Interactions between dendritic cells and T lymphocytes in pathogenesis of nasal polyps

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Abstract. The aim of the present study was to investigate the functional status of dendritic cells (DCs) in nasal polyps (NP) and their interactions with T lymphocytes. The interactions between DC and T lymphocytes in the pathogenesis of NP was also studied. The expression of cluster of differentiation (CD)1a and CD83 in NP was detected using immunohistochemistry and the ratio of CD83 DC/CD1a+DC was counted. The distribution of DCs in NP and normal inferior turbinate mucosa (nITM) was evaluated using double immunostaining (CD1a/CD40) and low illumination fluorescence microscopy. The number of CD1a+ cells, CD83+ cells and CD1a/CD40-dual positive cells in was significantly higher in NP tissues compared with nITM. Furthermore, the density of DCs observed in NP was significantly greater than that observed in nITM. The ratio of CD83 DC/CD1a+DC in NP was significantly higher compared with in nITM tissues. The results of the present study revealed significant infiltration of DCs in NP, with the majority being mature DCs. DCs are able to interact with T cells via the CD40/CD40L costimulatory factor, thus serving an important role in the development and progression of NP.

Introduction

Dendritic cells (DCs) are potent antigen presenting cells (APCs) that stimulate naïve T cell proliferation to initiate the *in vivo* immune response (1). T cell negative selection may be induced by the continuously unstable interaction between immature DCs and T cells, due to a deficiency of mature DCs, which may further lead to immune tolerance (2). DCs are considered to be important cells for studying the initiation and development of various inflammatory diseases (3).

Nasal polyps (NP) are one of the most common diseases in otorhinolaryngology, as well as head and neck surgery (4). The pathogenesis of NP remains to be elucidates; however, it is thought a combination of factors, including inflammation and allergies, cause this disease (5). Some scholars classify NP into inflammatory NP and allergic NP, with inflammatory NP characterized by the infiltration of lymphocytes and neutrophils, and allergic NP characterized by the infiltration of lymphocytes, plasma cells, eosinophils or neutrophils (5). Eosinophil infiltration is the most characteristic pathological change of NP and is caused by interactions between various cytokines, of which interleukin (IL)-5 is the main cytokine that causes eosinophil infiltration. During the initial stages of NP, the main source of IL-5 is Th2 cells (6). Lymphocyte infiltration in NP tissues may induce an imbalance of Th1/Th2, leading to the over expression of Th2 compared with Th1 (7). However, inflammatory cell infiltration and inflammatory mediators in NP tissues are observed during the middle and high-stages of the inflammatory response and the characteristics of early pathogenesis of NP remain unclear (8).

A previous study by our group revealed that NP tissues are characterized by the infiltration of a large number of DCs, which are mainly distributed in the submucosa and are concentrated in the epithelium (9). The role of DCs in the pathogenesis of NP and the interaction between DCs and T cells remain to be elucidated. The aim of the present study was to investigate the expression of cluster of differentiation (CD)1a and CD83, as well as the ratio of CD83+ DCs/CD1a+DCs, in NP tissues and the distribution of DCs in NP and normal inferior turbinate mucosa (nITM) tissues.

Materials and methods

Patients. A total of 30 patients underwent endoscopic NP resection (experiment group, E), 10 patients underwent simultaneous nasal septal construction and inferior turbinate resection (control group, C), and 1 patient (male, 53 years old) underwent esophageal cancer resection (positive control group) (10) between October 2015 to September 2016 at Shantou Central Hospital (Shantou, China). Group E included 16 males and 14 females, aged 14-72 years old (mean, 36 years).

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Group C included 7 males and 3 females, aged 16-60 years old (mean, 34 years). Exclusion criteria were as follows: History of allergic rhinitis and application of systemic or local glucocorticoids, immunosuppressive agents, or any kinds of nasal spray in the 2 weeks prior to the study. All specimens were confirmed as NP or nITM tissues by HE staining.

All tissues were washed three times with PBS, fixed with 4% paraformaldehyde at 4˚C for >24 h, dehydrated with a graded series of ethanol at room temperature, cleared with xylene and embedded in paraffin. Sections were then cut (5 μm) and stained at room temperature with haematoxylin (ZLI-9610; ZSGB-Bio Inc., Beijing, China) for 15 min and eosin solution for 10-30 sec at room temperature (ZLI-9613; ZSJQ Biotechnology, Inc.). Each slide was examined at a magnification of 40x using a light microscope (Axioplan 2; Carl Zeiss AG; Oberkochen, Germany).

