

Original article

Seasonal dynamics of chemokine receptors and CD62L in subjects with asymptomatic skin sensitization to birch and grass pollen

Background: Asymptomatic skin sensitization (AS) has been shown to be a risk factor for respiratory allergic disease. CCR4, CXCR1 and CD62L have all been assigned a role in the immunopathogenesis of allergy. Memory T-cell expression of CCR4, CXCR1 and CD62L has not hitherto been investigated in subjects with AS.

Methods: We investigated seasonal CD4 memory T-cell expression of the chemokine receptors CCR4, CXCR1 as well as L-selectin (CD62L) in fresh cultures derived from symptomatic atopics (SAs), subjects with AS and healthy controls (HCs). Peripheral blood mononuclear cells from all three groups were isolated during birch and grass pollination as well as in the following winter. CD4 memory T-cell expression of CCR4, CXCR1 and CD62L was determined by flow-cytometry.

Results: During spring and summer, a significantly increased proportion of memory T cells expressed CCR4, CXCR1 and CD62L in SAs when compared with subjects with AS and HCs. Only SAs exhibited seasonal fluctuations in numbers of CCR4, CXCR1 and CD62L positive memory T cells.

Conclusion: Although clearly IgE sensitized, subjects with AS have significant diminished numbers of CCR4, CXCR1 and CD62L positive memory T cells, during pollination, when compared with SAs. In contrast to SAs, cultures derived from subjects with AS did not display seasonal variation. Our findings explain the lack of clinical symptoms, during pollination, in subjects with AS.

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Key words: asymptomatic skin sensitization; CCR4; CD62L; CXCR1; memory CD4+ T cells; seasonal.

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Subjects with asymptomatic skin sensitization (AS) have positive allergen-induced skin tests to one or several allergens, without exhibiting allergic symptoms. It has been demonstrated that AS, in general, has a greatly increased risk for subsequent respiratory allergic disease, certain clinical and paraclinical features are associated with a more favourable prognosis (1, 2). T helper 2 (Th₂) cells arise from the pool of naïve precursor T cells as a result of activation with antigen (allergen) presenting cells (APC). As fully differentiated cells, Th₂ cells express CD45R0, the short isoform of the CD45 phosphotyrosine phosphatase, associated with the memory phenotype (3). CD45R0 may, because of transient expression, be viewed as a marker for recent T-cell activation (4). Chemokines and adhesion molecules direct activated Th₂ lymphocytes to the sites of allergic inflammation (5). The chemokine receptor CCR4 has in several studies been associated with allergen-induced lower airway disease (6, 7). Increased expression of CXCR1, the receptor for the inflammatory cytokine IL-8, has recently been found in T cells from symptomatic atopics (SA) (8). CXCR1 and CCR4 are predominantly expressed on CD4+ CD45R0+ memory T cells (henceforth memory T cells) and are associated with the Th₂/phenotype (8, 9). L-selectin (CD62L) facilitates roll-

ing adhesion of leukocytes along the blood vessel wall and has been associated with atopic diseases, both as a marker for extravasation (10, 11), and in adhesion of memory T cells to allergen-exposed nasal mucosa (12). As Th₂ cells will be found within the pool of memory T cells, we aimed at describing the seasonal expression of the mentioned surface antigens on memory T cells isolated from SA, AS and healthy controls (HC), so as to describe the potential for memory T-cell-mediated local inflammation in all three groups, however, with special emphasis on AS.

Materials and methods

Patients

Patients were included in 2005. All patients were clinically and paraclinically characterized at several visits in our clinic. During the birch pollen season (April until end of May) and grass pollen season 2003 (end of May until end of July) all participants completed daily diary cards, delivered at the second visit (autumn 2003), where symptoms from eyes, nose and lungs were graded (0 = none, 1 = mild, 2 = moderate, 3 = severe symptoms). In agreement with Bodtger et al. (2), the symptom score was calculated as the sum of daily organ (eyes + nose + lungs)-related scores during the relevant season. Symptoms were considered as pollen allergy when

lasting ≥ 7 days or when symptoms were repeatedly elicited when pollen counts exceeded a certain (individual) level (2). The skin prick test (SPT) was performed according to the EAACI guidelines (13). Based on SPT, patients were divided into three groups: the first group numbered 10 HC (three males), without any clinical history of hay fever and asthma, and with negative SPT against 10 standard aero-allergens (Soluprick, ALK Abello, Hørsholm, Copenhagen, Denmark) comprising: birch, grass, mugwort, horse, dog, cat, two house dust mite species and two mould species. The group of AS consisted of 11 mono-sensitized (SPT ≥ 3 mm to birch or grass) subjects (two males) with no former histories of hay fever, asthma, atopic dermatitis or Oral Allergy Syndrome (OAS) (14). The third group, numbering 11 mono- or poly-sensitized SA (six males), all suffered from rhinoconjunctivitis during the birch and/or grass pollen season. None of the patients had used corticosteroids within the last 3 months. None of the patients had received allergen immunotherapy or treatment with montelukast. All persons gave informed consent. The study was approved by the local Ethics Committee.

Specific IgE determination, histamine release and late phase reaction

Allergy class ≥ 2 was considered as positive (CAP System, Pharmacia Diagnostics, Uppsala, Sweden). Histamine release (HR) was performed according to Ostergaard et al. (15). The late phase reaction (LPR) was performed by injecting 0.05 ml of 1000 SQ birch or grass pollen extract (Aquagen, ALK Abello) intradermally (16). The size of a positive LPR was outlined after 24 h, by resistance to the movement of a sharpened pencil, and the mean wheal diameter was calculated.

