Shuiping Jiang *Editor*

T_H17 Cells in Health and Disease

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Part I
The Immunology of IL-17 Family and T_H17 Cells

From T_H1/T_H2 Paradigm to T_H17 Cells: Le Roi Est Mort, Vive Le Roi

Amit Awasthi and Vijay K. Kuchroo

Abstract Upon activation, naïve $CD4^+$ T cells differentiate into distinct T helper cell subsets with specific cytokine profiles and distinct effector functions. Until recently, effector T cells were classified into T_H1 or T_H2 subtypes depending on the cytokines they produced. However, this paradigm had to be revised with the discovery of a third subset of effector T cells called T_H17 cells. IL-17-producing T_H17 cells play an important role in clearing extracellular pathogens and tissue inflammation. TGF-β and IL-6 are the factors that induce differentiation of naïve T cells into T_H17 cells. Differentiated T_H17 cells produce IL-17, IL-17F, IL-21 and IL-22, and thereby mediate distinct effector functions compared to T_H1 and T_H2 cells. While IL-17, IL-17F and IL-22 induce tissue reaction, IL-21 produced by T_H17 cells is essential for amplification of T_H17 cells and B cell function. Whereas the maturation and stabilization of differentiated T_H17 cells are mediated by IL-23; the transcription factors (STAT3, RORγt, RORα and c-Maf) are involved in the development and transcription of various molecules expressed by T_H17 cells. The requirement of TGF-β in generation of both T_H17 cells and $CD4^+CD25^+Foxp3^+$ regulatory T cells (Tregs) suggest a close developmental relationship between these two cell types. Here, we summarize the current information on the differentiation and effector functions of the T_H17 lineage and their interplay with other T helper subsets during tissue inflammation.

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1 Introduction

Antigenic stimulation of naïve T cells in the presence of specific cytokines produced by innate cells induces activation, expansion and differentiation of T cells into distinct effector T cells (Abbas et al. 1996). About 25 years ago, Mosmann and Coffman introduced the T_H1/T_H2 paradigm of T helper cell differentiation, which helped explain many aspects of adaptive immunity from eliminating intracellular vs. extracellular pathogens to induction of different types of tissue inflammation (Mosmann et al. 1986; Mosmann and Coffman 1989). IFN-γ and IL-12 induce the differentiation of naïve T cells into a T cell subset that predominantly produces IFN-γ and classified as T_H1 cells (Hsieh et al. 1993). T_H1 cells are indispensable for clearing intracellular pathogens by activating effector functions of macrophages and inducing antibody class switching to IgG2a (Mosmann and Coffman 1989). The presence of IFN-γ during T_H1 differentiation induces STAT-1 dependent expression of T-bet, a master transcription factor required for IFN-γ production (Szabo et al. 2000). Both STAT-1 and T-bet deficient mice lack IFN-γ induction and are susceptible to intracellular pathogens such as *Leishmania* and *Mycobacteria* (Szabo et al. 2003). During the T_H1 development, T-bet induces the expression of IL-12Rβ2, which makes developing T_H1 cells responsive to IL-12 in a STAT-4 dependent manner (Mullen et al. 2001). IL-12 exposure to developing T_H1 cells stabilizes their T_H1 phenotypes, and this loss of STAT-4 in STAT-4 deficient mice enhances the susceptibility against intracellular pathogens (Kaplan et al. 1996b). Altogether, these observations suggest the sequential transcriptional requirements (STAT-1, T-bet, STAT-4) for the development of effector T_H1 cells (Szabo et al. 2003). In contrast, IL-4 inhibits the functions of T_H1 cells and induces the differentiation of T cell subsets that predominantly produce IL-4, IL-5, IL-13 and IL-25. The effector functions of this subset are opposite to that of T_H1 cells in that they eliminate extracellular pathogens (like helminthes) and promote antibody class switching to IgG1 and IgE. IL-4 initiates T_H2 development by inducing phospho-STAT-6, which induces GATA-3, a T_H2 specific transcription factor that binds and trans-activates IL-4 promoter to initiate T_H2 developmental program (Ansel et al. 2006; Takeda et al. 1996; Zheng and Flavell 1997). STAT-6 deficient mice are defective in generating T_H2 cells and are susceptible to extracellular pathogens (Kaplan et al. 1996a). Similarly, loss of GATA-3 in mice results in defective T_H2 development with enhanced susceptibility to T_H2 associated pathogens (Zhu et al. 2004). Moreover, overexpression of GATA-3 induces T_H2 development and inhibits the generation of T_H1 cells (Zheng and Flavell 1997). These observations suggest that both STAT-6 and GATA-3 are the transcription factors essential for the development of effector T_H2 cells (Ansel et al. 2006). One of the striking features of T_H1 and T_H2 cells is that they cross-regulate functions of each other by producing antagonizing cytokines (Mosmann and Coffman 1989). For instance, IFN-γ, a T_H1 cytokine, regulates the differentiation of T_H2 cells, while IL-4 produced by T_H2 cells inhibits the generation of T_H1 cells (Mosmann and Coffman 1989). Both T_H1 and T_H2 cells are indispensable for eliminating both intracellular and extracellular pathogens. Therefore, lack of appropriate differentiation in response to infection would result

