

Allergic humans are hyporesponsive to a CXCR3 ligand-mediated Th1 immunity-promoting loop¹

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SPECIFIC AIMS

CXCL10 (IP-10), a chemokine which binds CXCR3, selectively augments antigen-stimulated IFN γ responses of healthy nonallergic humans, suggesting a role for this pathway in maintenance of clinical tolerance to environmental allergens and raising the possibility of a therapeutic role for CXCR3 ligands in rebalancing the Th2-dominated immune responses that are responsible for generation and maintenance of allergic disorders. We tested the hypothesis that exogenous CXCR3 ligands CXCL9 (Mig), CXCL10, and CXCL11 (I-TAC) directly promote allergen-selective enhancements in Th1 recall responses (IFN γ) in allergic individuals and investigated the cellular mechanisms underlying these responses.

PRINCIPAL FINDINGS

1. In contrast to normal healthy individuals, PBMC from allergic individuals are hypo responsive to the capacity of CXCR3 ligands to selectively enhance established, allergen-specific IFN γ recall responses

Redirecting pre-established Th2 skewed responses to common environmental antigens has long been the objective of immunotherapeutic approaches to mitigating the clinical manifestations of allergy. The finding that the CXCR3 binding chemokine CXCL10, as a costimulus with antigen but not alone, selectively enhances recall IFN γ production in established Th1-responses of healthy individuals suggested the existence of an in vivo Th1-enforcing feedback loop between IFN γ and the chemokines it stimulates (all three being exclusive CXCR3 binding ligands: CXCL9, CXCL10, and CXCL11). Given that CXCR3 is highly expressed on a large proportion of circulating CD4⁺/CD45RO⁺ T cells, this receptor represents an attractive target for attempted modulation of the excessively intense Th2 responses responsible for induction and maintenance of human allergic diseases.

We compared the effect of CXCR3 ligands on ex vivo allergen recall responses of 1) PBMC from healthy control individuals and 2) PBMC of allergic individuals identified based on their positive skin prick tests for cat or grass allergen and clinical history of allergic rhinitis. In the

presence of allergen and rCXCR3 ligands, at concentrations typical of leukocyte migration and calcium mobilization assays, the majority of healthy study participants (26 of 32) demonstrated substantially augmented recall IFN γ responses (median 15-fold increased allergen-specific Th1 recall responses compared with cat or grass antigen stimulation alone) in short term primary culture directly ex vivo. Enhanced antigen-specific IFN γ production was ablated by a blocking antibody to CXCR3. Ligands for CCR5, also characteristic of Th1 cells, failed to emulate this selective enhancement of IFN γ , reinforcing the CXCR3-dependence of this pathway.

In stark contrast, grass or cat antigen-stimulated PBMC from allergic individuals were markedly hyporesponsive to CXCR3 agonists' capacity to enhance the IFN γ responses that dominated the recall response of nonallergic individuals upon exposure to these allergens. Median alterations in antigen-driven IFN γ production due to CXCR3 ligands in this population was only threefold compared with 15 fold in the controls ($P=0.0004$, Mann-Whitney U test; **Fig. 1**). Only 12% of the allergic individuals, as compared with 53% of controls, were capable of a strong increase in chemokine-enhanced IFN γ secretion (arbitrarily defined as a 10 fold increase; $P=0.001$, Fisher's Exact test). Thus, allergic individuals demonstrate markedly lower capacity to respond to CXCL9-11 with augmented Th1 responses than do healthy subjects exposed to the same environmental allergens.

