

Letter to the Editor

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

To the Editor:

Although T-cell-derived cytokines have been extensively studied in asthma and allergies, the contribution of the cytokine 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 Goswami and Kaplan¹). Moreover, IL-9 is induced by allergen challenge in bronchoalveolar lavage lymphocytes and by allergen stimulation of PBMCs isolated from atopic adults and preteens.²⁻⁴ To determine whether 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) who were recruited in the study on the basis of a diagnosis of dermatitis and subsequently characterized for atopic status. Atopic status was defined as having allergen-specific IgE to 1 of 10 allergens assayed, and nonatopic infants were negative for IgE to all 10 allergens^{5,6} (see [Tables E1 and E2](#) in this article's Online Repository at www.jacionline.org).

To examine cytokine production from atopic and nonatopic infants, we stimulated total PBMCs nonspecifically (phorbol 12-myristate 13-acetate [PMA]/ionomycin) and tested cytokine production by 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 with those from nonatopic patients ($n = 37$, $P = .029$) (see [Fig E1](#) in this article's Online Repository at www.jacionline.org). The increase in IL-9 was specific because no significant differences were identified in the production of T_H2 cytokines

(IL-4, -5, -13), T_H17 cytokines (IL-17A, -17F, -22), or the T_H1 cytokine (IFN- γ) between atopic and nonatopic patients ([Fig E1](#)). To more specifically look at T-cell responses, we stimulated PBMCs from nonatopic infants or atopic infants having specific IgE for egg, cat, or house dust mite allergen, the most common positive reactions, with anti-CD3 ([Table E2](#)). In each subpopulation with specific IgE, IL-9 production was also significantly increased compared with that in nonatopic controls ([Fig 1, A](#)). The production of IL-9 by PBMCs correlated with the serum level of total IgE ($r = 0.319$; $P = .002$). The production of T_H2 cytokines by PBMCs did not significantly correlate with the 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 differences in demographics or total serum IgE among subjects used for specific antigen stimulation compared with the entire population. Both cat dander and house dust mite extract (Invivogen, San Diego, Calif) stimulation induced significantly more IL-9 production in cultures from atopic infants who had IgE specific for that allergen ($n = 5-10$) compared with nonatopic ($n = 10-11$) infants who lacked IgE specific to any of the allergens tested ($P < .05$; [Fig 1, B](#)). Although there was a trend toward increased T_H2 cytokine production, there were no significant differences between atopic and nonatopic patients ([Fig 1, C](#)). 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 nonatopic cultures.

A novel subset of T_H cells that predominantly produce IL-9 was recently described and termed T_H9 cells.^{8,9} Human and mouse T_H9 cells rely on the expression of the transcription factor PU.1 for IL-9 production.⁷ Mouse T_H cells require STAT6 for the development of T_H2 and T_H9 cells. To investigate the underlying mechanism of IL-9 production and the development of T_H9 cells