The present study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Shantou Central Hospital (Shantou, China). Written informed consent was obtained from all participants.

Immunohistochemistry. Tissues were fixed with 4% paraformaldehyde at 4˚C for >24 h, dehydrated with a graded series of ethanol at room temperature, cleared in xylene and embedded in paraffin. Serial 5 μm sections were then cut and underwent conventional dewaxing and rehydration, followed by incubation for 10 min at room temperature with 3% hydrogen peroxide to block endogenous peroxidase activity. Sections were rinsed and incubated with citrate buffer (pH 6.0) at room temperature for 15 min. The solution was allowed to cool to room temperature and sections were rinsed three times with PBS for 2 min. Sections were subsequently incubated overnight at 4˚C with primary antibodies against CD1a (1:50; cat. no. EP3622; Neomarkers, Inc., Portsmouth, NH, USA) and CD83 (1:100; cat. no. LP0306577; Bio-Rad Laboratories, Inc., Hercules, CA, USA). Sections were rinsed with PBS three times for 2 min followed by incubation with mouse anti-rabbit horseradish peroxidase-conjugated secondary antibodies (1:100; cat. no. L111327; KPL, Inc., Gaithersburg, MD, USA) at 37˚C for 30 min. Sections were rinsed again with PBS three times for 2 min and subjected to DAB staining for 5 min at room temperature, hematoxylin counter-staining for 2 min at room temperature, decolored in 1% HCl-alcohol, lithium carbonate red-blue-staining for 15 sec at room temperature, conventional dehydration and hyalinization, followed by sealing in natural resin. Sections were digested in the 1 mM EDTA (pH 8.0) containing CD1a in an autoclave for 7.5 min, followed by digestion in the solution containing CD83 (cat. no. LP0306577; Bio-Rad Laboratories, Inc., Hercules, CA, USA) and 0.1% trypsin-containing CaCl2 (Shanghai Huajing Biotech Corp., Shanghai, China) at 37˚C for 10.3 min. PBS was used to replace the primary antibodies as the negative control and one esophageal cancer sample was selected as the positive control.

Result determination and observation indexes. CD1a+ and CD83+DCs were identified according to the staining and morphology of cells. For each section, the number of DCs was counted in five high-magnification fields at a magnification of x40 with a light microscope (Axioplan 2; Carl Zeiss AG) and the mean number of the positive cells in these fields was calculated to determine the degree of DC infiltration in this sample. The observation indices included the following: i) The infiltration of CD1a+DCs; ii) the infiltration of CD83+DCs; and iii) the ratio of CD83+/CD1a+DCs.

Double immunostaining. The 4-μm paraffin-embedded sections were subjected to the following treatments: Gradual alcohol dewaxing, rinsing with PBS, incubation in 3% H_2O_2 to block the endogenous peroxidase activity, PBS rinsing, antigen repair in in 0.01 M citrate buffer and further PBS rinsing. Sections were then incubated with primary antibodies against CD1a (cat. no. MA119314; rat anti-human monoclonal antibody; LAbVision, AB, Värmdö, Sweden; 1:50) and CD40 (cat. no. PA137334; rabbit anti-human monoclonal antibody Santa Cruz Biotechnology, Inc., Dallas, TX, USA; 1:50) at 37.5˚C for 2 h and washed with PBS. Subsequently, sections were incubated with secondary anti-rat antibody IgG-fluorescein isothiocyanate (FITC; Seracare, Milford, MA, USA; 1:100) and secondary anti-rabbit IgG-phycoerithrin (PE; Santa Cruz Biotechnology, Inc.; 1:100) at 37.5˚C for 30 min and mounted. Control tissues were treated as above with PBS in place of the antibodies. In addition, the esophageal cancer sample was used as the positive control.

Low-illumination fluorescence microscopy analysis. An Axioplan 2 imaging multi-function automatic fluorescence microscope, Axio Cam digital camera (magnification, 40x; resolution 3,900x 3,090 pixels) and a KS400 image analysis system (version 3.0) were used in the present study (all Zeiss AG, Oberkochen, Germany). The three-fluorescence-excitation light filter (Zeiss AG) was used to observe the cells. CD1a+cells exhibited yellow-green fluorescence and CD40+ cells exhibited red fluorescence. The area positive for double immunostaining per section was measured using the KS400 image analysis system and distribution density of double-positive cells was calculated.

