A novel galectin cDNA (galectin-14) was cloned from ovine eosinophil-rich leukocytes by low stringency reverse transcriptase-PCR and cDNA library screening. Data base searches indicate that this gene encodes a novel prototype galectin that contains one putative carbohydrate recognition domain and exhibits most identity to galectin-3/ecalectin, a potent eosinophil chemotactic. The sugar binding properties of the recombinant molecule were confirmed by a hemagglutination assay and lactose inhibition. The mRNA and protein of galectin-14 are expressed at high levels in eosinophil-rich cell populations. Flow cytometry and cyto spot staining demonstrate that the protein localizes to the cytoplasmic, but not the granular, compartment of eosinophils. In contrast, galectin-14 mRNA and protein were not detected in neutrophils, macrophages, or lymphocytes. Western blot analysis of bronchoalveolar lavage fluid indicates that galectin-14 is released from eosinophils into the lumen of the lungs after challenge with house dust mite allergen. The restricted expression of this novel galectin to eosinophils and its release into the lumen of the lung in a sheep asthma model indicates that it may play an important role in eosinophil function and allergic inflammation.

The immune response of mammals to multicellular parasite infections and allergens is characterized by the recruitment of eosinophils (1, 2). The role eosinophils play in both parasite infections and allergic reactions remains controversial, and little is known of the specific function of eosinophil constituents in combating multicellular parasites or exacerbating allergic responses. The presence of eosinophils and eosinophil-derived products in the respiratory tract does, however, correlate with the pathological manifestations of allergic asthma (2–4).

One of the limitations in the study of eosinophils is the scarcity of this cell population in normal individuals and the difficulty in obtaining sufficient numbers of unmanipulated cells from allergic tissues. Sheep offer a unique experimental system in which large numbers of eosinophils can be obtained using the relatively non-invasive procedure of mammary infusion of allergens followed by “milking” of the mammary gland to obtain the inflammatory cells recruited into the lumen (5, 6). Using this experimental system, the present study describes the identification of a novel galectin (galectin-14) specifically expressed by eosinophils. The expression of galectin-14 was up-regulated in the lung tissue of sensitized sheep challenged with house dust mite extract (HDM), and the protein was released into the bronchoalveolar lavage (BAL) fluid.

Galectins are carbohydrate binding proteins that have been increasingly implicated in both adaptive and innate immune responses. The eosinophil-specific expression of galectin-14 and its secretion into the lumen of the lung in a sheep asthma model indicates that it may play an important role in regulating the activity of eosinophils during allergic responses and further highlights the importance of carbohydrate binding proteins during inflammation.

**EXPERIMENTAL PROCEDURES**

All experimental animal procedures and collection of tissues and cells were approved by the Animal Experimental Ethics Committee of the University of Melbourne.

**Collection of Mammary Lavage (MAL) Samples**—To induce eosinophil migration into the mammary gland, mature non-lactating Merino ewes were primed every 2 weeks by intramammary infusions of 1 mg of solubilized house dust mite extract (HDM, Dermatophagoides pteronyssinus, Commonwealth Serum Laboratories Ltd., Melbourne, Victoria, Australia), rested for 3–4 weeks and challenged with an intramammary infusion of 1 mg of solubilized HDM. MAL was collected 2 days post-HDM challenge by infusion of sterile pyrogen-free saline (PFS, Baxter Healthcare Pty. Ltd., New South Wales, Australia) followed by milking of the gland as described previously (3, 4). Cells were pelleted by centrifugation and washed in PFS. The proportion of eosinophils in the leukocyte suspensions, as determined by Giemsa-stained cyto spots, varied from 75 to 90%.

Other sheep received a single intramammary infusion of lipopolysaccharide, and MAL cells were collected at 24 h and 5 days, which results in an initial influx of predominantly neutrophils (24 h), followed by macrophage infiltration at day 5 (3, 7).

**Collection of Lung Tissue and Bronchoalveolar Lavage (BAL) Samples**—4 to 5-month-old parasite-free female merino-cross lambs were sensitized by three subcutaneous injections of 50 μg of HDM, solubilized in PFS with aluminum hydroxide as adjuvant (1:1). Sheep that showed a high HDM-specific IgE serum response were challenged 2–3 weeks later with 1 mg of solubilized HDM, in the lower left lung lobe using a fiber optic bronchoscope (Pentax FG-H11003, 5.5 mm OD). The

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† The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AF443208.

