Selective Eosinophil Adhesion to Fibroblast Via IFN-γ-Induced Galectin-9

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Among galectin family members, galectin-9 was first described as a potent eosinophil chemoattractant derived from Ag-stimulated T cells. In the present study a role of galectin-9 in the interaction between eosinophils and fibroblasts was investigated using a human lung fibroblast cell line, HFL-1. RT-PCR, real-time PCR, and Western blot analyses revealed that both galectin-9 mRNA and protein in HFL-1 cells were up-regulated by IFN-γ stimulation. On the one hand, IL-4, known as a Th2 cytokine, did not affect the galectin-9 expression in HFL-1 cells. We further confirmed that IFN-γ up-regulated the expression of galectin-9 in primary human dermal fibroblasts. Flow cytometric analysis revealed that IFN-γ up-regulated surface galectin-9 expression on HFL-1 cells. Stimulation of HFL-1 cells with IFN-γ up-regulated adhesion of eosinophils, but not neutrophils, to HFL-1 cells. This adherence of eosinophils to HFL-1 cells was inhibited by both lactose and anti-galectin-9 Ab. These findings demonstrate that IFN-γ-induced galectin-9 expression in fibroblasts mediates eosinophil adhesion to the cells, suggesting a crucial role of galectin-9 in IFN-γ-stimulated fibroblasts as a physiological modulator at the inflammatory sites. The Journal of Immunology, 2002, 169: 5912–5918.

Galectin-9 belongs to a rapidly growing family whose members bind to carbohydrates in a Ca2+-independent fashion. Members of the family are composed of 14- to 15-kDa carbohydrate recognition domains (CRDs), which have an affinity for β-galactosides and share certain conserved sequence elements (1–6). To date, 14 galectins (galectin-1 to -14) in mammals have been cloned and sequenced. They have been shown to play roles in diverse biological events, including adhesion and proliferation of cells, apoptosis, mRNA splicing, and modulation of immune responses (1, 4, 7). However, the mechanisms by which galectins exert these diverse effects are largely unknown.

Human galectin-9 was first cloned from a cDNA library derived from tissue associated with Hodgkin’s disease. It has two CRDs connected by a linker peptide (8). Due to the difference in their linker length, there are three types of isoforms (long, medium, and short) in galectin-9 (9–11). We have independently purified and cloned a novel eosinophil chemoattractant (ECA) from T lymphocytes, and found it is identical with the galectin-9 molecule (12). Further, we have shown that galectin-9 requires divalent galactoside-binding domains to exhibit its potent ECA activity (13).

We hypothesized that galectin-9 was involved in the accumulation of eosinophils at inflammatory sites because galectin-9 was an eosinophil chemoattractant. In the present study we first examined the distribution of galectin-9 in inflammatory lung disease with eosinophil infiltration. Immunohistochemical analysis showed that galectin-9 was expressed in inflammatory cells around eosinophils, including fibroblasts (Fig. 1). Eosinophils play a role in various pathological situations, including allergic inflammation, parasitic infections, and neoplastic diseases (14). Previous studies have shown the effects of eosinophils on fibroblast properties. For example, human eosinophils stimulate DNA synthesis and matrix production of dermal fibroblasts (15, 16), and guinea pig peritoneal eosinophils enhance the replication of fetal lung fibroblasts (17). Several reports have examined the relation between galectins and fibroblasts. Galectin-3 could induce the proliferation of human lung fibroblasts (18). Galectin-1 had the ability to convert dermal fibroblasts into the myogenic lineage (19). Taken together, these results lead to the hypothesis that galectins, especially galectin-9, might be involved in eosinophil-fibroblast interaction. However, to our knowledge, no previous report has described the regulation and functional significance of galectin-9 expression in fibroblasts, and thus in the present study we addressed the correlation among fibroblast, galectin-9, and eosinophils.

