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Interactions of a Mammalian β-Galactoside-Binding Lectin With Hamster Fibroblasts

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A β-galactoside-binding endogenous lectin extracted from bovine heart binds to the surface of baby hamster kidney (BHK) cells. The binding to and agglutination of cells is reduced in certain ricin-resistant mutants (Ric cells) in parallel with the decreased number of binding sites for the selective agent, ricin, a galactose-specific plant lectin. However, clear differences in the binding specificities of bovine lectin and ricin are shown by the effect of neuraminidase. BHK cells and Ric mutant cells treated with neuraminidase bind similar amounts of the bovine lectin compared with untreated cells, and ricin binding is greatly increased.

The mammalian lectin immobilised on inert glass mediates the attachment and spreading of normal BHK cells and agglutinates these cells in solution. Ricin-resistant mutant cells respond poorly. These results are consistent with a role of endogenous lectins in cellular adhesiveness and show that cell adhesion may be regulated by the density of specific surface receptors for lectins.

Cell-surface carbohydrates have often been proposed as factors modulating cellular recognition and adhesion [1]. Assuming a lock-and-key mechanism, several types of carbohydrate-binding molecules are likely candidates to participate in intercellular adhesion, including glycosyl transferases [2], glycosidases [3], or proteins (lectins) with no known enzymatic activities. Lectins have been isolated from a variety of mammalian tissues [reviewed in 4], but to our knowledge there has been no direct demonstration of any activity in mediating adhesive interactions.

In this communication we report that the β-galactoside-binding lectin of bovine heart [5–7], which exists at least in part in intercellular spaces [8] binds to, and agglutinates normal hamster kidney fibroblasts (BHK cells). Furthermore, these cells adhere strongly to the lectin coupled to inert glass surfaces. By contrast, certain ricin-

resistant BHK cell lines [9] deficient in surface galactose-containing carbohydrate sequences bind reduced amounts of the lectin, are not agglutinated by moderate concentrations of the lectin and respond poorly to the lectin-derivatized matrices.

These results are consistent with proposals [1] for a role of galactose-binding endogenous lectins in cellular adhesiveness and show that cell adhesion may be regulated by the density of specific cell surface receptors.

MATERIALS AND METHODS

Cell Culture

BHK cells were grown [9] at 37°C in plastic flasks or multiwell plates (Nunc, Copenhagen) in Eagle's medium supplemented with 10% foetal calf serum, penicillin, streptomycin, and 10% tryptose phosphate broth. Cells were removed from monolayer cultures by brief trypsin treatment [9], isolated by centrifugation, and suspended in Eagle's medium without serum. In experiments using bovine lectin the medium contained 1 mM dithiothreitol [5].

Lectins

Ricin was from Miles Laboratories. Bovine heart lectin was obtained from calf heart [5–7] using affinity chromatography on a column of asialofetuin linked to Sepharose 4B. Lectin bound to the column was eluted with 0.2 M lactose–0.15 M NaCl–0.02 M ammonium bicarbonate–1 mM dithiothreitol. Eluted fractions were dialysed against the same solution but lacking lactose and freeze-dried. SDS-polyacrylamide gel electrophoresis of the iodinated lectin showed a major band migrating under reducing conditions with a molecular weight of 13,000 (results not shown) in agreement with other published data [10].

Radio-iodination of ricin (100 μg) or bovine lectin (100 μg) used the iodogen method [11]. Bovine lectin was dialysed free of dithiothreitol before iodination. Iodinated lectins were purified by affinity chromatography on Sepharose 6B for ricin and asialofetuin–Sepharose 4B for bovine lectin as before. Materials eluted by lactose were dialysed against phosphate-buffered saline alone for ricin and with 1 mM dithiothreitol for bovine lectin. Bovine serum albumin was added to 50 μg/ml final concentration. Specific activities of $10^5 - 10^6$ counts/min/mg lectin were obtained.

