The Journal of Immunology

Human Galectin-3 Is a Novel Chemoattractant for Monocytes and Macrophages

Hideki Sano, Daniel K. Hsu, Lan Yu, John R. Apgar, Ichiro Kuwabara, Tohru Yamanaka, Mitsuomi Hirashima and Fu-Tong Liu

J Immunol 2000; 165:2156-2164; ;
doi: 10.4049/jimmunol.165.4.2156
http://www.jimmunol.org/content/165/4/2156

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

Human Galectin-3 Is a Novel Chemoattractant for Monocytes and Macrophages[1]

Hideki Sano,* Daniel K. Hsu,* Lan Yu,* John R. Apgar,† Ichiro Kuwabara,* Tohru Yamanaka,‡ Mitsuomi Hirashima,§ and Fu-Tong Liu[2]*

Galectin-3 is a β-galactoside-binding protein implicated in diverse biological processes. We found that galectin-3 induced human monocyte migration in vitro in a dose-dependent manner, and it was chemotactic at high concentrations (1.0 μM) but chemokinetic at low concentrations (10–100 nM). Galectin-3-induced monocyte migration was inhibited by its specific mAb and was blocked by lactose and a C-terminal domain fragment of the protein, indicating that both the N-terminal and C-terminal domains of galectin-3 are involved in this activity. Pertussis toxin (PTX) almost completely blocked monocyte migration induced by high concentrations of galectin-3. Galectin-3 caused a Ca^{2+} influx in monocytes at high, but not low, concentrations, and both lactose and PTX inhibited this response. There was no cross-desensitization between galectin-3 and any of the monocyte-reactive chemokines examined, including monocyte chemotactic protein-1, macrophage inflammatory protein-1α, and stromal cell-derived factor-1α. Cultured human macrophages and alveolar macrophages also migrated toward galectin-3, but not monocyte chemotactic protein-1. Finally, galectin-3 was found to cause monocyte accumulation in vivo in mouse air pouches. These results indicate that galectin-3 is a novel chemoattractant for monocytes and macrophages and suggest that the effect is mediated at least in part through a PTX-sensitive (G protein-coupled) pathway. *The Journal of Immunology,* **2000, 165: 2156–2164.**

Galectin-3 is a 26-kDa β-galactoside-binding protein belonging to the galectin family, which now contains >10 members (1–6). This protein is composed of a carboxyl-terminal carbohydrate recognition domain (CRD)[3] and amino-terminal tandem repeats (7–9). Galectin-3 normally distributes in epithelia of many organs and various inflammatory cells, including macrophages, as well as dendritic cells and Kupffer cells (10). The expression of this lectin is up-regulated during inflammation (10), cell proliferation (11, 12), and cell differentiation (13, 14) and through *trans*-activation by viral proteins (15). Its expression is also affected by neoplastic transformation: up-regulated in certain types of lymphomas (15, 16) and thyroid carcinoma (17, 18), while down-regulated in other types of malignancies, such as colon (19, 20), breast (21), ovarian (22), and uterine (23) carcinomas. Recently, it has been reported that the expression of this lectin has a strong correlation with the grade and malignant potential of primary brain tumors (24). Increased galectin-3 expression has also been noted in human atherosclerotic lesions (25). These findings suggest that galectin-3 expression is affected during these physiological and pathological responses.

Galectin-3 has been shown to function through both intracellular and extracellular actions. Related to its intracellular functions, galectin-3 has been identified as a component of heterogeneous nuclear ribonuclear protein (hnRNP) (26), a factor in pre-mRNA splicing (27), and has been found to control cell cycle (28) and prevent T cell apoptosis; the latter is probably mediated through interaction with the Bcl-2 family members (29, 30). On the other hand, this protein, which is secreted from monocytes/macrophages (31, 32) and epithelial cells (33, 34), has also been demonstrated to function as an extracellular molecule in activating various types of cells, including monocytes/macrophages (13), mast cells (35, 36), neutrophils (37), and lymphocytes (15, 38). Galectin-3 has been shown to mediate cell-cell and cell-extracellular matrix interactions (39–41).

Recently, it has been shown that galectin-9, another member of the galectin family with two CRDs, is a selective chemoattractant for eosinophils (42). The activity is dependent on both CRDs, suggesting that cross-linking of cell surface molecules is involved in the chemoattraction (43). Galectin-3 is known to form dimers through the amino-terminal nonlectin domain and thus has the potential to cross-link appropriate cell surface glycoproteins (9). Therefore, it is conceivable that galectin-3 also has similar activity for certain cell types.

In the present study we focused on an extracellular function of galectin-3 on monocyte/macrophage, because 1) galectin-3 is known to bind to the cell surfaces of monocytes/macrophages (44), and 2) high expression of galectin-3 is seen in human and rat lungs (45, 46), where macrophages are one of the dominant cell types. Moreover, we recently found that macrophage recruitment in peritonitis is attenuated in galectin-3-deficient mice (47). As inflammatory macrophages are known to result from the recruitment of peripheral blood monocytes, these facts led us to examine whether galectin-3 attracts monocytes and macrophages. We found that

*Division of Allergy, La Jolla Institute for Allergy and Immunology, San Diego, CA 92121; †Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, CA 92037; ‡First Department of Internal Medicine, Kumamoto University School of Medicine, Kumamoto, Japan; and §Department of Immunology and Immunopathology, Kagawa Medical School, Kagawa, Japan

Received for publication January 11, 2000. Accepted for publication May 31, 2000.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This is Publication 353 from La Jolla Institute for Allergy and Immunology. This work was supported by National Institutes of Health Grants RO1AI-20958 and RO1AI-39620. The General Clinical Research Center at The Scripps Research Institute is supported by National Institutes of Health Grant MO1RR00833.

[2] Address correspondence and reprint requests to Dr. Fu-Tong Liu, La Jolla Institute for Allergy and Immunology, Division of Allergy, 10355 Science Center Drive, San Diego, CA 92121. E-mail address: ftliu@liai.org

[3] Abbreviations used in this paper: CRD, carbohydrate-recognition domain; galectin-3C, the C-terminal domain fragment of galectin-3; MCP-1, monocyte chemotactic protein-1; MIP-1α, macrophage inflammatory protein-1α; PTX, pertussis toxin; SDF-1α, stromal cell-derived factor-1α.

0022-1767/00/$02.00

galectin-3 induced migration of monocytes and macrophages both in vitro and in vivo through a pertussis toxin (PTX)-sensitive pathway. Our present study suggests a novel role for galectin-3 in inflammation and malignancy through its ability to recruit inflammatory cells and APCs into the affected sites to modify the biological responses. These results together with the finding that galectin-9 is a potent eosinophil chemoattractant suggest that galectins are a new class of chemoattractants.

Materials and Methods

Materials

Recombinant human galectin-3 (48), the C-terminal domain fragment of galectin-3 (galectin-3C) (29), a mouse mAb against galectin-3 (B2C10) (49), and mouse monoclonal anti-DNP IgG1 (50) were prepared as described previously. Recombinant MCP-1, MIP-1α, and SDF-1α were obtained from PeproTech (Rocky Hill, NJ). Indo-1/AM was purchased from Molecular Probes (Eugene, OR). HBSS and RPMI 1640 were purchased from Life Technologies (Grand Island, NY). Ficoll Paque and Percoll solution were obtained from Amersham Pharmacia Biotech (Uppsala, Sweden). Unless otherwise stated, all other reagents were purchased from Sigma (St. Louis, MO).

Preparation of human monocytes

Human monocytes were purified from venous blood of normal volunteers essentially as described previously (51). In brief, after erythrocytes were sedimented by addition of 6% dextran saline solution (1 part to 5 parts heparinized blood), and the leukocytes were collected, washed twice, and resuspended in Ca^{2+} and Mg^{2+}-free HBSS containing 5% autologous serum. Mononuclear cells were acquired by centrifugation of the leukocyte suspension on Ficoll Paque at 800 $\times$ *g* for 15 min. The cells were resuspended in RPMI 1640 containing 10% autologous serum and allowed to adhere to sterile tissue culture plates for 30 min in a humidified incubator at 5% CO_2 and 37°C. After incubation, nonadherent cells were removed by washing the plates three times with PBS at 37°C. Greater than 98% of the adherent cells showed the characteristic appearance of monocytes when examined by light microscopy following Wright staining or neutral red staining. To detach and harvest the adhered monocytes, 1 mM EDTA-PBS containing 5% serum was added, and the plates were incubated on ice for 30 min. The monocytes were washed twice with HBSS and resuspended in RPMI 1640 with 0.1% autologous serum for the migration assay. The viability of monocytes was determined by trypan blue exclusion and was >98%. In some experiments monocytes were purified according to another method using a Percoll discontinuous gradient described previously (52). No difference was noted in the purity and viability of the cells prepared by these two different methods.

