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Galectin-8 Functions as a Matricellular Modulator of Cell Adhesion*

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The interaction of cells with the extracellular matrix regulates cell adhesion and motility. Here we demonstrate that different cell types adhere and spread when cultured in serum-free medium on immobilized galectin-8, a mammalian β-galactoside-binding protein. At maximal doses, galectin-8 is equipotent to fibronectin in promoting cell adhesion and spreading. Cell adhesion to immobilized galectin-8 is mediated by sugar-protein interactions with integrins, and galectin-8 triggers integrin-mediated signaling cascades including Tyr phosphorylation of focal adhesion kinase and paxillin. Cell adhesion is potentiated in the presence of Mn^{2+}, whereas it is interrupted in the presence of soluble galectin-8, integrin β_1 inhibitory antibodies, EDTA, or thiodigalactoside but not by RGD peptides. Furthermore, cells readily adhere onto immobilized monoclonal galectin-8 antibodies, which are equipotent to integrin antibodies in promoting cell adhesion. Cell adhesion to immobilized galectin-8 is partially inhibited by serum proteins, suggesting that complex formation between immobilized galectin-8 and serum components generates a matrix that is less supportive of cell adhesion. Accordingly, cell motility on immobilized galectin-8 readily takes place in the presence of serum. Truncation of the C-terminal half of galectin-8, including one of its two carbohydrate recognition domains, largely abolishes its ability to modulate cell adhesion, indicating that both carbohydrate recognition domains are required to maintain a functional form of galectin-8. Collectively, our findings implicate galectin-8 as a physiological modulator of cell adhesion. When immobilized, it functions as a matrix protein equipotent to fibronectin in promoting cell adhesion by ligation and clustering of cell surface integrin receptors. In contrast, when present in excess as a soluble ligand, galectin-8 (like fibronectin) forms a complex with integrins that negatively regulates cell adhesion. Because of its dual effects on the adhesive properties of the cells and its association with fibronectin, galectin-8 might be considered a novel type of matricellular protein.

Extracellular matrix (ECM)[1] proteins have an important function in providing structural integrity to tissues and in presenting proper environmental cues for cell adhesion, migration, growth, and differentiation (1–3). All of these aspects rely on the spatiotemporal expression of adhesive as well as anti-adhesive components in extracellular matrices and on the cell surface (4). These include "classical" ECM proteins like fibronectin (5–7) and laminin (8) that act as anti adhesive ligands under certain experimental conditions. Many proteins that inhibit cell adhesion were classified as matricellular proteins (9). This group embodies proteins such as thrombospondin (10), tenascin (11), and hevin (12) that do not serve as integral components of matrix elements but rather function through binding to matrix proteins as well as to cell surface receptors. Diverse mechanisms were proposed to account for the anti adhesive effects of ECM (5–7) and matricellular proteins (9). Still, the underlying mode of action of most proteins inducing anti adhesive effects is unknown. Cell-matrix interactions depend to a large extent upon the engagement of specific ECM proteins with cell surface integrins (2, 13). Integrins are a family of α and β subunits that create heterodimer receptors with characteristic binding specificities. Both ligand occupancy and integrin clustering are critical for the activation of integrins and for triggering intracellular integrin-mediated signaling cascades (2, 13, 14).

Cell adhesion also depends upon carbohydrate-protein interactions, mediated by mammalian lectins of different families (15). Selectins function as mediators of cell-cell interactions (16) through calcium-dependent recognition of sialylated glycans (17, 18). Similarly, galectins, animal lectins that specifically bind β-galactoside residues (19), were implicated as modulators of cell adhesion. Although lacking a signal peptide and found mainly in the cytosol, galectins are externalized by an atypical secretory mechanism (20) to mediate cell growth, cell transformation, embryogenesis (reviewed in Ref. 21), and apoptosis (22). In accordance with their proposed functions, galectins enhance (23, 24) or inhibit (25, 26) cell-matrix interactions. Although little is known about the exact role of galectins in regulating cell adhesion, current models suggest that binding of galectins to matrix proteins elicits an inhibitory effect due to a steric hindrance of cell-matrix interactions (25, 26).

Galectin-8 (27–29), a member of the galectin family, is a 34-kDa secreted protein that is widely expressed. It is made of two homologous (38% identity) carbohydrate recognition domains (CRDs) joined by a short (~26 amino acids) linking peptide. We have recently shown that upon secretion, galectin-8 binds to a selective subset of cell surface glycoproteins of

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[1] The abbreviations used are: ECM, extracellular matrix; CRD, carbohydrate recognition domain; FAK, focal adhesion kinase; FCS, fetal calf serum; CHO, Chinese hamster ovary; PBS, phosphate-buffered saline; BSA, bovine serum albumin; GST, glutathione *S*-transferase; SDS-PAGE, SDS-polyacrylamide gel electrophoresis; TDG, thiodigalactoside; P-, phospho; CHO-P, Chinese hamster ovary parental cells; CHO-T, CHO-P cells overexpressing the insulin receptor.

the integrin family (29). These include $\alpha_3\beta_1$ and $\alpha_6\beta_1$, but not $\alpha_4\beta_1$, integrins. Binding of soluble galectin-8 to integrins presumably accounts for its inhibitory effects on cell adhesion (29). In the present study we set out to analyze the molecular basis for the anti adhesive properties of galectin-8. We demonstrate that galectin-8 is a matricellular protein that positively or negatively regulates cell adhesion, depending on the extracellular context. When immobilized onto matrix, galectin-8 can be classified as a novel ECM protein equipotent to fibronectin in promoting cell adhesion, spreading, and migration. Accordingly, cell adhesion to galectin-8 triggers integrin-mediated signaling cascades such as Tyr phosphorylation of FAK and paxillin. In contrast, excess soluble galectin-8 interacts both with cell surface integrins and with other soluble ECM proteins and inhibits cell-matrix interactions. These findings implicate members of the galectin family as a novel class of matricellular proteins that modulate cell adhesion.

EXPERIMENTAL PROCEDURES

Materials—Bacterially expressed recombinant galectin-8 (galectin-8) and GST-galectin-8 were generated as previously described (27). Restriction enzymes were purchased from New England Biolabs, Inc. (Beverly, MA). Echistatin, tetramethylrhodamine isothiocyanate-labeled phalloidin, crystal violet, fibronectin, and glutathione-agarose beads were purchased from Sigma. Agarose was purchased from Life Technologies, Inc.

Antibodies—Affinity-purified polyclonal antibodies (1.1) against galectin-8 were generated as described (27). Monoclonal antibodies (106.1) against recombinant galectin-8 were generated by established procedures (30) and were purified over protein-G coupled to Sepharose. Murine monoclonal antibodies against human β_1 integrin antibodies (CD-29, MCA1189) were purchased from Serotec. Peroxidase-conjugated affinity-purified goat anti-mouse IgG (H+L) and Cy3-conjugated and fluorescein isothiocyanate-conjugated affinity-purified $F(ab')_2$ fragment of goat anti-mouse IgG (H+L) were purchased from Jackson Immunoresearch Laboratories, Inc. Monoclonal anti-vinculin (hVin-1) and monoclonal anti-P-Tyr (PT-66) were purchased from Sigma. Anti-paxillin and anti-FAK monoclonal antibodies were obtained from Transduction Laboratories (Lexington, KY). Monoclonal anti-fibronectin antibodies were generated by B. Geiger (Weizmann institute, Rehovot, Israel). Affinity-purified polyclonal antibodies directed toward the insulin receptor α subunits were purchased from Santa Cruz Biotechnology (Santa Cruz, California). Monoclonal antibodies (IgG fraction), directed toward the extracellular domain of ErbB-2 (31), were a generous gift of Y. Yarden (Weizmann institute, Rehovot, Israel).

Cell Cultures—Human non-small cell lung carcinoma H1299 cells and rat hepatoma (Fao) cells were grown in RPMI medium containing 10% fetal calf serum (FCS). Chinese hamster ovary (CHO-P) cells and CHO-T cells (transfected with human insulin receptor (32)) were grown in F12 medium containing 10% FCS. HeLa cells were grown in minimum Eagle's medium containing 10% FCS. Human keratinocytes (HaCaT cells), human hepatoma Hep-G2 and NIH-hIR mouse fibroblasts (overexpressing the insulin receptor (33)) were grown in Dulbecco's modified Eagle's medium containing 10% FCS. N87 human gastric tumor cells were grown in Dulbecco's modified Eagle's medium containing 10% FCS supplemented with 1 mM sodium pyruvate.

Cell Adhesion Assay—Bacterial or tissue culture plates were precoated for 2 h at 22 °C with the indicated ligands (in PBS) or antibodies. Cells, grown on tissue culture plates, were detached from the plates with 5 mM EDTA, washed with PBS, resuspended in serum-free medium, and reseeded on the coated plates. At the indicated times cells were washed, and the adherent cells were counted. Alternatively, adherent cells were stained with 0.2% crystal violet in PBS containing 20% methanol for 15 min at 22 °C. Excess dye was removed by three washes with water, and cells were solubilized in 1% SDS for 1 h at 22 °C. Cell binding was quantified by measuring the absorbance at 540 nm in a TECAN enzyme-linked immunosorbent assay plate reader (Spectra, Austria). Specific binding was defined as the difference between the absorbance of cells binding to ligand-coated wells and the absorbance of cells binding to BSA-coated wells. All assays were performed in triplicate.

