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RESEARCH ARTICLE

Intraepithelial lymphocytes in celiac disease immunopathology

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Abstract Celiac disease is a T cell-mediated immune disorder induced by dietary gluten that is characterized by the development of an inflammatory anti-gluten CD4 T cell response, anti-gluten antibodies, and autoantibodies against tissue transglutaminase 2 and the activation of intraepithelial lymphocytes (IELs) leading to the destruction of the intestinal epithelium. Intraepithelial lymphocytes represent a heterogeneous population of T cells composed mainly of cytotoxic CD8 T cells residing within the epithelial layer, whose main role is to maintain the integrity of the epithelium by eliminating infected cells and promoting epithelial repair. Dysregulated activation of IELs is a hallmark of CD and is critically involved in epithelial cell destruction and the subsequent development of villous atrophy. In this review, we compare and contrast the phenotype and function of human and mouse small intestinal IELs under physiological conditions. Furthermore, we discuss how conditions of epithelial distress associated with overexpression of IL-15 and non-classical MHC class I molecules induce cytotoxic IELs to become licensed killer cells that upregulate activating NKG2D and CD94/NKG2C natural killer receptors, acquiring lymphokine killer activity. Pathways leading to dysregulated IEL activation could eventually be targeted to prevent villous atrophy and treat patients who respond poorly to gluten-free diet.

Keywords Celiac disease · Intraepithelial lymphocytes · NKG2D · CD94/NKG2C · TCRγδ T cells · IL-15

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Introduction

Intraepithelial lymphocytes (IELs) represent an abundant and heterogeneous population of T cells that reside within the intestinal epithelium. They are composed of antigen-experienced memory-effector T cell subtypes bearing the αβ or the γδ T cell receptor (TCRαβ$^+$ or TCRγδ$^+$). The gut is constantly submitted to a high antigenic load and therefore has developed numerous strategies to maintain tolerance to food and microbial antigens while controlling insults from pathogens and deleterious tissue inflammation. In fitting with their role as sentries, IELs have features of adaptive and innate immune cells that endow them with the ability to survey tissues based not only on recognition of specific antigens but also stress signals. However, they also contribute to inflammatory and tissue destructive reactions such as those seen in celiac disease (CD). Indeed, in conditions of epithelial stress and inflammation, IELs can be activated by innate signals released by tissue cells, acquiring a natural killer (NK)-like phenotype and cytotoxic effector functions. In the context of CD, this dysregulated activation

has deleterious consequences for the intestinal tissue, ultimately leading to villous atrophy. Here, we will review the composition of IELs and their role within the small intestinal compartment in both steady-state and pathological conditions, and we will discuss the impact of environmental signals in fine-tuning their phenotype and functional properties in the stressed and inflamed mucosa of CD patients.

Small intestinal intraepithelial lymphocytes in homeostasis conditions

IELs represent a large population of antigen-experienced "innate-like" T cells that reside between enterocytes in the intestinal epithelium. They are strategically located within the intestinal mucosa where they can interact with enterocytes to maintain epithelium integrity and prevent pathogenic incursion. In contrast to IELs of the colon, which are present in smaller numbers and are mainly TCR$\alpha\beta^+$CD4$^+$ T cells, small intestinal IELs are abundant and comprised mostly (>70%) of TCR$\alpha\beta^+$ or TCR$\gamma\delta^+$ CD8$^+$ T cells [1, 2]. Importantly, IELs have an effector phenotype as demonstrated by their ability to be activated through their TCR in absence of costimulation [3–5]. This is in sharp contrast to peripheral blood T cells, which are essentially comprised of naïve and memory T cells. In humans, much of what are thought to be unique functional features of IELs can actually be attributed to their effector phenotype and are not the consequence of IELs being a separate T cell lineage.

Mouse and human small bowel IELs share a number of features but also differ in their composition, phenotype, and function [6] (Table 1). Murine and human small intestines contain similar amounts of IELs, with an average of ten IELs per 100 intraepithelial cells (IECs) in mice [1, 7] and 10–20 IELs per 100 IECs in humans [8]. The chemokine receptor CCR9 is expressed on the surface of IELs, and its ligand CCL25 (thymus-expressed chemokine) is produced by IECs, playing a critical role in the homing of IELs to the intestinal epithelium [9, 10]. In addition, IELs express the integrin $\alpha E\beta 7$ (CD103), which interacts with E-cadherin expressed on enterocytes, allowing for cell–cell interactions with epithelial cells [11–14]. In addition, it has been proposed that CD103 might have signaling properties and could enhance IEL activation [12].

Studies in both human and murine intestines have demonstrated that the repertoires of IELs are polyclonal in the newborn individuals and become oligoclonal in adults (reviewed in [15]). Luminal antigens, and particularly the microbial composition of the gut lumen, influence the population size and the repertoire of IELs [15]. Indeed, analysis of the repertoire in human infants demonstrated that seeding of T cells to the intestine is not influenced by any specific antigenic stimulation, but, in that found in adults, there is a diffuse distribution of oligoclonally expanded IELs [15]. The influence of environmental antigens in skewing the repertoire of IELs was described in rodent studies comparing specific-pathogen-free (SPF) and germ-free (GF) adult animals. Overall, these studies showed that both the expansion and oligoclonal repertoire of $\alpha\beta$ TCR IELs are driven by microbial colonization [15]. As in mice, IELs in humans are highly oligoclonal [16, 17] and show evidence of being driven by antigens [18]. In addition, other external environmental factors such as dietary vitamins and phytochemicals can likely influence the development and expansion of IELs. For instance, vitamin D and its receptor have been shown to be important for the development of TCR$\alpha\beta^+$CD8$\alpha\alpha^+$ T cells in mice [19]. The aryl hydrocarbon receptor (AhR), whose ligands are metabolized from the vegetable-derived phytochemical indole-3-carbinol, also plays a crucial regulator role in maintaining the intestinal IEL population [20]. Small intestine TCR$\alpha\beta^+$CD8$\alpha\alpha^+$ and TCR$\gamma\delta^+$ IELs express high levels of AhR, and its activation directly affects their upkeep. Indeed, AhR deficiency results in the selective disappearance of TCR$\gamma\delta^+$ T cells and TCR$\alpha\beta^+$ CD8$\alpha\alpha^+$ IELs and, in the absence of AhR ligands, the majority of intestinal IELs are lost [20]. How these findings apply to human IELs remains to be defined.

Both mouse and human small intestines house a considerable number of $\gamma\delta$ T cells. However, in humans, the proportion of $\gamma\delta$ TCR T cells is smaller (about 10–14%) [2, 21] compared with mice where it is around 60% [22]. In addition, intestinal $\gamma\delta$ T cells use preferential variable segments of γ and δ genes. In mice, Vγ1 or Vγ7 are commonly found [23, 24] while, in humans, Vδ1 or, to a lesser extent, Vδ2 and Vδ3 are present at the highest frequencies [25–27].

Furthermore, murine IELs contain a large fraction of TCR$\alpha\beta^+$CD8$\alpha\alpha^+$ IELs [22, 28, 29] that have not been formally identified in humans. In addition, the nonclassical major histocompatibility complex (MHC) class I molecule thymic leukemia, which is abundantly expressed by IECs in mice and is recognized by CD8$\alpha\alpha$-expressing T cells [30], has not been documented in humans. Overall, innate and adaptive immune functions are mediated by different IEL T cell subsets in mice, whereas in humans adaptive and innate immune properties are combined within the same IEL subset. Shared and divergent phenotypic and functional properties between mouse and human IELs are further discussed below.

