Systemic chemokine and chemokine receptor responses are divergent in allergic versus non-allergic humans

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Abstract

Th1- and Th2-polarized human T cell clones display distinct patterns of chemokine receptor expression and selective chemokine responsiveness in vitro. We hypothesized that natural exposure to environmental grass pollen would induce differential systemic chemokine and chemokine receptor expression patterns in individuals with allergic rhinitis compared to healthy controls with type 2- and type 1-dominated responses to allergen respectively. To this end, we compared chemokine receptor expression on peripheral blood T cells directly ex vivo and plasma chemokine levels between these two groups of study participants prior to and during the grass pollen season. Th1-associated CXC chemokine receptor (CXCR) 3 was strongly expressed on >50% CD4+/CD45RO+ cells of all subjects. When examined longitudinally, CXCR3 expression increased over the grass pollen season (P < 0.0001), solely in non-allergic subjects. In contrast, for both allergic and non-allergic subjects, CC chemokine receptor (CCR) 5 (Th1-associated) and CCR3 (Th2-associated) were weakly expressed on <10% of CD4+CD45RO+ cells both prior to and during the grass pollen season. Type 1 chemokines CXC chemokine ligand (CXCL) 9 and CXCL10 (monokine induced by IFN-γ and IFN-γ-inducible protein of 10 kDa: CXCR3 ligands), and type 2 chemokines CC chemokine ligand (CCL) 11 (eotaxin: CCR3 ligand), CCL17 (thymus and activation-regulated chemokine: CCR4 ligand) and CCL22 (monocyte-derived chemokine: CCR4 ligand) were readily detectable in the plasma of most participants. Systemic CXCL9 levels decreased from pre- to grass pollen season in allergics (P < 0.05), whereas CCL17 decreased in non-allergics (P < 0.05) over the same period. Taken together, these longitudinal data suggest a systemic shift to more intensely type 1-dominated responses in non-allergic individuals and, conversely, to more type 2-dominated responses in allergic individuals upon natural re-exposure to grass pollen.

Introduction

The chemokine family and their receptors regulate diverse aspects of immune homeostasis and inflammation. Chemokines direct cell trafficking and recruitment, have anti-tumor effects, activate inflammatory cells, and regulate T1 and T2 development and function (1). In vitro analysis of human T cell lines and clones demonstrates an association between T1 phenotype and expression of the chemokine receptors CXC chemokine receptor (CXCR) 3 and CC chemokine receptor (CCR) 5. Conversely, CCR4, CCR8 and, initially, CCR3 expression has been associated with the T2 phenotype of T cell clones in vitro (2,3). The extent to which this polarized pattern of chemokine receptor expression is seen in vivo among antigen-experienced T cells during type 1- or type 2-associated disease remains to be determined. T cell CXCR3 and CCR5 and their respective ligands CXC chemokine ligand (CXCL) 10 [IFN-γ-inducible protein of 10 kDa (IP-10)] and CC chemokine ligand (CCL) 3 (MIP-1α) appear to be highly expressed at sites of inflammation and systemically in diseases of type 1-associated pathology such as multiple sclerosis (4). In contrast, in atopic dermatitis, an...
allergic disease with clear type 2-associated pathology, studies show both local and systemic up-regulation of T cell CCR3 and CCR4, as well as increases in their respective ligands CCL11 (eotaxin) and CCL17 [thymus and activation-regulated chemokine (TARC)] (5–10).

