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Policing the intestinal epithelial barrier: Innate immune functions of intraepithelial lymphocytes

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Abstract

Purpose of review—This review will explore the contribution of IELs to mucosal innate immunity and highlight the similarities in IEL functional responses to bacteria, viruses and protozoan parasite invasion.

Recent findings—IELs rapidly respond to microbial invasion by activating host defense responses, including the production of mucus and antimicrobial peptides to prevent microbes from reaching the epithelial surface. During active infection, IELs promote epithelial cytolysis, cytokine and chemokine production to limit pathogen invasion, replication and dissemination. Commensal-induced priming of IEL effector function or continuous surveillance of the epithelium may be important contributing factors to the rapidity of response.

Summary—Impaired microbial recognition, dysregulated innate immune signaling or microbial dysbiosis may limit the protective function of IELs and increase susceptibility to disease. Further understanding of the mechanisms regulating IEL surveillance and sentinel function may provide insight into the development of more effective targeted therapies designed to reinforce the mucosal barrier.

Keywords

intraepithelial lymphocytes; mucosal immunity; intestine; microbiota; virus; pathogen

Introduction

The intestinal lumen contains up to 4 trillion commensal bacteria in addition to other microbes, dietary antigens and metabolic waste products. Separating this harsh environment from the intestinal immune system is a single layer of epithelial cells, which serves as the primary barrier against microbial invasion or exposure to foreign antigens. As part of the

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Conflicts of Interest

Madeleine Hu, Luo Jia and Karen Edelblum declare that they have no conflict of interest.

Compliance with Ethics Guidelines

Conflict of Interest

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Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors

immune surveillance of the intestinal epithelium, intraepithelial lymphocytes (IEL) are located between adjacent epithelial cells in the lateral intercellular space and along the basement membrane, which separates the epithelial compartment from the underlying lamina propria. Possessing the characteristics of both innate and adaptive immunity, IELs serve as an effective first line of defense against invasive microorganisms. IELs are maintained in a partially activated state, thus allowing a rapid response to epithelial damage or microbial invasion.[1] Further, IELs also exhibit the cytotoxic effector functions commonly associated with conventional T cells,[2] which are highly regulated to ensure an efficient response to luminal microbes while simultaneously limiting aberrant epithelial cytotoxicity. IELs also resemble tissue-resident effector memory cells, as antigen-specific IELs expand and respond to secondary microbial infection (reviewed in [3]). However, this review will explore the contribution of IELs to mucosal innate immunity, and discuss how the protective phenotype of IELs may be useful for the development of therapeutic interventions for enteric infection and other inflammatory or autoimmune diseases in which microbial invasion plays a role in disease pathogenesis.

IEL ontogeny and differentiation

Distributed along the length of the small intestine at a density of 1 per 10 intestinal epithelial cells, IELs constitute one of the most abundant T cell populations of barrier immune cells.[4, 5] The murine small intestinal IEL compartment is comprised of equal numbers of $\text{TCR}\alpha\beta^+$ and $\text{TCR}\gamma\delta^+$ cells, whereas $\text{TCR}\alpha\beta^+$ cells account for nearly 80% of colonic IELs. While all IELs can be described as antigen-experienced, IEL subtypes can be further classified into two phenotypic subtypes based on antigenic reactivity. $\text{CD8}\alpha\beta^+ \text{TCR}\alpha\beta^+$ IELs are referred to as induced IELs since these lymphocytes are recruited to the epithelium after systemic priming following antigen presentation in secondary lymphoid organs.[6] These cells are considered to be tissue-resident memory (T_{RM}) cells and share phenotypic characteristics with peripheral conventional CD8^+ T cells.[3, 7] In contrast, natural IELs typically express the $\text{CD8}\alpha\alpha$ receptor along with either $\text{TCR}\alpha\beta$ or $\text{TCR}\gamma\delta$, but not $\text{CD8}\beta$ or CD4 co-receptors. Although the ontogeny of natural IELs remains controversial, these cells are thought to recognize self-antigen and traffic directly to the intestinal epithelium following thymic or extrathymic education.[8–12] In contrast, CD4^+ IELs often acquire $\text{CD8}\alpha\alpha$ after homing to the intestinal epithelium.[13, 14] Recently, a proportion of these double positive cells were shown to convert from lamina propria Foxp3^+ Tregs through downregulation of ThPOK , a helper T cell transcription factor.[15] Interestingly, these $\text{CD4}^+ \text{CD8}\alpha\alpha^+$ IELs exhibit both cytolytic effector and regulatory potential, but do not share a functional overlap with their Foxp3^+ precursors.[15] Regardless of subtype, all IEL populations are maintained in a state of immunological quiescence, which is essential to maintain an effective host defense response while limiting the potential for self-destruction.

