Materials and Methods

Patient details

Bronchoscopies: Three aliquots of normal saline (each 1ml/kg, to a maximum 40 ml) were instilled in the right middle lobe after wedging of the bronchoscope, then bronchoalveolar lavage (BAL) fluid retrieved by mechanical wall suction.

In vivo study Patient Baseline Characteristics: Asthma was defined based on American Thoracic Society criteria (reversible obstruction (>15%) of the airways). The patient baseline characteristics were: (mean (SD)): age 54 (15) years, basal FEV_1 55% (20%) predicted. Patients were diagnosed as "severe" asthmatics on the basis of reversibility, basal FEV_1 and a failure to respond to corticosteroid therapy – i.e. less than 15% improvement of FEV_1 from a baseline of less than 75% after 14 days of 40 mg/day oral prednisolone [15].

Cell purification and culture

PBMC were isolated as previously described [15]. Disaggregated tonsils and nasal polyps were digested in endotoxin-free collagenase (2 mg/ml; Liberase C1; Roche, IN, USA) for 1 hour at 37°C, and then filtered using a 100μm pore nylon mesh filter to obtain a single cell suspension. BAL cells were obtained by filtration of BAL fluid. PBMC-derived CD4+ and CD8+ T cells were purified by positive selection using Dynabeads (Dynal, Oslo; typical purity 98.5%). Blood CD14+ monocytes and tonsillar CD19+ B cells were negatively isolated using MACS sorting systems (Miltenyi, Gladbach, Germany). CD3+ T cells and CD326+ epithelial cells were purified from nasal polyp cell suspensions by cell sorting using a FACSARIA flow cytometer (Becton Dickinson, UK).

Real time RT-PCR

Real time RT-PCR was performed as previously described [15], in triplicate, using an Applied Biosystems 7900 HT system and FAM labelled Assay-on-Demand reagent sets for CD200: Hs00245978_m1, CD200R: Hs00990599_m1, CD200R1L: Hs02339410_m1 and hCAP-18: Hs00189038_m1. Real time RT-PCR reactions were multiplexed using VIC labelled 18s primers and probes (Hs99999901_s1) as an endogenous control and analyzed using SDS software version 2.1 (Applied Biosystems), according to the 2-(ΔΔCt) method.

CD200 / CD200R Staining

Peripheral T cells were stained for CD200 or CD200R using mouse anti-human CD200-APC (IgG1κ isotype, clone OX104) (eBioscience, Hatfield, UK) or mouse anti-human CD200R-PE (IgG1κ isotype, clone OX108) (eBioscience). Relevant isotype control antibodies were obtained from BD (Becton Dickinson, UK). Samples were analysed using a FACScalibur (Becton Dickinson, UK).

Statistics

Results are presented either as individual results of independent experiments or summarised as mean ± standard deviation (SD) for normally distributed data or median ± interquartile range (IQR) for non-normal data as indicated in the figure legend. Summarised paired data were statistically tested by paired or unpaired t test or repeated measures ANOVA for normally distributed data, or using the Wilcoxon,

Mann-Whitney U or Friedman test for non-normal data as indicated in specific figure legends. All p values were corrected for multiple comparisons using the Bonferroni test or Dunns test respectively. Differences were considered significant at the 5% confidence level. All statistical analyses were carried out using Graphpad Prism version 5.0 for Macintosh OS X.