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Development, ontogeny and maintenance of TCRαβ+ CD8αα IEL

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Abstract

The intestinal epithelium is the outermost cellular layer that separates the body from the gut lumen. The integrity of this protective mucosal barrier is crucial and maintained by specialized cells—intraepithelial lymphocytes (IEL). Much research has been conducted on these cells and our overall understanding of them is increasing rapidly. In this review we focus on the TCRαβ$^+$ subset of CD8αα IEL. We discuss recent studies that shed light on the development, ontogeny, maintenance and functional characteristics of CD8αα IEL, and highlight yet unanswered questions for future studies.

Introduction

Intestinal intraepithelial lymphocytes (IEL) are comprised of innate lymphoid cells and T cells within the gut epithelium [1–3]. The T cell IEL include conventional resident memory TCRαβ$^+$ CD4$^+$ and CD8αβ$^+$ T cells, as well as unconventional TCRαβ$^+$CD4$^-$CD8αα$^+$β$^-$ and TCRγδ$^+$ IEL [1,4]. Despite these differences in their origin, IEL bear notable functional and phenotypic commonality, including expression of T-bet, NK receptors of the Ly49 family, IL-15 receptors, and an activated phenotype with high expression of CD69 [1,5]. IEL patrol the epithelium and help to maintain homeostasis and integrity of the barrier surface between gut lumen and the body [1,6,7]. Effector mechanisms include stimulation of antimicrobial peptide production in epithelial cells through HVEM stimulation [8] and epithelial cell proliferation via an AHR regulated pathway [9]. IEL further have a cytolytic profile, including Granzyme B expression [1,4,10]. Within IEL, this review will focus on the TCRαβ$^+$CD4$^-$ CD8αα$^+$β$^-$ IEL (hereafter called CD8αα IEL) and emerging concepts about their development.

Intestinal CD8αα IEL

The two main features that distinguish CD8αα IEL from conventional CD8$^+$ or CD4$^+$ IEL are: 1) self-reactive TCRs and 2) a co-receptor that consists solely of CD8αα homodimers.

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CD8αα homodimers bind the nonclassical MHC class I molecule thymus leukemia antigen (TL), which is expressed by intestinal epithelial cells [11,12]. Some research suggests CD8αα regulates TCR signaling strength, and TL-deficient mice develop more severe disease in experimental models of inflammatory bowel disease (IBD) [11,13]. CD8αα homodimer expression is also driven by distinct control elements at the *Cd8a* locus [14], although what upstream signals regulate it has not been fully flushed out. Regarding TCR self-reactivity in IEL, even though no specific self-peptide ligands for CD8αα IEL have yet been identified, several lines of evidence support the idea that this population is self-reactive. First, superantigen reactive "forbidden" Vβs are present amongst IEL [15]. Second, in TCR transgenic systems, CD8αα IEL numbers are increased by agonist self peptides [16]. Third, CD8αα IEL and their precursors are increased in Bim-deficient and in Bcl-XL transgenic mice, in which normally deleted self-reactive cells are rescued [17–19]. Lastly, the thymic precursors for CD8αα IEL (IELp) have an activated phenotype and express high levels of $Nur77^{GFP}$ [18,19]. Interestingly, hydrophobic amino acids such as cysteine at certain positions within the complementary determining region 3 (CDR3) of the TCR have recently been suggested to promote self-reactivity in cells such as IELp [20,21].

Regarding the MHC restriction of these cells, it has long been known that gut CD8αα are strongly reduced in number in β2M deficient mice that lack expression of all classical and non-classical MHC class I molecules [19,22,23]. However the abundance of thymic IELp was only mildly reduced by β2M deficiency [19], suggesting that class I molecules (including TL) may have a role in the tissue maintenance of CD8αα IEL, as well as act as a restricting element for TCR specificity and selection in the thymus. Of CD8αα IEL TCRs characterized to date, many are restricted to nonclassical MHC class-I molecules, but clones restricted to classical MHC class-I and MHC class-II were also found [18,24], and MHC-crossreactivity was common amongst thymic CD8αα IEL precursors [25].

