Characterization of T-cell subpopulations in patients with chronic rhinosinusitis with nasal polyposis

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ABSTRACT

Background: *There is an ongoing discussion concerning the potential origins of chronic rhinosinusitis with nasal polyposis (CRSwNP).*

Objective: *The aim of this study was to quantify subpopulations of T cells in peripheral blood and nasal polyps in CRSwNP to examine their influence on the etiology of this disease.*

Methods: *Tissue and blood samples were collected from 11 patients who underwent nasal sinus surgery, and these samples were analyzed by multicolor flow cytometry.*

Results: *There was a significantly lower frequency of $CD4^+$ T-helper (Th) cells and a significantly higher frequency of $CD8^+$ T cells among lymphocytes isolated from nasal polyps compared with peripheral blood mononuclear cells (PBMC). In both T-cell subpopulations, a shift mainly from naive T cells among peripheral blood lymphocytes toward an effector memory and terminally differentiated subtype predominance in nasal polyps was observed. Among $CD4^+$ T cells, the frequencies of cluster of differentiation (CD) 45RA- Forkhead-Box-Protein P3high ($FoxP3^{high}$) cytotoxic T-lymphocyte-associated Protein 4high ($CTLA\text{-}4^{high}$) activated regulatory T (T_{reg}) cells, and CD45RA- Forkhead-Box-Protein P3low ($FoxP3^{low}$) memory T cells were significantly increased in nasal polyps compared with PBMC.*

Conclusion: *In this study, we presented a detailed characterization of $CD4^+$ and $CD8^+$ T-cell subpopulations in patients with CRSwNP. $CD8^+$ T cells were more prominent in nasal polyps than in $CD4^+$ T cells. Both nasal $CD8^+$ T cells and $CD4^+$ T cells predominantly had an effector memory phenotype. Among $CD4^+$ T cells, activated T_{reg} cells were increased in nasal polyps compared with PBMC. The data point toward a local regulation of T-cell composition within the microenvironment of nasal polyps, which might be further exploited in the future to develop novel immunotherapeutic strategies.*

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Chronic rhinosinusitis (CRS) is an inflammatory condition of the mucosal nasal tissue that persists over at least 12 weeks or that demonstrates more than four episodes of infections per annum. The development of nasal polyps subdivides CRS into two different groups: CRS with nasal polyposis (CRSwNP) and CRS without nasal polyposis (CRSsNP).[1] CRSwNP can be further subclassified into two endotypes, depending on the histologic findings with either eosinophilic or fibrotic (noneosinophilic) cell infiltration. Among white patients in Europe and the United States, the most common subtype is eosinophilic, whereas Asian patients most often develop fibrotic nasal polyps.[2] The statistics on CRS are heterogeneously discussed in the literature: the prevalence of CRS is described to be ~5% (range, 1–19%) whereas the subgroup CRSwNP has a prevalence of ~2%.[3] There is still an ongoing discussion about the potential origins of nasal polyps, including a chronic inflammatory reaction of the nasal mucosa, anatomic variations that lead to constrictions of draining passages, or an allergic diathesis.

Recently, noninvasive colonization with fungi, including *Aspergillus fumigatus* and *Candida albicans*[4–6] or with the bacterium *Staphylococcus aureus,* has been indicated to act as triggers for the development of nasal polyps.[7,8] Moreover, it is believed that fungal proteins[4] and/or bacterial superantigens[9–11] induce recruitment of T cells into the nasal mucosa. Apart from the sheer number of T cells recruited, variations in T-lymphocyte subpopulations, especially of regulatory T (T_{reg}) cells,[12]

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are also considered to crucially contribute to the development of nasal polyps. Immunomodulatory properties that induce variations in T-lymphocyte subpopulations, indeed, have been noted both for fungal[13,14] and for staphylococcal proteins.[15] It is possible to classify T_{reg} cells into two subpopulations, depending on their origin: natural T_{reg} cells from the thymus and peripherally induced T_{reg} cells, which develop from peripheral naive T-helper (Th) cells.[16,17] A deficiency of peripheral T_{reg} cells can generate a chronic T-cell–mediated immunopathology. Reduced numbers and/or the function of T_{reg} cells were demonstrated to be responsible for different kinds of autoimmune disorders and chronic infections.[16,18] Even in oncologic studies, the importance of T_{reg} cells has been shown, and many researchers have focused on their influence.[19,20]

For many years, T_{reg} cells were identified by the surface markers CD4, CD25, and the intracellular transcription factor Forkhead-Box-Protein P3 (FoxP3).[21] FoxP3 is a key regulator of T_{reg} cell function and development.[16] Miyara *et al.*,[22] however, managed to further differentiate T_{reg} cells into subpopulations of $CD4^+$ $FoxP3^+$ T cells based on the surface marker CD45RA and the checkpoint inhibitor cytotoxic T-lymphocyte-associated Protein 4 (CTLA-4). In this context, resting T_{reg} (rT_{reg}) cells, activated T_{reg} (aT_{reg}) cells, and conventional nonsuppressive memory T cells with a low expression of Forkhead-Box-Protein P3 ($FoxP3^{low}$) were defined.[22,23] For identifying these subpopulations and analyzing the differences between peripheral and edaphic T cells, flow cytometry analysis is the method of choice.[18,22,24,25] The aim of this study was to compare subpopulations of T cells in peripheral blood and nasal polyps of patients with CRSwNP by using an up-to-date panel of markers for effector and T_{reg} cell subsets to elucidate their influence on the etiology of this disease.