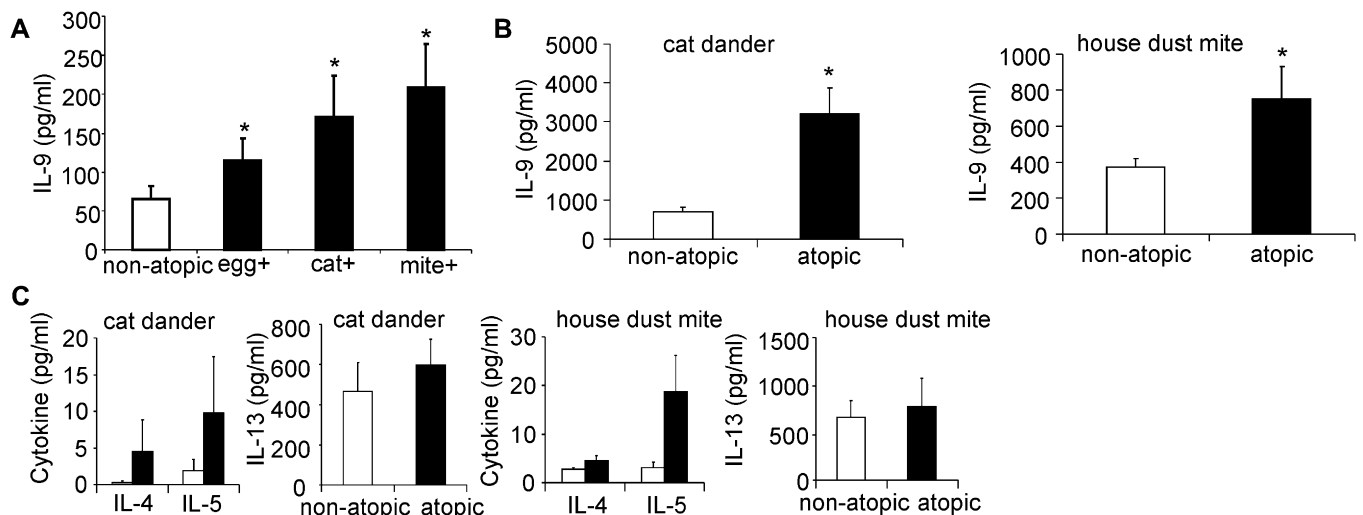


FIG 1. IL-9 concentration in anti-CD3- or allergen-stimulated PBMC from atopic and nonatopic patients. **A**, PBMCs were stimulated with anti-CD3 for 72 hours before supernatants were assayed for IL-9 concentration by using ELISA. Samples were divided into nonatopic patients ($n = 34$) or patients who had allergen-specific IgE for egg ($n = 20$), cat ($n = 29$), or house dust mite ($n = 28$). IL-9 (**B**) or T_H2 cytokine (**C**) concentration was determined in supernatants of PBMC stimulated for 7 days of culture with cat dander extract or house dust mite extract (10 μ g/mL) by using cells isolated from atopic ($n = 5-10$, black bars) or nonatopic ($n = 10-11$, white bars) patients. * $P < .05$ by Student t test.

