Human Ecalectin, a Variant of Human Galectin-9, Is a Novel Eosinophil Chemoattractant Produced by T Lymphocytes*

(Received for publication, December 19, 1997, and in revised form, April 17, 1998)

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A 1.6-kilobase pair cDNA was isolated from a human T-cell-derived expression library that encodes a novel eosinophil chemoattractant (designated ecalectin) expressed during allergic and parasitic responses. Based on its deduced amino acid sequence, ecalectin is a 36-kDa protein consisting of 323 amino acids. Although ecalectin lacks a hydrophobic signal peptide, it is secreted from mammalian cells. Ecalectin is not related to any known cytokine or chemokine but is rather a variant of human galectin-9, a member of the large family of animal lectins that have affinity for β-galactosides. Recombinant ecalectin, expressed in COS cells and insect cells, exhibited potent eosinophil chemoattractant activity and attracted eosinophils in vitro and in vivo in a dose-dependent manner but not neutrophils, lymphocytes, or monocytes. The finding that the ecalectin transcript is present in abundance in various lymphatic tissues and that its expression increases substantially in antigen-activated peripheral blood mononuclear cells suggests that ecalectin is a potent T-cell-derived regulator of eosinophil recruitment in tissues during inflammatory reactions. We believe that this is the first report of the expression of an immunoregulatory galectin expressed by a T-cell line that is selective for eosinophils.

Eosinophil accumulation is a common feature of many inflammatory diseases such as bronchial asthma, allergic rhinitis, helminth infection, and atopic dermatitis (1). Although eosinophils are proinflammatory cells that normally reside in the circulation, they are induced to extravasate into tissues by a diverse array of chemoattractants and viability-enhancing factors. The eotaxins (2–8) are the most specific of the known eosinophil chemoattractants (ECA), but granulocyte-macrophage colony-stimulating factor, interleukin (IL)-2, IL-3, IL-5, IL-8, IL-16, RANTES, macrophage inflammatory protein 1α, monocyte chemotactic peptide (MCP)-2, MCP-3, MCP-4, complement component 3a (C3a), and C5a also can regulate the chemotaxis, viability, growth, differentiation, and/or activation of certain populations of eosinophils (9–18, 20, 22–25). Platelet-activating factor (26) and 5-lipoxygenase metabolites of arachidonic acid (27–29) are lipid mediators with potent chemotactic activity for eosinophils. Because these regulatory factors often work in concert, an eosinophil generally has to be cytokine-primed to respond to an ECA.

Increased numbers of eosinophils in tissues that contain antigen-activated T-cells have been observed by several groups (30–34). However, it has been assumed that T-cells do not directly recruit eosinophils into tissues because they do not produce substantial amounts of an eotaxin. Most ECA are positively charged proteins that are small in size. For example, human eotaxin-1 consists of only 74 amino acids, yet 16 of them are either Lys or Arg (5, 6). We previously noted that concanavalin A-activated or antigen-activated CD4+ T-cells from patients infected with Schistosoma mansoni (35) produce an apparently novel 30–40-kDa ECA with a relatively neutral isoelectric point. The virus-transformed human T-cell line STO-2 also appears to spontaneously produce this ECA (36). Using blocking antibodies, we concluded that the T-cell-derived ECA was not IL-3, IL-5, or granulocyte-macrophage colony-stimulating factor. We now describe the purification, molecular cloning, and functional expression of ecalectin, a novel ECA produced by T-cells. Ecalectin is a variant of a protein, designated human galectin-9 (37), of unknown function recently found in the tumor of a patient with Hodgkin’s lymphoma.