METHODS

Preparation of Human Lymphocytes

Heparinized blood samples (10 mL) were obtained during surgery by venous puncture from 11 patients who were undergoing nasal sinus surgery, and the samples were transferred to the laboratory. Lymphocytes were separated by density-gradient centrifugation (10 minutes, $1000 \times g$) at room temperature on equal amounts of Ficoll (Biochrom GmbH, Berlin, Germany) by using a membrane-containing 10-mL cell tube (Greiner Bio-One GmbH, Frickenhausen, Germany). After washing twice in phosphate-buffered saline (PBS) solution (Gibco; BRL Life Technologies, Eggenstein, Germany), the cell number and viability were determined by using a cell counter plus analyzer system (CASY TT; Roche Innovatis AG, Reutlingen, Germany) according to the manufacturer's protocol. After centrifugation at 1600 rpm, the cells were frozen to $-80°C$ with 1 mL of freezing medium, which contained 10 parts of fetal calf serum (Linaris Biologische Produkte GmbH, Dossenheim, Germany) and one part of Dimethyl sulfoxide (DMSO) (Carl Roth GmbH + Co. KG, Karlsruhe, Germany).

Preparation of Tissue Samples

All tissue samples were collected during surgery from the 11 patients who were undergoing regular nasal sinus surgery. Polyp tissue specimens were processed to obtain tissue-associated lymphocytes; healthy nasal mucosa served as the control tissue. The polyps were cut into small fragments and mashed through a cell stainer (Greiner Bio-One, Frickenhausen, Germany) from 100 to 40 μm in PBS solution (Gibco; BRL Life Technologies). After washing twice in PBS solution, the cell number and viability were determined by using a CASY TT system according to the manufacturer's protocol. After centrifugation (5 minutes, 1600 rpm), the cells were frozen to $-80°C$ with 1 mL of freezing medium.

Flow Cytometry Analysis

The following antibodies were used: anti-CD45 Pacific Orange, anti-CD3 R-Phycoerythrin.cyanine dye 7 (PE.Cy7), anti-CD4 Pacific Blue, anti-CD8a Alexa 700, anti-CD28 R-Phycoerythrin (PE), anti-CD45RA Peridinin.Chlorophyll protein-Cyanin5.5 (Per.CP-Cy5.5), anti-C-C chemokine receptor type 7 (CCR7) Alexa 488, anti-CD25 Allophycocyanin (anti-CD25 APC), anti-Human Leukocyte Antigen - antigen D Related (HLA-DR) Alexa 700, anti-CD4 Fluorescein isothiocyanate (FITC), anti-Forkhead-Box-Protein P3 (FoxP3) Pacific Blue and anti-cytotoxic T-lymphocyte-associated Protein 4 (CTLA-4) R-Phycoerythrin (PE) (all from BioLegend, San Diego, California). Gating started on forward and side scatter properties, then CD45 was identified. Within this population, $CD3^+$ cells were detected, in addition, with Viability Dye 780 (eBioscence, Inc., San Diego, CA) in this step to detect apoptotic cells. Afterward, gating on viable $CD3^+$ $CD4^+$ or $CD3^+$ $CD8^+$ T cells followed, and $CD4^+$ and $CD8^+$ T-cell subsets were identified. Isotype control was performed by using mouse immunoglobulin G (mouse-IgG) Allophycocyanin and mouse-IgG R-Phycoerythrin (BioLegend) for a better discrimination of CTLA-4 and CD25.

The other populations could be easily discriminated. After blocking with 25 $\mu g/mL$ of normal mouse IgG (Sigma-Aldrich, Co., St. Louis, MO) for 15 minutes on ice, all the cells underwent cell surface staining, followed by intracellular staining. For intracellular staining of FoxP3 and CTLA-4, all the cells were treated with fixation buffer for 30 minutes at room tempera-

Table 1 **Baseline characteristics of the study group**

Clinical Feature	Study Group
Patients, no.	11
mean age, mean ± SD, y	53.27 ± 15.84
Women/men	4/7
Previous surgery, no. (%)	3 (27.27)
Eosinophilic polyps, no. (%)	10 (90,01)
Allergy, no. (%)	4 (36,36)
Samter triad, no. (%)	1 (9.09)

SD = Standard deviation.

ture (eBioscence). Afterward, permeabilization buffer was applied (eBioscence), followed by staining with anti-FoxP3 and anti–CTLA-4 for 45 minutes at room temperature. All the antibodies were used according to the manufacturers' instructions. Flow cytometry analysis was performed by using an LSR II flow cytometer (Becton, Dickinson and Co., San Diego, CA), and data were analyzed by using FlowJo software (TreeStar, Ashland, OR).