Mouse IELs subsets

IELs are extremely heterogeneous, and they first can be distinguished based on expression of either an $\alpha\beta$ or $\gamma\delta$ TCR. Furthermore in mice, IELs have been categorized into two main subsets based on the cognate antigen they recognize and the nature of the CD8 co-receptor expressed (α or β

Table 1 Major mouse and human intraepithelial lymphocytes subsets

Denomination		Subsets	Frequency	T cell receptor repertoire	NKR expression
MOUSE	Type a Conventional	$TCR\alpha\beta^+CD4^+$	<15%	MHC class II restricted & oligoclonal	-
		$TCR\alpha\beta^+CD8\alpha\beta^+$ $TCR\alpha\beta^+CD8\alpha\beta^+CD8\alpha\alpha^+$	20-30%	MHC class I restricted & oligoclonal	-
	Type b Unconventional	$TCR\alpha\beta^+CD8\alpha\alpha^+$ $TCR\alpha\beta^+CD8^-CD4^-$	20-50%	Non-classical MHC class I restricted & oligoclonal	+ (ITAM+)
		$TCR\gamma\delta^+CD4^-CD8^-$ $TCR\gamma\delta^+CD8\alpha\alpha^+$	40-70%	Non -classical MHC class I? Other?	+ (ITAM+)
HUMAN		$TCR\alpha\beta^+CD8\alpha\beta^+$	70-80%	MHC class I restricted & oligoclonal	+ (<5% ITAM+)
		$TCR\alpha\beta^+CD4^+$	10-15%	MHC class II restricted & oligoclonal	-
		$TCR\gamma\delta^+$ $CD4^-$ $CD8^-$ $TCR\gamma\delta^+$ $CD8^+$	5-20%	Non-classical MHC class I restricted CD1, MIC?, other ? & oligoclonal	-
		$CD3^-$ $CD103^+$ (<10%)	<10%		+ (ITAM+)

chains) [22, 28]: The type-a IELs also referred to as the 'conventional' CD8αβ αβ TCR subset or alternatively as 'induced' IELs, and the type-b CD8αα αβ TCR subset also known as the 'unconventional' or 'natural' IEL subset (reviewed in [29, 31]). IELs expressing γδ TCRs are included in the latter group.

Type-a IELs encompass conventional αβ TCR MHC class II-restricted $CD4^+$ T cells and MHC class I-restricted $CD8\alpha\beta^+$ T cells. The large majority of type-a IELs are $TCR\alpha\beta^+$ $CD8\alpha\beta^+$ T cells that, like their systemic $CD8^+$ counterparts, are absent from mice lacking MHC class I [32, 33]. While the TCR repertoire of type-a IELs is normally oligoclonal [34], under GF conditions, it becomes polyclonal [15], suggesting that IELs recognize intestinal commensal antigens under physiological conditions. $TCR\alpha\beta^+CD8\alpha\beta^+$ T cells display cytolytic effector functions upon antigenic challenge, killing via granzymes or by engagement of Fas [31], thus providing immediate cytotoxic responses to local infection. In addition, they also secrete T helper 1 (T_H1) cytokines [31].

Type-b IELs consist of either $TCR\alpha\beta^+$ T cells or $TCR\gamma\delta^+$ T cells, which are double-negative for CD4 and CD8 (DN) or express a homomeric CD8αα molecule. $TCR\alpha\beta^+CD8\alpha\alpha^+$ T cells can be found in mice lacking classical MHC class I molecules and in MHC class I/CD1-double-deficient mice [33], suggesting that they are not restricted by CD1 but rather by non-classical MHC class I molecules [32, 33, 35]. Furthermore, unconventional type-b IELs display an unusual T cell repertoire and antigen specificity. The αβ TCR is oligoclonal [15] and may contain numerous self-reactive TCRs [36]. Intriguingly, $TCR\alpha\beta^+CD8\alpha\alpha^+$ type-b T cells also have an oligoclonal TCR repertoire that is driven by the presence of microbiota ([34] and personal unpublished data). This indicates that they recognize microbial antigens and/or self-antigens induced in presence of commensal bacteria. Additionally, type-b IELs do not express some of the typical T cell markers expressed by type-a IELs, including CD2, CD5, CD28, lymphocyte-function-associated antigen 1, and THY1 [31]. Like type-a IELs, type-b IELs are cytolytic, expressing high levels of effector molecules that include granzyme and Fas ligand [31]. They also have proinflammatory properties, particularly through the ability to secrete interferon (IFN)-γ and tumor necrosis factor (TNF)-α [37].

Strikingly, the expression of NK receptors (NKRs) is restricted to the $TCR\alpha\beta^+CD8\alpha\alpha^+$ and $TCR\gamma\delta^+$ T cell subset in mice [4], suggesting that only type-b IELs possess innate properties under physiological conditions. This is in sharp contrast to human IELs, where conventional $TCR\alpha\beta^+CD8\alpha\beta^+$ T cells express NKRs. As in humans, the vast majority

of NKRs found on mouse IELs belong to the C-type lectin family; these include Ly49, CD94/NKG2, NKG2D, and 2B4 (CD244). Even though NKRs in mice and humans are comparable to an extent, there are some striking differences. Human IELs do not have Ly49, but instead express receptors of the killer-cell immunoglobulin-like receptors family [38]. Furthermore, important differences between the two species reside in the nature of the ligands for these receptors. While human NKG2D binds the self MHC-class-I-polypeptide-related sequence A (MICA) and MICB protein family as well as the UL16-binding protein (ULBP; also known as RAET1) family, mouse NKG2D ligands include five retinoic acid early transcript (RAE1) proteins, the major histocompatibility protein H60 and MULT1 (murine UL16-binding-protein-like transcript 1) [39]. Murine CD94/NKG2A recognizes the non-classical class I molecule Qa-1 that is not a histocompatibility antigen alpha chain E (HLA-E) ortholog [40]. Although cytolytic effector responses might be differently regulated, the parallels between species demonstrate the importance of this system for immunoregulation and immune defense.

Human IELs subsets

In humans, the IEL population of the normal small intestine consists of approximately 75% TCR$\alpha\beta^+$CD8$\alpha\beta^+$ T cells, 10% TCR$\alpha\beta^+$CD4$^+$, and 15% TCR$\gamma\delta^+$ T cells, which are either DN or CD8$^+$ and <10% (Table 1) [21]. In addition, under physiological conditions, one can find in the intestinal epithelium <10% of NKR-expressing CD3$^-$ cells that remain to be characterized (Table 1). Unlike TCR$\alpha\beta^+$CD8$\alpha\alpha^+$ IELs that can only be found in mice, CD8$\alpha\alpha$ $\gamma\delta$ TCR IELs have also been reported in humans [2, 21]. Most TCR$\alpha\beta^+$CD8$\alpha\beta^+$ cells are thought to be restricted by classical MHC class I molecules, except for one IEL subset recognizing non-classical CD1 molecules [41]. As in mice, the TCR repertoire of human IELs is highly oligoclonal [17, 42] and shows evidence for antigen drive [18], reflecting the chronic antigenic stimulation to which IELs are constantly exposed.

