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TL and CD8 α : Enigmatic Partners in Mucosal Immunity

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Abstract

The intestinal mucosa represents a large surface area that is in contact with an immense antigenic load. The immune system associated with the intestinal mucosa needs to distinguish between innocuous food antigens, commensal microorganisms, and pathogenic microorganisms, without triggering an exaggerated immune response that may lead to excessive inflammation and/or development of inflammatory bowel disease. The thymus leukemia (TL) antigen and CD8 α are interacting surface molecules that are expressed at the frontline of the mucosal immune system: TL is expressed in intestinal epithelial cells (IEC) whereas CD8 α is expressed in lymphocytes, known as intraepithelial lymphocytes, that reside in between the IEC. In this review we discuss the significance of the interaction between TL and CD8 α in mucosal immunity during health and disease.

Keywords

Thymus leukemia antigen; CD8 α ; mucosal immunology; immune regulation

1. Introduction

The large and small intestines constitute two major organs dedicated to the processing of food and adequate absorption of nutrients. Along with the appropriate enzymatic and biomechanical machinery needed to accomplish these functions, the exposed surface of the intestines has evolved to cover as much area as physically possible. It is estimated that the surface area of human intestines ranges between 260 and 300m², an area as large as a tennis court [1,2], whereas in mice, it reaches 1.4m² [3]. A large surface area, however, entails a greater exposure of the intestinal mucosa to a rich source of antigens, including innocuous food antigens, commensal bacteria and pathogenic microorganisms. How does the host maintain an appropriate balance between normal cellular homeostasis and induction of effective immune responses against pathogenic microorganisms? The answer lies in the specialized immune system of the intestinal mucosa.

The mucosal immune system is an intricate collection of organs, tissues and cells spread along or associated with the intestines, which includes, the mesenteric lymph nodes, the lamina propria (LP), Peyer's patches, lymphoid aggregates, appendix, and the intestinal intraepithelial lymphocytes (IEL). At least two layers of protection against microorganisms are present in these organs. First, components of the innate immune system provide immune

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surveillance in the mucosa that may lead to a rapid pro-inflammatory response. Second, antigen-specific components of the adaptive immune response are directed towards the invading pathogens.

In this review, we will focus primarily on the role of IEL in mucosal immunity and emerging evidence indicating a critical role for the interaction of CD8 $\alpha\alpha$ on IEL with the thymus leukemia (TL) antigen on intestinal epithelial cells (IEC) in controlling IEL functions.

2. Intestinal Intraepithelial lymphocytes

The intestinal epithelium is comprised of a single layer of IEC, and IEL reside in between these cells. In mice, IEL are present at a density of one IEL per 5–10 IEC in the small intestine and one IEL per 40 IEC in the large intestine [4], making this one of the largest immunological compartments in the body.

IEL can be divided in two large groups based on their differential expression of T cell receptors (TCR): TCR $\alpha\beta$ ⁺ cells and TCR $\gamma\delta$ ⁺ cells. In mice, depending on the particular strain, more than 50% of small intestine IEL correspond to TCR $\gamma\delta$ ⁺ cells, whereas in humans these cells only reach around ~10% or more depending on the disease status [5]. The remaining IEL are TCR $\alpha\beta$ ⁺ cells and a small fraction of IEL are negative for the expression of both TCR $\alpha\beta$ and TCR $\gamma\delta$, which may be non-T cells or B cells. Not all IEL are evenly spread along the intestines. For example, ~60 to 70% of IEL in the proximal and medium small intestine are TCR $\gamma\delta$ ⁺, whereas TCR $\alpha\beta$ ⁺ cells comprise ~70% of IEL in the distal region of the intestines [6], implying specific functions for each IEL subset in particular anatomical areas.

IEL expressing $\alpha\beta$ TCR chains can be subdivided into three main groups: TCR $\alpha\beta$ ⁺CD8 $\alpha\alpha$ ⁺, TCR $\alpha\beta$ ⁺CD8 $\alpha\beta$ ⁺, and TCR $\alpha\beta$ ⁺CD4⁺ cells. In the small intestine, the great majority of IEL is comprised of TCR $\alpha\beta$ ⁺CD8 $\alpha\alpha$ ⁺ cells, followed by TCR $\alpha\beta$ ⁺CD8 $\alpha\beta$ ⁺ cells, with just a small fraction of TCR $\alpha\beta$ ⁺CD4⁺ cells. It is important to note, however, that the proportion of TCR $\alpha\beta$ ⁺CD4⁺ cells increases in the distal region of the small intestine and may reach as much as 30% of the total IEL population in that area of the colon, suggesting again a compartmentalization of the different IEL subsets along the intestines in order to accomplish specific immunological functions. Conversely, TCR $\gamma\delta$ -expressing IEL are mostly CD8 $\alpha\alpha$ ⁺ and the remaining cells are negative for the expression of either CD8 or CD4.

