

Original article

Ratio of myeloid and plasmacytoid dendritic cells and T_H2 skew in CRS with nasal polyps

Background: The role of myeloid and plasmacytoid dendritic cells and its consequences for the T_H2 skew in chronic rhinosinusitis (CRS) with nasal polyps (CRSNP⁺) should be detailed.

Methods: In 18 CRS patients without nasal polyps (CRSNP⁻), 35 CRSNP⁺ patients and 22 patients with nasal structural abnormalities without rhinosinusitis (controls), dendritic cells (DC) were differentiated into myeloid (mDC) and plasmacytoid (pDC) subtypes using an antibody cocktail including CD1c (BDCA-1) and CD303 (BDCA-2) in peripheral blood mononuclear cells (PBMC) and single cell preparations of sinonasal mucosa by flow cytometry. Moreover, cells were analysed for expression of CD45, CD3, CD4, CXCR3 (T_H1) and CCR4 (T_H2) and IFN- γ , IL-5, TGF- β 1, TGF- β 2, ECP and total IgE in nasal secretions were determined. As a possible confounder, *Staphylococcus aureus* in nasal lavages was detected.

Results: The tissue mDC/pDC-ratio was 1.7 (1.0–2.4) in controls, 3.0 (1.8–4.0) in CRSNP⁻ and 0.8 (0.6–1.0) in CRSNP⁺ ($P < 0.01$). In tissue samples, the T_H1/T_H2 ratio was 12.6 (6.4–16.0) in controls, 12.5 (6.9–21.2) in CRSNP⁻ and 1.8 (1.3–3.6) in CRSNP⁺ (median and interquartile range, $P < 0.001$). Less pronounced differences were found in PBMC. *S. aureus* detection rates or TGF- β levels did not differ between patient groups and *S. aureus* detection had no influence on the parameters investigated.

Conclusion: A significant T_H2 skew in CRSNP⁺ could be confirmed on the cellular level. It was driven by low myeloid dendritic cell numbers. The T_H2 skew did not correlate with *S. aureus* detection. The data support the concept that CRSNP⁺ and CRSNP⁻ are pathophysiologically distinct.

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Key words: cytokines; dendritic cells; Helper-Inducer; nasal polyps; sinusitis; *Staphylococcus aureus*; T-lymphocytes.

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Chronic rhinosinusitis (CRS) is a symptomatic mucosal inflammation of the nose and paranasal sinuses lasting for more than 12 weeks (1). Clinically, CRS can appear without (CRSNP⁻) or with nasal polyps (CRSNP⁺). Various immunologic differences between CRSNP⁻ and CRSNP⁺ have been reported. In CRSNP⁺, polyclonal IgE-activation (2), high numbers of eosinophils and plasma cells (3) and nasal cytokine profiles indicating a T_H2-driven inflammatory process (4) were found. T_H2 cells are believed to contribute to the pathogenesis of CRSNP⁺ as IL-5 promotes the differentiation and activation of eosinophils and basophils, while IL-4 promotes immunoglobulin switching to the IgE-isotype (5).

Based on cytokine mRNA and transcription factor detection, T_H2 cells were identified in CRSNP⁺ mucosa (6, 7). T_H1 and T_H2 cells differ in the expression of some chemokine receptors. The chemokine receptor CXCR3 is preferentially expressed on T_H1 cells while CCR4 is predominantly expressed on T_H2 cells (8). In peripheral blood mononuclear cells (PBMC), flow cytometric inves-

tigations revealed a correlation of CCR4⁺ cells and serum IgE in asthma patients (9).

The T_H1/T_H2 ratio is substantially driven by dendritic cells (DC) (10). In nasal mucosa, DC have been identified by immunohistochemistry and flow cytometry (11, 12). In peripheral blood, two major DC subsets were distinguished (13) and each represent about 0.2%–0.4% of PBMC. Myeloid dendritic cells (mDC) and plasmacytoid dendritic cells (pDC) can be differentiated employing CD1c (BDCA-1) and CD303 (BDCA-2) antibodies (14). Although mDC/pDC differentiation reveals some plasticity (15), pDC have a propensity to elicit a T_H2 polarization of naive T cells (10). Studies in asthma showed that the mDC/pDC ratio tends towards pDC, associated with a T_H2 polarization (16).