Ethics Issues

The study was approved by the ethics board of the Medical Faculty, Julius-Maximilian-University Wuerzburg (12/06), and all the participants gave written informed consent. All the authors significantly contributed to this work.

Statistics

Data are presented as mean ± standard deviation (SD). Statistical significance was analyzed by a two-tailed paired *t*-test by using GraphPad Prism Software 6.0c (GraphPad Software Inc., La Jolla, California). For nonparametric distribution, the Wilcoxon test was applied. Values of $p < 0.05$ were considered to be statistically significant.

RESULTS

Patient Characteristics

Eleven patients were included in the study group (seven male and four female patients). All the patients received intranasal topical steroids before surgery. The treatment started weeks before surgery, and, if the patients did not benefit from this medical therapy, then a surgical intervention was planned, according to the European guideline suggestions.[26] Patients with Churg-Strauss syndrome, primary ciliary dyskinesia, or cystic fibrosis were excluded. The mean ± SD age was 53.27 ± 15.84 years. Eosinophilic polyposis was described in the histologic evaluation of most of the patients (10/11). The characteristics are summarized in Table 1.

Predominance of Effector and/or Memory Cells among $CD4^+$ and $CD8^+$ T-cell Subsets in Nasal Polyps

The number of isolated lymphocytes from healthy nasal mucosa was too low for the multicolor flow cytometry panel. Lymphocytes of nasal polyps and peripheral blood from the 11 patients were analyzed by flow cytometry. After separating all apoptotic cells, there was a significantly lower amount of $CD3^+$ $CD4^+$ T cells and a significantly higher amount of $CD3^+$ $CD8^+$ T cells in nasal polyps compared with peripheral blood mononuclear cells (PBMCs) (Fig. 1 *A;* Table 2).

Further analyses of $CD3^+$ $CD4^+$ T cells showed significantly lower frequencies of $CCR7^+$ $CD45RA^+$ naive and $CCR7^+$ $CD45^-$ central memory T cells in nasal polyps compared with PBMCs (Table 2). The proportion of $CCR7^-$ $CD45RA^-$ effector memory Th cells was significantly higher in nasal polyps (Fig. 1 *B;* Table 2). There was no statistically significant difference in the frequency of $CCR7^-$ $CD45RA^+$ terminally differentiated T cells (Fig. 1 *B;* Table 2). This result was consistent with the amounts of $CD45RA^+$ $CD28^-$ terminally differentiated T cells without statistical significance (0.43 ± 0.75% PBMC, 1.6 ± 2.79% nasal polyps) between both study groups. $CD4^+$ $CD25^-$ $CD45RA^-$ memory Th cells were significantly higher in nasal polyps (44.11 ± 13.20% PBMC, 77.33 ± 8.00% nasal polyps), and a significantly lower fraction of $CD4^+$ $CD25^-$ $CD45RA^+$ naive Th cells was observed in the polyps (36.60 ± 15.57% PBMC, 7.71 ± 2.65% nasal polyps).

Quantitative analysis of $CD3^+$ $CD8^+$ T cells showed significantly lower frequencies of $CCR7^-$ $CD45RA^+$ terminally differentiated and $CCR7^+$ $CD45RA^+$ naive subpopulations among these cells in nasal polyps, whereas $CCR7^-$ $CD45RA^-$ effector memory (cytotoxic) T cells were significantly higher in nasal polyps (Fig. 1 *C;* Table 2). $CCR7^+$ $CD45RA^-$ central memory cytotoxic T cells were significantly lower in nasal polyps (Fig. 1 *C;* Table 2). $CD45RA^+$ $CD28^-$ terminally differentiated T cells were also significantly higher in PBMC (12.73 ± 6.94% PBMC, 4.97 ± 4.95% nasal polyps). However, there was no statistically significant difference in the upregulation of the marker for activation HLA-DR[24] on $CD3^+$ T cells (Fig. 1 *D*) between both groups (2.69 ± 1.16% PBMC, 4.06 ± 1.9% nasal polyps).