The purpose of this study was to clarify the galectin-9 expression in fibroblasts stimulated with growth factors or IFNs and to investigate the possible role of galectin-9 in eosinophil adhesion to fibroblastic cells.

Materials and Methods

Reagents

IFN-γ was purchased from Roche Molecular Biochemicals (Mannheim, Germany). IFN-β was obtained from Strathmann Biotech (Hamburg, Germany). Basic fibroblast growth factor (FGF) was purchased from Genzyme (Cambridge, MA). Platelet-derived growth factor AB (PDGF-AB), IL-4, and PMA were obtained from Sigma-Aldrich (St. Louis, MO).
**Cell culture**

Normal diploid human fetal fibroblasts (HFL-1, Riken Gene Bank, Ibaraki, Japan) were cultured in Ham's F-12 medium supplemented with 15% FBS, 100 U/ml penicillin, 100 µg/ml streptomycin, and 250 ng/ml amphotericin B at 37°C in an atmosphere of 5% CO2 and 95% air.

**RNA extraction, RT-PCR, and real-time PCR**

To verify the expression of human galectin-9 mRNA, HFL-1 cells (1–2 × 10^6 cells/75 cm²) were cultured in the presence of or the absence of various cytokines for 24 or 48 h. Total RNA was extracted with TRizol (Life Technologies, Paisley, U.K.) according to the manufacturer's instructions. RNA (0.5 µg) was reverse transcribed for 15 min at 42°C with 2.5 U of Moloney murine leukemia virus reverse transcriptase (Perkin Elmer, Norwalk, CT), using oligo(dT)12-18. The galectin-9 mRNA and GAPDH mRNA were detected by RT-PCR assay.

The primers 5′-GCCATCAATGGACCTCTTATGGA-3′ and 5′-ACGGAGGGCATTGCAGTGGAC-3′ were used to detect GAPDH mRNA expression. The expected fragment size was 260 bp.

The RT-PCR program consisted of 30 cycles with denaturation at 94°C for 30 s, annealing at 60°C for 15 s, and extension at 72°C for 1 min. Amplification was conducted using a DNA thermal cycler (Perkin Elmer).

For the quantitative real-time PCR analysis of human galectin-9 mRNA levels, a Light Cycler System and reagents (Roche Molecular Diagnostics) were used with a dsDNA binding dye, SYBR Green 1, according to the procedures provided by the manufacturer. The real-time PCR program for galectin-9 consisted of 40 cycles with denaturation at 95°C for 15 s, annealing at 60°C for 5 s, and extension at 72°C for 10 s. For GAPDH it consisted of 40 cycles with denaturation at 95°C for 15 s, annealing at 66°C for 5 s, and extension at 72°C for 20 s.

**Generation and purification of a polyclonal Ab for human galectin-9**

As described previously (10), anti-galectin-9 IgG was generated and purified from sera of rabbits immunized with a mixture of recombinant C-terminal CRD of human galectin-9 (galectin-9 CT) and CFA.

**Immunohistochemistry**

Histopathological samples obtained from a 56-year-old woman with interstitial pneumonia and from a 21-year-old woman with eosinophilic pneumonia were stained with Giemsa reagent. Distribution of galectin-9 in these samples was examined by immunohistochemical staining. Tissue sections mounted on aminopropyltriethoxy-silane-coated glass slides were deparaffinized, rehydrated, and boiled by microwave irradiation in 0.01 M citrate buffer (pH 6.0) for Ag retrieval. The slides were incubated for 30 min with 0.3% hydrogen peroxide in methanol to quench endogenous tissue peroxidase activity. Immunostaining was performed using 5 µg/ml anti-galectin-9 Ab. After overnight incubation at 4°C in a moist chamber, the samples were incubated with ENVISION™ (DAKO, Glostrup, Denmark), according to manufacturer's instructions. The immunohistochemical reactions were visualized using diaminobenzidine as a chromogenic peroxidase substrate. Sections were counterstained with hematoxylin after immunostaining.

