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The light and dark sides of intestinal intraepithelial lymphocytes

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Abstract

The intraepithelial lymphocytes (IELs) that reside within the epithelium of the intestine form one of the main branches of the immune system. As IELs are located at this critical interface between the core of the body and the outside environment, they must balance protective immunity with an ability to safeguard the integrity of the epithelial barrier: failure to do so would compromise homeostasis of the organism. In this Review, we address how the unique development and functions of intestinal IELs allow them to achieve this balance.

The epithelium of the intestine digests and absorbs nutrients and fluids, and in adult humans it spans an area of about 200–400 m² (REF. 1). This huge surface is made up of a single cell layer of epithelial cells, which lines the lumen of the intestine to form a physical barrier between the core of the body and the environment and forms the largest entry port for pathogens. Probably as a consequence of this, sophisticated and complex innate and adaptive immune networks intensely communicate and synergize to tightly control the integrity of this critical interface. Although a major task of the mucosal immune system is to provide protection against intestinal pathogens, it is important that excessive or unnecessary immune responses are avoided.

In this Review, we focus on the intraepithelial lymphocytes (IELs), which, by their direct contact with the enterocytes and by their immediate proximity to antigens in the gut lumen, form the front line of immune defence against invading pathogens. IELs essentially comprise antigen-experienced T cells belonging to both the T cell receptor- $\gamma\delta$ (TCR $\gamma\delta$)⁺ and TCR $\alpha\beta$ ⁺ lineages. We discuss the thymic (natural) and peripheral (induced) differentiation of various IEL subpopulations, as well as their beneficial roles (that is, their ‘light side’) in preserving the integrity of the mucosal barrier and in preventing pathogen entry and spreading. Conversely, we also expose the ‘dark side’ of IELs and discuss how these cells can contribute to immune pathology and inflammatory diseases.

Mucosal IELs are unique and heterogeneous

IELs are extremely heterogeneous, and the various IEL subsets are distributed differently in the epithelium of the small and large intestine (TABLE 1). This pattern of distribution is probably influenced by the distinct digestive functions and the physiological conditions that allow these two compartments to cope with infections while simultaneously maintaining

tolerance to innocuous antigens from the diet or from resident non-invasive commensals. Nevertheless, these IELs also share characteristics that distinguish them from the conventional T cell pools in the periphery.

Gut IELs are almost exclusively T cells, and estimates based on histological sections indicate that there are more T cells in the intestinal epithelium than in the spleen². IELs include a significant proportion of TCR $\gamma\delta^+$ cells, which can constitute up to 60% of small intestinal IELs^{3–5}. These IELs are antigen-experienced cells that typically express activation markers, such as CD44 and CD69 (REF. 6). Furthermore, studies using parabiotic mice and intestinal grafting indicate that these antigen-experienced T cells do not recirculate^{7,8}. The majority of IELs contain abundant cytoplasmic granules for cyto-toxic activity, and they can express effector cytokines, such as interferon- γ (IFN γ), interleukin-2 (IL-2), IL-4 or IL-17 (REFS 9–16). Furthermore, they characteristically express both activating and inhibitory types of innate natural killer (NK) cell receptors, which typify them as stress-sensing (activated) yet highly regulated (resting) immune cells^{9,11,17–20}.

IELs constitutively express CD103 (also known as the α E integrin), which interacts with E-cadherin on intestinal epithelial cells^{21,22}, and most of them, especially in the small intestine, express CD8 $\alpha\alpha$ homodimers, which is a hallmark of their activated phenotype^{18,23–25} (BOX 1). A ligand for CD8 $\alpha\alpha$, the thymus leukaemia antigen (TLA), which is a non-classical MHC class I molecule, is abundantly expressed on mouse small intestinal epithelial cells^{26,27}. Many TCR $\alpha\beta$ IELs express CD8 $\alpha\alpha$ together with CD4 or CD8 $\alpha\beta$; however, a large fraction expresses CD8 $\alpha\alpha$ alone¹⁶. Finally, under normal conditions, and in contrast to systemic and lamina propria lymphocytes (LPLs), CD4⁺ cells are greatly under-represented in the IEL compartment, especially in the small intestine^{28,29}.

Box 1

CD8 $\alpha\alpha$ as a repressor

CD8 $\alpha\alpha$, which is composed of two CD8 α subunits, can be induced on activation through the T cell receptor (TCR)–CD3 complex, with the level of its expression being proportional with the signal strength. Therefore, CD8 $\alpha\alpha$ can be used as an activation marker for T cells⁴³. CD8 $\alpha\alpha$ has the ability to recruit TCR–CD3 signalling components, including LCK (also known as p56^{LCK}) and linker for activation of T cells (LAT)¹⁶⁵. However, owing to its physical separation from the TCR-activation complex¹⁶⁶, CD8 $\alpha\alpha$ neither functions as a TCR co-receptor nor can it substitute for the CD4 or CD8 $\alpha\beta$ co-receptors (see the figure, part **a**). Consistent with this, CD8 $\alpha\alpha$ is unable to support positive selection of MHC class I-restricted thymocytes or promote activation of mature MHC class I-restricted T cells¹⁶⁷. Instead, co-expression of CD8 $\alpha\alpha$ on CD8 $\alpha\beta^+$ (or CD4⁺) T cells downmodulates, rather than enhances, the functional avidity of the MHC–TCR activation complex¹¹³. The ability of CD8 $\alpha\alpha$ to function as a negative regulator is partly due to its exclusion from the TCR activation complex (see the figure, part **b**). Consequently, CD8 $\alpha\alpha$ can sequester signalling components outside the immunological synapse, thereby interfering with their recruitment to the CD8 $\alpha\beta$ or the CD4 co-receptor–TCR–CD3 activation complex. Thus, CD8 $\alpha\alpha$ serves as a TCR repressor (see the figure, part **b**) rather than as a TCR co-receptor (see the figure, part **a**). See REF. 43 for further details.

Ag, antigen; APC, antigen-presenting cell; ITAM, immunoreceptor tyrosine-based activation motif.

Although all IELs have an antigen-experienced phenotype, they can be divided into two major subsets based on the mechanisms by which they become activated and on the cognate antigens that they recognize. The ‘natural’ IELs (which were previously known as ‘type b’ IELs)⁵ acquire their activated phenotype during development in the thymus in the presence of self antigens, whereas the ‘induced’ IELs (which were previously known as ‘type a’ IELs)⁵ are the progeny of conventional T cells that are activated post-thymically in response to peripheral antigens (FIG. 1; TABLE 1).

