REVIEW

Human intraepithelial lymphocytes

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The location of intraepithelial lymphocytes (IEL) between epithelial cells, their effector memory, cytolytic and inflammatory phenotype positions them to kill infected epithelial cells and protect the intestine against pathogens. Human TCR $\alpha\beta$ ⁺CD8 $\alpha\beta$ ⁺ IEL have the dual capacity to recognize modified self via natural killer (NK) receptors (autoreactivity) as well as foreign antigen via the T cell receptor (TCR), which is accomplished in mouse by two cell subsets, the naturally occurring TCR $\alpha\beta$ ⁺CD8 $\alpha\alpha$ ⁺ and adaptively induced TCR $\alpha\beta$ ⁺CD8 $\alpha\beta$ ⁺ IEL subsets, respectively. The private/oligoclonal nature of the TCR repertoire of both human and mouse IEL suggests local environmental factors dictate the specificity of IEL responses. The line between sensing of foreign antigens and autoreactivity is blurred for IEL in celiac disease, where recognition of stress ligands by induced activating NK receptors in conjunction with inflammatory signals such as IL-15 can result in low-affinity TCR/non-cognate antigen and NK receptor/stress ligand interactions triggering destruction of intestinal epithelial cells.

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INTRODUCTION

Intraepithelial lymphocyte(s) (IEL) were first described by Weber in 1847 as small round cells within the epithelium of the small intestine whose primary function was in nutrient absorption.¹ Later on, Fichtelius reported the presence of these cells in the epithelium of a variety of species and suggested that their primary function was related to dealing with antigens present at body surfaces.² Guy-Grand and collaborators established in the early 1970s that IEL were mainly comprised of T lymphocytes.³ During the same period, they were reported in human^{4,5} and their potential role in celiac disease (CeD) was put forward by Ferguson and Holmes,^{6,7} and in tropical sprue by Montgomery and Shearer.⁸ Extensive studies of IEL in mice, especially because of the identification of naturally occurring innate-like T cell receptor (TCR) $\alpha\beta$ ⁺CD8 $\alpha\alpha$ ⁺ IEL that are autoreactive and undergo atypical thymic development, have suggested that IEL constitute a unique subset of T lymphocytes that are unique when compared to all other lymphocytes in the body.^{9–13} While CD3⁺, TCR $\gamma\delta$ ⁺, TCR $\alpha\beta$ ⁺CD8 $\alpha\beta$ ⁺, and TCR $\alpha\beta$ ⁺CD4⁺ IEL subsets are present in both human and mouse,^{9,14–16} the existence in human of the mouse naturally occurring autoreactive TCR $\alpha\beta$ ⁺CD8 $\alpha\alpha$ ⁺ IEL subset remains controversial.¹⁶

This review will focus on human tissue-resident TCR $\alpha\beta$ ⁺CD8 $\alpha\beta$ ⁺ IEL. We will compare and contrast them in the context of murine naturally occurring TCR $\alpha\beta$ ⁺CD8 $\alpha\alpha$ ⁺ and adaptively induced TCR $\alpha\beta$ ⁺CD8 $\alpha\beta$ ⁺ IEL while describing their distribution along the intestine, phenotype, TCR repertoire, autoreactivity, and function in homeostasis and disease.

CATEGORIZING THE TISSUE-RESIDENT IEL COMPARTMENT

T cells within the IEL compartment are distinct from peripheral lymphocytes^{17,18} and have been categorized primarily based on ontogeny into the naturally occurring (Type B) IEL and the

adaptively induced (Type A) IEL.^{11,13} Naturally occurring IEL are composed of TCR $\gamma\delta$ ⁺ T cells as well as the unique mouse subset of TCR $\alpha\beta$ ⁺CD8 $\alpha\alpha$ ⁺ T cells. Naturally occurring IEL are tissue-resident lymphocytes that seed the tissue early in life and independently of microbial colonization of the gut,^{19–22} whereas adaptively induced IEL are classical TCR $\alpha\beta$ ⁺CD8 $\alpha\beta$ ⁺ and TCR $\alpha\beta$ ⁺CD4⁺ T cells that are generated in response to local tissue insults²³ and gain residence within the IEL compartment not unlike tissue-resident memory cells.²⁴ To that end both naturally occurring and adaptively induced IEL were shown to be stable tissue-resident populations with little to no capacity to recirculate in the periphery in parabiosis experiments.²⁵ Finally, both human and mouse IEL express the tissue-resident hallmark marker CD103^{26,27} and have a cytolytic effector profile characterized by expression of the lymphocyte activation marker CD69 as well as granzyme and perforin cytolytic granules.^{17,18,28}

REGIONAL COMPOSITION OF THE IEL COMPARTMENT IN HUMAN VS. MOUSE

The intestine is classically separated into two anatomically distinct regions starting proximally at the small intestine defined by the duodenum, jejunum, and ileum, followed by the large intestine defined by the colon.²⁹ IEL can be found along the full length of the intestinal tract with the density of IEL relative to intestinal epithelial cells (IEC) being higher in the small intestine relative to the colon.^{30,31} These IEL are composed of T cells and innate lymphoid cells (ILCs). In an attempt to provide a resource, we present primary data side by side on the intraepithelial T cell compartments of both human and murine small intestine (duodenum) and large intestine (colon) (Fig. 1).

