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An interferon-γ-producing Th1 subset is the major source of IL-17 in experimental autoimmune encephalitis

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Abstract

$Th_{IL\text{-}17}$ (IL-17+/IFN-γ−) cell lines are significantly more encephalitogenic than Th1 (IL-17−/IFN-γ+) cell lines in adoptive transfer EAE models. In actively induced EAE short *ex vivo* peptide stimulation identifies an IL-17+/IFN-γ+ population of CD4+ CNS-infiltrating MOG_{35-55}-specific T cells, which outnumber IL-17+/IFN-γ− cells by approximately 3:1 as disease develops. A decrease in numbers of IL-17+/IFN-γ+ cells following *in vitro* culture is accompanied by an increase in IL-17−/IFN-γ+ cell numbers. Together these *ex vivo* and *in vitro* observations imply that the Th1 lineage is more encephalitogenic than is suggested by adoptive transfer of Th1 (IL-17−/IFN-γ+) cell lines which have been terminally differentiated *in vitro*.

Keywords: T cell differentiation; IL-17; Interferon-γ; EAE

1. Introduction

Recent studies have demonstrated that the pro-inflammatory cytokine IL-17 has a significant role in the development of pathological changes that accompany central nervous system (CNS) inflammation in experimental autoimmune encephalitis (EAE). IL-$17^{-/-}$ mice have a delayed disease onset and reduced disease severity with early recovery (Komiyama et al., 2006), while administration of neutralizing anti-IL-17 antibodies immediately prior to the expected onset of actively induced clinical disease in wild type animals reduces both disease incidence and severity (Langrish et al., 2005; Park et al., 2005). IL-17 production by T cells is dependent on IL-23, a heterodimeric cytokine composed of a p40 subunit, shared with IL-12, and an IL-23 specific p19 subunit (Oppmann et al., 2000). Both $p19^{-/-}$ and $p40^{-/-}$ mice are resistant to actively induced EAE (Cua et al., 2003) and CNS-infiltrating CD4+ T lymphocytes derived from $p19^{-/-}$ mice do not produce IL-17 following *in vitro* re-stimulation (Langrish et al., 2005). Additional *in vitro* studies have demonstrated that IL-23 supports the differentiation of a unique T cell subset, termed $Th_{IL\text{-}17}$. $Th_{IL\text{-}17}$ differentiation is also CD28 and ICOS dependent and occurs independently of STAT4 or STAT6 signaling (Harrington et al., 2005; Park et al., 2005). The $Th_{IL\text{-}17}$ subset is characterized by the production of IL-17, IL-17F, IL-6 and TNF, but not IFN-γ, and adoptive transfer of myelin-specific $Th_{IL\text{-}17}$ cell lines produces symptomatic EAE in a dose-dependent fashion (Langrish et al., 2005). In contrast, adoptively transferred IL-17−/IFN-γ+ Th1 cell lines only have a mildly encephalitogenic action (Langrish et al., 2005). It has also been demonstrated that in certain situations IFN-γ is a potent suppressor of IL-17 production (Harrington et al., 2005; Park et al., 2005) and loss of IFN-γ-mediated inhibition of IFN-γ− T cell proliferation (Chu et al., 2000) accounts for the observations that IFN-$\gamma^{-/-}$ (Ferber et al., 1996; Wensky et al., 2005), IFN-$\gamma R^{-/-}$ (Willenborg et al., 1996) and IL-12$p35^{-/-}$ (Becher et al., 2002) mice develop a more severe EAE disease course than that seen in wild type animals. Moreover, since studies in rat models of optic nerve crush injury and spinal cord contusion

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have demonstrated that myelin-specific Th1 cell lines can promote neural repair (Hauben et al., 2000; Moalem et al., 1999), understanding the mechanisms that regulate IL-17 and IFN-γ production in the course of CNS inflammation is relevant to refining immunomodulatory strategies used in the treatment of human neurological diseases.