Preparation of human cultured peripheral blood macrophages and alveolar macrophages

Human macrophages were obtained by culturing peripheral blood monocytes in vitro for 7 days as previously described (53). Human alveolar macrophages were obtained from bronchoalveolar lavage fluid according to a previously described protocol (54). The purity of the macrophages was >90%, and the viability was >99%.

Migration assay in vitro

Monocyte migration was examined by using 96-well micro-Boyden chambers with 5 μm pore size filters (Neuro Probe, Gaithersburg, MD) as described previously (55, 56). Briefly, after the indicated concentrations of galectin-3 or MCP-1, in RPMI 1640 were applied to the lower chambers, purified monocyte suspensions (2.5–5.0 $\times$ 10^4/well) were applied to the upper chambers. After incubation of the chambers for 1 h in a humidified incubator at 5% CO_2 and 37°C, the filters were washed once with PBS and processed with Wright stain. The number of monocytes on the bottom side of the filters was counted in 5–10 high power fields. Monocyte migration was calculated from the average numbers of the counted cells and expressed as the percentage of input cells in a well.

In assays using inhibitory reagents, the purified monocytes were pretreated with or without the indicated concentrations of B2C10 (49) or anti-DNP IgG1 (50) as an isotype-matched control mAb, galectin-3C, or PTX at 37°C for 30 min. Then the cells were applied to the upper chambers in the presence of these inhibitors at the same concentrations used in the pretreatment. Galectin-3 or MCP-1 was applied to the lower chambers as described above. In the assays using lactose and sucrose, the sugars were added to the lower chambers at the initiation of the migration assay.

Migration assay in vivo

The mouse air pouch experiments were performed according to a method described previously (57). Briefly, an air pouch was induced on the back of BALB/c mice by injecting 3 ml of air intradermally three times (one time each on 6, 4, and 2 days before the experiments). Then, 1 ml of 0.9% sodium chloride (USP grade saline; Baxter Healthcare, Deerfield, IL) containing 1 μM galectin-3 was injected into the pouch. As positive and negative controls, 100 ng/ml of recombinant MCP-1 and diluent only, respectively, were injected. Four hours afterward, recruited cells were recovered by gently lavaging the pouch with 1 ml of PBS containing 1 mM EDTA. Cell number was determined, and the distribution of leukocyte types was analyzed after cytospin preparation and Wright staining.

Measurement of Ca^{2+} influx in monocytes

Intracellular concentrations of Ca^{2+} were measured using indo-1/AM according to a previously described method (58). Purified monocytes were resuspended in HBSS containing 1 mM Ca^{2+}, 1 mM Mg^{2+}, and 5% autologous serum and incubated with 10 mM indo-1/AM for 45 min at 37°C. The cells were washed once and resuspended in the same buffer, and stimuli and inhibitors were added at the time points specified in the figure legends. Intracellular Ca^{2+} concentration was measured by monitoring light emission at 405 and 485 nm to an excitation wavelength of 355 nm, using an AMINCO-Bowman series 2 luminescence spectrometer (Rochester, NY).

Data analysis

Data are summarized as the mean $\pm$ SD. The statistical examination of the results was performed by the variance analysis using Fisher's protected least significant difference test for multiple comparisons. The analysis of the results from the mouse air pouch experiments was conducted with the Mann-Whitney test. Values of $p < 0.05$ were considered significant.

Results

Galectin-3 induces monocyte migration in vitro

Using a micro-Boyden chamber assay, we found that human recombinant galectin-3 induced monocyte migration in a dose-dependent manner. Galectin-3 significantly increased monocyte migration at concentrations >100 nM compared with diluent and showed a bell-shaped dose-dependent pattern like many chemoattractants (control, 3.54 $\pm$ 2.2%; 100 nM, 6.25 $\pm$ 1.3%; 300 nM, 9.8 $\pm$ 0.33%; 1 μM, 12.4 $\pm$ 1.2%; 3 μM, 21.6 $\pm$ 0.071%; 10 μM, 17.04 $\pm$ 0.28%, $p < 0.05$ vs control; $n = 4$ experiments; Fig. 1). While the difference in the effect between lower concentrations of galectin and control was not statistically significant in these initial experiments, in a number of subsequent ones, 10 nM galectin-3 also significantly increased monocyte migration (control, 4.26 $\pm$

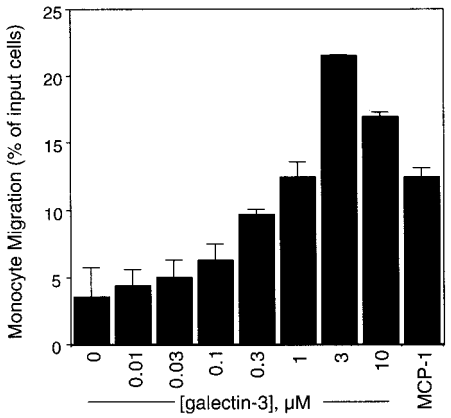

FIGURE 1. The effect of galectin-3 on human peripheral blood monocyte migration in vitro. Various concentrations of galectin-3 (and MCP-1 (100 ng/ml) as a positive control) were applied to the lower chambers, purified monocytes were applied to the upper chambers, and the migration assay was performed as described in *Materials and Methods*. Data are the mean $\pm$ SD of four individual experiments.

1.3%; 10 nM, 7.01 ± 2.1%; $p < 0.001$ vs control; $n = 21$). The effect of 3 μM galectin-3 on monocyte migration was greater than that of human recombinant MCP-1, a strong chemoattractant for monocytes (59), at 100 ng/ml (11.6 nM; Fig. 1), which was determined in dose-response experiments to be the concentration that induced maximum monocyte migration in this assay (data not shown).

To rule out the possibility that the above results were due to contaminating bioactive substances such as heat-stable endotoxins in the recombinant galectin-3 preparations, we conducted experiments using galectin-3 samples pretreated at 100°C for 5 min, which is known to inactivate this lectin (37). These samples did not induce monocyte migration at any of the concentrations used (10 nM to 1 μM; data not shown). Furthermore, we studied the effect of an anti-galectin-3 mAb B2C10, which has been shown to block the lectin activities of galectin-3 (49), on monocyte migration. We found that 10 μg/ml of B2C10, but not an isotype-matched control mAb, completely inhibited monocyte migration induced by galectin-3 at all concentrations examined ($p < 0.05$; $n = 3$; Fig. 2). B2C10 did not affect MCP-1-induced monocyte migration significantly under identical conditions (data not shown). These results indicate that exogenous galectin-3 induces migration of human monocytes in vitro.

Galectin-3 is chemotactic at high concentrations and chemokinetic at low concentrations for monocytes

The observed monocyte migration induced by galectin-3 could be due to a chemotactic or chemokinetic effect. The former means that the cell migration is strictly a consequence of the lectin's chemoattraction activity, whereas the latter implies that the cell migration is due to activation of the cells by the lectin that results in an enhanced movement of the cells in all directions. To differentiate between these two possibilities, we performed a checkerboard analysis. Various concentrations of galectin-3 were applied to the upper and/or lower chambers, and monocyte migration was examined. As shown in Table I and Fig. 3, when 10 or 100 nM galectin-3 was used, no significant difference in monocyte migration was observed regardless of whether the protein was added to the lower chambers or to both chambers. In contrast, when 1 μM galectin-3 was added to both chambers, no significant increase in monocyte migration over the background was observed. These results indicate that the effect of galectin-3 in vitro is chemokinetic at low concentrations (10 and 100 nM), but chemotactic at high concentrations (1 μM).

