Presentation of Galectin-1 by Extracellular Matrix Triggers T Cell Death*

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Apoptotic elimination of T cells at sites of inflammation or infiltration into tumors limits an effective immune response. T cell apoptosis can be initiated by a variety of triggers, including galectin-1, a soluble, secreted lectin that binds to oligosaccharide ligands on cell surface glycoproteins, or to oligosaccharide ligands on extracellular matrix glycoproteins in tissue stroma. Although galectin-1 has no transmembrane domain and is secreted from cells that make it, it is not clear if galectin-1 functions as a soluble death trigger in vivo. We examined the ability of stromal cells secreting galectin-1 to kill T cells. Although the stromal cells synthesized abundant galectin-1, the majority of the galectin-1 remained bound to the cell surface, and stromal cell-associated galectin-1 killed bound T cells. In contrast, insufficient amounts of functional galectin-1 were released from the stromal cells into the media to kill T cells in the absence of contact with stromal cells. However, when stromal cells were grown on Matrigel, a mixture of extracellular matrix proteins, or on permeable membranes above Matrigel, secreted galectin-1 bound to Matrigel and killed T cells without stromal cell contact. Ten-fold less galectin-1 on Matrigel was sufficient to kill adherent T cells compared with soluble galectin-1. These results demonstrate that galectin-1 in extracellular matrix is able to directly kill susceptible T cells. Because increased galectin-1 deposition in tumor stroma occurs with tumor progression in various types of cancer, galectin-1 in stroma may act locally in the apoptotic elimination of infiltrating T cells during an immune response.

Various factors regulate lymphocyte survival. This regulation may have positive effects, e.g. prevention of self-recognition and autoimmune disease, or negative effects, e.g. cancer cells can kill infiltrating lymphocytes that would attack the tumor (1–3). Galectins are a family of mammalian lectins with a variety of immunoregulatory functions, including control of lymphocyte death (4–9). Galectin-1, the first member of the family to be described, has a broad repertoire of immunoregulatory effects. Galectin-1 regulates the inflammatory responses of neutrophils, mast cells, and macrophages and also associates with components of the complement system (10, 11). Galectin-1 induces apoptosis of macrophages, thymocytes, T cells, and B cells (9). Pircher and co-workers (12) detected an increase in galectin-1 synthesis after activation of murine T cells and suggested that galectin-1 can act as an autocrine negative regulatory “cytokine,” killing T cells to terminate an immune response. Galectin-1 is highly expressed in CD4+/CD25+ regulatory T cells that suppress immune responses compared with conventional CD4+ T cells (13). In vivo, galectin-1 therapy ameliorated disease in models of hepatitis, nephritis, arthritis, inflammatory bowel disease, and multiple sclerosis (9, 14).

Galectin-1 is expressed in a variety of cell types, including thymic epithelial cells, endothelial cells, dendritic cells, macrophages, fibroblasts, and bone marrow stromal cells (4–6, 8, 9, 15, 16). There is increased galectin-1 expression in many types of cancer, including colon, breast, ovary and prostate carcinomas, and aggressive glioblastomas (8) and increased accumulation of galectin-1 in stroma surrounding tumor cells in ovarian and prostate carcinoma (17, 18). Numerous ligands for galectin-1 have been described in different tissues; in extracellular matrix, galectin-1 binds to laminin, fibronectin, and vitronectin and is proposed to facilitate tumor cell invasion and migration through stroma (4, 5, 8, 17–19). Although galectin-1 may function in tumorigenesis and metastasis, galectin-1 expression by tumors may also modulate the immune response to the tumor (4).

The mechanisms by which galectin-1 mediates these immunomodulatory effects have not been elucidated. In particular, it is not known whether galectin-1 can diffuse away from the cell that secretes it to act as a soluble “cytokine” or whether galectin-1 requires direct cell-cell contact to exert its effects. In the former case, secretion of large amounts of soluble galectin-1 by tumors could have a global immunosuppressive effect. In the latter case, whereas galectin-1 synthesis may be increased in a tumor, the immunosuppressive effects would be limited to the vicinity of the tumor. Moreover, although several studies have documented accumulation of galectin-1 in extracellular matrix, the ability of extracellular matrix-associated galectin-1 to effect T cell death is not known.