The objective of this study was to investigate the distribution of mDC and pDC cells in well characterized patients with CRSNP⁺, CRSNP⁻ and controls by flow cytometric analysis of sinus specimens and PBMC. The hypothesis was that the T_H2 skew in CRSNP⁺ is driven

by a low mDC/pDC ratio. To better characterize patients in addition to nasal endoscopy and radiological findings, levels of IL-5, IFN- γ , TGF- β 1, TGF- β 2, ECP and total IgE in nasal secretions and the number of eosinophils as well as the number of CXCR3 and CCR4 positive cells in tissue specimens were determined. Moreover, *Staphylococcus aureus* detection rates and the influence on the outcome parameters were studied.

Patients and methods

Study population

Patients with CRS irresponsive to conservative treatment referred to the Department of Otorhinolaryngology – Head and Neck Surgery of the University Hospital Ulm (Germany) between October 2005–May 2007 were consecutively screened for this prospective study. The diagnosis was confirmed by medical and clinical history, sinus CT scans and nasal endoscopy. CRS patients with a modified Lund-Mackay CT-score (17) of 15 or more (maximum score = 40) were eligible. CRS patients without any nasal polyps at endoscopic examination were grouped as CRSNP⁻. To be categorized as CRSNP⁺, a bilateral endoscopic polyp score of at least 4 (maximum = 6) was required (18). Patients with a bilateral polyp score of 1–3 were considered not classifiable with sufficient reliability and excluded. Further exclusion criteria were unilateral sinus disease, systemic or topical steroid medication during the last 4 weeks or sinusitis of dental origin or cystic fibrosis. Patients with nasal structural deformities without clinical and radiological evidence of rhinosinusitis served as controls.

Patients were additionally asked for the duration of disease and the presence of asthma, acetylsalicylic acid (ASA) intolerance and atopy. Patients using asthma medication were categorized as asthmatics. ASA intolerance was diagnosed if acute symptom exacerbation or an asthma attack followed nonsteroidal anti-inflammatory drug intake or by oral provocation tests (19). Atopy was defined as positive skin prick tests to inhalant allergens common in Central Europe and tested according to the recommendations of the EAACI (20). Sinus symptoms were scored on verbal rating scales. The study was approved by the Ethic Committee of the University of Ulm, Germany, No. 243/2005 and written informed consent was obtained from all participants.

Sample asservation and processing

Before surgery, blood samples were obtained from each patient and PBMC were isolated on Ficoll and then cryoconserved following a standardized freezing procedure. For nasal lavage, 5 ml sterile saline solution (0.9%) was instilled into each nostril and left for 10 s during voluntary nasopharyngeal occlusion. Presence of *S. aureus* was detected with standard tests in the Department of Microbiology, University of Ulm, Germany (21).

During surgery, mucosa samples were obtained from the anterior ethmoid in CRSNP⁻ and CRSNP⁺ patients or as close as possible from the adjacent inferior turbinate in controls. For single cell preparation, one part of the collected tissue was immediately stored on ice, given in a mixture of X-vivo and DNase [80 μ l DNase (10 mg/ml) in 10 ml X-vivo, BioWhittaker, Walkersville, MD, USA], separated in a tissue disaggregation chamber (30 s, Medicon, Dako GmbH, Hamburg, Germany), given through a cell strainer (mesh size 70 μ m, BD Biosciences GmbH, Heidelberg, Germany) and centrifuged (1400 rpm, 10 min) for thrombocyte depletion.

After treatment with erythrocyte-lysis-buffer (incl. NH₄Cl, KHCO₃ and EDTA) for 60 s, cells were washed twice in X-vivo medium and then cryoconserved following a standardized freezing procedure using cryomedium containing RPMI, FCS (20%) and DMSO (10%), respectively (Sigma-Aldrich, Munich, Germany). The other part of the collected tissue was sent to the Department of Pathology, University of Ulm, Germany, for hematoxylin-eosin stain.