2. Hyporesponsiveness of allergic individuals is not mediated by Th2 cytokines or IL-10

As PBMC from allergic individuals respond to allergens with sharply Th2 skewed cytokine responses, we investigated the effect of neutralizing Th2 cytokines on CXCR3 ligand-enhanced IFN γ production. We reasoned that the elevated levels of Th2 cytokines in allergic individuals' PBMC cultures may inhibit the capacity of CXCL9-11 to

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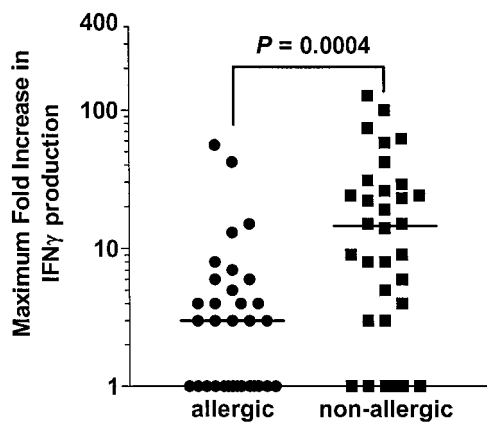
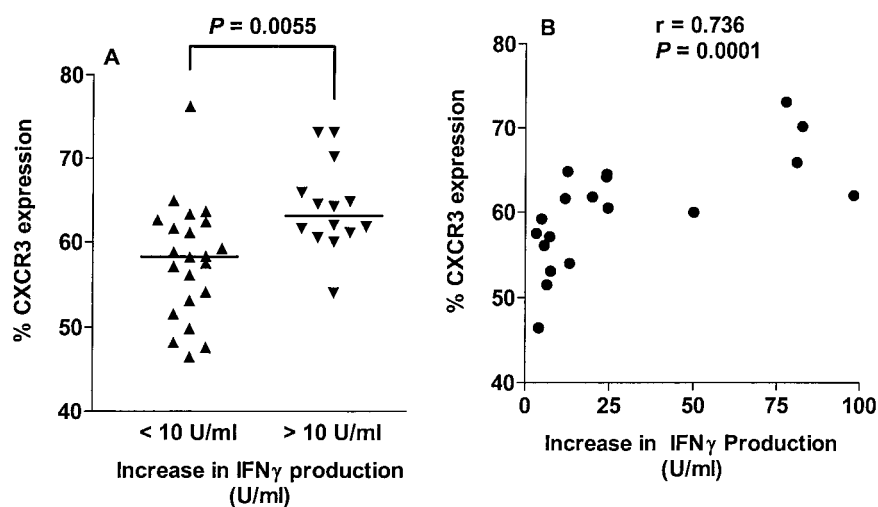


Figure 1. Hyporesponsive allergen-specific recall responses in allergic study participants. Each point represents the maximal fold increase in IFN γ production for each individual. This was determined by comparing IFN γ production from grass or cat allergen-stimulated PBMC after 4 days culture to that produced by cells stimulated with allergen and CXCL9, CXCL10, or CXCL11. As a group, allergic individuals had decreased intensity of response to CXCR3 ligands compared with non-allergic individuals (median 3- and 15-fold respectively; $P=0.0004$, Mann-Whitney U test, $n=64$). Fewer allergic individuals were capable of a strong response to CXCL9-11 (>10 fold increase in IFN γ production) than nonallergics (12% and 53%, respectively; $P=0.0011$ Fisher's Exact test).

redirect ongoing Th2 dominated responses. However, neutralization of the activity of endogenous IL-4, IL-5, and IL-13 or blocking the regulatory cytokine IL-10 did not restore or augment CXCL9-11-induced IFN γ responses in either cultures of cells from the allergic study participants that were moderate responders or from the allergic nonresponders to CXCR3 ligand-enhanced IFN γ synthesis. These data argue that the reduced ability of PBMC from allergic individuals to respond to CXCR3 ligands with enhanced Th1 cytokine responses is not attributable to allergen-driven production of Th2 cytokines or IL-10.

