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Protein-Tyrosine Kinase Syk Expressed in Human Nasal Fibroblasts and Its Effect on RANTES Production

Takechiyo Yamada,† Shigeharu Fujieda,2* Shigeru Yanagi, † Hirohei Yamamura, † Ryoko Inatome,† Hiroshi Sunaga,* and Hitoshi Saito*†

Fibroblasts, a rich source of chemokines, interact with eosinophils and play a key role in the pathogenesis of airway disease. RANTES is produced by fibroblasts to attract and activate eosinophils. LPS is known to induce RANTES and cause protein tyrosine phosphorylation. Nonreceptor protein tyrosine kinase Syk is widely expressed and an important role in intracellular signal transduction in hematopoietic cells. In the present study, we examined whether Syk was expressed in a number of primary human nasal polyp tissue-derived fibroblast lines and whether it played some role in cellular function. Syk proteins were expressed in human nasal fibroblasts, but the expression level varied. There were positive correlations between the level of Syk expression and RANTES production induced by LPS. Overexpression of wild-type Syk by gene transfer enhanced RANTES production from nasal fibroblasts stimulated with LPS. The decrease of Syk expression by the administration of Syk antisense inhibited RANTES production. These results suggest that Syk expression affects RANTES production in fibroblasts of nasal polyps. The Journal of Immunology, 2001, 166: 538–543.

The pathogenetic findings of nasal polyps show an increase in infiltrating cells including eosinophils (1, 2). Nasal fibroblasts play an important role in both nasal polyposis and allergic rhinitis through the release of biologically active factors (3, 4). RANTES is one of the active factors in nasal mucosa that attracts human eosinophils and induces eosinophil cationic protein release (5, 6). High amounts of RANTES in nasal polyp specimens were found (7) and cultured nasal polyps release RANTES (8). LPS causes expression of RANTES mRNA of cultured nasal fibroblasts and secretion of RANTES protein (9). LPS enhances immune responses by activating macrophages, B lymphocytes, and other cells of the immune system through the elevation of protein tyrosine phosphorylation, which appears to be a major intracellular signaling event that mediates cellular responses (10–14).

Syk, one of the well-known tyrosine kinases, is widely expressed and plays an important role in intracellular signal transduction in hematopoietic cells: B cells (15, 16), mast cells (17), platelets (18), macrophages (19), monocytes (20, 21), basophils (22), neutrophils (23), T cells (24), NK cells (25), eosinophils (26), and erythrocytes (27). However, there has been no study that demonstrates the expression of Syk in human nonhemopoietic cells.

In the present study, we sought the Syk expression in human nasal polyp tissue-derived fibroblast lines and measured RANTES production after stimulation with LPS. We also examined the correlation between Syk expression and RANTES production in nasal fibroblasts.

Materials and Methods

Reagents
Anti-Syk mAbs (4D10, C20, N19), anti-Lyn Ab, anti-c-Jun N-terminal kinase 1 (JNK1)3 Ab, anti-phosphorylated JNK Ab (G7), and anti-phosphotyrosine Ab (PY99) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). LPS (Escherichia coli 055:B5) was obtained from Difco (Detroit, MI). ELISA kits for RANTES were purchased from Bio-source (Camarillo, CA). The adenovirus expression vector kit was purchased from Takara Biomedicals (Tokyo, Japan). Chamber slide-glasses, LAB-TEK II, were obtained from Nalge Nunc International (Naperville, IL).

Nasal polyp
Nasal polyps were obtained during surgery from 14 patients with chronic paranasal rhinosinusitis. Chronic rhinosinusitis was strictly diagnosed via endoscopic findings, paranasal sinus x-ray tomography, clinical history, and symptoms by a specialist of otorhinolaryngology. All patients had nasal polyps, which were fluid-filled sacks formed from the upper part of the nasal cavity and composed of edematous tissue with infiltrating cells.

Cell preparation and stimulation
Human nasal fibroblasts were grown from small pieces of nasal polyp and regular passages, as previously described (28). Fibroblasts were used at passage number 4–10. No contamination of epithelial cells was confirmed by immunohistochemical examination using cytokeratin marker. The fibroblasts were stimulated by LPS in RPMI 1640 medium supplemented with 10% FCS and in humidified atmosphere of 10% CO2 in air at 37°C for 24 h. The supernatants were harvested and stored at −80°C.