Natural IELs

Natural IELs are either $CD8\alpha\alpha^+$ or $CD8\alpha\alpha^-$ T cells that express $TCR\gamma\delta$ or $TCR\alpha\beta$ but do not express either CD4 or $CD8\alpha\beta$. Typically, they are also negative for expression of CD2, CD5, CD28, lymphocyte function-associated antigen 1 (LFA1; also known as $\alpha L\beta 2$ integrin) and THY1 (REFS 9,28,30,31). In addition, the majority of $TCR\alpha\beta^+$ natural IELs are $B220^+CD44^{low/mid}CD69^+$, a phenotype that is rarely found among peripheral T cells^{32,33}. Many of the natural IELs also share molecules expressed by NK cells, such as CD16, CD122, DNAX-activation protein 12 (DAP12; also known as TYROBP), lymphocyte antigen 49A (Ly49A), Ly49E, Ly49G2, and NK1.1 (REFS 9,18,34,35). Lastly, they frequently express a CD3 complex that is composed of either $CD3\zeta$ - $Fc\epsilon RI\gamma$ heterodimers or $Fc\epsilon RI\gamma$ - $Fc\epsilon RI\gamma$ homodimers instead of the $CD3\zeta$ - $CD3\zeta$ homodimers that are expressed by conventional T cells^{9,36–38}.

Induced IELs

Induced IELs arise from conventional $CD4^+$ or $CD8\alpha\beta^+$ $TCR\alpha\beta^+$ T cells, which are MHC class II-restricted and MHC class I-restricted, respectively. In contrast to the natural IELs, induced IELs acquire an activated phenotype in response to cognate antigens encountered in the periphery^{33,39–42}. Consistent with this, they typically express a ‘memory-like phenotype’ ($CD2^+CD5^+CD44^+LFA1^+THY1^+$)^{9,28,30}, together with the activation marker $CD8\alpha\alpha$ ⁴³ (BOX 1). Contrary to the polyclonal nature of the conventional T cells in the periphery, the TCR repertoire of induced IELs is oligoclonal and does not significantly overlap with the limited TCR repertoire of the natural $TCR\alpha\beta^+$ IEL compartment⁴⁴.

Thymic development of IEL precursor cells

Similarly to peripheral T cells, all IEL subsets are progeny of bone marrow precursor cells that initially develop in the thymus⁴⁵. However, thymocyte maturation is not uniform, and multiple pathways exist that ultimately determine the diversity of mature T cells, including IELs.

Development of natural IELs

The origin and development of TCR $\gamma\delta$ and TCR $\alpha\beta$ natural IELs are the subjects of long-standing debates^{24,46–49} (BOX 2). For an overview of recent advances on this topic, we refer the reader to previously published reviews^{6,45,50,51}. Briefly, the term ‘natural’ refers to the ontogeny of the precursor cells. For the natural IELs, these precursors go through an ‘alternative’ self-antigen-based thymic maturation process that results in the functional differentiation of mature CD4 and CD8 $\alpha\beta$ double-negative, TCR $\gamma\delta$ -expressing or TCR $\alpha\beta$ -expressing T cells that directly migrate to the intestinal epithelium^{23,24,52} (FIG. 1).

Box 2

The distinct pathways of CD8 $\alpha\alpha^+$ TCR $\alpha\beta^+$ IEL differentiation

This Review does not aim to discuss the debates surrounding the importance of the thymic versus the extrathymic differentiation pathway for CD8 $\alpha\alpha^+$ T cell receptor- $\alpha\beta$ (TCR $\alpha\beta^+$) intestinal epithelial lymphocytes (IELs), as this has been extensively reviewed elsewhere^{47,48,168}. Here, we give a brief overview of the data that have challenged or supported the thymic differentiation pathway. CD8 $\alpha\alpha^+$ IELs were originally thought to differentiate locally in the gut. This attractive idea was fuelled, in part, by the presence of self-reactive TCRs in their TCR repertoire, by supporting data derived from studies using athymic mouse models, and by the presence of haematopoietic immature cells in the small intestine that display characteristics of T cell precursor cells^{169,171}. Nevertheless, ample data indicate that the absolute numbers of CD8 $\alpha\alpha^+$ TCR $\alpha\beta^+$ IELs in athymic mice are extremely reduced compared with euthymic mice^{16,172–174}. These data therefore support the notion that the vast majority of CD8 $\alpha\alpha^+$ TCR $\alpha\beta^+$ IELs are the progeny of cells with a thymic origin. Other data suggest that, under certain experimental conditions, immature T cell-committed precursors may leave the thymus prematurely, before TCR rearrangements have occurred, and complete their maturation locally in the gut¹⁷⁵. In conclusion, if indeed the gut contains precursors of bone marrow and thymic origin that have the potential under physiological conditions to further differentiate locally, multiple crucial questions remain unanswered. For example, under what circumstances is this local pathway functional? And how can the gut environment support selection? However, there is currently no evidence available to answer these questions.

Development of induced IELs

Induced IELs are the progeny of conventional CD4 $^+$ or CD8 $\alpha\beta^+$ TCR $\alpha\beta^+$ T cells that are selected in the thymus. The development of conventional TCR $\alpha\beta^+$ thymocytes, including selection and lineage commitment has been extensively reviewed elsewhere^{53–55}. Following positive selection, mature thymocytes exit the thymus and reach the periphery as conventional naive CD4 $^+$ or CD8 $\alpha\beta^+$ TCR $\alpha\beta^+$ T cells. In response to cognate antigens, they can further mature into antigen-experienced cells, including induced IELs (FIG. 1).

Local differentiation of IELs

Because of the antigen-experienced phenotype that they acquire in the thymus, one can assume that the repertoire of the natural IELs is predominantly tuned to self antigens, whereas induced IELs are mainly shaped by non-self antigens encountered in the periphery. Consequently, induced IELs are sparse early in life, but the population steadily increases with age in response to exposure to exogenous antigens^{56–59} (FIG. 2; TABLE 1). The gradual accumulation of induced IELs allows the mucosal immune system to adapt and develop an almost ‘personalized’ mucosal immune repertoire that is directed against those environmental antigens that are most likely to be re-encountered by a particular individual. The development of these antigen-specific immune cells not only provides focused

protective immunity at this mucosal interface but, at the same time, also reduces the risk of unwanted immune responses directed against innocuous antigens. By contrast, natural IELs do not depend on exogenous antigen-driven differentiation and so they are the first type of antigen-experienced T cells to populate the gut, even before birth⁶⁰ (FIG. 2). Although direct evidence is still lacking, it is likely that the early accumulation of natural IELs provides a self-antigen-based, stress-sensing surveillance mechanism that is tolerant to dietary antigens and colonizing microbiota but provides protective immunity against stress-inducing invasive pathogens. With time, the population of induced IELs gradually becomes larger than the natural IEL population, which remains steady in actual numbers but represents a minor IEL population at later stages in life (FIG. 2).