Biomarker determination in nasal secretions and eosinophil counts in tissue

IL-5, IFN- γ , TGF- β 1, and TGF- β 2 levels in nasal lavages were measured with the Quantikine[®] Kit (R&D-Systems, Wiesbaden, Germany) complying with the manufacturer's recommendations. The detection limit for IL-5 was 3.0 pg/ml, 8.0 pg/ml for IFN- γ , 4.61 pg/ml for TGF- β 1, and 7.0 pg/ml for TGF- β 2. Spiking controls performed for IL-5 and IFN- γ in advance by our group (4) obtained loss of recovery <20% in worst conditions. IL-5 and IFN- γ were measured undiluted, dilution for TGF- β 1 was 2.4-fold and for TGF- β 2 1.4-fold. ECP and total IgE levels were measured using an automated fluorescence-enzymeimmunoassay (UniCAP100 Diagnostic System, Phadia, Freiburg, Germany) according to the manufacturers' recommendations. The limit of detection was 0.7 μ g/l for ECP and 2 kU/l for total IgE. Dilution was 5-fold for ECP. Total IgE was measured undiluted. Hematoxylin-eosine stained tissue slices were analysed in high-power fields (HPF) at 40 $\times$ objective magnification (10 $\times$ ocular). Eosinophils were identified and counted in 10 HPF per slice. For subsequent analysis, mean cell number per specimen was determined.

Flow cytometry from PBMC and tissue samples

PBMC as well as single cell suspensions from tissue samples were thawed and incubated with FcR blocking reagent (Miltenyi Biotech, Bergisch Gladbach, Germany). Dendritic cells were differentiated into myeloid (mDC) and plasmacytoid (pDC) subtypes (Fig. 1) with the MACS[®] dendritic cell enumeration kit (Miltenyi Biotech, Bergisch Gladbach, Germany). For each specimen, two samples with at least 1×10^6 PBMC (blood) or 1×10^6 separated cells (tissue) were needed. MDC and pDC were detected in one sample by staining with Anti-BDCA-1-PE and Anti-BDCA-2-FITC, respectively. The other sample was stained with FITC- and PE-conjugated isotype control monoclonal antibodies. Both samples were simultaneously stained with CD19-PE-Cy5 and CD14-PE-Cy5 for exclusion of B cells and monocytes. All monoclonal antibodies were supplied as premixed cocktails (Anti-BDCA and Control Cocktail). Simultaneously to antibody staining, samples were stained with a fluorescent cell-impermeant dye (dead cell discrimination). After cell staining, erythrocytes were lysed with ammonium chloride buffer and washed and fixed using formaldehyde. Finally, cells were analysed by 3-color (FITC, PE, PE-Cy5) flow cytometry (FACScan, BD Biosciences GmbH, Heidelberg, Germany) for enumeration of mDC and pDC after strategical gating (14). In addition, each specimen was stained with antibodies against CD3, CD4, CD8, CD19, CD45, CXCR3 and CCR4 (BD Biosciences GmbH, Heidelberg, Germany), respectively. Both CXCR3-positive and CCR4-positive subpopulations were identified in CD3⁺/CD4⁺ populations by 3-color staining (Fig. 2).

Data analysis

Categorical data were tabulated and tested with the Chi-Square test. For description of ordinal and continuous data, medians and

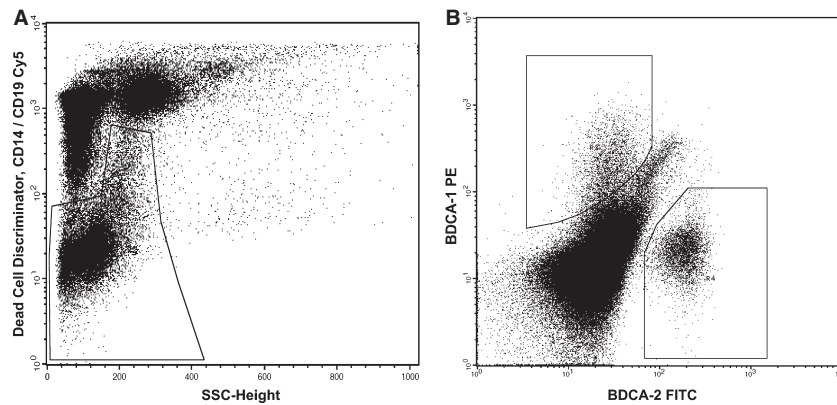


Figure 1. Determination of mDC/pDC in PBMC of a CRSNP⁻ patient. Identification of alive CD19⁺/CD14⁻ cells (panel A, lower gate, Cy5 = cyanine5) and discrimination between mDC (BDCA-1, y-axis) (panel B, upper left gate) and pDC (BDCA-2, x-axis) (panel B, lower right gate) subsets.