Challenge tests

Clinical susceptibility to birch and/or grass was assessed using titrated challenges of the conjunctivae with birch or grass pollen extracts (Aquagen) in 10-fold dilution from 100 to 100 000 SQ or until a positive test (17). A positive test was defined as red and itchy eyes within 10 min after allergen challenge (17). Results were expressed as the highest dose not eliciting symptoms.

Reagents and Abs

Tetanus toxoid (TT) (State Serum Institute, Copenhagen, Denmark) was used at a final concentration of 10 $\mu\text{g/ml}$. Phorbol 12-myristate 13-acetate (PMA) and ionomycin (both from Sigma Chemical Corp., St Louis, MO, USA) were used at final concentrations of 10 ng/ml and 0.5 $\mu\text{g/ml}$ respectively. So as to simulate natural allergen exposure, grass allergen (*Phleum pratense*) and birch allergen extracts (*Betula verrucosa*) (a kind gift from ALK Abello) were used at a final concentration of 15 $\mu\text{g/ml}$ (total protein). The lipopolysaccharide (LPS) content in both allergen extracts was < 7 EU/mg of protein (according to ALK Abello).

Flow cytometry

Peripheral blood mononuclear cells (PBMCs) were isolated from birch atopics during the birch pollen peak season 2004 and from grass atopics during the grass pollen season 2004 as well as from HC (during the birch season). Apart from one single drop out (subject with AS), blood samples were drawn from the same patients during winter (January and February 2005). Blood cells were separated by density centrifugation on Lymphoprep (Nyegaard, Oslo, Norway).

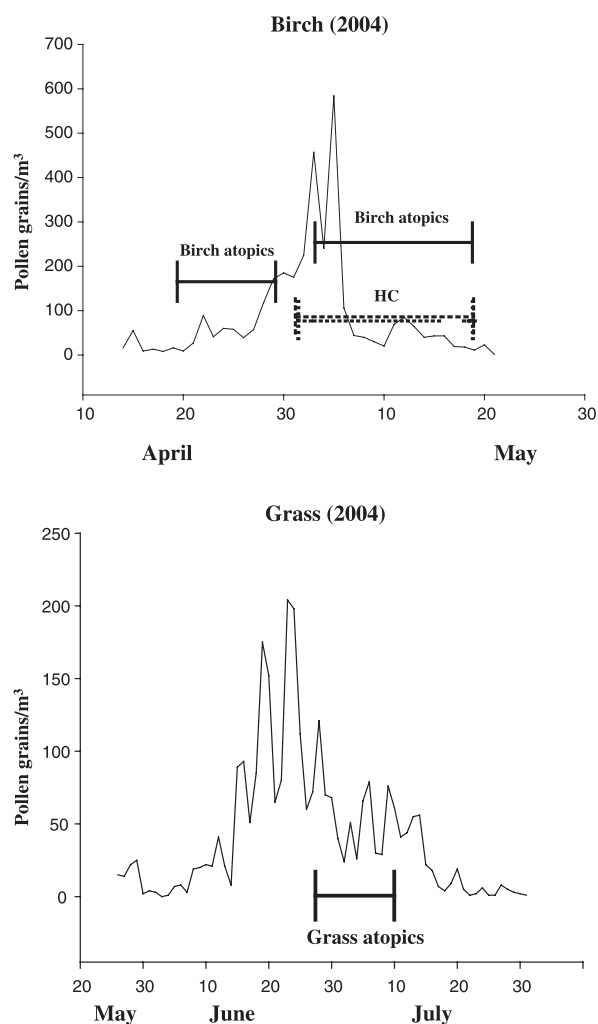


Figure 1. Blood samples were drawn from birch atopics (symptomatic atopic, SA and asymptomatic skin sensitization, AS) as well as healthy controls in the period of birch pollination where pollen counts, apart from 3 days, exceeded 10 grains/m³ (median: 131 grains/m³; range: 1–958 grains/m³). In the period of blood sampling from grass atopics (SA and AS) the corresponding grass pollen counts were (20 grains/m³; 15–78 grains/m³). All SA to birch and grass were symptomatic when blood samples were drawn.

The cells were washed, counted and labelled with primary antibodies: CCR4-PE and CXCR1-PE and CD 62 L-PE were obtained from (Becton-Dickinson, Rutherford, NJ, USA). CD4-RPE-Cy5 and CD45R0-FITC were purchased from (Dako-Cytomation, Copenhagen, Denmark). Antibodies were used at recommended concentrations. Three-colour flow cytometry was performed on day 0 (in duplicate). Isotype control cut-off values were set to $> 98\%$. A BD FACS scan flow cytometer (Becton-Dickinson, Rutherford, NJ, USA) was used, and 10 000 mononuclear cells per sample were acquired. Forward- and side-scatter characteristics identified the lymphocyte population. The percentage of CCR4, CXCR1 and CD62L positive memory T cells were estimated for all three groups of patients. In agreement with previous studies (3, 18) we defined memory T cells as CD4+ and CD45R0^{high} (Fig. 2).

