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Effect of IFN-λ2 on combined allergic rhinitis with nasal polyps

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Abstract. – OBJECTIVE: This study sought to investigate the expression of interferon-λ2 (IFN-λ2) in patients with combined allergic rhinitis and nasal polyps (AR+NP), analyze the correlation between IFN-λ2 and tryptase, interleukin 10 (IL-10), and interleukin 12 (IL-12), and identify its peripheral blood cell origins.

PATIENTS AND METHODS: ELISA kits were used to investigate plasma levels of IFN-λ2, tryptase, IL-10, and IL-12 in AR+NP patients and healthy controls (HC). Flow cytometry analysis was carried out to detect IFN-λ2 expression in peripheral blood leukocytes. Immunocytochemical staining was performed to detect nasal polyp IFN-λ2 expression in AR+NP patients.

RESULTS: Elevated plasma IFN-λ2 levels and positive correlations between plasma IFN-λ2 and tryptase levels in AR+NP patients indicated that IFN-λ2 likely contributes to AR+NP pathogenesis. IFN-λ2 expression was upregulated in cytotoxic T cells and eosinophils in AR+NP patients. Nasal polyp mast cells and macrophages in AR+NP patients expressed IFN-λ2.

CONCLUSIONS: The close correlation between IFN-λ2 expression and AR+NP may provide experimental evidence for a possible effect of IFN-λ2 against the allergic inflammatory reaction. Therefore, IFN-λ2 actions may have a potential utility for the treatment and prevention of AR+AP.

Key Words:
Allergic rhinitis with nasal polyps, IFN-λ2, Tryptase, IL-10, IL-12.

Introduction

Allergic rhinitis (AR) is an allergic disease that causes inflammation of the nasal mucosa mainly through immunoglobulin E (IgE), resulting in nasal symptoms after allergen exposure[1,2]. Some patients contract allergic rhinitis due to long-term repeated stimulation-induced allergic inflammation, resulting in allergic rhinitis with nasal polyps (AR+NP)[3]. AR pathogenesis includes IgE-mediated and IgE-independent mechanisms, although the IgE-mediated mechanisms are currently better recognized[4]. In addition, cytokines, as potent pro-inflammatory factors, are involved in the occurrence and development of AR, and have been identified as a unique marker of trypsin in mast cell degranulate. A research[5] found that IL-10 had anti-inflammatory and immunosuppressive effects in inhibiting Th2 cell proliferation, thus attenuating allergic disease progression. Moreover, IL-12 induced naive T cell differentiation to the Th1 subset, and increased IFN-r production in Th1 cells, thereby controlling the Th1/Th2 balance[6]. Human IFN-λ2 (IL-28A) is an IFN-λ subtype, which is comprised of IFN- λ1, IFN-λ2, IFN-λ3, and IFN-λ4[7]. It is a relatively newly uncovered cytokine, similar to the IL-10 family in genetic and protein structure[8,9]. Meager et al[10] have found that IFN-λ2 plays a central role in innate immunity (i.e., it induces antiviral activity in cell lines), although it is less potent than other interferons. In addition to this, IFN-λ2 has demonstrated an effective anti-tumor effect on lung cancer cells. Scholars also have found that IFN-λ2 can aggravate T cell-mediated autoimmune diseases such as uveitis[11]. Ren et al[12] and Blazek et al[13] have indicated that IFN-λ2 administration reduced joint and inguinal lymph node secretion of the pro-inflammatory mediators IL-17, as well as Th17 cell and γδ T cell accumulation, and limited IL-1β expressing neutrophil recruitment in a mouse model of systemic polyarthritis, thereby completely inhibiting or even reversing the

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development of collagen-induced arthritis. Lewis et al[14] have shown that the expression of IFN-λ2 mRNA is significantly elevated after naturally occurring respiratory tract viral infection in asthmatic children (p <0.05), and that IFN-λ2 has been shown to inhibit airway allergic disease via the regulation of pulmonary dendritic cell function[15]. These studies suggested that IFN-λ2 was not only involved in autoimmune diseases, but also associated with airway allergies[16,17]. We investigated the plasma levels of IFN-λ2, tryptase, IL-10, and IL-12 in AR+NP patients and healthy controls (HC). Next, we explored the correlation between IFN-λ2, tryptase, IL-10, and IL-12 in the plasma of patients with AR+NP. Moreover, the expression of IFN-λ2 in peripheral blood leukocytes was detected.