Nevertheless, regardless of the nature of the cognate antigens or the location of the initial differentiation, all IELs are directly influenced by the intestinal environment⁶¹. This was demonstrated with elegant studies in 'germ-free' and 'antigen-free' mice (the germ-free mice were fed an elementary diet), which showed that both the microbiota and dietary proteins have a crucial role in the establishment of a normal IEL repertoire, as virtually all IEL populations, with the exception of TCR $\gamma\delta$ -expressing T cells, were markedly reduced in such an antigen-deprived environment^{62–64}. Interestingly, although more than 95% of the commensal bacteria in the body normally reside in the large intestine, the small intestine contains at least ten times more IELs than the colon⁶. Furthermore, mice fed an amino-acid-based, protein-free diet, displayed a poorly developed intestinal immune system, similar to that of germ-free mice, with a strong decrease in most IEL populations⁶⁵. These effects could be due to direct effects of the diet on the immune system or could result from diet-induced shaping of the intestinal microbiota. This highlights the importance of the diet, in addition to the microbiota, as a major driving factor in establishing and shaping this mucosal immune branch.

Migration of natural IELs

In addition to their antigen-experienced phenotype, natural IEL precursors may also acquire expression of gut-homing receptors (including $\alpha E\beta 7$ integrin and CC-chemokine receptor 9 (CCR9)) in the thymus^{21,22,66–68}. The expression of the ligands for $\alpha E\beta 7$ integrin and CCR9, E-cadherin and CC-chemokine ligand 25 (CCL25), respectively, by small intestinal epithelial cells results in the direct recruitment of mature natural IEL precursors to the small intestinal epithelium^{24,66}. Consistent with this, the seeding of mucosal tissues with these cells is independent of sphingosine-1-phosphate receptor 1 (S1P₁, also known as S1PR1), which is required for the migration of conventionally-selected T cells⁶⁹. The direct and early population of the mucosal barrier with these stress-sensing self-reactive effector cells provides a layer of pre-existing immunity before the development of antigen-specific immune cells in response to exogenous antigens (FIG. 2). Additional local endogenous and exogenous factors, such as the cytokine IL-15 and vitamins A and D, further expand and adapt the natural IEL compartment. In light of this, a recent study showed that vitamin D receptor (VDR)-deficient mice had reduced numbers of CD8 $\alpha\alpha^+$ IELs and that this coincided with low levels of IL-10 in the small intestine and increased inflammation in the steady state⁷⁰.

Migration of induced IELs

One study showed that, similarly to natural IELs, CD8 $\alpha\beta^+$ recent thymic emigrants (RTEs) can directly migrate to the small intestinal compartment⁶⁶. However, in general, conventionally selected T cells do not express mucosal homing receptors and naive T cells are normally not detected within the intestinal epithelium. Instead, naive T cells acquire gut-homing capacity following priming in gut-associated lymphoid tissues (GALTs), such as Peyer's patches, and in mesenteric lymph nodes (MLNs)⁷¹. Specialized intestinal epithelial

cells or microfold cells (M cells), resident CX₃C-chemokine receptor 1 (CX₃CR1)⁺ macrophages and migratory CD103⁺ dendritic cells (DCs) all have the capacity to sample antigens in the gut and promote appropriate T cell responses^{42,72–75}. On priming in mucosal sites, naive T cells upregulate homing receptors that allow them to enter intestinal tissues in response to specific ligands expressed by the tissues⁷⁶. These receptor–ligand pairs include: LFA1 and intercellular adhesion molecule 1 (ICAM1); very late antigen 1 (VLA1; also known as α 1 β 1 integrin) and collagen; and α 4 β 7 integrin and mucosal vascular addressin cell adhesion molecule 1 (MADCAM1). α 4 β 7 integrin can also be induced on T cells primed in other sites, allowing peripherally activated cells to migrate to the intestine⁷¹. Upregulation of CCR9 expression further directs T cells to the small intestine compartment in response to its ligand, CCL25, which is constitutively expressed by intestinal epithelial cells.

Induction of the receptors that promote migration of antigen-experienced T cells to the intestine can be promoted by environmental factors that are unique to the gut. It was shown that a diet-derived factor, the vitamin A metabolite retinoic acid, is an important inducer of gut-homing molecules. Retinoic acid promotes upregulation of α 4 β 7 integrin and CCR9, thereby directing activated T cells mainly to the small intestine⁷⁷. Although migration to the colon is also dependent on α 4 β 7 integrin⁷⁸, retinoic acid seems to be neither necessary nor sufficient to induce migration to this site^{78,79}. The ability to produce retinoic acid and to imprint gut-homing receptors during T cell priming is characteristic of the CCR7⁺CD103⁺ migratory DC subset⁷⁷, which have been programmed previously by retinoic acid from intestinal epithelial cells and stromal cells^{80,81}. It is important to note that mucosal imprinting is not absolutely required for entering the intestinal tissues, and T cells primed in the periphery also readily migrate to the gut as effector cells. However, in response to gut-derived antigens, the imprinting process might greatly focus the immune response to the mucosal effector site.

The main adhesion molecule that is involved in the specific localization of IELs in the epithelial layer is composed of CD103 and β 7 integrin and interacts with E-cadherin on the basolateral surface of enterocytes. Expression of this integrin is induced by transforming growth factor β (TGF β) and runt-related transcription factor 3 (RUNX3) and promoted by signalling through CCR9 (REFS 67,82–84). Nearly all activated cells that migrate to the intestinal epithelium gradually upregulate α E β 7 integrin, which coincides with the reciprocal downregulation of α 4 β 7 integrin⁸⁵. Upregulation of CD69 also occurs on entry into the intestinal mucosa; however, although CD69 has a role in inhibiting S1P₁-mediated egress from the thymus and lymphoid tissues, its function on intestinal IELs remains elusive⁶⁹.

The light side of IELs

The location of IELs at the intersection between the external environment and the core of the body prompts teleological predictions about their functions. These cells are geared to provide immediate and heightened immune protection to avoid initial entry and spreading of pathogens. However, because of their proximity to the fragile single cell layer of the intestinal epithelium, IELs also need to display regulatory functions and avoid excessive or unnecessary inflammatory immune responses that could jeopardize the integrity of the barrier (FIG. 3; TABLE 1).

Protective functions of TCR $\gamma\delta$ ⁺ natural IELs

Although the exact functions and behaviour of natural IELs remain elusive, their primary role seems to be to ensure the integrity of the intestinal epithelium and to maintain local immune quiescence^{86–89}.

TCR $\gamma\delta^+$ natural IELs display dual roles: first, they condition and repair the epithelial barrier and control intestinal epithelial cell growth and turnover^{90,91}, and second, they provide front-line defence against enteric pathogens⁸⁹. TCR $\gamma\delta^+$ natural IELs have been implicated in various regulatory roles, including in antibody class switching and immunoglobulin A (IgA) production, in IL-10-dependent oral tolerance, and in clearing necrotic epithelium and repairing damaged epithelium^{90,92–94}. Furthermore, TCR $\gamma\delta$ -deficient mice show abnormalities in gut epithelial morphology, reduced MHC class II expression by enterocytes and impaired mucosal IgA production^{89,94}.