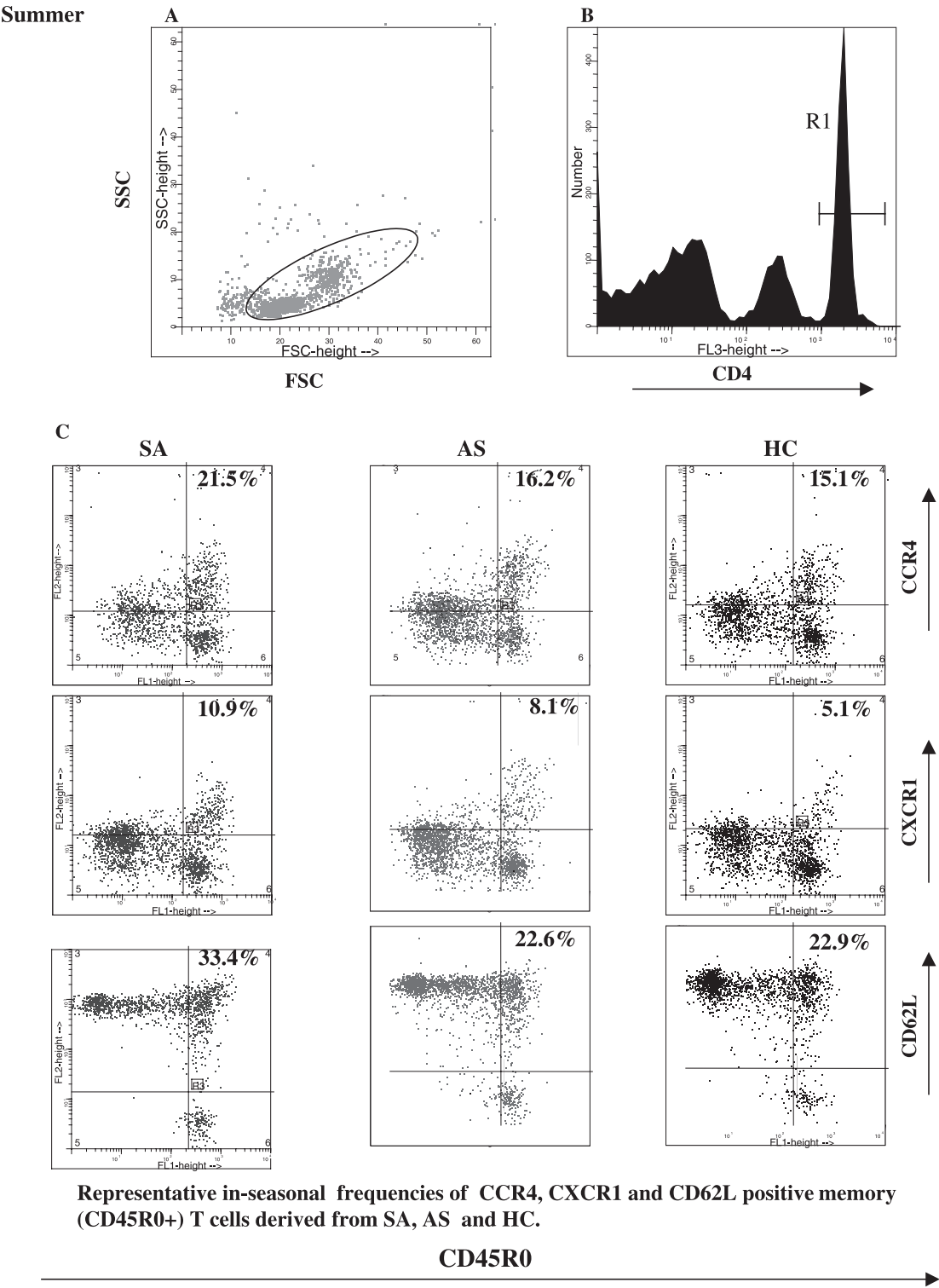


Figure 2. Memory T-cell expression of CCR4, CXCR1 and CD62L. (A) Mononuclear cells were identified by forward scatter/side scatter (FCS/SSC) characteristics. (B) Histogram showing the CD4 distribution among mononuclear cells, gate R1 representing lymphocytes. (C) In the period of pollination (summer) and off-season (winter), representative expressions of CD45R0^{high}, CCR4, CXCR1 and CD62L by CD4⁺ lymphocytes (gate R1) isolated from SA, AS, and HC are shown.