Contribution of IELs to innate antibacterial immune responses

Several studies have shown that enteric bacterial infection influences overall IEL number within the intestinal mucosa. *Salmonella typhimurium* or *Listeria monocytogenes* infection promoted the expansion of $\gamma\delta$ IELs, whereas *Listeria* also enhanced the $\text{CD8}\alpha\alpha^+ \text{TCR}\alpha\beta^+$ IEL population.[16, 17] This is not surprising since their close proximity to the intestinal

lumen places IELs among the first immune cells to encounter invasive bacteria or their metabolites. With this unique positional advantage, IELs function as first responders through the rapid initiation of innate immune responses within first hour or two after bacterial exposure.[18–22] IEL-mediated protection against bacterial invasion and systemic infection is conferred through a variety of different mechanisms; this section aims to summarize the current literature describing the interactions between enteric bacteria and the IEL compartment and the implications of these interactions on host innate immunity.

Commensal bacteria influence IEL maintenance and function

The human intestine is populated with 10^{13} – 10^{14} microorganisms and approximately 1,100 different species.[23] The intestinal microbiota and dietary metabolites directly influence maintenance of the IEL compartment; all IELs except those expressing TCR $\gamma\delta$ are significantly reduced in gnotobiotic and antibiotic-treated mice, as are mice fed an elemental diet.[24–26]. Monocolonization with segmented filamentous bacteria (SFB) or *Clostridia* spp. was sufficient to alter the ratio of CD8 $\alpha\beta^+$ TCR $\alpha\beta^+$ to CD8 $\alpha\alpha^+$ TCR $\alpha\beta^+$ IELs;[27] however, the mechanism by which these commensals influence the $\alpha\beta$ IEL population remains unknown. Further, it was recently reported that *Lactobacillus reuteri* contributes to Foxp3 $^+$ Treg/CD4 $^+$ IEL plasticity through the generation of bacterial metabolites.[28] The activation of aryl hydrocarbon receptor (AhR) by tryptophan-derived ligands was shown to promote the conversion of Tregs to CD4 $^+$ CD8 $\alpha\alpha^+$ IELs and maintain CD8 $\alpha\alpha^+$ IELs populations.[29, 28] This is one of the first reports detailing a distinct mechanism by which a single commensal species directly stimulates IEL differentiation. Together, these data begin to shed light on how bacterial metabolism of dietary antigens influence IEL populations and function within the intestinal mucosa. This rapidly expanding area of investigation will provide significant insight into how the microbiota shapes IEL-mediated innate immune responses.

The communication between IELs and commensal bacteria suggests that microbial recognition by host innate immunity is an important regulator of IEL homeostasis. In support of this, multiple reports demonstrate that host recognition of microbe associated molecular patterns (MAMP) is required to maintain an intact IEL compartment. Mice deficient in Toll-like receptor (TLR) signaling fail to develop CD8 $\alpha\alpha^+$ IELs, due to reduced epithelial interleukin (IL)-15 expression that is essential for the proliferation and survival of natural IELs. [30–33] In contrast, mice lacking the intracellular bacterial pattern recognition receptor, nucleotide-binding oligomerization domain-containing protein 2 (Nod2) exhibit impaired IL-15 production by macrophages or dendritic cells leading to a selective loss of CD8 $^+$ IELs.[34] Exogenous administration of the Nod2 ligand muramyl dipeptide to antibiotic-treated mice rescued CD8 $\alpha\alpha^+$, but not CD8 $\alpha\beta^+$ TCR $\alpha\beta^+$ IELs. Moreover, a significant proportion of the remaining IELs present in Nod2-deficient mice produced increased IFN γ , which was shown to adversely affect small intestinal goblet cell morphology and mucin production.[35] Interestingly, this intestinal phenotype was dependent on the presence of *Bacteroides vulgatus*,[35] thus providing a novel link between a specific commensal to the development of a pathological IEL phenotype. Adoptive transfer of wildtype CD8 $^+$ IELs partially reduced the susceptibility of Nod2-deficient mice to TNBS-induced colitis demonstrating that microbial recognition is critical for CD8 $^+$ IEL-mediated

