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Elevated presence of myeloid dendritic cells in nasal polyps of patients with chronic rhinosinusitis

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Abstract

Background—Although chronic rhinosinusitis with nasal polyps (CRSwNP) is characterized by Th2 inflammation, the mechanism underlying the onset and amplification of this inflammation has not been fully elucidated. Dendritic cells (DCs) are major antigen presenting cells, central inducers of adaptive immunity and critical regulators of many inflammatory diseases. However, the presence of DCs in CRS, especially in nasal polyps (NPs), has not been extensively studied.

Objective—The objective of this study was to characterize DC subsets in CRS.

Methods—We used real-time PCR to assess the expression of mRNA for markers of myeloid DCs (mDCs; CD1c), plasmacytoid DCs (pDCs; CD303) and Langerhans cells (LCs; CD1a, CD207) in uncinate tissue (UT) from controls and patients with CRS as well as in NP. We assayed the presence of DCs by immunohistochemistry and flow cytometry.

Results—Compared to UT from control subjects (n=15) and patients with CRS without NP (CRSsNP) (n=16) and CRSwNP (n=17), mRNAs for CD1a and CD1c were significantly elevated in NPs (n=29). In contrast, CD207 mRNA was not elevated in NPs. Immunohistochemistry showed that CD1c⁺ cells but not CD303⁺ cells were significantly elevated in NPs compared to control subjects or patients with CRSsNP. Flow cytometric analysis showed that CD1a⁺ cells in NPs might be a subset of mDC1s, and that CD45⁺CD19[−]CD1c⁺CD11c⁺CD141[−]CD303[−]HLA-DR⁺ mDC1s and CD45⁺CD19[−]CD11c⁺CD1c[−]CD141^{high} mDC2s were significantly elevated in NPs

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Author contribution

AK designed the study. JAP, SP, KW and AK performed the experiments; AK analyzed the data. KEH helped in the experiments and data analysis for flow cytometry. RPS, KEH, ATP, JEN, LAS, RC, KEH, LCG, BKT, RKC, DBC, and RCK helped in sample collection and evaluation. AK and JAP wrote the manuscript. All authors have read and approved the final form of the manuscript.

Conflict of interest

The authors declare no conflict of interest as to the interpretation and presentation of this manuscript.

compared to UT from controls and CRSsNP, but CD45⁺CD11c⁻CD303⁺HLA-DR⁺ pDCs were only elevated in NPs compared to control UT.

Conclusion & Clinical Relevance—Myeloid DCs are elevated in CRSwNP, especially in NPs. Myeloid DCs thus may indirectly contribute to the inflammation observed in CRSwNP.

Keywords

Chronic rhinosinusitis; Myeloid dendritic cells; Nasal polyps; Plasmacytoid dendritic cells

Introduction

Chronic rhinosinusitis (CRS) is a heterogeneous disease characterized by local inflammation of the upper airways and sinuses that persists for at least 12 weeks. CRS is a common chronic disease in adults in the United States, affecting over 10 million Americans, and has a severe impact on patients' quality of life [1-3]. CRS is frequently divided into two groups based on histology and physical examination: CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP). In general, CRSwNP is associated more closely with clinical complaints of nasal obstruction and olfactory loss and is characterized by eosinophilia and Th2-related inflammation, especially in Western countries [4-6]. However, the mechanisms underlying the amplification of Th2-related inflammation in CRSwNP have not been identified.