Significant Increase in aT_{reg} Cells and Nonsuppressive Conventional $FoxP3^{low}$ Memory T Cells in Nasal Polyps compared with PBMC

Most of the $CD3^+$ $CD4^+$ T cells were $FoxP3^-$ in both groups (Fig. 2 *A;* Table 3). In detail, there were significantly lower frequencies of $CD3^+$ $CD4^+$ $CD45RA^+$ $FoxP3^-$ naive T cells and significantly higher frequencies of $CD3^+$ $CD4^+$ $CD45RA^-$ $FoxP3^-$ memory T cells

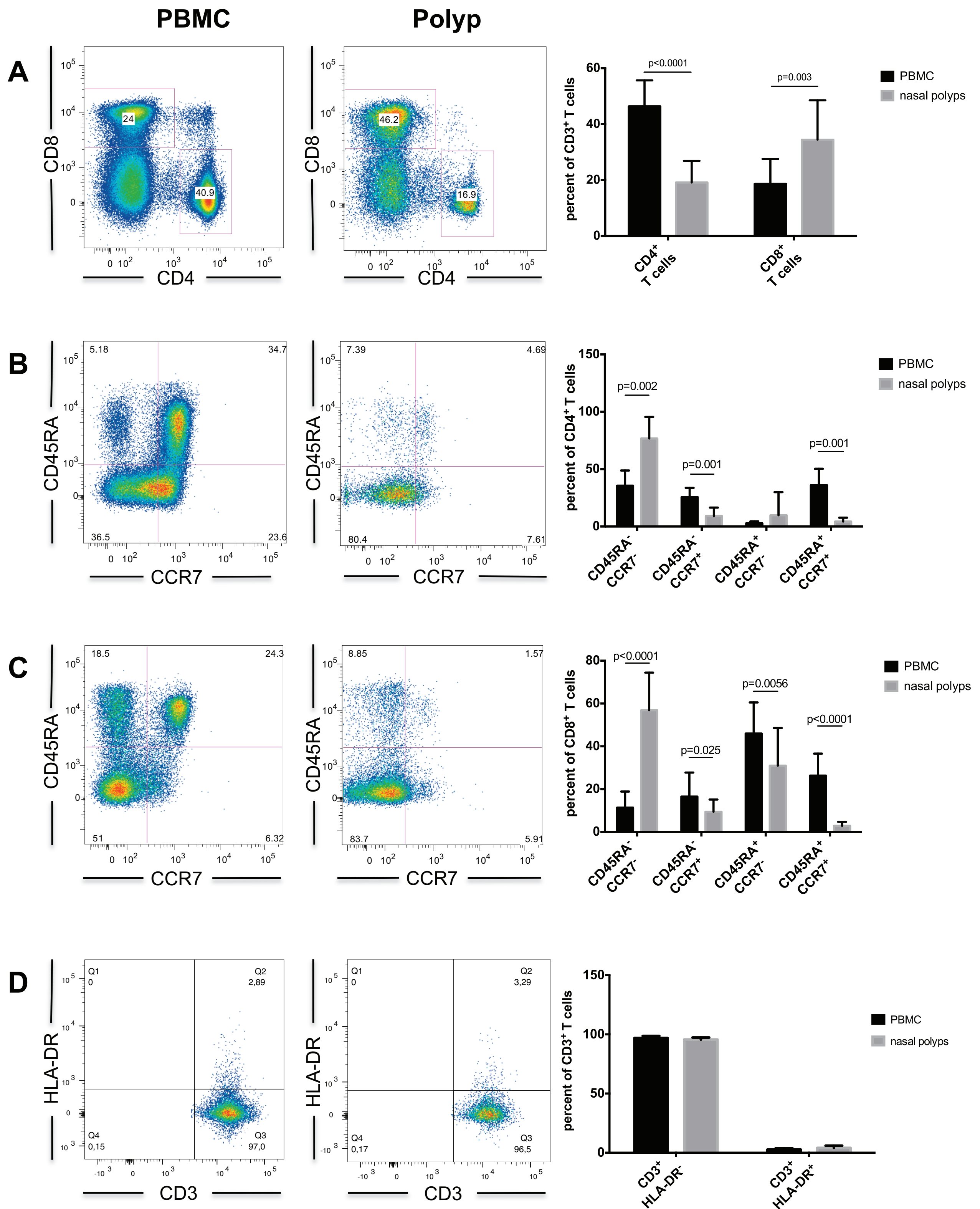

Figure 1. *(A) $CD8^+$ and $CD4^+$ T cells in PBMC and nasal polyps. (B) $CD4^+$ T cell subsets in PBMC and nasal polyps. (C) $CD8^+$ T cell subsets in PBMC and nasal polyps. (D) Upregulation of HLA-DR on $CD3^+$ T cells in PBMC and nasal polyps. Data are shown as means ± standard deviations (SD) of the 11 patients. PBMC = peripheral blood mononuclear cells; HLA-DR = human leukocyte antigen - antigen D related.*

