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Human Monocytes Recognize Porcine Endothelium via the Interaction of Galectin 3 and α-GAL

Rongyu Jin,* Allen Greenwald,* Mark D. Peterson,† and Thomas K. Waddell²*

Monocytes are one of the key inflammatory cells recruited to xenografts and play an important role in delayed xenograft rejection. Previous studies have demonstrated the ability of monocytes to bind to the major xenoantigen Gal-α(1,3)Gal-β(1,4)GlcNAc-R; however, the receptor that mediates this interaction has yet to be identified. We provide evidence that it is Galectin-3, a ~30-kDa lectin that recognizes β-galactosides (Gal-β(1–3/4)GlcNAc) and plays diverse roles in many physiological and pathological events. Human monocyte binding is strikingly increased on porcine aortic endothelial cells (PAEC), which express high levels of Gal-α(1,3)Gal-β(1,4)GlcNAc-R, compared with human aortic endothelial cells. Human monocytes obtained from healthy donors bind to Gal-α(1,3)Gal-β(1,4)GlcNAc-R at variable intensities. This variation of binding intensity was consistent and reproducible in individual monocytes. Galectin-3 is mainly expressed in human monocytes, not lymphocytes. Purified Galectin-3 is able to bind directly to Gal-α(1,3)Gal-β(1,4)GlcNAc-R. Galectin-3 can also be affinity isolated from monocytes (and not lymphocytes) using an Gal-α(1,3)Gal-β(1,4)GlcNAc-R-biotin/streptavidin-bead pull-down system. Soluble Galectin-3 binds preferentially to PAEC vs human aortic endothelial cells, and this binding can be inhibited by lactose, indicating dependence on the carbohydrate recognition domain of Galectin-3. Gal-α(1,3)Gal-β(1,4)GlcNAc-R is at least partly responsible for this phenomenon, as binding decreased after digestion of PAEC with α-galactosidase. Furthermore, monocytes pretreated with a blocking anti-Galectin-3 Ab show decreased adhesion to PAEC when compared with isotype control in a parallel plate flow chamber perfusion assay. Thus, we conclude that Galectin-3 expressed in human monocytes is a receptor for the major xenoantigen (Gal-α(1,3)Gal-β(1,4)GlcNAc-R), expressed on porcine endothelial cells. The Journal of Immunology, 2006, 177: 1289–1295.

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Materials and Methods

Abs and reagents

Mouse anti-human Galectin-3 (clone B2C10) was purchased from BD Biosciences. Purified recombinant human Galectin-3 was obtained from PeproTech. Immobilized streptavidin-agarose beads and ECL kit were purchased from Pierce. Monocyte separation kits were purchased from Miltenyi Biotec. The secondary Ab used for flow cytometry, PE-conjugated F(ab’)_2 of affinity-purified anti-mouse IgG (F(ab’)_2 goat Ig), was purchased from Biolegend.
from Rockland. The secondary Ab used for Western immunoblotting (goat anti-mouse HRP) was from Bio-Rad. Endothelial mitogen was purchased from Biomedical Technologies.

Soluble polymeric Gal-(1,3)Gal-(1,4)GlcNAc-R derivatives conjugated to poly(N-(2-hydroxyethyl)acrylamide) (PAA) were purchased from Lectinex: HOCH2(HOCH)2CH2NH-(control-PAA, 0000), HOCH2(HOCH)2CH2NH-PAA-FITC (control-PAA-FITC, 0000-FP), and Galel–3Gal–3Gal–3Gal–3GlcNAc–3β-OCH2CH2NH-PAA-FITC (α-Gal-PAA-FITC, 0070-FP). The GAS914 reagent (Galel–3Gal–3Gal–3GlcNAc–3β-polysilane) was a gift from G. Levy (University of Toronto, Toronto, Canada) and biotinylated in our lab using standard techniques.

**PBMC and monocye isolation**

Whole human blood from healthy donors was collected into syringes containing heparin and carefully layered onto Ficoll-Paque 1077 (Sigma-Aldrich). PBMCs were obtained from the interface between human plasma and the Ficoll-Paque following centrifugation at 400 × g. Monocytes were then isolated from platelet-depleted PBMCs by negative selection based on the depletion of neutrophils, dendritic cells, basophils, granulocytes, and B, T, and NK cells with a mixture of biotinylated mAbs, followed by a secondary anti-biotin Ab conjugated to magnetic beads. Flow cytometry routinely demonstrated that greater than 85% of the cells in the monocyte fraction were CD14+.

