Requirement of Divalent Galactoside-binding Activity of Ecalectin/Galectin-9 for Eosinophil Chemoattraction*

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Nobuko Matsushita‡‡, Nozomu Nishig, Masako Seki, Ryoji Matsumoto, Ichiro Kuwabara**, Fu-Tong Liu‡‡, Yuiro Hata, Takanori Nakamura†, and Mitsuomi Hirashima‡ ‡‡

From the Departments of Immunology and Immunopathology, §Plastic and Reconstruction Surgery, and ¶Endocrinology, Kagawa Medical School, Kagawa 761-0793, Japan, the Department of Immunology, Mayo Clinic, Rochester, Minnesota 55905, and the **La Jolla Institute for Allergy and Immunology, San Diego, California 92121

We have previously isolated and cloned a novel eosinophil chemoattractant (ECA) from a human T-cell-derived expression library. This ECA, termed ecalectin, is a variant of human galectin-9, a member of the β-galactoside-binding animal lectin family, which contains two conserved carbohydrate recognition domains (CRDs). In the present study, we addressed whether carbohydrate binding activity is required for the ECA activity of ecalectin and whether both CRDs are essential for this activity. Recombinant full-length wild-type ecalectin (ecalectin-WT) and N-terminal and C-terminal CRD (ecalectin-NT and -CT, respectively) were generated. All of these recombinant proteins exhibited affinity for lactose, a property shared by galectins, but ecalectin-WT exhibited substantially higher hemagglutination activities than ecalectin-NT and -CT. Furthermore, ecalectin-WT showed over 100-fold higher ECA activity than ecalectin-NT and -CT; combination of recombinant domain fragments did not reconstitute the ECA and hemagglutination activities of the full-length protein. ECA activity of ecalectin-WT was inhibited by lactose in a dose-dependent manner. Site-directed mutation of positions Arg⁶⁵ of ecalectin-NT and Arg⁹⁹ of ecalectin-CT to an aspartic acid residue resulted in the loss of both lactose-binding and ECA activities. We conclude that divalent galactoside-binding activity is required for eosinophil chemoattraction by ecalectin.

Accumulation of eosinophils in the sites, tissue eosinophilia, is frequently observed in allergic diseases as well as helminthic infection and certain malignant neoplasm (1). Eosinophils may play a role as principal effector cells in the inflammatory process in these diseases. Selective eosinophil chemoattractants (ECAs)¹ are believed to be the key molecules produced at the inflamed sites that induce eosinophil accumulation. Although a variety of ECAs have been described, most of them are not selective for eosinophils with the exception of eotaxin-1 and -2 (2–6). In our previous papers, we have shown that antigen-activated T cells and T cells stimulated with solubilized membrane proteins derived from a B-cell line produce an ECA (7, 8). The isolation, cloning, and expression of the ECA (now termed ecalectin) have revealed that it is a variant of galectin-9, a member of the β-galactoside-binding animal lectin family (9).

Animal lectins can be classified into a number of different families, of which the C-type lectins that bind carbohydrate in a Ca²⁺-dependent manner is best known (10). Galectins are members of another rapidly growing family of lectins that bind carbohydrate in a Ca²⁺-independent manner. The family members are composed of 14–15-kDa carbohydrate recognition domains (CRDs), which have affinity for β-galactosides and share certain conserved sequence elements (11, 12). In regard to the number of CRDs, galectins can be divided into two groups. One group, including galectin-1, -2, -3, -5, -7, and -10, has a single CRD (13–21), and the other group, including galectin-4, -6, -8, and -9, has N- and C-terminal CRD connected by a linker peptide (15, 22–24).