EXPERIMENTAL PROCEDURES

Partial Purification of Ecalectin and Generation of Ecalectin-specific Antibodies—The STO-2 cell line was previously derived by transformation of normal human T-cells with human T-cell lymphoma-leukemia virus (35). Fluorescence-activated cell sorting analysis revealed that the cell line is of T-cell origin. It expresses CD2, CD3, CD4, CD5, and CD8 but lacks granulocyte/macrophage and B-cell markers such as CD16, CD19, and Leu7. Supernatants from STO-2 cells (2 × 10⁷/ml) that had been cultured for ~72 h in SF-92 serum-free medium (Sanko-Junyaku Co., Tokyo, Japan) supplemented with 0.1% human serum albumin, 100 units/ml IL-2 (Tokushima Research Institute, Tokushima, Japan), 50 μM 2-mercaptoethanol, 100 μg/ml streptomycin, 100 units/ml penicillin, and 5 μg/ml fungizone were adjusted to pH 5.0 with 50 mM sodium acetate buffer. The resulting conditioned medium (5 liters) that had been equilibrated with 2.6 × 40-cm columns of CM-Sepharose (Amersham Pharmacia Biotech) that had been equilibrated with 50 mM sodium acetate, pH 5.0. Each ion exchange column was washed extensively with equilibration buffer to remove the major albumin contaminant in the conditioned medium. Bound ecalectin was eluted by exposing the
column to 20 mM sodium phosphate and 0.1 mM NaCl, pH 7.0. After dialysis of the pooled eluate (~300 ml) against 1% glycine, the material was subjected to preparative isoelectric focusing. Those 40-ml fractions having proteins with a pI of 7–8 were collected on a Rotofor system (Bio-Rad). The ecalectin-enriched material was then concentrated ~10-fold by Vivaspin 20 ultracentrifugation from Amersham Pharmacia Biotech (Buckinghamshire, England) and reisolated from the protein precipitation buffer. After the material was dialyzed against phosphate-buffered saline (PBS), it was fractionated further on a 1 × 30-cm column of Superose-12 (Amersham Pharmacia Biotech) equilibrated with the same buffer. Based on a calibration of the gel filtration column, most of the ECA activity was 30–40 kDa. The ecalectin-enriched fractions were precipitated with 60% ammonium sulfate and dissolved in 20 mM sodium phosphate (pH 7.0) containing 30% ammonium sulfate, and applied to a 4.6 cm × 7.5-cm reverse phase column of TSKgel phenyl-5PW (Tozoh Co., Tokyo, Japan) equilibrated in the same buffer. The column was subjected to a linear gradient, decreasing the ammonium sulfate concentration in the buffer from 30 to 0%. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) analysis of a portion of the final biologically active fraction failed to reveal a Coomassie Blue-stainable protein of a defined molecular weight. Nevertheless, New Zealand White rabbits were immunized with the resulting partially purified ecalectin in Freund’s complete adjuvant, and after booster injections in Freund’s incomplete adjuvant, sera were collected.

Once the ecalectin cDNA described below was isolated and its amino acid sequence was deduced from the deduced amino acid sequence of the generated antibodies in rabbits using an anti-peptide approach. A 13-mer peptide (AFSSQAPYPLSPA) corresponding to residues 2–14 of human ecalectin was synthesized by Peptron Co. (Osaka, Japan). A Cys residue was then added to the C terminus of the ecalectin peptide to facilitate the coupling of the resulting 14-mer peptide through its thiol group to m-maleimidobenzoic acid N-hydroxysuccinimide ester-activated keyhole limpet hemocyanin (Sigma). A rabbit was immunized with the coupled peptide, sera were collected, and the relevant antibodies were purified using an affinity column consisting of the same peptide coupled to 2-fluoro-1-methylpyridinium toluene 4-sulfonate cellulose (Seikagaku Corp., Tokyo, Japan).