**Western blot analysis**

After 24-h preincubation, HFL-1 cells were treated with IFN-γ for 24 h unless otherwise specified. Lysis buffer (10 mM Tris-HCl (pH 7.2), 0.15 M NaCl, 2 mM EDTA, 5 mM benzamidine-HCl, and 1 mM PMSF) plus 100 µM sucrose was added to the cells. Then the mixtures were homogenized and centrifuged. One milliliter of buffer containing 100 mM lactose was added, and the mixture was centrifuged twice. The secondary supernatants were used as cell lysates for electrophoresis. SDS sample buffer was added to the cell lysates, and samples were incubated for 5 min at 95°C, then placed on ice. Samples were run on 10% acrylamide-SDS gels and transferred to a polyvinylidene difluoride membrane (Bio-Rad, Hercules, CA). Nonspecific binding was blocked using 5% skim milk in TBS containing 0.05% sodium azide. The membranes were then incubated at room temperature for 1 h with 2 µg/ml of affinity-purified rabbit anti-human galectin-9 in blocking buffer. Membranes were washed, followed by incubation with 1% skim milk in TBS containing HRP-conjugated anti-rabbit IgG Ab (Amersham Pharmacia Biotech, Piscataway, NJ) at room temperature for 1 h. Finally, membranes were incubated with ECL-HRP substrate solution included in the ECL kit (Amersham Pharmacia Biotech), and immunoblotting was visualized by exposing the membrane to x-ray film (Fuji, Tokyo, Japan).

**Flow cytometric analysis**

To assure the surface galectin-9, cells were collected by centrifugation and washed with HBSS, followed by a 30-min incubation on ice with 50 µg/ml rabbit anti-human galectin-9. After being washed twice, the cells were incubated on ice with FITC-conjugated goat anti-rabbit Ab (Santa Cruz Biotechnology, Santa Cruz, CA) for 30 min. The amount of surface galectin-9 was analyzed using a flow cytometer (EPICS XL-MCL; Coulter, Hialeah, FL).

**Eosinophil chemotactic activity**

EACA activity was evaluated in vitro as described previously (12, 20). In brief, CD16-negative eosinophils were enriched by subjecting PBL from healthy volunteers to a discontinuous density gradient of Percoll (Amer- sham Pharmacia Biotech), followed by immunomagnetic treatment of the cells with anti-CD16 IgG (Dako). The purity and viability of the purified eosinophils were >97 and >95%, respectively. EACA activity was evaluat- ed using a 48-well chamber (NeuroProbe, Cabin John, MD) containing a polynvinyl pyrolidone-free membrane with a pore size of 5 µm. Human eosinophils (0.5–1 × 10^7/ml) and a test chemoattractant were placed in the top and bottom chambers, respectively. Each assay was performed in triplicate. The membrane separating the two chambers was removed and placed in Diff-Quick stain (Baxter Healthcare Corp., McGaw Park, IL) after 2 h of incubation at 37°C in a humidified atmosphere of 5% CO2. Stained eosinophils were counted under a microscope. Human C5a (Sigma) was used as a positive chemotactant.

EACA activity was represented by a chemotactic index (CI = migrated eosinophil number related to the test chemoattractant/the number related to the supernatant of nonstimulated HFL-1 cells). Data represented the mean CI ± SD of experiments performed in triplicate.

