Role of the intestinal cytokine microenvironment in shaping the intraepithelial lymphocyte repertoire

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ABSTRACT
Local resident IELs are composed of distinct subsets of T cells with potent cytolitic and immunoregulatory capacities. As IELs are located within this unique interface between the core of the body and the outside environment, the specific development and function of intestinal IELs must be tightly regulated. To accomplish this, the cytokine microenvironment of the intestine has evolved sophisticated mechanisms that modulate the phenotype, ontogeny, and function of these cells. In this review, we summarize the evidence demonstrating the origin of certain intestinal cytokines, including IL-7, IL-15, IL-2, TGF-β, and SCF and discuss what influence such cytokines may have on IELs. Moreover, we review data suggesting that the abnormal expression of cytokines that leads to the heightened activation of IELs may also contribute to immunopathological responses or exacerbate inflammatory diseases, such as IBD and celiac disease, or promote cancer development and progression.

Introduction
The intestine is the largest immune organ in the body and forms the main protective barrier between the internal milieu and a large number of nonself antigens and potential pathogens in the intestinal lumen. To maintain immune homeostasis, the intestinal immune system must guard the body against invasion by pathogens while avoiding aberrant responses to commensal microbiota or food antigens. Generally, the intestinal immune response occurs at the GALT, which contains ~70% of the total lymphocytes in the body, and based on location, the GALT can be divided into effector sites, which consist of the lamina propria, the IEL compartment, and inductive sites that are responsible for the initiation of immune responses and tolerance [1]. These sites include isolated lymphoid follicles, PPs, and the MLNs [2]. The GALT is separate from, yet connected to, the systemic immune system and is locally specialized and compartmentalized, in large part, as a result of adaptation to the microenvironment [3]. The development and functional programming of the intestinal immune system are regulated by sophisticated mechanisms and characterized by regionalization at multiple levels.

An important player in the gastrointestinal immune system is the IEL population, which resides at the basolateral site of the intestinal EC layer. The IELs are the first immune cells to encounter intraluminal foreign pathogens that have entered the body via the epithelial surface. In the small and large intestine, IELs mainly consist of T cells, whose number can represent up to half of the total T cell population in the organism [4]. Although primarily CD8+ T cells, IELs are composed of antigen-experienced memory-effector subtypes bearing the αβ or the γδ TCR (TCRαβ+ or TCRγδ+) [5]. Virtually, all TCRγδ+ and many TCRαβ+ IELs in the small intestine are known to express the CD8αα, which is rarely detected in peripheral lymphoid tissues [6]. Furthermore, IELs include a greater proportion of TCRγδ- cells than is found in the systemic circulation of mice or humans [7–9]. Based on their phenotype and developmental origin, IELs can be classified into 2 cell types: “a” and “b” [10]. These 2 types of cells are discussed in detail below. The physical location of IELs at the intersection between the internal and luminal (external) environments suggests that the regulation of IELs is carefully mediated and that the development, activation, and functional specialization of IEL subsets are influenced by other cell types and soluble factors, particularly the cytokines released by immune and nonimmune cells in the local microenvironment.

In this review, we summarize the evidence detailing the origin of certain intestinal cytokines and discuss what influence such cytokines may have on IELs. Furthermore, we present studies showing that the abnormal expression of cytokines observed in diseased intestines could be associated with IEL dysfunction and discuss how these cells may contribute to immune pathology and inflammatory diseases.

Abbreviations: /−/− = deficient, β/γ = β/γ-chain, Bcl-2 = B cell lymphoma 2, Bim = B cell lymphoma 2-interacting mediator of cell death, CD8αα = homodimeric form of cluster of differentiation 8α, DC = dendritic cell, EC = epithelial cell, FasL = Fas ligand, IR = ischemia/reperfusion, IBD = inflammatory bowel disease, IEL = intraepithelial lymphocyte, IL-7Rα = IL-7R α-chain, IFN = IFN regulatory factor, KGF = keratinocyte growth factor, (continued on next page)
SUBSETS OF INTESTINAL IELS

Type a IELs consist of the conventional or "induced" T cells, which express an αβ TCR together with CD4- or CD8αβ as TCR coreceptors. Type b IELs encompasses TCRαβ+ T cells or TCRγδ+ T cells, which do not express a TCR coreceptor but typically express CD8αα [10]. Although TCRγδ+ and TCRαβ+CD8αα IELs are sometimes classified into the type b group, these 2 unconventional populations have some fundamental differences, which will be detailed in the sections that follow [11].

