



Published in final edited form as:

Semin Immunol. 2009 June ; 21(3): 130–138. doi:10.1016/j.smim.2009.03.003.

Intestinal T cells: Facing the mucosal immune dilemma with synergy and diversity

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Abstract

The epithelium of the gastrointestinal tract, which represents the greatest body surface area exposed to the outside environment, is confronted with a plethora of foreign and potentially harmful antigens. Consequently, the immune system of the gut faces the daunting task of distinguishing harmless dietary proteins and commensal bacteria from potentially dangerous pathogens, and of then responding accordingly. Mucosal T cells play a central role in maintaining barrier function and controlling the delicate balance between immune activation and immune tolerance. This review will focus on the unique features of mucosal T cell subsets that reside in the epithelium and lamina propria of the gut.

Keywords

Intestinal barrier; Intraepithelial lymphocytes; Lamina propria lymphocytes; CD8 $\alpha\alpha$; Regulatory T cell

1. Introduction

The intestine represents a major immune organ with several specialized lymphoid structures and cell types and has been roughly divided into the inductive organized gut-associated lymphoid tissue (GALT) and effector sites. Organized GALT includes Peyer's Patches (PP), isolated lymphoid follicles, the appendix, and the gut draining mesenteric lymph nodes (MLN), whereas effector cells accumulate in the lamina propria (LP) as lamina propria lymphocytes (LPL) and within the epithelium as intraepithelial-lymphocytes (IEL).

The challenging function of the gut immune system is to prevent penetration and spreading of commensals and pathogens while avoiding excessive or unnecessary immune responses. Several levels of protective barriers can be distinguished that act to avert microbial invasion of the host. The first line of passive defense attempts to prevent intact antigens and pathogens from entering the body and encountering the immune system (immune exclusion). The second immune barrier is formed by an active innate immune sensing system that provides a combination of maintaining homeostasis and initiating active pro-inflammatory immune responses to microbial invasion by immune surveillance at the mucosal surface. Finally, a highly developed adaptive immune system regulates the responses to antigens that have crossed the epithelial barrier.

The mucosal epithelium is formed by a single layer of tightly connected intestinal epithelial cells (IEC) and acts as a physical protective wall, separating luminal antigens from the underlying tissue compartments. The microvilli of the brush border and the tight junctions between the IEC are essential structural components in regulating permeability of the mucosal border. Specialized IEC, such as the paneth cells and associated innate immune cells also secrete several defensive compounds including mucins, proteolytic enzymes, nitric oxide, and anti-microbial peptides, both constitutively and in response to microbes [1]. In addition to these physical defense systems, antibody-secreting B cells play a key role in maintaining immunological quiescence (reviewed in Ref. [2]). At least 70% of all plasma cells are found in the gut LP and there is more IgA secreted than the total of all other Ig isotypes combined [3]. IgA class-switching is promoted by TGF- β , a cytokine that is abundantly present in the gut mucosa and the secretion of IgA is controlled by several factors including IL-6 and retinoic acid [4]. Most plasma cells produce dimers of secretory IgA (sIgA) antibodies that are exported to the gut lumen and function by entrapping dietary antigens and micro-organisms in the mucus leading to their excretion and by preventing microbial components from attaching to the epithelium. In the gut wall, locally produced IgA can also interact with antigens that have reached the LP and the resulting immune complexes are either taken up by phagocytosis or transcytosed back to the lumen, again enforcing immune exclusion [5].

The barrier is not absolute however and under normal conditions there is extensive crosstalk between the luminal microbes and all arms of the mucosal immune system, coordinated by the IEC and dendritic cells (DC) as messengers. The importance of these interactions is mirrored by the fact that without bacterial colonization of the intestine the structure and function of the intestine itself, including the mucosal immune system, are highly impaired. The crosstalk between the outside environment and concealed immune system is executed via continuous sampling of luminal antigens. The so-called M cells, which are specialized enterocytes that are located in the follicle-associated epithelium, constantly transport intact antigens to the mucosal lymphoid tissue underneath for processing and antigen presentation [6]. Their primary function of trans-cellular endocytosis is facilitated by several distinctive morphological features including a reduced brush border, the absence of a thick glycocalyx, and lack of enzymatic activity. Inevitably perhaps, M cells are also used by many pathogens as a route of entry into the body [6]. Besides these “gateways to the mucosal immune system”, DC have been shown to take up apoptotic IEC and their contents (such as endocytosed antigens) in addition to direct sampling of luminal antigens across the mucosal epithelia without compromising the integrity of the barrier. These processes are mainly associated with promoting tolerance and immune suppression in order to prevent damage of the intestinal barrier and for maintenance of immune homeostasis. In addition, the controlled sampling may also allow for the generation of immune memory before invasion by the pathogen. Overall, the sampling of the gut content may be a mechanism to specifically “adapt” the mucosal immune system to the environment (and therefore also the pathogens likely to be encountered) by the individual.

In the event however that pathogens cross the barrier and gain uncontrolled access to the mucosal immune system, strong and protective immune responses may be initiated that eliminate infections before they become apparent or spread systemically. The rapid and effective protection is ensured by the presence of numerous DC, macrophages, and plasma cells as well as various subsets of effector T cells. Both the LP and the epithelial compartment contain large numbers of antigen-experienced T cells that play a crucial role in protection of the barrier and the host. These memory T cell subsets differ significantly from each other in their ontogeny, the type of antigens they recognize, the signals they received for their differentiation, and the specific effector and/or regulatory function they exert. In this review we will focus on the diverse subsets of mucosal T cells. We will address issues regarding their development, differentiation, function, and interaction with other cell types in the intestinal mucosa.