protection against enteric inflammation.[34] Similarly, expression of PGLYRP2, which is involved in peptidoglycan recognition, is upregulated in cecal CD8 $\alpha\alpha^+$ IELs following *Salmonella typhimurium* infection.[36] Loss of PGLYRP2 increases susceptibility to salmonellosis, demonstrating a key role for intracellular bacterial recognition in IEL-mediated antibacterial immunity.[36] In the next section, we will explore how IELs confer protection against bacterial infection and discuss the links between bacterial recognition and IEL effector function.

Mechanisms of IEL-mediated innate immune defense against bacteria

Cytotoxicity—All intestinal IEL populations possess cytotoxic effector functions, including CD4 $^+$ IELs, suggesting that the development of this phenotype is intrinsic to the intestinal epithelial microenvironment.[37] As a result, IELs are able to directly induce the apoptosis of infected epithelial cells through the production of granzymes, perforin and Fas ligand [38, 37] to prevent the further spread of infection. Since IELs are partially activated, licensing of IEL lytic activity is tightly regulated to prevent aberrant or excessive epithelial cytolysis, which could disrupt the epithelial barrier resulting in increased bacterial translocation.[1] Although microbial or cellular stress serves as the primary inducer of IEL cytolytic activity, IEL-induced epithelial cytolysis does not require the presence of an intact microbiota.[1, 2]

While cytolysis can be activated in both induced and natural IELs via TCR activation, only natural IELs initiate cytolysis through natural killer receptors such as natural killer group 2D (NKG2D)(Figure 1).[39, 40] NKG2D is upregulated downstream of IL-1 β and IL-15 signaling following bacterial infection.[41, 34, 42, 43] *S. typhimurium* also induces the expression of Fas ligand and IFN γ , as well as cellular stress ligands that bind to NKG2D on the surface of infected epithelial cells,[41, 16] thus effectively marking these cells for destruction. Conversely, TLR9 activation and commensal bacteria negatively regulate the expression of these stress ligands to limit indiscriminate IEL-induced cytotoxicity.[41, 44]

The human ortholog for murine NKG2D ligands is MHC class I polypeptide-related sequence A (MICA), which is also upregulated in the epithelium in response to bacterial infection.[45, 46] MICA activates the NKG2D receptor as well as a variety of V δ 1 TCRs derived from human $\gamma\delta$ T cell clones, with binding to $\gamma\delta$ TCR being sufficient to induce $\gamma\delta$ T cell cytolytic activity.[45, 47, 39] Together with data from murine models, these findings suggest that epithelial recognition of pathogenic bacteria is necessary to license IEL cytotoxicity in an effort to facilitate clearance of intracellular bacteria.

Regulation of mucus production—A layer of mucus secreted by goblet cells serves as a physical barrier to prevent microbes from directly interacting with the epithelial surface, and therefore, loss of goblet cells or altered mucin expression increases susceptibility to intestinal infection and inflammation.[48] $\gamma\delta$ T-cell-deficient mice exhibit reduced goblet cell number along with altered mucin expression and glycosylation in both the small intestine and colon.[49] This leads to a reduction in mucosal sialic acid, which is a nutrient source and critical for mucosa-associated bacterial niche formation.[50, 51] These data suggest that the impaired development of specific IEL subsets may influence mucus

composition that can in turn promote microbial dysbiosis leading to increased disease susceptibility. This may provide an additional mechanistic explanation for the small intestinal abnormalities observed in Nod2-deficient mice, as the absence of an intact IEL population correlated to changes in goblet cell morphology, mucin production and the expansion of the mucosa-associated commensal, *B. vulgatus*. [35] Further investigation into the mechanisms by which $\gamma\delta$ IELs regulate mucin production and secretion are warranted, since this may have profound effects on the diversity and abundance of specific mucosal bacterial populations.

