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REVIEW

Intestinal intraepithelial lymphocytes: Maintainers of intestinal immune tolerance and regulators of intestinal immunity

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Abstract

Intestinal immune tolerance is essential for the immune system, as it prevents abnormal immune responses to large quantities of antigens from the intestinal lumen, such as antigens from commensal microorganisms, and avoids self-injury. Intestinal intraepithelial lymphocytes (IELs), a special group of mucosal T lymphocytes, play a significant role in intestinal immune tolerance. To accomplish this, IELs exhibit a high threshold of activation and low reactivity to most antigens from the intestinal lumen. In particular, CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ IELs, TCR$\gamma\delta^+$ IELs, and CD4$^+$CD8$\alpha\alpha^+$ IELs show great potential for maintaining intestinal immune tolerance and regulating intestinal immunity. However, if the intestinal microenvironment becomes abnormal or intestinal tolerance is broken, IELs may be activated abnormally and become pathogenic.

KEYWORDS

CD4$^+$CD8$\alpha\alpha^+$IELs, CD8$\alpha\alpha^+$TCR$\alpha\beta^+$IELs, CD8$\alpha\beta^+$TCR$\alpha\beta^+$ IELs and celiac disease, TCR$\gamma\delta^+$ IELs

1 | INTRODUCTION

Immune tolerance is the process by which the immune system mounts a weak specific immune response or no response to a specific antigen under certain conditions. Although the generation of immune tolerance has been studied for years, the mechanisms are still not completely understood. In previous studies, the mechanisms of immune tolerance were divided into central and peripheral tolerance. Central tolerance includes clonal deletion, receptor editing, forbidden clones, clonal abortion, and clonal anergy. Peripheral tolerance is mainly related to clonal anergy, clonal ignorance, clonal deletion, inhibitory regulation, and so on. The intestine is the largest immune organ and can protect the body from pathogen invasion. However, under normal circumstances, from birth to death, the intestinal mucosa is constantly exposed to commensal microorganisms, food, and environmental agents, but most foreign antigens do not stimulate an excessive immune response. This phenomenon is called intestinal immune tolerance, which limits inflammatory responses to resident commensal microbes, and provides tolerance to food antigens.[1] Even under pathologic conditions, the intestinal immune system can also regulate itself to avoid self-injury. In fact, through long-term coevolution, the intestine has developed a set of mechanisms to tolerate these foreign antigens and maintain intestinal homeostasis.

The intestinal mucosal immune system, which is part of the intestinal barrier, mainly includes organized tissues, such as the mesenteric lymph nodes and Peyer's patches; diffuse lymphoid tissue, such as the cryptopatches of the lamina propria in the intestinal mucosa; and groups of immune cells, such as intraepithelial lymphocytes (IELs).[2] It is essential to maintain the balance between immune tolerance and immune clearance. In the intestinal wall, a monolayer of intestinal epithelial cells (IECs) comprises the intestinal epithelium, with an area between 200 and 400 m^2. IELs, with approximately 1 IEL per 10 IECs,

Abbreviations: AhR, Aryl hydrocarbon receptor; CD, celiac disease; CLP, cecal ligation and puncture; CT, cholera toxin; CTL, cytotoxic T lymphocyte; Ctla4, cytotoxic T-lymphocyte-associated protein 4; DAP12, DNAX-activation protein 12; GPRs, G protein-coupled receptors; IBD, inflammatory bowel disease; iCD3, intracellular CD3; IECs, intestinal epithelial cells; IELs, intraepithelial lymphocytes; LAG-3, lymphocyte activating 3; Ly49, lymphocyte antigen 49; MICA, MHC class I polypeptide-related sequence A; NKG2A, natural killer receptor group 2, member A; NKG2D, natural killer receptor group 2, member D; NKR, NK cell receptor; PD-1, programmed cell death protein 1; PDK1, phosphoinositide-dependent protein kinase 1; SRBCs, sheep red blood cells; T-bet, a member of the T-box family, encoded by the Tbx21 gene; Th1, T helper 1; ThPOK, ZBTB7B (zinc finger and BTB domain containing 7B); Thy1, thymus cell antigen 1; TL, thymic leukemia antigen; Tnfrsf18, TNF receptor superfamily member 18; Tregs, regulatory T cells; VDR, vitamin D receptor.

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are resident in the small intestinal epithelium.[3] This large group of T cells, as the first immune cells that contact foreign antigens that pass through the intestinal epithelium, accounts for almost 50% of T lymphocytes in the body and are called sentinels of the intestinal mucosal barrier.[2,4] Consistent with their large quantities and special locations, IELs play vital roles in the intestinal immune response to foreign antigens and in maintaining intestinal immune homeostasis.

