

Differences in cytokine levels between the nasal polyps of adolescents with local allergic and adolescents with allergic or nonallergic rhinitis

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ABSTRACT

Background: Local allergic rhinitis (LAR) is rhinitis with a localized nasal allergic response in the absence of systemic allergy. This study aimed to evaluate the pathogenesis specific to LAR compared with allergic rhinitis (AR) and nonallergic rhinitis (NAR) by using cytokines from polypous tissues.

Methods: We recruited 43 patients with AR ($n = 15$; mean age, 17.4 years), LAR ($n = 12$; mean age, 15.9 years), and NAR ($n = 16$; mean age, 15.6 years) who underwent polypectomy. Atopic status was defined as presenting a sufficiently high total immunoglobulin E (IgE) serum concentration and skin-prick test or serum allergen test. Immunoassays were performed by using polyp tissue homogenates to quantify the levels of regulated on activation of normal T cell expressed and secreted (RANTES), tumor necrosis factor (TNF) alpha, interleukin (IL) 5, and sera to assess total IgE and eosinophil cationic protein.

Results: RANTES levels were higher in patients with LAR than in patients with AR and NAR. There was a significant correlation in the concentration of RANTES between polyp tissue homogenates and serum ($R^2 = 0.51$, $p < 0.05$). The levels of IL-5, TNF alpha, and interferon gamma also demonstrated positive correlations between polyp tissue homogenates and serum; however, they were not significantly different.

Conclusion: Results of our study indicated that RANTES may play an important role and contribute to allergic reaction in LAR, and RANTES may be related to the pathogenesis of LAR

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Allergic rhinitis (AR) is the most common form of noninfectious rhinitis, but local allergic rhinitis (LAR) also affects a large number of patients with rhinitis.¹ LAR is a localized nasal allergic response in the absence of systemic allergy, characterized by local production of specific immunoglobulin E (IgE) antibodies, especially the allergic response during natural exposure to aeroallergens, and a positive nasal allergen provocation test (NAPT) response. Localized IgE-mediated inflammation may be suspected in a small subgroup of the idiopathic rhinitis group with negative skin-prick testing (SPT) results, whereas allergen-specific IgE is measurable in patients' nasal secretions.² Results of studies have proven that localized cellular pathogenesis is analogous to that in patients with AR.^{1–4}

Patients with nonallergic rhinitis (NAR) have been considered to be nonallergic because they have negative SPT

responses and serum IgE is absent.^{3,4} However, over the past decade, a number of studies have shown that many patients with negative SPT responses and who lack serum sIgE and would otherwise have been categorized as having NAR, but some patients presented nasal symptoms after nasal allergic provocation tests with common aeroallergens, such as house-dust mites, grass, and some pollens.^{1–3} In addition, results of recent studies indicated that the local production of sIgE occurs in these patients. LAR has been proposed in these cases, which led to a new classification of rhinitis. The possibility that IgE is produced locally in the target organ was first proposed after an experiment in children with AR to house-dust mites and asthma.^{2–4} IgE was measured in the serum and in nasal secretions in the steady state, and kinetic studies were conducted. It was observed that, after reexposure, local IgE levels increased more rapidly than did serum IgE.^{2–6} Although precise prevalence data are not available, according to several reports, LAR may be present in 47 to 62.5% of patients with perennial and seasonal symptoms. Many of these patients were previously diagnosed with idiopathic rhinitis or NAR with eosinophilia syndrome.^{1–3} These early studies included patients with NAR with eosinophilia syndrome and, possibly, inpatients with LAR.^{6–9}

Nasal polyps are a chronic inflammatory disease that affects the nasal cavity and paranasal sinuses, which are

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characterized by the presence of a number of inflammatory cells with infiltration of eosinophils, T-helper type 2 (Th2) cells, mast cells, and macrophages.¹⁰ The cytokines of endothelin-1 (ET-1), thymus- and activation-regulated chemokine (TARC), and/or chemokine ligand 17 (CCL 17), and α -defensin were significantly increased in patients with chronic rhinosinusitis and with polyps compared with those without polyps, as in patients with AR, which indicates that the pathogenesis of polyp may be associated with Th2-based inflammation, *e.g.*, the pathogenesis of AR.¹¹ Although patients with nasal polyps frequently exhibit concomitant allergies and patients with eosinophilic polyps respond favorably to nasal corticosteroid steroid as with patients with AR, there is limited information about the impact of allergies on the severity of nasal polyps.¹² Nasal polyposis often coexists with asthma, rhinitis, and sinusitis.¹⁰ When nasal polyps and atopy occur together, a special interaction occurs and total and specific IgEs are found at a significantly greater concentration in nasal polyp tissue than in sera and tonsil tissue.^{10–14} Polyps recur frequently during specific pollen seasons in individuals who are sensitive.¹⁵