It should be noted that the overwhelming majority of research on CD8αα IEL has been conducted in mice. A recent report however indicates that a similar subset might also exist in humans, albeit 'masked' by CD8αβ coreceptor expression in addition to the CD8αα homodimer [26], which should spur more research on these cells in humans in the future.

Thymic development of CD8αα IEL precursors

Recent work has shed much light on the developmental pathways of CD8αα IEL. Although there was controversy in the past about the site of CD8αα IEL development [15,27,28], there now is agreement that the majority of these cells arise in the thymus [1,4]. IELp have been identified within the $CD4^{-}CD8^{-}$ (DN) $TCR\alpha\beta^{+}$ pool [17–19,29,30]. During thymic maturation, IELp, like conventional T cells, upregulate MHC class-I (i.e. H-2Kb in C57BL/6 mice) and also CD122 [18,19,31]. CD122 is a shared subunit of the IL-15 receptor, a cytokine that is essential for CD8αα IEL survival. Nonetheless, thymic IELp do not depend on IL-15 [19,30,32], implying that the relevant source of IL-15 is in the gut [33].

IELp have high expression of CD69, $Nur77^{GFP}$, PD-1, and Egr2, indicative of strong and recent TCR activation[18,19]. But how do IELp escape deletion when they are strongly signaled in the thymus? Interestingly, IELp numbers were greatly enhanced in Bim-deficient

mice, wherein strongly TCR-signaled thymocytes escape apoptosis [19,34,35]. These results suggest a model where strongly signaled thymocytes are sometimes deleted and sometimes give rise to CD8αα IEL. Indeed, retrogenic expression of TCRs cloned from CD8αα IEL supported both clonal deletion and IEL generation in the thymus, with an estimated event outcome of 97:3, respectively [18]. This fate decision could be stochastic as has been suggested for self-reactive regulatory T cells (T_{reg}) [36,37] or directed by non-TCR factors, a process referred to as “clonal diversion”. In this context, the presence or absence of CD28 costimulation was shown to influence the fate of strongly signaled self-reactive thymocytes: While CD80/86-CD28 signaling normally results in deletion of self-reactive T cells in the thymus, the absence of CD80/86 or CD28 induced differentiation into the CD8αα IEL pathway and increased gut CD8αα IEL numbers [23]. Further, recent work showed that PD-1 inhibits CD28 signaling [38,39], suggesting the possibility that PD-1 facilitates IELp survival and differentiation by blocking CD28 signaling. Nonetheless, this remains to be experimentally proven, and the relevant APC that express CD80 and CD86, which presumably reside in the thymic cortex, have not been identified. An absence of CD28 signaling may not be the only factor favoring IEL differentiation. Like T_{reg} and invariant natural killer T (iNKT) cells, IELp selection is dependent on store-operated Ca^{2+} entry [40], where conventional T cell selection is not.

A model of thymic IELp development is shown in Figure 1.

A Second Thymic Precursor for CD8αα IEL

While the PD-1^+ self-reactive IELp is a prominent and well-studied precursor, recent reports indicate that an alternative and potentially functionally distinct precursor also exists [17,19]. Within the TCRαβ$^+$ DN population, IELp were reported to express either PD-1 [18] or Tbet [30], yet these two populations are mutually exclusive [19]. The PD-1^+Tbet$^-$ (Type A) IELp reside in the thymic cortex, and were reduced in both classical- and nonclassical MHC class-I knockout models (suggesting restriction/crossreactivity to these MHC molecules) and were localized in the thymic cortex. PD-1^-Tbet$^+$ (Type B) IELp on the other hand were reduced in MHC class-II and nonclassical MHC class-I knockout mice (suggesting restriction/crossreactivity to these MHC molecules), and were mainly found in the medulla. Further, studies with Bim$^{-/-}$ and Nur77^{GFP} mice revealed that Type A cells fall into the classic self-reactive IELp group, while Type B IELp were apparently nonself-reactive [19]. This would fit with observations that some CD8αα IEL recognize microbiota [41], although such cells may also have been derived from CD4$^+$ IEL [42]. Molecular timestamp experiments further indicated that there was no immediate precursor-product relationship between Type A and B IELp. But which of these precursors populate the gut CD8αα IEL pool the most? One study cloned a number of TCRs from CD8αα IEL in the adult gut and expressed them as retrogenics [18]. All of them gave rise to PD-1^+ cells in the thymus, leading those authors to conclude that all CD8αα IEL develop from Type A precursors. However, another study compared the TCR repertoires of gut CD8αα IEL to thymic Type A precursors and found a large fraction of specificities that were present in the gut IEL CD8αα IEL but not present in the Type A thymic precursor [21]. Thus, further work on this topic is warranted, as the two populations may be functionally distinct, and there might be disproportionate contributions from each precursor at different ages.