**Endothelial cells**

Cultured human aortic endothelial cells (HAEC) were obtained from Cambrex. Detailed methods for isolating porcine aortic endothelial cells (PAEC) were described in our previous study (15). In brief, freshly harvested porcine aortas (from a local slaughterhouse) were treated with 0.1% collagenase (Sigma-Aldrich) at 37°C to liberate endothelial cells. These cells were then collected and washed with DMEM (Invitrogen Life Technologies) + 10% FBS to inactivate the collagenase and resuspended in DMEM + 10% FBS (with Pen/Strep and 25 μg/ml endothelial mitogen) and plated in a Corning T25 tissue culture flask. PAEC and HAEC were grown to confluence and used for experiments between the second and sixth passage. For adhesion assays, both HAEC and PAEC were cultured to confluence on a gelatin (2%)-coated 96-well tissue culture plate (Falcon).

**Static adhesion assay**

HAEC and PAEC were seeded onto a 96-well plate and incubated for 2 days until confluent. On the day of the experiment, human monocytes were isolated and resuspended in HBSS at 2 × 105 cells/mL. Cells were labeled with Calcein-AM at 4 μM at 37°C for 30 min. After being washed twice in HBSS buffer, 5 × 104 labeled monocytes were applied to each of the preseeded wells of HAEC or PAEC in triplicate. Fluorescence was measured with a Cytofluor 4000 II multiwell plate reader (Applied Biosystems) using 485 nm excitation and 530 nm emission filters. Readings were performed with a CytoFluor plate reader.

**Parallel plate flow chamber assay**

Adhesion assays were performed using a parallel plate flow chamber (Glyco-vac) at a shear stress of 0.8 dyne/cm². Confluent monolayers of PAEC were mounted in the flow chamber and placed on the stage of a Nikon Diaphot 300 inverted phase-contrast microscope. Experiments were videotaped with a Sony DXC-151A color video camera and Sony SVT-S3100 time-lapse video cassette recorder for later offline analysis. Purified monocytes (0.5 × 106) were resuspended in 500 μl of HBSS (with 1 mM CaCl2/ MgCl2) supplemented with 10 mM Hepes and 0.5% FBS (assay buffer) and injected into the flow chamber. Adherent monocytes were counted in six consecutive high-power fields after 4 min of flow. Monocytes were considered firmly adherent if they remained stationary for >4 s. It was unusual for adherent monocytes to subsequently release.

**Flow cytometric analysis**

The capacity of human monocytes to bind to α-gal was determined by incubating PBMCs obtained from healthy donors with soluble α-gal-FITC (0070-PAAP-FP) or control glyconjugate-FITC (0000-PAAP-FP) for 30 min at 4°C. Before incubation with glyconjugates, nonspecific binding sites were blocked by incubating with 500 mg/ml 0000-PAAP for 20 min at 4°C. The correct flow cytometer gating for monocytes was previously established using Abs specific for CD14. The mean fluorescence intensity (MFI) of each gated population was then determined by flow cytometry.

The surface staining of PBMCs with Galectin-3 was performed by incubating the cells with 25 μg/ml mouse anti-human Galectin-3 Ab on ice for 30 min, followed by the incubation of PE-conjugated goat anti-mouse Ab on ice for 30 min. For intracellular staining, the PBMCs were fixed with 4% paraformaldehyde and permeabilized with 0.05% saponin in PBS before the primary Ab incubation. MFI was then measured for both monocytes and lymphocytes.

Binding of Galectin-3 to endothelial cells was performed by incubating the cells with 50 μg/ml purified recombinant human Galectin-3 and either 30 mM sucrose or lactose for 20 min at 4°C. Cells were subsequently stained for surface Galectin-3 as above. The glycosylation of PAEC was modified by treating a confluent monolayer with 2 U/ml of an α-exogalactosidase (Sigma-Aldrich) intact or heat inactivated (100°C for 20 min), or β-exogalactosidase (Sigma-Aldrich) in HBSS for 2 h at 37°C. Verification of digestion of Gal-(1,3)Gal-(1,4)GlcNAc-R was shown by flow cytometry staining with 10 μg/ml FITC-labeled Bandeiraea simplicifolia isoelectin B4 (BS-IB4)-FITC before and after exogalactosidase treatment.