Dendritic cells (DCs) are major professional antigen presenting cells that play a critical role in host immunity by linking innate and adaptive immune responses. Human peripheral blood DCs can be divided into two major subsets, CD11c⁺ myeloid DCs (mDCs) and CD123⁺ plasmacytoid DCs (pDCs) [7]. Both mDCs and pDCs are also present in peripheral tissues. Myeloid DCs act as strong antigen presenting cells. In contrast, pDCs are less effective at antigen presentation than mDCs but strongly promote antiviral immunity. Human DCs are defined as cells that lack lineage (Lin) markers (CD3, CD14, CD19, CD20, CD56, and glycophorin A) but express class II MHC (HLA-DR) [7]. CD11c⁺ mDCs can be further divided into two subtypes, blood dendritic cell antigen-1 (BDCA-1, also known as CD1c)⁺ mDC type 1 (mDC1) and BDCA-3 (CD141)⁺ mDC type 2 (mDC2) [8]. In general, mDC2s are a minor population of mDCs in the peripheral blood [8, 9]. Human pDCs are characterized as Lin⁻HLA-DR⁺CD123⁺BDCA-2 (CD303)⁺ BDCA-4 (CD304)⁺ cells, and CD303 and CD304 are very specific markers of pDCs [8]. Another subset of human DCs is CD1a⁺CD207⁺ Langerhans cells (LCs) that are mainly present in the skin epidermis. LCs can be detected in other tissues, including lung, but are not present in the circulation [7, 10]. DCs are known to be critical regulators of many inflammatory diseases including atopic dermatitis and asthma [11]. However, the role of DCs in CRS is still largely unknown. We hypothesized in this study that DC subsets are elevated in CRSwNP and that DC subsets control adaptive immunity and inflammation in CRSwNP.

Although CRSwNP is a Th2-related disease in Western countries, and DCs are sufficient and necessary for induction of Th2 cell differentiation, there are few studies investigating the presence of DCs in CRS and in NPs in particular [12-14]. In this study, we characterized the presence of DC subsets in CRS and found that mDCs were elevated in NPs.

Materials and methods

Patients and biopsies

CRS patients were recruited from the Allergy-Immunology clinic, the Otolaryngology clinic and the Northwestern Sinus Center of Northwestern Medicine. Sinonasal and NP tissues were obtained from routine functional endoscopic sinus surgery in patients with CRS. All subjects met the criteria for CRS as defined by the American Academy of Otolaryngology-Head and Neck Surgery Chronic Rhinosinusitis Task Force [2]. Patients with an established immunodeficiency, pregnancy, coagulation disorder or diagnosis of classic allergic fungal sinusitis, Churg-Strauss syndrome or cystic fibrosis were excluded from the study. Sinus tissues from disease-free control subjects were obtained during endoscopic skull base brain tumor excisions as well as intranasal procedures for obstructive sleep apnea and facial fracture repairs on patients without a history of CRS, nasal inflammation or asthma, recruited from the Otolaryngology clinic of Northwestern Medicine. Subjects were skin-tested for pollens, dust mites, pets, molds, and cockroach using Hollister-Stier (Spokane, WA) extracts. Patients were taking a variety of medications, including nasal corticosteroids, antihistamines, decongestants, and short- or long-acting β -agonists, among others. Details of subjects' characteristics are included in Table 1. All subjects signed informed consent forms and the protocol governing procedures for this study was approved by the Institutional Review Board of Northwestern University Feinberg School of Medicine.

Real-time PCR

Nasal epithelial scrapings were collected from the uncinate process tissues (UT) or nasal polyps (NP) by curettage with a Rhinoprobe and were transferred in RNeasy Protect (Qiagen, Valencia, CA) and stored at -20°C . A portion of nasal tissues for isolation of RNA was transferred in RNeasy Lysis Buffer (Qiagen, Valencia, CA) and stored at -80°C . Total RNA from sinus tissue was extracted using RNeasy Spin (Qiagen, Valencia, CA) and was cleaned and treated with DNase I using a RNeasy Spin RNA II (MACHEREY-NAGEL, Düren, Germany) kit according to the manufacturer's instructions. The quality of total RNA from sinus tissue was assessed with a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA) using a RNA 6000 Nano LabChip. Single-strand cDNA was synthesized with SuperScript II reverse transcriptase (Invitrogen, Carlsbad, CA) and random primers. Semi-quantitative real-time RT-PCR was performed using the TaqMan method on an Applied Biosystems 7500 Sequence Detection System (Foster City, CA) in 15 μl reactions as described previously [15]. Primer and probe sets for CD1a (Hs00233332_m1), CD1c (Hs00233509_m1), CD207 (Hs00210453_m1), CD303 (Hs01092462_m1), IL-5[6], IL-13[6], CLC (Hs00171342_m1) and β -glucuronidase (PN; 4326320E) were purchased from Applied Biosystems or Integrated DNA Technologies (Coralville, IA). To determine the exact copy number of the target genes, quantified aliquots of purified PCR fragments of the target genes were serially diluted and used as standards in each experiment. Aliquots of cDNA equivalent to 10 ng of total RNA were used for real-time PCR. The mRNA expression levels were normalized to the median expression of the housekeeping gene, β -glucuronidase. Expression of β -glucuronidase was not significantly different among the 4 groups (data not shown).