CD4αβTCRαβ+ and CD8αβTCRαβ+ IELs

Type a IELs arise from CD4αβ or CD8αβ TCRαβ+ T cells, which are MHC class II restricted and MHC class I restricted, respectively. These IELs are thought to have followed conventional thymic selection and reached the gut after antigenic stimulation in the periphery [12]. For this reason, type a IELs have a memory-phenotype (CD2−CD5+CD4+CD28+Thyl+LFA-1+) [7, 13]. In addition, because of the positive and negative selection in the thymus, one can assume that type a IELs are specific for nonself antigens. Unlike the mainstream naïve T cells in the periphery, this group of cells expresses an oligoclonal TCR repertoire [14]. Interestingly, the ratio of CD8αβ+ to CD4αβ+ T cells among the small intestinal IELs is much higher than that seen in the spleen [15]. Another feature of the small intestine type a IELs is the frequent coexpression of CD8αα, a hallmark of activated mucosal T cells and their adaptation to the gut microenvironment [16]. However, these double-positive cells (CD4αβCD8αα or CD8ααβCD8αα−) are much less prevalent in the large intestine epithelium and lamina propria, but their number can increase under inflammatory conditions. CD8ααβTCRαβ+ IELs are primarily cytolytic upon antigenic challenge, killing via granymes or by engagement of Fas, and may also secrete Th type 1 cytokines [10].

TCRγδ+ IELs

TCRγδ+ lymphocytes are the first T cells to emigrate from the thymus, and most of these cells take up residence in the epithelial tissues [17]. Upon migration into the intestinal epithelium, they do not exchange with other γδ T cell populations [18]. The vast majority of TCRγδ+ IELs expresses CD8αα, but a small proportion lacks CD8α and CD8β [1]. This is in striking contrast to TCRγδ+ T cells located in lymphoid tissues, which predominantly lack CD8 expression entirely. In humans, the γδ TCRs expressed by IELs predominantly use the Vγ1 gene and Vδ1 gene; however, in mice, Vγ5 T cells are enriched in the intraepithelial tissues of the gut [19]. The ratio of TCRγδ+ to TCRαβ+ in the IEL population varies among species. In mice, TCRγδ+ IELs can constitute up to 60% of small intestinal IELs, whereas in humans, TCRγδ+ IELs constitute a relatively small (~10%) proportion to the total IEL population [20]. In sharp contrast to TCRαβ+ IELs, TCRγδ+ cells recognize nonclassic MHC molecules, such as thymus leukemia antigen or the human MHC class I-like molecules, MICA and MICB, and may help down-regulate excessive immune responses [21, 22]. Additionally, TCRγδ+ IELs do not express some "typical" T cell markers, such as CD2, CD28, LFA-1, and Thy1 [23]. Similar to the type a IELs, these IELs are cytolytic, expressing high levels of effector molecules, including granzyme and Fasl [5]. TCRγδ+ IELs also contribute to EC proliferation by secreting KGF, suppressing CD4+ T cell expansion through TGF-β and IL-10 production, and provide help for Ig class-switching [24].

