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Lectins Inhibit Cell Binding and Spreading on a Laminin Substrate

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Summary: This study examined the effects on cell binding and spreading of the exposure of laminin substrates to the lectins Wheat Germ Agglutinin or Concanavalin A. Exposure of laminin to Wheat Germ Agglutinin inhibited binding of mouse B16 F1 melanoma cells in a dose dependent manner. Exposure to Concanavalin A had no deleterious effects on binding but did inhibit cell spreading. Both effects were completely prevented by the specific sugars for each lectin. These effects may be due to the proximity of N-linked oligosaccharides to the known cell binding sites of laminin.

Laminin is a large glycoprotein which is a prominent component of basement membranes. It is composed of three polypeptide chains, A (MW = 400,000), B1 (MW = 210,000) and B2 (MW = 200,000), held together in a cruciform shape by disulfide bonds (1). The laminin molecule is known to be about 15% carbohydrate by weight (2). Many different epithelial, neural and malignant cell lines are known to bind or otherwise respond to laminin. At least three different cell surface receptors for this large glycoprotein have been found (3,4,5) as well as two high-affinity cell adhesion sites (6,7). In this study we have attempted to examine some of the ways in which the carbohydrate components of laminin may influence its biological properties.

MATERIALS AND METHODS

Preparation of Laminin Substrates:
Laminin was isolated from Englebreth-Holm-Swarm (EHS) sarcoma by the method of Timpl et al (8). In Tris-buffered saline solution it was allowed to dry overnight at various concentrations (1 to 25 ug per well), in non-tissue culture treated 96 well flat-bottom plates (Becton Dickinson, Oxnard, CA).

Lectin Treatment of Laminin Substrates:
The laminin coated wells were rinsed twice with phosphate buffered saline (PBS), and then incubated for 2 hours at room temperature on a shaking platform with either of the two lectins

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tested, Wheat Germ Agglutinin (WGA) or Concanavalin A (Con A) (Sigma, St. Louis, MO), at various concentrations in PBS. As controls the laminin coated wells were incubated for 2 hours with PBS alone, a lectin plus its specific sugar in PBS, a lectin plus a non-specific sugar in PBS, or sugar alone in PBS. These solutions were dumped out of the wells which were then washed three times with PBS (binding experiments with [^{125}I] labeled laminin indicated that less than 10% of the glycoprotein was washed away under these conditions (data not shown). To each well was added 100 ul of serum-free Dulbecco's modified Eagle medium (DMEM; GIBCO, Grand Island, NY).

Cell Culture and Binding Assay:

Cells used in all assays were mouse B16 F1 melanoma (the generous gift of Dr. I. Fidler, Univ. Texas Cancer Center), grown in DMEM (GIBCO) supplemented with 10% fetal bovine serum (CC Laboratories, Cleveland, OH) and 1% antibiotic-antimycotic (GIBCO) in 100mm plastic petri dishes (Becton Dickinson) under standard temperature and atmospheric conditions.

Cells were released from culture plates using an Earle's basic salt solution containing 2mM EDTA. Cells were counted using a Coulter counter (Coulter Electronics, Hialeah, FL) and seeded 5000 cells per well, in serum-free DMEM, in triplicate. Plates were placed back in the incubator for 1 hour under standard culture conditions. Cell viability was assessed on the basis of trypan blue exclusion and was found to be >85%. Medium was dumped out of the plates and all wells were washed 2 times with PBS. The adherent cells were then fixed, stained, and counted essentially by the method of Ruoslahti et al (9). Briefly, the cells were fixed overnight at 4^{0}C with 3% formaldehyde in PBS, cells were then stained with 1% toluidine blue for 2 hours at room temperature, rinsed 3 times with PBS and counted using an inverted microscope (Olympus, Japan)with a video camera (Optomax, Hollis, NH) connected to a digital image analyzer(Optomax).

RESULTS

In Figures 1a and 1b it is evident that exposure of laminin-coated plastic to WGA for 2 hours has a marked effect upon cell binding. This effect seems to be dependent upon the amount of WGA, from 25 to 100 ug per well (data not shown). Use of N-acetylglucosamine, a specific sugar for WGA, completely reverses the inhibition of B16 F1 melanoma cell binding.

Figures 2a and 2b indicate that Con A has essentially no effect on cell binding, as measured by our assay system, at any concentration tested (from 25 to 250 ug per well). However, upon examination of the fixed, stained cells it was found that Con A treatment of the laminin substrates had prevented cell spreading as compared with controls, as shown in Figures 3a and 3b. Addition of the specific sugar for Con A completely reversed the anti-spreading effect.