Table 2 **CD3$^+$ T cell subpopulations**

T Cells	PBMC, mean ± SD*	Nasal Polyps, mean ± SD*	*p* Value#
CD4$^{+(\%)}$	46.38 ± 9.27	19.15 ± 7.73	<0.0001
CD45RA$^-$ CCR7$^-$ effector memory	35.57 ± 13.27	76.72 ± 18.84	0.002
CD45RA$^+$ CCR7$^-$ terminal differentiated	2.81 ± 1.63	9.76 ± 20.23	0.052
CD45RA$^-$ CCR7$^+$ central memory	25.62 ± 8.04	9.18 ± 7.28	0.001
CD45RA$^+$ CCR7$^+$ naive	36.00 ± 14.40	4.35 ± 3.36	0.001
CD8$^{+(\%)}$	18.65 ± 8.91	34.44 ± 14.11	0.003
CD45RA$^-$ CCR7$^-$ effector memory	11.34 ± 7.52	56.82 ± 17.62	<0.0001
CD45RA$^+$ CCR7$^-$ terminally differentiated	45.96 ± 14.55	30.94 ± 17.59	0.0056
CD45RA$^-$ CCR7$^+$ central memory	16.49 ± 14.55	9.39 ± 5.73	0.025
CD45RA$^+$ CCR7$^+$ naive	26.31 ± 10.28	2.83 ± 1.90	<0.0001

PBMC = peripheral blood mononuclear cells; SD = standard deviation; CD45RA = cluster of differentiation 45RA; CCR7 = C-C chemokine receptor type 7.
**Of the 11 patients.*
#Paired t-test.

in nasal polyps compared with peripheral blood (Fig. 2 *A;* Table 3). CD3$^+$ CD4$^+$ CD45RA$^-$ FoxP3^{high} aT_{reg} and CD3$^+$ CD4$^+$ CD45RA$^-$ FoxP3^{low} memory T cells were significantly higher in nasal polyps, but CD3$^+$ CD4$^+$ CD45RA$^+$ FoxP3^{low} rT_{reg} cells were not elevated in nasal polyps (Fig. 2 *A;* Table 3). The mean fluorescence intensity of CTLA-4 was high on aT_{reg} cells and low on rT_{reg} cells in both groups (Fig. 3 *A*). To determine whether there is a mismatch of conventional CD45RA$^-$ FoxP3^{low} memory, CD45RA$^-$ FoxP3$^-$ memory, and CD45RA$^+$ FoxP3$^-$ naive CD4$^+$ T cells or CD8$^+$ T cells to T_{reg} cells (aT_{reg} and rT_{reg}), the ratio of these cells was calculated (Fig. 3 *B*). The ratio of conventional CD4$^+$ T cells to T_{reg} cells was significantly higher in peripheral blood than in nasal polyps.

DISCUSSION

In this study, a detailed quantification of the subpopulations of T lymphocytes in peripheral blood and nasal polyps in CRSwNP was presented. As previously shown, there was a switch from mainly CD4$^+$ T cells among peripheral blood $\alpha\beta$ T cells to mainly cytotoxic CD8$^+$ T cells in nasal polyps, with a significant domination of effector T cells among both CD4$^+$ and CD8$^+$ T cells in nasal polyps compared with peripheral blood.[27–29] Moreover, aT_{reg} cells were significantly increased among CD4$^+$ T cells in nasal polyps compared with peripheral blood in patients with CRSwNP.

CD4$^+$ Th cells can exhibit different properties, depending on their differentiation into one of the following Th1, Th2 or Th17 subsets. However, newer studies showed a variation in different T-cell subtypes present in the infected nasal mucosa but with a predominant Th1 subset in CRSsNP and Th2 subset in CRSwNP.[29–31] Th17 T cells act synergistically with the other subsets and produce, especially interleukin (IL) 17, IL-21, IL-22, and IL-26. Among patients with CRSwNP, demographic variation seemed to play an additional role with respect to T-cell subdivision: Th2-biased cytokine profiles were mostly found in patients from Europe and the United States, whereas Th1:Th17 profiles were predominantly found in Asian patients.[25]

In the present study, the majority of T lymphocytes in nasal polyps were CD8$^+$ T cells with an effector memory phenotype. These findings underscored the importance of CD8$^+$ cytotoxic T-cell responses in the pathogenesis of nasal polyps. The role of CD8$^+$ T cells in the defense against viruses and intracellular bacteria is clearly understood, but their role in allergic diseases is not yet completely known. Tang *et al.*[32] described effector CD8$^+$ T cells as dampening allergic responses in the effector phase. In contrast, there are other studies that identify effector memory CD8$^+$ T cells as pathogenic in allergic responses.[33,34]

Because fungal and bacterial colonization of the local nasal mucosa has been identified to have at least contributed to T-cell recruitment into polyps,[4–11] we were curious to study HLA-DR expression as a marker of recent T-cell activation. In contrast to their overall activated phenotype, the edaphic CD3$^+$ T cells did not show any increased HLA-DR expression compared with blood T cells. This lack in HLA-DR upregulation might be caused by differentiation of the CD4$^+$ and CD8$^+$ T-cell subsets outside the polyp tissue in lymphoid organs without a restimulation of the T cells within the nasal polyp itself. Tissue-resident CD8$^+$ T cells have been shown in mice to be maintained *in situ,* without local antigenic restimulation,[35] and antifungal memory CD8$^+$ T cells have also been shown to survive in the absence of antigen or CD4$^+$ T-cell help.[36] Alternatively, the preoperative treatment with local or systemic corticoids inhibits the upregulation of HLA-DR