2. Mucosal T cell subsets

In the intestine, large populations of T cells reside in three main compartments; the organized GALT, the LP, and the epithelium. Whereas organized structures of the GALT, such as the MLN, contain naïve T cells, almost all T cells in the LP and epithelium display characteristics of an effector/memory phenotype. Although these frontline T cells are notoriously heterogeneous with regard to their phenotype and function, two major subsets can be distinguished based on T cell receptor (TCR) and co-receptor expression [7]. The first group, or “type a” cells, consists of TCR $\alpha\beta$ ⁺ MHC class II-restricted CD4⁺ and MHC class I-restricted CD8 $\alpha\beta$ ⁺ lymphocytes that resemble conventional thymus-selected antigen-experienced T cells also found in the blood, spleen and other secondary lymphoid organs. After priming in response to their cognate antigen encountered in the periphery or at the local induction sites, these activated T cells migrate to the effector site of intestine, where some reside long-term as effector memory T cells. The second group, which is referred to as “type b” cells, express either TCR $\alpha\beta$ or TCR $\gamma\delta$ and they frequently express CD8 $\alpha\alpha$ molecules but lack expression of the typical TCR co-receptors CD4 or CD8 $\alpha\beta$. The different mucosal T cell subtypes are present to a varying degree in different anatomical mucosal compartments (LP versus epithelium, small versus large intestine) and their distribution depends on age, strain, and housing conditions. However, in general the T cell component of the LP is largely composed of type a cells, whereas type b cells are far more prevalent in the mucosal epithelium (for an overview see Fig. 1). In the following sections we will discuss different aspects, including function, of the type a and b LPL and IEL.

3. IEL and LPL

Intraepithelial lymphocytes reside within the columnar epithelial layer. When they have been looked for, they have been found in all vertebrates that possess a thymus, in both small and large intestine. However, their frequencies vary along the gut and from species to species. In the mouse, it is estimated that there is approximately one IEL per 5–10 IEC in the small intestine *versus* one IEL per 40 IEC in the colon [8]. In the small intestine of mice a large population of IEL expresses TCR $\gamma\delta$, while the remaining population consists of TCR $\alpha\beta$ ⁺ IEL that are mainly TCR $\alpha\beta$ ⁺CD8 $\alpha\alpha$ ⁺IEL, a subset of TCR $\alpha\beta$ ⁺CD8 $\alpha\beta$ ⁺ and fewer TCR $\alpha\beta$ ⁺CD4⁺ T cells. In human the proportion of TCR $\gamma\delta$ ⁺ T cells in the small intestine is smaller (about 10%) but numbers greatly increase under certain allergic and/or inflammatory conditions such as celiac disease [9]. Conventional type a CD4⁺ IEL are more abundant in the large intestine (about 30% of total IEL). The different distributions of T cell subtypes between the small and large intestine may be a reflection of different roles of IEL in these two compartments, with regions of high bacterial load presumably demanding strong protective barrier function, whereas other relatively sterile, highly absorptive regions may require greater immune regulation in response to luminal (food) antigens and for the induction of oral tolerance.

T lymphocytes scattered throughout the LP consist of mainly type a CD4⁺ T helper (Th) cells and (less) cytotoxic CD8 $\alpha\beta$ ⁺ T cells. Few non-conventional T cell subsets populate the LP but interesting subsets include invariant NKT [10] and mucosal-associated invariant T cells [11] that interact with the non-classical MHC molecules CD1d and MR1 respectively.

4. Type a mucosal T cells

Type a mucosal T cells are progeny of conventional naïve T cells and they have much in common with the antigen-induced memory cells in the periphery although they also display some distinct features. Type a cells gradually increase with age when more and more antigen-experienced T cells migrate and accumulate in the gut mucosa as long-lived memory cells. In contrast to type b mucosal T cells, type a cells are not confined to the epithelial compartment and they are abundant in the LP and the thoracic duct lymph as well [12]. Initial priming of

many (but perhaps not all) type a precursor cells, takes place in the organized lymphoid structures of the GALT. Upon luminal antigen uptake, DC migrate to the T cell areas of the PP and MLN in a CCR7-dependent fashion where they initiate T cell activation. The activated T cells then home to the gut and their recruitment is regulated by distinct sets of adhesion molecules expressed by the T cells and their respective ligands on vascular endothelial cells (reviewed in Ref. [13]). Several adhesion receptors have been implicated in regulating T cell entry and localization within the intestinal epithelium. These receptor–ligand pairs include LFA-1 and ICAM-1, integrin $\alpha_4\beta_7$, and MADCAM-1, the integrin $\alpha_E\beta_7$ (CD103) whose ligand E-cadherin is expressed on the basolateral surface of IEC and the chemokine CCR9, whose ligand CCL25 is constitutively expressed by small intestine IEC.

How are these gut homing T cells generated? The local mucosal lymphoid environment, rather than the nature of the antigen, plays a major role in imprinting homing properties of the primed T cells [14]. It was demonstrated that DC from PP and MLN have enhanced ability to generate gut-tropic T cells compared to splenic and peripheral lymph node DC. A seminal study by Iwata et al. further showed that this unique capacity of the mucosal DC was mediated through the selective release of the Vitamin A metabolite, retinoic acid (RA) by mucosal DC during priming [15]. RA induces $\alpha_4\beta_7$ and CCR9 on activated CD4⁺ and CD8⁺ T cells and is pivotal for the imprinting of gut-homing cells as shown by the severe reduction in T cells in the small intestine of vitamin A deficient mice [15]. More recent studies suggest that the RA-producing gut IEC and MLN stromal cells may also contribute to the induction of gut homing by the mucosal DCs [16,17]. Expression of CD103 on IEL is initiated by TGF- β and the transcription factor Runx3 [18,19] and facilitated by signaling through CCR9 [20].

