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Thymic maturation determines $\gamma\delta$ T cell function, but not their antigen specificities

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Abstract

$\gamma\delta$ T cells contribute uniquely to host immune defense, but how they do so remains unclear. Recent work suggests that thymic selection does little to constrain $\gamma\delta$ T cell antigen specificities, but instead determines their effector fate. When triggered through the T cell receptor, ligand-experienced cells make IFN γ , whereas ligand-naïve $\gamma\delta$ T cells produce IL-17, a major initiator of inflammation. These advances warrant a fresh look at how $\gamma\delta$ T cells may function in the immune system.

Introduction

$\gamma\delta$ T cells, like $\alpha\beta$ T cells, develop in the thymus before entering the periphery. In the case of $\alpha\beta$ T cells, thymic development entails endogenous ligand driven positive and negative selection, which determine what $\alpha\beta$ T cell can recognize and whether these T cells will develop into CD4⁺ helper or CD8⁺ cytolytic T cells. However, the role of ligand-mediated selection in $\gamma\delta$ T cell development and function has been less clear. In the past year this issue has been reexamined, and the emerging picture is fundamentally different from what we know about $\alpha\beta$ T cell development and differentiation. Not only is encountering ligand in the thymus not required for $\gamma\delta$ T cells to mature and exit to the periphery, but antigen naïve $\gamma\delta$ T cells appear to constitute a large fraction of the peripheral repertoire. Furthermore, regardless of antigen experience, $\gamma\delta$ T cells can be triggered to produce cytokines without requirement for antigen specific priming. Importantly, antigen naïve $\gamma\delta$ T cells make IL-17. In this review, we will discuss these recent findings and their implications.

The development of $\gamma\delta$ T cells with invariant TCRs

Murine $\gamma\delta$ T cells can be divided into two categories based on their receptor diversity, ontogeny and anatomical location. The first group, the invariant $\gamma\delta$ T cells, are generated from the first two waves of T cells in the fetal thymus and later found in either the epidermis of the skin, or the epithelium of the reproductive tract. The first wave produces the skin resident dendritic epidermal T cells (DETCs), which express V γ 5 and V δ 1 TCRs [1], whereas the second wave produces the $\gamma\delta$ T cells that populate the vaginal epithelium and express V γ 6 and V δ 1 TCRs [2]. The corresponding TCR chains essentially lack N regions or other types of junctional diversity [3], and the particular timing and choice of V γ gene rearrangement correlates with its location 5' to the C γ region [4]. All experimental results suggest that ligand driven positive

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selection is required for DETCs to migrate to the skin and to acquire their ability to react to keratinocytes [5–7]. In general, it is believed that these invariant $\gamma\delta$ T cells recognize host antigens and play a role in epithelial cell maintenance.

Recently, the expression of an immunoglobulin-like transmembrane protein, encoded by *Skint1* (*selection and upkeep of intraepithelial T cells 1*), on fetal thymic stromal cells was identified as necessary for the positive selection of the DETCs [8]. In the absence of proper *Skint1* expression, the epidermal layer lacks the invariant V δ 1+V γ 5+ DETCs (iDETCs) found in normal mice and is instead populated by $\gamma\delta$ T cells that express diverse TCR V genes. However, *Skint1* mutant mice develop spontaneous ear inflammation, suggesting that the ‘replacement’ DETCs function differently than the iDETCs [7]. Although neither the invariant TCR-antigen, nor the molecular entity on the iDETC which interacts with *Skint1* has been identified, several indirect evidences indicate that the ‘replacement’ DETCs do not recognize the same ligand as the iDETCs. Given the observations made by Jensen et al. (discussed below), it would be interesting to determine whether or not these ‘replacement’ DETCs have encountered ligand during development in the thymus and if these cells function as antigen naïve $\gamma\delta$ T cells.