CD8αα+TCRαβ+ IELs

Over the past decade, the origin and development of CD8αα+ TCRαβ+ IELs have been the subject of longstanding debate. Here, we provide a brief overview of the data that attempt to unify the divergent views on the subject. Initially, based on the self-reactivity of the TCRs in their repertoire and data from athymic mice, all CD8αα+ IELs, including the CD8αα+TCRαβ+ subset, were thought to differentiate locally in the gut [11]. However, more recent approaches have led to the conclusion that the thymus appears to be the major source of CD8αα+TCRαβ+ IELs in normal euthyemic mice. The presence of a thymus during the neonatal stage plays a critical role in the homeostasis of the CD8αα+ IELs, as neonatal, thymectomized mice and nude mice harbor few CD8αα+TCRαβ+ IELs, and neonatal thymus transplantation can restore this subset in athymic mice [25-27]. Interestingly, this unique IEL subset requires an alternative selection process in the thymus, similar to other Treg populations, including forkhead box p3-positive natural Treg and CD1d-reactive NKt cells [28-30]. This alternative selection process is also called "agonist selection," reflecting the fact that these types of T cells require relatively strong interactions with self-antigens for positive selection and differentiation into specialized T lymphocytes [11]. In the thymus, CD8αα+TCRαβ+ IELs originate from immature CD4αβCD8αα+ (triple-positive) thymocytes; thereafter, they undergo agonist selection and migrate to the intestine as double-negative (CD4αβ−CD8αα+) T cells, which reside in the IL-15-rich environment induces the up-regulation of CD8αα expression [31]. Unlike the TCRγδ+ IELs, CD8αα+TCRαβ+ IELs are greatly under-represented in the IEL compartment. In C57BL/6J mice, 50% of small intestine IELs are CD8αα single positive, of which, TCRαβ+ and TCRγδ+ cells are found at a ratio of 1:4 [25]. Although the selection of these CD8αα+TCRαβ+ IELs is based on self-reactivity, they are not self-destructive and have a regulatory function within the mucosal immune system [32, 33]. These IELs do not express some of the typical T cell markers that are expressed by type a IELs, including CD2, CD5, CD28, Thy1, and LFA-1 [34]. However, similar to the type a IELs, CD8αα+TCRαβ+ IELs are cytolytic, expressing high levels of effector molecules, which include granzyme and Fasl [35].

INTESTINAL CYTOKINE MICROENVIRONMENT AND IELS

Although IELs are extremely heterogeneous, they are all the progeny of bone marrow precursor cells that develop initially in
the thymus [36]. This heterogeneity strongly suggests that their thymic education is not uniform and that multiple development pathways exist. The thymic microenvironment provides a unique combination of cellular interactions and cytokine stimulation that directs lineage commitment and functional differentiation. This topic has been reviewed extensively elsewhere [11, 37, 38]. Regardless of the mechanisms of selection or the location of initial differentiation, all IELs are influenced directly by the intestinal microenvironment. Given the complex environmental conditions faced in the intestine, the IELs are tightly regulated through cell survival, activation, and death [39]. The specific signals that regulate T cell homeostasis are numerous. Cytokine signaling is believed to be particularly important for the function and differentiation of IELs (Fig. 1).

**IL-7**

IL-7 was first described as a potent hematopoietic growth factor of B cell progenitors [40]. Subsequent studies have demonstrated that IL-7 plays an important role in the differentiation of pre-T cells into mature thymocytes and controlling the survival and homeostatic proliferation of naive T cells in the periphery [41–43]. This cytokine is constitutively secreted by nonhematopoietic stromal cells, such as thymic ECs and intestinal ECs, and in turn, IL-7Rs have been detected on the surface of thymocytes and IELs [44]. The IL-7R consists of the common cytokine receptor γc (CD132) and the unique IL-7Rα. Interestingly, IL-7Rα is shared by the receptor for TSLP, and the common γc is shared by the receptors for IL-2, IL-4, IL-9, IL-15, and IL-21 [45]. Two main pathways are activated by IL-7: the JAK/STAT5 pathway and the PISK/Akt/mammalian target of rapamycin pathway [46]. Originally, it was demonstrated that normal human and murine intestinal ECs express IL-7 [47]. Subsequently, several studies have shown that rIL-7 stimulates a significant increase in DNA synthesis and prevents the caspase-dependent and -independent pathways that lead to spontaneous apoptosis of IELs [48, 49]. Following these studies, other investigators have demonstrated that IL-7 is crucial for the development of TCRγδ+ T cells in the intestinal mucosa of the mouse. IL-7−/− or IL-7R−/− mice did not have TCRγδ+ IELs but possessed small numbers of TCRβ+ IELs [50, 51]. Laky et al. [52] observed that local IL-7 transgene expression by the ECs of IL-7−/− mice restored the development of TCRγδ+ IELs; thus, they considered that IL-7 expression by enterocytes was sufficient for extrathymic development of TCRγδ+ IELs. Confocal microscopic analysis also confirmed the colocalization of IEL subpopulations to EC-derived IL-7, which suggests that there is close IEL-EC cross-communication mediated by EC-derived IL-7 expression [53]. Additionally, TCRγδ+ IELs could be preferentially impaired in the deficient mice, at least in part, as IL-7-mediated signaling is essential for the rearrangement of the TCRγ genes [54]. Although the experiments cited above showed extrathymic lymphopoiesis, recent studies support the notion

