importance, relatively little is known about the biology of this enigmatic cell population other than their tissue distribution and cell-surface marker expression. The field is therefore still in its infancy compared to, for example, the wealth of data on the many dendritic cell subsets and their role in $CD4^+$ and $CD8^+$ T-cell activation. This is partly due to the difficulties associated with purifying these infrequent tissue-resident cells from their native environment and the paucity of reagents to perturb them selectively without causing inflammatory changes to the lymph node. In this regard, a recent study that phenotyped the cells by flow cytometry as $CD169^{hi}CD11c^{int}F4/80^-$ showed that they were less prone to rapidly degrade immune complexes and were dependent on B-cell-derived lymphotoxin signals for their development.[6] In this study, Batista and colleagues used similar cell surface markers to purify SCS macrophages and showed that they were indeed able to internalize, process and present α-galactosidase-dependent lipid antigens via CD1d to activate iNKT cells to secrete IL-2 and proliferate marking them as bona fide antigen-presenting cells.

These new findings[4] raise some interesting questions about the regulation of iNKT cell trafficking in resting and inflamed lymph nodes and the functional consequences of their activation by SCS macrophages. In particular, how iNKT cells arrive at the SCS (for example, through the lymph or high endothelial venules) and their intranodal migration pattern remain to be fully elucidated. Intravital microscopy of lymph node preparations with intact lymphatic vessels and blood flow will help resolve this. Another intriguing question arising from this study is whether SCS macrophages release specific factors to recruit iNKT cells to the SCS and if they can discriminate between lipid and non-lipid antigens. In terms of function, the timing of iNKT cell activation by SCS macrophages and cytokine secretion appears out of synchrony with the timing of B-cell and $CD8^+$ T cell activation reported in earlier studies. For example, at 16 h iNKT cells are arrested on SCS macrophages by which time antigen has already been transported into the follicle and activated B cells have migrated away from the SCS toward the T-B border.[2] Similarly, $CD8^+$ T cell arrest on SCS macrophages after challenge with virus[11] and protozoan parasites[12] occurred after 2–6 h whereas intracellular cytokines were detectable in iNKT cells after 12 h. The observed asynchrony may be due to differences in the antigen systems and experimental models used by these studies. Further two-photon experiments to visualize the coordinate activation of iNKT and B and T cells in the same experimental model will be able to answer these and other questions.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Human $CD4^+$ T cell subsets

Human Th9 cells: inflammatory cytokines modulate IL-9 production through the induction of IL-21

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The T helper (Th)1-Th2 hypothesis was first described in 1986.[1,2] It proposed that a naive $CD4^+$ T cell had the potential to differentiate into distinct effector subsets that were specialized to protect against infection by specific pathogens.[1] As such, interferon-γ (IFN-γ)-producing Th1 cells were implicated in immunity against viruses and intracellular pathogens, whereas IL-4-producing Th2 cells were proposed to have a function in immunity against extracellular pathogens and mediating humoral immune responses.[2] Subsequent studies revealed that the cytokine environment at the time of $CD4^+$ T cell activation was the key determinant in generating these effector subsets. This was on account of the ability of a particular cytokine to activate specific transcription factors required for the differentiation of the Th subsets. In the case of Th1 and Th2 cells, this process was dependent on IL-12 acting on *T-bet* and IL-4 acting on *Gata3*, respectively.[3–5] Applying this same concept, whereby a specific cytokine exerts its effect on a transcription factor to mediate lymphocyte differentiation, has led to the identification of other Th subsets believed to have distinct functions in the immune response, namely regulatory T cells (Tregs), Th17 cells and follicular helper T cells.[5–7]

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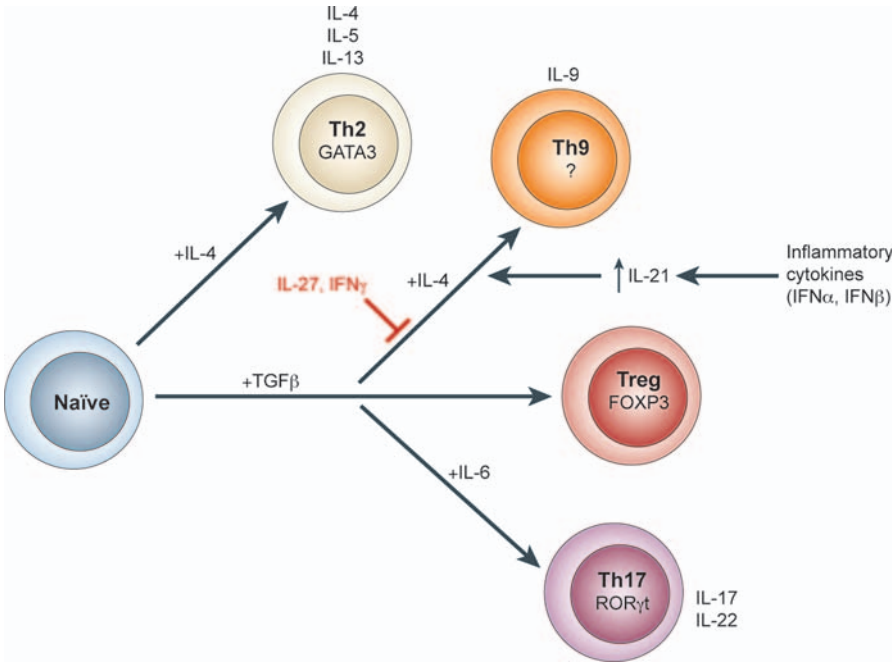