Construction and Screening of a cDNA Library from a T-cell Line—A STO-2 cell cDNA expression library was constructed with the ZAP ExpressTM cDNA Gigapack cloning kit (Stratagene, La Jolla, CA) according to the manufacturer’s instructions. In brief, ~5 μg of poly(A)+ mRNA was isolated from 1 × 106 STO-2 cells with the FastTrack RNA isolation kit from Invitrogen (San Diego, CA). The first DNA strand was synthesized with Moloney murine leukemia virus reverse transcriptase (Amersham Pharmacia Biotech, San Diego, CA) downstream of the promoter of the polyhedrin gene. Plasmid DNA, purified by CsCl density gradient centrifugation, was mixed with linearized BaculoGoldTM DNA (PharMingen) and calcium phosphate precipitation. The resulting DNA solution was added to Sf9 insect cells. The recombinant baculovirus that contained the ecalectin cDNA was identified by a plaque assay, amplified, and used to infect fresh Sf9 cells. Sf9 cells were cultured at 27 °C in TMF-HF medium (Life Technologies) supplemented with 10% heat-inactivated FCS. After 3 days of culture, the infected cells (~7 × 106) were collected by centrifugation and suspended in 1 ml of 20 mM Tris-HCl, pH 7.5, containing 150 mM NaCl (TBS) and varied protease inhibitors (100 μg/ml phenylmethylsulfonyl fluoride (Sigma), 2 μg/ml leupeptin (Sigma), 1 μg/ml pepstatin A (Sigma), 2 μg/ml aprotinin (Sigma), and 1 μM EDTA). The cell suspension was sonicated on ice and centrifuged to remove the debris, and the resulting supernatant was applied to a 1-mL lactose column (Seikagaku Corp.). After the affinity column was washed extensively with TBS and the column was eluted with TBS containing 100 mM NaCl, the mixture was dialyzed against 1% glycine, the material was then loaded on a 30-cm 300-mL Superose-12 column of Superose-12 (Amersham Pharmacia Biotech) followed by immuno-magnetic treatment of the obtained proteins with anti-CD16 immunoglobulin (DAKO A.S., Glostrup, Denmark). The purity and viability of the obtained ecalectin were >95%, respectively. The ECA activity was evaluated with a 48-well chamber (Neuro Probe Inc., Cabin John, MD) containing a polyvinylpyrrolidone-free polycarbonate membrane with 5-μm standard pore sizes. Human ecalectin (0.5–1 × 109/ml) and various concentrations of a test chemotactic agent 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% CO2, the membrane separating the two chambers was removed and placed in Diff-Quik® stain (Baxter Healthcare Corp., Irvine, CA), fixed, and the neutrophils that had migrated through the membrane were counted under the microscope. Human C5a (Sigma), IL-5 (Genzyme, Cambridge, MA), RANTES (Genzyme), and eotaxin-1 (Seikagaku Corp.) were used as positive controls.

The ability of ecalectin to attract peripheral blood human neutrophils, monocytes, and lymphocytes in vitro was evaluated in a similar fashion. Neutrophils were obtained by centrifuging human peripheral blood cells at 400 × g for 20 min on a discontinuous density gradient of Percoll. After the contaminating erythrocytes were lysed with ammonium chloride, the purity of the resulting neutrophils was ~99%. Monocytes and lymphocytes were separated from one another by briefly culturing the resulting monocyte/lymphocyte-enriched fraction in 75-cm2 plastic culture flasks. The nonadherent lymphocytes were separated from the monocytes by a second plastic adherence step. As assessed immunohistochemically with fluorescence-activated cell sorting and anti-CD14 immunoglobulin (DAKO A.S.), the monocyte contamination in the resulting lymphocyte preparation was 1% or less. The flasks containing the adherent monocytes were washed several times with PBS. Ice-cold RPMI 1640 containing 5% FCS and 0.5% EDTA were added, and the flasks were shaken on ice for 30 min. The recovered cells were almost pure monocytes. Chemotaxis was assayed as described above for eosinophils except that the membranes used in some of the assays had 3- and 8-μm standard pore sizes. Human C5a and IL-8 (Genzyme) served as positive controls in the neutrophil chemotaxis assay. Human lymphotactin (Genzyme) and MCP-1 (Genzyme) served as positive controls in the lymphocyte and monocyte chemotaxis assays, respectively.
DNA and RNA Blot Analyses—To determine if there are multiple ecalectin-like genes in the human genome, 6-μg samples of human blood-derived genomic DNA (Noven, Madison, WI) were digested with 3 M HClO4. The digests were size-fractionated by centrifugation. The obtained cells (5 × 10⁶ cells) were incubated overnight at 37 °C with 30–60 units of BamHI, BglII, EcoHI, HindIII, KpnI, NotI, PsiI, SalI, XhoI, or XhoI (New England Biolabs). After electrophoretic separation of the digests in a 0.9% agarose gel (41), a DNA blot was prepared and probed under conditions of high stringency with [α-32P]dCTP-labeled probes that correspond to residues 1–460 or residues 1073–1579 (sequence corresponding to the 3′-untranslated region) of the human ecalectin cDNA.

