Accumulation of CD69+ tissue-resident memory T cells in the nasal polyps of patients with chronic rhinosinusitis

PASCAL IKKRATH1, NORBERT KLEINSASSER2, XIN DING3, CHRISTIAN GINZKEY4, NIKLAS BEYERSDORF3, RUDOLF HAGEN1, THOMAS KERKAU3 and STEPHAN HACKENBERG1

1Department of Otorhinolaryngology, Plastic, Aesthetic and Reconstrucive Head and Neck Surgery, University of Wuerzburg, D-97080 Wuerzburg, Germany; 2Department of Otorhinolaryngology, Head and Neck Surgery, Kepler University Hospital, A-4021 Linz, Austria; 3Institute for Virology and Immunobiology, University of Wuerzburg, D-97080 Wuerzburg; 4Department of Otorhinolaryngology, Head and Neck Surgery ‘Otto Körner’, University Medical Center Rostock, D-18057 Rostock, Germany

Received December 3, 2017; Accepted April 16, 2018

DOI: 10.3892/ijmm.2018.3653

Abstract. In patients with chronic rhinosinusitis with nasal polyps (CRSwNP), a relative accumulation of cluster of differentiation (CD)8+ T cells over CD4+ T cells occurs in nasal polyps compared with the peripheral blood. Nasal CD8+ T cells and CD4+ T cells predominantly present an effector memory phenotype. Immunological studies have reported that memory T cells recirculate from the tissues to the peripheral blood and a high percentage of these T cells persist within the tissue. The aim of the present study was to characterize CD69+ sphingosine-1-phosphate receptor 1 (S1PR1) tissue resident memory T cells (Trm) in the polyps of patients with CRSwNP. Tissue and blood samples were collected from 10 patients undergoing nasal sinus surgery. Expression of specific extracellular and intracellular molecules were analyzed using multicolor flow cytometry. A significantly higher level of CD8+ T cells than CD4+ T cells was present in nasal polyps, while significantly more CD4+ T cells than CD8+ T cells were detected in the peripheral blood of patients with CRSwNP. The frequency of CD69+ T cells was significantly higher in CD8+ and CD4+ T cells in nasal polyps compared with the peripheral blood. The frequency of CD69+ S1PR1+ Trm was also significantly higher in CD4+ and CD8+ T cells from nasal polyps compared with the peripheral blood. Within polyps, the frequency of CD69+ S1PR1+ Trm was again significantly higher in CD8+ compared with CD4+ T cells. In summary, a significantly higher frequency of CD69+ S1PR1+ T cells was observed in the nasal polyps compared with the peripheral blood in patients with CRSwNP. The results of the present study suggest that local regulation of the immune response occurs within nasal polyps. As such, Trm should be considered a potential stimulus in the pathogenesis of nasal polyps. However, the role of Trm in nasal polyps as a pathogenic trigger of the local inflammatory reaction requires further investigation.

Introduction

Chronic rhinosinusitis (CRS) can be divided into two subtypes: CRS without nasal polyps (CRSsNP) and CRS with nasal polyps (CRSwNP). CRSwNP is a heterogeneous disease with an unclear pathophysiology (1). CRSwNP is subdivided into different endotypes by inflammatory markers and/or cells that serve a role in the disease (1). One theory about the maintenance of the inflammatory reaction is a variation of T cells within the polypoid tissue (2). In a previous study (2), cluster of differentiation (CD)4+ and CD8+ T cell subsets were characterized by multicolor flow cytometry, which revealed a predominance of CD8+ T cells in nasal polyps compared with the peripheral blood mononuclear cells (PBMCs) in patients with CRSwNP. There was a significantly higher amount of CD8+ T cells compared with CD4+ T cells in nasal polyps, whereas there were significantly more CD4+ T cells compared with CD8+ T cells in the PBMCs (2). These data suggest a local regulation of the immune response within nasal polyps. Furthermore, both CD4+ and CD8+ T cells were able to differentiate into an effector memory phenotype. It was postulated that variations in regulatory T cells are responsible for a number of autoimmune diseases (3). A previous study reported a significant increase in activated regulatory T cells (Treg) in polypoid tissue compared with the PBMCs in patients with CRSwNP (2). Specific triggers, including fungal colonization (4-6) or Staphylococcal...
superantigens (7,8) influence T cell recruitment in CRSwNP and may also influence T cell subset composition within the polyps. Additionally, local changes in B cell subpopulations in the nasal polyps compared with PBMCs has been reported and underlines the role of lymphocytes in this disease (9).