TCR $\gamma\delta^+$ natural IELs also secrete several factors, including TGF β 1, TGF β 3 and prothymosin β 4, that have direct or indirect roles in protecting the integrity of the epithelium⁹. In addition, their ability to secrete keratinocyte growth factor (KGF) is crucial for restoring the integrity of the epithelium in response to physical and inflammatory damage^{92,95–97}. Exaggerated tissue damage is observed in TCR $\gamma\delta$ -deficient mice following *Listeria monocytogenes* infection^{98–100} or during chemically induced colitis^{87,95,101}; similarly, KGF-deficient mice are also more susceptible to dextran sulphate sodium (DSS)-induced colitis. This suggests that, at least in mice, TCR $\gamma\delta^+$ cells control intestinal homeostasis partly through the production of KGF⁹⁵. TCR $\gamma\delta^+$ natural IELs also express the co-stimulatory molecule junctional adhesion molecule-like (JAML), which is known to upregulate KGF expression following ligation by coxsackievirus and adenovirus receptor (CAR), thereby linking this co-stimulatory pathway to the repair and maintenance functions of TCR $\gamma\delta^+$ natural IELs¹⁰².

Many TCR $\gamma\delta^+$ natural IELs express the inhibitory NK receptor NK group 2, member A (NKG2A), which, when triggered in conjunction with TCR activation, increases the production of intracellular and secreted TGF β ¹⁹, suggesting that these cells may also have suppressive roles. Consistent with this, TCR $\gamma\delta^+$ natural IELs were shown to control the number and activation of cytotoxic CD8 $\alpha\beta^+$ TCR $\alpha\beta^+$ induced IELs and to reduce the expression of the activating NK receptor NKG2D¹⁹. Furthermore, levels of IFN γ were greatly increased in TCR $\gamma\delta$ -deficient mice infected with the protozoan parasite *Eimeria veriformis* and in various models of chemically induced colitis⁸⁹, whereas depletion of TCR $\gamma\delta^+$ cells in IFN γ -deficient mice did not affect disease development during DSS-induced colitis⁸⁷.

In addition to their maintenance and regulatory roles, TCR $\gamma\delta^+$ natural IELs are also cytotoxic and they can contribute to protective immunity. This was shown in a model of *Toxoplasma gondii* infection, in which the presence of TCR $\gamma\delta^+$ natural IELs greatly synergized with the resistance mediated by pathogen-specific CD8 $\alpha\beta^+$ T cells^{103,104}. Furthermore, TCR $\gamma\delta^+$ natural IELs also produce protective cytokines, such as tumour necrosis factor (TNF) and IFN γ ¹⁰⁵. Similarly to other TCR $\gamma\delta^+$ T cells, TCR $\gamma\delta^+$ natural IELs have a highly restricted TCR repertoire; however, substantial junctional and combinatorial diversity indicates that they have the potential to recognize a large variety of antigens, although these are still poorly defined^{106,107}. Triggering of their TCRs does not seem to require antigen processing and presentation by MHC molecules, and they may recognize molecular patterns generated by bacterial non-peptide antigens¹⁰⁸ or conserved unprocessed protein antigens (such as heat-shock proteins) produced by bacteria or stressed epithelial cells¹⁰⁹.

The ability of TCR $\gamma\delta^+$ natural IELs to, on the one hand, produce IFN γ in response to infections and, on the other hand, to control the production of IFN γ by other inflammatory cells, suggests that TCR $\gamma\delta^+$ natural IELs are capable of protecting the mucosal barrier against exogenous insults as well as from self-induced damage during excessive immune responses.

Protective functions of CD8 α ⁺TCR α β ⁺ natural IELs

Although self-reactive CD8 α ⁺TCR α β ⁺ natural IELs are normally immunologically quiescent, they have a potent antigen-experienced cytotoxic effector phenotype, which is characterized by high levels of granzyme, CD95 ligand (CD95L; also known as FASL) and CD69 expression, but low levels of IFN γ production^{9,18}. A specific function for these cells has not been described yet. However, as they differentiate in response to self antigens, possess a cytotoxic phenotype and show early localization to the gut epithelium, they might have important functions in maintaining and protecting the mucosal barrier at the time of gut colonization and before protective immunity towards exogenous antigens has been established in the intestine (FIG. 2). Furthermore, their proximity to the rapidly renewing intestinal epithelium suggests potential roles for CD8 α ⁺TCR α β ⁺ natural IELs in sensing and eliminating cancerous or injured epithelial cells.

Nevertheless, although self reactive, these natural IELs are not self destructive, and evidence indicates that self tolerance and regulatory features are pre-programmed during agonist selection of their precursors in the thymus^{17,18,24}. This notion is supported by the finding that when exposed *in vitro* to their cognate agonist antigens, surviving immature thymocytes induce an almost identical transcriptional signature as that described for CD8 α ⁺TCR α β ⁺ natural IELs, including the expression of NK receptors, Ly49 members, CD94, SLAM-related receptor 2B4 (also known as CD244), DAP12 and Fc ϵ RI γ , together with the intestinal homing receptors CD103 and CCR9 (REFS 17,110). These observations underscore the importance of the thymic agonist selection process as a central mechanism to drive the antigen-experienced effector yet regulatory differentiation of these self-specific natural IELs.

We identified TCR α β ⁺ double-negative (CD4⁻CD8⁻) thymocytes that express co-receptor-independent high-affinity TCRs^{33,111} as the agonist-selected mature thymocytes, suggesting that agonist selection preserves and functionally imprints precursor cells with high-affinity TCRs. Agonist-selected CD4⁻CD8⁻ cells that migrate to the intestine re-induce and maintain CD8 α expression. The re-induction of CD8 α expression on agonist-selected natural IELs is in part driven by IL-15 and does not require additional activation²⁴. Furthermore, local interaction with TLA may further stabilize the constitutive expression of CD8 α and control the responsiveness of these cells^{23,25}. In contrast to the conventional TCR co-receptors, CD4 and CD8 α β , CD8 α does not enhance, but instead suppresses TCR activation⁴³ (BOX 1). Therefore, CD8 α expression by these high-affinity self-reactive natural IELs probably sets a new threshold for activation, which prevents or reduces the potential for unnecessary or auto-aggressive immune responses⁴³. However, inhibition by CD8 α is not definite and can be overcome by high levels of antigen stimulation^{112,113}.

CD8 α ⁺TCR α β ⁺ natural IELs also express other molecules associated with immune regulation, including lymphocyte activation gene 3 (LAG3), TGF β , fibrinogen-like 2 (FGL2), prothymosin β 4, 2B4, and several killer cell immunoglobulin-like receptors (KIRs), such as Ly49A, Ly49E and Ly49G¹⁸. However, some of these receptors, such as 2B4, can also function as activating receptors and trigger cytotoxic responses¹¹⁴. The concomitant expression of effector and regulatory molecules implies that these 'activated yet resting' natural IELs may gain a full activation status to serve self-antigen-directed protective and/or regulatory functions⁹. Indeed, it was shown in a cell-transfer model of induced colitis that, when transferred in high numbers, self-reactive CD8 α ⁺TCR α β ⁺ natural IELs could prevent inflammation induced by non-self-reactive naive CD4⁺CD45RB^{hi} T cells⁸⁶.