According to much research, the classification and basic features of IELs have been well clarified. On the basis of TCR, IELs are divided into TCR^+ IELs and TCR^- IELs.[3] TCR^+ IELs are composed of induced IELs (type-α IELs) and natural IELs (type-β IELs). Induced IELs, including $CD4^+TCR\alpha\beta^+$ IELs and $CD8\alpha\beta^+TCR\alpha\beta^+$ IELs, such as conventional T cells that originate from thymocytes and undergo double positive and negative selection, migrate from the GALT and peripheral lymphoid tissues to the intestinal epithelium while expanding and acquiring an activated phenotype via stimulation by antigens.[5] They express CD2, CD5, CD28, LFA-1, and thymus cell antigen 1 (Thy1), and some of them also express $CD8\alpha\alpha$.[2] Similar to conventional T cells, $CD4^+TCR\alpha\beta^+$ IELs have T helper (Th) characteristics, and $CD8\alpha\beta^+TCR\alpha\beta^+$ IELs exhibit cytotoxicity in response to mucosal pathogens.[6] However, some $CD4^+TCR\alpha\beta^+$ IELs that express $CD8\alpha\alpha$ show cytotoxicity with expression of granzymes and display immunomodulatory functions that we describe in the following text.[7] The functions of $CD8\alpha\alpha^+CD8\alpha\beta^+TCR\alpha\beta^+$ IELs are still not completely understood because of their small numbers and the difficulties of research. Natural IELs, composed of $TCR\gamma\delta^+$ IELs and $CD8\alpha\alpha^+TCR\alpha\beta^+$ IELs, settle in the intestinal epithelium after their development.[2] They lack expression of CD2, CD5, CD28, Thy1, and LFA-1.[6] Previous research has shown that this group of IELs has great potential to regulate intestinal immunity by producing the inhibitory cytokines, such as IL-10 and TGF-β. TCR^- IELs, a small population of IELs, include subsets similar to innate lymphoid cells that are found peripherally and subsets that express intracellular CD3 (iCD3) chains, and some TCR^-iCD3^+ IELs express $CD8\alpha\alpha$.[3] Although these IELs have been identified, their proportion among IELs is small, and reports about these IELs are limited.

Given previous studies, under physiologic conditions, all IELs, including induced IELs and natural IELs, maintain a state of tolerance to most foreign antigens from the intestinal lumen. Induced IELs prefer a protective immune response to defend against the invasion of pathogens, and natural IELs tend to mediate tolerance. However, under pathologic conditions, it seems that IELs are activated excessively or the abilities of IELs are enhanced; thus, induced IELs damage autologous tissue and natural IELs regulate abnormal immune responses. Especially in CD, a classical autoimmune disorder in which intestinal immune tolerance is broken and patients become sensitive to gluten, induced IELs play an important role in self-injury.[8] More surprisingly, natural IELs are sometimes self-injured, and induced IELs are self-protective; they exchange functions in some exceptional circumstances. In this review, we focus on TCR^+ IELs and summarize evidence supporting the significance of IELs in immune tolerance and immunomodulation, providing additional perspectives to understand IELs.

Box 1. CD8

IELs, a group of special immune T cells, notably express $CD8\alpha\alpha$, which is also one of the features that distinguish them from peripheral immune T cells. Subsets of IELs express two isoforms of CD8: $CD8\alpha\alpha$ homodimers or $CD8\alpha\beta$ heterodimers. Although the $CD8\alpha\alpha$ molecule is similar to $CD8\alpha\beta$, numerous studies have supported the view that $CD8\alpha\alpha$ plays a different role in T cell activation. In terms of structure, the α chain of CD8 binds to the α3 conserved domain of the MHC class I molecule and the cytoplasmic region of the α chain that contains the docking site for the tyrosine kinase p56lck required for initiation of early TCR signaling events, but it is the β chain that acts as a coreceptor of the TCR.[89] Some studies also show that $CD8\alpha\beta$ is a more effective coreceptor of the TCR than $CD8\alpha\alpha$.[26,89] In terms of function, IECs universally express thymic leukemia antigen (TL), which is a nonclassical MHC class I molecule but has not been considered an antigen-presenting molecule until now. TL has a high affinity for $CD8\alpha\alpha$ rather than $CD8\alpha\beta$, and TL binding to $CD8\alpha\alpha$ alters TCR activation so that T cells exhibit attenuated proliferation and cytotoxicity, but enhanced cytokine production.[90,91] As a result, TL-$CD8\alpha\alpha$ interactions attenuate the injury caused by IELs to the gut epithelium and may promote the renewal of gut epithelium favoring IFN-γ production.[90,91] In addition, $CD8\alpha\alpha$ is expressed not only by IELs but also by other cell lineages, such as subsets of human NK cells and dendritic cells.[89,92] Recent research shows that the $CD8\alpha\alpha$ homodimer also functions as a coreceptor for KIR3DL1 (an inhibitory receptor of NK cells) and that this interaction augments KIR3DL1-mediated inhibition of NK cell activation.[92]

2 | $CD8\alpha\alpha^+TCR\alpha\beta^+$ IELS

$CD8\alpha\alpha^+TCR\alpha\beta^+$ IELs, a group of IELs that dominantly express $CD8\alpha\alpha$ and lack CD4 or $CD8\beta$, have self-reactivity and diverse MHC class I restriction,[2] making up approximately 20–50% of the total IELs in mice and <1% in humans.[3] This group of IELs does not express CD2, CD5, CD28, Thy1, or LFA-1,[6] but it expresses activating receptors such as natural killer receptor group 2, member D (NKG2D), and 2B4 (CD244), and inhibitory receptors such as lymphocyte antigen 49 (Ly49A) and Ly49G2.[9] In newborn mice and humans, $CD8\alpha\alpha^+TCR\alpha\beta^+$ IELs represent a polyclonal TCR repertoire,[10] but in mature mice and humans, TCR repertoire of these IELs becomes slightly oligoclonal, and the degree of oligoclonality is significantly higher in IELs than in peripheral lymph node T cells.[10-11]