Immunohistochemistry

Nasal tissue was dehydrated, infiltrated and embedded with paraffin, and tissue was sectioned at 3 μ m using a Leica RM2240 Cryostat. Sections were rehydrated and blocked for endogenous peroxidase activity with 3% H₂O₂/methanol. For CD303 staining, tissue sections were incubated with trypsin solution (Thermo Fisher Scientific, Fremont, CA) for 5 min at 37°C to induce antigen retrieval. After washing, tissue sections were blocked for non-specific binding with 5% horse serum/0.3% Tween-20/PBS. Tissue sections were then incubated with 10 μ g/ml mouse anti-human CD303 mAb (Imgenex, San Diego, CA, clone 124B3) or 1.25 μ g/ml mouse anti-human CD1a (Thermo Fisher Scientific, clone O10) at 4°C overnight. For staining of CD1c, we used slides prepared from frozen tissue. After fixation in cold acetone and blocking endogenous peroxidase activity in 3% H₂O₂/methanol, tissue sections were blocked with 5% horse serum/PBS and treated with Avidin/Biotin Blocking Kit (Vector Laboratories, Burlingame, CA). The slides were then incubated with 10 μ g/ml mouse anti-human CD1c mAb (BioLegend, San Diego, CA, clone L161) in blocking buffer at 4°C overnight. After treatment with the primary antibody, tissue sections were incubated with biotinylated horse anti-mouse IgG for 1 hour and then ABC reagent (avidin-biotin-HRP complex; Vector Laboratories,) for 1 hour at room temperature. Sections were rinsed and incubated in DAB reagent (Invitrogen) for 5 minutes at room temperature. They were then rinsed in deionized H₂O, counterstained with hematoxylin, dehydrated, cleaned, mounted and coverslipped using Cytoseal 60 (Richard-Allan Scientific, Kalamazoo, MI) in preparation for microscopic analysis. Slides were blinded and ten pictures were randomly taken from each slide. The number of positive cells in nasal mucosa was counted by two independent observers.

Cell isolation and flow cytometric analysis

Tissue samples obtained during surgery were weighed, fragmented and then incubated with 30 μ g/ml DNase I and 1 mg/ml type I collagenase at 4°C overnight. Following this, tissues were minced using a gentleMACS dissociator (Miltenyi Biotec, Auburn, CA) and the cells were filtered through 70 μ m nylon mesh (BD Biosciences, San Jose, CA). Cells were then treated with red blood cell lysis solution (Miltenyi Biotec) and washed with dPBS before counting and staining for flow cytometric analysis. We obtained 1.31 ± 0.39 million live cells (CRSsNP UT, n=10) and 8.49 ± 2.79 million live cells (NP, n=10) from 145.7 ± 16.9 mg and 350.0 ± 95.3 mg tissue respectively. Values were expressed as total numbers of cells obtained per mg of tissue.