Chemokine assay
Amounts of RANTES in the cell culture supernatant were measured using ELISA kit. Measurements were performed according to the manufacturers’ protocol.

Gel electrophoresis and Western blots
The fibroblasts or immunoprecipitants were boiled with electrophoresis sample buffer for 3 min and separated using 12.5% SDS-PAGE. The separated proteins were transferred electrophoretically to polyvinylidene difluoride membranes at the same protein concentration per lane. The membranes were blocked at room temperature for 1 h in pH 7.4 PBS with 10% 

3 Abbreviations used in this paper: JNK, c-Jun N-terminal kinase; MAP, mitogen-activated protein; TLR, Toll-like receptor.
BSA, and were probed with Abs, as described previously (15). Band quantities of Syk expression of 14 fibroblast lines were analyzed from the findings of immunoblotting, using the Bio Image System (Genomic Solution, Ann Arbor, MI).

Immunoprecipitation

Cells were collected by centrifugation and lysed in 1 ml of Triton buffer (2% Triton X-100, 0.15 M NaCl, 5 mM EDTA, 100 μM Na₂VO₃, 10 μg/ml leupeptin, 1 mM PMSF, and 50 mM Tris-HCl, pH 7.5). The lysate was clarified and incubated with excess protein A-Sepharose 4B (50% slurry). The cleared sample was immunoprecipitated with Abs and protein A-Sepharose 4B at 4°C. The immune complexes were processed for immunoblot as above.

Immunohistochemical staining

Immunohistochemical staining was performed to detect Syk in human nasal fibroblasts using the traditional avidin-biotin-peroxidase complex technique (29). After purified fibroblasts were seeded and cultured on chamber slide-glasses, the cells were fixed with 70% ethanol. After washing in pH 7.4 PBS, inhibition of endogenous peroxidase activity was accomplished by incubation in 0.3% H₂O₂ solution, dissolved in absolute methanol, at room temperature for 15 min. Chamber slide-glasses were washed in distilled water, rinsed with PBS, and incubated with normal sheep serum (Dako LSAB Kit; Dako, Carpentaria, CA) for 5 min at room temperature to block the background absorption of antiserum, then with mouse or rabbit anti-Syk Abs at 4°C overnight. All specimens were treated with goat anti-mouse or rabbit biotinylated IgG (Dako). Specimens were then rinsed with PBS, and peroxidase color visualization was conducted with 3,3′-diaminobenzidine tetra-hydrochloride solution (DAB; Dojin, Kumamoto, Japan; 30 mg dissolved in 150 ml PBS, added to 10 ml of 30% H₂O₂ solution).

Preparation of recombinant adenoviruses

Wild-type Syk vector is an adenovirus vector encoding porcine Syk, which was constructed using an adenovirus expression kit. A 2.7 kb cDNA fragment containing the entire coding sequence of porcine Syk was ligated into the pAXCAwt cosmid vector, which contains the modified chicken β-actin promoter with immediate early promoter of CMV enhancer (CAG promoter). Then the recombinant adenovirus pAXCAwt-Syk was prepared by homologous recombination of the expression cosmid cassette and parental viral genome, and amplified to achieve a stock with a titer of 10⁹ PFUs/ml. Inactive Syk vector contained the ATP binding site mutant of Syk (K395R). We used 1 × 10⁸ PFUs of adenovirus vector encoding Syk for 1 × 10⁵ cells, and Syk expressions were detected from day 2 to day 8 after the infection.