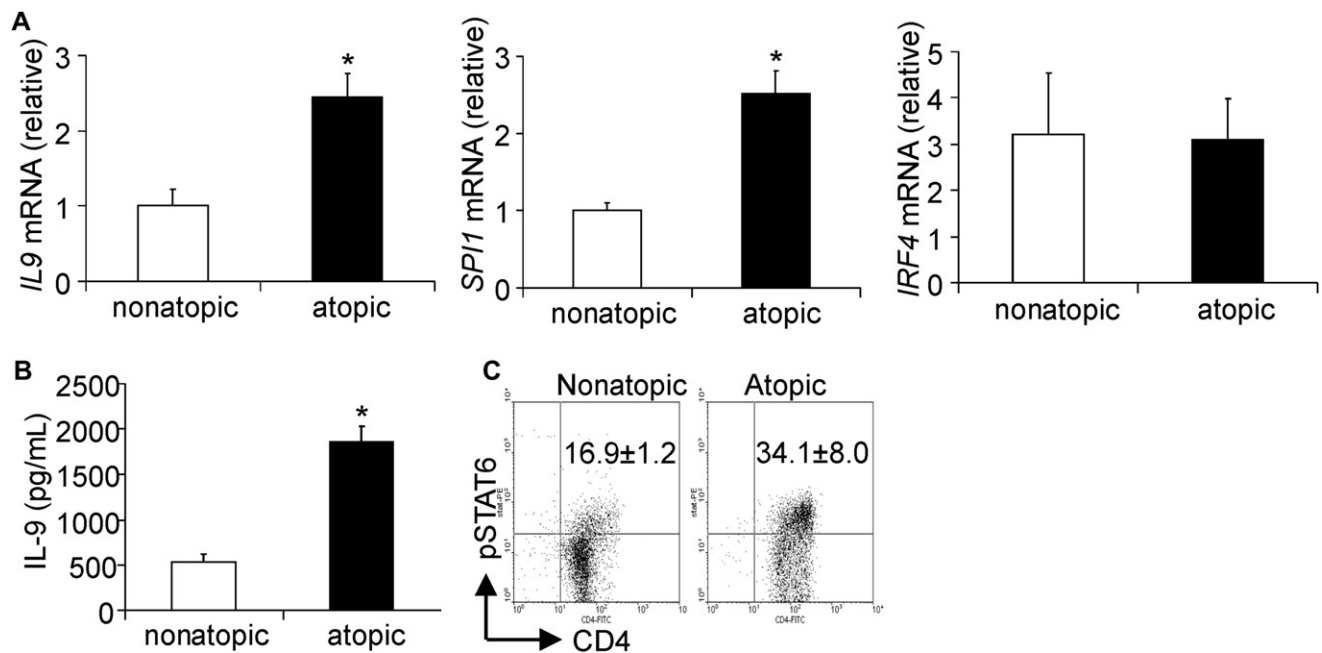


FIG 2. *In vitro* T_H9 differentiation of naive (CD4⁺ CD45RA⁺) T cells from atopic and nonatopic patients. **A**, IL9, PU.1 (*SP11*), and *IRF4* gene expression in T_H9 cells derived from naive CD4⁺ T cells isolated from nonatopic (n = 11) and atopic patients (n = 10) patients and cultured for 5 days with TGF- β and IL-4. **B**, IL-9 production from T_H9 cells derived as in panel A. **C**, The percentage of pSTAT6-positive cells was assessed at day 4 of differentiation under T_H9 conditions in cultures from atopic and nonatopic patients (n = 6). *P < .05 by Student t test.

in this patient population, we differentiated T_H9 cells from naive CD4⁺ CD45RA⁺ T cells isolated from atopic and nonatopic patient samples by using methods previously described.⁷ Following differentiation in culture for 5 days, T cells were then stimulated with anti-CD3. T_H9 cells derived from atopic patient T cells (n = 10) secreted significantly higher amounts of IL-9 than did those from nonatopic patients (n = 11) at the level of both mRNA and protein (Fig 2, A and B). Increased IL-9 production was paralleled by increased *SP11* (PU.1) expression in the T_H9 cultures derived from atopic patients, but not with altered expression of *IRF4* (Fig 2, A), another transcription factor that promotes IL-9 expression, nor with altered expression of other T_H lineage factors including *GATA3*, *TBX21*, *FOXP3*, or *RORC* (see Fig E2, A, in this article's Online Repository at www.jacionline.org). There was no difference in IL-5 or IL-13 production from T_H9 cultures from atopic and nonatopic infants (Fig E2, B), and T_H1 or T_H17 cytokines were expressed in low amounts. As with the polyclonal analysis described earlier, the effects on T_H9 development were specific. No significant differences were detected in the production of T_H2 cytokines (IL-4, -5, -13) or the T_H17 cytokine IL-17 between cultures from atopic (n = 6-8) and nonatopic patients (n = 3-10) differentiated under the respective conditions (Fig E2, C and D). It is possible that IL-9 production was also increased in T_H2 cultures from atopic infants, but this was not tested, and although T_H9 cultures produce more IL-9 than do T_H2 cultures, our observations may extend to all IL-9-secreting T cells. Since STAT6 is one of the essential transcription factors for T_H9 differentiation, we compared the kinetics of phospho-STAT6 (pSTAT6) during T_H9 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 T_H9 cultures derived from atopic

patients demonstrated higher percentages of pSTAT6-positive cells than did cells in T_H9 cultures derived from nonatopic patients (Fig 2, C).

