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IL-17+ CD8+ T cells: Differentiation, phenotype and role in inflammatory disease

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ABSTRACT

The pro-inflammatory cytokine interleukin-17A (IL-17) has been the subject of research by many groups worldwide. IL-17 expression is often associated with a specific subset of CD4+ T cells (the so-called Th17 cells); however various other immune cell subsets can also synthesise and express IL-17, including CD8+ T cells. Here we review recent data regarding the presence of IL-17+ CD8+ T cells (also known as Tc17 cells) in human inflammatory disease, discuss current knowledge regarding the culture conditions required for the differentiation of these cells in humans and mice, and describe key phenotypic and functional features. Collectively, this information may shed light on the potential pathogenic role that IL-17+ CD8+ T cells may play in human inflammatory disease.

1. Introduction

Since its discovery in 1993 [1], the pro-inflammatory cytokine interleukin (IL)-17A (in this review generally referred to as IL-17) has been the subject of intense research. The interest in this cytokine increased considerably when it was found to be produced by a specific subset of CD4+ T cells, the so-called Th17 cells. However, it is well established that other immune cell subsets can also synthesise and express IL-17, including CD8+ T cells. In this review, we summarise current data regarding the presence of IL-17+ CD8+ T cells in human inflammatory disease, discuss the differentiation and polarisation protocols reported to induce these cells in humans and mice, and describe current knowledge regarding their phenotype and function. We also discuss how these cells may contribute to immunopathology in human inflammatory diseases.

2. Presence of IL-17+ CD8+ T cells in human inflammatory disease

The presence of IL-17-expressing CD8+ T cells (also referred to as Tc17 cells) has been described in several human inflammatory diseases. An early study reported the presence of IL-17 mRNA in CD8+ T cell clones derived from psoriatic lesional skin [2]. Later studies using flow cytometry, demonstrated that psoriatic skin plaques contain increased numbers [3] or proportions of IL-17+ CD8+ T cells [4–6], whilst this was not observed in control skin samples. Our own lab showed that synovial fluid from the inflamed joints of patients with psoriatic arthritis, but not rheumatoid arthritis, contains increased frequencies of IL-17+ CD8+ T cells compared to matched peripheral blood [7]. In active lesions in brain tissue from patients with multiple sclerosis, IL-17 expression was detected in both CD8+ and CD4+ T cells with equal distribution, and both cell types were present at higher levels compared to inactive lesions [8]. In children with new onset type I diabetes, an increased percentage of IL-17+ cells within peripheral blood CD8+ and CD4+ T cell populations was found following 3 days of *in vitro* stimulation compared to age-matched healthy controls [9]. IL-17+ CD8+ T cells were found to be enriched in the liver of patients with chronic hepatitis C virus (HCV) infection or nonalcoholic steatohepatitis [10] and in the pleural effusion of tuberculosis patients [11]

Abbreviations: EAE, experimental autoimmune encephalomyelitis; HCV, hepatitis C virus; IRF, interferon regulatory factor; RORγ, retinoic acid receptor-related orphan receptor gamma.

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compared to peripheral blood. Finally, using immunofluorescence staining, CD8+ T cells expressing IL-17A and IL-17F were detected in bronchoscopic biopsies from the subsegmental bronchi of patients with chronic obstructive pulmonary disease, at percentages similar to CD4+ T cells [12]. Together, these data demonstrate that IL-17+ CD8+ T cells are present in inflamed tissue in various human inflammatory diseases suggesting these cells may contribute to immune pathology.

3. IL-17+ CD8+ T cell differentiation and polarisation in humans and mice

It is well established that transforming growth factor (TGF)-β, IL-6, IL-1β, IL-21 and IL-23 can promote IL-17+ CD4+ T cell differentiation in humans [13–16] and mice [17–20]. Since IL-17+ CD8+ T cells have a similar cytokine profile to IL-17+ CD4+ T cells, this provides a rationale for applying IL-17+ CD4+ T cell polarising conditions to induce or expand IL-17+ CD8+ T cells. Table 1 summarises the *in vitro* culture conditions reported thus far to expand human or mouse IL-17+ CD8+ T cells and IL-17+ interferon (IFN)-γ+ dual producing CD8+ T cells. A limited number of human IL-17+ CD8+ T cell differentiation studies are published to date compared to those in mice. One study reported that human IL-17+ CD8+ T cells were induced upon culture of naïve CD8+ T cells with recombinant TGF-β, IL-6, IL-1β, IL-23 and α-IFN-γ mAb for 5 days, followed by IL-2 addition for a further 4 days [21]. However, a representative figure showed 0.11% of IL-17+ CD8+ T cells indicating that only a limited percentage of these cells was induced. Another protocol involved culturing human bulk CD8+ T cells with TGF-β and IL-6 for 3 days [22]. IL-17+ CD8+ T cell induction frequencies were not reported, but low IL-17 levels were detected by ELISA.