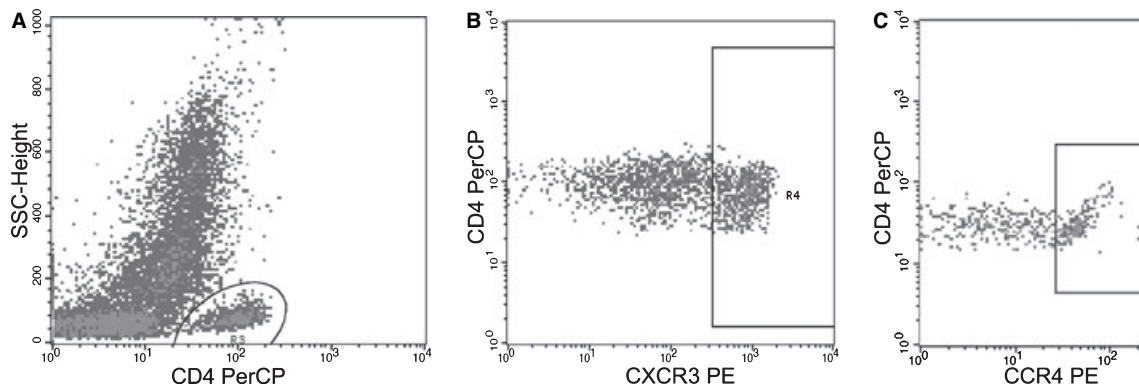


Figure 2. Identification of CXCR3-positive T cell subsets in a CRSNP⁺ tissue sample by determination of CD4 positive cells (panel A, SSC = sideward scatter, PerCP = peridinin chlorophyll protein) and detection of CXCR3/CD4 (panel B, PE = phycoerythrin) and/or CCR4/CD4 positive cells by 3-color-staining in a single tube respectively [(A) and (C) determined in the same run].

interquartile ranges were tabulated. Kruskal–Wallis test served to calculate probabilities. *P* values ≤ 0.05 were stated significant. For correlations, Spearman's rank test was employed. The applied statistical methods respected floor effects in case of left-skewed distribution. Calculations were performed with SYSTAT 12.0 (Systat Software Inc., San Jose, CA, USA) and STATXACT 8.0 (Cytel Inc. Cambridge, MA, USA).

Results

Study population

Of 235 screened patients, 75 patients were eligible. Main reasons for noninclusion were bilateral polyp scores of 1, 2 or 3, systemic or topical steroid medication during the last 4 weeks and unilateral sinus disease. Control patients were younger (32.0; 25.3–37.3 years, median and interquartile range) than CRSNP⁻ patients (43.1; 30.5–51.3 years) and CRSNP⁺ patients (44.8; 38.1–59.8 years; *P* = 0.001). Symptom scores were highest in CRSNP⁻ patients (138; 113.5–159.8), followed by CRSNP⁺ patients (109.5; 91.5–149) and patients with nasal structural deformities

(controls), who scored 98.5 (68–112; *P* = 0.009). Median CT-score in controls was 4 (2–6) compared to 18 (16–23) in CRSNP⁻ and 27 (19.5–36.3) in CRSNP⁺ (*P* < 0.001). Differences were also observed concerning the number of previous sinus surgeries, CRS recurrence and bronchial asthma (Table 1). The three investigated groups did not significantly differ concerning gender, smoking, ASA intolerance and atopy. Duration of disease did not differ significantly between CRSNP⁻ (median duration more than 3 years) and CRSNP⁺ (median duration more than 3 years), *P* = 0.449.