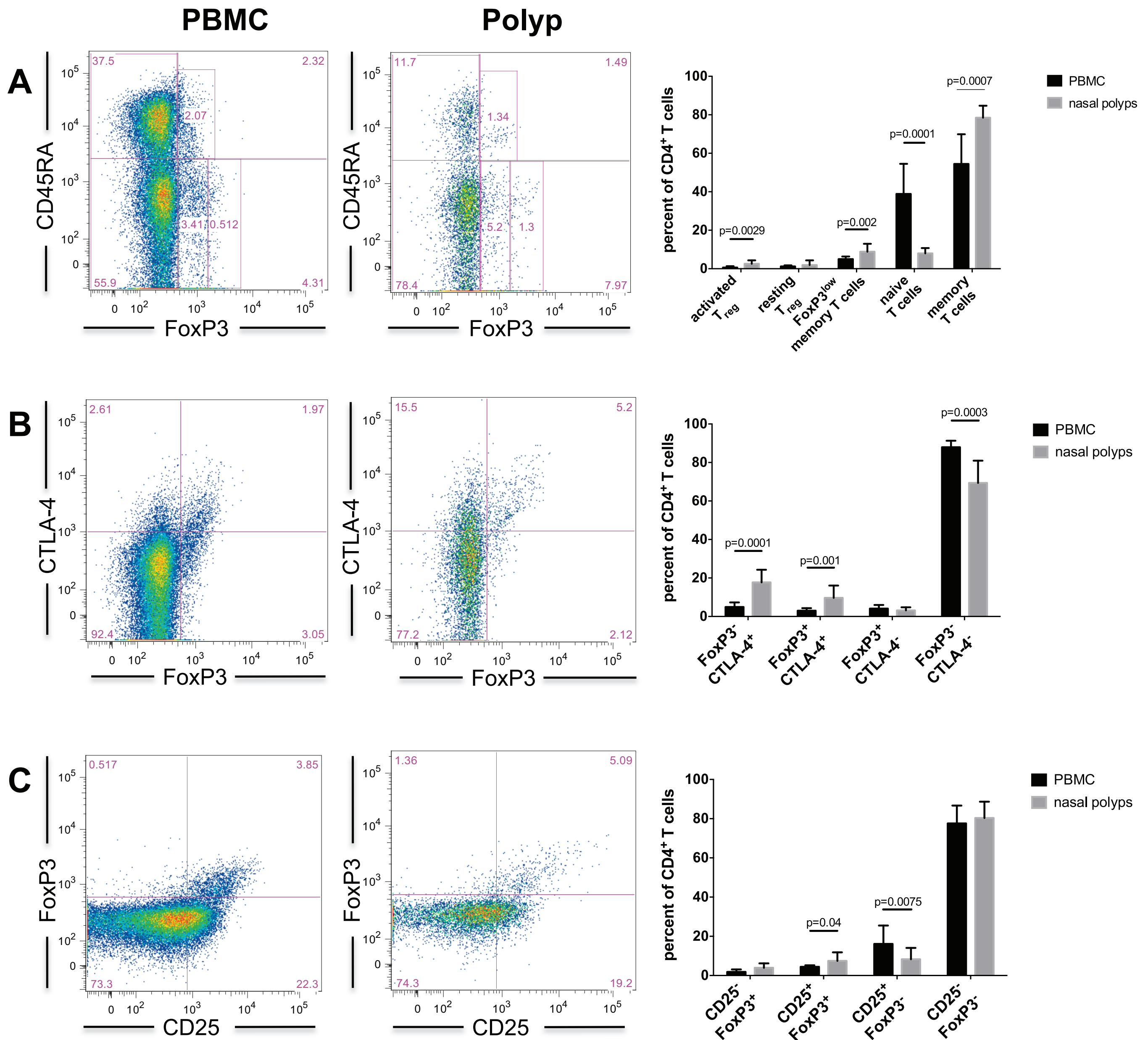

Figure 2. *Regulatory T cell (T_{reg}) subpopulations in PBMC and nasal polyps. (A) Frequency of $CD4^+$ resting T_{reg} (rT_{reg}), activated T_{reg} (aT_{reg}), and $FoxP3^{low}$ memory T cells in PBMC and nasal polyps. (B) Expression of CTLA-4 by $FoxP3^+$ cells in PBMC and nasal polyps. (C) $CD4^+$ $CD25^+$ $FoxP3^+$ activated T_{reg} in PBMC and nasal polyps. Data are shown as means ± standard deviations (SD) of the 11 patients. PBMC = peripheral blood mononuclear cells; $FoxP3^{low}$ = low expressive Forkhead-Box-Protein P3; CTLA-4 = cytotoxic T-lymphocyte-associated Protein 4; $FoxP3^+$ = Forkhead-Box-Protein P3.*