The intraepithelial T cell compartment in human is composed of TCR $\gamma\delta$ ⁺, TCR $\alpha\beta$ ⁺CD8 $\alpha\beta$ ⁺, and TCR $\alpha\beta$ ⁺CD4⁺ T cells (Fig. 1). In addition to the three subsets above, the murine intraepithelial T

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Fig. 2 Here we compare the human $\text{TCR}\alpha\beta^+\text{CD8}\alpha\beta^+$ IEL subset with the $\text{TCR}\alpha\beta^+\text{CD8}\alpha\beta^+$ and $\text{TCR}\alpha\beta^+\text{CD8}\alpha\alpha^+$ IEL subsets found in mouse for NK receptor expression and cytokine producing capacity. **a** The human $\text{TCR}\alpha\beta^+\text{CD8}\alpha\beta^+$ IEL expresses NK receptors such as the activating NKG2D and inhibitory NKG2A/CD94 in the steady state, whereas the $\text{TCR}\alpha\beta^+\text{CD8}\alpha\alpha^+$ IEL and not the $\text{TCR}\alpha\beta^+\text{CD8}\alpha\beta^+$ IEL subset is enriched for NK receptor expression in mouse. These $\text{TCR}\alpha\beta^+\text{CD8}\alpha\alpha^+$ IEL express NK receptors that can be found in steady-state human IEL such as NKG2D in addition to receptors such as those of the Ly49 family and NKG2C/CD94 which can associate with the ITAM adapter molecule DAP12. Interestingly, human IEL can also gain expression of ITAM bearing NK receptors such as NKG2C/CD94 in patients with CeD. The propensity for autoreactivity of IEL TCRs is shown with more autoreactive TCRs, such as those attributed to the development of $\text{TCR}\alpha\beta^+\text{CD8}\alpha\alpha^+$ IEL, illustrated in red and less autoreactive TCRs illustrated in green. The threshold for TCR/non-cognate antigen interactions to result in activation can be met in the case of human $\text{TCR}\alpha\beta^+\text{CD8}\alpha\beta^+$ IEL in CeD via the costimulatory impact of inflammatory signals such as IL-15 and the engagement of activating NK receptors. **b** Freshly isolated IEL were treated with 50 ng/mL of PMA and 500 ng/mL of Ionomycin for 3 h to assess the expression of Granzyme B and IFN- γ at steady state for human $\text{TCR}\alpha\beta^+\text{CD8}\alpha\beta^+$ IEL from a 30-year-old individual and $\text{TCR}\alpha\beta^+\text{CD8}\alpha\beta^+$ and $\text{TCR}\alpha\beta^+\text{CD8}\alpha\alpha^+$ IEL from 4-week and 11-week-old C57BL/6 SPF mice, revealing human $\text{TCR}\alpha\beta^+\text{CD8}\alpha\beta^+$ IEL are potent cytokine producers and simultaneously express Granzyme B, while cytokine production is age dependent and exclusive to the mouse $\text{TCR}\alpha\beta^+\text{CD8}\alpha\beta^+$ IEL with $\text{TCR}\alpha\beta^+\text{CD8}\alpha\alpha^+$ IEL being more potent expressors of Granzyme B. Representative flow cytometry contour plots with outliers and large dots are shown for intracellular staining with fluorescently labeled antibody against IFN- γ and Granzyme B for human (left) and mouse (right)

cell compartment contains the naturally occurring $\text{TCR}\alpha\beta^+\text{CD8}\alpha\alpha^+$ IEL (Fig. 1). The proportions of these subsets and how they vary across the intestine between human and mouse is summarized in Fig. 1. The human small and large intestine is dominated by $\text{TCR}\alpha\beta^+$ IEL. In contrast, the mouse small intestine has a more or less equal distribution of $\text{TCR}\gamma\delta^+$ and $\text{TCR}\alpha\beta^+$ IEL with a shift toward $\text{TCR}\alpha\beta^+$ IEL in the colon.^{30,31} Interestingly, $\text{TCR}\gamma\delta^+$ IEL increase proportionally from the small to large intestine in human while decreasing proportionally from small to large intestine in mouse (Fig. 1). Among $\text{TCR}\alpha\beta^+$ IEL, $\text{TCR}\alpha\beta^+\text{CD4}^+$ IEL are a minor population in the small intestine but increase proportionally in the large intestine in both mouse and human³³ (Fig. 1). Of note, the population of $\text{TCR}\alpha\beta^+\text{CD4}^+$ IEL in the human colon is primarily CD103^- , a phenotype which has been described for IEL exposed to chronic antigen challenge,³⁴ however, we cannot rule out contaminating CD103^- cells from the lamina propria (Lp). A key difference is the sizeable proportion of the $\text{TCR}\alpha\beta^+\text{CD8}\alpha\alpha^+$ IEL in mouse, a population which is seemingly absent in human (Fig. 1). The existence of this population in human has been speculated on²⁹ based on misinterpretation of data that failed to exclude $\text{TCR}\gamma\delta^+$ IEL, which can express $\text{CD8}\alpha\alpha$,