### Table: Intestinal Cytokines and Their Effects on IELs

<table>
<thead>
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<th>Cytokine</th>
<th>Target cells</th>
<th>Process</th>
<th>Related diseases</th>
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| IL-7     | Type a IEL, Type b IEL | Increasing DNA synthesis, Preventing apoptosis, Rearrangement of TCRγ genes, Upregulation of TNF-α, INF-γ, KGF Enhancement of activation | • Total parenteral nutrition ↓  
• Ischemia/reperfusion↑  
• Chronic colitis↑ |
| IL-15    | CD8α+CD4−TCRγδ+ IEL, CD8α−CD4+TCRγδ+ IEL | Modulating Th1/Th2 balance, Modulating histone acetylation, Increasing IFN-γ, TNF-α, IL-10, Upregulation of cytotoxicity, Uptregulation of NKG2D | • Celiac disease↑  
• Inflammatory bowel disease↑ |
| IL-2     | Type a IEL, CD8α−TCRγδ+ IEL | Supporting TCRγδ+ IELs growth, Restoration of CD4+ CD8+ IELs | • Ischemia/reperfusion? |
| TGF-β    | Type a IEL, CD8α−TCRγδ+ IEL | Maintaining CD8α expression, Inhibiting mitosis, Inducing CD103 | • Graft-versus-host disease?  
• Colorectal cancer↑ |
| SCF      | CD8α+TCRγδ+ IEL | Inducing proliferation and IFN-γ, Uptregulation of TCRγδ, Inducing cytotoxic activity |  

**Figure 1.** Intestinal cytokines and their effects on IELs.
that in the presence of a functional thymus, all IELs, including the TCRγδ+ IELs, develop intrathymically. These questions have been addressed by assessing the expression of RAG through the use of RAG2-GFP transgenic mouse models. The use of this reporter system, it was shown that athymic mice show receptor rearrangements predominantly in the MLNs and PPs [55]. Interestingly, these alternative pathways were totally suppressed in euthymic mice. Nevertheless, in euthymic GFP TCRγδ−/− transgenic mice, GFP−/−TCRγδ−/− precursors were present in the MLNs and PPs, suggesting that the lack of extrathymic T cell development in euthymic mice is not merely a result of the presence of a thymus but rather, to the thymus-derived TCRαβ+ T cells, which appear to suppress lymphopoiesis actively in the gut [20, 56].

Several factors are known to be involved in the regulation of IL-7 production in the gut. Shalapour et al. [57] found that commensal-driven IFN-γ production by intestinal lymphocytes promoted steady-state IL-7 production by intestinal EC and the subsequent maintenance of IELs. Likewise, previous work has shown that KGF, secreted by TCRγδ+ IELs, could up-regulate IL-7 expression in enterocytes through the STAT1/IRF-1 and IRF-2 signaling pathway [58]. It is worthwhile to note that IL-7 from maternal milk crosses the intestinal barrier and modulates T cell development in offspring [59]. The tight regulation of IL-7 availability is crucial for intestinal mucosal homeostasis. For instance, the lack of IL-7 leads to immunodeficiency, whereas its overexpression causes aberrant IEL activation and autoimmunity. Previous experiments have shown that TPN significantly decreased EC-derived IL-7 expression, along with a marked decline in the CD8αβ+, CD4+, and TCRαβ+ IEL subpopulations, decreased IEL proliferation, and the resultant, marked decrease in IEL numbers [60]. This change in IEL function resulted in a loss of epithelial barrier function and intestinal mucosal atrophy [61]. Subsequently, we generated a transgenic mouse model that overexpressed intestinal IL-7 overexpression signiﬁcantly attenuated the postadministration, TPN-associated changes in IEL phenotype and function [62]. Thus, the above evidences indicate the existence of cross-talk between IEL and EC, which could potentially regulate the mucosal homeostasis. In contrast, it was observed that intestinal I/R up-regulated IL-7 expression significantly; increased the CD8αβ+, CD4+, and TCRαβ+ IEL subpopulations; and enhanced IEL activation [63]. Coincidentally, IL-7 expression was increased during the mild ischemia seen in small intestinal tissues of patients with intestinal obstruction [64]. These findings provide profound insight into potential IEL-mediated epithelial barrier dysfunction after intestinal I/R. In UC patients, IL-7 protein expression is increased significantly and mediates the persistence of chronic colitis through IL-8αα expression, specifically on CD4+ T cells but not on other cell types [65]. Moreover, the systemic overexpression of IL-7 has resulted in the expansion of lymphoid populations and development of spontaneous chronic colitis. Flow cytometric analysis of IELs showed that CD4+ IELs were increased significantly in the colitis lesions; however, IEL functional changes were not assessed in the study [66].

