Published in final edited form as:

Curr Gastroenterol Rep. 2010 December ; 12(6): 513–521. doi:10.1007/s11894-010-0148-6.

Intraepithelial Lymphocytes: To Serve and Protect

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Abstract

The mucosal immune system is constantly exposed to a wide range of commensal and potentially pathogenic microbial species. Chronic exposure to foreign organisms makes generation of an appropriate immune response critical in maintaining a balance between elimination of harmful pathogens, peaceful coexistence with commensals, and prevention of autoimmunity. Intestinal intraepithelial lymphocytes provide a first line of defense at this extensive barrier with the outside world, and as such, understanding their role in immunity is critical.

Keywords

Mucosal immunology; Intraepithelial lymphocyte; CD8 $\alpha\beta$ T cell; $\gamma\delta$ T cell; T-cell migration; Immunologic memory; Intestinal homeostasis; Microbial surveillance

Introduction

The intestinal immune response is typically subdivided into events that occur at intestinal inductive sites, namely the Peyer's patches (PP) and mesenteric lymph nodes (MLN), or the intestinal effector sites, the lamina propria and intraepithelial lymphocyte (IEL) compartment. To generate a mucosal T-cell response, antigen must first gain access to antigen-presenting cells by penetrating the mucus layer and then the intestinal epithelial cell (IEC) barrier. Numerous mechanisms operate to prevent the invasion of microbial species at the intestinal epithelial surface. The rapid movement of intestinal contents through peristalsis, secretion of a thick layer of mucus by goblet cells, and release of extensive antimicrobial products into the lumen by Paneth cells all function to limit the invasion of pathogenic organisms. Despite this, continual exposure to potentially pathogenic microorganisms and the ability of microbial populations to rapidly adapt to different environmental constraints require the immune system to handle numerous and often repeated insults. To handle repeated exposures to the same pathogen, our immune system has the ability to generate clonotypic T-cell responses with the capacity to self-renew and rapidly perform effector functions. Often, this protection lasts for the life of the individual. In this manner, antigen-experienced as well as "innate type" IELs serve as gateway guards at the intestinal epithelial surface constantly monitoring the epithelial layer for infection or disruption. Distinct IEL subsets serve a wide range of functions in the intestinal mucosa. IELs may regulate intestinal homeostasis, maintain epithelial barrier function, rapidly respond to infection, and mount as well as regulate adaptive and innate immune responses.

These frontline lymphocytes residing within the epithelial layer are incredibly heterogeneous with regard to their function and phenotype and are represented by conventional and nonconventional T-cell subsets. In the mouse small intestine, it is estimated that one IEL is present for every 10 intestinal villous epithelial cells, making intestinal IELs one of the most abundant T-cell populations [1, 2]. Two major groups of lymphocytes that reside within the murine intestinal epithelial layer can be distinguished based on expression of either an $\alpha\beta$ T-cell receptor (TCR) or a $\gamma\delta$ TCR, and are retained in almost equal proportions. These lymphocyte subsets can be further subdivided on the basis of CD8 coreceptor expression. The vast majority of TCR $\gamma\delta^+$ T cells in the IEL compartment express the CD8 $\alpha\alpha$ coreceptor, but a small proportion lacks both CD8 α and CD8 β . This is in striking contrast to TCR $\gamma\delta^+$ T cells located in lymphoid tissues, which predominantly lack CD8 expression. In humans, TCR $\gamma\delta^+$ IELs represent about 10% of the small intestinal IEL, but drastically increase under certain allergic or inflammatory conditions [3]. The greater prevalence of TCR $\alpha\beta^+$ IEL in human intestines may reflect increased exposure to pathogenic microbes. Indeed, even in mice, the distribution of different mucosal T-cell subtypes varies based on anatomic location, age, strain, microbial flora, and housing conditions.