**Binding capacity of Galectin-3 to α-gal**

Pure protein samples of BSA, BSA-IB4, or Galectin-3 were added to a Nunc Maxi-sorp plate (Nalge Nunc International) at 100 μg/ml at 4°C overnight. On the following day, the plate was washed with PBS three times and blocked with 1% BSA at room temperature for 1 h. Glycoconjugates (0070-PAAP-FP or 0000-PAAP-FP) were then applied to the precoated wells at room temperature for 30 min. The plate was then washed three times with PBS, and the fluorescent signal from each well was measured using a CytoFluor plate reader.

**Western blotting**

Human monocytes and lymphocytes were isolated, as described above. The cells were washed twice with PBS and then lysed using lysis buffer (PBS plus 1% Nonidet P-40 and 10 μg/ml aprotinin, leupeptin, and pepstatin A). The cell lysates were then separated by electrophoresis on a 10% SDS-PAGE gel at 100 V for 2 h, transferred to nitrocellulose membrane using an electric transfer cell at 18 V for 45 min, and blocked with 5% nonfat milk in PBS. The blot was then probed with mouse anti-human Galectin-3 mAb, followed by detection using goat anti-mouse HRP and an ECL kit to expose photographic film (Pierce Biotechnology).

**Affinity precipitation assay**

Cell lysates from human monocytes or lymphocytes were incubated with or without GAS914-biotin at 4°C overnight. On the following day, streptavidin beads were applied to each sample and incubated for another 4 h at 4°C. Beads were washed three times in PBS with 1% Nonidet P-40, and bound protein was eluted with SDS-PAGE sample buffer (100 mM Tris [pH 6.8], 2% SDS, 0.01% bromphenol blue, and 10% glycerol). The samples were then separated by SDS-PAGE, and Galectin-3 expression was detected with anti-human Galectin-3 Ab described as above.

**Statistical analysis**

Statistical analysis was performed with SPSS software (SAS version 8.1; SAS Institute). Comparisons of variables between groups were performed with unpaired Student’s t tests or ANOVA. A two-tailed p value <0.05 was considered statistically significant.

**Results**

Human monocytes adhere to porcine, but not human endothelium

Gal-(1,3)Gal-(1,4)GlcNAc-R is expressed on many different cell types, including endothelial cells from nonhuman species. To confirm this pattern of expression, we first examined the level of expression on HAEC and PAEC using the BS-IB4. As expected, HAEC exhibited no detectable BS-IB4-FITC fluorescence, whereas a tremendously high level of Gal-(1,3)Gal-(1,4)GlcNAc-R was detected on the surface of PAEC (Fig. 1A, −31 ± 23 vs 3685 ± 178 arbitrary fluorescence units; p < 0.0001). We then examined the interaction between human monocytes and endothelial cells under static adhesion conditions. Specifically, after coculturing human monocytes with either HAEC or PAEC at 37°C for 30 min, a much higher proportion of human monocytes bound to

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PAEC as opposed to HAEC (23.51 ± 1.94 vs 4.03 ± 1.63% adhesion; p = 0.0001; Fig. 1B). Moreover, previous studies from our group have documented that the high expression level of Gal-α(1,3)Gal-β(1,4)GlcNAc-R on porcine endothelial cells contributes to the elevated levels of firm adhesion to xenogeneic endothelial cells by human monocytes (15).

**FIGURE 1.** Human monocytes adhere to porcine, but not human endothelial cells in static adhesion conditions. A. The major xenotaintgen α-Gal is highly expressed in PAEC, but not in HAEC: 3685 ± 178 vs −31 ± 23 arbitrary fluorescence units; *, indicates p < 0.0001. B. Static adhesion of human monocyte to HAEC or PAEC: data are expressed as percentage of adherent human monocyte to the total amount of loading. *, Indicates p = 0.0001. The graph is expressed as the mean ± SEM of at least three experiments.

**α-Gal is a ligand for human monocyte lectins**

We hypothesized that Gal-α(1,3)Gal-β(1,4)GlcNAc-R could bind directly to human monocytes through a lectin-type recognition pathway. Indeed, we have previously demonstrated specific and saturable binding of a soluble Gal-α(1,3)Gal-β(1,4)GlcNAc-R fluorescent probe to monocytes in suspension (15). Interestingly, monocytes obtained from different donors showed a variation in MFI, while there was essentially no binding to lymphocytes (Fig. 2A). Confocal microscopy of the cells indicates significant uptake of this fluorescent probe (Fig. 2Bi), with most of the signal located intracellularly, providing cells are allowed to warm up after binding the probe. Control glycoconjugate was not evident either on the cell surface or intracellularly, using the same exposure time and gain to obtain the microscopic images (Fig. 2Biv). Interestingly, this variable binding was reproducible when the same donors were examined repeatedly over a span of months to years. Among the donors that were tested, the lowest, median, and highest are presented in Fig. 3A. As previously reported, there is minimal staining of the lymphocyte population, perhaps representing NK cells. The monocytes show a second population shifted to the right compared with the unstained sample, and the size and MFI of this high α-gal-binding population varied among different donors. This variability between donors was quite reproducible when donors were re-examined. Fig. 3B represents all nine donors that have undergone repeat examination. Note the small SD within a donor compared with the large variability between donors.