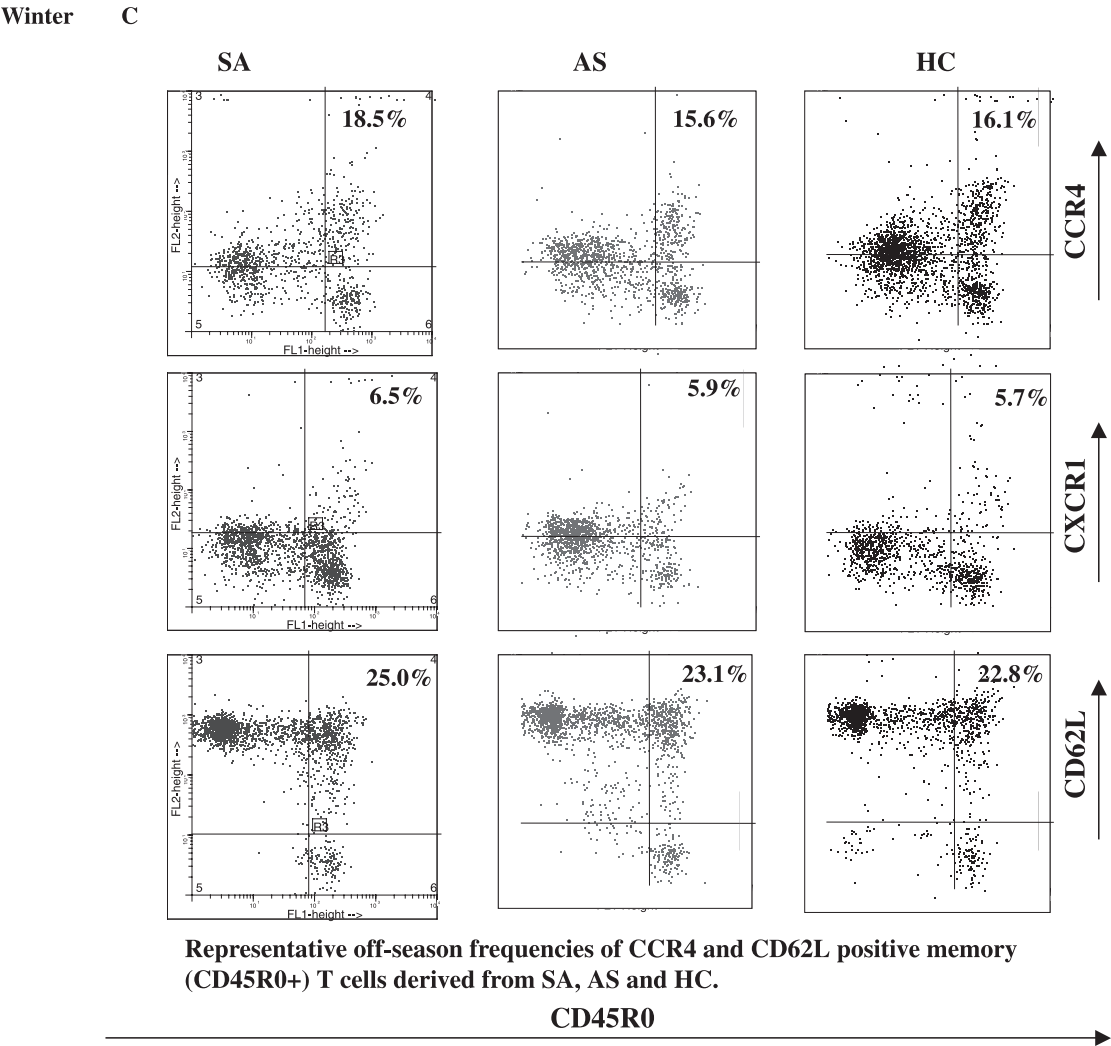


Figure 2. Continued.

Cell culture and carboxyfluorescein succinimidyl ester staining

In brief, 5×10^7 cells/ml were resuspended in phosphate buffered saline (PBS) and incubated at 37°C for 10 min with carboxyfluorescein succinimidyl ester (CFSE; Molecular Probes Inc, Eugene, OR, USA) at a final concentration of 2 μ M, followed by RPMI washing steps. PBMCs (3×10^6) were incubated in six well plates with antigen in 6 ml of low toxin-containing RPMI-1640 with 25 mM HEPES (Biological Industries, Kibbutz, Beit Haemek, Israel) and supplemented with 2 mM L-glutamine, and 100 U/ml penicillin/streptomycin and 50 μ M β -mercaptoethanol (all reagents from Gibco, Paisley, UK) in 10% heat inactivated autologous serum. The allergen to which a SA subject had symptoms was used for stimulation. Furthermore, the allergen to which a subject with AS was sensitized was used for stimulation. Cultures from nonatopic donors were stimulated with both birch and grass extracts (concentrations unaltered). At day 6, PMA and ionomycin were added to some wells. Supernatants from these wells were collected at day 7 for cytokine measurements. Cells from the remaining wells were harvested for further flow cytometric analysis. As a control for LPS-induced activation of monocytes, PBMCs from healthy donors were incubated with both allergen extracts for 24 h.

Cytokine measurements

Interferon- γ , TNF- α , IL-2, IL-4, IL-5 and IL-10 were measured using the Cytometric Bead Array (CBA) system (Becton-Dickinson, Rutherford, NJ, USA). BD CBA software version 1.1 (Becton-Dickinson, Rutherford, NJ, USA) was used for analysing cytokine responses to antigen. For facilitated detection of Th₂ cytokines, we used a protocol developed by Kurtzhals et al. (19). Changes in cytokine production were calculated by subtracting the cytokine values from 'nonstimulated' cells (no antigen at day 0, but PMA and ionomycin added at day 6) from the values obtained from antigen-stimulated cells (allergen or TT at day 0, plus PMA and ionomycin added at day 6). Incubation (24 h) of both allergens with PBMCs derived from healthy donors resulted in undetectable levels of TNF- α (data not shown).

Statistics

Groups of data were compared by Wilcoxon's matched paired test or Mann-Whitney *U*-test using SPSS version 11.0 (SPSS Inc., Chicago, IL, USA). Two-tailed *P* < 0.05 were considered significant.

Results

Characteristics of SA and AS and HC

Clinical and paraclinical characteristics of SA (median: 25.1 years; range: 22.2–42.2 years), AS (24.5 years; 20.5–29.9 years) and HC (23.2 years; 21.5–42.1 years) are depicted in Table 1. In contrast to SA, the majority of AS had no detectable allergen-induced LPRs (Table 1). The blood sampling time points for SA, AS and HC, during birch and grass pollination, are shown in Fig. 1.

Expression of the chemokine receptors CCR4, CXCR1 and expression of L-selectin (CD62L) on CD4+CD45RO+ memory T cells

The expression of CCR4, CXCR1 and CD62L by memory T cells isolated from SA, AS and HC during pollination and off-season is shown in Fig. 2A,B). In the period of birch and grass pollination, the percentage of peripheral CCR4+ memory T cells was significantly higher in SA (median: 22.3%; range: 18.3–31.1%) when compared with AS (16.7%; 5.8–30.1%) and HC (16.6%; 7.5–22.5%) (Fig. 3). In spring and summer, the median value of CXCR1+ memory T cells was significantly higher in PBMCs isolated from SA (11.5%; 2.1–26.2%) than in those isolated from AS (7.9%; 3.4–24.9%) and

HC (5.5%; 2.0–22.5%) (Fig. 3). During the birch and grass season, the proportion of CD62L+ memory T cells was significantly increased in SA (33.2%; 23.1–43.1%) when compared with AS (22.5; 12.4–37.6%) and HC (23.3%; 14.1%–36.5%). During winter, all three groups were statistically nondistinguishable with regard to the percentage of CCR4+, CXCR1+ and CD62L+ memory T cells.