Whereas the regulation of T cell migration to the small intestine is well described, the mechanisms underlying recruitment of T cells to the large intestine remain poorly defined. Although colon-tropism is dependent on $\alpha_4\beta_7$ (but not CCR9) [21], RA seems neither necessary nor sufficient to induce T cell migration to the large intestine [21,22].

5. Type a IEL function and regulation

In the small intestine type a IEL are mostly CD8 $\alpha\beta^+$ TCR $\alpha\beta^+$ memory cells that show cytolytic effector function upon antigen challenge. Compared to central memory T cells in the spleen, these effector memory T cells can be rapidly activated and they may provide initial immediate cytotoxic responses to local infection [23] (Fig. 2). Their TCR repertoire is more restricted than that of peripheral memory CD8 $\alpha\beta^+$ T cells suggesting that repeated re-stimulation in the intestine may lead to TCR focusing or that a TCR-dependent mechanism drives the selective migration and/or differentiation of mucosal CD8 $\alpha\beta$ effector memory T cells [24]. While antigen stimulation can drive their proliferation, cytokines such as IL-7 and IL-15 mediate their slow turnover in the absence of TCR triggering [25].

After activation and migration to the intestine some CD8 $\alpha\beta^+$ effector T cells reside as effector memory IEL and they can be rapidly activated in response to local re-infections (reviewed in Ref. [26]). Transfer of antigen-specific type a CD8 $\alpha\beta^+$ IEL to infected hosts has demonstrated indeed that these memory T cells play an important role in providing immune protection to several infections including LCMV [27], rotavirus [28], *Toxoplasma gondii* [29], and *Giardia lamblia* [30].

Although most memory CD8 $\alpha\beta^+$ IEL retain an activated phenotype (e.g. CD69 expression and maintenance of a heightened cytolytic state) that enables them to rapidly respond to antigen re-exposure, they also display reduced ability to proliferate or produce inflammatory cytokines [31]. These unique features might have evolved in order to maintain the balance of immunological protection without compromising organ integrity. In this respect, most type a CD8 $\alpha\beta^+$ IEL co-express CD8 $\alpha\alpha$. In contrast to CD8 $\alpha\beta$ however, which functions as a TCR co-

receptor to enhance TCR signals, CD8 $\alpha\alpha$ functions as a co-repressor of TCR signaling [24]. We showed before that the transient expression of CD8 $\alpha\alpha$ during primary activation rescues the cells from activation induced cell death (AICD) and allows for their further differentiation to memory T cells [32]. CD8 $\alpha\alpha$ is readily re-induced on migrating effector cells in the gut environment and it is possible that constitutive expression of CD8 $\alpha\alpha$ on these CD8 $\alpha\beta^+$ IEL increases the threshold for TCR activation to prevent aberrant activation of these effector type T cells. In addition, CD8 $\alpha\alpha$ may interact with its ligand TL expressed on IEC to control homeostatic proliferation [33], survival and T cell activation [31,34]. Another example of how the tissue environment may influence local T cell responses is the expression of the P2X7 purinoreceptor by IEL. It was recently shown that CD8 $\alpha\beta^+$ IEL selectively express high levels of P2X7, probably induced by RA in the gut environment [35]. Expression of P2X7 was associated with increased ATP-induced apoptosis and in P2XY-deficient mice enhanced intestinal, but not spleen CD8 T responses, were observed following *Listeria monocytogenes* infection [35].

Although most type a IEL are CD8 $\alpha\beta^+$ CTL, CD4 $^+$ T cells are also present within the epithelium, especially in the colon however they are more prevalent among the LPL.

6. Type a LPL function and regulation

All classical CD4 $^+$ Th subsets can be found in the intestinal LP including the Th1 subset that drives cell-mediated immune responses associated with intracellular infection and cytotoxicity and the Th2 subset that is involved in IgE production, clearance of helminth infection, and allergic sensitization. However, in the past few years it has become apparent that the LP is also home to (1) a Th cell population that constantly produces pro-inflammatory cytokines such as IL-17A, IL-22, and IL-17F, the so-called Th17 cells and (2) high percentages of Foxp3-expressing regulatory T cells (Treg) (Fig. 2).

Foxp3 $^+$ Treg are known to play an important role in intestinal homeostasis and one of the most pronounced features of the immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome, caused by a mutation in the *FOXP3* gene, is intestinal inflammation [36]. Furthermore, it was demonstrated that transfer of Treg can inhibit and even cure colonic inflammation in a naïve T cell transfer colitis model [37]. Mechanisms of action by Treg include production of regulatory cytokines (IL-10 and TGF- β) and expression of inhibitory receptors such as CTLA-4 [38]. Both thymus-derived natural Treg and peripherally induced Treg preferentially accumulate in the gut. The mechanism of induced Treg differentiation in response to peripherally encountered antigens may be of particular benefit to the intestine, where the immune system has to deal with an enormous load of innocuous foreign antigens derived from the food and the flora. The environment of the gut is indeed thought to favor Treg conversion and CD103 $^+$ DC that are enriched in the LP and MLN are highly effective in inducing Foxp3 $^+$ Treg in an antigen-specific manner [39,40]. This induction is depending on TGF- β which is abundantly present in the intestine. Although TGF- β , along with its unique ability to induce Foxp3 $^+$ Treg, has been mainly associated with immune suppression, a key study from Veldhoen et al. showed that TGF- β in the context of the pro-inflammatory cytokine IL-6, supports *de novo* differentiation of pro-inflammatory effector Th17 cells [41]. In the gut, IL-17 producing cells play an important role in the host defense against extracellular bacterial and fungal pathogens [42]. Interestingly, under steady-state conditions “naturally occurring” Th17 cells are also (selectively) enriched in the LP of the gut, demonstrating that the mutually exclusive pathways of Treg and Th17 development co-exist in, and probably contribute to, intestinal immune homeostasis. We recently identified RA as a key regulator in controlling the pro- and anti-inflammatory TGF- β -driven immune balance [43]. In addition to promoting induced-Treg conversion [39,43], RA is also capable of inhibiting the TGF- β /IL-6-driven induction of Th17 cells [43]. Distinct intestinal APC subsets that have the capacity to release