Binding Experiments

Cells growing as monolayers in multiwell tissue culture plates were washed with phosphate-buffered saline and incubated with various concentrations of (^{125}I)-ricin in saline, pH 7.2 (0.5 ml) or (^{125}I)-bovine lectin in saline–1 mM dithiothreitol (0.5 ml). Controls contained the lectins in solution supplemented with 20 mM lactose or 20 mM thiodigalactoside. After incubation at room temperature for 1 hr the cell monolayers were washed with saline, pH 7.2, extensively. Monolayers were dissolved in hot (80°) N–NaOH (0.5 ml), and portions were taken for protein and radioactivity determinations [9]. Specific binding was obtained by correcting values for the non-specific binding of lectins to the cells in the presence of the sugar inhibitors. In some experiments cell monolayers were treated first with Vibrio cholerae neuraminidase (Cal Biochem) in phosphate-buffered saline (0.5 ml, 0.1 IU/ml). After a 30-minute incubation the cell monolayers were washed with phosphate-buffered saline and used for binding experiments.

Cell Agglutination

Haemagglutination tests were performed using trypsinised rabbit erythrocytes [5, 6]. Microtitre plates contained in each well erythrocytes (25 μl, 1% suspension in phosphate-buffered saline) and bovine lectin (25 μl) of various concentrations in 0.15 M NaCl–0.01 M ammonium bicarbonate–1 mM dithiothreitol. Serial twofold dilutions were used and agglutination was assessed microscopically by counting the number of single cells. Agglutination is expressed as the percentage of single cells relative to the total number of cells added. Agglutination could be inhibited completely by 20 mM thiodigalactoside (not shown).

Agglutination of trypsinised BHK cells (total 2–3 x 10^4 per well) was assessed as described above except that the lectin was diluted in Eagle's medium containing 1 mM dithiothreitol.

Adhesion Experiments

Bovine lectin was covalently attached to glass as previously described [12]. Glass coverslips (8 × 22 m) were first treated with aminopropyltriethoxysilane and (^{125}I)-labeled lectin solutions (up to 2 mg/ml^{-1}) were added in the presence of glutaraldehyde. After incubation at room temperature for 1 hr, the coverslips were rinsed in phosphate-buffered saline and used for adhesion experiments within 24 hr. Aliquots of trypsinised cell suspensions (0.7 ml, approximately 10^6 cells) were added and incubated at 37°C for 4 hr. The coverslips were rinsed in buffered saline, and the extent of cell attachment and spreading was assessed [12]. Finally, the coverslips were heated at 90°C for 5 min in 1% SDS in phosphate-buffered saline and counted in a gamma-scintillation spectrometer to estimate the amount of covalently bound lectin.

RESULTS

Binding of Bovine Lectin to Hamster Fibroblasts

The bovine lectin iodinated using (^{125}I) by the iodogen method was used to measure the extent of binding to cells as shown in Figure 1A. At increasing concentrations of iodinated lectin the amount bound to the intact cells increased to near saturation (Fig. 1A). The ricin-resistant cell line Ric 14 bound negligible amounts of the lectin, and three other ricin-resistant cell lines bound reduced amounts (Fig. 1A) compared with parental BHK cells.

These results and their quantitation by the Scatchard method [13] are summarised in Table I, which also shows the abilities (Fig. 1B) of the various cell lines to bind ricin. These latter data are in general agreement with previously published results [9, 14] showing the greatly reduced binding of ricin to Ric 14, 17, and 21 cells compared with parental BHK cells. Some interesting differences in the binding properties of the various cell lines to the bovine lectin and to ricin are apparent (Table I). Thus, parental BHK cells bind significantly more bovine lectin than ricin. Furthermore Ric 14 cells bind significantly to ricin (approximately 4% compared with normal BHK cells) but carry essentially no detectable binding sites for bovine lectin. The Ric 21 cell line binds the bovine lectin to a slightly greater extent than ricin compared to the normal BHK cells. These results suggest that although bovine lectin and ricin bind to terminal galactosyl residues [5, 7], there are clear differences in their affinity for galactose-containing sequences.