Memory T cells represent the main subset of CD4+ and CD8+ T cells in polypoid tissue in patients with CRSwNP (2). These memory T cells can be classified into two subsets based on the expression of homing receptors, including C-C chemokine receptor 7 (CCR7) (10), into CCR7+ central memory T cells (Tcm) and CCR7 effector memory T cells (Tem). After antigen presentation and differentiation into Tem, T cells migrate towards non-lymphoid tissue (NLT). It was previously assumed that these T cells recirculate into the PBMCs; however, immunological studies have reported persistent populations of tissue-resident memory T cells (Trm) in NLT (11). These T cells may be identified by a high expression of CD69 and a down-regulation of the sphingosine-1 phosphate receptor 1 (SIPR1). SIPR1 is required for naïve T cells to circulate and exit the thymus and peripheral lymphoid organs (11). SIPR1 down-regulation is an essential marker for Tem (12). In contrast, CD69 upregulation is a major signal for the persistence of Tem in NLT (13). Both signals are necessary for the persistence of Tem (14) in the local tissue. Chemoattractant receptors, including CCR7, also serve a role in the egress of T cells from NLT (15), therefore a downregulation in CCR7 is a sign that T cells persist in the tissue and do not recirculate. These Tem cells are described as being more potent in protection against local infections compared with memory T cells residing elsewhere (16). Furthermore, Tem have been reported to serve a role in drug hypersensitivity reactions (17).

The aim of the present study was to quantify the number of CD4+ and CD8+ Tem cells in the nasal polyps compared with PBMCs in patients with CRSwNP and to determine whether there were differences between these subpopulations.

Materials and methods

Ethical approval. The study was approved by the Ethics Board of the Medical Faculty, Julius-Maximilian-University, Wuerzburg, Germany. Ethics approval and written informed consent was obtained from all patients.

Preparation of human lymphocytes. A total of 10 ml of heparinized blood samples were obtained intraoperatively from 10 patients undergoing regular paranasal sinus surgery due to CRSwNP. Additionally, nasal mucosa was collected from 3 patients diagnosed with CRSwNP undergoing paranasal sinus surgery between August and September 2016 at the local university. Exclusion criteria were as above. All patients were female and mean age was 45.33±17.44. The polyps and nasal mucosa samples were cut into small fragments and mashed through a cell strainer (Greiner Bio-One) from 100 to 40 µm in PBS. Tissues were washed twice in PBS and the cell number and viability were determined using a CASY TT system according to the manufacturer's protocol. Following centrifugation for 5 min at 1.600 rpm, cells were frozen at -80°C in 1 ml freezing medium.

Fluorescence-activated cell sorting. The following antibodies were used: Anti-CD45 Pacific Orange (1:300; MHCD4550; Thermo Fisher Scientific Inc., Waltham, MA, USA), anti-CD3 phycoerythrin (PE)-Cy7 (1:300; 300420); anti-CD4 Pacific Blue (1:50, Nr. 300521), anti-CD8α Alexa 700 (1:50, 301028) anti-CD45RA peridinin chlorophyll protein complex-Cy5.5 (1:50; 301422), anti-CCR7 Alexa488 (1:80; 353206), anti-CD69 Alexa 488 (1:50, Nr. 301996), anti CD69 allophycocyanin (APC; 1:50; 310909), anti-CD4 fluorescein isothiocyanate (FITC) (1:40; 300506), anti-FoxP3 Pacific Blue (1:25; 320216) anti-CD52 (CTLA-4) PE (1:400; 349906; all BioLegend, Inc., San Diego, CA, USA) and anti-SIPR1 eFluor 660 (1:20; 50-3639-41; eBioscience; Thermo Fisher Scientific Inc., Inc.), isotype control staining was performed using mouse-IgG APC (1:80; 137214) and mouse-IgG PE (1:25; 400140) (BioLegend, Inc.). Viability Dye780 (1:10; 65-0865-14; eBioscience; Thermo Fisher Scientific, Inc.) was used to detect apoptotic and dead cells. Following blocking with 25 µg/ml normal mouse immunoglobulin G (1:50, Nr. I5381, Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) for 15 min on ice, all cells underwent cell surface staining on ice for 30 min, followed by intracellular staining. For intracellular staining of Foxp3 and CTLA-4, cells were treated with fixation buffer (eBioscience; Thermo Fisher Scientific, Inc.) for 30 min at room temperature. Permeabilisation buffer was subsequently applied (eBioscience; Thermo Fisher Scientific, Inc.) followed by staining with anti-Foxp3 and anti-CTLA-4 for 45 min at room temperature. All antibodies were used according to the manufacturer's protocol. FACS analysis was performed using an LSR II flow cytometer and the data were analyzed using FlowJo software (FlowJo LLC, Ashland, OR, USA).