Most human conventional TCR$\alpha\beta^+$CD8$\alpha\beta^+$ express inhibitory and co-activating NKRs [5, 43, 44], which is in contrast to murine IELs where NKR expression is limited to the TCR$\alpha\beta^+$CD8$\alpha\alpha^+$CD8$\alpha\beta^-$ and TCR$\gamma\delta^+$ T cell subsets [4]. TCR$\alpha\beta^+$CD8$\alpha\beta^+$ IELs express three main co-activating C-type lectin NKRs: NKG2D, the CD94/NKG2 complex, and NKR-P1A. NKG2D recognizes MICA, MICB, and ULBP proteins [45–48]. NKG2D is constitutively expressed by TCR$\alpha\beta^+$CD8$\alpha\beta^+$ and TCR$\gamma\delta^+$ T cells, albeit at low levels in control IELs compared with peripheral T cells, probably due to the presence of transforming growth factor (TGF)-β in the gut environment [5]. This is in contrast to the murine NKG2D, which is induced following T cell receptor (TCR) stimulation [49]. NKG2D associates selectively with the adaptor molecule DAP10 in humans [50], which has a PI3-kinase binding motif but lacks an immunoreceptor tyrosine-based activation motif (ITAM). Another difference between humans and mice is in the nature of adaptor proteins that associate with and transduce signals from NKG2D. In mice, in addition to the long NKG2D isoform that associates solely with DAP10, a short NKG2D splice variant capable of associating with both DAP10 and DAP12 proteins exists [51], thus endowing NKG2D with the ability to promote cytokine secretion and proliferation. The complex CD94/NKG2 binds the non-classical class Ib molecule HLA-E [52, 53] and exerts inhibitory or activating functions depending on the nature of the NKG2 molecule associated with CD94 [54]. Less than 30% of normal IELs express co-activating CD94 receptors [18, 44]. However, the nature of the activating NKG2 molecule associated with CD94 remains to be determined. CD94/NKG2A represents the main inhibitory NKR expressed on IELs [18, 44]. NKG2A/B contains an immunoreceptor tyrosine-based inhibitory motif in the cytoplasmic domain that can recruit the SH2 domain-containing phosphatase (SHP)-1 or SHP-2 upon receptor engagement, delivering a negative signal that inhibits NK effector function and blocks TCR activation [48]. The fact that HLA-E, the ligand for CD94/NKG2A, is not constitutively expressed on enterocytes suggests that CD94/NKG2A exerts a regulatory role under inflammatory conditions, preventing immunopathology secondary to chronic activation. Conversely, NKG2C lacks a signaling motif but requires the presence of the adaptor molecule DAP12 [48]; DAP12 contains an ITAM motif in its cytoplasmic domain, and therefore confers the CD94/NKG2 heterodimer activating properties [55, 56]. Nevertheless, NKG2C is barely detectable in IELs under physiological conditions [18, 56]. NKR-P1A (CD161) recognizes the lectin-like transcript 1 (LLT1) [48, 57, 58]. In contrast to NK cells, NKR-P1A selectively exerts costimulatory functions in T cells [57], and we have evidence that this function is preserved in IELs and that its ligand LLT1 is expressed on IELs at very low levels under physiological conditions (personal unpublished data).

Altogether, human IELs selectively express C-type lectin NKRs. Furthermore and most importantly, IELs lack expression of activating NKRs that associate with adaptor molecules bearing an ITAM motif, and they do not express DAP12. Interestingly, this regulation operates at the transcriptional as well as translational level [56]. Thus, NKRs in IELs selectively play a co-stimulatory role by modulating TCR-mediated-effector functions but are incapable of inducing transcriptional programs resulting in proliferation and cytokine secretion on their own [48].

Functions of IELs

The main role of IELs is to promote immune protection by preventing the entry and spread of pathogens while avoiding unwanted and excessive inflammatory reactions capable of

damaging the intestinal epithelium (Fig. 1). To that end, IELs exert both cytolytic functions to eliminate infected and damaged cells, and regulatory functions that contribute to epithelium healing and repair.

Studies in mice have demonstrated that TCR$\alpha\beta^+$ CD8$\alpha\beta^+$ IELs function as inflammatory effector T cells against primary infection with *Eimeria vermiformis*, as well as other pathogens including *Encephalitozoon cuniculi* and *Toxoplasma gondii* [59–63]. They are involved in parasite killing through strong cytolytic activity and the production of IFN-γ and TNF-α. TCR$\gamma\delta^+$ T cells are also cytotoxic [64] and are required to achieve maximal intestinal protection against various enteric pathogens. During *T. gondii* and *Salmonella* infections, they restrict the epithelial transmigration of pathogens by maintaining the integrity of intercellular tight junctions, thus preventing leakage between enterocytes [65]. They can secrete cytokines such as interleukin (IL)-13 that help to control intestinal nematodes such as *Nippostrongylus brasiliensis* [59]. Finally, it has been proposed that TCR$\alpha\beta^+$CD4$^+$ IELs preserve epithelial integrity during mucosal infection with Simian Immunodeficiency Virus or Human Immunodeficiency Virus [66, 67]. The role played by NKRs in these settings has not been delineated in mice. Under physiological conditions, human IELs, despite being in an effector state, are only weakly cytotoxic and express inhibitory NKRs and low levels of activating NKRs [5]. Furthermore, IECs do not express ligands for NKRs [56, 68, 69]. However, under conditions of inflammation and infection, NKRs are likely involved in the preservation of epithelial integrity by modulating TCR activation positively and negatively, allowing for the elimination of infected cells while preventing chronic inflammation (reviewed in [48]). The role played by TCR$\gamma\delta^+$ T cells in the elimination of stressed/infected IECs in humans remains elusive and may involve cytolytic NK-like functions as those described for TCR$\alpha\beta^+$CD8$\alpha\beta^+$ IELs [70].

An anti-inflammatory role has been consistently attributed to murine TCR$\gamma\delta^+$ IELs, which preserve the integrity of the mucosal barrier by dampening inflammation that can occur after acute injury and promoting the healing of epithelial cells [65, 71–73]. They also regulate the generation and differentiation of IECs through the production of keratinocyte growth