IEL can also be divided into two groups based on their expression of co-receptor molecules and, currently, this division appears to be the most widely accepted and used. The first group is called the “type a” cells, which includes MHC class I- and class II-restricted TCR $\alpha\beta$ ⁺ T lymphocytes, expressing CD8 and CD4, respectively. These cells are conventional, thymus-derived, antigen-experienced CD4⁺ and CD8⁺ T cells that, following activation, migrate into the mucosa to accomplish their effector function and may remain there as long-term memory cells. These TCR $\alpha\beta$ ⁺ cells, once in the mucosa, may upregulate the expression of CD8 $\alpha\alpha$ homodimers [7–9], becoming either TCR $\alpha\beta$ ⁺CD4⁺CD8 $\alpha\alpha$ ⁺ or TCR $\alpha\beta$ ⁺CD8 $\alpha\beta$ ⁺CD8 $\alpha\alpha$ ⁺ cells. Akin to conventional T cells, type a IEL are involved in acquired immunity against pathogens, most probably those transmitted orally [10–12]. Further, as reported for a specific TCR $\alpha\beta$ CD4⁺CD8 $\alpha\alpha$ ⁺ IEL population, some of the type b IEL might mediate other immunoregulatory functions such as protection against Th1-dependent colitis via production of IL-10 [13].

The second group of IEL is comprised of “type b” IEL, which includes TCR $\alpha\beta$ ⁺CD8 $\alpha\alpha$ ⁺ and TCR $\gamma\delta$ ⁺CD8 $\alpha\alpha$ ⁺ cells. The origin of these cells has been a point of debate for several years, with some authors suggesting an extra-thymic, intestine-based origin [14–16]. However,

these cells do not develop in athymic mice, providing substantial evidence for a thymic origin [17]. Indeed, recent evidence indicates that type b IEL develop in the thymus where they interact with strong agonistic self-peptides, endowing these cells with natural autoreactivity [18–20].

The specific functions of type b IEL are not very well defined, but due to their natural autoreactivity, these cells are most likely involved in innate immune responses and tissue homeostasis. For example, it has been reported that mice deficient in TCR $\gamma\delta$ cells are more susceptible to dextran sodium sulphate- and hapten-induced colitis [21,22], most likely reflecting a defect in the regeneration of IEC, suggesting that TCR $\gamma\delta$ IEL have an important role in the homeostasis of the epithelium. Indeed, under homeostatic conditions, TCR $\gamma\delta$ IEL regulate the turnover of IEC [23,24] and can boost IEC growth via production of keratinocyte growth factor [25]. On the other hand, in a T cell adoptive transfer system, TCR $\alpha\beta^+$ CD8 $\alpha\alpha^+$ IEL were capable of preventing development of inflammatory bowel disease (IBD) [13,26].

Although our understanding of the function of type b IEL may be limited, it is clear that these cells reside in the intestine in a partially activated state [27]. For example, IEL bear markers such as granzymes, Fas ligand, RANTES, and CD69 that are related to effector activity [28], and can rapidly acquire cytotoxic activity without prior exposure to antigen [29]. However, despite their partially activated state, IEL proliferate poorly in response to mitogenic signals [29–31]. Each of these features favors the notion that type b IEL have an immunoregulatory role and contribute to IEC homeostasis.

3. The Thymus Leukemia Antigen

In 1963, using serologic studies, Old and Boyse discovered a new antigenic moiety expressed on spontaneous and radiation-induced leukemias, which they named the thymus leukemia (TL) antigen [32]. Further genetic linkage studies mapped the TL product to a locus within the murine MHC region of chromosome 17, referred to as the H2-T locus [33]. The H2-T locus of the MHC encodes for the heavy chains of a subset of unconventional MHC class I molecules [34]. In most mouse strains, there are about 20 genes within the H2-T locus, some of which are the result of large duplication events [35–38]. Several genes within this locus are non-functional or pseudogenes. The TL heavy chain is encoded by the *T3/T18* gene duplication pair and shares approximately 70% sequence homology with classical MHC class I heavy chains [39,40].