The type 2 immunity-associated chemokines CCL11 and CCL17 are highly expressed in the nasal mucosa of allergic rhinitis patients (11,12) as is CCL5 (RANTES) (13), a chemokine associated with type 1 immunity (14). However, the extent to which systemic chemokine and chemokine receptor expression is altered in response to natural exposure to grass pollen in allergic rhinitis patients and healthy individuals has not been determined. While type 2 immune responses clearly orchestrate the pathological manifestations of allergic rhinitis as such as the symptoms of allergic rhinitis, resistance to allergy development is associated with moderate type 1 responses to allergens (15–17). Hence, we hypothesized that non-allergic individuals would respond to natural grass pollen exposure with type 1-dominated chemokine and chemokine receptor expression, while allergies would display enhanced type 2 expression patterns under these conditions. To test this hypothesis, we examined representative T1p1 (CXCR3 and CCR5)- and T1p2 (CCR3)-associated chemokine receptor expression on CD4+CD45RO+ T cells longitudinally prior to and during the grass pollen season in grass-allergic and non-allergic individuals. In parallel, plasma levels of representative type 1 [CXCL9 (monokine induced by IFN-γ; Mig) and CXCL10] and type 2 immunity-associated chemokines [CCL11, CCL17 and CCL22 (monocyte-derived chemokine; MDC)] were determined.

Methods

Human subjects

This study was approved by the University of Manitoba Research Ethics Boards and written informed consent was obtained from each participant. Twenty-seven subjects ranging from 18 to 35 years of age were included in the study. Grass-allergic individuals (n = 13) were identified on the basis of (i) a positive epicutaneous test (wheal diameter >4 mm that of the negative control) to grass pollen mix (grass mix 1649: Kentucky blue, orchard, redtop and timothy grass; Hollister-Stier, Spokane, WA) and (ii) a history of seasonal allergic rhinitis of at least 2 years duration. Allergic individuals had not previously received immunotherapy. Healthy participants (n = 14) had no history of allergic rhinitis or asthma, and exhibited negative epicutaneous tests to grass pollen and 14 other common environmental antigens. All of the subjects were free from known concurrent infections or conditions that might influence the parameters measured in the study. Blood was obtained from all participants prior to (preseason) and during (seasonal) the grass pollen season in Winnipeg, Canada. Peak atmospheric grass pollen levels (20–70 grains/m3) were recorded from 6 June to 12 July (18).

Blood collection and plasma isolation

Peripheral blood (5 ml) was collected by venepuncture into 0.25 ml of 2.7% EDTA. A 0.5-ml aliquot of each sample was used for flow cytometry and the remaining blood was centrifuged at 200 g for 8 min to obtain platelet-poor plasma. Plasma was separated from cells within 30 min of blood collection, treated with NP-40 (0.5% final; Sigma, Oakville, Ontario, Canada) or left untreated and stored at –20°C until analysis of plasma chemokine levels. NP-40 treatment or freezing did not affect plasma chemokine levels observed (data not shown).

Flow cytometry

Chemokine receptor expression on CD4+ antigen-experienced (CD45RO+) peripheral blood mononuclear cells (PBMC) was quantified by three-color flow cytometry, CyChrome-labeled anti-human CD4 (clone RPA-T4) and phycoerythrin-labeled anti-human CD45RO (clone UCHL1; PharMingen, Mississauga, Ontario, Canada) or left untreated and stored at –20°C until analysis of plasma chemokine levels. NP-40 treatment or freezing did not affect plasma chemokine levels observed (data not shown).

Statistical analysis

Plasma chemokine levels were converted to base-10 logarithms to satisfy normality assumptions for statistical analysis using paired Student’s t-test (SPSS version 9.0; SPSS, Chicago, IL). Chemokine receptor expression was analyzed using Repeated Measures ANOVA (SAS version 8.0; SAS Institute, Cary, NC). All P values shown are two-tailed.
Results

CD4+/CD45RO+ T cell chemokine receptor expression prior to and during environmental exposure to grass pollen

Representative expression of chemokine receptors CXCR3, CCR3 and CCR5 on CD4+/CD45RO+ T cells is shown in Fig. 1. The T4,1/0-associated receptor CXCR3 was strongly expressed on >50% CD4+/CD45RO+ cells for all subjects. In contrast, T4,1-associated CCR5 and T4,2-associated CCR3 were weakly expressed on <10% of CD4+/CD45RO+ cells prior to and during the grass pollen season for both grass-allergic and non-allergic individuals. All three chemokine receptors were predominantly expressed on the antigen-experienced (CD45RO+) subset of CD4+ cells (data not shown).

