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$\gamma\delta$ T Cells: the Overlooked T cell Subset in Demyelinating Disease

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Abstract

$\gamma\delta$ T cells represent a small subpopulation of T cells that express a restricted repertoire of T cell receptors and, unlike $\alpha\beta$ T cells, function more as cells of the innate immune system. These cells are found in skin and mucosal sites as well as secondary lymphoid tissues and, frequently act as first line of defense sentinels. $\gamma\delta$ T cells have been implicated in the pathogenesis of demyelinating disease although little was known regarding their trafficking and effector functions. In this brief review we highlight recent studies demonstrating that $\gamma\delta$ T cells migrate rapidly to the CNS during experimental autoimmune encephalomyelitis (EAE), the animal model for multiple sclerosis. $\gamma\delta$ T cell trafficking to the CNS is independent of β_2 -integrins and occurs well before onset of clinical signs of disease, peaking early during the acute phase of disease. $\gamma\delta$ T cell-mediated production of inflammatory cytokines including IFN- γ and TNF- α appears critical for EAE development, suggesting these cells may set the stage for activation of other subsets of infiltrating effector cells. These data suggest that $\gamma\delta$ T cells or subsets of $\gamma\delta$ T cells may represent a new therapeutic target in demyelinating disease.

Keywords

neuroimmunology; $\gamma\delta$ T cells; experimental autoimmune encephalomyelitis

$\gamma\delta$ T CELLS - ORIGIN, TISSUE-SPECIFIC HOMING AND EFFECTOR FUNCTIONS

$\gamma\delta$ T cells are an important link between innate and adaptive cellular immunity. These cells develop from lymphocyte precursors at an early stage in T cell development, under the influence of cytokines, including IL-7 and the transcription factor Sox13 and, do so in a fashion independent of T cell receptor (TCR) signaling (Hayes and Love 2007; Narayan and Kang 2007; Xiong and Raulet 2007). Although it has been controversial, recent data suggest that $\gamma\delta$ T cells are selected in a ligand-independent manner in the thymus and that thymic selection events regulate the effector fate of $\gamma\delta$ T cells (Jensen et al. 2008). In contrast to $\alpha\beta$ T cells, $\gamma\delta$ T cells represent a minor portion of the T cell population (1–5%; although they

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can expand to up to 50% of the T cell population (Eberl et al. 2003)). The rearrangement of $\gamma\delta$ T cell antigen receptor genes results in the generation of functional $\gamma\delta$ subsets based on the usage of specific V γ or V δ gene segments (see Table 1). The resulting $\gamma\delta$ subsets are further divided based on tissue-specific homing and the level of the restriction of TCR diversity. Tissue-specific $\gamma\delta$ T cells generally, but not always, have restricted TCR diversity and are found in the epithelial layers of organs or in the mucosa. These cells provide protection from infection in the skin, lung, intestine, oral cavity and genitourinary tract and, within this subset of $\gamma\delta$ T cells, there is further evidence of heterogeneity based on responsiveness to certain cytokines. $\gamma\delta$ T cells are also found in blood and lymphoid tissues and this subset displays marked TCR diversity relative to those found in other tissues (reviewed in (Carding and Egan 2002; Chen and Letvin 2003; Hayday and Tigelaar 2003; O'Brien et al. 2007)). Although it has been suggested that $\gamma\delta$ T cells with restricted V γ usage are present in the normal mouse CNS, this remains an isolated finding (Szymanska et al. 1999). We have not seen T cells, of any subset, in the normal mouse spinal cord (S. Smith, J. Wohler and S. Barnum, unpublished observations).