EXPERIMENTAL PROCEDURES

Chemicals and Reagents—Recombinant human galectin-1 and polyclonal rabbit antiserum to human galectin-1 were prepared as described (15). The following reagents were purchased as indicated: annexin V-propidium iodide (PI) and galectin-3 (R&D Systems, Minneapolis, MN), dithiothreitol (Fisher), 10% PBS and bovine serum albumin (Sigma), Ficol-Paque (Amersham Biosciences), horseradish peroxidase-chromogen kit (Biomedia, Foster, CA), Matrigel (BD Biosciences), mouse anti-rabbit-horseradish peroxidase (Jackson ImmunoResearch Laboratories, West Grove, PA), Texas Red-goat anti-rabbit IgG, FITC-annexin V (Molecular Probes, Eugene, OR), and sulfo-NHS-biotin (Pierce).

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Cells and Culture Conditions—The BW5147PhaR2.1 cell line (PhaR2.1), gift of Dr. M. Pierer, and the δ35 thymic stromal epithelial cell line, a gift of Dr. K. Dorshkind, were cultured as described (20). Chinese hamster ovary (CHO) cells and Lec8-CHO (American Type Tissue Collection, Manassas, VA) were grown in α-minimal essential medium (Invitrogen) with 10% fetal bovine serum. For transwell experiments, 4.5 × 10^3 δ35 cells, CHO, or Lec8 cells in 3 ml of media were plated in the bottom chamber of transwell plates (Costar, Corning, NY) for 24 h. To assess T cell death, viability, and proliferation, fresh medium was added to the stromal wells, and 2.25 × 10^5 PhaR2.1 cells in 1.5 ml of media were placed in the upper inserts in 0.4 μm pore size, BD Biosciences; cell counts and trypan blue exclusion assays were performed at the indicated times. PhaR2.1 cell death was assessed by flow cytometry using annexin V and PI as described (20).

To deposit galectin-1 on Matrigel, 2 × 10^5 stromal cells in 1.5 ml of media were plated directly on solidified Matrigel or placed in the upper inserts, and 3 ml of medium was added to the lower wells over 100 μl of solidified Matrigel on glass coverslips (Fisher). 100 μl of liquid Matrigel was pipetted onto coverslips in each well at 4 °C. Plates were brought to room temperature to solidify the gel, and the gel was air-dried for 1 h. 100 μl of media was added to the solidified gel to keep the gel hydrated.

Galectin-1 bound to Matrigel was detected by immunohistochemistry and quantitated by ELISA (below).

To bind recombinant galectin-1 or galectin-3 directly to Matrigel, 100 μl of galectin at the indicated concentrations in PBS, 0.1% bovine serum albumin was added to the surface of the solidified Matrigel for 1 h. The Matrigel was washed once with PBS before T cell binding assays. No unbound galectin-1 or galectin-3 was detected in the wash buffer after Matrigel binding, indicating that all added galectin bound to the Matrigel.

**Cell Conjugate Assays and Confocal Microscopy**—To assess T cell death by cell-cell contact, 10^5 PhaR2.1 cells were incubated for 1 h with subconfluent (~50%) monolayers (~2 × 10^5 cells) of δ35 cells, CHO, and Lec8 cells plated on coverslips in 6-well plates. To assess T cell death by T cell matrix contact, 10^5 PhaR2.1 cells were added to Matrigel solidified on coverslips and allowed to bind to the Matrigel for 1 h. Unbound cells were removed by washing once with PBS. 100 μl of annexin V-FITC/PI in binding buffer (10 mM HEPES, pH 7.4, 150 mM NaCl, 2.5 mM CaCl_2, 1 mM MgCl_2) was added to the wells for 20 min at 20 °C. Coverslips were washed with PBS, fixed with 2% paraformaldehyde for 30 min at 4 °C, washed with PBS, quenched with 0.2 M glycine in PBS for 10 min at 4 °C, and mounted onto slides with 25 μl of Prolong anti-fade mounting medium (Molecular Probes).

For immunohistochemistry cell conjugates or cell-Matrigel layers were prepared on coverslips as above, blocked with 10% goat serum, and incubated with either anti-galectin-1 antisera or with normal rabbit serum, all diluted 1:100 in PBS with 2% goat serum. After washing, bound antibody was detected with Texas Red-goat anti-rabbit IgG for 1 h at 20 °C for fluorescence microscopy or with mouse anti-rabbit IgG (Jackson ImmunoResearch) and rhodamine-9-amino-6-phenylcarbazole for light microscopy. After washing, coverslips were mounted to slides as above.

