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From Cell Biology to Tissue Engineering

The role of dendritic cells in immune regulation of nasal polyps

Zhuoping Liang, Ting Yang, Wei Xu, Ying Huang, Liang Jiang, Zedeng Yin and Gang Qin

Department of Otolaryngology Head and Neck Surgery,
the Affiliated Hospital of Southwest Medical University, Sichuan, Luzhou, China

Summary. Nasal polyps (NPs) are caused by a variety of immune cells and inflammatory cells. However, as the most potent antigen-presenting cells in the immune system, the role of dendritic cells (DCs) in NPs is still unclear. In the present research, we studied the role of DCs in immune regulation of NPs. Thirty patients with NPs, who served as the experimental group, received systemic and local glucocorticoids for 4-7 d, and specimens were collected prior to hormone treatment and during surgery. Normal middle turbinate mucosa tissues from 18 patients who underwent nasal septum surgery were collected as controls. The expression levels of CD83, tumor necrosis factor-α (TNF-α), interleukin-4 (IL-4) and eosinophils (EOS) in NP tissues before and after glucocorticoid therapy and in control middle turbinate mucosa tissues were studied. After glucocorticoid therapy, the expression levels of CD83, TNF-α, IL-4 and EOS decreased significantly. In addition, the expression of IL-4 was lower than that of TNF-α, reversing the Th2 cytokine-dominant condition. CD83 and EOS showed a positive correlation. DCs participated in the development and progression of NPs and could promote the generation of Th2 cytokines. After interference by glucocorticoid therapy, DCs could inhibit the expression of Th2 cytokines and induce secretion of Th1 cytokines. DCs and EOS thus might both play roles in promoting the development and progression of NPs, but the underlying mechanism requires further study.

Key words: Dendritic cells, Nasal polyp, Tumor necrosis factor-α, Interleukin-4, Eosinophils

Offprint requests to: Dr. Gang Qin, MD, Department of Otolaryngology Head and Neck Surgery, the Affiliated Hospital of Southwest Medical University, NO.25, Taiping Road, Jiangyang District, Luzhou City, 646000, China. e-mail: qing_lzmc@163.com

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Introduction

Nasal polyps (NPs) represent a common disease in otolaryngology and head and neck surgery. The most recent epidemiological statistics show that the incidence and the recurrence rate of NPs are high. This directly or indirectly increases socioeconomic burdens and severely affects the quality of life of patients (Halawi et al., 2013). The clinical manifestations of NPs include the formation of a single polyp or multiple polyps in the nasal mucosa of the middle nasal meatus, with extreme edema. The disease usually presents as persistent nasal obstruction with mucoid or purulent nasal discharge, hyposmia, and head and face fullness (Georgy and Peters, 2012). The pathological features of the disease include coverage of the mucosal surface by pseudostratified ciliated columnar epithelium, a large amount of inflammatory cell infiltration, obvious interstitial edema, changes in the shapes of glands, and hyperplasia of goblet cells in the submucosal lamina propria (Perić et al., 2011). However, the pathomechanism of NPs is still unclear. At present, most scholars agree that NPs are caused by a variety of factors and that they constitute a chronic inflammatory disease involving various immune cells and inflammatory cells (Casale et al., 2011).

As the most potent antigen-presenting cells in the immune system, dendritic cells (DCs) use specific

antigen uptake and processing to present antigens to T cells, thus regulating the direction of T cell differentiation and inducing humoral and cellular immunity. An increasing number of studies have shown that the expression of DCs is significantly increased in NP tissues. DCs possess relatively specific surface recognition markers, including CD1a, CD83, and S-100, among others. The CD83 molecule, a typical marker of DC maturation, is expressed only after maturation of the cells in vitro culture. The maturation of DCs is primarily indicated by the expression of CD83 molecule. Mature DCs express high surface levels of major histocompatibility complex II (MHC II) molecules and co-stimulatory molecules, which can effectively stimulate the activation and proliferation of naïve T cells to produce immune responses (Schuler and Steinman, 1997). Immature DCs primarily take up and process antigens, then become mature DCs after they migrate to peripheral lymphoid organs. B7-1 and B7-2 molecules on the DC surfaces are activated, and the secretion of cytokines is promoted. In addition, through the CD28/cytotoxic T lymphocyte-associated antigen 4 (CTLA4) pathway, B7 molecules and T cell receptors (TCRs) together activate T cells to induce immune responses (Grewal et al., 1996; Jiang et al., 2011). DCs and T cells have mutual interactions, and these interactions are the major factor that determines whether Th0 cells differentiate into Th1 or Th2 cells. Currently, the majority of scholars recognize that DCs cause an imbalance of the Th1/Th2 ratio in NPs and that these cells promote the development and progression of NPs by affecting the direction of Th0 differentiation.

Eosinophils (EOS) are the most common type of inflammatory cells in human NP tissues, accounting for more than 60% of inflammatory cells. EOS play important roles in the development and progression of NPs and are closely associated with the postoperative recurrence of NPs (Gelardi et al., 2010). The activation rate of EOS in NPs is very high. In addition, it is thought that only activated EOS can induce inflammatory responses in the nasal cavity (Jeong et al., 2011). The activation of EOS is a complex microenvironment-controlled process involving many cytokines, among which interleukin (IL)-4, IL-5, and interferon-γ (IFN-γ) play pivotal roles (Sun et al., 2009). Studies have shown that the degree of epithelial damage, the thickness of the basal layer, and the number of EOS are significantly greater in NP tissues than in control tissues. In addition, certain authors have reported that in patients with NPs, the infiltration depth of EOS shows a strong relationship with the epithelial damage and remodeling processes, thus, the major mechanism might be associated with the release of a variety of mediators by EOS. Epithelial damage might occur due to the release of various inflammatory cytokines that facilitate cell mitosis and fiber growth and cause collagen deposition, angiogenesis, and smooth muscle proliferation, thus promoting the growth of NPs (Saitoh et al., 2009).

Glucocorticoid therapy has become an important method for the comprehensive treatment of NPs. Studies have shown that fluticasone propionate aqueous nasal spray and beclomethasone dipropionate aqueous nasal spray are effective in treating the symptoms of NPs, with certain evidence that fluticasone propionate aqueous nasal spray has a faster onset of action and is tolerated at least as well as beclomethasone dipropionate aqueous nasal spray at the same dose (Holmberg et al., 1997). The major molecular mechanism underlying the treatment of NPs with glucocorticoids is regulation of the expression of glucocorticoid receptor genes, which influences molecular information transmission and directly or indirectly interferes with the transcription and translation of many inflammatory mediators. In addition, glucocorticoid therapy influences cell migration and epithelial cell hyperplasia through an increase in the expression of vascular endothelial growth factor (VEGF) in the nasal mucosa (Grzanka et al., 2011). One study reported that the clinical efficacy of glucocorticoids in NPs may be due to 1) induction of apoptosis in both EOS and T lymphocytes that infiltrate NPs and 2) down-regulation of epithelial granulocyte macrophage colony-stimulating factor (GM-CSF) production, which prolongs EOS survival (Watanabe et al., 2004). In recent years, studies have shown that glucocorticoids can significantly decrease the ability of DCs to take up and process antigens in the airways, without affecting the antigen presentation process (Sokolovska et al., 2007). In a guinea pig model of asthma, fluticasone propionate could reduce antigen-induced airway hyperreactivity and pulmonary inflammation, and the results suggested that DCs are the targets of the anti-inflammatory effects of fluticasone propionate in the airways (Lawrence et al., 1998).