Table I. *Checkerboard analysis of the effect of galectin-3 on the attraction of human peripheral blood monocytes* in vitro[a]

Below	Above 0 nM	Above 10 nM	Above 100 nM	Above 1000 nM
0 nM	4.23 ± 0.75	7.55 ± 0.79	10.7 ± 0.86	3.30 ± 2.82
10 nM	8.66 ± 0.22	8.88 ± 1.09	11.4 ± 2.11	3.25 ± 3.11
100 nM	9.96 ± 0.72	9.23 ± 2.23	10.5 ± 2.10	4.55 ± 3.69
1000 nM	13.1 ± 1.33	11.5 ± 3.49	12.5 ± 2.87	3.50 ± 2.41

[a] Various concentrations of galectin-3 were applied to the lower chambers, and purified monocytes mixed with various concentrations of galectin-3 were applied to the upper chambers, as described in *Materials and Methods*. Monocyte migration is expressed as the percentage of migrated cells of the total cells. Data are the mean ± SD of four individual experiments.

Both N- and C-terminal domains of galecin-3 are necessary for the monocyte chemoattractant activity

As mentioned above, galectin-3 is composed of a C-terminal lectin domain and an N-terminal nonlectin part. To determine whether the chemoattractant activity of galectin-3 is dependent on its lectin properties, we tested the effect of saccharides on the lectin's induction of monocyte migration. As shown in Fig. 4*A*, 5 mM lactose significantly decreased monocyte migration induced by 10 nM, 100 nM, and 1 μM galectin-3 by 63.8, 71.5, and 57.6%, respectively ($p < 0.05$; $n = 3$). Similarly, 10 mM lactose significantly inhibited the migration by 78, 74.1, and 71.1%, respectively ($p < 0.05$; $n = 3$). These concentrations of lactose did not affect the monocyte migration induced by MCP-1 (100 ng/ml MCP-1, 20.0 ± 3.2%; 100 ng/ml MCP-1 plus 10 mM lactose, 20.2 ± 2.1%; $n = 3$). As a negative control, we also tested the effect of sucrose, which dose not bind to galectin-3. As shown in Fig. 4*B*, sucrose had no significant effect on monocyte migration. These results indicate that the C-terminal lectin domain of galectin-3 is involved in the induction of monocyte migration.

We then examined the effect of a recombinant C-terminal domain fragment of galectin-3 (galectin-3C) on monocyte migration. Monocytes were preincubated with various amounts of galectin-3C for 30 min at 37°C, the mixture was then applied to the upper chambers, and a standard migration assay was performed. As shown in Fig. 5, 1 μM galectin-3C alone did not have any chemokinetic effect on monocytes, but it significantly inhibited cell migration induced by 100 nM and 1 μM galectin-3 by 77.4 and 45.0%, respectively ($p < 0.05$; $n = 3$). Galectin-3C pretreated

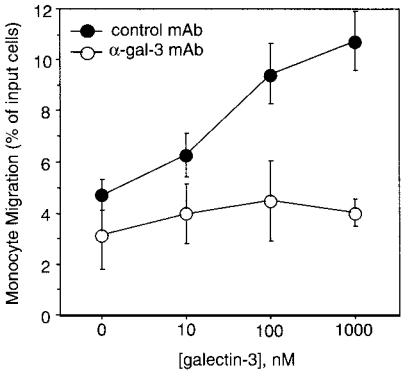

FIGURE 2. Effect of anti-galectin-3 mAb on monocyte migration. After treatment with control (●) or anti-galectin-3 (○) mAb, purified monocytes were added to the upper chambers, and the migration assay was performed as described in *Materials and Methods*. Data are the mean ± SD of three individual experiments.

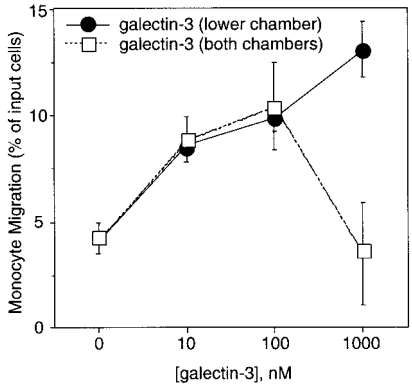

FIGURE 3. Chemotaxis vs chemokinesis in galectin-3-activated monocytes. The data from the checkerboard experiment in Table I are expressed graphically in this figure. ●, monocyte migration when galectin-3 was added only to the lower chambers; □, monocyte migration when equal concentrations of galectin-3 were added to both chambers.

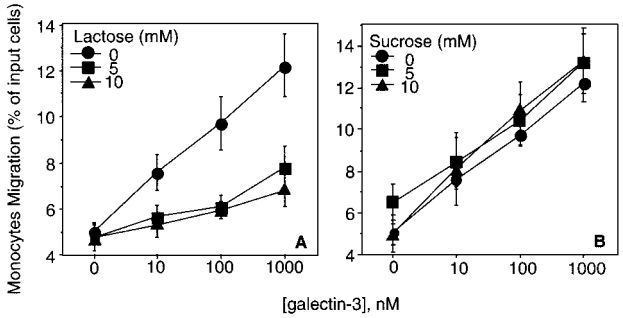

FIGURE 4. Effect of sugars on galectin-3-induced monocyte migration. Various concentrations of galectin-3 were mixed with 0 mM (●), 5 mM (■), or 10 mM (▲) lactose (*A*) or sucrose (*B*) and placed in the lower chambers. Purified monocytes were added to the upper chambers, and a standard migration assay was then performed. Data are the mean ± SD of four individual experiments.

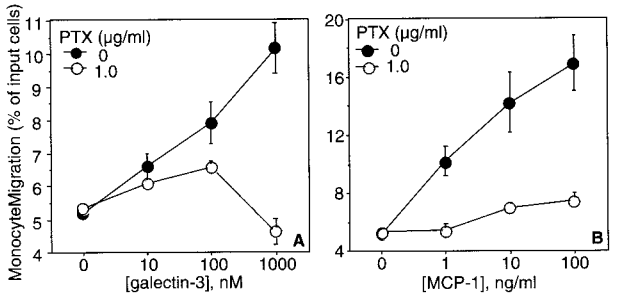

FIGURE 6. Effect of PTX on monocyte migration. After monocytes were treated with PTX, the cells were added to the upper chambers and the migration toward galectin-3 (*A*) or MCP-1 (*B*) was performed as described in *Materials and Methods*. Data are the mean ± SD of four individual experiments.

at 100°C showed no effect on galectin-3-induced monocyte migration (data not shown). We also tested the effect of 100 nM galectin-3C, but no influence on monocyte migration was observed (Fig. 5). These results further confirm the involvement of the lectin domain in the chemoattractant activity and suggest that the N-terminal domain is also necessary for this activity.

Galectin-3 induces monocyte migration by a PTX-sensitive pathway

We tested the possibility that G proteins might be involved in galectin-3-induced monocyte migration using the inhibitor PTX, because it is well known that many chemoattractants, including all chemokines, use G protein-coupled receptors to transduce signals into the cell (60). Before the experiments, we confirmed that 1 μg/ml of PTX did not decrease the viability of monocytes (data not shown). We found that PTX decreased monocyte migration induced by 1 μM galectin-3 by 91.2% ($p < 0.01$; $n = 5$; Fig. 6*A*). However, PTX only partially inhibited monocyte migration induced by 10 or 100 nM galectin-3 ($p = 0.8501$ and 0.3093, respectively; $n = 5$). In contrast, 1 μg/ml of PTX significantly inhibited monocyte migration induced by MCP-1 at all concentrations examined (Fig. 6*B*). These results suggest that a PTX-sensitive G protein-coupled receptor(s) is involved in monocyte migration induced by galectin-3.

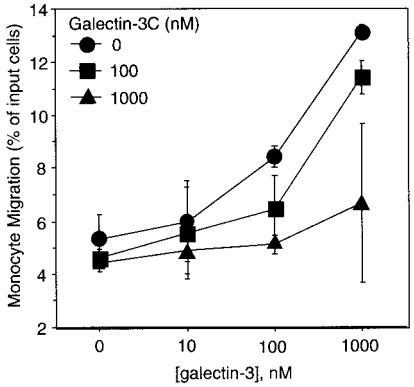

FIGURE 5. Effect of a C-terminal domain fragment of galectin-3 (galectin-3C) on galectin-3-induced monocyte migration. After monocytes were incubated with the indicated concentrations of galectin-3C, the cells were added to the upper chambers, and a standard migration assay was performed. Data are the mean ± SD of four individual experiments.

