Transforming Growth Factor-β: An Indirect Inducer of Th17 Cell Differentiation both in Human and Murine System

J. Schumann
Institute of Physiological Chemistry, Faculty of Veterinary Medicine, University of Leipzig, An den Tierkliniken 1, 04103 Leipzig, Germany

Abstract: Since their first description only some years ago Th17 cells have become of vital importance in immunological research. However, the role of TGF-β in Th17 cell development is still a matter of controversial discussion. Predicted to have a key function in Th17 cell differentiation in the mouse TGF-β was shown to inhibit IL-17 production of human CD4+ T cells. Moreover, recent data indicate TGF-β signaling in T cells to be dispensable for Th17 cell differentiation in the murine system. Hence, rather being a specie-specific factor TGF-β is likely to act as an indirect inducer of Th17 cell differentiation both in mice and men.

Key words: Transforming growth factor, T helper cell, mice and men, murine system

INTRODUCTION

Th17 cells are a subset of T helper cell lymphocytes producing IL-17, TNF-α and IL-6, but not IFN-γ or IL-4 (McKenzie et al., 2006; Iwakura and Ishigame, 2006). Via secretion of IL-17 Th17 cells enhance the expression of G-CSF (Fossiez et al., 1996; Cai et al., 1998; Schwarzenberger et al., 1998; 2001), IL-8 (Fossiez et al., 1996; Laan et al., 1999; Yao et al., 1995a; Kawaguchi et al., 2000; Katz et al., 2001; Jones and Chan, 2002; Prause et al., 2003) and ICAM-1 (Yao et al., 1995a; Albanesi et al., 1999) thereby promoting chemotaxis of neutrophils and monocytes to sites of inflammation. In addition, IL-17 stimulates the production of IL-6 (Fossiez et al., 1996; Yao et al., 1995a; 1995b; Katz et al., 2001; Chabaud et al., 1998), matrix metalloproteinases (Chabaud et al., 2000), prostaglandin E2 (Fossiez et al., 1996), NO (Shalomb-Barak et al., 1998; Attur et al., 1997) as well as the complement components C3 and factor B (Katz et al., 2000). Hence, Th17 cells represent potent pro-inflammatory mediators crucial in the regulation of inflammatory responses.

In a number of inquiries using murine models of host defense Th17 cells have been described to be important in the pathogenesis of chronic inflammatory diseases including arthritis (Lubberts et al., 2000; Latham et al., 2005), colitis (Zhang et al., 2006; Ogawa et al., 2004), encephalitis (Komiyama et al., 2006; Hofstetter et al., 2005) as well as asthma and allergy (Hellings et al., 2003). In humans IL-17 producing T cells have been isolated from synovial membranes and synovial fluid of patients suffering from rheumatoid arthritis (Chabaud et al., 1999). At this, severity of inflammation is reported to be consistent to the IL-17 concentration detectable in serum and tissue of the arthritis patients. Furthermore, a correlation between IL-17 serum levels and the degree of inflammatory processes has been described in the context of autoimmune encephalomylititis (Lock and Heller, 2003) and inflammatory bowel disease (Fujino et al., 2003). Thus, IL-17 especially if produced by T cells is a likely candidate for intervention in chronic inflammatory diseases in men.

Th17 cell differentiation: Proceeding specie-specific?

In the murine system the synergistic action of TGF-β and IL-6 has been considered to be essential for Th17 cell development, so far (Veldhoen et al., 2006; Mangan et al., 2006; Bettelli et al., 2006). In contrast, differentiation of human naïve T helper cells into Th17 cells has been reported not to depend on TGF-β (Chen et al., 2007; Wilson et al., 2007; Acosta-Rodriguez et al., 2007; Manel et al., 2008; Santarlasci et al., 2009; Cosmi et al., 2008). Moreover, TGF-β has been predicted to act suppressive on IL-17 production of human CD4+ T cells (Acosta-Rodriguez et al., 2007; Manel et al., 2008). Due to the divergent observations made in murine and human systems Th17 cells development in mice and men has been assumed to be regulated differentially (Annunziato et al., 2009; Annunziato and Romagnani, 2009). However, recent data provide evidence that TGF-β is not directly required for the molecular orchestration of Th17 cell differentiation in...
the mouse as well (Das *et al.*, 2009; Schumann, 2008). Thus, the situation seen in the murine system is closer related to the human situation as previously thought.

**What role for TGF-β in Th17 differentiation?** TGF-β initially has been published as a growth factor (De Larco and Todaro, 1978). Thereinafter it was found, that TGF-β not only influences differentiation of cells but is also crucial in inflammatory processes (Kehrl *et al.*, 1986a; 1986b). TGF-β regulates the innate and the adaptive immunity affecting macrophages (Ashcroft, 1999), dendritic cells (Strobl and Knapp, 1999), T cells (Cerwenka and Swain, 1999), B cells (Lebman and Edmiston, 1999) and NK cells (Horwitz *et al.*, 1999). The cytokine acts both pro- and antiinflammatory depending on genetic background, cell type, state of differentiation, extracellular matrix as well as the presence of other regulatory factors (McCartney-Francis and Wahl, 1994; Wahl, 1994). Hence, TGF-β shows a distinctive pleiotropy playing a complex role in immunomodulation.