from the analysis.^{14,35} Additionally, a recent report showing a $\text{TCR}\alpha\beta^+\text{CD8}\alpha^+/\text{CD8}\beta^-$ population in human intestine³⁶ attempted to extend this observation to suggest the existence of $\text{TCR}\alpha\beta^+\text{CD8}\alpha\alpha^+$ IEL in human; however, the data suggests the same cell may express both $\text{CD8}\alpha\alpha$ and $\text{CD8}\alpha\beta$ dimers, a cell type that would not phenocopy the bonafide $\text{TCR}\alpha\beta^+\text{CD8}\alpha\alpha^+$ IEL found in mouse. Finally, the $\text{TCR}\alpha\beta^+\text{CD8}/\text{CD4}$ double negative population which is enriched in the mouse colon^{30,32} and considered to be similar in nature to $\text{TCR}\alpha\beta^+\text{CD8}\alpha\alpha^+$ IEL^{37,38} as well as the $\text{TCR}\alpha\beta^+\text{CD4}^+\text{CD8}\alpha^+$ IEL³⁹ whose inflammatory potential is modulated in the intestine via the upregulation of Runx3 and downregulation of ThPOK^{40,41} are both still poorly characterized in human. Single cell ex vivo transcriptional profiling of these subsets will help establish the extent to which these populations exist in human and whether or not they play a role in health or disease.

The physiological significance of the difference in distribution of various T cell subsets across the gut is still to be determined. Nonetheless, a complex array of local signals may play a role in shaping each compartment locally as can be appreciated from a study comparing the small and large intestine IEL compartments

in neonatal mice vs. adult mice.²¹ It is well accepted that the microbial burden in the colon is higher than that in the small intestine; however, the mucus layer produced in the colon is thicker and keeps the microbiota farther from the hosts epithelial cells than in the small intestine.^{42,43} Furthermore, the impact of the microbiota on adaptively induced IEL is well established as these cells decrease drastically in absolute numbers in germ-free mice.^{19,20,22} Additionally, food is primarily absorbed in the small intestine therefore providing a unique pool of antigens that may impact IEL in the small intestine that are absent in the large intestine.

ARE TWO CELL SUBSETS BETTER THAN ONE?

There seems to be a clear division of labor for mouse IEL in that naturally occurring $\text{TCR}\alpha\beta^+\text{CD8}\alpha\alpha^+$ IEL and induced $\text{TCR}\alpha\beta^+\text{CD8}\alpha\beta^+$ IEL have been shown to have different transcriptional and functional profiles.^{17,37} One critical difference is the vast array of natural killer (NK) receptors that have been shown to be enriched on $\text{TCR}\alpha\beta^+\text{CD8}\alpha\alpha^+$ IEL when compared to $\text{TCR}\alpha\beta^+\text{CD8}\alpha\beta^+$ IEL in mouse^{37,44} (Fig. 2). In contrast to $\text{TCR}\alpha\beta^+\text{CD8}\alpha\alpha^+$ IEL which seem to be geared toward cytolytic function and express high levels of Granzyme B, $\text{TCR}\alpha\beta^+\text{CD8}\alpha\beta^+$ IEL in mouse have a substantial capacity to produce cytokines⁴⁵ such as IFN- γ when probed *ex vivo* (Fig. 2). Of note, $\text{TCR}\alpha\beta^+\text{CD8}\alpha\alpha^+$ IEL express similar levels of Granzyme B in both young and adult mice indicative of their unique developmental program whereas the capacity of $\text{TCR}\alpha\beta^+\text{CD8}\alpha\beta^+$ IEL to produce Granzyme B and IFN- γ is gained with age (Fig. 2), presumably as the gut microbiota matures leading to the generation of more adaptively induced $\text{TCR}\alpha\beta^+\text{CD8}\alpha\beta^+$ IEL. Interestingly, $\text{TCR}\alpha\beta^+\text{CD8}\alpha\beta^+$ IEL at steady state in human have dual capacity in that they express NK receptors such as NKG2D^{46,47} and CD94 receptors,^{48,49} while also carrying the capacity to produce Granzyme B and inflammatory cytokines such as IFN- γ ⁵⁰ (Fig. 2). However, several important differences with respect to the nature of NK receptors expressed exist between $\text{TCR}\alpha\beta^+\text{CD8}\alpha\beta^+$ IEL in human and $\text{TCR}\alpha\beta^+\text{CD8}\alpha\alpha^+$ IEL in mouse. First, both human $\text{TCR}\alpha\beta^+\text{CD8}\alpha\beta^+$ IEL and mouse $\text{TCR}\alpha\beta^+\text{CD8}\alpha\alpha^+$ IEL carry NK receptors such as NKG2A/CD94 and NKG2D that can recognize non-classical MHC class I molecules,^{51,52} however, mouse IEL also express the Ly49 family of receptors which endows them with the capacity to recognize classical MHC class I molecules⁵³ (Fig. 2). Second, under steady-state conditions, human $\text{TCR}\alpha\beta^+\text{CD8}\alpha\beta^+$ IEL do not express any NK receptors with immunoreceptor tyrosine-based activation motif (ITAM) adapter molecules such as DAP12,^{46,54} whereas mouse $\text{TCR}\alpha\beta^+\text{CD8}\alpha\alpha^+$ IEL can express receptors associated with DAP12^{44,53,54} (Fig. 2). Therefore, the only activating receptor with the capacity to induce cell proliferation and cytokine production on healthy human $\text{TCR}\alpha\beta^+\text{CD8}\alpha\beta^+$ IEL is the TCR. However, under inflammatory conditions as is associated with CeD,⁵⁵ a subset of aberrant $\text{TCR}\alpha\beta^+\text{CD8}\alpha\beta^+$ IEL can gain expression of activating NK receptors that can pair with DAP12, such as NKG2C/CD94⁵⁶ (Fig. 2). These cells also displayed elevated transcript levels for a variety of killer immunoglobulin-like receptors⁵⁶ thus bridging the gap in functional capacity that exists between mouse and human IEL at steady state.

