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Predisposition to the development of IL-9-secreting T cells in atopic infants

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To the Editor

Although T cell derived cytokines have been extensively studied in asthma and allergies, the contribution of the cytokine interleukin (IL)-9, which is associated with the development of allergic disease, has not been well defined. Transgenic expression of IL-9 in mice results in allergic inflammation, antibodies to IL-9 block the development of allergic disease in mice, and polymorphisms in the *IL9* and *IL9R* genes are associated with atopic phenotypes in patients (reviewed in ¹). Moreover, IL-9 is induced by allergen challenge in BAL lymphocytes, and by allergen stimulation of PBMCs isolated from atopic adults and pre-teens ²⁻⁴. To determine if increased IL-9 production was an early indicator of allergic disease, we examined IL-9 production from PBMCs isolated from infants (18–30 months old) that were recruited into the study based on a diagnosis of dermatitis and subsequently characterized for atopic status. Atopic status was defined as having allergen-specific IgE to one of ten allergens assayed, and non-atopic infants were negative for IgE to all 10 allergens ^{5, 6}(Table E1 and E2).

To examine cytokine production from atopic and non-atopic infants, we stimulated total PBMCs non-specifically (PMA/Ionomycin) and tested cytokine production using multiplex bead assay as previously described ^{6, 7}. PMA/ionomycin stimulation resulted in significantly more IL-9 in cultures from atopic patients (n=58) compared to those from non-atopic patients (n=37, p=0.029) (Fig. E1). The increase in IL-9 was specific because no significant differences were identified in the production of Th2 cytokines (IL-4, -5, -13), Th17 cytokines (IL-17A, -17F, -22), or in the Th1 cytokine (IFN- γ) between atopic and non-atopic patients (Fig. E1). To more specifically look at T cell responses, we stimulated PBMCs with anti-CD3 and divided the population into non-atopic, or those having specific IgE for egg, cat or house dust mite allergen, the most common positive reactions (Table E2). In each subpopulation with specific IgE, IL-9 production was also significantly increased compared to non-atopic controls (Fig. 1A). The production of IL-9 by PBMCs correlated with the serum level of total IgE (r=0.319; p=0.002). The production of Th2 cytokines by PBMCs did not significantly correlate with total serum IgE.

In a subset of patient samples where there were sufficient numbers of cells available, we next tested the responses of total PBMCs to specific allergens. There were no significant

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differences in demographics or total serum IgE among subjects used for specific antigen stimulation compared to the entire population. Both cat dander and house dust mite extract (Invivogen) stimulation induced significantly more IL-9 production in cultures from atopic infants that had IgE specific for that allergen (n=5–10) compared to non-atopic (n=10–11) patients that lacked IgE specific to any of the allergens tested ($p<0.05$) (Fig. 1B). Although there was a trend towards increased Th2 cytokine production, there were no significant differences between atopic and non-atopic patients (Fig. 1C). It is likely that the allergen extracts also stimulate IL-9 production from non-CD4+ T cells and that may account for IL-9 production in the non-atopic cultures.