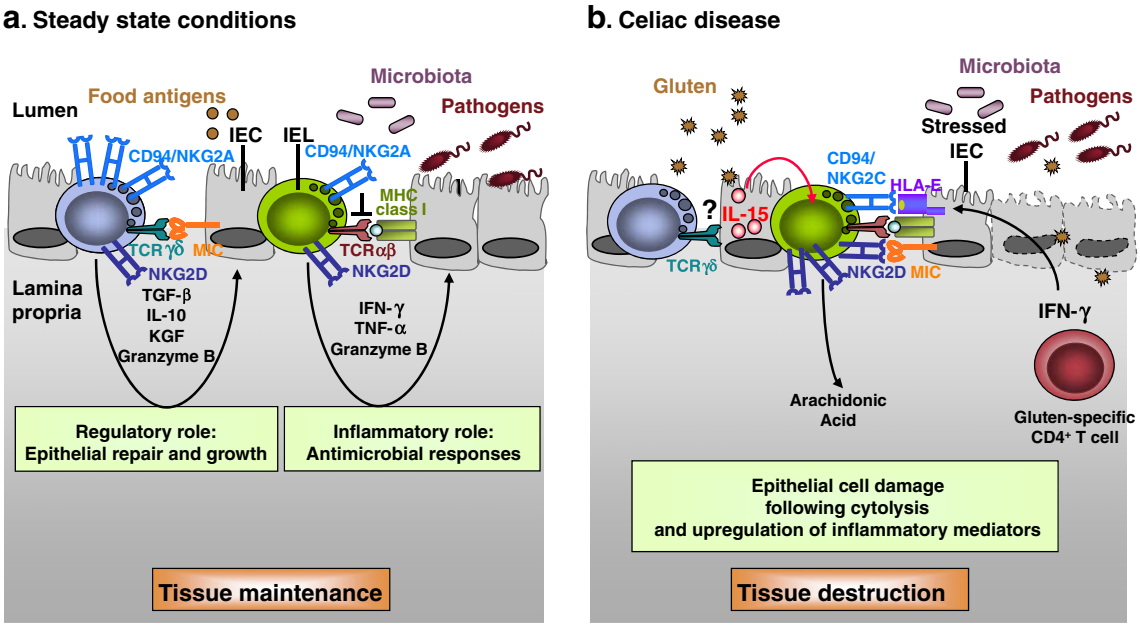

Fig. 1 Protective and damaging roles of intraepithelial lymphocytes. **a** Under steady-state conditions, TCR$\gamma\delta^+$ intraepithelial lymphocytes (IELs) respond to stress signals produced by intestinal epithelial cells (IECs) and produce anti-inflammatory cytokines (IL-10, TGF-β) and keratinocyte growth factor (KGF) to participate in IECs growth, homeostasis, and wound repair. TCR$\alpha\beta^+$ and to a lesser extent TCR$\gamma\delta^+$ IELs also play a key role in immune protection to prevent pathogens from breaching the intestinal barrier. They combat infectious agents by lysing infected cells through cytotoxic mechanisms involving the release of granzyme B and perforin, and by producing inflammatory cytokines such as IFN-γ and TNF-α. Nevertheless, in a healthy tissue, normal IELs have low cytolytic capacities. They express the inhibitory natural killer receptor (NKR) CD94/NKG2A, low levels of the activating NKR NKG2D, and low levels of perforin. **b** In the context of celiac disease, distressed IECs express high levels of interleukin-15 (IL-15), together with MHC class I polypeptide-related molecules (MICA and MICB) and HLA-E, the ligands for NKG2D and CD94/NKG2C, respectively. IL-15 leads to the upregulation of NKG2D expression in TCR$\alpha\beta^+$ intraepithelial cytotoxic T cells (IE-CTLs) allowing them to kill IECs expressing NKG2D ligands in a TCR-independent manner. Furthermore, IE-CTLs express the CD94/NKG2C NKR that recognizes HLA-E on IECs and can promote proliferation and secretion of inflammatory cytokines in addition to cytolysis. IL-15 also acts as a costimulatory molecule for the NKG2D cytolytic pathway, leading to the release of arachidonic acid, which in turn promotes activation and recruitment of granulocytes and the generation of additional intestinal inflammation. Whether TCR$\gamma\delta^+$ IELs actively participate in celiac disease pathogenesis is still unknown

factor (KGF) [74–76]. In addition, γδ IELs have the ability to produce anti-inflammatory cytokines in vivo [77] and participate in the maintenance of oral tolerance in an IL-10-dependent manner [78, 79]. Importantly, they also play an immune regulatory role in the infected intestinal mucosa. Indeed, following *E. vermiformis* infection, they sustain epithelial integrity through the release of KGF, expression of junctional molecules, and production of the anti-inflammatory cytokines IL-10 and TGF-β [60, 72]. Whether γδ TCR IELs also play roles in epithelial healing and blocking inflammation in humans remains to be fully determined. In accordance with an immune regulatory role, however, a subset of TCRγδ$^+$ NKG2A$^+$ IELs was shown to inhibit cytolysis via the production of TGF-β [80]. In addition, it has been suggested that TCRαβ$^+$CD8αα$^+$ T cells could play an immunoregulatory role by repressing the induction of unnecessary immune responses [81]. Nonetheless, TCRαβ$^+$CD8αα$^+$ T cells have not been identified in humans, and their origin and precise functions in the mouse intestine is still unclear.

Overall, to protect the intestinal barrier, TCRαβ$^+$ IELs are involved in the killing of infected IECs while TCRγδ$^+$ may be responsible for the resolution of inflammation that takes place in the gut during and after infection, and additionally for the growth, homeostasis, and repair of IECs. The chronic activation of IECs by locally produced inflammatory signals is a critical event in overcoming the protective functions of IELs to shift the balance towards sustained and potentially excessive IEL-mediated cytolysis as seen in chronic intestinal inflammatory conditions such as CD. The expression of inhibitory CD94/NKG2A receptors that recognize inflammation-induced HLA-E/Qa-1 on IECs may play an important role in preventing chronic activation of IELs in humans and mice.

Intraepithelial lymphocytes in celiac disease

CD is an inflammatory disorder with autoimmune features that occurs in genetically susceptible individuals expressing HLA-DQ2 or HLA-DQ8 molecules. CD patients develop inflammatory T cell and antibody responses against dietary gluten, a protein present in wheat, rye, and barley. In addition, CD patients develop autoantibodies directed against the enzyme tissue transglutaminase. The typical histopathological picture of CD is a small intestine enteropathy characterized by crypt hyperplasia, a massive increase in IELs, and villous atrophy as a consequence of surface IEC destruction.

Intraepithelial lymphocytes subsets in different stages of CD

Along with villous blunting, an increase in IELs is one of the main features of CD. This epithelial infiltration is often the only phenotypic difference in early stage untreated disease. Additionally, it correlates with gluten ingestion, and therefore, it is considered to be the most sensitive histopathological marker of CD. However, the exact threshold that can be used to consider a biopsy "normal" is still debated. The most recent study suggests that a cut-off value of 20–25 IELs per 100 enterocytes can be used to define intraepithelial lymphocytosis [82]. Previously, a count of 25–29 IELs per 100 IECs was considered to be "borderline intraepithelial lymphocytosis" with values above 30 being "pathologic intraepithelial lymphocytosis" [83]. The distribution of IELs along the villi, particularly at the villus tip, also represents a sensitive morphological feature for the diagnosis of CD [84]. In addition, lymphocytic infiltration of the small intestinal epithelium in the absence of villous atrophy has been observed in patients with dermatitis herpetiformis [85], an autoimmune skin manifestation of CD, supporting the concept that intraepithelial lymphocytosis represents a marker of CD even in the absence of intestinal damage. Nevertheless, intraepithelial lymphocytosis is not a CD exclusive feature and therefore cannot be considered a CD-specific diagnostic tool. Intraepithelial lymphocytosis is observed in several other clinical conditions, such as autoimmune disorders, food intolerances, gastroenteritis, duodenitis, and autoimmune enteropathy [86]. Therefore, it has to be evaluated in a broader clinical context while taking into account serum levels of CD-specific autoantibodies, presence of HLA-DQ CD-predisposing alleles and clinical responsiveness to a gluten-free diet (GFD) [87].

In untreated celiac patients, intestinal epithelial infiltrates are mainly composed of TCRαβ$^+$ IELs, even though a higher relative density of TCRγδ$^+$ IELs is also observed [27, 88], representing around 20% (ranging between 11% and 53%) of the whole IEL population in CD patients compared with 2% (ranging between 0% and 39%) in controls [27]. In contrast, NK and NKT cells do not expand in untreated celiac patients [89]. As expected, expansion of both TCRγδ$^+$ and TCRαβ$^+$ IELs subsets is observed upon gluten-challenge in organ-cultures [90] and in vivo after rectal gluten challenge [91]. Of note, the increase in the density of intraepithelial TCRγδ$^+$ T cells in active CD patients is more pronounced in children than in adults [92]. Interestingly, gluten withdrawal has a different impact on TCRαβ$^+$CD8αβ$^+$ and TCRγδ$^+$ T IELs. Although both populations decrease [93], only the density of TCRαβ$^+$ CD8αβ$^+$ IELs returns to basal levels [89, 93, 94]. Overall, a significant increase in TCRγδ$^+$ IELs can be found in the intestinal epithelium of both treated and untreated CD patients compared with non-celiac subjects [27], and in some cases, there is a slightly larger proportion of TCRγδ$^+$ IELs in active than in treated CD patients [95]. No correlation between TCRγδ$^+$ IEL density and GFD duration has yet been observed [95], though a reduction of the γδ

population was observed in a cohort of CD patients followed prospectively over a 2-year period after starting a GFD [96]. Therefore, the impact of GFD on TCRγδ$^+$ IELs remains unclear. Furthermore, unlike what has been reported for TCRαβ$^+$ T cells, there is no correlation between the density of TCRγδ$^+$ IELs and the degree of intestinal tissue damage [89, 94]. Accordingly, an increase in the density of TCRγδ$^+$ IELs has been described at all stages of CD, including in latent celiac patients [97] and healthy first-degree family members carrying the predisposing HLA haplotype [98]. It has been proposed that the intraepithelial infiltration of TCRγδ$^+$ T cells could be used as a diagnostic marker to identify early stage CD [93], or to predict the risk of CD development among at risk subjects with positive CD-specific autoantibodies and normal intestinal architecture [99].