**IL-15**

Much attention has been focused recently on IL-15, a cytokine that is produced by several kinds of cells, including DCs and intestinal ECs, and that acts on virtually every cell type of the innate and adaptive immune system [67–69]. The IL-15Rα is a heterotrimeric receptor composed of 3 subunits: the IL-15Rα is specific for IL-15, for which it possesses a high affinity; whereas the β, (CD122) and γ, (CD123) are common to the IL-15R and IL-2R, binding soluble IL-2 and IL-15 with intermediate affinity, and are expressed mainly by hematopoietic cells [25]. IL-15 and IL-15Rα could form a complex intracellularly and exist in a transmembrane form [70]. The transmembrane IL-15−IL-15Rα complex is then “in trans presented” to the IL-15Rβγ on neighboring cells for use [71]. The common receptor components (β, and γ) mediate the use of the JAK1/JAK3/STAT5 signaling pathway, which supports T cell and NK cell homeostasis and expansion [72]. In addition, IL-15 triggers other signaling pathways in T lymphocytes, including the PI3K/AKT, and the Ras/MAPK pathways, which lead to mitogenic and antiapoptotic signals [73, 74]. The discovery that IL-15 expression can be influenced by several factors, including innate-immune signaling, dietary nutrients, and lumen microbiota, and that IL-15 is a potent stimulant of IELs focused attention on the role of IL-15 in the interplay between ECs and IELs [75]. Indeed, TLR4 activation was shown to up-regulate IL-15 on DCs, whereas intestinal ECs require MyD88 for the expression of IL-15 and the promotion of CD8αα “TCRαβ” and TCRγδ+ IEL maintenance in an IL-15-dependent manner [76]. Jiang et al. [77] reported that NOD2 signaling might maintain the expression of IL-15 via recognition of commensal bacteria, as reduced IL-15 expression contributes to the loss of IELs (CD8αα+TCRαβ+ and TCRγδ+ subsets) in NOD2−/− mice. Furthermore, microbial changes induced by antibiotic treatments could modify the intestinal cytokine balance. A significant IL-15 increase was observed in ampicillin-treated mice; however, vancomycin treatment, which propagated the bacterium *Akkermansia muciniphila*, reduced the level of IFN-γ and IL-15 expression in the intestine [78]. Finally, consumption of a diet high in polyunsaturated fat led to a decrease in IL-15 expression and decreased the proportion of CD8αα “TCRαβ” cells and IEL-derived TNF-α, IFN-γ, IL-4, and IL-10 [79]. Taken together, these studies strongly suggest that an IL-15-rich environment in the gut is crucial for the development and maintenance of type b IELs. Mice lacking the IL-15 system, including IL-15−/−, IL-15Ra−/−, and IL-15Rb−/− mice, show a severe reduction in CD8αα+TCRαβ+ and TCRγδ+ IELs [80–82]. The mechanism underlying IL-15-mediated survival of type b IELs involves the activation of the Jak3-Jak1-P190-Akt-ERK pathway to modulate the balance between Bcl-2 and Bim expression [25, 83, 84]. Interestingly, the intestinal ECs expressing the IL-15 and IL-15Ra are considered to be parenchymal cells, which *trans*-present IL-15 to IELs and direct the development of the CD8αα+ subset [85]. Additionally, it has been suggested that the preferential Vγ5 gene use among TCRγδ+ IELs is controlled by IL-15, which specifically modulates Vγ5 gene segment-associated histone acetylation [86]. Regardless, IL-15 does not seem to be critical for the survival of CD8αβ+TCRαβ+ IELs, whose numbers are maintained in the absence of IL-15Ra.
[85, 87]. We discuss below how, according to its pleiotropic function, IL-15 impacts distinct IELs subsets and pathways to disrupt intestinal immune homeostasis.