Encountering thymic ligand is neither required, nor inhibitory for the generation of antigen specific $\gamma\delta$ T cells in adult animals

The second group of $\gamma\delta$ T cells appears postnatally after the first two waves of fetal $\gamma\delta$ thymocyte development. Unlike the invariant $\gamma\delta$ T cells, this group expresses TCRs with various V δ s and V γ s, and diverse CDR3 regions. In adult mice, these cells are found in all secondary lymphoid organs and below the epithelium or mucosal surfaces of many tissues, including the small intestine and lung. Only a few ligands have been identified for these cells, which include both host- and pathogen- derived antigens. However, it is clear that MHC molecules are not obligatory components of $\gamma\delta$ T cell ligands, and there is no apparent common structural motif shared by defined $\gamma\delta$ T cell antigens (reviewed in [9]).

The impact of thymic ligand expression on the development of this second group of $\gamma\delta$ T cells was first examined in transgenic mice expressing the TCRs of two independently derived $\gamma\delta$ T cell clones, KN6 and G8, which recognize the closely related β_2 -microglobulin associated MHC class Ib molecules, T10 and T22. It was reported that development of KN6 and G8 transgenic T cells was inhibited in both C57BL/6 (B6) (which express both T10 and T22) and β_2 -microglobulin deficient mice (*B2m*^{−/−}, which do not have cell surface T10 or T22 expression), but occurred normally in BALB/c mice (which only express T10). These observations lead to the conclusion that $\gamma\delta$ T cells, similar to $\alpha\beta$ T cells, undergo ligand driven positive and negative selection in the thymus [10–13]. However, analyzing the same G8 transgenic mice, Schweighoffer and Fowlkes found that G8 T cells were able to mature in *B2m*^{−/−} mice, contradicting the conclusion that positive selection is required [14].

Subsequently, T10/T22 were found to be natural ligands for a sizable population (~0.2–2%) of $\gamma\delta$ T cells in normal mice [15]. Surprisingly, using a T22 tetrameric staining reagent, a comparable frequency (~0.2–1%) of T10/T22 specific $\gamma\delta$ T cells were found in B6, BALB/c, and *B2m*^{−/−} mice [16]. A similar frequency of T10/T22 specific $\gamma\delta$ T cells was also found in the thymuses and spleens of mice lacking both β_2m and class II MHC molecules, and in the thymuses of these mice treated with cyclosporin A [16]. In addition, normal numbers of $\gamma\delta$ thymocytes are found in calcineurin deficient animals [17]. Thus, the ligands and signaling pathways required for positive selection of $\gamma\delta$ T cells are not required for $\gamma\delta$ thymocyte development.

Furthermore, T10/T22 specific $\gamma\delta$ thymocytes from different genetic backgrounds have similar levels of phosphorylated ERK1/2 (extracellular signal-regulated kinase) and CD5, both of

which are downstream indicators of TCR-signaling and/or signaling-threshold in the thymus. While sphingosine-1-phosphate receptor 1 (S1P₁) up-regulation is necessary for mature $\alpha\beta$ thymocytes to exit the thymus [18] and is highly expressed in positively selected fetal thymocyte DETC-precursors [6], there was no observable difference in the S1P₁ surface expression between tetramer-positive and tetramer-negative $\gamma\delta$ thymocytes from B6 and *B2m*^{-/-} mice. Also, T10/T22 specific $\gamma\delta$ thymocytes are lineage committed, as evidenced by remaining CD4-CD8- [16] (Figure 1). Taken together, these results indicate that endogenous thymic ligand expression does very little to constrain $\gamma\delta$ thymocyte development and thymic exit. Indeed, 0.85% of 'non-selected' TCR δ sequences from CD3 ϵ -deficient murine thymocytes and from out-of-frame VDJ recombination events contain the T10/T22 recognition motif [19] (Figure 2). This frequency is well within the range of the 0.2–2% T10/T22 specific $\gamma\delta$ T cells observed in normal mice. These observations represent a significant departure from what has been known for $\alpha\beta$ T cell development and what has been thought previously about $\gamma\delta$ T cell selection through the analysis of $\gamma\delta$ TCR transgenic mice. Skewed thymocyte development by prearranged TCR transgenes [20], and extrapolating results from the analysis of a single transgenic TCR to populations of T cells with a range of affinity for the same ligand may have contributed to this discrepancy.