in dissemination of infection (Mosmann and Coffman 1989). However, deregulated T_H1 and T_H2 cells can mediate tissue inflammation and immunopathology (Kuchroo et al. 2002). T_H1 cells have been associated with multiple auto-immune diseases such as multiple sclerosis, rheumatoid arthritis, type-1 diabetes, and inflammatory bowel disease (IBD). Similarly, a deregulated T_H2 response promotes asthma, allergies, and atopy. Although T_H1 cells with specificity of self antigens were considered to be the pathogenic T cells involved in inducing auto-immune diseases, loss of IFN-γ or T_H1 differentiation factors, IL-12, did not inhibit auto-immune diseases, but paradoxically enhanced the incidence and severity of disease (Cua et al. 2003; Ferber et al. 1996). Similarly, T_H2 cells or cytokines were not able to induce auto-immunity (Kuchroo et al. 1995) and this raised the issue of whether there were other cell types involved in inducing auto-immune tissue reactions. With the discovery of IL-23, a cytokine that shares a common chain with IL-12, it became clear that the loss of IL-23 and not IL-12 made mice resistant to the development of multiple auto-immune diseases (Cua et al. 2003; Oppmann et al. 2000). IL-23 induced IL-17 from T cells and this led to the discovery of IL-17-producing T cells, which were shown to induce massive tissue inflammation and auto-immunity (see below) (Korn et al. 2009). T_H17 cells are a subset of T cells that have differentiation and transcription factors that distinguish them from T_H1 and T_H2 cells (Bettelli et al. 2008; Korn et al. 2009). The discovery of T_H17 cells has expanded the T_H1–T_H2 paradigm, and the integration of T_H17 cells with T_H1 and T_H2 effector T cells is beginning to explain the underlying mechanisms of tissue inflammation in a number of infections and auto-immune disease settings. The highly pathogenic nature of T_H17 cells makes them the primary effector cells type in eliminating specific pathogens that are not adequately handled by T_H1 or T_H2 cells. Besides their role in clearing infections, T_H17 cells are potent inducers of tissue inflammation since IL-17 receptors are present on all parenchymal cells including endothelium, fibroblasts, and epithelial cells. Plus, activation with IL-17 results in the production of IL-1, IL-6, TNF-α, matrix metalloproteinases and chemokines, including IL-8 which makes tissue susceptible to infiltration to other pro-inflammatory cells and propagate tissue inflammation (Bettelli et al. 2008). IL-17 and T_H17 cells have been associated with the pathogenesis of many auto-immune diseases both in mice and man. In the last 5 years, cytokines that are required for differentiation, amplification and expansion of T_H17 cells have been identified and transcription factors that induce T_H17 cells differentiation have been elucidated.

In this chapter, we summarize the findings that led to the identification of T_H17 cells, their differentiation factors, and we describe their helper and effector functions.

2 T_H17 Cells Differentiation

Identification of the differentiation and transcription factors for T_H17 cells has classified T_H17 cells into a unique subset of helper T cells, distinct from T_H1 or T_H2 cells (Korn et al. 2009). The discovery of IL-23 was instrumental in identifying T_H17 cells and studying their role in inducing tissue inflammation and auto-immunity (Cua et al. 2003). IL-23, a member of the IL-12 family of cytokines, was discovered

in 2000 and is composed of a unique sub-unit called IL-23p19 which associates with the p40 sub-unit of IL-12 to form a hetero dimeric cytokine (Oppmann et al. 2000). Cua et al. showed that p19 deficient mice, in contrast to p35 deficient mice, were resistant to the development of experimental auto-immune encephalomyelitis (EAE) (Cua et al. 2003). Further analysis showed that the central nervous system (CNS) of IL-23p19 deficient mice harbors less T_H17 cells and therefore suggested that IL-23 may be the differentiation factor for the generation of pathogenic T_H17 cells (Cua et al. 2003). In fact, IL-23 was able to expand IL-17 producing T cells from immunized mice further supporting the interpretation that IL-23 may indeed induce differentiation of T_H17 cells (Awasthi et al. 2009). As IL-23R is predominantly expressed on activated/memory T cells, it was possible that IL-23 might act on previously differentiated T_H17 cells rather than promoting de novo differentiation of naïve T cells (Awasthi et al. 2009; McGeachy et al. 2009; Parham et al. 2002). Furthermore, addition of IL-23 to sorted naïve T cells was not able to induce differentiation of T_H17 cells, confirming that IL-23 is not the diversity factor for the differentiation of T_H17 cells (Bettelli et al. 2006). In 2006, three independent studies reported that instead of IL-23, a combination of the immuno-regulatory cytokine TGF-β and a pro-inflammatory cytokine IL-6 is required to induce the differentiation of T_H17 cells from naïve T cells (Bettelli et al. 2006; Mangan et al. 2006; Veldhoen et al. 2006a). Interestingly, TGF-β induces the generation of $Foxp3^+$ iTregs cells from naïve T cells which are able to suppress immune responses in vitro and in vivo (Fig. 1) (Bettelli et al. 2006).

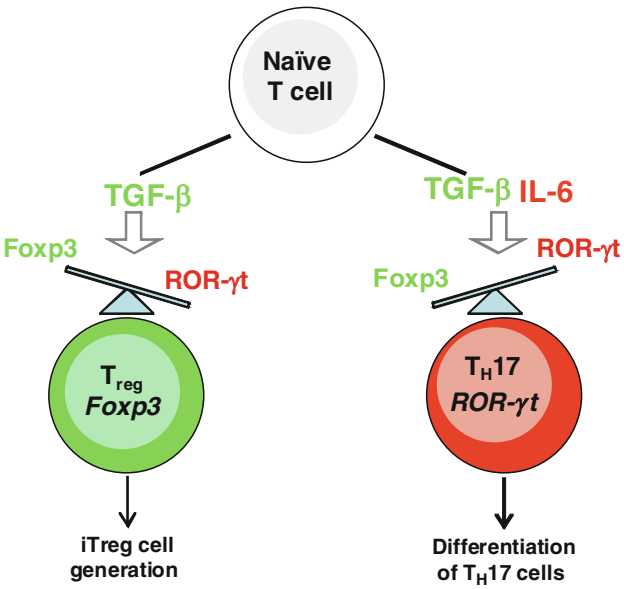

Fig. 1 Reciprocal generation Tregs and T_H17 cells. Activation of naïve T cells with TGF-β induces the generation of Foxp3+ Tregs cells while the addition of IL-6 not only inhibited the induction of TGF-β-induced Foxp3 but also concomitantly induced the generation of T_H17 cells