RA during priming play a prominent role in this immune deviation. CD103⁺ DC from the LP and MLN are most efficient in generating Treg (compared to CD103⁻ and splenic DC) and the induction of Treg can be blocked by inhibition of the retinal-converting enzyme retinal dehydrogenase (*Aldh*), which is highly expressed in this DC subset. In contrast, a population of CD11c^{hi}CD11b^{hi} LP DC has been shown to promote Th17 differentiation [44] and the presence of the TLR5 ligand flagellin further increased this ability [45]. Finally, LP resident macrophages seem to favor Foxp3⁺ Treg differentiation via a mechanism depending on IL-10, RA and exogenous TGF- β [44].

The preferential accumulation of both Treg and Th17 cells in the LP of the intestine seems to be at least in part regulated by commensal bacteria. The presence of commensal DNA was shown to limit the conversion of Treg in a TLR9-dependent fashion [46]. TLR9-deficient mice displayed increased frequencies of Foxp3⁺ Treg and reduced IL-17 producing cells in the LP. In contrast, it has also been reported that commensals can induce Treg, which protects the host from pathogen-induced inflammatory responses [47]. In line with this, induction of Treg has been suggested as one of the mechanisms of action of probiotics.

Data from several papers indicate that commensal bacteria are required for IL-17 production by intestinal type a cells, since germ-free mice contain virtually no Th17 cells in the LP [48–50]. Surprisingly, the “spontaneous” production of IL-17 in the LP was independent of TLR (MyD88 or Trif) signaling [49,50]. It was suggested that ATP produced by commensals can drive Th17 differentiation by stimulating a subset of LP DCs to produce TGF- β , IL-6, and IL-23 [50].

7. Type b mucosal T cells

Most type b mucosal T cells reside in the epithelial compartment of the small intestine and express either TCR $\alpha\beta$ or TCR $\gamma\delta$. Although these type b TCR $\gamma\delta$ ⁺ and TCR $\alpha\beta$ ⁺ IEL are clearly different from one another, they share many “unconventional” characteristics that distinguish them from type a IEL [51]. Type b IEL contain a large number of “self”-reactive T cells and in addition to their activated phenotype they also typically express CD8 $\alpha\alpha$ homodimers in the absence of the TCR co-receptors CD4 or CD8 $\alpha\beta$ and they lack expression of some “typical” T cell markers including CD2, CD28, and Thy-1. They are already present at birth and remain the dominant IEL population in young animals. In older mice type b IEL are gradually replaced by an expanding pool of type a effector/memory T cells. Nonetheless, abundant type b IEL were detected when wild mice were sampled. Although some IEL can be detected at fetal stages, the major colonization of the gut epithelium by (mostly type b) IEL takes place perinatally. Two important events shortly after birth have a huge impact on IEL numbers: first the microbial colonization of the gut immediately after birth and second the changes in antigen load and composition occurring at weaning [52]. These events are associated with waves of IEL seeding. The importance of exogenous antigen challenge in the development of TCR $\alpha\beta$ ⁺ IEL is demonstrated by the fact that in germ-free mice IEL numbers are greatly reduced and skewed toward $\gamma\delta$ T cells. However, early migration of type b IEL to the intestine seems, in contrast to type a IEL, to be independent of antigen-stimulation but crucially depending on a CCR9/CCL25- and $\alpha 4\beta 7$ -integrin-mediated pathway [53]. In addition, whereas conventional type a IEL trafficking crucially depends on sphingosine-1 phosphate (S1P), S1P does not play a role in the migration pathways of type b IEL [54].

Even though it is clear that CD8 $\alpha\alpha$ ⁺ IEL do not follow the pathway of conventionally selected thymocytes, their precise ontogeny, intermediate stages of development, as well as the sites where they develop have long been a matter of debate (reviewed in Ref. [55]). CD8 $\alpha\alpha$ ⁺ IEL were initially classified as thymus-independent T cells, however more recent studies showed that under normal conditions all gut IEL, including the type b IEL, are of thymic origin.