In search of a mechanism that allows $\gamma\delta$ thymocytes to signal through the TCR without encountering ligand, Jensen et al. used a cellular dimerization assay worked out for the preT α receptor and found that dimerization is a general feature of many V δ 's. Whether or not $\gamma\delta$ TCRs expressed on T cells do dimerize or multimerize, and its biological consequences remains to be determined. However, this provides an attractive hypothesis as to why $\gamma\delta$ thymocytes do not need a 'pre-T $\gamma\delta$ receptor' to sense properly folded and surface expressed $\gamma\delta$ TCRs, and can signal without ligand recognition (Figure 3). In the periphery, $\gamma\delta$ TCR dimerization might also serve to enhance antigen specific stimulation, even though most of these cells do not express either CD4 or CD8 co-receptors and do not recognize MHC molecules.

A large fraction of lymphoid $\gamma\delta$ T cells may not have encountered ligand during development or in the periphery

T10/T22 specific $\gamma\delta$ T cells that developed in B6, BALB/c, and *B2m*^{-/-} mice are similar in number, but phenotypically different, leading to several insights. First, it was obvious that the majority of T10/T22 specific $\gamma\delta$ T cells from B6 and BALB/c mice have encountered antigen whereas those from the *B2m*^{-/-} mice have not. In particular, T10/T22 specific cells from B6 and BALB/c mice expressed higher levels of the IL-2 and IL-15 receptor common β chain (CD122) than those from *B2m*^{-/-} mice (Figure 1). The upregulation of CD122 has been used as an indicator of self-ligand recognition for $\alpha\beta$ thymocytes [21] and during DETC development [6].

Secondly, the surface expression pattern of a variety of markers (e.g. CD122, CD25, CD44, CD127, NK1.1, HSA, DX5, etc.) on tetramer-negative $\gamma\delta$ T cells (i.e. >99% of the total $\gamma\delta$ T cell population) in all strains of mice were more similar to tetramer-positive cells from *B2m*^{-/-} mice than from B6 and BALB/c mice, suggesting that a large fraction of lymphoid $\gamma\delta$ T cells may not have encountered ligand during development or in the periphery. Finally, analysis of the turnover rates of these $\gamma\delta$ T cell populations was consistent with this supposition [16]. However, these differences did not substantially bias the repertoire, as similar frequencies of T10/T22 specific cells can be found in the periphery of *B2m*^{-/-} and wild type mice.

Antigen naïve $\gamma\delta$ T cells make IL-17 without requirement for antigen specific priming

Jensen et al. found that upon stimulation through the TCR, CD122^{lo} cells make IL-17, and CD122^{hi} $\gamma\delta$ T cells make IFN γ . The ability to differentially make IL-17 versus IFN γ can be observed in $\gamma\delta$ T cells from the spleen, lymph nodes [16], and peritoneal cavity [22]. Importantly, CD122^{lo} $\gamma\delta$ T cells have already gained the ability to make IL-17 in the thymus [16,22]. One exception is in the inter-epithelial lymphocyte (IEL) compartment of the small intestine, where $\gamma\delta$ IELs are mainly CD122^{lo}, constitutively cytolytic, and were found to secrete IFN γ but not IL-17 when stimulated. Whether or not CD122 expression on $\gamma\delta$ T cells is induced only by TCR-ligand engagement will require more extensive analysis. Nevertheless, these results are consistent with the hypothesis that at least for $\gamma\delta$ T cells of the lymphoid organs, antigen recognition drives effector function development, where antigen naïve cells make IL-17 and antigen experienced cells make IFN γ (Figure 3).