Human galectin-9 was first cloned from a cDNA library derived from the disease-involved spleen of a patient with a nodular sclerosis type of Hodgkin’s disease (25). The clone was identified by immunoscreening with autologous serum, suggesting that the patient had developed autoimmune antibodies to this protein. Previously, these investigators have shown that autoantibodies reactive to galectin-9 were detectable in the sera from 10 of 18 patients with Hodgkin’s disease (26). Northern blot analysis revealed that galectin-9 expression is restricted to peripheral blood leukocytes and lymphatic tissues (25). Murine galectin-9 has also been cloned, and the protein was found to be expressed by thymic epithelial cells, among other tissues, and to cause apoptosis of murine thymocytes in vitro, suggesting a possible role in T cell ontogeny in the thymus (24, 28). Our recent findings revealed an important function of galectin-9; we found that a T-cell line-derived ECA (termed ecalectin) has a nearly identical amino acid sequence to galectin-9 (differing only in 5 amino acids); recombinant ecalectin has potent ECA activity, as demonstrated both in vitro and in vivo; the corresponding mRNA is highly induced in peripheral blood mononuclear cells from patients allergic to mites when the cells are treated with a mite allergen in vitro (9). These findings have provided a possible explanation for the accumulation of eosinophils in lymphatic tissues involved in Hodgkin’s disease. Furthermore, they have suggested that ecalectin may play an important role in recruiting eosinophils in allergic inflammation.

The mechanism underlying ecalectin’s ECA activity remains to be elucidated. In this study, we generated recombinant ecalectin and its N- and C-terminal CRDs to determine the role of each domain in this activity. We also addressed whether the ECA activity is dependent on lectin-carbohydrate interactions by testing the inhibition of the activity by lactose, which is a...
saccharide ligand recognized by galectins, and by studying ecalectin mutants lacking the lectin activity. We demonstrate that ecalectin’s bivalent carbohydrate binding property is essential for its ECA activity.

**EXPERIMENTAL PROCEDURES**

**Reagents—**Nonidet-P40, 2-mercaptoethanol, Tris-HCl, Coomassie Brilliant Blue R-250, SDS, and bovine serum albumin were from Sigma.

**Construction of Expression Vectors for Galectin-1, -3, and -8; Ecalectin (ecalectin-WT); and Ecalectin-N- and C-terminal CRD (Ecalectin-NT and -CT, Respectively)**—The following forward (S) and reverse (AS) primers were used to amplify cDNAs for galectin-1, -3, and -8; ecalectin; and ecalectin-NT and -CT from plasmids containing the respective cDNA: galectin-1-S (5'-CCCGGATCCATGGCTTGTGGTCTGGTC), galectin-1-AS (5'-CCCCCTCGAGTACGCTAAGGACACACACA), galectin-3-S (5'-CCCGGATCCATGGCTTGTGGTCTGGTC), galectin-3-AS (5'-CCCCCTCGAGTACGCTAAGGACACACACA), galectin-3-AS (5'-CCGGTACCCTACTACCCGATCTGTTCC), ecalectin-NT-S (5'-CCGGTACCCTACTACCCGATCTGTTCC), ecalectin-NT-AS (5'-CCGGTACCCTACTACCCGATCTGTTCC), ecalectin-NT-S (5'-CCGGTACCCTACTACCCGATCTGTTCC), ecalectin-NT-AS (5'-CCGGTACCCTACTACCCGATCTGTTCC), ecalectin-CT-S (5'-CCGGTACCCTACTACCCGATCTGTTCC), ecalectin-CT-AS (5'-CCGGTACCCTACTACCCGATCTGTTCC), ecalectin-CT-S (5'-CCGGTACCCTACTACCCGATCTGTTCC), ecalectin-CT-AS (5'-CCGGTACCCTACTACCCGATCTGTTCC).

Expression vectors were constructed by inserting cDNAs into the BamHI/Kpn1 site of pTrcHisB (galectin-8; Invitrogen) or BglII/HindIII site (galectin-1) or EcoRI/HindIII site (others) of pGEX-4T-2 (a glutathione S-transferase (GST) fusion vector; Amersham Pharmacia Biotech).