At present time the role of TGF-β in T helper cell differentiation has become of special interest. Depending on cytokine milieu naïve T helper cells differentiate into four known T helper cell subsets. TGF-β interacts in this process by several ways. Both Th1 (Gorelik *et al.*, 2002; Lin *et al.*, 2005) and Th2 cells (Chen *et al.*, 2003a; Gorelik *et al.*, 2000; Heath *et al.*, 2000) are inhibited by TGF-β. Concomitantly, TGF-β represents the direct inducer of Treg cells, which themselves are considerable producers of the cytokine (Chen *et al.*, 2003b).

Beside its clear role in Treg cell development conflicting data have been published concerning the relevance of TGF-β in Th17 cell generation in mice and men. In murine system cytokines critically for Th17 cell differentiation were propagated to be TGF-β and IL-6 (Veldhoen *et al.*, 2006; Mangan *et al.*, 2006; Bettelli *et al.*, 2006). However, TGF-β has not been shown to directly promote the generation of IL-17 producing T cells from naïve precursors. Rather, a beneficial effect of TGF-β was seen, when naïve CD4+ T cells were co-cultivated with Treg cells plus dendritic cells and stimulated with anti-CD3-antibody as well as Lipopolysaccharid (LPS) (Veldhoen *et al.*, 2006). LPS is known as strong inducer of Th1 reactions via a release of IL-12 by dendritic cells (Pearce *et al.*, 2006). The effect of TGF-β in such a setting might therefore rather be based on the prevention of a Th1 cell development. Consistently to this assumption, using a mouse model unable to generate Th1 and Th2 cells, the cytokine IL-6 has been reported to be sufficient to drive Th17 cell generation (Das *et al.*, 2009). In addition, TGF-β was shown not to affect the expression of the retinoic acid receptor-Related Orphan Receptor γt (ROR γt), a Th17 cell specific transcription factor (Das *et al.*, 2009). Beyond that, Th17 cells could be detected in a transgene mouse strain T cell specific insensitive for TGF-β signaling (Schumann, 2008). Visualization of Th17 cells succeeded both ex vivo after re-stimulation of lymph node cells of *B. burgdorferi*-infected mice as well as in vitro after T cell specific stimulation of splenocytes and purified CD4+ lymphocytes (Schumann, 2008). In contrast, stimulation of T cells over-expressing TGF-β resulted in decreased Th17 cell numbers in comparison to the wild type (Schumann, 2008). Hence, there is compelling evidence that TGF-β does not act as a direct inducer of Th17 cell development in the mouse.

The dispensability of TGF-β signaling on T cells for Th17 cell development in the murine system is consistent to publications dealing with effects of TGF-β on human T helper cell differentiation. In human system TGF-β has been shown to inhibit IL-17 production by T cells (Acosta-Rodriguez *et al.*, 2007; Manel *et al.*, 2008). According hitherto existing literature rather than TGF-β, cytokines such as IL-1β and IL-23 drive Th17 cell development from human CD4+ precursors (Chen *et al.*, 2007; Wilson *et al.*, 2007; Acosta-Rodriguez *et al.*, 2007).

**TGF-β affects Th17 cell differentiation in an indirect way:** TGF-β is part of a complex regulatory system critically in immune homeostasis. The cytokine is of vital importance in T helper cell differentiation influencing all four known T helper cell subsets. In addition to interference in T helper cell development TGF-β represents the main product of Treg cells thereby mediating the immunosuppressive and anti-inflammatory function of these cells (Chen *et al.*, 2003b).

Of note, the cytokine accomplishes its effects both directly and indirectly. TGF-β directly induces Treg cell differentiation via promotion of the transcription factor FoxP3 (Chen *et al.*, 2003b). TGF-β directly blocks Th1 as well as Th2 cell development via inhibition of T-bet (Gorelik *et al.*, 2002; Lin *et al.*, 2005) and GATA-3 (Chen *et al.*, 2003a; Gorelik *et al.*, 2000; Heath *et al.*, 2000) expression, respectively. By contrast, Th17 cell differentiation is affected indirectly by TGF-β. IFN-γ and IL-4, the cytokines produced by Th1 and Th2 cells, are well known inhibitors of Th17 cell development. Hence, blocking of Th1 and Th2 cells linked with the absence of IFN-γ and IL-4 provides a milieu optimal for naïve CD4+ precursors to
differentiate into Th17 cells (Veldhoen et al., 2006; Mangan et al., 2006; Bettelli et al., 2006; Infante-Duarte et al., 2000). Taken together, the impact of TGF-β in T helper cell specification both in human and murine system is due to its inhibitory action on Th1 and Th2 cells in addition to its direct promotion of Treg cell amplification.

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