Regulated on activation of normal T cell expressed and secreted (RANTES) is a member of the chemokine family of proinflammatory basic chemoattractant polypeptides.⁴ This molecule has been indicated as playing an important role in inflammatory recruitment, which leads to cell activation and directional migration of specific T-cell subsets and contributes to the activation of integrin-mediated adhesive events necessary for transendothelial migration.^{16,17} RANTES is a potent mediator of eosinophilic chemotaxis *in vitro* but also acts on a wider range of other cells. Hence, mechanisms by which Th2 cell subsets are recruited to the inflamed tissue were examined, with a focus on chemotactic factors.^{3,17–19}

Tumor necrosis factor (TNF) α is a proinflammatory cytokine produced by macrophages, eosinophils, epithelial cells, and mast cells in airways.²⁰ TNF- α along with Th2 cytokines, such as interleukin (IL) 5 may stimulate nasal polyp fibroblasts to produce inflammatory mediators. TNF- α is also involved in the production of antigen-specific IgE and the adhesion molecules responsible for the attraction of eosinophil to allergic inflammatory sites. However, the specific role and source of TNF- α in the nasal mucosa is not well known. TNF- α is responsible for smooth-muscle activation and for inducing late-phase plasma exudation, which is associated with increased nasal mucosal output of granulocyte mediators.^{20,21} The goal of the present study was to evaluate the pathogenesis specific to LAR compared with AR and NAR by using cytokines from nasal polypous tissues. We evaluated differences and compared the contents of RANTES, TNF- α , IL-5, and interferon (IFN) γ among the nasal polyps of children with LAR, AR, and NAR.

METHODS

Subjects

We recruited children with rhinitis who underwent polypectomy between June 2009 and July 2013 in the Department of Otolaryngology at Hanyang University Guri Hospital. Among them, children with congenital anomalies in the head and neck or with systemic diseases, or those suspected of having congenital or acquired immune deficiency were excluded. Cases of accompanying sinusitis diagnosed by radiologic findings of paranasal series or endoscopic findings were excluded. A total of 43 subjects were included. Cases of polypectomy in infants or young age groups were rarely found in the present study. The age range of the subjects with polypectomy was 10–20 years old.

Participation was by parental informed consent, and the study was approved by the local ethics committee of Hanyang University Guri Hospital. The parents or foster parents of the patients were asked to complete an extensive questionnaire. Data were collected on symptoms and signs; medications; and family history with respect to allergy, such as rhinoconjunctivitis, asthma, eczema, smoking habits, and contact with pets. Atopy is defined as predisposition toward developing certain allergic hypersensitivity reactions, which is also defined as any IgE-mediated reaction, even in those that are appropriate and proportional to the antigen. Therefore, atopic status constitutes presenting a sufficiently high total IgE serum concentration and positivity in a SPT or an serum allergen test, such as ImmunoCAP system (Phadia, Uppsala, Sweden). Patients were excluded if medications that contained antihistamines were used during surgery or within the preceding week, or if immunosuppressive agents, including steroids, were used during surgery or within the preceding 6 weeks.

The patients were classified into one of the following three groups: (1) AR: patients who, with an allergen SPT result that had skin swelling of >3 mm larger than that caused by histamine or showed a positive result (>0.35 kU/L) on the specific IgE test; (2) LAR: patients who, with an SPT result, had no skin swelling caused by an allergen larger than that caused by histamine, had a negative result (<0.35 kU/L) on the specific IgE test, and who tested positive on the nasal allergen provocation test (NAPT); and (3) NAR: patients who, with an SPT result of an allergen, had no skin swelling larger than that caused by histamine, had a negative result (<0.35 kU/L) on the IgE test, and who also had a negative result on the nasal provocation test.

Nasal Allergen Provocation Test

A specific, unilateral NAPT was performed according to current recommendations in our otolaryngology department. Challenges were performed during the pollen-free season in a room with constant temperature

and humidity. The participants underwent provocation between 9:00 AM and noon, and were acclimatized for 30 minutes before starting the procedure. All the tests were performed by the same investigator (YJC). All the patients were asymptomatic at the time of testing. No decongestants were used during NAPT.

The same batch of a standardized nasal extract of house-dust mites, *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* (Aquagen SQ; ALK-Abello, Hørsholm, Denmark), was used throughout the study. The allergen solution contained 5,000 standardized biologic units/mL of the allergen mixture was delivered through the nostril into the inferior turbinate region by using a nasal pump sprayer, insufflating one puff (0.04–0.05 mL) into the challenged nasal cavity. During insufflation, the patients held their breath and kept their head in a forward prone position to avoid swallowing or aspirating allergen into the lower respiratory tract.^{14,15} To interpret results, the total nasal symptom scores were determined for sneezing, runny nose, and nasal blockage, and their sum was used as a total score. A positive result was defined as increasing the post-provocation total score by >30% compared with the preprovocation score.

Tissue Collection

Polyps were removed with a curette. They were thawed and dispersed in radioimmunoprecipitation lysis buffer (Sigma-Aldrich, St. Louis, MO) that contained a protease inhibitor (Roche, Indianapolis, IN). Cells were harvested by centrifugation and the supernatants were stored at -70°C before use. To obtain tissue homogenates, nasal tissue specimens were weighed, and 1 mL of 0.9% NaCl solution was added for every 0.1 g of tissue. The mixture was homogenized with a mechanical homogenizer (B. Braun, Melsungen, Germany). All the parameters measured in the polyp tissue homogenate are presented as ratios to albumin concentration to correct for differences in dilution of the individual tissues. Albumin levels in adenoid tissue homogenates were measured by using a Quanti Chrom BCP Albumin Assay Kit (Bio Assay Systems, Hayward, CA).

