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Yoking OX40 to regulation of IL-9

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Abstract

The secretion of cytokines by helper T cells is affected by the cytokine environment and by costimulatory signals. Engagement of the receptor OX40 on T cells increases expression of the transcription factor TRAF6, activates the alternative transcription factor NF- κ B pathway and induces the production of interleukin 9.

The development of helper T cells that express discrete subsets of cytokines is critical for the development of effective immunity to a wide array of pathogens and environmental toxins. The cytokine micro environment is a principal trigger that promotes the differentiation of naive CD4⁺ helper T cells that acquire the ability to secrete a restricted panel of cytokines. Among the specialized subsets of helper T cells are the interleukin 9 (IL-9)-secreting T_H9 cells^{1,2}. T_H9 differentiation requires transcription factors that include PU.1, downstream of transforming growth factor- β (TGF- β), and a STAT6-IRF4 pathway downstream of IL-4 (refs. 3–5). The transcription factors NFAT and NF- κ B are also required in differentiated T_H9 cells for the induction of IL-9 after stimulation of antigen receptors⁶. However, other signaling pathways that specifically induce IL-9 have not been described. In a study published in this issue of *Nature Immunology*, Xiao and colleagues now show that the receptor OX40 potently promotes T_H9 cell generation⁷.

OX40 (CD134) is a member of the tumor-necrosis factor (TNF) receptor superfamily that is rapidly induced on T cells after activation. Engagement of OX40 by its ligand OX40L (CD252) expressed on antigen-presenting cells inhibits development of regulatory T cells and promotes inflammation in many organ systems⁸. OX40 is required for the development of allergic lung disease in mouse models⁹ and is activated by OX40L expressed on dendritic cells to support T helper type 2 (T_H2)-mediated inflammation^{10,11}. Xiao *et al.* demonstrate that engagement of OX40 results in much more production of IL-9 by T_H9 cultures⁷ (Fig. 1a). Moreover, stimulation of OX40 *in vivo* results in IL-9-dependent allergic lung inflammation.

In trying to define how OX40 contributes to the development of inflammatory disease, Xiao *et al.* demonstrate that the addition of antigen-presenting cells with transgenic expression of OX40L to cultures of differentiating helper T cells results in less development of regulatory

T cells that express the transcription factor Foxp3 and IL-17-secreting cells of the T_H17 subset of helper T cells but a much greater frequency of IL-9⁺ cells in T_H9 cultures⁷. A greater abundance of IL-9 is a direct effect of OX40L-OX40 interaction, as it is replicated with antibody to OX40 and is dependent on the expression of OX40 by T cells. Notably, although T_H9 culture conditions are required for the effects of OX40 stimulation on IL-9 production, OX40 engagement does not affect phosphorylation of the signal transducers Smad2, Smad3, STAT5 or STAT6, which are molecules downstream of TGF- β , IL-2 or IL-4. The expression of PU.1, IRF4 or GATA-3, three factors required for the development of T_H9 cells, is not altered by OX40 signaling. Moreover, the authors use PU.1-deficient T cells to demonstrate that the effects of OX40 engagement are independent of PU.1. Although the PU.1 independence could be a result of OX40L overexpression, as both PU.1 expression in T cells and OX40 are required for the development of allergic lung inflammation^{3,7,9}, these results suggest that OX40 stimulates IL-9 production through a previously unknown pathway.

Members of the TNF superfamily are known to stimulate the activation of NF- κ B, and Xiao *et al.* demonstrate that the engagement of OX40 on T_H9 cells by OX40L activates both the canonical and noncanonical NF- κ B pathways⁷ (Fig. 1b). Using a combination of pharmaceutical and dominant-negative approaches, they demonstrate that the canonical pathway is not required for the effects of OX40 stimulation and that the NF- κ B subunit RelA (in the canonical pathway) does not bind the *Il9* promoter. In contrast, the induction of IL-9 by OX40L is dependent on the NF- κ B subunit p52, and retroviral transduction of p52 and the NF- κ B subunit RelB results in the induction of IL-9 production, but retroviral transduction of p50 and RelA does not. In agreement with those results, RelB is bound to the *Il9* promoter after stimulation with OX40, and coexpression of RelB and p52 activates an *Il9* promoter reporter. Activation of the noncanonical pathway is dependent on the OX40L-induced expression of the transcription factor TRAF6, and transduction of TRAF6-deficient T_H9 cultures to express RelB and p52 restores IL-9 production, which suggests that this represents the main pathway of induction. The results by Xiao *et al.*⁷ contrast somewhat with a published report showing that RelA is required for antigen receptor-induced IL-9 (ref. 6), although such differences could be due to distinct stimuli or culture conditions.