Patients and Methods

Patients

Thirty-four patients with AR+NP and 22 healthy controls (HC) were recruited for our study. The criteria for asthma diagnosis were conformed to the Global Initiative for Asthma[18], and the criteria for allergic rhinitis diagnosis were conformed to Allergic Rhinitis and its Impact on Asthma (ARIA)[3]. All subjects were asked to terminate anti-allergy medication usage for at least 2 weeks beforeo the study. Recruited patients did not have airway infections lasting longer than one month. Informed consent was confirmed according to the Declaration of Helsinki, and the agreement of the Ethical Committee of Jining First People's Hospital was granted.

Sample Preparation

Peripheral venous blood samples (5 ml) were collected from patients and HCs, and were immediately treated for the collection of plasma and cells for subsequent analysis. Enzyme-linked immunosorbent assay (ELISA) kits were used to detect plasma levels of IFN-λ2, tryptase, IL-10, and IL-12. The correlation between IFN-λ2 and the cytokines was explored in AR+NP patients. Flow cytometry analysis was carried out to measure IFN-λ2 expression in peripheral blood leukocytes. Nasal polyps were collected from eight AR+NP patients.

ELISA

Human ELISA kits were allowed to equilibrate at room temperature for 30 min. Sample diluent and standard (50 µl each) were added to each well. The plates were then covered and incubated at room temperature for 2 h. After washing 4 times, 100 µl human-specific antibody were added to each well. The plate was covered and incubated for 1 h. After washing 4 times again, 100 µl of avidin-horseradish peroxidase solution was added, upon which the plate was sealed and incubated for 30 min. Finally, 100 µl of TMB substrate was added to each well, incubated for 15 min in the dark, and 100 µl Stop Solution was added. Absorbance was read at 450 nm.

Flow Cytometry Analysis

To detect IFN-λ2 expression on non-T cell leukocytes, the following antibodies were used: PerCP anti-human CD16, PE/Cy7 anti-human CD14. Antibodies were added to 200 µl whole blood at 37°C and incubated for 15 min in the dark. Following red blood cell lysis, the remaining white blood cells were fixed and permeabilized. Cell pellets were washed and then resuspended, upon which rabbit anti-human IFN-λ2 followed by PE- or FITC-conjugated goat anti-rabbit IgG antibody was incubated for 30 min at 4°C. Finally, cells were resuspended in fluorescence-activated cell sorting (FACS) flow solution and analyzed with a FACSVerse flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA). To detect T cell IFN-λ2 levels, mononucleated cells in peripheral

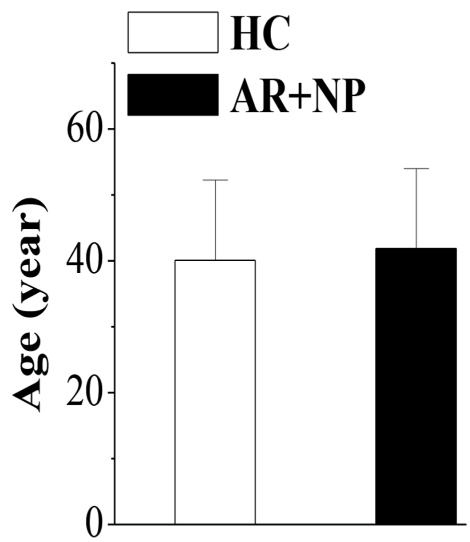

Figure 1. Clinical data comparison between the AR+NP and HC groups. The clinical data was compared between the AR+NP and HC groups to ensure comparability between the two groups. There were no statistically significant differences in the age characteristics between the two groups (p>0.05).