Antisense oligodeoxynucleotides

Human nasal fibroblasts were cultured in RPMI 1640 in the presence of 10% fetal calf serum, 100 units/ml penicillin, and 100 μg/ml streptomycin. We assayed human nasal fibroblasts using the traditional avidin-biotin-peroxidase complex technique (29). After purified fibroblasts were seeded and cultured on chamber slide-glasses, the cells were fixed with 70% ethanol. After washing in pH 7.4 PBS, inhibition of endogenous peroxidase activity was accomplished by incubation in 0.3% H₂O₂ solution, dissolved in absolute methanol, at room temperature for 15 min. Chamber slide-glasses were washed in distilled water, rinsed with PBS, and incubated with normal sheep serum (Dako LSAB Kit; Dako, Carpentaria, CA) for 5 min at room temperature to block the background absorption of antiserum, then with mouse or rabbit anti-Syk Abs at 4°C overnight. All specimens were treated with goat anti-mouse or rabbit biotinylated IgG (Dako). Specimens were then rinsed with PBS, and peroxidase color visualization was conducted with 3,3′-diaminobenzidine tetra-hydrochloride solution (DAB; Dojin, Kumamoto, Japan; 30 mg dissolved in 150 ml PBS, added to 10 ml of 30% H₂O₂ solution).

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Antisense oligodeoxynucleotides

Human nasal fibroblasts were cultured in RPMI 1640 in the presence of phosphorothioate oligodeoxynucleotides (Nisshinbo Industries, Tokyo, Japan) at 10 μM. The sequences used were as follows: antisense Syk, CAT GCTTCAGGGGCCGG; sense Syk, CCGGCCCTGAAGCATG (26).

Statistical analyses

Statistical analysis was performed using Mann-Whitney’s U test and Wilcoxon signed-ranks test to assess the differences in RANTES production levels. The correlation between RANTES production and the level of Syk expression was examined using the Spearman’s correlation coefficient by rank. Macintosh computers (Stat view software; Abacus Concepts, Berkeley, CA) were used for all statistical analyses.

Results

Syk expression of human nasal fibroblasts

We examined the expression of Syk in human nasal fibroblasts by Western blots. Syk proteins were detected in all cases. In cases 1, 2, 3, 5, 6, 9, and 14, Syk expression was high, although every case had the same protein concentration in each lane. However, the expression of Syk was low in cases 4, 7, 8, 10, 11, 12, and 13, as shown in Fig. 1. Additionally, we examined whether protein tyrosine kinase Lyn was expressed in human nasal fibroblasts. The expression of Lyn was found in fibroblasts of all cases by Western blots. However, there were no differences in the levels of Lyn expression (Fig. 1). The expression of Syk in nasal fibroblasts was also found by the immunohistochemical technique. Syk expression was detected in the cytoplasm of nasal fibroblasts. Three different anti-Syk Abs revealed the same results showing Syk expression in the fibroblasts (data not shown).

LPS-induced RANTES production of human nasal fibroblasts

Production of RANTES by nasal fibroblasts from patients with nasal polyps was measured using ELISA. As shown in Table I, RANTES production by fibroblasts in the presence of 5 μg/ml LPS was significantly elevated, compared with those in the absence of LPS (p < 0.005). However, stimulation of LPS had little effect on RANTES production in cases 4, 8, 10, and 11. The increase in RANTES production was detectable with LPS at concentrations as low as 1 ng/ml, and was dose dependent up to 10 μg/ml. The half-maximum effect of LPS was obtained at 5 μg/ml in triplicates of experiments examined (data not shown). Based on these findings, all subsequent experiments were performed using LPS concentrations of 5 μg/ml. We also examined whether LPS induced tyrosine phosphorylation in human nasal fibroblasts. Using an- tiphosphotyrosine immunoblotting, we observed tyrosine phosphorylation of proteins that occurred from 1 min after stimulation with LPS and reached maximum by 10 min and then decreased gradually (data not shown).