Altogether, these observations indicate that TCRαβ$^+$ but not TCRγδ$^+$ IELs can be linked to villous atrophy. We lack information on whether TCRγδ$^+$ IELs subsets are the same in normal individuals and at different stages of CD and understand very little about what drives TCRγδ$^+$ IELs expansion in CD.

Intraepithelial TCRγδ$^+$ lymphocytes in celiac disease

The increase of TCRγδ$^+$ IELs in active CD patients, their persistence in patients on a GFD, and the lack of elevation in patients with other small bowel enteropathies [88] suggest a specific relationship between CD pathogenesis and TCRγδ$^+$ IELs. Studies in monozygotic twins with CD that have a distinct, non-overlapping TCR δ repertoire in the intestine suggest that TCRγδ repertoire is not influenced by genetic factors in CD [100]. Although the exact implication of TCRγδ$^+$ IELs in CD pathogenesis remains elusive, CD represents a unique model to study their function in the intestine and to address their potential role in maintaining epithelium integrity and contributing to immune surveillance against foreign antigens.

TCR repertoire of intraepithelial TCRγδ$^+$ lymphocytes

In contrast to αβ T cells, the γδ TCR repertoire does not seem to depend on classical MHC class I and class II molecules, and γδ T cells do not recognize peptides presented in their context [101, 102]. A reduction in the clonality of intestinal TCR δ repertoire has been shown during ontogenesis [103], suggesting that the expansion of a selected number of γδ T cell clones during intestinal development determines the γδ TCR oligoclonality observed in the gut. Even though the range of ligands recognized by the human γδ TCR is still unclear, thus far, ligands identified for Vδ1$^+$ T cells include CD1c molecules [104] and the non-classical MHC class I molecule MIC [105, 106]. The ability of the microbiota to influence the repertoire of TCRγδ$^+$ T cells is still unclear. Although studies comparing GF and SPF mice have not found any appreciable impact of the microbiota on the repertoire of γδ T cells [107, 108], the microbiota seems to influence their cytolytic activity [64, 108]. Further studies are needed to assess the role of commensal bacteria on shaping the repertoire and function of TCRγδ$^+$ T cell in the gut mucosal immune system.

In contrast to peripheral blood T cells, most γδ T cells in the human intestinal epithelium use the TCR Vδ1 gene segment [26, 27]. Vδ1-bearing γδ T cells have a highly restricted repertoire that remains stable over time [25]. Accordingly, both freshly isolated and cultured intestinal intraepithelial TCRγδ$^+$ T cells from CD patients are predominantly Vδ1$^+$ (Vγ9$^-$/Vδ1$^+$/Vδ2$^-$) [70]. Besides Vδ1$^+$, Vδ2$^+$ T cells are also found in the small intestinal epithelium of controls and CD patients [70, 95]. The only reported change in TCR usage between controls and active CD patients is a relative increase in Vδ2$^+$ and a decrease in Vγ9$^+$ T cells [95]. Interestingly, on a GFD, Vδ2$^+$ IELs return to a normal mean frequency, whereas Vγ9$^+$ IELs remain, albeit at low percentage [95]. Furthermore, there seems to be a relative increase in Vδ1$^+$ IELs in CD patients undergoing a GFD [95]. Further analysis of the Vγ-gene usage in TCRγδ$^+$ T cells revealed that a large proportion of IELs express the Vγ8 chain together with Vδ1 (two out of three clones generated from the intestinal epithelium of CD patients were Vδ1$^+$Vγ8$^+$) or in fewer cases with Vδ3 [109]. Intriguingly, the γδ T cell subset expressing Vγ8 together with Vδ1 was also highly prevalent in the synovial tissue of patients with rheumatoid arthritis [109], suggesting that this particular γδ T cell subset could play a role in tissue inflammation in the context of autoimmune disorders. Finally, it is interesting to note that there is an overlap between the TCR δ repertoire in the small intestine and the colon of CD patients [100] and an increase of TCRγδ$^+$ IELs in the distal intestine [110] and rectal mucosa of subjects developing a severe complication of CD called refractory sprue (RCD) [111]. These observations suggest a possible alteration of the γδ T cell compartment all along the small and large intestine in CD.

Whether celiac patient TCRγδ$^+$ IEL subsets are antigen-driven and the exact nature of the antigen recognized have yet to be elucidated. Since IL-15 drives the expansion of intraepithelial TCRγδ$^+$ IELs, it is possible that the density of TCRγδ$^+$ IELs is a surrogate marker for IL-15 expression in the epithelium. In line with this hypothesis, over-expression of IL-15 drives the expansion of TCRγδ$^+$ IELs in intestinal organ cultures treated with IL-15 [112] and in transgenic mouse models where IL-15 is overexpressed in IECs [113]. Whether IL-15 overexpression in the epithelium is also associated with an increase of γδ T cells in the context of other intestinal disorders remains to be determined.

Phenotype of intraepithelial TCRγδ$^+$ lymphocytes

Cytofluorometry analysis of TCRγδ$^+$ T cell clones generated from CD patients IELs showed that the γδ T cell population is heterogeneous, as T cell clones could express CD8αα, CD8αβ, or CD4 coreceptors [70]. Interestingly and in contrast to TCRγδ$^+$Vδ1$^+$ peripheral T cells [114], intraepithelial TCRγδ$^+$Vδ1$^+$ T cells isolated from CD patient biopsies expressed the CD45RO memory T cell marker [70], suggesting that they were activated in vivo. A functional analysis highlighted the cytotoxic properties of intraepithelial TCRγδ$^+$ T cell clones derived from CD patients, with Vγ9$^+$/Vδ2$^+$ T cell clones showing higher lytic activity compared with those expressing Vγ9$^-$/Vδ1$^+$/Vδ2$^-$ [70]. Furthermore, TCRγδ$^+$ IELs isolated from CD patients express NKG2D and CD94 NKRs [44]. These observations suggest that TCRγδ$^+$ IELs may be involved in tissue destruction. Alternatively, it was suggested that TCRγδ$^+$ NKG2A$^+$ IELs could play a protective role in CD due to their ability to secrete anti-inflammatory cytokines that could contribute to the resolution of the intestinal inflammation [80].

Clearly, the exact role played by TCRγδ$^+$ IELs in the epithelium of CD patients is still up for debate. As discussed above, their persistence in patients on a GFD would suggest that they do not play a pathogenic role; however, functional comparison of GFD and active CD patients TCRγδ$^+$ T cells is lacking, and whether a correlation exists between the persistence of TCRγδ$^+$ IELs and responsiveness to GFD has not been evaluated.

Intraepithelial cytotoxic TCRαβ$^+$ CD8αβ$^+$ lymphocytes (IE-CTLs) and NKRs in the pathogenesis of celiac disease

Despite the fact that expansion of cytotoxic IELs (IE-CTLs) correlates with villous atrophy, their role in CD pathogenesis has been questioned due to genetic and functional observations. Although CD8$^+$ gluten-specific T cells were identified in the lamina propria [115], no gluten-specific CD8$^+$ T cells could be detected in the gut epithelium. Moreover, there was no genetic association found between MHC class I genes and CD [116]. The idea that IE-CTLs that did not recognize gluten peptides could kill IECs based on stress signals in CD was put forward when it was discovered that IELs expressing the activating NKR NKG2D could exert cytolytic activity independently from TCR specificity based on recognition of stress-induced molecules [5, 117]. Subsequent studies analyzing IELs and IECs of CD patients provided direct evidence for this hypothesis [56, 68, 69]. The general concept that has emerged beyond CD is that inflammatory and stress signals provided by the tissue environment dictate the activation status of IE-CTLs and license them to become effective killer cells [48, 118]. In the context of CD, the expression of stress molecules on the surface of IECs, the over-expression of the pro-inflammatory cytokine IL-15 in the intestinal mucosa, the expression of activating NKRs, and the loss of the inhibitory CD94/NKG2A receptor promote the sustained activation of cytotoxic IELs (IE-CTLs) that lead to the destruction of IECs and villous atrophy [118] (Fig. 1). In accordance with a role for IE-CTLs in CD pathogenesis, genome-wide association studies (GWAS) have identified a large number of genes implicated in CD, including various genes potentially involved in the development, migration, activation, and cytotoxic/NK functions of IELs [119] (Fig. 2).