**Site-directed Mutagenesis of Ecalectin**—The following S and AS primers were used to generate cDNAs for site-directed mutants of ecalectin-NT and -CT: NT-R655-S (5'-CCCTGACCTTGACCTTACG), NT-R655-AS (5'-CCCTGACCTTGACCTTACG), CT-R239D-S (5'-CCTCGTCAACCCGACATTTGAGTGAAG), and CT-R239D-AS (5'-ATTCTCTCAACCCGACATTTGAGTGAAG).

Site-directed mutagenesis of ecalectin-NT residue Arg-305 to Asp was carried out as follows. In two separate reactions, cDNA fragments upstream and downstream of the point mutation were amplified using ecalectin-NT-S plus NT-R655-AS and NT-R655-S plus ecalectin-NT-AS. The amplified fragments were then mixed and subjected to a second round of PCR using ecalectin-NT-S plus ecalectin-NT-AS to generate full-length cDNA. The cDNA carrying a point mutation was inserted into the EcoRI/HindIII site of pGEX-4T-2. Site-directed mutagenesis to generate ecalectin-CT-R239D was carried out using a Quickchange Kit (Stratagene) and CT-R239D-S and CT-R239D-AS according to the manufacturer’s instructions. The resultant mutations were confirmed by DNA sequencing.

**Expression and Purification of Recombinant Proteins**—Escherichia coli BL-21 cells carrying each expression plasmid were grown in 2% YT medium supplemented with 10% (v/v) glucose and 100 μg/ml ampicillin to an optical density of 0.7 at 600 nm. The induction of fusion protein expression was induced by the addition of 0.1 mm isopropyl-β-D-thiogalactopyranoside, and the cultures were continued for 6 h at 30 °C (ecalectin-WT, ecalectin-NT, and ecalectin-NT-R239D) or for 2 h at 37 °C (galectin-1, -3, and -8; ecalectin-CT; and ecalectin-CT-R239D). The cell pellet obtained from a 1-liter culture was suspended in 100 ml of 10% Tris-HCl (pH 7.5) containing 0.5 mM NaCl, 5 mM 2-mercaptoethanol, 1 mM EDTA, and 1 mM phenylmethylsulfonyl fluoride and then sonicated for 4 min. The sonicate was supplemented with 1% (v/v) Triton X-100 and then stirred for 30 min at 4 °C, followed by centrifugation. The resulting supernatant was subjected to affinity chromatography on a glutathione-Sepharose column (Amersham Pharmacia Biotech). The affinity-puriﬁed fusion proteins were digested with thrombin, and the released ecalectin and galectins were purified by lactose-agarose affinity chromatography (Seikagaku Corp., Tokyo, Japan). To purify galectin-8, E. coli extract was directly subjected to lactose-agarose affinity chromatography. In the case of site-directed mutants, which lack affinity for lactose (see “Results”), the GST moiety was removed from the thrombin digest with glutathione-Sepharose. Protein concentration was determined using BCA protein assay reagent (Pierce) and bovine serum albumin as a standard.

**In Vitro Chemotaxis**—ECA activity was evaluated in vitro as described previously (8, 9). In brief, CD16-negative eosinophils were enriched by subjecting peripheral blood leukocytes from healthy volunteers to a discontinuous density gradient of Percoll (Amersham Pharmacia Biotech) followed by immunomagnetic treatment of the cells with anti-CD16 immunoglobulin (DAKO, Glostrup, Denmark). The purity and viability of the purified eosinophils were >97 and >95%, respectively. ECA activity was evaluated using a 48-well chamber (Neuro Probe Inc.) containing a polyvinylpyrrolidone-free membrane with 5-μm pore sizes. Human eosinophils (0.5–1 × 10^6/ml) and varied concentrations of a test chemotacticant were placed in the top and bottom chambers, respectively. Each assay was performed in triplicate. After a 1–2-h incubation at 37 °C in a humidified atmosphere of 5% CO_2, the membrane separating the two chambers was removed and placed in Dif-Quick stain (Baxter Healthcare Corp.). Stained eosinophils were counted under a microscope. Human eotaxin-1 (Seikagaku Corp.) was used as a control.