Biomarkers in nasal secretions and tissue eosinophil counts

Consistent with the results of previous studies (4), higher concentrations of IL-5, TGF- β 1, ECP and total IgE were observed in CRSNP⁺ patients than in controls or CRSNP⁻ patients. No differences between the three groups were found for IFN- γ and TGF- β 2 (Table 2A and B). In controls, median number of eosinophils was 0.9 (0.6–1.1, *n* = 9), in CRSNP⁻ patients 1.4 (0.7–4.6,

Table 1. Distribution of clinical data of study participants

	Controls (<i>n</i> = 22)	CRSNP ⁻ (<i>n</i> = 18)	CRSNP ⁺ (<i>n</i> = 35)	<i>P</i> -value
Female/male	7/15	7/11	11/24	0.848
Smoker/nonsmoker	9/13	6/12	9/26	0.484
Asthma/no asthma	3/19	2/16	14/21	0.024
ASA intolerance/no ASA intolerance	0/22	0/18	3/32	0.168
Atopy/no atopy	8/14	6/12	11/24	0.929
Previous surgery/no previous surgery	1/21	3/15	15/20	<0.001
Recurrent CRS/no recurrent CRS	0/22	1/17	14/21	<0.001

Controls = patients with nasal structural anomalies without chronic rhinosinusitis (CRS); CRSNP⁻ = CRS patients without nasal polyps; CRSNP⁺ = CRS patients with nasal polyps. *P*-value calculated with chi-square test of association.

n = 11) and in CRSNP⁺ patients 6.1 (2.6–19.5, *n* = 22). Eosinophil counts differed significantly between controls and CRSNP⁺ (*P* = 0.000) and between CRSNP⁻ and CRSNP⁺ (*P* = 0.009) respectively, but not between controls and CRSNP⁻ (*P* = 0.160).

Flow cytometric analysis of PBMC

The mDC/pDC ratio was highest in CRSNP⁻ patients (2.5; 1.6–3.0), followed by CRSNP⁺ patients (1.5; 0.8–2.0) and controls (1.1; 1.0–1.5; *P* = 0.009). *P*-value between CRSNP⁻ and CRSNP⁺ was calculated 0.019, between controls and CRSNP⁻ 0.003 and between controls and CRSNP⁺ 0.447. The CD3/CD19 ratio served as a marker for the proportion of T cells and B cells. The median T cell/B cell ratio was higher in CRSNP⁻ patients (7.5; 5.2–8.5; *n* = 11) than in controls (4.7; 4.1–5.2; *n* = 14) and CRSNP⁺ patients (5.5;

4.2–8.6; *n* = 18), with significant differences between controls and CRSNP⁻ (*P* = 0.016). The median CD4/CD8 ratio did not differ between the three disease groups. It was 1.8 (1.3–2.4) in controls, 2.2 (1.7–2.7) in CRSNP⁻ and 2.7 (1.9–3.2) in CRSNP⁺ (*P* = 0.179). The median CXCR3/CCR4 (T_H1/T_H2) ratio was highest in CRSNP⁻ patients (535; 413–680), lowest in CRSNP⁺ patients (294; 224–467), and 421 (246–814) in controls. Compared with controls, *P* values of CRSNP⁻ and CRSNP⁺ were not significant respectively, but between CRSNP⁻ and CRSNP⁺ (*P* = 0.012).

Flow cytometric analysis of single cell suspensions of tissue samples

Disease specific differences of mDC/pDC ratios were more pronounced in tissue samples than in PBMC. Median mDC/pDC ratio was 1.7 (1.0–2.4) in controls, 3.0 (1.8–4.0) in CRSNP⁻ and 0.8 (0.6–1.0; *P* < 0.001) in CRSNP⁺ (Fig. 3). *P*-value for CRSNP⁺ when compared to controls and CRSNP⁻ was 0.010 and <0.001 respectively, *P*-value between controls and CRSNP⁻ was 0.079. T cell/B cell ratio revealed no significant differences between the three patient groups (controls: 12.2; 9.6–19.1; *n* = 15, CRSNP⁻: 17.5; 9.0–23.4; *n* = 10, CRSNP⁺: 12.1; 9.5–14.7; *n* = 18). The median CD4/CD8 ratio was 0.7 (0.4–0.8) in controls, 0.8 (0.8–1.7) in CRSNP⁻ and 0.9 (0.6–1.2) in CRSNP⁺ (*P* = 0.105). Changes in the ratio between CD4/CD8 were observed thoroughly, tended towards CD8 in CRSNP⁺ but were not found significantly (*P* = 0.076). CXCR3/CCR4 (T_H1/T_H2) ratio in tissues was substantially lower than in PBMC and group specific differences were more pronounced. Median