Cell Migration Assay—Cellular migration was assayed essentially as described (34). 60-mm bacterial or tissue culture plates were precoated for 2 h at 22 °C with galectin-8 or with fibronectin (in PBS). Cells, grown on tissue culture plates, were detached from the plates with 5 mM EDTA and washed with PBS. 2% low melting point agarose stock (maintained at ~38 °C) was adjusted to 0.2% agarose using medium containing 10% serum and was mixed with the cells. Cells (3.3×10^4) were plated in 1–2-μl droplets on the coated plates. After the agarose was gelled by incubation at 4 °C for 10 min, medium containing 10% fetal calf serum was added, and the cells were incubated at 37 °C for the rest of the experiment. At the indicated days, the medium was aspirated, and the cells were fixed with 4% formaldehyde for 10 min. Following rinsing, cells were stained with 1% crystal violet in 95% ethanol for 15 min at 22 °C. Excess dye was removed with water, and the plates were photographed.

Binding of Serum Proteins to GST-Galectin-8—GST-galectin-8 or GST (0.5 mg each) was immobilized on 200-μl glutathione-agarose beads. Following 2 h of incubation at 4 °C, the beads were washed and further incubated for 2 h at 4 °C with 5 ml of 100% fetal calf serum. The beads were intensively washed three times with 1% Triton X-100 in PBS, and the bound proteins were eluted by 2 h of incubation at 4 °C with 100 μl of lactose, glucose, or NaCl (150 mM each). The eluted proteins were suspended in Laemmli sample buffer and resolved by 10% SDS-PAGE.

Preparation of Cell Extracts—Cell extracts were prepared in buffer A (25 mM Tris/HCl, 25 mM NaCl, 0.5 mM EGTA, 2 mM sodium orthovanadate, 10 mM NaF, 10 mM sodium pyrophosphate, 80 mM β-glycerophosphate, 1% Triton X-100, 0.5% deoxycholate, 0.5% SDS, 5 μg/ml leupeptin, 10 μg/ml trypsin inhibitor, and 1 mM phenylmethylsulfonyl fluoride, pH 7.5). Insoluble material was removed by a 15-min centrifugation ($12{,}000 \times g$) at 4 °C. Supernatants were mixed with 5× concentrated Laemmli sample buffer (35), boiled for 5 min, resolved on 10% SDS-PAGE under reducing conditions, transferred to nitrocellulose membrane (Schleicher and Schuell), and Western immunoblotted with the indicated antibodies.

Immunoprecipitation—Cell extracts (1–2 mg of protein) were incubated for 16 h at 4 °C with monoclonal FAK or paxillin antibodies, followed by additional incubation for 2 h at 4 °C with 30 μl of protein G-agarose beads. Immunocomplexes were washed twice with buffer B (25 mM Tris/HCl, 25 mM NaCl, 0.5 mM EGTA, 2 mM sodium orthovanadate, 10 mM NaF, 10 mM sodium pyrophosphate, 80 mM β-glycerophosphate, 1% Triton X-100, 5 μg/ml leupeptin, 10 μg/ml soybean trypsin inhibitor, and 1 mM phenylmethylsulfonyl fluoride, pH 7.5) and once with PBS. Samples were mixed with Laemmli sample buffer, boiled for 5 min, resolved by means of 10% SDS-PAGE, and immunoblotted with the indicated antibodies.

Peptide Sequencing—Proteins were resolved by means of 7.5% SDS-PAGE. Following staining with Coomassie Blue, the bands were excised from the gel, destained, and trypsinized. The resulting peptides were subjected to matrix-assisted laser desorption ionization analysis as described (36).

Immunofluorescence Microscopy—Cultured cells, plated on glass coverslips, were washed and fixed with paraformaldehyde (3%) containing 0.5% Triton X-100. Following several washes with PBS, cells were incubated for 1 h at 22 °C with anti-paxillin, anti-vinculin, or anti-P-Tyr monoclonal antibodies or with tetramethylrhodamine isothiocyanate-labeled phalloidin. Cells were washed and incubated for 1 h at 22 °C with secondary Cy3-conjugated goat anti-mouse antibodies in PBS. The specimens were washed, mounted onto glass microscope slides, and examined on a Zeiss fluorescence microscope.

Generation of Recombinant Truncated Galectin-8 (N-galectin-8)—Site-directed mutagenesis was performed using a QuikChange kit (Stratagene) according to the manufacturer's instructions. pET-3a-galectin-8 encoding rat galectin-8 (27) served as a template. The N-terminal fragment of galectin-8 (amino acids 1–158), in which Glu-159 was replaced by a stop codon, was generated sequentially using the following set of overlapping primers: 5′-GCTCGGATTTACAGAGTATGTAAAGATCTACTCTGGGACTG-3′ and 5′-CAGTCCCAGAGTAGATCTTTACATACTCTGTAAATCCGAGC-3′ (an additional restriction site for *Bgl*II is *underlined*). The polymerase chain reaction products were digested with *Bgl*II to confirm the mutation and then introduced into the pET-3a expression plasmid. To express N-galectin-8, pET-3a was transformed in the pLysS bacterial host, and the expressed soluble N-galectin-8 was purified over lactosyl-Sepharose as described (27).

RESULTS

Soluble Galectin-8 Attenuates Cell Adhesion—We have previously shown that incubation of soluble recombinant galectin-8 with suspended H1299 cells resulted in a marked attenuation in their rate of adhesion (29). Cells seeded on culture plates in the presence of 10% FCS started to spread after 1 h,

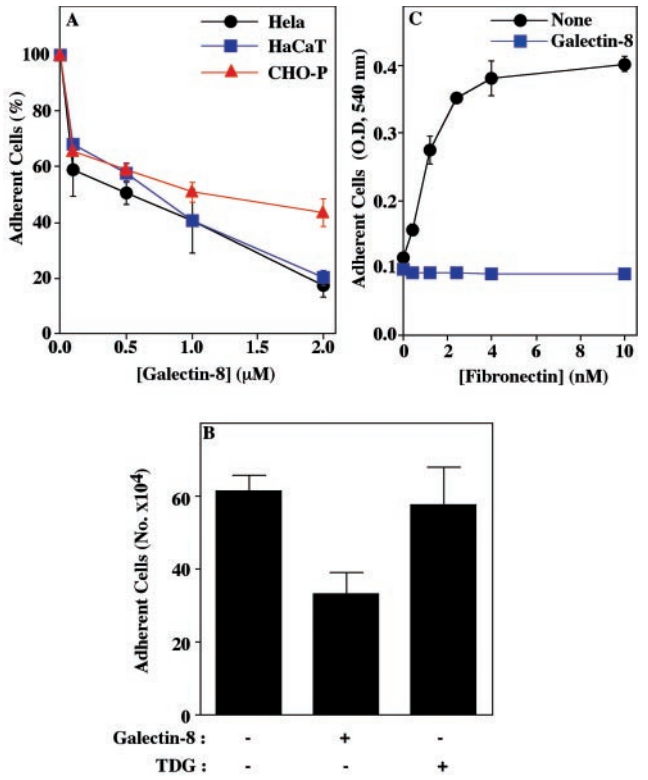


FIG. 1. **Inhibition of cell adhesion to culture plates by soluble galectin-8.** *A*, HeLa (●), HaCaT (■), and CHO-P (▲) cells (3×10^5) were detached from culture plates with 5 mM EDTA, washed with PBS, and resuspended in medium containing 10% FCS. Cells were seeded on 24-well Costar plates in the absence or presence of the indicated concentrations of galectin-8. Following 2 h of incubation at 37 °C, cells were washed, and the number of adherent cells was counted. Values are the mean ± S.D. of duplicate measurements of a representative experiment. *B*, HeLa cells (3×10^5) were detached from culture plates with 5 mM EDTA, washed with PBS, and seeded on 24-well Costar plates in the absence (control) or presence of galectin-8 (1 μM) or galectin-8 and TDG (10 mM). Following 2 h of incubation at 37°C, cells were washed, and the number of adherent cells was counted. Values are the mean ± S.D. of quadruplicate measurements of a representative experiment. *C*, 96-well tissue culture plates were precoated with 0.1 ml of the indicated concentrations of fibronectin for 1 h at 37 °C and further blocked with 1% BSA for 1 h at 37 °C. HeLa cells (1×10^5) were detached from culture plates with 5 mM EDTA and washed with PBS. Cells were incubated in suspension for 1 h at 37 °C in serum-free medium in the absence (●) or presence (■) of galectin-8 (2.5 μM). At the end of incubation, cells were washed before being seeded on the fibronectin-coated wells. Following 2 h of incubation at 37 °C, cells were washed and stained with crystal violet, and the number of adherent cells was determined using an enzyme-linked immunosorbent assay reader at 540 nm. Values are the mean ± S.D. of triplicate measurements of a representative experiment

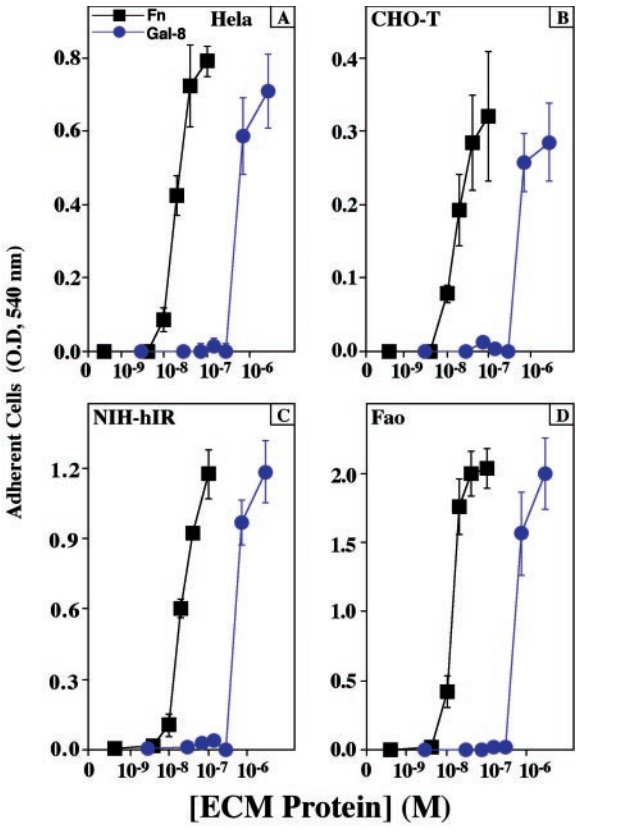


FIG. 2. **Cell adhesion to plates coated with galectin-8 or fibronectin.** 96-well bacterial plates were precoated with 0.1 ml of the indicated concentrations of galectin-8 (●) or fibronectin (■) for 2 h at 22 °C. HeLa (*A*), CHO-T (*B*), NIH-hIR (*C*), or Fao cells (*D*) (4×10^5 each) were detached from culture plates with 5 mM EDTA, washed with PBS, and seeded in serum-free medium on the coated wells. Following 2 h of incubation at 37 °C, cells were washed and stained with crystal violet, and the number of adherent cells was determined. Values are the mean ± S.D. of six measurements of a representative experiment.

