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NEWS AND COMMENTARY

T helper cell differentiation

IL-21 and T helper cell differentiation: Jack of all trades?

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The study of the role of interleukin-21 (IL-21) in immune regulation is a rapidly evolving field, and one of the most important issues pertaining to IL-21 is its role in T helper (T_H) cell differentiation. The production of IL-21 by $CD4^+$ T cells occurs following their activation and continues through their differentiation into more specialized T_H subsets. In addition to producing IL-21, T_H cells are known to utilize IL-21 for their growth and differentiation. For instance, IL-21 has been shown to be necessary for the generation of T_H2 responses[1] but reports of a role for IL-21 in the generation of IL-17-producing T_H17 cells[2–4] have come under scrutiny.[1,5,6] A recent study by Suto *et al.*,[7] published in the *Journal of Experimental Medicine*, focuses on this relevant yet unresolved issue and has alleviated some of the earlier problems arising from mixed $CD4^+$ T-cell cultures by detecting cells producing IL-21 and IL-17 at the single-cell level with dual intracellular immunostaining. This study demonstrates that, while defined *in vitro* culture conditions can induce naïve $CD4^+$ T cells to make both IL-17 and IL-21, IL-21- and IL-17-producing $CD4^+$ T cells are largely distinct. Instead, returning to the theme of earlier studies, $IL\text{-}21^+$ cells were found to retain a closer affiliation with IL-4-producing T_H2 cells.[8,9]

IL-21 was identified almost a decade ago as a cytokine that costimulates lymphocyte proliferation and drives the differentiation of NK cells *in vitro*.[10] The receptor for IL-21 is expressed on various immune cells, including T, B, NK and dendritic cells.[11,12] By contrast, IL-21 synthesis is restricted to activated $CD4^+$ T cells and NKT cells.[10,13] The production of this cytokine by $CD4^+$ T cells makes it a candidate for mediating T_H cell function, and a number of studies support a central role for IL-21 in humoral responses and $CD8^+$ T-cell responses. The observation that IL-21 costimulates Ag receptor-induced activation of T cells is germane to our understanding of the role of IL-21 in T_H cell differentiation. Highly differentiated T_H cell subsets are generated following the integration of multiple costimulatory signals during their interaction with peptide MHC ligands on Ag-presenting cells, and IL-21 appears to provide one of these signals.

IL-21 is necessary for T_H2 differentiation but IL-21 does not readily fit within the established Th1/Th2 paradigm.[1] The production of IL-21 is common to recently activated $CD4^+$ T cells and differentiated subsets (Figure 1). Studies that have analysed IL-21 message and protein in mixed populations suggest that a number of T_H subsets produce IL-21, including T_H2, T follicular helper (T_{FH}) and T_H17 cells. T_H17 cells are a pro-inflammatory T_H subset that produce IL-17A and IL-17F (as well as IL-21, among other cytokines) and elicit the production of cytokines and chemokines that attract neutrophils and other inflammatory cells to the site of the immune response.[14] The study by Suto *et al.* raises the relatively unexplored possibility that IL-17 is made by T_H cells prior to their commitment to the T_H17 lineage.

An important advance in our understanding of T_H differentiation has been the discovery of transcription factors that determine T_H lineage commitment. The transcription factor RORγt directs the differentiation of the T_H17 subset, distinguishing them from T_H1 and T_H2 cells the differentiation of which is controlled by expression of T-bet and GATA3, respectively.[15] T_H17 cells express IL-21 in response to IL-6 but are unlikely to be the major source of IL-21 for immune responses as $ROR\gamma t^{-/-}$ mice express normal levels of IL-21.[16] Instead, some of the highest levels of IL-21 mRNA transcript and protein have been found in the T_{FH} cell subset of $CD4^+$ T cells that are capable of providing help to B cells in germinal centres of lymphoid follicles.[17,18]

The search for cytokines that induce mouse T_H17 cell differentiation *in vitro* has revealed a number of candidates, including IL-6, IL-23, TGF-β and IL-21 (Figure 1).[19] Similarly, in humans, TGF-β, IL-23, IL-1β and IL-6 are important, and the combination of TGF-β and IL-21 was recently reported to be capable of promoting the differentiation of human naive $CD4^+$ T cells into T_H17 cells.[20–22] However, TGF-β has also been shown to inhibit human T_H17 generation by antagonizing RORγt,[23] highlighting the uncertainty of the exact role of TGF-β in the development of human T_H17 cells. Similarly, studies of the effects of cytokines on mouse T_H17 differentiation *in vitro* versus that *in vivo* have revealed inconsistencies. IL-17 production can be induced in response to TGF-β and IL-6 alongside TCR signals (Figure 1), and IL-21 can substitute for IL-6 in T_H17 differentiation *in vitro*. It is of interest in this regard that both IL-6 and IL-21 strongly activate Stat3, which, in turn, is important for induction of RORγt expression and IL-17 production.[4,16] However, IL-21 was found to be dispensable for T_H17 generation—both during infection and in an autoimmune setting where IL-6 was present.[1,5] Thus, which cytokines drive *de novo* differentiation of human and mouse T_H17 cells requires further clarification and an effort to standardize culture conditions to best reflect physiological conditions would clearly benefit this effort.

