Table 1: Patient characterization.

			Birch				Grass			
Patients	**Age** (range)	**Sex** Male/Female	Symptoms	Spt	IgE class Median(range)	HR class Median(range)	Symptoms	Spt	IgE class Median(range)	HR class Median(range)
Healthy	25 (22–43)	3/7	0/10	0/10	0 (0)	0 (0)	0/10	0/10	0 (0)	0 (0)
AS Birch	25 (24–27)	1/4	0/5	5/5	0 (0–2)	2 (0–3)	0/5	0/5	0 (0)	0 (0)
AS Grass	25 (22–31)	1/4	0/5	0/5	0 (0)	0 (0)	0/5	5/5	0 (0–2)	0 (0–3)
Allergic Birch	27 (25–43)	4/1	5/5	5/5	3 (2–4)	3 (2–3)	2/5	2/5	0 (0–4)	2 (0–3)
Allergic Grass	26 (24–41)	3/0	1/3	1/3	0 (0–3)	0 (0–3)	3/3	3/3	4 (2–4)	3 (0–3)

AS: asymptomatically sensitized. Spt: skin prick test. HR: histamine release
Skin prick tests (performed in duplicate) were considered positive when mean wheal diameter >3 mm. Allergic symptoms were reported in diary cards during the relevant pollen 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 was performed according to the guidelines of European Academy of Allergy and Clinical Immunology [18]. n = 10 for the healthy controls, n = 10 for the asymptomatically sensitized individuals and n = 8 for the allergic individuals.

The chemokines and their receptors play a pivotal role in leukocyte migration and chemotaxis. It is still controversial whether these receptors can function as phenotypic markers on certain cell subsets. CCR3 and CCR8 have been suggested as Th2 markers whereas CXCR3 is mentioned in the literature as a Th1 marker [4-6]. The CCR3 ligand CCL11/eotaxin is upregulated in nasal mucosa of allergic rhinitis patients during the pollen season [7]. CCL1/I-309, which is the only CCR8 ligand, is upregulated in patients with atopic dermatitis [8] and IL-12 inhibit its production [9]. Also, CCL1/I-309 is released by mast cells in response to IgE cross-linking [10] indicating a role in allergic inflammation. On the contrary, the IFN-γ-inducible CXCR3 ligands and some CCR5 ligands are increased in autoimmune diseases [11-14]. However, other findings show that no correlation exists between Th1/Th2 cytokine profile and chemokine receptor expression on a single cell level [15] and also suggest that the chemokine receptor profile can be changed without a concomitant change in cytokine profile [16] questioning the use of chemokine receptors as markers for T cell subsets.

As the chemokine receptor profile determines the migratory patterns of leukocytes, we wanted to compare this profile with respect to CCR3, CCR5, CCR8 and CXCR3 in memory Th cells from allergic, asymptomatically sensitized and healthy individuals to obtain knowledge about their migratory potential and any differences in expression patterns that might exist between these three groups.

Methods

Patients

10 healthy, 5 asymptomatically birch pollen sensitized, 5 asymptomatically grass pollen sensitized, 5 birch pollen allergic and 3 grass pollen allergic volunteers with seasonal hay fever symptoms were examined during the birch or grass pollen season respectively. Skin prick test (Soluprick, ALK-Abello, Hørsholm, Copenhagen), histamine release [17] and specific IgE against birch and grass pollen using the CAP-system (Pharmacia, Uppsala, Sweden) were determined for all volunteers (Table 1). The skin prick test was performed according to the guidelines of European Academy of Allergy and Clinical Immunology [18]. Three of the allergic patients had allergic asthma. The allergic subjects received no corticosteroid treatment for three months prior to the study. The asymptomatically sensitized and healthy control subjects took no hay fever medicine. All subjects came from the area of greater Copenhagen (Storkøbenhavn). The study was approved by the local Ethical Committee and the clinical features of the patients are described in detail elsewhere [19].

Cell stimulation

Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood by gradient centrifugation on Lymphoprep (Nycomed, Roskilde, Denmark). The PBMCs were cultured (3×10^6) in 6 well plates with antigen in 6 ml of low endotoxin RPMI1640 medium containing 10% heat-inactivated autologous serum, 25 mM HEPES, 2 mM L-glutamine, 50 μM β-mercaptoethanol, 100 U/ml streptomycin/penicillin for 7 days in the presence of either 15 μg/ml birch or grass allergen (ALK-Abello, Hørsholm, Copenhagen), 10 μg/ml Tetanus toxoid (TTx) (Statens Serum Institut, Copenhagen, Denmark) or no antigen as a control. On day 7, the cells were harvested and used for flow cytometric analysis. The lipopolysaccharide level in both allergen extracts was < 7 EU/mg and after 24 hours of stimulation of PBMCs from