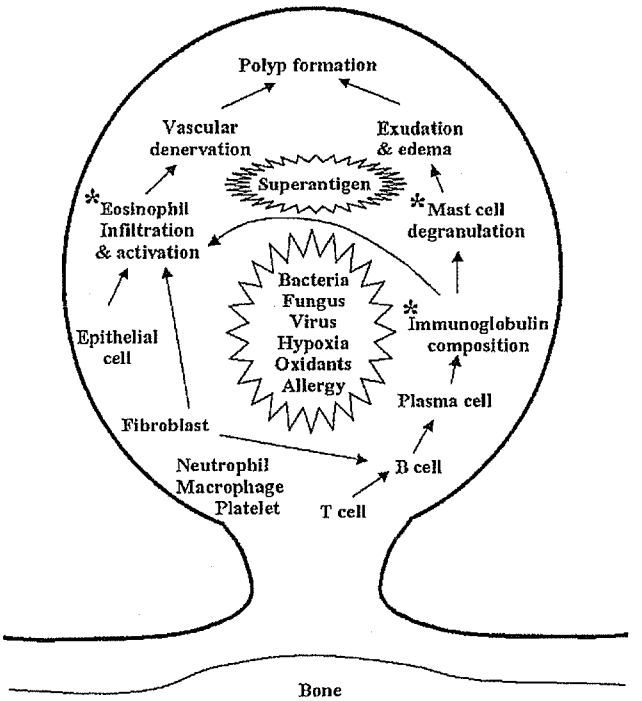

Fig. 1. Mechanism of nasal polyp formation. *Some commonly observed pathological findings in nasal polyps.

We demonstrated an association between B cell antigen receptor (BCR) and Syk and activation of Syk by cross-linking [12]. The BCR is a complex between membrane Ig and the Ig-α and Ig-β heterodimer. The cytoplasmic domains of Ig-α and Ig-β each contain an ITAM. Cross-linking activates Syk through ITAMs and thereby induces vigorous signalling reactions [24] (Fig. 2b). Specific IgE to *S. aureus* enterotoxins is produced in nasal polyps through this signalling because foreign antigens are recognized by BCR as an obligatory early step in B cell activation.

Nasal fibroblasts

Fibroblasts, a rich source of chemokines, interact with eosinophils and thereby play a key role in the pathogenesis of airway disease. Human nasal fibroblasts cultured from nasal polyp tissue express a variety of cytokines that induce differentiation of human haemopoietic progenitor cells [25]. Figure 3a shows Syk expression in the cytosol of nasal polyp-derived fibroblasts by immunohistochemical staining using the traditional ABC technique [26].

High concentrations of regulated on activation, normal T cell expressed and secreted (RANTES) have been demonstrated in nasal polyp specimens [27], and cultured nasal polyps have been shown to release RANTES spontaneously [28]. Stimulation with lipopolysaccharide (LPS) induces expression of RANTES mRNA in cultured nasal fibroblasts and secretion of RANTES protein [29]. The level of Syk expression is associated with RANTES production induced by LPS stimulation in nasal fibroblasts [11]. Overexpression of wild-type Syk increases RANTES production from human nasal fibroblasts. However, fibroblasts transfected with inactive Syk vector fail to produce high levels of RANTES.

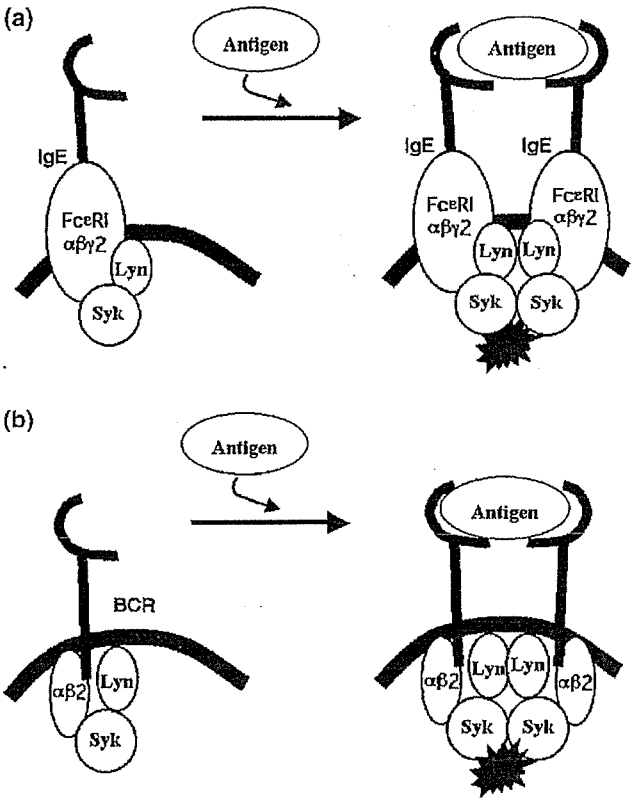

Fig. 2. Syk plays critical roles in intracellular signal transduction in human mast cells (a) and B cells (b).

Pre-treatment of antisense oligodeoxynucleotides to Syk inhibits RANTES production and activation of c-Jun N-terminal kinase 1 (JNK1) stimulated with LPS.

IL-1 induces interaction of TNF receptor-associated factor 6 (TRAF6) with IL-1 receptor-associated kinase that is rapidly recruited to the IL-1 receptor after IL-1 induction. We found that Syk plays an important role in IL-1-induced chemokine production through a signalling complex involving Syk and TRAF6. Overexpression of wild-type Syk by gene transfer enhanced RANTES production from nasal fibroblasts stimulated with IL-1. Decrease of Syk expression by administration of Syk-antisense inhibited RANTES production in response to IL-1. Syk is required for the IL-1-induced chemokine production with TRAF6 in fibroblasts of nasal polyps through JNK and p38 phosphorylation [30] (Fig. 3b).

Roles of Syk in various other cells

Table 1 shows some recently reported roles of Syk in other cells located in nasal polyp tissues. In human eosinophils, Syk is essential for the activation of the antiapoptotic pathway(s) induced through the IL-3/IL-5/granulocyte-macrophage colony-stimulating factor (GM-CSF) receptor β-subunit [15]. Furthermore, eosinophils derived from Syk (−/−), but not wild-type mice, were incapable of generating reactive oxygen intermediates in response to Fcγ receptor engagement, although eosinophil differentiation and survival were not affected [31].

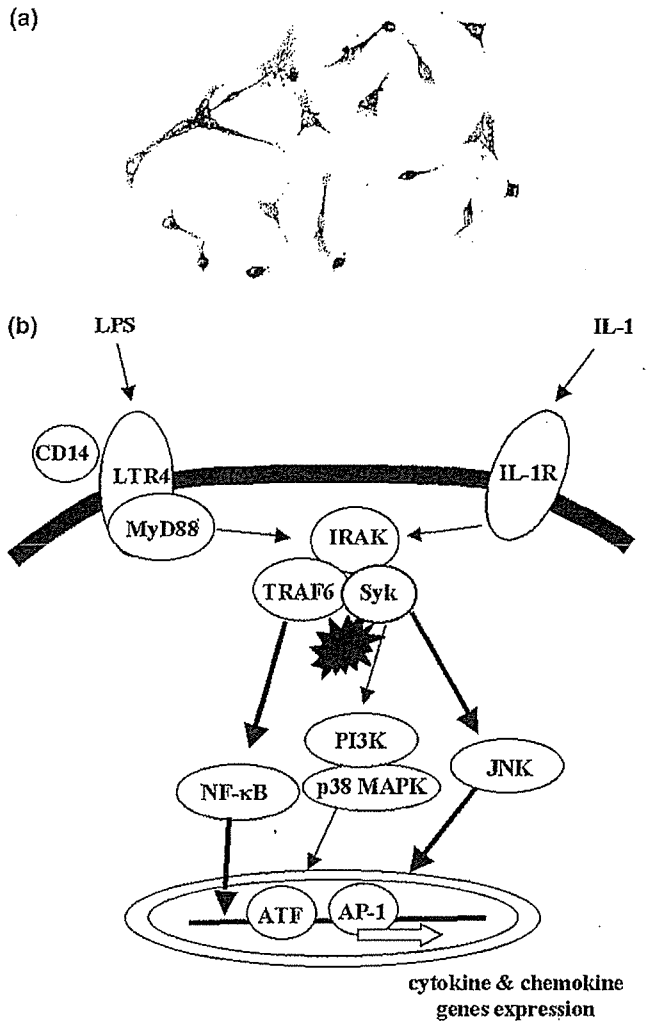

Fig. 3. Syk expressed in human nasal fibroblasts (a) and its signal transduction (b).