Epithelial cells in active celiac disease

Initially, CD is triggered by gluten-derived peptides which have numerous and diverse effects on intestinal cells. Among them is the upregulation of the stress-inducible non-classical MHC class I MIC molecules on IECs [68] and the upregulation of the pro-inflammatory cytokine IL-15 by lamina propria mononuclear cells [120]. Furthermore, gluten peptides could increase cell surface expression of the non-classical MHC class I molecule HLA-E [121]. Altogether, these studies suggest that gluten-derived peptides possess innate immune-stimulating properties, leading to the expression of non-classical MHC class I molecules recognized by NKRs and the induction of IL-15. Despite the suggestions of numerous studies, definitive proof of the innate-stimulating properties of gluten has yet to be obtained because these results have not been reproduced and no specific molecular mechanism has been defined [118]. It has been conclusively established that IECs in the inflamed intestinal mucosa of CD patients express high levels of MIC molecules and HLA-E, which are the main ligands for NKG2D and CD94/NKG2C, respectively [56, 68, 69]. One major inducer of HLA-E is IFN-γ [122], which is highly secreted by CD IE-CTLs [123] and inflammatory lamina propria CD4$^+$ T cells in the presence of IL-15 [124].

NK receptors expression on intraepithelial lymphocytes

Several sequential events are required to induce IE-CTL-mediated killing of IECs. The expression and activity of inhibitory and activating NKRs are greatly modulated by antigen exposure and the interplay between cytokines locally produced in the intestine. The frequency of IE-CTLs expressing the inhibitory CD94/NKG2A NKR is significantly decreased in CD patients, including in patients on a gluten-free diet [56]. The mechanism underlying the major decrease in IE-CTLs expressing CD94/NKG2A remains to be identified and cannot be explained by upregulation of IL-15 alone [18]. Conversely, while NKG2C expression at a protein level is rarely found in normal IELs [18, 56], it is

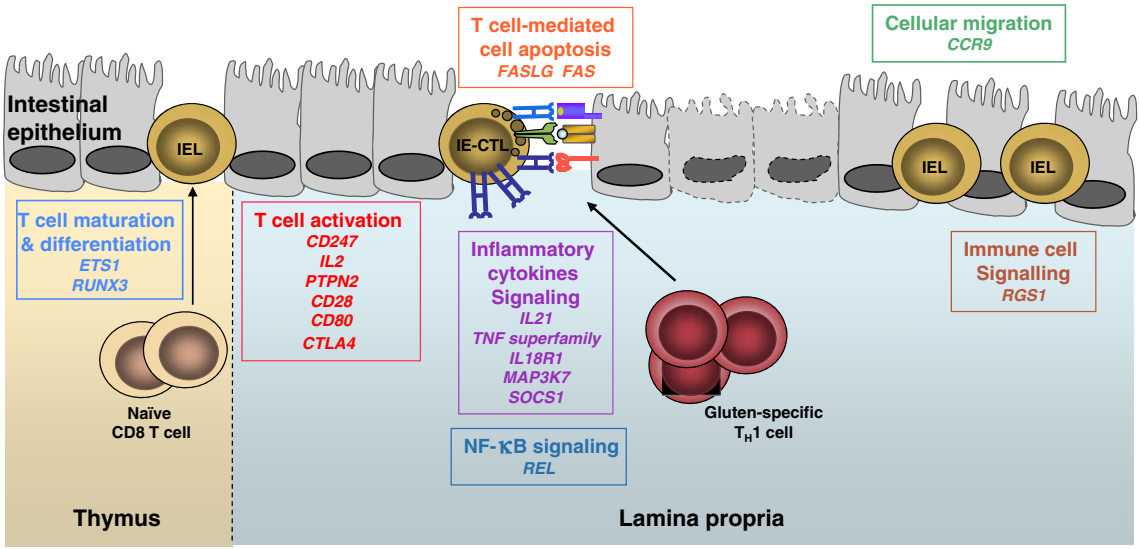

Fig. 2 Celiac disease-associated genes involved in intraepithelial lymphocytes development, migration, activation, and functions. CD susceptibility genes identified by GWAS and their potential implications in IEL biology are listed. Notably, *RUNX3* and *ETS1* are involved in the regulation of CD8 T cell differentiation in the thymus. Several other genes play a role in T cell activation and costimulation (e.g., *CD28, CD80, CTLA4, CD247, PTPN2, IL2*). *REL* is a gene that participates in NFκB signaling. *SOCS1*, *MAP3K7*, *IL18R1*, and members of the *TNF* superfamily are genes involved in inflammatory cytokine signaling. Other genes regulate the activation and migration of cytotoxic IELs (*IL-21, CCR9, RGS1).* Finally, *FAS* and *FASLG* are directly involved in cytotoxic T cell-mediated apoptosis

induced in IE-CTLs of patients with CD, even when gluten has been withdrawn [56]. In addition, another co-activating CD94/NKG2 receptor (non-NKG2A, non-NKG2E, non-NKG2H) is highly upregulated in CD patients [44]. Finally, NKG2D expression is significantly increased in IE-CTLs of active CD patients and returns to subnormal levels in patients on a GFD [5, 69]. The functional implications of an increase in activating NKRs and a concurrent loss of inhibitory NKRs are significant. NKG2D [5, 68, 69] and co-activating CD94/NKG2 receptors [18] lower the TCR activation threshold, thus rendering IE-CTLs capable of potentially recognizing low-affinity self-antigens expressed by IECs or microbial antigens present in the lumen [117, 118]. Furthermore, in the presence of IL-15, NKG2D acquires the ability to mediate TCR-independent cytolysis [5, 69] (see below), endowing IE-CTLs with the ability to kill IECs expressing the stress-induced MIC molecules. Finally, in addition to mediating TCR-independent cytolysis, NK reprogramming of CD IE-CTLs leads to the expression of activating NKRs such as CD94/NKG2C associated with ITAM-bearing adaptor molecules allowing for TCR-independent proliferation and cytokine secretion [56, 118]. Thus, CD IE-CTLs have acquired the capacity to kill IECs in a non-cognate antigen non-specific manner, based on the recognition of stress signals. This may also explain why several months of GFD are required for normalization of the intestinal mucosa. NK-reprogramming of IE-CTLs may also be the basis for poor responses to GFD and, ultimately, in some cases, may lead to the development of RCD.

Impact of IL-15 and IL-21 in licensing IELs in active celiac disease

IL-15 is expressed at low levels in IECs in the small bowel, but its expression can be upregulated under conditions of inflammation and epithelial distress, including those typical of CD [44, 125]. IL-15 stimulation induces CD94 expression in IELs [44]. To do so, IL-15 increases CD94 transcription and its expression on the cell surface [44]. However, it does not affect the expression of NKG2A, NKG2C, or DAP12 [44, 56]. Moreover, IL-15 induces the expression of NKG2D [5, 69], by increasing NKG2D and DAP10 transcription [69] and by opposing the effects of TGF-β via activation of c-Jun N-terminal kinase (JNK) and subsequent phosphorylation of c-jun [126, 127].