The origin and development of $CD8\alpha\alpha^+TCR\alpha\beta^+$ IELs is still under debate. The latest accepted view is that the majority of these cells originate from thymocytes. Double-positive thymocytes undergo agonist positive selection that contributes to the self-reactivity and escape

from negative selection,[12] and develop into CD4$^-$CD8$^-$TCRβ^+ thymocytes, the precursors of CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ IELs.[13] These precursors may mainly consist of programmed cell death protein 1 and a member of the T-box family, encoded by the Tbx21 gene (PD-1$^-$T-bet$^+$) and PD-1$^+$T-bet$^-$ cells.[14] Interestingly, the PD-1$^-$T-bet$^+$ precursors are mainly located in the medulla, and their population includes cells that have nonclassical MHC class I restriction. However, PD-1$^+$T-bet$^-$ precursors are mainly found in the cortex, have a classical MHC class I, and are enriched in self-reactive thymocytes.[14] PD-1$^+$T-bet$^-$ precursors preferentially expressed the G-protein-coupled receptor sphingosine 1-phosphate receptor 1 and its related transcription factor Krüppel-like factor 2, which is required for lymphocyte egress from tissues.[14,15] It seems that PD-1$^+$T-bet$^-$ cells rather than PD-1$^-$T-bet$^+$ cells are the main population that migrates from the thymus to the periphery and develops into IELs. However, in other studies, it was demonstrated that T-bet was induced in thymic IEL precursors of CD8$\alpha\alpha^+$ IELs as a result of agonist selection and IL-15 receptor signaling, which indicated that PD-1$^-$T-bet$^+$ cells were also the precursors of CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ IELs.[16] The roles of these proteins in the generation and development of CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ IELs remain unclear and require further confirmation. It was proposed that agonist positive selection gives these IELs self-tolerance and immune regulatory features.[17,18] This notion is supported by the finding that when exposed to their cognate agonist antigens in vitro, surviving immature thymocytes induce transcriptional signature almost identical to that described for CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ IELs, including the expression of NK receptors, Ly49 members, and CD94.[18]

When these cells migrate into the intestinal epithelium, the special intestinal microenvironment promotes IEL differentiation and maturation. The interaction between IL-15 expressed by IECs and T-bet promotes the differentiation and maturation of these IELs, for example, as indicated by the expression of CD8$\alpha\alpha$.[16,19,20] TGF-β is also important for extrathymic development.[21] Aryl hydrocarbon receptor (AhR), a vitamin D receptor (VDR) expressed by CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ IELs, also plays an important role in the survival and maintenance of this group of cells.[22,23] The development of CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ IELs has been described previously.[13,15]

Normally, CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ IELs have high expression of granzyme and FasL, which means that this group of natural IELs may have cytotoxicity.[9] However, interestingly, in the case of intestinal infection, CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ IELs do not display cytotoxicity or proinflammatory activity based on self-reactivity.[24] In contrast, a previous study indicated that exposure to self-antigen in vivo increased the threshold of TCR activation to the same self-antigen in IELs,[25] which might be related to the expression of CD8$\alpha\alpha$,[26] the lack of costimulatory molecules such as CD2, CD28, and LFA-1, and the tendency to express NK inhibitory receptors such as Ly49A and Ly49G2 under physiologic conditions.[9] In a comparison of germ-free mice and conventional mice, the repertoire of CD8$\alpha\alpha^+$ IELs in conventional mice was more oligoclonal.[27] These results suggest that CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ IELs are selected by intestinal environmental factors such as commensal microorganisms and exhibit low reactivity to limited antigens, which may favor the tolerance of these IELs in the intestinal environment.