Statistical analysis. Data are presented as mean ± standard deviation. Statistical significance was analyzed by a two-tailed paired t-test using GraphPad Prism Software 6.0c (GraphPad Software, Inc., La Jolla, CA, USA). For non-parametric distribution the Wilcoxon test was applied. P<0.05 was considered to indicate a statistically significant difference.
Results

Patient characteristics. A total of 10 patients with CRSwNP were included in the present study (8 male and 2 female). The mean age was 45 ± 9.48 years. Eosinophilic polyposis was described in the histological evaluation of most of the patients (7/10). Patient characteristics are summarized in Table I.

<table>
<thead>
<tr>
<th>Clinical feature</th>
<th>Study group (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (standard deviation)</td>
<td>44 (9.48)</td>
</tr>
<tr>
<td>Sex, female/male</td>
<td>2/8</td>
</tr>
<tr>
<td>Previous surgery, n (%)</td>
<td>2 (20)</td>
</tr>
<tr>
<td>Eosinophilic polyps, n (%)</td>
<td>7 (70)</td>
</tr>
<tr>
<td>Allergy, n (%)</td>
<td>4 (40)</td>
</tr>
<tr>
<td>Samter's triad, n (%)</td>
<td>1 (10)</td>
</tr>
</tbody>
</table>

Higher frequency of CD69+ cells in CD4+ and CD8+ T cells in CRSwNP. The amount of CD3+ CD4+ T cells among CD45RA leukocytes was significantly higher in PBMCs compared with in nasal polyps from patients with CRSwNP (Table II). In contrast, CD3+ CD8+ T cells were significantly increased in nasal polyps compared with PBMCs (Table II). A significant increase in the frequency of CD69-expressing cells was observed among CD3+ CD4+ and CD3+ CD8+ T cells in the nasal polyps compared with PBMCs (Table II; Fig. 1). However, these cells did not constitute recently activated T cells, as T cells from nasal polyps do not express elevated levels of human leukocyte antigen-antigen D related (HLA-DR) compared with PBMCs (2). In PBMCs, a majority of cells were CD69+ (Table II; Fig. 1). The percentage of CD69+ cells was significantly higher among CD8+ compared with CD4+ T cells in PBMCs and polyps (Table II).

SIPR1 expression in CD4+ and CD8+ T cells in CRSwNP. The percentage of SIPR1+ between CD4+ and CD8+ T cells was significantly higher in nasal polyps compared with PBMCs (Table II; Fig. 2). This is most likely attributable to down modulation of SIPR1 expression by its ligand S1P, which is abundantly present in PBMCs (18). In PBMCs the proportion of cells expressing SIPR1 was significantly higher among CD8+ T cells compared with CD4+ T cells, whereas no significant difference in SIPR1 expression was observed between CD8+ and CD4+ T cells in nasal polyps (Table II).

CD69+ SIPR1+ Trm was significantly increased in nasal polyps compared with PBMCs. In patients with CRSwNP, the frequency of CD69+ SIPR1+ Trm in CD4+ and CD8+ T cells was significantly higher in nasal polyps compared with PBMCs (Table II; Fig. 3). CD8+ T cells contained more CD69+ SIPR1+ Trm compared with CD4+ T cells, irrespective of the anatomical compartment analyzed (Table II).