and most of the cells were spread after 2 h at 37 °C. In contrast, cells treated with soluble galectin-8 retained a rounded morphology and did not spread on the culture plates after 2 h at 37 °C. The inhibitory effect was not restricted to H1299 cells. As shown in Fig. 1*A*, soluble galectin-8 effectively inhibited (albeit with a different potency) the adhesion of several cell types, assayed 2 h after plating. High doses (2 μM) of galectin-8 inhibited by 50–80% adhesion of HaCaT, HeLa, and CHO-P cells (Fig. 1*A*), as well as adhesion of Hep-G2 and PC-12 cells (data not shown). Galectin-8 effects were abolished upon addition of 10 mM thiodigalactoside (TDG), which blocks lectin-carbohydrate interactions (Fig. 1*B*). TDG alone (10 mM) did not affect cell adhesion (data not shown). These results indicate that the inhibitory effects of galectin-8 on cell adhesion are a widespread phenomenon observed in a number of cell lines and involve protein-sugar interactions.

Soluble galectin-8 effectively inhibited cell adhesion even in the absence of serum. When galectin-8 was preincubated with HeLa cells, and unbound lectin was removed by washing, a marked inhibition of cell adhesion to fibronectin-coated plates was observed (Fig. 1*C*). These results indicate that soluble galectin-8, like soluble fibronectin (5) and laminin (8), could negatively regulate cell-matrix interactions by generating an anti adhesive complex upon binding to cell surface receptors.

Immobilized Galectin-8 Functions as an Extracellular Matrix Protein and Supports Cell Adhesion—Because soluble ECM proteins such as fibronectin (5) were shown to inhibit cell adhesion, we explored the possibility that when immobilized, galectin-8, like fibronectin, can support cell adhesion. To this end, adhesion of HeLa cells to bacterial plates coated with galectin-8 was studied. Whereas bacterial plates fail to support cell adhesion under serum-free conditions, galectin-8 immobilized on the plates promoted cell adhesion in a dose-dependent manner (Fig. 2*A*). Higher doses of added galectin-8 than of fibronectin were required to support cell adhesion. Still, when applied to the plates at a maximal dose, galectin-8 (2.5 μM) was as effective as fibronectin (0.1 μM). Similar results were obtained when adhesion of CHO-T, NIH-hIR, or Fao cells was studied (Fig. 2, *B–D*). These findings indicate that galectin-8 could function as an ECM protein for different cell types. The kinetics of adhesion of HeLa cells to galectin-8- *versus* fibronectin-coated plates was evaluated next. As shown in Fig. 3*A*, adhesion to galectin-8 or fibronectin occurred at comparable rates. The adhesion to immobilized GST-galectin-8 was decreased upon addition of either TDG or galectin-8-specific poly-

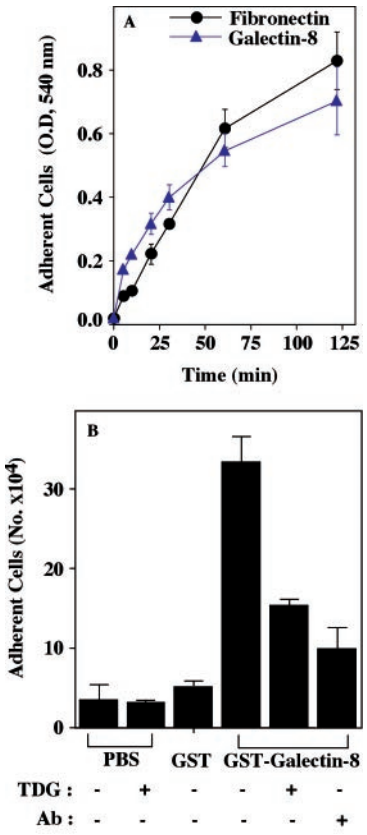


FIG. 3. **Kinetics and specificity of cell adhesion to galectin-8.** *A*, 96-well bacterial plates were precoated with 0.1 ml of galectin-8 (▲, 2.9 μM) or fibronectin (●, 0.1 μM) for 2 h at 22 °C. HeLa cells (4×10^5) were detached from cultured plates with 5 mM EDTA, washed with PBS, and seeded at 37 °C in serum-free medium on the coated wells. At the indicated times cells were washed and stained with crystal violet, and the number of adherent cells was determined. Values are the mean ± S.D. of six measurements of a representative experiment. *B*, 24-well Costar plates were precoated with 0.5 ml of GST (1.0 μM) or GST-galectin-8 (0.4 μM) for 2 h at 37 °C. The plates were then blocked with 5% BSA for 2 h at 37 °C. HeLa cells (3×10^5) were detached with 5 mM EDTA, washed with PBS, and seeded in serum-free medium onto the plates in the absence or presence of TDG (10 mM) or anti-galectin-8 polyclonal (1.1) antibodies (10 μg/ml), as indicated. Following 2 h of incubation at 37 °C, cells were washed, and the number of adherent cells was counted. Values are the mean ± S.D. of duplicate measurements of a representative experiment. *Ab*, antibody.

clonal antibodies (Fig. 3*B*). TDG alone did not affect cell adhesion (data not shown). These findings suggest that the adhesive functions of galectin-8, like its anti adhesive effects, involve protein-sugar interactions.

Cell Attachment, Spreading, and Cytoskeletal Organization Mediated by Immobilized Galectin-8—The ability of immobilized galectin-8 to induce cell spreading was evaluated next. Immobilized galectin-8, like fibronectin, induced both attachment and spreading of HeLa, NIH-hIR (Fig. 4), and CHO-P cells (Fig. 5) plated in serum-free medium on cover glasses precoated either with galectin-8 or fibronectin. Although the cells adhered to both matrices, marked differences were noted in their cytoskeletal organization. The differences were not prominent following 30 min of incubation (Fig. 5) but became more evident as the adhesion process progressed for 2 h. Prominent stress fibers that traverse the cell body were readily observed in fibronectin-adherent cells but were less abundant in cells adherent to galectin-8 (Fig. 4, *A versus C* and *E versus G*, and enlargement of Fig. 5). Second, whereas vinculin and paxillin were associated with large focal contacts in cells adherent to fibronectin, the number and size of vinculin- and paxillin-containing focal contacts was reduced in cells attached to galectin-8. In fact, many adhesion sites, especially in HeLa cells, were devoid of paxillin altogether. Similarly, NIH-hIR, HeLa, or CHO-P cells seeded on fibronectin formed many focal adhesions throughout their entire ventral surface, whereas vinculin- or paxillin-containing plaques were rather small in size and were primarily limited to the cell periphery in cells seeded on galectin-8 (Figs. 4 and 5).

Signal Transduction Mediated by Cell Adhesion to Galectin-8—To study the signaling cascades induced upon cell adhesion to galectin-8, we compared the tyrosine phosphorylation profile of cytoskeletal proteins in cells adherent to galectin-8 or fibronectin. In accordance with the observed differences in cytoskeletal organization, CHO-P cells adherent onto galectin-8 showed a diffused staining of P-Tyr, which was not confined to focal adhesion sites (Fig. 5). To better characterize the nature of the cytoskeletal proteins whose Tyr phosphorylation was stimulated upon adhesion to galectin-8, HeLa and CHO-P cells were detached from their culture plates and reseeded in serum-free medium on bacterial plates precoated with galectin-8 or fibronectin. Consistent with previous studies (*cf.* Ref. 37) Tyr phosphorylation of FAK and paxillin was largely diminished upon detachment of the cells from culture plates (*cf.* Fig. 6, time 0 and 60 min without galectin or fibronectin}. Readhesion of CHO-P or HeLa cells onto fibronectin-coated plates induced Tyr phosphorylation of FAK and paxillin that remained elevated for at least 60 min post-adhesion. Reduced levels of Tyr phosphorylation of FAK and paxillin were observed at all time points when HeLa cells adhered onto galectin-8-coated plates (Fig. 6, *A* and *C*). This reduction was less prominent when CHO-P cells adherent to galectin-8 were studied. The identity of pp125 and pp74 as FAK and paxillin, respectively, was confirmed by immunoprecipitation with FAK- or paxillin-specific antibodies (Fig. 6, *B* and *D*).

