Exclusion criteria

Patients in the following categories were excluded: i) Smoking within 3 months prior to screening or total smoking history 10 pack years, ii) Pregnant or lactating females or those at risk of pregnancy, iii) Patients taking >20mg of prednisolone daily, iv) Hospitalisation for asthma or exacerbation requiring systemic corticosteroid therapy within 3 months of the screening visit, v) History of life-threatening asthma, defined as an asthma episode that required intubation and/or was associated with hypercapnia, respiratory arrest and/or hypoxic seizures within the 6 months prior to screening, vi) Pre-bronchodilator $FEV_1 < 40\%$ predicted, vii) Patients in whom omalizumab therapy was conventionally contraindicated or should be used with caution according to the omalizumab SmPC.

Immunofluorescence

Bronchial biopsies were fixed in 4% PFA for 2 hours at room temperature, washed overnight in PBS containing 15% sucrose, embedded in O.C.T. compound (Tissue-Tek®, VWR International LLC) and stored at -80°C. Eight micron thick sections were cut using a cryostat (Bright OTF 5000, Bright Instruments Co Ltd), air dried overnight onto Polysine slides (Thermo Scientific) and stored at -80^{0}C. PFA fixed sections were thawed at room temperature for 30 minutes and rehydrated in PBS for 5 minutes.

Total tryptase^{+} mast cells, CD20^{+} B cells, CD138^{+} plasma cells, CD38^{+} plasmablast cells, IgE^{+} cells and cells of these phenotypes co-expressing IgE (with the exception of CD38^{+} plasmablasts) were sought and enumerated from digital images of the sections by two observers ignorant of the origin of the sections, using single and double immunofluorescence and confocal microscopy. Sections were stained with primary antibodies diluted in PBS containing 1% normal human serum and 1% normal goat serum (antibody buffer) or PBS/0.1% saponin for anti-CD20cy. The following primary antibodies were used: mouse anti-human tryptase (1:100, Dako), mouse anti-human CD20cy (1:120, Dako), mouse anti-human CD38 (1:100, Abgent), mouse anti-human CD38 (1:100, Abgent) and rabbit anti-IgE (1:500, Dako) and incubated overnight at room temperature. Excess antibodies were washed off in PBS (5 min X 3 times), then the sections stained with anti-human tryptase, anti-human CD20 and anti-human CD138^{+} plasma and rabbit anti-IgE were incubated with secondary antibodies anti-mouse-IgG-FITC (1:200; Life Technologies) and anti-rabbit-IgG-Alexa Fluor 594 (1:200; Life Technologies) diluted in antibody buffer for 1 hour at room temperature in the dark. Sections stained with anti-human CD38 were incubated anti-mouse-IgG-Alexa

Fluor 594 (1:200; Life Technologies) as second antibody to reduce autofluorescence. After washing with PBS, the sections were mounted with Prolong Gold Antifade Reagent with Dapi blue nucleic acid background stain (Life Technologies). The images were captured using a Nikon Eclipse Ti-E inverted confocal microscope (Nikon Imaging Centre, King's College London). The images were processed under identical conditions of illumination using ImageJ (http://rsb.info.nih.gov/ij/) and the total numbers of positively stained cells (green fluorescing CD20^{+}, CD138^{+} and tryptase^{+} cells and red fluorescing IgE^{+} cells and CD38^{+} plasmablasts, and those expressing both red and green fluorescence measured confocally) counted and expressed as per mm^{2} of the entire area of the submucosa. The typical mean co-efficient of variability between the sections was 67.46% for tryptase^{+} mast cells and 93.01% for CD138^{+} plasma cells.

Immunohistochemistry

Bronchial biopsies were fixed, frozen and stored as described in the immunofluorescence section. Total BMK-13^{+} cells were sought and enumerated using single immunohistochemistry stain and confocal microscopy. Sections were stained with BMK-13 (1:30, Abcam) diluted in PBS containing 5% normal human serum and incubated overnight at room temperature. Excess antibodies were washed off in PBS (5 min x 3 times), then the sections were incubated with rabbit anti-mouse secondary antibody (1:50; Dako) in PBS containing 5% normal human serum for 30 mins at room temperature in a humidifier chamber. After washing with PBS, the sections were incubated with mouse anti-AP-APAAP (1:30, AbD Serotec) in PBS containing 5% normal human serum for 30 minutes at room temperature and then washed again with PBS (5 min x 3 times). Fast Red solution, prepared by dissolving Fast Red tablets (Sigma) in dH_2O, was applied to the sections for 20 mins or until a suitable colour change was observed under the microscope. The Fast Red reaction was stopped with H_2O for 5 minutes. Haematoxylin was applied for 30 seconds, then rinsed off under running water for 5 minutes. The slides were then dried for an hour, mounted with Glycerol Gel (Dako) and dried overnight. The images were captured using a Nikon Eclipse Ti-E inverted confocal microscope (Nikon Imaging Centre, King's College London). The images were processed under identical conditions of illumination using ImageJ (http://rsb.info.nih.gov/ij/) and the total numbers of BMK-13 positively stained cells (red colour) counted and expressed as per mm^{2} of the entire area of the submucosa.