Table 1 outlines some of the distinguishing characteristics of Type A and B IELp.

Emigration and tissue seeding of CD8αα IEL

After selection in the thymus, conventional T cell progenitors upregulate the transcription factor KLF2 and the sphingosine-1 phosphate receptor 1 (S1PR1) and migrate along an S1P gradient through thymic perivascular spaces and into the vasculature [31,43–45]. The same emigration mechanism seems to apply to IELp, as the presence of gut CD8αα IEL was dependent on both KLF2 and S1PR1, even though neither of these molecules were expressed by CD8αα IEL in the gut [46]. Instead, KLF2 and S1PR1 are present on thymic IELp, and direct examination of recent thymic emigrant IELp showed their egress to be dependent on these factors [17,19,47]. In fact, thymic S1PR1$^+$ IELp already expressed the mucosal-homing integrin α4β7, suggesting that thymic events program the cell for trafficking to the gut [17,19]. Of interest to the question above, Type A IELp contributed around 90% to the thymic emigrants in adult mice, while Type B only contributed around 10%, despite numeric equivalence in the thymus. Perhaps as a consequence of the slower thymic emigration of Type B compared to Type A IELp, they were found to accumulate in the thymus with age [19].

The ontogeny of CD8αα IEL and their seeding of the intestine is an intriguing subject that warrants further research. It has been shown that CD8αα IEL are established in the small intestine early in life [47,48]. The TCR repertoire of CD8αα IEL is somewhat oligoclonal in nature, being quite variable between each individual [18,49], evoking a scenario where the niche is seeded by a relatively small number of progenitors. The rate of expansion and turnover in early life, and the possibility of temporal differences in the seeding of the gut with qualitatively different CD8αα IEL progenitors is yet unclear. It is conceivable that the onset of solid food uptake, colonization of the gut by microbiota in early life, and/or pathogen exposure could all influence the CD8αα IEL repertoire.

Regarding their maintenance, intestinal epithelial cell-derived IL-15 is known to result in Tbet upregulation, and both are required to maintain CD8αα IEL numbers [30,50]. In addition, aryl hydrocarbon (AH) receptor (AHR)-stimulation through food-derived AH is necessary for the maintenance of CD8αα IEL [9]. Interestingly, components of the microbiota may also activate the AHR, as recently demonstrated in CD4$^+$ IEL for *Lactobacillus reuteri*-derived products [51]. Another component that deserves further investigation is the β2m-dependent molecules expressed by epithelial cells, such as Qa2, TL, Hfe, or the neonatal Fc receptor, that as mentioned above, may promote local survival and homeostasis.

Future Questions for the Study of CD8αα IEL

In the last decade or so, much effort has been put into better understanding the developmental pathways and functional values of CD8αα IEL. Traditionally, studies of IELp populations heavily rely on adoptive transfer experiments, in which donor populations are injected into congenically distinct T cell deficient (i.e. Rag-1° or Rag-2°) hosts. This approach can rightly be criticized as compromised, as only a small number of IELp can be

isolated from thymi, and because they need to be transferred into lymphopenic mice to enable engraftment. A more recent approach is the above mentioned retrogenic mouse model, in which isolated IEL TCR were expressed in Rag° bone marrow cells from a retroviral vector [18,24,25]. While this is an elegant model, studies with retrogenic mice have focused on a limited number of TCRs, usually the most expanded ones. This helps to better understand the big picture but likely does not catch potential minor subpopulations within the heterogeneous IEL pool, which may be different from the dominant populations. To confirm or disprove the existence of various thymic IELp populations, their contribution to total intestinal CD8αα IEL, and for functional studies, retrogenic mice could be utilized by investigating TCR selected from different thymic precursor populations in question. Similarly, receptors from very young versus adult mice could be studied.