However, the addition of IL-6 to TGF-β abrogated the induction of Foxp3 expression and resulted in the induction of T cells that predominantly produced IL-17 (Bettelli et al. 2006). The function of TGF-β in differentiation of T_H17 cells was confirmed using TGF-β transgenic mice, where TGF-β overexpression was controlled under IL-2 promoter. Immunization of TGF-β transgenic mice with myelin antigen emulsified in CFA induced severe inflammation in the CNS with the development of overly aggressive EAE (Bettelli et al. 2006). Further analysis of CNS of these mice revealed a higher frequency of T_H17 cells and reduction in frequency $FoxP3^+$ Treg cells (Bettelli et al. 2006). Conversely, when T cells are deficient in a functional receptor of TGF-β and cannot signal to TGF-β, the generation of T_H17 cells was abolished and mice were completely protected from development of EAE (Veldhoen et al. 2006b). Immunization with CFA induces IL-6 from innate immune cells, which in combination with TGF-β induces the generation of T_H17 cells. This data confirmed the in vitro data that TGF-β and IL-6 are essential in generating T_H17 cells and indicated that IL-6 is the key switch factor in determining whether an immune response is dominated by $Foxp3^+$ Tregs or by T_H17 cells (Bettelli et al. 2008). TGF-β is a pleiotropic cytokine produced by multiple lineages of leukocytes and stromal cells (Li and Flavell 2008). However, the cellular source of TGF-β for the induction of T_H17 cells has not been identified. Deletion of the TGF-β1 gene specifically in T cells is detrimental in that the mice develop lethal immunopathology associated with uncontrolled T_H1 and T_H2 responses in multiple organs (Li et al. 2007). TGF-β-deficient $Foxp3^+$ T cells, in contrast to wild type $Foxp3^+$ T cells, fail to control T_H1 mediated inflammation in a T cell transfer model of colitis (Li et al. 2007). In addition, ablation of TGF-β1 production in T cells resulted a defective generation of T_H17 cells and these mice were protected from developing EAE, implying that T cells which produced TGF-β contributed to the differentiation of T_H17 cells (Li et al. 2007). In addition to T cells, DCs express sufficient amounts of TGF-β to drive the differentiation T_H17 cells (Veldhoen et al. 2006b). In fact, conditional deletion of αvβ8 (an integrin required for processing inactive forms of TGF-β into active forms) in DCs resulted in lymphoproliferation and tissue inflammation that was reminiscent of TGF-β1 deficiency in T cells (Travis et al. 2007). These observations indicate that DCs play a role in generating active TGF-β1 locally to support the generation of T_H17 cells in vivo (Veldhoen et al. 2006b). Furthermore, DCs from lamina propria produce TGF-β and retinoic acid induces de novo conversion of T cells into $Foxp3^+$ Tregs cells (Denning et al. 2007). T_H17 cells are also highly enriched in the lamina propria, which raises the possibility that DC-produced TGF-β in the gut in combination with IL-6 might drive T_H17 differentiation in this context.

Similar to TGF-β, IL-6 is also a pleiotropic cytokine induced by infection, inflammation or injury, and mediates a variety of functions in both immune and non-immune compartments (Kishimoto 2005). IL-6 is mainly produced by activated DCs, monocytes, and macrophages in response to TLRs activation. Different approaches led to the identification of IL-6 as a critical player in the differentiation of T_H17 cells: (1) LPS stimulated DC/T cell co-cultures induced the de novo generation of T_H17 cells when TGF-β was present and neutralization of IL-6 by an IL-6

antibody abolished the generation of T_H17 cells (Veldhoen et al. 2006a), (2) the addition of IL-6 together with TGF-β inhibited the expression of $Foxp3^+$ iTregs and resulted in the generation of T_H17 cells (Bettelli et al. 2006). The in vivo function of IL-6 in the generation of T_H17 cells was emphasized in both IL-6 deficient and gp130, a signaling sub-unit of IL-6 receptor complex, and conditional "knock-out" mice (Korn et al. 2007, 2008). These mice were completely resistant to EAE, and in fact, the peripheral repertoire in these mice was dominated by a high frequency of $Foxp3^+$ T_{regs} (Korn et al. 2007, 2008). The reciprocal developmental pathways of T_H17 and iT_{regs} cells suggest that these cells share a common precursor and depending on the cytokines present at the time of their activation, T cells can differentiate into Tregs or T_H17 cells depending on the availability of IL-6 (Fig. 1). It suggests that TGF-β and IL-6 are essential in the differentiation of T_H17 cells both in vitro and in vivo.