Statistical analysis. SPSS10.0 (SPSS, Inc., Chicago, IL, USA) was used for data analysis. All data are expressed as the mean ± standard deviation and analyzed using Student's t-test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Light microscopy. The DCs exhibited irregular shapes with dendrites of varying lengths unevenly distributed in NP and nITM. Commonly concentrated and formed foci were observed and DCs were mainly distributed in T cell-concentrated areas. Many dendrites were in physical contact with the surrounding lymphocytes (Fig. 1).

Distribution density of CD1a+DCs and CD83+DCs. As presented in Fig. 2 and Table I, the distribution density [total number of CD1a+ cells in the unit area (mm^2)] of CD1a+DCs in NP tissues was significantly higher compared with nITM tissues (39.8±3.5 and 11.1±4.9 cells/mm^2, respectively). Similarly, the distribution density of CD83+ DCs in NP tissues were also significantly higher compared with nITM

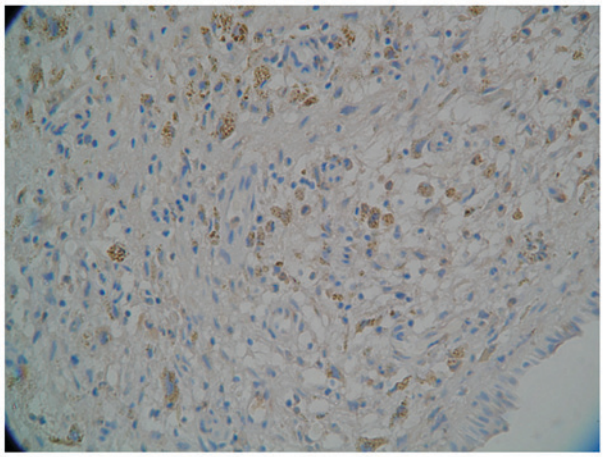

Figure 1. Dendritic cells distribute unevenly in the inferior turbanate mucosa tissue and more aggregate in T cell-concentrated regions Magnification, x40.

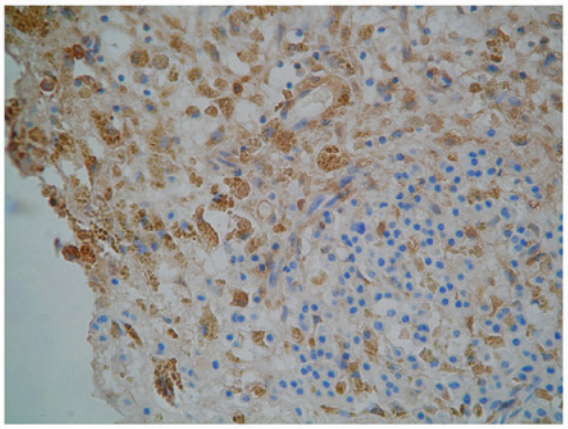

Figure 2. CD1a is mainly expressed in the cytoplasm and cell membrane of DCs in NP. Magnification, x40.

tissues (distribution density, 26.9±2.3 and 8.7±3.3 cells/mm^2, respectively).

Number of DCs. The results of the present study revealed that the ratio of CD83+DCs/CD1a+DCs in Group C was significantly higher compared with Group E (Fig. 3A, Table I). The number of CD83+DCs was significantly higher in nITM tissues compared with NP tissues.

Comparison of distribution area, number and density of CD1a/CD40DC. As presented in Fig. 3 and Table II, the total area of CD1a/CD40 double stained DCs in nITM tissues (285.2±169.6 μm^2) was significantly lower than compared with NP tissues (3,417.3±755.1 μm^2). Furthermore, the number and density of CD1a/CD40 double stained DCs in nITM tissues (42.9±33.5 and 566±389 cells/cm^2, respectively) were significantly lower compared with NP tissues (692.3±247.1 and 7,327±2,429 cells/cm^2, respectively).

Discussion

CD1a is a marker of DCs and the double phenotypes of CD1a and CD40 used in the present study are specific DC markers.