CD69 overexpression, activated Treg (aTreg) and conventional memory T cells (Tconv) in CRSwNP. Further analysis of CD4+ T cell subsets with respect to CD45RA and FoxP3 expression revealed significantly more CD3+ CD4+ CD45RA+ FoxP3− naïve T cells in PBMCs compared with nasal polyps in patients with CRSwNP (Table III). However, CD69 expression was significantly higher in phenotypically naïve CD4+ T cells in nasal polyps compared with PBMCs (Table III; Fig. 4). The number of CD45RA+ FoxP3− conventional Tconvs, cells was significantly higher among CD4+ T cells in nasal polyps with significantly higher expression of CD69 in these cells compared with PBMCs (Table III; Fig. 4). The proportion of CD45RA+ FoxP3low memory T cells with Th17 potential was significantly elevated among CD4+ T cells in nasal polyps, while CD69 expression was also significantly higher in these cells in nasal polyps compared with PBMCs (Table III; Fig. 4). Percentages of CD4+ CD45RA+ FoxP3low resting Treg (Treg) were not significantly different in PBMCs and polyps (Table III). In contrast, CD4+ T cells in nasal polyps from patients with CRSwNP contained significantly more CD45RA− FoxP3hi aTreg compared with in PBMCs (Table III). tTreg and aTreg cells harbored significantly more CD69+ cells in nasal polyps compared with in PBMCs (Table III; Fig. 4). In nasal polyps, aTreg contained significantly more CD69+ cells compared with tTreg (Fig. 4). Among the different CD4+ T cell subsets in nasal polyps, aTreg had the highest number of CD69-expressing cells followed by Tconvs (Table III).

Homing receptor CCR7 on CD4+ and CD8+ T cells in CRSwNP. A significantly reduced proportion of CCR7+ cells among CD3+ CD4+ and CD3+ CD8+ T cells was apparent in nasal polyps compared with PBMCs (Table II). CD8+ T cells had a significantly lower incidence of CCR7+ cells compared with CD4+ T cells in nasal polyps and PBMCs (Table II).

Lack of CD4+ and CD8+ T cells in CRSsNP. Evaluation of lymphocytes in the nasal mucosa of patients with CRSsNP was not possible due to the low amounts of these cells in the tissue harvested intraoperatively. Only cell counts between 3 and 207 were found for CD4+ and CD8+ T cells, thus a statistically appropriate analysis was not possible. For this reason, a comparison of T cell subsets in samples from patients with CRSsNP and CRSwNP was not possible in the present study.

Discussion

In the present study, a detailed quantification of Trm in PBMCs and nasal polyps from patients with CRSwNP is presented. Percentages of CD69+ cells were significantly increased in nasal polyps compared with PBMCs. Furthermore, the incidence of CD69+ cells was significantly higher among CD8+ T cells compared with CD4+ T cells in polypoid tissue. Extending the analysis to SIPR1 expression, the proportion of CD69+ SIPR1+ Trm cells was significantly increased among both CD4+ and CD8+ T cells in nasal polyps compared with PBMCs in patients with CRSwNP. Furthermore, the number of CD69+ SIPR1+ Trm cells was significantly higher among CD8+ compared with CD4+ T cells. The frequency of SIPR1+ cells was also significantly increased in edaphic CD4+ and CD8+ T cells compared with PBMCs. Thus, the number of Trm identified by double staining of CD69 and SIPR1 was lower compared with CD69 alone. Nonetheless, the percentage of CCR7+ cells was significantly increased among CD4+ and CD8+ T cells in edaphic lymphocytes compared with PBMCs in patients with CRSwNP.
IcKRATH et al: ACCUMULATION OF CD69+ TISSUE-RESIDENT MEMORY T CELLS IN NASAL POLYPS

Effector memory T cells migrate from PBMCs into the local tissue as a result of acute infection. Following further differentiation into T<sub>rm</sub>, a high percentage of these cells will remain in the local tissue (19). Ma et al (19) discussed transforming growth factor (TGF)-β as one of the major signals for the differentiation of kidney-resident T cells. However, TGF-β-independent differentiation of T<sub>rm</sub> in the intestinal lamina propria has also been reported (20). In CRSwNP, an accumulation of effector CD4<sup>+</sup> and CD8<sup>+</sup> T cells has been discussed (2). TGF-β concentrations in CRSwNP differ from CRSsNP and vary between patients from different countries (21). Therefore, future studies should focus on the possible factors that drive T<sub>rm</sub> generation in CRSwNP.