For fluorescence microscopy samples were excited at 488 and 568 nm with argon and krypton lasers for FITC and Texas Red or PI, respectively, and the light emitted between 525 and 540 nm was recorded for FITC and above 630 nm for Texas Red and PI. Slides were visualized on a Fluoview laser scanning confocal microscope (Olympus America Inc., Melville, NY) using the 100× objective. Dual emission fluorescent images were collected in separate channels. Images were processed using the Fluoview image analysis software (version 2.1.39). To compensate that were not in direct contact with the thymic stromal cells.

**ELISA Assay for Galectin-1—Anti-galectin-1 IgG was purified from rabbit polyclonal anti-galectin-1 antiseraum on a Protein A spin column (Pre-Chem, Acton, MA). Purified anti-galectin-1 IgG was diluted in PBS to 10 μg/ml, and 100 μl/well was added to 96-well enhanced protein binding ELISA plates (Immulon 2, Thermo Electron Corp., Franklin, MA) overnight at 4 °C. Wells were washed 3 times with wash buffer (1× PBS, 0.05% Tween 20) and blocked with blocking buffer (5% skim milk in PBS) for 1 h at room temperature. After blocking, recombinant galectin-1, conditioned media, or cell lysates were added for 1 h at room temperature. Wells were washed 3 times, biotinylated anti-galectin-1 IgG (10 μg/ml in blocking buffer) prepared as in Amano et al. (21) was added for 1 h, and wells were washed 3 times. Streptavidin-horseradish peroxidase (1:500 in blocking buffer) was added for 30 min at room temperature. After washing, 200 μl of 0.4 mg/ml o-phenylenediamine dihydrochloride was added to each well, and the absorbance of each well was read at 490 nm using a microplate reader (Bio-Rad Model 550). Absorbance values were converted to protein concentrations based on a galectin-1 standard curve. To quantify galectin-1 bound to Matrigel, biotinylated anti-galectin-1 IgG was added directly to the Matrigel and allowed to bind for 1 h. Color was developed as above, and 200-μl aliquots were transferred to 96-well ELISA plates.

**Statistical Analysis**—Data were analyzed by student’s t test, one-way analysis of variance analysis. Significance was considered at p < 0.05, and data are presented as the mean ± S.E.

**RESULTS**

Direct T cell binding to cells that express galectin-1, such as human and murine thymic stromal cells and activated endothelial cells, can trigger T cell death (15, 20). However, it has been suggested that galectin-1 can act as a cytokine or soluble factor in vivo to trigger T cell death and inhibit T cell proliferation (12, 22). To directly examine whether cells that synthesize galectin-1 can kill T cells in the absence of cell-cell contact, we examined the ability of the murine δ35 thymic stromal cells to kill murine PhaR2.1 T cells (Fig. 1A). We observed that >50% of PhaR2.1 T cells in contact with δ35 cells became annexin V-positive within 1 h of binding to δ35 cells. The annexin V-positive cells also demonstrated membrane blebbing and clustering of annexin V on apoptotic blebs, as we have described previously (20). In addition to annexin V staining, cells were also labeled with propidium iodide to detect the later stage of cell death characterized by loss of membrane integrity. 45% of the annexin V-positive cells were also labeled with propidium iodide (data not shown), demonstrating that several hallmarks of cell death were present after the T cells bound to the thymic stromal cells.

PhaR2.1 cell death appeared to require direct contact with δ35 cells, as T cells on the plastic tissue culture surface adjacent to, but not in contact with the δ35 cells showed no cell death above background (Figs. 1, A and B). This suggested that the δ35 cells did not secrete sufficient gal-1 to kill nearby T cells that were not in direct contact with the δ35 stromal cells. In addition, T cell death was galectin-1-dependent, as death was virtually abolished by the addition of anti-galectin-1 antisera (Fig. 1A).

To examine the requirement for contact between galectin-1-expressing cells and T cells for T cell death, we plated δ35 thymic stromal cells in the bottom wells of transwell plates and added PhaR2.1 T cells to the top wells. This would allow any galectin-1 secreted by the δ35 cells to diffuse through the semi-permeable membrane separating the upper and lower wells but would not allow T cell-stromal cell contact. In contrast to experiments in which direct T cell-stromal cell contact triggered T cell death within 1 h (Fig. 1), we detected no loss of T cell viability when T cells were grown in the upper wells of transwell plates above δ35 cells for 24, 48, or 72 h; the viability of T cells cultured over δ35 cells was essentially identical to the viability of T cells cultured over bottom wells containing media alone (Table I). Similar results were seen at 1, 3, 6, 12, and 144 h of culture (data not shown).