TCR $\alpha\beta^+\text{CD8}\alpha\alpha^+$ /TCR $\alpha\beta^+\text{CD8}\alpha\beta^+$ IEL TCR REPERTOIRE

Analysis of the TCR repertoire based on measurement of the length of the hypervariable - complementary determining region 3 (CDR3), using littermate controls and genetically identical mice, concluded the TCR repertoires of both naturally occurring $\text{TCR}\alpha\beta^+\text{CD8}\alpha\alpha^+$ and adaptively induced $\text{TCR}\alpha\beta^+\text{CD8}\alpha\beta^+$ IEL are highly restricted and non-overlapping, indicating these two subsets are not clonally related.⁵⁷ Furthermore, there was no

evidence for shared/public T cell clones even in mice born from the same mother and living in the same cages. Finally, the degree of oligoclonality was significantly higher in IEL than peripheral lymph node T cells. All together, these results suggest IEL undergo considerable expansion and/or are selectively accumulated, the antigens driving their expansion are diverse, and there is a stochastic component to the selection of particular TCRs undergoing oligoclonal expansion in a given mouse. Interestingly, an elegant study comparing the TCR repertoire of adaptively induced $\text{TCR}\alpha\beta^+\text{CD8}\alpha\beta^+$ T cells in the thoracic duct and the epithelial compartment of the same mouse showed T cells sharing the same TCR are polyclonal in the thoracic duct and oligoclonal in the epithelium.⁵⁸ Given that naive $\text{TCR}\alpha\beta^+\text{CD8}\alpha\beta^+$ T cells primed in the Peyer's patches and mesenteric lymph nodes transit through the thoracic duct before returning through the blood stream in the intestine, these results suggest that the major clonal expansion takes place within the intestine. Furthermore, identification of similar T cell clones across the length of the small intestine suggests that T cells primed at one location of the gut seed the whole intestine after they have circulated through the thoracic duct.⁵⁷ More recent studies show indeed that $\text{TCR}\alpha\beta^+\text{CD8}\alpha\alpha^+$ IEL subsets can be selected by a diverse set of MHC and MHC-like molecules.^{38,59} Furthermore, there is evidence for antigen-driven expansion of naturally occurring $\text{TCR}\alpha\beta^+\text{CD8}\alpha\alpha^+$ and adaptively induced $\text{TCR}\alpha\beta^+\text{CD8}\alpha\beta^+$ IEL. In H-Y TCR transgenic mice, $\text{TCR}\alpha\beta^+\text{CD8}\alpha\alpha^+$ IEL are expanded in the gut of male but not female mice,⁶⁰ and the TCR repertoire of naturally occurring and adaptively induced IEL is less oligoclonal in the absence of microbiota.⁶¹ In germ-free mice, this polyclonality is accompanied by a significant decrease in the number of IEL, in particular of adaptively induced $\text{TCR}\alpha\beta^+\text{CD8}\alpha\beta^+$ and $\text{TCR}\alpha\beta^+\text{CD4}^+$ IELs, while the number of naturally occurring IEL is less decreased,²⁰ with $\text{TCR}\gamma\delta^+$ IEL being preserved in particular.^{19,22} These results suggest the highly oligoclonal nature of the IEL TCR repertoire results from antigen-driven selection. Whether oligoclonality of IEL, in particular of naturally occurring $\text{TCR}\alpha\beta^+\text{CD8}\alpha\alpha^+$ IEL, is antigen driven remains to be determined. Non-antigen-driven expansions can be potentially explained by epithelial factors such as IL-15.^{62,63} Dietary factors that are required for the generation of ligands for the aryl hydrocarbon receptor⁶⁴ have also been shown to play an important role in the expansion and maintenance of naturally occurring IEL.⁶⁵ The more significant decrease of $\text{TCR}\alpha\beta^+\text{CD8}\alpha\alpha^+$ IEL in food antigen-free mice as compared to germ-free mice²² may point to the critical role dietary factors play in their TCR-independent expansion.