**Hemagglutination Assays**—Hemagglutination activity was assessed by the method of Nowak et al. (29). Briefly, assay samples were prepared by serial 2-fold dilutions of recombinant proteins in a 96-well microtiter plate. After the addition of bovine serum albumin and glutaraldehyde-fixed trypsin-treated rabbit erythrocytes to a final concentration of 0.25% (w/v) and 1% (v/v), respectively, the reaction mixture was incubated for 1 h at room temperature. The minimum concentration required for hemagglutination was visually determined.

**RESULTS**

**Purification of Recombinant Proteins**—Ecalectin has previously been shown to have affinity for β-galactosides and to be a selective eosinophil chemotaactant (9). In order to examine the structure-function relationships of ecalectin, we produced three types of ecalectin: ecalectin-WT, ecalectin-NT, and ecalectin-CT (Fig. 1A). GST-ecalectin-WT, -NT, and -CT were expressed in E. coli BL21 and purified by glutathione affinity chromatography followed by lactose affinity chromatography. After cleavage of glutathione by thrombin, GST-free ecalectin and domain fragments were purified by lactose affinity chromatography (Fig. 1B).

In the affinity purification of ecalectin and derivatives, the proteins bound to lactose-agarose were eluted with a gradient of lactose (0–200 mM). We found that all of the ecalectin preparations (with or without the GST moiety) were eluted at 20–23 mM lactose, suggesting that they have similar affinity for lactose.

**Eosinophil Chemotactic Activity of Recombinant Proteins**—Since the recovery of ecalectin-WT after thrombin digestion was low, we compared ECA activity between GST-containing and GST-cleaved preparations to clarify whether the GST moiety affects ECA activity of ecalectin-WT, -NT, and -CT. We failed to detect any difference in ECA activity between GST-containing ecalectin and GST-cleaved ecalectin at any concentration tested (Fig. 2). Therefore, we used GST-containing ecalectin-WT, -NT, and -CT for further analysis.

Ecalectin-WT, -NT, -CT exhibited ECA activity in a dose-dependent fashion (Fig. 3). ECA activity of ecalectin-WT at 1 × 10^{-7} M was similar to that of eotaxin-1, a potent chemotaactant specific for eosinophils, at 1 × 10^{-7} M. In contrast, ecalectin-NT and -CT and galectin-8 did not exhibit evident ECA activity at 1 × 10^{-7} M, although the activity was about two-thirds of that of ecalectin-WT (Fig. 3). In contrast, galectin-1 and -3, which have one CRD, exhibited detectable ECA activity even at 3 × 10^{-6} M (Fig. 3).

We also tested the ECA activity of a mixture of ecalectin-NT and -CT at 1 × 10^{-7} M, and the results showed that the ECA activity of ecalectin was not reconstituted by the combination of isolated individual domains. In addition, we found that the ECA activity of ecalectin-WT was not affected by the presence of equal amounts of ecalectin-NT and -CT (Fig. 4). Galectin-1 and -3 also had no effect on ECA activity of ecalectin-WT (Fig. 4).

Novel, experiments were done to clarify whether lactose could inhibit the ECA activity of ecalectin-WT. ECA activity of ecalectin-WT was inhibited by lactose in a dose-dependent manner, while sucrose at 20 mM failed to inhibit ECA activity. ECA activity of ecalectin-WT was inhibited almost completely by 20 mM lactose, whereas that of eotaxin-1 was barely inhibited (Fig. 5).
**Hemagglutination Activity on Rabbit Erythrocytes**—One of the characteristics of galectins is their hemagglutination activity, which is attributable to their bivalent carbohydrate-binding property. In the case of galectins with two CRDs, the bivalency is probably due to carbohydrate binding manifested by each domain. Therefore, hemagglutination activity of recombinant ecalectin and domain fragments was assessed and compared with galectin-1. As shown in Table I, the minimum concentration of ecalectin-WT required for the hemagglutination was 0.0125 μM, which was comparable with that for galectin-1 (0.025 μM). Ecalectin-NT and -CT also exhibited hemagglutination activity, although the activity was clearly lower than that of ecalectin-WT (Table I). The results also showed that the presence of the GST moiety had no effect on the activity.

