# Helping the Helpers!

Cindy S. Ma[1] and Stuart G. Tangye[1,2,*]
[1]Immunology Program, Garvan Institute of Medical Research, Darlinghurst, NSW 2010, Australia
[2]St Vincent's Clinical School, University of NSW, Kensington, NSW 2052, Australia
*Correspondence: s.tangye@garvan.org.au
DOI 10.1016/j.immuni.2009.06.009

**T follicular helper (Tfh) cells are crucial for generating humoral immune responses. In this issue of *Immunity*, Schmitt et al. (2009) reveal the differentiation of human Tfh cells is dependent on dendritic cell-derived interleukin-12.**

It has been over 20 years since Mosmann and Coffman first published the T helper (Th) 1-Th2 cell hypothesis. By studying murine $CD4^+$ T cell clones, they demonstrated that $CD4^+$ T cells could differentiate into distinct subsets with unique effector function (reviewed in Dong, 2008; Romagnani, 1994; Zhou et al., 2009). Subsequent studies established that this process was dependent on the cytokines present at the time of activation. Thus, interleukin-12 (IL-12) was required for the generation of interferon (IFN)-γ- and tumor necrosis factor-α-producing Th1 cells, which function in cellular immune responses to viruses and bacteria. Alternatively, IL-4-primed naive $CD4^+$ T cells became Th2 cells producing IL-4 and IL-13 that are important for protection against extracellular parasites and allergic responses and may also have a role in humoral immunity (Dong, 2008; Romagnani, 1994; Zhou et al., 2009). IL-12 and IL-4 achieve these effects through the action of the transcription factor pairs signal transducer and activator of transcription 4 (STAT4)-T box transcription factor (T-bet) and STAT6-GATA binding protein 3 (GATA3), respectively (Dong, 2008; Zhou et al., 2009). Although Th1 and Th2 cell populations were verified in humans, a degree of plasticity was also revealed as $CD4^+$ T cells producing both IFN-γ and IL-4 were detected (Romagnani, 1994). Nevertheless, this simplified view of naive $CD4^+$ T cell differentiation whereby a predominant cytokine induced defined transcription factors resulting in the generation of a distinct effector subset has been used for the identification of other Th cell populations with specialized functions. This includes regulatory T (Treg) cells, which are important in immune regulation and are induced by transforming growth factor (TGF)-β acting through the transcription factor Forkhead box P3 (FoxP3), and more recently Th17 cells, which are important in controlling infection with extracellular pathogens but are also pathogenic in certain autoimmune conditions (Annunziato et al., 2008; Dong, 2008; Zhou et al., 2009). Although there is some controversy surrounding the requirements for the generation of human Th17 cells, it is likely to involve IL-23 and IL-6 (and possibly TGF-β) acting through STAT3 and retinoic acid-related orphan receptor (ROR) γt (Annunziato et al., 2008; Dong, 2008).

Another subset of Th cells is T follicular helper (Tfh) cells. Tfh cells express the chemokine receptor CXCR5 and high amounts of the costimulatory molecules CD40 ligand (CD40L) and inducible costimulator (ICOS) and the cytokine IL-21. This suite of effector molecules allows Tfh cells to localize to B cell follicles and germinal centers (GCs) where they are positioned to guide the differentiation of B cells into memory and plasma cells (King et al., 2008). Although Tfh cells were first described in 2000, the mechanisms underlying their development remain incompletely defined. For instance, although it was recently reported that IL-6, IL-21, and STAT3 are required for the generation of murine Tfh cells (King et al., 2008; Nurieva et al., 2008; Suto et al., 2008), it is unknown whether the same factors are involved in the development of human Tfh cells.