Measurement of Cytokines in Polyp Tissue Homogenates

Inflammatory markers, eosinophil cationic protein (ECP) (diluted 1:5) and albumin levels, in polyp tissue homogenates were quantified by fluorimmunoassay by using the ImmunoCAP system (Phadia, Uppsala, Sweden). The minimal detection limits for ECP were 2 ng/mL. Biomarker concentrations were corrected for albumin content and expressed as nanograms per milligram of albumin. Levels of IL-5, IFN- γ , TNF- α , and

RANTES were quantified by commercial enzyme immunoassay (R&D System Inc., Minneapolis, MN).

Statistical Analysis

Statistical analyses were performed by using SPSS version 15 (SPSS Inc., Chicago, IL). The data are expressed as mean $\pm$ standard deviation (SD). Statistical comparisons were made with paired *t*-tests. A *p* value of <0.05 indicated statistical significance. Parametric one-way analysis of variance was used to compare levels of cytokines among the study groups.

RESULTS

Characteristics of Subjects

In the present study, we recruited 43 patients with rhinitis, which comprised AR ($n = 15$), LAR ($n = 12$), and NAR ($n = 16$). The respective IgE concentrations, total eosinophil counts in peripheral blood, and serum ECP concentrations of patients with AR and NAR are shown in Table 1. These were as follows mean $\pm$ SD: AR, 727.8 ± 501.7 IU/mL; LAR, 94.3 ± 87.5 IU/mL; and NAR, 79.2 ± 47.6 IU/mL (AR versus NAR, $p < 0.01$). Total mean $\pm$ SD eosinophil counts in peripheral blood were AR, $804.5 \pm 452.8/\text{mm}^3$; LAR, $233.4 \pm 421.6/\text{mm}^3$; and NAR, $67.6 \pm 42.5/\text{mm}^3$ (AR versus NAR, $p < 0.01$). Mean $\pm$ SD serum ECP concentrations were for AR, 134.4 ± 69.9 $\mu\text{g/L}$; LAR, 79.2 ± 34.9 $\mu\text{g/L}$; and NAR, 22.8 ± 10.4 $\mu\text{g/L}$ (AR versus LAR versus NAR, $p < 0.01$).

Concentrations of Cytokines in Polyp Tissue Homogenates

RANTES levels were significantly higher in patients with LAR than those with AR and NAR, but there was no significant difference between AR and NAR (mean $\pm$ SD RANTES: LAR, 37.0 ± 12.5 pg/mL; AR, 27.9 ± 7.7 pg/mL; and NAR, 19.7 ± 7.9 pg/mL; $p > 0.05$). TNF- α and IL-5 levels were higher in AR and LAR than in NAR (mean $\pm$ SD TNF- α : LAR, 105.3 ± 43.3 pg/mL; AR, 117.8 ± 96.8 pg/mL; and NAR, 63.8 ± 38.7 pg/mL; AR and LAR versus NAR, $p < 0.05$). Mean $\pm$ SD IL-5 values were the following: LAR, 83.2 ± 48.5 pg/mL; AR, 110.7 ± 86.3 pg/mL; and NAR, 38.7 ± 28.8 pg/mL; AR and LAR versus NAR, $p < 0.01$, but there was no significant difference in mean $\pm$ SD IFN- γ levels (IFN- γ : AR, 116.7 ± 66.0 pg/mL; LAR, 138.6 ± 43.3 pg/mL; NAR, 141.8 ± 97.7 pg/mL; $p > 0.05$) (Table 2).

Serum Cytokine Concentrations

RANTES, TNF- α , and IL-5 levels were higher in both AR and LAR than in NAR (mean $\pm$ SD RANTES levels: LAR, 30.2 ± 7.83 pg/mL; AR, 39.4 ± 14.43 pg/mL; and NAR, 20.7 ± 7.53 pg/mL; and for TNF- α values: LAR,

Table 1 Comparison of characteristics of subjects with LAR and with polyps versus subjects with AR and with polyp and those with NAR and with polyp

Group	No. Patients (boys/girls)	Age, mean $\pm$ SD, y	IgE Level, mean $\pm$ SD, IU/mL	Eosinophils, mean $\pm$ SD, no./mm ³	ECP Level, mean $\pm$ SD, μ /L
AR with polyps	15 (12/3)	17.4 $\pm$ 4.2	727.8 $\pm$ 501.7*	804.5 $\pm$ 452.8*	134.4 $\pm$ 69.9*
LAR with polyps	9 (5/4)	15.9 $\pm$ 5.5	94.3 $\pm$ 87.5	233.4 $\pm$ 421.6	79.2 $\pm$ 34.9#
NAR with polyps	14 (8/6)	15.6 $\pm$ 2.9	79.2 $\pm$ 47.6	67.6 $\pm$ 42.5	22.8 $\pm$ 10.4
<i>p</i> Value	NS	NS	<i>p</i> < 0.01*	<i>p</i> < 0.01*	<i>p</i> < 0.01*#

LAR = Local allergic rhinitis; AR = allergic rhinitis; NAR = nonallergic rhinitis; SD = standard deviation; IgE = immunoglobulin E; ECP = eosinophil cationic protein; NS = nonsignificant.