There is increasing evidence from several sources that both $\gamma\delta$ and $\alpha\beta$ type b IEL are at last in part functionally programmed during thymic differentiation. Moreover, there are data strongly supporting the hypothesis that $\text{TCR}\alpha\beta^+\text{CD8}\alpha\alpha^+$ IEL precursors undergo a unique self-antigen-dependent agonist selection process. In the thymus, conventional T cells are selected based on an intermediate, positive signal through their $\text{TCR}\alpha\beta$, which allows further maturation (positive selection), whereas cells that have a too strong affinity for self-antigens are clonally deleted (negative selection). Elimination of auto-reactive T cell by negative selection is believed to represent one of the main mechanisms for the establishment of central tolerance. However, recent evidence suggests that alternative pathways exist that specifically select for auto-reactive T cells (agonist selection) [56–58]. Like $\text{CD4}^+\text{CD25}^+$ regulatory T cells and NKT cells, $\text{TCR}\alpha\beta^+\text{CD8}\alpha\alpha^+$ IEL precursor cells preferentially accumulate under conditions that lead to the deletion of conventional $\text{TCR}\alpha\beta^+$ T cells [56,59,60]. Consistent with this, “forbidden” TCR combinations can be found within the $\text{TCR}\alpha\beta^+\text{CD8}\alpha\alpha^+$ IEL repertoire that are normally purged from the thymic repertoire by stromal presentation of “super-antigens” [61]. Thymic selection of $\text{TCR}\alpha\beta^+\text{CD8}\alpha\alpha^+$ IEL precursor cells seems to be most effective in the presence of high-affinity self-antigens [58,60,62] and the selection process drives their initial functional and phenotypic differentiation [58]. Agonist selected $\text{TCR}\alpha\beta^+\text{CD8}\alpha\alpha^+$ IEL precursor cells exit the thymus as $\text{TCR}\alpha\beta^+\text{CD4}^-\text{CD8}^-$ double negative (DN), and they migrate directly to the intestine in a SIP independent fashion. Further maturation, including the induction of $\text{CD8}\alpha\alpha$, occurs extra-thymically in the IL-15-rich environment of the gut [63].

Normal $\text{CD8}\alpha\alpha^+$ IEL homeostasis requires several cytokines and whereas IL-7 is indispensable for the development of $\text{TCR}\gamma\delta^+$ IEL, in mice lacking IL-15, both $\text{CD8}\alpha\alpha^+$ $\text{TCR}\alpha\beta^+$ and $\text{TCR}\gamma\delta^+$ IEL are strongly reduced. In a recent paper it was demonstrated that IL-15 does not affect IEL development in the thymus however, but mainly controls homeostasis of both $\text{TCR}\gamma\delta^+$ and $\text{TCR}\alpha\beta^+$ $\text{CD8}\alpha\alpha^+$ IEL in the intestine [64]. Furthermore, it was shown that toll-like receptor (TLR) signaling via MyD88, induces IL-15 production by IEC and plays a crucial role in the maintenance of $\text{TCR}\gamma\delta^+$ and $\text{TCR}\alpha\beta^+$ $\text{CD8}\alpha\alpha^+$ IEL [65]. This suggests that crosstalk between commensal bacteria and the epithelium is also important for type b IEL homeostasis.

8. Type b IEL function and regulation

The unique localization of IEL is associated with an unusual T cell repertoire and antigen-specificity. The $\text{TCR}\alpha\beta$ repertoire of type b is oligoclonal [66,67] and contains numerous self-reactive TCR [61]. Results showing that the TCR repertoire of $\text{TCR}\alpha\beta^+\text{CD8}\alpha\alpha^+$ IEL from the fetal intestine or from littermates in the same cage or from germ-free mice showed the same degree of random oligo-clonality suggest that the microflora is not responsible for the antigen specificity of the repertoire of these cells [67,68]. However, $\text{TCR}\alpha\beta^+\text{CD8}\alpha\alpha^+$ IEL numbers are highly reduced in germ-free mice and after initial seeding of these cells in the intestine, the microflora is thought to induce initial oligoclonal expansion and continuous renewal. The $\text{TCR}\gamma\delta^+$ T subset is the only intestinal IEL population that is present under germ-free conditions and has been reported to display a restricted TCR repertoire in mice [69].

$\text{TCR}\alpha\beta^+\text{CD8}\alpha\alpha^+$ IEL are enriched for self-antigens [61], which would be consistent with the proposal that they are agonist-selected during ontogeny. However, both identity and nature of the possible auto-antigens for type b IEL are unknown and research is complicated by the fact that $\text{TCR}\alpha\beta^+\text{CD8}\alpha\alpha^+$ IEL may be reactive with either classical class I molecules, non-classical class I molecules, or even class II molecules [56]. $\text{TCR}\alpha\beta^+\text{CD8}\alpha\alpha^+$ IEL are drastically reduced in $\beta 2$ -microglobulin ($\beta 2m$)-deficient mice [70], but they are present in mice lacking transporter associated with antigen-processing (TAP) [71] or classical MHC class I molecules [72]. Therefore, it has been hypothesized that one or more $\beta 2m$ -dependent, TAP-independent non-classical MHC class I molecules are responsible for their generation and/or maintenance. Proposed candidates include Qa-2 [73] and thymus leukemia antigen (TL), both abundantly

expressed by IEC, although an interaction between these molecules and the TCR $\alpha\beta$ of CD8 $\alpha\alpha^+$ IEL has never been shown. Nevertheless, CD8 $\alpha\alpha$ does bind with high affinity to TL and this interaction does not lead to activation, but has been shown to restrain proliferation and modulate reactivity of CD8 $\alpha\alpha$ IEL [24,34,74].

Although ligand recognition by TCR $\gamma\delta^+$ IEL also remains elusive, data suggest that most $\gamma\delta$ T cells are activated upon TCR- or natural killer receptor (NK)-mediated recognition of a restricted set of conserved yet poorly defined endogenous stress determinants [75]. In some cases, they can recognize unprocessed antigens directly. In humans, the (V δ 1 γ) TCR and activating NK-receptor NKG2D expressed on $\gamma\delta$ IEL both directly recognize the highly polymorphic class I-like molecules MICA and MICB that are induced on IEC upon stress [76]. Activation of the $\gamma\delta$ T cell by this interaction may lead to fast cytolysis of the stressed (e.g. damaged or infected) IEC thereby hampering systemic dissemination of pathogens. In a recent study by Jensen et al. it was shown that antigen-expression in the thymus followed by thymic selection has little influence on antigen specificity of a subpopulation of $\gamma\delta$ T cells [77]. This finding suggests that TCR $\gamma\delta$ recognition may also include pathogen-derived or stress-induced self-antigens that may not be expressed in the thymus.