In the absence of environmental antigenic stimulation, in vivo chemokine and chemokine receptor expression is longitudinally stable in healthy individuals (19). To determine if seasonal grass pollen exposure had differential effects on systemic T4,1- and T4,2-associated chemokine receptor expression in grass-allergic versus non-allergic populations, CXCR3, CCR5 and CCR3 expression on circulating T cells was quantified longitudinally: prior to and during the grass pollen season. Exposure to environmental grass pollen coincided with a highly significant increase in T4,1/0-associated CXCR3 expression on CD4+/CD45RO+ cells of non-allergic individuals (P < 0.0001) (Fig. 2a). CXCR3 expression of grass-allergic participants was unchanged by seasonal allergen exposure. In contrast, T4,1-associated CCR5 levels remained low from preseason to mid grass pollen season (Fig. 2b). Fewer than 5% of peripheral blood CD4+/CD45RO+ cells stained (weakly) positive for CCR5.

Chemokine receptors CCR3, CCR4 and CCR8 are preferentially expressed on human T4,2 cell lines and clones in vitro (2,3). However, at the time of this study, mAb to CCR4 and CCR8 were not commercially available. CCR3 has been reported on CD4+ T cells in allergic conditions (20,21) and its ligand eotaxin is highly expressed in response to allergen challenge in the nasal mucosa of subjects with allergic rhinitis (11,22). Hence, we examined T4,2-associated systemic CCR3 expression prior to and during grass pollen season in allergic and non-allergic study participants. CCR3 expression was slightly higher than that of CCR5 on CD4+/CD45RO+ cells of both grass-allergic and non-allergic subjects, but did not differ between groups and remained at low levels (<10% cells) from pre- to mid-grass pollen season in both subject populations (Fig. 3).

Type 1- and type 2-associated plasma chemokine levels prior to and during environmental exposure to grass pollen

While most chemokines are promiscuous in receptor binding, CXCL9 and CXCL10 both bind exclusively to the T4,1-associated receptor CXCR3. CCL11 binds only CCR3 (T4,2 associated), while CCL17 and CCL22 bind exclusively to CCR4 (T4,2 associated). Thus, through selective binding, these chemokines tend to participate selectively in type 1 or type 2 immune responses. As allergic and non-allergic individuals respond to the sensitizing environmental allergens with respective type 2- and type 1-dominated responses, we determined whether differential type 1 (CXCL9 and CXCL10) or type 2 (CCL11, CCL17 and CCL22) systemic chemokine responses were evident in our grass-allergic and grass-non-allergic subjects, and whether these responses were altered longitudinally in response to natural grass pollen exposure.

All five chemokines were readily detectable in the plasma of both grass-allergic and healthy individuals (Figs 4 and 5). Cross-sectional analysis of the data demonstrated no significant differences in plasma chemokine levels between the two groups at either time point. In contrast, longitudinal analysis of plasma levels of the type 1 chemokine CXCL9 were decreased upon natural allergen re-exposure, but, interestingly, only in the allergic group (P < 0.05), with no changes evident in non-sensitized individuals (Fig. 4). Conversely, levels of the type 2 chemokine CCL17 were reduced in non-allergic participants (P < 0.05), but not in grass-allergics, over the same time period (Fig. 5). There were no longitudinal changes in CCL11 or CXCL10 levels. Collectively, these data on chemokine and chemokine receptor expression patterns suggest that...
seasonal exposure to grass pollen promotes a subtle shift to a more type 1-dominated response in non-allergic individuals, but a shift to an increasingly type 2-dominated chemokine response in grass pollen-sensitized individuals.
Discussion