*Expressed as *p* values when AR with polyps compared with NAR with polyps.

#Expressed as *p* values when NAR with polyps compared with NAR with polyps.

Table 2 Comparison of cytokines in polyp tissues of patients with AR and polyps, LAR and polyps, and NAR with polyps

Group	IFN- γ Level, mean $\pm$ SD, pg/mL	TNF- α Level, mean $\pm$ SD, pg/mL	IL-5 Level, mean $\pm$ SD, pg/mL	RANTES Level, mean $\pm$ SD, pg/mL
AR with polyps	116.7 $\pm$ 66.0	117.8 $\pm$ 96.8*	110.7 $\pm$ 86.3*	27.9 $\pm$ 7.7
LAR with polyps	138.6 $\pm$ 43.3	105.3 $\pm$ 43.3#	83.2 $\pm$ 48.5#	37.0 $\pm$ 12.5*
NAR with polyps	141.8 $\pm$ 97.7	63.8 $\pm$ 38.7	38.2 $\pm$ 28.8	19.7 $\pm$ 7.9
<i>p</i> Value	NS	<i>p</i> < 0.05*#	<i>p</i> < 0.01*#	<i>p</i> < 0.01*

AR = Allergic rhinitis; LAR = local allergic rhinitis; NAR = nonallergic rhinitis; IFN = interferon; SD = standard deviation; TNF = tumor necrosis factor; IL = interleukin; RANTES = regulated on activation of normal T cell expressed and secreted; NS = nonsignificant.

*Expressed as *p* values when AR with polyps compared with NAR with polyps.

#Expressed as *p* values when LAR with polyps compared with NAR with polyps.

Table 3 Comparison of cytokines in sera of AR with polyp, LAR with polyp, and NAR with polyp

Group	IFN- γ Level, mean $\pm$ SD, pg/mL	TNF- α Level, mean $\pm$ SD, pg/mL	IL-5 Level, mean $\pm$ SD, pg/mL	RANTES Level, mean $\pm$ SD, pg/mL
AR with polyps	197.7 $\pm$ 92.0	156.1 $\pm$ 143.3*	126.1 $\pm$ 108.1*	39.4 $\pm$ 14.4*
LAR with polyps	244.7 $\pm$ 145.9	133.6 $\pm$ 85.4#	83.6 $\pm$ 61.1#	30.2 $\pm$ 7.8#
NAR with polyps	226.9 $\pm$ 164.2	37.6 $\pm$ 30.9	55.7 $\pm$ 21.3	20.7 $\pm$ 7.5
<i>p</i> Value	NS	<i>p</i> < 0.05*	<i>p</i> < 0.01*#	<i>p</i> < 0.05*#

AR = Allergic rhinitis; LAR = local allergic rhinitis; NAR = nonallergic rhinitis; IFN = interferon; SD = standard deviation; TNF = tumor necrosis factor; IL = interleukin; RANTES = regulated on activation of normal T cell expressed and secreted; NS = nonsignificant.

*Expressed as *P* values when AR with polyps compared with NAR with polyps.

#Expressed as *P* values when LAR with polyps compared with NAR with polyps.

133.6 $\pm$ 85.4 pg/mL; AR, 156.1 $\pm$ 143.3 pg/mL; and NAR, 37.6 $\pm$ 30.9 pg/mL; LAR and AR versus NAR, *p* < 0.05); for IL-5 values: LAR, 83.6 $\pm$ 61.1 pg/mL; AR, 126.1 $\pm$ 108.1 pg/mL; and NAR, 55.7 $\pm$ 21.3 pg/mL, but mean $\pm$ SD INF- γ levels did not differ (IFN- γ levels: AR, 197.7 $\pm$ 92.0 pg/mL; LAR, 244.7 $\pm$ 145.9 pg/mL; NAR, 226.9 $\pm$ 164.2 pg/mL; *p* > 0.05) (Table 3).