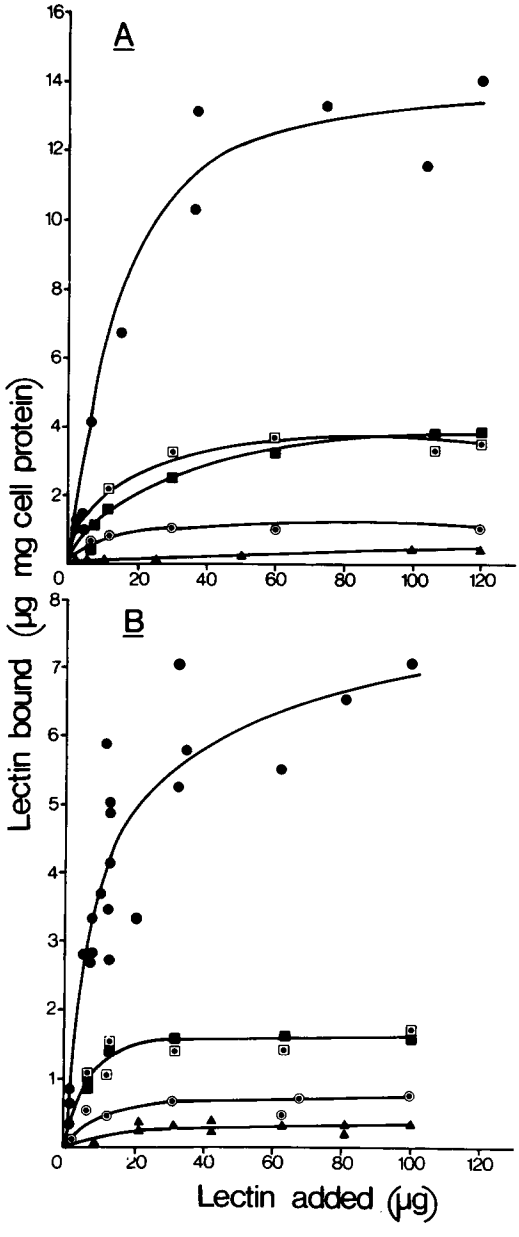

Fig. 1. Binding of bovine lectin (A) and ricin (B) to wild-type BHK (●) cells and ricin-resistant mutants; Ric 14 (▲), 15 (◉), 17 (■), and 21 (◙). Monolayer cultures were incubated with increasing concentrations of (^{125}I)-lectins and the cell-bound radioactivity was estimated. Nonspecific binding was estimated in the presence of 20 mM lactose (A) or 20 mM thiodigalactoside (B), and the results show the specific binding.

This conclusion is confirmed by experiments using neuraminidase (Table I). As shown by Rosen and Hughes [14], treatment of normal BHK cells with neuraminidase to remove terminal sialic acid from the carbohydrate sequences of surface-exposed glycoproteins reveals new binding sites for ricin. The stimulation of ricin binding by neuraminidase treatment of normal BHK cells was at least threefold according to previous work [14], a value in good agreement with the present determination (Table I). Similarly, as described [14], neuraminidase treatment of Ric 14 cells which bind ricin poorly induced a greatly increased binding (14-fold) which then approximated that of normal BHK cells before neuraminidase treatment. The other ricin-resistant cell lines also bound significantly more ricin after neuraminidase (Table I) as described previously [14]. By contrast, neuraminidase treatment had no effect on the binding by the cells of bovine lectin (Table I). In particular, neuraminidase treatment of Ric 14 cells produced no appearance of binding sites for bovine lectin.

Agglutination of Cells

The bovine lectin strongly agglutinated trypsin-treated rabbit erythrocytes (Fig. 2), and the minimum concentration at which the lectin gave significant haemagglutination was in the range 1 μg/ml. This agglutination was specifically inhibited by the sugar hapten, 20 mM thiodigalactoside (results not shown). Agglutination activity occurred only in the presence of a reducing agent—for example, dithiothreitol. Concentrations of the reducing agent less than 1 mM resulted in little or no haemagglutination, and this concentration was therefore included in the agglutination tests with trypsinised BHK cells.