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1 The abbreviations used are: HDM, house dust mite extract; BAL, bronchoalveolar lavage; MAL, mammary lavage; PFS, pyrogen-free saline; GST, glutathione S-transferase; mAb, monoclonal antibody; TF, transcription factor; STAT, signal transducer and activator of transcription; CRD, carbohydrate recognition domain; MALDI, matrix-assisted laser desorption ionization; RT, reverse transcriptase; BSA, bovine serum albumin; UTR, untranslated region; PBS, phosphate-buffered saline.
right lung lobe of the same sheep was challenged with PFS only as a control. BAL samples were collected from each challenge and control lung site before and 6–48 h post-challenge, by gently adding and aspirating 5 ml of PFS through the bronchoscope port. Sheep were sacrificed, and lung tissue samples were collected after the final BAL sample collections (~48 h post-challenge) for histology. Cells within the BAL were quantified using a Neubauer hemacytometer, and eosinophil numbers were determined on Giemsastained cytopsots.

Larger BAL leukocyte populations required for RNA preparation were collected from whole lung lavage of left and right lung lobes by aspirating 5 ml of PFS through the bronchoscope port. Sheep were given intraperitoneal injections of Freund’s adjuvant. Spleen cells from immune mice were fused with NS-1 myeloma cells using 50% polyethylene glycol 4000 (Merck, Darmstadt, Germany), and supernatants screened for galectin-14 binding by enzyme-linked immunosorbent assay. Positive hybridomas were cloned by limiting dilution and supernatants were separated by 12.5% SDS-PAGE and transferred to m nitrocellulose membranes (MSI, Melbourne, Australia) by elec troblotting at 100 V for 1 h. Membranes were incubated with mAb against galectin-14 (ovalbumin) and the strongest of these (clone 1:2) was used in all subsequent studies.

**Production of Galectin-14 Monoclonal Antibodies—BALB/c mice were given intraperitoneal injections of ~5 μg of cleaved and purified recombinant galectin-14 once a month for 3 months, initially in complete Freund’s adjuvant and subsequently in incomplete Freund’s adjuvant. Spleen cells from immune mice were fused with NS-1 myeloma cells using 50% polyethylene glycol 4000 (Merck, Darmstadt, Germany), and supernatants were screened for galectin-14 binding by enzyme-linked immunosorbent assay. Positive hybridomas were cloned by limiting dilution at least three times before being converted to DM10 media alone. Ascitic fluid was produced by giving pristine primed BALB/c mice an intraperitoneal injection of 1 × 10⁶ hybridoma cells. Four mAbs were generated that showed negative reaction with another recombinant ovine galectin (Gogall1 (11)), and the strongest of these (clone 1:2) was used in all subsequent studies.**

**Western Blot Analysis—Proteins from recombinant protein preparations, leukocyte protein preparations, or cell-free MAL and BAL supernatants, were separated by 12.5% SDS-PAGE and transferred to 0.45-μm nitrocellulose membranes (MSI, Melbourne, Australia) by electroblotting at 100 V for 1 h. Membranes were incubated with mAb supernatant for 2 h at room temperature, followed by incubation with secondary antibody, horseradish peroxidase-conjugated rabbit anti mouse Ig (Dako, Carpinteria, CA), for at least 1 h at room temperature. Signals were detected using 1.5 mM 3,3'-diaminobenzidine tetrahydrochloride (Sigma Chemical Co.) or ECL (Amersham Biosciences, Inc.).**

**Immunocytochemistry—For cytopsots, 1 × 10^⁶ cells were added to a cytospin chamber and centrifuged at ~900 g for 5 min. The slides were air dried and fixed in 95% ethanol for 10 min. Endogenous peroxidase was quenched by submerging slides in 1.5% H₂O₂/ PBS for 10 min. Slides were incubated with mAb for 1–2 h at room temperature in a humidified chamber, followed by the secondary antibody, for 1 h. Conjugate binding was detected by incubating slides with 3,3'-diaminobenzidine tetrahydrochloride for 10 min at room temperature. The slides were counterstained with hematoxylin and eosin Y.
Fig. 1. Nucleotide and predicted amino acid sequence of ovine galectin-14. Three putative casein kinase II sites and two putative protein kinase C sites are shown (dark and light shading, respectively). A putative N-glycosylation site is underlined, and a Cbl-2-like motif is double underlined. RT-PCR primers used to amplify the cDNA and confirm the coding region from three individual animals are boxed. Putative binding sites for transcription factors within the untranslated regions are also shown (TCFII and STAT5). These putative binding sites were detected using MatInspector V2.2, available at www.gsf.de/cgi-bin/matsearch.pl. Please note, the untranslated regions have not been confirmed in multiple animals, but have been confirmed in multiple cDNA from one cDNA library.