Antimicrobial peptides—As an additional layer of protection, IELs and epithelial cells secrete antimicrobial peptides (AMPs) into the intestinal lumen. AMPs exhibit bactericidal activity and are essential to maintain the spatial segregation between luminal bacteria and the epithelial apical surface. [52] The first demonstration of IEL bactericidal AMP production was observed in response to DSS-induced injury in which colonic $\gamma\delta$ IELs were shown to upregulate expression of RegIII γ , lysozyme, and complement proteins Iqa and Iqb. [53] Whereas colonic $\gamma\delta$ IEL-induced RegIII γ expression was MyD88-independent, [53] epithelial MyD88 signaling was required for $\gamma\delta$ IEL AMP production in the small intestine under steady-state conditions. [22] Loss of $\gamma\delta$ T cells or epithelial MyD88 signaling was sufficient to enhance the translocation of *S. typhimurium* or resident pathobionts across the epithelial barrier. [22]

In addition to producing antimicrobial factors, $\gamma\delta$ IELs have been shown to reciprocally stimulate epithelial AMP production. Following acute *Salmonella* exposure, the activation of epithelial pattern recognition receptors (PRR) promotes IL-23 expression, which can upregulate $\gamma\delta$ IEL IL-22 production *in vitro*. [54] IL-22 production by V γ 7⁺ IELs induces Paneth cell production of angiogenin 4 (Ang4) to limit bacterial invasion in the small intestine. [54] It should be noted that innate lymphoid cells (ILC3) are the primary producers of IL-22 in the intestinal mucosa; [55] however, IELs also influence epithelial AMP production through direct ligand/receptor interactions. For example, in the early stages of *Citrobacter rodentium* infection, colonic CD8 $\alpha\alpha$ ⁺ IELs rapidly upregulate the herpes virus entry mediator (HVEM) ligand, CD160. [56] Upon CD160/HVEM binding, epithelial RegIII β / γ production is enhanced through Stat3- or IL-22-dependent mechanisms. Similar mechanisms may be involved in the regulation of AMP expression in response to a variety of bacterial pathogens since RegIII γ and/or RegIII β have been implicated in protection against *L. monocytogenes*, *S. enteritidis* and *Y. pseudotuberculosis* [57, 58, 52, 59]. Taken together, these findings highlight IEL-mediated regulation of AMP production as an important mechanism by which innate immunity responds to invasive bacterial species.

$\gamma\delta$ IEL motility and epithelial surveillance—Although many of the innate immune functions discussed above rely on IEL generation of paracrine factors to signal neighboring cells, the intimate association between IELs and the epithelium implies that direct contact modulates the function of both cell types. While IELs were presumed to be sessile, [60] we found that $\gamma\delta$ IELs provide extensive surveillance of the villous epithelium by continuously surveying the basement membrane and migrating between adjacent epithelial cells. [61] $\gamma\delta$ IEL migration and distribution along the crypt-villus axis was recently characterized.

Throughout the small intestine, $\gamma\delta$ IELs were shown to accumulate mid-villus and their migration and localization along the length of the villus was influenced by the presence of commensal bacteria.[62] The dynamics of this migration is mediated via direct interactions between $\gamma\delta$ IEL CD103 ($\alpha_E\beta_7$ integrin) and epithelial E-cadherin, as well as between occludin expressed by both $\gamma\delta$ IELs and epithelial cells.[61] Interestingly, luminal exposure of *S. typhimurium* resulted in enhanced $\gamma\delta$ IEL migration toward bacterial-adjacent enterocytes.[63] $\gamma\delta$ IEL localization in the lateral intercellular space is essential for $\gamma\delta$ IEL-mediated protection against acute *Salmonella typhimurium* invasion, as bacterial translocation was significantly enhanced in mice with non-motile occludin-deficient $\gamma\delta$ T cells.[63, 64, 22] Further, *Salmonella* infection shifted the distribution of $\gamma\delta$ IELs toward the base of the villus, which was shown to be dependent on epithelial MyD88 expression.[62] Increased glycolysis was observed in $\gamma\delta$ IELs in response to infection, and inhibition of this process prevented the infection-induced changes in $\gamma\delta$ IEL migratory behavior.[62] Together, these findings suggest that $\gamma\delta$ IEL migration may provide a targeted and localized effector response to ensure the maximal efficiency of host defense responses. Moreover, these studies indicate a role for innate immune signaling in attracting $\gamma\delta$ IELs to sites of bacterial invasion, but continued investigation is required to elucidate the cellular mechanisms regulating $\gamma\delta$ IEL epithelial surveillance both at steady-state and in response to microbial pathogens.

