

Letter to the Editor

Cross-linking IgE augments human conventional dendritic cell production of CC chemokine ligand 28

To the Editor:

It has been well documented that infants hospitalized with a severe respiratory viral infection have an increased risk of atopic disease.¹ Whether the viral infection imparts a change in the individual that leads to atopic disease or whether it simply reveals an underlying predisposition to atopic disease remains controversial.² However, initial mechanistic information has been obtained by using rodent models of human disease. Recently, we used a mouse model of viral induced atopic disease (the Sendai virus model) to show that expression of the high-affinity receptor for IgE, FcεRI, on lung conventional dendritic cells (cDCs) was critical for accumulation of IL-13-producing T_H2 cells in the lung postviral infection.³ Cross-linking IgE on the cDCs led to production of the T_H2-cell and regulatory T-cell chemoattractant CC chemokine ligand (CCL)–28. Neutralization of this chemokine inhibited translation of the viral infection into atopic disease.

CC chemokine ligand 28 is a ligand for CC chemokine receptor 3 and CC chemokine receptor 10 and is a chemoattractant for IgA secreting plasmablasts, T_H2 cells, and regulatory T cells.^{4,5} Human CCL28 is 83% homologous with mouse CCL28 and has been identified in the sputum and lung biopsies from individuals with asthma.⁶ The major source of this chemokine has been assumed to be epithelial cells, primarily on the basis of studies of the gut (where it is highly produced and was first discovered).⁵ To begin to determine the relevance of the mouse model with human disease, we explored whether cross-linking human peripheral blood cDC FcεRI would produce CCL28, and whether this would be related to atopic status.

Atopic and nonatopic, nonpregnant individuals 18 years of age or older were eligible to participate in the study provided they had no chronic disease. The Washington University Human Studies Committee approved this study. After informed consent was obtained, peripheral blood was drawn, and subjects underwent epicutaneous skin testing to a routine panel of aeroallergens (cat pelt, *Cladosporium*, oak, ragweed, Kentucky blue grass, and dust mite mix, all from Greer Laboratories, Lenoir, NC). Subjects with 1 or more positive skin tests were considered atopic. Of the 23 subjects enrolled, 12 were women (75% of whom were atopic), and 11 were men (64% of whom were atopic). The median age was 34 years (range, 21–57 years). Demographic data were not significantly different between the atopic and nonatopic groups, nor were they different between sexes.

Leukocytes were isolated from peripheral blood by density gradient centrifugation (Histopaque-1077; Sigma-Aldrich, St Louis, Mo). Conventional DCs were enriched from peripheral blood leukocytes by 2 serial positive immunomagnetic selections with anti-CD1c (clone AD5-8E7) beads according to the manufacturer's instructions (Miltenyi Biotec, Auburn, Calif). The final cDC purity was 20% ± 5% (mean ± SEM), with the majority (≥95%) of contaminating cells being CD19⁺ B cells (data not shown). As shown in Fig 1, we found that the only cells expressing IgE in this preparation were cDCs. The enriched cDCs were cultured at 10⁵ cells/mL in Dulbecco modified Eagle medium

supplemented with 2-mercaptoethanol and 10% FCS for 18 hours, with 10 μg/mL of either an antihuman IgE cross-linking goat antibody (Kirkegaard & Perry, Gaithersburg, Md) or control goat IgG (Sigma-Aldrich). CCL28 protein in the culture supernatants was determined by using a commercially available ELISA kit (R & D Systems, Minneapolis, Minn). The Wilcoxon signed-rank test was used for statistical analyses with significance set at $P < .05$.

After culture for 18 hours, measurable quantities of CCL28 were found in the supernatant, as shown in Fig 2, A. Treatment with the IgE cross-linking antibody led to a significant increase in protein (median value of 999 pg/mL, which increased to 2208 pg/mL; $P = .007$). Although we cannot definitively state that CCL28 came from cDCs, B cells are reported to not make CCL28.⁵ Furthermore, only the cDCs expressed IgE (Fig 1) and thus would be the only cell population responsive to IgE cross-linking. Thus, it appears that the human and rodent systems are similar in cDC production of CCL28 as a result of IgE cross-linking. However, we cannot exclude the possibility that cross-linking IgE on cDCs indirectly stimulated contaminating cells to produce CCL28.

We next explored what role atopic status played in CCL28 production as a result of cross-linking of IgE. Surprisingly, as shown in Fig 2, B, the modest increase in CCL28 production in cDCs from atopic individuals did not reach statistical significance (median values of 931.5 pg/mL to 1911 pg/mL; $P = .16$). However, cDCs isolated from nonatopic individuals showed a greater increase, with median values of 1054 pg/mL (control IgG) increasing to 3180 pg/mL after cross-linking of IgE ($P = .016$; Fig 2, C). These findings suggest that although cDCs from both atopic and nonatopic individuals make CCL28, this level of protein can be augmented by cross-linking IgE, with the level of increase much greater in nonatopic subjects. Interestingly, the baseline level of CCL28 did not correlate with atopic status, age, or subject sex; however, levels post-IgE cross-linking were associated with atopic status ($P = .04$, Spearman rank).