Correlations among Concentrations of RANTES, IL-5, TNF- α , and IFN- γ in Nasal Polyp Tissue Homogenates and Serum

There was a significant correlation between the concentrations of RANTES in polyp tissue homogenates and sera (R^2 = 0.51, *p* < 0.05). TNF- α , IL-5, and IFN- γ values also demonstrated positive correlations. However, there were no significant correlations in polyp

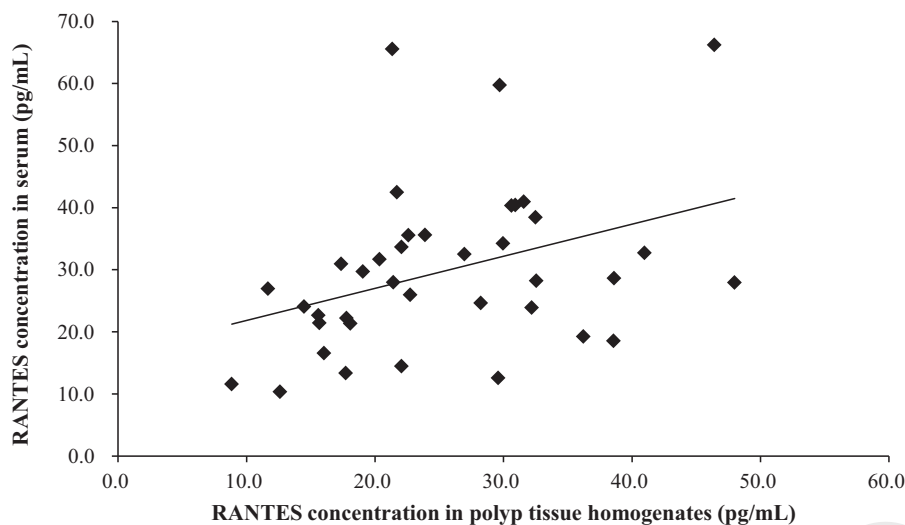


Figure 1. The correlation between polyp tissue homogenates and serum concentration of regulated on activation of normal T cell expressed and secreted (RANTES) ($R^2 = 0.51$, $p < 0.05$, $n = 38$).

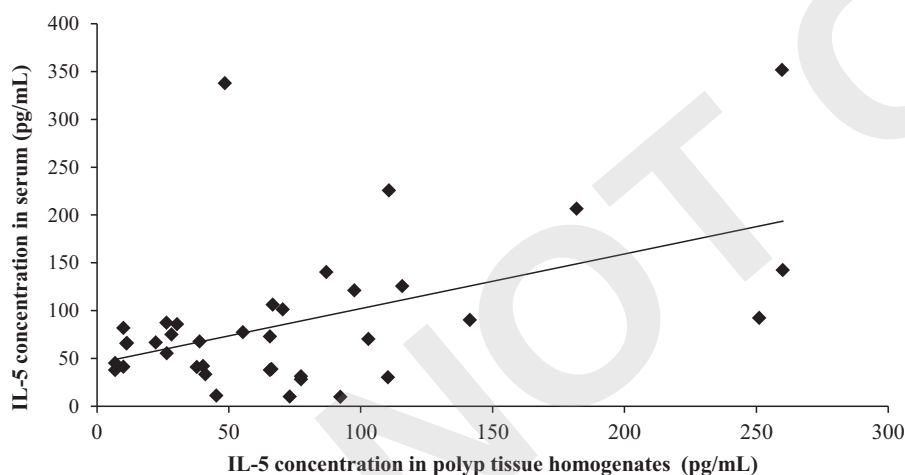


Figure 2. The correlation between polyp tissue homogenates and serum concentration of interleukin (IL) 5 ($R^2 = 0.34$, $p < 0.05$, $n = 38$).

tissue homogenates or sera (IL-5: $R^2 = 0.34$, $p < 0.05$; TNF- α : $R^2 = 0.11$, $p < 0.05$; and IFN- γ : $R^2 = 0.26$, $p < 0.05$) (Figs. 1–4).

DISCUSSION

The concept of LAR is both intriguing and controversial. Although some of the aforementioned studies found nasal-specific IgE, positive NAPT results, and inflammatory cells in a subset of patients, the results are still conflicting.^{1–4} The failure to detect IgE in serum from a large proportion of patients with LAR with positive NAPT responses might be due to the existence of an immunologic mechanism, such as the stimulation of allergen on airway innate immune cells by a non-specific protease activity. Intrinsic allergen-binding specific for allergens is found in the nasal mucosa of 30% of patients with idiopathic rhinitis, as further evidenced by a localized allergic response and creating the term “entopy” to describe the process of a localized allergic mucosal response that is independent of systemic atopic responses.²²

Rondon *et al.*^{23,24} demonstrated that tryptase and eosinophilic cationic protein were detected in the nasal secretions of 40% and 43% patients with LAR, and specific IgE to grass pollen was detected in 30% of patients after the NAPT; in contrast, an increase in total serum IgE levels was not detected. Hence, the researchers concluded that patients with LAR had not only local, specific IgE production but also mast cell and eosinophil activation induced by nasal exposure to grass pollen.^{6,24} The existence of an inflammatory mechanism in patients with LAR has been controversial. Although several histologic and *in situ* hybridization studies found a Th2 inflammatory pattern with increased numbers of mast cells, eosinophils, B cells, and T cells, other studies found no significant differences between patients with NAR and control subjects.^{17,22–25}

Several investigators noted that patients with a subset of idiopathic rhinitis have positive specific NAPT results, but other investigators found the results to be negative on repeated challenging.^{24–28}

Figure 3. The correlation between polyp tissue homogenates and serum concentration of tumor necrosis factor (TNF) α ($R^2 = 0.11$, $p < 0.05$, $n = 38$).