Numerous distinctions exist between IELs and their systemic counterparts in the spleen, lymph nodes, and circulation. The majority of small intestinal IELs are composed of CD8 $^+$ T cells, whereas most peripheral tissues are composed of roughly equal portions of CD4 $^+$ and CD8 $^+$ lymphocytes. TCR $\alpha\beta^+$ IELs can also be divided into two major subsets based on the composition of the CD8 chains expressed. The CD8 $\alpha\beta^+$ TCR $\alpha\beta^+$ IELs are the progeny of conventional naïve CD8 $^+$ T cells. Moreover, a large fraction of IELs express the CD8 $\alpha\alpha$ homodimer and lack CD8 β expression, unlike circulating T cells that primarily express both CD8 chains [4]. The CD8 $\alpha\alpha$ homodimer expressed on intestinal IELs interacts with high affinity to the nonclassical major histocompatibility complex (MHC) class I molecule, thymus leukemia antigen, expressed by many IEC. The role of the CD8 $\alpha\alpha$ homodimer on intestinal IEL remains poorly defined. It has been reported to promote cytokine production by IEL without modulating lytic activity, proliferation, survival, or migration [4, 5]. CD8 $\alpha\alpha$ expression on CD8 $\alpha\beta^+$ T cells may regulate the threshold of T-cell activation to prevent aberrant effector function [6]. However, its role on IELs that do not express CD8 $\alpha\beta$ remains to be clarified. The majority of lymphocytes residing in the intestinal epithelium also share some phenotypic characteristics with peripheral effector/memory CD8 $\alpha\beta^+$ T-cell subsets, suggesting previous antigen encounter. In addition, the majority of intestinal IELs express the early activation marker CD69 but lack CD25 (interleukin [IL]-2R α), suggesting an altered activation state likely because of factors present in the gut microenvironment. Indeed, memory CD8 T cells migrating into the intestinal epithelium upregulate CD69 even in the absence of cognate antigen [7]. In addition, most IELs also express the lytic granule component granzyme B and are cytolytic directly ex vivo [8, 9].

CD8 $\alpha\alpha^+$ T-cell Receptor $\gamma\delta^+$ Intraepithelial Lymphocytes

Several effector functions have been attributed to TCR $\gamma\delta^+$ T cells, including rapid production of cytokines that may regulate pathogen clearance, inflammation, and intestinal homeostasis. TCR $\gamma\delta^+$ T cells isolated from lymphoid tissues may promote inflammation by the production of IL-17 or IFN- γ and lysis of infected cells through lytic granules or death receptors [10–12], dampen inflammation by the production of IL-10 [13], and regulate epithelial cell integrity and healing by production of keratinocyte growth factor [14, 15]. However, individual subsets have more limited effector functions based on TCR expression, tissue localization, and activation status. The precise role of TCR $\gamma\delta^+$ IEL has remained elusive. However, TCR $\gamma\delta^+$ IEL are directly lytic ex vivo [8] and have the capacity to produce IL-10 in vivo [16] and keratinocyte growth factor [15, 17]. In mice, IELs

predominately express the V γ 5 and V γ 1 gene segments together with a variety of V δ genes and have extensive junctional and CDR3 region diversity in their TCRs. Human TCR $\gamma\delta^+$ IELs predominately express the V δ 1 gene segment, and TCR $\gamma\delta^+$ T cells can interact with the stressed induced nonclassical MHC class I molecules MIC-A and MIC-B [18], which could play a role in maintaining epithelial cell health and barrier integrity. Unlike TCR $\alpha\beta^+$ T cells, little is known about TCR $\gamma\delta^+$ specificity and antigen recognition capabilities, impeding our complete understanding of TCR $\gamma\delta^+$ T-cell behavior.

Murine studies have demonstrated that TCR $\gamma\delta^+$ IEL maintenance and activation may be dependent on IEC-derived MyD88-dependent IL-15 production [19]. This result suggests that normal intestinal microbial flora may be important in TCR $\gamma\delta^+$ IEL regulation, but whether TCR recognition of bacterial products is involved is unknown. In the case of pathogenic infections, TCR $\gamma\delta^+$ IELs are required for optimal protection against several intestinal pathogens, including the *Encephalitozoon cuniculi* [20], *Eimeria vermiciformis* [21, 22], *Toxoplasma* spp, and *Salmonella* spp [23]. However, even in these pathogenic settings, protection is conferred by distinct mechanisms. For example, loss of protection against *E. cuniculi* in TCR $\gamma\delta$ T-cell-deficient mice is the result of a reduced antigen-specific CD8 $\alpha\beta^+$ T-cell response, although not necessarily linked to a defect in the IEL response, because this study used intraperitoneal infections. Nevertheless, the response to oral *E. cuniculi* infection is characterized by the rapid appearance of antigen-specific TCR $\alpha\beta^+$ CD8 $\alpha\beta^+$ IEL [24]. In contrast, in the absence of TCR $\gamma\delta$ T cells, protection against *Toxoplasma* and *Salmonella* spp is the result of elevated transmigration of these pathogens across the epithelial barrier because of defects in tight junction formation resulting in increased intestinal permeability [23]. Clearly, TCR $\gamma\delta^+$ T cells are capable of a diverse array of effector functions, yet their precise role in inflammation and disease will continue to be enigmatic until their TCR specificity is more clearly defined.