We do not know why the response to IgE cross-linking was greater in nonatopic individuals. Both atopic and nonatopic individuals expressed IgE on their cDCs, although expression was higher on cDCs from atopic subjects (20.4 ± 7.1 vs 12.3 ± 9.6 , mean fold mean fluorescence intensity (MFI) ± SEM, atopic vs nonatopic), similar to what has been reported.⁷ Perhaps anti-IgE treatment cross-linked a greater percentage of FcεRI on the cDCs from nonatopic individuals, translating into increased CCL28 production. Constitutive production of CCL28 was high in our cDC cultures, suggesting that factors other than IgE are important in baseline release of CCL28. Perhaps association of CCL28 and atopic status is a result of these other factors or cell types, especially in diseases like atopic dermatitis.⁸

This is the first study to demonstrate that human cDCs are a potent source of CCL28, and cross-linking cDC IgE increases CCL28 production by nearly 3-fold. Because our data show the FcεRI-CCL28 pathway is intact in human beings, it provides initial evidence that the virus-driven IFNαR–FcεRI–CCL28–IL-13 axis found in mice might also be operative in human beings.³ Unexpectedly, our data show that the level of CCL28 production is not a predictor of atopic status. Future studies will need to be

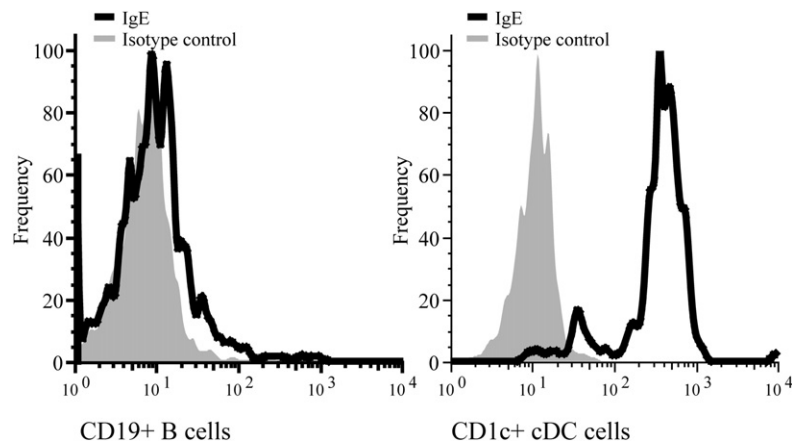
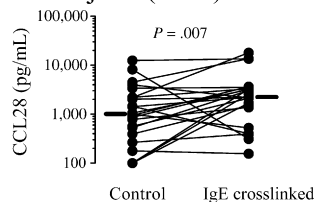
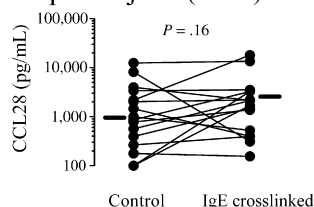


FIG 1. Expression of IgE is limited to enriched population of cDCs. Representative histograms of IgE expression (dark line) on CD19⁺ B cells and CD1c⁺ cDCs from an enriched cDC preparation before cell culture. Gray histogram is isotype control staining.

A All subjects (n=23)



B Atopic subjects (n=16)



C Nonatopic subjects (n=7)

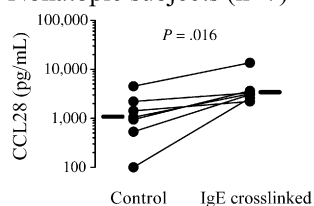


FIG 2. Cross-linking IgE augments peripheral blood cDC CCL28 production. **A**, cDCs were cultured with either a control or IgE cross-linking antibody and CCL28 production measured. Each dot represents an individual, with lines representing median values. **B**, Response as outlined in **A** of cDCs from atopic subjects. **C**, Response as outlined in **A** of cDCs from nonatopic subjects.

performed to determine whether upstream or downstream events in this cascade impart a risk of atopic disease development.

We thank Drs Xiuxu Chen, Dorothy Cheung, and Monica Vasudev for their critical review of the manuscript and Dr H. James Wedner for his support and help with this study.

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Supported by the AAAAI Third Year Research Grant (S.H.K.), NIH HL087778 (M.H.G.), and the CHW Children's Research Institute (M.H.G.).

Disclosure of potential conflict of interest: M. H. Grayson receives research support from the NIH and Genentech. S. H. Khan has received a Third Year Fellowship Research Grant from the AAAAI.

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