More detailed information stems from mouse studies, in which TGF-β and IL-6 have been used to drive IL-17+ CD8+ T cell differentiation from CD8+ T cells [23–29], leading to frequencies ranging from 19%–64% (Table 1). TGF-β decreases IFN-γ production, while reducing cytolytic activity and expression of the cytolytic marker granzyme B within *in vitro* cultured CD8+ T cells [24,25]. TGF-β also inhibits CD8+ T cell proliferation and division, but in concert with IL-6, these TGF-β-mediated actions are opposed while maintaining reduced cytolytic activity, a characteristic of IL-17+ CD8+ T cells [25]. A role for IL-6 in IL-17+ CD8+ T cell induction was also shown in mice *in vivo*, since after allogeneic stem cell transplantation IL-6R blockade reduced IL-17+ CD8+ T cell frequencies [30]. In contrast to IL-6, the effects of TGF-β may vary between *in vitro* and *in vivo* conditions. TGF-β removal from the IL-17+ CD8+ T cell differentiation cocktail containing IL-1β, IL-2, IL-6, IL-21, IL-23, α-IL-4 and α-IFN-γ mAbs led to a strong reduction in IL-17+ CD8+ T cell percentages *in vitro* [23]. However, *in vivo* TGF-β neutralisation in mice did not considerably affect IL-17+ CD8+ T cell frequencies [30]. Furthermore, TGF-βRIIDN mice with impaired TGF-β signalling still exhibited IL-17+ CD8+ T cell differentiation, whilst IL-17+ CD4+ T cell differentiation was inhibited [31], suggesting that TGF-β may not be critical for *in vivo* IL-17+ CD8+ T cell differentiation, and that cytokines required for IL-17 induction in CD4+ versus CD8+ T cells may differ.

IL-21 has also been shown to be important for IL-17+ CD8+ T cell differentiation in mouse cells, either as part of a cytokine cocktail (TGF-β, IL-6, IL-1β, IL-2, IL-21, IL-23, α-IL-4 and α-IFN-γ mAb) [23] or in combination with TGF-β [32]. Increased *Il21* mRNA expression was observed in mouse CD8+ T cells cultured with TGF-β and IL-21, with TGF-β and IL-6, or with IL-21 alone [24]. IL-21 production by IL-17+ CD8+ T cells may promote a positive feedback loop to expand IL-17+ CD8+ T cells further, an autocrine mechanism reported in IL-17+ CD4+ T cells [20,33]. Human stimulated IL-17+ CD8+ T cells from psoriatic lesions express IL-21 [5], however it remains to be established whether IL-21 is important for human IL-17+ CD8+ T cell differentiation.

IL-23 is often used to expand human IL-17+ CD4+ T cells [14,15]. IL-23 addition to hapten-primed mouse CD8+ T cell and dendritic cell co-cultures induced IL-17 production [34], yet IL-23 alone only slightly induced *Il17a* expression in mouse naïve CD8+ T cell cultures [27]. Thus, IL-23 may maintain the IL-17+ CD8+ T cell phenotype rather than drive differentiation, similar to its role in IL-17+ CD4+ T cells [35]. In humans, a role for IL-23 in IL-17+ CD8+ T cell differentiation is not yet elucidated, however one study revealed that heterozygous carriers of the R381Q IL23R variant exhibited reduced IL-17+ CD8+ T cell frequencies compared to carriers of the common variant [36], indicating a potential role of IL-23 in human IL-17+ CD8+ T cell development. IFN-γ neutralisation expanded mouse IL-17+ CD8+ T cells *in vitro* [23,26,32] and α-IFN-γ mAb removal from the polarising cocktail also containing IL-1β, IL-2, IL-6, IL-21, IL-23, TGF-β and α-IL-4 mAb, reduced IL-17+ CD8+ T cell frequencies [23]. These data indicate that IFN-γ reduces IL-17+ CD8+ T cell expansion, as seen in mouse IL-17+ CD4+ T cell studies [37]. In support of this, Type I IFN signalling-deficient mice (used to inhibit IFN-γ+ CD8+ T cell induction) treated with neutralising IFN-γ Abs showed higher IL-17+ CD8+ T cell levels *in vivo,* as compared to wild type mice [27]. IL-17+ CD8+ T cell frequencies were also expanded *in vivo* when allogeneic mice were injected with α-IFN-γ mAb 7 days post-stem cell transplant [30]. Additionally, higher IL-17+ CD8+ T cell frequencies were reported in IFN-γ-deficient OT-I mice compared to wild-type mice [23], further indicating that IFN-γ inhibition *in vivo* enhances IL-17 production by CD8+ T cells in mice. IFN-γ was neutralised in one *in vitro* human IL-17+ CD8+ T cell differentiation study [21], however further investigations are required to establish its exact role in the human context.

Collectively, the findings reported thus far indicate that similarities exist between the culture conditions used for mouse IL-17+ CD8+ and IL-17+ CD4+ T cell differentiation. However, there are still significant gaps in our knowledge regarding the exact conditions required for human IL-17+ CD8+ T cell induction or expansion. It will be important to address these gaps in future, given the accumulating evidence of the presence of these cells in human inflammatory disease, which warrants detailed investigation of their function.

4. Phenotype of IL-17+ CD8+ T cells in humans and mice

To date, phenotypic profiling of human IL-17+ CD8+ T cells has been limited at both protein and molecular level, although some characterisation has been performed. Furthermore, variation in the inflammatory sites from which cells are sourced combined with disparity between *in vitro* induction or expansion protocols makes comparison of individual studies challenging. Despite these challenges some phenotypic features have been described for human IL-17+ CD8+ T cells including surface marker, cytokine and transcription factor expression. A summary of current mouse and human IL-17+ CD8+ T cell phenotype data is shown in Fig. 1. Several of these features are shared with IL-17+ CD4+ T (Th17) cells, indicating some similarities between these cell types which may give an insight into the functional potential of IL-17+ CD8+ T cells

The most definitive feature of human IL-17+ CD8+ T cells is their ability to produce the pro-inflammatory cytokine IL-17A (IL-17) but concurrent expression of several other cytokines has been shown. The most well-described of these is the pro-inflammatory cytokine IFN-γ,