CD8 $\alpha\beta^+$ T-cell Receptor $\alpha\beta^+$ Intraepithelial Lymphocytes

Although a large proportion of IELs found in the small intestine are resident, a significant population of antigen-specific CD8 $\alpha\beta^+$ T cells can migrate to the intestinal tissues and intercalate within the epithelial cell layer following antigen exposure. This compartment of IELs increases with age, consistent with the notion that most CD8 $\alpha\beta^+$ IELs are pathogen-specific CD8 $^+$ T cells [25]. Moreover, these cells share many qualities consistent with antigen-experienced CD8 $^+$ T cells isolated from other peripheral tissues. A thorough examination of antigen-specific memory CD8 $\alpha\beta^+$ IEL suggests that although they demonstrate distinct differences from central or effector memory CD8 $^+$ T-cell subsets, they closely resemble effector memory CD8 $^+$ T cells, albeit with some phenotypic differences [9]. Moreover, the anatomic location directly impacts the memory T-cell differentiation program, because donor memory cells isolated from the spleen or IEL adopt the memory phenotype of their new location upon migration [9].

The analysis of pathogen-specific CD8 $\alpha\beta^+$ IELs has largely been performed following systemic infection in which T-cell priming occurs predominately in the spleen or other peripheral lymphoid tissues. Following interaction of a naïve CD8 $\alpha\beta^+$ T cell with its cognate antigen in the context of the proper inflammatory environment, antigen-specific CD8 $\alpha\beta^+$ T cells undergo expansion, emigrate from the site of priming, and infiltrate effector tissues [26]. During this process, CD8 $\alpha\beta^+$ T cells acquire homing receptors early following priming, which instructs their migration to the appropriate tissues [27]. Despite this knowledge, little information exists concerning the generation of adaptive immunity within the intestinal mucosa, particularly given its importance as an inductive site following exposure to microbial pathogens. Moreover, the intestine contains specialized sites designed for antigen presentation (PP and MLN) and maintenance of effector and memory CD8 $\alpha\beta^+$ T

cells, which may impart distinct requirements for T-cell generation, expansion, and maintenance of memory populations. Distinct populations of dendritic cells (DCs) located within the intestinal mucosa, particularly within the PP and MLN, but also within the lamina propria may differentially prime T cells. Indeed, PPs are unique among T-cell inductive sites. PP contain specialized M cells capable of capturing and transporting antigen from the luminal side of the intestine into the PP for acquisition by other DC subsets contained within the subepithelial dome. Three main DC subsets reside within the intestinal mucosa and are defined by the expression of CD11b and CD8 α : CD11b⁺CD8 α ⁻, CD11b⁻CD8 α ⁺, and CD11b⁻CD8 α ⁻ [28, 29]. Following oral infection with *Salmonella*, pathogen-specific CD4⁺ T cells are activated by CCR6 expressing DCs (CD11b⁺), which migrate into the follicle-associated epithelium of the PP [30]. These DCs are restricted to the PP, and anti-*Salmonella* CD4⁺ T-cell responses are poorly generated in their absence [30].