Figure 1 Role TGF-β in Th differentiation. TGF-β can influence the generation of Tregs and, in combination with other cytokines, Th17 and Th9 effector subsets of CD4$^+$ T cells. In the context of Th9 cells, the effect of TGF-β/IL-4 can be enhanced by inflammatory cytokines through the production of IL-21 and attenuated by IFN-γ and IL-27. As to IL-21, it remains to be determined whether it is derived from the IL-9-expressing cells themselves, or IL-9-negative cells that are present in the same culture. See text for more details.

In late 2008, two groups showed that the combination of transforming growth factor-β (TGF-β) and IL-4 induced the differentiation of naive murine CD4$^+$ T cells into a unique IL-9-producing subset termed Th9 cells.[8,9] This confirmed the observations made 15 years earlier by Schmitt *et al.*[10] that IL-4/TGF-β could induce IL-9 production from CD4$^+$ T cells. Although the exact function of this subset of effector Th cells has yet to be completely determined, functions in immunity against helminth infections and/or the promotion of inflammatory diseases were proposed.[8,9] Furthermore, the requirements for the generation of Th9-type cells from human naive CD4$^+$ T cells have not been elucidated. In this issue of *Immunology and Cell Biology*, Wong and colleagues confirm that similar to mice,[8–10] TGF-β and IL-4 can induce the differentiation of human Th9 cells (Figure 1).[11] Both human and murine Th9 cells do not co-express cytokines that are characteristic of other Th subsets, specifically IFN-γ (Th1), IL-4, IL-5 and IL-13 (Th2) or IL-17 (Th17). However, in contrast to murine Th9 cells,[8] IL-10 is not expressed by human Th9 cells.[11] Similar findings have also recently been reported by another group of investigators.[12]

This study also investigated other factors that regulate the generation of human Th9 cells. It was found that IFN-γ or IL-27 inhibited IL-9 production by human naive CD4$^+$ T cells cultured with TGF-β and IL-4. As IFN-γ and IL-27 can both activate STAT1,[5] it will be interesting to determine whether these cytokines suppress formation of Th9 cells by a similar signaling pathway. Overall, these findings suggest that a Th1 response could potentially negatively regulate human Th9 cell differentiation (Figure 1),[11] as originally shown for the secretion of IL-9 by TGF-β/IL-4-stimulated murine CD4$^+$ T cells.[10] However, this is somewhat inconsistent with the finding that the addition of the IFN-γ-inducing cytokine IL-12 to TGF-β/IL-4-stimulated human CD4$^+$ T cells had little effect on IL-9 secretion. This may reflect reduction in the secretion of IFN-γ by CD4$^+$ T cells cultured under 'Th9' polarizing conditions in the presence of IL-12, compared with IL-12 alone, or the existence of other regulatory mechanisms.

Conversely, the addition of inflammatory cytokines, such as IL-1β, IL-6, IL-10, IFN-α, IFN-β and IL-21, to Th9 culture conditions enhanced IL-9 expression by human CD4$^+$ T cells (Figure 1).[11,12] Furthermore, although TGF-β/IL-4 induced IL-9 expression, it also induced a very small population of IL-9$^+$IL-21$^+$ cells although overall, IL-9$^-$IL-21$^+$ cells were inhibited. Interestingly, augmentation of IL-9 expression by IFN-α and IFN-β was found to be IL-21 dependent because neutralizing the endogenous IL-21 secreted into cultures of CD4$^+$ T cells stimulated with TGF-β/IL-4 and either IFN-α or IFN-β inhibited IL-9 expression. It is unclear whether the IL-21 that promotes IL-9 expression is derived from the Th9 cells themselves, because in the presence of IL-12, IFN-α or IFN-β, IL-21 was produced by both IL-9$^+$ and IL-9$^-$ cells. This creates a complex situation whereby TGF-β+IL-4 induces IL-9 and inhibits IL-21 expression, but at the same time IL-21—induced by inflammatory cytokines—can increase IL-9 expression. However, a role for IL-21 in enhancing differentiation of human Th9 cells is not supported by the investigators' findings that IL-12, which induces high levels of IL-21,[13,14] had no effect on IL-9 expression.[11] It is possible that the lack of an effect of IL-12 (through the induction of IL-21) results from the ability of IFN-γ (also induced by IL-12) to inhibit IL-9 production in some of the experiments presented.[11] Despite this uncertainty, there appears to be a complex regulatory network involved in the differentiation of human Th9 cells.