and reactivation of the T cells. Moreover, corticoid treatment might also have had a differential impact on T_{reg} cells and conventional T cells, although information about the effects of glucocorticoids on functional properties of T_{reg} cells is inconsistent in the literature. Some investigators see an increase in T_{reg} cells after glucocorticoid therapy.[37] However, in contrast, de Paz *et al.*[38] described interindividually different responses of T_{reg} cells to glucocorticoids among different patients, and Tabares *et al.*[39] showed T_{reg} cells to be relatively resistant to the immunosuppressive actions of glucocorticoids, which seemed to be in line with the present study findings.

T_{reg} cells play an important role in maintaining peripheral tolerance to self-antigens and in counteracting the inflammatory activity of effector Th cell subsets.[37] Classically, T_{reg} cells were analyzed by staining for CD4, CD25, and FoxP3 expression. As previously mentioned, Miyara *et al.*[22] described a revised classification of $CD4^+$ $FoxP3^+$ T cells into $FoxP3^{high}$ aT_{reg}, $FoxP3^{low}$

Table 3 **T_{reg} subpopulations analyzed by staining of CD4, CD45RA, FoxP3, and CTLA-4**

$CD3^+CD4^+$ T Cells	PBMC, mean ± SD*, %	Nasal Polyps, mean ± SD*, %	p Value#
$CD45RA^+$ $FoxP3^{low}$ CTLA-4^{low} resting T_{reg} cells	1.24 ± 0.51	1.93 ± 2.44	0.38
$CD45RA^-$ $FoxP3^{high}$ CTLA-4^{high} activated T_{reg} cells	0.60 ± 0.70	2.57 ± 1.81	0.0029
$CD45RA^-$ $FoxP3^{low}$ memory T cells	5.03 ± 1.34	8.87 ± 4.08	0.002
$CD45RA^-$ $FoxP3^-$ memory T cells	54.45 ± 15.44	78.49 ± 6.20	0.0007
$CD45RA^+$ $FoxP3^-$ naive T cells	38.85 ± 15.65	8.00 ± 2.78	0.0001

T_{reg} = regulatory T; CD45RA = cluster of differentiation 45RA; FoxP3 = Forkhead-Box-Protein P3; CTLA-4 = cytotoxic T-lymphocyte-associated Protein 4; PBMC = peripheral blood mononuclear cell; SD = standard deviation; $FoxP3^{low}$ = low expression of Forkhead-Box-Protein P3; $FoxP3^{high}$ = high expression of Forkhead-Box-Protein P3; CTLA-4^{low} = low expression of cytotoxic T-lymphocyte-associated Protein 4; CTLA-4^{high} = high expression of cytotoxic T-lymphocyte-associated Protein 4.
**Of the 11 patients.*
#Paired t-test.