Unlike classical MHC class I molecules that are expressed by nearly all nucleated cells, expression of TL shows a restricted pattern. The *T3* gene product is only expressed on the surface of IEC, whereas the *T18* gene is expressed in both the intestine and immature thymocytes [41–43]. Based on the expression of TL in thymocytes, mouse strains have been classified as either TL⁺ or TL[−]. C57BL/6, one of the most commonly used strain of mice, fails to express TL in the thymus, as they have a defective *T18* gene [44]. Dendritic cells and T cells have also been reported to express TL upon activation [45,46], and in some instances, T cells can “snatch” TL from IEC and present it in their own surface membrane [47].

Structurally, TL consists of a heavy chain comprised of three extracellular domains, a transmembrane domain and a short cytoplasmic tail. Expression of TL on the cell surface is also dependent upon the non-covalent association with β_2 -microglobulin (β_2m) [48]. Resolution of the crystal structure of TL has shown that, in contrast to classical MHC class I molecules, the putative peptide-binding groove in TL is occluded [49]. Consistent with this finding, *in vitro* folding of recombinant TL protein occurs in the absence of peptide [50]. Moreover, TL is expressed *in vivo* in the absence of the TAP peptide transporter [51,52].

These findings suggest that TL does not function in antigen presentation in the same way as classical MHC class I molecules, and warrants research focused on determining the role of TL in the mucosa.

4. CD8 $\alpha\alpha$ and the Mucosa

CD8 is a transmembrane glycoprotein encoded by the *CD8 α* and *CD8 β* genes located on chromosome 2 in humans and chromosome 6 in mice. For proper surface expression and function, CD8 requires to be expressed as a dimer composed either of disulphide-linked CD8 α and CD8 β chains (CD8 $\alpha\beta$ heterodimer) or a CD8 α chain paired with another CD8 α chain (CD8 $\alpha\alpha$ homodimer). Expression of CD8 $\beta\beta$ homodimers on the surface has not been reported. Both chains are part of the immunoglobulin superfamily, with an immunoglobulin-like variable extracellular domain connected to the membrane by a stalk and intracellular tail [53]. Contrary to CD8 $\alpha\beta$, a dimer mostly expressed in conventional CD8⁺ T cells found in peripheral lymphoid organs, the expression pattern of CD8 α homodimers is limited to IEL and a fraction of activated CD8 [54] and CD4 T cells (our unpublished results). Expression of the CD8 α chain has also been reported in a subset of monocytes and dendritic cells [55,56].

In terms of immunological function, CD8 $\alpha\beta$ and CD8 $\alpha\alpha$ share similar binding affinities to classical MHC class I molecules [57]. CD8 $\alpha\beta$ has been considered a co-receptor molecule that enhances the interaction between the TCR-MHC/peptide complex and boosts the signaling machinery. Indeed, the intracellular tail of CD8 α associates with p56^{lck} and linker of activated T cells (LAT), coupling CD8 $\alpha\beta$ signaling with the TCR and other downstream events [58–60]. Although CD8 $\alpha\beta$ appears to be a *bona fide* co-receptor, there is ample evidence to suggest a different role for CD8 $\alpha\alpha$ in T cell activation [61]. For example, despite the association of CD8 α with p56^{lck} and LAT, CD8 α homodimers cannot provide the required signals for thymic selection in CD8 β -deficient mice, although mature CD8 T cells in these mice are capable of mounting a cytolytic response [62–64]. It has been postulated that the intracellular domain of CD8 β is responsible for associating CD8 $\alpha\beta$ heterodimers with lipid rafts and, therefore, allowing a better interaction between the TCR and the signaling machinery [65,66].

If CD8 $\alpha\alpha$ does not serve as a conventional TCR co-receptor, what, then, is its function? The available evidence suggests that CD8 $\alpha\alpha$ functions as a repressor of T cell activation. For example, van Oers et al. showed that overexpression of CD8 α homodimers in double-negative thymocytes leads to weakened signal transduction events during TCR stimulation [67]. It has also been reported that expression of transgenes coding for high-affinity peptides induces development of CD8 $\alpha\alpha$ IEL, whereas expression of low-affinity peptides induces CD8 $\alpha\alpha$ and CD8 $\alpha\beta$ IEL [68]. Finally, it has been shown that CD8 $\alpha\alpha$ can be induced in CD8 T cells, in a manner proportional to the strength of TCR stimulation [46]. Considering these observations, it is attractive to postulate that CD8 $\alpha\alpha$ expression is needed in those cells with high affinity for their ligands to quench the response. This effect could be accomplished either by abducting CD8 α chains away from CD8 β and lipid rafts or by direct inhibition of TCR stimulation mediated by CD8 $\alpha\alpha$ -coupled p56^{lck}. Definitive evidence for either of these two hypotheses is still needed.