Premade blots containing either total RNA (OriGene Technologies, Rockville, MD) or poly(A)+ RNA (CLONTECH, Palo Alto, CA) from various human tissues were hybridized with the radiolabeled ecalectin probes. RNA blots were washed twice for 15 min each at room temperature in 2 × SSC containing 0.1% SDS and then twice for 30 min each at 60 °C in 0.1% SDS. After autoradiography, the images were analyzed with a BAS-2000 32P imaging apparatus (Fuji Film Co, Tokyo, Japan).

To determine if the steady-state level of the ecalectin transcript in a T-cell can be antigen-regulated, heparinized peripheral blood was obtained from two volunteers sensitive to Dermatophagoides farinae, and the peripheral blood mononuclear cells were isolated by Ficoll-Hypaque density gradient centrifugation. The obtained cells (5 × 10⁶/ml) were suspended in RPMI 1640 medium supplemented with 5% FCS and cultured for 48 h in the absence or presence of 500 ng/ml of D. farinae. Under these in vitro conditions, the T-cells in the peripheral blood mononuclear cell preparation should be the cells that are preferentially activated. Total RNA was isolated from control and D. farinae-stimulated cell RNase easy Midi kits from Agencourt. For blot analysis of each sample, ~5 μg of denatured total RNA were electrophoresed in a 1.4% agarose/formaldehyde gel, and the separated RNA was blotted onto Hybond™ nylon membranes (Amer sham Pharmacia Biotech) (42). After UV-cross-linking of the RNA, the membranes were incubated at 68 °C for 30 min in QubitHyb (Stratagene) containing 42 μg/ml of denatured salmon sperm DNA (Stratagene) alone and then for 2 h in the same buffer containing radiolabeled ecalectin probes. To ensure that approximately equal amounts of RNA were loaded in the individual lanes of the blot, the same blot generally was reprobed with the same blots generally were reprobed with the peptide antibody directed against the N terminus of ecalectin revealed that immunoreactive ecalectin was exocytosed from these nontransformed cells.