In this issue of *Immunity*, Schmitt et al. (2009) show that Toll-like receptor (TLR)- or CD40L-stimulated DCs induce the in vitro differentiation of human naive $CD4^+$ T cells into Tfh-like cells, defined by IL-21 production (Schmitt et al., 2009). Functional analysis of DC-derived cytokines demonstrated that IL-12 and the related cytokine IL-23 were responsible for this process. Similar to studies in mice (Nurieva et al., 2008; Suto et al., 2008), IL-21 could also induce its own production (Figure 1). Interestingly, the effects of IL-21 and IL-23 were dramatically less than that of IL-12 (Schmitt et al., 2009). These in vitro-generated IL-21-expressing human $CD4^+$ T cells functionally resembled Tfh cells in vivo inasmuch that they induced Ig secretion by cocultured B cells in an IL-21- and ICOS-dependent manner (Figure 1). In light of the prominent role of IL-12 in generating Th1 cells, the finding that it also induced Tfh-like cells may seem unexpected. These results however are actually consistent with DCs being a prominent producer of IL-12 and their pivotal role in priming naive $CD4^+$ T cells for differentiation into specific effector cell fates. In contrast to IL-12 and IL-23, IL-21 is not produced by DCs. Thus, IL-21 secreted by IL-21-stimulated $CD4^+$ T cells may act in a paracrine manner to promote IL-21 synthesis by additional naive $CD4^+$ T cells or in an autocrine manner to sustain endogenous IL-21 production (Figure 1).

The population of $IL\text{-}21^+CD4^+$ T cells induced by IL-12 contained $IFN\text{-}\gamma^+$ and $IFN\text{-}\gamma^-$ subsets. The functional similarities and differences between the $IL\text{-}21^+IFN\text{-}\gamma^+$ and $IL\text{-}21^+IFN\text{-}\gamma^-$ $CD4^+$ T cells, and whether one subset can give rise to the other, remain to be determined. It is possible that $IL\text{-}21^+IFN\text{-}\gamma^+$ Th1-Tfh-like cells could convert into $IL\text{-}21^+IFN\text{-}\gamma^-$ Tfh-like cells in response to appropriate signals provided by the microenvironment within B cell follicles. This would resemble murine Treg and Th2 cells that have recently been reported to differentiate into Tfh cells in vivo during responses to specific pathogens or in compartmentalized lymphoid tissues (reviewed in Zhou et al., 2009). Interestingly, $IL\text{-}21^+IFN\text{-}\gamma^+$