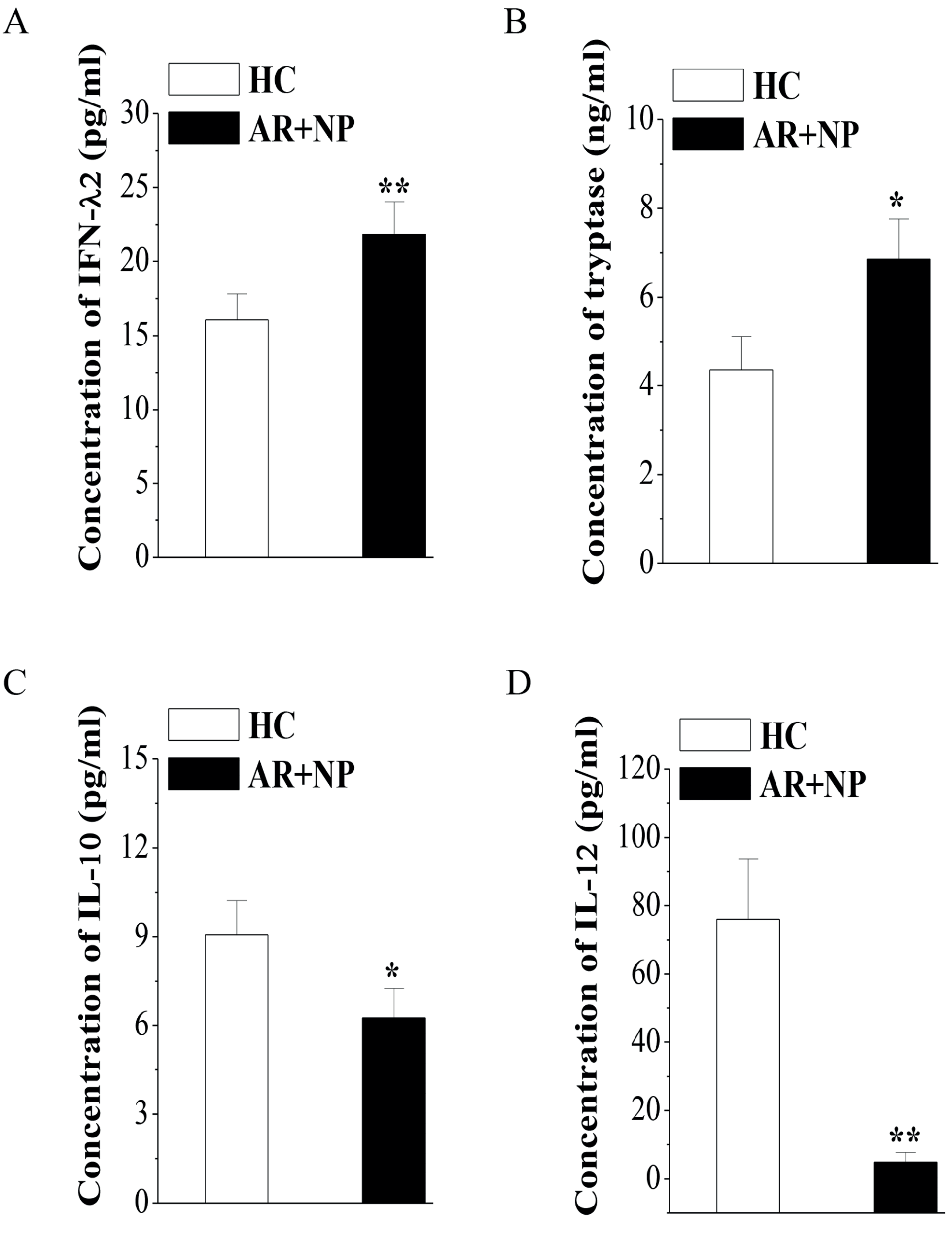

Figure 2. Comparison of IFN-λ2, tryptase, IL-10, and IL-12 plasma levels in AR+NP patients and HCs. ELISA was used to measure the plasma levels of IFN-λ2 *(A)*, tryptase *(B)*, IL-10 *(C)*, and IL-12 *(D)* in AR+NP patients and HCs. *p<0.05, **p<0.01 between AR+NP and HC groups.

blood were isolated using Lymphoprep according to the manufacturer's instructions. PE/Cy7 anti-human CD8 and PE-conjugated goat anti-rabbit IgG antibodies were used to detect CD8+ T cells.