Galectin-3 increases intracellular calcium concentration by a PTX-sensitive pathway(s)

As mentioned above, galectin-3 can dimerize and cross-link cell surface receptors. This suggests that the reason why galectin-3 is chemotactic is because it is able to activate chemokine receptors. Therefore, to further analyze galectin-3-mediated signaling, we tested the effect of this lectin to induce a Ca^{2+} influx in monocytes, because many chemoattractants are known to cause Ca^{2+} influx. We found that 1 μM galectin-3, but not lower concentrations, induced a Ca^{2+} influx in human monocytes similar to MCP-1 (Fig. 7, *A* and *B*), although the extent of the Ca^{2+} influx caused by the lectin was lower than that caused by the chemokine in all three separate experiments. We found that heat-inactivated galectin-3 did not produce any response (data not shown). The specificity of this activity was also demonstrated by the complete inhibition of galectin-3-induced, but not MCP-1-induced, Ca^{2+} influx by 5 mM lactose, but not sucrose (Fig. 7, *C* and *D*). Furthermore, both the galectin-3- and the MCP-1-induced Ca^{2+} influx were blocked by PTX (Fig.7, *E* and *F*). These results indicate that galectin-3 causes a Ca^{2+} influx, which is probably mediated by a PTX-sensitive G protein coupled receptor(s).

Galectin-3 does not use the presently known chemokine receptors on monocytes to induce Ca^{2+} influx

Among various chemoattractants, the monocyte/macrophage-reactive chemokines, including MCP-1, MIP-1α, and SDF-1α, are known to cause a Ca^{2+} influx in the cells (61–63) by binding to their receptors, such as CCR2/9, CCR1/5/9, and CXCR-4, respectively, all of which are coupled with PTX-sensitive G proteins (60, 64, 65). To determine the possibility that galectin-3 interacts with these receptors to transduce activation signal(s) into monocytes, we performed Ca^{2+} influx experiments to study cross-desensitization. This method is known to be useful in identifying the usage of the chemoattractant receptors, although cross-desensitization occurs at multiple levels and can affect signals mediated by other receptors (66, 67). We found that all these chemokines (100 ng/ml) induced a Ca^{2+} influx in human monocytes (Fig. 8, *A*, *C*, and *E*). We also observed that such responses were desensitized by pretreatment with the same, but not other, chemokines (data not shown), consistent with previous results from other investigators (61–63). We found, however, that there was no cross-desensitization between galectin-3 and any of the above-mentioned monocyte-reactive chemokines (Fig. 8). These results suggest that galectin-3 does not interact with any of these presently known chemokine receptors expressed on monocytes for signal transmission into the cell.

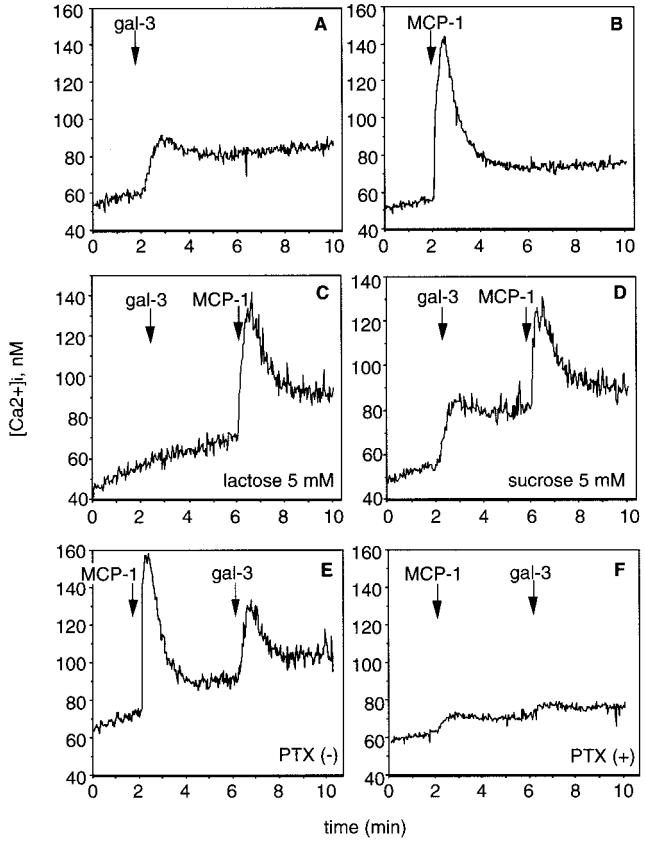

FIGURE 7. Effects of galectin-3 and MCP-1 on Ca^{2+} mobilization in monocytes. Traces represent the average mobilized intracellular concentrations of Ca^{2+} in the examined monocytes. The final concentrations of galectin-3 and MCP-1 in the cell suspensions were 1 μM and 100 ng/ml, respectively. *A* and *B*, Effects of galectin-3 (*A*) and MCP-1 (*B*) on Ca^{2+} influx in monocytes, respectively. These reagents were added to the cell suspensions at 2 min after the initiation of the measurement. *C* and *D*, Effects of two different sugars on galectin-3-induced Ca^{2+} influx in monocytes. After 5 mM lactose (*C*) or sucrose (*D*) was mixed with the cell suspension, galectin-3 and MCP-1 were added as the first and second stimulants at 2 and 6 min after the start of the measurement. *E* and *F*, Effect of PTX on galectin-3-induced Ca^{2+} influx in monocytes. Monocytes were incubated in the presence or the absence of 1 μg/ml of PTX (together with indo-1/AM) for 45 min before the assay. MCP-1 and galectin-3 were sequentially added to the monocyte suspensions in the presence of the same concentration of PTX. Each figure shows representative data from three individual experiments using different donors.

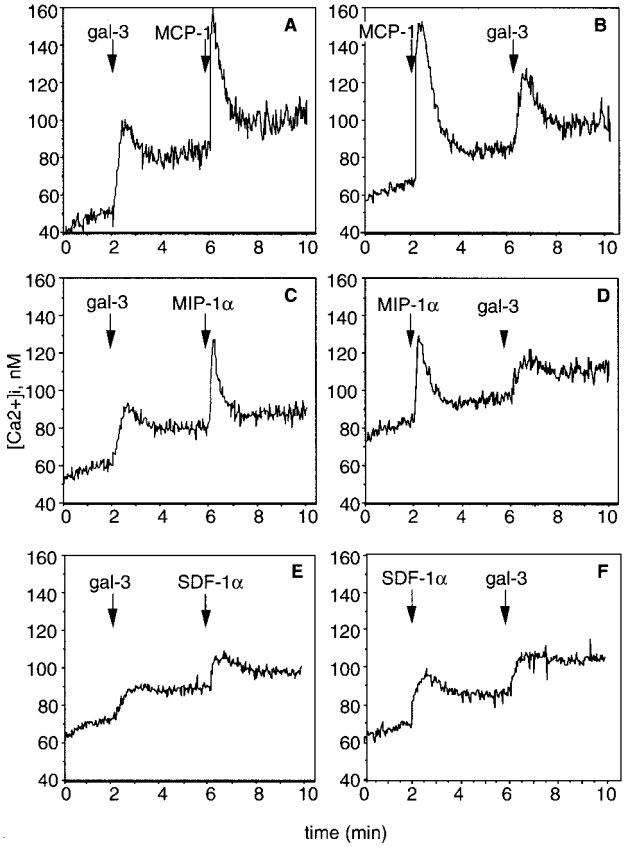

FIGURE 8. Effects of chemokines on galectin-3-induced Ca^{2+} mobilization in monocytes. Traces represent the average intracellular concentrations of Ca^{2+} in the examined monocytes. Monocytes were stimulated first with galectin-3 and then with MCP-1 (*A*), MIP-1α (*C*), or SDF-1α (*E*) or first with MCP-1 (*B*), MIP-1α (*D*), or SDF-1α (*F*) and then with galectin-3. The final concentrations of galectin-3 and each chemokine in the cell suspensions were 1 μM and 100 ng/ml, respectively. The first and second stimulants were added to the cell suspension at 2 and 6 min after the start of the measurement. Each figure shows representative data from three individual experiments using different donors.

Galectin-3, but not MCP-1, induces migration of macrophages

Unlike monocytes, there are fewer chemokines that have been shown to attract mature macrophages (65). To determine the effect of galectin-3 on mature macrophages, we used human macrophages obtained from culturing peripheral blood monocytes as well as alveolar macrophages. A previous study showed that cultured human macrophages do not express a detectable amount of CCR2 and do not respond to its ligand, MCP-1 (53), which we also confirmed (Fig. 9). In contrast, we found that galectin-3 induced macrophage migration in a dose-dependent manner, and 1 μM galectin-3 enhanced migration by 190% over that induced by the control medium ($p < 0.05$; $n = 3$; Fig. 9). Similarly, human alveolar macrophages migrated toward galectin-3 in two separate experiments (Fig. 10). In these experiments, bell-shaped dose-response curves were obtained, which is commonly observed for many chemokines. In contrast, MCP-1 had no effect (Fig. 10, Expt 1) or a negligible effect (Fig. 10, Expt 2) on macrophage migration.