In summary, DCs have been shown to play a pivotal role in inflammatory immune responses. However, how the interaction between DCs and T cells affects the development and progression of NPs requires further investigation. The following additional questions must be answered: How are the expression and functions of DCs in NP tissues altered by glucocorticoid treatment? Given that EOS are the most common inflammatory cells in NP tissues, what is the relationship between EOS and DCs? With these questions in mind to further investigate the pathological mechanism of NPs, the present study measured the expression of the CD83 cell surface marker on mature DCs, tumor necrosis factor-α (TNF-α), IL-4 and EOS in NP tissues before and after glucocorticoid treatment. The results will contribute to a better understanding of the pathological mechanism of NP development and progression and provide a theoretical basis and more effective targets for the prevention and treatment of NPs.

Materials and methods

Patient selection and study design

A total of 30 patients hospitalized with NPs between

June 2013 and December 2013 in the Department of Otolaryngology Head and Neck Surgery at the Affiliated Hospital of Southwest Medical University who met the inclusion criteria were selected as the experimental group. The experimental group received combined systemic and local glucocorticoid therapy for 4-7 d. Specimens were collected prior to hormone treatment and during surgery. There were 30 patients in each group before and after hormone treatment, with 19 male patients and 11 female patients. Their ages ranged from 15-67 years, and the median age was 41 years. The specimens were diagnosed as NPs by conventional hematoxylin-eosin (HE) staining. The control group included normal middle turbinate mucosa tissues (Pezato et al., 2014) from 18 hospitalized patients (10 male and 8 female) who underwent nasal septum surgery during the same time period. The age of the control group ranged from 22-45 years, and the median age was 36 years. Specimens were prepared using conventional HE staining, and the diagnosis of the middle turbinate mucosa was confirmed. NPs and middle turbinate mucosa specimens were obtained only from patients who had given their written informed consent according to a study protocol approved by the ethics committee of the Affiliated Hospital of Southwest Medical University.

Inclusion criteria

1. The patients had no history of allergic diseases such as allergic rhinitis or asthma, and no history of hypertension, diabetes mellitus, or cancer.
2. The patients had not received systemic or local glucocorticoids or immunosuppressants within 2 weeks of hospitalization.
3. The experimental group had received preoperative systemic dexamethasone (10 mg) and local fluticasone propionate nasal spray in the nasal cavity for 4-7 d, with an average of 5d.

Reagents

Rabbit anti-human TNF-α polyclonal antibody; rabbit anti-human IL-4 polyclonal antibody; rabbit anti-human CD83 polyclonal antibody; a streptavidin-biotin complex (SABC) reagent kit; and CD-83, TNF-α, and IL-4 in situ hybridization reagent kits were purchased from Wuhan Boster Bio-engineering, Hubei, Wuhan, China.

Methods

All specimens were fixed in 4% paraformaldehyde containing 1% DEPC and were conventionally dehydrated, embedded in paraffin, and sectioned. Conventional HE staining was performed. Immunohistochemistry (SABC method) and in situ hybridization of CD83, TNF-α, and IL-4 were performed using reagent kits according to the manufacturer's instructions. The positive section specimens provided by the manufacturer of the reagent kits were used as positive controls in the experiment. As a negative control, the primary antibody was replaced with PBS.

Interpretation of results

The localization and quantity of CD83, TNF-α, and IL-4 were determined by immunohistochemistry, in situ hybridization, and HE staining followed by imaging using a LEICA microscopic imaging system (Leica Microsystems Wetzlar GmbH, Germany). Two pathologists performed blind interpretation of the slides. For interpretation of the tissue section results for CD83, TNF-α, and IL-4 from immunohistochemistry and in situ hybridization, five random fields in each section were selected and photographed under high-power magnification (×400). The average light density value (MOD=Sum IOD/Sum Area) was analyzed using the Image-Pro Plus 6.0 image analysis system. The mean value was used as the final result for the section. For EOS counting, five random fields in each section were selected under high-power magnification (×400). A counting grid was installed on the eyepiece for counting, and the average number of positive cells was calculated.

Statistical analyses

The experimental results were statistically analyzed using SPSS17.0 software. The values are presented as the mean±standard deviation ($\bar{\chi}$±s). All measurement data underwent the normality test. Pairwise comparison of data obtained before and after glucocorticoid therapy and from the control group was performed using Dunnett's t3 test. Correlation analysis of detection indicators in the samples was performed using linear correlation analysis. A significance level of α=0.05 was used, and $p<0.05$ indicated that the results were statistically significant.

Table 1. Expression of CD83 in the experimental and the control groups.

Group/Number of cases		Staining method	CD83 mean optical density value
①before glucocorticoid therapy			
	30	immunohistochemistry	0.1618±0.0578
		in situ hybridization	0.2048±0.0380
②after glucocorticoid therapy			
	30	immunohistochemistry	0.0359±0.0149
		in situ hybridization	0.0505±0.0189
③control group			
	18	immunohistochemistry	0.0075±0.0027
		in situ hybridization	0.0059±0.0013
t value [a]	①②11.551[c]	①③14.547[c]	②③10.214[c]
t value [b]	①②20.039[c]	①③28.841[c]	②③12.771[c]

[a]: immunohistochemistry, [b]: in situ hybridization, [c]: $p<0.05$.

Results

Expression of CD83 in NPs and normal middle turbinate mucosa tissues

CD83-positive cells were mainly distributed in the epithelial layer and the lamina propria of the nasal mucosa. A small proportion of these cells were found surrounding glands and blood vessels. Immunohistochemistry and in situ hybridization detection produced light yellow or brown-yellow staining in the cytoplasm. The expression level of CD83 cells was higher before glucocorticoid therapy than after the treatment, and the difference was statistically significant ($p<0.05$). The expression levels of CD83-positive cells both before and after glucocorticoid therapy were significantly higher than the expression levels in the control group (both $p<0.05$) (Fig. 1, Table 1).