Tissues were fixed in 10% neutral-buffered formalin and processed to paraffin. Sections were pre-blocked with normal sheep serum/PBS for 20 min prior to immunohistochemical staining as above.

Flow Cytometry—Cells were processed for surface expression of galectin-14 using standard procedures (3, 4). For intracellular staining, cells were washed twice in ice-cold PBS then fixed in 4% formaldehyde for 25 min at room temperature. Cells were resuspended in wash buffer (1% BSA/0.05% azide/PBS) and transferred in 50-μl aliquots to a 96-well V-bottomed plate. Following centrifugation, 100 μl of permeabilization buffer (wash buffer supplemented with 0.5% saponin; Sigma) was added to each well, and the cells were incubated for 10 min. Primary and secondary antibody incubations were performed in 0.5% saponin at room temperature. Cells were incubated with 50 μl of the primary antibody for 20 min, washed twice with permeabilization buffer, resuspended in 10 μl of 10% normal sheep serum/0.1% saponin/PBS, and incubated for 10 min, followed by incubation with phycoerythrin-conjugated sheep anti-mouse Ig (Silenus) for 30 min. Cells were then washed twice with permeabilization buffer, once with wash buffer and resuspended in PBS for analysis on a FACSCalibur instrument (Becton-Dickinson, Mountain View, CA) using CellQuest software (Becton-Dickinson).

Hemagglutination Assays—Trypsin-treated, glutaraldehyde fixed rabbit erythrocytes were prepared and tested in an agglutination assay according to the method of Nowak et al. (12). Agglutination assays were performed in 96-well V-shaped microtiter plates with serial 2-fold dilutions of samples in 25 μl of DES (0.15 M NaCl, 2 mM EDTA, 2 mM dithiothreitol), 50 μl of 0.5% (w/v) BSA in 0.15 M NaCl and 25 μl of a 4% suspension of rabbit erythrocytes. The plates were shaken vigorously for 30 s, and agglutination was read after the plates had stood at room temperature for 1 h. Agglutinated erythrocytes formed a “mat” on the bottom of the well. For assessment of the inhibitory effects of saccharides, lactose, galactose, and N-acetyl-glucosamine were serially diluted in 25 μl of 0.15 M NaCl prior to the addition of recombinant protein in DES, 25 μl of 1% (w/v) BSA in 0.15 M NaCl and 4% erythrocytes in PBS. The minimum concentration of GST-galectin-14, which gave mat formation (0.28 μM) was used in the saccharide inhibition assay.

RESULTS

Isolation of a Novel Galectin cDNA—While screening for cDNA clones that were differentially expressed in fresh and cultured eosinophil-rich mammary lavage (MAL) cells, a partial cDNA clone of 325 bp was isolated showing similarity to the potent human eosinophil chemotactic ecalectin/galectin-9. Northern blot analysis confirmed that this clone was expressed at relatively high levels by the eosinophil-rich leukocyte population. Hence an eosinophil-rich MAL cell cDNA library was screened to isolate the full-length clone. Literature and nucleotide data base searches indicate that this molecule is a galectin but does not show enough identity to known galectins to be classified as the sheep homologue. This galectin can therefore be classified as a novel galectin, and, as it is the fourteenth

| Species | Name | Amino acid identity |
|---------|------|---------------------|
| Rat     | Galectin-9      | 58.00               |
| Human   | Galectin-9/ecalectin | 57.33         |
| Mouse   | Galectin-9      | 55.33               |
| Mouse   | Galectin-9      | 41.45               |
| Human   | Galectin-4      | 40.13               |
| Human   | Galectin-8      | 38.56               |
| Porcine | Galectin-4      | 38.16               |
| Human   | Galectin-7      | 29.93               |
| Rat     | GRAFIN          | 27.54               |
| Ovine   | Galectin-1      | 27.34               |
| Rat     | Galectin-5      | 26.85               |
| Human   | Galectin-10     | 24.65               |
| Human   | Galectin-3      | 24.10               |
| Ovine   | Galectin-11     | 22.70               |
| Pig     | Galectin-2      | 22.66               |

TABLE II Amino acid identities between known galectins and galectin-14
mammalian galectin published in the data bases, we suggest this molecule be called galectin-14.