We then examined CHO and Lec8 cells as galectin-1-secreting cells. CHO cells make abundant galectin-1 that is secreted from the cell and binds back to oligosaccharide ligands on the cell surface (23). Lec8 cells are a CHO mutant lacking UDP-galactose transporter activity (24); as no galactose residues are added to cell surface glycoproteins or glycolipids, there are no available oligosaccharide ligands for galectin-1 on the surface of Lec8 cells, and all the galectin-1 is secreted into the media (23). δ35, CHO, and Lec8 cells all make abundant galectin-1, between 40 and 60 μg/mg of total cellular protein; we detected
galectin-1 on the surface of \( \text{H9258} \) cells for 1 h. Cell death was assessed by annexin V-FITC binding. The % annexin V⁺ T cells in contact with or adjacent to \( \text{H9258} \) cells was determined for 50–100 T cells in 5–8 microscopic fields. The top bar in A indicates % annexin V⁺ T cells in the absence of \( \text{H9258} \) cells. Death was inhibited by the addition of anti-galectin-1 antiserum (anti-Gal-1). Panel B is a phase image of an adherent \( \text{H9258} \) cell and a T cell (upper left), annexin V-FITC staining of the T cell (lower left), overlay of the phase and fluorescent images (upper right), and galectin-1 expression by a \( \text{H9258} \) cell (red) and annexin V-FITC binding by an adherent T cell (green) (lower right). C and D, \( \text{H9258} \) cell death was assessed as above on CHO or Lec8 cells. The top bar in C indicates the percent annexin V⁺ T cells in the absence of CHO or Lec8 cells. Panel D shows phase images of T cells bound to CHO and Lec8 cells; numerous annexin V⁺ T cells were bound to CHO cells, whereas T cells bound to Lec8 cells did not become annexin V⁺. In A and C values are the mean of triplicate samples in a representative experiment.

Table I: Galectin-1 secretion and effect on T cell viability

|       | 24 h Viab | 24 h Gal-1 | 48 h Viab | 48 h Gal-1 | 72 h Viab | 72 h Gal-1 |
|-------|-----------|------------|-----------|------------|-----------|------------|
| Media | 100       | 100        | 100       | 100        |
| \( \text{H9258} \) 35 | 97.9      | 7.3        | 98.5      | 1.9        | 97.0      | 0.9        |
| CHO   | 100.5     | 48.8       | 100.7     | 53.6       | 102.7     | 47.4       |
| Lec8  | 100.6     | 40.5       | 99.1      | 79.5       | 99.7      | 106.8      |

a % Pha².1 viability compared to control.
b Galectin-1 concentration (\( \mu g/ml \)) in medium.

>70% of Pha².1 T cells that adhered to CHO cells became annexin V-positive within 1 h of contact with CHO cells. In contrast, we detected no annexin V binding to T cells in contact with Lec8 cells that had no detectable cell surface galectin-1 (Figs. 1, C and D). As we had observed with the \( \text{H9258} \) cells, in addition to annexin V binding, we observed membrane blebbing of T cells in contact with CHO cells (Fig. 1D) but no membrane blebbing of T cells in contact with Lec8 cells. When the T cells were added to the CHO cells, 61% of the annexin V-positive cells also labeled with propidium iodide, indicating loss of membrane integrity (data not shown).