3 T_H17 Amplification and IL-21

IL-21, a member of the IL-2 family of cytokines, uses a common γ chain of IL-2 receptor expressed on all T and B cells (Leonard et al. 2008). T_H17 cells produce high amount of IL-21 in addition to IL-17A, IL-17F, and IL-22 (Korn et al. 2007; Nurieva et al. 2007). IL-21, a pleiotropic cytokine, induces a variety of functions on $CD4^+$, $CD8^+$, and B cells (Leonard et al. 2008). Analysis of differentiation in IL-21R deficient T_H17 cells showed that IL-21 is essential to amplify T_H17 differentiation (Korn et al. 2007; Nurieva et al. 2007). This observation is consistent with findings that IL-21, in combination with TGF-β, induces de novo T_H17 differentiation (Korn et al. 2007). These findings imply that T_H17 differentiation can be induced in the absence of IL-6, and cells such as NKT and NK cells that produce IL-21 can support T_H17 differentiation in the absence of IL-6. However, IL-6 still remains the dominant factor in the differentiation of T_H17 cells such that IL-6 deficient mice are completely resistant to EAE development with a defect in T_H17 generation (Korn et al. 2007; Okuda et al. 1998). IL-6 induces IL-21 in T_H17 cells in a STAT-3 dependent manner, which further amplifies the generation of T_H17 cells. IL-21 also helps T_H17 cells in stabilizing their T_H17 phenotype by inducing the expression of IL-23R, and makes T_H17 cells responsive to IL-23 exposure to attain their effector phenotype (Korn et al. 2007; Nurieva et al. 2007). IL-6 also induces IL-23R expression on T_H17 cells sequentially by inducing IL-21 in differentiating T_H17 cells (Littman and Rudensky 2010). These in vitro observations were further confirmed using IL-21 or IL-21R deficient mice showing a diminished expression of IL-23R, resulting in the blunt T_H17 generation. Therefore, IL-21 not only amplifies T_H17 differentiation, but also helps T_H17 cells to attain a mature T_H17 phenotype (Coquet et al. 2008). An initial study showed that IL-21-deficient mice are resistant to EAE and two subsequent studies showed development of a more severe EAE than the wild type cohorts (Coquet et al. 2008). These studies questioned whether IL-21 is critical in vivo for the generation of T_H17 cells. Using Complete Freund's Adjuvant (CFA) for immunization to induce EAE in IL-21 or IL-21R deficient mice might

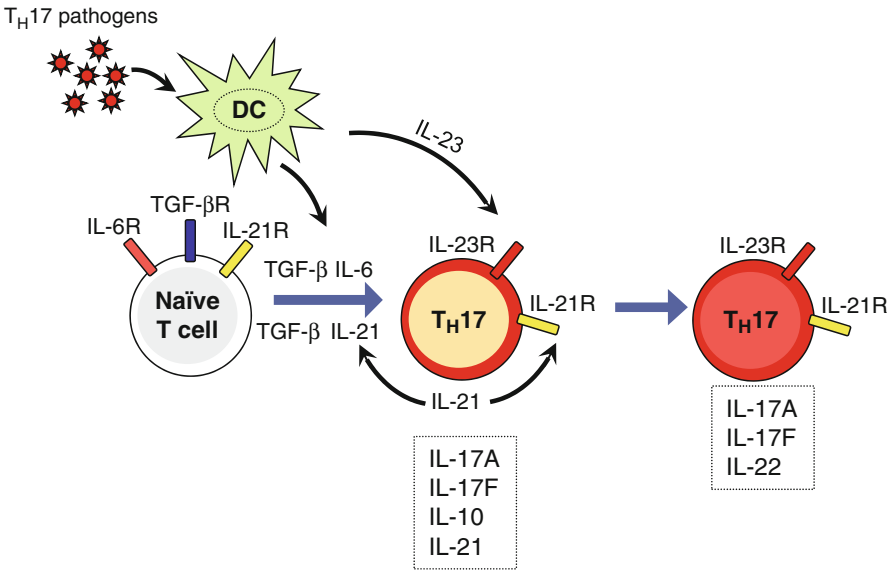

Fig. 2 Developmental pathway of T_H17 cells. Activated DC provide IL-6 that induce T_H17 differentiation together with TGF-β. Differentiated T_H17 cells produce IL-21, which in combination of TGF-β, further enhances the generation of T_H17 cells. IL-21 also induces the expression of IL-23R on T_H17 cells. Innate immune cells (DC and macrophages) produced IL-23 stabilizes T_H17 differentiation

override the need for the induction of IL-21 because massive amounts of IL-6 produced in vivo by immunization with CFA most likely compensates for IL-21 deficiency. This argument is further supported by the observation that NOD mice are completely resistant to the development of type 1 diabetes; if they are deficient in IL-21 or IL-21R induction of auto-immunity occurs spontaneously without any immunization with CFA (Spolski et al. 2008) (Sutherland et al. 2009). In addition to the generation of T_H17 cells, it has been observed that IL-21 is an essential growth factor for IL-27-induced IL-10 producing Tr1 cells (Awasthi et al. 2007; Pot et al. 2009) (Spolski et al. 2009). Therefore, IL-21, or lack of protection against EAE observed in IL-21R deficient mice may be partly due to loss of IL-10 producing Tr1 cells compensating for a defect in T_H17 cells in IL-21 or IL-21R deficient mice. Altogether, it suggests that IL-21 is an essential feed forward loop in self-amplification of T_H17 cells (Fig. 2).