We found that Syk plays an important role in LPS-induced chemokine production from nasal fibroblasts. In neutrophils, Syk associates with Toll-like receptor 4 (TLR4) following LPS stimulation, and plays a pivotal role in the LPS-induced signalling pathway and monocyte chemoattractant protein (MCP)-1 expression [32]. Syk (−/−) neutrophils fail to undergo respiratory burst, degranulation or spreading in response to pro-inflammatory stimuli while adherent to immobilized integrin ligands or when stimulated by direct cross-linking of integrins [33]. In response to Fcγ receptor engagement, Syk (−/−) neutrophils were incapable of generating reactive oxygen intermediates [34]. Syk (−/−) macrophages were also defective in phagocytosis induced by the Fcγ receptor [34]. In monocytes, Syk is essential for β_2 integrin signalling and cell spreading [35]. Collagen induces tyrosine phosphorylation of Syk in platelet aggregation [36], and Syk phosphorylation is required for dendritic cell maturation induced by Fc receptor-mediated antigen presentation [37].

While we have demonstrated the roles of Syk in nasal fibroblasts, it has also been reported that Syk proteins are expressed in other non-haematopoietic cells such as endothelial and epithelial cells. The proliferation and migration of human umbilical vein endothelial cells are severely impaired by adenovirus-mediated expression of Syk dominant-negative mutants [38]. In Jurkat T cells, TNF activates Syk protein tyrosine kinase, leading to TNF-induced mitogen-activated protein kinase (MAPK) activation, nuclear factor-κB (NF-κB) activation and apoptosis [39].

Table 1. Roles of Syk in various cells

Cell	Cellular function or signal transduction	References
Eosinophils	Antiapoptotic pathway(s) through the IL-3/IL-5/GM-CSF-Rβ	[15]
	Generating reactive oxygen mediators in response to FcγR engagement	[31]
Neutrophils	Association with TLR4 (LPS stimulation), MCP-1-expression	[32]
	Degranulation or spreading (proinflammatory stimuli)	[33]
	Generating reactive oxygen mediators in response to FcγR engagement	[34]
Platelets	Collagen-induced signal transduction	[36]
Macrophages	Phagocytosis induced by FcγR engagement	[34]
Monocytes	β2 integrin signalling and cell spreading	[35]
Dendritic cells	FcR-mediated antigen presentation and cell maturation	[37]
Endothelial cells	Proliferation and migration	[38]
Epithelial cells	TNF-induced NF-κB	[39]

GM-CSF, granulocyte-macrophage colony-stimulating factor; TLR, Toll-like receptor; LPS, lipopolysaccharide; MCP, monocyte chemoattractant protein; NF-κB, nuclear factor-κB.

Conclusion

Fibroblasts are a rich source of chemokines, cytokines and other inflammatory mediators, and as such are known to play a major role in the pathogenesis of airway diseases including bronchial asthma, cystic fibrosis and rhinosinusitis with polyps. Nasal fibroblasts produce RANTES [9, 10], eotaxin [40], MCP-1 and GM-CSF [41]. On LPS stimulation, RANTES expression leads to eosinophilic recruitment and activation [28, 42, 43]. Syk is required for this process [11]. LPS also increases IL-4-induced production of eotaxin, which is a potent mediator in the development of tissue eosinophilia [44], and significantly induces gene expression and production of GM-CSF and IL-8 in nasal tissue-derived fibroblasts [45]. We have demonstrated that IL-8 may be an important aspect of the effect of treatment on nasal polyps [46].

LPS induces tyrosine phosphorylation of Syk and activates JNK1 in nasal fibroblast lines. The Syk-generated signal cooperates to enhance JNK activation in T lymphocytes [47], and MAPK activation has been shown to be compromised in the macrophages of Syk (−/−) mice after Fcγ receptor stimulation [35]. Syk is an important component leading to activation of NF-κB in human monocytic cell lines [48]. Decreased Syk expression has been shown to attenuate JNK1 activation in nasal fibroblast lines in the same way that oxidative stress-induced JNK activation is significantly

decreased in B cells that do not express Syk [49]. Experiments on the roles of src homology 2 (SH2) domains of Syk have revealed that the C-terminal SH2 domain of Syk is required for induction of JNK activation in oxidative stress [50]. Recently, TLR has been implicated in the recognition of various bacterial cell wall components including LPS [51]. Syk associates with TLR4 upon LPS stimulation [32]. TRAF6 mediates both IL-1- and LPS-induced signalling. We found a signalling complex involving Syk and TRAF6 after IL-1 induction, leading to chemokine production and subsequent eosinophil infiltration [30].

An Syk-negative variant of rat basophilic leukemia-2H3 cells failed to release histamine by FcεRI aggregation, whereas reconstituted cells with stable expression of Syk could release histamine [52]. Syk-deficient mast cells failed to degranulate, synthesize leukotrienes and secrete cytokines [53]. Furthermore, Syk may be critical in cell survival after damage in inflammatory diseases, as antiapoptotic pathways involve Syk-dependent signalling [15, 49].

Syk expression affects chemokine production in airway diseases. Syk antisense oligodeoxynucleotides delivered by aerosol to the lungs *in vivo* depressed Syk expression and pulmonary inflammation [54]. Syk is associated with Fc receptors and the B cell receptor involved in allergic diseases, antibody-mediated autoimmune diseases and nasal polyps. Syk inhibition might control the levels and function of specific IgE to *S. aureus* enterotoxins in nasal polyps. Because the role of Syk in regulating vascular homeostasis and other housekeeping functions is minimal or masked by redundant Syk-independent pathways, targeting Syk may be an optimal approach to the effective treatment of a multitude of chronic inflammatory diseases without undue toxicity [55]. In conclusion, manipulation of Syk expression may prove to be a useful strategy in the treatment of airway diseases such as asthma and nasal polyposis.

Acknowledgements

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Expression of Syk is associated with nasal polyp in patients with allergic rhinitis

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Abstract

Objective: Numerous signalings are involved in allergic inflammation. The non-receptor protein tyrosine kinase, Syk, is widely expressed in immune-potentiated cells and plays critical roles in initiating signal transduction in response to the activation of cytokine, chemokine and other types of receptors. It has been hypothesized that Syk expression in allergic nasal mucosa and polyps with allergy is different from non-allergic mucosa, and that changes in Syk expression contribute to the activation of allergic reactions.
Methods: We examined whether the expression of Syk is found in allergic nasal mucosa and polyps. We investigated the expression of Syk in 46 nasal mucosa and polyps (14 samples from patients with allergic rhinitis and 32 samples with non-allergic chronic sinusitis) using an immunohistochemical technique.
Results: Allergic polyps had more Syk positive cells than non-allergic polyps. Syk positive cells were determined to mainly be eosinophils. There was no difference in Syk expression in the lamina propria and nasal gland between allergic mucosa and non-allergic mucosa.
Conclusion: Eosinophils in allergic polyps receive an intracellular signal, although the signal is not able to determine the function in the present state. Syk appears to be a promising target molecule for anti-allergic inflammation in allergic rhinitis.