In winter, SA displayed significantly lower percentages of CCR4+ memory T cells (18.1%; 8.3–30.3%) and CD62L+ memory T cells (24.7%; 7.7–39.7%) than during pollination ($P < 0.01$ and 0.001 respectively) (Fig. 3). The relative numbers of CXCR1+ memory T cells, isolated from SA, tended to be lower during winter (6.2%; 1.5–31.6%) when compared with the period of pollination ($P = 0.09$). Neither memory T cells derived from AS nor from HC, exhibited significant seasonal differences in CCR4, CXCR1 and CD62L expression (percentage positive cells) (Fig. 3).

In vitro expansion of CCR4+, CXCR1+ and CD62L+ memory T cells

In CD4 T-cell cultures derived from SA, AS and HC, significant differences in CCR4, CXCR1 and CD62L receptor levels expressed as percentage of positive cells or

Table 1. Clinical and paraclinical characteristics of birch-/grass-pollen-sensitized subjects

	Skin prick test			Specific IgE		Histamine release		CPT	Symptoms	Late phase reaction
	Inclusion (mm)	Visit 1 (mm)	Visit 2 (mm)	Visit 1 (class)	Visit 2 (class)	Visit 1 (class)	Visit 2 (class)	Visit 1 (SQ – U)	Season (Score)	Visit 1 (mm)
Symptomatic atopics										
1 M/B	4.5	7.0	9.0	4	4	3	3	1000	70	62.0
2 F/B	8.0	8.5	5.5	3	3	3	3	1000	7	43.5
3 M/B	7.5	11.0	6.0	3	3	3	3	1000	28	56.0
4 M/B	3.5	5.5	5.5	2	3	2	3	1000	68	44.5
5 M/B	11.5	9.0	8.0	4	4	3	2	1000	48	0.0
6 F/G	16.0	14.5	13.5	3	3	3	3	1000	140	135.0
7 M/G	8.5	19.0	10.5	3	3	3	3	100	176	82.0
8 F/G	10.0	20.0	14.0	4	3	3	3	100	59	100.0
9 M/G	14.5	11.5	12.5	4	4	3	3	100	182	93.0
10 F/G	8.0	5.0	12.0	2	2	0	3	1000	44	43.5
11 F/B	7.0	11.0	7.5	3	3	3	3	1000	40	56.0
Asymptomatic sensitized										
1M/B	6.5	4.5	7.5	0	1	2	3	10 000	0	0.0
2 F/B	3.5	5.0	3.5	1	1	3	2	100 000	0	0.0
3 F/B	3.5	3.5	6.0	0	0	2	3	10 000	0	0.0
4 F/B	3.5	3.5	4.0	0	0	2	1	100 000	0	0.0
5 F/B	4.0	4.0	6.0	0	0	3	2	100 000	0	49.5
6 F/B	4.5	3.5	Neg.	0	0	2	2	10 000	0	0.0
7 F/G	5.0	4.5	6.5	0	0	0	0	100 000	0	0.0
8 F/G	6.0	Neg.	6.0	2	2	0	1	100 000	0	0.0
9 F/G	3.5	5.5	4.5	0	0	0	0	100 000	0	0.0
10 F/G	5.5	4.0	4.0	0	0	0	0	100 000	0	0.0
11 M/G	4.0	3.5	3.5	1	2	3	2	10 000	0	23.5

CPT, conjunctival provocation test F, female; M, male; B, sensitized (SA: also symptoms) to birch and challenged with birch; G, sensitized (SA: also symptoms) to grass and challenged with grass. The symptom score was calculated as the sum of daily organ (eyes + nose + lungs)-related scores during the relevant season. Symptoms were considered as pollen allergy when lasting ≥ 7 days or when symptoms were repeatedly elicited when pollen counts exceeded a certain (individual) level.