The entire coding region of galectin-14 (including the stop codon) is 489 bp and encodes a predicted protein of 162 amino acids (Fig. 1). The 5′-untranslated region is only 35 bp and contains a putative binding site for the transcription factor TCF11. The 3′-untranslated region is 260 nucleotides (not including the poly(A) tail) and contains another putative TCF11 binding site as well as a putative signal transducer and activator of transcription (STAT) 5 binding site (Fig. 1).

A PROSITE data base search (13) indicates that the predicted protein contains three potential casein kinase II sites, two putative protein kinase C sites, and a possible N-glycosylation site (Fig. 1). Hydrophobicity plots (obtained from ProtScale, available at expasy.proteome.org.au/cgi-bin/protscale) also indicate that the protein contains four short hydrophobic regions but is not likely to span a membrane.

Both a nucleotide BLAST data base search and a protein BLAST search (using the predicted amino acid sequence) indicate that the clone encodes a novel galectin (Table II and Fig. 2). The nucleotide and predicted protein sequences of galectin-14 demonstrate most identity to ecalectin/galectin-9 (58% and 57% amino acid identity to rat and human galectin-9, respectively; see Table II).

Galectin-14 contains six of the seven residues (His74, Asn76, Arg78, Asp82, Trp95, Glu98; Fig. 2) reported to be important for sugar recognition and binding by galectin-1 (14, 15). Additionally, the amino acid alteration detected in one of these seven

![MAL cell](image1)
![Treated Sheep](image2)
![Controls](image3)

![Galectin-14](image4)

![18S rRNA](image5)
residues is a conservative change (Arg to Lys 100; Fig. 2). Like residues in the CRD (NWGP residues 94–99), the highly conserved monomeric galectin-14 is a monomer (A and B), but after storage at high concentrations recombinant galectin-14 often self-aggregates into oligomers (C). Western blot analysis using galectin-14 mAb was also performed on endogenous proteins prepared from cell suspensions (~2 × 10^6 cells/lane) containing high proportions (>80%) of MAL eosinophils (E), MAL neutrophils (N), lymph node (LN), BAL fluid (L), or BAL macrophages (M) from control lungs, compared with BAL cells containing 5% eosinophils after local HDM challenge (HDM). The far right panel shows the presence of galectin-14 in cell-free MAL fluid of a sensitized sheep before (S) and after (SC) HDM challenge of the mammary gland. The arrow points to the position of monomeric galectin-14. All samples were run under reducing conditions.

Galectin-14, a Novel Eosinophil-specific Galectin

Recombinant galectin-14 was produced as a GST fusion protein in E. coli. During storage, particularly at high concentrations, recombinant galectin-14 appeared to aggregate (Fig. 4). To study expression of the galectin-14 protein, galectin-14 mAbs were raised against cleaved and purified recombinant galectin-14. A mAb with high reactivity for galectin-14 but no cross-reactivity with another ovine galectin (OVGAL11 (11)) was selected and used to study endogenous galectin-14 protein expression.

Eosinophil-rich MAL and BAL cells solubilized in sample buffer were run on SDS-PAGE, transferred to nitrocellulose, and probed with the galectin-14 mAb (Fig. 4). This clearly detected a protein of similar size to recombinant galectin-14 under both reducing and non-reducing conditions (apparent molecular mass ~17 kDa). The expected molecular mass of galectin-14 calculated from the predicted amino acid sequence is only slightly larger (18.2 kDa). In concentrated samples or after storage, higher molecular weight bands could often be observed in both recombinant and endogenous samples, probably due to aggregation. These aggregates did not dissociate even when samples were run on gels under reducing conditions (Fig. 4). Occasionally, higher molecular weight bands were detected by galectin-14 mAb that did not correspond to the predicted mass of oligomers, especially in samples that contain relatively large amounts of monomeric galectin-14 (Fig. 4). These may be the result of post-translational processing of galectin-14 or due to galectin-14 forming stable complexes with other cellular proteins.