The ligands for $\gamma\delta$ T cells remain elusive although there is evidence for both microbial and self-antigen recognition, including several self-antigens recently characterized on macrophages such as nonclassical MHC Class I molecules (T22 and T10 in mice and MIC-A and MIC-b in humans) (Aydintug et al. 2008; Chien and Konigshofer 2007; Moser and Eberl 2007). In the CNS it is not clear what ligands may be reacting with $\gamma\delta$ T cells, however, citrullinated myelin basic protein (the so-called C8 form), modified arginine residues (NG-dimethylarginines) and p-cresol sulfate are potential candidates (Cao et al. 2000; Rawal et al. 1995; Whitaker et al. 1992). Modified versions of other myelin-derived proteins may also serve as ligands, although there is no experimental evidence for this as yet. The absence of CD4 or CD8 on most $\gamma\delta$ T cell subsets, combined with crystal structure data suggests that $\gamma\delta$ T cells recognize antigen in a MHC-independent manner and phosphoantigens remain strong candidate ligands (reviewed in (Carding and Egan 2002; Girardi and Hayday 2005; Wilson and Stanfield 2001)). $\gamma\delta$ T cells uniquely express WC1 molecules (workshop cluster 1), a group of transmembrane glycoproteins that are members of the scavenger receptor cysteine-rich family (Hanby-Flarida et al. 1996; Wijngaard et al. 1994). Expression of WC1.1 versus WC1.2 appears to define functional $\gamma\delta$ T cell subsets, in part by their capacity to respond to certain stimuli (Rogers et al. 2005a; Rogers et al. 2005b). $\gamma\delta$ T cells are thought to play an important role in many aspects of the host immune response depending on their location. For example $\gamma\delta$ T cells in the skin (also termed dendritic epithelial T cells) produce growth factors required for tissue repair, while $\gamma\delta$ T cells in the gut (also known as intraepithelial lymphocytes) are often cytolytic and produce cytokines and chemokines to regulate local immune responses to infection (reviewed in (Carding and Egan 2002; Hayday and Tigelaar 2003; Jameson et al. 2003)). $\gamma\delta$ T cells have also been shown to play important roles in tumor regression, systemic infections and in modulating inflammation and adaptive immune responses (Beetz et al. 2008; Lamb and Lopez 2005; Liu et al. 2008; Moser and Eberl 2007; O'Brien et al. 2007; Pennington et al. 2005). More recently it has become clear that the mechanism of $\gamma\delta$ T cell modulation of immune responses is, in part, dependent on developmental competition and interdependence with $\alpha\beta$ T cells and NK cells (French et al. 2005; Pennington et al. 2003; Silva-Santos et al. 2005).

T CELL SUBSETS AND DEMYELINATING DISEASE

The contribution of T cells as a whole to the development and progression of experimental autoimmune encephalomyelitis (EAE) and multiple sclerosis (MS) is well established (reviewed in (Sospedra and Martin 2005)). The relative contribution of the proportionally smaller T cell subsets, such as $\gamma\delta$ T cells, to the development and regulation of demyelinating disease remains unclear. Deletion of individual T cell subsets by gene targeting methods has clearly demonstrated that $\alpha\beta$ and $\gamma\delta$ T cells contribute in unique ways to the pathogenesis of EAE (Elliott et al. 1996; Sospedra and Martin 2005; Spahn et al. 1999), while the absence of Tregs results in exacerbated disease (Kohm et al. 2002; McGeachy et al. 2005; O'Connor and Anderton 2008; Reddy et al. 2004). Multiple sclerosis and EAE are classified as CD4⁺ Th1 diseases based largely on the predominance of this subset in CNS leukocyte infiltrates and the ease, in the case of EAE, of inducing disease using encephalitogenic CD4⁺ T cells in multiple rodent model systems. In MS, CD8⁺ T cells are readily seen in lesions, however the extent and mechanisms of their contribution, along with Th17 cells, remains less established (Sospedra and Martin 2005; Stockinger and Veldhoen 2007; Stockinger et al. 2007; Weiss et al. 2007). Although deletion of cell subsets is a relatively blunt approach and does not fully address subtle mechanistic differences utilized by each T cell subset, it remains a valuable technical approach, especially when paired with reconstitution studies using cells with single molecule deficiencies.