One of the most prominent features of type b IEL is their “activated yet resting” phenotype. Although they are cytolytic upon isolation from the intestinal epithelium in the absence of overt stimulation they do not seem to behave as activated T cells in other ways. They have a limited capacity to proliferate and their presence often correlates with immune quiescence instead of productive immunity. This dual identity was confirmed by serial analysis of gene expression (SAGE) and subsequent gene microarray data showing that CD8 $\alpha\alpha^+$ IEL express high levels of particular effector molecules including Granzyme, Fas ligand, RANTES, and CD69, whereas common cytokines such as IFN- γ , IL-2 and IL-4, and cytokine-receptors (IL-2R α , IL12-R) were underrepresented [78,79]. Furthermore in addition to CD8 $\alpha\alpha$, they also highly express other molecules associated with immune regulation including LAG-3, CTLA-4, PD-1, TGF- β , and the inhibitory NK receptors 2B4, Ly-49 A, E, and G [78]. Together these findings imply a continuous balancing act for type b IEL between an activation and hyporesponsive state and although these partially activated cells may gain a full activation status upon the appropriate signal, their main function may not entail destructive effector immunity.

What is then the physiological function of these cytotoxic unconventional T cells residing at the front line of the intestinal mucosa? How do they behave and survive in the harsh environment of the gut? Are they effector or regulatory cells, warriors or peacekeepers, front runners or bystanders, or all of them in one? Very few answers to these questions are available and addressing function and behavior has been hampered by the unavailability of defined antigen-specific type b IEL clones and of mice exclusively lacking type b IEL. However, one of the major known functions of IEL, particularly of TCR $\gamma\delta^+$ IEL, is the control of IEC homeostasis. Under homeostatic conditions TCR $\gamma\delta^+$ IEL regulate the continuous turnover of IEC [80,81]. In addition, $\gamma\delta$ T cells can enhance epithelial cell growth *in vitro* through secretion of keratinocyte growth factor [82], which indicates that they may be involved in epithelial damage repair elicited by inflammation. In line with this, TCR $\delta^{-/-}$ mice show increased susceptibility to DSS- and hapten-induced colitis [83,84].

Although type b IEL have minimal pathogen-specificity they may allow for immune protection and prevention of microbial invasion by elimination of infected IEC through direct recognition of self-antigen and stress-induced receptors. Accordingly, data from an intracellular *Eimeria vermiciformis* infectious model suggest that IEL are involved in killing infected IEC while sustaining barrier function through the expression of junctional molecules and the production of TGF- β [85]. Furthermore, $\gamma\delta$ IEL have been shown to restrict epithelial transmigration of *Toxoplasma* and *Salmonella* [86] by transforming tight junctions, thereby maintaining

epithelial barrier function. $\gamma\delta$ IEL have also been demonstrated to enhance protective immunity of pathogen-specific CD8 $\alpha\beta^+$ T cells in *Toxoplasma gondii* infection [29] and stimulate innate immunity by rapidly producing IL-17 upon bacterial infection [87]. However, in general the absence of type b IEL does not seem to significantly compromise protective immunity against a range of pathogens [88]. Alternatively, type b IEL have been implicated as immune regulators in several infectious and inflammatory models. In the case of infection with the IEC-specific protozoa *E. vermiformis*, TCR $\delta^{-/-}$ mice were able to effectively clear the infection but also displayed exaggerated intestinal damage, apparently due to a failure to regulate the (consequences of) the TCR $\alpha\beta^+$ T cell response [89]. In an elegant study by Saurer et al. the impact of a potent LCMV infection on the potentially self-reactivity TCR $\alpha\beta^+$ CD8 $\alpha\alpha^+$ IEL was assessed [90]. They demonstrated by using a double transgenic mice expressing both LCMV epitope-specific TCR (under control of the MHC class I promoter) and the cognate antigen transgene that upon LCMV infection, TCR $\alpha\beta^+$ CD8 $\alpha\alpha^+$ IEL display signs of virus-induced activation. However, unlike the conventional virus-specific CD8 $\alpha\beta^+$ T cells, the responding self-reactive CD8 $\alpha\alpha^+$ IEL did not induce antigen-cytotoxicity nor did they promote an inflammatory response. Furthermore, it was also shown that TCR $\alpha\beta^+$ CD8 $\alpha\alpha^+$ IEL could prevent inflammation in the CD4 $^+$ CD45RB hi T cell transfer model of colitis [91]. Together these findings are in support of an immune regulatory role for type b IEL in the intestinal mucosa.

Although we are still far from fully understanding the physiological function of type b IEL subsets, they seem to be principally involved in (self-reactivity-induced) immune regulation and maintenance of gut immune homeostasis as has been hypothesized more than 30 years ago [92] (Fig. 2).