Again, as we observed with \( \text{H9258} \) cells, there was no loss of viability of Pha².1 T cells in the upper wells of the transwell plates when either CHO or Lec8 cells were plated in the bottom wells compared with bottom wells containing media alone (Table I). We measured the amount of galectin-1 secreted from \( \text{H9258} \), CHO, and Lec8 cells into the media in the bottom of the transwell plates (Table I). The \( \text{H9258} \) cells secreted barely detectable amounts of galectin-1 into the media, whereas both the CHO and Lec8 cells secreted appreciable quantities of galectin-1 into the media (47 and 107 \( \mu g/ml \), respectively). Indeed, galectin-1 secretion by Lec8 cells was increased compared with CHO cells, because the Lec8 cells did not bind back the secreted galectin-1. However, although these cells made abundant galectin-1, the amount of soluble galectin-1 secreted by either CHO or Lec8 cells into the media was not sufficient to reach the concentration required for Pha².1 T cell death. We have determined that concentrations above 150 \( \mu g/ml \) (10 \( \mu M \)) soluble galectin-1 are optimal for triggering T cell death, most likely because the \( K_d \) of the galectin-1 dimer is in this range, and dimeric galectin-1 is required to induce death (15, 19). Therefore, the requirement for stromal cell-T cell contact demonstrated in Fig. 1 may reflect an ability of the stromal cells to concentrate the galectin-1 at the cell-cell interface, rather than an active process by stromal cells in triggering T cell death.

To confirm that soluble galectin-1 could kill Pha².1 T cells in the transwell system, we added increasing concentrations of soluble galectin-1 to the lower wells of the transwell plates and measured the death of T cells in the upper wells. As shown in
Fig. 2A, no death of PhaR2.1 T cells in the upper wells of the transwell chambers was observed until the concentration of soluble galectin-1 in the wells was in excess of 150 μg/ml, which was also the concentration at which T cell agglutination began to occur, confirming multimeric binding of galectin-1. In these experiments appreciable T cell death was seen at galectin-1 concentrations of 380 μg/ml and above. Thus, very high concentrations of galectin-1 would have to be released from stromal cells in the bottom wells to kill T cells only 0.9 mm across the transwell plate.

Fig. 2B, secreted galectin-1 had no effect on PhaR2.1 T cell proliferation. PhaR2.1 cells were cultured in the upper well inserts, with CHO, Lec8, or no cells in the bottom wells. PhaR2.1 cell number was determined at indicated times. Results in A and B are the mean of triplicate samples.

In addition, there was no positive or negative effect on T cell proliferation over 72 h by galectin-1 secreted from stromal cells. The numbers of PhaR2.1 cells in the upper wells of the transwell plates were essentially identical at 24, 48, and 72 h whether the lower wells contained CHO cells, Lec8 cells, or no stromal cells (Fig. 2B). Galectin-1 stimulates neural and hepatic cell proliferation at concentrations as low as 5 pg/ml (25, 26); although CHO and Lec8 cells secreted 40–100 μg/ml of galectin-1 (Table I), we detected no effect on T cell proliferation.

Thus, the ability of stromal cells to kill T cells via galectin-1
may relate to the ability of the stromal cell surface to concentrate and present galectin-1. Because several extracellular matrix glycoproteins, such as laminin, fibronectin, and vitronectin bind galectin-1, cells secreting galectin-1 could also deposit the lectin on the surrounding extracellular matrix; increased galectin-1 accumulation in tumor stroma could kill infiltrating T cells (17, 18). To examine galectin-1 secretion into extracellular matrix, we used Matrigel, which contains laminin, collagen IV, entactin, nidogen, and heparan sulfate proteoglycans. By immunoblot analysis we did not detect any endogenous galectin-1 in Matrigel (data not shown).

CHO and Lec8 cells were plated on Matrigel, and the deposition of galectin-1 on the Matrigel was detected immunohistochemically. Abundant galectin-1 was deposited on Matrigel cultured with either CHO or Lec8 cells (Fig. 3A). We added T cells to plates containing Matrigel and either CHO or Lec8 cells. Matrigel coated by galectin-1 produced by CHO cells killed a significant fraction of bound Pha^2.1 T cells (Fig. 3B).

Importantly, T cells analyzed in Fig. 3B were adjacent to, but did not appear to be in contact with the adherent CHO or Lec8 cells (Fig. 3C). Thus, galectin-1 in extracellular matrix as well as on the cell surface could trigger T cell death, although the level of T cell death on the matrix was less than that observed for direct T cell-CHO cell contact (Fig. 1, A and C).

Surprisingly, we also observed T cell death when T cells were added to Matrigel coated by galectin-1 from Lec8 cells (Fig. 3B), although no death above background was observed when T cells bound directly to Lec8 cells (Fig. 1C). Growth of Lec8 cells directly on Matrigel apparently allowed the secreted galectin-1 to bind to glycoconjugate ligands in the extracellular matrix and retain carbohydrate binding activity (Fig. 3A). On Matrigel coated with galectin-1 from either CHO or Lec8 cells, galectin-1 was responsible for T cell death, since there was no death of T cells plated on Matrigel alone, and anti-galectin-1 antiserum reduced T cell death to background levels.

