert, the complementary groups of ILC and effector T cells orchestrate the generation of the different types (types 1-3) of immunity. Recently, subsets of TCR^- intraepithelial lymphocytes that resemble subsets of ILC have been identified in the intestine (see main text).

Outstanding Questions Box

- Are all subsets of intestinal IEL currently identified conserved between mice, humans, and other mammals?
- Does the intestinal epithelium contain additional IEL subsets yet to be discovered?
- How does a major fraction of TCR$\gamma\delta^+$ IEL develop extrathymically?
- Are distinct subsets of TCR^- IEL, especially CD8$\alpha\alpha^-$ and CD8$\alpha\alpha^+$ subsets of $iCD3^+$ IEL, developmentally related?
- How do different members of the gut microbiota influence IEL development, homeostasis and function?
- What are the molecular interactions between IEL and intestinal epithelial cells that regulate intestinal homeostasis and disease?
- How do distinct subsets of TCR^+ and TCR^- IEL interact to enhance mucosal barrier integrity during diverse exogenous and endogenous insults?
- How do IEL influence immune responses outside the intestinal epithelium, within the mucosal lamina propria and beyond?
- How can distinct subsets of IEL be targeted for disease prophylaxis and therapy?
- Can the composition of the intestinal microbiota be manipulated to enhance the protective functions of IEL against breaches of the mucosal barrier?

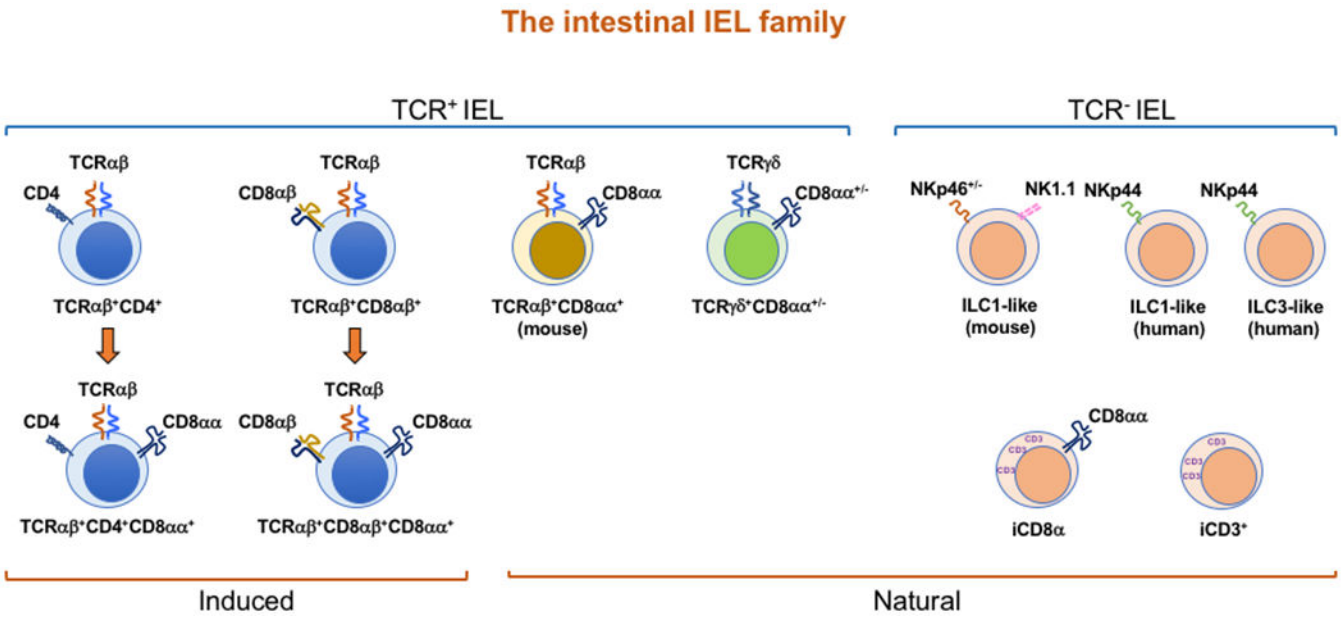

Figure 1. Intestinal IEL Classification.

Intestinal IEL can be classified according to their TCR expression profile (i.e., TCR$\alpha\beta^+$, TCR$\gamma\delta^+$ or TCR$^-$). TCR$^+$ IEL can be further subdivided into induced TCR$^+$ IEL that are derived from conventional TCR$\alpha\beta^+$ T cells activated outside of the intestinal epithelium, and natural TCR$^+$ IEL that home naturally to the intestinal epithelium following their development. Many induced TCR$^+$ IEL become positive for CD8$\alpha\alpha$ expression upon entry into the intestinal epithelium (see arrows). TCR$^-$ IEL comprise subsets of cells resembling innate lymphoid cells (ILC), intracellular CD3$^+$ (iCD3$^+$) cells, and iCD8α cells. Some differences in the prevalence and phenotype of mouse versus human IEL are indicated. Of note, TCR$\alpha\beta^+$CD8$\alpha\alpha^+$ IEL are rare in adult humans, and ILC3-like IELs have not yet been described in mice. It is likely that additional intestinal IEL subsets are yet to be discovered.

Table 1:

Development, Effector Properties and Functions of Intestinal IEL.

IEL Subset	Development	Effector Properties	Functional Roles	References
TCRαβ$^+$CD4$^+$	Conventional T cell development Antigen-experienced cells	T helper properties	Effector memory Th1 and Th17 responses against mucosal pathogens	3,14
TCRαβ$^+$CD4$^+$CD8αα$^+$	Derived from antigen-experienced cells Dependent on TGF-β, IFN-γ, IL-27, T-bet, Runx3	Cytotoxicity? Others?	MHC-class II dependent cytotoxicity Regulatory functions	13,22–26,28,29,32
TCRαβ$^+$CD8αβ$^+$	Conventional T cell development Antigen-experienced cells	Cytotoxicity NKG2D-mediated cytotoxicity	Effector memory responses against mucosal pathogens Pathogenic in celiac disease	18,33,40,41
TCRαβ$^+$CD8αβ$^+$CD8αα$^+$	Derived from antigen-experienced cells	Cytotoxicity?	Not well understood	35
TCRαβ$^+$CD8αα$^+$	Thymic development Agonist TCR specificity Classical or non-classical MHC class I restriction Dependent on TGF-β, IFN-γ, IL-15, T-bet, Runx3	NK-like properties	Immunoregulatory	25,37,44–47,49–55,59
TCRγδ$^+$CD8αα$^{+/-}$	Extra-thymic development Dependent on butyrophilin-like molecules	Cytotoxicity Produce IFN-γ, TGF-β, IL-10, IL-13, KGF	Anti-microbial Promote healing and protect integrity of the intestinal epithelium	65–66,68–70,71–76
ILC1-like	Extra-thymic development Dependent on Nfil3, Tbet, Id2	Produce IFN-γ	Involved in intestinal inflammatory responses	10,88–89
iCD8α	Extra-thymic development Dependent on IL-15, Notch-1, E8$_I$, TL	Produce osteopontin, IFN-γ, granzymes Phagocytic, antigen presentation	Promote bacterial clearance Involved in intestinal inflammatory responses	11–12,91
iCD3$^+$	Extra-thymic development Dependent on IL-15, Notch-1	NK-like cytotoxicity	Involved in refractory celiac disease lymphomas	11,92