Cell Adhesion to Galectin-8 Is Mediated by Integrins—Secreted galectin-8 remains surface-bound and forms complexes with the extracellular domains of specific integrin subunits (*e.g.* α_3, α_6, and β_1) (29). These findings implicate integrins as potential receptors that could mediate the adhesive effects of galectin-8. To test this hypothesis, HeLa cells were incubated with anti-β_1 integrin antibodies, known to inhibit integrin-mediated adhesion (38). As shown in Fig. 7*A*, adhesion of HeLa cells to fibronectin or galectin-8 was inhibited 50 and 40%, respectively, when the cells were pre-incubated with anti-β_1 integrin antibodies. The effect of the antibodies on cell adhesion to galectin-8 was specific and could not be mimicked by anti-β_5 or anti-α_v integrin antibodies (data not shown). In contrast, whereas echistatin, which contains an RGD motif (39), effectively inhibited (60%) adhesion of HeLa cells to fibronectin, it did not inhibit cell adhesion to galectin-8 (Fig. 7*B*). Similarly, EDTA effectively inhibited adhesion of HeLa cells to fibronectin, but it only partially inhibited (60%) cell adhesion to galectin-8 (Fig. 7*C*). Conversely, Mn^{2+}, which increases the affinity of integrins to their substrates (2, 13), induced a 2-fold increase in the number of cells adherent to fibronectin, whereas it had a more modest effect (50% increase) on cell adhesion to galectin-8. Combined with the fact that TDG effectively inhibited cell adhesion to galectin-8 (Fig. 3), the above results indicate that the interactions of galectin-8 with integrins do not involve the RGD-binding domain of the integrin molecule but rather are mediated by glycoconjugates that form part of the integrin extracellular domain. These findings support the notion that galectin-8 functions as a matrix-associated protein whose interaction with integrins mediates, at least in part, both its adhesive and anti adhesive functions.

Monoclonal Anti-galectin-8 Antibodies Promote Cell Adhesion—Certain integrin antibodies are characterized by their

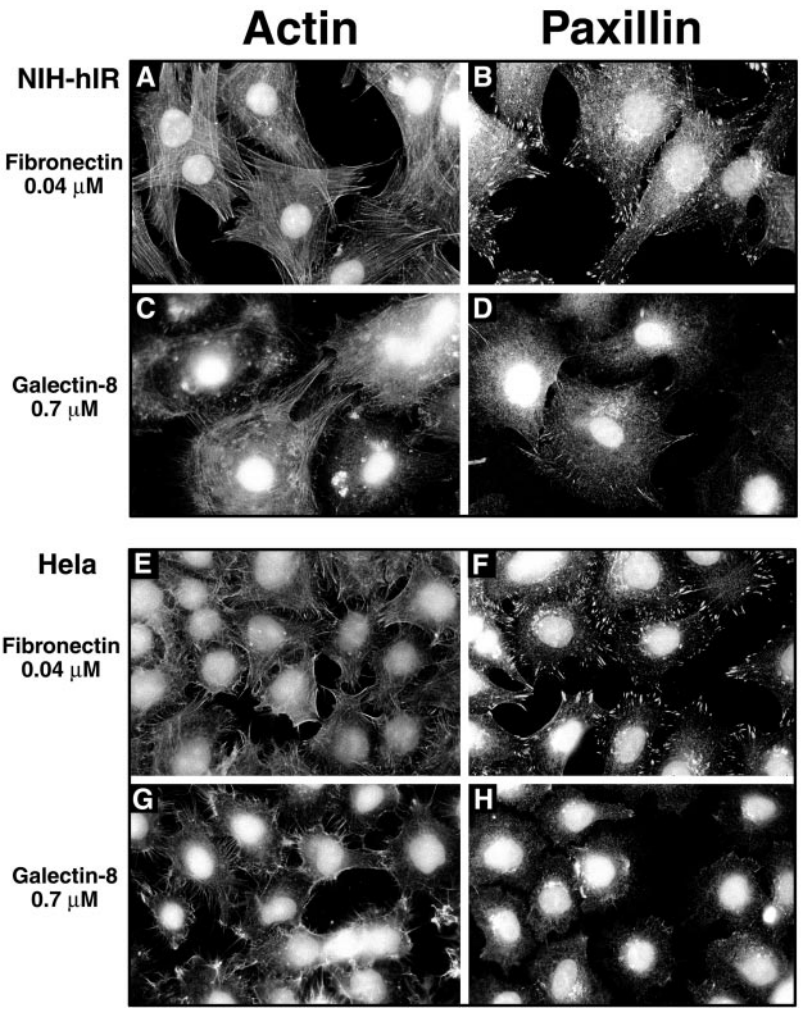


FIG. 4. **Cytoskeletal organization of cells adherent to galectin-8 or fibronectin.** Cover glasses were precoated for 2 h at 22 °C with 1 ml of fibronectin (0.04 μM) (*A*, *B*, *E*, and *F*) or galectin-8 (0.7 μM) (*C*, *D*, *G*, and *H*). HeLa or NIH-hIR cells were grown on tissue culture plates and incubated for 16 h in serum-free medium. Cells were detached from the culture plates with 5 mM EDTA, washed, and incubated in suspension for 30 min at 37 °C in serum-free medium. Cells were then seeded in serum-free medium on the coated cover glasses. Following 2 h of incubation at 37 °C, cells were fixed and incubated with tetramethylrhodamine isothiocyanate-labeled phalloidin (*A*, *C*, *E*, and *G*) for actin staining or immunostained for paxillin (*B*, *D*, *F*, and *H*) as described under "Experimental Procedures."

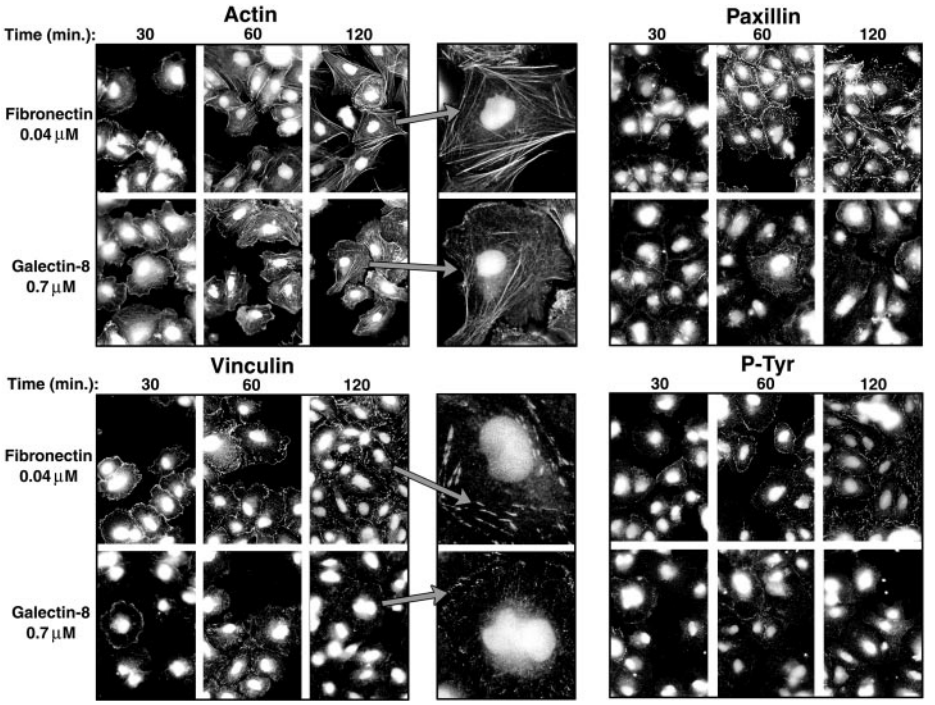


FIG. 5. **Kinetics of cytoskeletal organization in cells adherent to galectin-8 or fibronectin.** CHO-P cells were seeded on cover glasses coated with fibronectin or galectin-8 as described in the legend to Fig. 4. Following incubation for the indicated times at 37 °C, cells were washed, fixed, and immunostained for actin, paxillin, vinculin, or P-Tyr as described under "Experimental Procedures."

unique ability to promote cell adhesion (40). Because galectin-8 forms cell surface complexes with integrins, the effects of galectin-8 antibodies on cell adhesion were studied. The adhesion conditions (bacterial plates, serum-free medium) were selected such that basal adhesion was negligible. Similar to integrin antibodies, immobilized monoclonal mouse antibodies to galectin-8 effectively promoted cell adhesion in a dose-dependent manner (Fig. 8*A*). The effects of the galectin-8 antibodies were

