Published in final edited form as:

J Allergy Clin Immunol. 2010 January ; 125(1): 265. doi:10.1016/j.jaci.2009.09.038.

Cross-linking IgE augments human conventional dendritic cell production of CCL28

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Keywords

dendritic cells; CCL28; IgE; atopy

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 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 (cDC) was critical for accumulation of IL-13 producing Th2 cells in the lung post viral infection.³ Cross-linking IgE on the cDC led to production of the Th2 and Treg chemoattractant CCL28. Neutralization of this chemokine inhibited translation of the viral infection into atopic disease.

CCL28 is a ligand for CCR3 and CCR10 and is a chemoattractant for IgA secreting plasmablasts, Th2 and Treg 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, based primarily on 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 if cross-linking human peripheral blood cDC FcεRI would produce CCL28, and if this would be related to atopic status.

Atopic and non-atopic, non-pregnant 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). Subjects with one or more positive skin test were considered

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atopic. Of the 23 subjects enrolled, 12 were female (of which 75% were atopic) and 11 were male (64% of which were atopic). The median age was 34 years (range of 21–57 years). Demographic data were not significantly different between the atopic and non-atopic groups, nor were they different between genders.

Leukocytes were isolated from peripheral blood by density gradient centrifugation (histopaque-1077, Sigma-Aldrich). Conventional DC were enriched from peripheral blood leukocytes by two serial positive immunomagnetic selections with anti-BDCA-1 beads according to manufacturer's instructions (Miltenyi Biotec). The final cDC purity was $20 \pm 5\%$ (mean $\pm$ sem), with the majority ($\geq 95\%$) of contaminating cells being CD19⁺ B cells (data not shown). As shown in figure 1, we found that the only cells expressing IgE in this preparation were cDC. The enriched cDC were cultured at 10^5 cells/ml in DMEM media supplemented with 2-ME, and 10% fetal calf serum for 18 hours, with 10 μ g/ml of either an anti-human IgE crosslinking goat antibody (Kirkegaard & Perry) or control goat IgG (Sigma-Aldrich). CCL28 protein in the culture supernatants was determined by using a commercially available ELISA kit (R & D Systems). Wilcoxon signed rank test was used for statistical analyses with significance set at $p < 0.05$.

After culture for 18 hours measurable quantities of CCL28 were found in the supernatant, as shown in Figure 2A. Treatment with the IgE cross-linking antibody led to a significant increase in protein (median values of 999 pg/ml, which increased to 2208 pg/ml, $p = 0.007$). While we cannot definitively state that CCL28 came from cDC, B cells are reported to not make CCL28.⁵ Furthermore only the cDC expressed IgE (Figure 1) and, thus would be the only cell population that was responsive to IgE cross-linking. Thus, it appears that the human and rodent systems are similar in cDC production of CCL28 due to IgE cross-linking. However, we cannot exclude the possibility that cross-linking IgE on cDC indirectly stimulated contaminating cells to produce CCL28.

We next explored what role atopic status played in CCL28 production due to cross-linking of IgE. Surprisingly, as shown in Figure 2B, the modest increase in CCL28 production in cDC from atopic individuals did not reach statistical significance (median values of 931.5 pg/ml to 1911 pg/ml, $p = 0.16$). However, cDC isolated from non-atopic individuals showed a greater increase, with median values of 1054 pg/ml (control IgG) increasing to 3180 pg/ml after cross-linking of IgE ($p = 0.016$; Figure 2C). These findings suggest that although cDC from both atopic and non-atopic individuals make CCL28 this level of protein can be augmented by cross-linking IgE, with the level of increase much greater in non-atopic 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 ($p = 0.04$, Spearman's rank).