**Expression and purification of recombinant proteins**

Recombinant proteins were obtained as described previously (13) with minor modifications. In brief, *Escherichia coli* BL-21 containing galectin-1 or galectin-9 expression plasmid was grown in 2xYT medium supplemented with 2% (w/v) glucose and 100 µg/ml ampicillin to an OD of 0.7 at 600 nm. Isopropyl-β-thiogalactopyranoside was added to a final concentra- tion of 0.1 M, and the culture was continued for 2 h at 37°C. The cells were harvested by centrifugation at 8000 × g for 10 min and resuspended in 90 ml of lysis buffer containing 10 mM Tris-HCl, 0.5 mM sodium chloride, 1 mM DTT, and 1 mM PMSF. The cells were lysed using a sonicator, and lysates were centrifuged at 12,000 × g for 30 min. The supernatant was collected and applied to a lactose-agarose column (Seikagaku Corp., To- kyo, Japan). The adsorbed proteins were eluted using TBS containing 200 mM lactose. Fractions were collected, and proteins were analyzed by SDS-PAGE. Fractions containing the expressed protein were pooled and dialyzed against PBS containing 0.1 mM DTT.

**Adhesion assay**

HFL-1 cells grown to ~70% confluence in a 35-mm diameter dish were stimulated with 100 U/ml IFN-γ for 24 h, washed with RPMI 1640 containing 5% FBS, and incubated for 2 h with human eosinophils or neutrophils (1.0 × 10^6/ml) and a test chemoattractant were placed in the top and bottom chambers, respectively. Each assay was performed in triplicate. The membrane separating the two chambers was removed and placed in Diff-Quick stain (Baxter Healthcare Corp., McGaw Park, IL) after 2 h of incubation at 37°C in a humidified atmosphere of 5% CO2. Stained eosinophils were counted under a microscope. Human C5a (Sigma) was used as a positive chemotactant.

EACA activity was represented by a chemotactic index (CI = migrated eosinophil number related to the test chemoattractant/the number related to the supernatant of nonstimulated HFL-1 cells). Data represented the mean CI ± SD of experiments performed in triplicate.

**Expression of galectin-9 in tissue eosinophilia**

We first examined the localization of galectin-9 in inflammatory lung disease with tissue eosinophilia. In the interstitial pneumonia, evident eosinophil infiltration was observed in the inflammatory sites, and immunoreactive galectin-9 was detected in fibroblasts, vascular endothelial cells, and inflammatory cells such as macrophages and eosinophils themselves (Fig. 1, A–C). In the tissue of...
eosinophilic pneumonia, potent staining of immunoreactive galectin-9 was also observed in the various types of cells, including fibroblasts and inflammatory cells around eosinophils (Fig. 1, D–F). From these results, we considered the possibility that the expression of galectin-9 in inflammatory cells, including fibroblasts, is directly involved in eosinophil infiltration. Therefore, additional experiments were performed to clarify the relationship among galectin-9, fibroblasts, and eosinophils.

**IFN-γ up-regulates the expression of galectin-9 in fibroblasts**

Based on the immunohistochemical analysis data, we conducted the experiment to clarify whether various fibroblast-affecting cytokines, including IFN-β, IFN-γ, basic FGF, PDGF-AB, and PMA, induce galectin-9 mRNA expression in HFL-1 cells. Among them, only IFN-γ up-regulated the expression of galectin-9 mRNA, while all other cytokines did not up-regulate it (data not shown).

The results of RT-PCR and real-time PCR analyses are shown in Fig. 2. HFL-1 cells did not express galectin-9 under resting conditions. IFN-γ, however, up-regulated the expression of galectin-9 mRNA (Fig. 2A). Such up-regulation of galectin-9 mRNA was detected by IFN-γ stimulation at concentrations of >3 U/ml. The results of the quantitative real-time PCR analysis were almost identical to those of conventional RT-PCR (Fig. 2B).

The kinetics of galectin-9 mRNA up-regulation by IFN-γ were assessed using RT-PCR (Fig. 2C) and real-time PCR analyses (Fig. 2D). Galectin-9 mRNA became evident 6 h after stimulation with 100 U/ml IFN-γ, reached its maximum at 24 h, and decreased thereafter.