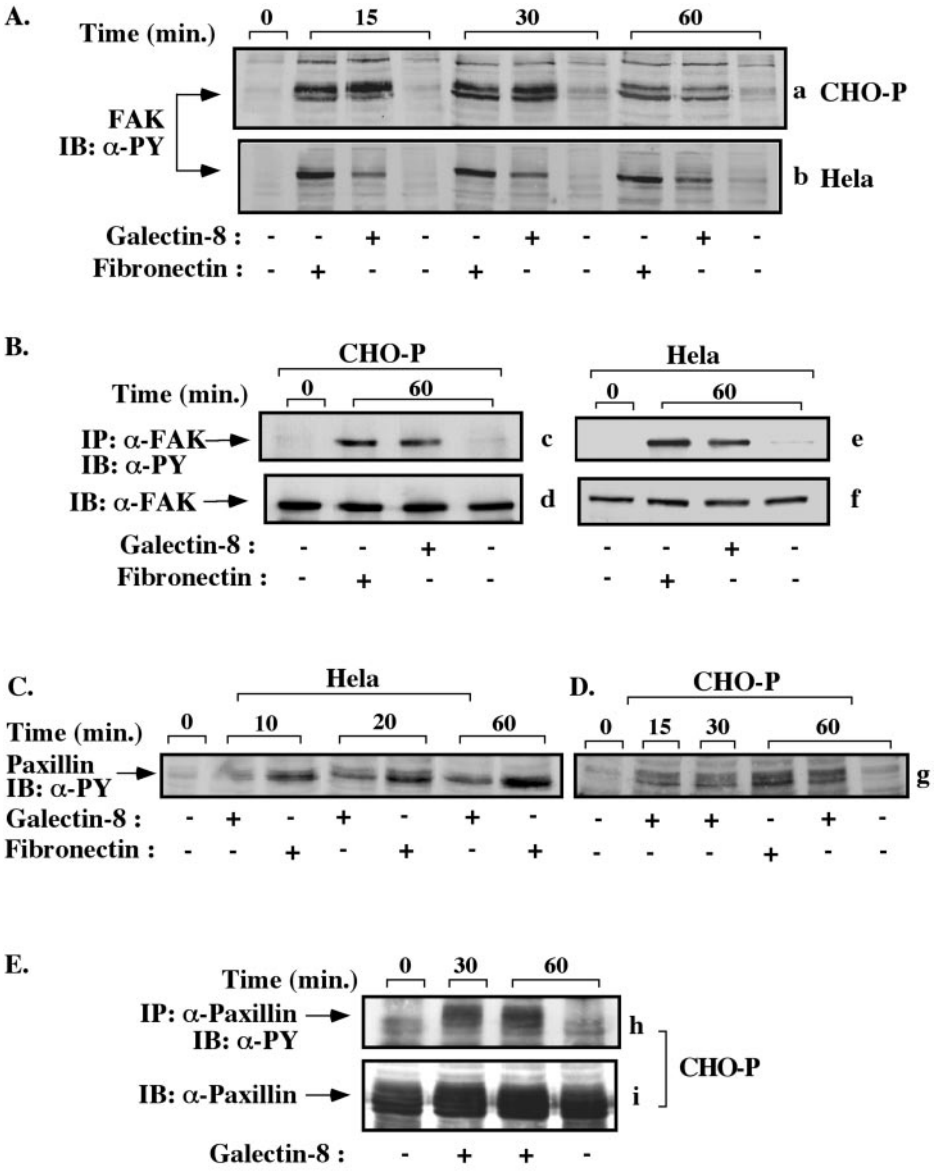


FIG. 6. **Tyr phosphorylation of FAK and paxillin, induced upon cell adhesion to galectin-8 or fibronectin.** Six-cm bacterial plates were precoated with 5 ml of galectin-8 (0.7 μM) or fibronectin (0.04 μM) for 2 h at 22 °C. HeLa or CHO-P cells were grown on tissue culture plates and incubated for 16 h in serum-free medium. Cells were detached from plates with 5 mM EDTA (*A*, *B*, *D*, and *E*) or with 0.05% trypsin and 2 mM EDTA (*C*), washed, incubated in suspension for 30 min (*A*, *B*, *D*, and *E*) or 60 min (*C*) at 37 °C in serum-free medium, and seeded in serum-free medium on the coated plates. Following incubation at 37 °C for the indicated times, cells were washed and extracted as described under "Experimental Procedures." Proteins (100 μg) were resolved by 10% SDS-PAGE, transferred to nitrocellulose membranes, and Western immunoblotted with anti P-Tyr antibodies (*a*, *b*, and *g*), anti-FAK antibodies (*f*), or anti-paxillin antibodies (*i*). Alternatively, cell extracts were subjected to immunoprecipitation with anti-FAK (*B*) or anti-paxillin (*E*) antibodies, resolved by 10% SDS-PAGE, transferred to nitrocellulose membranes, and Western immunoblotted with anti-P-Tyr (*c*, *e*, and *h*) or anti-FAK antibodies (*d*). This is one of six experiments that yielded essentially similar results. *IB*, immunoblot; *α-PY*, α-P-Tyr; *IP*, immunoprecipitation.

specific and could not be mimicked by P-Tyr antibodies or antibodies toward other cell surface proteins such as the extracellular α subunit of the insulin receptor or the extracellular domain of Erb2 (Fig. 8*B*). Moreover, the adhesive effects of the antibodies were abolished upon addition of goat anti-mouse antibodies (Fig. 8*C*). These findings further establish the role of galectin-8 as a potential physiological modulator of cell adhesion.

Serum Components Inhibit Cell Adhesion to Immobilized Galectin-8—Cell adhesion to immobilized galectin-8 is inhibited in the presence of serum components (29). In the following example (Fig. 9), adhesion of HeLa cells (in serum-free medium) to culture plates precoated with galectin-8 (25 μg/ml) was inhibited in a dose-dependent manner when the plates were further coated with increasing concentrations of serum (0–10%) before addition of the cells. 50% inhibition was observed when 1% serum was added to galectin-8-coated plates. Coating of the plates with serum alone or with galectin-8 alone promoted cell adhesion. To better characterize the nature of the inhibitory effects exerted by serum components, the kinetics of cell adhesion, in the presence of serum, to plates coated with galectin-8 was studied. As shown in Fig. 9*B*, immobilized galectin-8 attenuated the rate of cell adhesion; still a significant fraction of the cells remained adherent to the galectin-8-coated plates even in the presence of serum. These results suggest that complex formation between serum components and immobilized galectin-8 generates a matrix that partially inhibits, but does not abolish, cell adhesion to the immobilized lectin.

Galectin-8 Promotes Cellular Migration—Because cell adhesion on immobilized galectin-8 was attenuated in the presence of serum, we studied whether the reduced adhesion enabled cells to better migrate on galectin-8-coated plates. As shown in Fig. 10, and consistent with previous findings (34), cells seeded in agar droplets readily sprouted and migrated on plates coated with fibronectin. Similarly, the cells readily migrated on plates coated with galectin-8 (in the presence of serum). Prominent cellular foci, which sprouted out of the agar droplet, were readily detected throughout the plates 6 days after implantation, and the foci further grew in size until day 8, when the whole 60-mm plate was covered. In contrast, cells implanted in agar droplets on uncoated tissue culture plates, even in the presence of serum, migrated at a much slower rate and failed to generate visible foci even following 8 days of growth. These results indicate that immobilized galectin-8 is equipotent to fibronectin in supporting cellular migration in the presence of serum.

Galectin-8 Binds ECM Proteins Present in Serum—To identify serum components that could form complexes with galectin-8, serum proteins were incubated with immobilized GST-galectin-8, and the bound proteins were studied. As shown in

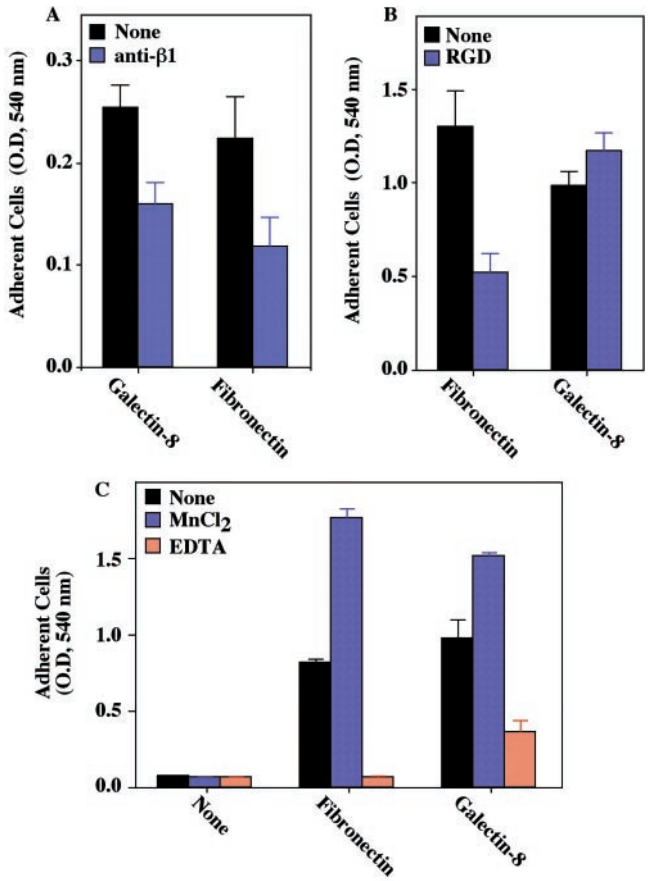


FIG. 7. **Effects of β1 integrin antibodies, RGD peptides, Mn^{2+}, and EDTA on cell adhesion to galectin-8.** 96-well bacterial plates (*A* and *B*) were coated for 2 h at 22 °C with 0.1 ml of galectin-8 (2.9 μM, *A*; 0.7 μM, *B*) or with fibronectin (0.1 μM, *A*; 0.04 μM, *B*). 96-well tissue culture plates (*C*) were coated with galectin-8 (2.5 μM) or fibronectin (0.01 μM) for 1 h at 37 °C, followed by blocking with 1% BSA for 1 h at 37 °C. HeLa cells (4×10^5, *A* and *B*; 1×10^5, *C*) were detached from culture plates with 5 mM EDTA and washed with PBS. Cells were incubated in suspension for 1 h at 37 °C in serum-free medium in the absence or presence of anti-β_1 integrin antibody (5 μg/ml) (*A*), echistatin (100 nM) (*B*), or 5 mM EDTA or 1 mM $MnCl_2$ (in 150 mM NaCl, 10 mM $MgCl_2$, 1 mM $CaCl_2$, 5 mM glucose, 10 mM Tris, pH 7.5) (*C*) before being seeded on the wells. Following 2 h of incubation at 37 °C, cells were washed and stained with crystal violet, and the number of adherent cells was determined. Values are the mean ± S.D. of triplicate measurements of a representative experiment.

Fig. 11*A*, three serum proteins having molecular masses of 270, 250, and 110 kDa, respectively that were retained by GST-galectin-8 could be selectively eluted with lactose but not with glucose or NaCl, indicating that they remained bound to galectin-8 due to specific protein-sugar interactions. Matrix-assisted laser desorption ionization analysis revealed that p250 contains eight peptides derived from calf fibronectin that corresponded to 6.6% of the total protein mass (amino acids 881–891, 928–945, 1099–1126, 1221–1243, 1380–1403, 1254–1270, 1494–1508, 1746–1759). The identity of p250 as fibronectin was confirmed by blotting the lactose-eluted proteins with specific fibronectin antibodies (Fig. 11*B*). The nature of the other two proteins is currently being investigated.