IEL response to parasites

IELs also confer protection against a wide range of intestinal protozoan parasites, including the apicomplexan *Eimeria vermiformis*. At the peak of infection, IEL number and production of IFN γ , TNF and TGF β were increased.[21, 65] Although several reports investigating the role of $\alpha\beta$ and $\gamma\delta$ T cells in *E. vermiformis* have yielded contradictory results,[21, 66–68] the majority of the data indicate an immunoprotective role for $\gamma\delta$ IELs through the production of IFN γ . [66] Yet it remains unclear as to whether IFN γ directly mediates killing of infected enterocytes [69] or if it promotes parasite clearance through the recruitment of professional phagocytes. Alternatively, IFN γ may activate cell autonomous killing of the parasite by epithelial cells.[70]

The role of IELs is also evident in oral infection with *Toxoplasma gondii*, an obligate intracellular parasite that induces acute ileitis in mice.[71] In mice infected with *Toxoplasma*, elevated CCR5 expression increases the recruitment of antigen-primed CD8 $\alpha\beta$ ⁺ IELs that exhibit lytic activity toward parasite-infected macrophages and enterocytes via IFN γ production.[72, 73] While acute IFN γ production may confer protection against initial parasite invasion and replication,[74] CD8 $\alpha\beta$ ⁺ IELs also produce TGF β to suppress the sustained activation of this pro-inflammatory response to prevent further mucosal damage. [73–75] In contrast, V γ 7⁺ IELs confer protection against *T. gondii* invasion as early as one hour post-infection.[64, 63] Further investigation showed that *Toxoplasma* induced the mislocalization of the epithelial tight junction protein occludin,[64, 76] which may adversely affect $\gamma\delta$ IEL migration and enhance epithelial permeability.[61] In support of this, we found that impaired $\gamma\delta$ IEL motility increased the initial translocation of *T. gondii* tachyzoites across the epithelial barrier. This finding in combination with intravital imaging studies demonstrating that the migratory response of $\gamma\delta$ IELs along the villus in response to

T gondii is similar to those observed with *Salmonella* infection suggests that $\gamma\delta$ IEL surveillance may represent a conserved mechanism for limiting enteric microbial invasion. [63, 62]

Intestinal IELs promote antiviral innate immune responses

As presented in the previous sections, many IEL responses appear to be conserved across multiple infectious pathogens. This idea is further supported by the observations that similar to the response to invasive bacteria, infection with lymphocytic choriomeningitis virus (LCMV) simian immunodeficiency virus (SIV), reovirus or rotavirus induces the expansion of CD8⁺ TCR $\alpha\beta$ ⁺ IELs.[77–82] In contrast, primary SIV infection also results in the depletion of natural IEL populations,[82] which likely creates favorable conditions for viral replication. Whereas rotavirus and norovirus can cause severe disease in neonates, adults often exhibit milder symptoms.[83] This may be partly attributed to a relatively low number of IELs in the neonatal gut immune compartment, which coincides with increased susceptibility to viral infection.[84–86] Considering the antigen-specificity of CD8 $\alpha\beta$ ⁺ IELs and the ability of these cells to generate an effector memory response, it stands to reason that this IEL population increases with age as a result of cumulative microbial exposure.[3] Although the generation of antigen-specific effector memory IEL populations is an important component of antiviral defense, much less is known regarding the contribution of IELs to innate antiviral immune responses.

IEL antiviral effector responses

Cytolysis—Similar to our discussion of IEL responses to other microbes, cytolytic activity in both the induced and natural IEL populations is essential for restricting viral replication within the intestinal mucosa (Figure 2). IELs upregulate perforin and granzyme B expression in response to LCMV infection;[87] however, whereas CD8 $\alpha\beta$ ⁺ IELs exhibited enhanced LCMV-specific cytotoxicity via IFN γ , TCR $\alpha\beta$ ⁺ CD8 $\alpha\alpha$ ⁺ IEL lytic activity was reduced in comparison.[87–89] Further, studies investigating the role of IELs in response to vaccinia virus showed that CD8 $\alpha\beta$ ⁺ IELs demonstrated lower avidity for viral antigen than cytotoxic lymphocytes in the lamina propria or spleen.[90] This suggests that the hyporesponsive nature of IELs may provide a rapid response to initial infection, yet ensures downregulation of these pro-inflammatory responses to prevent further epithelial damage. Alternatively, CD4⁺ IELs may detect MHC class II molecules upregulated on the epithelial surface, allowing them to target virus-infected enterocytes that may have evaded MHC class-I restricted cytotoxic IELs.[91, 92] However, the role of natural IELs in antiviral immunity and the mechanisms by which these IELs recognize and respond to virus-infected enterocytes remains an area of active investigation.