In human, the oligoclonal nature of the $\text{TCR}\alpha\beta^+\text{CD8}\alpha\beta^+$ IEL TCR repertoire has been demonstrated in the small intestine⁶⁶ and colon.⁶⁷ As for mice, no public TCRs could be identified. Importantly, concomitant analysis of Lp lymphocytes showed a significantly more polyclonal TCR repertoire.⁶⁷ Frequently, a unique CDR3 amino acid sequence accounted for more than 50% of all IEL sequences for a given TCR V β -chain.⁶⁷ These observations pose the question of whether the highly oligoclonal nature of the TCR repertoire of human $\text{TCR}\alpha\beta^+\text{CD8}\alpha\beta^+$ IEL is dependent on antigen. The antigen-driven nature of a T cell expansion can be shown in different ways such as presence of conserved amino acids in the CDR3 that are encoded by distinct nucleotide sequences, presence of a conserved amino acid motif flanked by different amino acid sequences, or association of the same V β -chain with different V α -chains. Using such criteria, existence of unequivocal antigen drive was not only shown in human $\text{TCR}\alpha\beta^+\text{CD8}\alpha\beta^+$ IEL but also linked to the expression of activating vs. inhibitory NK receptors.⁴⁹ The combination of major clonal expansion with evidence for antigen-driven selection suggests that IEL respond to specific antigens that drive their expansion. However, many questions remain unanswered in both human and mouse. How can one explain the extremely clonal repertoire of IEL that contrasts not only with the repertoire of

peripheral T cells but also with Lp T cells? Such a clonal repertoire was described during the *Listeria* recall response but not in the primary effector and memory $\text{TCR}\alpha\beta^+\text{CD8}\alpha\beta^+$ T cell response.⁶⁸ This suggests that $\text{TCR}\alpha\beta^+\text{CD8}\alpha\beta^+$ IEL may undergo chronic recall responses. In both human and mouse, it is extremely rare to identify IEL in mitosis. Human $\text{TCR}\alpha\beta^+\text{CD8}\alpha\beta^+$ IEL are Ki67^- under steady-state conditions.⁶⁹ In mice, 0.2–3% of IEL are in mitosis.^{70,71} Using electron microscopy, it was estimated that 5% of IEL were immunoblast.⁷² Furthermore, an elegant study suggested that while adaptive $\text{TCR}\alpha\beta^+\text{CD8}\alpha\beta^+$ IEL divide in the GALT and the thoracic duct lymph, naturally occurring $\text{TCR}\alpha\beta^+\text{CD8}\alpha\beta^+$ IEL divide after they have entered the epithelium.⁷¹ Where these recall responses take place and in response to what antigens remain to be determined as does the nature and location of the memory T cells that feed the response.

$\text{TCRAB}^+\text{CD8AA}^+/\text{TCRAB}^+\text{CD8AB}^+$ IEL AND AUTOREACTIVITY

Studies in mouse suggest that autoreactivity is primarily a characteristic associated with the naturally occurring $\text{TCR}\alpha\beta^+\text{CD8}\alpha\alpha^+$ IEL subset. This IEL subset was shown to be selected by self-antigens restricted by non-classical and classical MHC class I and II molecules during thymic development.^{38,59,73–75} The current line of thought is self-reactive T cells that failed to undergo negative selection are destined to preferentially migrate and expand in the intestine,^{76,77} where they acquire $\text{CD8}\alpha\alpha$ and granzyme.⁷¹ In addition to having an autoreactive TCR, these naturally occurring innate-like lymphocytes express activating NK receptors^{37,44,53} that enable them to recognize self-antigens induced under conditions of stress and inflammation⁷⁸ (Fig. 2). This latter autoreactivity is destined to recognize modifications of self that signal the presence of pathogens and transformed cells. Interestingly, $\text{TCR}\alpha\beta^+\text{CD8}\alpha\alpha^+$ IEL are poorly reactive through their TCR,³⁷ which is in line with the proposed role for the $\text{CD8}\alpha\alpha$ homodimer as a corepressor,⁷⁹ suggesting they respond mainly to innate signals.