Complex Formation between Fibronectin and Galectin-8 Does Not Interfere with Cell Adhesion—To further study the interactions of galectin-8 and fibronectin, their combined effects on cell adhesion were explored. As shown in Fig. 12*A*, when plates coated with fibronectin were further coated with galectin-8 and vice versa, no inhibitory effect of the immobilized galectin-8 on adhesion of HeLa cells could be detected. In fact, a higher number of cells adhered to plates coated with galectin-8 when these plates were pre-coated with fibronectin, indicating that the effects of galectin-8 and fibronectin might be partially additive. These findings indicate that interactions of galectin-8 with serum fibronectin do not account for the anti adhesive effects observed when cells adhere onto plates coated with galectin-8 in the presence of serum.

A Truncated Galectin-8 Is Impaired in Its Ability to Mediate the Adhesive and Antiadhesive Functions of Galectin-8—Galectin-8 contains two homologous domains, joined by a link peptide, each having a single CRD (27). To determine whether each domain can independently mediate the adhesive or the anti adhesive activities of galectin-8, a truncated form of galectin-8, which contains only the N-terminal half of the protein (amino acids 1–158), was constructed. This 14-kDa truncated module, termed N-galectin-8, contains one CRD and is therefore capable of binding sugars, as evident by its ability to bind to, and be eluted from, a lactosyl-Sepharose column. As shown in Fig. 12*B*, immobilized N-galectin-8 was about 5-fold less potent than wild-type galectin-8 in promoting cell adhesion. In contrast, N-galectin-8 was essentially ineffective in inhibiting cell adhesion to galectin-8- or fibronectin-coated plates (Fig. 12*C*). The ability of N-galectin-8 to partially mimic the effects of immobilized galectin-8, when applied at high doses (20 μM), stems from the fact that N-galectin-8 is structurally related (34% identity) (27) to monomeric galectins (*e.g.* galectins 1 and 2), which tend to form homodimers when present at high enough concentrations (41). These findings indicate that the isolated N-terminal domain, having a single CRD, is markedly less potent than the full-length protein, suggesting that occupancy of glycoconjugates by both CRDs of galectin-8 is required to modulate cellular adhesion and migration.

DISCUSSION

The present study provides evidence that galectin-8, a mammalian β-galactoside-binding lectin, modulates cellular adhesion and migration depending on its mode of interaction with the cells. Whereas immobilized galectin-8 functions as an ECM protein, excess soluble galectin-8 blocks cell adhesion. Hence, because of its dual effects on the adhesive properties of the cells and its association with fibronectin, we refer to galectin-8 as a novel type of matricellular protein (9), capable of binding both ECM proteins and cell surface receptors. Several lines of evidence support such a conclusion. Like other galectins (21), galectin-8 is a secreted protein that accumulates in culture medium in a time-dependent manner (29). Soluble galectin-8 binds to the cell surface and inhibits subsequent cell adhesion, similar to soluble fibronectin (5), indicating that galectin-8 binds receptors that mediate cell matrix interactions. Conversely, when immobilized, galectin-8 readily supports cellular adhesion and migration, similar to fibronectin or other ECM proteins. Finally, cell adhesion onto galectin-8, like cell adhesion onto fibronectin, induces transmembranal signaling events manifested by Tyr phosphorylation of cytoskeletal proteins like FAK and paxillin. Combined with the ability of galectin-8 to interact with serum fibronectin, these findings implicate galectin-8 as a novel form of matricellular protein.

Cell adhesion to galectin-8 was studied by gravity assay (no centrifugal force applied), in which cell attachment is followed by cellular spreading. The ability of immobilized galectin-8 to promote both processes is evident from immunofluorescence studies, which demonstrate the elaborated spreading of cells that takes place on immobilized galectin-8. In fact, cells adhere and spread onto plates coated with galectin-8 with kinetics and efficacy similar to that found when cells adhere to fibronectin. Moreover, the overall morphology of the adherent cells is essentially indistinguishable when the cells are plated over the two types of surfaces. Despite its functional similarity to other

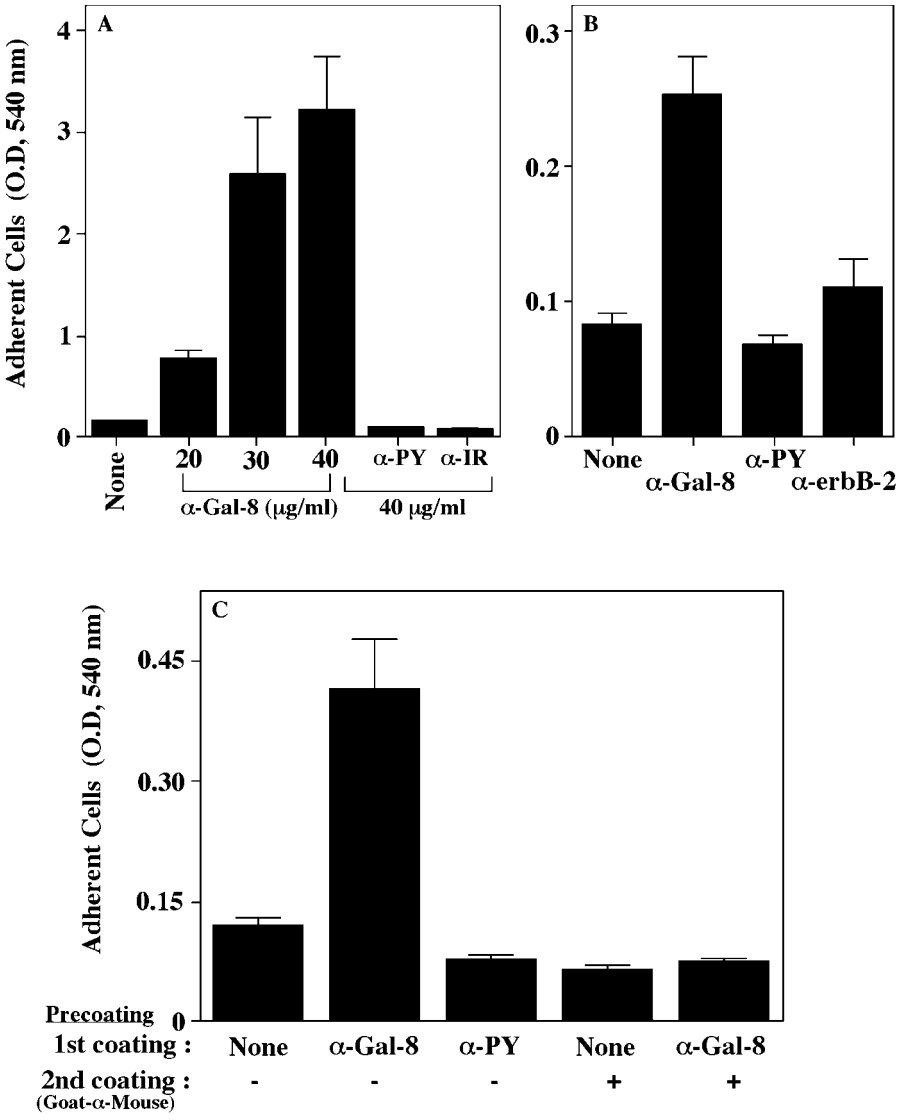


FIG. 8. **Effects of anti-galectin-8 monoclonal antibodies (106.1) on cell adhesion.** 96-well bacterial plates were precoated (2 h at 22 °C) with 100 μl of monoclonal antibodies (106.1), anti-P-Tyr (*α-PY*), anti-insulin receptor (extracellular) α subunit (*α-IR*), goat anti-mouse antibodies, or anti-erb2 monoclonal antibodies, each added at 40 μg/ml unless indicated otherwise. When indicated, plates coated with monoclonal antibodies (106.1) were further coated (2 h at 22 °C) with 50 μl of goat anti-mouse antibodies. Fao (*A*), N87 (*B*), or HeLa cells (*C*) (4×10^5) were detached from cultured plates with 5 mM EDTA, washed with PBS, and seeded at 37 °C in serum-free medium on the coated wells. Following 2 h of incubation at 37 °C, cells were washed and stained with crystal violet, and the number of adherent cells was determined. Values are the mean ± S.D. of quadruplicate measurements of a representative experiment.

ECM proteins, galectin-8 manifests several distinct features. First, when applied to culture plates, much higher concentrations of galectin than of fibronectin are required to support cell adhesion. This could be attributed to the fact that immobilized galectin-8 is less efficacious in binding to the culture plates or that it is presented at less than optimal configuration, when immobilized *in vitro*. Still, most striking are the differences in cytoskeletal organization and focal contact formation induced by galectin-8, compared with those induced by fibronectin. Whereas cells adherent onto fibronectin develop an elaborated network of actin bundles associated with well developed focal contacts, cells attached to galectin-8 manifest a poorly organized network of actin microfilaments, with small focal contacts distributed mainly at the cell periphery. More importantly, many of these adhesion sites contain minimal amounts of vinculin or paxillin, which correlates with the reduced Tyr phosphorylation of paxillin, suggesting that other proteins presumably act in conjunction with integrins to propagate the intracellular signals evoked upon cell adhesion to galectin-8. This idea is not surprising in view of the fact that adhesion complexes show extraordinary structural and molecular diversity, and sites of cell adhesion to the ECM can be mediated by a variety of matrix molecules and integrin proteins (42).

A related aspect is the Tyr phosphorylation of FAK, which takes place upon cell adhesion to galectin-8. This observation is consistent with the fact that galectin-8 by virtue of its two CRDs binds (29) and presumably aggregates β_1 integrin subunits, the main upstream activators of FAK (43). Still, galectin-8-induced activation of FAK is independent of focal adhesion formation, a phenomenon already observed in other cellular systems (44). The apparent dissociation of FAK phosphorylation from focal adhesion formation mirrors findings showing that FAK can be displaced from focal adhesions and that the Tyr phosphorylation in focal adhesions can be reduced to undetectable levels without affecting their assembly or stability (45). Hence, FAK phosphorylation that takes places during early stages of cell-matrix interactions is a common signal emitted upon cell adhesion to fibronectin or galectin-8. Thereafter, bifurcation of signals mediated by fibronectin and galectin-8 presumably takes place. Fibronectin recruits additional cytoskeletal elements like vinculin and paxillin and induces their Tyr phosphorylation, whereas galectin-8 fails to do so and presumably emits a different set of signals. Of relevance to this argument is the fact that galectin-8 effectively stimulates, better than fibronectin, the activities of protein kinase B and p70S6 kinase as well as mitogen-activated protein kinase,[2] which is undetected in focal adhesions (43).