**FIGURE 2.** α-Gal is a ligand for human monocyte lectins. A. Binding of control (■) or soluble α-Gal-FITC (■) to PBMC from 15 tests on 11 different donors at the dose of 100 μg/ml. Data are presented as the MFI ± SEM. The results for the monocyte and lymphocyte are 11.7 ± 9.4 and 48.3 ± 34.8 vs 3.4 ± 1.7 and 9.3 ± 7.2, respectively. *, Indicates p < 0.005. B. Human monocytes were incubated with α-gal-FITC (ii) or control (iv), after washing; the confocal fluorescent images through the middle of the cells are shown in ii or iv; i and iii show the brightfield images.

**FIGURE 3.** Binding of α-Gal is variable between individuals. A. Binding of soluble α-Gal-FITC (solid line) or control (dotted line) to PBMC from three different donors at the dose of 100 μg/ml. Data are presented as flow cytometry histograms of PBMC gated on monocytes or lymphocytes. Specific binding of α-Gal-FITC is represented by a shift in the peak (solid line) vs control (dotted line). The MFI and the percentage of positive population were: 5.9, 15.6%; 19.0, 36%; and 79.7, 75.3% for the low, medium, and high-binding donors, respectively. However, the binding to human lymphocyte was very weak, 1.1, 2.5%; 2.1, 4.4%; 7.2, 9.4% for those three donors. B. Summary of the variation of α-gal-FITC-binding capacity of human monocytes from different donors: reproducible binding (at the dose of 100 μg/ml) is reflected by the small SE within a donor compared with the large variability between donors in the MFI (ordinate). Data are presented as the MFI ± SEM; n ≥ 2.
Galectin-3 is expressed in human monocytes and can bind α-gal.

Cell lysates from human monocytes and lymphocytes were subjected to SDS-PAGE and immunoblotted for Galectin-3. As shown in Fig. 4A, a band ~30 kDa was detected in human monocyte cell lysates, but not lymphocytes. Furthermore, we examined where Galectin 3 is expressed in the monocyte by complementary surface (Fig. 4, B and C) and intracellular (Fig. 4, D and E) staining, followed by flow cytometry. The intracellular staining was very consistent with the Western blot data with high expression of Galectin-3 in monocytes, but not lymphocytes. As expected, lymphocyte surface Galectin-3 expression was essentially undetectable. Monocyte surface expression was also very low, but definitely above isotype control Ab staining.

**FIGURE 4.** Galectin-3 is expressed in human monocytes, but is not found in lymphocytes. A, Western blot analysis of the Galectin-3 expression in human monocytes and lymphocytes: lane P, 20 ng of pure Galectin-3 as positive control; lanes M and L, cell lysate from human monocyte and lymphocyte, respectively (50 μg of total protein/lane), was loaded into a 10% SDS-PAGE gel and immunoblotted with anti-Galectin-3 mAb (top panel) and anti-G3PDH (bottom panel). The Galectin-3 immunoreactive band shows a molecular mass of ~30 kDa. B–E, Galectin-3 staining of human monocytes and lymphocytes from four different healthy donors by flow cytometry. Galectin-3 surface staining for monocytes and lymphocytes; the MFI (B) is 4.3 ± 1.0 vs 1.1 ± 0.2, and percentage of positive population (C) is 9.5 ± 1.0% vs 3.9 ± 0.7% for monocytes and lymphocytes, respectively; intracellular staining of Galectin-3 after cells were fixed and permeabilized; the MFI for monocytes and lymphocytes. D, 7.4 ± 2.0 vs 1.5 ± 0.4 and percentage positive; E, 88.4 ± 3.6% vs 54.2 ± 1.8%. *, Indicates p < 0.05.