Cells were first treated with Aqua LIVE/DEAD fixable dead cell staining reagent (Invitrogen) as a live/dead discriminator. Cells were then incubated with an Fc Block reagent (Miltenyi Biotec) for 5 minutes at room temperature. All antibodies were obtained from BioLegend, unless otherwise stated. The following antibodies and dilutions were used to stain the surface of the cells: 0.6 μ g/ml Brilliant Violet 421 anti-CD45 (HI30), 2.5 μ g/ml PE-Cy7 anti-CD19 (HIB19), 5 μ g/ml Alexa Fluor 488 anti-CD1c (L161), 2 μ g/ml APC-Cy7 anti-CD11c (Bu15), PE anti-CD303 (AC144, 1:20, Miltenyi Biotec), 5 μ g/ml PerCP-Cy5.5 anti-CD141 (M80), 2.5 μ g/ml PerCP-Cy5.5 anti-HLA-DR (L243), 2.5 μ g/ml Alexa Fluor 647 anti-HLA-DR (L243) and 10 μ g/ml Alexa Fluor 647 anti-CD1a (HI149). Cells were stained for 30 minutes at 4°C in the dark, and washed with FACS buffer (BD Biosciences).

Cells were fixed with a BD Cytofix/Cytoperm Kit, resuspended in FACS buffer and stored at 4°C in the dark before analysis on an LSRII (BD Biosciences). 141,385 ± 27,211 events (UT, n=13), 328,800 ± 52,579 events (NP, n=10) or 260,000 ± 54,160 events (tonsil, n=6) were collected. A minimum of 38,000 (UT) or 64,000 (NP) events was collected for each sample. All analysis and compensation were performed with FlowJo software, version 7.6.5 (TreeStar, Ashland, OR), and each experiment contained the proper single-stained control beads (BD Biosciences) and fluorescence minus one (FMO) negative controls. Aqua⁺ dead cells and multiplets were excluded from the analysis. Representative images of FMO controls and gating strategy are shown in the online Supporting information (Fig. S1-S3).

Statistics

All data are reported as the median (25-75% interquartiles, in the Figures) or the mean (± SEM, in the text). Differences between groups were analyzed using the 1-way ANOVA Kruskal-Wallis with Dunn's *post hoc* testing (PCR and immunohistochemistry) or the Mann-Whitney *U* test (flow cytometry). All statistical analyses were performed using GraphPad prism 5.04 software (La Jolla, CA). A *p* value of less than 0.05 was considered significant.

Results

Expression of DC markers in CRS

To estimate the levels of DCs in nasal mucosa, we first assessed the expression of mRNA for markers of mDCs (CD1c), pDCs (CD303) and LCs (CD1a and CD207) in uncinate process tissues (UTs) from controls, CRSsNP and CRSwNP, and in nasal polyps (NPs) by real-time PCR. CD1c and CD1a levels were significantly elevated in NP tissues (Fig. 1a,c). However, the LC marker CD207 was not elevated in NPs (Fig. 1d). This suggests that CD1a⁺ DCs might not be LCs in NPs. Expression of CD303 was significantly elevated in NPs compared to UTs from controls and CRSwNP, although expression of CD303 was significantly lower than CD1a and CD1c (Fig. 1 and data not shown). We initially hypothesized that DCs might be accumulated in the epithelium of NPs. We therefore analyzed the expression of DC markers in epithelial cells derived from nasal scrapings. However, we found that mRNAs for CD1c, CD303 and CD1a were not elevated in NP epithelium (Fig. S4).

Increased number of mDCs in NPs

To further characterize the DCs in the nasal mucosa, we performed immunohistochemistry on UT and NP tissue. As shown in Fig 2, 3 and S5, DCs were primarily located in the submucosal area of nasal tissues. Semiquantitative analysis suggested that CD1c⁺ mDCs were significantly elevated in NPs compared with UTs in patients with CRSsNP and in UTs from controls (Fig. 2). CD1c⁺ mDCs were also elevated in UTs of patients with CRSwNP compared with UTs from controls (Fig. 2). We also found that CD1a⁺ DCs were elevated in NPs compared with UTs from controls and CRSwNP (Fig. S5). However, there was no difference in the presence of CD303⁺ pDCs among the groups (Fig. 3).

Determination of DC subsets in NPs

Immunohistochemistry is not widely considered to be a reliable quantitative assay. In addition, mDCs can be classified into two subsets, mDC1 and mDC2 which are not easy to distinguish by immunohistochemistry. To further characterize the DC subsets and to obtain a more accurate assessment of their numbers in nasal mucosa, we performed flow cytometry.