In this report, we demonstrate differential longitudinal changes in peripheral blood chemokine levels and chemokine receptor expression in mildly grass pollen-allergic versus non-allergic individuals in response to seasonal allergen exposure. Seasonal grass pollen challenge resulted in elevated CXCR3 expression, a chemokine receptor associated with Th1 clones, on peripheral blood T cells of non-allergic subjects only. In contrast, CCR3 and CCR5 levels remained low prior to and during the grass pollen season. Concomitantly, plasma CXCL9 levels (an IFN-γ-regulated chemokine) significantly decreased longitudinally in the allergic group, while CCL17 levels (a type 2 immunity-associated chemokine) significantly decreased in the non-allergic group over the same time period. Collectively, these data demonstrate: (i) the existence of systemic expression of type 1 and type 2 chemokines both in allergic individuals and in individuals not clinically allergic or sensitized, and (ii) differential chemokine/chemokine receptor recall responses in these populations as a consequence of natural allergen re-exposure. Elevated CXCR3 expression and reduced CCL17 levels from pre- to mid-grass pollen season are indicative of an increased type 1-dominated response in non-allergic individuals, whereas decreased plasma CXCL9 levels in grass pollen-allergic subjects suggest a shift to a more type 2-dominated response in vivo in sensitized individuals exposed to similar environmental stimuli. While such changes may be even more pronounced in severely atopic individuals, this study was restricted to mildly allergic subjects because analysis of severely asthmatic populations would likely be confounded by the effects of medications used for clinical management of disease (i.e. corticosteroids).

How the Th1- and Th2 polarized patterns of chemokine receptor expression seen with human T cell lines and clones in vitro relates to the maintenance of allergy in vivo is unclear. Panina-Bordignon et al. (23), using endobronchial biopsies from atopic asthmatics, observed that virtually all T cells were CCR4+ and that CCR8 was co-expressed on ~28% of the CCR4+ cells. In contrast, T cells from lung biopsies of patients with chronic obstructive pulmonary disease expressed CXCR3, but not CCR4 or CCR8. However, a separate study of T cell chemokine receptor expression in atopic asthma revealed preferential expression of CXCR3 and CCR5 on lung T cells, and no difference between asthmatics and control subjects (24).

T cell expression of CCR3 is somewhat controversial. Neither of the above studies detected CCR3 expression on lung T cells of asthmatics (23,24). However, double-stained CD3+/CCR3+ cells were identified in nasal polyp tissue (21) and CD4+/CCR3+ cells have been identified in atopic dermatitis skin biopsies by serial staining (5). In our study, CCR3 expression was at very low intensity and on a low percentage of peripheral blood cells. It is likely that, at most, only a small proportion of Th2 cells express CCR3 in contrast to its high expression on eosinophils (25).

In contrast to CXCR3, the other Th1 clone-associated receptor CCR5 was expressed at weak intensity on few CD4+/CCR5+ T cells. While systemic T cell CCR5 is strongly up-regulated in highly polarized type 1 inflammatory diseases such as multiple sclerosis (4), our data suggest that it may be
of less value than CXCR3 as a marker of moderate type 1 responses to environmental allergens (i.e. in healthy subjects). Kim et al. (29) recently demonstrated that ~88% of T\(_\text{h}1\) phenotype cells in blood are CXCR3\(^+\) and that other chemokine receptors such as CCR5 do not show preferential T\(_\text{h}1\)-association unless co-expressed with CXCR3.

We were unable to measure expression of the putative T\(_\text{h}2\)-associated chemokine receptor CCR4 because of lack of suitable commercial reagents at the time of this study. Recent studies demonstrate that CCR4 is highly expressed on almost all skin (but not intestinal)-homing memory T cells expressing the cutaneous lymphocyte antigen (26–27). This may explain the high systemic and local levels of T cell CCR4 expression recently reported in atopic dermatitis (28). Interestingly, atopic dermatitis is also associated with a decreased frequency of CXCR3 expression on circulating T cells (28).