Because Galectin-3 has been previously found to have a Ca$^{2+}$-independent affinity for the Galilli Ag and has been found to be expressed on macrophages, we theorized that it could potentially be a receptor of Gal-α(1,3)Gal-β(1,4)GlcNac-R in human monocytes. To test this hypothesis, we first examined the ability of Galectin-3 to bind Gal-α(1,3)Gal-β(1,4)GlcNac-R and the requirement for Ca$^{2+}$, BSA, Galectin-3, or BS-IB$_4$ (as a positive control) was coated to a plate, which was then washed and incubated with α-Gal-PAAG-FITC or PAA-FITC in the presence or absence of Ca$^{2+}$ and Mg$^{2+}$. As shown in Fig. 5, Gal-α(1,3)Gal-β(1,4)GlcNac-R bound to both BS-IB$_4$ and Galectin-3 (71 ± 10 and 36 ± 2 arbitrary fluorescence units), but not to BSA (2 ± 2). The absence of Ca$^{2+}$ had no effect on the interaction of Galectin-3 and α-Gal (46 ± 3).

**FIGURE 5.** Galectin-3 binds to α-Gal in a Ca$^{2+}$-independent manner. Control glycoconjugate ([]) or α-Gal-PAAG-FITC ([]) was applied to BS-IB$_4$ or Galectin-3-precoated 96-well plate. α-Gal-PAAG-FITC bound to both BS-IB$_4$, and Galectin-3 (71 ± 10 and 36 ± 2 arbitrary fluorescence units), but not to BSA (2 ± 2). The absence of Ca$^{2+}$ had no effect on the interaction of Galectin-3 and α-Gal (46 ± 3).

α-Gal directly interacts with Galectin-3

Based on the capacity of Galectin-3 to bind to Gal-α(1,3)Gal-β(1,4)GlcNac-R and its expression in and on human monocytes, we hypothesized that Galectin-3 is the human monocyte receptor for Gal-α(1,3)Gal-β(1,4)GlcNac-R. To prove that monocyte Galectin-3 still possesses the ability to bind Gal-α(1,3)Gal-β(1,4)GlcNac-R (as opposed to the commercial recombinant Gal-α(1,3)Gal-β(1,4)GlcNac-R used above), we performed affinity purification. Cell lysates from monocytes and lymphocytes were incubated with or without α-Gal-containing GAS914-biotin overnight. The next day, the proteins that could interact with the α-gal reagent were pulled down using streptavidin beads. The bound protein was eluted and subsequently detected using anti-Galectin-3 Ab in a Western immunoblot. As shown in Fig. 6, a strong positive band (lane 3) was present in the monocyte cell lysates that were preincubated with GAS914-biotin reagents, whereas those that were not incubated with the GAS914-biotin reagent didn’t manifest such a band. This effectively rules out the possibility of nonspecific binding between

**FIGURE 6.** α-gal directly interacts with Galectin-3. Cell lysate from human lymphocytes or monocytes was incubated with or without GAS914-biotin (lane 1 vs 2 for lymphocytes; lane 3 vs 4 for monocytes) plus streptavidin-agarose beads. After washing, bound proteins were eluted with SDS-PAGE sample buffer. Samples were analyzed using Western blot, as described above, using anti-Galectin-3 Ab. Pure Galectin-3 (2 ng in lane 5) was loaded as a positive control.
the proteins and the streptavidin beads. Additionally, lymphocyte cell lysates did not show any positive band. Thus, we conclude that human monocyte Galectin-3 can specifically bind α-gal.

**Galectin-3 binds preferentially to porcine endothelium**

To further prove that galectin-3 is the monocyte receptor for Gal-α(1,3)Gal-β(1,4)GlcNAc-R, we showed that soluble Galectin-3 binds very highly to PAEC, while almost no binding is seen in HAEC (MFI, 21.07 ± 2.46 vs 1.9 ± 0.61; Fig. 7A; p < 0.001) when compared with the baseline levels. To confirm that this binding was a phenomenon of the lectin activity of Galectin-3, endothelial cells were incubated with Galectin-3 in the presence of sucrose, a control disaccharide, or lactose, which has a strong affinity for Galectin-3. The binding of Galectin-3 is almost completely diminished in the presence of lactose (MFI, 0.8 ± 0.17; p < 0.001).

To determine whether this lectin interaction is due to endothelial Gal-α(1,3)Gal-β(1,4)GlcNAc-R, PAEC were treated with exogalactosidases that specifically cleave terminal α-galactoside sugars. As the amount of Gal-α(1,3)Gal-β(1,4)GlcNAc-R is reduced (shown by percentage of baseline MFI), the binding capacity of Galectin-3 decreases (100 to 43.0 ± 6.78% for baseline vs α-galactosidase; Fig. 7B; p < 0.001).