To exclude the possibility that death of T cells on galectin-
1-coated Matrigel involved a brief T cell-stromal cell contact step before T cell adhesion to the matrix, we plated CHO or Lec8 cells in the upper wells of transwell plates with a layer of Matrigel in the bottom wells for 48 h. The galectin-1 concentration bound to the Matrigel from CHO or Lec8 cells was determined (under the respective bars). In parallel wells, $10^5$ PhaR2.1 cells were bound in the presence or absence of anti-gal-1 for 1 h, and the percent annexin V $^+$ T cells was calculated. B, indicated concentrations of galectin-1 or galectin-3 were bound directly to solidified Matrigel for 1 h. $10^5$ PhaR2.1 T cells were added to the Matrigel for 1 h, and % annexin V $^+$ T cells were determined. In A and B, values are the mean of triplicate samples.

![Graph A](image)

**Fig. 4.** Galectin-1 on Matrigel is sufficient to kill T cells in the absence of stromal cells. A, CHO, Lec8, or no stromal cells were placed in the upper well inserts over solidified Matrigel, in the bottom wells for 48 h. The galectin-1 concentration bound to the Matrigel from CHO or Lec8 cells was determined (under the respective bars). In parallel wells, $10^5$ PhaR2.1 cells were bound in the presence or absence of anti-gal-1 for 1 h, and the percent annexin V $^+$ T cells was calculated. B, indicated concentrations of galectin-1 or galectin-3 were bound directly to solidified Matrigel for 1 h. $10^5$ PhaR2.1 T cells were added to the Matrigel for 1 h, and % annexin V $^+$ T cells were determined. In A and B, values are the mean of triplicate samples.
death of T cells on galectin-3-coated Matrigel at concentrations as high as 100 μg/ml (Fig. 4B).

These results demonstrate that T cell contact with galectin-1 either on the surface of another cell or on extracellular matrix in the absence of stromal cells can kill adherent T cells. Moreover, the amount of galectin-1 secreted by CHO and Lec8 cells was sufficient to kill adherent T cells when the galectin-1 was presented on the surface of the extracellular matrix, whereas we observed no T cell death when galectin-1 was secreted from CHO or Lec8 cell into media surrounding the T cells.

**DISCUSSION**

Galectin-1 participates in development, in immune system homeostasis, and in tumor progression (4–9). Pharmacologic administration of galectin-1 is effective in decreasing T cell responsiveness to antigens and inducing T cell apoptosis in several autoimmune disease models (9, 14); however, in these models immunosuppressive effects were not seen unless galectin-1 was administered at very high doses, typically 10 mg/kg, usually via an intraperitoneal route.

In contrast, although galectin-1 is very abundant in many cells and tissues (e.g. 35–40 μg/g of wet tissue in the spleen) (27), the serum concentration of galectin-1 in healthy women is only ~100 ng/ml (roughly equivalent to 420 μg of total serum galectin-1 in a 60-kg person), whereas patients with ovarian carcinoma (a type of tumor that expresses galectin-1 at high levels compared with normal tissue) have even lower serum levels of galectin-1, ~20 ng/ml (28). The reduced serum concentration of galectin-1 in ovarian carcinoma patients with tumors that synthesize high levels of galectin-1 may result from the increased deposition of galectin-1 on glycoproteins such as CA125 on the tumor cell surface as well as increased galectin-1 deposition in the ovarian carcinoma-associated stroma (29). The relatively low serum concentrations of galectin-1 in both healthy controls and cancer patients, in the ng/ml range, are far lower than the concentration of soluble, recombinant galectin-1 required for T cell death in *in vitro* assays (150 μg/ml). Thus, although galectin-1 has been called a cytokine (12), it is unlikely that increased galectin-1 synthesis in tumors would result in sufficiently elevated serum levels of galectin-1 to cause systemic immunosuppression.