**Effect of Point Mutation on Hemagglutination and ECA activities of Ecalectin**—Recent crystal structure studies of galectins suggest that the amino acids directly interacting with the bound carbohydrate are highly conserved among members of the galectin family. Highly conserved residues His\(^{158}\), Asn\(^{160}\), and Arg\(^{162}\) of galectin-3 may play a central role in binding (30, 31). We therefore focused on Arg\(^{162}\) in the N-terminal CRD and Arg\(^{239}\) in the C-terminal CRD of ecalectin, which correspond to Arg\(^{162}\) of galectin-3. We generated site-directed mutants of ecalectin-NT and -CT, and galectin preparations that had been purified by lactose affinity chromatography. The number of eosinophils that had migrated through the membrane is indicated on the y axis. The results represent the mean ± S.D. of two experiments in triplicate. ECA activity of GST-ecalectin-WT (●), -NT (○), and -CT (▲) was similar to that of ecalectin-WT (□), -NT (○), and -CT (▲).
DISCUSSION

In our previous paper (9), we demonstrated that ecalectin is a potent and selective eosinophil chemoattractant. We noted previously that ecalectin represents a variant form of human galectin-9, because there are as many as five amino acids that are different between the two proteins (9). Subsequently, we generated ecalectin cDNA from a human T-cell line, Jurkat, by reverse transcriptase-PCR using oligonucleotide primers that correspond to internal sequences of ecalectin. DNA sequencing revealed that all of the 12 independent clones share a common sequence, and the deduced protein sequence differs from our previously published ecalectin sequence (9) by 1 amino acid and from the published galectin-9 sequence (25) by 4 amino acids (Table II). In the meantime, we found over 17 EST clones in the database for which partial sequences are available. The amino acids for positions 5, 88, 135, and 238 are known for some of them, and they are invariably Gly, Lys, Ser, and Pro, respectively, and are identical to the Jurkat sequence (Table II). We know that Jurkat cells express ecalectin, because the cell supernatant contains ECA that is absorbable by lactose-agarose and anti-ecalectin antibodies (data not shown). Therefore, the Ser to Gly substitution at position 5 does not affect the eosinophil chemoattractant activity. The fact that none of the EST clones has amino acids at positions 88, 135, and 238 that match those originally described for galectin-9 suggests that the latter probably contains sequences that are a result of allelic variation.

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**FIG. 4.** Effect of GST-ecalectin-NT, GST-ecalectin-CT, GST-galectin-1, and GST-galectin-3 on ECA activity of GST-ecalectin-WT and the activity of a mixture of ecalectin-NT and -CT. ECA activity of a mixture of GST-ecalectin-NT (10⁻⁷ M) and GST-ecalectin-CT (10⁻⁷ M) and the activity of ecalectin-WT (10⁻⁷ M) in the presence of GST-ecalectin-NT (10⁻⁷ M), GST-ecalectin-CT (10⁻⁷ M), GST-galectin-1 (10⁻⁶ M), or GST-galectin-3 (10⁻⁷ M) were determined. ECA activity of GST-ecalectin-WT (10⁻⁷ M) was regarded as 100%. The depicted results are the mean ± S.D. of three experiments in triplicate.

**FIG. 5.** Effect of lactose on ECA activity of GST-ecalectin. We examined ECA activity of recombinant ecalectin-WT in the presence of lactose (○) or sucrose (●) and the activity of eotaxin in the presence of lactose (■). The number of eosinophils that had migrated through the membrane is indicated on the y-axis. The depicted results are the mean ± S.D. of three experiments in triplicate. ECA activity of ecalectin-WT was inhibited by only lactose in a dose-dependent manner.