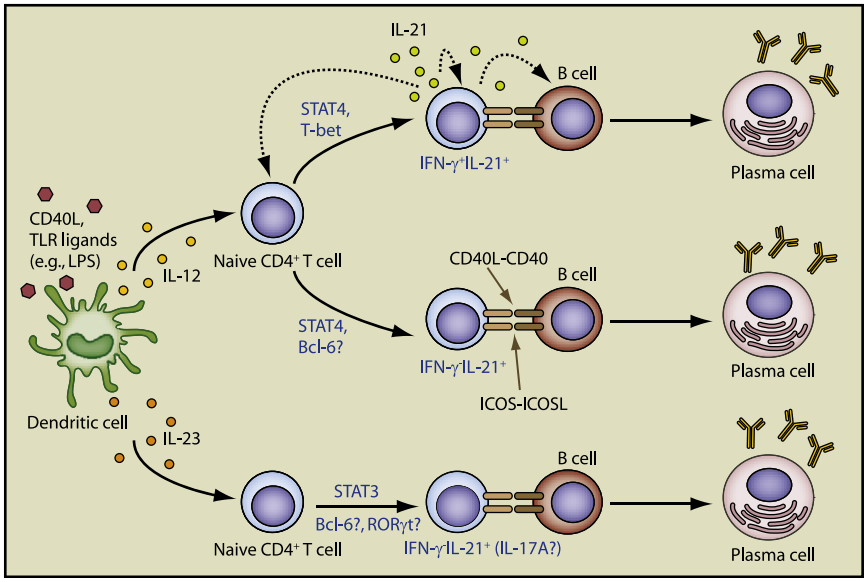


**Figure 1. Differentiation of Human Tfh Cells Is Dependent on DC-Derived IL-12 and IL-23**
CD40 and TLR-stimulated DCs secrete a large spectrum of cytokines. Of these, Schmitt et al. (2009) showed that IL-12 and IL-23 induced the differentiation of naive $CD4^+$ T cells into IL-21-producing effector cells. IL-12 generated IFN-$\gamma^+$IL-$21^+$ and IFN-$\gamma^-$IL-$21^+$ cells via STAT4. The differentiation of IFN-$\gamma^+$IL-$21^+$ T cells involves T-bet, whereas that of IFN-$\gamma^-$IL-$21^+$ cells probably depends on Bcl-6. In contrast, IL-23 acts via STAT3 and perhaps Bcl-6 and/or RORγt to induce IFN-$\gamma^-$IL-$21^+$ cells, which may also exhibit features of Th17 cells. IL-21 produced by Tfh cells may act in an autocrine manner to further promote Tfh cells. The signals required to maintain CXCR5 expression that allow precursor Tfh cells to migrate to follicles and interact with B cells are unknown. The two-way interaction that ensues results in the generation of bona fide Tfh cells that mediate the differentiation of B cells into plasma cells through the combined actions of IL-21, ICOS and presumably CD40L.

T cells expressed T-bet, whereas IL-$21^+$IFN-$\gamma^-$ T cells did not (Schmitt et al., 2009), suggesting that IL-12 acts via a T-bet-dependent pathway to generate IL-$21^+$IFN-$\gamma^+$ cells, whereas IL-$21^+$IFN-$\gamma^-$ $CD4^+$ T cells arise independently of T-bet. B cell lymphoma-6 (Bcl-6) is highly expressed by both human and murine Tfh cells and has been proposed to be a "master regulator" of Tfh cell differentiation (King et al., 2008). Thus, although expression of Bcl-6 by IL-12-stimulated $CD4^+$ T cells was not assessed (Schmitt et al., 2009), it is tempting to speculate that the IL-12-mediated differentiation of naive CD4 T cells into IL-$21^+$IFN-$\gamma^-$ cells involves this transcription factor (Figure 1). This would be consistent with the induction of Bcl6 expression in murine $CD4^+$ T cells cultured under "Tfh" cell-polarizing conditions (Nurieva et al., 2008). However, the specific role of Bcl-6 in regulating commitment to a Tfh cell fate awaits formal examination.

Cytokines mediate their effects by activating specific STAT signaling pathways. By reducing expression of STAT3 and STAT4 in primary human $CD4^+$ T cells by siRNA transfection, it was found that IL-12 induced IL-21 via a STAT4-dependent pathway, whereas the effects of IL-21 and IL-23 required STAT3 (Schmitt et al., 2009) (Figure 1). The fact that IL-12 and IL-23 generate IL-21-expressing $CD4^+$ T cells via distinct mechanisms raises the possibility that the effector cells arising from these cultures may have functional or molecular differences. IL-23 has an important role in generating Th17 cells from naive human $CD4^+$ T cells (Annunziato et al., 2008). Although Schmitt et al. (2009) reported that IL-23-induced IL-$21^+CD4^+$ T cells did not express IL-17A, human $CD4^+$ T cells activated by IL-23 express RORγt, yet lack IL-17 (Annunziato et al., 2008). Because IL-23 can induce RORγt and IL-21 in a STAT3-dependent manner in murine Th17 cells (Dong, 2008), it is possible that the IL-$21^+$ cells arising from human naive $CD4^+$ T cells cultured with IL-23 are related to Th17 cells. This is not incompatible with their ability to provide B cell help in vitro because human Th17 cells are as efficient as Th1 and Th2 cells at inducing T cell-dependent B cell differentiation (Annunziato et al., 2008). On the basis of this scenario, it will be interesting to determine whether RORγt has a role in inducing IL-21 by these STAT3 activating cytokines (Figure 1).