4 T_H17 Stabilization and IL-23

IL-23, a member of the IL-12 cytokine family, is a heterodimer consisting of the p40 sub-unit shared with IL-12 and the IL-23 specific p19 sub-unit (Oppmann et al. 2000). It is expressed by cells of the myeloid lineage, including DCs and macrophages,

and is identified as a cytokine that induces expansion of T_H17 cells from activated T cells (Awasthi et al. 2009). The in vivo function of IL-23 in shaping T_H17 responses came from the analysis of IL-23p19 deficient mice in that the IL-23p19 deficient mice were resistant to the development of EAE and collagen-induced arthritis (Cua et al. 2003; Langrish et al. 2005). This protection against auto-immune inflammation was associated with a defective T_H17 response while the T_H1 immune response was unaltered. Consistent with this observation, IL-23R deficient mice were also resistant EAE and showed a defect in T_H17 development (Awasthi et al. 2009; McGeachy et al. 2009). Using IL-23R-GFP reporter mice, we have identified that in addition to T cells, innate immune cells including gd T cells, NK, NK T cells, DCs, and macrophages express IL-23R and respond to IL-23 by producing IL-17 (Awasthi et al. 2009). Our data indicated that IL-17 is mainly produced by IL-23R$^+$/GFP$^+$ cells, suggesting an absolute requirement of IL-23 to induce/maintain IL-17 production from both T cells and non T cells (Awasthi et al. 2009). Initial studies suggested that TGF-β induces IL-23R expression and further analysis revealed that IL-6 and IL-21 also induce IL-23R expression in a STAT-3 dependent manner (Zhou et al. 2007). IL-23R-GFP reporter mice revealed that IL-23 is the best inducer of its own receptor, even more so than TGF-β, IL-21 and IL-6 (Awasthi et al. 2009). However, activation of naïve T cells requires exposure of IL-6 or IL-21, instead of IL-23 to induce initial expression of IL-23R, as IL-23 does not induce IL-23R expression on naïve T cells (Zhou et al. 2007). Once activated in the presence of TGF-β, IL-6 and/or IL-21, T cells express IL-23R and IL-23 further enhances IL-23R expression to induce T_H17 generation (Awasthi et al. 2009; Zhou et al. 2007). Studies using IL-23R-deficient mice revealed that while IL-23R deficient T cells are able to differentiate into T_H17 cells, they fail to maintain their effector functions (Awasthi et al. 2009; McGeachy et al. 2009). Taken together, these observations suggest that the exposure of T_H17 cells to IL-23 matures/stabilizes their pro-inflammatory phenotype. Initial studies suggest that IL-23 not only upregulates expression of IL-23R, but also enhances the expression of T_H17-lineage specific cytokines IL-17A, IL-17F, IL-21, and IL-22 (Awasthi et al. 2009; McGeachy et al. 2009). It also suppresses production of cytokines like IL-10, which do not belong to T_H17 lineage (McGeachy et al. 2007) (Fig. 2). How IL-23 mediates this function at a molecular level is not well understood.

The role of IL-23/IL-23R in human auto-immune diseases is being increasingly recognized. A genome-wide association scan (GWAS) recently identified the IL-23R gene as a susceptibility gene for human inflammatory bowel disease. A single nucleotide polymorphism of arginine 381 to glutamine confers protection against Crohn's disease (Duerr et al. 2006), which is consistent with the mouse model that indicates a pathogenic role of IL-23 in intestinal inflammation (Buonocore et al. 2010; Kullberg et al. 2006). IL-23p19 deficient CD4$^+$ T cells failed to transfer colitis in a T cells transfer model. IL-23 treatment enhances the development of colitis with the increased expression of IL-17 and IL-6 (Yen et al. 2006). These observations indicate that IL-23–T_H17 pathway plays an important role in development of gut inflammation (Wu et al. 2009). Similarly, other genome-wide association studies revealed an association of SNPs in IL-23R with ankylosing spondylitis and

psoriasis, and treatment with a monoclonal antibody specific for IL-12/IL-23p40 or IL-17 showing promising results in psoriasis patients, further strengthening the idea that IL-23 and T_H17 cells may be involved in inducing human auto-immune tissue inflammation (Rahman et al. 2009).

5 Transcriptional Regulation of T_H17 Cells

Recently, T_H17 lineage was specified by the expression of a specific factor, ROR-γt, a retinoids-related orphan transcription factor identified as an early element required for T cell and lymphoid organ development (Ivanov et al. 2008; Korn et al. 2009). The gene profiling analysis of T_H17 cells identified ROR-γt as a highly expressed transcription factor in this T cell lineage. Distinction of naïve T cells with TGF-β and IL-6 or IL-21 induces the manifestation of ROR-γt in developing T_H17 cells (Ivanov et al. 2006). Neither T_H1 nor T_H2 cells showed any expression of this transcription factor, suggesting a lineage specific appearance of ROR-γt induces T_H17 differentiation. T_H17 cells are highly enriched in the lamina propria in naïve mice and further analysis revealed that ROR-γt$^+$, but not ROR-γt$^-$ T cells produce IL-17, and forced expression of ROR-γt is sufficient to encourage IL-17 expression without the addition of exogenous cytokines (TGF-β plus IL-6) (Ivanov et al. 2006). These observations confirm the ability of ROR-γt to initiate the development of T_H17 cells. The analysis of ROR-γt-GFP reporter mice revealed the co-expression of T_H17 cytokines with ROR-γt (Lochner et al. 2008). In line with its critical functions in differentiation of T_H17 cells, ROR-γt-deficient T cells were defective in T_H17 cell differentiation and developed less severe auto-immune diseases (Ivanov et al. 2006).

The two cytokines (TGF-β and IL-6) required for the differentiation of T_H17 cells have antagonizing effects on one another, e.g. TGF-β strongly upregulates the appearance of Foxp3, while addition of IL-6 or IL-21 inhibits the expression of Foxp3 and induces the expression of IL-17 by upregulation of ROR-γt expression (Bettelli et al. 2006; Korn et al. 2007; Zhou et al. 2008). TGF-β induces both ROR-γt and Foxp3 without expressing IL-17 unless combined with the pro-inflammatory cytokines require for T_H17 differentiation (IL-6, IL-21 or IL-23) (Zhou et al. 2008). In fact, Foxp3 was shown to associate with ROR-γt, and thereby inhibit its transcriptional activity. Addition of pro-inflammatory cytokines suppresses induction of Foxp3, and relieves ROR-γt from the ROR-γt-Foxp3 complex to induce IL-17 transcription (Zhou et al. 2008). Further insight between Foxp3 and ROR-γt came from the analysis of a truncated form of Foxp3, which lacks exon 2 (Zhou et al. 2008). The truncated Foxp3 was unable to interact with ROR-γt, and thus ROR-γt was functional and able to prompt the expression of IL-17 (Zhou et al. 2008). This data clearly demonstrates the role of Foxp3 in inhibiting the development of T_H17 cells. However, the consequences and relevance of this interaction in vivo are not clear. Runx1, a transcription factor required for both T_H17 cell and Treg functions, interacts with both Foxp3 and ROR-γt (Ono et al. 2007; Zhang et al. 2008).