Different subsets of tissue-resident lymphocytes have previously been described (22). Tissue residency was mainly attributed to CD8<sup>+</sup> T cells and they were observed in many different organs (23,24). Memory T cells were subdivided into T<sub>rm</sub> and T<sub>em</sub> by the homing receptor CCR7 (25). In the present study, high numbers of CD8<sup>+</sup> CCR7<sup>-</sup> T<sub>em</sub> were identified in nasal polyps compared with PBMCs from patients with CRSwNP. The characterization of CD8<sup>+</sup> T<sub>rm</sub> is heterogeneous, often lacking CCR7 and highly expressing CD69 (15,26). In the present study, significantly more CD8<sup>+</sup> T cells were observed in nasal polyps compared with in PBMCs. Almost 97% lacked the homing receptor CCR7 and ~63% of these CD8<sup>+</sup> T cells were CD69+. Whether T<sub>rm</sub> depend (27) on specific antigen presentation or not (24) remains controversial. However, a low incidence of HLA-dR-expressing T cells in nasal polyps (2) suggests that repeated antigenic stimulation is not responsible for maintaining T cells within the polyps. Rather, multiple triggers serve a role in this chronic disease, including fungal (4–6) infections or staphylococcal (7,8) superantigens, which may

---

Table II. Comparison of CD<sup>4</sup> and CD<sup>8</sup> T<sub>rm</sub> cells in patients with CRSwNP.

<table>
<thead>
<tr>
<th>T cells</th>
<th>PBMCs</th>
<th>Nasal polyps</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3&lt;sup&gt;+&lt;/sup&gt; CD4&lt;sup&gt;+&lt;/sup&gt; T cells</td>
<td>37.47±10.18</td>
<td>20.67±8.71</td>
<td>0.0002</td>
</tr>
<tr>
<td>CD4&lt;sup&gt;+&lt;/sup&gt; CD69&lt;sup&gt;+&lt;/sup&gt; T&lt;sub&gt;rm&lt;/sub&gt;</td>
<td>0.27±0.13</td>
<td>38.25±14.23</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CD4&lt;sup&gt;+&lt;/sup&gt; CD69&lt;sup&gt;+&lt;/sup&gt; S1PR1&lt;sup&gt;-&lt;/sup&gt; T&lt;sub&gt;rm&lt;/sub&gt;</td>
<td>0.28±0.13</td>
<td>23.61±15.26</td>
<td>0.0013</td>
</tr>
<tr>
<td>CD4&lt;sup&gt;+&lt;/sup&gt; CCR7&lt;sup&gt;-&lt;/sup&gt; T&lt;sub&gt;em&lt;/sub&gt;</td>
<td>66.06±20.21</td>
<td>85.16±14.29</td>
<td>0.0093</td>
</tr>
<tr>
<td>CD4&lt;sup&gt;+&lt;/sup&gt; S1PR1&lt;sup&gt;-&lt;/sup&gt; T cells</td>
<td>4.56±6.46</td>
<td>33.82±27.98</td>
<td>0.0098</td>
</tr>
<tr>
<td>CD3&lt;sup&gt;+&lt;/sup&gt; CD8&lt;sup&gt;+&lt;/sup&gt; T cells</td>
<td>23.7±7.24</td>
<td>40.2±15.6</td>
<td>0.0089</td>
</tr>
<tr>
<td>CD8&lt;sup&gt;+&lt;/sup&gt; CD69&lt;sup&gt;+&lt;/sup&gt; T&lt;sub&gt;rm&lt;/sub&gt;</td>
<td>1.14±0.36</td>
<td>63.24±18.83</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CD8&lt;sup&gt;+&lt;/sup&gt; CD69&lt;sup&gt;+&lt;/sup&gt; S1PR1&lt;sup&gt;-&lt;/sup&gt; T&lt;sub&gt;rm&lt;/sub&gt;</td>
<td>0.95±0.23</td>
<td>35.36±23.57</td>
<td>0.0017</td>
</tr>
<tr>
<td>CD8&lt;sup&gt;+&lt;/sup&gt; CCR7&lt;sup&gt;-&lt;/sup&gt; T&lt;sub&gt;em&lt;/sub&gt;</td>
<td>79.04±13.43</td>
<td>97.18±5.48</td>
<td>0.0018</td>
</tr>
<tr>
<td>CD8&lt;sup&gt;+&lt;/sup&gt; S1PR1&lt;sup&gt;-&lt;/sup&gt; T cells</td>
<td>9.51±13.44</td>
<td>38.08±31.52</td>
<td>0.0488</td>
</tr>
</tbody>
</table>

PBMCs, peripheral blood mononuclear cells; CD, cluster of differentiation; T<sub>rm</sub>, tissue-resident memory T cells; S1PR1, sphingosine-1-phosphate receptor 1.