Table 2. Biomarkers in nasal secretions

(A) Median and interquartile range				
	Controls (<i>n</i> = 16)	CRSNP ⁻ (<i>n</i> = 13)	CRSNP ⁺ (<i>n</i> = 23)	Units
IFN- γ	4.7 (3.5–29.6)	21.8 (5.2–58.3)	5.2 (4.3–38.3)	pg/ml
IL-5	2.1 (1.7–2.5)	2.4 (1.9–3.2)	3.5 (2.6–6.2)	pg/ml
TGF- β 1	14.9 (10.3–62.7)	18.5 (8.8–206.7)	161.4 (14.2–571.3)	pg/ml
TGF- β 2	22.8 (19.7–30.1)	23.7 (18.4–32.3)	23.1 (18–35.2)	pg/ml
ECP	2.5 (0–6.2)	4.2 (0–10.6)	10.4 (3.1–46.4)	μ g/l
Total IgE	5.5 (5.3–5.7)	6 (5.2–6.4)	7.3 (5.7–10)	kU/l

(B) *P*-value by Kruskal–Wallis test, for all and among each groups

	1: <i>P</i> -value All groups	2: <i>P</i> -value CRSNP ⁻ vs controls	3: <i>P</i> -value CRSNP ⁺ vs controls	4: <i>P</i> -value CRSNP ⁺ vs CRSNP ⁻
IFN- γ	0.246	0.105	0.376	0.307
IL-5	0.002	0.202	0.001	0.046
TGF- β 1	0.130	0.759	0.043	0.242
TGF- β 2	0.840	0.965	0.493	0.843
ECP	0.028	0.447	0.015	0.067
Total IgE	0.003	0.148	0.013	0.024

Controls = patients with nasal structural anomalies without chronic rhinosinusitis (CRS); CRSNP⁻ = CRS patients without nasal polyps; CRSNP⁺ = CRS patients with nasal polyps.

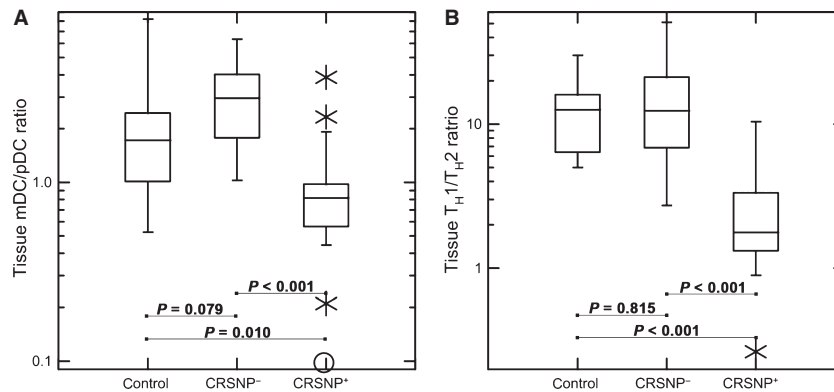


Figure 3. (A) Ratio of myeloid (mDC) and plasmacytoid (pDC) dendritic cells and (B) ratio of CXCR3⁺ and CCR4⁺ cells (TH1/TH2) in sinonasal tissues of controls ($n = 14$), CRSNP⁻ ($n = 10$) and CRSNP⁺ ($n = 17$); $P =$ Kruskal–Wallis test, * = outside value, O = far outside value.

CXCR3/CCR4 ratio was 12.6 (6.4–16.0) in controls, 12.5 (6.9–21.2) in CRSNP⁻ and 1.8 (1.3–3.6; $P < 0.001$) in CRSNP⁺. P -value for CRSNP⁺ when compared to controls and CRSNP⁻ was <0.001 respectively, P -value between controls and CRSNP⁻ was 0.815. Neither the mDC/pDC ratio nor the CXCR3/CCR4 ratio in CRSNP⁺ was influenced by previous surgery ($P = 0.965$ and $P = 0.386$ respectively).

Tissue mDC-deficit and tissue TH2-excess

The low mDC/pDC ratio in CRSNP⁺ patients was due to a mDC deficit ($P < 0.001$), whereas the number of pDC cells did not differ between the three groups ($P = 0.311$). In contrast, low TH1/TH2 ratio in CRSNP⁺ was due to a TH2 excess ($P < 0.001$), whereas the number of TH1 cells did not differ significantly ($P = 0.059$). Consistently, an inverse correlation between tissue mDC cells and TH2 cells was observed ($R_{\text{Spearman}} = -0.63$; $P < 0.001$, Fig. 4), whereas no correlations between mDC and TH1 ($P = 0.9$) and pDC and TH2 cells ($P = 0.3$) were found. Moreover, low mDC was inversely correlated with high IL-5 ($R_{\text{Spearman}} = -0.47$; $P = 0.008$) and eosinophil counts ($R_{\text{Spearman}} = -0.56$; $P = 0.006$).