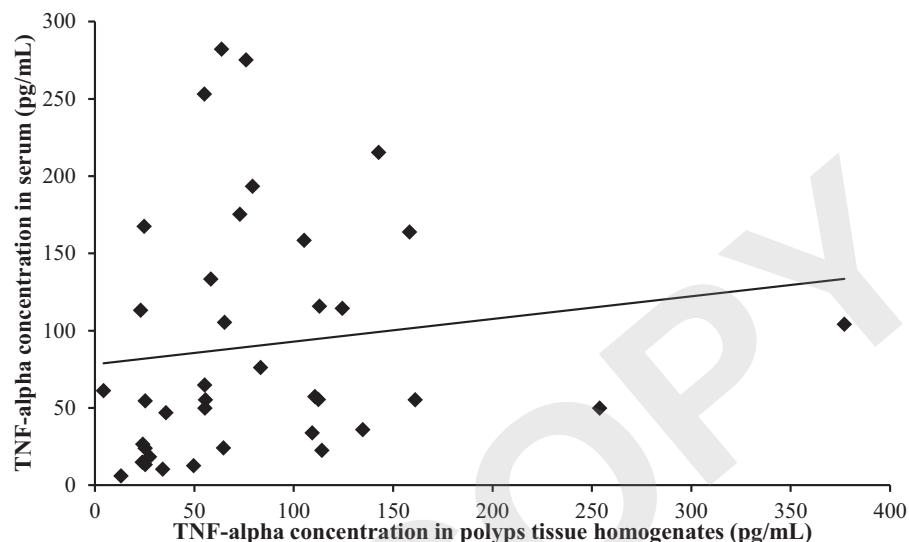
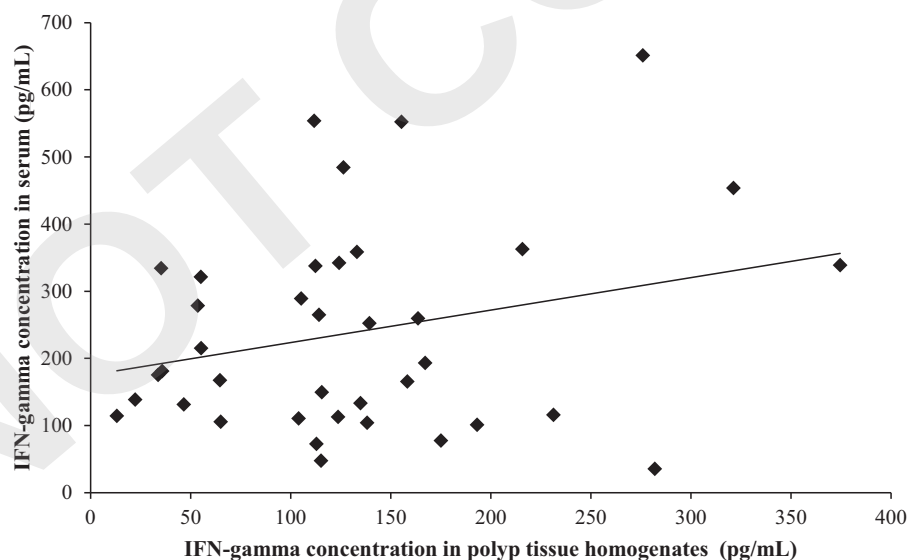


Figure 4. The correlation between polyp tissue homogenates and serum concentration of interferon (IFN) γ ($R^2 = 0.26$, $p < 0.05$, $n = 38$).



Powe *et al.*⁸ demonstrated an increase in mast cells, eosinophils, and IgE cells, together with a similar T-cell phenotype in patients with AR. In contrast, van Rijswijk *et al.*²⁹ found that patients with idiopathic rhinitis had a similar inflammatory profile to controls with NAR. Differences in biopsy specimens and patient selection may explain these conflicting results.³⁰

In the present study, RANTES levels were significantly higher in patients with LAR than in patients with AR and those with NAR, but there was no significant difference between AR and NAR. Detectable levels of RANTES antigen in nasal polyps of both patients who were allergic and patients who were not allergic support the hypothesis that local production of RANTES could be an important factor in sustaining recruitment of leukocytes in nasal polyps.^{31,32} RANTES is a potent mediator of eosinophilic chemotaxis but also acts on a wider range of other cells. Inflammatory infiltrates may contribute to

tissue injury through local production of a number of inflammatory mediators, such as RANTES and monocyte chemoattractant protein-1, and mechanisms by which T lymphocytes are recruited to the inflamed tissue. Therefore, RANTES may play an important role in local inflammatory recruitment, which leads to directional migration of specific leukocyte subsets and may activate integrin-mediated adhesion necessary for transendothelial migration. RANTES is also produced locally within the nasal microenvironment of polyps and may be responsible for the eosinophil recruitment. The level of RANTES expression is also higher in allergic polyps than in allergic nasal mucosae.^{18,31,32}