Keywords: Nasal polyp; Syk; Eosinophil; Mast cell; Nasal allergy

1. Introduction

Nasal polyps are recurrent protrusions of the nasal sinus mucosa that prolapses into the nasal cavity. The origin of nasal polyps can be divided into two categories: allergic rhinitis (AR) and non-allergic chronic sinusitis (NACS). In AR, exposure to the allergen promotes the cross-linking of IgE molecule on mast cells and mast cells release histamine and other proinflammatory mediators within minutes. The released chemical mediators induce many signals in the cells of the nose and the sum of signals causes allergic inflammation. Chemoattractants play a dual role by triggering integrin activation and directing leukocyte migration. Some chemokines and cytokines (RANTES, Eotaxin, IL-5, etc.) have been shown to attact and activate eosinophils *in vitro* and to recruit eosinophils into the inflammatory region in the nasal mucosa. The addition of several factors (ex. edema, a change in architecture of the epithelium, a large influx of water, an alteration of the structure of gland) to the AR contributes to the development of nasal polyp [1].

NACS may originate from or be perpetuated by local or systemic factors that predispose one to sinus ostial obstruction and infection [2]. Japanese NACS is different from Western NACS, and has a characteristic that neutrophils are the predominant infiltrating cells in nasal mucosa [3]. A high level of IL-8 concentration in the nasal lavage from patients with nasal polyps is typical in Japanese NACS [3–5]. Persistent inflammation due to bacterial

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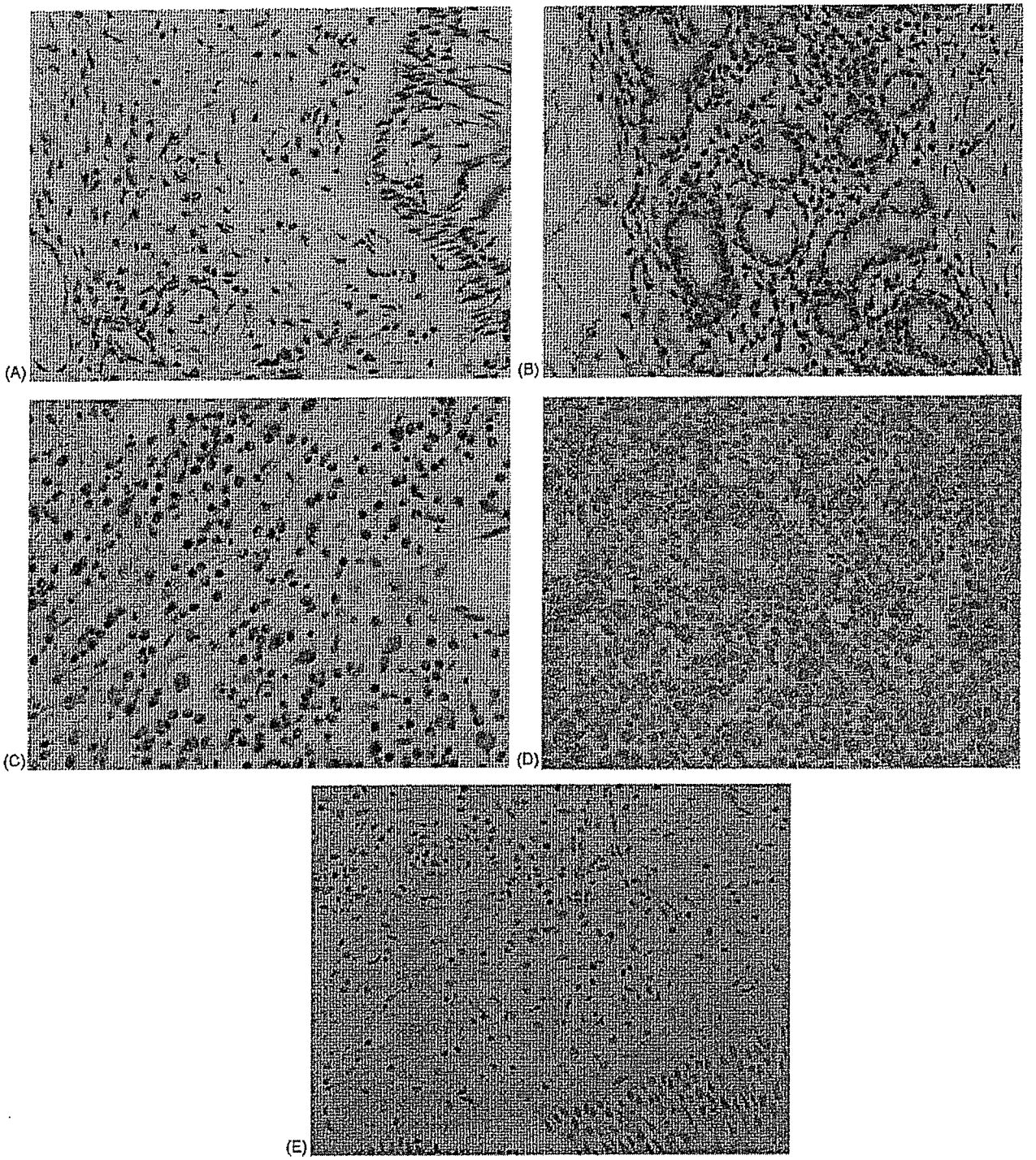

Fig. 1. Immunohistchemical staining of Syk in the nasal mucosae, intestinum of lamina propria (a: Syk score = 29.5), the nasal grand (b: Syk score = 46.5), nasal polyp (c: Syk score = 57.5), the tonsil was for the positive staining of Syk (d: Syk score = 48.8) nasal polyp (e: negative control) (magnification 200×).

infection and sequential cytokine secretion (IL-1β, TNFα, IL-8, TGFα and TGFβ) induced many signals in inflammatory cells, fibroblasts and epithelial cells [6,7]. The activation of epidermal growth factor receptor kinase and platelet-derived growth factor receptor kinase leads to hyperplasia of epithelial cells and nasal glands for the pathogenesis of nasal polyps in NACS [8,9].

The complicated activation in cells through cytokine and chemokine receptors is associated with protein tyrosine kinase. Protein tyrosine kinases can be divided into two

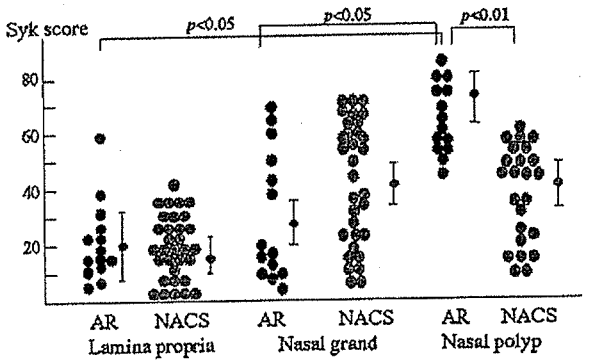

Fig. 2. The number of Syk positive cells. The Syk score determined the number of Syk positive cells in all cells. The Syk score in the nasal polyp with allergic rhinitis (AR) was higher than those with non-allergic chronic sinusitis (NACS).

families: receptor tyrosine kinases and non-receptor tyrosine kinases. Syk has non-receptor protein tyrosine kinases function as critical components in signaling cascades from membrane receptors lacking intrinsic tyrosine kinase activity, and is widely expressed on hematopoietic cells: B cells [10,11], mast cells [12], eosinophils [13], T cells [14], neutrophils [15] and other cells. Recently, it has been shown that Syk is also expressed in non-hematopoietic cells; human nasal fibroblasts [16,17], breast epithelium [18] and human hepatocytes [19]. The level of Syk expression in nasal polyp-derived fibroblasts was correlated with RANTES production by LPS [16].

In this study, we investigated the Syk expression on nasal polyps between AR and NACS using an immunohistochemical technique, since there are few reports about the expression of signal molecules in nasal polyps.

2. Materials and methods

2.1. Patients and sample collections

We studied the inferior turbinate mucosa and nasal polyps of 14 patients (10 males and 4 females) with perennial AR caused by *Dermatophagoides Pteronyssinus* (DP), and 32 control subjects (20 males and 12 females) with NACS. All of the patients underwent endoscopic sinus surgery, because all conservative treatments had no effect on their nasal congestion. We obtained informed consent from all patients. Both the inferior turbinate mucosa and the nasal polyps were excised during surgery. All of the patients with AR had a high titer of anti-DP-specific IgE without Japanese Cider and Ragweed in the serum. All patients with NACS had negative evidence for these allergies. No patients in either group had histories of aspirin-induced asthma. There were no significant differences in the background of the patients in AR and NACS groups except for nasal allergy. The operation was performed under local anesthesia by injection with 0.5% lidocaine with 1:100,000 adrenalin and 10% cocaine.