RESULTS

Isolation and Characterization of Human Ecalectin Transcript and Protein—32 immunoreactive clones were obtained when the expression cDNA library prepared from the human T-cell line was screened with anti-ecalectin immunoglobulin directed against the semi-purified factor. When the cDNAs from these clones were individually transfected into COS cells and the supernatants of the transfec tants were analyzed 2 days later, three of the clones exhibited potent ECA activity in the in vitro chemotaxis assay. Subsequent nucleotide sequence analysis revealed that these clones encoded the same protein. The consensus nucleotide and deduced amino acid sequences of the STO-2 cell-derived ecalectin cDNAs are depicted in Fig. 1. If the translation-initiation and translation-termination sites begin at residues 60 and 1028, respectively, the open reading frame of the transcript encodes a 36-kDa protein consisting of 323 amino acids. The ecalectin transcript has a typical polyadenylation signal sequence at residues 1573–1581. 59 and >560 nucleotides are present in its 5′- and 3′-untranslated regions, respectively. Repetitive sequences are often found in the 3′-untranslated regions of transcripts that have short half-lives (44). Although no repetitive “AUAUA” motif resides in the 3′-untranslated region as it does in eotaxin-1 and most other chemokine and cytokine transcripts, four copies of a “CCCUCC” motif reside between residues 1341 and 1484 in the 3′-noncoding portion of the ecalectin transcript. Native ecalectin is secreted from the STO-2 cell line. Although the deduced amino acid sequence of the STO-2-derived transcript indicates that ecalectin contains large numbers of Phe, Leu, and Val residues, this ECA lacks a hydrophobic signal peptide at its amino terminus. Ecalectin is also predicted to have a pl of ~8.1. Ecalectin has four Cys residues and three potential Asn-linked glycosylation sites at positions 34, 79, and 137.

Expression of the Ecalectin Transcript in Cells and Tissues—Blot analysis of genomic DNA digested separately with 10 different restriction enzymes indicated multiple genes in the human genome encode ecalectin-like transcripts. Nevertheless, the 5′ (Fig. 2, lanes 3, 5, 6, 8, and 10) and 3′ (data not shown) ecalectin probes often hybridized strongly to one major DNA fragment in the digested genomic DNA. When varied human tissues were analyzed with these probes, the ~1.8-kb ecalectin transcript or its homolog was found in relative abundance in lymphoid tissues (Fig. 3). Because of the prominence of the ecalectin transcript in T-cell-enriched tissues, we investigated whether or not its level increases in peripheral blood mononuclear cells after antigen stimulation. As shown in Fig. 4 for one of the two allergic patients, these cells contained a small amount of ecalectin mRNA. However, the amount of ecalectin transcript increased ~50- and ~90-fold in the two experiments after the cells from the patients were exposed to D. farinae for 48 h. Although the steady-state level of a transcript does not always correlate with the amount of protein that the cell produces, these RNA blot findings suggest that the T-cells in the preparation dramatically increased their expression of ecalectin when they were exposed to the relevant antigen. SDS-PAGE/immunoblot analysis with the peptide antibody directed against the N terminus of ecalectin revealed that immunoreactive ecalectin was present in the conditioned medium of activated, nontransformed human T-cells (Fig. 4).

Bioactivity of Recombinant Ecalectin in Vitro and in Vivo—Recombinant ecalectin was spontaneously secreted from COS cells but not insect cells (Fig. 5). Nevertheless, because insect cells express measurable amounts of ecalectin by silver staining analysis of an SDS-PAGE gel (Fig. 5A), we attempted to affinity-purify the immunoreactive (Fig. 5B) recombinant protein from the lysates of these cells. The observation that the ecalectin cDNA appears to encode a protein with carbohydrate-binding domains suggested that the recombinant protein probably would bind to a lactose column. Thus, soluble lysates of insect cells were subject to lactose affinity chromatography. After the column was exposed to 200 ml lactose, a single ~36-kDa silver-stained protein (Fig. 5C) was eluted, which was recognized by anti-ecalectin antibody (Fig. 5D). The culture supernatant of COS cells transfected with an ecalectin cDNA (data not shown) and purified, recombinant, insect cell-derived ecalectin (Fig. 6) exhibited dose-dependent ECA activity. In our in vitro system, the optimal chemotactic activity of IL-5, RANTES, eotaxin-1, and C5a for freshly isolated peripheral blood eosinophils was achieved at 10⁻¹¹ M, 10⁻⁸ M, 10⁻¹⁰ M, and 10⁻⁸ M, respectively. Insect cell-derived ecalectin was active at 10⁻¹⁰ M, and its activity at 10⁻⁸ M was approximately one-half of that of eotaxin-1. Although IL-5 was active at a lower molar concentration, in comparative assays carried out on replicate eosinophils from...
the same patients, recombinant ecalcin induced ~4-fold more eosinophils to migrate through the membrane than the optimal dose of recombinant IL-5. COS cell-derived (data not shown) and insect cell-derived (Fig. 7) recombinant ecalcin did not induce chemotaxis of peripheral blood neutrophils, lymphocytes, or monocytes. 4–24 h after C57BL/6 mice were given insect cell-derived ecalcin, 15.1 ± 2.7% (n = 3; mean ± S.D.) of the cells in the peritoneal cavity exudates consisted of eosinophils. In contrast, the number of eosinophils was below detection (<1 eosinophil/500 cells) in the exudates of control mice given buffer lacking ecalcin. Thus, ecalcin has potent ECA activity in vitro and in vivo.