Because it is known that a subset of B cells in peripheral blood express CD1c [16], we first examined whether any of the CD1c staining seen by immunohistochemistry was due to a subset of B cells in sinuses that expresses CD1c. We found that CD19⁺ B cells were a minor population of CD1c⁺ cells in NP ($5.2 \pm 0.6\%$, n=10) and UT from CRSsNP ($11.0 \pm 3.2\%$, n=10), compared to tonsils ($84.3 \pm 2.6\%$, n=6) and peripheral blood mononuclear cells (PBMC, $48.9 \pm 5.1\%$, n=5) (Fig. S6), indicating that CD1c⁺ staining was due to the presence of mDCs in the sinus mucosa. We identified DC populations by the gating strategy shown in the Online Repository (Fig. S2). Myeloid DC1s were defined as CD45⁺CD1c⁺CD11c⁺CD19⁻CD141⁻CD303⁻HLA-DR⁺ cells and pDC as CD45⁺CD1c⁻CD11c⁻CD19⁻CD141⁻CD303⁺HLA-DR⁺ cells in UT and NP (Fig. 4a, S2 and S3). We then calculated the total number of cells in each DC subset normalized to mg of tissue they were isolated from. We found that mDC1s were significantly elevated in NPs compared to UTs from control subjects and patients with CRSsNP (Fig. 4a). Plasmacytoid DCs were also significantly elevated in NPs compared to UTs from control subjects (Fig. 4a). However, pDCs were not elevated in NPs compared to UTs from CRSsNP even after the normalization (Fig. 4a). We next assessed mDC2s. Since mDC2s are rare in peripheral blood and may not be detectable in NPs and UTs, we used PBMC as a control to gate the mDC2 population. We detected mDC2s (CD45⁺CD1c⁻CD11c⁺CD19⁻CD141^{high}CD303⁻HLA-DR⁺ cells) and found they were significantly elevated in NPs compared to UTs from control subjects and patients with CRSsNP (Fig. 4b, S2 and S3). However, the frequency of mDC2 was significantly lower than mDC1 in NP and UT (Fig. 4). Finally, we looked at CD1a⁺ DCs and found they were significantly elevated in NPs compared to UTs from controls and CRSsNP (Fig. 4c). CD1a⁺ DCs were also significantly higher in CRSsNP than control subjects (Fig. 4c). Flow cytometric analysis showed that CD1a⁺ cells also expressed CD1c and CD11c, suggesting that they might be a subset of mDC1s (Fig. 4c and S7). Supporting this notion, we also found that $20.8 \pm 4.5\%$ of mDC1 in NPs (n=10) expressed CD1a (Fig. S2 and not shown).

Correlation of DC marker and Th2 inflammation in NPs

Since mDCs were increased in NPs, and mDC1 in particular control Th2 differentiation, we examined whether the expression of markers of mDC1 correlated with Th2 inflammation in NPs. CD1c expression positively correlated with markers of Th2 inflammation including IL-5 ($r=0.482$, $p=0.008$) and IL-13 ($r=0.517$, $p=0.004$) and also with markers of eosinophilia including Charcot-Leyden crystal protein (CLC; $r=0.452$, $p=0.014$) in NPs (n=29, Fig. 5). In contrast, expression of the pDC marker CD303 did not correlate with IL-5 ($r=0.076$, $p=0.696$), IL-13 ($r=0.231$, $p=0.228$) or CLC ($r=0.248$, $p=0.195$) (n=29, Fig. 5).