Solutions of the bovine lectin agglutinated trypsinised BHK cells (Fig. 2), and the agglutination was inhibited by thiodigalactoside (results not shown). In comparison with normal BHK cells trypsinised ricin-resistant cell line Ric 15 was agglutinated appreciably less well (Fig. 2). The trypsinised cells of the Ric 14 line were hardly agglutinated by any concentration of the lectin (Fig. 2).

Cell Adhesion to Bovine Lectin

Using a method to link proteins chemically to glass coverslips, it has been shown previously [12, 15] that trypsinised BHK cells do not attach to bare glass or

TABLE I. Ricin and Bovine-Lectin Binding Sites in Wild-Type and Ricin-Resistant Variants

	Number of binding sites for			
	Bovine lectin		Ricin	
Cells	Untreated[a]	Increase[b] after neuraminidase treatment	Untreated[a]	Increase[b] after neuraminidase treatment
BHK	30 (100)[c]	1.01	6.2 (100)[c]	3.80
Ric 14	Undetectable	Undetectable	0.23 (4.19)	14.00
Ric 15	4.0 (13.3)	1.00	1.6 (25.8)	10.00
Ric 17	8.6 (28.6)	—[d]	1.35 (24.63)	—[d]
Ric 21	9.9 (33.0)	—	1.42 (22.5)	—

[a]Number of binding sites $\times 10^3$ per mg of cell protein, as determined by the Scatchard [14] relation. Molecular weights of 60,000 (ricin) and 26,000 (bovine lectin) were used for these calculations.
[b]Ratio relative to untreated cells.
[c]Percent of binding sites relative to wild-type BHK cells.
[d]Not determined.

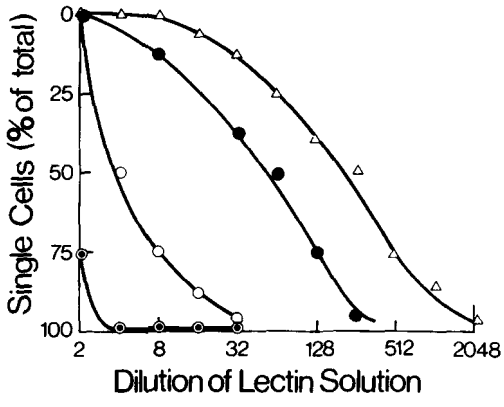

Fig. 2. Agglutination of BHK cells by heart lectin. Trypsinised BHK cells (●), Ric 14 (⊙), or Ric 15 (○) cells were incubated with various dilutions of heart lectin (initial concentration 1 mg ml^{-1}) and the extent of agglutination was assessed after 1 hr. Titration of the agglutination of trypsinised rabbit erythrocytes by heart lectin is also shown (△).

glass surfaces coated with nonspecific proteins such as bovine serum albumin (Fig. 3b). Several proteins such as fibronectin (Fig. 3a), and certain plant lectins such as ricin (Fig. 3c) facilitate the attachment of trypsinised BHK cells. Attached cells rapidly convert into a well-spread configuration, provided a threshold concentration of protein is attached to the glass [15].

When trypsinised BHK cells were added to coverslips carrying adequate concentrations of purified bovine lectin, the cells rapidly attached and spread out (Fig. 3d). The minimum concentration of glass-immobilised bovine lectin required to trigger the transformation of BHK cells into a fibroblastic morphology was approximately 40 ng/cm^2 (Fig. 4), which converts to about 10^{12} lectin molecules/cm^2. It is interesting that the threshold concentration of bovine lectin required to induce the morphological transformation is much higher [15] than the corresponding values for fibronectin (2×10^{10} molecules/cm^2) but similar to ricin (10^{12} molecules/cm^2).

The ricin-resistant cell lines Ric 14 and 15 lacking or carrying reduced amounts of surface receptors for bovine lectin attached weakly (Fig. 3e) to the lectin-coated glass coverslips, similarly to albumin-coated glass (Fig. 3f), at any concentration of matrix-attached lectin (Fig. 4).