Immunocytochemical Staining

Tissues were fixed, dehydrated, and embedded. Tissue sections (5 μm) were dewaxed, rehydrated, and incubated with 0.5% H_2O_2 in methanol for 10 min followed by sodium azide for another 10 min. 5% bovine serum albumin (BSA) dissolved in phosphate-buffered saline (PBS) was added for 1 h. Sequential sections of nasal polyps were incubated with biotinylated rabbit anti-human IFN-λ2 for 2 h, washed with Tris-buffered saline and Tween 20 (TBS-T) 5 times, and incubated with ExtrAvidin peroxidase conjugate for 1 h. DAB (diaminobenzidine) chromogen system staining was developed over 4 min.

Statistical Analysis

All data were expressed as the mean ± standard error of the mean (SEM), and were analyzed using a one-way analysis of variance followed by Bonferroni-Dunn correction. Statistical analysis was performed using SPSS 20.0 (IBM Corp. IBM SPSS Statistics for Windows, Armonk, NY, USA). $p<0.05$ was considered statistically significant.

Results

Clinical Data Comparison Between AR+NP and HC Groups

The clinical parameters of the AR+NP and HC groups were compared. As shown in Figure 1 and Table I, there was no significant difference in the age and sex of the two groups ($p>0.05$).

Plasma IFN-λ2, Tryptase, IL-10, and IL-12 Levels in AR+NP and HC Groups

ELISA was used to measure IFN-λ2, tryptase, IL-10, and IL-12 plasma levels. IFN-λ2 and tryptase levels were significantly elevated in AR+NP patients (Figure 2A-B) ($p<0.05$), while plasma IL-10 and IL-12 levels were markedly decreased in the AR+NP group (Figure 2C-D).

The Correlation Between IFN-λ2 and Tryptase, IL-10, and IL-12 Plasma Levels in AR+NP Patients

As shown in Table II, there was a positive correlation between IFN-λ2 and tryptase levels (r = 0.603, $p<0.05$) and a negative correlation between IFN-λ2 and IL-10 levels (r = -0.601, $p<0.05$). There was no correlation between IFN-λ2 and IL-12 levels (r = -0.387, $p>0.05$).

IFN-λ2 Expression in Peripheral Blood Leukocytes

We found that IFN-λ2 was predominately expressed in neutrophils and monocytes in HCs. However, in AR+NP patients, IFN-λ2 was mainly expressed in cytotoxic T cells, eosinophils, monocytes, and neutrophils. Compared with HC, IFN-λ2 expression was upregulated in cytotoxic T cells and eosinophils, but downregulated in monocytes and neutrophils in AR+NP patients, and all of these differences were statistically significant ($p<0.05$) (Figure 3).

Expression of IFN-λ2 in AR+NP Patient Nasal Polyps

Immunohistochemical staining showed that IFN-λ2 was expressed in several large cells within nasal polyps (most likely mast cells or macrophages). Flow cytometry analysis indicated that both mast cells and macrophages in dispersed human nasal polyps expressed IFN-λ2 (Figure 4).

Discussion

In our present research, elevated levels of IFN-λ2 and a positive correlation between IFN-λ2 and tryptase in the plasma of AR+NP patients indicated that IFN-λ2 likely contributed to the pathogenesis of combined allergic rhinitis with nasal polyps. IFN-λ2 expression was upregulated in cytotoxic T cells and eosinophils

Table I. Sex ratio comparison between the AR+NP group and the HC group.

	Male	Female	X^2-value	*p*-value
HC	9	13	0.204	0.753
AR+NP	16	18		

$p > 0.05$, there was no statistically significant difference in the sex ratio of the two groups.

Table II. Correlation index among IFN-λ2, Tryptase, IL-10, and IL-12 in AR+NP group.

	IFN-λ2	Tryptase	IL-10	IL-12
IFN-λ2	1	0.603*	-0.601*	-0.387
Tryptase	0.603*	1	-0.301	0.144
IL-10	-0.601*	-0.301	1	0.367
IL-12	-0.387	0.144	0.367	1

*Correlation is significant at the 0.05 level.