In the present study, TNF- α and IL-5 levels were higher in polyp tissue homogenates from patients with LAR and patients with AR than in those from patients with NAR, but IFN- γ levels did not differ among them. TNF- α is a well-known proinflammatory cytokine that is relevant to the pathogenesis of allergic mucosal in-

flammation. TNF- α is required for the migration of Th2 cells to the sites of allergic inflammation, which has been demonstrated to increase the effects of IL-4 on IgE production.³³ Some studies reported that TNF- α is localized to nasal mucosal mast cells and is released in acute AR.^{20,21} IFN- γ is also an important activator of macrophages and an inducer of major histocompatibility complex-II molecule expression, and there is recent evidence that IFN- γ is a potent inhibitor of IgE synthesis in animal models.²² Hence, we compared differences in the contents of RANTES, TNF- α , IFN- γ , and IL-5 among the nasal polyps of children with LAR, AR, and NAR for the unique biomarker for the LAR mechanism.

The present study may support the hypothesis that local production of RANTES could be a crucial factor in sustained recruitment of eosinophils and leukocytes in nasal polyps. RANTES is locally produced within the nasal microenvironment of polyps and may be partially responsible for the eosinophil recruitment of AR. RANTES likely plays a more significant role in the pathogenesis of LAR than of AR, although the role of RANTES in inflammatory disorders might induce T-cell infiltration, directly inducing local inflammation. Otherwise, RANTES probably plays a more significant role in the pathogenesis of local inflammation on nasal mucosa than do other preinflammatory cytokines, such as TNF- α , IFN- γ , and IL-5.

The present study had some limitations. First, the study had a small sample size, of 43 subjects, with only 12–16 subjects per group. Second, we used polyp tissue instead of turbinate mucosa. It was not easy to collect nasal tissues or nasal turbinate tissues of children with AR; therefore, tissues from children with AR or LAR who had polypectomy were used in the present study. Nonetheless, we validated a biomarker that was important in LAR. It is expected that further studies will identify and evaluate the pathogenesis specific to LAR.

CONCLUSION

The present study indicated that RANTES may have played a critical role of pathogenesis of LAR. RANTES was not only significantly increased in the polyp tissue of patients with LAR compared with the patients with AR or with NAR but also had significantly higher levels in serum of patients with AR and LAR compared with patients with NAR. IL-5 and TNF- α levels were significantly increased in polyp tissues and serum of patients with AR and LAR compared with patients with NAR, but there were no differences between AR and LAR. Thus, there are still further evaluations for the clarification of the mechanism of LAR.

REFERENCES

1. Rondón C, Campo P, Galindo L, et al. Prevalence and clinical relevance of local allergic rhinitis. *Allergy*. 2012; 67:1282–1288.

2. De Schryver E, Devuyst L, Derycke L, et al. Local immunoglobulin E in the nasal mucosa: clinical implications. *Allergy Asthma Immunol Res*. 2015; 7:321–331.
3. Powe DG, Jagger C, Kleinjan A, et al. 'Entropy': localized mucosal allergic disease in the absence of systemic responses for atopy. *Clin Exp Allergy*. 2003; 33:1374–1379.
4. Rond C, Bogas G, Barrionuevo E, et al. Nonallergic rhinitis and lower airway disease. *Allergy*. 2017; 72:24–34.
5. Sensi LG, Piacentini GL, Nobile E, et al. Changes in nasal specific IgE to mites after periods of allergen exposure-avoidance: a comparison with serum levels. *Clin Exp Allergy*. 1994; 24:377–382.
6. Carney AS, Powe DG, Huskisson RS, Jones NS. Atypical nasal challenges in patients with idiopathic rhinitis: more evidence for the existence of allergy in the absence of atopy? *Clin Exp Allergy*. 2002; 32:1436–1440.
7. Perić A, Sotirović J, Špadijer-Mirković C, Matković-Jožin S, Perić AV, Vojvodić D. Nonselective chemokine levels in nasal secretions of patients with perennial nonallergic and allergic rhinitis. *Int Forum Allergy Rhinol*. 2016; 6:392–397.
8. Powe DG, Huskisson RS, Carney AS, et al. Mucosal T-cell phenotypes in persistent atopic and nonatopic rhinitis show an association with mast cells. *Allergy*. 2004; 59:204–212.
9. Ellis AK, Keith PK. Nonallergic rhinitis with eosinophilia syndrome and related disorders. *Clin Allergy Immunol*. 2007; 19: 87–100.
10. Stoop AE, van der Heijden HA, Biewenga J, van der Baan S. Eosinophils in nasal polyps and nasal mucosa: an immunohistochemical study. *J Allergy Clin Immunol*. 1993; 91:616–622.
11. Tsybikov NN, Egorova EV, Kuznik BI, Fefelova EV, Magen E. Biomarker assessment in chronic rhinitis and chronic rhinosinusitis: Endothelin-1, TARC/CCL17, neopterin, and α -defensins. *Allergy Asthma Proc*. 2016; 37:35–42.
12. Wise SK, Ahn CN, Schlosser RJ. Localized immunoglobulin E expression in allergic rhinitis and nasal polyposis. *Curr Opin Otolaryngol Head Neck Surg*. 2009; 17:216–222.
13. Brescia G, Barion U, Zanotti C, Parrino D, Marioni G. Pre- and postoperative blood neutrophil-to-lymphocyte and eosinophil-to-lymphocyte ratios in patients with sinonasal polyps: A preliminary investigation. *Allergy Asthma Proc*. 2017; 38:64–69.
14. Baba S, Kondo K, Toma-Hirano M, et al. Local increase in IgE and class switch recombination to IgE in nasal polyps in chronic rhinosinusitis. *Clin Exp Allergy*. 2014; 44:701–712.
15. Han DY, Cho JS, Moon YM, et al. Effect of prostaglandin e2 on vascular endothelial growth factor production in nasal polyp fibroblasts. *Allergy Asthma Immunol Res*. 2013; 5:224–231.
16. Kimura S, Pawankar R, Mori S, et al. Increased expression and role of thymic stromal lymphopoietin in nasal polyposis. *Allergy Asthma Immunol Res*. 2011; 3:186–193.
17. Artis D, Humphreys NE, Bancroft AJ, Rothwell NJ, Potten CS, Grencis RK. Tumor necrosis factor alpha is a critical component of interleukin 13-mediated protective T helper cell type 2 responses during helminth infection. *J Exp Med*. 1999; 190:953–962.
18. Dairaghi DJ, Soo KS, Oldham ER, et al. RANTES-induced T cell activation correlates with CD3 expression. *J Immunol*. 1998; 160:426–433.
19. Broide DH, Stachnick G, Castaneda D, Nayar J, Sriramarao P. Inhibition of eosinophilic inflammation in allergen-challenged TNF receptor p55/p75- and TNF receptor p55-deficient mice. *Am J Respir Cell Mol Biol*. 2001; 24:304–311.
20. Widegren H, Korsgren M, Andersson M, Greiff L. Effects of TNFalpha on the human nasal mucosa in vivo. *Respir Med*. 2007; 101:1982–1987.
21. Iwasaki M, Saito K, Takemura M, et al. TNF-alpha contributes to the development of allergic rhinitis in mice. *J Allergy Clin Immunol*. 2003; 112:134–140.