Interferon signaling—Interferons (IFN) are a pleiotropic family of cytokines that play a key role in the innate immune response to bacterial and viral infection. IFNs are broadly categorized into three families based on receptor specificity. Type I IFNs consist of multiple IFN α isoforms and one IFN β , which bind to interferon α/β receptor (IFNAR).[93] The microbiota induces tonic type I IFN production to maintain mucosal homeostasis and maintain host responsiveness to viral infection.[94–96] Although depletion of the microbiota

results in impaired mucosal immunity, norovirus infection compensated for these defects through an IFNAR-dependent mechanism.[97] Whereas type II IFN (IFN γ) facilitates the response to intracellular bacteria, type III IFNs (IFN λ) largely affect the intestinal epithelium and are critical for host antiviral defense.[98] Recently, activated $\alpha\beta$ and $\gamma\delta$ IELs were shown to produce all three types of IFN to induce epithelial activation of IFN-stimulated genes (ISGs).[99] As a result, IEL production of IFN was sufficient to protect against viral infection of enterocytes. Moreover, IEL-mediated induction of epithelial ISGs markedly improved the host antiviral response to norovirus *in vivo*. [99] While these studies elucidate a novel role for IELs in innate antiviral host defense, much still remains unknown regarding the direct influence of viral infection and IFN signaling on IELs. Based on the functional similarities of IEL effector responses to various pathogens, it is interesting to speculate that IEL-mediated IFN signaling may also contribute to protective immunity against bacterial or protozoan invasion. Reciprocally, it is plausible that tonic IFN signaling induced by the microbiota may condition or prime host responsiveness to pathogen invasion.

Immune cell recruitment—Although IELs provide an initial antiviral response, it is unlikely that IELs alone are sufficient to eliminate infection. Transcriptional analysis showed that IELs express chemokines including RANTES (CCL5), lymphotactin (XCL1), macrophage inflammatory protein (MIP)-1 α , and MIP-1 β (CCL3 and CCL4).[100, 101] Further, interferon produced by IELs was shown to stimulate epithelial production of multiple chemokines.[99] Activation of epithelial ISGs results in the upregulation of T cell chemoattractants such as CXCL10 and CXCL11, for which IELs express the cognate receptor CXCR3,[102, 101] suggesting that IFNs may also modulate IEL/epithelial interactions. These studies indicate that IELs induce local chemokine production to recruit additional leukocytes to sites of infection as a means to amplify the innate and adaptive arms of the antiviral response.[103]

IEL recognition of virus-infected cells

As discussed in earlier sections, IELs respond to the presence of microbial antigens or stress ligands via TCR-dependent and -independent pathways, including the activation of PRRs or NKG2D. Acute intraluminal administration of the TLR ligand poly I:C resulted in an increase in IEL number and small intestinal enteropathy characterized by villous blunting. [104] This small intestinal injury was attributed to TLR3-induced CD8 $\alpha\alpha^+$ IEL-mediated epithelial cytolysis.[105, 106] Rotavirus infection, poly I:C or purified viral double-stranded RNA were all able to promote epithelial IL-15 production, which is essential for IEL homeostasis.[106, 33] Likewise, poly I:C induced the expression of IEL NKG2D and its ligand Rae-1 leading to enhanced CD8 $\alpha\alpha$ cytotoxicity.[105] Viral infection is thought to be associated with the initiation of celiac disease, an intestinal disorder mediated by abnormal IELs that develop a natural killer cell-like phenotype.[107] Recently, experimental evidence demonstrated that reovirus infection induces the loss of tolerance to dietary antigen through the activation of Th1 immunity and simultaneous inhibition of peripheral Treg differentiation.[108] Given the association between viral PRR-induced IL-15 production and increased IEL lytic activity,[105, 106] reovirus infection may also influence IEL function in the context of celiac disease.[108]