In all experiments, incubation of the laminin substrates with either PBS or lectin-specific sugar alone gave the same cell

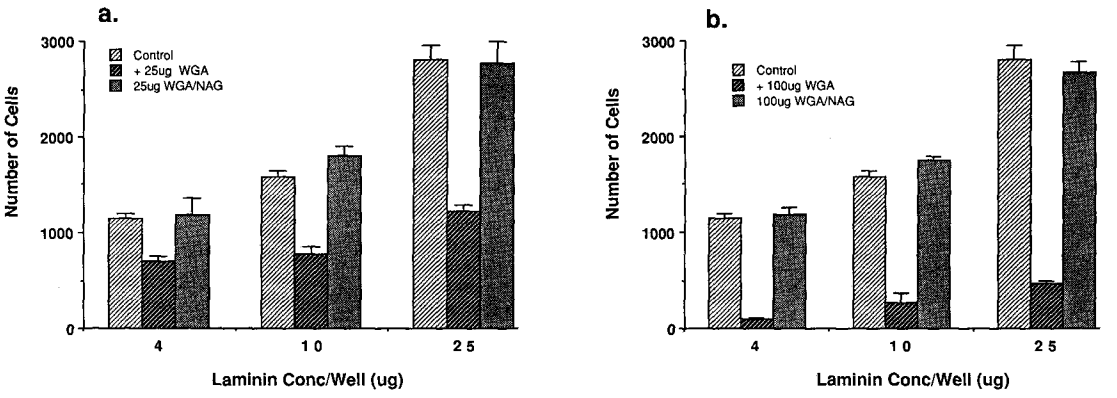

Figure 1. Number of mouse B16 F1 melanoma cells bound to laminin coated plastic (Control) or laminin coated plastic incubated with Wheat Germ Agglutinin (WGA) or incubated with WGA and N-acetylglucosamine, in triplicate wells (see Materials and Methods for detailed procedures). a. Lectin concentration is 25 ug WGA, with standard deviation bars. b. Lectin concentration is 100 ug WGA with standard deviation bars.

binding as the untreated controls (data not shown). Incubation of the lectin-treated laminin substrates with non-specific sugars gave the same results as found for lectin treatment alone (data not shown).

DISCUSSION

The laminin isolated from EHS sarcoma has been shown to possess approximately 40 N-linked oligosaccharides (10,11). We have examined how these carbohydrate moieties may influence cell

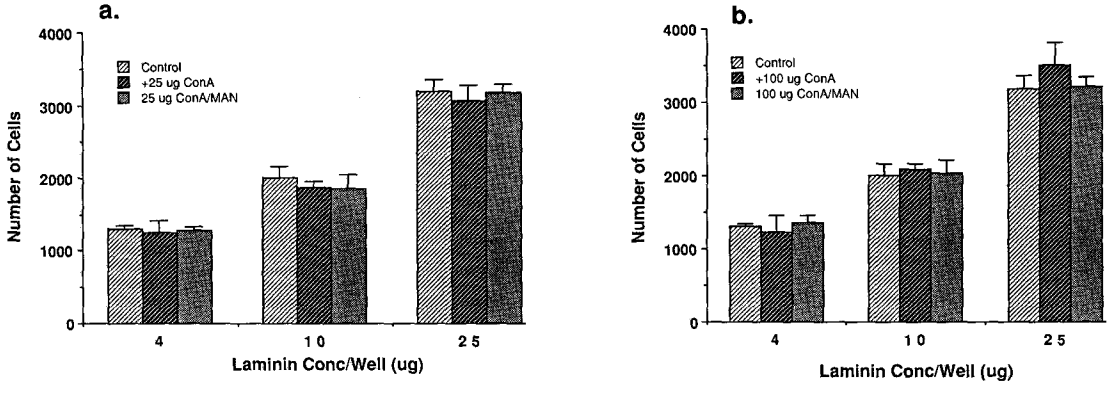

Figure 2. Number of mouse B16 F1 melanoma cells bound to laminin coated plastic (Control) or laminin coated plastic incubated with Concanavalin A (Con A) or incubated with Con A and alpha-methyl mannoside, in triplicate wells (see Materials and Methods for detailed procedures). a. Lectin concentration is 25 ug Con A, with standard deviation bars. b. Lectin concentration is 100 ug Con A, with standard deviation bars.