Expression of TNF-α in NPs and normal middle turbinate mucosa tissues

TNF-α was expressed in epithelial cells, various inflammatory cells, vascular endothelial cells, and gland cells of NP tissues, and immunohistochemistry and in situ hybridization methods produced light yellow and brown-yellow staining in the cytoplasm. TNF-α expression was significantly higher before glucocorticoid therapy than after hormone treatment, and the expression levels of TNF-α positive cells before and after glucocorticoid therapy were significantly higher than the expression levels in the control group (all $p<0.05$) (Fig. 2, Table 2).

Expression of IL-4 in NPs and normal middle turbinate mucosa tissues

Before and after glucocorticoid therapy, light yellow or brown-yellow IL-4 positive cells could be observed in NP specimens. IL-4 positive cells were mainly distributed in the cytoplasm of the tissue epithelial layer, the basement membrane, vascular endothelial cells, and inflammatory cells. Following glucocorticoid therapy, IL-4 expression decreased, and the difference was statistically significant ($p<0.05$). The expression levels of IL-4-positive cells in the experimental group were significantly higher both before and after glucocorticoid therapy than the expression levels in the control group (both $p<0.05$) (Fig. 3, Table 3).

Association between TNF-α and IL-4

Immunohistochemistry and in situ hybridization showed that the expression level of IL-4 in NP tissues before glucocorticoid treatment was higher than the level

Table 2. Expression of TNF-α in the experimental and the control groups.

Group/Number of cases		Staining method	TNF-α mean optical density value
①before glucocorticoid therapy			
	30	immunohistochemistry	0.1520±0.0557
		in situ hybridization	0.0668±0.0201
②after glucocorticoid therapy			
	30	immunohistochemistry	0.0576±0.0232
		in situ hybridization	0.0388±0.0134
③control group			
	18	immunohistochemistry	0.0061±0.0022
		in situ hybridization	0.0075±0.0032
t value [a]	①②8.536[c]	①③14.255[c]	②③12.000[c]
t value [b]	①②6.364[c]	①③16.027[c]	②③12.038[c]

[a]: immunohistochemistry, [b]: in situ hybridization, [c]: $p<0.05$.

Table 3. Expression of IL-4 in the experimental and the control groups.

Group/Number of cases		Staining method	IL-4 mean optical density value
①before glucocorticoid therapy			
	30	immunohistochemistry	0.2314±0.0434
		in situ hybridization	0.1419±0.0552
②after glucocorticoid therapy			
	30	immunohistochemistry	0.0228±0.0059
		in situ hybridization	0.0293±0.0148
③control group			
	18	immunohistochemistry	0.0031±0.0014
		in situ hybridization	0.0056±0.0024
t value [a]	①②26.075[c]	①③28.899[c]	②③17.909[c]
t value [b]	①②10.827[c]	①③13.495[c]	②③8.464[c]

[a]: immunohistochemistry, [b]: in situ hybridization, [c]: $p<0.05$.

Table 4. Expression of EOS in the experimental and the control groups.

Group	Number of cases	The number of EOS	
①before glucocorticoid therapy	30	45.24±14.80	
②after glucocorticoid therapy	30	14.99±5.72	
③control group	18	4.08±1.49	
t value	①②10.445[c]	①③15.111[c]	②③9.908[c]

[c]: $p<0.05$.

Table 5. Correlation between CD83 and EOS expression (r value).

Group	Number of cases	Immunohistochemistry	In situ hybridization
①before glucocorticoid therapy	30	0.626[c]	0.539[c]
②after glucocorticoid therapy	30	0.74[c]	0.638[c]

[c]: $p<0.05$.

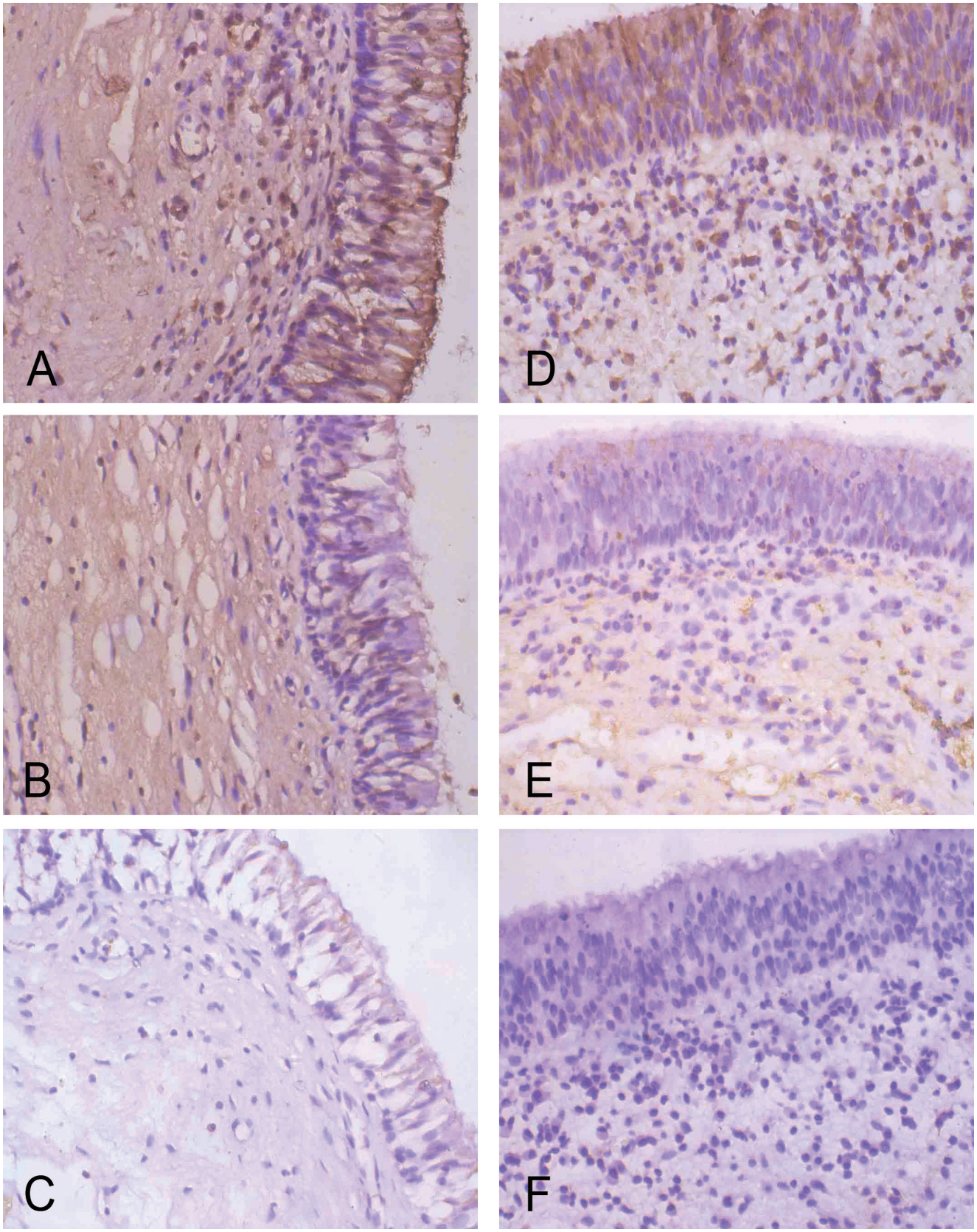

Fig. 1. Immunohistochemistry and in situ hybridization detected CD83 expression in the epithelial layer and the lamina propria of the nasal mucosa, with light yellow or brown-yellow cytoplasmic staining. **A, B and C** refer to the experimental group before glucocorticoid therapy, the experimental group after glucocorticoid therapy, and the control group, respectively (immunohistochemistry). **D, E and F** refer to the experimental group before glucocorticoid therapy, the experimental group after glucocorticoid therapy, and the control group, respectively (in situ hybridization). × 400

