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 FEV₁ 55% (20%) predicted. Patients were diagnosed as “severe” asthmatics on the basis of reversibility, basal FEV₁ and a failure to respond to corticosteroid therapy – i.e. less than 15% improvement of FEV₁ 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.