Role of Staphylococcus aureus

Staphylococcus aureus was detected in nasal lavages of five out of 11 controls, one out of 11 CRSNP⁻ patients and eight out of 25 CRSNP⁺ patients ($P = 0.165$). The detection of *S. aureus* in nasal lavages had no significant influence on the parameters investigated with the exception of a higher PBMC CXCR3/CCR4 ratio ($P = 0.043$).

Discussion

The aim of this prospective study was to investigate the role of myeloid and plasmacytoid dendritic cells in

CRSNP⁻ and CRSNP⁺ and its relation to the ratio of TH1/TH2 cells. Symptomatic CRS patients with substantial alterations in sinus CT scans were allocated to CRSNP⁺ and CRSNP⁻ groups depending on the presence of polyps on nasal endoscopy. Patient selection aimed to obtain two fairly homogenous groups who clearly differed in the presence or absence of nasal polyps. Patients with minor nasal polyps were excluded, possibly suffering of a noneosinophilic sinusitis with marginal nasal polyps. To confirm accurate patient allocation, eosinophils in nasal tissue specimens and biomarker profiles in nasal secretions were determined. The demographic, clinical, histological and laboratory data indicate that patient allocation complied with the aims of this study.

MDC/pDC and TH1/TH2 ratios were determined in PBMC and single cell suspensions of tissue samples by flow cytometric analysis. To obtain a single cell solution, tissue samples were mechanically disaggregated following brief DNase pretreatment. In preliminary experiments, this treatment did not relevantly alter the cell surface receptors investigated in this trial. Dendritic cell subsets were characterized by their differential expression of BDCA-1 (CD1c) and BDCA-2 (CD303). The absolute numbers of mDC and pDC cells in PBMC observed in this study was consistent with the findings of others (22). The mDC/pDC ratio in PBMC was highest in CRSNP⁻ patients (2.5; 1.6–3.0), followed by CRSNP⁺ patients (1.5; 0.8–2.0) and controls (1.1; 1.0–1.5; $P = 0.009$). In tissue samples, differences were more pronounced. Patients with CRSNP⁺ revealed a low mDC/pDC ratio due to a low number of mDC cells ($P < 0.001$, Fig. 4, panel A) whereas the number of pDC cells did not differ between the three groups ($P = 0.31$). Methodological problems, e. g. a bias due to high mDC vulnerability, cannot explain the observed differences in mDC counts, because tissue specimens of all three groups were treated identically. The CXCR3/CCR4 ratio was calculated as an indicator for the TH1 or TH2 prevalence. In PBMC,