In agreement with the Northern blot analysis, Western blots did not, or only weakly, detect galectin-14 in neutrophil- or macrophage-rich cell populations, or in lymph node lymphocytes (Fig. 4). The weak reactions observed in some neutrophil and lymphocyte preparations were probably due to contaminating (1–2%) eosinophils present in these populations detected by the highly sensitive ECL assay, because no staining was observed in these cells on cytospots (not shown).

Detailed examination of cytospots prepared from circulating blood cells (not shown) and eosinophil-rich MAL or BAL cells of HDM-sensitized and challenged sheep (Fig. 5, A and B) confirmed the localization of galectin-14 to eosinophils and not neutrophils or lymphocytes. The galectin-14 staining in eosinophils was patchy and widespread within the cytoplasm, with occasional staining of the nuclei, but did not appear to localize to the granules.

Flow-activated cell sorting analysis detected strong galectin-14 intracellular staining in more than 95% of eosinophils isolated from mammary lavage after allergen challenge. In contrast, no intracellular staining was detected in neutrophils and macrophages, and only weak nonspecific staining in lymphocytes (Fig. 6). The nonspecific nature of the absorbance shift in lymphocytes was confirmed by negative staining of lymphocytes in both cytospots and lymph node sections (not shown). No galectin-14 surface staining was detected on eosinophils or any other class of leukocytes (not shown).

Expression of Galectin-14 in Lung Tissue and Its Release into the Lumen of the Lung after Allergen Challenge—To study galectin-14 expression and release in lung tissue, a sheep asthma model was developed. Sheep were sensitized to HDM and then challenged with HDM or PFS in the left and right lung lobes, respectively. Relatively high levels of galectin-14 mRNA were detected in lung tissue and BAL cells of HDM-sensitized and lung challenged sheep (Fig. 3). There were consistently higher levels of galectin-14 mRNA in the lung tissue and BAL of the left, challenged lung lobe, compared with the samples from the right control lobe (Fig. 3). The level of expression was associated with lung eosinophilia, with the sheep known to have the greatest number of BAL eosinophils (38%) exhibiting the highest levels of galectin-14 mRNA. Weak or no expression was observed in the lungs of control, unchallenged sheep (Fig. 3).

Lung tissue sections of both left and right lung lobes were stained with galectin-14 mAb (Fig. 5, C and D). Numerous positive staining eosinophils were present around the bronchiol-es and blood vessels of the left HDM-challenged lung lobes (Fig. 5C), whereas the right, saline-challenged lungs of the same sheep showed only sporadic eosinophils (Fig. 5D).

Western blot analysis detected galectin-14 in cell-free MAL fluid (Fig. 4), indicating that galectin-14 may be released into the extracellular environment. The presence of galectin-14 in the MAL fluid was associated with eosinophilia and was only detected in the MAL fluid of sheep challenged with allergen (Fig. 4). The identity of this band to galectin-14 was confirmed by peptide mass fingerprinting (MALDI mass spectrometry performed by Australian Proteome Analysis Facility, Sydney) using the predicted amino acid sequences, resulting in a five-peptide match (not shown). To determine if the galectin-14 molecule was also released into the lung, HDM-sensitized sheep were challenged in the left and right lung lobes with HDM and PFS, respectively, and small BAL samples collected from the challenge sites by gentle deposition and aspiration of PFS at different time points. Western blot analysis of cell-free BAL fluid (Fig. 7) clearly detected the presence of galectin-14 protein in the left lung at 24 and 48 h after local HDM challenge, whereas galectin-14 was not detected at any time in the
BAL fluid of the right, control lung lobe. The amount of galectin-14 present in the cell free BAL fluid correlated with the numbers of eosinophils present in the lavage, with the time point corresponding to most eosinophil infiltration (48 h) exhibiting the highest level of galectin-14 release in the BAL fluid (Fig. 7). No eosinophils were observed in the right, control lung lobe at any of the time points.

**Recombinant Galectin-14 Exhibits Hemagglutination Activity**—Fresh cleaved and purified galectin-14 was found to exhibit agglutination activity on rabbit erythrocytes at a minimum concentration of 0.16 μM (not shown). However, this activity was rapidly lost upon storage, even in the presence of reducing agents. In contrast, the agglutination activity of recombinant GST-galectin-14 fusion protein did not deteriorate upon storage, and the GST moiety did not appear to interfere with agglutination. Hence GST-galectin-14 was used in subsequent saccharide inhibition studies. These studies confirmed the agglutination activity of recombinant galectin-14 and demonstrated that this hemagglutination could be inhibited by the addition of sugars (Fig. 8). The presence of 0.28 μM GST-galectin-14 clearly induces hemagglutination, whereas GST alone does not. However, this agglutination is readily inhibited by lactose, with doses as low as 0.78 mM capable of reducing agglutination. The activity could also be inhibited to a lesser extent by galactose and N-acetyl-glucosamine. (Inhibition becoming evident at 12.5 and 50 mM, respectively.)