In the future, tanscriptomic and epigenetic analysis may be employed to better understand the differentiation, trafficking, and function of CD8αα IEL. Such studies might reveal specific genes that enable the generation of mice allowing for fate mapping studies and selective deficiency models, and allow for more informed comparisons to human IEL populations.

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Highlights

- Intestinal IEL are a heterogeneous lymphocyte population.
- In adult animals, the majority of CD8αα IEL precursors are self-reactive thymocytes that undergo agonist selection.
- Alternative, and likely functionally distinct, precursors exist.

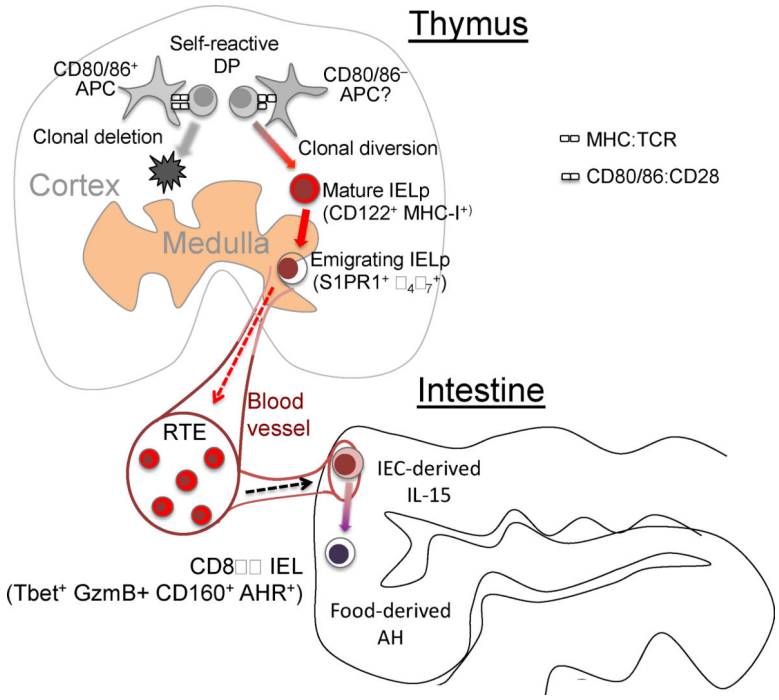

Figure 1. Model of Type A IELp thymic development.

Self-reactive DP thymocytes can be either clonally deleted or undergo agonist-selection and differentiate to the CD8αα IEL lineage. Costimulation through CD80/86-CD28 is a key factor wherein costimulated cells are deleted, while absence of costimulation can allow clonal diversion of self-reactive thymocytes. Unlike developing conventional T cells, IELp are CCR7 negative and do not migrate to the medulla for further selection processes, but mature in the cortex of the thymus. Maturation of IELp is accompanied by upregulation of CD122 and MHC class-I. Prior to exiting the thymus, IELp become emigration-competent ($S1PR1^+$) and express the mucosal-homing $\alpha_4\beta_7$ integrin. Emigration occurs in an S1P-dependant fashion via perivascular spaces at the corticomedullary junction. Once in the intestine, factors such as intestinal epithelial cell (IEC)-derived IL-15 and food-derived aryl hydrocarbon (AH) ensure the maintenance of CD8αα IEL. Besides Tbet and AH-receptor (AHR), CD8αα IEL express a range of activation and functional markers such as Granzyme B (GzmB), NK receptors of the Ly49 family, and CD160.

Table 1.

Comparison of two thymic IEL precursors.

Besides self-reactive IELp (Type A), an alternative precursor of CD8αα IEL was defined (Type B). This cell does not show evidence of being self reactive, and expresses distinct cell surface molecules. While the Tbet^{-} Type A IELp are localized in the cortex, Tbet^{+} Type B IELp are found in the medulla. A majority of the thymic emigrant IELp in adult animals are Type A cells.

IELp subset	Markers of mature cells	Thymic localization	TCR-ligand	Emigration rate in adults
Type A	PD-1^{high}Tbet^{-}α4β7^{+}	Cortex	Self	+++
Type B	PD-1^{-}Tbet^{+}NK1.1^{+}CD103^{+}	Medulla	Non-self?	+