Recently, it has been shown that ROR-γt and Runx1 together bind to the *il-17a* promoter and lead to increased expression of IL-17 (Zhang et al. 2008) (Fig. 3).

In addition to ROR-γt, differentiation of T_H17 cells also requires ROR-α, another ROR family of transcription factors. ROR-α is also upregulated during T_H17 development. Forced manifestation of ROR-α is able to induce IL-17. On the other hand, overexpression of ROR-γt in ROR-α deficient T cells was not able to induce optimal expression of IL-17 in comparison to control T cells (Yang et al. 2008). The role of ROR-α in T_H17 differentiation is further emphasized by the fact that ROR-α deficient T cells are unreliable in making the development of T_H17 cells (Yang et al. 2008). These observations suggest that ROR-α synergizes the functions of ROR-γt in T_H17 cells differentiation (Yang et al. 2008).

Cytokines act through Jak-Stat pathways to initiate signaling events necessary to induce effector functions. The T_H17 inducing cytokines, IL-6, IL-21, and IL-23 activate STAT-3 to initiate T_H17 cells differentiation (Mathur et al. 2007; Yang et al. 2008). The central role of STAT-3 in T_H17 differentiation was revealed by the observation that STAT-3 deficient $CD4^+$ T cells were flawed in inducing T_H17 differentiation (Liu et al. 2008). Further analysis of STAT-3 deficient T cells showed a defect in the expression of IL-21 and IL-23R, both essential for optimal development of T_H17 cells (Liu et al. 2008). Furthermore, mice lacking STAT-3 in T cells were protected from the development of EAE as they generated lower frequencies of T_H17 cells (Liu et al. 2008). The role of STAT-3 in T_H17 differentiation was also confirmed on a molecular level as chip-based analysis showed that STAT-3 directly binds to the *IL-17a* and *IL-17f* promoter (Chen et al. 2006). Taken together, this data suggests that STAT-3 is essential for the creation of T_H17 cells (Fig. 3).

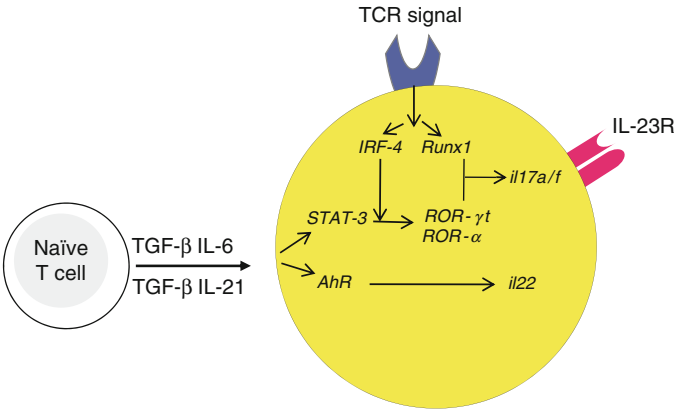

Fig. 3 Transcriptional regulation of T_H17 cells. The activation of naïve T cells in the presence of TGF-β and IL-6 or IL-21 initiate the T_H17 differentiation by inducing RORγt and RORα expression in a STAT-3 dependent manner. T_H17 cells produce IL-21. T_H17 cells also induces AhR expression, which enhances the production of IL-17 and IL-22. Both IRF-4 and Runx1 induced by TCR activation, which further promotes T_H17 differentiation

Suppressor of cytokine signaling- (SOCS) 3 is a major negative feedback regulator of STAT-3 activation and inhibits the generation of T_H17 cells. SOCS-3 negatively regulates IL-23 signaling and thus adjusts the generation of T_H17 cells (Chen et al. 2006). SOCS-3 deficient T cells showed an enhanced generation of T_H17 cells, which might be due to heightened STAT-3 signaling necessary for the development of T_H17 cells (Qin et al. 2009; Taleb et al. 2009). Consistent with the idea, STAT-3 also plays a crucial role in human T_H17 differentiation; a genetic mutation in STAT-3 in Job's syndrome leads to a hyper IgE production, defective T_H17 differentiation with recurrent *Candida albicans* and *Staphylococcus aureus* infection in the lungs of the affected patients (Ma et al. 2008).