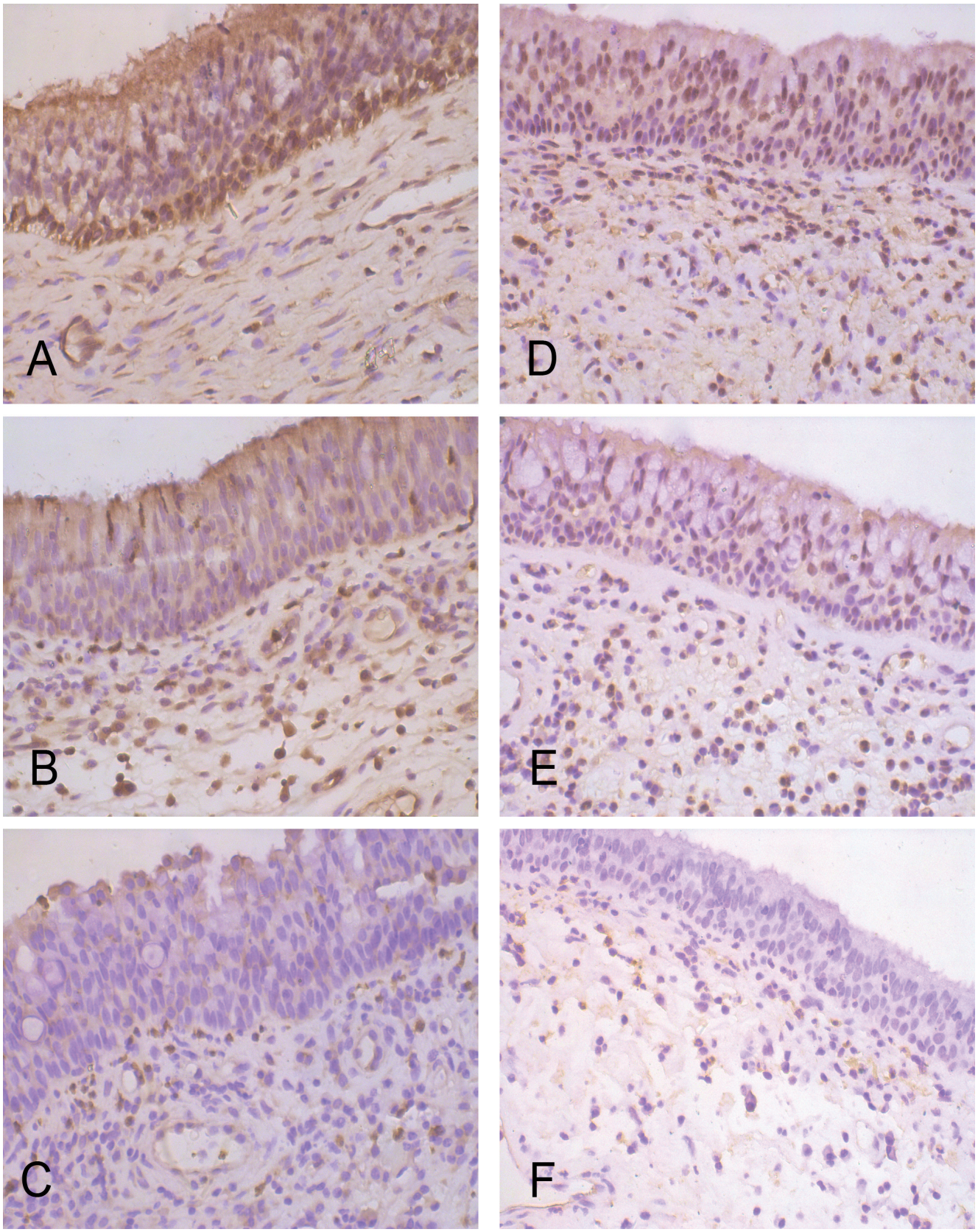

Fig. 2. TNF-α was expressed in epithelial cells, various inflammatory cells, vascular endothelial cells, and gland cells in NP tissues, with light yellow and brown-yellow staining in the cytoplasm, as detected using immunohistochemistry and in situ hybridization methods. **A-C** refer to the experimental group before glucocorticoid therapy, the experimental group after glucocorticoid therapy, and the control group, respectively (immunohistochemistry). **D-F** refer to the experimental group before glucocorticoid therapy, the experimental group after glucocorticoid therapy, and the control group, respectively (in situ hybridization). × 400

