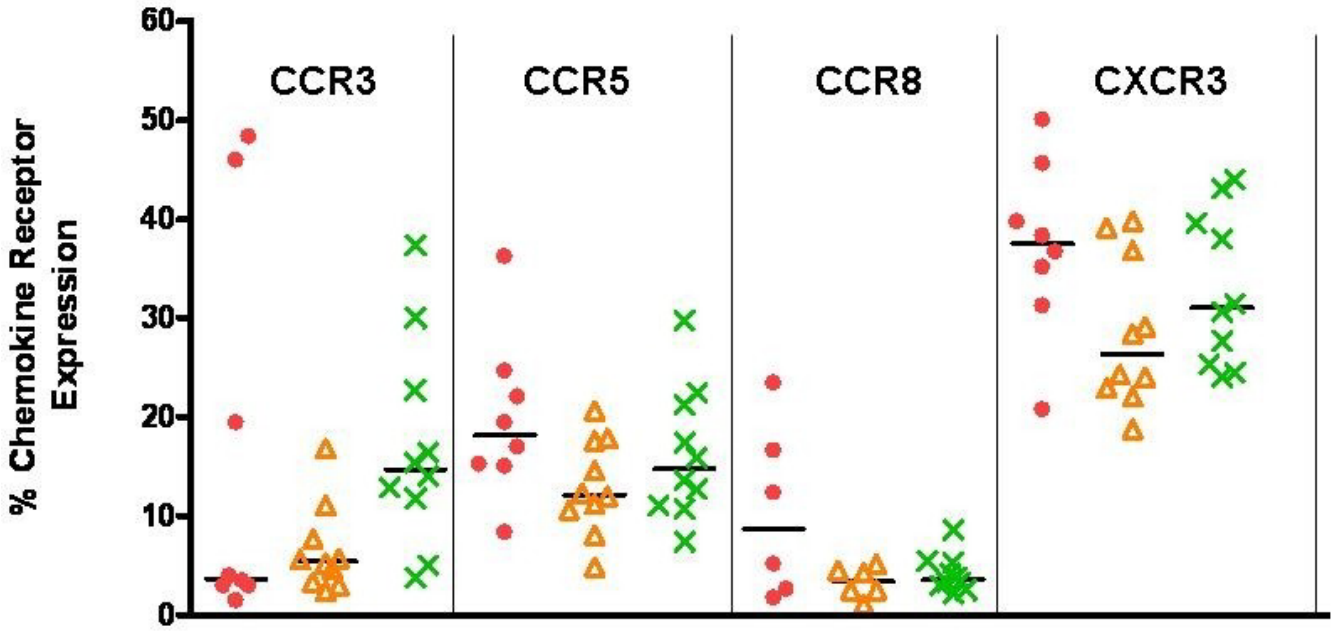

Figure 1

Percentage chemokine receptor positive memory T helper cells day 0. Percentage CCR3+, CCR5+, CCR8+ and CXCR3+ memory Th cells from allergic (dots), asymptomatically sensitized (triangles) and healthy control (crosses) individuals on day 0 immediate *ex vivo*. - = median value. n = 10 for the healthy controls, n = 10 for the asymptomatically sensitized 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.

healthy individuals no detectable amounts of TNF-α were observed.

Flow cytometry

Surface markers were detected using primary labeled antibodies: CCR3-FITC, CCR5-FITC, CCR8-FITC, CXCR3-FITC (R&Dsystems, Abingdon, UK), CD4-PE-Cy5 and CD45RO-PE (Dakocytomation, Glostrup, Denmark). Three-color flow cytometry was performed on day 0 and day 7 on a FACScan (Becton Dickinson, Heidelberg, Germany) using WinList (Verity Software House, Topsham, USA) software for analysis. Isotype control cut-off values were set to > 98% and 10.000 PBMC were acquired. Gating was done by firstly applying a CD4+ gate followed by determination of the percentage and mean fluorescence intensity (MFI) of the CD45RO and chemokine receptor double positive population. CD45RO is a marker of effector and memory T cells, however throughout this article these CD4+ CD45RO+ T cells will be mentioned as memory T helper cells for convenience.

Statistical analysis

Samples were compared using non-parametric statistics (Kruskall-Wallis test or Wilcoxon's test for matched pairs). Values of $P < 0.05$ were considered significant.

Results

Day 0

Immediate after isolation of the PBMCs, the cells were subjected to flow cytometric analysis. No significant differences in the percentage of CCR3+, CCR5+, CCR8+ and CXCR3+ memory Th cells from allergic, asymptomatically sensitized and healthy individuals were observed (Figure 1). Likewise, no differences in MFI were observed between the three donor groups (results not shown).

Day 7

Effect of stimulation

No significant differences in chemokine receptors (neither expressed as the percentage of positive cells nor as MFI) were observed between day 0 and the cells having been kept in antigen-free medium for 7 days as controls. Thus the medium alone and the experimental set-up did not influence the chemokine receptor expression. After TTx stimulation a significant increase in MFI was observed for CCR3 in the allergic and asymptomatically sensitized individuals, but not in the healthy control group (Table 2). However, no increases in the percentage of CCR3+ memory Th cells were observed in any of the groups. CCR5 increased both as MFI and the percentage of CCR5+ memory Th cells in the asymptomatically sensitized and