**TABLE I**

Hemagglutination activity of ecalectin-WT, -NT, and -CT and galectin-1 and -8

| Recombinant proteins | Minimum concentration for hemagglutination (µM) |
|----------------------|-----------------------------------------------|
| GST-ecalectin-WT/(ecalectin-WT) | 0.0125/(0.0125) |
| GST-ecalectin-NT/(ecalectin-NT) | 0.2/(0.2) |
| GST-ecalectin-NT-R65D | ND |
| GST-ecalectin-CT/(ecalectin-CT) | 0.2/(0.2) |
| GST-ecalectin-CT-R239D | ND |
| GST-galectin-1 | 0.025 |
| GST alone | ND |
| Galectin-8 | 0.0084 |

*ND, not detected.

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**FIG. 6.** Lactose binding ability of site-directed mutants. Recombinant proteins with or without site-directed mutation (ecalectin-NT, ecalectin-NT-R65D (Arg65Asp) (a), ecalectin-CT, and ecalectin-CT-R239D (Arg239Asp) (b)) were expressed in E. coli and purified by glutathione affinity column chromatography. Site-directed mutants and the parental proteins were subjected to lactose affinity column chromatography. Proteins bound to lactose-agarose were eluted with 0.2 M lactose and then analyzed by SDS-polyacrylamide gel electrophoresis. Molecular weight markers are shown on the left of each column. The gel depicted was stained with Coomassie Blue. GST column, eluate from glutathione-Sepharose column; Lac column, eluate from lactose-agarose column.

**FIG. 7.** Comparison of ECA activity of site-directed mutants and their parental proteins. a, ECA activities of ecalectin-NT (●) and ecalectin-NT-R65D (E) were assessed. b, those of ecalectin-CT (●) and ecalectin-CT-R239D (E) were assessed. The number of eosinophils that had migrated through the membrane is indicated on the y-axis. The depicted results are the mean ± S.D. of two experiments in triplicate.
These putative galectin-9 EST clones were identified by BLAST sequence search (27) using the ecalectin nucleotide sequence as query. The sequence identity of these clones with the ecalectin is usually 97–99%. The EST clones from different sources are demonstrated without Gly (G), Lys (K), Ser (S), Pro (P), and Glu (E) at positions 5, 88, 135, 238, and 281, respectively. The sequence identity of these clones with the ecalectin is usually 97–99%. The EST clones from different sources are demonstrated without Gly (G), Lys (K), Ser (S), Pro (P), and Glu (E) at positions 5, 88, 135, 238, and 281, respectively. The sequence identity of these clones with the ecalectin is usually 97–99%. The EST clones from different sources are demonstrated without Gly (G), Lys (K), Ser (S), Pro (P), and Glu (E) at positions 5, 88, 135, 238, and 281, respectively. The sequence identity of these clones with the ecalectin is usually 97–99%. The EST clones from different sources are demonstrated without Gly (G), Lys (K), Ser (S), Pro (P), and Glu (E) at positions 5, 88, 135, 238, and 281, respectively.

| Amino acids at positions | 5 | 88 | 135 | 238 | 281 |
|-------------------------|---|----|-----|-----|-----|
| Gly (G)                 |   |    |     |     |     |
| Lys (K)                 |   |    |     |     |     |
| Ser (S)                 |   |    |     |     |     |
| Pro (P)                 |   |    |     |     |     |
| Glu (E)                 |   |    |     |     |     |

Because each CRD contains only one carbohydrate-binding site, the hemagglutination activity exhibited by ecalectin-NT and -CT suggest that these CRDs may exist as dimers and/or multimers. This is different from the case of a 32-kDa galectin isolated from the nematode Caenorhabditis elegans, which is also composed of two homologous domains. While the full-length protein exhibits hemagglutination activity, the N- and C-terminal CRDs of the protein fail to exhibit evident hemagglutination activity even at the concentration of 100 μg/ml. Gel permeation chromatography of these domain fragments showed that they do not form dimer (32). On the other hand, galectin-1 contains one CRD but exists as noncovalently linked homodimers. However, ecalectin-NT and -CT clearly have lower hemagglutination activity than galectin-1, suggesting that they may not form stable dimers and/or multimers.