Implications for maintenance of the epithelial barrier and autoimmunity

Studies investigating the role of IELs have been hampered by the inability to specifically delete this subpopulation of lymphocytes without disrupting systemic T cell-mediated immunity; however, study of induced and natural IELs in response to intestinal infection and inflammation has provided the best functional characterization of these innate-like adaptive immune cells to date. In the most highly colonized mucosal surface in the body, IELs ensure a rapid response to luminal microbes while also contributing to the maintenance of mucosal homeostasis. As we continue to define the role of IELs in mucosal innate immunity, the next step is to translate these findings into the context of autoimmunity, or other inflammatory diseases in which epithelial barrier integrity is compromised. IEL biology has been well-characterized in the context of celiac disease;[4] however, relatively little is known about the role of IELs in inflammatory bowel disease (IBD), where epithelial barrier dysfunction is thought to precede disease development.[109–111] This is of particular interest since microbial dysbiosis, diet and defects in microbial recognition are contributing factors to the pathogenesis of IBD.[112] Recent reports have begun to explore how IBD-associated defects in innate immunity affect IEL function,[35] yet this is still a largely underappreciated area of investigation that has been recently reviewed elsewhere.[111] Although further studies are needed to elucidate the underlying functional regulation of natural IELs, both in animal models and humans, the modulation of IEL surveillance or innate immune function may prove to be a valuable therapeutic tool to reduce intestinal inflammation and strategically reinforce the mucosal barrier.

Conclusions

While the initial stimulus varies, IEL effector function is similar following the invasion of commensal and pathogenic bacteria, viruses and protozoan parasites suggesting that IELs respond to microbial invasion via the activation of signaling downstream of conserved pathogen recognition motifs. While several studies have described a mechanism by which PRR signaling by epithelial cells or myeloid cells can contribute to IEL homeostasis or effector function,[34, 32, 30, 31, 22, 53] whether microbes or MAMPs directly stimulate IELs remains unclear. Recent evidence demonstrates that there are multiple mechanisms by which the microbiota and IELs interact, indicating that the integration of multiple innate immune signals regulates IEL-mediated effector function. As a result, impaired microbial recognition, dysregulated innate immune signaling or microbial dysbiosis may limit the protective function of IELs and increase susceptibility to diseases such as IBD. Elucidation of the cellular and molecular mechanisms regulating IEL surveillance and sentinel function may provide insight into the development of novel therapeutics to treat autoimmune or inflammatory disease.

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Figure 1. IELs and innate antibacterial immune responses

In the small intestine, pathogenic bacteria (red) induce upregulation of NKG2D ligands and release TCR-activating antigens, which signal via NKG2D and TCR to trigger the release of cytotoxic factors such as granzymes and Fas ligand (FasL). While TCR activation induces cytotoxicity in all IELs, NKG2D activation only promotes killing by natural IELs. Both pathogenic bacteria and invading commensals (green) promote $\gamma\delta$ IEL epithelial surveillance, induce $\gamma\delta$ IEL production of RegIII γ and upregulate epithelial interleukin (IL)-23 production. In turn, IL-23 promotes $\gamma\delta$ IEL production of IL-22 to further stimulate epithelial antimicrobial peptide (AMP) production. In the colon, HVEM/CD160 binding in response to pathogenic bacteria induces epithelial AMP production. Finally, DSS-induced injury enhances the translocation of commensal bacteria, resulting in $\gamma\delta$ IEL production of AMPs and chemokines that serve to recruit myeloid cells to sites of damage.

Figure 2. Intestinal IELs promote antiviral innate immune responses

Induced (CD8 $\alpha\beta$ ⁺) and natural (CD8 $\alpha\alpha$ ⁺) IELs promote the cytolysis of virus-infected IECs through the production of perforin, granzyme B and IFN γ . This can be mediated in part through activation of TLR3, which promotes the expression of IEL NKG2D and its ligand Rae-1 leading to enhanced CD8 $\alpha\alpha$ cytotoxicity. Further, activated CD8 $\alpha\alpha$ ⁺ IELs produce type I, II, III interferons (IFN) to enhance the epithelial antiviral response via induction of interferon-stimulated genes (ISG). This includes the production of chemokines CXCL10, CXCL11, CCL2 and CCL5, which can recruit both IELs and myeloid cells to facilitate viral clearance.