**Blocking Galectin-3 inhibits monocyte adhesion**

If Galectin-3 is involved in the increased adhesion of human monocytes to PAEC, we hypothesized that the monocytes would show decreased adherence to PAEC if their surface Galectin-3 was blocked. Indeed, upon treatment of human monocytes with anti-Galectin-3 Ab vs an isotype control before perfusion over PAEC in a parallel plate flow chamber assay, adhesion was inhibited by ~39% (68.48 ± 5.16 vs 41.82 ± 2.58; Fig. 8; *, p = 0.001).

**Discussion**

Our current investigations provide new insight into a biological function of human Galectin-3 as a receptor for the major xenograft Gal-α(1,3)Gal-β(1,4)GlcNAc-R. Galectin-3 is expressed by human monocytes and binds Gal-α(1,3)Gal-β(1,4)GlcNAc-R. Our previous study demonstrated that adhesion to PAEC was much greater than to HAEC in a laminar flow model. This effect was dependent upon Gal-α(1,3)Gal-β(1,4)GlcNAc-R, and Gal-α(1,3)Gal-β(1,4)GlcNAc-R was found to induce high-affinity ligand binding of monocyte β2 integrins (15). Galectin-3 plays diverse roles in inflammation, including the recruitment of leukocytes and the promotion of adhesion (21–24). These characteristics prompted us to investigate the role of Galectin-3 in the context of xenotransplantation. Our current observations are that Galectin-3 is expressed in and on monocytes, but not lymphocytes, can bind Gal-α(1,3)Gal-β(1,4)GlcNAc-R, and, more importantly, binds extensively to PAEC, much more than to human cells. These findings provide additional support for the concept of a lectin-recognition mechanism that is involved in the attraction and accumulation of monocytes in a xenograft during the course of DXR.

A key difference exists between our current study and the previous one. In the previous study, we found that the fluorescent signal after incubation of polymeric α-gal and human monocytes was significantly decreased in the absence of Ca²⁺/Mg²⁺. This led us to the assumption of the involvement of C-type lectins (15). Conversely, we demonstrated in the current study that the binding of Galectin-3 to its ligand is calcium independent. We postulate that calcium is, in fact, required for internalization of polymeric α-Gal, as shown in Fig. 2B, or potentially in regulating Galectin-3 distribution within the cell, i.e., its delivery to the cell surface. Although Galectin-3 has no signal sequence, many different types of cell in culture have been found to secrete it (25–27). The mechanism behind this apparent secretion of Galectin-3 is thought to occur through an endoplasmic reticulum/Golgi complex-independent pathway that remains unclear. The studies on regulation of Galectin-3 secretion by Sato and Hughes (28) demonstrated that the secretion of Galectin-3 by murine peritoneal macrophages was strongly stimulated by treatment with calcium ionophores, suggesting that Ca²⁺ influx into the cell might play a role in the mechanism of Galectin-3 secretion.
Galectin-3 primarily binds to the C4 and C6 hydroxyl groups of the galactose molecule next to GlcNAc and the C3 hydroxyl group on the GlcNAc molecule. Other bonds can be made to the N-acetyl group of GlcNAc, as well as to the terminal galactose molecule. Conversely, the anti-gal Ab binds mainly to the 2C and 4C hydroxyl groups of the nonreducing terminal galactose molecule.

Sano et al. (29) have shown that Galectin-3 acts as a chemotactic factor for human monocytes in a much more potent fashion than even MCP-1. Stimulation of monocytes with Galectin-3 induces a Ca^{2+} influx. Monocytes migrate in response to Galectin-3 in a dose-dependent manner. Both monocyte migration and the intracellular Ca^{2+} signal can be completely blocked with pertussis toxin, which implies the involvement of G protein-coupled pathway.

Macrophages, the major phagocytes of the human immune system, play a very important role in the internalization and later degradation of pathogens. Galectin-3 has been shown to play a critical role in macrophage phagocytosis of both IgG-opsonized erythrocytes and apoptotic cells using Galectin-3-deficient cells from Galectin-3 knockout mice (30).

We believe that the interaction between Galectin-3 and the xenograft target by which DXR may be inhibited. Galectin-3 could provide another pharmacological target by which DXR may be inhibited.

Disclosures
The authors have no financial conflict of interest.

References

FIGURE 9. Anti-Gal Ab and Galectin-3 bind to different sites on α-gal. Galectin-3 mediates interaction of α-Gal and human monocytes.