The differences in cytoskeletal organization between cells adherent to fibronectin *versus* galectin-8 could be attributed to

[2] Levy *et al.*, unpublished observations.

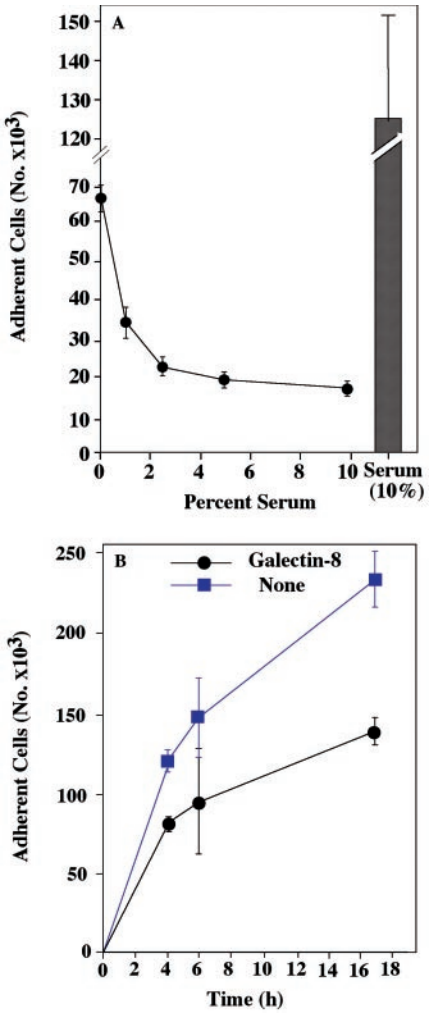


FIG. 9. **Effects of serum on cell adhesion to immobilized galectin-8.** *A*, 24-well Costar plates were coated for 2 h at 37 °C with GST-galectin-8 (25 μg/ml) (●) or with 10% FCS (*hatched bar*). The galectin-8-coated plates were further coated with the indicated serum concentrations (0–10%) for 2 h at 37 °C. All plates were blocked with 5% BSA for 2 h at 22 °C. HeLa cells (3×10^5) in serum-free medium were added to the coated plates. Following 2 h of incubation at 37 °C, cells were washed, and the number of adherent cells was counted. Values are the mean ± S.D. of duplicate measurements of a representative experiment. *B*, 24-well Costar plates were coated for 2 h at 37 °C with GST-galectin-8 (25 μg/ml) or with 10% FCS. The plates were further coated with 10% FCS for 2 h at 37 °C. All plates were then blocked with 5% BSA for 16 h at 4 °C. CHO-P cells (3×10^5) in serum-free medium were added to the coated plates. At the indicated time cells were washed, and the number of adherent cells was counted. Values are the mean ± S.D. of duplicate measurements of a representative experiment.

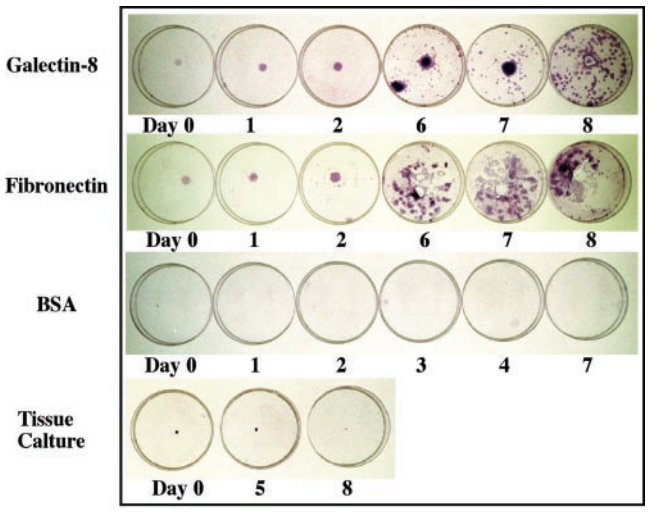


FIG. 10. **Kinetics of cellular migration on galectin-8.** Six-cm bacterial plates were precoated with 5 ml of galectin-8 (0.7 μM), fibronectin (0.04 μM), or BSA (12 μM) for 2 h at 22 °C. CHO-P cells were grown on tissue culture plates, detached with 5 mM EDTA, washed with PBS, and seeded within an agarose droplet on the coated wells as described under "Experimental Procedures." Cells seeded within an agarose droplet on plain 6-cm tissue culture plates served as controls. All cultures were then incubated in medium containing 10% FCS. At the indicated days cells were washed, fixed with formaldehyde, and stained with crystal violet. Results are of a representative experiment carried out three times.

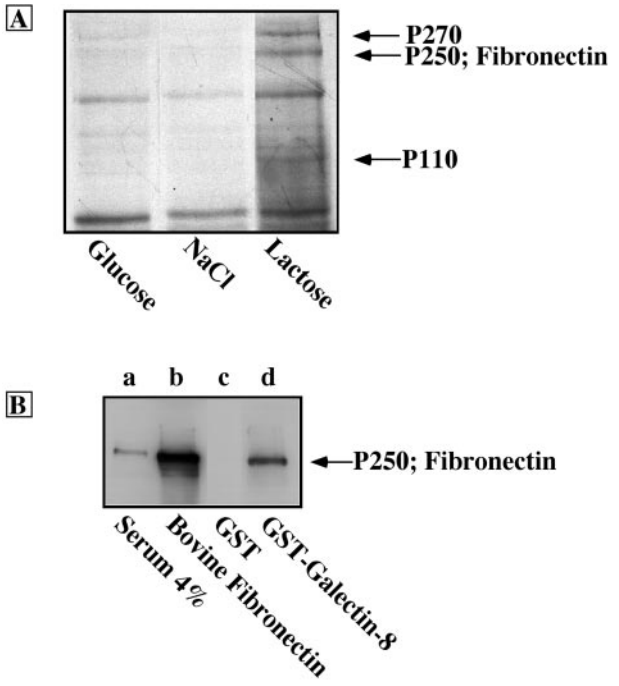


FIG. 11. **Identification of galectin-8-associated serum proteins.** *A*, a sample (5 ml) of 100% fetal calf serum was incubated with immobilized GST-galectin-8 as described under "Experimental Procedures." Bound proteins were eluted with 150 mM lactose, glucose, or NaCl and resolved by 10% SDS-PAGE. Following staining with Coomassie Blue, the 250-kDa band was excised and subjected to matrix-assisted laser desorption ionization analysis as described under "Experimental Procedures." *B*, proteins (33 μl), eluted with 150 mM lactose from columns of GST-galectin (*d*) or GST (*c*), were transferred to nitrocellulose membranes and subjected to Western immunoblotting with anti-fibronectin antibodies. A 40-μl sample of 4% fetal calf serum (*a*) or 2 μg of purified fibronectin (*b*) were run as controls.

their different mode of interaction with integrins. Unlike fibronectin, which binds most integrins (46), galectin-8 selectively interacts with a subgroup of cell surface integrin subunits that include α_3, α_6, and β_1, whereas it interacts to a very limited extent with α_4 and β_3 integrins (29). Moreover, galectin-8 interactions with integrins involve binding to sugar moieties rather than the ligand-binding site on the extracellular domain of the integrin molecules. This idea is supported by the fact that β_1 integrin antibodies, directed toward protein epitopes, are less efficacious in inhibiting cell adhesion to galectin-8; adhesion to galectin-8 is only partially sensitive to depletion of divalent cations (with EDTA) and is completely insensitive to the presence of RGD peptides. Still, the ability of EDTA and Mn^{2+} to inhibit or potentiate, respectively, cell adhesion to galectin-8 suggests that the metal-bound conformation of integrins is the preferred conformation that promotes adhesion to galectin-8. Hence, formation of protein-protein complexes upon binding of integrins to fibronectin *versus* the formation of protein-sugar complexes between galectin-8 and

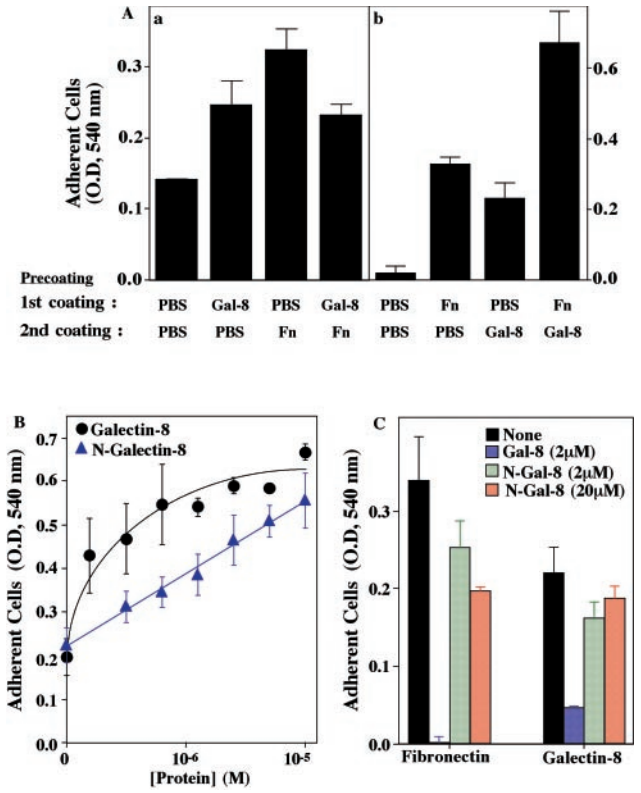
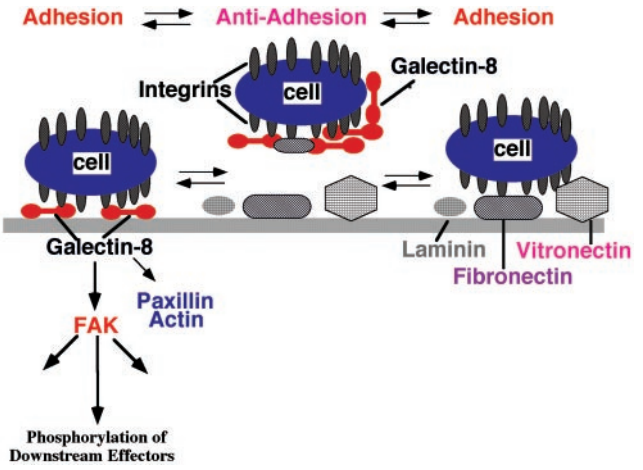