These results indicate that galectin-3, but not MCP-1, is a chemoattractant for macrophages. The results also corroborate the conclusion made above that the signaling pathway induced by galectin-3 is not mediated through CCR2.

Galectin-3 induces monocyte migration in vivo

Lastly, we examined the effect of galectin-3 on cell recruitment into mouse air pouches to determine whether galectin-3 induces migration of cells in vivo. As shown in Fig. 11, galectin-3 increased the numbers of monocytes, neutrophils, and eosinophils in the air pouch by 15.7, 5.30, and 4.63 times, respectively, over those induced by vehicle (saline) only ($p < 0.05$; $n = 6$). In contrast, the number of lymphocytes was not augmented significantly by the treatment ($p = 0.309$). We tested galectin-3 samples pretreated at 100°C for 5 min and found that these samples caused a background level of inflammatory cell accumulation comparable to that induced by saline (data not shown). These results indicate that galectin-3 can recruit monocytes in vivo.

Discussion

A major conclusion of this study is that galectin-3 is a novel chemoattractant for monocytes and macrophages. Recombinant human galectin-3 induces monocyte migration in vitro, and it is chemotactic at high concentrations (1.0 μM), but chemokinetic at low

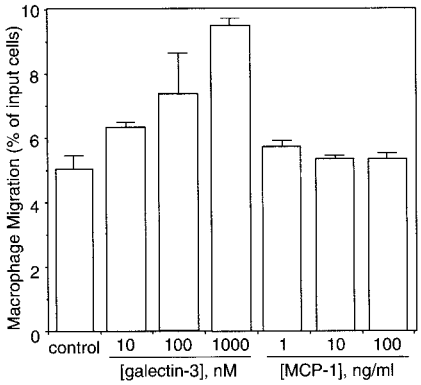

FIGURE 9. Effects of galectin-3 and MCP-1 on the migration of cultured human peripheral blood macrophages in vitro. The assays were performed as described in Fig. 1. Data are the mean ± SD of three individual experiments.

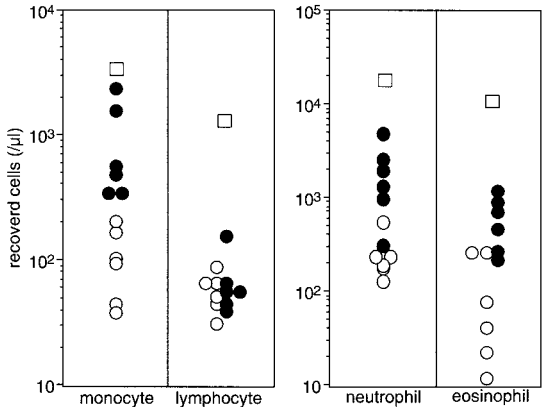

FIGURE 11. Effect of galectin-3 on monocyte/macrophage recruitment in mouse air pouches. Galectin-3 (1 μM; ●; $n = 6$), vehicle only (○; $n = 6$), or 100 ng/ml of MCP-1 (□; $n = 1$) was injected into the pouches as described in *Materials and Methods*. Each mark represents the cell number from an individual mouse. After a 4-h incubation, the recruited cells were recovered, counted, and analyzed after cytospin preparation and Wright staining.

concentrations (10–100 nM). Both the N-terminal and C-terminal domains of this protein appear to be involved in the activity, and the specificity is demonstrated by the inhibition of this activity by lactose, specific mAb, and the C-terminal domain fragment. In addition, similar to many chemoattractants, galectin-3 causes Ca^{2+} influx in monocytes, and both the chemotactic effect and the induction of Ca^{2+} influx involve a PTX-sensitive pathway(s). However, cross-desensitization experiments suggest that the signaling pathway(s) is different from that of the presently known chemokine receptors on monocytes. Finally, the physiological relevance of the findings is supported by the fact that galectin-3 also recruits monocytes in vivo in a mouse air pouch model.

The finding that galectin-3 is a chemoattractant for macrophages in addition to monocytes is noteworthy, because unlike monocytes, there are fewer chemokines that have been shown to attract mature macrophages (65). The major monocyte chemoattractant, MCP-1, for example, is inactive in this respect. Therefore, it is possible that galectin-3 is one of the major factors involved in the influx of macrophages to inflammatory sites. Based on the results from the present study, we propose that galectin-3 produced by epithelial cells, a major source of this lectin, can contribute to the attraction of monocytes and macrophages during inflammation. Because monocytes and macrophages also produce galectin-3, this lectin may mediate a continued influx of these cell types once the inflammatory process is initiated. Recently, we found that galectin-3-deficient mice developed significantly reduced numbers of peritoneal macrophages compared with wild-type mice when treated with thioglycolate i.p. (47). These results are highly consistent with the present findings, and together they suggest that galectin-3 released by the peritoneal cells in thioglycolate-treated mice is responsible at least in part for recruiting monocytes and macrophages to the peritoneal cavity, and that galectin-3-deficient mice exhibit a lower macrophage response due to the absence of this chemoattractant.

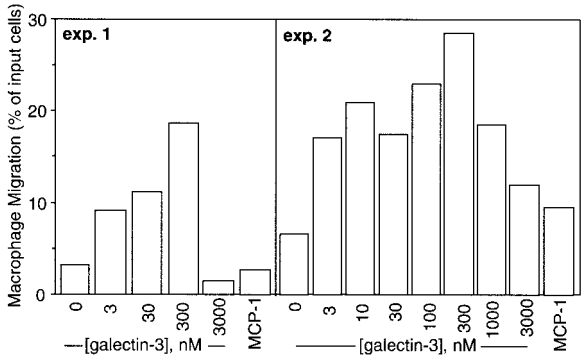

FIGURE 10. Effects of galectin-3 and MCP-1 on the migration of human alveolar macrophages in vitro. Alveolar macrophages obtained from bronchoalveolar lavage fluid were used in a standard migration assay. The results from two separate experiments are shown.

It has been shown previously that galectin-3 can activate various cell types, including induction of superoxide production by monocytes/macrophages (13). Although the precise mechanisms remain to be determined, these activities are probably related to the dimerization or oligomerization of galectin-3 through intermolecular interactions involving the amino-terminal domain (48). The lectin thereby becomes bivalent or multivalent functionally and capable of activating cells by effectively cross-linking cell surface glycoproteins (3–7). We believe that this process also contributes to the monocyte chemoattractant activity of galectin-3, and this possibility is supported by our finding that both the N-terminal and C-terminal domains of galectin-3 are required for this activity. However, an unusual feature of galectin-3's chemoattractant activity is that the response is both qualitatively and quantitatively dependent on the concentration of the lectin. First, galectin-3 appears to be chemokinetic at low concentrations, but chemotactic at high concentrations. One possible explanation is that galectin-3 at high concentrations can cause cell aggregation, and thus in the checkerboard analysis, when galectin-3 was added to the upper chambers together with the cells, the cells were prevented from migrating toward the lower chambers because they were aggregated. Therefore, it might be possible that galectin-3 is actually chemokinetic for monocytes at both high and low concentrations.

However, only monocyte migration induced by high concentrations of galectin-3 was completely inhibited by PTX. Also, only high concentrations of galectin-3 caused a Ca^{2+} influx in monocytes, and this occurred through a PTX-sensitive mechanism(s). The most likely explanation for these findings is that galectin-3 binds to and activates different (or different sets of) cell surface molecules depending on its concentration. At lower concentrations, it preferentially binds to glycoproteins that interact with the lectin relatively strongly, while only after reaching a certain threshold concentration does it begin to recognize other cell surface glycoproteins that interact with the lectin relatively weakly.

Our data suggest that the latter include a PTX-sensitive, G protein-coupled receptor(s). Galectin-3 has already been shown to bind to a number of different cell surface glycoproteins on macrophages (68), and based on a recent study with galectin-1 (69), it is likely that the lectin can cause segregation of these different glycoproteins. It is entirely possible that the lectin binds to these different glycoproteins with variable affinity, because they are differentially glycosylated, and the lectin exhibits a fine specificity for oligosaccharides (45, 46, 70).

