Supplementary data

Detailed Material and Methods

1- Subjects and samples

- Subjects

We collected nasal and bronchial brushings from 39 subjects: 12 with both allergic rhinitis and asthma, 14 with allergic rhinitis only, and 13 healthy controls.

A power calculation was performed to determine the sample size using the results of our previous work published in the ERJ[1]. Briefly a power of 90% to detect a log2 fold change >1 at a FDR of 5% for Th2 marker genes would be achieved with experimental of groups of 6-7 patients.

Allergic rhinitis was defined according to 2010 ARIA (*Allergic Rhinitis and its Impact on Asthma*) recommendations[2]. Asthma was defined according to 2012 GINA (Global Initiative for Asthma)[3] and 2007 EPR3 (Expert Panel Report)[4] recommendations.

Three experimental groups were defined: (i) Group AR: allergic rhinitis with asthma. The inclusion criteria were: allergic rhinitis defined with clinical ARIA criteria; mild to moderate persistent controlled asthma defined with clinical GINA and EPR3 criteria; (ii) Group R: isolated allergic rhinitis. The inclusion criteria were: allergic rhinitis defined with clinical ARIA criteria; no asthma defined with clinical GINA and EPR3 criteria; (iii) Group C: healthy controls. The inclusion criteria were: no clinical criteria of allergic rhinitis according to ARIA recommendation, no clinical criteria for asthma according to GINA and EPR recommendations.

Our noninclusion criteria were: rhinitis or bronchial infection during the previous 6 weeks; asthma exacerbation during the previous 6 weeks; chronic disease that could modify the results or expose to risk; intranasal, inhaled or oral corticosteroids during the previous 6 weeks; current tobacco use; smoking cessation for less than 12 months; cumulative smoking

above five pack-years; intellectually disabled; vulnerable person; pregnancy or breast feeding; woman not using contraception.

Our exclusion criteria were: respiratory functional test criteria: severe obstructive pulmonary disease with VEMS < 30% predictive value, severe obstructive pulmonary disease not significantly reversible, obstructive pulmonary disease not corresponding to asthma; serious adverse event; withdrawal of informed consent; violation of protocol; pregnancy.

- Clinical work-up:

We organized a preselection visit and two hospital visits for participants.

The preselection visit consisted of the collection of clinical data and performance of cutaneous allergy tests. Data were collected via allergy interview and physical examination.

Patients' nasal symptoms were investigated through a five-item (PAREO) score derived from [5], which consisted of three levels (0 = no complaint, 1 = light-to-moderate complaints, 2 = high level of complaints) about five clinical parameters: itchy nose (P), anosmia (A), rhinorrhea (R), sneezing (E) and nasal obstruction (O). The total nasal symptom score was calculated by summing the scores of each symptom. The SNOT-22 score[6] was also determined.

All patients were required to discontinue antihistamine medication for at least 5 days before the skin prick test (SPT). The SPT, a total of 18 common standardized allergen extracts (SPT: Alyostal Stallergenesgreer, London, UK and Lancet, Stallerpoint®) was performed on the medial side of the forearm. In our study, the allergens used were house-dust mite (*Dermatophagoides pteronyssinus* (Dp), *Dermatophagoides farinae* (Df)), cockroach (German cockroach), molds (*Alternaria alternata*, Aspergillus mix), animal dander (cat, dog), latex, a five-grasses mixture (*Dactilis glomerata, Phleum pratense, Anthoxanthum odoratum, Lolium perenne*, *Poa pratensis*), *Parietaria officinalis*, *Plantago lanceolata*, sweet wormwood, ambrosia, olive, oak, cypresses, plane tree, trees mixture (alder, birch, hornbean,

hazel). Histamine (1% histamine phosphate) and 0.9% saline were used as positive and negative controls, respectively.

The number of sensitized allergens was calculated as the sum of positive results for the list of allergens given above. An allergen was interpreted as positive if the largest wheal induced was ≥ 3 mm in diameter as recommended[7].

The results were communicated to the participant at the time of testing.

The investigator checked the inclusion and noninclusion criteria and proposed to suitable participants to enter the study.

The first visit included the practice of functional respiratory tests in accordance with ATS (American Thoracic Society)/ERS (European Respiratory Society) recommendations[8]. This visit was realized within two months of the preselection visit.

At the second visit, bronchial endoscopy was performed. This visit was realized within three weeks of visit one.