9. Concluding remarks

The immune system of the gut constantly faces a delicate balancing act between fighting pathogenic intruders on the one hand, and preventing excessive immune responses to harmless antigens and commensals on the other hand. This dilemma seems to be reflected in the functionally diverse mucosal T cell pallet. At the frontline of the intestinal barrier, cytotoxic effector memory cells and pro-inflammatory Th subsets provide rapid protective immunity, whereas Treg and unique self-reactive IEL maintain barrier integrity and immune homeostasis and control excessive inflammation. The differentiation, activation, and function of these intestinal T cell subsets are tightly regulated by interaction with other cell types and soluble factors in the gut environment. However, we are only starting to understand the mechanisms involved in the development and functions of this complex and sophisticated network of mucosal T cells and their cross-talk with other cells and the exterior. Further knowledge on these key players of mucosal immunology may provide novel strategies for the development of mucosal vaccines and new therapeutic avenues for the treatment of inflammatory bowel disease, celiac disease and food allergy.

Acknowledgments

This work was supported by a Ter Meulen Fonds fellowship from the Royal Netherlands Academy of Arts and Sciences (FW) and by Grant RO1AI050265 from the National Institute of Allergy and Infectious Diseases. The content is solely the responsibility of the authors and does not necessarily represent the official views of National Institute of Allergy and Infectious Diseases or the National Institutes of Health. This is manuscript 1103 from the La Jolla Institute for Allergy and Immunology.

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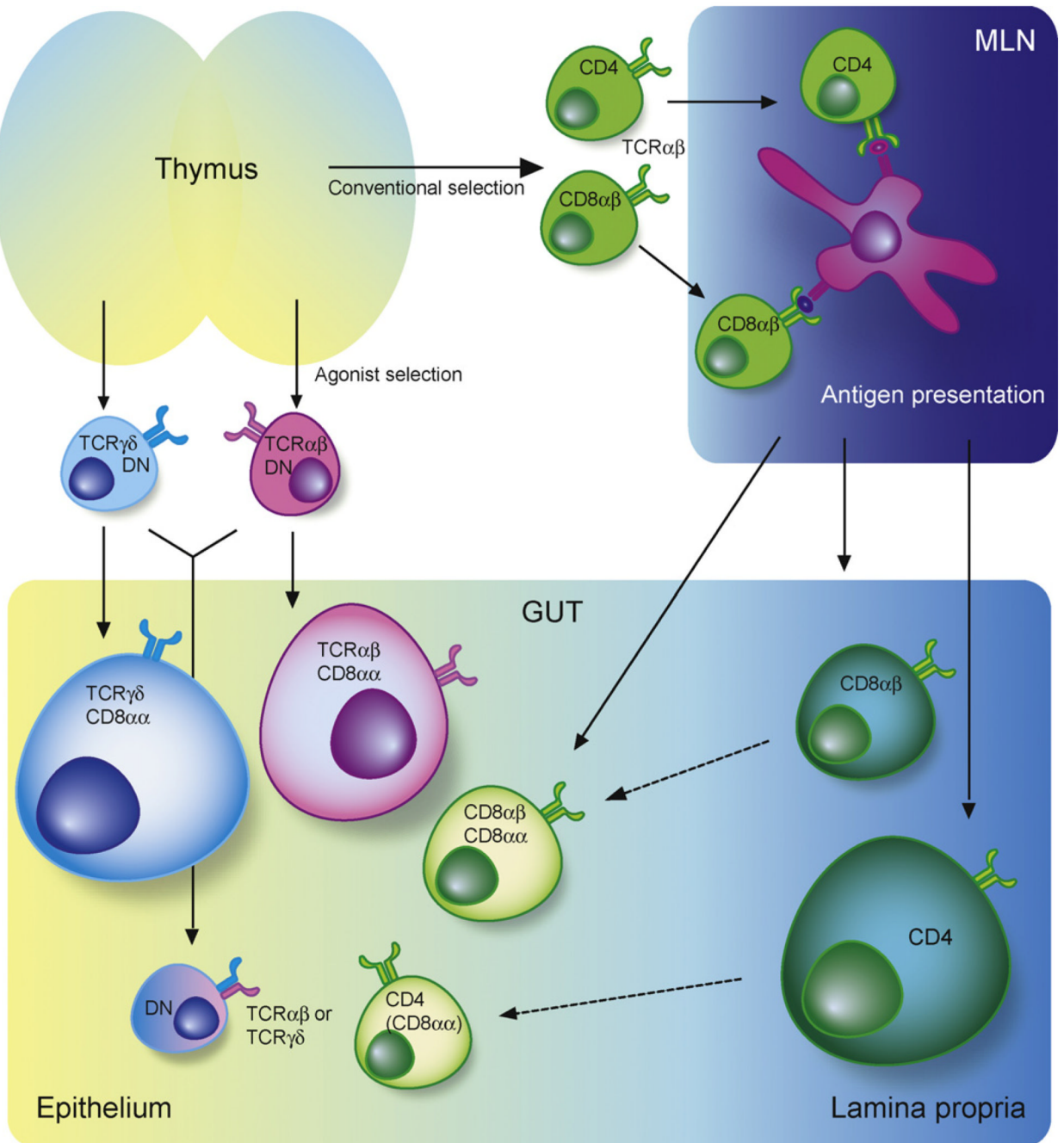


Fig. 1. Intestinal T cell subsets

The lamina propria (LP) and epithelium of the intestine harbor diverse populations of T cells. Conventional or “type a” mucosal T cells that have matured in the thymus along the conventional selection pathway migrate, after antigen priming in the mesenteric lymph nodes (MLN), to mainly the LP but also the epithelium. Upon entry in the epithelium these cells often co-express CD8αα. Most intraepithelial lymphocytes (IEL) however belong to two subsets of unconventional or “type b” mucosal T cell populations: the TCRγδ⁺CD8αα⁺ IEL that are thymus derived and develop along the double-negative pathway and the TCRαβ⁺CD8αα⁺ IEL that have matured and differentiated in the thymus along the agonist-selection pathway. Both

subsets migrate as antigen-experienced directly to the intestine where the majority of cells upregulate CD8 α , whereas some remain double-negative (DN).