Relatively high concentrations of galectin-3 are needed for the demonstration of optimal chemoattractant activity. The situation is analogous to other activities demonstrated for this lectin previously, such as activation of inflammatory cells (13, 35, 37), and is probably related to the concentrations that are required for the dimerization or oligomerization of the lectin to take place. However, galectin-3 is known to exist at relatively high concentrations in the cytosol of many cell types (e.g., 5 μM in a human colon adenocarcinoma cell line, T84 (71)). Therefore, a high local concentration of the lectin may be achieved when there is a burst release of the protein from these cells. In fact, galectin-3 has been found to be present in significant amounts in biological fluids. For example, the concentrations of galectin-3 in bronchoalveolar lavage fluid from mice with airway inflammation were >20 nM (our unpublished observation). Considering the dilution factor introduced in obtaining the lavage fluid, it is easily conceivable that the initial local concentrations of the lectin are in the micromolar range. On the other hand, the effective concentrations of galectin-3 for attracting alveolar macrophages are much lower (Fig. 10), approaching those typically found for many chemokines. It is possible that the putative receptor for galectin-3 on these cells either exists in higher numbers or interacts with the lectin more strongly. Alternatively, the putative receptor on these cells transmits signals more effectively upon interacting with the lectin.

Galectin-3 probably activates PTX-sensitive, G protein-coupled receptors similar to those recognized by many known chemokines (60, 64). This lectin does not have significant sequence similarity with any of these chemokines, and thus it appears unlikely that it recognizes these receptors through protein-protein interactions, but it could do so via lectin-carbohydrate interactions. Chemokine receptors expressed on monocytes include CCR-1, CCR-2, CCR-5, and CXCR-4 (60, 64). However, we found that there was no cross-desensitization between galectin-3 and any of the monocyte-reactive chemokines that use these receptors, including MCP-1 for CCR-2, MIP-1α for CCR-1 and CCR-5, and SDF-1α for CXCR-4 (60–64). We also did not detect interactions between galectin-3 and these four chemokine receptors by immunoprecipitation and immunoblotting using specific Abs (data not shown). It has been reported that CCR-3 may also be expressed on human monocytes and macrophages (53). However, we did not analyze the usage of this receptor, because we observed that galectin-3 does not attract eosinophils (which are known to express CCR-3) in vitro (our unpublished data), and these cells are known to selectively express CCR-3, suggesting that galectin-3 does not act on this receptor. Therefore, in this study we could not determine the exact receptor(s) for galectin-3, but it is not any of the known receptors, such as CCR-1, CCR-2, CCR-3, CCR-5, and CXCR-4.

Other types of chemoattractant receptors, including those for fMLP, platelet-activating factor, leukotrienes, and C5a, could mediate the effects of galectin-3. Galectin-3 is also known to recognize CD11b, LAMP1 and -2, Mac-3, and CD98 on thioglycolate-stimulated mouse peritoneal macrophages (68). Stimulation and/or cross-linking of CD11b and CD98 could enhance adhesion and transendothelial migration of monocytes (72, 73). However, we tested the effects of anit-CD11b and anti-CD98 Abs in our monocyte migration assay and found that they do not inhibit galectin-3's activity (data not shown), suggesting that these two do not mediate the observed chemotaxis function of galectin-3.

It should be noted that while galectin-3 is likely to bind to a number of different cell types through lectin-carbohydrate interactions, its chemoattractant activity is cell type-specific, as it does not induce migration of lymphocytes and eosinophils in vitro (our unpublished data) and lymphocytes in vivo (Fig. 11). This selectivity could be explained by the differential expression of the putative galectin-3 receptor on different cell types. For example, galectin-3 is known to cause a Ca^{2+} influx in Jurkat T cells, but the effect was sustained and insensitive to PTX (38), in contrast to the effect in monocytes (Fig. 7). Thus, this lectin can use different receptors on different cell types, resulting in the activation of selected types of cells, or cause a similar effect(s) on different types of cells by alternative pathways. On the other hand, although this study was focused on monocytes/macrophages, preliminary in vitro data suggest that galectin-3 may be a chemoattractant for neutrophils as well (data not shown). We also found that lower concentrations of this lectin were required for maximum migration of neutrophils compared with monocytes. In addition, galectin-3-induced recruitment of neutrophils in the mouse air pouch experiments (Fig. 11). The neutrophil chemoattractant activity of galectin-3 is also consistent with the results obtained from studies of galectin-3-deficient mice by other investigators (74), who noted that galectin-3 deficiency results in a significantly lower degree of neutrophil response in the peritoneal cavity following thioglycolate stimulation. Finally, galectin-3 was shown to also attract eosinophils in vivo (Fig. 11). However, it is possible that the lectin stimulates certain resident or recruited cells, such as monocytes, macrophages, and neutrophils, to release eosinophil chemoattractant(s). This proposed mechanism appears to be operative for MCP-1 also, which is known not to be an eosinophil chemoattractant, but appears to recruit this cell type into the air pouches (Fig. 11).

In conclusion, galectin-3 is a novel chemoattractant for monocytes and macrophages as well as neutrophils by a PTX-sensitive pathway, which is probably different from that used by the presently known monocyte-reactive chemokines. The expression of this lectin at the sites of inflammation and malignancy may modify the biological responses through the control of recruitment and activation of monocytes/macrophages, which act as inflammatory effector cells and APCs. It is interesting to note that galectin-3 has multiple functions in addition to chemoattraction, much like many of the chemokines, which possess a broad spectrum of other activities (75–77). Finally, our present work together with the recent studies by other investigators on the eosinophil chemoattractant activity of galectin-9 (42) suggest the possibility that galectins in general could act as cross-linkers of cell surface molecules, through their structurally conserved CRDs, to induce chemoattraction. While the underlying mechanisms await elucidation, a picture that emerges is that the galectin family is a new class of chemoattractants.

References

1. Cooper, D. N. W., and S. H. Barondes. 1999. God must love galectins; He made so many of them. *Glycobiology 9:979.*
2. Rabinovich, G. A. 1999. Galectins: an evolutionarily conserved family of animal lectins with multifunctional properties; a trip from the gene to clinical therapy. *Cell Death Differ. 6:711.*
3. Barondes, S. H., D. N. W. Cooper, M. A. Gitt, and H. Leffler. 1994. Galectins: structure and function of a large family of animal lectins. *J. Biol. Chem. 269: 20807.*
4. Kasai, K., and J. Hirabayashi. 1996. Galectins: a family of animal lectins that decipher glycocodes. *J. Biochem. 119:1.*
5. Perillo, N. L., M. E. Marcus, and L. G. Baum. 1998. Galectins: versatile modulators of cell adhesion, cell proliferation, and cell death. *J. Mol. Med. 76:402.*