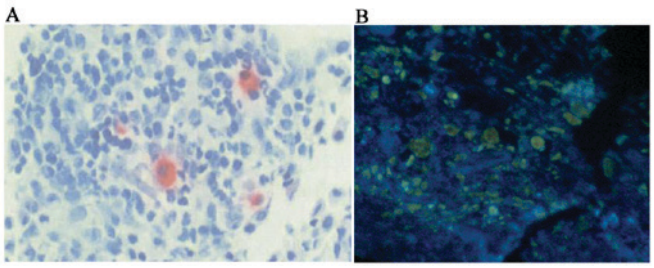

Figure 3. (A) CD83 is mainly expressed in the cell membrane of DCs in NP (x40). (B) CD1a/CD40 double-stained cells are more distributed in the submucous layer of NP Magnification, x40.

Furthermore, CD40+ staining indicates that DCs are in the mature phase and interacting with the T cells (11). The results of the present study indicate that the number of DCs in nITM tissue is significantly lower compared with NP tissues, which is consistent with previous reports (12) and suggests that DCs may serve an important role in the onset and development of NP. CD40+ staining suggests that DCs may be associated with the pathogenesis of NP, mainly via impacting the differentiation of T cells (13). In addition, the results of the present study revealed that CD83 was overexpressed in the DCs of nITM tissues compared with NP, which was consistent with previous studies (9,14). In addition, CD83 is a specific surface molecule expressed in mature DCs, suggesting that DCs in NP immature. Partially mature DCs express CD40, which then interacts with T cells and mediates the immune responses (15).

In the present study it was demonstrated that DCs are mainly distributed in the submucosal layer in NP tissues and DCs near the epithelium are relatively dense. DCs most likely infiltrate the lamina propria, explaining the gradual decrease from the outside to the inside (14). Such distribution characteristics of DCs may be due to the effect of chemotactic adhesion factor; Yoshimi *et al* (16) observed that DCs are mainly located in the squamous epithelium of NP and rarely distributed in the pseudo stratified ciliated columnar epithelium. Yoshimi *et al* considered that this may be due to the fact that keratinocytes are mainly constructed by the squamous epithelium and are able to secrete DC chemokines, including IL-1 and GM-CSF, to attract DCs onto NP tissues via chemotaxis. The submucosal lamina propria is the site in which DCs interact with Eosnophils (EOS) and T cells: DCs are mainly distributed in the submucosal lamina propria and EOS, T cells and other inflammatory cells mainly infiltrate the submucosal lamina propria of NP (17). It has also been reported that (the expression of Toll-like receptors (TRL-2/TRL-4) in NP are significantly enhanced and mainly expressed in the submucosa (18). DCs are able to upregulate TRLs (19). The present study, combined with these previous reports, suggests that DCs upregulate TRLs in NP to identify pathogen associated molecular patterns (PAMPs). Interactions of PAMPs with TLRs on DCs leads to the maturation of DCs via the NF-κB pathway during an immune response, which may result in the secretion of major histocompatibility complex class II, over-expression of costimulatory molecules and the secretion of proinflammatory cytokines (IL-1, TNF-α, IL-6, or IL-12). T cells may then become activated by these costimulatory

Table I. Comparison of distribution density and ratio of CD1a+DCs and CD83+DCs between NP and nITM.

		Distribution density of CD1a+ and CD83+DCs (cell/mm^2)		Ratio of CD83+ and CD1a+DCs
Group	n	CD1a+DCs	CD83+DCs	CD83+/CD1a+DCs
C	10	11.1±4.9	8.7±3.3	0.78±0.67
E	30	39.8±3.5[a]	26.9±2.3[a]	0.68±0.66[a]

[a]P<0.01 vs. group C. CD, cluster of differentiation; DC, dendritic cell; NP, nasal polyp; nITM, normal inferior turbinate mucosa; C, control group; E, experimental group.

Table II. Comparison of distribution area, number and density of CD1a/CD40 DCs between NP and nITM.

Group	n	Total area (μm^2)	Total number (cells)	Density (cells/cm^2)
C	10	285.2±169.6	42.9±33.5	566±389
E	30	3,417.3±755.1	692.3±247.1	7,327±2,429
P-value		<0.01	<0.01	<0.01

CD, cluster of differentiation; DC, dendritic cell; NP, nasal polyp; nITM, normal inferior turbinate mucosa; C, control group; E, experimental group.

molecules and cytokines, which further promotes the immune response (20).