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 T_H2 cytokines, is stimulated by allergen challenge in atopic adults and preteens.²⁻⁴ Our study demonstrates that in atopic infants, IL-9 production from polyclonal or allergen-stimulated PBMCs is increased compared with production from nonatopic controls. Importantly, this is observed in the absence of concomitant increases in T_H2 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 naive CD4⁺ T cells to differentiate into IL-9-secreting T cells, in the presence of T_H9-skewing conditions *in vitro*, suggests intrinsic differences in these cells. We observed greater percentages of cells during culture that were pSTAT6-positive as well as 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 suggest that IL-9 may be an early target for therapy in individuals predisposed to atopy.

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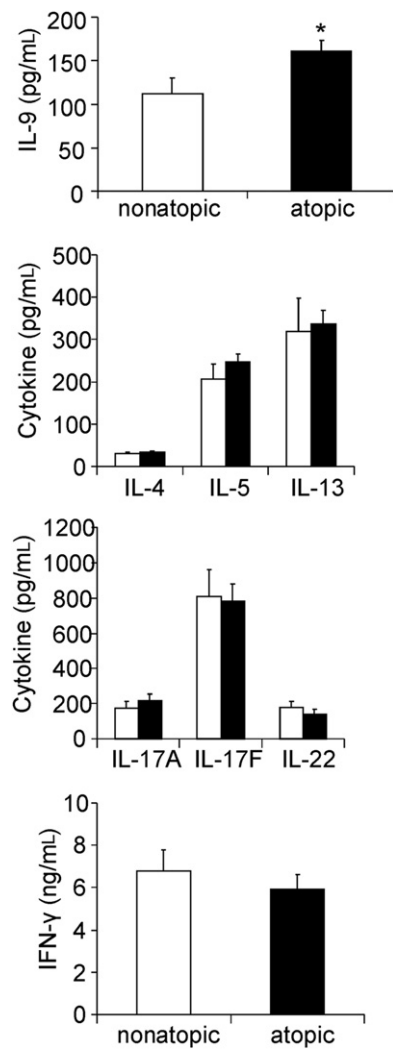


FIG E1. Cytokine levels in PMA/ionomycin-stimulated PBMC. T_H9 , T_H2 , T_H17 , and T_H1 cytokines were compared in PMA/ionomycin-stimulated PBMC from atopic ($n = 58$, black bar) and nonatopic ($n = 37$, white bar) patients. The concentration was determined in supernatants following 72 hours of PMA/ionomycin stimulation.