DISCUSSION

We previously reported that antigen-activated T-cells produce an ECA with unique biochemical properties (35, 36).
now describe the isolation, cloning, and expression of this ECA, which is discovered to be a member of the galectin family of lectins. Polyclonal antibodies prepared against the partially purified factor from T-cell conditioned medium were used to screen a cDNA expression library prepared from a T-cell line to obtain candidate cDNAs that encoded the novel ECA (Fig. 1). To confirm that the isolated cDNAs encoded a protein possessing ECA, the cDNAs were expressed in COS cells, and the resulting supernatants from the transfectants were evaluated for their chemotactic activity. Because native ecalectin was isolated from the conditioned medium of a T-cell line and because recombinant ecalectin was isolated from the conditioned medium of a COS cell transfectant, ecalectin can be secreted by two very different types of mammalian cells. Nevertheless, because we were unable to induce insect cells to exocytose recombinant ecalectin, we cannot rule out the possibility that some ecalectin is retained by activated T-cells in vivo. Recombinant ecalectin induced the specific chemotaxis of eosinophils (Figs. 6 and 7). On a weight or molar basis, ecalectin exhibited an ECA that is approximately one-half as potent as that of eotaxin-1. Native ecalectin was isolated from a human T-cell line, whereas recombinant ecalectin was isolated from both COS cells and insect cells. Only one silver-stained protein was detected after insect cell-derived ecalectin was purified by lac-tose affinity chromatography (Fig. 5). Although this finding suggests that ecalectin does not require an insect cell-derived cofactor to exert its chemotactic activity, we still cannot rule out the possibility that an unrecognizable low molecular weight peptide, oligosaccharide, or glycolipid stays tightly bound to the ecalectin during the affinity chromatography step or after it is dissolved in medium.

Genomic blot analysis revealed that there are a number of closely related ecalectin-like genes in the human genome (Fig. 2). As assessed by RNA blot analysis, ecalectin (Figs. 3 and 4) is expressed by T-cell-rich lymphoid tissues or cells. However, a relatively high level of the ecalectin transcript was also found in the stomach RNA sample. Patients with chronic gastritis often have large numbers of activated T-cells and eosinophils in their stomach (45). Because the stomach RNA was obtained from a commercial source, it was not possible for us to ascertain whether some of the transcripts in the sample originated from activated T-cells. Nevertheless, because the ecalectin was found in the stomach, as well as in the lung and small intestine (Fig. 3A), it is likely that T-cells are not the only cell type that expresses ecalectin.