Another important finding from this study (Schmitt et al., 2009) is that substantial differences appear to exist for the generation of human versus murine Tfh cells, inasmuch that IL-12 failed to induce IL-21 production from murine $CD4^+$ T cells in vitro (Suto et al., 2008). Similarly, although IL-6 induces IL-21 in murine $CD4^+$ T cells (Nurieva et al., 2008; Suto et al., 2008), this was not the case for humans (Schmitt et al., 2009). This raises the logistical issue of determining the physiological significance of the IL-12-STAT4 pathway in regulating Tfh cell formation and subsequent humoral immune responses in humans in vivo. Although this may appear challenging it is certainly achievable because individuals have been identified with specific immunodeficiencies due to mutations in *IL-12B*, *IL-12RB1*, and *TYK2* (which is downstream of IL-12-IL-23 signaling) (Marodi and Notarangelo, 2007). Furthermore, $CD4^+$ T cells in patients recovering from stem cell transplants are STAT4 deficient; however, the mechanism underlying this has not been determined (Robertson et al., 2005). Thus, such "experiments of nature" will provide a valuable opportunity to determine the role of the *IL-12-STAT4* pathway in generating human Tfh cells in vivo and will establish the feasibility of targeting this pathway to modulate Tfh cells in cases of autoimmunity, immunodeficiency, and vaccination.

One outstanding question is to understand exactly what a Tfh cell is and how it fits into the scheme of $CD4^+$ T cell differentiation. Tfh cells may belong to a specific lineage of effector $CD4^+$ T cells, similar to Th1, Th2, Th17, and Treg cells. However, numerous types of human $CD4^+$ T cells can produce IL-21 and provide help to B cells: tissue-resident $CXCR5^+$ Tfh cells, IL-12-induced IFN-$\gamma^+$IL-$21^+$ Th1-Tfh-like and IFN-$\gamma^-$IL-$21^+$ Tfh-like cells, NKT cells, Th17 cells, and γδ T cells. Furthermore, CXCR5 can be expressed on subsets of these cells and is induced on all T cells after activation in vivo and in vitro (King et al., 2008). Thus, an alternative possibility is that Tfh cells correspond to a discrete differentiation stage of other effector cell

lineages, thereby highlighting the plasticity of $CD4^+$ T cell differentiation (Zhou et al., 2009). This is supported by the ability of murine Treg and Th2 cells to develop into Tfh cells in vivo. A precise definition of a bona fide Tfh cell is predicated on their positioning within GCs where they can then deliver "help," a process that involves the coordinated expression and function of chemokine receptors, but still remains incompletely defined (King et al., 2008). Although the identification by Schmitt et al. of IL-12 (and IL-23) as key inducers of IL-21 production by naive human $CD4^+$ T cells represents an important advance in our understanding of the requirements for generating human Tfh-like cells, these cytokines did not influence expression of CXCR5. Thus, to improve our knowledge of the complexities of Tfh cells, future studies will need to focus on elucidating the mechanisms that underlie the migration and positioning of IL-21-producing effector cells within B cell follicles and GCs such that they can function as genuine Tfh cells.

## REFERENCES

Annunziato, F., Cosmi, L., Liotta, F., Maggi, E., and Romagnani, S. (2008). Int. Immunol. *20*, 1361–1368.

Dong, C. (2008). Nat. Rev. Immunol. *8*, 337–348.

King, C., Tangye, S.G., and Mackay, C.R. (2008). Annu. Rev. Immunol. *26*, 741–766.

Marodi, L., and Notarangelo, L.D. (2007). Nat. Rev. Immunol. *7*, 851–861.

Nurieva, R.I., Chung, Y., Hwang, D., Yang, X.O., Kang, H.S., Ma, L., Wang, Y.H., Watowich, S.S., Jetten, A.M., Tian, Q., and Dong, C. (2008). Immunity *29*, 138–149.

Robertson, M.J., Chang, H.C., Pelloso, D., and Kaplan, M.H. (2005). Blood *106*, 963–970.

Romagnani, S. (1994). Annu. Rev. Immunol. *12*, 227–257.

Schmitt, N., Morita, R., Bourdery, L., Bentebibel, S.E., Zurawski, S.M., Banchereau, J., and Ueno, H. (2009). Immunity *31*, this issue, 158–169.

Suto, A., Kashiwakuma, D., Kagami, S., Hirose, K., Watanabe, N., Yokote, K., Saito, Y., Nakayama, T., Grusby, M.J., Iwamoto, I., and Nakajima, H. (2008). J. Exp. Med. *205*, 1369–1379.

Zhou, L., Chong, M.M., and Littman, D.R. (2009). Immunity *30*, 646–655.