In addition to upregulating NKRs, IL-15 also lowers the overall activation threshold of IELs [3, 5, 68] and endows IE-CTLs with lymphokine killer activity [5, 69]. The ability of IL-15 to act as a costimulatory molecule for antigen receptors has been studied in particular in the context of the NKG2D cytolytic pathway [69, 128]. The NKG2D cytolytic signaling pathway involves the NKG2D–DAP10 complex binding to distinct adaptor proteins, including phosphatidylinositol 3 kinase (PI3K) and growth factor receptor-bound protein 2/nucleotide exchange factor Vav-1 [129]. NKG2D-initiated PI3K activation promotes the mitogen-activated protein (MAP) kinases, extracellular signal-regulated kinase (ERK), and MAP kinase kinase (MEK) phosphorylation, which are required for cytotoxicity. The upstream signaling molecule Vav-1 is also required for optimal calcium release and

cytotoxicity [129]. In addition to activating ERK, Vav-1 has been shown to promote JNK phosphorylation [128]. Both PI3K–ERK and Vav–JNK signaling pathways are involved in NKG2D-mediated cytotoxicity by regulating $cPLA_2$ activation, which in turn critically regulates NKG2D-mediated degranulation and cytolysis. IL-15 upregulates ERK and JNK phosphorylation and synergizes with NKG2D to induce $cPLA_2$ phosphorylation and the release of arachidonic acid, a precursor of the pro-inflammatory compounds called leukotrienes [128] (Fig. 3). Activation of IE-CTLs by NKG2D and IL-15 could therefore not only contribute to the destruction of distressed IECs but also promote the production of inflammatory mediators that could participate in the exacerbation of non-specific inflammation in the intestinal mucosa of CD patients. The involvement of IL-15 in multiple steps of the NKG2D cytolytic pathway, together with its confirmed roles in abrogating oral tolerance to dietary gluten [124] and interfering with the suppressive activity of intestinal regulatory T cells [126, 130], makes this cytokine a key player involved in the dysregulation of IELs activity in CD.

IL-21 is another locally produced cytokine that shares some functional properties with IL-15 (Fig. 4). Similarly to IL-15, IL-21 renders effector $CD4^+$ T cells resistant to the suppressive effects of regulatory T cells [131] and instead induces the production of IFN-γ [132]. Although in vitro studies have demonstrated that IL-21 by itself has very little effect, if any, on the proliferation of $CD8^+$ T cells, IL-21 synergizes with IL-15 to promote $CD8^+$ T cell activation and expansion, production of IFN-γ, and upregulation of granzyme B and perforin [133]. Moreover, IL-21 was shown to directly impact the function of human IELs by upregulating their perforin-mediated cytotoxic activity and serine-esterase release [134]. Although additional studies are required to confirm the effects of IL-21 on IELs and thus its involvement in CD pathogenesis, the ability of IL-21 to increase CTL activity, to upregulate the expression of perforin and granzyme B, and to influence NKG2D and CD94/NKG2A expression in many systems [134] support the hypothesis that IL-21 could contribute to the induction of inflammation and tissue damage in CD in addition to or perhaps even in place of IL-15. Accordingly, potential CD patients who have normal mucosa despite the presence of an adaptive anti-gluten immune response lack IL-21 upregulation in the intestinal mucosa [135].

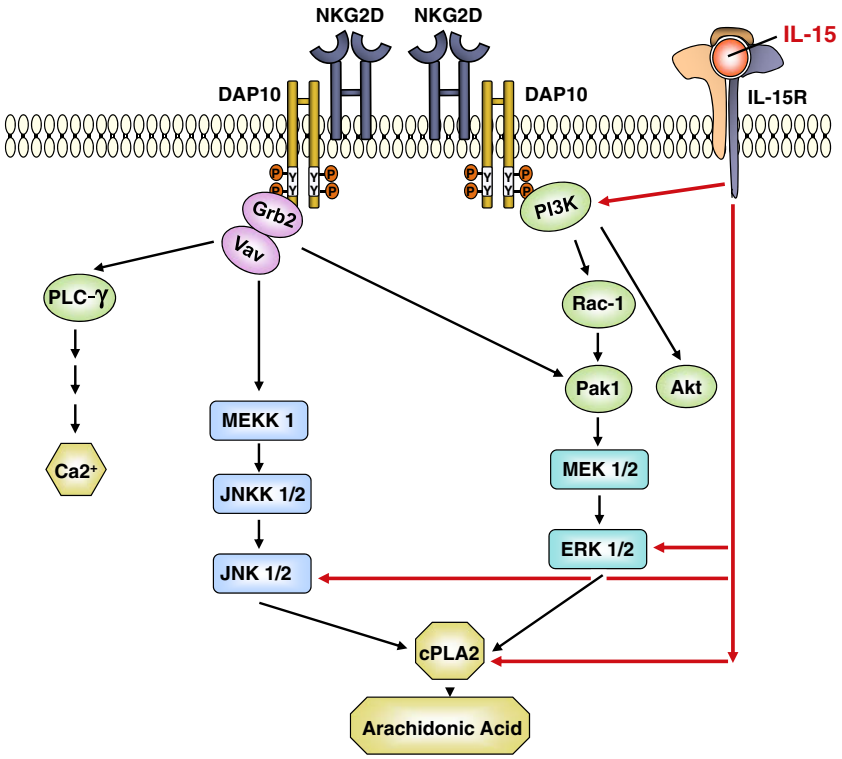

Fig. 3 Impact of IL-15 on the NKG2D cytolytic pathway in cytotoxic intraepithelial lymphocytes. The NKG2D receptor complex is a hexameric structure consisting of two NKG2D molecules, each coupled to a DAP10 homodimer. Upon ligand binding, DAP10 is phosphorylated by a Src-family kinase. The phosphorylated NKG2D–DAP10 complex binds multiple adaptor proteins, in particular, a subunit of phosphatidylinositol 3 kinase (PI3K) and the growth factor receptor-bound protein 2 (Grb-2). Both are implicated in the activation of $cPLA_2$. PI3K activation initiates the PI3K → Rac1 → Pak1 → MEK1/2 → ERK1/2 → $cPLA_2$ pathway, while Grb2 directly binds Vav1 and initiates the Vav1 → MEKK1 → JNKK1/2 → JNK1/2 → $cPLA_2$ pathway. In addition, binding of the Grb2-Vav1 intermediate to DAP10 is both necessary and sufficient for PLC-γ tyrosine phosphorylation. IL-15 synergizes with NKG2D to induce PI3K, ERK, JNK, and $cPLA_2$ activation, which in turn leads to the production and secretion of arachidonic acid