Kisielow and colleagues used a cDNA subtraction screening approach to determine genes that are involved in $\gamma\delta$ thymocyte development. They identified two novel genes, which belong to the scavenger receptor superfamily B, *SCART1* and 2, to be highly expressed in adult $\gamma\delta$ thymocytes and lymph node $\gamma\delta$ T cells [23]. Interestingly, when triggered through the TCR, SCART^{hi} but not SCART2⁻, $\gamma\delta$ T cells make IL-17 without previous priming. Furthermore, activating SCART2^{hi} $\gamma\delta$ T cells through the TCR, and in the presence of IL-2, down-regulates the expression of SCART2. Therefore, it appears that SCART2 expression marks $\gamma\delta$ T cells which have not encountered ligand (Figure 3). However, this possibility remains to be tested.

Conclusion

Whether or not the development of T10/T22 specific $\gamma\delta$ T cells is typical of the entire adult $\gamma\delta$ T cell repertoire will require further studies when additional $\gamma\delta$ T cell antigens are identified. Until then, these recent developments seem to indicate that $\gamma\delta$ T cells differ from $\alpha\beta$ T cells in how thymic development influences their TCR specificities and effector-fate development. In particular, the absence of positive selection, and the lack of antigen specific priming, seems ideal for $\gamma\delta$ T cells to function in the first line of defense. Importantly, antigen naïve $\gamma\delta$ T cells do so by mounting a robust IL-17 response. One of the main functions of IL-17 is to promote the expansion and maturation of neutrophils in the bone marrow [24,25]. In order to replenish circulating neutrophils that deploy within hours to the site of infection, a swift IL-17 response must be elicited, days before the development of CD4⁺ Th17 $\alpha\beta$ T cells. Therefore, the rapid IL-17 response mounted by antigen-inexperienced $\gamma\delta$ T cells would play a critical role at the onset of an acute inflammatory response to pathogens that the host encounters for the first time, or to host antigens that are only revealed by injury. Indeed, $\gamma\delta$ T cells are the major early producers of IL-17 after CFA immunization [16] and in several murine models of infection and autoimmunity (reviewed in [26]).

Consistent with a role for $\gamma\delta$ T cells in the regulation of neutrophil homeostasis, mice lacking $\gamma\delta$ T cells fare worse in neutrophil-dominated inflammatory responses, such as in heat-, ozone- or chlorine-induced injuries [27–29] and in bacterial infections (*Nocardia asteroides* [27], *Klebsiella pneumoniae* [30]). In these cases, fewer infiltrating neutrophils, increased bacterial load, early dissemination and higher mortality rates are noted. These observations may explain reports showing increased numbers of $\gamma\delta$ T cells in the peripheral blood of patients with acute bacterial and viral infections (from <5% in healthy individuals up to >45% in patients with these diseases), and the abundance of infiltrating $\gamma\delta$ T cells in primary lesions of the central nervous system in Multiple Sclerosis patients (up to 20–30% of the total number of T cells) [31].

In addition, by acting early in the inflammatory response, $\gamma\delta$ T cells may affect the development of antigen specific $\alpha\beta$ T cell and B cell responses during the priming phase. Thus, $\gamma\delta$ T cells may play a much larger role in the adaptive immune response than previously recognized. This may be the key to understanding how $\gamma\delta$ T cells contribute to host immune competence and why these cells have been maintained throughout vertebrate evolution, wherever $\alpha\beta$ T cells and B cells are found.