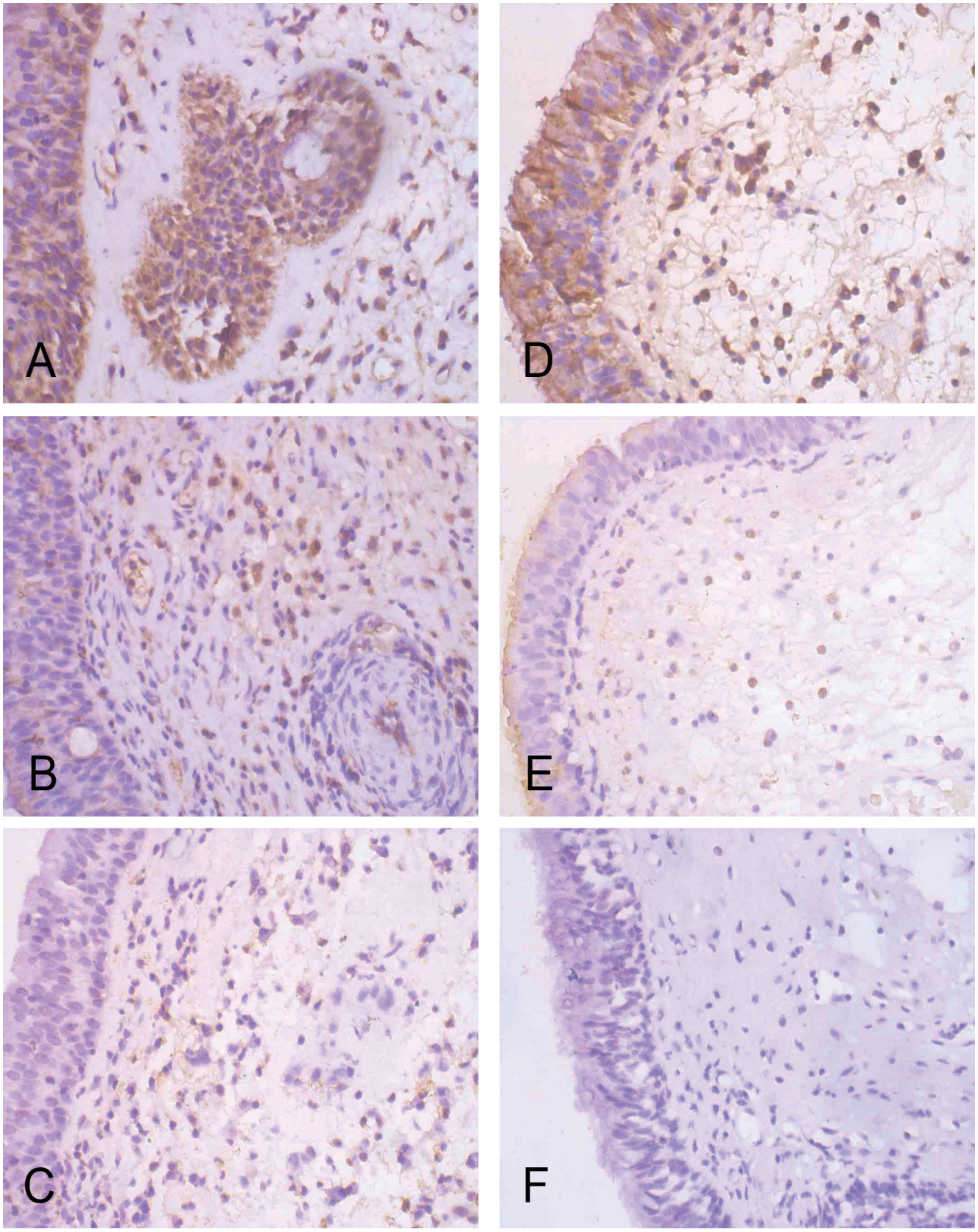

Fig. 3. Before and after glucocorticoid therapy, light yellow or brown-yellow IL-4 positive cells could be observed in NP specimens. IL-4 positive cells were mainly distributed in the cytoplasm of the epithelial layer, the basement membrane, vascular endothelial cells, and inflammatory cells. **A-C** refer to the experimental group before glucocorticoid therapy, the experimental group after glucocorticoid therapy, and the control group, respectively (immunohistochemistry). **D-F** refer to the experimental group before glucocorticoid therapy, the experimental group after glucocorticoid therapy, and the control group, respectively (in situ hybridization). × 400

of TNF-α. Statistical analyses showed that the t values were 6.197 and 6.995 for the immunohistochemistry and in situ hybridization results, respectively (both $p<0.05$). The level of expression of IL-4 in NP tissues after glucocorticoid therapy was lower than the level of TNF-α. The t values were 7.996 and 2.623 for the immunohistochemistry and in situ hybridization results, respectively (both $p<0.05$). The difference was statistically significant.

EOS infiltration

HE-stained sections revealed varying degrees of hyperplasia and thickening of the epithelial layers of NP tissues, which were mostly pseudostratified ciliated columnar epithelia and a few squamous epithelia. The glands showed irregular expansion changes and obvious interstitial edema. A large amount of inflammatory cell infiltration, consisting primarily of EOS and neutrophils, could be observed. EOS usually showed 2-3 lobes in the nucleus and a large number of bright red granules in the cytoplasm. Ciliated columnar epithelia could be observed on the surface of normal nasal mucosal tissues, which contained abundant goblet cells. Loose connective tissues containing more glands and blood vessels could be observed in the interstitia, but obvious inflammatory cell infiltration was not observed, and EOS were almost undetectable. The number of EOS decreased significantly after glucocorticoid therapy ($p<0.05$), and there were significant differences between the control and the experimental groups (both $p<0.05$) (Fig. 4, Table 4).

Correlation analysis of CD83 and EOS in the experimental groups

The levels of expression of CD83 and EOS in NP tissues before and after hormone treatment were measured using immunohistochemistry and in situ hybridization technology. The r values of the correlation analysis of CD83 and EOS were shown below (Table 5), both showed a positive correlation (both $p<0.05$).

Discussion

Expression of DCs in NP tissues

DCs, which are the most potent antigen-presenting cells with an ability to sensitize naive T lymphocytes and induce a primary immune response, were first