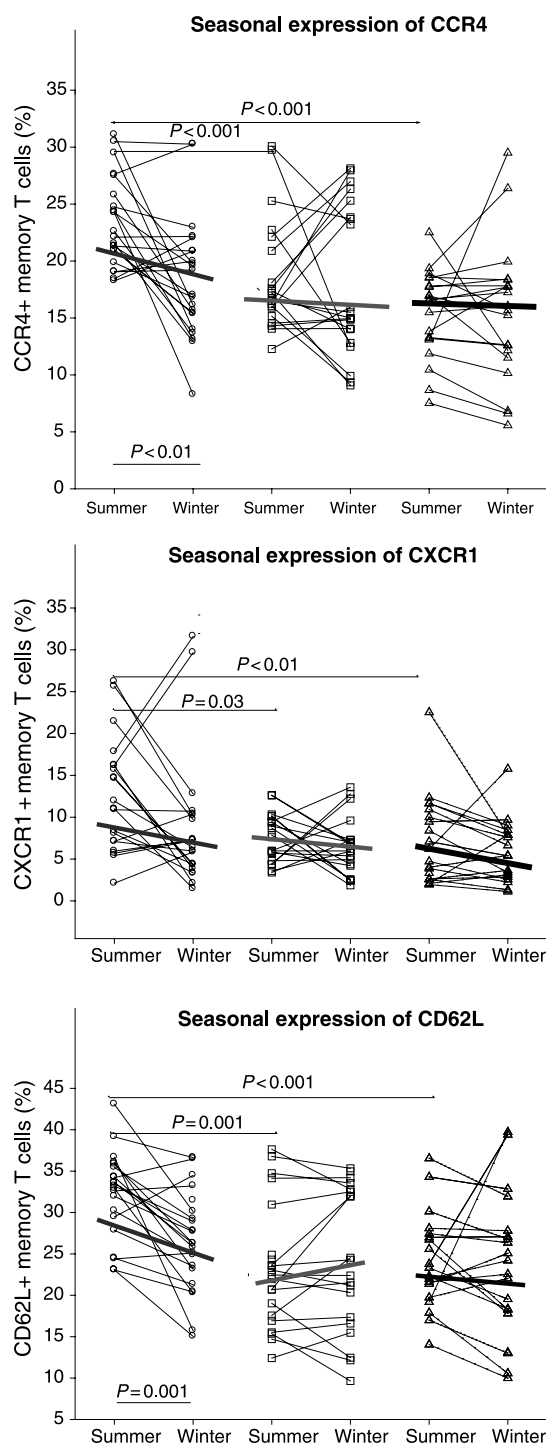


Figure 3. Seasonal memory T-cell expression of CCR4, CXCR1 and CD62L. During the period of pollen exposure and during winter, peripheral blood mononuclear cells from the same SA, AS and HC were isolated and immediately analysed. Statistical significant intragroup (spring/summer vs winter) and intergroup (summer vs summer/winter vs winter) changes in memory T-cell expression of CCR4, CXCR1 and CD62L for all three groups are shown.

as mean fluorescence intensity (MFI) were not observed between day 0 and antigen-free cultures at day 7. Among SA, the examination of allergen-driven proliferating cells revealed significant expansions of CCR4 (44.5%; 23.7–69.8%), CXCR1 (16.8%; 6.5–17.4%) and CD62L (70.2%; 56.3–88.1%) positive memory (CD45R0+) T cells, when compared with nonproliferating cells (13.3%; 5.4–15.7%, $P = 0.02$), (4.1%; 3.1–5.2%, $P = 0.03$), (28.1%; 19.8–40.2%, $P = 0.01$) respectively (Fig. 4). The numbers of proliferating CCR4, CXCR1 and CD62L positive memory T cells in allergen-stimulated cultures isolated from AS and HC were few (<1%), to allow for a consistent comparison with nonstimulated cultures. Statistical differences in memory T-cell proliferation in response to TT, between the three groups, were not observed.

Cytokine responses to allergen and TT

Allergen-induced levels of Th₁ cytokines (IFN- γ , TNF- α) and Th₂ cytokines (IL-4, IL-5, IL-10) differed significantly between SA and the two other groups (Fig. 5). Concerning allergen-induced production of Th₁ and Th₂ cytokines, AS and HC were statistically nondistinguishable (Fig. 5). Significant differences in TT-induced Th₁ and Th₂ cytokine levels, between these three groups, were not observed (data not shown).

Discussion

To our knowledge, this is the first description of seasonal memory T-cell expression of CCR4, CXCR1 and CD62L in clinically and paraclinically well-characterized AS. Activated memory Th₂ cells have the potential to migrate, via the blood, into tissues in which an allergic inflammatory response is developing, exerting their influence through a cascade of different mediators and cytokines (18). This migration is among other factors governed by a complex interplay between various chemokines and their receptors. According to the literature, we chose a panel of obvious receptor candidates for the study of memory T-cell chemotaxis in AS. One of the receptors for IL-8, CXCR1, has recently been shown to be differentially expressed on Th₂ skewed memory T lymphocytes from SA (8). Moreover, the CXCR1 positive memory T cells from atopic donors responded functionally to CXCL8 with increased actin polymerization when compared with normal donors (8). IL-8 (CXCL 8) plays a vital role in delayed hypersensitivity and has been associated with the development of allergic rhinitis (20). Thus, the increased frequency of CD4+ CXCR1+ memory T cells observed in SA during pollination, very likely reflects the ongoing mucosal inflammation in these patients. Interestingly, this pattern was not observed among AS. The lack of symptoms in these patients and the fewer positive conjunctival provocation tests (values <100 000 SQ)