The project received the approval of the CPP Sud Méditerranée V Ethics Committee (ref 13.032) on July 23, 2013 and all volunteers gave their written informed consent.

- Brushings

We performed a same-day upper and lower endoscopy sampling during the same procedure first the nasal epithelium and then bronchial epithelium.

The brush was passed down the working channel of a bronchoscope and kept concealed within the bronchoscope tip. For nasal brushings: the bronchoscope was passed through a nostril and directed and positioned, either to the medial part of the middle third (the anterior third being squamous) of the inferior turbinate, or to the medial part of the medium turbinate (if the inferior was scarred). For bronchial brushings: the bronchoscope was passed down through the nasopharynx and positioned at the entrance of the right main bronchus. In cases

where the brush was passed down the working channel after aspiration of mucus, the working channel was flushed carefully with sterile saline in order not to plug the brush with mucus.

The cytology brush was advanced, unsheathed and directed until resistance was encountered, either on the medial part of the turbinate or on the lateral wall of right main bronchus.

About twenty rotational movements were performed to sample the cells. The cytology brush was then retracted to just beyond the tip of the bronchoscope without being resheathed (to avoid massive loss of cells when the bristles of the brush directly slid along the interior wall of the sheath at retraction). The bronchoscope and the retracted brush were withdrawn together. The cytology brush was then pushed out from the bronchoscope and transferred into 1.5 ml 4°C Hank's Buffered Salt Solution (HBSS) culture medium (Invitrogen, Carlsbad, CA 92008, United States) in a sterile 15 mL conical polypropylene Falcon tube and its handle cut 3 cm over the top of the tube. The brush was first stirred manually for about one minute and then resheathed-unsheathed about five times in order to remove cells. This procedure was repeated once more for each sampling site with a second cytological brush deposited in the same conical tube. Samples for each site were then split into two parts: 750 µL were used for cell counting and flow cytometry analyses, 750 µL for RNA extraction. The part used for RNA extraction was mixed with 2.25 mL Trizol LS and the tube was vortexed for one minute. The two 15 mL conical polypropylene Falcon tubes were transported on ice to the laboratory.

2- Cell counting

Thoma cell counting (cf http://insilico.ehu.es/counting_chamber/thoma.php)

The large central square of a Thoma cell (1 mm^2, entirely visible with a 10X objective) is divided into 16 medium squares (each one entirely visible with a 40X objective). The height of a cell suspension inserted under a coverslip is equal to 0.1 mm. The volume of the largest square thus corresponds to 10^{-4} ml. The cellular concentration in the sample is equal to f x N x

10^4 cell/ml, where f is the dilution factor introduced at the addition of the Trypan blue, and N is the number of cells detected in the large square.

3- RNA extraction

Total RNA was isolated from brushings using using Trizol LS (Invitrogen), miRNeasy Mini kit (Qiagen, France), according to the manufacturer's instructions. RNA quantity was estimated by spectrophotometry with a Nanodrop ND-1000 UV spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA). RNA quality was checked by electrophoresis on a Bioanalyser 2100 (Agilent Technologies, Santa Clara, CA 95051, USA) with an RNA Pico LabChip® (Agilent Technologies, Santa Clara, CA 95051, USA).

4- RNAseq analysis

Mapping was performed with STAR_2.4.0a versus hg19 followed by read counting using featureCounts (subread-1.4.6-p1-Linux-x86_64) with "--primary -g gene_name -p -s 1 -M " options. Quality control of RNAseq count data was assessed using in-house R scripts. Normalization was performed using the voomWithQualityWeights function from the Bioconductor package *limma*[8]. Differential gene expression was assessed using linear models. A paired design was used for the direct comparison between nasal and bronchial tissues. To compare the different combinations of tissue types and clinical conditions, we used a model including a term for batch and sex. For each comparison, differential expression was assessed using moderated *t*-statistics[9]. P values were adjusted for multiple testing using the Benjamini and Hochberg method. Heatmaps were generated using the R package *pheatmap*. Hierarchical clustering of nasal and bronchial samples based on the 63 most differentially expressed genes

was performed using Euclidean distance and complete linkage. Clustering of samples based on the Th2, Th17 or IFN gene signature was performed using 1- abs (Spearman correlation) as distance for genes, Euclidean distance for samples, and complete linkage. RNAseq data are archived in GEO under reference GSE101720. Molecular function and biological networks analysis were performed using Ingenuity Pathway Analysis software (http://www.ingenuity.com/).

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