DISCUSSION

These results show that a lectin known to be present, at least in part, at the surface of some cells [6, 8] can mediate the adhesion of hamster fibroblasts carrying a sufficient complement of cell surface receptors. In vivo, therefore, the galactoside-binding bovine lectin might mediate the adhesion of homologous cells secreting the lectin, and if it is attached to connective tissue it may be involved in the attachment of cells to substratum and the organization of cardiac tissue. The results are broadly in agreement with a model previously proposed [16] for BHK cell adhesion: Cell adhesion may involve recognition of surface-exposed galactose residues by a carbohydrate-binding cell surface protein. Thus, aggregation of BHK 21 cells is inhibited by glycopeptides sensitive to galactose oxidase, isolated from the surface of BHK

cells [17], and ricin-resistant BHK mutants lacking surface galactose residues are poorly adhesive to one another as well as ricin-coated matrices [15, 16]. It is interesting that at least one endogenous lectin of similar specificity has already been identified in BHK cells [18].

Attachment of BHK cells to glass derivatized with bovine lectin and the subsequent spreading into fibroblastic forms requires a threshold density of lectin molecules on the glass surface. Similar results were obtained for ricin and other lectins as well as for fibronectin [15].

The present results show that BHK cell adhesion is also regulated by the density of cell-surface receptors for the bovine lectin. Mutant cell lines carrying fewer receptors fail to spread significantly even at the highest concentration of matrix-immobilised lectin. This finding is consistent with models for cell adhesion involving multiple and complementary interactions between galactose-containing sequences and endogenous lectins occurring on the surfaces of apposed cells or absorbed to a substratum [1, 16]. Thus in vivo, cells with numerous receptors and synthesising large amounts of endogenous lectins will show preferential adhesive specificity, suggesting a possible biochemical basis of cell sorting.

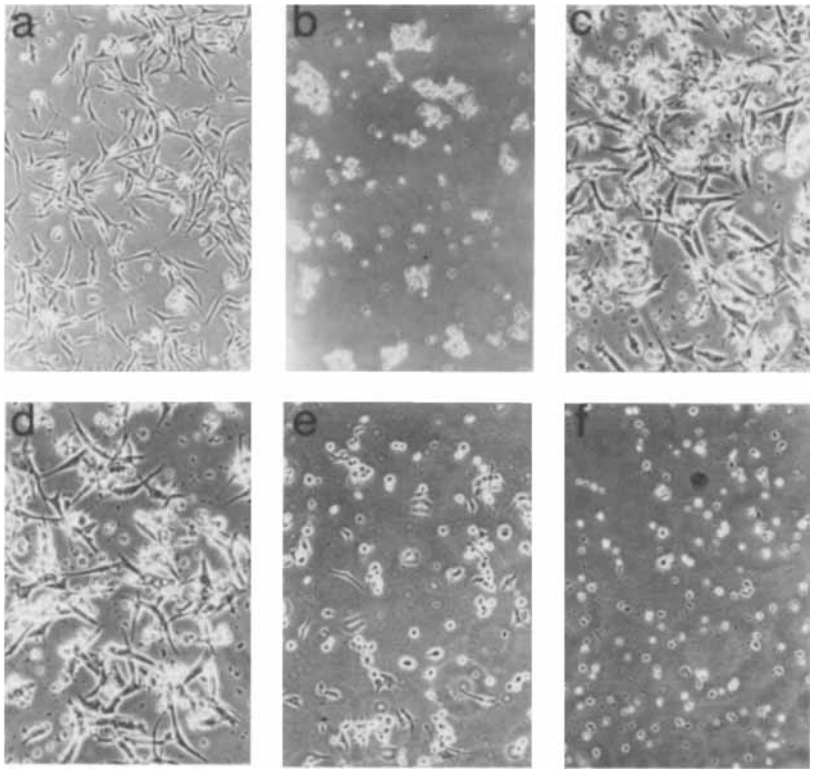

Fig. 3. Adhesion of trypsinised BHK cells to glass coverslips derivatized with 1 mg ml^{-1} solutions of fibronectin (a); bovine serum albumin (b); ricin (c), or bovine lectin (d). The adhesion of trypsinised Ric 14 cells to bovine lectin (e) or bovine serum albumin (f) is also shown. Magnification × 120 (a, b, e, f); × 260 (c, d).