Figure 2. CXCR3 agonist-enhanced IFN γ production is proportional to CXCR3 expression. A) Frequency of ex vivo CXCR3 expression (%) on CD4 $^{+}$ /CD45RO $^{+}$ T cells in whole blood of individuals who were weak or nonresponders to CXCR3 ligands (<10 U/mL increase in antigen-stimulated IFN γ production) and individuals who demonstrated marked increases in IFN γ production (>10 U/mL increase in antigen-stimulated IFN γ production) in response to CXCR3 ligands. Increases in IFN γ production represent additional cytokine produced by PBMC in response to grass pollen or cat antigen and CXCR3 ligands (CXCL9, CXCL10 or CXCL11) compared with cytokine produced in response to antigen alone. Individuals who were strong responders to CXCR3 ligands had significantly higher CXCR3 expression compared with weak/nonresponders ($P=0.0055$, Mann Whitney U test, $n=36$). B) Correlation between CXCR3 expression on CD4 $^{+}$ /CD45RO $^{+}$ T cells in whole blood and increase in IFN γ production in response to CXCR3 ligands. Among CXCR3 responsive nonallergic study participants, there was a highly significant correlation between CXCR3 expression and augmented IFN γ production ($P=0.0001$, Spearman's rank correlation coefficient, $n=19$).



3. Ex vivo responsiveness to CXCR3 ligands for elevated IFN γ is associated with CXCR3 expression on PBMC

To determine whether differential responsiveness to CXCL9-11 in allergic and healthy controls reflects inherently differential CXCR3 expression on circulating T cells, we examined the relationship between CXCR3 expression on peripheral blood CD4 $^{+}$ /CD45RO $^{+}$ cells and the intensity of CXCR3 agonist-enhanced IFN γ recall responses to allergens. The percentage of CXCR3 $^{+}$ CD4 $^{+}$ /CD45RO $^{+}$ T cells in peripheral blood, directly ex vivo, was significantly higher for individuals demonstrating strong CXCL9, CXCL10 or CXCL11-induced increases in antigen-specific IFN γ production (>10 U/mL) compared with that of weak and nonresponding individuals (<10 U/mL IFN γ) (Fig. 2A; $P=0.0055$; Mann-Whitney U test). Moreover, among CXCR3 ligand-responsive nonallergic individuals, a highly significant correlation between the percentage of CXCR3-expressing CD4 $^{+}$ /CD45RO $^{+}$ T cells in circulation and the magnitude of CXCR3 agonist-enhanced antigen-specific IFN γ recall responses was evident (Fig. 2B; $P=0.0001$, $r=0.736$; Spearman's rank correlation coefficient). Thus, the degree of responsiveness of PBMC to CXCL9-11 for enhanced IFN γ production in culture correlates closely with CXCR3 expression on peripheral blood T cells examined directly ex vivo.

CONCLUSIONS

Multiple reports have demonstrated elevated T cell expression of CXCR3 and CCR5 and their ligands in Th1 skewed human diseases such as rheumatoid arthritis and multiple sclerosis. Conversely, high expression of CCR4 and ligands TARC and MDC has been noted in allergic conditions. However, beyond selective recruitment of Th1 or Th2 cells to sites of inflammation, there is little

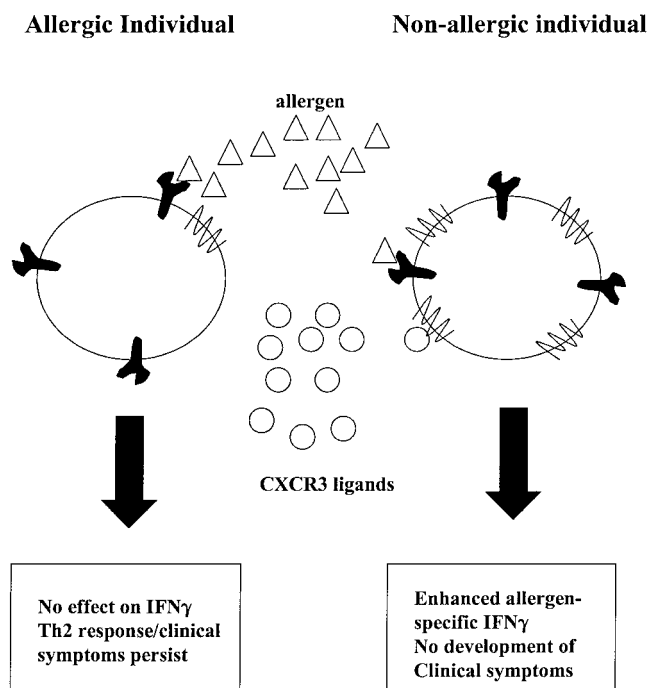


Figure 3. Schematic representation of the effect of differential CXCR3 expression on ongoing environmental allergen-driven cytokine production and clinical outcome. We speculate that endogenous production of CXCR3 ligands, CXCL9-11, similar in allergic and nonallergic individuals, actively reinforces maintenance of a balanced Th1/Th2 response in healthy subjects but fails to enhance Th1 immunity in allergic individuals as a consequence of weaker CXCR3 expression and function.