The results of the present study show that the DCs in NP are significantly increased and are CD40-positive, suggesting that DCs may activate the expression of B7-1 (CD80) and B7-2 (CD86) by binding to T cells' CD40L; the B7 molecule expressed by DCs may then activate the T cells via the CD28/CTLA-4 pathway (21). This antigen presentation process is a two-way process, in which DC mediates the activation of T cells and receives an activation signal feedback from T cell to enter the so-called terminal mature stage, during which DCs highly express CD80/CD86 on their surface and lose their adhesiveness and phagocytosis (22). The interaction between DCs and T cells may lead to T cell maturation and cytokine secretion, which may induce a greater infiltration of DCs and T cells into NP tissues, resulting in the increased number of DCs and T cells observed in the present study, which further exacerbates NP.

Increase numbers of DCs and T cells in NP tissues produces several effects. The increase of DCs and T cells in NP is crucial to maintain the Th1/Th2 ratio in NP (23), with predominant expression of the Th2 cytokine. This may be achieved via various mechanisms: i) Jahnsen *et al* (24) demonstrated that an increase in the number of DC2 in the mucosa induces Th2, thus resulting in a significant increase in Th2 cells in NP and upregulation of the Th2 cytokines; ii) DC-induced maturation and differentiation of T cells may occur via connecting to surface molecules and secreting cytokines. DC-expressed CD86 is the costimulatory molecule that causes Th0 to differentiate into Th2. CD80 mainly induces the differentiation of Th1 (25), and DC is able to cause an imbalance of Th1/Th2 in NP by altering the CD80/CD86 ratio; iii) When T cells interact with DCs, they are able to induce DCs to secrete IL-12 via CD40/CD40L and IL-12 is the most important cytokine during Th0 differentiation into Th1 cells (26). However, IL-10 has been reported to be highly expressed in NP (27). Reider *et al* (28) also confirmed that IL-10 acting on DCs may cause defective IL-12 secretion, thus inhibiting the differentiation of T cells into Th1 cells while inducing their polarization toward Th2; iv) DCM is able to induce the production of IL-4 in DCs (29). IL-4 is the most important cytokine during Th0 cell differentiation into Th2 cells. IL-4 is increased in NP tissues, thus prompting the Th0 cells to differentiate toward the Th2 cells. The Th2 cells can also secrete IL-4 and this cycle can promote the dominance of Th2 in NP. In addition, IL-4 can promote the B cells to transform toward the plasma cells and secrete IgE (30); IgE overexpression is known to be one of the factors associated with NP onset and its increase is positively correlated with EOS infiltration (31).

The increased number of DCs and T cells in NP is also responsible for the overexpression of IL-5 in NP. It has been reported that DCs are able to induce T cells to secrete IL-5 (32). IL-5 is one of the most important cytokines and has been confirmed to be associated with the onset and development of NP (33). During the early stages of NP, Th2 cells are the main source of IL-5; however, as the disease progresses, EOS gradually replaces Th2 and becomes a direct source of IL-5 in NP (34). It has been reported that IL-5 is able to directly inhibit the differentiation and maturation of DCs (35). Therefore, DCs may promote the infiltration of EOS by inducing the IL-5 secretion pathway in T cells in NP. The infiltration of EOS may then inhibit the differentiation and maturation of DCs by secreting IL-5, thus keeping the DCs in an immature stage to promote the development of NP.

In summary, the present study demonstrated that the submucosal layer of NP has a large number of DCs, whereas

the ratio of CD83+ DCs is relatively low in NP, indicating that more DCs are in non-mature stages. Partially mature DCs interact with T cells via the CD40/CD40L costimulatory factor, thus serving an important role in the development and progression of NP. This process may be regulated by the immune response signaling pathway. The results of the present study indicate that the interaction between DCs and T cells is associated with the pathogenesis of NP, which might further contribute to Th1/Th2 imbalance and cytokine secretion. However, there were a number of limitations in the present study, including the small sample size, which needs to be addressed.

Future studies should investigate the signaling pathways involved in the interaction between DC and T-cells in NP at the cytological and molecular levels. Closer attention should be paid to the interaction between DCs and T cells to develop targets for the prevention and treatment of NP, thus helping to improve our understanding of the pathogenesis of NP.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

XL collected the samples; XZ performed immunohistochemistry and immunostaining; CL performed statistical analysis; XW planned the study and wrote the manuscript.

Ethics approval and consent to participate

The present study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Shantou Central Hospital (Shantou, China). Written informed consent was obtained from all participants.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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