### Table I. LPS-induced RANTES production of human nasal fibroblasts

<table>
<thead>
<tr>
<th>Fibroblast Lines</th>
<th>RANTES (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>1 T.T.</td>
<td>24.0 ± 13.0</td>
</tr>
<tr>
<td>2 K.S.</td>
<td>24.2 ± 6.1</td>
</tr>
<tr>
<td>3 H.H.</td>
<td>17.5 ± 12.7</td>
</tr>
<tr>
<td>4 S.K.</td>
<td>24.3 ± 0.6</td>
</tr>
<tr>
<td>5 R.F.</td>
<td>22.0 ± 7.0</td>
</tr>
<tr>
<td>6 T.M.</td>
<td>21.4 ± 8.6</td>
</tr>
<tr>
<td>7 N.S.</td>
<td>19.9 ± 4.0</td>
</tr>
<tr>
<td>8 Y.T.</td>
<td>18.8 ± 6.5</td>
</tr>
<tr>
<td>9 M.N.</td>
<td>21.1 ± 11.3</td>
</tr>
<tr>
<td>10 R.S.</td>
<td>17.6 ± 4.3</td>
</tr>
<tr>
<td>11 H.T.</td>
<td>22.5 ± 8.2</td>
</tr>
<tr>
<td>12 K.O.</td>
<td>19.9 ± 2.6</td>
</tr>
<tr>
<td>13 K.K.</td>
<td>21.7 ± 7.5</td>
</tr>
<tr>
<td>14 H.K.</td>
<td>18.4 ± 6.7</td>
</tr>
<tr>
<td>Total</td>
<td>209.3 ± 2.4*</td>
</tr>
</tbody>
</table>

*a Cultured fibroblast lines (1 × 10⁷/ml) from nasal polyps of 14 patients were incubated in the presence or absence of LPS (5 μg/ml). Supernatants were harvested for analysis of RANTES secretion by ELISA 24 h after LPS induction. The values are expressed as the mean ± SD for triplicate experiments. *p < 0.005.”
Correlation between Syk expression and LPS-induced RANTES production in human nasal fibroblasts

We analyzed the correlation of LPS-induced RANTES production and Syk expression in fibroblasts (Fig. 2). The levels of Syk expression in 14 fibroblast lines were measured using the densitometry method from the findings of Western blots, as shown in Fig. 1. The measurement of intensity was the semiquantitative analysis of Syk. It ranged from 0.22 to 0.88, and its average was 0.50. A significant positive correlation was found between Syk expression and RANTES production ($\gamma = 0.863, p < 0.01$, Fig. 2).

As shown in Table II, 14 fibroblast lines were divided into two groups according to the grade of expression: high Syk expression group and low Syk expression group from the findings in Fig. 2. In the high Syk expression group, band intensity of Syk was more than 0.50, and in the low Syk expression group, it was less than 0.50 (as in Table II). The levels of LPS-induced RANTES production were significantly higher by fibroblasts in the high Syk expression group than by those in the low Syk expression group ($p < 0.005$). In the high Syk expression group, LPS induced RANTES production about 60-fold for 24 h compared with that in the absence of LPS. In contrast, in the low Syk expression group, RANTES production by fibroblast lines stimulated with LPS was only about 7-fold greater than that by unstimulated cells.

Effect of antisense oligodeoxynucleotides on Syk expression and LPS-induced RANTES production by nasal fibroblasts

Although our compelling statistical analysis could support a correlation between Syk and RANTES production, some other molecules besides Syk might vary on different fibroblast lines. Therefore, to examine the possibility that Syk plays a role in LPS-induced RANTES production, we used the same fibroblast line whose Syk expression was artificially changed using antisense oligodeoxynucleotides to Syk. As shown in Fig. 3A, human nasal fibroblasts exposed to 10 $\mu$M phosphorothionated Syk-antisense oligodeoxynucleotides for 6 h inhibited the expression of Syk protein, whereas the exposure of Syk-sense oligodeoxynucleotides did not change Syk protein levels. We examined Lyn expression in these fibroblasts using anti-Lyn Ab. There were no differences in the levels of Lyn expression. LPS failed to induce RANTES production by fibroblasts not exhibiting Syk protein (Fig. 3B). In contrast, sense oligodeoxynucleotides did not alter LPS-induced RANTES production. There was significant difference in LPS-induced RANTES production between fibroblasts treated with Syk-antisense oligodeoxynucleotides and those with sense oligodeoxynucleotides ($p < 0.05$). No difference was found in cell viability and cell number among three fibroblasts. The same results were obtained, using every other fibroblast line from the nasal polyps in the high Syk expression group (data not shown).