A novel subset of T helper cells that predominantly produce IL-9 was recently described, and termed Th9 cells^{8,9}. Human and mouse Th9 cells rely on the expression of the transcription factor PU.1 for IL-9 production⁷. Mouse Th cells require STAT6 for the development of Th2 and Th9 cells. To investigate the underlying mechanism of IL-9 production and the development of Th9 cells in this patient population, we differentiated Th9 cells from naïve CD4+ CD45RA+ T cells isolated from atopic and non-atopic patient samples using methods previously described⁷. Following differentiation in culture for five days, T cells were then stimulated with anti-CD3. Th9 cells derived from atopic patient T cells (n=10) secreted significantly higher amounts of IL-9 than those from non-atopic patients (n=11) at the level of both mRNA and protein (Fig. 2A–B). Increased IL-9 production was paralleled by increased *SP11* (PU.1) expression in the Th9 cultures derived from atopic patients, but not with altered expression of *IRF4* (Fig. 2A), another transcription factor that promotes IL-9 expression, nor with altered expression of other Th lineage factors including *GATA3*, *TBX21*, *FOXP3* or *RORC* (Fig. E2A). There was no difference in IL-5 or IL-13 production from Th9 cultures between cultures from atopic and non-atopic infants (Fig. E2B), and Th1 or Th17 cytokines were expressed in low amounts. As with the polyclonal analysis described above, the effects on Th9 development were specific. There were no significant differences detected in the production of Th2 cytokines (IL-4, -5, -13) or the Th17 cytokine IL-17 between cultures from atopic (n=6–8) and non-atopic patients (n=3–10) differentiated under the respective conditions (Fig. E2C–D). It is possible IL-9 production was also increased in Th2 cultures from atopic infants, but this was not tested, and although Th9 cultures produce more IL-9 than Th2, our observations may extend to all IL-9-secreting T cells. Since STAT6 is one of the essential transcription factors for Th9 differentiation, we compared the kinetics of phospho-STAT6 (pSTAT6) during Th9 differentiation. Although pSTAT6 was only modestly activated at day 2 of culture (data not shown), there was an increase in the percentage of cells staining for pSTAT6 at day 4 of differentiation and cells in Th9 cultures derived from atopic patients demonstrated higher percentages of pSTAT6-positive cells than non-atopic patients (Fig. 2C).

Although IL-9 has been associated with allergic inflammation, when it is expressed during the development of atopic disease is only beginning to be understood. IL-9, similar to Th2 cytokines, is stimulated by allergen challenge in atopic adults and pre-teens^{2–4}. Our study demonstrates that in atopic infants, IL-9 production from polyclonal or allergen-stimulated PBMCs is increased compared to production from non-atopic controls. Importantly, this is observed in the absence of concomitant increases in Th2 cytokine production, suggesting that increased IL-9 production may be an earlier event in the development of atopic disease than increases in the production of other cytokines. The increased propensity of naïve CD4+ T cells to differentiate into IL-9-secreting T cells, in the presence of Th9 skewing conditions in vitro, suggests intrinsic differences in these cells. We observed greater percentages of cells during culture that were pSTAT6-positive, and greater expression of the transcription factor PU.1 in differentiated cultures, which indicates an altered ability of cells from atopic infants to respond to their environment. Together, these data define a predisposition to the

development of IL-9-secreting T cells in atopic infants and suggests that IL-9 may be an early target for therapy in individuals predisposed to atopy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

IL-9 concentration in anti-CD3- or allergen-stimulated PBMC from atopic and non-atopic patients. A. PBMCs were stimulated with anti-CD3 for 72 hours before supernatants were assayed for IL-9 concentration using ELISA. Samples were divided into non-atopic patients (n=34), or patients that had allergen-specific IgE for egg (n=20), cat (n=29) or house dust mite (n=28). B–C, IL-9 (B) or Th2 cytokine (C) concentration was determined in supernatants of PBMC stimulated for seven days of culture with cat dander extract- or house dust mite extract (10 µg/ml) using cells isolated from atopic (n=5–10, black bars) or non-atopic (n=10–11, white bars) patients. *:p<0.05 by Student's *t* test.

Fig. 2.

In vitro Th9 differentiation of naïve (CD4⁺ CD45RA⁺) T cells from atopic and non-atopic patients. A. *IL9*, PU.1 (*SPI1*), and *IRF4* gene expression in Th9 cells derived from naïve CD4⁺ T cells isolated from non-atopic (n=11) and atopic patients (n=10) and cultured for five days with TGFβ and IL-4. B. IL-9 production from Th9 cells derived as in (A). C. The percentage of pSTAT6-positive cells was assessed at day 4 of differentiation under Th9 conditions in cultures from atopic and non-atopic patients (n=6). *:p<0.05 by Student's *t* test.