Protective functions of CD8 $\alpha\beta$ ⁺TCR $\alpha\beta$ ⁺ induced IELs

The intestinal tissue is particularly enriched for antigen-experienced cytotoxic CD8 $\alpha\beta$ ⁺TCR $\alpha\beta$ ⁺ T cells¹¹⁵. Consistent with a protective role for these induced IELs, a recent study demonstrated that rhesus macaques inoculated with rhesus cytomegalovirus vectors containing simian immunodeficiency virus (SIV) epitopes were protected from progressive SIV infection following repeated low-dose intrarectal challenge¹¹⁶. The protected animals showed no evidence of systemic infection months later, suggesting that the infection was contained locally, preventing extensive viral replication or systemic spreading of the virus. Also, in mouse systems, experiments involving transfer of antigen-specific CD8 $\alpha\beta$ ⁺ induced IELs to infected hosts demonstrated a protective role for these cells against various infectious agents, including lymphocytic choriomeningitis virus (LCMV)¹³, rotavirus¹¹⁷, *T. gondii*¹⁰³ and *Giardia lamblia*¹¹⁸.

CD8 $\alpha\beta$ ⁺TCR $\alpha\beta$ ⁺ induced IEL effector memory cells are distinct from memory T cells in other tissues. For example, compared to splenocytes, CD8 $\alpha\beta$ ⁺TCR $\alpha\beta$ ⁺ induced IELs show limited homeostatic proliferation, but they are functionally more mature and show stronger and longer effector responses¹¹⁹. Furthermore, whereas a contraction phase is normally observed during T cell responses, induced IELs appear to maintain a long-term effector phase, and repeated challenges result in an enhanced and sustained cytotoxic effector phenotype¹²⁰. Also, in the case of *L. monocytogenes* infection, oral infections seem to induce larger numbers of *L. monocytogenes*-specific CD8 $\alpha\beta$ ⁺TCR $\alpha\beta$ ⁺ induced IELs than do intravenous infections^{120,121}. Moreover, *L. monocytogenes*-specific CD8 $\alpha\beta$ ⁺TCR $\alpha\beta$ ⁺ induced IELs display a distinct oligoclonal V β TCR repertoire, indicating that there is either selective migration or differential expansion and/or maintenance of primary and secondary CD8 $\alpha\beta$ ⁺TCR $\alpha\beta$ ⁺ effector IELs¹²². The co-stimulatory requirements for induced IELs are also distinct. CD40L triggering is required for the CD8 $\alpha\beta$ ⁺TCR $\alpha\beta$ ⁺ induced IEL response, but the absence of CD40–CD40L interactions has little or no effect on splenic memory T cells¹²⁰. These observations indicate that a second level of regulation dictated by intestine-specific cues controls the nature, magnitude, efficacy and specificity of these mucosal T cells. Furthermore, they imply that the unique functions of primary and memory CD8 $\alpha\beta$ ⁺ induced IELs is greatly influenced by multiple factors, including the route of infection, the origin and nature of the antigen and other poorly defined cues that are present in the gut environment itself.

Protective functions of CD4⁺TCR $\alpha\beta$ ⁺ induced IELs

Although most induced IELs are CD8 $\alpha\beta$ ⁺ cytotoxic T lymphocytes (CTLs), CD4⁺ T cells are also present within the epithelium, especially in the large intestine. The preferential loss of mucosal CD4⁺ T cells from both the lamina propria and epithelial compartments during SIV or HIV infections^{123–127} impairs the integrity of the mucosal barrier and leads to translocation of enteric bacteria and increased local and systemic infections¹²⁵. This highlights the importance of these T cells for protective immunity in the mucosa. Furthermore, recent data indicate that the HIV-mediated depletion of CD4⁺ T cells is initially preceded by an enhanced infiltration of systemic cytolytic effector CD4⁺ and CD8⁺ T cells that directly target the epithelium. These observations might indicate an important role for local IELs to not only protect against pathogen-induced damage of the epithelium but also prevent tissue destruction mediated by infiltrating peripheral effector T cells¹²⁷. Furthermore, the suppressive functions of induced CD4⁺ T_{reg} cells and the observation that most CD4⁺ T helper (T_H) cell subsets (including T_H1 and T_H17 cells) display strong plasticity and can produce IL-10 under certain conditions, especially during chronic stimulation^{128,129}, also support the notion that CD4⁺ IELs may contribute to regulatory mechanisms that prevent immunopathology in the intestine.

In the steady state, the gut environment is thought to favour anti-inflammatory immune responses, and migratory DCs, which produce TGF β and retinoic acid, promote the induction of forkhead box P3 (FOXP3)⁺ T_{reg} cells^{130–133}. Nevertheless, under inflammatory conditions, T_H-type induced IELs participate in protective immunity, especially in host defence against extracellular bacterial and fungal pathogens¹³⁴. Finally, recent data have highlighted the intriguing phenomena of plasticity of CD4⁺ effector and regulatory T cells^{135,136}, although protective and/or maintenance roles for such plastic CD4⁺ induced IELs remain to be investigated.

In this context, we recently generated data indicating that CD4⁺ effector T cells can upregulate CD8 α and adopt a cytotoxic yet regulatory induced IEL phenotype *in vivo* (D.M., F. van Wijk, S. Muroi, R. Shinnakasu, Y. Naoe, J.-W. Shui, Y. Huang, M. Docherty, A. Attinger, G. Kim, C. J. Lena, O. Turovskaya, F.L., S. Sakaguchi, K. Atarashi, C. Miyamoto, W. Ellmeier, K. Honda, M. Kronenberg, I. Taniuchi and H.C., unpublished observations). This lineage-differentiation mechanism represents a new form of immune regulation that diverts effector CD4⁺ T cells away from becoming pro-inflammatory T_H-type IELs. Therefore, we have defined a new subtype of CD4⁺ T cells that lose T_H-like functions but gain adaptive and innate-like effector and regulatory functions. This restraint of the T_H effector functions of CD4⁺ IELs but preservation of their protective and regulatory functions makes teleological sense at this mucosal interface, where effective protective immunity has to coincide with the least inflammatory damage. As the TCRs of these adapted cytotoxic CD4⁺ IELs remain MHC class II-restricted, they are capable of detecting infected intestinal epithelial cells that have upregulated expression of MHC class II molecules¹³⁷ and they may also provide immediate protection against viruses that have developed mechanisms to evade detection by the MHC class I-restricted CD8 $\alpha\beta$ ⁺TCR $\alpha\beta$ ⁺ induced IELs^{138,139}. Furthermore, under steady-state conditions, these CD4⁺ cytotoxic IELs may also contribute to tolerance by eliminating MHC class II-expressing migratory DCs, thereby preventing these DCs from priming T cell responses against innocuous antigens derived from the diet or commensal bacteria.