The chronic up-regulation of IL-15 in the intestinal mucosa is a hallmark of celiac disease and correlates with the degree of mucosal damage [88]. In addition, untreated celiac disease IELs, characterized by higher IL-15Ra expression, showed increased production of IFN-γ and TNF-α, perforin/granzyme-dependent cytotoxicity, and a decreased propensity to apoptosis in response to IL-15 [89], which was shown to up-regulate the activating NKG2D receptor, endowing these cytotoxic IELs with the ability to recognize the intestinal ECs expressing the stress-induced MICA [90]. The NKG2D/MICA interaction led to an innate-like cytotoxicity toward epithelial targets and villus atrophy [91]. When human IELs were cultured with IL-15, an increasing number of IELs became CD94+ and produced massive quantities of IL-10, which promoted FasL-mediated cytotoxicity [92].

Furthermore, a recent study indicated that IL-21 was produced at high levels by IELs and lamina propria lymphocytes in active celiac disease; mucosal T cells were treated with IL-15, resulting in the activation of Akt and STAT3, thus enhancing synthesis of IL-21, which amplifies the Th1 and Th17 cell responses [98]. As a result of its crucial pathogenetic role and counter-regulated the immune-suppressive activities of Tregs [93].

Responses to IL-2 are mediated through its interaction with the high-affinity trimeric IL-2R complex (IL-2Rα, CD122, and CD132). The combination of IL-2Rβ (CD122) and IL-2Rγ (CD152) together forms an IL-2Rβ/γc complex, mainly expressed on memory T cells and NK cells, which binds IL-2 with intermediate affinity [100]. IL-2 signals via the heterodimerization of the IL-2Rβ/γc cytoplasmic domains, which leads to the activation of at least 3 major signaling pathways: PI3K/AKT, Ras-MAPK, and JAK-STAT. In contrast to the multiple cellular sources of IL-15 (including ECs, DCs, and IELs), IL-2 is produced primarily by CD4+ T cells following their activation by antigen [101]. León et al. [102] demonstrated that TCRαβ IELs are the main source of IL-2 and IFN-γ, followed by NK IELs and TCRγδ IELs. Consistent with an immunoregulatory role in the intestinal mucosa, CD8αα+ TCRαβ IELs down-regulate IL-2 and IFN-γ expression after the specific self-antigen challenge [103].

Secreted by the neighboring TCRαβ IELs, IL-2 supports the growth of restimulated TCRγδ IELs [104]. This notion is supported by the finding that IL-2−/− mice demonstrated a marked reduction in CD8αα+ TCRγδ IELs to nearly the same level as IL-2−/− mice [105]. Furthermore, Nüssler et al. [106] found that IL-2 treatment does not prevent intestinal I/R but promotes the restoration of the IEL subset distribution via an increase of CD4+CD8− IELs.