We emphasize that cross-sectional analysis was much less powerful in revealing differences in chemokine or chemokine receptor expression between atopic and control subjects than was the longitudinal analysis performed here. Grass pollen exposure increased T cell CXCR3 expression in non-allergics, whereas allergic subjects showed no change, emphasizing the importance of comparing CXCR3 expression of allergic and non-allergic subjects longitudinally to identify differential effects of natural allergen challenge. Similar improvements in sensitivity were evident upon longitudinal analysis of plasma chemokine levels. This is particularly important in examining systemic responses directly ex vivo (i.e. in plasma), as chemokine levels will reflect consequences of all environmental stimuli, not solely the allergens of interest. Despite the fact that these subjects were exposed to a great number of immunologic stimuli over the course of this study, seasonal allergen exposure resulted in a clearly observed shift towards expression of more type 1-dominated immunity in healthy subjects and more type 2-dominated in atotics. At the same time, multi-year studies will be required for dissection of the mechanisms underlying these differential immunoregulatory responses. Collectively, the data suggest that this approach will provide a much more sensitive strategy for identifying in vivo regulatory events associated with maintenance and exacerbation of immediate hypersensitivity than cross-sectional studies. The data also suggest that it will prove a useful approach for evaluation of therapeutic candidates that aim to re-orient ongoing maladaptive immune responses in vivo.

Finally, the utility of examination of plasma chemokine levels deserves comment. Multiple studies have examined local chemokine expression/production in allergic rhinitis patients. Collectively, they demonstrated up-regulation of nasal CCL11, CCL17, CCL5, CCL7 [monocyte chemotactic protein (MCP)-3] and CCL13 (MCP-4) in response to acute experimental allergen challenge (12,13,30,31). However, how systemic chemokine levels compare between healthy controls and subjects with allergic rhinitis has not been determined. Here, our cross-sectional and longitudinal analyses evaluated the effects of chronic natural allergen exposure on type 1 versus type 2 chemokines in allergic and non-allergic subjects by quantifying plasma levels of CXCL9 and CXCL10 (both type 1 associated) and circulatory levels of CCL11, CCL17, and CCL22 (type 2 associated). These chemokines were chosen based on their exclusivity in binding putative T\(_\text{h}1\)- and T\(_\text{h}2\)-associated receptors. Moreover, CXCL9 and CXCL10 are induced by IFN-\(\gamma\) (32), whereas CCL11 and CCL17 production can be stimulated by type 2 cytokines such as IL-4 (12,33). We excluded CCR5 ligands from the study because of their promiscuous receptor binding. CCL5, for example, binds CCR3 as well as CCR5. Interestingly, while significant longitudinal changes in CXCL9 (in allergics only) and CCL17 (in non-allergics) were evident, there were no significant differences in chemokine expression between the allergic and non-allergic groups, and no longitudinal changes in CCL11 or CCL10 levels. This may reflect the diverse sources and stimuli of chemokine production. Plasma levels comprise chemokine produced locally (e.g. by epithelial cells) as well as that secreted by circulating cells. Natural grass pollen exposure reduced CXCL9 levels in the plasma of allergic subjects and CCL17 levels in the plasma of non-allergic subjects, suggesting enhanced skewing to pathogenic type 2 and putatively protective type 1 responses respectively. The longitudinal reduction in CXCL9 levels within the grass-allergic group may be particularly indicative of a declining type 1 response as CXCL9 production has been suggested as a surrogate marker for IFN-\(\gamma\)-producing PBMC (34). The extent to which such changes reflect a cause or an effect of ongoing immune processes remains to be determined. However, while much research focuses on the capacity of T\(_\text{h}1\) and T\(_\text{h}2\) cytokines to shape chemokine responses, we, and others, have demonstrated that this communication is two way. Chemokines can act as potent stimuli for initiation (35) or amplification (36) of allergen-specific type 1 versus type 2 immunity.

In conclusion, this is the first report to examine both systemic T\(_\text{h}1\)- and T\(_\text{h}2\)-associated chemokine and chemokine receptor responses in allergic rhinitis. The data indicate that exposure to allergen in the airways translates to distinct systemic patterns of chemokine and chemokine receptor expression depending on atopic status, and that longitudinal analysis provides markedly enhanced sensitivity for characterizing the nature and intensity of these changes. Specifically, longitudinal changes in CXCR3 expression as well as CXCL9 and CCL17 levels suggest a shift to a more type 1-dominated response in non-allergic individuals while eliciting a shift to more intensely type 2-dominated chemokine responses in grass-allergic individuals following natural exposure to grass pollen.

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Abbreviations

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<td>CCL</td>
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<td>CXCL</td>
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<td>IP-10 (CXCL10)</td>
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References