6. Hughes, R. C. 1997. The galectin family of mammalian carbohydrate-binding molecules. *Biochem. Soc. Trans. 25:1194.*
7. Liu, F.-T. 1993. S-type mammalian lectins in allergic inflammation. *Immunol. Today 14:486.*
8. Hughes, R. C. 1994. Mac-2: a versatile galactose-binding protein of mammalian tissues. *Glycobiology 4:5.*
9. Liu, F.-T. 2000. Role of galectin-3 in inflammation. In *Lectins and Pathology*. M. Caron and D. Seve, eds. Harwood Academic Publishers, Amsterdam, The Netherlands, p. 51.
10. Flotte, T. J., T. A. Springer, and G. J. Thorbecke. 1983. Dendritic cell and macrophage staining by monoclonal antibodies in tissue sections and epidermal sheets. *Am. J. Pathol. 111:112.*
11. Moutsatsos, I. K., M. Wade, M. Schindler, and J. L. Wang. 1987. Endogenous lectins from cultured cells: nuclear localization of carbohydrate-binding protein 35 in proliferating 3T3 fibroblasts. *Proc. Natl. Acad. Sci. USA 84:6452.*
12. Agrwal, N., J. L. Wang, and P. G. Voss. 1989. Carbohydrate-binding protein 35: levels of transcription and mRNA accumulation in quiescent and proliferating cells. *J. Biol. Chem. 264:17236.*
13. Liu, F.-T., D. K. Hsu, R. I. Zuberi, I. Kuwabara, E. Y. Chi, and W. R. Henderson, Jr. 1995. Expression and function of galectin-3, a β-galactoside-binding lectin, in human monocytes and macrophages. *Am. J. Pathol. 147:1016.*
14. Nangia-Makker, P., J. Ochieng, J. K. Christman, and A. Raz. 1993. Regulation of the expression of galactoside-binding lectin during human monocytic differentiation. *Cancer Res. 53:1.*
15. Hsu, D. K., S. R. Hammes, I. Kuwabara, W. C. Greene, and F.-T. Liu. 1996. Human T lymphotropic virus-1 infection of human T lymphocytes induces expression of the β-galactose-binding lectin, galectin-3. *Am. J. Pathol. 148:1661.*
16. Konstantinov, K. N., B. A. Robbins, and F.-T. Liu. 1996. Galectin-3, a β-galactoside-binding animal lectin, is a marker of anaplastic large-cell lymphoma. *Am. J. Pathol. 148:25.*
17. Fernádez, P. L., M. J. Merino, M. Gómez, E. Campo, T. Medina, V. Castronovo, A. Cardesa, F.-T. Liu, and M. E. Sobel. 1997. Galectin-3 and laminin expression in neoplastic and non-neoplastic thyroid tissue. *J. Pathol. 181:80.*
18. Xu, X. C., A. K. El-Naggar, and R. Lotan. 1995. Differential expression of galectin-1 and galectin-3 in thyroid tumors: potential diagnostic implications. *Am. J. Pathol. 147:815.*
19. Lotz, M. M., C. W. Andrews, Jr., C. A. Korzelius, E. C. Lee, G. D. Steele, Jr., A. Clarke, and A. M. Mercurio. 1993. Decreased expression of Mac-2 (carbohydrate binding protein 35) and loss of its nuclear localization are associated with the neoplastic progression of colon carcinoma. *Proc. Natl. Acad. Sci. USA 90: 3466.*
20. Castronovo, V., E. Campo, F. A. van den Brûle, A. P. Claysmith, V. Cioce, F.-T. Liu, P. L. Fernandez, and M. E. Sobel. 1992. Inverse modulation of steady state mRNA levels of two non-integrin laminin binding proteins in human colon carcinoma. *J. Natl. Cancer Inst. 84:1161.*
21. Castronovo, V., F. A. Van den Brule, P. Jackers, N. Clausse, F. T. Liu, C. Gillet, and M. E. Sobel. 1996. Decreased expression of galectin-3 is associated with progression of human breast cancer. *J. Pathol. 179:43.*
22. van den Brûle, F. A., A. Berchuck, R. C. Bast, F.-T. Liu, C. Pieters, M. E. Sobel, and V. Castronovo. 1994. Differential expression of the 67-kD laminin receptor and 31-kD human laminin-binding protein in human ovarian carcinoma. *Eur. J. Cancer 30A:1096.*
23. van den Brûle, F. A., C. Buicu, A. Berchuck, R. C. Bast, M. Deprez, F. T. Liu, D. N. W. Cooper, C. Pieters, M. E. Sobel, and V. Castronovo. 1996. Expression of the 67-kD laminin receptor, galectin-1, and galectin-3 in advanced human uterine adenocarcinoma. *Hum. Pathol. 27:1185.*
24. Bresalier, R. S., P. S. Yan, J. C. Byrd, R. Lotan, and A. Raz. 1997. Expression of the endogenous galactose-binding protein galectin-3 correlates with the malignant potential of tumors in the central nervous system. *Cancer 80:776.*
25. Nachtigal, M., Z. Al-Assaad, E. P. Mayer, K. Kim, and M. Monsigny. 1998. Galectin-3 expression in human atherosclerotic lesions. *Am. J. Pathol. 152:1199.*
26. Laing, J. G., and J. L. Wang. 1988. Identification of carbohydrate binding protein 35 in heterogeneous nuclear ribonucleoprotein complex. *Biochemistry 27:5329.*
27. Dagher, S. F., J. L. Wang, and R. J. Patterson. 1995. Identification of galectin-3 as a factor in pre-mRNA splicing. *Proc. Natl. Acad. Sci. USA 92:1213.*
28. Kim, H.-R. C., H.-M. Lin, H. Biliran, and A. Raz. 1999. Cell cycle arrest and inhibition of anoikis by galectin-3 in human breast epithelial cells. *Cancer Res. 59:4148.*
29. Yang, R.-Y., D. K. Hsu, and F.-T. Liu. 1996. Expression of galectin-3 modulates T cell growth and apoptosis. *Proc. Natl. Acad. Sci. USA 93:6737.*
30. Akahani, S., P. Nangia-Makker, H. Inohara, H. R. C. Kim, and A. Raz. 1997. Galectin-3: a novel antiapoptotic molecule with a functional BH1 (NWGR) domain of Bcl-2 family. *Cancer Res. 57:5272.*
31. Cherayil, B. J., S. J. Weiner, and S. Pillai. 1989. The Mac-2 antigen is a galactose-specific lectin that binds IgE. *J. Exp. Med. 170:1959.*
32. Sato, S., and R. C. Hughes. 1994. Regulation of secretion and surface expression of Mac-2, a galactoside-binding protein of macrophages. *J. Biol. Chem. 269: 4424.*
33. Sato, S., I. Burdett, and R. C. Hughes. 1993. Secretion of the baby hamster kidney 30-kDa galactose-binding lectin from polarized and nonpolarized cells: a pathway independent of the endoplasmic reticulum-Golgi complex. *Exp. Cell Res. 207:8.*
34. Lindstedt, R., G. Apodaca, S. H. Barondes, K. E. Mostov, and H. Leffler. 1993. Apical secretion of a cytosolic protein by Madin-Darby canine kidney cells: evidence for polarized release of an endogenous lectin by a nonclassical secretory pathway. *J. Biol. Chem. 268:11750.*
35. Frigeri, L. G., R. I. Zuberi, and F.-T. Liu. 1993. ϵBP, a β-galactoside-binding animal lectin, recognizes IgE receptor (FcϵRI) and activates mast cells. *Biochemistry 32:7644.*
36. Zuberi, R. I., L. G. Frigeri, and F.-T. Liu. 1994. Activation of rat basophilic leukemia cells by, an IgE-binding endogenous lectin. *Cell. Immunol. 156:1.*
37. Yamaoka, A., I. Kuwabara, L. G. Frigeri, and F.-T. Liu. 1995. A human lectin, galectin-3 (ϵBP/Mac-2), stimulates superoxide production by neutrophils. *J. Immunol. 154:3479.*
38. Dong, S., and R. C. Hughes. 1996. Galectin-3 stimulates uptake of extracellular Ca^{2+} in human Jurkat T-cells. *FEBS Lett. 395:165.*
39. Kuwabara, I., and F.-T. Liu. 1996. Galectin-3 promotes adhesion of human neutrophils to laminin. *J. Immunol. 156:3939.*
40. Inohara, H., S. Akahani, K. Koths, and A. Raz. 1996. Interactions between galectin-3 and Mac-2-binding protein mediate cell-cell adhesion. *Cancer Res. 56: 4530.*
41. Sato, S., and R. C. Hughes. 1992. Binding specificity of a baby hamster kidney lectin for H type I and II chains, polylactosamine glycans, and appropriately glycosylated forms of laminin and fibronectin. *J. Biol. Chem. 267:6983.*
42. Matsumoto, R., H. Matsumoto, M. Seki, M. Hata, Y. Asano, S. Kanegasaki, R. L. Stevens, and M. Hirashima. 1998. Human ecalectin, a variant of human galectin-9, is a novel eosinophil chemoattractant produced by T lymphocytes. *J. Biol. Chem. 273:16976.*
43. Matsushita, N., N. Nishi, M. Seki, R. Matsumoto, I. Kuwabara, F.-T. Liu, Y. Hata, T. Nakamura, and M. Hirashima. 2000. Requirement of divalent galactoside-binding activity of ecalectin/galectin-9 for eosinophil chemoattraction. *J. Biol. Chem. 275:8355.*
44. Frigeri, L. G., and F.-T. Liu. 1992. Surface expression of functional IgE binding protein, an endogenous lectin, on mast cells and macrophages. *J. Immunol. 148: 861.*
45. Sparrow, C. P., H. Leffler, and S. H. Barondes. 1987. Multiple soluble β-galactoside-binding lectins from human lung. *J. Biol. Chem. 262:7383.*
46. Leffler, H., and S. H. Barondes. 1986. Specificity of binding of three soluble rat lung lectins to substituted and unsubstituted mammalian β-galactosides. *J. Biol. Chem. 261:10119.*
47. Hsu, D. K., R.-Y. Yang, L. Yu, Z. Pan, D. R. Salomon, W.-P. Fung-Leung, and F.-T. Liu. 2000. Targeted disruption of the galectin-3 gene results in attenuated peritoneal inflammatory responses. *Am. J. Pathol. 156:1073.*
48. Hsu, D. K., R. Zuberi, and F.-T. Liu. 1992. Biochemical and biophysical characterization of human recombinant IgE-binding protein, an S-type animal lectin. *J. Biol. Chem. 267:14167.*
49. Liu, F.-T., D. K. Hsu, R. I. Zuberi, A. Shenhav, P. N. Hill, I. Kuwabara, and S.-S. Chen. 1996. Modulation of functional properties of galectin-3 by monoclonal antibodies binding to the non-lectin domain. *Biochemistry 35:6073.*
50. Liu, F.-T., J. W. Bohn, E. L. Ferry, H. Yamamoto, C. A. Molinaro, L. A. Sherman, N. R. Klinman, and D. H. Katz. 1980. Monoclonal dinitrophenyl-specific murine IgE antibody: preparation, isolation and characterization. *J. Immunol. 124:2728.*
51. Nakagawara, A., C. F. Nathan, and Z. A. Cohn. 1981. Hydrogen peroxide metabolism in human monocytes during differentiation in vitro. *J. Clin. Invest. 68: 1243.*
52. Chuluyan, H. E., and Issekutz. 1993. The VLA-4 integrin mediate CD11/CD18 independent transendothelial migration of human monocytes. *J. Clin. Invest. 92: 2768.*
53. Fantuzzi, L., P. Borghi, V. Ciolli, G. Pavlakis, F. Belardelli, and S. Gessani. 1999. Loss of CCR2 expression and functional response to monocyte chemotactic protein (MCP-1) during the differentiation of human monocytes: role of secreted MCP-1 in the regulation of the chemotactic response. *Blood 94:875.*
54. Sugimoto, M., H. Nakashima, M. Matsumoto, E. Uyama, M. Ando, and S. Araki. 1989. Pulmonary involvement in patients with HTLV-1 associated myelopathy: increased soluble IL-2 receptors in bronchoalveolar lavage fluid. *Am. Rev. Respir. Dis. 139:1329.*
55. Falk, W., Jr. RH. Goodwin, and EJ. Leonard. 1980. A 48-well micro chemotaxis assembly for rapid and accurate measurement of leukocyte migration. *J. Immunol. Methods 33:239.*
56. Chertov, O., D. F. Michiel, L. Xu, J. M. Wang, K. Tani, W. J. Murphy, D. L. Longo, D. D. Taub, and J. J. Oppenheim. 1996. Identification of defensin-1, defensin-2, and CAP37/azurocidin as T-cell chemoattractant proteins released from interleukin-8-stimulated neutrophils. *J. Biol. Chem. 271:2935.*
57. Perretti, M., A. Ahluwalia, J. G. Harris, N. J. Goulding, and R. J. Flower. 1993. Lipocortin-1 fragments inhibit neutrophil accumulation and neutrophil-dependent edema in the mouse: a qualitative comparison with an anti-CD11b monoclonal antibody. *J. Immunol. 151:4306.*
58. Lopez, M., D. Olive, and P. P. Mannoni. 1989. Analysis of cytosolic ionized calcium variation in polymorphonuclear leukocytes using flow cytometry and indo-1 AM. *Cytometry 10:165.*
59. Zachariae, C. O. C., A. O. Anderson, H. L. Thompson, E. Apella, A. Mantovani, J. J. Oppenheim, and K. Matsushima. 1990. Properties of monocyte chemotactic and activating factor (MCAF) purified from a human fibrosarcoma cell line. *J. Exp. Med. 171:2177.*
60. Baggiolini, M. 1998. Chemokines and leukocyte traffic. *Nature 392:565.*
61. Sozzani, S., M. Molino, M. Locati, W. Luini, C. Cerletti, A. Vecchi, and A. Mantovani. 1993. Receptor-activated calcium influx in human monocytes exposed to monocyte chemotactic protein-1 and related cytokines. *J. Immunol. 150: 1544.*