The dark side of IELs

As noted above, the differentiation, activation and functional specialization of all IEL subsets are regulated by interactions with other cell types and soluble factors, and are highly influenced by dietary and microbial products in the gut. The dynamic interactions between environmental cues and the mucosal adaptive immune system help maintain a stable equilibrium and sustain barrier function. Nevertheless, the heightened activation status of IELs and their close proximity to the intestinal epithelium suggest that these cells may contribute to immunopathological responses and initiate and/or exacerbate inflammatory diseases, such as inflammatory bowel disease (IBD) and coeliac disease, or promote cancer development and progression (FIG. 3; TABLE 1).

Pathological functions of natural IELs

Although TCR $\gamma\delta$ ⁺ natural IELs have important regulatory and protective roles, other evidence exposes a rather dark side of these IELs as well. Several reports indicate that there is a direct correlation between the numbers of TCR $\gamma\delta$ ⁺ cells in the intestinal mucosa and disease severity in patients with IBD^{140–142}. Also, direct evidence showing that $\gamma\delta$ ⁺ cells promote immunopathology in various mouse models of IBD suggests a pro-inflammatory role for $\gamma\delta$ ⁺ natural IELs^{105,143,144}. However, as some of these studies used TCR α -deficient mice or TCR β -deficient mice, both of which contain aberrant $\gamma\delta$ ⁺ T cell populations, it is possible that the pro-inflammatory nature of $\gamma\delta$ ⁺ natural IELs in those particular studies was an experimental artefact. In another model of spontaneous colitis induced by deletion of

phosphoinositide-dependent protein kinase 1 (PDK1) in T cells, $\gamma\delta^+$ IELs were shown to be responsible for the colitis induction, presumably through the secretion of IL-17 (REF. 145).

Unlike conventionally selected T cells, $CD8\alpha\alpha^+TCR\alpha\beta^+$ natural IELs tend to express self-reactive TCRs. Despite this, in the steady state, $CD8\alpha\alpha^+TCR\alpha\beta^+$ natural IELs do not promote autoimmune tissue damage but instead show self-antigen-driven regulatory functions⁸⁶. However, it remains possible, under inflammatory conditions or on recognition of foreign antigens with high similarity to self, that these auto-reactive T cells may drive autoimmune pathology. Furthermore, although normally these IELs show a higher threshold for TCR activation compared with conventional T cells¹⁴⁶, increased or uncontrolled inflammatory conditions, or excessive production of IL-15, may trigger their autoreactive cytotoxicity, thereby jeopardizing the integrity of the mucosal barrier.

Pathological functions of $CD8\alpha\beta^+$ induced IELs

Although recent data indicate important roles for cytotoxic $CD8\alpha\beta^+$ induced IELs in protecting against invading pathogens, these cells have also been implicated in the progression¹⁴⁷, or even initiation, of IBD. For example, in a transfer model of induced colitis, naive $CD8\alpha\beta^+$ T cells were shown to induce colitis in an IL-17-dependent fashion¹⁴⁸. Furthermore, in a hapten-induced model of colitis, previously sensitized hapten-specific $CD8^+$ effector CTLs were found to initiate the inflammatory response¹⁴⁹. Interestingly, in this study, the pathology induced by the $CD8^+$ CTLs did not induce a chronic inflammation and the colitic mice gradually recovered¹⁴⁹. Hapten-specific $CD8^+$ effector T cells were not all deleted and some differentiated into long-lived IELs, which caused relapse of disease on secondary challenge of recovered animals. Despite their destructive cytotoxic attacks on the intestinal epithelium, these IELs had differentiated post-thymically and were not self-reactive natural IELs¹⁴⁹.

Induced IELs have also been shown to exacerbate coeliac disease. In this setting, TCR-activated $CD8\alpha\beta^+TCR\alpha\beta^+$ induced IELs cause severe villous atrophy by targeting intestinal epithelial cells that express stress-induced MHC class I polypeptide-related sequence (MIC) antigens, in an NKG2D-dependent fashion^{150,151}. Furthermore, IL-15, a cytokine that is over-expressed by enterocytes from individuals with Crohn's disease, is known to trigger potent cytotoxic responses by $CD8\alpha\beta^+$ induced IELs through the NKG2D–DAP10 signalling pathway¹⁵⁰.

$CD4^+$ induced IELs and intestinal disease

Aberrant differentiation and/or functions of mucosal $CD4^+$ T_H cell subsets are major contributing factors to immunopathology at mucosal sites. Perhaps the most significant detrimental effect of $CD4^+$ induced IELs is their ability, in conjunction with $CD4^+$ T cells in the lamina propria, to promote the development of small intestinal inflammation in patients with coeliac disease (reviewed in REF. 152). Contrary to other common inflammatory bowel diseases, such as ulcerative colitis and Crohn's disease, which mostly affect the large intestine, coeliac disease primarily affects the small intestine. The disease develops in genetically susceptible individuals that express HLA-DQ2 and/or HLA-DQ8. These MHC class II molecules bind gluten peptides with strong affinity and, under inflammatory conditions, are greatly induced on enterocytes, which then become targets for gluten-specific $CD4^+$ effector T cells¹⁵².

The exact role of $CD4^+$ T cells in coeliac disease is still not clear, but the excessive production of IFN γ , which may enhance HLA-E expression by intestinal epithelial cells and promote bystander cytotoxic responses by $CD8\alpha\beta^+$ and/or $CD4^+$ $TCR\alpha\beta^+$ induced IELs through the innate CD94–NKG2D pathway, is likely to be a key mechanism involved in the

disease development¹⁵³. IFN γ and IL-21 double-producing CD4⁺ T cells¹⁵⁴, and more recently T_H17 cells¹⁵⁵, have been implicated in coeliac disease as well. The differentiation of pathogenic CD4⁺ T cells is thought to be fuelled by high levels of pro-inflammatory cytokines, including IL-15 and IFN- α , that are present in the intestinal mucosa from patients with coeliac disease¹⁵². IL-15 may also interfere with immune regulation by disrupting TGF β -mediated signalling through SMAD3 (REF. 156). In this context, a recent study reported that IL-15 may also synergize with retinoic acid to enhance inflammatory responses¹⁵⁷. This is in sharp contrast to the anti-inflammatory effects of retinoic acid in conjunction with TGF β , which in the absence of IL-15 promotes T_{reg} cell differentiation and suppresses inflammatory T_H17 cell differentiation¹³⁰.