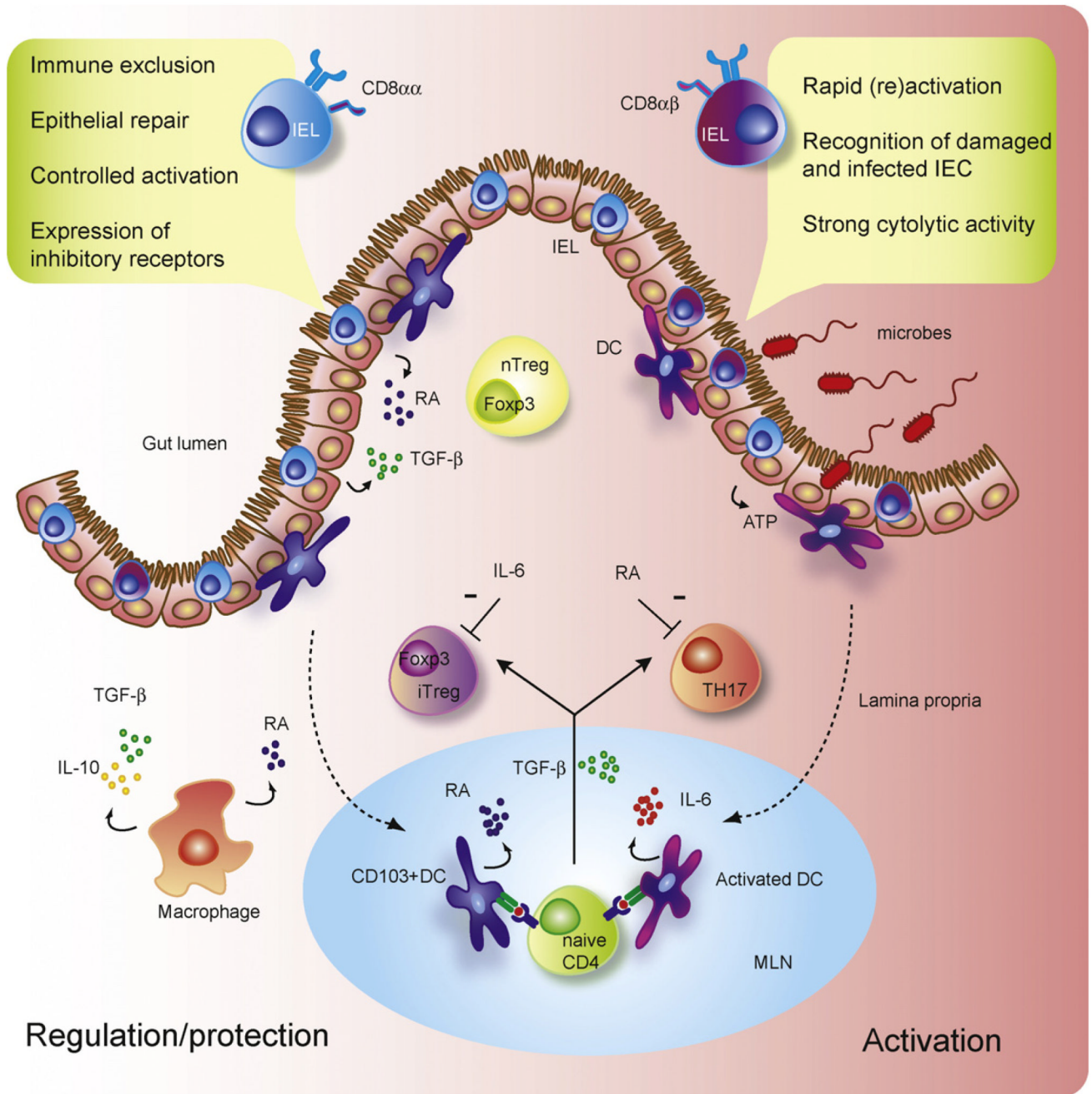


Fig. 2. Mucosal T cell regulation and activation

The functionally diverse T cell populations of the intestine that are shaped by the gut environment are important players in sustaining the delicate immune balance between activation and regulation. The CD8 $\alpha\alpha$ ⁺ intraepithelial cells (IEL) of the intestine play a crucial role in protection of the mucosal barrier. They are involved in maintaining and restoring barrier homeostasis by stimulating intestinal epithelial cell (IEC) turnover. Upon pathogen entry rapid activation and high cytolytic activity of the CD8 $\alpha\beta$ ⁺ IEL contribute to the prevention of pathogen spreading by killing infected IEC. The activation of IEL is highly controlled through the expression of inhibitory receptors that may alter the threshold for activation. Under the influence of IEC and IEL, lamina propria (LP) dendritic cells (DC) acquire ability to produce

retinoic acid (RA) thereby inducing gut-homing receptors during CD4 T cell priming in the mesenteric lymph nodes (MLN). Additionally, gut-derived CD103⁺ DC and LP macrophages favor the conversion of Foxp3⁺ induced regulatory T cells (iTreg) in a RA, TGF- β , and IL-10 (in the case of LP macrophages)-mediated fashion, whereas activated DC promote the differentiation of IL-17-producing Th17 cells via a combination of IL-6 and TGF- β . This pro- and anti-inflammatory immune deviation of iTreg and Th17 is reciprocally controlled by RA and IL-6. Finally, the LP is also home to agonist-selected Foxp3-expressing naturally occurring regulatory T cells (nTreg).