In human, as previously discussed, $\text{TCR}\alpha\beta^+\text{CD8}\alpha\alpha^+$ IEL are, if not non-existent, extremely rare. Furthermore, reactivity to self-antigens such as CD1 was not detected in humans *ex vivo*, but rather seen in IE-CTL lines generated after multiple *in vitro* stimulations with PHA and peripheral blood monocytes.⁶⁶ In contrast, $\text{TCR}\alpha\beta^+\text{CD8}\alpha\beta^+$ IEL show signs of antigen-driven expansion,⁴⁹ suggesting that likewise to $\text{TCR}\alpha\beta^+\text{CD8}\alpha\beta^+$ IEL in mice, they are adaptively induced in response to exogenous antigens. However, unlike murine $\text{TCR}\alpha\beta^+\text{CD8}\alpha\beta^+$ IEL, all human $\text{TCR}\alpha\beta^+\text{CD8}\alpha\beta^+$ IEL express the activating NK receptor NKG2D ,^{46,47} in addition to other activating NK receptors such as CD94 and NKR-PIA ,^{48,49} indicating that they are poised to recognize modified self and respond to stress and inflammatory signals. Importantly, it was shown that NKG2D and CD94 receptors have the ability to kill targets through engagement of these receptors and in absence of TCR engagement.^{46,80} Finally, because both NKG2D ^{46,47,80} and CD94 ⁴⁹ can act as costimulatory molecules for the TCR they can significantly reduce the TCR activation threshold, hence potentially enabling the TCR to recognize non-cognate, low-affinity antigens *in vivo*. Conversely, induction of the $\text{CD8}\alpha\alpha$ homodimer on $\text{TCR}\alpha\beta^+\text{CD8}\alpha\beta^+$ IEL in mouse is thought to increase the TCR activation threshold.⁷⁹ Such a function for the $\text{CD8}\alpha\alpha$ homodimer based on the observation of a $\text{TCR}\alpha\beta^+\text{CD8}\alpha^+/\text{CD8}\beta$ -dim population in human intestine³⁶ is possible but yet to be demonstrated. The ability to recognize self-antigens is further enhanced when IL-15 ,⁸¹ a cytokine induced under conditions of stress, inflammation and infection, is upregulated.^{80,82,83} Of note, IL-15 ^{46,48} and NKG2D ⁴⁶ signaling in $\text{TCR}\alpha\beta^+\text{CD8}\alpha\beta^+$ IEL induces effector programs that are not observed in memory $\text{TCR}\alpha\beta^+\text{CD8}\alpha\beta^+$ T cells. These observations led us to propose that activation of human $\text{TCR}\alpha\beta^+\text{CD8}\alpha\beta^+$ IEL is driven by recognition of non-classical MHC class I molecules by NK receptors and IL-15 ,

signifying the presence of ongoing tissue distress.^{80,84} Concomitantly, a role for NKG2D in the rejection of skin tumors expressing non-classical MHC class I molecules Rae-1 and H60 by skin epithelial $\text{TCR}\gamma\delta^+$ T cells was reported.⁸⁵

In summary, both human and mouse IEL display autoreactive properties. However, whereas in mice this property is innately displayed by $\text{TCR}\alpha\beta^+\text{CD8}\alpha\alpha^+$ IEL that have been selected by self-antigens, in human adaptively induced $\text{TCR}\alpha\beta^+\text{CD8}\alpha\beta^+$ IEL can display these properties via the activating NK receptors they express and their ability to respond to IL-15 . Because mouse IEL have been mainly studied in specific pathogen-free conditions, it remains possible that mouse $\text{TCR}\alpha\beta^+\text{CD8}\alpha\beta^+$ IEL could acquire expression of NK receptors under infectious and inflammatory conditions and gain the capacity to respond to stress signals.

IEL FUNCTION DURING HOMEOSTASIS

It is important to preserve the integrity of the intestinal epithelium given the burden of foreign antigens in the lumen so it is no surprise that tissue-resident lymphocytes are enriched in the epithelium. Given this proximity to the barrier, IEL are thought to participate in tissue surveillance and maintenance of barrier function. To accomplish this task, the IEL compartment is endowed with the capacity to respond to tissue stress via NK receptor/non-classical MHC-like molecule interactions as well as pathogen invasion via classical antigen-specific TCR/MHC interactions (Fig. 2). These functions can be accomplished by a singular cell in human in the $\text{TCR}\alpha\beta^+\text{CD8}\alpha\beta^+$ IEL but are differentially distributed in mouse between naturally occurring and adaptively induced IEL as can be appreciated by the predominant expression of NK receptors on $\text{TCR}\alpha\beta^+\text{CD8}\alpha\alpha^+$ IEL (Fig. 2). One of the primary functions of IEL is cytotoxicity^{37,50,86–88} and can be inferred from their potent expression of granzymes,^{17,18,71} which endow them with the capacity to lyse infected or aberrant cells.^{88,89} However, establishing requirement for a given function of IEL has been challenging due to the lack of models where IEL can be selectively ablated in addition to the redundancy in functional capacity between different IEL subsets. For instance, $\text{TCR}\alpha\beta^+$ IEL are the primary producers of $\text{IFN-}\gamma$ in response to *Listeria monocytogenes* infection, but in β_2 -Microglobulin-deficient mice, which lack adaptively induced $\text{TCR}\alpha\beta^+\text{CD8}\alpha\beta^+$ IEL, this response is compensated for by the β_2 -Microglobulin-independent $\text{TCR}\gamma\delta$ and $\text{TCR}\alpha\beta^+\text{CD8}\alpha\alpha^+$ IEL subsets.⁸⁷

Naturally occurring IELs have also been suggested to exert an immuno-regulatory role under homeostatic conditions. Interestingly, transfer of naturally occurring $\text{TCR}\alpha\beta^+\text{CD8}\alpha\alpha^+$ IEL but not adaptively induced $\text{TCR}\alpha\beta^+\text{CD8}\alpha\beta^+$ IEL is protective against the development of colitis in a $\text{CD4}^+\text{CD45RBhi}$ T cell transfer colitis model.⁹⁰ In line with this observation, $\text{TCR}\delta^{-/-}$ mice, which lack naturally occurring $\text{TCR}\gamma\delta^+$ IEL, infected with *Eimeria vermiformis* show increased epithelial cell damage,⁹¹ an observation that fits with the proposed role for $\text{TCR}\gamma\delta^+$ IEL in tissue repair mediated via secretion of keratinocyte growth factor.⁹² Furthermore, naturally occurring $\text{TCR}\gamma\delta^+$ IEL actively survey epithelial cells at homeostasis and respond to *Salmonella* infection by increasing their movement speed and localization while enhancing expression of antimicrobial genes to participate in the early phase of the immune response.⁹³