$\gamma\delta$ T CELLS AND EAE

Experimental autoimmune encephalomyelitis shares many features of the human disease multiple sclerosis (Gold et al. 2006; Sospedra and Martin 2005; Steinman and Zamvil 2006). A hallmark feature of EAE is cellular infiltration of the brain and spinal cord by many leukocyte subsets including macrophages, dendritic cells, and lymphocyte subsets including B and T cells (Gold et al. 2006; Sospedra and Martin 2005). The function of $\gamma\delta$ T cells in EAE remained controversial for some time (see Table 2 for overview). The majority of initial studies were performed using anti- $\gamma\delta$ T cell antibodies. Although most of these studies showed attenuated disease (Dandekar and Perlman 2002; Rajan et al. 1996), antibody treatment also had no effect in one case (Matsumoto et al. 1998), or exacerbated disease in another (Kobayashi et al. 1997). The contradictory nature of initial findings for $\gamma\delta$ T cells in EAE is due, in large part, to the use of different rodent species and strains and technical approaches (antibody-mediated $\gamma\delta$ T cell depletion versus δ -chain deletion). In addition, comparison of results between these studies is complicated by differences in the specificity, avidity and biological half-life of the different antibody clones used to deplete $\gamma\delta$ T cells. Nevertheless, the consensus of all the studies, particularly those using δ -chain^{-/-} mice (and thus $\gamma\delta$ T cell deficient mice), is that $\gamma\delta$ T cells make an important contribution to the pathogenesis of EAE. Phenotypically, C57BL/6 $\gamma\delta$ T cell^{-/-} mice have attenuated EAE during the chronic phase of disease, but little to no difference in disease onset or during the acute phase of disease (Spahn et al. 1999; Wohler et al. 2009). It is not known if this phenotype would hold in other murine strains using the appropriate myelin-derived protein or peptide to induce disease. Interestingly, reconstitution of $\gamma\delta$ T cell^{-/-} mice with as few as 5 x 10⁵ wild type $\gamma\delta$ T cells or with wild type splenocytes restores disease severity to that seen in wild type mice (Spahn et al. 1999; Wohler et al. 2009).

$\gamma\delta$ T CELL TRAFFICKING DURING EAE

The observation that onset of EAE in $\gamma\delta$ T cell^{-/-} mice is similar to wild type mice suggests that $\gamma\delta$ T cells contribute to pathogenic events late in the disease process and perhaps play an important role in disease maintenance. This observation sharply contrasts with the early migration of $\gamma\delta$ T cell into the CNS during EAE. Several studies have shown that $\gamma\delta$ T cells can be detected in the CNS in a week or less post-induction of disease, after which they peak rapidly in number prior to the height of disease severity and then quickly leave the CNS (Gao et al. 2001; Rajan et al. 1996; Smith and Barnum 2008; Wohler et al. 2009). These data are more consistent with $\gamma\delta$ T cells shaping the early inductive events in EAE rather than modulating chronic disease. It is not known if similar trafficking kinetics occur in MS, although it is well known that $\gamma\delta$ T cells are present in active and chronic active lesions (Battistini et al. 1997; Droogan et al. 1994; Hvas et al. 1993; Poggi et al. 1999; Selmaj et al. 1991; Traugott 1992; Wucherpfennig et al. 1992). The adhesion molecules required for $\gamma\delta$ T cell migration into the CNS have not been identified. VLA-4 would seem a prime candidate as it contributes to $\gamma\delta$ T cell adhesion to endothelium, epithelium and fibroblasts, but it does not mediate migration into the CNS (Galea et al. 1994; Mohaghehpour et al. 1992; Nakajima et al. 1995; Watkins et al. 1996). Studies from our lab have demonstrated that β_2 -integrins also do not play a significant role in the development and progression of EAE (Wohler et al. 2009). In these experiments $\gamma\delta$ T cell^{-/-} mice reconstituted with wild type $\gamma\delta$ T cells or $\gamma\delta$ T cells deficient in either CD11a, CD11b or CD11c, all developed EAE with comparable onset and severity. The absence of these adhesion molecules did not hinder the capacity of $\gamma\delta$ T cells to migrate to sites of MOG injection, to brachial lymph nodes or the CNS, although the loss of LFA-1 appears to affect retention at sites of $\gamma\delta$ T cell priming (Wohler et al. 2009). Clearly, $\gamma\delta$ T cells use a distinct repertoire of adhesion molecules for trafficking compared to $\alpha\beta$ T cells, at least in the context of EAE.