In addition to exhibiting tolerance to antigens, CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ IELs show many similarities with thymus-derived regulatory T cells (Tregs). Perhaps CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ IELs are intestinal epithelial "Tregs" that maintain intestinal immune tolerance. Some evidence supports this viewpoint. First, both types of special T cells have similar thymic development processes. Thymus-derived Tregs, the main subset of natural Tregs, are a cluster of special immune cells that specifically express Foxp3 and possesses self-reactivity.[28] Comparing thymus-derived Tregs with CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ IELs, thymic development and agonist-driven positive selection determine both T cell self-reactivity and fate to regulate immunity.[9] Second, both groups of special T have similar major transcription factors. Foxp3 is identified as the key transcription factor of Tregs, which is the major regulatory gene for the development of Tregs.[29] Foxp3 modulates the transcription of many genes in Tregs, for instance, stabilizing and amplifying the expression of genes conferring Treg cell suppressor function, such as *Il2ra*, *Ctla4* (cytotoxic T-lymphocyte-associated protein 4), and *Tnfrsf18* (TNF receptor superfamily member 18), and genes repressing proinflammatory cytokine gene expression, such as IL-17.[30] In CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ IELs, T-bet, a characteristic T helper 1 (Th1) transcription factor, is crucial for the differentiation of these IELs, such as the expression of CD8$\alpha\alpha$.[19] CD8$\alpha\alpha$ is an important molecule that participates in the immunomodulation of IELs (Box 1). T-bet is also expressed by subsets of Tregs, and it was proposed that activation of T-bet optimizes Treg cells to counteract Th1-type inflammation.[31] Whether T-bet has the same function in CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ IELs and in Tregs may be confirmed by research showing that CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ IELs prevent colitis induced by TCR$\alpha\beta^+$CD4$^+$CD45RB^{high} T cells.[32] Perhaps Foxp3 can be induced in CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ IELs under some special conditions and gives these IELs more properties to maintain immune tolerance; how T-bet regulates these natural T cells still needs more discussion. Third, both types of special T cells have similar expression levels. CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ IELs are enriched for the expression of lymphocyte activating 3 (LAG-3), which participates in immune-suppression by Tregs.[9] LAG-3, a coinhibitory molecule, inhibits T cell responses by counteracting TCR and costimulatory signals, resulting in the inhibition of proliferation and effector function and the down-regulation of activation and adhesion molecules at the cell surface.[33] Both CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ IELs and Tregs express fibrinogen-like protein 2, suppressing DC maturation,[9] and some inhibitory cytokines, such as TGF-β and IL-10. The similar origins, developmental pathways, and expression profiles of CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ IELs and Tregs might provide more inspiration to explain the intestinal immune tolerance of CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ IELs.

Under some pathologic conditions, special intestinal epithelial "Tregs" show more potential to regulate immunity. In sepsis induced by cecal ligation and puncture (CLP), CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ IELs are reduced. Glutamine, an amino acid with immunomodulatory effects, prevents apoptosis of these IELs, which may ameliorate sepsis-induced inflammatory reactions.[34] Colitis induced by dextran sulfate sodium also diminishes CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ IELs. However, 6-formylindolo (3, 2-b) carbazole, an endogenous agonist of the AhR, prevented this reduction by increasing the expression of IL-15 and AhR, which relieved

colitis and improved the histology and length of the colon.[35] These pieces of evidence suggest that CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ IELs might keep from self-injury resulted from other T cells and play a protective role. Nevertheless, how does this group of IELs regulate immunity? As mentioned earlier, CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ IELs prevent colitis induced by TCR$\alpha\beta^+$CD4$^+$CD45RB^{high} T cells in an IL-10-dependent manner, which is demonstrated by reconstitution of CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ IELs in SCID mice and IL-10$^{-/-}$ mice.[32] Consistent with this finding, CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ IELs also highly express TGF-β3, which promotes intestinal epithelial healing in an in vitro model system.[9] These results imply that cytokines secreted by CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ IELs play a suppressive or protective role.

CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ IELs seem to be a group of IELs with a stable phenotype and function, and there is no direct evidence that supports supporting the idea that CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ IELs act as protective immune or pathogenic immune cells. However, these cells with high expression of granzyme and FasL express some activating receptors, such as NKG2D and 2B4, which can trigger cytotoxic responses.[36,37] Cheroutre et al. also supported the view that CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ IELs perform an important function of protecting the intestinal epithelium before establishing adaptive immunity, sensing, and eliminating cancerous or injured epithelial cells.[37] If these cells are excessively or abnormally stimulated, they might damage the autologous tissue and lead to inflammation.

3 | TCR$\gamma\delta^+$ IELS

TCR$\gamma\delta^+$ IELs, which make up approximately 40–70% of the total IELs in mice and approximately 5–20% in humans,[3] express predominantly CD8$\alpha\alpha$ and lack CD8β compared with TCR$\gamma\delta^+$ T cells resident in lymphoid tissue.[38] Many TCR$\gamma\delta^+$ IELs express the inhibitory natural killer receptor group 2, member A (NKG2A).[39] The TCR repertoire of TCR$\gamma\delta^+$ IELs is highly limited, with a dominant Vγ4 subset in humans and a dominant Vγ7 subset in mice, and lacks MHC restriction.[40] In addition, TCR$\gamma\delta^+$ IELs have cytotoxicity and high motility between the lamina propria and intestinal epithelium, and produce abundant cytokines such as antimicrobial proteins (RegIIIγ), profibrotic factors (IL-13), anti-inflammatory cytokines (TGF-β and IL-10), proinflammatory cytokines (IFN-γ and TNF-α), and factors associated with wound healing (keratinocyte growth factor, prothymosin β4, and TGF-β).[2,3] Associated with such features, TCR$\gamma\delta^+$ IELs produce a marked effect in immunologic surveillance, resisting the invasion of gut microbes and promoting intestinal epithelial repair, and have an important function that mediates immune tolerance and immune regulation.