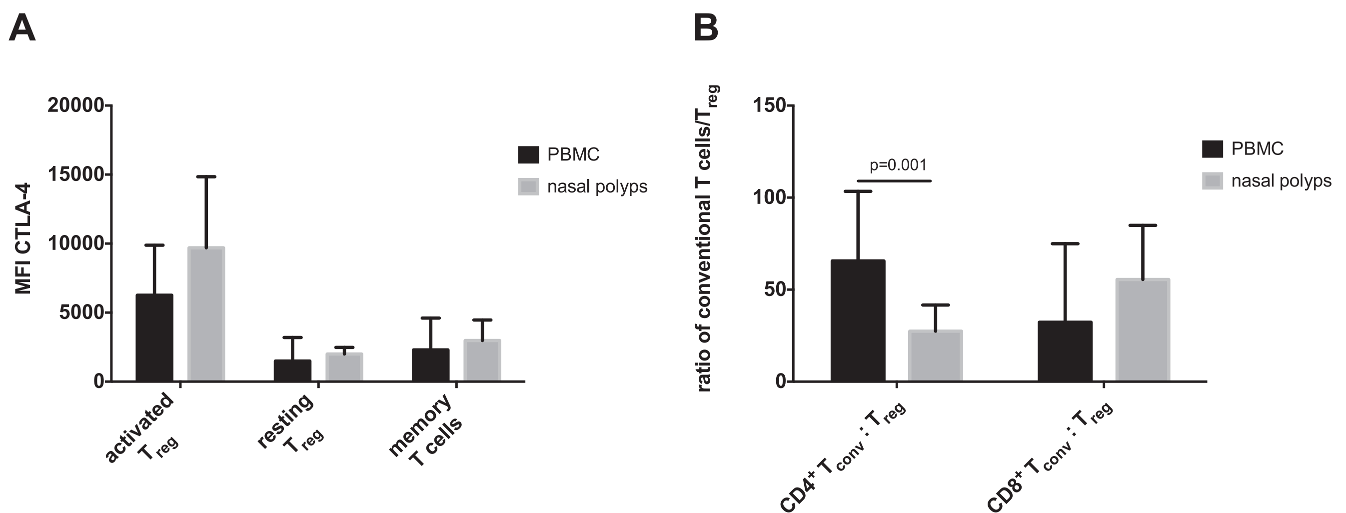

Figure 3. *(A) Mean fluorescence intensity (MFI) of CTLA-4 on $FoxP3^+$ T cells in PBMC and nasal polyps. (B) Ratio of conventional T cells to regulatory T cells in $CD4^+$ and $CD8^+$ T cells. Data are shown as means ± standard deviations (SD) of the 11 patients. CTLA-4 = cytotoxic T-lymphocyte-associated Protein 4; $oxP3^{high}$ = high expression of Forkhead-Box-Protein P3.*

rT_{reg}, and conventionally nonsuppressive $Foxp3^{low}$ memory T cells. The $FoxP3^{low}$ memory T cells contain Th17 T cells and are not suppressive. Moreover, within the three subpopulations described, $FoxP3^{low}$ memory T cells had the highest frequencies in our study, which indicated that the Th17 pathway might also be important in the CRSwNP pathogenesis, even in patients of European descent. Apart from $FoxP3^{low}$ memory T cells, we found significantly more aT_{reg} cells among $CD4^+$ T cells in polyp tissue compared with T cells from peripheral blood (Fig. 2). Furthermore, we found a significantly lower ratio of conventional memory and naive $CD4^+$ T cells to T_{reg} cells in nasal polyps than in PBMC. This indicated that T_{reg} cells should be able to efficiently suppress local $CD4^+$ T-cell responses in the polyp and might thereby prevent further exacerbation of the disease. In contrast, the $CD8^+$:T_{reg} ratio was not significantly different in nasal polyps compared with peripheral blood, which indicated that T_{reg} cells might be less able to control $CD8^+$ T cells than conventional $CD4^+$ T cells in polyps.

To our knowledge, this study provided the first comparison of different T_{reg} cell subpopulations in peripheral blood and nasal polyps when using multicolor flow cytometry. A previous study on T_{reg} cells in CRSwNP used flow cytometry to characterize T_{reg} cells in peripheral blood and two-color immunohistochemistry to detect T_{reg} in nasal polyps.[17] Other studies focused on the nasal mucosa and/or polyps by using immunohistochemistry to detect T_{reg} cells[2,12] Although T_{reg} cell frequencies in peripheral blood in healthy subjects and in patients with CRSwNP do not seem to differ,[17] the two studies that analyzed T_{reg} cells in the mucosa reported a decrease in absolute

as well as relative numbers compared with patients with CRSsNP and mucosal tissue from controls.[2,12] In these studies, however, FoxP3 expression alone was used to identify T_{reg} cells. Because our own analyses revealed that the majority of $CD4^+$ $FoxP3^+$ cells in nasal polyps were nonregulatory memory T cells, further studies are needed to determine the abundance of T_{reg} cells in polyps versus mucosal tissues of patients with CRSsNP and "healthy" controls.

The results of this study were limited by (1) the small number of subjects, and (2) there not being a control group of healthy nasal mucosa. The study was declared to be a pilot project with a limited amount of samples. Because there was no control group with healthy mucosa, interpretation of the data was affected. For example, a significantly lower ratio of conventional memory and naive $CD4^+$ T cells to T_{reg} cells in nasal polyps compared with PBMC in patients with CRSwNP was observed. But, if the ratio of conventional T cells to T_{reg} cells were much lower in healthy control tissue than in polyps, this would indicate that there are not enough T_{reg} cells in the polyps. Without a control group of healthy mucosa, it was difficult to provide further mechanistic insight. Unfortunately, analysis of lymphocytes from healthy nasal mucosa failed due to low amounts of these cells in this noninflammatory tissue, and high cell amounts are necessary for this multicolor flow cytometry analysis. When assuming that most of the lymphocytes in healthy nasal mucosa were circulating intravascular cells, a control group of PBMC seemed to be equivalent, and, furthermore, a comparison of isolated T cells from tissue with PBMC is well established in the literature.[23,40]

CONCLUSION

In this study, a detailed contemporary characterization of T-cell subpopulations was presented. $CD8^+$ (cytotoxic) T cells are the main subpopulation in nasal polyps. Both $CD8^+$ T cells and $CD4^+$ T cells showed differentiation into a more effector memory phenotype. T_{reg} cells are important for immune homeostasis and aT_{reg} cells were increased relative to conventional $CD4^+$ T cells but not to $CD8^+$ T cells in nasal polyps compared with peripheral blood, which indicated that a failure of T_{reg} cells to control the $CD8^+$ T cell response in the polyp might contribute to the development of nasal polyposis.

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