2.2. Immunohistochemical staining

Specimens were immediately fixed in 4% paraformaldehyde for over 48 h. After fixation, samples were embedded in paraffin and sectioned at 2–4 μm thickness. They were deparaffinated and treated with ethanol, then rinsed with pH7.4 phosphate-buffered saline (PBS). Immunohistchemical staining was performed using the avidin–biotin-complex technique [20].

Specimens were washed in distilled water, and rinsed with pH 7.4 PBS, and incubated in 0.3% H_2O_2 solution dissolved in absolute methanol at room temperature for 15 min to inhibit endogenous peroxidase activity. After washing, specimens that would be stained for Syk were treated by microwave irradiation for 10 min in distilled water. They were rinsed with PBS and incubated with rabbit anti-human Syk polyclonal antibodies diluted at 1:200 at 4 °C for 24 h. After rinsing with PBS, all specimens were treated with polymerized peroxidase anti rabbit-IgG (DAKO, Glostrup, Denmark) for 1 h at room temperature. After rinsing with PBS, peroxidase color visualization was carried out with 15 mg of 3–3′-diaminobenzidine tetrahydrochroride (WAKO, Tokyo, Japan) dissolved in 100 ml PBS with 8 μl of 30% H_2O_2 for 10 min. Nuclear counter staining was carried out with Mayer's haematoxylin for 2 min before mounting. For positive controls, we used the tissue of the tonsil, which was already known to be positive for Syk. For the negative control, we used rabbit anti-human IgG for the first antibody.

2.3. Double immunofluorescence techniques

The standard double immunofluorescence technique was employed. Mouse anti-human EG2 monoclonal antibody (mAb) as a marker of eosinophils, anti-CD30 mAb for T cells, anti-CD14 mAb for macrophages, anti-elastase mAb for neutrophils, anti-tryptase for mast cells, and anti-CD20 mAb for B cells were used. After being incubated with anti-human Syk antibody, rinsing with tris buffered saline (TBS), we applied swine FITC conjugated anti rabbit immunoglobulins for 2 h at room temperature. After rinsing with TBS, we applied the second antibody (for example: CD20) at 4 °C for 24 h. After rinsing with TBS, we treated rabbit RPE conjugated anti-mouse immunoglobulin for 2 h at room temperature. After washing with TBS, nuclear counter staining was carried out with Mayer's haematoxylin for 2 min before mounting. Then, we counted the positive cells with fluorescence microscopy. In this experiment, 7 NACS samples were randomly selected.

2.4. Evaluation of Syk expression

For microscopic analysis, we randomly selected five images of strongly stained sections of Syk in each specimen. The mean number of Syk positive cells per field, that had infiltrated the intestinum of the lamina propria, goblet cell,

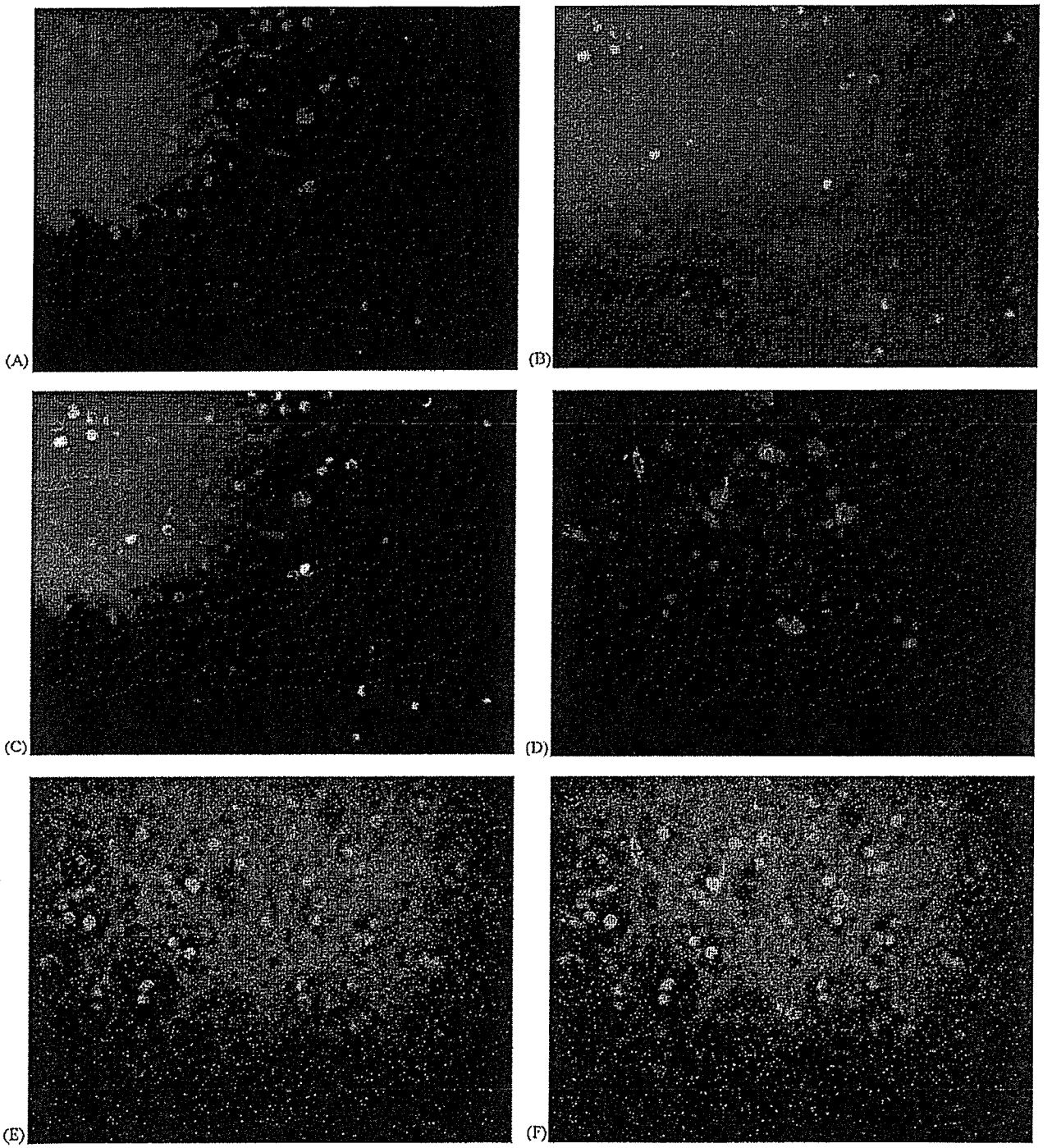

Fig. 3. Double immunohistchemical staining. (A) EG2 positive cells (red); (B) Syk positive cells (green); (C) merged view Syk score is 0.44 in this section (magnification 200×); (D) mast cell positive cells (red); (E) Syk positive cells (green); (F) merged view Syk score is 0.16 in this section (magnification 200×) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.).

epithelial layer were counted. We counted at least 1000 cells, including Syk positive cells, and estimated the positive cells as the Syk score [20].

For fluorescence analysis, we used a microscope (BX51-33-FL-3, Olympus, Tokyo, Japan). We randomly selected three images of strongly stained sections of Syk. The mean number of Syk and other per field that had infiltrated the nasal polyp were counted. These analyse were performed at a magnification of 400× (Syk positive cells). Macintosh computers (Stat view software; Abacus Concepts Inc., Berkeley, CA) were used for all statistical analysis.