A homology search of the GenBank™ protein and nucleotide data bases revealed that STO-2 cell-derived ecalectin is not related to any known cytokine or chemokine. Rather, it is related to the galectin family of carbohydrate-binding proteins (46). Based on a comparison of its deduced amino acid sequence (Fig. 8), ecalectin most closely resembles the proteins designated human (37) and mouse (54) galectin-9. Mouse galectin-9 cDNA was isolated from an embryonic kidney cDNA library, and expression studies revealed that its transcript is particularly abundant in liver. Although the mouse galectin-9 transcript is present in the thymus, in situ hybridization and immunohistochemical analyses revealed that this galectin is expressed primarily by thymus epithelial cells rather than by T-cells (54). Micromolar concentrations of recombinant mouse galectin-9 induced cultured T-cells to undergo apoptosis (55). Because the ecalectin-expressing STO-2 cell line is a T-cell line that is not undergoing apoptosis and because the ecalectin transcript is not abundant in human liver (Fig. 3A), either ecalectin is not the human homolog of mouse galectin-9 or this
Galectins differ from Ca²⁺-dependent C-lectins. Galectins have conserved sequences that enable them to interact with β-galactoside residues. As noted in Fig. 7, those residues thought to be the most important for carbohydrate binding in galectin-2 (57) are present in ecalectin. Galectin-1 and galectin-2 are dimers, whereas the other galectins are monomers. Galectin-1, -2, -3, -5, and -7 have a single carbohydrate-binding domain, whereas galectin-4, -8, and -9 have two homologous carbohydrate-binding domains. In this regard, ecalectin resembles the latter family of galectins. Recombinant ecalectin readily binds to a lactose column (Fig. 5, C and D), as do other galectins. How ecalectin selectively induces eosinophil chemotaxis remains to be determined at the molecular level. However, the finding that ecalectin has two putative domains that both bind carbohydrate suggests that this galectin might function as a bridging protein interacting with two or more galactoside-containing molecules on the surface of the eosinophil. Because ecalectin has four Cys residues (Fig. 1), it could have two disulfide bonds. However, structural analysis of crystallized galectin-2 (57) suggests that these residues do not form disulfide bonds.

Because all galectins lack a hydrophobic signal peptide, they are often found in the cytosol. It has been proposed that galectins have intracellular functions. However, galectins can reside on the surface of a cell and often are spontaneously secreted by a mechanism that has not been deduced. For example, galectin-3 (49) is the Mac-2 antigen found abundantly expressed on the surface of inflammatory macrophages (58). When phag-
eral blood mononuclear cells are exposed to antigen, ecalectin mRNA increases dramatically (Fig. 5), and the amount of ECA recovered in the conditioned medium increases accordingly (35). Exocytosis of ecalectin could occur via a pathway that does not involve post-translational modification of the protein. However, ecalectin has two sites in its amino terminus at residues 27 and 57 that might be susceptible to IL-1β-converting enzyme (59). Thus, some ecalectin may be released into the conditioned medium by the same proteolytic mechanism that T-cells use to generate soluble IL-1β.

Many galectins are developmentally regulated (46). An ecalectin-like expressed sequence tag has been identified in Jurkat T-cells. However, our finding that the steady-state level of ecalectin mRNA is low in human peripheral blood mononuclear cells (Fig. 4) until these cells are activated with antigen may explain why there has been no published in depth report of ecalectin expression in a T-cell line. Although members of the large galectin family are expressed in numerous cell types, some galectins exhibit immunoregulatory activities, and some galectins are expressed by certain hematopoietic cells. Galectin-1, expressed in abundance in muscle, can induce T-cells to undergo apoptosis (60). Galectin-3 has been localized in macrophages, basophils (61), eosinophils (62), and mast cells (61). Immunoelectron microscopic studies have indicated that much of the galectin-3 found in a mast cell is granule-associated. Because it readily binds to the oligosaccharides attached to both IgE and its receptor, galectin-3 can induce mast cells to degranulate (63). Galectin-3 prevents T-cells from undergoing apoptosis (64), induces neutrophils to increase their production of superoxide (65) and to adhere to laminin (66), and potentiates the production of IL-1 by human monocytes (67). Because both galectin-1 and galectin-3 bind to laminin, these two galectins could influence cell proliferation, cell-cell interactions, and cell-matrix interactions in vivo. Erythrocytes express galectin-5, which