Thus, IL-6, a cytokine involved in the acute-phase response and produced by several

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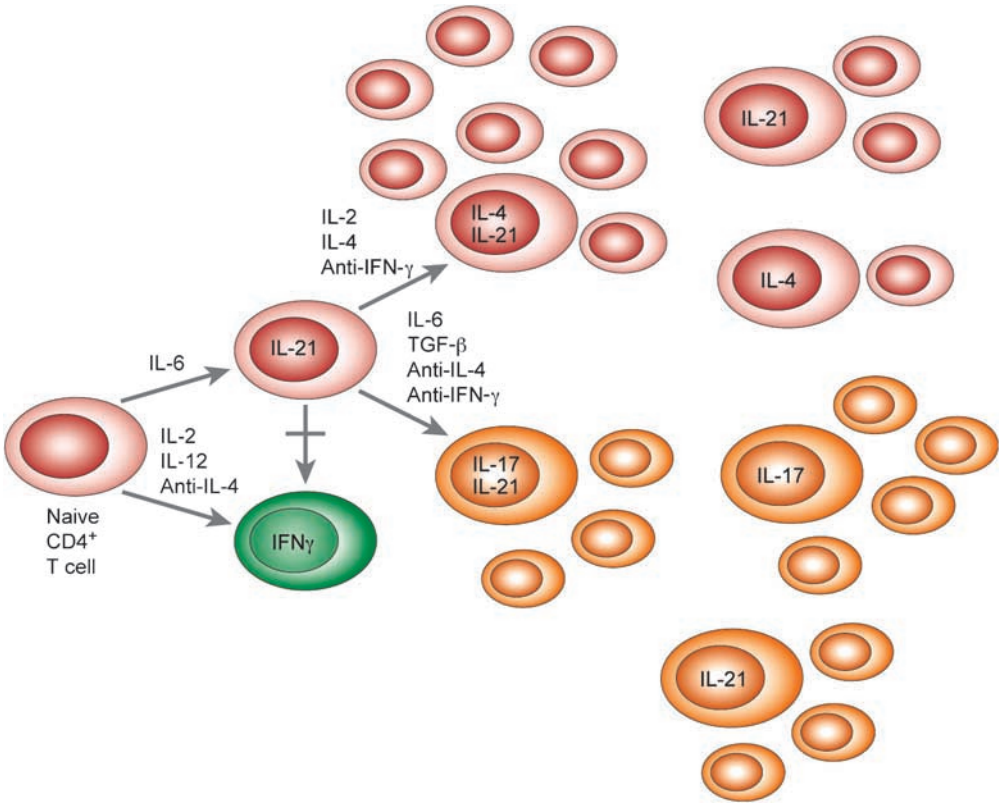

Figure 1 CD4$^+$ T cells produce interleukin-21 (IL-21) upon ligation of the T-cell receptor with peptide/MHC ligand. Activated CD4$^+$ T cells have the potential to produce a wide array of cytokines including IL-21, and this profile is determined by cell-to-cell interaction and soluble factors within the microenvironment or within the defined culture conditions as shown. IL-21 costimulates CD4$^+$ T cells for their further activation and differentiation and as the CD4$^+$ T cell differentiates its array of cytokines produced becomes more limited.

immune cell types (including T_H2 cells), is a common denominator for generating both IL-21-producing and T_H17 cells[16] (Figure 1). The study by Suto *et al.* supports this notion, as they found that IL-6 induced IL-21$^+$ cells as well as IL-17$^+$ cells. However, the majority of IL-21$^+$ cells did not coexpress IL-17. If IL-6 acts to induce IL-21$^+$ and IL-17$^+$ cells, then TGF-β appears to switch *in vitro* differentiation in favour of T_H17 cells.[7] Interestingly, although the findings supported the notion of an autocrine effect of endogenous IL-21 on the further generation of IL-21$^+$ cells, neutralization of IL-21 with an IL-21R-Fc chimaera barely inhibited the generation of IL-21$^+$ cells in the presence of IL-6 but did reduce IL-21$^+$ cells under T_H17-polarizing conditions.[7] This finding requires clarification.

Another interesting theme that emerges from the study by Suto *et al.*, and which is likely to be explored in future studies, is that IL-21$^+$ cells align more closely with T_H2 than with T_H17 cells. T_H17-polarizing conditions favoured IL-21 production from CD4$^+$ T cells but most IL-21$^+$ CD4$^+$ T cells did not coexpress IL-17. In contrast, under T_H2-polarizing conditions, a significant number (>70%) of IL-21$^+$ cells coexpressed IL-4 and, moreover, restimulating IL-21$^+$ cells under T_H2-polarizing conditions resulted in half of them acquiring expression of IL-4. However, interferon-γ was not produced from IL-21$^+$ cells restimulated under T_H1-polarizing conditions (Figure 1). These findings suggested that IL-21$^+$ cells remain capable of further differentiation into the T_H2 (and T_H17) lineages but are functionally distinct from T_H1 cells (Figure 1). T_H subsets are known to develop according to the type of pathogen encountered, and are capable of eliciting production of unique immunoglobulin isotypes by Ag-specific B cells. Indeed, the humoral immune response is an arena in which a common IL-21$^+$ precursor to T_H cells that provide help to B cells for antibody production (T_{FH}, T_H2 and T_H17 cells) may have some functional merit.

One potential caveat of the study is that the phenotype of IL-21$^+$ cells generated under their culture conditions was considered to be stable, but the cultured IL-21$^+$ cells were not fully differentiated and thus revealed a remarkable degree of plasticity. The range of phenotypes within the IL-21$^+$ population may reflect that CD4$^+$ T cells produce IL-21 following activation and that these cells are likely to differentiate further into specialized T_H subsets in response to cell-to-cell interactions and factors in the local microenvironment (Figure 1). It is possible that a subset of phenotypically stable, highly differentiated IL-21$^+$ cells does exist but that the culture conditions in the study by Suto *et al.*, did not produce those cells. As activated CD4$^+$ T cells have an early capacity to produce a wide range of cytokines (a characteristic that is lost during differentiation), future studies will need to utilize single-cell analyses of IL-21-, IL-17- and IL-4-producing cells with a more formal demonstration of T_H lineage commitment.

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