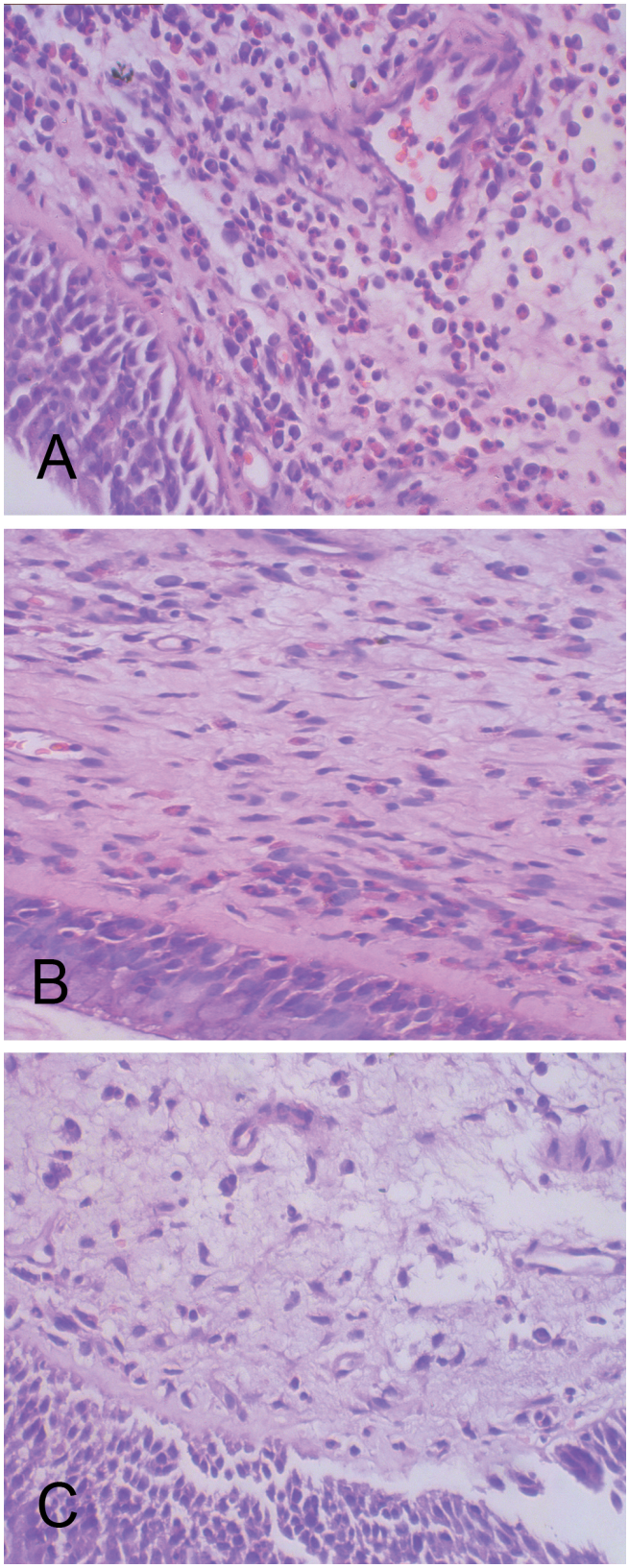

Fig. 4. HE staining revealed that a large amount of inflammatory cell infiltration, consisting primarily of EOS and neutrophils, could be observed. EOS usually showed 2-3 lobes in the nucleus and a large number of bright red granules in the cytoplasm. **A.** Strong EOS infiltration in the experimental group before glucocorticoid therapy. **B.** Weak EOS infiltration in the experimental group after glucocorticoid therapy. **C.** EOS were nearly undetectable in the control group. (HE). × 400

discovered by the Canadian scholar Steinman. These cells can be isolated from secondary lymphoid organs or peripheral sites, demonstrate considerable heterogeneity in both phenotype (i.e., cell surface marker expression) and function, and can be categorized into distinct subtypes (Reid et al., 2000; Hao et al., 2008). Human DCs can be divided into two main subtypes: myeloid DCs (mDCs) and plasmacytoid DCs (pDCs) (Ueno et al., 2007; Segura and Amigorena, 2014); the latter also comprise resident and migratory DCs (Segura et al., 2012). Whereas mDCs are $CD11c^{+}DC\text{-}SIGN^{+}$ cells, pDCs express CD123, BDCA-2, and BDCA-4, but no CD11c, with each playing distinct and complementary roles in the induction of immune responses (Rissoan et al., 1999; Cella et al., 1999; Ito et al., 2005). mDCs are efficient in the uptake, processing, and presentation of foreign antigens. Following Toll-like receptor stimulation, mDCs produce TNF-α, and matured mDCs produce proinflammatory cytokines such as IL-12. Conversely, pDCs are less effective in these processes and are mainly known for their function in antiviral immunity and rapid production of type I interferon (Hayashi et al., 2013). In the process of differentiation and development, DCs exist in two different phases, immature DCs and mature DCs. After stimulation by pathogens or tissue injury, immature DCs eventually differentiate into mature DCs in peripheral lymphoid organs. With the maturation of DCs, the expression of many surface molecules, such as MHC II, CD83, and the co-stimulatory factors CD80 and CD86 increases (Banchereau and Steinman, 1998; Kadowaki, 2007). CD83 is currently the best-known surface marker for mature DCs, and it is the only molecule that is stably expressed on the surface of mature DCs. In addition, CD83 can activate T cells and B cells (Prechtel et al., 2007). The present study used immunohistochemistry and in situ hybridization technology to determine the level of expression of CD83 in NP tissues. The results showed that CD83-positive cells were primarily expressed in the epithelial layer and the lamina propria of the nasal mucosa and that a small amount of CD83-positive cells were present around glands and blood vessels. Via the detection of DCs in NP tissues, certain scholars have found that the number of DCs in NP tissues was relatively dense in the epithelium and the submucosal lamina propria and that it showed a decreasing trend from outside to inside. These results are consistent with our experimental results (Lin et al., 2013).

Mutual functional interactions between DCs and T lymphocytes

Under general conditions, after presenting antigens to T cells, DCs provide specific signals that permit interactions between CD40L distributed on the surface of T cells and CD40 on the surface of DCs; these interactions stimulate DCs to highly express co-stimulatory molecules such as B7-1 and B7-2. CD28 interacts with the B7 molecule expressed by DCs and functions together with the TCR to activate T cells (Pletinckx et al., 2011), whereas T cells influence the survival of DCs through the expression of cytokines and receptors. In in vitro cultures of DCs, TNF-α significantly promotes the expression of markers of DC maturation, such as CD80, CD86, and CD83, and significantly increases the ability of DCs to stimulate T lymphocyte proliferation. After removal of TNF-α, the quantity, phenotype, and function of DCs significantly decrease (Ito et al., 2012; Son et al., 2013). In addition, CD83 not only is a marker of mature DCs but also regulates T lymphocyte proliferation to achieve regulation of immune function (Aerts-Toegaert et al., 2007). DC subtypes are capable of driving CD4 and CD8 responses, thus regulating Th1/Th2 bias (Hancock et al., 2013). DCs are also divided into functional subtypes, such as a Th1-inducible DCs subtype (DC1s) and a Th2-inducible DCs subtype (DC2s). Over the past few decades, mDCs and pDCs have been thought to represent DC1s and DC2s, respectively (Rissoan et al., 1999; Moser and Murphy, 2000). However, recent studies have shown that both DC1s and DC2s differentiate from mDCs, and in certain reports, the terms “mDC1” and “mDC2” have been used to describe the mDC subtypes that promote naive T cell differentiation into Th1 and Th2 cells, respectively (Autissier et al., 2010; Hayashi et al., 2013). Th1 cells mainly secrete TNF-α, IFN-γ, and IL-12, and Th2 cells mainly secrete IL-4, IL-5, and IL-6 (Chen et al., 1998; Kidd, 2003). The present study measured the expression of TNF-α and IL-4 in NP tissues and showed that the expression of both TNF-α and IL-4 in these tissues was significantly increased prior to hormone treatment and that the expression level of IL-4 was higher than that of TNF-α. These results confirm the immune status of mixed Th1/Th2 expression and Th2 cell dominance in NP tissues (Kirsche et al., 2010). Certain studies using allergic respiratory disease animal models have reported that the expression of IL-4 and IL-5 is significantly downregulated and that the Th1/Th2 ratio is significantly increased after glucocorticoid intervention; under these conditions, Th1 cells become dominant (Zhang et al., 2009). The present study produced the same results, i.e., after glucocorticoid therapy, CD83 expression in NP tissues decreased, and the expression of mature DCs decreased. In addition, the expression of TNF-α and IL-4 also decreased significantly, and the expression level of IL-4 was lower than that of TNF-α, thus promoting a Th1 cytokine dominant environment in the NP tissues. We speculate that the differentiation and maturation processes and the survival ability of DCs are influenced by glucocorticoids. DCs inhibit excessive secretion of Th2 cytokines and promote the expression of Th1 cytokines, thereby restoring the balance of Th cell populations in NP tissues. In this study, high levels of CD83 and TNF-α expression were detected in NP tissues. Based on this result, we speculate that TNF-α might promote DC maturation and increase the immune

regulation functions of DCs in NP tissues. However, the detailed regulatory and molecular mechanisms of DC subtypes that induce Th1 and Th2 responses in NP tissue, require further experimental research. Treatment of NPs using steroid hormone drugs could change the inflammatory cell-cytokine network effect in the NP microenvironment. This finding may provide theoretical and experimental bases for improving the clinical treatment of NPs.