Although ROR-γt is the master transcription factor of the T_H17 cells lineage, other aspects cooperate with RORγt for optimal distinction of T_H17 cells and their development. IRF-4, a factor previously shown to enhance T_H2 development was recently shown to also play an important role in the generation of T_H17 cells (Rengarajan et al. 2002). IRF-4 deficient mice were protected from EAE, and T cells from IRF-4-deficient mice failed to separate into T_H17 cells with an attenuated expression of ROR-γt and ROR-α, suggesting that IRF-4 is upstream of these nuclear receptors (Brustle et al. 2007; Huber et al. 2008). Studies using IRF-4 deficient T cells also revealed a shortcoming in induction of IL-21 and IL-23R, factors necessary for T_H17 cell development (Huber et al. 2008; Xiao et al. 2008). Interestingly, IRF-4-deficient T cells showed enhanced expression of Foxp3, suggesting that IRF-4 might be essential in regulating the IL-6 or IL-21 mediated inhibition of Foxp3, and thus improve the development of T_H17. cMaf, a proto-oncogene previously described to be a T_H2-specific transcription factor, is highly expressed in T_H17 cells (Ho et al. 1998). Comparatively, T_H17 cells express cMaf at higher levels than T_H2 cells (Bauquet et al. 2009). Whereas loss of cMaf does not result in a deficiency in T_H17 differentiation, c-Maf lacking T cells are unable to make IL-21 and sustain expression of IL-23R (Bauquet et al. 2009). Therefore, there is a gradual loss of T_H17 cells in cMaf deficient mice. Whereas cMaf is initially induced by IL-6 during T_H17 differentiation, later on the inducible stimulatory molecule (ICOS), which is expressed at higher levels on T_H17 cells, maintains its appearance throughout the life-span of T_H17 cells (Bauquet et al. 2009). Loss of ICOS in ICOS-deficient mice, results in defective T_H17 differentiation by virtue of lower cMaf, IL-21 and IL-23R expression (Bauquet et al. 2009). cMaf has been shown to bind to promoter elements in both IL-21 and IL-23R and specifically trans activate both of these genes (Pot et al. 2009).

Further analysis of T_H17 cells revealed the involvement of aryl hydrocarbon receptors (AHR), a ligand-dependent transcription factor required to mediate the effects of environmental toxins such as 2,3,7,8-Tetrachlorodibenzodioxin (known as TCDD or dioxin). Upon binding to its ligands (FICZ, TCDD), AHR transfers from the cytosol to the nucleus and binds to DNA in order to trans-activate AHR-dependent genes. Activation of AHR with a high affinity natural ligand FICZ during T_H17 differentiation enhances the development of T_H17 cells, but AHR seems to specifically regulate production of IL-22 in T_H17 cells (Quintana et al. 2008; Veldhoen et al. 2008).

Taken together, multiple transcription factors facilitate separation of T_H17 cells and production of various cytokines of T_H17 lineage, but of these, RORγt is the more crucial lineage specific transcription factor required for the development of T_H17 cells (Fig. 3).

6 Inhibition of T_H17 Cells

T_H17 cells are protective against certain extracellular pathogens and a deregulated T_H17 response can induce severe immunopathology of host tissues. Therefore, the regulation of T_H17 cells is essential to prevent tissue damage. Here we described the cytokines that antagonize the differentiation of T_H17 cells.

6.1 IL-27 and Inhibition of T_H17 Response

IL-27, a member of IL-12 cytokine family, is produced by innate immune cells and induces IFN-γ production from T cells (Pflanz et al. 2002). IL-27R consists of the gp130 sub-unit of the IL-6 receptor with a unique chain called WSX-1 and is predominantly expressed on T cells (Pflanz et al. 2004). Initial work suggested that IL-27 is essential to initiate T_H1 response by upregulating IL-12Rβ2, a sub-unit require for IL-12 signaling in a T-bet manner (Hibbert et al. 2003; Takeda et al. 2003). However, soon after its discovery, it become evident that by using different T_H1 and T_H2 associated pathogens, IL-27 negatively regulates both T_H1 and T_H2 responses (Holscher et al. 2005; Rosas et al. 2006; Villarino et al. 2003; Yoshimoto et al. 2007). Toxoplasmosis is a parasitic infection that requires T_H1 mediated effector functions for inhibiting parasite growth within the host. Mice deficient in the T_H1 cells inducing cytokines IL-12 and IFN-γ, die because of high parasitic burden (Liu et al. 2006; Scharton-Kersten et al. 1995, 1996). IL-27 was shown to clear parasites efficiently by mounting a massive T_H1 response that can eliminate them. However, the mice died due to fatal and severe immunopathology (Villarino et al. 2003). This observation was further extended for other intracellular pathogens such as *Leishmania* and *Mycobacterium* (Holscher et al. 2005; Rosas et al. 2006). After the discovery of pathogenic T_H17 cells, the severe immunopathology associated with *Toxoplasma gondii* infection in IL-27R deficient mice was revisited in the context of T_H17 cells. Analysis of *T. gondii* infected IL-27R deficient mice revealed an exaggerated T_H17 response, suggesting that IL-27 adversely adjusts the generation of T_H17 cells (Stumhofer et al. 2006). Furthermore, immunization of IL-27R deficient mice with MOG peptide in CFA induced EAE associated with exaggerated T_H17 response (Batten et al. 2006). An increased number of T_H17 cells were found in the CNS in both models of the IL-27R deficient mice (Batten et al. 2006; Stumhofer et al. 2006). Similarly, addition of IL-27 together with TGF-β and IL-6 also inhibited the variation of T_H17 cells in vitro (Batten et al. 2006; Stumhofer et al. 2006). Since IL-27 significantly induces production of IFN-γ, it is possible that