Two fundamental events must occur for the initiation of an adaptive immune response in the intestinal mucosa. First, antigen must be transported from the intestinal epithelium to PP or MLN, which contains specialized T-cell compartments for priming. Secondly, antigen must be processed and presented to T cells in the context of costimulation and inflammation to activate naïve T cells. Although these events have been extensively studied in vitro and following intravenous infection, the capacity to examine mucosal generated CD8 T-cell responses only recently became available. Recent studies suggest that DCs are capable of imprinting a “homing” phenotype on T cells to instruct migration to particular tissues [27]. In this manner, DCs located in intestinal tissues induce the upregulation of the heterodimer integrin $\alpha_4\beta_7$ and the chemokine receptor CCR9, factors critical for intestinal infiltration. Alternatively, DCs that acquire antigen from the skin induce a “skin-homing” phenotype characterized by E- and P-selectin ligands and CCR4 and/or CCR10 expression [31]. The ability of DCs to instruct T cells with a gut-homing phenotype appears to be imparted by a subset of MLN DCs that express the α_E integrin (CD103), paired with the β_7 integrin chain, and is mediated, at least in part, by retinoic acid [32, 33]. Indeed, the ability to produce retinoic acid may be sufficient to induce $\alpha_4\beta_7$ and CCR9 expression on responding CD8⁺ T cells, because bone marrow-derived DCs, when cocultured with intestinal IECs, can instruct gut-homing receptor expression in a retinoic acid-dependent manner [34]. However, it should be noted that although mucosal lymphoid tissues preferentially prime CD8⁺ T cells to induce a gut-homing phenotype, this requirement is not absolute because systemic infections induced by lymphocytic choriomeningitis virus, vesicular stomatitis virus, or *Listeria monocytogenes* also lead to intestinal infiltration of antigen-specific CD8⁺ T cells [26, 35].

Gut-homing T cells express integrins that mediate cell-cell attachments and are therefore essential for IEL homing to the intestine and retention thereafter. Moreover, homing of CD8 $\alpha\beta$ ⁺ T cells to the intestinal epithelium has a dramatic impact on intestinal barrier integrity by reducing transepithelial resistance and increasing paracellular flux of fluorescein isothiocyanate–dextran in an in vitro coculture model [36, 37]. Therefore, it is not surprising that blockade of the interactions which mediate intestinal infiltration by leukocytes has been a promising strategy for the treatment of inflammatory bowel disease [38]. One of the most extensively studied integrins is $\alpha_4\beta_7$, which binds the addressin MAdCAM-1 that is constitutively expressed by intestinal lamina propria vascular endothelial cells [39]. In vivo antibody blockade or adoptive transfer of lymphocytes that lack this integrin demonstrate its importance for CD8 $\alpha\beta$ ⁺ T-cell localization to the intestinal mucosa and to the epithelium [40, 41]. Therapeutic approaches using antibody blockade against all α_4 integrins (natalizumab) as well as specifically against $\alpha_4\beta_7$ integrin (vedolizumab/MLN0002) hold promise in the treatment of Crohn’s disease and ulcerative colitis [42–44], but are not without potential negative side effects [45, 46] (Table 1). Of significant concern, a few individuals undergoing treatment with natalizumab experienced JC polyomavirus reactivation and subsequent progressive multifocal leukoencephalopathy, a potentially fatal

disease. It is unclear whether vedolizumab has a similar effect, because clinical trials have not reached a large enough size to carefully evaluate this issue. Despite these concerns, further characterization of the factors regulating infiltration of effector T-cell subsets into the intestinal mucosa will likely provide novel therapeutic approaches to specifically limit T-cell migration into the intestinal mucosa without impairing T-cell migration into other tissues, and therefore potentially limit adverse side effects.

Although $\alpha_4\beta_7$ expression is required for lymphocyte infiltration into the intestinal epithelium, once there, most CD8 $\alpha\beta^+$ T cells downregulate $\alpha_4\beta_7$ and upregulate CD103 [7, 47]. Indeed, almost all CD8 $\alpha\beta^+$ IEL (as well as all other IEL) express high levels of CD103 on their surface, whereas peripheral CD8 $\alpha\beta^+$ T cells lack or express low CD103 levels, suggesting a prominent role for this integrin in infiltration or retention of IEL in the epithelium (Table 1). Mice deficient for CD103 have reduced numbers of TCR $\alpha\beta^+$ intestinal IEL consistent with expression of the CD103 ligand, E-cadherin, by intestinal epithelial cells [48]. However, migration of transferred TCR transgenic CD103 $^{-/-}$ T cells to the epithelium in vivo was unaffected following systemic viral infection, indicating that CD103 is not required for effector CD8 $\alpha\beta^+$ T-cell migration into the IEL compartment. Further, CD103 $^{-/-}$ T cells were retained within the epithelium for 3 weeks without any appreciable decline, though long-term maintenance of pathogen-specific CD103 $^{-/-}$ T cells has not been determined for intestinal IEL [41]. CD103 was similarly shown to be dispensable for infiltration of CD8 $\alpha\beta^+$ T cells into the intestinal epithelium in a TCR transgenic model of graft-versus-host disease (GVHD). However, in this model system, CD103 was essential for mediating GVHD because it was required for retention of CD8 $\alpha\beta^+$ T cells within the intestinal epithelium [49]. CD103 interaction with E-cadherin also influences cellular shape and motility of lymphocytes by conferring dendrite formation and cellular sprawl within the skin epidermal layer [50]. These abilities suggest a potential costimulatory role for CD103 expression. Indeed, a recent study showed that CD103 expression on tumor-infiltrating lymphocytes (TIL) played a major role in tumor clearance. Blockade of CD103 or RNA interference of E-cadherin expression prevented tumor lysis by impairing TIL lytic granule polarization and exocytosis [51•]. Moreover, TGF- β and TCR engagement on tumor-specific T-cell clones induced CD103 expression and potentiated tumor cell lysis. Whether CD103 upregulation on intestinal IEL serves a similar function and facilitates lytic granule polarization and target cell lysis is unknown, but TGF- β expression is also required for induction of CD103 expression by intestinal IEL [49]. In this manner, TGF- β may serve as an essential mediator between inflammation and suppression in the small intestine.