The findings that ECA activity of ecalectin is inhibitable by lactose and that mutated domains lacking carbohydrate-binding activity do not exhibit ECA activity suggest that galactoside-binding activity is critically involved in ecalectin’s ECA activity (Figs. 6 and 7). Based on these findings, we postulate that ecalectin functions by cross-linking galactoside-containing glycoprotein molecules on the surface of eosinophils, resulting in eosinophil chemotraction. The fact that both ecalectin-NT and -CT retain partial ECA activity suggests that both domains contribute to the activity of ecalectin by binding to the appropriate cell surface glycoconjugates. Two possibilities exist: 1) the two domains bind to two distinct glycoproteins, and thus ecalectin functions by cross-linking these two different glycoprotein species; and 2) the two domains bind to different oligosaccharide moieties on the same glycoprotein, and thus ecalectin functions by cross-linking the same glycoprotein species. The finding that ecalectin-NT and -CT at 10⁻⁷ M do not affect ECA activity of ecalectin at the same concentration (Fig. 4) is consistent with the fact that the domains individually or in combination do not exhibit ECA activity at this concentration, although they are active at higher concentrations, e.g. 10⁻⁶ M (Figs. 3 and 4). The results suggest that the domains are not as effective as the full-length protein in binding to and/or cross-linking the relevant cell surface receptor(s). We found that ecalectin-NT or -CT at 10⁻⁶ M has an additive effect, rather than an inhibitory effect, on ECA activity of ecalectin at the same concentration (data not shown). The results suggest that binding sites for each domain on the cell surface are at such high densities that binding by ecalectin and the domains is not mutually exclusive at the concentration being studied (i.e. 10⁻⁶ M). The possibility that the isolated domains bind to different glycoconjugates than the ones recognized by the full-length protein is also consistent with the data, but it is less likely.

The receptor for ecalectin that mediates the observed ECA of this lectin is presently unknown. There are a number of proteins in the C-, CC-, or CXC-chemokine families that have been identified to be eosinophil chemoattractants (33, 34), and they function through binding to the CCR1 or CCR3 receptors (35–38). Ecalectin does not have significant sequence similarity with any of these chemokines (1, 25), and thus it appears unlikely that it is recognized by these receptors through protein-protein interaction. However, an interesting possibility exists that its ECA activity is also mediated through these chemokine receptors, which may be recognized by ecalectin via lectin-carbohydrate interaction (39–41). Alternatively, ecalectin’s ECA activity may be mediated through a novel cell surface receptor.

Although galectins are defined by their affinity for galactoside, in addition to their sharing of a consensus sequence, it is likely that different galectins have different fine carbohydrate specificities (42–45). Therefore, it is expected that each galectin recognizes different glycoproteins on surfaces of various cells and thus has different effects on the cell. Indeed, we found that ECA appears to be unique to ecalectin and is not exhibited by galectin-1 and -3 (Fig. 3). Also, ECA activity of ecalectin is not inhibitable by galectin-1 and -3 (Fig. 4), suggesting that the receptor for ecalectin is not recognized by these other two...
gaitectins. ECA activity of gaitectin-8 was less than 10% of that of ecalectin-WT but was comparable with those of ecalectin-NT and -CT at concentrations of 1–3 × 10⁻⁶ M. It is possible that gaitectin-8 does not bind to any glycoprotein that effectively transmits chemotactic activity or that it recognizes the same receptor as ecalectin does but does not engage it as effectively. It is notable also that while ecalectin is likely to bind to a number of different cell types through lectin-carbohydrate interactions, its chemotactic activity is cell type-specific, since it does not induce chemotaxis of lymphocytes, neutrophils, and monocytes (9). On the other hand, the possibility exists that other gaitectins may possess chemotaxis activity for different cell types.

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Fu-Tong Liu, Yuiro Hata, Takanori Nakamura and Mitsuomi Hirashima

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