**Fig. 6.** ECA activity of recombinant ecalectin. In the in vitro assay used in these experiments, a porous membrane separates the top chamber that contains human eosinophils from the bottom chamber that contains medium with or without an ECA. After the incubation, the membrane that separates the two compartments in each well was removed and stained with Diff-Quik. Depicted in A is the stained side of the membrane that faces the bottom chamber of cells exposed to conditioned medium from a mock transfectant (left panel), conditioned medium from an ecalectin transfectant of COS cells (center panel), or culture medium supplemented with an optimal amount of eotaxin-1 (right panel). The colored cells are the eosinophils that had migrated through the black and white holes in the membrane. Depicted in B are the quantitative chemotaxis data from multiple experiments using insect cell-derived ecalectin that had been purified by lactose affinity chromatography. In these experiments, the ECA activity of ecalectin was similar to that obtained with an optimal dose of RANTES and IL-5 and was approximately one-half of that obtained with an optimal dose of eotaxin-1. The number of eosinophils that had migrated through the membrane is indicated on the y axis.

**Fig. 7.** Analysis of the chemotactic activity of recombinant human ecalectin for neutrophils, lymphocytes, and monocytes. The ability of purified, insect cell-derived ecalectin to attract peripheral blood neutrophils (A), lymphocytes (B), and monocytes (C) in the in vitro assay was determined. PBS was used as a negative control in each assay. IL-8, lymphotactin, and MCP-1 were used as positive controls for the chemotaxis of neutrophils, lymphocytes, and monocytes, respectively. The numbers of neutrophils, lymphocytes, and monocytes that had migrated through the membranes are indicated on the y axis. The depicted results are the mean ± S.D. of an experiment in which each assay was carried out in triplicate.
exhibits weak agglutinin activity (51).

While our study was in progress, galectin-9 was identified during an analysis of the antigens that are selectively expressed by a patient with Hodgkin’s lymphoma (37). Although the cellular origin of the galectin-9 transcript was not identified in the patient’s tumor, galectin-9 mRNA or a related transcript also was found to be expressed in an undefined, peripheral blood leukocyte. Hodgkin’s disease, which accounts for nearly 1% of all cancers in United States, is a neoplastic disorder primarily involving lymphoid tissue (68). Neoplastic Reed-Sternberg cells are found in affected tissue and are surrounded by a variable inflammatory infiltrate of B-cells, histiocytes, plasma cells, stromal cells, neutrophils, T-cells, and eosinophils. Although different types of Hodgkin’s lymphoma have been identified, eosinophils regularly appear in those patients with the “nodular sclerosis” form of the disease. Interestingly, the galectin-9 transcript was isolated from a cDNA library prepared with mRNA isolated from a patient with the “nodular sclerosis” form of Hodgkin’s lymphoma (37). While it has been proposed that the increased number of eosinophils in this subtype of Hodgkin’s lymphoma is primarily a consequence of increased production of IL-5 and/or granulocyte-macrophage colony-stimulating factor by the Reed-Sternberg cells (19, 21, 68, 69), galectin-9 and/or its variant ecalectin could work in concert with the two viability-enhancing cytokines to cause the prominent eosinophilia. If galectin-9 and ecalectin are allelic variants of the same gene, galectin-9 probably originates, in part, from the antigen-activated T-cells present in Hodgkin’s lymphoma. Whatever its mechanism of action at the molecular level, ecalectin appears to be a newly recognized factor by which antigen-activated T-cells regulate eosinophil extravasation into tissues. Because the eotaxins are expressed primarily by epithelial and endothelial cells rather than by T-cells (3), ecalectin could be the dominant ECA expressed during certain T-cell-mediated inflammatory reactions in humans.

Acknowledgments—We thank Drs. Takashi Yokota, Hisao Masai, Tastuo Kinashi, and Maiko Fukuoka for valuable suggestions and Junko Ohmoto for technical assistance.

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J. Biol. Chem. 1998, 273:16976-16984.
doi: 10.1074/jbc.273.27.16976

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