**TGF-β**

TGF-β exists in 3 isoforms (TGF-β1, TGF-β2, and TGF-β3), with TGF-β1 as the most common within the immune system. TGF-β signaling is mediated primarily through the Smad family of transcription factors. Therefore, the inhibition of the canonical TGF-β signaling pathway through deletion of Smad3 or Smad4 or by the increased expression of inhibitory Smad7 promotes gut inflammation [107, 108]. The gut is a TGF-β-rich environment, in which most cell types (including intestinal ECs, lymphocytes, monocytes, macrophages, and DCs) can produce and respond to this cytokine [109]. Nagafuchi et al. [110] showed that dietary nucleotides fed to mice enhanced the secretion of TGF-β from intestinal ECs. Their subsequent study suggested that this increased secretion of TGF-β from intestinal ECs was a result of indirect effects of the nucleotides, which may affect intestinal microflora or cells other than ECs, which in turn, influence intestinal EC cytokine secretion [111]. Furthermore, type b IELs can also be a source of TGF-β in the intestinal mucosa [112]. Similar to the developmental pathways of other Treg populations (Treg and CD1d-reactive NKT), CD8αα+ TCRαβ IEL development is TGF-β dependent. TGF-β1−/− mice and mice with a T cell-specific deletion of TCRαR I both lacked CD8αα+ TCRαβ IELs, whereas transgenic mice that overexpressed TGF-β1 have an increase in this population. Notably, it has been demonstrated that TGF-β maintains CD8α expression, not only in TCRαβ IELs but also in peripheral CD8+ T cells [113]. In an in vitro study with the use of IELs derived from the proximal jejunum of patients, TGF-β inhibited the mitosis of IELs in response to mitogens, IL-7, and CD2 and CD3 stimulation [114]. Once in the gut, expression of CD103 (also known as αε integrin) is important for the retention of IELs in the gut environment. Failure to induce CD103 on T cells by TGF-β results in reduced numbers of intestinal IELs [115]. In the absence of signaling through the TGF-βR, host-specific CD8+ effectors infiltrating the intestinal epithelium during graft-versus-host disease do not

**IL-2**

IL-2 is a pleiotropic cytokine that drives T cell growth, augments NK cytolytic activity, and mediates activation-induced cell death. Responses to IL-2 are mediated through its interaction with the glycoprotein IL-2/IL-15R, which enhances local T cell activation, in contrast to the anti-inflammatory effects of retinoic acid in intestinal ECs from 2,4,6-trinitrobenzene sulfonic acid-treated mice [96]. On the contrary, higher levels of IL-15 are expressed by untreated celiac disease IELs, but also synergize with retinoic acid to enhance intestinal EC cytokine secretion [111]. Furthermore, type b IELs microarray analysis of the nucleotides, which may affect intestinal immune homeostasis.

Through the TGF-β/Smad signaling pathway, Smad3 and Smad4 transcription factors. Therefore, the inhibition of the canonical TGF-β signaling pathway through deletion of Smad3 or Smad4 or by the increased expression of inhibitory Smad7 promotes gut inflammation [107, 108]. The gut is a TGF-β-rich environment, in which most cell types (including intestinal ECs, lymphocytes, monocytes, macrophages, and DCs) can produce and respond to this cytokine [109]. Nagafuchi et al. [110] showed that dietary nucleotides fed to mice enhanced the secretion of TGF-β from intestinal ECs. Their subsequent study suggested that this increased secretion of TGF-β from intestinal ECs was a result of indirect effects of the nucleotides, which may affect intestinal microflora or cells other than ECs, which in turn, influence intestinal EC cytokine secretion [111]. Furthermore, type b IELs can also be a source of TGF-β in the intestinal mucosa [112]. Similar to the developmental pathways of other Treg populations (Treg and CD1d-reactive NKT), CD8αα+ TCRαβ IEL development is TGF-β dependent. TGF-β1−/− mice and mice with a T cell-specific deletion of TCRαR I both lacked CD8αα+ TCRαβ IELs, whereas transgenic mice that overexpressed TGF-β1 have an increase in this population. Notably, it has been demonstrated that TGF-β maintains CD8α expression, not only in TCRαβ IELs but also in peripheral CD8+ T cells [113]. In an in vitro study with the use of IELs derived from the proximal jejunum of patients, TGF-β inhibited the mitosis of IELs in response to mitogens, IL-7, and CD2 and CD3 stimulation [114]. Once in the gut, expression of CD103 (also known as αε integrin) is important for the retention of IELs in the gut environment. Failure to induce CD103 on T cells by TGF-β results in reduced numbers of intestinal IELs [115]. In the absence of signaling through the TGF-βR, host-specific CD8+ effectors infiltrating the intestinal epithelium during graft-versus-host disease do not
up-regulate CD103 and are less pathogenic [116]. In this context, a study reported that elevated Smad4 expression, increased tumor cell proliferation, and increased tumor cell secretion of TGF-β are independent predictors of increased tumor IEL infiltration. Together, this suggested that the TGF-β signaling pathway may represent an important therapeutic target in the battle against colorectal cancer [117].