Discussion

DCs play a critical role in inflammatory diseases as well as in host immunity. Several groups have reported the presence of DCs in CRS. Ayers *et al.* reported that CD209 (DC-SIGN)⁺ DCs were elevated in the osteomeatal complex of CRSwNP [12]. O'Connell *et al.* reported that peripheral blood CD209⁺ DCs were elevated in CRSwNP [14]. In contrast, Kirsche *et al.* reported that CD1c⁺ mDC1s were reduced in the anterior ethmoid sinus of CRSwNP [13]. Thus, there is some discrepancy in the literature regarding the presence of DCs in CRSwNP. In addition, the presence of DC subsets in NP tissues has not been well characterized. In the current study we carefully studied DC subsets in NP tissues and compared them to DC subsets in UT from controls, CRSsNP and CRSwNP by real-time PCR, immunohistochemistry and flow cytometry. We found that mDCs, both the mDC1 and mDC2 subsets were significantly increased in NPs. In contrast, pDCs were increased in NPs compared to control, but there was no difference compared to CRSsNP. Since the frequency of pDCs in CRSsNP was variable and CRSsNP is known to be a heterogeneous disease, sub-classification of CRSsNP and the impact of such classification on the presence of pDC and disease phenotypes require further investigation.

Initially we identified CD1c⁺ mDC1 by immunohistochemistry and found that CD1c⁺ cells were significantly elevated in CRSwNP compared with control subjects (Fig. 2). However, it is well known that subsets of human B cells, but not plasmablasts or plasma cells, express CD1c, especially marginal zone B cells and IgM^{high}IgD^{low}CD27⁺ blood B cells [16]. We therefore sought to confirm the presence of mDC1 by flow cytometry and found that mDC1s were significantly elevated in NP compared with UT from controls and CRSsNP (Fig. 4). Since the presence of CD1c⁺ B cells in NP has not been reported, we further analyzed the population of CD1c⁺ cells in sinus mucosa compared with tonsils and PBMC. We found that B cells were a very minor population of CD1c⁺ cells in nasal mucosa compared to tonsils and PBMC (Fig. S6). Indeed, the majority of CD1c⁺ cells in NP ($92.7 \pm 0.7\%$, n=10), CRSsNP UT ($86.7 \pm 3.5\%$, n=10) and control UT ($82.3 \pm 7.25\%$, n=3) were mDC1 (Fig. S6). The flow cytometric data reinforce the results of our CD1c immunohistochemistry data and establish the presence of mDC1 in sinus mucosa. Since there was a trend toward elevation of CD1c mRNA and CD1c⁺ cells were significantly elevated in CRSwNP UT compared to control UT (Fig. 1 and 2), mDC1 may also be elevated in UT from CRSwNP. Further study will be required to confirm the relative presence of mDC1 in CRSwNP UT by flow cytometric analysis.

Myeloid DC type 2 is a very minor population of immune cells comprising only 0.01-0.04% of PBMC in healthy individuals [8, 9]. Although the data is limited, several groups reported the presence of mDC2 in the airways including in lung and bronchoalveolar lavage fluid [10, 17]. In contrast to the circulation, mDC2 might be the predominant DC subset in the lungs and were reported to be 1.9-3.2% of all lung leukocytes [10, 18]. However, the presence of mDC2 in sinus and nasal mucosa was still not clear. We found that mDC2s were present in CRSsNP UTs ($0.15 \pm 0.03\%$ of the CD45⁺ population) and NPs ($0.24 \pm 0.05\%$ of the CD45⁺ population). Unlike in the lung, mDC2s were a minor population of DC in sinuses compared to mDC1 ($1.53 \pm 0.26\%$ of the CD45⁺ population) or pDC ($1.02 \pm 0.28\%$ of the CD45⁺ population). However, we found that mDC2s were significantly elevated in NPs

compared to UT from controls and CRSsNP. Yerkovich *et al.* reported that mDCs were significantly elevated in patients with atopic asthma, and that mDC2s induced strong Th2-polarized cytokine responses by allergen specific T cells [19]. Dua *et al.* reported that mDC2s were increased in the sputum of subjects with asthma after allergen challenge [20]. This suggests that the elevation of mDC2 in NPs may contribute to the local Th2-polarized environment. Future study will be required to assess the role of mDC2 in Th2 inflammation in NPs.