Many researchers support the view that most TCR$\gamma\delta^+$ IELs originate from the thymus and are selected in the thymus, but some studies also show that a portion of the population originates and develops extrathymically.[41-43] When TCR$\gamma\delta^+$ IELs migrate into the intestinal epithelium, some molecules play a crucial role in the selection and retention of TCR$\gamma\delta^+$ IELs. AhR, a transcriptional regulator that can regulate the development and function of immune cells by binding cellular and dietary ligands,[44] is important for maintaining and expanding TCR$\gamma\delta^+$ IELs.[45] AhR deficiency or the lack of AhR ligands affects the maintenance of innate immune cells such as TCR$\gamma\delta^+$ IELs and the control of the microbial load and composition; as a result, the immune system is overactivated and damages tissues.[22,45] Butyrophilin-like proteins, which are selectively expressed by IECs, are structurally related to CD80 costimulatory and PD-L1 inhibitory molecules.[41] These proteins promote the maturation and expansion of IELs during TCR-dependent responses, which has been described earlier.[2,3] G protein-coupled receptors (GPRs) expressed by TCR$\gamma\delta^+$ IELs also contribute to this process. The orphan receptor GPR18 promotes the accumulation of TCR$\gamma\delta^+$ cells in the epithelium, but GPR55 counteracts this accumulation in response to lysophosphatidylinositol.[46,47] How these molecules mediate selection and retention is still not clear and needs further exploration.

As mentioned earlier, the lack of costimulatory receptors such as CD28, LFA-1, and CD2 means an increased threshold of TCR$\gamma\delta^+$ IEL activation. The inhibitory NK cell receptor (NKR) NKG2A are expressed by many TCR$\gamma\delta^+$ IELs and in the cytoplasmic domain of NKG2A, there is an immunoreceptor tyrosine-based inhibitory motif that can recruit SH2 domain-containing phosphatase (SHP)-1 or SHP-2 upon receptor engagement and mediate a negative signal that blocks the TCR activation function and inhibits NK effectors.[48] These pieces of evidence suggest low reactivity to antigens under physiologic conditions, which supports the tolerance of TCR$\gamma\delta^+$ IELs to most antigens from the intestinal lumen.

In addition to exhibiting tolerance to antigens, the significance of TCR$\gamma\delta^+$ IELs in maintaining intestinal immune tolerance has been identified in previous research. In C57BL/6 mice treated with GL3 anti-$\gamma\delta$m Ab or TCR$\gamma\delta$ gene knockout mice, oral mucosal tolerance is significantly impaired.[49-51] Mice lacking TCR$\gamma\delta$ cells display exaggerated intestinal damage, apparently because of a failure to regulate the consequences of the TCR$\alpha\beta$+ T cell response,[52] Nevertheless, how these IELs maintain tolerance is still not understood. In research, TCR $\gamma\delta^+$ T cells cloned from intestinal IELs inhibited the development of cytotoxic T lymphocyte (CTL) responses ex vivo, which might be mediated by the expression of TGF-β1 and macrophage migration inhibitory factor, as well as IL-10 mRNA.[51] This suggests that TCR$\gamma\delta^+$ IELs may tend to produce anti-inflammatory cytokines to maintain a tolerant intestinal microenvironment.

Despite a lack of direct evidence concerning how TCR$\gamma\delta^+$ IELs mediate intestinal immune tolerance, investigation of TCR$\gamma\delta^+$ IELs under pathologic conditions may provide additional perspectives. For instance, TCR$\gamma\delta^+$ T cells are significantly reduced in septic patients, and this large decrease is associated with disease severity and mortality.[53] In sepsis induced by CLP, there was an increase in small intestinal TCR$\gamma\delta^+$CD8$^+$ IELs and these cells appeared to improve survival compared with $\gamma\delta^{-/-}$ mice.[54] In CD, TCR$\gamma\delta^+$ IELs are significantly increased. Based on these studies, some clues may explain the regulation of TCR$\gamma\delta^+$ IELs. First, TCR$\gamma\delta^+$ IELs restrain some immune responses by secreting inhibitory cytokines or inhibiting immune cells directly. As mentioned earlier, TCR$\gamma\delta^+$ IELs probably inhibit the development of CTL responses by expressing inhibitory