We also tested the effect of IL-4, known as a Th2 cytokine, on galectin-9 mRNA expression in HFL-1 cells. Incubation of HFL-1 with IL-4 (1–100 ng/ml) alone had no or little effect on galectin-9 expression, and IL-4 did not synergize with IFN-γ to increase galectin-9 mRNA expression (Fig. 2E).

We further examined whether IFN-γ stimulation up-regulates the galectin-9 mRNA expression in primary human dermal fibroblasts. Galectin-9 mRNA expression in primary human dermal fibroblasts was up-regulated by IFN-γ stimulation at concentration of 100 U/ml for 24 h (Fig. 2F).

**Galectin-9 protein expression in fibroblasts stimulated with IFN-γ**

Western blot analysis of galectin-9 is shown in Fig. 3A. In accordance with the RT-PCR results, no evident band of galectin-9 protein in HFL-1 cells was detected without IFN-γ stimulation. Among three isoforms, the medium-sized (35.9 kDa) and long-sized (39.5 kDa) galectin-9 proteins were up-regulated by IFN-γ stimulation for 24 h.

We also confirmed that galectin-9 protein expression in primary human dermal fibroblasts was up-regulated by IFN-γ stimulation at concentration of 100 U/ml for 24 h (Fig. 3B). In primary human dermal fibroblasts, medium-sized galectin-9 protein was up-regulated by IFN-γ stimulation, and a small amount of short-sized (34.7 kDa), rather than long-sized, galectin-9 was induced by IFN-γ stimulation.

Flow cytometric analysis was used to assess the expression of cell surface galectin-9. Fig. 3C showed that HFL-1 cells expressed a low level of galectin-9 protein on the surface under resting conditions, and that the level of galectin-9 was up-regulated by stimulation with IFN-γ for 24 h.

**Eosinophil chemoattractant activity**

Experiments were performed to assess whether the culture supernatants from HFL-1 cells stimulated with IFN-γ exhibited ECA activity. Fig. 4 shows that no ECA activity was detected in the supernatants of
FIGURE 2. Expression of galectin-9 mRNA in fibroblasts. HFL-1 cells were stimulated with various concentrations of IFN-γ (A and B) or IL-4 (E). HFL-1 cells were also cultured with IFN-γ (100 U/ml) for the indicated periods (C and D). Total RNA was extracted, and single-strand cDNA was synthesized from total RNA. Specific cDNAs for galectin-9 and GAPDH were amplified by PCR. A, C, and E, RT-PCR analysis was performed using a DNA thermal cycler. The products were analyzed by electrophoresis on an agarose gel containing ethidium bromide. B and D, Quantitative real-time PCR analysis of galectin-9 mRNA was performed. The ratio of galectin-9/GAPDH amplimers was calculated based on PCR results using the Light Cycler System and SYBR Green 1 dye. Values shown are the mean ± SD (n = 4). *, p < 0.01 vs unstimulated control (−) by Student’s t test. n.s., not significant. Primary human dermal fibroblasts were also stimulated with IFN-γ at a concentration of 100 U/ml, and RT-PCR analysis was performed as described above (F).

IFN-γ-stimulated HFL-1 cells. On the basis of these data, we hypothesized that the galectin-9 protein of fibroblasts exhibits biological functions other than eosinophil chemotraction.