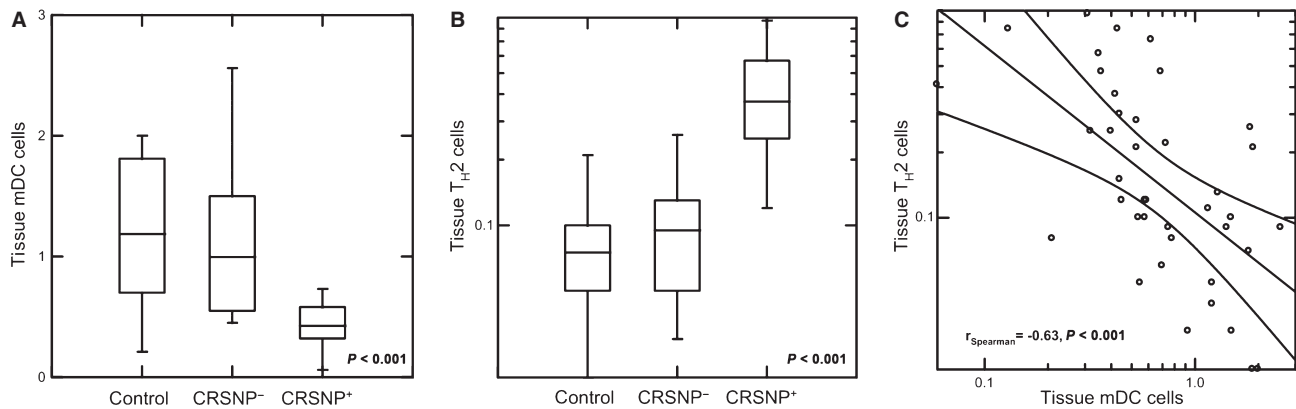


Figure 4. Quantity of (A) myeloid dendritic cells (mDC) and (B) CCR4⁺ cells (T_H2) in sinonasal tissues of controls ($n = 14$), CRSNP⁻ ($n = 10$) and CRSNP⁺ ($n = 17$). $P =$ Kruskal–Wallis test. Low numbers of mDC were correlated with high numbers of T_H2 cells (panel C).

CRSNP⁻ patients had a trend towards higher T_H1/T_H2 ratios than controls, whereas CRSNP⁺ patients had a trend towards lower ratios than controls. In tissue samples, disease related differences were more pronounced and did not differ between controls and CRSNP⁻ samples whereas a striking T_H2 skew in CRSNP⁺ patients ($P < 0.001$) was observed. The biological relevance of this observation was supported by the correlation between a low T_H1/T_H2 ratio and other indicators of a T_H2 biased immune response including high concentrations of IL-5 ($P < 0.05$), total IgE ($P < 0.05$) and tissue eosinophil counts ($P = 0.001$). The reason for the low T_H1/T_H2 ratio in CRSNP⁺ patients was not low numbers of T_H1 cells but five times higher amount of T_H2 cells in CRSNP⁺ patients ($P < 0.001$, Fig. 4, panel B). In atopic CRS patients, high CCR4 expression has already been shown by immunohistochemistry and polymerase chain reaction (23) and an accumulation of CCR4-positive-cells in airway epithelium has previously been observed in asthmatic patients (24).

We hypothesized that the T_H2 skew in CRSNP⁺ is driven by a low mDC/pDC ratio. Low numbers of mDC cells were correlated with high T_H2 cell numbers ($P < 0.001$) suggesting that a mDC deficit underlies the observed T_H2 skew in CRSNP⁺ patients, possibly by insufficient IL-12 production (25). The reason for the observed mDC/pDC imbalance in CRSNP⁺ remains unclear. Atopy was not more frequent in CRSNP⁺ patients and in this population, the mDC/pDC ratio was even higher in atopic than in nonatopic individuals ($P = 0.01$). Neither the number of previous surgery nor the duration of the disease showed a significant influence on the mDC/pDC or CXCR3/CCR4 ratio,

especially not between CRSNP⁻ and CRSNP⁺. Recent data suggest that *S. aureus* may play a role in the etiology of CRSNP⁺ and *in vitro* it was shown that different microbial compounds may selectively induce T_H1 cell-promoting or T_H2 cell-promoting dendritic cells (15). In this study, *S. aureus* detection had no influence on DC subsets or any other indicator of T_H2 bias nor did the frequency of *S. aureus* detection differ between the three groups (21). However, missing detection of *S. aureus* in nasal lavage does not exclude the presence of *S. aureus* endotoxins or other species, which might be pathophysiologically important (26). TGF- β maintains tolerance via the regulation of lymphocyte and dendritic cell proliferation, differentiation, and survival. Increased TGF- β 1 concentrations or activity have been found in CRSNP⁺ by some (27–29), but not all authors (7, 30, 31). We could observe elevated levels of TGF- β 1 in CRSNP⁺, produced by the increased eosinophils found in CRSNP⁺ most likely. TGF- β 2 levels between the three patient groups did not differ significantly. Coherence with the presence of *S. aureus* has already been investigated but not found significant (32).

In conclusion, the results of this study support the concept that CRSNP⁺ and CRSNP⁻ are two immunologically distinct subgroups of CRS. The dissimilarity is already detectable on the basic immunological level of DC differentiation. It is hypothesized that a relative deficit of myeloid dendritic cell subsets in CRSNP⁺ favors priming of naïve T cells towards T_H2 cells. This effect is not influenced by the presence of *S. aureus*. As a consequence, a T_H2 dominated cytokine profile characterizes CRSNP⁺ and results in an eosinophilic mucosal inflammation.

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