$\gamma\delta$ T CELLS AND CYTOKINES IN EAE

To determine potential mechanisms whereby $\gamma\delta$ T cells contribute to EAE, several groups have examined cytokine and chemokine production during the course of EAE. Rajan and colleagues (Rajan et al. 2000; Rajan et al. 1998) reported significantly reduced levels of several pro-inflammatory cytokines and chemokines, in mice depleted of $\gamma\delta$ T cells by antibody treatment, compared to untreated controls. Although this approach was informative, it has limitations in that $\gamma\delta$ T cell effector functions in the course of EAE can only be inferred rather than directly determined. For example, in $\gamma\delta$ T cell-depleted mice, the expression of several cytokines and chemokines including IFN- γ , TNF- α , IL-1, IL-6, IL-12 and others, was reduced. However since spinal cord homogenate was used to quantitate cytokine mRNA levels in these studies, it is unclear which cell types produced (or didn't produce) these cytokines, a concern that extends to $\gamma\delta$ T cell-sufficient control mice. Other studies have shown that $\gamma\delta$ T cells producing IFN- γ and IL-4 are significantly more numerous during EAE (Jensen et al. 1999). Gao and colleagues reported similar results (Gao et al. 2001), however in this study it was shown that in the spleen and CNS of *normal* mice, there were substantial numbers of CD3⁺ and $\gamma\delta$ T cells secreting both IFN- γ and IL-4. Since the CNS of normal mice is usually devoid of any lymphocyte subset, particularly $\gamma\delta$ T cells, the meaning of these results is unclear. More recent studies have indicated that $\gamma\delta$ T cells act

in an antigen-independent fashion to modulate cytokine production (IL-12 and IFN- γ) and thus the early effector phase of the immune response in EAE (Odyniec et al. 2004; Ponomarev et al. 2004). Ponomarev and colleagues have suggested that the immunomodulatory effect of $\gamma\delta$ T cells in EAE is independent of their ability to produce IFN- γ (Ponomarev et al. 2004). These studies were performed using bone-marrow chimeric mice and unfortunately the extent of reconstitution was not documented nor was CNS-specific production of IFN- γ protein. In contrast, we have shown by flow cytometry and intracellular cytokine staining that production of IFN- γ by $\gamma\delta$ T cells is critical to the development of EAE and, that the majority of the $\gamma\delta$ T cells infiltrating the CNS produce IFN- γ at early time points well before clinical signs of disease. $\alpha\beta$ T cell production of IFN- γ at these same time points was substantially lower (Smith and Barnum 2008) (Wohler et al. 2009). A critical role for IFN- γ was further shown in reconstitution experiments in which $\gamma\delta$ T cell^{-/-} mice were reconstituted with wild type or IFN- γ ^{-/-} $\gamma\delta$ T cells. Mice reconstituted with IFN- γ ^{-/-} $\gamma\delta$ T cells developed significantly attenuated EAE that mimicked the course of disease seen in control $\gamma\delta$ T cell^{-/-} mice. In fact, EAE onset was modestly delayed in these mice, a finding never seen in wild type $\gamma\delta$ T cell reconstitutions (Wohler et al. 2009). In contrast, while reconstitution with wild type cells resulted in disease identical to wild type mice (Wohler et al. 2009). Similar results were obtained in identical EAE experiments examining the role of TNF- α production by $\gamma\delta$ T cells. Clearly the studies to date implicate IFN- γ , TNF- α and other cytokines in $\gamma\delta$ T cell-mediated pathogenic mechanisms in EAE, however the role of $\gamma\delta$ T cell-produced cytokines in EAE is far from being understood and requires additional study.

$\gamma\delta$ T CELLS: SIGNIFICANCE AND RELEVANCE TO MS

There is substantial evidence to support a significant contribution by $\gamma\delta$ T cells to human demyelinating disease. As mentioned earlier, $\gamma\delta$ T cells are found in active and chronic active lesions along with other T cell subsets and macrophages (Battistini et al. 1997; Droogan et al. 1994; Hvas et al. 1993; Poggi et al. 1999; Selmaj et al. 1991; Traugott 1992; Wucherpfennig et al. 1992). In addition, oligoclonal expansion or activation of $\gamma\delta$ T cells in lesions or CSF has been reported, indicating local responses to currently unknown antigens or stimuli (Bieganowski et al. 1996; Hvas et al. 1993; Michalowska-Wender et al. 1998; Murzenok et al. 2002; Shimonkevitz et al. 1993; Stinissen et al. 1998; Wucherpfennig et al. 1992). Functionally, $\gamma\delta$ T cells from MS patients have also been shown to produce cytokines, chemokines and cytokine receptors in CSF from MS patients (Murzenok et al. 2002; Romagnani 1994; Stinissen et al. 1998) and mediate cytotoxic responses (Freedman et al. ; Saikali et al. 2007; Zeine et al. 1998). The extent to which these functional responses contribute to the severity of MS or to various MS clinical classifications remains poorly understood.