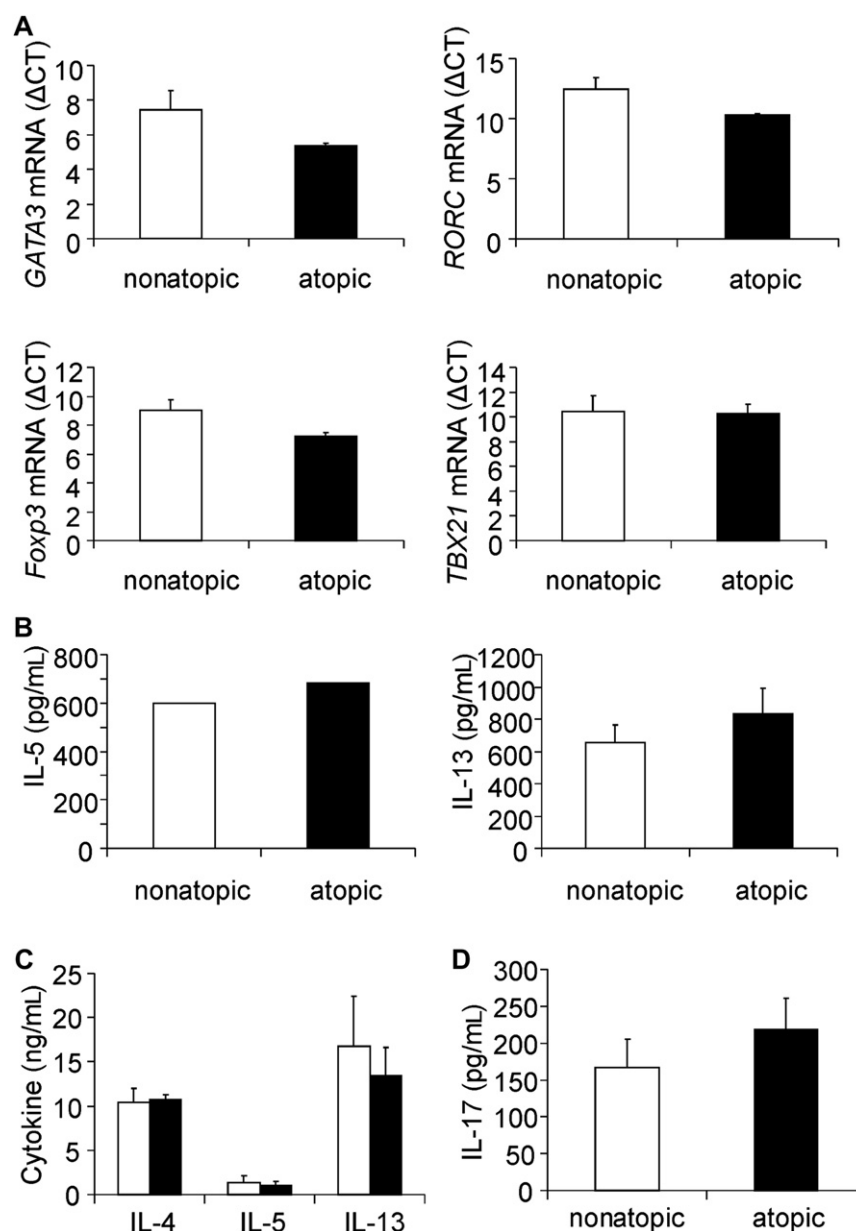


FIG E2. *In vitro* T_H cell differentiation of naive (CD4⁺ CD45RA⁺) T cells from atopic and nonatopic patients. **A and B.** After 5 days of differentiation in T_H9 conditions, cells were isolated for analysis of gene expression by qPCR (**A**) or were stimulated by anti-CD3 alone for 24 hours before supernatants were tested for T_H2 cytokines by using ELISA (**B**). **C.** After culture for 5 days under T_H2 conditions (IL-4), cells were washed and stimulated with anti-CD3 alone for 24 hours before T_H2 cytokine production was compared between cultures from nonatopic (n = 3, *white bar*) and atopic (n = 6, *black bar*) patients. (**D**) T_H17 cytokine was compared with T_H17 cells differentiated from atopic (n = 8) and nonatopic (n = 10) patients.

TABLE E1. Demographics of patient population

	Nonatopic (n = 37)	Atopic (n = 58)	P value
Age (mo)	24.7 ± 0.88	23.6 ± 0.61	.28
Gender (% male)	21/37 (57%)	41/58 (71%)	.39
Race (% Caucasian)	18/37 (49%)	29/58 (50%)	.75
SCORAD index	5.2 ± 1.0	7.6 ± 0.9	.08
IgE (IU/mL) (log)	1.44 ± 0.81	2.32 ± 1.77	.02
History of immediate family allergy or asthma	28/37 (75.7%)	53/58 (91.4%)	.15

Atopic status was defined as previously described^{E1,E2} on the basis of the presence or absence of allergen-specific IgE to milk, egg, wheat, house dust mite, cat dander, *Alternaria*, timothy grass, Bermuda grass, ragweed, and cedar.

SCORAD, SCORing Atopic Dermatitis.

TABLE E2. Frequency and titers of allergen-specific IgE in the patient population

	IgE (IU/mL)	Patient count
Nonatopic	<0.35	36
Egg ⁺	8.812	21
Mite ⁺	4.758	29
Cat dander ⁺	7.495	32

The number of patients with IgE for each allergen is indicated with the average IgE titer. Some patients were positive for multiple allergens.

⁺, Positive for allergen-specific IgE.