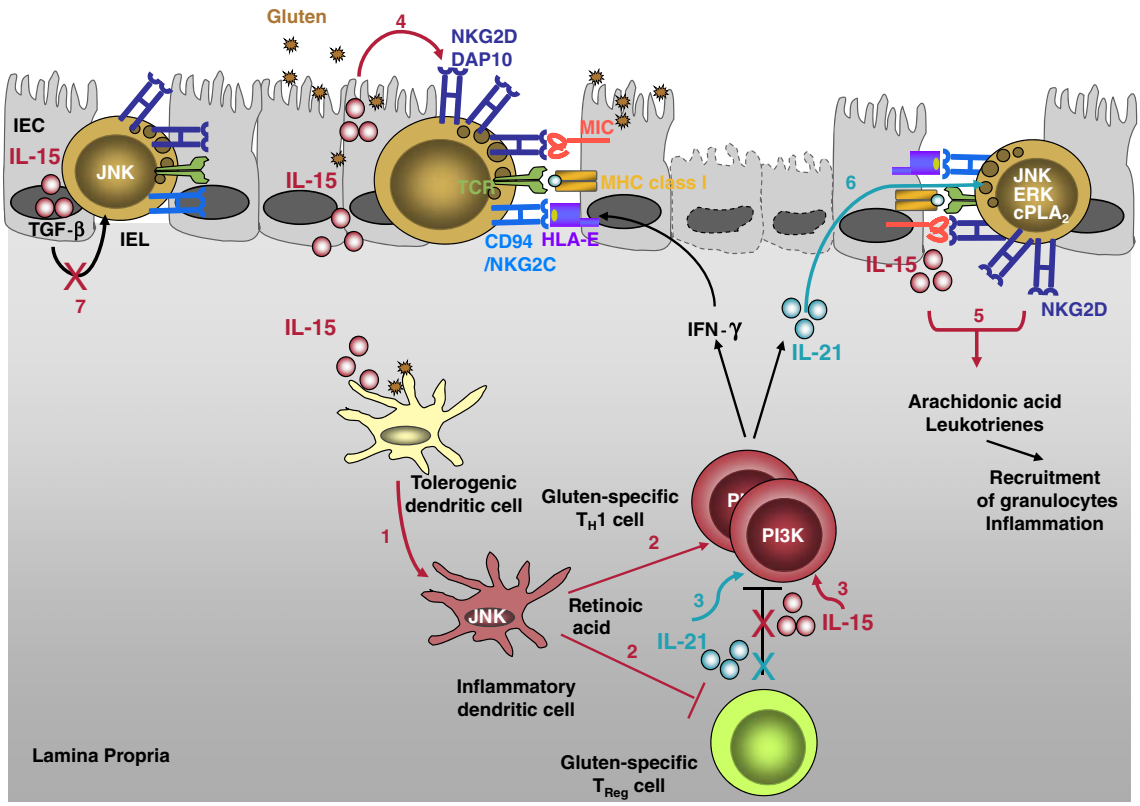

Fig. 4 Role of IL-15 and IL-21 in celiac disease pathogenesis. IL-15 and IL-21 act on various cell types and multiple immunological pathways, leading to destructive immune responses. *1* In the lamina propria, dendritic cells acquire inflammatory properties in the presence of IL-15. *2* Through the synergistic action of IL-15 and retinoic acid, differentiation of regulatory T cells is inhibited and inflammatory T helper 1 (T_H1) cells are induced. *3* IL-15 and IL-21 render effector $CD4^+$ T cells resistant to the suppressive functions of regulatory T cells through a mechanism involving phosphatidylinositol 3 kinase (PI3K). *4* IL-15 induces the expression of NKG2D by increasing NKG2D and DAP10 transcription in intraepithelial lymphocytes (IELs) and costimulates the cytolytic signaling pathway associated with this receptor. *5* Activation of the NKG2D cytolytic pathway in conjunction with IL-15 leads to the release of arachidonic acid and possibly leukotrienes by IELs, which in turn can promote the recruitment and activation of granulocytes and inflammation. *6* IL-21 promotes expression of perforin and granzyme B. *7* IL-15 renders IELs insensitive to the anti-inflammatory effects of TGF-β

In some extreme situations, sustained expression of high levels of IL-15 in the epithelium [125, 136] leads to the expansion of a subset of $CD3^-$ IE-CTLs that have undergone profound genetic reprogramming of their NK functions, ultimately acquiring an aberrant and highly activated NK cell-like phenotype [137]. This severe complication of CD called RCD is characterized by a persistent villous atrophy despite a GFD [137, 138]. Such a situation is reflected in mouse models where high upregulation of IL-15 is associated with expansion of activated NK-like IE-CTLs and villous atrophy [139, 140]. Whether dysregulated expression of IL-21 can lead to a similar phenotype remains to be determined.

Is there a role for the TCR in intraepithelial lymphocyte-mediated pathology?

The role of the TCR in IEL-mediated CD pathogenesis should be analyzed considering its role in (1) the activation status of IELs, (2) the type of NKRs expressed on IELs, and (3) the specificity that drives IEL activation and IEC killing. The TCR plays a critical role in promoting an effector phenotype in IELs, which is required for IL-15 to upregulate NKRs and endow IE-CTLs with lymphokine killer activity [44, 69, 128]. In addition, TCR specificity determines whether IE-CTLs express inhibitory or activating CD94/NKG2 receptors [18]. However, this does not signify that the TCR specificity of IE-CTLs is directly involved in driving destruction of IECs. As discussed above, activating NKRs that recognize stress- or inflammation-induced molecules kill IECs without direct involvement of the TCR. Furthermore, IL-15 can lower the activation threshold of the TCR in IELs, allowing them to recognize very-low-affinity antigens that did not originally drive their activation and expansion as naïve T cells.

In summary, although the direct contribution of the TCR in IECs killing may seem minimal compared with NKRs, its participation in the acquisition of activating NKRs and a suitable activation status seems essential. Further analysis of the characteristics of the TCR repertoire of IELs in the intestinal epithelium of CD patients and controls will help

us decipher whether IE-CTLs kill IECs based on recognition of self or microbial (commensal bacteria) antigens and therefore whether there is a role for TCR specificity in IE-CTL-mediated pathology.

Conclusions and opened issues

TCR$\alpha\beta^+$CD8$\alpha\beta^+$ IELs represent the effector T cell subset that mediates IEC destruction, ultimately leading to villous atrophy in CD. The basis for IEL-mediated epithelial destruction is the recognition of stress ligands expressed by IECs. Whether this killing is mediated mainly by NKRs or whether activating NKRs lower the activation threshold of the TCR allowing IELs to recognize low-affinity self-antigens expressed by IECs or intestinal commensal bacteria remains unanswered. The role of TCR$\gamma\delta^+$ IELs in CD pathogenesis also remains unclear. We lack studies to definitively conclude whether they play no decisive role (and, by extension, that their expansion is due solely to upregulated IL-15 expression in the epithelium), have a protective role, or contribute to the pathogenesis of CD. In order for IELs to be licensed to kill epithelial cells, they require (1) changes in the epithelium resulting in the upregulation of IL-15 and non-classical MHC class I molecules that are recognized by activating NKRs and (2) expansion and activation of HLA-DQ2 or HLA-DQ8-restricted anti-gluten CD4$^+$ T cells that produce IL-21, IFN-γ, and other yet-undefined factors. How the cross-talk between adaptive anti-gluten immunity, epithelial cells, and IELs in CD is orchestrated is an outstanding question that will need to be addressed to better delineate the different stages of CD and gluten-sensitivity, design preventive strategies, and develop new therapeutic approaches. Moreover, the relationship between normal IELs and RCD IELs remains poorly understood. It is important to distinguish between RCD type I (RCD I) IELs that express a TCR on the surface and RCD type II (RCD II) IELs that lack surface TCR expression despite having rearranged TCR chains. We would like to suggest that RCD I is at the end of the spectrum of CD where IELs have undergone NK-reprogramming and hence have become independent from anti-gluten CD4 T cells. Whether RCD II IELs are derived from IE-CTLs and represent the final step of NK reprogramming or whether they derive from a subset of CD3$^-$ IELs that are present at low frequency (<10%) in normal individuals (Table 1) is still unclear. It is also intriguing that GWAS studies have been unable to identify epithelial-specific genes associated with CD, in contrast to other inflammatory disorders like asthma [141]. Understanding the basis for alterations of epithelial cells in CD is a critical piece of the puzzle that is still missing from our understanding of CD and RCD pathogenesis. Finally, characterizing the signaling and transcriptional pathways in the diseased mucosa of CD patients that lead to dysregulated activation of IELs will allow for the development of new therapies that prevent tissue damage and malignant transformation of IELs in CD.

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