SCF
The kit ligand, also termed SCF, is a hematopoietic growth factor that augments the responses of early progenitor cells to other growth factors and acts as an important growth factor for human and murine mast cells [118]. The binding of SCF induces the homodimerization of the c-kit receptor, resulting in the phosphorylation of selective tyrosine residues in c-kit, which has been shown to play several roles in a wide range of physiologic functions. SCF and IL-7 mRNA are abundant in all 3 tissues (fetal intestine, fetal thymus, and fetal liver) during fetal development [119]. Puddington et al. [120] have shown that IELs express c-kit and that intestinal ECs produce SCF, suggesting that SCF-c-kit interactions could play an important role in regulating IEL homeostasis. With the use of mice that are mutated for SCF (SI/SI(fl)) and c-kit (W/WV), the number of TCRγδ IELs was found to be greatly reduced, whereas T-lymphocyte populations outside of the intestine (spleen, lymph nodes, and skin) were comparable with those in control mice [120]. Enhanced production of SCF is a potentially important response of the intestinal tract following exposure to cholera toxin or Salmonella typhimurium [121]. SCF has been shown to act synergistically with a number of cytokines to augment IEL differentiation and function. It has been shown that SCF acts synergistically with anti-TCRγδ and with Con A to induce proliferation and IFN-γ production in IELs [122]. Furthermore, SCF can up-regulate the expression of TCRγδ and the common γ chain on IELs and act synergistically with IL-2, IL-7, and IL-15 to induce IEL proliferation, IFN-γ production, and non-MHC-restricted cytotoxic activity [123]. Overall, the fact that SCF, IL-7, and IL-15 can all be produced by intestinal ECs and that IL-2 is produced by IELs suggests that these cytokines, alone or in combination, may play a significant role in regulating IEL development and function.

CONCLUDING REMARKS
IELs are known as peripheral T cells with marked specificity and heterogeneity, and whether their homeostasis is regulated by the ECs and other intestinal mediators is always an important and hot topic in the mucosal immunology field. The data summarized in this review highlight that intestinal cytokines are fundamental modulators that impact IELs, especially type b IELs, on several levels. As most IELs of the small intestine but only a small fraction of colon IELs are type b IELs, it is at least partly dependent on the presence of these cytokines. The common γc cytokines, IL-2, IL-7, and IL-15, play critical roles in supporting IEL (especially TCRγδ and TCRαβ CDSaa+) proliferation, survival, and function during extrathymic differentiation and immune responses. Furthermore, SCF has been shown to have potent synergistic activity with the hematopoietic growth factors (e.g., IL-2, IL-7, and IL-15) in mediating proliferation and activation. We also cannot ignore the emerging roles of TGF-β in IEL immune suppression and adhesion modulation. This increased understanding of multiple IEL subsets coexisting in a similar environment underscores the need for further understanding of the cytokines that balance immune responses. Remarkably, the luminal environment of the bowel is distinct; small intestinal IELs are mainly exposed to various food antigens, whereas large intestinal IELs encounter antigens of the bacterial flora. These environmental factors may affect intestinal cytokine production and may also account for the differences observed in the large intestine and the small intestine on the phenotype and function of IELs. Therefore, the understanding of the role of cytokines in IEL shaping is of paramount importance for elucidating mechanisms underlying distinct patterns of IEL distribution.

Whereas great strides have been made in understanding the properties of IELs in recent years, many questions still remain about their maintenance and differentiation. Memory CD8αβ+ T cells within small intestine epithelium (type a IELs) are well-characterized examples of Trm, and they maintain a long-lived, effector-like phenotype. However, numerous studies have revealed that the homeostatic cytokines IL-7 and IL-15 are important for maintenance of type b IELs. Do these cytokines also play a role in Trm (type a IELs) survival? It is noteworthy that Trm express less IL-7Ra and IL-15Ra [124]. Thus, additional survival factors might also play a role in Trm longevity. The definition of the factors mediating different IEL subset maintenance will help provide insight into leveraging IELs for the design of new and effective mucosal vaccines and for the development of therapies to treat inflammatory diseases, food allergies, and cancers.

AUTHORSHIP
H.Y. designed the review. Y.Q. wrote the manuscript. W.W. and W.X. participated in the modification of grammar.

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REFERENCES


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