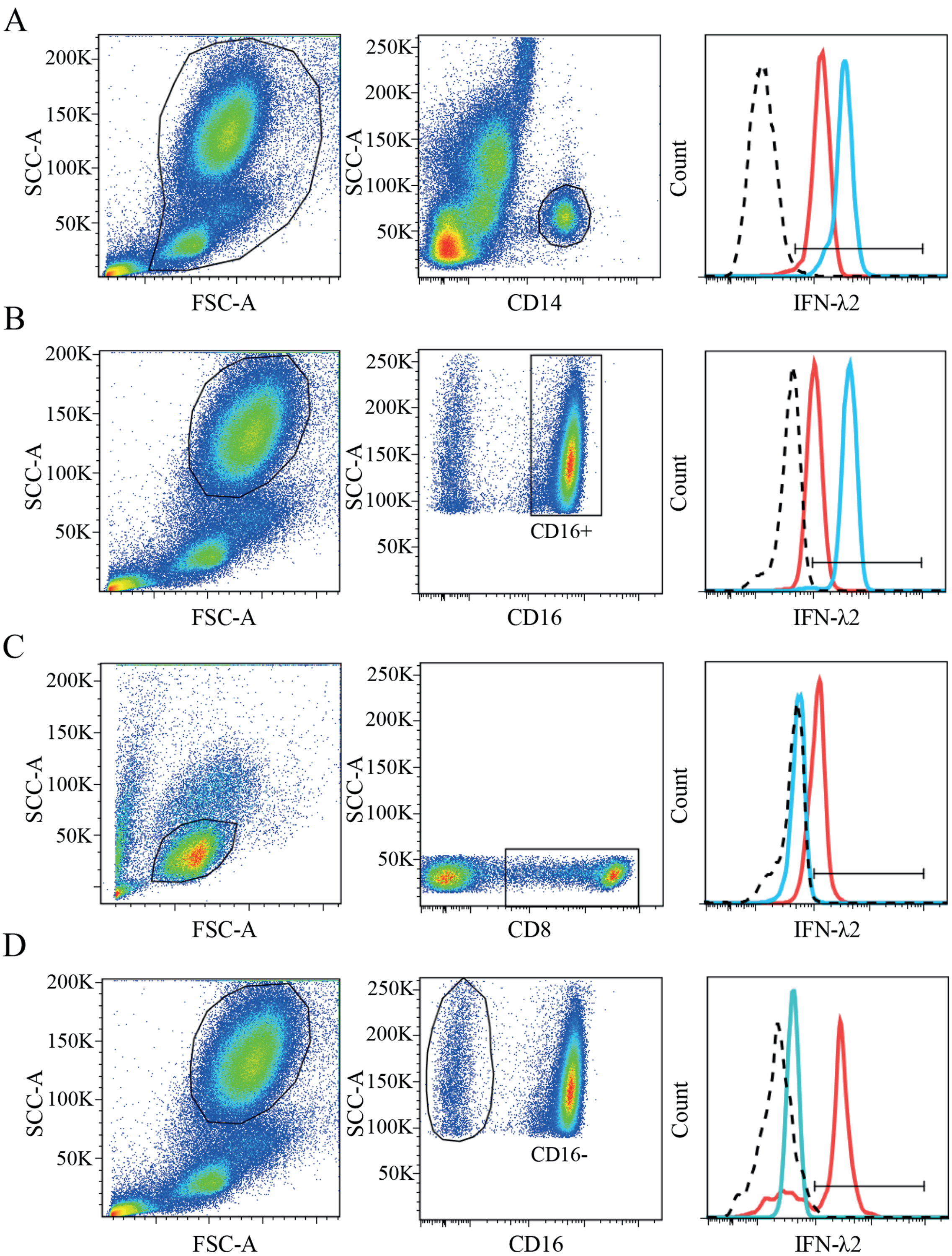

Figure 3. Expression of IFN-λ2 in peripheral blood leukocytes. IFN-λ2 was predominately expressed in monocytes ***(A)*** and neutrophils (B) in the HC group. In AR+NP patients, IFN-λ2 was mainly expressed in cytotoxic T cells (C), eosinophils (D), monocytes (A), and neutrophils (B). Compared with HC, IFN-λ2 in expression in AR+NP patients was upregulated in cytotoxic T cells and eosinophils, but downregulated in monocytes and neutrophils (p<0.05).