Our current understanding of $\gamma\delta$ T cells suggests they make an important contribution to the pathogenesis of MS and therefore may represent an unexplored therapeutic opportunity. We still know little regarding the infiltration kinetics, trafficking patterns or roles in modulation of CNS inflammation and demyelination for $\gamma\delta$ T cells relative to $\alpha\beta$ T cells during disease development. The most successful therapeutic approach for MS to date has been targeting of the adhesion molecule, $\alpha 4\beta 7$ (VLA-4), which is broadly expressed on T

cell subsets. Unfortunately, that success has not come without risk in the form of progressive multifocal leukoencephalopathy (PML) (Kleinschmidt-DeMasters and Tyler 2005; Langer-Gould et al. 2005; Miller et al. 2003; Ransohoff 2005). At this time it is not clear if the protective mechanism of anti- α_4 -integrin therapy is derived from altering the pathogenic effects of multiple T cell subsets, including $\gamma\delta$ T cells, or primarily through $CD4^+$ T cells. Clearly additional therapies and/or co-therapies are needed for treatment of MS and $\gamma\delta$ T cells may represent an untested but viable therapeutic option for MS.

CONCLUDING REMARKS

The immunobiology of $\gamma\delta$ T cells in demyelinating disease remains poorly explored relative to other T cell subsets. We know, for example, a great deal regarding IFN- γ and $\gamma\delta$ T cells, but recent studies have shown that $\gamma\delta$ T cells also produce IL-17 (Lockhart et al. 2006; Roark et al. 2007; Roark et al. 2008; Shibata et al. 2007), a cytokine strongly implicated in the pathogenesis of demyelinating disease (Aranami and Yamamura 2008; Bettelli et al. 2008; Dardalhon et al. 2008). To date there is no evidence demonstrating IL-17 production by $\gamma\delta$ T cells in EAE or MS. However, given that IL-17 appears to contribute to early inflammatory events, production of this cytokine by $\gamma\delta$ T cells, coupled with their early and rapid entry in the CNS in EAE, raises the possibility that $\gamma\delta$ T cells are a critical source of this cytokine in the development of disease. A broader profile of $\gamma\delta$ T cell cytokine-mediated biology in EAE, using targeted approaches (i.e., not antibody-mediated depletion of $\gamma\delta$ T cells) is required to better appreciate the role of $\gamma\delta$ T cells in demyelinating disease.

Although there are many T cell subsets, particularly within the $CD4^+$ T cell population, $\gamma\delta$ T cells represent a much smaller and discrete subpopulation, uniquely defined by restricted TCR usage and tissue-specific localization. These latter features may offer a powerful therapeutic advantage over pan-T cell therapeutics currently in use for MS (anti-VLA-4 antibodies). Should future studies determine that $\gamma\delta$ T cell subsets expressing a given TCR are primarily responsible for the $\gamma\delta$ T cell-mediated mechanisms in EAE, targeting an extremely small percentage of T cells would leave patients with a largely intact immune system and potentially reduce the risk of PML.

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Table 1Comparison of $\gamma\delta$ and $\alpha\beta$ T cell features

Function	$\gamma\delta$ T cells	$\alpha\beta$ T cells
TCR diversity	limited	broad
CD4/8 expression	rarely	yes
MHC-restricted recognition	No	Yes
Antigen recognition	Self-Ags, some microbial	Microbial, limited self-Ag
Co-receptors	CD81, WC1	CD4/8, LFA-1

Table 2Role of $\gamma\delta$ T cells in EAE

Gene Deficiency/Treatment	Effect in EAE	Species/strain
Anti- $\gamma\delta$ T cell Mab (clone GL3) (Rajan et al. 1996)	Protection	Mouse/SJL
α chain-KO mice (Elliott et al. 1996)	Protection	Mouse/SJL/NOD
Anti- $\gamma\delta$ T cell Mab (clone UC7 –13D5) (Kobayashi et al. 1997).	Exacerbation	Mouse/B10PL
Adoptive transfer in δ -chain KO mice (Clark and Lingenheld 1998)	No effect	Mouse/C57BL/6
Anti- $\gamma\delta$ T cell Mab (clone V65) (Matsumoto et al. 1998)	No effect	Rat/Lewis
Active EAE in δ -chain KO mice (Spahn et al. 1999)	Protection	Mouse/C57BL/6
Anti- $\gamma\delta$ T cell Mab (clone UC7 –13D5) (Dandekar and Perlman 2002).	Protection	Mouse/C57BL/6