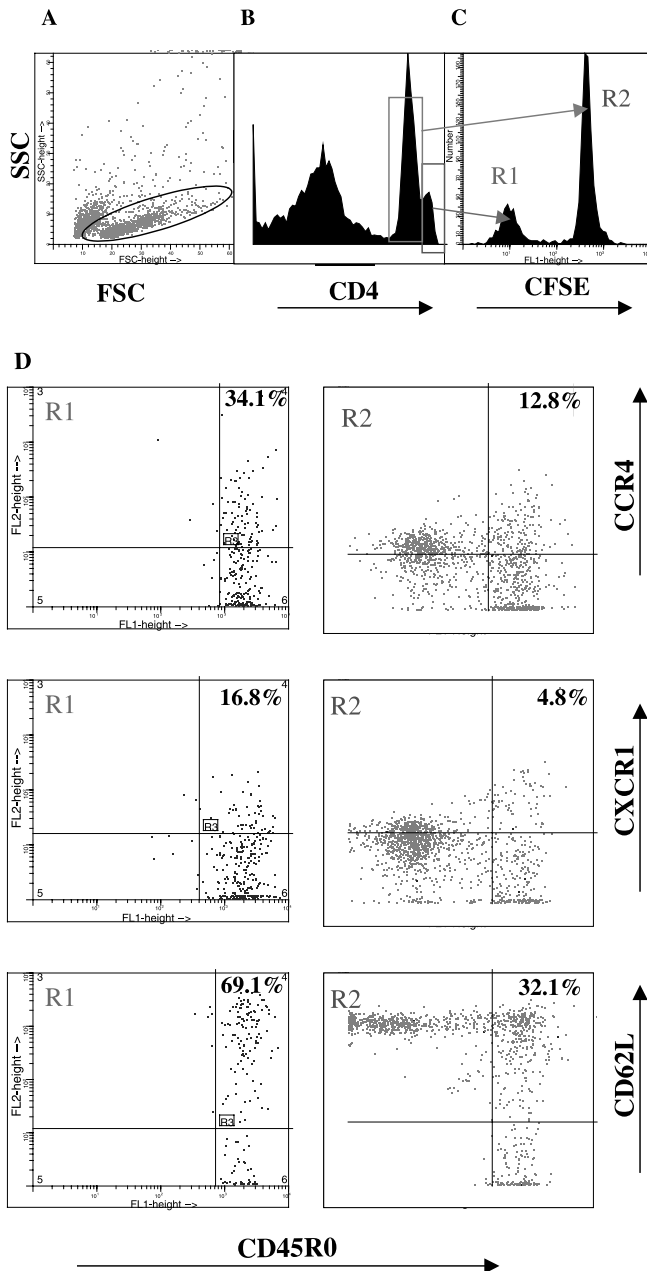


Figure 4. Expression of CCR4, CXCR1 and CD62L among proliferating CD4⁺ CD45R0⁺ T cells vs nonproliferating CD4⁺ CD45R0⁺ T cells. Carboxyfluorescein succinimidyl ester (CFSE)-loaded peripheral blood mononuclear cells from a representative symptomatic atopic were stimulated with allergen for 7 days. (A) The lymphocyte population (including proliferating cells) was identified by FCS/SSC characteristics. (B) The CD4⁺ histogram showing two peaks – the red gate corresponding to R1, the blue gate corresponding to R2 in histogram C. (C) Histogram showing the CFSE content of the gated CD4⁺ lymphocyte population. R1 and R2 represent divided and nondivided cells respectively. (D) The red and blue gates (histogram B) identified the memory T-cell (CD45R0⁺) expression of CCR4, CXCR1 and CD62L by the divided cell population (corresponding to R1), respectively, the nondivided cell population (corresponding to R2).

could be related to reduced levels of IL-8. As increased expression of CXCR1 is not dependent upon T-cell activation (21), local immune regulating mechanisms suppressing allergen-induced inflammation in the eyes, and respiratory organs (22) could very well be the principal mechanism for the abolished upregulation of CXCR1 seen in AS. Although not an exclusive Th₂ marker, the majority of Th₂ cells (90%) express CCR4 (9). Migration of CCR4⁺ T cells into allergen-exposed mucosa has been associated with severity of symptoms in SA (6, 7). Consistent with these studies, we found significantly elevated levels of CCR4⁺ memory T cells in SA compared with AS and HC in the period of birch and grass pollination. As CCR4 expression seems to be coupled with activation (23), the sparse allergen-induced memory T-cell proliferation, which we observed in cultures derived from AS (manuscript submitted), could explain the lack of increased expression of CCR4 in this group. Thus, among AS, at least two (probably more) different mechanisms may account for the impeded influx of harmful allergen-specific memory T (h₂) cells into potential shock organs. Until recently, CD62L expression was associated with naïve T cells, being a requirement for their recirculation between blood and lymph nodes (immune surveillance) (24). However, new evidence suggests, that a major subset of memory T cells express CD62L, perhaps also fulfilling a role in immune surveillance (25). These CD62L positive memory T cells proliferate well in response to recall antigen, differentiating into CD62L⁻ memory effector cells (26). Adding to the complexity, Symon et al. demonstrated CD62L-mediated memory T-cell adhesion to allergen-exposed nasal epithelium (12). In contrast to the group of SA, increased memory T-cell expression of CD62L among AS, during the period of birch and grass pollination, was not observed. Assuming functional diversity of the CD62L⁺ memory T-cell pool, memory T cells derived from AS not only had a reduced potential for homing to the respiratory mucosa, but also exhibited a reduced potential for allergen-induced activation in secondary lymphoid tissue, when compared with SA.

Substantiating the association between allergic symptoms and the increase in the relative numbers of CCR4, CXCR1 and CD62L positive memory T cells, only freshly isolated memory T cells from SA, exhibited highly significant seasonal variations with respect to expression of CCR4 and CD62L, while the relative numbers of CXCR1⁺ memory T cells tended to decrease during winter (Fig. 2). Furthermore, in agreement with McNeill et al. (4) the relative increase in CCR4, CXCR1 and CD62L expression among CD45R0 positive T cells, observed in SA during pollination, was consistent with recent T-cell activation.

Contrary to the immunological characteristics observed among SA, the lack of a Th₂ skewed cytokine secretion pattern (Fig. 5) and the absence of proliferation of CCR4, CXCR1 and CD62L positive memory cells in

