Online supplementary material

Persistence of asthma following avoidance of occupational allergens is associated with proTh2 myeloid dendritic cell activation.

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Material and methods

Cell staining and analysis

The following monoclonal antibodies (mAbs) were used for flow cytometry: FITC-conjugated Abs to Lin 1 (CD3, 14, 16, 19, 20, 56) (clones NCAM16.2, MϕP9, L27, SJ25C1, 3G8, SK7), CD4 (clone RPA-T4), isotype-matched control IgG (clone MOPC-21, BD Pharmingen, San Diego, CA, USA); PE-conjugated Ab to isotype matched control (clone MOPC-21, BD Pharmingen, San Diego, CA, USA), CD80 (clone L307.4), CD86 (clone 2331 -FUN-1-); PerCP-conjugated Ab to HLA-DR (clone G46-6) and isotype-matched control IgG (clone X39, BD Pharmingen, San Diego, CA, USA); APC-conjugated Ab to CD1c (clone L161, eBioscience, San Diego, CA, USA), CD11c (clone B-ly6); PE-Cy7-conjugated Ab to CD3 (clone HIT3a, Biolegend, San Diego, CA, USA), and isotype-matched control IgG (clone MOPC-21, BD Pharmingen, San Diego, CA, USA).

For immunostaining, 5-10 x 10^5 cells per sample were saturated with a solution of PBS-20% human serum for 30 minutes, to block Fc-receptors. After washes, cells were stained with fluorochrome-labelled antibodies (at concentrations according to manufacturer's guidelines) for 45 minutes at 4°C, and fixed with 0.625 % formaldehyde.

Flow cytometry analysis was performed using a FACS Canto™ II (BD Biosciences, San Diego, CA, USA). At least 10,000 events were read and results were analyzed with Flowjo software vX.0.7 (Tree Star, Ashland, OR, USA).

Cytokine assays

Cytokine levels were assayed in culture supernatants by sandwich ELISA, using paired antibodies for detection of human IL-5 (MAB405 / BAM6051), IL-13 (MAB213 / BAF213) (R&D, Minneapolis, MN USA), IFN-γ (MAB2852/BAF285), and IL-10 (BD554705 / BD554499, BD Pharmingen, San Diego, CA, USA). Detection limits were 8 pg/mL for IL-13 and 16 pg/mL for IFN- γ, IL-10 and IL-5.

96-wells plates were coated with capture antibody overnight and then saturated for 1h with PBS containing 1% bovine serum albumin (BSA). After 3 washes with PBS-Tween 20 0.05%, 50 µL of each sample were incubated for 2 hours at room temperature. Biotinylated antibodies were added and incubated for 1 hr, and reaction was revealed by adding 50 µL of 0.01% streptavidin conjugated with horseradish peroxidase (BD Pharmingen, San Diego, CA, USA) for 30 minutes, followed by TMB (TetraMethylBenzidine, Thermo fisher, Meridian, Rockford, USA). After stopping the reaction with 100µl of 1.8M H_2SO_4/well, absorbance was read at 450 nm, using an ELISA microreader (Bio-Rad, Model 3550, Hercules, CA, USA). Concentrations were expressed in pg or ng/mL as referred to recombinant standards.

Supplementary table

Table SI: Characteristics of patients for blocking experiments

Patients' characteristics (N=7)	
Causal allergen, flour/latex	4/3
Gender, M/F	4/3
Age, years	47 (39-50)
Atopy (N, %)*	5 (71)
Current or ex-smokers	2 (28)
Smoking history, pack-years (at follow-up)	3, 7
Duration of exposure before removal (months)	107 (51-210)
Time elapsed since removal from exposure, years	8 (5-13)