Wild-type Syk vector transfection magnified LPS-induced RANTES production from nasal fibroblasts

We transfected expression vector from wild-type Syk and from inactive Syk into the nasal fibroblast line (case 8), respectively. The transfection of wild-type Syk vector and inactive Syk enhanced the expression of Syk on day 2, whereas the control vector did not do so (Fig. 4A). There was no difference in the spontaneous RANTES production in the absence of LPS among the fibroblasts transfected with different vectors. As shown in Fig. 4B, the fibroblasts transfected with wild-type Syk vector produced high levels of RANTES after stimulation. However, those transfected with inactive Syk vector failed to do so. Stimulation with LPS enhanced RANTES production by fibroblasts transfected with the wild-type Syk vector, significantly more than that by fibroblasts transfected with the control vector and inactive Syk vector ($p < 0.01$). In parent fibroblasts and control vector-transfected fibroblasts, LPS had little effect on RANTES production. The fibroblasts transfected with inactive Syk vector failed to produce high levels of RANTES. No difference was found in spontaneous RANTES production and cell number among four fibroblasts even after LPS stimulation (data not shown). We examined Lyn expression using anti-Lyn Ab in these fibroblasts transfected with vectors. There were no differences in the levels of Lyn expression, as shown in Fig. 4A.

LPS-induced phosphorylation of Syk and JNK1

To investigate whether LPS induces tyrosine phosphorylation of Syk in nasal fibroblasts, antiphosphotyrosine immunoprecipitates of cell lysates from the cells treated with or without LPS were subjected to immunoblotting with anti-Syk Ab. As shown in Fig. 5A (top), the exposure of nasal fibroblasts to LPS triggered a rapid and sustained tyrosine phosphorylation of Syk. This increase in tyrosine phosphorylation of Syk began to rise and reached maximum at 2 min and decreased thereafter. Immunoblot analysis with an anti-Syk Ab revealed that total amounts of Syk from treated or untreated cells were comparable (Fig. 5A, bottom).

At the same time, the cell lysates were subjected to immunoblotting with anti-phosphorylated JNK Ab to address the question of cell viability and cell number among three fibroblasts. The same results were obtained, using every other fibroblast line from the nasal polyps in the high Syk expression group (data not shown).

### Table II. Syk expression and RANTES production

<table>
<thead>
<tr>
<th>Syk / RANTES (pg/ml)</th>
<th>Control</th>
<th>24-h Supernatant</th>
</tr>
</thead>
<tbody>
<tr>
<td>High expression group</td>
<td>21.2 ± 2.6</td>
<td>132.12 ± 1222.5*</td>
</tr>
<tr>
<td>Low expression group</td>
<td>20.7 ± 2.3</td>
<td>155.6 ± 193.7*</td>
</tr>
</tbody>
</table>

*Fourteen fibroblast lines were divided into two groups according to the findings of Syk expression in nasal fibroblasts. In the high expression group, band intensity of Syk was >0.50. In the low expression group, it was <0.50. RANTES levels of supernatants were measured as shown in Table 1. $p < 0.005$.
of whether JNK is activated in the fibroblasts by LPS. JNK1 was activated by LPS, because the anti-phosphorylated JNK Ab reacted with active JNK1 phosphorylated on Thr183 and Tyr185 (Fig. 5B, top). JNK1 phosphorylation was almost maximal within 10 min and remained elevated throughout the 30-min incubation. Total amounts of JNK1 were the same in all lanes (Fig. 5B, bottom).

Decreased Syk expression attenuated JNK1 activation

To analyze whether Syk plays a role in regulating JNK1 activation in response to LPS, the fibroblasts were exposed to Syk-antisense oligodeoxynucleotides, and then treated with LPS for 10 min. The cell lysates were subjected to immunoblotting with anti-phosphorylated JNK Ab. LPS failed to induce JNK1 phosphorylation of the fibroblasts treated with Syk-antisense oligodeoxynucleotides (Fig. 6). In contrast, sense oligodeoxynucleotides did not alter LPS-induced JNK1 phosphorylation. Immunoblot analysis revealed that Syk-antisense oligodeoxynucleotides inhibited the expression of Syk protein. Syk expression affected JNK1 activation, but not JNK1 expression.