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Figure 1. Ligand expression is not required for the development of antigen specific $\gamma\delta$ thymocytes

a) T10/T22 specific $\gamma\delta$ thymocytes that developed in the presence (B6, top row) or absence of ligand (*B2m*^{-/-} bottom row) were analyzed for basal (i.e. non-stimulated) intracellular levels of phosphorylated-ERK1/2 (basal level of pERK1/2 in CD8+CD4+ DP thymocytes for comparison in grey), or the surface expression of CD5 (isotype staining control in grey), S1P₁, CD4 and CD8. T22-tetramer negative thymocytes are also shown (blue). For comparison, the S1P₁ surface expression on DP (black) and mature $\alpha\beta$ thymocytes (CD4+ CD62L^{hi}, purple); as well as the CD4/8 surface expression profiles on thymocytes from B6 and *B2m*^{-/-} mice are plotted. Data is from Jensen et al. [16]. **b)** The CD122 surface expression on T10/T22 specific $\gamma\delta$ thymocytes and splenocytes (red) that developed in the presence (B6, BALB/c) or absence (*B2m*^{-/-}) of ligand. Tetramer-negative $\gamma\delta$ T cells are shown in blue. Data is from Jensen et al.

Figure 2. Frequency of the T10/T22 recognition motif in $\gamma\delta$ T cells from different genetic backgrounds

a) The T10/T22 recognition motif (amino acids in red, W SEGYE L) expressed by T10/T22 specific $\gamma\delta$ T cells from B10.BR mice, are also found in T10/T22 specific $\gamma\delta$ T cells isolated from B6, B2m^{-/-} and BALB/c mice (analyzed at a single cell level). Underlined, are the amino acids found in all T10/T22 specific $\gamma\delta$ T cells from B10.BR mice as reported by Shin, et al. [19]. The recognition motif is largely encoded by an intact D δ 2 (SEGYE) gene segment with contributions from a V-, D δ 1- or N-nucleotide encoded tryptophan (W) and a P-nucleotide encoded leucine (L). Diverse TCR V γ and V δ genes are associated T10/T22 specific TCRs.

b) Frequency of the T10/T22 recognition motif observed in $\gamma\delta$ T cell populations derived from different genetic backgrounds that vary in TCR expression and/or ligand recognition. *CDR3 sequences are from CD3ε^{-/-} thymocytes and out of frame VDJ δ TCR rearrangements, both of which undergo gene rearrangement but for different reasons fail to express TCRs on the cell surface. Presumably, these thymocytes are not subjected to endogenous ligand selection events. B2m^{-/-} mice fail to express T10/T22 on the cell surface and consequently, $\gamma\delta$ TCR expressing cells are not exposed to T10/T22. The frequency of the recognition motif in wild type and B2m^{-/-} mice was confirmed by tetramer staining and direct single cell sequence analysis [16,19].

Figure 3. A model for the development of a functional $\gamma\delta$ T cell repertoire

a) V δ 2, 4, 5, 6, 7, 8, 10, etc. (not V δ 1)-DJ δ and V γ 1, 2, 4, 7-J γ rearrangements occur in DN2/3 thymocytes. The Terminal Deoxynucleotidyl Transferase (Tdt) is expressed in adult thymocytes and thus, the resulting TCRs have considerable CDR3 diversity. **b)** TCR expression is initially low (TCR^{lo}), but progression to a TCR^{hi} phenotype requires the CD3 and LAT signaling subunits [32]. We hypothesize that $\gamma\delta$ TCR dimerization may drive this progression, and possibly serves as a checkpoint for correctly folded and surface expressed TCR units. The high pERK1/2 levels may lower the signaling threshold and allow thymocytes to sense dimerization events mediated through the TCR. **c)** $\gamma\delta$ T cells that bind self-ligand through their TCR (+ TCR ligand) in the thymus up-regulate the expression of CD122, and down-regulate SCART1 (extrapolated from Kisielow and colleagues data [23]). **d)** In peripheral lymphoid organs, antigen naïve and experienced cells, when stimulated through their TCR, secrete IL-17 and IFN γ respectively; T $\gamma\delta$ -IFN γ s are preferentially found in the spleen, whereas T $\gamma\delta$ -17s are abundantly found in both the spleen and lymph nodes. In the periphery, the pERK1/2 levels in un-stimulated $\gamma\delta$ T cells are low [16]. Thus, TCR-ligand engagement is required to activate the cytokine production program. However, in the periphery, TCR-ligand interactions could also be augmented by TCR δ dimerization events.