Interaction between DCs and EOS

EOS are the most common inflammatory cell type in NP tissues, and they play important roles in the development and progression of NPs. EOS have a close relationship with DCs. Together, EOS and DCs participate in the regulation of immune function. Studies have confirmed that CpG DNA can stimulate EOS to induce DC maturation. DC maturation is directly associated with EOS, and the mechanism of this association may depend on the release of major basic proteins by EOS. It has been noted that viral and bacterial products represent potential activators that may promote EOS-mediated induction of DC maturation (Lotfi and Lotze, 2008). Certain scholars have also speculated that the mechanism underlying the promotion of DC maturation by EOS involves the activation of DCs by EOS -derived neurotoxin (EDN). Studies have additionally shown that EDN significantly enhances the immune responses of Th2 cells (Yang et al., 2008). EOS act on DCs to activate T cells, and they participate in the regulation of the inflammatory immune response by Th2 cells in the respiratory tract. It has also been found that in EOS-deficient rats, DCs increase Th17/Th1 expression in neutrophils. These results suggest that EOS play an important role in the regulation of the balance of Th1, Th2, and Th17 expression (Jacobsen et al., 2011). Several scholars have already confirmed that IL-5 can directly inhibit DCs differentiation and maturation. It is generally believed that the role of DCs in the mechanism underlying the development of NP tissues is that DCs promote EOS infiltration into tissues through induction of IL-5 secretion by T cells, whereas EOS inhibit DC maturation through the IL-5 pathway, affecting the production of effective immune responses and thus facilitating the formation of NPs (Sokolovska et al., 2007; Yi et al., 2007). The results of the present study show that the expression of CD83 and EOS is positively correlated and that these components together participate in immune regulation of NP tissues. These results also provide a novel basis for the study of immune responses in NP tissues. However, the specific mechanisms of the DCs and EOS that participate in immune regulation of NP tissues require further study.

In summary, in-depth study of the relationship between DCs, related cytokines, and EOS in NP tissues can increase our understanding of the pathological mechanisms associated with the development and progression of NPs. Studies of the effects of steroid hormone drugs on DCs and cytokine levels in NP tissues are expected to provide a theoretical basis for the prevention and treatment of NPs and for the identification of more effective intervention targets in NPs.

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References

Aerts-Toegaert C., Heirman C. Tuyaerts S. Corthals J. Aerts J.L. Bonehill A. Thielemans K. and Breckpot K. (2007). CD83 expression on dendritic cells and T cells:correlation with effective immune responses. Eur. J. Immunol. 37, 686-695.

Autissier P., Soulas C., Burdo T.H. and Williams K.C. (2010). Evaluation of a 12-color flow cytometry panel to study lymphocyte, monocyte, and dendritic cell subsets in humans. Cytometry 77, 410-419.

Banchereau J. and Steinman R.M. (1998). Dendritic cells and the control of immunity. Nature 392, 245-252.

Casale M., Pappacena M., Potena M., Vesperini E., Ciglia G., Mladina R., Dianzani C., Degener A.M. and Salvinelli F. (2011). Nasal polyposis: from pathogenesis to treatment, an update. Inflamm. Allergy. Drug. Targets 10, 158-163.

Cella M., Jarrossay D., Facchetti F., Alebardi O., Nakajima H., Lanzavecchia A. and Colonna M. (1999). Plasmacytoid monocytes migrate to inflamed lymph nodes and produce large amounts of type I interferon. Nat. Med. 5, 919-923.

Chen B., Shi Y., Smith J.D., Choi D., Geiger J.D. and Mulé J.J. (1998). The role of tumor necrosis factor alpha in modulating the quantity of peripheral blood-derived, cytokine-driven human dendritic cells and its role in enhancing the quality of dendritic cell function in presenting soluble antigens to $CD4^+$ T cells *in vitro*. Blood 91, 4652-4661.

Gelardi M., Russo C., Fiorella M.L., Fiorella R. and Ciprandi G. (2010). Inflammatory cell types in nasal polyps. Cytopathology 21, 201-203.

Georgy M.S. and Peters A.T. (2012). Chapter 7: Nasal polyps. Allergy Asthma Proc. 33 (suppl 1), s22-23.

Grewal I.S., Foellmer H.G., Grewal K.D., Xu J., Hardardottir F., Baron J.L., Janeway C.A. Jr and Flavell R.A. (1996). Requirement for CD40 ligand in costimulation induction, T cell activation,and experimental allergic encephalomyelitis. Science 273, 1864-1867.

Grzanka A., Misiołek M., Golusiński W. and Jarząb J. (2011). Molecular mechanisms of glucocorticoids action: implications for treatment of rhinosinusitis and nasal polyposis. Eur. Arch. Otorhinolaryngol. 268, 247-253.

Halawi A.M., Smith S.S. and Chandra R.K. (2013). Chronic rhinosinusitis: epidemiology and cost. Allergy Asthma Proc. 34, 328-334.

Hancock D.G., Guy T.V., Shklovskaya E. and Fazekas de St Groth B. (2013). Experimental models to investigate the function of dendritic cell subsets: challenges and implications. Clin. Exp. Immunol. 171, 147-154.

Hao X., Kim T.S. and Braciale T.J. (2008). Differential response of respiratory dendritic cell subsets to influenza virus infection. J. Virol. 82, 4908-4919.

Hayashi Y., Ishii Y., Hata-Suzuki M., Arai R., Chibana K., Takemasa A. and Fukuda T. (2013). Comparative analysis of circulating dendritic cell subsets in patients with atopic diseases and sarcoidosis. Respir. Res. 14, 29.

Holmberg K., Juliusson S., Balder B., Smith D.L., Richards D.H. and Karlsson G. (1997). Fluticasone propionate aqueous nasal spray in the treatment of nasal polyposis. Ann. Allergy Asthma Immunol. 78, 270-276.