**DISCUSSION**

A full-length cDNA encoding a novel galectin was isolated from ovine eosinophil-rich leukocyte populations by RT-PCR and cDNA library screening. Previously some galectins have been referred to as carbohydrate binding proteins, S-type lec-
Galectin-14, a Novel Eosinophil-specific Galectin

The predicted protein sequence of galectin-14 demonstrates most similarity to galectin-9/ecalectin (17). However, galectin-14 is not likely to be the ovine homologue of galectin-9/ecalectin, because it demonstrates a different pattern of expression, and the highest overall amino acid identity it exhibits to any galectin-9/ecalectin isoform is only 58%. A comparison between galectin-14 and galectin-9 variants also reveals that there are 23 non-conservative substitutions of residues that are conserved in the galectin-9 sequences cloned from three different species (human, mouse, and rat (18–21)).

Galectins are carbohydrate binding proteins and can be classified as proto, tandem-repeat, or chimera types. Unlike the galectin-9 variants, which are tandem-repeat-type galectins, galectin-14 is clearly a proto-type galectin containing only one CRD. In addition, galectin-14 has an extended NH₂ terminus (12 residues longer than that of the galectin-9 variants). This extended NH₂ terminus is unusual for galectins, currently the only other galectin reported to have an extended NH₂ terminus is galectin-3 (Mac2, epsilon binding protein (22)). However, the NH₂ terminus of galectin-14 is much shorter than that of galectin-3 and does not contain a proline and glycine-rich repetitive sequence, indicating that galectin-14 should not be classified as a chimera-type galectin.

Galectin-14 is expected to exhibit a similar carbohydrate binding specificity to the most well characterized galectins. It contains six of the seven residues reported to be important for sugar recognition and binding by galectin-1 (14, 15), and the amino acid substitution that does occur is a conservative change. Additionally, the arginine residues known to be important for sugar binding, hemagglutination, and eosinophil chemotactic activity of ecalectin/galectin-9 are also conserved in galectin-14 (Arg⁶⁵ and Arg²³⁹ of ecalectin (23)). The chemotactic activity of ecalectin/galectin-9 appears to require both CRDs (23). Hence, galectin-14, which only contains a single CRD, is not likely to exhibit chemotactic activity via a similar mechanism to ecalectin. However, recombinant galectin-14 does exhibit hemagglutination activity, and studies using GST-galectin-14 fusion protein demonstrate that this activity can be inhibited by sugars. This suggests that galectin-14 contains a functional CRD and that it may homodimerize. Recently, it has been shown that phosphorylation regulates the carbohydrate binding activity of galectin-3 (24). It remains to be determined if the putative post-translational processing of galectin-14 also regulates carbohydrate binding specificity.

**FIG. 7.** Western blot analysis of cell-free BAL fluid using galectin-14 mAb. BAL fluid was collected before (0 hr), or 6, 24, or 48 h post-local lung challenge of HDM-sensitized sheep. The left lung lobe was challenged with HDM, and the right lung lobe with sterile PFS as a control. The total number of cells in the BAL fluid and the number of eosinophils present at each time point was calculated (A), and cell-free BAL fluid was probed with galectin-14 mAb (B).

**FIG. 8.** Hemagglutination assay using GST-galectin-14 fusion protein. Agglutination of rabbit erythrocytes was assayed in a 96-well microtiter plate. Assays were conducted in the presence (2, 3, 4, and 5) or absence (1 and 6) of 0.28 μM recombinant GST-galectin-14, the minimum concentration to induce agglutination. Increasing concentrations (0.78–100 mM) of lactose (2), galactose (3), or N-acetyl-glucosamine (4) were added to inhibit agglutination. In the presence of GST-galectin-14 without sugar (5) the rabbit erythrocytes form a “mat” at the bottom of the wells. The assay buffer DES alone (1) and 3 μM rGST (6) were included as negative controls.