---

Figure 1. Expression of CD69 in (A) CD8<sup>+</sup> T cells and (B) CD4<sup>+</sup> T cells in PBMCs and nasal polyps in patients with chronic rhinosinusitis with nasal polyps. Data are presented as the mean ± standard deviation of 10 patients. ****P<0.0001. CD, cluster of differentiation; PBMC, peripheral blood mononuclear cell.
generate a niche for T rm development and maintenance independent of antigens.

Similar to CD8+ T rm, CD4+ T rm are described as CD69+ T cells and lack the homing receptor CCR7. In the present study, ~38% of the CD4+ T cells were also positive for CD69 and 85% lacked the homing receptor CCR7, which suggests a high percentage of CD4+ T rm in nasal polyps. Interestingly, CD8+ CD69+ T rm were significantly increased compared with CD4+ CD69+ T cells in nasal polyps. The reason for higher CD8+ than CD4+ T rm numbers in polyps remains unclear and should be the focus of future studies. Besides identical expression of CD69, CCR7 and S1PR1 in CD4+ and CD8+ T cells, the signals for tissue residency are differentially described for CD4+ compared with CD8+ T rm in the literature (28). The precise mechanisms for keeping these T cells inside the tissue are still unclear.
Another subdivision of CD4+ T cells which are responsible for several autoimmune disorders are Treg cells (29,30). Lynch et al (31) reported that Treg do not recirculate in the blood. In contrast, Luo et al (32) demonstrated that Treg do not persist in the local tissue for a long period of time. Like CD4+ and CD8+ Tem, Treg require the expression of CD69 as a signal to remain in the local tissue (22). In the present study, Treg were differentiated into CD3+ CD4+ CD45RA+ FoxP3low rTreg and CD3+ CD4+ CD45RA FoxP3high aTreg. rTreg and aTreg exhibited a significantly higher expression of CD69 in nasal polyps compared with PBMCs. In nasal polyps, aTreg had a significantly higher CD69 expression compared with rTreg. These findings suggest that Treg populations in the polyps primarily consist of tissue-resident cells.

Skon et al (12) critically remarked that CCR7 downregulation alone is not a reliable marker for T rm. For a more appropriate characterization of T rm, evidence of S1PR1 downregulation is required (12,13). Following detection of its ligand, SIP, S1PR1 is a necessary signal for naïve lymphocytes to exit the local tissue and recirculate (11). CD69/S1PR1 double staining revealed significantly more CD3+ CD4+ and CD3+ CD8+ CD69+ S1PR1 T rm in nasal polyps compared with PBMCs in patients with CRSwNP. Furthermore, the frequency of CD69+ S1PR1 T rm was significantly higher among CD8+ compared with CD4+ T cells, which underscores the dominating role of CD8+ T cells in CRSwNP. Interestingly, the expression of S1PR1 alone was significantly higher in total CD3+ CD4+ and CD3+ CD8+ T cells from nasal polyps compared with PBMCs. This may be because S1PR1 expression is modulated by the binding of SIP and there is a high concentration of this ligand in PBMCs, with a downregulation on lymphocytes in PBMCs (18).

The pathophysiological function of T rm has been described in the literature. They are regarded as a potent sentinel mechanism against acute re-infections, thereby supporting protective immunity (14). In contrast, whether a high percentage of T rm in chronic diseases, including CRSwNP, may act as a possible pathogenic trigger of the disease or of early-onset recurrence following therapy remains unclear. Schmidt et al discussed allergen-specific CD8+ T rm as key mediators for acute contact dermatitis (33). In addition, they may be responsible for the development of novel sensitizations (33). Park et al (34) outlined the important role of accumulating resident memory T cells in various diseases of barrier and non-barrier tissues. Furthermore, pathological accumulation of hyperactive T rm as a response to an extended inflammatory reaction may lead to further disease (34).