cytokines, such as TGF-β1 and IL-10. In mice with sepsis induced by CLP, cytokines such as IL-6 and IL-12 in macrophages were more suppressed and Th1 cytokine release by anti-CD3-stimulated splenocytes was significantly reduced in $\gamma\delta^{+/+}$ mice compared with $\gamma\delta^{-/-}$ mice, but the expression of IL-10 was not different. This implied that TCR$\gamma\delta^{+}$ IELs might inhibit macrophages or the Th1 response, but the mechanisms are not clear.[54] In a model of infection induced by the protozoan parasite *Eimeriavermiformis*, TCR$\gamma\delta^{+}$ IELs suppressed the inflammatory reaction by inhibiting the activation of other immune cells.[52] Second, TCR$\gamma\delta^{+}$ IELs may induce or influence some immune-regulating cells, such as Tregs, that are significant in intestinal immune tolerance. In previous research, exposure of the nasorespiratory mucosa of NOD mice (a model of autoimmune or type 1 diabetes) to insulin, an autoantigen that drives T cells to destroy pancreatic cells in type 1 diabetes,[56] induces TCR$\gamma\delta^{+}$CD8$\alpha\alpha^{+}$ T cells, which are regulatory and antidiabetogenic.[56] Locke et al.,[57] found that CD8$\alpha\alpha^{+}$ TCR$\gamma\delta^{+}$ IELs were required for the induction of CD4^{+}CD25^{+} Tregs by oral insulin to prevent diabetes in neonatal thymectomy-NOD mice. They thought that CD8$\alpha\alpha^{+}$TCR$\gamma\delta^{+}$ IELs might promote the induction of tolerant mucosal dendritic cells, which in turn induce CD4^{+}CD25^{+} Tregs rather than produce anti-inflammatory cytokines to mediate them. Nevertheless, cholera toxin (CT)-activated TCR$\gamma\delta^{+}$ IELs display some characteristics of APCs, such as expressing MHC class II molecules, CD80 and CD86,[58] which may explain the interaction between CD8$\alpha\alpha^{+}$TCR$\gamma\delta^{+}$ IELs and CD4^{+}CD25^{+} Tregs. Third, the interactions between TCR$\gamma\delta^{+}$ IELs and IECs, such as the CD94/NKG2A-HLA-E interaction, give these IELs immunomodulatory functions. Although HLA-E, the ligand for CD94/NKG2A, is not constitutively expressed by IECs, its expression is increased under inflammatory conditions such as CD.[8] This interaction may induce TCR$\gamma\delta^{+}$ IELs to secrete TGF-β against CD.[39] However, how these IELs participate in CD is still not clear, and TCR$\gamma\delta^{+}$ IELs tend to be a diagnostic marker of CD.[59] In summary, the functions of TCR$\gamma\delta^{+}$ IELs in maintaining intestinal immune tolerance under normal conditions may be enhanced under some abnormal conditions, so TCR$\gamma\delta^{+}$ IELs can maintain the intestinal immune balance.

However, the immune tolerance mediated by TCR$\gamma\delta^{+}$ IELs is limited and can be broken. TCR$\gamma\delta^{+}$ T cells play a significant immunoregulatory role in IL-10-mediated, low-dose oral tolerance induction but are not essential participants in the induction of systemic tolerance to orally introduced antigens given in larger doses.[60] Some TCR$\gamma\delta^{+}$ IELs from mice orally immunized with sheep red blood cells (SRBCs) transferred to mice with oral tolerance to SRBCs reversed oral tolerance and resulted in antibody responses to SRBCs.[61] CT-activated TCR$\gamma\delta^{+}$ IELs also broke oral tolerance to the food allergen β-lactoglobulin in mice.[58] Under some conditions, TCR$\gamma\delta^{+}$ IELs may even promote inflammation and cause self-injury. In research, CD8α^{+}TCR$\gamma\delta^{+}$ T cells were markedly increased in phosphoinositide-dependent protein kinase 1-deficient mouse colonic IELs and were related to inflammatory colitis, but the adoptive transfer of wild-type Treg cells prevents the spontaneous activation and proliferation of intestinal TCR$\gamma\delta^{+}$ T cells.[62] In some other studies, TCR$\gamma\delta^{+}$ T cells also promoted inflammation.[63,64]

4 | CD4^{+}TCR$\alpha\beta^{+}$ IELS

CD4^{+}TCR$\alpha\beta^{+}$ IELs make up approximately 10–15% of the total IELs in mice and humans.[3] Generally, they are considered a group of Th-type cells with strong plasticity,[37] especially Th1 cells.

Although these IELs tend to be peripheral CD4^{+}TCR$\alpha\beta^{+}$ T cells, when these cells migrate from GALT and peripheral lymphoid tissues to the intestinal epithelium, they not only gain an activated phenotype but also acquire some properties, such as the expression of CD8$\alpha\alpha$, that depend on the intestinal environment. CD4^{+}CD8$\alpha\alpha^{+}$ IELs, a group of special CD4^{+} IELs with distinct MHC class II-restricted CD4^{+} cytotoxicity,[7,65] have also been popular and controversial group of IELs in recent years. The development of CD4^{+} T cells into CD4^{+}CD8$\alpha\alpha^{+}$ IELs is mainly related to up-regulation of the transcription factor ThPOK (encoding *Zbtb7b*), which suppresses the CD8^{+} T cell lineage while promoting the CD4^{+} T cell lineage, and down-regulation of RUNX family transcription factor 3, which acts in the opposite manner of ThPOK.[66,67] The transcription factors T-bet and AhR also promote differentiation and maturation, which has been discussed in detail.[19,65] Intestinal vitamin D and VDR expressed by CD4^{+}TCR$\alpha\beta^{+}$ IELs are also responsible for intestinal development of CD4^{+}CD8$\alpha\alpha^{+}$ IELs. Without the VDR, CD4^{+} T cells cannot acquire the expression of CD8$\alpha\alpha$ and fail to home to the gastrointestinal tract, which contributes to the normal bacterial flora becoming unbalanced.[23] The intestinal development of CD4^{+}CD8$\alpha\alpha^{+}$ IELs has been described previously.[68] Similar to CD8$\alpha\alpha^{+}$TCR$\alpha\beta^{+}$ IELs, the TCR repertoire of CD4^{+}TCR$\alpha\beta^{+}$ IELs is highly oligoclonal in mature mice and adults, which might be a result of intestinal factor selection, such as microbial colonization.[69,70]