Ulcerative colitis and Crohn's disease are also generally thought to be driven by aberrant CD4⁺ IEL and LPL responses, in this case directed against the intestinal microbiota (for extensive reviews on this topic, see REFS 158,159). Although both Crohn's disease and ulcerative colitis share some important end-stage pathways of tissue damage, they represent immunologically different diseases with distinct effector CD4⁺ T cell types involved. Crohn's disease is considered to be a classical T_H1-cell-mediated inflammatory disorder that is characterized by elevated levels of IFN γ and IL-12. However, the more recent findings that inflamed colons from both mouse models and patients with Crohn's disease show considerable T_H17 cell infiltrates, suggests a more complex disorder. In addition, IL-23, which promotes T_H17 cell responses, seems to be a major player in IBD pathogenesis^{160,161}, and genome-wide association studies in humans defined *IL-23R* as one of the major IBD-susceptibility genes¹⁶². By contrast, ulcerative colitis has been associated with T_H2 cell responses and is connected to elevated levels of IL-5 and IL-13 (REF. 163). Recent studies have also pointed to roles for thymic stromal lymphopoietin (TSLP) and the IL-17 family member IL-25 in the induction of CD4⁺ T cell-driven intestinal inflammation¹⁶⁴; this warrants further exploration in the context of ulcerative colitis. Further studies are also needed to distinguish the exact contribution made by IELs in the inflamed intestine from that made by infiltrating systemic and lamina propria T cells.

Overall, although all effector T cells, including the various IEL subsets, have the potential to initiate or propagate gut inflammation, aberrant or uncontrolled T_H-type induced IELs are almost always involved and play a big part in the destructive immune pathology that jeopardizes the barrier function of the mucosal epithelium.

Conclusions and perspective

IELs are engaged in a constant, complex, multidirectional dialogue with epithelial cells and other cells in the gut environment. Their uniqueness, in terms of their location, their development pathways, their diverse self and non-self antigen specificity and their innate and adaptive functional specialization, allows these extraordinary effector T cells to provide the first line of defence at the most vulnerable entry port for pathogens. At the same time, they also protect the integrity of the mucosal border by preventing uncontrolled immune cell infiltration and excessive or unnecessary immune responses mediated by the systemic conventional T cells (FIG. 3; TABLE 1). Although IELs have many beneficial roles geared toward maintaining a stable equilibrium and sustained barrier function, they also have a dark side, and the combination of potent effector cells that are in direct contact with the epithelial target cells provides the IELs with the opportunity to mediate pathological responses that drive and exacerbate detrimental inflammatory diseases (FIG. 3; TABLE 1).

Understanding the differentiation pathways and communication networks that programme, wire and control these immune cells may provide new insights for the design of new and

effective mucosal vaccines to prevent and combat infections and for the development of therapies to prevent and/or treat inflammatory diseases, food allergies and cancers.

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Glossary

Pathogens	Opportunistic organisms that cause acute or chronic disease following host infection. Derived from the Greek word 'pathos', which means 'suffering'
Intraepithelial lymphocytes	(IELs). These lymphocyte populations consist mostly of T cells and are found within the epithelial layer of mammalian mucosal linings, such as the gastrointestinal tract and reproductive tract. However, unlike conventional naive T cells, IELs are antigen-experienced T cells and, on encountering antigens, they immediately release cytokines or mediate killing of infected target cells
Thymus leukaemia antigen	(TLA). A non-polymorphic, non-classical MHC class I molecule (MHC class I-b family) with a restricted expression pattern. It is constitutively expressed on intestinal epithelial cells and can be induced on antigen-presenting cells. TLA is structurally incapable of binding or presenting peptide antigens and it does not engage with T cell receptors. However, the $\alpha 3$ extracellular domain of TLA interacts with CD8 α . TLA displays stronger affinity for CD8 $\alpha\alpha$ homodimers compared with CD8 $\alpha\beta$ heterodimers, and CD8 $\alpha\alpha$ expression can be detected with TLA-specific tetramers
Lamina propria	Connective tissue that underlies the epithelium of the mucosa and contains various myeloid and lymphoid cells, including macrophages, dendritic cells, T cells and B cells
Microbiota	The microorganisms present in normal, healthy individuals. These microorganisms live mostly in the digestive tract but are also found in some other tissues
Germ-free mice	Mice born and raised in sterile isolators. They are devoid of colonizing microorganisms, but after they have been experimentally colonized by known bacteria, they are said to be gnotobiotic
Gut-associated lymphoid tissues	Lymphoid structures and aggregates associated with the intestinal mucosa, specifically the tonsils, Peyer's patches, lymphoid follicles, appendix and caecal patch. Enriched in lymphocytes and specialized dendritic cell and macrophage subsets
Peyer's patches	Groups of lymphoid nodules present in the small intestine (usually the ileum). They occur in the intestinal wall, opposite the line of attachment of the mesentery. They consist of a dome area, B cell follicles and interfollicular T cell areas. High endothelial venules are present mainly in the interfollicular areas

Mesenteric lymph nodes	Lymph nodes, located at the base of the mesentery, that collect lymph (including cells and antigens) draining from the intestinal mucosa
Microfold cells	(M cells). Specialized antigen-sampling cells that are located in the follicle-associated epithelium of the organized mucosa-associated lymphoid tissues. M cells deliver antigens by transepithelial vesicular transport from the aero-digestive lumen directly to subepithelial lymphoid tissues of nasopharynx-associated lymphoid tissue and Peyer's patches
NKG2D	(Natural killer group 2, member D). A lectin-type activating receptor that is encoded by the NK complex and is expressed at the surface of NK cells, NKT cells, natural and induced intraepithelial lymphocytes and conventional T cell receptor- $\gamma\delta$ (TCR $\gamma\delta$) T cells, as well as some conventional cytolytic CD8 $\alpha\beta$ ⁺ TCR $\alpha\beta$ ⁺ T cells. The ligands for NKG2D are MHC class I polypeptide-related sequence A (MICA) and MICB in humans, and retinoic acid early transcript 1 (RAE1) and H60 in mice. Such ligands are generally expressed at the surface of infected, stressed or transformed cells
Inflammatory bowel disease	A chronic condition of the intestine that is characterized by severe inflammation and mucosal tissue destruction. The most common forms in humans are ulcerative colitis and Crohn's disease
Coeliac disease	Coeliac disease is a condition that damages the lining of the small intestine and interferes with nutrient absorption. The damage is due to an aberrant immune response to gluten-derived antigens, which are found in wheat, barley, rye and possibly oats
Crohn's disease	A form of chronic inflammatory bowel disease that can affect the entire gastrointestinal tract but is most common in the colon and terminal ileum. It is characterized by transmural inflammation, strictures and granuloma formation, and it is thought to result from an abnormal T cell-mediated immune response to commensal bacteria
Ulcerative colitis	A chronic disease that is characterized by inflammation of the mucosa and sub-mucosa tissues, mainly of the large intestine

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Figure 1. Thymic and peripheral differentiation of natural and induced IELs

In the thymus, immature $CD4^+CD8\alpha\beta^+CD8\alpha\alpha^+$ (triple positive) thymocytes undergo agonist ('alternative') selection and differentiate into double-negative T cell receptor- $\alpha\beta$ ($TCR\alpha\beta$) $^+$ cells that are the precursors of natural $TCR\alpha\beta^+$ intraepithelial lymphocytes (IELs). The $TCR\alpha\beta^+$ natural IEL precursor cells partly acquire their antigen-experienced phenotype 'naturally' during selection with self antigens. In addition, the precursor cells for $TCR\alpha\beta^+$ and $TCR\gamma\delta^+$ natural IELs upregulate intestinal homing receptors during their maturation in the thymus, which allows these cells to directly seed the intestinal epithelium after they leave the thymus. $CD4^+CD8\alpha\beta^+$ (double positive) thymocytes undergo 'conventional' thymic selection and differentiate into naive $CD4^+$ and $CD8\alpha\beta^+$ $TCR\alpha\beta^+$ T cells that migrate to the periphery. These naive T cells can differentiate into effector T cells in response to peripheral antigens and subsequently migrate to the gut and become incorporated into the induced IEL compartment. APC, antigen-presenting cell; MLNs, mesenteric lymph nodes.