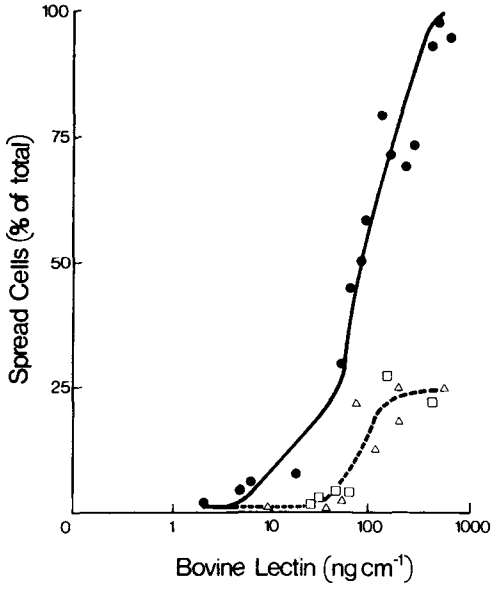

Fig. 4. Relationship of cell spreading to the matrix density of glass-immobilised bovine lectin. Glass coverslips were derivatized with (^{125}I)-labeled lectin as described in the text. Metabolically labeled, trypsinised cells were added to the coverslips and the extent of cell spreading was assessed after 4 hr at 37°C. Wild-type cells, ●; Ric 14, △; Ric 15, □.

The bovine lectin, like ricin, reacts well with glycoproteins having asparagine-linked carbohydratre chains (N-glycans) with Gal β1→3 GlcNAc or Gal β1→4 GlcNAc sequences at their nonreducing termini [5, 7, 10]. Hence, selection affecting biosynthetic assembly of ricin-binding sequences [9] would be expected to induce alterations in the binding properties of ricin-resistant cell lines toward the bovine lectin. However, the binding specificities of bovine lectin and ricin are clearly different, a conclusion that is consistent with other data [7, 19]. Thus, the number of binding sites at the BHK cell surface is greater for the bovine lectin than for ricin. In addition, neuraminidase treatment of normal BHK cells or the ricin-resistant mutants exposes additional binding sites for ricin [14] but not for the bovine lectin. This difference is possibly explained by the poor affinity of the bovine lectin for O-glycans containing the Gal β1→3 GalNAc sequence [5]. Ricin binds with high affinity to proteins substituted with such sequences, but only after neuraminidase treatment [19]. These structures thus probably do not contribute significantly to the binding of ricin to untreated cells, and the assembly of these chains would be expected to be unchanged after selection with ricin. It is interesting that neuraminidase treatment of normal BHK cells revealed no additional binding sites for the bovine lectin. By contrast, the affinity of several soluble glycoproteins for the lectin in increased by desialylation [5]. These disparate results suggest significant differences between the galactose-containing sequences of the soluble glycoproteins examined by others [5] and the glycoprotein receptors present on the BHK cell surface. It is of particular interest to note the relative binding of bovine lectin [7] and ricin [9] to branched oligosaccharides containing only one Gal-GlcNAc terminal sequence. The affinity of this structure for the bovine lectin is hardly affected, but the affinity for ricin is

reduced 10- to 20-fold. The reduced binding of ricin to Ric 21 cells, which retain a more significant complement of surface binding sites for the bovine lectin, may therefore suggest a similar alteration in the surface N-glycans of these cells, in agreement with analysis of the levels of glycosyl transferase in the cells compared with normal BHK cells [20] and glycopeptide analysis (Hughes, unpublished).

Future work will be directed toward identifying the nature of the determinants on the BHK cell surface binding to the lectin, in particular their relationship to galactose-containing components with antigenic activity [7]. The interaction of the bovine lectin with the carbohydrate chains of glycoproteins known as Ii antigens has been described previously [7]. Preliminary evidence suggests that these determinants are expressed at the surface of BHK cells but are absent from the surface of several of the ricin-resistant mutants (unpublished). Since the expression of Ii antigens appears to be developmentally regulated [21] as are some mammalian lectins [22], the interactions between the two systems offer intriguing possibilities for specific intercellular interactions occurring during growth and development.

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