Although the transcription factor necessary for the differentiation of human Th9 cells is not known, it does not appear to be T-BET, RORγt or FOXP3.[11] In fact, similar to the mouse setting,[8,9] IL-4 inhibited TGF-β-induced *Foxp3* expression and in doing so presumably drives CD4$^+$ T cells away from a Treg phenotype and toward that of Th9 cells. Despite this, the suppression of TGF-β-induced *Foxp3* expression by IL-4 in human

$CD4^+$ Th9 cells was much less ($\sim$50%) than that observed for mouse $CD4^+$ T cells (that is, $\sim$100%).[8,11] Another potential difference between the differentiation of human and murine Th9 cells is the role of the Th2-associated transcription factor *GATA3*. Although murine Th9 cells do not express Gata3,[8,9] Wong *et al.* report that GATA3 is upregulated in human Th9 cultures. Furthermore, they showed that there was no difference in GATA3 expression by IL-9^+ and IL-9^- cells present in cultures of naive $CD4^+$ T cells stimulated with TGF-β/IL-4.[11] Consistent with this observation, human Th9 cells secreted low amounts of the Th2 cytokines IL-5 and IL-13.[11] Taken together, these results suggest *GATA3* may have a role in the differentiation of human Th9 cells, or alternatively, these human Th9 cells do not show the same level of polarization as murine Th9 cells. Consistent with this observation, memory $CD4^+$ T cells positive for the Th2 marker, CRTH2, showed threefold more IL-9 expression than total memory $CD4^+$ T cells. However, this elevated production of IL-9 by CRTH2 cells may reflect the well-known association between IL-9 and Th2 cells, rather than CRTH2 expression being a feature of human Th9 cells. As the human Th9 cells generated from naive precursors *in vitro* do not produce appreciable quantities of IL-4, yet CRTH2-expressing memory $CD4^+$ T cells secrete both IL-4 and IL-9, it will be important to determine whether the same subset of $CRTH2^+$ cells co-expresses both cytokines, or if each cytokine is produced by a separate subpopulation. This will also allow delineation of the relationship between IL-4^+IL-9^- Th2 and IL-4^-IL-9^+ Th9 human memory $CD4^+$ T cells.

An interesting point that has come from recent studies on $CD4^+$ T cell differentiation is the role of TGF-β. Historically TGF-β has been classified as a regulatory cytokine with adverse effects on cell proliferation. Subsequent studies proved that it had favorable effects on the differentiation of Tregs, attributable to its ability to upregulate *Foxp3*.[4] Recently, TGF-β has been implicated in the induction of Th17 cells.[4,6,7] Although it is generally accepted that TGF-β is essential for the differentiation of murine Th17 cells, there is still much controversy surrounding the requirements for TGF-β in the differentiation of human Th17 cells.[15] TGF-β now seems to be involved in the differentiation of both human and murine Th9 cells.[8–12] It will be interesting to determine how one cytokine can be important for the generation of so many $CD4^+$ T cell fates with distinct effector function, and whether these subsets are all part of a common pathway (Figure 1). The observation that TGF-β induces *Foxp3* and *Rorc* and the addition of IL-4 promotes differentiation toward Th9 would support a common pathway for the differentiation of Treg, Th17 cells and Th9 cells. In the instance of human Th9 cells it seems that Th2 cells are also intertwined in the Th9 differentiation.

Another unanswered question is the role of Th9 cells in the immune system. Studies in mice have implicated Th9 cells in tissue inflammation,[8] immunity against helminth infections as well as allergy,[9] the latter of which are typically associated with Th2 cells.[2] The identification of cytokines that promote and attenuate human Th9 cells[11] represents an important step in facilitating future investigation into the molecular requirements for this Th cell lineage, such as specific transcription factors necessary for their development. This will shed more light on the function of Th9 cells and reveal how they fit into the complex scheme of $CD4^+$ T cell differentiation.

NOTE ADDED IN PROOF

It has recently been suggested that the transcription factor PU.1 is required for the generation of murine Th9 cells (Chang *et al.*, 2010. *Nat Immunol* **11**: 527). It will be important therefore to establish the role of PU.1 in the differentiation of human Th9 cells.

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