62. Bizzari, C., R. Bertini, P. Bossu, S. Sozzani, A. Mantovani, J. V. Damme, A. Tagliabue, and D. Boraschi. 1995. Single-cell analysis of macrophage chemotactic protein-1-regulated cytosolic Ca^{2+} increment in human adherent monocytes. *Blood 86:2388.*
63. Oberlin, E., A. Amara, F. Bachelerie, C. Bessia, J.-L. Virelizier, F. Arenzana-Seisdedos, O. Schwartz, J.-M. Heard, I. Clark-Lewis, D. F. Legler, et al. 1996. The CXC chemokine SDF-1 is the ligand for LESTR/fusin and prevents infection by T-cell-line-adapted HIV-1. *Nature 382:833.*
64. Sallusto, F., A. Lanzavecchia, and C. R. Mackay. 1998. Chemokines and chemokine receptors in T-cell priming and Th1/Th2- mediated responses. *Immunol. Today 19:568.*
65. Zlotnik, A., J. Morales, and J. Hedrick. 1999. Recent advances in chemokines and chemokine receptors. *Crit. Rev. Immunol. 19:1.*
66. Richardson, R. M., H. Ali, E. D. Tomhave, B. Haribabu, and R. Snyderman. 1995. Cross-desensitization of chemoattractant receptors occurs at multiple levels. *J. Biol. Chem. 270:27829.*
67. Tomhave, E. D., R. M. Richardson, J. Didsbury, L. Menard, R. Snyderman, and H. Ali. 1994. Cross-desensitization of receptors for peptide chemoattractants. *J. Immunol. 153:3267.*
68. Dong, S., and R. C. Hughes. 1997. Macrophage surface glycoproteins binding to galectin-3 (Mac-2-antigen). *Glycoconjugate J. 14:267.*
69. Pace, K. E., C. Lee, P. L. Stewart, and L. G. Baum. 1999. Restricted receptor segregation into membrane microdomains occurs on human T cells during apoptosis induced by galectin-1. *J. Immunol. 163:3801.*
70. Feizi, T., J. C. Solomon, C.-T. Yuen, K. C. G. Jeng, L. G. Frigeri, D. K. Hsu, and F.-T. Liu. 1994. The adhesive specificity of the soluble human lectin, IgE-binding protein toward lipid-linked oligosaccharides: presence of the blood group A, B, B-like, and H monosaccharides confers a binding activity to tetrasaccharide (lacto-*N*-tetraose and lacto-*N*-neotetraose) backbones. *Biochemistry 33:6342.*
71. Huflejt, M. E., E. T. Jordan, M. A. Gitt, S. H. Barondes, and H. Leffler. 1997. Strikingly different localization of galectin-3 and galectin-4 in human colon adenocarcinoma T84 cells: galectin-4 is localized at sites of cell adhesion. *J. Biol. Chem. 272:14294.*
72. Meerschaert, J., and M. B. Furie. 1995. The adhesion molecules used by monocytes for migration across endothelium include CD11a/CD18, CD11b/CD18, and VLA-4 on monocytes and ICAM-1, VCAM-1, and other ligands on endothelium. *J. Immunol. 154:4099.*
73. Fenczik, C. A., T. Sethi, J. W. Ramos, P. E. Hughes, and M. H. Ginsberg. 1997. Complementation of dominant suppression implicates CD98 in integrin activation. *Nature 390:81.*
74. Colnot, C., M.-A. Ripoche, G. Milon, X. Montagutelli, P. R. Crocker, and F. Poirier. 1998. Maintenance of granulocyte numbers during acute peritonitis is defective in galectin-3-null mutant mice. *Immunology 94:290.*
75. Ward, S. G., K. Bacon, and J. Westwick. 1998. Chemokines and T lymphocytes: more than an attraction. *Immunity 9:1.*
76. Kunkel, S. L. 1999. Through the looking glass: the diverse in vivo activities of chemokines. *J. Clin. Invest. 104:1333.*
77. Nickel, R., L. A. Beck, C. Stellato, and R. P. Schleimer. 1999. Chemokines and allergic disease. *J. Allergy Clin. Immunol. 104:723.*