ONLINE SUPPLEMENT

METHODS

Patients

Tissues were obtained at the Department of Otorhinolaryngology, Ghent University Hospital, Belgium during routine endonasal sinus surgery and approved by the local ethical committee. All patients gave their written informed consent before collecting material. All patients stopped oral and topical application of corticosteroids for at least 1 month before surgery. The diagnosis of chronic sinus disease was based on history, clinical examination, nasal endoscopy, and computed tomography of the paranasal cavities according to the current European [1, 2] and American Guidelines [3]. The patients' clinical data can be found in Table 1 of the main document.

Single cells preparation

1.5g of freshly resected human nasal mucosa was transferred to 10 ml tissue culture medium (RPMI 1640 (Invitrogen, Ghent, Belgium)), containing 2 mM LGlutamine (Invitrogen), antibiotics (50 IU/ml penicillin and 50 μg/ml streptomycin) (Invitrogen) and 2% FBS (Fetal Bovine Serum, Invitrogen) and roughly fragmented with scissors into small pieces, followed by a mixing step in the Gentle MACs (Miltenyi Biotec, Leiden, The Netherlands). Gentle MACs tubes with tissue were centrifuged for 5 min at 300 g and supernatant was discarded and replaced by RPMI medium containing 2 mg/ml collagenase (Worthington, USA) and 0.04 mg/ml DNAse1 (Roche Diagnostics, Belgium). After a 45 min incubation period at 37 °C, an additional mixing step in the GentleMACs was performed. The cell suspension was dispersed and passed through a 70 μm cell strainer (BD Bioscience, Erembodegem, Belgium). Red

blood cells were lysed by resolving the pellet in Versalyse (Beckman Coulter, Suarlée, Belgium) and cells were incubated for 10 min at RT and after centrifugation the cell pellet was dissolved in RPMI with 2% FBS. For the descriptive part of the study, the single cells were used immediately for flow cytometry staining as described in the paragraph "Flow cytometry staining" below and in the main document. For the functional part of the study, the single cells were processed as reported in the paragraph "in vitro assessment of the functional responses by freshly isolated ILC2s" in both the main document.

Flow cytometry staining

Flow cytometry data were acquired on an LSR II (BD, Erenbodegem, Belgium) and were analyzed with FlowJo software (TreeStar). The following antibodies to human proteins were used for staining freshly resected tissues: fluorescein isothiocyanate (FITC)-conjugated anti-CD3 (BW264/56), anti-CD11c (MJ4-27G12), anti-CD19 (LT19), anti-CD34 (AC136), anti-CD303 (AC144), anti-FceRIa (AER-37) (all from BioLegend, San Diego, CA, USA), anti-CD94 (DX22) (ebioscience, San Diego, CA, USA), anti-CD14 (MφP9) (BD), phycoerythrin (PE)-conjugated anti-ILRI (Polyclonal Goat IgG) (R&D, Oxon, United Kingdom), PECy7-conjugated anti-CD117 (104D2) (ebioscience, San Diego, CA, USA), allophycocyanin (APC)-conjugated anti-CRTH2 (BM16) (Biolegend), APCCy7-conjugated anti-CD45 (2D1) (Miltenyi, Leiden, The Netherlands), peridinin chlorophyll (PerCP) efluor 710-conjugated anti-NKp44 (44.189) (ebioscience) and brilliant violet (BV)421-conjugated anti-CD127 (A019D5) (Biolegend).

To investigate the contradictory data present in the literature regarding the expression of IL-1RI on ILC2s [4-6] and to evaluate the relative contributions of the documented CD117 positive and negative fractions within the ILC2 population [4, 7], we extended our gating

strategy including antibodies against IL-1RI and CD117 in the ILC2 gating strategy. as shown in figures E1 and E2 in this Online Repository.

After in vitro stimulation, additional IL-5 cytokine expression was assessed using BV421-conjugated anti-IL5 (TRFK5) (Biolegend). For labeling cells of the in vitro series, all FITC-conjugated antibodies remained identical to the staining on the freshly resected tissues. The other antibodies in this series were conjugated as follows: PECy7-conjugated anti-CD117 (104D2) (ebioscience, San Diego, CA, USA), allophycocyanin (APC)-conjugated anti-CRTH2 (BM16) (Biolegend), APCFire-conjugated anti-CD45 (BM16) (Biolegend) and Horizon BV510-conjugated anti-CD161 (HP-3G10) (Biolegend).

Fluorescence Minus One (FMO) data for CRTH2, CD117, IL1RI and NKp44 were used in the gating strategy ensuring accurate setting of gates for correct determinations of positive and negative populations. This is also indicated in figure E1 as grey overlays in the histograms explaining the gating strategy used in this study. During protocol optimization, also an isoclonal control was used for IL1RI.

Tissue homogenates

Snap-frozen tissue specimens were weighed and suspended in a 10 times volume of 0.9% NaCl solution with protease inhibitor Complete Roche (Mannheim, Germany). In order to prepare soluble protein fractions, the frozen tissue was pulverized by means of a mechanical TissueLyser LT (Qiagen, Hilden, Germany) at 50 oscillations per second for 2 minutes in pre-chilled eppendorf tubes. The tissue homogenates were centrifuged at 1800 g for 5 minutes at 4°C. The supernatants from the tissue homogenates were stored at –20°C until further analysis

RESULTS

In vitro assessment of the functional responses by freshly isolated ILC2s

After 3h on 37°C in the presence of IL-2 without stimulation, the single cell fractions from nasal polyps of CRSwNP patients (all allergic or asthmatic) contained 0.08 ± 0.05 % $CD117^+$ ILC2s and 0.09 ± 0.04 % $CD117^-$ ILC2s (n=3) of the living cell fraction, being similar to what was observed for the ex-vivo part of the study on 35 CRSwNP patients (see fig 5 in the main document). However, in PMA/Ionomycin stimulated single cell fractions from the same patients the number of $CD117^+$ ILC2s was almost abolished to 0.01 ± 0.004 % of living cells, while the number of $CD117^-$ ILC2s remained unaffected at 0.08 ± 0.02 % of living cells. The latter cell fraction contained no IL5+ cells at baseline conditions, while in the PMA/Ionomycin stimulated single cell fraction, 16.80 ± 5.11 % of the cells were IL-5 positive.

The % of counted cells in the ILC1 gate increased from 0.05 % ± 0.02 % of living cells under basal non-stimulated conditions to 0.19 % ± 0.08 % of living cells when the cells were stimulated by PMA/Ionomycin. While under basal conditions, the intracellular IL-5 expression in these cells was virtually absent, the stimulated cells in the ILC1 gate contained 15,5 ± 3.66 % $IL\text{-}5^+$ cells. The cells found in the ILC1 gate could be recovered to the ILC2 fraction by intracellular staining for CRTH2 and CD117 in addition to the membrane staining for these markers; by doing so, the number of CD117+ ILC2 cells restored to 0.05 ± 0.01 % of living cells (of which 12.63 ± 4.05 % were $IL\text{-}5^+$), while the number of cells in the ILC1 gate reduced again to 0.04 ± 0.01 % of living cells, all lacking intracellular IL-5 expression.

After 3h on 37°C in the presence of IL-2 without stimulation, the single cell fractions from nasal polyps of CRSsNP patients contained an equal number of $CD117^+$ ILC2s and $CD117^-$

ILC2s (0.04 ± 0.005 % vs 0.03 ± 0.002 % of the living cell fraction (n=2) respectively, which contrasts the virtual absence of CD117+ ILC2s in freshly resected tissue of CRSsNP patients (see fig 5 in the main document)

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