We do not know why the response to IgE cross-linking was greater in non-atopic individuals. Both atopic and non-atopic individuals expressed IgE on their cDC, although expression was higher on cDC from atopic subjects (20.4 ± 7.1 versus 12.3 ± 9.6 mean fold MFI $\pm$ sem atopic versus non-atopic), similar to what has been reported.⁷ Perhaps anti-IgE treatment cross-linked a greater percentage of Fc ϵ RI on the cDC from non-atopic 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 due to these other factors or cell types, especially in diseases like atopic dermatitis.⁸

This is the first study to demonstrate that human cDC are a potent source of CCL28 and cross-linking cDC IgE increases CCL28 production by nearly 3 fold. Since our data show the Fc ϵ RI-CCL28 pathway is intact in humans, it provides initial evidence that the virus driven IFNAR-Fc ϵ RI-CCL28-IL13 axis found in mice might also be operative in humans.³

Unexpectedly, our data show that the level of CCL28 production is not a predictor of atopic status. Future studies will need to be performed to determine if upstream or downstream events in this cascade impart a risk of atopic disease development.

Acknowledgments

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

This research supported by the AAAAI 3rd year Research Grant (SHK), NIH HL087778 (MHG), and the CHW Children's Research Institute (MHG).

Abbreviations

CCL	CC chemokine ligand
CCR	CC chemokine receptor
cDC	conventional dendritic cells
Th2	CD4+ T helper 2 cells
Treg	CD4+ T regulatory cells
FcεRI	high affinity receptor for IgE

References

1. Sigurs N. A cohort of children hospitalised with acute RSV bronchiolitis: impact on later respiratory disease. *Paediatr Respir Rev* 2002;3:177–83. [PubMed: 12376053]
2. Stensballe LG, Simonsen JB, Thomsen SF, Larsen AM, Lysdal SH, Aaby P, et al. The causal direction in the association between respiratory syncytial virus hospitalization and asthma. *J Allergy Clin Immunol* 2009;123:131–7. e1. [PubMed: 19130934]
3. Grayson MH, Cheung D, Rohlfing MM, Kitchens R, Spiegel DE, Tucker J, et al. Induction of high-affinity IgE receptor on lung dendritic cells during viral infection leads to mucous cell metaplasia. *J Exp Med* 2007;204:2759–69. [PubMed: 17954569]
4. Lazarus NH, Kunkel EJ, Johnston B, Wilson E, Youngman KR, Butcher EC. A common mucosal chemokine (mucosae-associated epithelial chemokine/CCL28) selectively attracts IgA plasmablasts. *J Immunol* 2003;170:3799–805. [PubMed: 12646646]
5. Wang W, Soto H, Oldham ER, Buchanan ME, Homey B, Catron D, et al. Identification of a novel chemokine (CCL28), which binds CCR10 (GPR2). *J Biol Chem* 2000;275:22313–23. [PubMed: 10781587]
6. O'Gorman MT, Jatoi NA, Lane SJ, Mahon BP. IL-1β and TNF-α induce increased expression of CCL28 by airway epithelial cells via an NFκB-dependent pathway. *Cell Immunol* 2005;238:87–96. [PubMed: 16581045]
7. Foster B, Metcalfe DD, Prussin C. Human dendritic cell 1 and dendritic cell 2 subsets express FcεRI: correlation with serum IgE and allergic asthma. *J Allergy Clin Immunol* 2003;112:1132–8. [PubMed: 14657872]
8. Ezzat MH, Sallam MA, Shaheen KY. Serum mucosa-associated epithelial chemokine (MEC/CCL28) in atopic dermatitis: a specific marker for severity. *Int J Dermatol* 2009;48:822–9. [PubMed: 19659860]

Figure 1.

Expression of IgE is limited to enriched population of cDC. Representative histograms of IgE expression (dark line) on CD19+ B cells and BDCA-1+ cDC from enriched cDC preparation prior to cell culture. Grey histogram is isotype control staining.

A. All subjects (n=23)

B. Atopic subjects (n=16)

C. Non-atopic subjects (n=7)

Figure 2.

Cross-linking IgE augments peripheral blood conventional dendritic cell (cDC) CCL28 production. **(A)** cDC 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 cDC from atopic subjects. **(C)** Response as outlined in (A) of cDC from non-atopic subjects.