Some debate exists as to the degree of overlap that exists between $Th_{IL\text{-}17}$ and Th1 effector cell developmental pathways. In one model it is proposed that a pre-Th1 intermediate is activated by IFN-γ or IL-27 induction of T-bet, via STAT1, leading to upregulation of IL-12Rβ2 and IL-23R. Both IL-12Rβ2 and IL-23R pair with constitutively expressed IL-12Rβ1, thereby resulting in coexpression of the IL-12R and the IL-23R. Further differentiation to a Th1 or $Th_{IL\text{-}17}$ phenotype then depends upon subsequent IL-12 or IL-23 stimulation (Bettelli and Kuchroo, 2005). An alternate hypothesis suggests that IL-17 and IFN-γ effector differentiation occurs at the naïve T cell stage. However, naïve T cells do not express the IL-23R (Aggarwal et al., 2003) and recent studies show that IL-6 and TGF-β1 are also important in the differentiation of the $Th_{IL\text{-}17}$ subset (Bettelli et al., 2006; Veldhoen et al., 2006), with IL-23R being induced by TGF-β1 (Mangan et al., 2006). While Betelli et al., were unable to induce differentiation of IL-17 producing T cells from naive 2D2 $MOG_{35–55}$ Tg T cells (Bettelli et al., 2006), Langrish et al., demonstrated that after 7 days in culture with anti-CD3/anti-CD28 antibodies and rIL-23 naïve TCR-transgenic D011.10Tg X $Rag^{-/-}$ cells had all transformed to a CD62Llo phenotype and 1% of cells produced IL-17 following stimulation with PMA/ionomycin (Langrish et al., 2005). Similarly, Harrington et al., were able to identify 2% of IL-17+/IFN-γ− cells after stimulation of naïve D011.10Tg X $Rag^{-/-}$ T cells with OVA peptide and splenic feeder cells in the presence of IL-23 and anti-IFN-γ antibody (Harrington et al., 2005).

Further evidence suggests that IL-23 is not essential for the priming of $Th_{IL\text{-}17}$ cell differentiation. T cells derived from the draining lymph nodes (DLN) of IL-23$p19^{-/-}$ mice 6 days after immunization with $MOG_{35–55}$ peptide emulsified in CFA demonstrate normal proliferative responses to $MOG_{35–55}$ restimulation (Cua et al., 2003) and following immunization of $p40^{-/-}$ mice, *in vitro* culture of DLN cells with IL-23 produces a $Th_{IL\text{-}17}$ phenotype (Langrish et al., 2005). However, while active immunization of $p19^{-/-}$ and $p40^{-/-}$ mice primes myelin-specific T cell responses in the absence of IL-23, systemic expression of IL-23 by treatment with gene transfer vectors is not sufficient to cause symptomatic disease (Cua et al., 2003), whereas reconstitution of IL-23 expression in the CNS alone induces the disease state (Cua et al., 2003). Taken together these data suggest that although $Th_{IL\text{-}17}$ cells have been induced from naïve T cells in certain *in vitro* conditions, IL-23 is not essential for *in vivo* priming of naïve T cells following immunization with myelin epitopes, but is critical for IL-17 production by activated/memory T cells when they re-encounter antigen. Therefore, mechanisms that regulate activated T cell differentiation and cytokine production within the CNS are different from those that result in priming of T cell responses in the periphery.

In this study we provide support for the concept that immunization with $MOG_{35–55}$ peptide results in the activation of a CD4+ T cell population capable of producing both IFN-γ and IL-17. We describe a hitherto unreported population of short-lived IL-17+/IFN-γ+ population of $MOG_{35–55}$-specific cells that can be isolated from the CNS and our data is consistent with this cell population representing a pre-Th1 intermediate phase of differentiation. Recent reports suggesting that the Th1 lineage is only mildly encephalitogenic are based on results of adoptive transfer studies that used Th1 cell lines which had been terminally differentiated *in vitro* to an IL-17−/IFN-γ+ phenotype. The observation that Th1 differentiation is associated with IL-17 production challenges the paradigm that the $Th_{IL\text{-}17}$ subset is the major pathogenic population in EAE.

2. Materials and methods

2.1. Mice

C57BL/6 were obtained from ARC, Perth, WA and housed under specific pathogen-free conditions. Animals were maintained according to regulations of the NH and MRC and all animal procedures were approved by the Garvan Institute Animal Ethics Committee.

2.2. EAE

EAE was induced in 8–10 week old female mice. Mice received a 50 μl injection into each flank, containing 50 μg $MOG_{35–55}$ (MEVGWYRSPFSRVVHLYRNGK) (>90% purity, Mimotopes, Clayton, Vic) emulsified with CFA supplemented with 5 mg/ml of H37 Ra Mycobacterium tuberculosis (Difco Laboratories), and received an i.p. injection containing 200 ng of pertussis toxin (List Biological Laboratories) on days 0 and 2. Animals were observed daily for signs of clinical disease. Disease severity was graded as follows: grade 0, normal; grade 1, loss of tail tone; grade 2, flaccid tail; grade 3, hindlimb paresis; grade 4, hindlimb paralysis; grade 5, forelimb paresis.

2.3. Isolation of CNS inflammatory cells

Mice were euthanized by i.p. injection of ketamine and perfused with ice-cold PBS. Inflammatory cells were recovered from the CNS (brain and spinal cord) by mechanical dissociation of tissues in 30% Percoll (4 °C), which was then layered over 70% Percoll (Amersham Biosciences). Inflammatory cells were separated from the interface after centrifugation at 500 *g* for 20 min and washed twice in PBS (4 °C). Total number of cells derived from each animal was determined using a haemocytometer.