E-cadherin is another known ligand for killer cell lectin-like receptor G1 (KLRG1), which is widely expressed on natural killer and CD8 T-cell effector subsets [52]. KLRG1 engagement by E-cadherin inhibits effector cell responses in natural killer cells, thereby preventing target cell lysis [53]. The role of KLRG1 is less clear on effector CD8 $\alpha\beta^+$ T cells but, in conjunction with CD127 (IL-7R α), is used to delineate effector CD8 $^+$ T-cell fates [54]. At the peak of the CD8 $^+$ T-cell response, cells that express CD127 but not KLRG1 are referred to as memory precursor effector cells (MPECs), and typically represent less than 10% of the antigen-specific CD8 $^+$ T-cell response. The majority of effector CD8 $^+$ T cells express KLRG1 but not CD127, and are referred to as short-lived effector cells (SLECs) [55, 56]. As their names imply, MPECs appear to represent a bona fide memory precursor population with stem cell-like qualities persisting for the life of the animal, whereas SLECs rapidly contract and are unable to form self-renewing memory populations. Although KLRG1 is not expressed on IELs from naïve mice, it may play an important role following intestinal infection [57]. The contradictory function of CD103 and KLRG1 interaction with their shared ligand may suggest that these receptors balance activation of CD8 $\alpha\beta^+$ T cells by regulating a threshold for lytic function in the intestinal epithelium. Moreover, TGF- β , which induces the expression of CD103, was also recently reported to promote SLEC

apoptosis by reducing the expression of the pro-survival molecule Bcl-2 [58]. The relationship of TGF- β , CD103, and KLRG1 with IEL retention and effector function is an attractive theme for future investigations (Table 1).

Although integrins are important for the infiltration of IEL into the epithelium and retention thereafter, important chemotactic factors are involved in the initial recruitment of effector or memory cells. CCR9 is upregulated on CD8 $\alpha\beta^+$ T cells primed by DCs in the MLN and PP, and is essential for CD8 $\alpha\beta^+$ and CD4 $^+$ TCR $\alpha\beta^+$ T-cell recruitment to the IEL compartment [27]. CCL25 appears to be the exclusive ligand for CCR9, is highly expressed by epithelial cells lining small but not large intestinal villi, and mediates activated T-cell localization to the intestinal epithelium [59]. Although CCL25 deficiency results in CD8 $\alpha\beta^+$ T cells that generate a gut-homing phenotype, these cells fail to accumulate in the small intestinal epithelium [59]. Lack of CD8 $\alpha\beta^+$ T-cell accumulation within the intestinal epithelium of CCL25 $^{-/-}$ mice may be partly regulated by reduced CD103 upregulation in the absence of CCR9-generated signals [60]. Recently, CCR9 antagonists were developed for the potential treatment of Crohn's disease and celiac disease, and have demonstrated some efficacy in a murine model of Crohn's-like intestinal inflammation [61]. Moreover, this compound is currently undergoing phase 2/3 clinical trials for the treatment of Crohn's and celiac disease, and offers a potentially exciting alternative to integrin blockade immunotherapies (Table 1).