3. Results

3.1. Syk expression in the nasal mucosa and polyp

Syk positive cells were observed in the lamina propria, nasal gland and nasal polyp. Syk staining was positive in the cells that had infiltrated the lamina propria and the nasal gland (Fig. 1A and B). The usual pattern of positive staining for Syk involved the cytoplasm. The mean Syk positive cells per field (Syk score) in the intestinum of lamina propria from all 46 patients was 15.9 ± 11.4 (mean ± S.D.), that in the nasal gland was 32.2 ± 22.4, and that in nasal polyp was 38.0 ± 19.0. In the AR group, the mean Syk score in the intestinum of lamina propria was 18.0 ± 11.7, that in the nasal gland was 25.8 ± 21.1, that in the nasal polyp was 50.9 ± 17.9. Syk score in the nasal polyp was higher than that in the lamina propria and nasal gland ($p < 0.05$, $p < 0.05$). In the NACS group, the mean of the Syk score in the intestinum of the lamina propria was 14.8 ± 11.2, that in the nasal grand was 35.3 ± 22.6, that in the nasal polyp was 30.2 ± 15.2 (no difference among the NACS group). In the AR group, the Syk score in the nasal polyp was significantly higher than that in the NACS group (50.9 ± 17.9 versus 30.2 ± 15.2, $p < 0.01$, Fig. 2). Few Syk positive cells were detected in the epithelial layer (data not shown).

3.2. Double staining

To clarify which cells expressed Syk in the nasal polyp from patients with AR, double immunostaining was performed. EG2 and Syk double positive cells were mainly observed in the allergic polyp (Fig. 3). In the AR group, the mean percentage of double positive staining for Syk and EG2 was higher than the NACS group (29.4 ± 21.4% versus 11.5 ± 8.7%, $p < 0.05$, Fig. 4). Since the Syk score of the nasal polyp in AR was higher than that in the NACS group, the absolute number of double positive nasal polyp cells in AR was also higher than that in NACS. Double positive cells

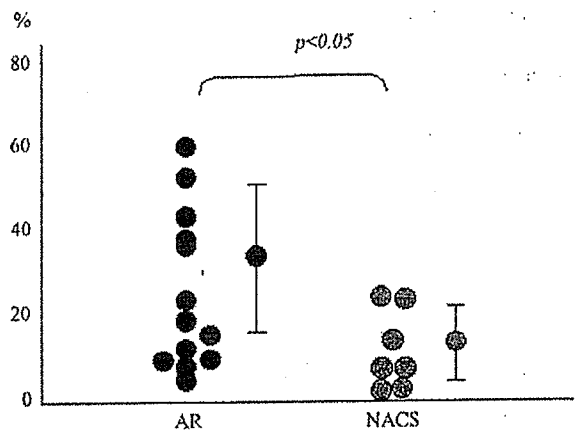

Fig. 4. The mean percentages of EG2 and Syk double positive cells. The double positive cells in AR weres higher than those in NACS ($p < 0.05$).

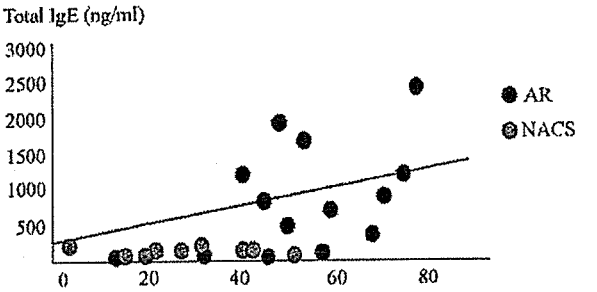

Fig. 5. The correlation with serum IgE titer and Syk score in AR. Total IgE in the serum was correlated with Syk score of nasal polyp from AR patients.

for Syk with each CD30 or elastase or CD14 were only minimally detected in both the AR and NACS group (data not shown). Although CD20 positive cells were stained in AR, the mean percentage of double positive cells in the CD20 cells was 1.1 ± 0.9%. And the mean percentage of double positive cells in the tryptase positive cells was 10.92 ± 16.85%.

3.3. Correlation of Syk score and IgE in the serum

Serum total IgE values in AR range from 16 to 2400, while those values in NACS range from 8 to 130. Total IgE in the sera is significantly associated with the Syk score in the nasal polyp ($r = 0.640$, $p < 0.01$, Fig. 5). There is no difference between the Syk score in the lamina propria and serum IgE (data not shown). Nine NACS samples which has IgE data were included.

4. Discussion

In this study, we demonstrated that Syk expression in nasal polyps from AR patients was significantly higher than that those from NACS patients. There was no difference in the Syk expression between nasal mucosa from AR and that from NACS. The Syk-positive cells are mainly eosinophils in the nasal polyp. Syk was stained in the cytoplasm of eosinophils. The mean percent of Syk positive cells in nasal polyps were associated with serum total IgE.

Eosinophils are well known to be induced and activated by several cytokines and chemokines. In allergic disease, the up-regulation of Interleukin-5 (IL-5), granulocyte/macrophage colony-stimulating factor (GM-CSF), eotaxin and RANTES cause blood and tissue eosinophilia. Patients with allergic nasal polyposis had significantly higher tissue densities of IL-4, IL-5, GM-CSF and IL-3 compared with those with non-allergic nasal polyposis [21,22]. Fan et al. also reported that EG2 and IL-5 positive cells were abundant in the submucosa of patients with allergic sinusitis, especially in the superficial layer. About half of the IL-5 producing cells were eosinophils and apoptotic eosinophils were less numerous in the superficial layer [23]. Human eosinophils have IL-3/IL-5/GM-CSF receptor on

the surface. IL-5 receptor activation in eosinophils has been shown to stimulate JAK2, STAT1, Lyn and Syk [24]. IL-5 and GM-CSF inhibited the apoptosis of eosinophils *in vitro* and *in vivo*. Both Syk and Lyn are essential signal molecules for the activation of the anti-apoptotic pathway(s) induced by the IL-3/IL-5/GM-CSF receptor subunit in human eosinophils [25]. Thus, one possibility is that Syk expression in the nasal polyps of AR may indicate the activation of Syk by IL-5 receptor to lead to the elongation of eosinophil-survival.

Conversely, the analysis of Syk-knock out mice ($Syk^{-/-}$) demonstrated that the anti-apoptotic effect of IL-5 in cells does not require Syk despite the activation of this tyrosine kinase upon IL-5 receptor ligation [26]. However, Syk is important in activation events (oxidative burst or phagocytosis) induced by Fcγreceptor (FcγR) stimulation [26]. FcγR is found on the surface of eosinophils and plays a critical role in eosinophil activation in cooperation with Syk phosphorylation. Among several FcγRs, allergen-specific IgG1 and IgG3 induces degranulation of eosinophils as inflammatory reaction through FcγRII (CD32) [27]. Also, FcγRII may pivotally regulate both the survival and death of eosinophils, depending on the manner of receptor ligation and β2 integrin involvement [28]. The integrin family of cell adhesion receptors mediates both cell–cell and cell–matrix interaction and plays critical roles in development, inflammation, angiogenesis, migration, metastasis and other important biological processes [29]. The binding of β2 integrin receptors to their ligands (ICAM-1) is critical for firm attachment, spreading and the transendothelial migration of eosinophils [30]. Syk is essential to activate signal transduction cascades initiated by the binding of β2 integrin receptors to their ligands [31]. Thus, Syk expression might suggest that signal transduction from β2 integrin receptors in eosinophils was working to migrate into the nasal polyp. As Syk is regulated by multiple classes of integrins, Syk is deeply associated with the integrin family [32]. Additionally, signaling by integrin and ICAM-1 prolong eosinophil survival [33].

Aggregation of the high affinity IgE receptor (FcεRI) by IgE binding results in the sequential activation of Syk and Lyn on mast cells [34]. Local IgE class switchings and local IgE syntheses were demonstrated in human allergic nasal mucosa [35,36]. IgE itself up-regulates FcεRI, which prevents protease digestion at the cell surface [37]. Recently, nasal polyps have been characterized by a high concentration of IgE in the nasal polyp associated with presence of *Staphylococcus aureus* enterotoxin-specific IgE [38]. These data led us to speculate that Syk-dependent FcεRI signaling is working well in nasal polyps of AR. However, the positive expression of Syk in mast cells was less than eosinophils in this study (Fig. 3D–F). Although eosinophils express FcεRI, most of the protein is confined to the cytoplasm [39]. Our data showed that IgE in the serum was correlated with the Syk score in nasal polyps in AR patients. However, there is little evidence for IgE-dependent function in eosinophils. IgG appears to be more important for eosinophil activation in allergic disease than IgE.