The function of adaptively induced IEL has been primarily studied in the context of infection, and although evidence for antigen-specific responses in naturally occurring IEL are still lacking, antigen-specific adaptively induced $\text{TCR}\alpha\beta^+\text{CD8}\alpha\beta^+$ IEL can be quantified by MHC-tetramer staining post infection with either vesicular stomatitis virus or *Listeria monocytogenes*.^{94,95} In accordance, the protective potential of adaptively induced $\text{TCR}\alpha\beta^+\text{CD8}\alpha\beta^+$ IEL is best demonstrated in models of adoptive transfer of antigen-specific IEL in various mouse infection models.^{88,96}

The role for human IEL under homeostatic conditions is still poorly understood. What can be said for certain is human IEL have potent cytolytic capacity which can be mediated via engagement of the TCR or NK receptors such as NKG2D.⁴⁶ Additionally, the potent cytokine production by TCR $\alpha\beta$ ⁺CD8 $\alpha\beta$ ⁺ IEL in healthy individuals (Fig. 2) strongly suggests they are responding to stimuli *in vivo*; however, the specificity of these responses and the degree to which functional redundancy between various IEL subsets exists is yet to be determined.

IEL FUNCTION DURING PATHOLOGY

A role for IEL in pathology was implied primarily because of their increase in enteropathies such as CeD, tropical sprue, and parasite infections.⁴ IEL were also reported to be increased in graft vs. host disease, allograft rejection, autoimmune enteropathies, and inflammatory bowel disease; however, their increase in these disorders is less significant and occurs late in the disease process.^{4,97,98} Because of the technical limitations that prevent the selective elimination of IEL in mouse models of disease, their requirement in immunopathology could not be established. In a mouse model expressing the antigen ovalbumin in the epithelium, TCR $\alpha\beta$ ⁺CD8 $\alpha\beta$ ⁺T cells specific for ovalbumin (OT-1 TCR Transgenic T cells) preferentially migrated to the inductive and effector sites of the intestinal mucosa without causing epithelial cell destruction. However, once mice were infected with a virus expressing ovalbumin, OT-1 T cells induced epithelial cell destruction and villous atrophy.⁹⁹ Using human fetal small intestinal explants, it was shown that activation of T cells could induce villous atrophy,¹⁰⁰ but the respective role of IEL and Lp lymphocytes could not be delineated, neither in the mouse nor the human experimental model.

In human, the role of TCR $\alpha\beta$ ⁺CD8 $\alpha\beta$ ⁺ IEL in disease is best established in CeD, a T cell-mediated small intestinal enteropathy induced by dietary gluten in genetically susceptible HLA-DQ2 or HLA-DQ8 individuals.^{55,84,98,101} Intraepithelial lymphocytosis is a hallmark of CeD and used in clinic for the diagnosis of CeD.¹⁰² However, the inability to identify gluten-specific TCR $\alpha\beta$ ⁺CD8 $\alpha\beta$ ⁺ IEL initially led scientists in the field to propose that the increase in IEL was secondary to the activation of dietary gluten-specific TCR $\alpha\beta$ ⁺CD4⁺T helper-1 (T_H1) cells and did not play a role in the pathogenesis of CeD.¹⁰³ However, similarly to patients with latent autoimmune diabetes of adults who preserve a functional pancreas despite the presence of an adaptive immune response against beta-islet antigens,^{104,105} potential CeD patients conserve a normal intestinal architecture despite having developed an adaptive immune response against gluten,^{106,107} suggesting that gluten-specific T_H1 cells are not the effector cells mediating tissue destruction. The discovery that activating NK receptors and their ligands are upregulated in TCR $\alpha\beta$ ⁺CD8 $\alpha\beta$ ⁺ IEL and epithelial cells in active CeD but not in patients on a gluten-free diet,^{46,47,56} provided a mechanism through which TCR $\alpha\beta$ ⁺CD8 $\alpha\beta$ ⁺ IEL can destroy epithelial cells despite not being gluten-specific.⁸⁴ The observation that activating NK receptors and their ligands are not upregulated in potential CeD¹⁰⁸ further supports that TCR $\alpha\beta$ ⁺CD8 $\alpha\beta$ ⁺ IEL are the key effector T cell subset mediating epithelial cell destruction and villous atrophy in CeD. In agreement with observations in human, mice in which gluten-specific T_H1 IFN- γ -producing T cells were induced in absence of TCR $\alpha\beta$ ⁺CD8 $\alpha\beta$ ⁺ IEL activation failed to develop villous atrophy,^{109–111} in contrast to mice in which TCR $\alpha\beta$ ⁺CD8 $\alpha\beta$ ⁺ IEL acquired lymphokine killer-like activity.¹¹² In addition to TCR $\alpha\beta$ ⁺CD8 $\alpha\beta$ ⁺ IEL, innate-like IEL lacking surface TCR expression were involved in the development of villous atrophy in patients with refractory CeD,^{113–115} an indolent or cryptic innate intraepithelial lymphoma that rarely complicates CeD. In both active and refractory CeD, upregulation of IL-15 in the epithelium is thought to play a critical role in the activation of IEL and epithelial cell