evidence that polarized chemokine and chemokine receptor expression has other modulatory effects on established in vivo human immune responses. Here, we present findings that demonstrate a clear regulatory effect of differential expression of CXCR3 on antigen-stimulated cytokine recall responses. Specifically, PBMC from grass or cat allergic rhinitis sufferers (Th2-biased), as compared with cells from healthy, nonallergic controls (Th1-biased), show marked hyporesponsiveness to the Th1 cytokine-enhancing effects of CXCR3 ligands. Furthermore, among CXCL9-11-responsive individuals, the intensity of the in vitro response strongly correlates with systemic CXCR3 expression.

We discovered that the capacity of allergic individuals to exhibit allergen-selective rebalancing of their Th2 dominated responses was markedly deficient. These findings suggest a lack of elasticity in chemokine receptor expression/responsiveness in established immune responses. To address this issue, we neutralized the activity of endogenous Th2 cytokines and IL-10 in PBMC cultures of allergic individuals. PBMC from allergic individuals remained hyporesponsive to CXCR3 ligands under these conditions. These findings suggest that Th2 cytokines and IL-10 are not regulating responsiveness to CXCR3 ligands. We recently found that in vivo (plasma) and after recall antigen exposure in primary culture, CXCL9 and CXCL10 production is substantial and indistinguishable in healthy and allergic populations. While the latter finding suggests that the Th2 skewed cytokine environment in allergic individuals is also not having appreciative effects on systemic levels of Th1-associated chemokines, it reinforces the possibility that deficient responsiveness to (rather than production of) CXCR3 ligands contributes to the maintenance of allergic disease in genetically predisposed individuals.

Deficient expression of CXCR3 by antigen-experienced CD4⁺ T cells may be a key cause limiting the ability of allergic individuals to respond to CXCL9-11

with augmented Th1 immunity upon reexposure to allergens. Recent reports of CXCR3 expression in the systemic condition allergic dermatitis noted decreased circulating CD4⁺/CD45RO⁺ T cells. Indeed, in our study of allergic rhinitis sufferers and controls, strongly responding individuals had higher CXCR3 expression on peripheral blood T cells than did weak or nonresponders. Among responding nonallergic individuals, there was a strong correlation between CXCR3 expression and degree of responsiveness in terms of absolute increase in IFN γ production in the presence of CXCR3 binding chemokines. Hence, determining the key factors involved in regulation of CXCR3 expression and responsiveness may facilitate future manipulation of T cell cytokine responses for therapeutic effect.

A number of reports have demonstrated a role for CCR5 ligands in initial polarization of T cell responses to the Th1 phenotype. Although both CXCR3 and CCR5 are Th1-associated chemokine receptors, CCR5 ligands consistently failed to enhance antigen-specific IFN γ production from PBMC of nonallergic individuals. This suggests that activation of CCR5 and CXCR3 may promote Th1 immunity at different stages of the immune response: at initial sensitization and during ongoing responses, respectively.

In summary, our data highlight a clear and functionally significant difference in the regulation of allergen-induced immune responses in allergic and nonallergic individuals. As establishment of Th2 cytokine responses to allergen is essential to the etiology of allergic rhinitis and allergic asthma, CXCR3 ligand-mediated augmentation of allergen-specific IFN γ responses may prevent the initiation of allergic inflammation in healthy individuals. Conversely, the hyporesponsiveness of allergic individuals to this potential Th1 immunity-promoting mechanism may be associated with the maintenance of allergen-specific Th2 responses and associated clinical disease (Fig. 3). [F]