Eosinophil and neutrophil adhesion assay

Eosinophils were added to HFL-1 cells that had or had not been stimulated with IFN-γ for 24 h (Fig. 5). The numbers of eosinophils adhering to nonstimulated and IFN-γ-stimulated HFL-1 cells were 3.0 ± 2.2 and 25.4 ± 9.8 cells/field, respectively (mean ± SD; p < 0.001, by Student’s t test). Both 10 and 30 mM lactose significantly reduced the number of adherent eosinophils. Lactose (30 mM) reduced it to 4.2 ± 2.7 cells/field, which was more effective than 10 mM lactose (10.3 ± 3.7 cells/field). In contrast, the same amount of sucrose did not reduce the number of adherent eosinophils (10 mM, 24.7 ± 6.7 cells/field; 30 mM, 23.2 ± 8.6 cells/field). Further, 10 μg/ml anti-galectin-9 Ab significantly reduced the number of adherent eosinophils to 2.8 ± 2.2 cells/field. Neither anti-galectin-1 Ab (10 μg/ml) nor normal rabbit IgG (10 μg/ml), which were used as negative controls, inhibited eosinophil adhesion to HFL-1. In contrast to eosinophils, neutrophils did not adhere to HFL-1 cells even after stimulation of HFL-1 cells with IFN-γ (adherence to nonstimulated HFL-1 cells, 1.4 ± 1.5; that to IFN-γ-stimulated cells, 1.6 ± 1.8). These findings suggested that galectin-9 on the surface of HFL-1 cells preferentially mediated eosinophil adhesion to the cells.

Furthermore, we performed experiments to confirm whether eosinophil adhesion is mediated by galectin-9. Eosinophil adherence to the culture dish coated with recombinant human galectin-9 was assessed. Fig. 6 showed that the numbers of eosinophils adherent to the control and to the galectin-9-coated dish were 2.1 ± 1.2 and 18.6 ± 6.6 cells/field, respectively (mean ± SD; p < 0.001, by Student’s t test). Lactose (30 mM) significantly reduced the number of adherent eosinophils to 4.6 ± 1.5 cells/field (p < 0.001), whereas sucrose (30 mM) did not (16.4 ± 9.6 cells/field). Recombinant galectin-1 was used as a control. In contrast, eosinophils failed to adhere to the galectin-1-coated dish (Fig. 6), indicating that galectin-9, but not galectin-1, selectively mediates eosinophil adhesion. Further, we also confirmed that neutrophils did not adhere to the galectin-9-coated dish (data not shown).

Discussion

IFN-γ exhibits diverse effects on cells of the immune system, serving as an important regulator of lymphocyte and macrophage functions (21, 22). IFN-γ also has anti-proliferative effects on many cell types (22), including fibroblasts (23, 24). In the present study we demonstrate that IFN-γ stimulated the expression of galectin-9 in fibroblasts. Neither basic FGF nor PDGF-AB, which are known fibroblast stimulators, induced the expression of galectin-9 mRNA in HFL-1 cells (data not shown). We have previously shown that stimulation of Jurkat T cells with PMA results in the up-regulation of galectin-9 expression (10). However, we have found that PMA does not enhance the expression of galectin-9 mRNA in HFL-1 cells (data not shown), indicating that the signal for galectin-9...
expression differs according to cell type, though the mechanism of regulation is still unclear.

There are three isoforms of galectin-9 that differ in the length of the linker peptide (9–11), and all of them have comparable ECA activities (11). In this study IFN-γ up-regulated mainly the medium-sized galectin-9 in both HFL-1 and primary human dermal fibroblasts (Fig. 3, A and B). However, IFN-γ also up-regulated the long galectin-9 in HFL-1 (Fig. 3A), while the short galectin-9 was up-regulated by IFN-γ in primary human dermal fibroblasts (Fig. 3B). This different regulation of galectin-9 isoforms may reflect the functional variance among them. Further study is required to ascertain the mechanisms of their different up-regulation between HFL-1 and primary human dermal fibroblasts.

