Physical attraction of Th9 cells is skin deep

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IL-9 was first identified as a growth factor for T cells and mast cells, and as an IL-2 cytokine family member with effects on hematopoietic progenitor cells and immune cells. The production of IL-9 by Th2 cells and by mast cells led to exploration of the role of IL-9 in allergic responses and parasite defense [see (1,2) for review]. Recent studies have resulted in identification of Th9 cells, a subset of CD4 Th cells that produce IL-9 and appear to be distinct from other CD4 T cell subsets (3,4). Thus, the identification of Th9 cells expanded the panel of CD4 Th subsets already including Th1, Th2, Th17, follicular helper T cells and regulatory T cells.

We first showed that IL-9-producing T cells can be primed *in vitro* by the combined presence of TGFβ and IL-4 (3) and Veldhoen *et al*. showed that Th9 cells can be generated by redirecting differentiated Th2 cells in the presence of TGFβ (4). Both papers concluded that the concomitant presence of IL-4 and TGFβ or the serial exposure to these two cytokines results in the generation of IL-9 producing Th9 cells. In addition to IL-9, Th9 cells have been described to produce other cytokines including IL-10 or IL-21, raising the question of whether these cells would be proinflammatory or regulatory in their function. Although several transcription factors such as IRF4 or PU.1 have been identified as crucial for the generation of IL-9-producing T cells, the master regulator unique to Th9 differentiation has not been identified as yet.

A number of studies have highlighted the contributions of Th9 cells to the development of immune responses in helminth and parasite infections. Studies in animal models of autoimmune and inflammatory disease also revealed a role for Th9 cells in EAE and in colitis models, as well as in animal models of allergy and asthma. Recent studies further suggest that Th9 cells can be critical effector T cells in mediating anti-tumor immune responses.

IL-9-production and IL-9-producing T cells have also been associated with different human pathological conditions such as asthma, atopic dermatitis, ulcerative colitis, allergic airway inflammation or other allergies [reviewed in (2,5,6)]. Nonetheless, besides these reports using *ex vivo* biopsies from patients, human Th9 cells have been studied almost exclusively using *in vitro* TGFβ differentiated Th9 cells. However, the *in vivo* importance and function of human Th9 cells has not been directly elucidated. Clark and colleagues have taken a major step forward in elucidating the role of the Th9 cells *in vivo* in humans.

Schlapbach and colleagues have had a long-standing interest in skin homeostasis, skin-directed and skin-resident immune responses, notably under diverse pathological conditions. In this study, they report the isolation of a human IL-9-producing T cell population from the blood and tissues of healthy donors (7). They use an elegant approach to sort memory skin- and gut-tropic Th cells based on the expression of the skin-homing receptor cutaneous lymphocyte antigen (CLA) and the gut-homing integrin α4β7. Sorted CLA^+ or $\alpha4\beta7^+$ CD4 T cells were stimulated with monocytes pulsed with a panel of antigens present at the interface of different types of epithelial and mucosal barriers. IL-9 was produced almost exclusively by skin-derived CLA^+ T cells following stimulation with *Candida albicans* (and to a lower extent with HSV1). This T cell population did not share a cytokine signature with the other effector Th subsets but co-produced IL-9, TNFα

granzyme B. Survival of these skin Th9 cells *in vitro* and cytokine production by them did not require the presence of either TGFβ or IL-2. These data clearly suggest that human Th9 cells can be induced in the skin in response to specific fungal antigens, suggesting a role for Th9 cells in fungal infection. Additionally, Schlapbach *et al.* found an increase in $CD4^+$ T cells that produce IL-9 in skin lesions from psoriasis patients, a disease that is clinically dependent upon production of IL-17 and TNFα. These findings support a potential role for Th9 cells in contributing to the pathology of psoriasis.

One particularly exciting but puzzling aspect of this work lies in the evidence of a limited tropism and/or residency for human memory Th9 cells. Since IL-9-producing T cells have been previously detected in many different tissues including lungs, why would human memory Th9 cells be mainly skin tropic and/or skin-resident? Are the IL-9-producing T cells present in other tissues/pathologies too transient or too plastic, such that they assume other phenotypes? This raises many questions and one of the future challenges will be a further understanding of the differentiation process and maturation of human Th9 cells; how/where they are generated, migrate and become effector/memory?

Interestingly, IL-9-production was rapidly but transiently induced in CLA^+Th9 T cells following activation. This initial expression was crucial not only for the autocrine regulation of IL-9 production by Th9 cells but also to activate paracrine loops resulting in the production of IL-17, IFNγ-13 from other Th cells. This specific feature was also highlighted in previous studies with murine models (8,9), where IL-9 was shown to be a potent switch for amplifying inflammatory responses by recruiting and regulating other Th subsets and innate immune cells (ILCs, mast cells, etc.). This would then explain why IL-9 plays an important role at the initial development of inflammatory responses, with its expression tightly regulated over time, as uncontrolled expression of IL-9 could lead to overwhelming and pathologic inflammatory responses. This hypothesis could also explain to a certain extent why IL-9 was difficult to visualize at established inflammatory sites following adoptive-transfer of Th9 cells (8,10).

Finally, considering its pleiotropic expression, understanding the distinct and redundant contribution of other IL-9-producing immune cells and their potential link with Th9 cells becomes an important question to investigate. More specifically the authors have found that IL-9 was also produced by $CD3^-CD4^-$ cells in psoriatic lesions. Earlier studies have pointed to the importance of IL-9-producing ILC2 cells, together with Th9 cells, in the maintenance of the gut barrier in the context of worm expulsion (11). This raises a point previously discussed in this commentary as to why no gut-tropic and/or resident IL-9-producing T cells were detected in this study? Are these mostly tissue resident cells? This also raises an important issue as to whether human IL-9-producing ILC2 cells are also present in healthy skin tissues?

In light of recent findings demonstrating their potent anti-tumor functions, Th9 cells represent a particularly promising novel immunotherapeutic strategy against cancer (12-15). The present study not only opens many new avenues for exploring the differentiation and function of human Th9 cells but, together with other studies, also highlights IL-9 and IL-9-producing cells as attractive therapeutic targets for treating skin-related inflammatory disorders. Furthermore, strategies designed to enhance the function of skin-resident Th9 cells may be useful in the treatment of skin cancers.

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