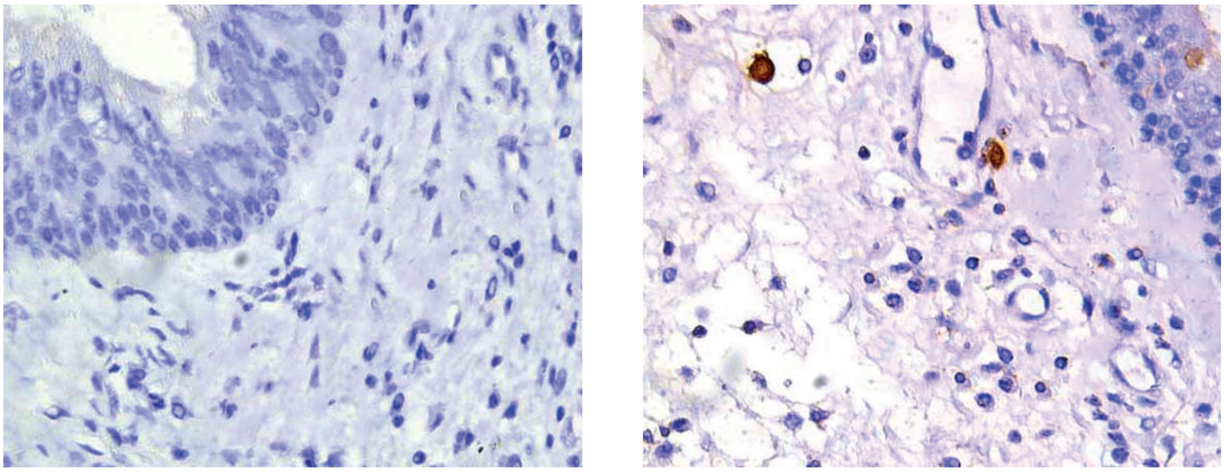

Figure 4. Expression of IFN-λ2 in nasal polyps of the patients with AR+NP. Immunohistochemical staining showed that IFN-λ2 was expressed in large nasal polyp cells (likely mast cells or macrophages) (40×).

of AR+NP patients, demonstrating that these two cell types were the main peripheral blood cell sources for elevated plasma IFN-λ2 levels in AR+NP patients. Nasal polyp mast cells and macrophages in AR+NP patients also expressed IFN-λ2, indicating that IFN-λ2 tissue cell origins derived from mast cells and macrophages. IgE-mediated mechanisms are now widely recognized to be involved in the pathogenesis of allergic diseases, including AR. Allergic pathological lesions and clinical symptoms are mainly initiated by mast cells. Soluble allergens, IgE, and mast cells are the three central factors in the pathology of allergic inflammatory responses[19], representing pathogenic factors, messengers, and main effector cells. When a soluble allergen enters the body for the first time, antigen-presenting cells (APCs) treat the allergens and present allergen fragments to activate T cells, which in turn secretes IL-4, IL-5, and IL-13. IL-4 and IL-13 then activate B cells to secrete IgE, which binds to the high-affinity IgE receptor on the surface of mast cells to form sensitized mast cells[20]. When the same allergen enters the body for the second time, mast cell membranes are sensitized on two adjacent cross-linking IgEs, activating mast cells, and releasing pro-inflammatory mediators or cytokines triggering a series of clinical symptoms[21]. Kotenko et al[22] first discovered IFN-λ in 2003. Since then, an increasing number of studies involving the anti-viral and anti-tumor effects of IFN-λ have been performed[23,24]. In recent years, IFN-λ studies[25] have gradually extended to allergic diseases, although we found that the majority of these studies involved asthma. The role of IFN-λ, especially IFN-λ2 in the development of allergic rhinitis with nasal polyps, has not been widely reported.

Conclusions

We have demonstrated that, as an anti-inflammatory molecule, IFN-λ2 may be involved in the development of allergic rhinitis with nasal polyps through inhibition of the allergic inflammatory reaction. When AR + NP occurs, mast cells degranulate, releasing trypsin and other inflammatory mediators resulting in an allergic inflammatory reaction. Alternatively, mast cells promote the production and release of IFN-λ2 to suppress the allergic inflammatory reaction. The close correlation between IFN-λ2 expression and allergic rhinitis with nasal polyp development may provide some experimental evidence for the possible effects of IFN-λ2 against allergic inflammatory reactions. The clear mechanisms underlying IFN-λ2 actions and its utility for the treatment and prevention of AR+NP in humans still need to be further investigated.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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