One limitation of the present study is the lack of a control group comprising the nasal mucosa of patients with CRSsNP. It is therefore difficult to assess whether the accumulation of T rm in nasal polyps is pathological or the normal physiological condition. An analysis of lymphocytes from the nasal mucosa of patients with CRSsNP was attempted, however the number of cells was too small for a reliable evaluation. As very few lymphocytes were able to be isolated from the nasal mucosa of patients with CRSsNP, an accumulation of T rm seems unlikely. Sathaliyawala et al (35) performed a unique analysis of human T cells in healthy lymphoid and mucosal tissue obtained from individual donors, thus describing a steady state of T cells. Interestingly, the majority of T rm identified, even in respiratory mucosae, were CD4+ memory T cells. This is in contrast to the present study in which the majority of T rm in polypoid tissue were CD8+ T cells. This suggests a pathological increase in the percentage of CD8+ T rm in comparison with healthy respiratory mucosae from patients with CRSwNP.

Summarizing the findings of this study and the data in the literature, there are two different T cell pools in nasal polyps: A high percentage of CD8+ T rm and a lower percentage of predominantly CD4+ T rm. Interestingly, these T cells are HLA-DR (2), therefore there are no recently activated T cells in the polypoid tissue. T rm may be important mediators of the chronic inflammatory process in CRSwNP. Selective inhibition, or eliminating these cells by modifying their ability to persistently reside in tissue, may be a possible approach for the development of novel therapeutic strategies (34). Hypothetically, targeting and blocking CD69 could, for example, support the elimination of pathogenic T rm in the tissue. The clinical impact of T rm in recurrent CRSwNP should be further investigated in the future.

Table III. CD4+ T cell subpopulations and their CD69 expression.

<table>
<thead>
<tr>
<th>CD3+ CD4+ T cells</th>
<th>PBMCs</th>
<th>Nasal polyps</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD45RA+ FoxP3low CTLA-4low resting Treg</td>
<td>0.52±0.30</td>
<td>1.24±1.41</td>
<td>0.275</td>
</tr>
<tr>
<td>CD69+</td>
<td>4.81±3.99</td>
<td>61.26±31.97</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CD45RA+ FoxP3high CTLA-4high activated Treg</td>
<td>1.29±0.82</td>
<td>5.74±2.18</td>
<td>0.0004</td>
</tr>
<tr>
<td>CD69+</td>
<td>3.78±2.42</td>
<td>86.01±10.68</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CD45RA+ Foxp3low memory T cells</td>
<td>3.63±1.26</td>
<td>6.6±1.45</td>
<td>0.0004</td>
</tr>
<tr>
<td>CD69+</td>
<td>1.99±0.76</td>
<td>67.75±13.74</td>
<td>0.002</td>
</tr>
<tr>
<td>CD45RA+ Foxp3 memory T cells</td>
<td>56.87±16.27</td>
<td>74.73±8.88</td>
<td>0.014</td>
</tr>
<tr>
<td>CD69+</td>
<td>2.23±0.64</td>
<td>64.15±8.15</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CD45RA+ Foxp3 naïve T cells</td>
<td>37.83±16.64</td>
<td>11.01±9.90</td>
<td>0.002</td>
</tr>
<tr>
<td>CD69+</td>
<td>2.33±0.72</td>
<td>14.69±10.27</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Data are presented as the mean ± standard deviation of 10 patients. CD, cluster of differentiation; PBMCs, peripheral blood mononuclear cells; Treg, regulatory T cells.
To the best of our knowledge, this is the first study to describe resident memory T cells in nasal polyps compared with PBMcs from patients with CRSwNP. CD8+ T rm dominated CD4+ T rm within nasal polyps. The role of T rm in nasal polyps as a pathogenic trigger of the local inflammatory reaction must be further investigated in future studies; however, the results of the present study suggest local regulation of the immune response within the nasal polyps. Thus, T rm can be a potential trigger in the pathogenesis of nasal polyps.
Acknowledgements

Not applicable.

Funding

Dr Niklas Beyersdorf was supported by the Deutsche Forschungsgemeinschaft (DFG) (SFB/TR 124 FungiNet, Project C6).

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

PI performed all experiments, analyzed the results and was the main author of the manuscript. XD, NB and TK conceived the study and analyzed the results. NK, RH and CG analyzed the data and were major contributors to the manuscript. SH conceived the study, analyzed the results and was a major contributor to the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Board of the Medical Faculty, Julius-Maximilian-University Wuerzburg (vote no. 12/06). Ethics approval and written informed consent have been obtained from every patient.

Consent for publication

Not applicable.

Competing interests

The authors declare that there are no competing interests.

References