IL-27 hinders the differentiation of T_H17 cells by enhancing IFN-γ production. Neutralization of IFN-γ in the T_H17 cells cultures did not reverse the IL-27 induced inhibition of T_H17 cells (Batten et al. 2006). IL-27 directly inhibits the differentiation of T_H17 cells in a STAT-1 dependent pathway. STAT-1 deficient mice failed to deter T_H17 differentiation induced by IL-27 (Batten et al. 2006; Stumhofer et al. 2006). Recent data suggests that IL-27 inhibits T_H17 differentiation at the transcriptional level by impeding the expression of ROR-γt (Diveu et al. 2009). IL-6, which inhibits the induction of TGF-β induced iT_{reg} generation with a concomitant, increases the induction of T_H17 cells, and IL-27 inhibits the generation of both T_H17 and iT_{regs} induction (Awasthi et al. 2007; Batten et al. 2006; Stumhofer et al. 2006). The identification of IL-27 as a destructive regulator for the generation of T_H1, T_H2, and T_H17 cells suggested a possibility that it might induce other factors or T cell variation that might directly or indirectly control other effector T cells responses. Recently, a series of papers identified that IL-27 is the differentiation factor for IL-10 producing T cells, raising the possibility that IL-27 might curbT_H17 generation and other effector T cells by inducing IL-10 producing Tr1 cells (Awasthi et al. 2007; Fitzgerald et al. 2007; Stumhofer et al. 2007). These observations might also explain the mechanism of uncontrolled immunopathology in IL-27R deficient mice and is perhaps due to a lack of IL-10-producing T cells. Consistent with this observation, in vivo administration of IL-27 was shown to suppress development of EAE with a significant decrease in the generation of pro-inflammatory effector T cells.

7 T_H1/T_H2 Paradigm and T_H17 Plasticity

Before the discovery of T_H17 cells, most of the inflammatory responses in various infections and auto-immune diseases were described to be associated with either T_H1 or T_H2 cells. In fact past 25 years, T_H1–T_H2 paradigm revolutionized our understanding of adaptive immune responses to various infections and allergic diseases. T_H1 cells are essential to eliminate intracellular pathogens such as *Leishmania*, *Mycobacteria* etc., and were described as the pathogenic effector T cells in many auto-immune diseases such as multiple sclerosis, type-1 diabetes, rheumatoid arthritis, and IBD. Similarly, T_H2 cells that eliminate extracellular pathogens are the effector T cells that induce allergic inflammation. Here we provide evidence that integrates T_H17 cells into the existing T_H1/T_H2 paradigm and explains the underlying mechanisms of tissue inflammation.

7.1 Integration of T_H17 Cells with T_H1/T_H2 Cells in Tissues Inflammation

It is well established that the T_H1 cells with specificity for the self-antigen can transfer auto-immunity. T_H1 clones specific for myelin antigens, MBP, MOG, and PLP were shown to transfer EAE in adoptive transfer mode (Kuchroo et al. 1992). However,

genetic deficiencies of molecules associated with T_H1 cells such as IL-12p35, IL-12Rβ2, STAT-1, IFN-γ, and IFN-γR did not abrogate EAE (Korn et al. 2009; McGeachy and Cua 2008). In fact, the incidence and severity of the disease in these genetically deficient mice was enhanced. It is well documented that IL-23, instead of IL-12, is essential for tissue inflammation in EAE (Awasthi et al. 2009; Cua et al. 2003). This observation challenged the notion of association of T_H1 cells with EAE. Further work revealed a clear association of IL-23–T_H17 axis in inducing organ-specific tissue inflammation. T_H1 cells have always been found at the target tissue together with T_H17 cells in EAE. Analysis of CNS infiltrating T cells revealed a population of T cells that co-expressed both IFN-γ and IL-17, suggesting a possibility that Th1 cells synergize the functions of T_H17 cells or vice versa in inducing inflammation. A recent report suggests that T_H17 cells breach the blood brain barrier to allow infiltration of other cell types including T_H1 cells in CNS (Kebir et al. 2007). In addition to a synergy between T_H17 and T_H1 cells, T_H17 cells may convert into IFN-γ-producing T_H1 cells in the target tissue and these IL-17$^+$ IFN-γ$^+$ T cells might be the most potent inducer of tissue inflammation.

Similar to EAE, T_H1 cells play a dominant role in gut inflammation. Both Crohn's disease and the animal model of IBD were shown to be associated with IFN-γ and TNF-α (Neurath et al. 2002a). The association of T_H1 cells in IBD was further evident with the detection of high amounts of IL-12 in patients with Crohn's disease and also in the animal model. Neutralization of IL-12 by anti-IL-12 antibodies enhances intestinal inflammation, presumably by preventing the generation of T_H1 cells (Neurath et al. 1995). Failure of STAT-4 deficient T cells in transferring T cells mediated colitis and the development of overly aggressive colitis in STAT-4 transgenic mice suggested that an IL-12 dependent pathway is dominant in inducing colitogenic T cells at the mucosal surface (Neurath et al. 1995) (Wirtz et al. 1999). Further evidence about the association of T_H1 cells in colitis came with the experiment that the loss of T-bet, a master transcription factor that induces IFN-γ, failed to transfer colitis in T cells (Neurath et al. 2002b). Discovery of a genetic association of IL-23R polymorphism with Crohn's disease in GWAS studies suggested the involvement of IL-23–T_H17 pathways in gut inflammation (Duerr et al. 2006). Consistent with human genetic studies, neutralization of IL-23 ameliorates gut inflammation in a number of mouse models of IBD including colitis induced by transfer of naïve T cells. These observations were further supported with the fact that T_H17 cells are highly enriched in gut associated lymphoid tissues at steady state (Ivanov et al. 2006), suggesting a possibility of their involvement in inducing gut inflammation in certain specific conditions. These observations suggest that both T_H17 and T_H1 cells are essential for inducing tissue inflammation in the gut. Exactly how these T_H1 and T_H17 cells generated in the gut is not clear. New emerging data suggests that the transfer of highly purified T_H17 cells from IL-17F reporter into a lymphopenic host induces T_H1 cells associated colitis, which clearly indicated the conversion of T_H17 cells into T_H1 cells in the gut environment (Lee et al. 2009). However, whether T_H17 cells can induce disease without conversion into IFN-γ producing cells or whether IFN-γ deficient T_H17 cells can also transfer colitis has