Recently, it was reported that enhanced IFN-α signaling and proinflammatory function were dependent on the tyrosine kinase Syk and on adaptor proteins that activate Syk through immunoreceptor tyrosine activation motifs [40]. IFN-α inhibited IL-5 and GM-CSF genreration of cord blood. IFN-α receptor was found on eosinophils collected from patients with various eosinophilic disorders and inhibited the release of eosinophil granule proteins, such as eosinophil cationic protein, neurotoxin, or IL-5 [41]. The oromucosal administration of IFN-α reduced allergen-specific IgE production and allergen-induced eosinophil recruitment in the absence of detectable toxicity for the treatment of allergic disease [42]. However, our previous study showed that IFN-α was not detected in the nasal lavage from patients with AR and nasal polyps [5] and in the supernatant of nasal polyp-derived fibroblasts (data not shown). There might be the possibility that Syk activation in eosinophils of allergic rhinitis polyp induces suppressive signaling for allergic disease, but this possibility was low.

The delivery of Syk antisense oligodeoxynucleotides (ASO) by aerosol to rat lungs *in vivo* has the potential to reduce Syk expression in infiltrated immune cells and to suppress Ag-pulmonary inflammation [43]. Additionally, the treatment of Syk ASO greatly inhibited the number of eosinophils in the lung parenchyma [44]. They suggested that Syk ASO may be a useful anti-inflammatory agent. Intranasal application of Syk inhibitor R112 improved allergic symptoms of seasonal allergy in a park setting [45]. They suggested that intranasal application of Syk inhibitor become a new treatment of a seasonal allergy.

The degree of Syk expression is not equal to the activity of Syk in cells, while no expression of Syk does not means any activities of Syk in cells. The autophosphorylation and activation of Syk (phosphorylation of adaptor molecule) produce the signal pathway in eosinophils. Constitutive phosphorylated Syk was detected in nasal polyps with a high Syk expression from patients with AR by Western blotting in this study (data not shown). Although further study is necessary to investigate how Syk works in nasal polyps with AR, Syk may be a target molecule for the treatment of nasal polyps with allergy.

Acknowledgements

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Ⅱ．耳鼻咽喉科
1）鼻粘膜由来線維芽細胞における RANTES・Eotaxin 制御

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気道粘膜の再構築，リモデリングの観点から，アレルギー疾患における線維芽細胞の役割が最近注目されている。鼻粘膜由来線維芽細胞は，いろいろな刺激により，細胞外基質(ECM)の他，ケモカイン，サイトカイン，成長因子を産生する。RANTES, Eotaxin, MCP-1, IL-8は，好酸球や好塩基球に作用があり，鼻粘膜由来線維芽細胞が産生するケモカインである。鼻粘膜由来線維芽細胞における RANTES・Eotaxin 産生の制御について，Toll-like receptor(TLR)3, TLR4, IL-4受容体，IL-13受容体の細胞内情報伝達系を中心に概説し，将来のアレルギー治療の展望について考察する。

鼻粘膜由来線維芽細胞／RANTES／Eotaxin／Syk

はじめに

細菌，ウイルス，抗原，酸化ストレスなどの影響を気道の最初に受ける下甲介には，樹状細胞，T細胞，B細胞，形質細胞，肥満細胞，好塩基球，好酸球，単球，気道上皮細胞，線維芽細胞，血管内皮細胞，神経細胞，血小板などが存在し，アレルギー性鼻炎の病態に関与する。特に線維芽細胞は，これらの他の細胞の刺激を受け，更に影響を与えることから，状況によって悪循環を引き起こし，最終的に下甲介の病的な肥厚の原因となる。RANTES（regulated upon activation normal T expressed and presumably secreted），Eotaxinは，好酸球や好塩基球に作用があり，鼻粘膜由来線維芽細胞から大量に産生される。抗Eotaxin抗体[1]，EotaxinとRANTESがリガンドとなるCCR3に対する抗体による治療効果がアレルギーの病態を改善することから，これらのケモカインが病態において重要であることが判明した。RANTES，Eotaxinの産生を制御できれば治療へと結びつく。細菌，ウイルス，Th2サイトカイン，脂質メディエーター，アセチルコリン作動性物質などは，RANTES，Eotaxinの

発現や産生に影響を与えることから，これらの細胞内情報伝達とその抑制の可能性について述べる。

Ⅰ．種々の刺激による鼻粘膜由来線維芽細胞の反応

アレルギー疾患における気道粘膜の再構築，リモデリングでは，基底膜直下のコラーゲンやエラスチンなどの異常な沈着が報告されており，そのほとんどが気道線維芽細胞から産生される。鼻粘膜由来線維芽細胞は，細菌感染，ウイルス感染，サイトカイン，成長因子，抗原に存在するプロテアーゼ，酸化ストレス，低酸素などの刺激により，ECM (extracelluar matrix) の他，RANTES, Eotaxin, MCP-1(macrophage chemoattractant protein-1), IL (interleukin)-8, TARC (thymus and activation-regulated chemokine) などのケモカイン，サイトカイン，成長因子を産生する（図1）。

Th2 細胞，好塩基球，肥満細胞からの

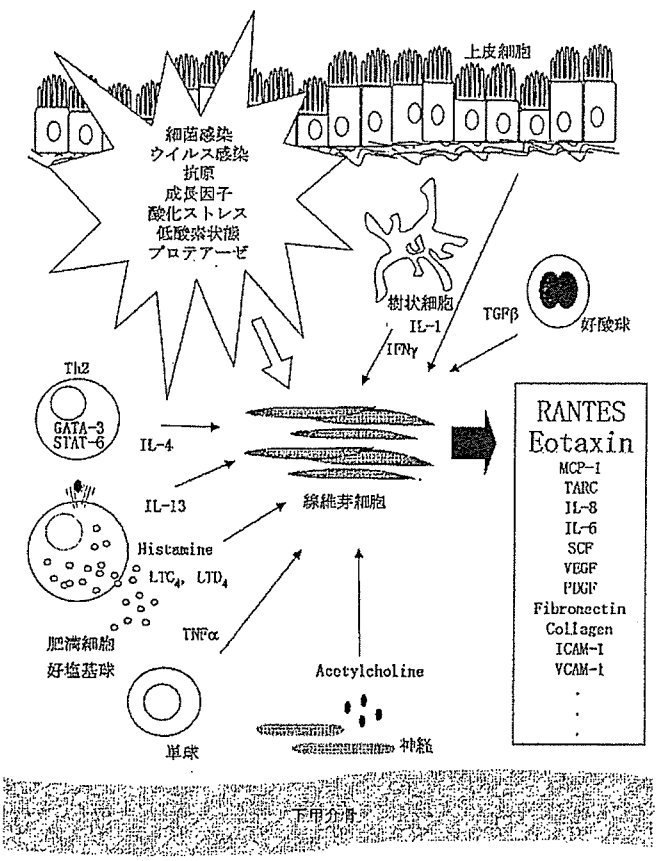

図1　種々の刺激による鼻粘膜由来線維芽細胞の反応
鼻粘膜由来線維芽細胞は，細菌・ウイルス・サイトカイン・抗原などの影響で，ケモカイン・サイトカイン・成長因子を産生する.