Figure 2. Kinetics of the accumulation of natural and induced IELs

Natural intraepithelial lymphocytes (IELs) gain their antigen-experienced phenotype and gut-homing capacity in the thymus during their initial agonist-based selection in response to self antigens. They populate the gut compartment early, before environmental antigens have been encountered. Conventional naive T cells differentiate into effector cells in response to peripherally encountered antigens and gradually accumulate over time as antigen-experienced induced IELs in the gut.

Figure 3. The 'light' and 'dark' sides of intestinal IELs

a | The 'light side' shows the beneficial roles of natural and induced intraepithelial lymphocytes (IELs) in preserving the epithelium and restoring tissue integrity after injury. **b** | The 'dark side' depicts the pathogenic roles of natural and induced IELs. IELs can exert uncontrolled and/or excessive cytotoxicity and promote inflammatory responses, which may initiate and/or exacerbate inflammatory bowel diseases and coeliac disease.

Table 1

Characteristics of the different intestinal T cells subsets in mice

Cell type	Characteristics		Frequency of TCR $\gamma\delta$ and TCR $\alpha\beta$ IELs	Kinetics of accumulation	Development and TCR repertoire	Potential beneficial functions	Potential pathogenic functions
Small intestinal epithelium							
Natural IELs	Self and non-self	CD2 ⁻ CD5 ⁻ CD28 ⁻ ; CTLA4 ⁻ THY1 ^{-low} ; B220 ^{+/} - CD69 ^{hi} ; NK receptors (++++)	TCR $\gamma\delta$ ⁺ (+++); - CD4 ⁻ CD8 ⁻ (++) - CD8 $\alpha\alpha$ ⁺ (++) + TCR $\alpha\beta$ ⁺ (++) +; - CD4 ⁻ CD8 ⁻ (+) - CD8 $\alpha\alpha$ ⁺ (++) +)	Present at birth; decrease with age	'Alternative' positive selection for CD8 $\alpha\alpha$ ⁺ TCR α β ⁺ IELs in the thymus; oligoclonal TCR repertoire; unknown MHC restriction	Anti-inflammatory responses; antimicrobial responses; tolerance to intestinal antigens; immune regulation; homeostasis of the epithelium	Promote epithelial damage during intestinal inflammation
Induced IELs	Non-self	CD2 ⁺ CD5 ⁺ CD28 ^{+/} ; CTLA4 ^{+/} - THY1 ^{hi} ; B220 ⁻ CD69 ^{hi} ; NK receptors (++++)	TCR $\alpha\beta$ ⁺ (++) +; - CD8 $\alpha\beta$ ⁺ (+) - CD8 $\alpha\beta$ ⁺ CD8 $\alpha\alpha$ ⁺ (++) +) - CD4 ⁺ (+) - CD4 ⁺ CD8 $\alpha\alpha$ ⁺ (++)	Absent at birth; increase with age	'Conventional' positive selection in the thymus; oligoclonal TCR repertoire; restricted on MHC class I or MHC class II	Oral tolerance; protective antimicrobial responses	Excessive inflammation and cytotoxicity in response to luminal antigens; exacerbate coeliac disease
Small intestinal lamina propria							
T cells	Non-self	Not applicable	TCR $\alpha\beta$ (+++); - CD8 $\alpha\beta$ ⁺ (+) + - CD8 $\alpha\beta$ ⁺ CD8 $\alpha\alpha$ ⁺ (+) - CD4 ⁺ (++) - CD4 ⁺ CD8 $\alpha\alpha$ ⁺ (+)	Absent at birth; increase with age	'Conventional' positive selection in the thymus; polyclonal TCR repertoire; restricted on MHC class I or MHC class II	Oral tolerance; protective antimicrobial responses	Ileitis; inflammation
Large intestinal epithelium							
Natural IELs	Self and non-self	CD2 ⁻ CD5 ⁻ CD28 ⁻ ; CTLA4 ⁻ THY1 ^{-low} ; B220 ^{+/} - CD69 ^{hi} ;	TCR $\gamma\delta$ ⁺ (+); - CD4 ⁻ CD8 ⁻ (++) +)	Present at birth; decrease with age	'Alternative' positive selection for CD8 $\alpha\alpha$ ⁺ TCR α β ⁺	Anti-inflammatory responses; antimicrobial responses; tolerance to intestinal antigens	Promote epithelial damage during intestinal inflammation

Cell type	Characteristics			
	Antigen dependency	IEL phenotype	Frequency of TCR $\gamma\delta$ and TCR $\alpha\beta$ IELs	Development and TCR repertoire
			Kinetics of accumulation	Potential beneficial functions
				Potential pathogenic functions
Induced IELs	Non-self	CD2 ⁺ CD5 ⁺ CD28 ^{hi} ; CTLA4 ⁺ THY1 ^{hi} ; B220 ⁻ CD69 ^{hi} ; NK receptors (++)	NK receptors (+++) - CD8 $\alpha\alpha^+$ (+) TCR $\alpha\beta$ (++) - CD4 ⁻ CD8 ⁻ (++) - CD8 $\alpha\alpha^+$ (+)	q β^+ IELs in the thymus; TCR repertoire and MHC restriction unknown 'Conventional' positive selection in the thymus; TCR repertoire not defined; restricted on MHC class I or MHC class II
<i>Large intestinal lamina propria</i>				
T cells	Non-self	Not applicable	TCR $\alpha\beta^+$ (++) +; - CD8 $\alpha\beta^+$ (++) - CD8 $\alpha\beta^+$ CD8 $\alpha\alpha^+$ (+) - CD4 ⁺ (++) - CD4 ⁺ CD8 $\alpha\alpha^+$ (+)	'Conventional' positive selection in the thymus; polyclonal TCR repertoire; restricted on MHC class I or MHC class II
				Drive excessive inflammation and cytotoxicity in response to microbiota-derived antigens; exacerbate IBD

Plus symbols represent frequency of expression, from low (+) to high (+++++). CTLA4, cytotoxic T lymphocyte A4; IBD, inflammatory bowel disease;

IEL, intraepithelial lymphocyte; NK, natural killer; TCR, T cell receptor.