Discussion

In the present study, Syk was expressed in nasal fibroblasts. The level of Syk expression was associated with RANTES production induced by LPS stimulation in nasal fibroblasts. Overexpression of wild-type Syk increased RANTES production from human nasal fibroblasts. However, the fibroblasts transfected with inactive Syk vector failed to produce high levels of RANTES. Pretreatment of antisense oligodeoxynucleotides to Syk inhibited RANTES production by fibroblasts stimulated with LPS. These findings suggested that Syk has a certain role in chemokine production by LPS stimulation.

In general, Syk expression has usually been found in hemopoietic cells. So, it has been suggested that nonhemopoietic cells do not exhibit Syk expression. As this manuscript was being completed, Wang et al. (30), using a strategy of reverse transcription and PCR, reported that 3T3-L1 mouse embryonic fibroblasts expressed Syk, and Tsuchida et al. (31) reported that hepatocytes expressed Syk. We also found Syk expression in human nasal polyp-derived fibroblasts.

FIGURE 3. Effect of antisense oligodeoxynucleotides on Syk expression and LPS-induced RANTES production from nasal fibroblasts. A, Nasal fibroblasts (case 9) were cultured in the presence of 10 μM Syk antisense (lane 3) or 10 μM Syk sense (lane 2) or in the absence of oligodeoxynucleotide (lane 1) for 6 h. Harvested proteins (30 μg) from cultured cells were reacted with anti-Syk Ab or anti-Lyn Ab, and then processed for immunoblotting. Arrows indicate the positions of Syk and Lyn. B, Nasal fibroblasts were transfected with Syk antisense or Syk sense, and then stimulated with 5 μg/ml LPS. RANTES levels in the supernatants were measured using ELISA. Increases in RANTES production were observed in human nasal fibroblasts pretreated with Syk sense. The pretreatment of Syk antisense inhibited RANTES production by fibroblasts stimulated with LPS (*, p < 0.05).

FIGURE 4. Wild-type Syk vector transfection magnified LPS-induced RANTES production by nasal fibroblasts. A, After the transfection with wild-type Syk vector into the fibroblasts (case 8) for 48 h (lane 3) or inactive Syk vector (lane 4) or control vector (lane 2) or no vector (lane 1), samples were harvested. The prepared proteins (30 μg) were processed and reacted with anti-Syk Ab (C20) or anti-Lyn Ab. Arrows indicate the positions of Syk and Lyn. B, Nasal fibroblasts infected with wild-type Syk vector or inactive Syk vector or control vector were incubated in the presence or absence of LPS (5 μg/ml), and supernatants were harvested for analysis of RANTES secretion by ELISA. LPS-induced RANTES levels from fibroblasts transfected with wild-type Syk vector were significantly higher than those from fibroblasts transfected with control vector and inactive Syk vector (*, p < 0.01).
Syk EXPRESSION AND RANTES PRODUCTION IN NASAL FIBROBLASTS

LPS augments tyrosine phosphorylation (11, 12, 14), which results in phosphorylation or activation of mitogen-activated protein (MAP) kinases: 44- and 42-kDa MAP kinases (extracellular signal-related kinase 1 and 2) (14, 32), stress-activated protein kinase/JNK (33), and p38 kinase (10, 14, 34). In this study, we demonstrated that LPS induced tyrosine phosphorylation of Syk and activated JNK1 in nasal fibroblast lines. Tyrosine phosphorylation of MAP kinase is associated with LPS-induced TNF-α production in mice (13). RANTES mRNA expressions were induced through the activation of MAP kinases in endothelial cells (32), or through JNK and NF-κB kinase cascades in macrophages stimulated with LPS (33). The Syk-generated signal cooperates to enhance Rac-induced JNK activation in T lymphocytes (35), and MAP kinase activation was compromised in the macrophages of Syk−/− mice after Fc-γ receptor stimulation (36). Syk is an important component, leading to activation of NF-κB in the human monocyte cell line (37). Furthermore, in response to LPS activation or Fc-γ receptor cross-linking of macrophages, tyrosine-phosphorylated 145-kDa protein associated with Syk (38). Concerning other cytokine production, IL-1α was produced by an early tyrosine phosphorylation of Syk in murine resident peritoneal macrophages (39). Consequently, these studies suggest that nonreceptor type protein kinase Syk has a specific role in LPS-induced RANTES production. In this study, it has also been revealed that decreased Syk expression attenuated JNK1 activation in nasal fibroblast lines in the same way that oxidative stress-induced JNK activation significantly decreased in B cell line that did not express Syk (40). Hiiura et al. (33) demonstrated that transcriptional activation of the human RANTES promoter by LPS was dependent on specific AP-1, which was regulated by JNK.