IL-4やIL-13の刺激で，線維芽細胞からEotaxin[2]やTARCが産生され，接着分子であるICAM-1（intercellular adhesion molecule-1），VCAM-1（vascular cell adhesion molecule-1）の発現が亢進する。Eotaxinは強力な好酸球遊走能を持ち，TARCはCCR4に結合しTh2細胞に作用する。樹状細胞，単球，気道上皮からのIL-1，単球から産生されるTNF（tumor necrosis factor）α，LPS（lipopolysaccharide），double-stranded RNA（dsRNA）で鼻粘膜由来線維芽細胞がRANTES，IL-8を産生する[3)~5)]。鼻粘膜由来線維芽細胞から産生されるフィブロネククチン，SCF（stem cell factor）は，好塩基球，肥満細胞の細胞遊走，増殖を促す。アレルギー性疾患では，好塩基球や肥満細胞の脱顆粒による即時相と，Th2細胞や好酸球などの浸潤による遅発相が存在するが，線維芽細胞は，好塩基球，肥満細胞，好酸球に作用し，それぞれの病態を悪化させている。

TGF β（transforming growth factor β），IL-4の刺激でコラーゲンなどのECMの産生が促される。低酸素状態により線維芽細胞から産生されるVEGF（vascular endotherial growth factor）は，内皮細胞増殖と透過性亢進の作用があるが，アセチルコリン作動性物質でも発現が増強する。アレルギー性鼻炎では非アレルギーに比べると基底膜の肥厚と血管新生が強く起こっていることから，リモデリングと類似の変化が認められ，線維芽細胞が重要な役割を果たしている。

Ⅱ．鼻粘膜由来線維芽細胞が産生するケモカインの比較

RANTES，Eotaxin，MCP-1，IL-8は，好酸球や好塩基球に作用があり，鼻粘膜由来線維芽細胞が産生するケモカインである（表1）。ケモカインは，最初の2つのシステインの位置関係から4つのサブファミリーとして，CXC，CC，C，CX_3Cに分類される。RANTES，Eotaxin，MCP-1はCCケモカインで，IL-8はCXCケモカインである。アレルギー疾患の肺胞洗浄液や鼻洗浄中には，RANTES，Eotaxin，MCP-1が多いことが知られている。CCR3をリガンドとするケモカインの中で，Eotaxinは最も高親和性で，CCR3以外のCCRには結合しない。Eotaxinは低濃度で好酸球と好

表1　鼻粘膜由来線維芽細胞が産生するケモカインの比較

好酸球と好塩基球への作用とケモカイン受容体	RANTES	Eotaxin	IL-8	MCP-1
好酸球遊走	++	+++	+	−
好酸球脱顆粒	++	++	+	−
好塩基球遊走	++	+++	+	+
好塩基球脱顆粒	++	−	+	++
ケモカイン受容体	CCR1 CCR3 CCR5	CCR3	CXCR1 CXCR2	CCR2 CCR4

塩基球の遊走を誘導する。RANTES は，CCR1，CCR3，CCR4，CCR5 を介して好酸球と好塩基球の遊走と脱顆粒を誘導する作用があり，また，B 細胞に働き IL-4 と CD40 刺激による Ig（immunoglobulin）E と IgG_4 の産生を亢進させる[9]。IL-8 は代表的な好中球走化性因子であるが，受容体としては CXCR1，CXCR2 があり，好塩基球や IL-5 でプライミングされた好酸球には CXCR2 が発現しており，IL-8 は遊走能を亢進させる。MCP-1 は，CCR2 を介して好塩基球の脱顆粒を誘導しヒスタミンの遊離を起こす。

Ⅲ．鼻粘膜由来線維芽細胞の RANTES 産生に関わる TLR3 と TLR4 からの細胞内情報伝達

細菌，ウイルス，寄生虫，植物などが体内に侵入した際にそれを排除しようとする免疫系には，自然免疫と獲得免疫があり，互いに関連しあい成り立っている。自然免疫は，抗体産生に関わる獲得免疫と比べて非特異的と思われてきたが，自然免疫の特異的な受容体である TLRs（Toll-like receptors）が発見され，それぞれ特異的な反応を示すことが判明した。鼻粘膜由来線維芽細胞にも，TLRs が発現しており，TLR2,

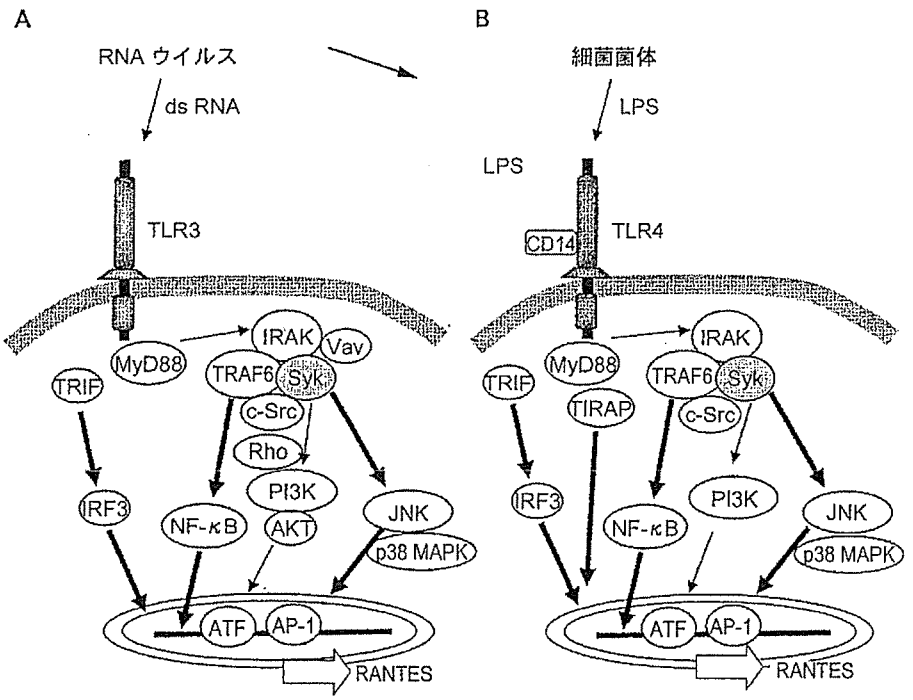

図２　鼻粘膜由来線維芽細胞の RANTES 産生に関わる TLR3 と TLR4 からの細胞内情報伝達
TLR3・TLR4 からの細胞内情報伝達には，TRIF・IRF3・MyD88・IRAK・NF-κB・Syk・PI3K・JNK・p38MAPK などが関与する．

TLR3, TLR4, TLR9が認められ，TLR3とTLR4の発現が最も強く，RANTES産生に関与している。poly ICやLPSで，TLR3とTLR4が活性化するとRANTESの産生が亢進する。

TLR3とTLR4の細胞内情報伝達は，TRIF，IRF3 (interferon regulatory factor-3)，MyD88，IRAK(IL-1 receptor associated kinase)，TRAF (tumor necrosis factor receptor associated factor) 6などを介して，NF-κB (nuclear factor-κB)，JNK (c-jun N-terminal kinase)，p38 MAPK (mitogen activated protein kinase)，PI3K (phosphoinositide 3-kinase)を活性化させる(図2)。我々は，IgE受容体(FcεRI〔Fc epsillon receptor I〕)のシグナルに中心的な働きをしておりアレルギー疾患や自己免疫疾患の治療の標的分子としても候補としてあげられているSykが，TLR4に関わるRANTES産生に重要な働きをしており，TRAF6に会合することを証明した。Sykに対するアンチセンスやmutant vectorを用いてLPSによるRANTES産生を抑制することに成功した[3),4)]。

鼻粘膜由来線維芽細胞をdsRNAで刺激すると，JNK，p38 MAPK以外にAKTがリン酸化され，細胞内情報伝達をタンパク質リン酸化アレイで検討すると，Rho，Syk，Vav，c-Src，TRAF6の関与が観察された。また，PI3K阻害剤やJNK阻害剤によりdsRNA誘導RANTES産生が抑制された[5)]。