Understanding the migratory nature of lymphocytes residing within the intestinal epithelial layer will facilitate the design of novel therapeutics for the treatment of human intestinal diseases. One technique that has been used to analyze the requirements for migration of T cells to the intestine is parabiosis. Parabiotic mice are surgically attached to one another, leading to shared circulation between pairs, and have been used to examine IEL population dynamics in vivo. In this manner, lymphocytes from congenically distinct, surgically conjoined mice can be distinguished from one another to examine lymphocyte migration. Early studies demonstrated that although lymphocytes in the spleen and lymph nodes rapidly equilibrated between parabionts, IELs retained the host congenic allele for several weeks [62, 63]. These studies demonstrate that little recirculation of CD8 $\alpha\beta^+$ IELs or other IEL subsets occur under steady-state conditions. More recent studies using immunized mice show a similar finding for memory CD8 $\alpha\beta^+$ IELs. After systemic virus infection, immune mice were surgically joined and monitored for several weeks. Small numbers of donor memory CD8 $\alpha\beta^+$ T cells gradually infiltrate the IEL compartment but never completely equilibrate with the resident population [7]. To a similar end, a small section of naïve-donor small intestine was engrafted into lymphocytic choriomeningitis virus-immune mice to examine recirculation of memory CD8 $\alpha\beta^+$ T cells to the IEL compartment. Minimal infiltration of host memory CD8 $\alpha\beta^+$ T cells occurred [35]. However, forced recirculation of IELs by adoptive transfer of donor IEL into lymphopenic mice demonstrate that IEL-derived CD8 $\alpha\beta^+$ T cells preferentially home back to the IEL compartment and undergo IL-15-dependent proliferation [64]. These studies collectively demonstrate that, under homeostatic conditions, memory CD8 $\alpha\beta^+$ T-cell migration to the IEL compartment is limited, whereas exit of IELs from the epithelium back into circulation is not likely to occur. Whether these events are altered in pathology or infection is not known, but these data suggest that therapeutic approaches in the treatment of human intestinal diseases could focus on limiting initial migration into the intestines (Table 1). However, it should be noted that the effect of continued inflammation on the recruitment of memory or memory-phenotype CD8 $\alpha\beta^+$ lymphocytes to the intestinal mucosa remains to be resolved.

Intraepithelial Lymphocyte Regulation by Intestinal Epithelial Cells

The ability of IEC-derived cytokines to modulate intestinal IELs has been a recent and exciting theme for mucosal immunologists. IECs express numerous pattern recognition

receptors, typically associated with activation of innate cells of the immune system and paramount for the detection of microbe-associated molecular patterns of pathogenic and nonpathogenic organisms of the small intestine [65]. Indeed, mixed bone marrow chimeras generated from normal and MyD88-deficient mice demonstrate the critical importance of nonhematopoietic-derived MyD88-dependent signals for the efficient control of oral *Listeria monocytogenes* and *Citrobacter rodentium* infections [66•, 67]. IL-15 expression in the intestine also appears to be regulated by MyD88-dependent IEC-derived signals [19] and IL-15 transpresentation via IL-15R α expression on intestinal parenchymal cells is required for CD8 $\alpha\beta$ ⁺ IEL development [68]. Moreover, aberrant IL-15 expression in the intestinal mucosa, particularly by IEC, is a hallmark of celiac disease in humans [69••]. Novel therapeutic approaches could specifically target IEC-derived IL-15 or downstream signals generated from aberrant IL-15 signals (Table 1). For example, IL-15 induces expression of the antiapoptotic molecules Bcl-2 and Bcl-xL in an IL-15R β -dependent but IL-15R α -independent manner in human IEL cell lines derived from patients with type 2 refractory celiac disease [69••]. Moreover, in a celiac disease model in which mouse enterocytes overexpress human IL-15, IEL apoptosis was induced by blockade of IL-15 signaling with a fully humanized human IL-15-specific monoclonal antibody (AMG714) or with a Bcl-2 homology domain 3 (BH3) mimetic, ABT-737 [69••]. An additional study was able to ameliorate autoimmune intestinal damage by targeting IL-15R β by antibody blockade in the same murine model [70••]. Together, these studies demonstrate the feasibility and potential of targeting IL-15 or its downstream mediators in the treatment of celiac disease (Table 1).