5. The TL-CD8 $\alpha\alpha$ Interaction and Its Significance

Due to its similarities with MHC class I molecules, it was originally believed that engagement of TL with CD8 would play a role in T cell activation similar to that for MHC class I. Early results based on cell-cell adhesion techniques and interaction of cells with plate-bound TL demonstrated that CD8 can indeed bind with TL [69], raising the hypothesis that TL plays a relevant role in antigen presentation, possibly presenting antigens to TCR $\gamma\delta$

T cells. However, when the structure of TL was solved by X-ray crystallography it became clear that TL could not bind an antigenic moiety due to occlusion of the putative antigen-binding groove [49]. Therefore, the idea that TL has a role in antigen presentation was abandoned. Nevertheless, a pivotal set of publications compellingly demonstrated that TL binds CD8, primarily CD8 $\alpha\alpha$ homodimers rather than CD8 $\alpha\beta$ heterodimers [50,54,70,71]. Cheroutre and colleagues further demonstrated that TL-transfected RMA tumor cells could modify the cytokine response of CD8 $\alpha\alpha$ -expressing IEL [54], establishing a basic model in which TL expressed on IEC modulates the immune response of IEL [72].

Evidence for a *bona fide* interaction between IEC and IEL via TL-CD8 $\alpha\alpha$ came from the studies of Pardigon et al. [47]. These authors observed that a fraction of IEL incubated overnight with TL-transfected P815 mastocytoma cells become TL⁺, an effect blocked by the addition of anti-CD8 α antibodies. Furthermore, when analyzing contaminant IEL present in the IEC fractions, it was found that ~15% of CD8 α ⁺ IEL were positive for TL expression. Moreover, IEL obtained from mice undergoing colitis showed a fraction positive for the expression of TL. The relevant conclusion from this report was that TL was not *de novo* expressed by IEL, but instead was “snatched” from the surface of IEC (or TL⁺P815 cells), indicating that IEC and IEL indeed interact *in vivo* via TL-CD8 $\alpha\alpha$.

What is the physiological relevance of the TL-CD8 $\alpha\alpha$ interaction? An initial attempt to determine the role of TL in the gut showed that mice deficient in β_2m (which is required for surface expression of TL) do not show a reduction in the percentages of IEL expressing CD8 $\alpha\alpha$ [73], an observation that was later confirmed using TL-deficient animals [74]. These reports clearly indicate that TL is not relevant for the generation of CD8 $\alpha\alpha$ ⁺ IEL. Furthermore, Pardigon et al. showed that TL expression is also not relevant for migration of IEL into the mucosa [73].

Because IEL are in a partially activated state in an environment rich in antigenic stimuli, there must be mechanisms or check points in place capable of halting unwanted, “full-throttle” IEL activation. The close proximity between TL-expressing IEC and CD8 $\alpha\alpha$ -expressing IEL, the great affinity between these two molecules, and the putative suppressor function of CD8 α homodimers point toward a potential role for the TL-CD8 $\alpha\alpha$ interaction as a modulator of IEL responses.

IEL, in comparison to conventional T cells present in the periphery, proliferate poorly *in vitro* when stimulated via the TCR [29–31]. These findings imply that IEL have an intrinsic low proliferative capacity and/or are tightly regulated by other factors present in crude IEL preparations, which usually contain a significant fraction of contaminant IEC. Yamamoto and colleagues reported that highly purified IEL preparations devoid of IEC responded more strongly to anti-CD3 stimulation than IEL co-cultured in the presence of IEC [75]. Interestingly, reduced IEL proliferation was restored when purified IEC membranes (but not soluble factors) were added to the culture. This effect could not be blocked by adding antibodies directed against TGF- β , CD1d, E-cadherin, or MHC class I or class II molecules [75]. Is TL the relevant molecule expressed by IEC responsible for this observed suppression of IEL proliferation? The results presented in our initial description of a mouse strain deficient in the expression of TL suggested that this is indeed the case [74]. We first confirmed that *in vitro* stimulation of crude IEL preparations from wild type mice with anti-CD3 antibodies resulted in reduced proliferative responses [74]. However, when IEL preparations derived from TL-deficient animals were stimulated under similar conditions, IEL proliferation was relieved from the suppressive effects of IEC, clearly suggesting that TL is the molecule responsible for preventing unwanted proliferation of the IEL compartment. Interestingly, suppression of IEL proliferation by TL appears to occur only during situations of low TCR stimulation, because strong TCR engagement was able to

override the effect of TL on IEL proliferation [74]. This TL-mediated regulatory mechanism might prevent IEL from proliferating when they encounter low antigenic stimuli, but allow a vigorous IEL response when the antigenic load becomes excessive, as could be the case during infection.