destruction.^{81,116,117} In line with a role for IL-15 in promoting tissue destruction, a study showed that when mice overexpressing IL-15 in the epithelium were crossed to ovalbumin-specific CD4 TCR transgenic mice, they developed villous atrophy when ingesting ovalbumin.¹¹⁸ Altogether, the observations in CeD point toward TCR $\alpha\beta$ ⁺CD8 $\alpha\beta$ ⁺ IEL being a key effector cell able to mediate epithelial cell destruction based on the recognition of stress and inflammatory signals. Intriguingly, TCR $\alpha\beta$ ⁺CD8 $\alpha\beta$ ⁺ IEL in active CeD share numerous functional features with TCR $\alpha\beta$ ⁺CD8 $\alpha\alpha$ ⁺ IEL in that they can exert NK-like properties⁴⁶ and even express NK receptors associated with ITAM-bearing adapter molecules⁵⁶ (Fig. 2a).

CONCLUSION

The tissue-resident intraepithelial T cell compartment is shaped by the local environment (oral antigen, microbial signals, region-specific IEL–IEC interactions) as can be appreciated by the different proportion of various cell subsets between the small and large intestine in both human and mouse. Therefore, studies involving IEL should consider the distribution of IEL subsets across the gut; especially those involving the design of mouse models to investigate human relevant questions as there are also differences in the distribution of subsets between the two species. One such difference is the relative absence of the naturally occurring mouse TCR $\alpha\beta$ ⁺CD8 $\alpha\alpha$ ⁺ IEL in human. A close comparison between the human and mouse IEL subsets highlights a critical aspect of IEL function which is the dual capacity to recognize both modified self and non-self. Whereas in mouse, recognition of modified self via NK receptors is restricted to the naturally occurring TCR $\alpha\beta$ ⁺CD8 $\alpha\alpha$ ⁺ IEL and recognition of non-self is best described for adaptively induced TCR $\alpha\beta$ ⁺CD8 $\alpha\beta$ ⁺ IEL, in human a singular TCR $\alpha\beta$ ⁺CD8 $\alpha\beta$ ⁺ IEL subset has the capacity to do both given the dual expression of NK receptors and the TCR. The pathogenic role and propensity for autoreactivity of the human TCR $\alpha\beta$ ⁺CD8 $\alpha\beta$ ⁺ IEL subset has been best characterized in CeD where inflammatory signals such as IL-15 can combine with NK receptor/stress ligand engagement to result in non-cognate antigen activation of IEL TCRs, ultimately resulting in tissue destruction.

The role for the local environment in shaping the IEL compartment is further highlighted by the observation that both naturally occurring TCR $\alpha\beta$ ⁺CD8 $\alpha\alpha$ ⁺ and adaptively induced TCR $\alpha\beta$ ⁺CD8 $\alpha\beta$ ⁺ IEL have private oligoclonal TCR repertoires in the steady state. To that point, it has been challenging to uncover the intimate specificities of IEL. This is most likely due to the rich source of antigen they encounter, be it microbial, dietary, or epithelial antigens and thus studies geared toward removal of one source of antigen may simply result in the expansion of IEL specific to the remaining source of antigen. The reduction in IEL numbers observed in both food antigen-free and germ-free mice suggests IEL may be reactive to dietary and microbial antigens; however, there has been no identification of dietary or commensal-specific IEL.

The reason for the absence of the TCR $\alpha\beta$ ⁺CD8 $\alpha\alpha$ ⁺ IEL in human is still unclear. Given the observations that TCR $\alpha\beta$ ⁺CD8 $\alpha\beta$ ⁺ IEL in mice increase with age and that germ-free mice have a significant reduction of TCR $\alpha\beta$ ⁺CD8 $\alpha\beta$ ⁺ IEL, it remains possible that wild mice and mice under chronic inflammatory conditions would lose the TCR $\alpha\beta$ ⁺CD8 $\alpha\alpha$ ⁺ IEL subset at the expense of the TCR $\alpha\beta$ ⁺CD8 $\alpha\beta$ ⁺ IEL that may acquire expression of NK receptors akin to those in human. Interestingly, TCR $\gamma\delta$ ⁺ IEL, which are also considered naturally occurring in mouse, are also significantly underrepresented in the human intestine.

The IEL compartment as a whole is comprised of many cell subsets, including non-T cell subsets of ILCs, and these subsets are influenced by their environment under steady (small intestine vs. colon) and pathogenic conditions. What remains to be elucidated is whether there is a clear division of labor between IEL subsets

and if not to what extent does redundancy exist in their functions. Finally, whether the tissue-resident IEL compartment is stable and the extent to which it can be reshaped as a result of chronic insults and inflammatory conditions is a question with important physiopathological consequences.

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AUTHOR CONTRIBUTIONS

T.M. and B.J. designed experiments. T.M. conducted experiments. T.M. and B.J. designed figures. T.M. and B.J. wrote the manuscript.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

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