22. Forester JP, Calabria CW. Local production of IgE in the respiratory mucosa and the concept of entropy: does allergy exist in nonallergic rhinitis? *Ann Allergy Asthma Immunol.* 2010; 105: 249–255; quiz 256–258.
23. Rondón C, Fernandez J, Lopez S, et al. Nasal inflammatory mediators and specific IgE production after nasal challenge with grass pollen in local allergic rhinitis. *J Allergy Clin Immunol.* 2009; 124:1005–1011.e1.
24. Rondón C, Romero JJ, López S, et al. Local IgE production and positive nasal provocation test in patients with persistent nonallergic rhinitis. *J Allergy Clin Immunol.* 2007; 119:899–905.
25. Powe DG, Groot Kormelink T, Sisson M, et al. Evidence for the involvement of free light chain immunoglobulins in allergic and nonallergic rhinitis. *J Allergy Clin Immunol.* 2010; 125:139–145.e1–3.
26. Melillo G, Bonini S, Cocco G, et al. EAACI provocation test with allergens. Report prepared by the European Academy of Allergology and Clinical Immunology Subcommittee on provocation tests with allergens. *Allergy.* 1997; 52(suppl):1–35.
27. Malm L, Geth van Wijk R, Bachert C. Guidelines for nasal provocations with aspects on nasal patency, airflow, and airflow resistance rhinology. International Committee on Objective Assessment of the Nasal Airways, International Rhinologic Society. *Rhinology.* 2000; 38:1–6.
28. Cohn L, Homer RJ, Marinov A, Rankin J, Bottomly K. Induction of airway mucus production by T helper 2 (Th2) cells: a critical role for interleukin 4 in cell recruitment but not mucus production. *J Exp Med.* 1997; 186:1737–1747.
29. Van Rijswijk JB, Blom HM, KleinJan A, Mulder PG, Rijntjes E, Fokkens WJ. Inflammatory cells seem not to be involved in idiopathic rhinitis. *Rhinology.* 2003; 41:25–30.
30. Staevska MT, Naramoil KM. Differential diagnosis of persistent nonallergic rhinitis and rhinosinusitis syndromes. In: Baraniuk JN, Shusterman D. *Nonallergic Rhinitis.* New York: Informa, 2007: p. 35–54.
31. Beck LA, Stellato C, Beall LD, et al. Detection of the chemokine RANTES and endothelial adhesion molecules in nasal polyps. *J Allergy Clin Immunol.* 1996; 98:766–780.
32. Marcella R, Croce A, Moretti A, Barbacane RC, Di Giocchino M, Conti P. Transcription and translation of the chemokines RANTES and MCP-1 in nasal polyps and mucosa in allergic and non-allergic rhinopathies. *Immunol Lett.* 2003; 90: 71–75.
33. Rudmann DG, Moore MW, Tepper JS, et al. Modulation of allergic inflammation in mice deficient in TNF receptors. *Am J Physiol Lung Cell Mol Physiol.* 2000; 279:L1047–L1057. □

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