Generally, consistent with Th features, CD4^{+}TCR$\alpha\beta^{+}$ IELs play an indispensable role in protective immunity in the intestinal mucosa. In SIV or HIV infection, there are prominent reductions in CD4 T cells in both the lamina propria and epithelial compartments, which impairs the integrity of the mucosal barrier and leads to translocation of enteric bacteria and increased local and systemic infections.[71,73] MHC class II-restricted CD4^{+} cytotoxicity also suggests that CD4^{+}TCR$\alpha\beta^{+}$ IELs might sense and eliminate pathologic or injured epithelial cells. However, under the normal conditions, the gut environment is thought to be tolerant to most foreign harmless antigens, so CD4^{+}TCR$\alpha\beta^{+}$ IELs are also tolerant of these antigens. The expression of CD8$\alpha\alpha$ and the limited TCR repertoire support such tolerance, but it is insufficient. The strong plasticity of Th cells may suggest that the unique intestinal microenvironment shapes these IELs into a state of low reactivity, but this process is still not clear.

Associated with strong plasticity, some CD4^{+}TCR$\alpha\beta^{+}$ IELs also show great potential to regulate the immune response. CD4^{+} T cells stimulated under Th2 but not Th1 differentiation conditions acquire CD8$\alpha\alpha$ expression and produce IL-10 upon reaching the intestinal epithelium.[74] These CD4^{+}CD8$\alpha\alpha^{+}$ IELs (Th2-type CD4^{+}CD8$\alpha\alpha^{+}$ IELs) appear to prevent T helper 1-induced intestinal inflammation in an IL-10-dependent fashion.[74] Foxp3^{+} Tregs in peripheral tissues that are resident in the lamina propria lose Foxp3 and convert to Foxp3^{-}CD8$\alpha\alpha^{+}$CD4^{+} T cells in a microbiota-dependent manner when migrating to the intestinal epithelium.[75] This group of IELs

also performs an anti-inflammatory function. It is possible that this group of IELs, such asTh2-type CD4$^+$CD8$\alpha\alpha^+$ IELs and CD4 IELs, gives CD4$^+$TCR$\alpha\beta^+$ IELs the ability to participate in intestinal immune tolerance and regulate the immune response.

However, once intestinal immune tolerance is broken, CD4$^+$TCR$\alpha\beta^+$ IELs may mediate and promote the development of inflammation. In CD characterized by distressed IECs and an inflammatory anti-gluten CD4 T cell response, overproduction of IL-21 promotes a T helper cell type 1 response, with marked production of IFN-γ.[76] Such excessive T helper cell type 1 activity exacerbates abnormal activation of cytotoxic CD8 T cells, which contributes to injury to the intestinal epithelium.[8] In refractory celiac disease (CD) type II, a severe complication of CD, gluten-specific CD4$^+$ T cells isolated from CD duodenal biopsy specimens produce cytokines such as TNF, IL-2, and IL-21, which are able to trigger the proliferation of malignant intraepithelial lymphocytes lacking classical B-, T-, and natural killer (NK)-cell lineage markers (Lin-IELs) lines.[78] These results suggest that CD4$^+$TCR$\alpha\beta^+$ IELs may have abnormal Th1-type activities and exacerbate bowel lesions. Although there has been a lack of direct evidence concerning the pathogenicity of CD4$^+$TCR$\alpha\beta^+$ IELs, recent research showed that the frequencies of granzymeB$^+$CD4$^+$CD8α^+ IELs were increased in pediatric CD patient biopsies, and in mice, gluten drove inflammatory CD4$^+$ and cytotoxic CD4$^+$CD8α^+ IEL infiltration in the absence of IL-10 signaling.[78] Costes et al. demonstrated that APC activation leads to inflammatory IFN-γ-producing CD4$^+$ T-cell differentiation and conversion to CD4$^+$ CTLs and that IL-10 primarily plays an anti-inflammatory role via APCs by preventing proinflammatory cytokine release.[78] In some other studies, CD4$^+$ T cells in the colon also mediated manifestations of colitis, such as inflammatory bowel disease (IBD).[79,80] The potential pathogenicity of CD4$^+$TCR$\alpha\beta^+$ IELs has been discussed earlier.[37]

5 | CD8$\alpha\beta^+$TCR$\alpha\beta^+$ IELS

CD8$\alpha\beta^+$TCR$\alpha\beta^+$ IELs account for approximately 70–80% of the total IELs in humans and 20–30% in mice.[3]