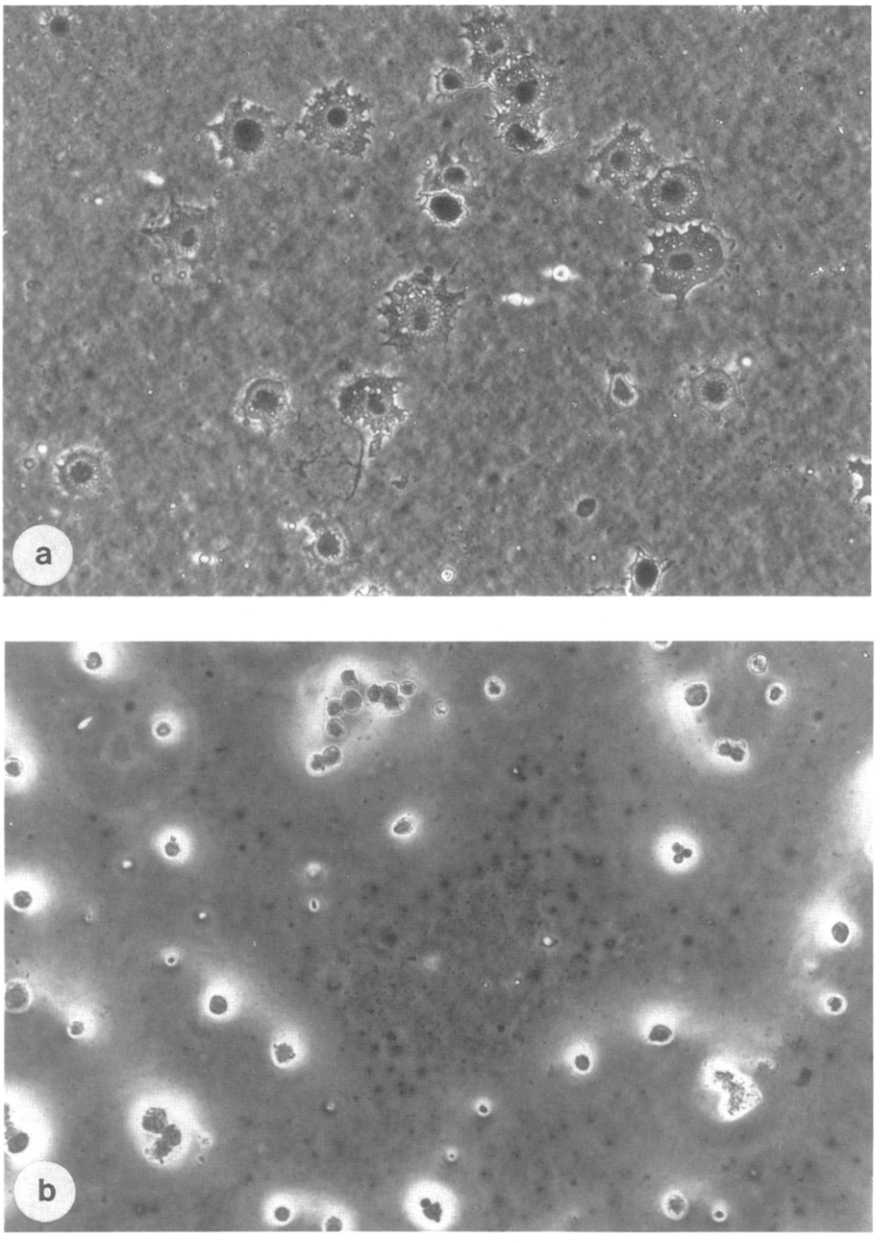

Figure 3. a. Photomicrograph of B16 F1 cells attached and spread on laminin coated plastic, 400x (appearance was the same when lectin plus its specific sugar was added). b. Photomicrograph of B16 F1 cells on laminin coated plastic that has been incubated with Con A, 400x.

binding to laminin. The results indicate that WGA, when bound to laminin substrates, blocks cell attachment sites. This interference is reversed only by N-acetylglucosamine, the specific sugar recognized by WGA. In contrast, Con A does not inhibit cell adhesion, but does prevent cell spreading. The effect is only

reversed by alpha-methyl mannose, the specific sugar recognized by Con A.

Work with fibronectin has shown that the type and extent of glycosylation of this glycoprotein can affect the way it binds to gelatin (12). Jones et al reported that lack of carbohydrates significantly enhanced fibroblast adhesion and spreading on fibronectin coated surfaces (13).

Our results correlate well with the findings of Trinkaus-Randall et al (14) on the effects of different lectins on the binding of rabbit basal corneal epithelial cells to freshly denuded corneal basal lamina. Their work indicates that WGA incubation of corneal basal lamina inhibits cell attachment, while Con A has no effect on cell binding, but prevents extensive cell spreading. The laminin present in the corneal basal lamina used in those experiments may account for their findings.

A possible explanation for the effects that we report on cell binding and spreading is that the bound lectin sterically blocks cell surface receptor binding sites on the laminin molecule. This argument is strengthened by the fact that the amino acid sequence of the laminin B1 chain (15) reveals a potential N-glycosylation site only ten amino acid residues away from the well known YIGSR cell binding domain (6). Further work may reveal the precise location of specific oligosaccharide chains on the native laminin molecule and how they might modulate cell attachment and spreading. The results presented suggest that the lectin, Con A, may be useful in separating these two phenomena of the cellular response to laminin substrates.

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