However, as galectin-1 produced by tumors may primarily deposit on the tumor cells and surrounding stroma, galectin-1 may have profound local effects on the immune response to the tumor. Our data demonstrate that the acellular matrix can bind galectin-1 secreted from stromal cells and kill adherent T cells within 60 min. Although apoptotic death of tumor-infiltrating T cells has been observed, this has been attributed to T cell encounters with death ligands directly on the tumor cell surface (2) rather than in the tumor-associated stroma. Although the level of T cell death on the galectin-1-coated matrix (25–40%) was typically lower than the level of T cell death initiated by T cell-CHO cell contact (50–75%) (Figs. 3 and 4 versus Fig. 1), matrix-associated galectin-1 was sufficient to kill bound T cells; the membrane fluidity in cellular presentation of galectin-1 may facilitate the clustering of T cell glycoprotein counter-receptors that participate in galectin-1-induced cell death (21) compared with a more static presentation of galectin-1 on Matrigel.

Thus, the present study indicates that, even before encountering with tumor cells, T cells that bind galectin-1 in tumor-associated extracellular matrix may be triggered to die. As proposed by van den Brule and Castronovo (18), increased deposition of galectin-1 in carcinoma stroma may act as an “immunologic shield” surrounding tumor cells. As galectin-1 is secreted by many types of tumors, including glioblastoma, breast, and prostate carcinoma (4, 8), immunotherapy approaches in these cancers may be thwarted by the rapid death of T cells as they encounter galectin-1-loaded matrix surrounding the tumor. Moreover, galectin-1-induced phosphatidylserine exposure on leukocytes is sufficient for phagocytosis of the cells by macrophages (30), suggesting that stromal macrophages could rapidly eliminate infiltrating T cells that encounter galectin-1.

It is intriguing that joint tissue stroma from patients with rheumatoid arthritis had decreased galectin-1 deposition compared with normal joint tissues, in contrast to the increased galectin-1 deposition seen in tumor stroma (31). Thus, the T cell infiltration and immune-mediated damage in rheumatoid arthritis may relate to the decreased level of galectin-1 in joint stroma. Of interest, CD4⁺/CD25⁺ regulatory T cells that express high levels of galectin-1 (13) typically require cell-cell contact to exert immunoregulatory function (3), consistent with our observation that galectin-1 bound to the cell surface or to the matrix is most effective at killing T cells.

The movement of galectin-1 from cells that produce it to the extracellular matrix is likely facilitated by the relatively low affinity (micromolar) of lectins for saccharide ligands, resulting in a rapid on-off rate on the surface of the cell (19, 32). However, the bivalency of galectin-1 may increase the likelihood of the secreted lectin remaining tethered to local glycoproteins, retaining the galectin-1 in the vicinity of cells that make it. Moreover, as the dissociation constant of the galectin-1 homodimer is in the micromolar range (19), the tethering of galectin-1 to matrix glycoproteins may increase the likelihood that the lectin remains in dimeric form, the form required to trigger T cell death. Of note, galectin-1 secreted from Lec8 cells will misfold and lose carbohydrate binding activity with a t1/2 of ~10 h, whereas galectin-1 bound to saccharide ligands retains binding activity for weeks (19, 23). Galectin-1 secreted from Lec8 cells onto Matrigel over 24–72 h retained binding activity, suggesting that the secreted lectin bound fairly rapidly to saccharide ligands in the Matrigel. In addition, a galectin-1 concentration as low as 45 μg/ml was sufficient to kill T cells when the galectin-1 was presented on the Matrigel surface, whereas a 10-fold higher concentration of soluble galectin-1 was required to kill an equivalent fraction of T cells.

Extracellular matrix glycoproteins may, thus, serve three roles in mediating galectin-1-triggered T cell death. First, the extracellular matrix may provide a rich source of saccharide ligands such as laminin, fibronectin, and vitronectin, which can contribute to maintaining galectin-1 carbohydrate binding activity. Second, matrix glycoproteins can concentrate galectin-1 secreted by surrounding cells to increase the fraction of galectin-1 molecules that are homodimers. Third, the matrix presents galectin-1 to the T cell surface in a two-dimensional array rather than in a three-dimensional space as when T cells encounter soluble galectin-1; this type of presentation may facilitate the interaction of galectin-1 with the large T cell surface glycoproteins CD43 and CD45 that form a glycoalyx surrounding the T cell to increase the rate and/or duration of galectin-1 binding to the cell (32). Understanding the mechanism by which galectin-1 interacts with extracellular matrix in tissues is critical for designing effective therapeutic strategies for galectin-1 in localized and systemic autoimmune disease and in manipulating the immune response to tumors.

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