FIG. 12. **Effects of immobilized galectin-8 on cell adhesion.** *A*, 96-well tissue culture (*a*) or bacterial plates (*b*) were left uncoated (PBS) or first coated for 1 h at 37 °C with 0.1 ml of galectin-8 (1 μM) or fibronectin (0.01 μM). The culture plates were blocked with 1% BSA for 1 h at 37 °C and then further coated for 1 h at 37 °C with fibronectin (0.01 μM) or galectin-8 (1 μM) as indicated. HeLa cells (1×10^5) were detached from culture plates with 5 mM EDTA, washed with PBS, and seeded in serum-free medium on the wells. Following 2 h of incubation at 37 °C, cells were washed and stained with crystal violet, and the number of adherent cells was determined. *B*, 96-well bacterial plates were precoated for 1 h at 37 °C with 0.1 ml of the indicated concentrations of galectin-8 (●) or its N-terminal truncated form (▲). HeLa cells (1×10^5) were detached from culture plates with 5 mM EDTA, washed with PBS, and seeded in serum-free medium on the wells. Following 2 h of incubation at 37 °C, cells were washed and stained with crystal violet, and the number of adherent cells was determined using an enzyme-linked immunosorbent assay reader at 540 nm. *C*, 96-well tissue culture plates were precoated with 0.1 ml of fibronectin (0.01 μM) or galectin-8 (2.5 μM) for 1 h at 37 °C and further blocked with 1% BSA for 1 h at 37 °C. HeLa cells (1×10^5) were detached from culture plates with 5 mM EDTA and washed with PBS. Cells were incubated in suspension for 1 h at 37 °C in serum-free medium in the absence or presence of galectin-8 (2 μM) or its N-terminal truncated form (2 or 20 μM). At the end of incubation cells were washed before being seeded on the coated wells. Following 2 h of incubation at 37 °C, cells were washed and stained with crystal violet, and the number of adherent cells was determined.



SCHEME 1. **Function of galectin-8 as a matricellular protein.** Immobilized galectin-8 functions as a matrix protein equipotent to fibronectin in promoting cell adhesion by ligation and clustering sugar moieties of cell surface integrin receptors. Adhesion to galectin-8 triggers integrin-mediated signaling cascades such as Tyr phosphorylation of FAK and paxillin. In contrast, when present in excess as a soluble ligand, galectin-8 interacts both with integrins and other soluble matrix proteins such as fibronectin to negatively regulate cell-matrix interactions in a sugar-dependent manner. Such a mechanism allows local signals emitted by galectin-8 to specify territories inaccessible for cell adhesion. This implicates members of the galectin family as a novel class of matricellular proteins.

integrins offers a molecular aspect for the differences in cytoskeletal organization and signaling induced by these two matrices. Indeed, the less developed pattern of actin filaments and focal contacts observed in cells seeded on galectin-8 resembles the appearance of cells whose integrins were aggregated in the absence of a ligand (*e.g.* RGD peptide) (47), suggesting that galectin-8 presumably fails to occupy the protein ligand-binding site of integrins, whereas it effectively induces aggregation of these receptors. The possibility that immobilized galectin-8 induces integrin clustering is consistent with the fact that a truncated form of galectin-8, which contains only its N-terminal half with a single CRD, is much less efficient (about 5-fold) in functioning as an ECM protein that promotes cell adhesion. These findings suggest that ligation of cell surface integrins is necessary but insufficient to trigger the biological functions of immobilized galectin-8, and receptor clustering, in addition to receptor occupancy, is required to promote the adhesive effects of galectin-8. In that respect, galectin-8 resembles classical ECM proteins that induce integrin aggregation to trigger cell adhesion and to initiate the signaling cascade generated thereby (2, 13).

Cell adhesion to immobilized galectin-8 is inhibited in the presence of serum. These findings suggest that complex formation between galectin-8 and serum components generates a matrix that attenuates cell adhesion. This phenomenon is compatible with the ability of galectin-8 to promote cell motility. Indeed, cells readily migrate on plates coated with galectin-8 (in the presence of serum), indicating that complexes of galectin-8 with matrix/serum components are presumably advantageous when cellular migration is in effect and that their formation might play a physiological role in the regulation of cellular motility.

The nature of the serum elements that constitute complexes with galectin-8 is presently unknown; however, generation of fibronectin-galectin-8 complexes does not seem to interfere with cell adhesion, because cell adhesion to immobilized galectin-8 is not interrupted in the presence of purified fibronectin. The galectin-8-binding proteins present in serum are themselves mediators of cell adhesion, because their biological activity is attenuated upon complex formation with immobilized galectin-8. Cell adhesion is also inhibited by exogenously added soluble galectin-8. In that respect galectin-8 resembles other soluble ECM proteins like laminin (8) and fibronectin (5, 7) that inhibit cell adhesion upon binding to integrins. We have already demonstrated (29) that surface-associated native galectin-8 forms complexes with a subgroup of integrin subunits. Hence, perturbation of the physiological galectin-integrin complexes, upon addition of excess soluble galectin-8, presumably masks the integrin ligand-binding sites and thus impairs cell adhesion to integrin ligands such as fibronectin. Inhibition of cell adhesion induced by soluble galectin-8 is further complicated in view of the fact that galectin-8 can also bind soluble fibronectin present in the serum. In fact, galectins, by virtue of their affinity for glycans carrying multiple *N*-acetyllactosamine units, bind to certain isoforms of laminin and fibronectin that express such glycans (26). Hence, the anti adhesive effects of

galectin-8 could be mediated either upon direct binding of excess soluble galectin-8 to cell surface integrins or upon binding and recruitment to the cell surface of other soluble ECM proteins such as fibronectin that could then exert an anti adhesive effect of their own (5, 7). This function requires the occupancy of both CRDs of the native galectin-8 and might account for the inability of the truncated monovalent soluble N-galectin-8 to inhibit cell adhesion. Hence, the function of soluble galectin-8 seems to be dictated by the combinatorial arrangement of available cell surface and extracellular ligands (Scheme 1).

Because of its anti adhesive functions, galectin-8 can be considered a novel member of adhesion-modulating proteins such as SPARC, thrombospondin, tenascin, hevin, and disintegrins (10–12), collectively known as matricellular proteins. Similar to semaphorins (48), connectin (49), and netrin (50) that inhibit neurite outgrowth and provide local cues that can divert the trajectories of growth cones, soluble galectin-8 added to explant cultures converts conditions permissive for axonal growth into ones that are inhibitory for adhesion of neurites of the spinal cord.[3] Hence, soluble galectin-8 may provide local signals to specify territories inaccessible for growing axons. Still, it should be emphasized that the action of matricellular proteins depends upon protein-protein interactions, whereas the function of galectin-8 depends upon sugar-protein interactions. Such usage of the glyco-code adds a novel biological function for lectin-carbohydrate interactions.

Several lines of evidence suggest that galectin-8 functions as a physiological modulator of cellular adhesion and migration. Galectin-8 is a secreted protein that remains associated with the cell surface (29). Cell surface galectin-8 forms tight complexes with a selective subset of integrins, as evident by the fact that complexes of $\alpha_3\beta_1$ integrins and native cellular galectin-8 are detected upon immunoprecipitation of cell extracts with α_3 antibodies (29). Accordingly, galectin-8 antibodies, like antibodies directed against other cell adhesion molecules (*e.g.* integrins (34) and CD44 (51)), promote cell adhesion and spreading, thus supporting the idea that engagement of immobilized antibodies either with integrins or with galectin-8 within galectin-integrin complexes is sufficient to promote cell adhesion and spreading. Conversely, immobilized galectin-8 readily promotes cell adhesion on its own, whereas complex formation between galectin-8 and serum/matrix proteins attenuates cell adhesion while supporting cellular migration. In that respect it should be noted that galectins are present in tumor cells intra- and extracellularly. Galectin-3 is overexpressed in human colon and gastric carcinomas (52), whereas prostate carcinoma tumor antigen-1, the human isoform of galectin-8, is highly expressed in certain forms of prostate carcinomas (53) and other tumor cells (54). Furthermore, galectin-8 localizes to the invasive parts of certain glioblastomas explanted into brains of nude mice (54). These findings implicate overexpression of galectin-8 as a key attribute associated with the development and metastatic potential of certain tumor types. Because interactions of a secreted galectin-8 with cell surface integrins inhibit cell adhesion, whereas immobilized galectin-8 has the potential to promote cellular attachment, spreading, and migration, galectin-8 may modulate cell-matrix interactions in a variety of physiological and pathological processes.

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[3] Bar-Peled *et al.*, unpublished observations.

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