Ito T., Liu Y.J. and Arima K. (2012). Cellular and molecular mechanisms of TSLP function in human allergic disorders-TSLP programs the "Th2 code" in dendritic cells. Allergol. Int. 61, 35-43.

Ito T., Liu Y.J. and Kadowaki N. (2005). Functional diversity and plasticity of human dendritic cell subsets. Int. J. Hematol. 81, 188-196.

Jacobsen E.A., Zellner K.R., Colbert D., Lee N.A. and Lee J.J. (2011). Eosinophils regulate dendritic cells and Th2 pulmonary immune responses following allergen provocation. J. Immunol. 187, 6059-6068.

Jeong W.J., Lee C.H., Cho S.H. and Rhee C.S. (2011). Eosinophilic allergic polyp: a clinically oriented concept of nasal polyp. Otolaryngol. Head Neck Surg. 144, 241-246.

Jiang X.D., Li G.Y., Li L. and Zhu D.D. (2011). The characterization of IL-17A expression in patients with chronic rhinosinusitis with nasal polyps. Am. J. Rhinol. Allergy 25, e171-175.

Kadowaki N. (2007). Dendritic cells: a conductor of T cell differentiation. Allergol. Int. 56, 193-199.

Kidd P. (2003). Th1/Th2 balance: the hypothesis, its limitations, and implications for health and disease. Altern. Med. Rev. 8, 223-246.

Kirsche H., Niederführ A., Deutschle T., Fuchs C. and Riechelmann H. (2010). Ratio of myeloid and plasmacytoid dendritic cells and TH2 skew in CRS with nasal polyps. Allergy 65, 24-31.

Lawrence T.E., Millecchia L.L. and Fedan J.S. (1998). Fluticasone propionate and pentamidine isethionate reduce airway hyperreactivity, pulmonary eosinophilia and pulmonary dendritic cell response in a guinea pig model of asthma. J. Pharmacol. Exp. Ther. 284, 222-227.

Lin X.S., Luo X.Y., Wang H.G., Li C.W., Lin X. and Yan C. (2013). Expression and distribution of dendritic cells in nasal polyps. Exp. Ther. Med. 5, 1476-1480.

Lotfi R. and Lotze M.T. (2008). Eosinophils induce DC maturation, regulating immunity. J. Leukoc. Biol. 83, 456-460.

Moser M. and Murphy K.M. (2000). Dendritic cell regulation of TH1-TH2 development. Nat. Immunol. 1, 199-205.

Perić A., Vojvodić D., Vukomanović-Đurđević B. and Baletićet N. (2011). Eosinophilic inflammation in allergic rhinitis and nasal polyposis. Arh. Hig. Rada. Toksikol. 62, 341-348.

Pezato R., Voegels R.L., Pinto Bezerra T.F., Perez-Novo C., Stamm A.C. and Gregorio L.C. (2014). Mechanical disfunction in the mucosal oedema formation of patients with nasal polyps. Rhinology 52, 162-166.

Pletinckx K., Dohler A., Pavlovic V. and Lutz M.B. (2011). Role of dendritic cell maturity/costimulation for generation, homeostasis, and suppressive activity of regulatory T cells. Front. Immunol. 2, 39.

Prechtel A.T., Turza N.M., Theodoridis A.A. and Steinkasserer A. (2007). CD83 knockdown in monocyte-derived dendritic cells by small interfering RNA leads to a diminished T cell stimulation. J. Immunol. 178, 5454-5464.

Reid S.D., Penna G. and Adorini L. (2000). The control of T cell responses by dendritic cell subsets. Curr. Opin. Immunol. 12, 114-121.

Rissoan M.C., Soumelis V., Kadowaki N., Grouard G., Briere F., de Waal Malefyt R. and Liu Y.J. (1999). Reciprocal control of T helper cell and dendritic cell differentiation. Science 283, 1183-1186.

Saitoh T., Kusunoki T., Yao T., Kawano K., Kojima Y., Miyahara K., Onoda J., Yokoi H. and Ikeda K. (2009). Relationship between epithelial damage or basement membrane thickness and eosinophilic infiltration in nasal polyps with chronic rhinosinusitis. Rhinology 47, 275-279.

Schuler G. and Steinman R.M. (1997). Dendrtic cells as adjuvants for immune-mediated resistance to tumors. J. Exp. Med. 186, 1183-1187.

Segura E. and Amigorena S. (2014). Cross-presentation by human dendritic cell subsets. Immunol. Lett. 158, 73-78.

Segura E., Valladeau-Guilemond J., Donnadieu M.H., Sastre-Garau X., Soumelis V. and Amigorena S. (2012). Characterization of resident and migratory dendritic cells in human lymph nodes. J. Exp. Med. 209, 653-660.

Sokolovska A., Hem S.L. and HogenEsch H. (2007). Activation of dendritic cells and induction of CD4 (+) T cell differentiation by aluminum-containing adjuvants. Vaccine 25, 4575-4585.

Son C.H., Bae J.H., Shin D.Y., Lee H.R., Choi Y.H., Yang K. and Park Y.S. (2013). Enhanced maturation and function of dendritic cells using hydrogel coated plate and antigen electroporation. Immunol. Invest. 42, 341-355.

Sun D.I., Joo Y.H., Auo H.J. and Kang J.M. (2009). Clinical significance of eosinophilic cationic protein levels in nasal secretions of patients with nasal polyposis. Eur. Arch. Otorhinolaryngol. 266, 981-986.

Ueno H., Klechevsky E., Morita R., Aspord C., Cao T., Matsui T., Di Pucchio T., Connolly J., Fay J.W., Pascual V., Palucka A.K. and Banchereau J. (2007). Dendritic cell subsets in health and disease. Immunol. Rev. 219, 118-142.

Watanabe K., Shirasaki H., Kanaizumi E. and Himi T. (2004). Effects of glucocorticoids on infiltrating cells and epithelial cells of nasal polyps. Ann. Otol. Rhinol. Laryngol. 113, 465-473.

Yang D., Chen Q., Su S.B. Zhang P., Kurosaka K., Caspi R.R., Michalek S.M., Rosenberg H.F., Zhang N. and Oppenheim J.J. (2008). Eosinophil-derived neurotoxin acts as an alarmin to activate the TLR2-MyD88 signal pathway in dendritic cells and enhances Th2 immune responses. J. Exp. Med. 205, 79-90.

Yi H., Zhang L., Zhen Y., He X. and Zhao Y. (2007). Dendritic cells induced in the presence of GM-CSF and IL-5. Cytokine 37, 35-43.

Zhang S., Shen Z., Hu G., Liu R. and Zhang X. (2009). Effects of endogenous glucocorticoids on allergic inflammation and T(H)1/T(H)2 balance in airway allergic disease. Ann. Allergy Asthma Immunol. 103, 525-534.

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