tins, S-Lac lectins, galaptins, or simply lectins (L) with a reported molecular weight; however, the current nomenclature is to name all members of this family of β-galactoside binding proteins “galectins,” numbered in sequential order as they are discovered (16). Hence we suggest this molecule be named galectin-14.
Western blot analysis of both recombinant and endogenous galectin-14 indicates that the protein can self-aggregate, which would provide a mechanism through which galectin-14 could cross-link binding partners such as mucins (see below). It has previously been shown that galectins 1, 2, and 3 homodimerize, and galectin-3 can self-aggregate into larger complexes (25, 26). However, how galectins self-associate and what effect this has on their function has been difficult to ascertain. In the case of galectin-3 both the NH₂ terminus and CRD may be involved in self-association, but the presence of ligand inhibits self-association through the CRD. The extended NH₂ terminus of galectin-14 does not contain a repetitive sequence like that of galectin-3, residues known to be necessary for galectin-1 homodimerization, nor any obvious motifs that may be involved in self-association such as a coil-coil domain. The galectin-14 aggregates are also unlikely to occur due to disulfide bonds, because these aggregates appeared to be resistant to reducing agents.

The distribution of galectin-14 mRNA and protein indicates that galectin-14 is specifically expressed by eosinophils and not other leukocytes, although expression on the rare basophil cannot at present be excluded. In contrast to the previously isolated ovine galectin-11, which was induced in epithelial tissue after allergic stimulation of the gastrointestinal tract (11), expression of galectin-14 seems to be constitutive in all eosinophils examined (blood, tissue, MAL, and BAL). The only other galectin known to be expressed at high levels in eosinophils is galectin-10, also known as the Charcot-Leyden crystal (27). However, galectin-14 exhibits little identity to this galectin (25% amino acid identity) and is not likely to have similar functions.

Immunocytochemistry and flow cytometry indicate that galectin-14 is localized to the cytoplasm (and possibly nuclei) of eosinophils and is not found on the cell surface of any leukocytes. This differs from galectin-3, which is expressed at high levels on the surface of macrophages (28), or galectin-1, which is released from thymic epithelial cells and then binds to carbohydrate motifs on the surface of thymocytes (29).

Like all other known galectins, galectin-14 lacks an obvious targeting peptide but appears to be externalized. Possibly, as postulated for galectins 1 and 3, galectin-14 may be released into the extracellular environment in vesicles after accumulating under the plasma membrane (30, 31). Once released at sites of inflammation, there are many carbohydrate motifs galectin-14 may interact with, including the highly glycosylated mucins, whose composition and expression is known to be altered in the lung lavage during allergic responses such as asthma (32, 33). Galectin-14 binding partners may also include extracellular matrix components such as laminin, which are already known to be bound by other galectins (34–36), and could modulate cell migration.

In addition to being up-regulated in the lung after allergen-challenge, galectin-14 mRNA and protein expression was also found to be up-regulated during helminth infections (not shown). Once released from eosinophils at the sites of infection galectin-14 may interact with carbohydrate motifs of multicular parasites and regulate the inflammation induced by such infections. Identification of foreign carbohydrate motifs appears increasingly important in eliciting appropriate immune responses (37).

Like other galectins, galectin-14 is likely to have both extracellular and intracellular functions. These intracellular functions may include the regulation of apoptosis. The galectin-14 sequence contains a Bcl-2-like motif shared with galectins 1 and 3. Recently it has been suggested that galectins 1 and 3 may regulate apoptosis through this domain (38–41). In particular, it has been postulated that Bcl-2 and galectin-3 may heterodimerize through this motif to inhibit Fas-antibody-mediated apoptosis (38–40). The other galectins have similar but not identical sequences. It remains to be determined if galectin-14 may regulate eosinophil apoptosis after their recruitment into inflammatory tissues or airways.

Some nuclear staining with galectin-14 mAb has been observed in eosinophils. The nuclear staining was variable and may depend on the nature of the stimuli or different stages of the cell cycle. Galectins 1, 3, 10, and 11 are all known to localize simultaneously to the nucleus and cytoplasm under various conditions (11). The role galectins 10 and 11 play in the nucleus remains unknown, but it has been postulated that galectins 1 and 3 regulate pre-mRNA splicing (11). The role galectins 10 and 11 play in the nucleus may depend on the nature of the stimuli or different stages of the cell cycle.
Galectin-14, a Novel Eosinophil-specific Galectin

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