Although we found elevation of mDC1 and mDC2 in NPs (Fig. 4), the mechanism of accumulation of mDCs is not clear. We recently reported that the chemokines CCL18 and CCL23 were significantly elevated in NPs [21, 22]. CCL23 is known to recruit DCs via the receptor CCR1. Lundberg *et al.* found that CCR1 was expressed on tonsillar mDC1 [23]. In addition, we found that levels of CCL23 significantly correlated with CCR1 and CD1c in sinus tissues [22]. CCL18 is also known to be chemotactic for immature mDC1 [24]. These results suggest that overproduction of CCL18 and CCL23 may contribute to the accumulation of mDC1 in NPs. In contrast, the expression of chemokine receptors on mDC2 is not clear. Lundberg *et al.* reported that mRNAs for CCR2, CCR5, CX3CR1, CXCR4 and XCR1 were detected in tonsillar mDC2 but CCR2, CCR5 and CX3CR1 were not detected in blood mDC2 by microarray [23]. Future study will be required to test whether these chemokine receptors are expressed on NP mDC2s and whether ligands for these particular receptors are elevated in NPs.

One of the key functions of DCs is to control polarization of T helper cells. The epithelial derived cytokine thymic stromal lymphopoietin (TSLP) is now recognized to be a master regulator of DC mediated Th2 inflammation. TSLP-stimulated mDC1s are known to induce naive CD4⁺ T cells to differentiate into Th2 cells through OX40 ligand [25]. It is highly relevant to the present study that we recently found that TSLP activity was significantly elevated in NPs [6]. In addition, Liu *et al.* reported that populations of OX40 ligand expressing CD11c⁺ mDCs were higher in NPs than control sinus tissue [26]. These results indicate that local production of TSLP might control Th2 inflammation in NPs via activation of mDC1 and induction of Th2 differentiation. In contrast to mDC, TSLP-activated pDC induce the generation of FOXP3⁺ regulatory T cells [27]. Although mDC1s constitutively express the TSLP receptor, resting pDC do not have it. However, pDC express the TSLP receptor complex upon activation, especially by TLR7 and 9 ligands, and become responsive to TSLP [27]. These results indicate that the mDC1/pDC ratio and the activation status of pDC may control TSLP-mediated immunity. In our current study, we found that pDCs were not elevated in NPs compared to CRSsNP and that the frequency of mDC1 was significantly higher than pDC in NPs (Fig. 4). In addition, markers of mDC1 but not pDC correlated with Th2 inflammation in NPs (Fig. 5). This suggests that mDC1 mediated inflammation might be predominant in NPs and mDC1 might control Th2 inflammation in NPs. Future study will be required to assess whether TSLPR is expressed on pDC and whether mDC1s control type 2 immunity in NPs.

When our study was completed, Pezato *et al.* reported that mDCs and pDCs were elevated in NPs from the Belgium population [28]. This suggests that the elevation of mDCs and pDCs in NPs may be conserved across the American and European populations. In contrast to our

study, Pezato *et al.* did not investigate the presence of DCs in CRSsNP. Future study will be required to examine whether mDCs are more elevated in NPs than CRSsNP in the European population.

More recently, Shi *et al.* reported that DCs were elevated in both eosinophilic and non-eosinophilic NPs from a Chinese population [30]. Importantly, they found in *in vitro* co-cultures that DCs isolated from either eosinophilic or non-eosinophilic NPs skewed autologous naïve helper T cells toward Th17 and Th1 phenotypes, but only DCs from eosinophilic NPs were able to skew naïve T helper cells toward a Th2 phenotype compared to DCs isolated from control inferior turbinate tissues [30, 31]. In addition, they found that OX40 ligand and programmed death ligand-1 (PD-L1) were significantly upregulated in DCs isolated from eosinophilic NP and these played an important role in the skewing of Th cells to produce type 2 cytokines [30]. Since over 80% of NPs in western countries showed eosinophilia, overexpression of OX40 ligand and PD-L1 on DCs may also be involved in the type 2 inflammation in the American and European populations. Future study will be required to identify the expression of OX40 ligand and PD-L1 and their role in type 2 inflammation in western countries.

