Table 2: Chemokine receptor expression in memory T helper cells induced by 7 days of antigenic stimuli. Median chemokine receptor expression in memory Th cells in percentage and MFI after 7 days of stimulation with antigen (allergen (15 μg/ml) or TTx (10 μg/ml)) or no antigen as a control. n = 10 for the healthy controls, n = 10 for the asymptomatically sensitized individuals and n = 8 for the allergic individuals except for CCR8 where n = 6 for the asymptomatically sensitized and allergic individuals. 10.000 PBMCs were acquired for the analysis. Isotype control cut-off values were set to >98%. Samples were run in monocates. For experimental design and analysis see Methods.

		% median (range)			MFI median (range)		
		No Ag	**Allergen**	**TTx**	***No Ag***	**Allergen**	**TTx**
CCR3	Allergic	*10.6 (4.7–32)*	17.3 (9.8–29.5)	15.6 (4.4–52.2)	*21 (16.2–32.3)*	19.4 (16.9–37.2)	**26.1* (16.6–40.5)**
	AS	*10.3 (3.3–19.7)*	8.7 (2.8–23.2)	11.9 (5–28.8)	*20.5 (14.7–27.2)*	18.6 (16.4–32.3)	**23.4 * (18.4–37.4)**
	Healthy	*8.7 (1.5–45)*	11.2 (3.2–36.8)	23.4 (4.5–44.3)	*19.7 (12.6–45.4)*	17.8 (12.5–32.2)	23.7 (12.5–38.4)
CCR5	Allergic	*21.3 (7.8–31.5)*	25 (4.1–53.9)	28.3 (12.3–61.1)	*21.4 (16.8–27.9)*	**25.9 * (22.4–48.5)**	26.1 (16.1–42.5)
	AS	*15.2 (2.6–36.6)*	17 (3–33.5)	**16.9 * (5.2–65.9)**	*18.3 (15.2–24.5)*	17.5 (15.4–20.5)	**22.5 * (18.2–58.2)**
	Healthy	*11.7 (5.6–34.5)*	**13.5 * (6.7–29.7)**	**20.2 * (10.1–74.1)**	*16.7 (12.7–22)*	17.3 (13.5–25.6)	**25.1 * (13.4–72)**
CCR8	Allergic	*10.4 (1.6–20.4)*	12.8 (3.3–20)	12.7 (2.2–27.4)	*24.3 (16.8–37.6)*	27.9 (18.8–40.3)	28.2 (17.8–82.6)
	AS	*3.7 (1.8–16.6)*	3.2 (1.7–14.7)	6.3 (1.4–34.9)	*21.9 (17–26.1)*	22.8 (17.2–44.6)	24.9 (16.2–36.6)
	Healthy	*5.7 (0.7–16.4)*	3.9 (1.5–14)	**9.3 * (3.8–35.7)**	*19 (12.6–49.6)*	19.2 (11.7–46.6)	23.9 (12.4–48.2)
CXCR3	Allergic	*42.9 (22–47.5)*	42.1 (19.2–67.4)	**49.2 * (27–74.2)**	*57.4 (33.1–65.3)*	54.4 (48.4–72.4)	60.6 (36.9–81.8)
	AS	*28.1 (18–56.6)*	27.8 (21–56.8)	**29 * (20.8–77.4)**	*39.7 (29.3–59.7)*	42.3 (30.6–54.5)	46.5 (33.5–115)
	Healthy	*28.8 (19.8–46.5)*	31.4 (17.3–46.9)	**35.9 * (25.8–82.9)**	*37.8 (32–65.2)*	39.3 (30.1–63.9)	**57.6 * (32–162.4)**

Bold text and * indicates significant differences between the antigen (allergen or TTx) stimulated samples and control samples where no antigen was added.
Ag: antigen AS: asymptomatically sensitized individuals.

healthy control group whereas no changes in CCR5 was observed in the allergic individuals. Also, an increase in the percentage of CCR8+ memory Th cells was observed in the healthy control group, but no significant changes in MFI were observed for this receptor. An increase in the percentage of CXCR3+ memory Th cells was observed in all three groups after TTx stimulation, however increases in MFI were only observed in the healthy control group.

After stimulation with allergen, increases in the percentage of CCR5+ memory Th cells were observed in healthy controls and in MFI in allergic individuals. Allergen stimulation did not induce any changes in CCR3, CCR8 and CXCR3 expression. When pooling all 28 patients in the statistical analysis, TTx was able to induce expression of all receptors both seen as a significant increase in the percentage of chemokine receptor positive cells and as MFI. Allergen stimulation only induced a significant increase in the percentage of CCR5+ memory Th cells.

Group differences

To compare the chemokine receptor expression between the three groups, the Day $7_{\text{no antigen}}$ receptor level was subtracted from either the Day 7_{allergen} or the Day 7_{TTx} sample to obtain the change in receptor expression (ΔChemokine receptor).

No differences in ΔChemokine receptor for the percentage of chemokine receptor positive cells were observed between the three groups after stimulation with TTx or allergen. When comparing the ΔChemokine receptor for the MFI, the change in the CCR5 after allergen stimulation was significantly different between the three groups (P = 0.02).

Discussion

Other studies have linked certain diseases with aberrant expression of one or more chemokine receptors [12,20,21]. However, very few studies have been conducted with regard to the phenotype of asymptomatically sensitized individuals and, to our knowledge none on chemokine receptor profiles.

In this study, no differences were found in receptor expression patterns immediate *ex vivo* for CCR3, CCR5, CCR8 and CXCR3 in memory Th cells from allergic, asymptomatically sensitized and healthy individuals despite the fact that the study was carried out in the pollen season.

Our findings are in agreement with other studies reporting equal mRNA levels of CCR3 and CCR5 in PBMCs [22] and same levels of CXCR3+ peripheral blood Th cells [21] in patients with atopic dermatitis and healthy controls, but in disagreement with other findings showing decreased percentage of CCR5+ and CXCR3+ memory Th cells in the blood from patients with atopic dermatitis compared to healthy controls [23].

Changes in chemokine receptor expression were observed after stimulation with both antigens (Table 2). CCR5 expression was induced after TTx stimulation, but only in