Fibroblasts are involved in the pathogenesis of fibrotic diseases in various organs. These diseases are characterized by the accumulation of extracellular matrix collagen as a consequence of the increased proliferation of fibroblasts. Eosinophils and fibroblasts are known to play major roles in the pathogenesis of such fibrotic diseases. For example, it has been shown that the infiltration of eosinophils precedes and parallels the development of bleomycin-induced pulmonary fibrosis (25). In patients with fibrotic lung disease, the presence of eosinophils has been found to correlate with a worse prognosis of the fibrotic process in the lung (26, 27). Thus, a better understanding of the interaction between eosinophils and fibroblasts may lead to clarification of the mechanisms of fibrotic diseases. Several studies have demonstrated that eosinophils adhered to fibroblasts stimulated with certain cytokines, such as IL-1α, TNF-α, or PMA (17, 28), and that eosinophils stimulated the replication of fibroblasts (17). In addition, fibroblasts induced prolonged eosinophil survival (29, 30). In the present study the level of cell surface galectin-9 protein was up-regulated by IFN-γ (Fig. 3C), and we found that eosinophils, but not neutrophils, adhered to HFL-1 cells stimulated with IFN-γ (Fig. 5). The eosinophil adhesion to HFL-1 cells was inhibited by lactose, but not by sucrose, indicating that adhesion is attributable to the galectin property. In fact, such eosinophil adhesion was inhibited by anti-galectin-9 Ab. These results suggest that eosinophil adhesion to HFL-1 cells was mediated by galectin-9. Further, we found that eosinophils adhered to galectin-9, but not to galectin-1 (Fig. 6), confirming the possibility mentioned above that galectin-9 is preferentially involved in eosinophil adhesion. From these findings we suggest a possible role for cell surface galectin-9 on the fibroblasts in eosinophil recruitment. Thus, galectin-9, expressed on fibroblastic cell surface stimulated with IFN-γ, adheres to eosinophils, resulting in eosinophils staying in tissues and allowing eosinophils to perform their effector function in tissues for extended times. The recent study demonstrated that galectin-9 had not only ECA activity, but also...
several unique effects on eosinophils, such as eosinophil activation, superoxide production, and the prevention of eosinophil apoptosis (31). Recently, we reported that galectin-9 suppressed the apoptosis of eosinophils from patients with hypereosinophilic diseases, whereas it enhanced the apoptosis of eosinophils from healthy volunteers. In contrast, galectin-9 accelerated Fas-induced apoptosis of both eosinophils (32). These findings suggested that galectin-9 had heterogeneous effects on eosinophils. Therefore, further investigation is required to clarify whether galectin-9 on the fibroblastic cell surface prolongs eosinophil survival by preventing the apoptosis of eosinophils.

In human lung fibroblasts, IFN-γ inhibits TGF-β-induced signaling and collagen deposition (33). Recently, fibroblasts have been found to produce eotaxin, which is a potent ECA, and Th2 cytokine IL-4 stimulates eotaxin production in fibroblasts (34, 35). IFN-γ, also known as a Th1 cytokine, inhibits the expression and production of eotaxin in fibroblasts (36, 37). Further, it has been shown that IFN-γ reduces tissue eosinophilia by regulating Th2 cytokines, such as IL-4 and IL-5, in various diseases, such as airway inflammation and atopic dermatitis (38–42). However, our findings imply that IFN-γ plays a role in eosinophil recruitment in tissues by enhancing galectin-9 expression on the surface of fibroblasts. IL-4 did not up-regulate the galectin-9 expression in fibroblasts, and there was no or little synergistic effect of IFN-γ and IL-4 on its expression (Fig. 2E). These results contradict those in previous reports regarding the correlation among Th1- and Th2-type cytokines and eosinophils and may reflect the complexity of the role of eosinophil infiltration at various inflammatory sites.

Recently, galectin-9 was reported as a urate transporter (43) that resides in plasma membranes as a transmembrane protein when expressed in renal epithelial cells, although galectins were commonly described as cytoplasmic or secreted proteins. We have found the cell surface localization of galectin-9 is required for eosinophil adhesion to fibroblasts (Fig. 3C). Further studies are required to ascertain whether galectin-9 on the surface of fibroblasts also acts as a urate transporter.
In conclusion, based on the results of the present experiments it is suggested that galectin-9 expression on the surface of fibroblasts plays a role in the infiltration of eosinophils and modulates the pathogenesis of eosinophilia-related diseases.

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