The development of CD8$\alpha\beta^+$TCR$\alpha\beta^+$ IELs is also attributed to the unique intestinal environment. Distinct from peripheral CD8$\alpha\beta^+$TCR$\alpha\beta^+$T cells, CD8$\alpha\beta^+$TCR$\alpha\beta^+$ IELs constitutively express granzymeB, CD69, CD103, and b7 integrin and produce low levels of IFN-γ and TNF-α.[2] In research, compared with effector memory CD8 T cells isolated from the spleen or blood, memory CD8 T cells from IELs showed a reduced ability to proliferate and produce inflammatory cytokines, and stronger and longer effector responses.[81] These memory CD8 T cells changed phenotype in response to the change in location after transfer and in vivo restimulation.[81] These results indicate that the unique intestinal environment gives these IELs a stronger ability to protect the body against pathogens with longer memory and the ability to maintain intestinal immune tolerance, and this memory quality seems to have plasticity. However, how the intestinal environment shapes these CD8 T cells is still not clear. A highly oligoclonal TCR repertoire of CD8$\alpha\beta^+$TCR$\alpha\beta^+$ IELs has been described in detail earlier, which results from intestinal factor selection, such as microbial colonization.[27]

Generally, consistent with their cytotoxicity and memory quality, CD8$\alpha\beta^+$TCR$\alpha\beta^+$ IELs may play a major protective role in sensing and eliminating pathologic or injured epithelial cells and resisting infection. In some infections of mice induced by different pathogens such as *lymphocytic choriomeningitis virus*, *rotavirus*, and *Toxoplasma gondii*, CD8$\alpha\beta^+$TCR$\alpha\beta^+$ IELs protect against invasion of these pathogens.[82-84] However, when exposed to large amounts of antigens that are not harmful to the body, why do these IELs coexist with them peacefully? There is also some evidence supporting intestinal immune tolerance of CD8$\alpha\beta^+$TCR$\alpha\beta^+$ IELs. Under normal conditions, human TCR$\alpha\beta^+$CD8$\alpha\beta^+$ IELs express inhibitory NKG2A/CD94 and activating NKG2D but do not express any NK receptors with ITAM adapter molecules such as DNAX-activation protein 12 (DAP12),[36] and some of these IELs express CD8$\alpha\alpha$,[85] which appears to increase the threshold of activation of TCR$\alpha\beta^+$CD8$\alpha\beta^+$ IELs and maintain low reactivity in the normal intestinal environment. A limited TCR repertoire and reduced inflammatory cytokines also mean that CD8$\alpha\beta^+$TCR$\alpha\beta^+$ IELs respond to a limited number of antigens with a less inflammatory response. Whether TCR$\alpha\beta^+$CD8$\alpha\beta^+$ IELs have an immunomodulatory function remains to be determined.

Although data indicate that CD8$\alpha\beta^+$TCR$\alpha\beta^+$ IELs have a stable status and an important role in protecting against invading pathogens, these cells have also been implicated in the progression of inflammation. Especially in CD, distressed IECs strongly express IL-15 and stress-induced ligands such as MHC class I polypeptide-related sequence A (MICA). A high level of IL-15 promotes the expression of activating NKRs such as NKG2D and DAP10. NKG2D-MICA can mediate innate-like cytotoxicity to kill IECs, and the expression of NKG2D reduces the threshold of the TCR signal during the CD8 T cell-mediated adaptive response.[86] IFN-γ and IL-21 are strongly expressed by anti-gluten CD4 T cells, and IL-21 synergism with IL-15 can promote CD8$^+$ T cell activation and expansion, IFN-γ production, and granzyme B and perforin up-regulation.[87] Such features may endow CD8$\alpha\beta^+$TCR$\alpha\beta^+$ IELs with a low threshold of activation and abnormal cytotoxicity and contribute to damage to IECs and villous atrophy. However, whether the damage is dependent on TCR or innate-like cytotoxicity is still not clear. Surprisingly, CD8$^+$ IELs show a significant increase in the expression of IL-10,[88] which may reflect self-regulation to resist CD. In addition, CD8$\alpha\beta^+$TCR$\alpha\beta^+$ IELs also participate in other inflammatory conditions, such as IBD.[37] CD8$\alpha\beta^+$TCR$\alpha\beta^+$ IELs are major IELs with cytotoxicity; they prevent the invasion of mucosal pathogens under normal conditions, but they are also an important cause of self-injury when intestinal immune tolerance is broken.

6 | CONCLUSION

There is no doubt that IELs play an important role in intestinal immune tolerance, especially natural TCR$\gamma\delta^+$ IELs and CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ IELs, which show great potential for immunomodulation; however, the

mechanism is not completely understood. The CD8$\alpha\alpha$ molecule may be an important clue. It seems that the expression of CD8$\alpha\alpha$, inhibitory NKRs and low levels of costimulatory molecules gives IELs a stable status in to the intestinal environment. IELs regulate immunity by a series of mechanisms, such as producing inhibitory cytokines. Nevertheless, their state is limited and changes in response to changes in the intestinal environment. Under some special conditions, natural TCR$\gamma\delta^+$ IELs and CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ IELs may promote self-injury, and induced IELs may mediate immunomodulation. Furthermore, IELs are a group of cells with potential plasticity, and intestinal environmental factors, such as intestinal microorganism and their products, shape the immune features of IELs. Perhaps we can adjust intestinal flora to shape IELs so that the intestinal immune status is better controlled. This provides more possibilities to treat intestinal immune diseases.

AUTHORSHIP

H.M. conceived and wrote the manuscript. H.Y. and Y.Q. contributed to the writing and revision of the manuscript.

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DISCLOSURES

The authors declare no conflicts of interest.

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