Ⅳ．鼻粘膜由来線維芽細胞のEotaxin産生に関わるIL-4受容体とIL-13受容体からの細胞内情報伝達

Th2細胞からのIL-4が(CD40LやBLySの存在下で) B細胞を活性化させるとIgEクラススイッチが起こり，最終的にIgEを産生させる。抗原特異的IgEはアレルギーの本体であるから，IL-4は病態の中で最も重要なサイトカインである。鼻粘膜由来線維芽細胞においては，IL-4とIL-13はEotaxinの産生を誘導する。IL-4受容体α鎖(IL-4 α R)はIL-4受容体とIL-13受容体の共有受容体であるため，IL-13はIL-13受容体のリガンドであるのに対し，IL-4はIL-4受容体とIL-13受容体に作用する(図3)。IL-4は受容体に結合するとJAK1，JAK3を介してSTAT (signal transducer and activator of transcription) 6をリン酸化させ，Eotaxin産生に関与する。IL-13受容体ではJAK1，JAK2，Tyk2，STAT6のリン酸化が起こる。

IL-4やIL-13によるEotaxin産生では共刺激による実験でその影響が報告されている。IL-4誘導EotaxinがLPSにて誘導される。ヒト由来線維芽細胞(HFL-1)では，IL-13によりLTC_4 (leukotriene C_4)，LTD_4 (leukotriene D_4)の受容体である$CysLT_1R$の発現が増強し，LTC_4によりIL-4誘導Eotaxin産生が増強され，$CysLT_1R$拮抗薬であるpranlukastにより抑制された。我々は，鼻粘膜由来線維芽細胞を用いて同様の実験を行い，LTC_4によりIL-4誘導Eotaxin産生が増強され，$CysLT_1R$拮抗薬で抑制することを確認した。また，dsRNA

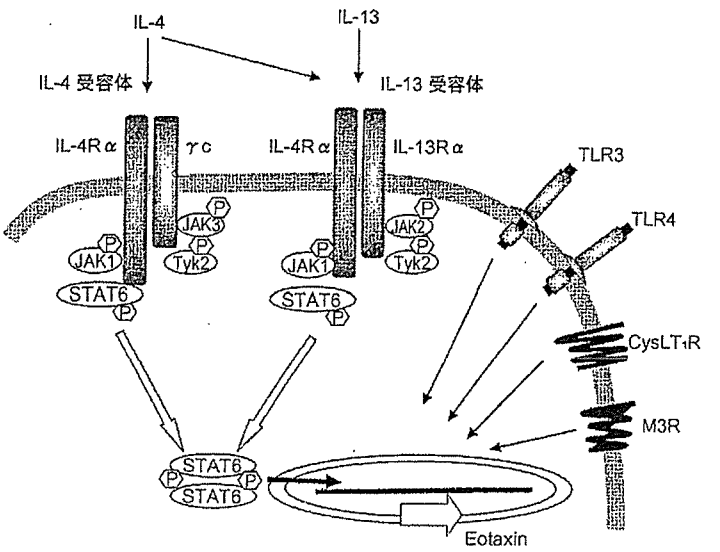

図３　鼻粘膜由来線維芽細胞の Eotaxin 産生に関わる IL-4 受容体と IL-13 受容体からの細胞内情報伝達

IL-4 受容体・IL-13 受容体からの細胞内情報伝達では，JAK1・JAK2・JAK3・Tyk2・STAT6 などの分子が関与する．

により IL-4 誘導 Eotaxin 産生も増強したことから，TLR3，TLR4，$CysLT_1R$ からのシグナルが IL-4 受容体と IL-13 受容体からシグナルを増強していると考えられる。TLR3，TLR4 のシグナルは図２で示したが，$CysLT_1R$ からのシグナルは，phospholipase Cβ，IP_3(inositoltriphosphate)，PKC (protein kinase C)，p34 MAPK，ERK (extracellular signal regulated kinase) が報告されているがあまり知られていない。B 細胞を活性化するために IL-4 は低濃度でよいが，鼻粘膜由来線維芽細胞が Eotaxin を産生するには IL-4 は高濃度である必要がある。Eotaxin 産生においては，IL-4 受容体と IL-13 受容体から，JAK1，JAK3，STAT6 以外のシグナルが関与している可能性が高い。実際，IL-4 受容体では，IRS-3，PI3K，Shc，PLCγ などのシグナル分子が報告されている。

アレルギー性鼻炎では，副交感神経が有意でセチルコリン受容体が過剰な鼻汁の分泌を促し，病態を悪化させている。ムスカリン性アセチルコリン受容体は $CysLT_1R$ と同様に７回膜貫通型の受容体であり，分泌腺や神経節に M1，平滑筋や心臓に M2，分泌腺や平滑筋に M3，中枢神経に M4 と M5 が発現している。鼻粘膜由来線維芽細胞でのこの受容体の発現を real time PCR (polymerase chain reaction) で検討すると，発現が認められ，M3 の発現が最も強く，M1，M2，M4，M5 に比べて 10 倍以上であった。鼻粘膜由来線維芽細胞にアセ

チルコリン作動性物質であるメサコリンを作用させると，IL-4誘導Eotaxin発現を増強した。

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IL-4シグナルを抑制する可能性のある細胞内シグナル分子としては，SH2 domain-containing PTPase-1（SHP-1），SOCS(suppressor of cytokine signaling) 1，SOCS3，SOCS5などが，細胞外の分子としては，TGFβ，IFN (interferon) α，IFNγ，CpG DNAなどが候補として挙げられるが，線維芽細胞では作用が不明な点も多い。

RANTESとEotaxinは好酸球・好塩基球・肥満細胞に作用する。これらの細胞を標的にした薬剤が中心であるが，将来期待できるアレルギー治療薬を表2に示した。抗Eotaxin抗体，EotaxinとRANTESがリガンドとなるCCR3に対する拮抗薬，抗IL-5抗体，細胞接着分子に対する抗VLA4 (very late antigen 4) 抗体などは好酸球の浸潤抑制の作用が期待されている。

抗IgE抗体は，喘息，通年性アレルギー性鼻炎，花粉症，アトピー性皮膚炎に対する有効性が報告されている[7)~9)]が，投与を中止すると血清IgE値が戻ることと高価であることから使用方法に制限がある。

RANTES産生に重要な働きをしているSykは，抗原と抗原特異的IgEによる好塩基球と肥満細胞の脱顆粒（ヒスタミン，プロテアーゼ），IL-4，IL-13，TNF αなどのサイトカイン産生，LTC_4，LTD_4，LTE_4，PGD_2 (prostaglandin D_2) などの放出に至るIgE受容体のシグナルで中心的な働きをしている。Sykに対する阻害剤は，花粉症に対するpark studyで，placebo薬に比べて有意に鼻の症状を抑制することが判明した[10)]。

IgG受容体であるFcγRIIb (Fc gamma receptor II b) のITIM (immunoreceptor tyrosine-based inhibition motif)は，リン酸化チロシン脱リン酸化酵素であるSHP-1とイノシトールリン脂質脱リン酸化酵素SHIPを会合し，Sykとその下流のシグナルが阻害する。この負のシグナルを利用したキメラ分子がアレルギー反応を抑えることを我々は確認した[11), 12)]。

その他，経口減感作療法，ペプチド免疫療法，CpG DNA抗原療法など，多くのアレ

表2　好酸球・好塩基球・肥満細胞に作用し将来期待できるアレルギー薬剤

薬剤	作用	名称	その他
抗eotaxin抗体	好酸球浸潤抑制	Bertilimumab	国外第II相
CCR3拮抗薬	好酸球浸潤抑制	GW-766944	国外第II相
IL-5拮抗薬	好酸球浸潤抑制	Mepolizmab	国外第III相
VLA4拮抗薬	好酸球浸潤抑制	RBx7796	国外第II相
抗IgE抗体	IgE受容体活性化抑制	Omalizumab	国内第II相
Syk阻害薬	IgE受容体活性化抑制	R-112	国外第II相
IgE-IgGキメラ分子	IgE受容体活性化抑制	GE2	開発中（文献11）
抗原-IgGキメラ分子	IgE受容体活性化抑制	GFD	開発中（文献12）

ルギー薬の効果が期待できる。効果の程度，効果の持続，価格，病態を考慮し，併用など使用方法を工夫していくことでアレルギーの治療はより進歩していくと考えられる。

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