Syk expression can affect a variety of cellular functions. The absence of Syk expression led peripheral blood basophils to fail to degranulate in response to cross-linking the high affinity IgE receptor FcεR-I (41). Also, TBL1A2 cells, a Syk-negative variant of basophilic leukemia RBL-2H3 cells, failed to release histamine by the cross-linking system (22). Syk-deficient mast cells failed to degranulate, synthesize leukotrienes, and secrete cytokines (42). In addition, Syk expression is required for signaling in development (24, 43, 44), differentiation (30), and phagocytosis (45) of cells. Furthermore, Syk may be critical in cell survival after damage in inflammatory diseases, because antiapoptotic pathway(s) requires a Syk-dependent signaling pathway (26, 46).

Syk is upstream in mediating high affinity IgE receptor (FceRI) (17), Fc receptor γ-chain RI (19, 47), IL-2R (48), and CD40 (49). Recently, Toll-like receptor (TLR)-2 or TLR-4 has been proven to mediate LPS-induced cellular signaling (50–52). Bacterial LPS activates NF-κB through a signal transduction molecule in the LPS receptor complex belonging to the IL-1R/TLR superfamily (52, 53). Some other molecules besides Syk might play an important role in the LPS-induced RANTES production from the nasal fibroblasts. Research concerning these signals in human nasal fibroblast lines and some structure motifs in this system as those defined for the B cell receptor or IgE receptor signaling system should be further investigated.

Eosinophils and fibroblasts are known to play a major role in the pathogenesis of airway diseases; bronchial asthma, cystic fibrosis, and rhinosinusitis with polyps, because structural cells like airway fibroblasts are a rich source of chemokines, cytokines, and inflammatory mediators. Fibroblasts can produce RANTES (3, 4), eotaxin (54), monocyte chemoattractant protein-1, IL-8, GM-CSF, G-CSF, or TGF-β (55). RANTES proteins were highly detected in nasal polyps compared with control normal mucosa (7). Cultured nasal polyps release RANTES to attract and activate eosinophils (5, 6, 8). Accordingly, the mechanism of RANTES production is a critical problem in the clinical management of nasal polyps.

In conclusion, Syk proteins are expressed in adult human nasal fibroblasts, which are nonhemopoietic or nonembryonic cells. Syk expression affects RANTES production in airway diseases. The regulation of Syk expression has a possibility to be one of the strategies for the treatment of nasal polyps.

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References


FIGURE 5. LPS induced phosphorylation of Syk and JNK1. A. The fibroblasts (case 9) were cultured without LPS (lane 1), or with LPS for 2 min (lane 2), for 10 min (lane 3), and for 30 min (lane 4). The sample was immunoprecipitated with anti-phosphotyrosine Ab and blotted with anti-Syk Ab. The same amount of lysate (30 μg) before immunoprecipitation was applied to each lane and blotted with anti-Syk Ab. The positions of phosphorylated Syk (P-Syk) and total amount of Syk were indicated to the right with the arrows. B. The same amount of lysate from the cells after LPS stimulation was applied to each lane, as shown above, and blotted with anti-phosphorylated JNK Ab (P-JNK1) and the expression of JNK1 or Syk were indicated to the right with the arrows. The lanes are the same as above.

FIGURE 6. Decreased Syk expression attenuated JNK1 activation. Nasal fibroblasts (case 9) were transfected with 10 μM Syk antisense (lane 3) or 10 μM Syk sense (lane 2) or no oligodeoxynucleotide (lane 1) for 6 h, and then stimulated with 5 μg/ml LPS for 10 min. The same amount of lysate was applied to each lane and blotted with anti-phosphorylated JNK Ab, anti-JNK1 Ab, and anti-Syk Ab. The position of phosphorylated JNK1 (P-JNK1) and the expression of JNK1 or Syk were indicated to the right with the arrows.

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