In summary, we report here that mDCs are significantly elevated in NP tissue. We also found that the level of markers of mDC1 correlated with Th2 inflammation in NPs. Our findings indicate that the accumulation of mDCs in NPs might contribute to the pathogenesis of CRSwNP.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

BDCA	blood dendritic cell antigen
CRS	Chronic rhinosinusitis
CRSsNP	CRS without nasal polyps
CRSwNP	CRS with nasal polyps
DCs	dendritic cells
FMO	fluorescence minus one
LCs	Langerhans cells
mDCs	Myeloid DCs

mDC1	mDC type 1
pDCs	Plasmacytoid DCs
TSLP	Thymic stromal lymphopoietin
UT	Uncinate tissue

Figure 1.

Increased expression of markers of DCs in NPs. Total RNA was extracted from whole uncinat tissue (UT) and nasal polyps (NP). Expression of mRNAs for CD1c, CD303, CD1a and CD207 was analyzed using real-time RT-PCR. * $p < 0.05$.

Figure 2.

Increased presence of CD1c⁺ cells in NPs. Representative immunostaining for CD1c is shown in UT from a control subject (a), a patient with CRSsNP (b), a patient with CRSwNP (c), and in NP (d). Negative control antibody staining in NP is shown (e). The number of CD1c positive cells in UT from control (n=7), CRSsNP (n=9) and CRSwNP (n=15) and in NPs (n=14) was counted (f). Magnification; $\times 400$. * $p < 0.05$.

Figure 3.

CD303⁺ cells are not elevated in NPs. Representative immunostaining for CD303 is shown in UT from a control subject (a), a patient with CRSsNP (b), a patient with CRSwNP (c), and in NP (d). Negative control antibody staining in NP is shown (e). The number of CD1c positive cells in UT from control (n=12), CRSsNP (n=11) and CRSwNP (n=12) and in NPs (n=30) was counted (f). Magnification; ×400.

Figure 4.

Myeloid DCs are elevated in NPs. Cells were isolated from sinus tissue biopsy specimens and analyzed by means of flow cytometry. Representative flow cytometric plots for mDC1 (a), pDC (a), mDC2 (b) and CD1a⁺ DC (c) in CRSsNP UT and NP were shown within the Aqua⁻CD45⁺CD19⁻ population. Numbers of mDC1s (CD1c), mDC2s (CD141, CD11c), pDCs (CD303) and CD1a⁺ DCs in control UTs (n=3), CRSsNP UTs (n=10) and NPs (n=10) were calculated and normalized by mg of tissue. * p < 0.05.

Figure 5. Correlation of markers for DCs and Th2 inflammation in NPs. Messenger RNA for CD1c, CD303, IL-5, IL-13 and CLC was assessed by real-time PCR (n=29). The correlations were assessed by using the Spearman rank correlation.

Table 1

Subject characteristics

	Control			CRSsNP			CRSwNP		
	Y	N	U	Y	N	U	Y	N	U
Total no. of subjects	n=34 (15M)			n=46 (14M)			n=104 (73M)		
Age (y), median (range)	45* (25-64)#			37 (21-72)			44 (26-74)		
Atopy	2	31	1	27	18	1	60	24	20
Asthma	0	34	0	7	38	1	50	52	2
Nasal steroid	0	34	0	13	33	0	15	89	0
Inhaled steroid	0	34	0	5	41	0	12	92	0
Oral steroid	2	32	0	3	43	0	19	85	0
Methodologies used	UT			UT			UT		
Tissue RNA	n=15 (7M)			n=16 (5M)			n=17 (11M)		
Age	48 (25-62)			33 (21-60)			40 (29-67)		
Immunohistochemistry	n=18 (8M)			n=20 (8M)			n=26 (16M)		
Age	45 (25-64)			43 (25-67)			45 (28-74)		
Flow cytometry	n=3 (2M)			n=10 (1M)			n=10 (6M)		
Age	44 (26-61)			52 (30-72)			46 (26-53)		

* median,
(range).

M; male, Y; yes, N; no, U; unknown, UT; uncinate process tissue, NP; nasal polyp.