Recently, thymic stromal lymphopoietin (TSLP) production by IEC was identified as a potential modulator of mucosal immunity. TSLP IEC expression is minimal in naïve mice, yet can be induced by Toll-like receptor ligation [71, 72]. One function of TSLP is to reduce levels of IL-12p40 production in DCs, thereby impairing their ability to promote antigen-specific CD4 T_H1 responses and limiting the expression of proinflammatory cytokines [71, 72]. Consistent with this, TSLP^{-/-} mice have more rapid onset and greater severity of disease in a mouse model of colitis, and colonic epithelium from Crohn's disease patients have lower expression of TSLP [71, 72]. TSLP may also regulate CD8 $\alpha\beta$ ⁺ T-cell responses directly within the intestinal epithelium. The TSLP-specific receptor chain is expressed by CD8 $\alpha\beta$ ⁺ T cells in vivo, is upregulated following activation, and regulates survival by increasing Bcl-2 expression [73]. Clarification of the role of TSLP in human intestinal disease is still required, but these cytokines provide potential and attractive targets for the design of future therapeutic modalities (Table 1). Thus, IEL do not exist as solitary soldiers among the vast epithelial cell network, but interact with IEC to provide as well as to receive signals to maintain epithelial integrity.

Conclusions

The immune system of the gut is constantly assaulted by microbial pathogens and commensal organisms and must rapidly respond to epithelial breach with sterilizing immunity while moderating overt inflammation. To accomplish this task, a diverse population of intestinal IELs—comprised of conventional and nonconventional T cells—exerts a range of vital effector functions. IEL subsets can provide immunosurveillance at the epithelial barrier to prevent or impair infection in the intestine either through innate-like mechanisms or as antigen-specific effector or memory CD8 $\alpha\beta$ ⁺ T cells. Moreover, these populations are vital for the regulation of intestinal homeostasis and epithelial cell healing and repair, and are in turn regulated by these cells. However, we are only now starting to unravel the complex and sophisticated network of mucosal immune responses, their regulation, and potential targets for novel therapies to alleviate intestinal diseases (Table 1). More work is required to understand how basic knowledge of the mucosal immune system can be translated into efficacious therapies to relieve human disorders, including IBD and

celiac disease, and used to design effective mucosal vaccines to prevent or treat infectious diseases.

Acknowledgments

Disclosure Conflicts of interest: Dr. Sheridan's institution has received grants from the Crohn's and Colitis Foundation of America. Dr. Lefrançois's laboratory has received grants from the National Institutes of Health.

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Table 1

Identification of potential therapeutic targets

Potential target(s)	Role for lymphocytes/IELs	Drug development	Clinical trials associated with target or potential for treatment
Integrin $\alpha 4$	Regulates migration to intestinal tissues but also other nonintestinal tissues (eg, CNS)	Natalizumab	Multiple sclerosis, Crohn's disease, and multiple myeloma
Integrin $\alpha 4\beta 7$	Regulates migration to the LP and IEL compartments	Vedolizumab/MLN0002	Crohn's disease and ulcerative colitis
IL-15	Promotes the survival of effector and memory lymphocytes by the upregulation of antiapoptotic molecules	AMG714	Rheumatoid arthritis and psoriasis; celiac disease
IL-15 receptor components	Required for IL-15 signaling; some IL-15 receptor components are shared with the IL-2 receptor	Mik- β -1 (specific for the shared IL-2/-15R β subunit)	Leukemia and HTLV-1; celiac disease
Bcl-2/Bcl-xL	Regulates T-cell survival by inhibiting apoptosis	Oblimersen, SPC2996, Obatoclox, and ABT-737	Numerous cancers including colorectal cancers
CCL25/CCR9	Chemokine/receptor pair that regulates lymphocyte trafficking to the IEL	GSK1605786 (CCR9 antagonist)	Crohn's disease
CD103	May function to retain lymphocytes in the intestinal epithelium	None known	No current trials but high potential
TSLP	Regulates inflammation by reducing proinflammatory cytokine production	None known	No current trials but high potential

CNS central nervous system; *HTLV* human T-cell lymphoma virus; *IEL* intraepithelial lymphocyte; *IL* interleukin; *LP* lamina propria; *TSLP* thymic stromal lymphopoietin

Information for this table was obtained from ClinicalTrials.gov and references listed throughout this review