Does TL-deficiency cause an increase in T cell numbers in the IEL compartment that leads to uncontrolled inflammation in the gut? To our surprise, the number and proportion of IEL and their subpopulations in both the small and large intestine of TL-deficient mice were indistinguishable from TL-competent animals [74]. Moreover, morphological and histological examination of the intestines of aged TL-deficient mice revealed no difference in comparison with TL-competent littermates. If TL modulates the effector functions of IEL, then its effect is likely masked by other “check-points” or regulatory factors (such as T_{reg}) capable of maintaining homeostasis in the gut. To circumvent the possible influence of regulatory T cells we crossed TL-deficient mice with $TCR\alpha^{-/-}$ animals, which are susceptible to the spontaneous development of colitis [76]. We observed that the incidence, severity and onset of colitis in double deficient mice was substantially increased when compared with $TCR\alpha$ single knockout mice [74].

Finally, a recent study has proposed that the TL-CD8 α interaction may have implications beyond the intestinal mucosa [46]. Two main observations led to this hypothesis: a) Recently activated conventional CD8 T cells transiently upregulate expression of CD8 α ; b) Professional antigen presenting cells such as dendritic cells and monocytes express TL upon activation. These authors further showed that the TL-CD8 α interaction provides survival signals to recently activated CD8 T cells allowing them to become committed to the memory cell lineage. Although this is an attractive hypothesis, a subsequent publication showed that TL expression is not required for the development of memory CD8 T cell responses [77]. Moreover, our own data also suggested that TL-deficient mice could mount efficient memory CD8 T cell responses to lymphocytic choriomeningitis virus infection [74]. Nevertheless, it remains possible that TL impacts the generation of CD8 T cell memory responses to antigens that enter the host via the intestinal mucosa.

In summary, recent advances in the study of TL and CD8 α interactions in the mucosa have revealed a potential function for this interaction as a molecular switch that halts the proliferation and pathogenic potential of IEL (Figure 1). Such a molecular switch might also modulate immune responses toward a more pathogenic pattern. Thus, it will be critical to further investigate the functional significance of TL-CD8 α interactions, as it may shed light on immune control mechanisms that could be targeted for prevention or treatment of intestinal inflammation and disease.

Despite the progress made in our understanding of the importance of the TL-CD8 α interaction in the intestinal mucosa, there are still many questions waiting to be answered. For example, considering the many populations of T cells present in the IEL compartment, it is imperative to determine how TL⁺ IEC modify their different functions. We hypothesize that each individual CD8 α ⁺ IEL population may be modulated differently when interacting with TL⁺ IEC. For example, it is possible that, besides controlling proliferation, TL⁺ IEC may also regulate cytokine production and other functions. Should this be the case, TL may serve as a molecular switch governing the individual effector functions of IEL. Future studies should also investigate the possible effects of TL-CD8 α interactions on IEC function and integrity. Such changes in the TL⁺ IEC population imprinted by CD8 α ⁺ IEL may be passed on to future generations of IEC and subsequently modulate secondary immune responses.

6. Concluding Remarks

The intestinal mucosa represents a location where the immune system is in constant contact with foreign antigens and pathogens, and where immune responses must be tightly regulated to prevent overactivation, inflammation and disease. One key pair of interacting molecules involved in controlling the immune response in the intestine is TL and CD8 α . We have just begun to uncover the importance of the interaction between these molecules in mucosal immunity. Further studies in this area should improve our understanding of the intricate cellular and molecular interactions in the intestinal mucosa and will help in the design of improved treatments and prophylactic measures for intestinal disorders. Importantly, however, because a direct homologue of murine TL has not been identified in humans [34], it will be imperative to investigate the surface molecules on human IEC that can modulate IEL function.

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Figure 1. Proposed model for the function of TL-CD8αα interactions in mucosal immunity

Top, under normal conditions, TL and CD8αα interact during IEL activation. While IEL perform their appropriate effector functions, tissue homeostasis is maintained. Bottom, in the absence of TL expression, activation of IEL results in an exuberant immune response that might yield, under certain conditions, excessive inflammation and/or development of inflammatory bowel disorders.