Notably, Xiao *et al.* demonstrate that engagement of OX40 *in vivo* promotes inflammation⁷. Mice with transgenic expression of OX40L, which have been shown to develop multiorgan inflammation, have lung inflammation characterized by mucus production, with a concomitant increase in *Il9* mRNA in lung tissue and IL-9-secreting T cells in the lungs. In parallel studies, antibody to OX40 is also shown to promote lung inflammation dependent on endogenous IL-9. The induction of allergic lung inflammation in a model of ovalbumin in alum is dependent on OX40, and OX40 deficiency is associated with less IL-9 in bronchoalveolar lavage fluid, as has been shown before^{9,11}. This final set of studies is critical, as it demonstrates the link from OX40 to IL-9 *in vivo* in the absence of transgenic expression of OX40L or injected antibody to OX40.

Both T_H2 and T_H9 cells are probably required for maximal allergic inflammation. The link between OX40 signaling and IL-9 raises the important question of the relative contributions of the OX40-T_H9 pathway and of the previously established OX40-T_H2 pathway to the

development of allergic inflammation. In the absence of OX40-OX40L signaling, the production of T_H2 cytokines is diminished, concomitant with the lower abundance of IL-9 observed by Xiao *et al.*⁷ in the ovalbumin model of allergic airway disease^{8–11}. The conditions in which OX40 would ‘preferentially’ promote T_H2 or T_H9 development are not clear, and this may depend entirely on the polarizing cytokine milieu. However, the amount of stimulation by OX40L on antigen-presenting cells might also contribute to the target cytokine. The ability of transgenically expressed OX40L to potently induce IL-9 production suggests that higher OX40L expression might favor the induction of IL-9 rather than T_H2 cytokines.

Another key issue is whether OX40-induced IL-9 is transient or whether stimulation induces programming of *Il9*. Xiao *et al.* analyze most of their *in vitro* data after only 3 days of culture, and kinetic data indicate the greatest induction of *Il9* mRNA and IL-9 protein occurs within the first 48 hours, with transcription waning thereafter⁷. Although many members of the TNF superfamily alter the production of helper T cell cytokines, none have been shown to be essential initiators of helper T cell differentiation. Similarly, although NF- κ B proteins can induce many cytokines, members of the NF- κ B family are not generally thought of as helper T cell lineage-promoting factors. Thus, engagement of OX40 might facilitate a short burst of IL-9 production without resulting in long-term changes in the ability of the stimulated cell to produce IL-9 after subsequent stimulation through the antigen receptor. These issues will need further experimentation to discern both *in vitro* and *in vivo* effects.

Finally, it will be important to determine if the effects of OX40 engagement are restricted to T_H9 cells or are observed in other helper T cell subsets and in non-T cells. Xiao *et al.* note that OX40 promotes IL-9 production when cells are cultured in the presence of TGF- β and IL-4, the T_H9-inducing cytokines, but not in the absence of polarizing cytokines⁷. However, they do not report whether stimulation by OX40 induces IL-9 as it represses expression of Foxp3 or IL-17 in culture conditions that promote regulatory T cells or T_H17 cells, respectively. Engagement of OX40 could explain the ability of several helper T cell subsets to produce IL-9 under some culture conditions. As additional IL-9-producing cell types are identified, such as innate lymphoid cells¹², this pathway could prove to be critical in establishing an IL-9-sufficient environment through many cell types.

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Figure 1.

OX40 induces IL-9 in differentiating T_H9 cells. **(a)** OX40L expressed on an antigen-presenting cell (APC) engages OX40 on the surface of a T cell. In the context of activation of the antigen receptor and T_H9 -polarizing cytokines, engagement of OX40 results in the induction of *Il9*. **(b)** Despite the engagement of OX40 in T_H9 cultures that results in activation of the canonical and noncanonical NF- κ B pathways, only the noncanonical pathway induces *Il9*. TRAF6 has an obligatory role in activation of the noncanonical pathway. RelB-p52 heterodimers are bound to NF- κ B consensus sequences in the *Il9* promoter, indicated by numbers that are relative to the transcriptional start site. NIK and IKK are kinases; I κ B is an inhibitor of NF- κ B; p100 is the precursor of p52.