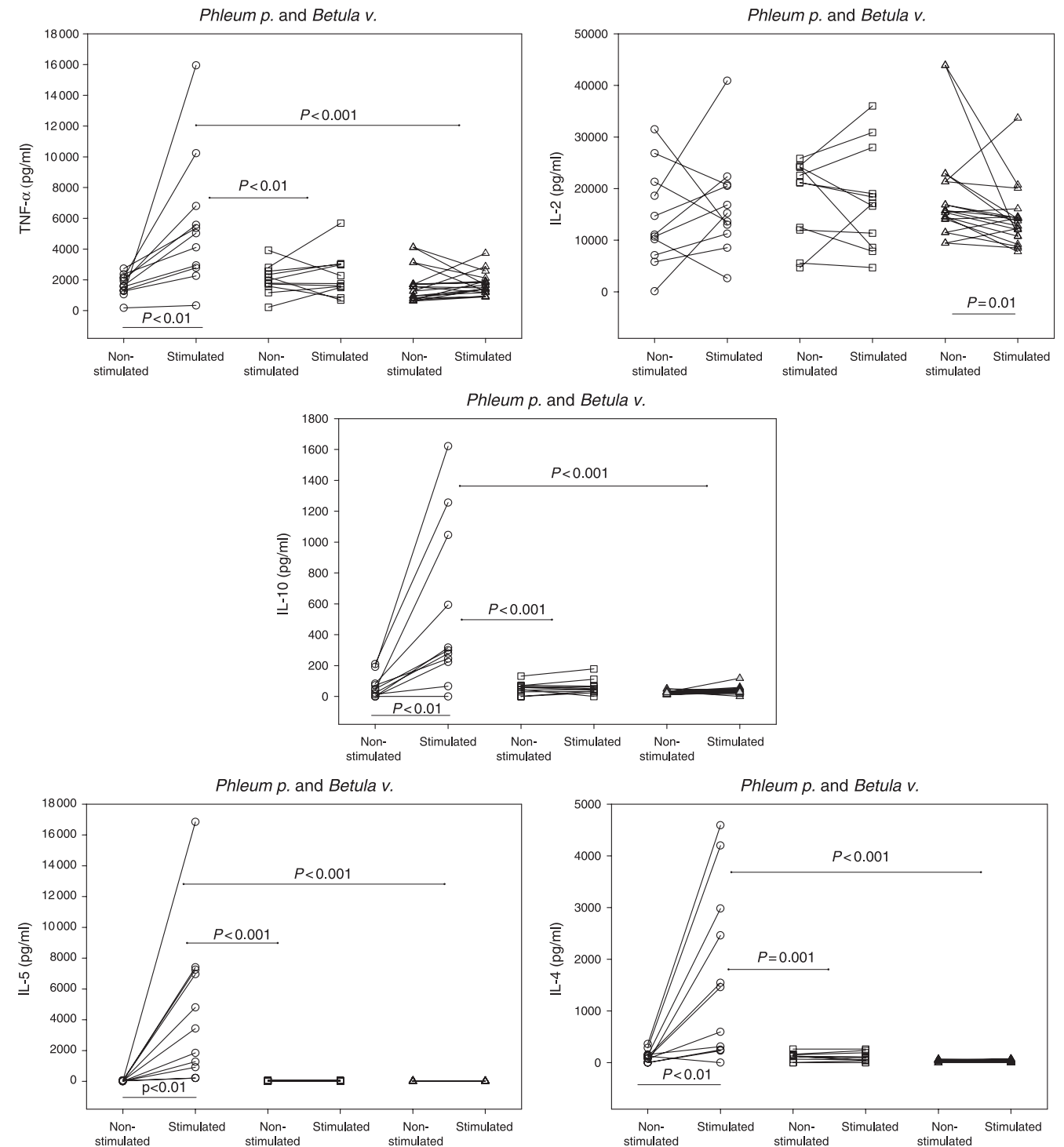


Figure 5. Allergen-driven cytokine production in peripheral blood mononuclear cell cultures. Cultures were stimulated with allergen from day 0 or left nonstimulated. At day 6, Phorbol 12-myristate 13-acetate and ionomycin were added, so as to amplify the cytokine response. At day 7, supernatants were harvested and analysed. Changes in the levels of Th₁ and Th₂ cytokines after 7 days are shown. Wilcoxon's paired test was used to test differences in proliferation between medium and allergen conditions. Mann-Whitney *U*-test was used to test differences between SA, AS and HC.

allergen-stimulated cultures derived from AS and HC, substantiates the observed patterns of seasonal memory T-cell expression of CCR4, CXCR1 and CD62L observed in these two latter groups.

Whether the inertness to allergen in cultures derived from AS and HC is due to CD4+CD25+ regulatory T cells is not clear; however, the lack of an allergen-induced IL-10 response in cultures derived from AS, may entail that other mechanisms (diminished numbers of allergen-specific memory T cell) are responsible for this indolent allergen-specific immunoresponse.

The total of our findings suggest that memory T cells positive for at least one of these three surface antigens, are not being recruited to the peripheral mucosa in AS during pollination. As the IL-5 produced by Th₂ cells promotes survival of eosinophils at sites of allergic inflammation (27), the abrogated migrational pattern of memory T(h₂) cells, in these otherwise clearly atopic patients, explains the general absence of allergen-induced LPRs. As our patients with AS were predominantly serum negative for IgE (Table 1), the observed positive SPTs as well as conjunctival provocation tests were presumably either a result of local IgE production in the mucosa/skin (28) or because of increased entrapment of small amounts of IgE in the skin (29). Because of the

impaired influx of Th₂ cells during pollination, local immune regulatory mechanisms (22) may be able to sustain suppression of allergen-induced mucosal inflammation, thereby preserving mucosal integrity and preventing symptoms.

In conclusion, AS did not exhibit seasonal memory T-cell expression of the chemokine receptors CXCR1 and CCR4 as well as CD62L, consistent with impeded peripheral chemotaxis in these patients. At least two different, possibly supplementing, mechanisms could account for this: first, the suppression of mucosal inflammation by local immune regulators; second, a reduced numbers of allergen-specific T cells. Our findings contribute not only to an increased understanding of why subjects with AS are asymptomatic, but may also be helpful in framing questions with regard to how the immune system, within the context of allergic diseases, can be manipulated for therapeutic ends.

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