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Chicken Tissue Binding Sites for a Purified Chicken Lectin

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A lactose-binding lectin previously purified from embryonic chicken muscle and adult chicken liver, and here referred to as chicken-lactose-lectin-I (CLL-I), was added to sections of various adult chicken tissues to detect available binding sites. Both the sites of binding of added CLL-I as well as the tissue distribution of endogenous CLL-I were determined by indirect immunofluorescence using a rabbit antibody to CLL-I followed by fluorescent goat anti-rabbit IgG. Some tissues such as intestine and kidney showed abundant extracellular binding sites for the lectin, primarily between cells, in basement membrane, and in material on the luminal surface. In contrast, adult heart showed no significant binding sites for CLL-I. Adult pancreas showed considerable endogenous CLL-I in an extracellular site surrounding exocrine lobules, but added CLL-I did not bind substantially. The distribution of CLL-I binding sites in intestine were mimicked by those of purpurin, another lactose-binding lectin. CLL-I binding sites were also detected on the surface of cultured chick embryo skin fibroblasts. The factors controlling the specific distribution of occupied and unoccupied CLL-I binding sites are not known.

Key words: lectins, lectin binding sites, cell surfaces, extracellular materials

A number of embryonic [1–3] and adult [4] chicken tissues contain an endogenous lactose-sensitive carbohydrate-binding protein which undergoes marked changes in activity with development. Because this protein may be assayed as a lactose-sensitive hemagglutinin it is referred to as a lectin. The role of this lectin in cellular differentiation or in other cellular functions has not yet been determined. The lectin has been purified from several embryonic and adult tissues [5–7]. It is a dimer of subunits with apparent molecular weight of 15,000. In this paper we will refer to this lectin as chicken-lactose-lectin-I (CLL-I) to distinguish it from another distinct lactose-binding lectin [7] from chicken tissues. This second lactose-binding lectin, like CLL-I, is found in adult chicken intestine, from which it has been purified [7].

Detailed comparisons of CLL-I purified from embryonic muscle and adult liver indicate that they are identical by all chemical and immunological tests that were used [7]. A potent antiserum has been raised by immunizing rabbits with this lectin [4]. Immuno-

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histochemical studies have shown that CLL-I is concentrated at different sites in different tissues. In developing muscle [5] and brain [8], some is detectable on the surface of myoblasts and neurons, but most is intracellular. Lectin levels in adult muscle and brain are low, but are high in several other adult tissues. CLL-I localization differs in three adult tissues studied [4]. It is concentrated in secretory granules of intestinal goblet cells, in the extracellular matrix surrounding pancreatic exocrine lobules, and in hepatic Kupffer cells.

The present study was designed to determine the location of binding sites for CLL-I in several adult chicken tissues and the relationship between these binding sites for added CLL-I and the localization of endogenous CLL-I. We found that in some tissues such as adult intestine and kidney there are many extracellular CLL-I binding sites not already occupied by endogenous CLL-I. Adult heart, in contrast, had neither endogenous lectin nor significant binding sites for CLL-I lectin, and the binding sites in adult pancreas were either absent or fairly well occupied by endogenous CLL-I which was abundant in the extracellular material surrounding lobules.

MATERIALS AND METHODS

Preparation of Lectins and Antisera

The lectin from adult chicken liver (CLL-I) was isolated and purified by affinity chromatography as described previously [4, 5]. This lectin is indistinguishable from the lactose-binding lectin previously purified from chick embryonic pectoral muscle [7].

Production of antiserum directed against this antigen and demonstration of its specificity have also been described [4]. Purpurin, the lactose-binding lectin from Dictyostelium purpureum, and its antiserum were obtained as described previously [9]. Concanavalin A and antiserum directed against it were purchased from Calbiochem.

Lectin Localization in Tissue Sections

Tissue fragments were fixed in 3% paraformaldehyde, 0.1% glutaraldehyde, and 0.5 to 1.0 μm frozen sections were cut as described previously [4]. Indirect immunofluorescent staining for endogenous lectin was done with immune gamma globulin followed by tetramethylrhodamine-conjugated goat anti-rabbit IgG (Cappel Labs) as described previously [4]. To determine binding of exogenous lectin to sections, they were incubated for 15 minutes with a small droplet of pure lectin (100 μg/ml). After unbound lectin was washed away, the sections were stained with anti-lectin gamma globulin and tetramethylrhodamine-conjugated goat anti-rabbit IgG as above. To verify specific binding of the lectin to sections, lectin was incubated in the presence of a high concentration (300 mM) of a hapten inhibitor.

Cell Culture

Primary cultures of chick embryo skin fibroblasts were grown on glass coverslips in Eagle's minimal essential medium supplemented with 10% fetal calf serum, as described previously [5, 10]. Cells fixed with 3% paraformaldehyde were stained by the same protocol as for the tissue sections.

Fluorescence Microscopy

Slides of sections or cells were examined with a Leitz Dialux epifluorescence microscope with a X40 (NA 1.3) oil immersion lens. Photomicrographs were taken with 30-sec exposures with Kodak Plus-X film and developed with Diafine developer. All photographs of each tissue were printed under identical conditions.

RESULTS

Lectin Binding to Tissue Sections

In adult chicken intestine, endogenous CLL-I was localized to the secretory granules of the mucus-secreting goblet cells (Fig. 1a) as observed previously [4]. Sections reacted with non-immune sera showed only very faint and diffuse labelling, and all specific staining could be adsorbed with purified CLL-I [4]. However, if 100 μg/ml CLL-I was incubated with the sections before antibody staining, much additional fluorescence was observed (Fig. 1b). This exogenous lectin bound to the epithelial basement membrane, the extracellular spaces between adjacent epithelial cells, the microvilli and their mucous coat, and extracellular materials in the lamina propria, especially surrounding smooth muscle bundles. CLL-I binding was all specifically abolished by incubation in the presence of the hapten inhibitor lactose (300 mM) (Fig. 1c). Endogenous CLL-I staining remained since this lectin was held in place by tissue fixation. It is possible that endogenous CLL-I in unfixed sections would also be solubilized by hapten, since lactose markedly facilitates extraction of this lectin from homogenates [5].

Since exogenous CLL-I bound so well to intestinal sections we compared the distribution of this bound lectin with that of two others. The distribution of bound Concanavalin A in intestinal sections differed somewhat from that of CLL-I in that it bound relatively more to diffuse cytoplasmic materials compared with intercellular substances (Fig. 2a). Concanavalin A also showed relatively less binding to the vesicles in goblet cells, although it did intensely stain materials on the intestinal luminal surface. In contrast, purpurin, a lactose-binding lectin from D purpureum [19] bound to the same structures as CLL-I, especially the goblet cell material and intercellular material between intestinal epithelial cells and muscle bundles (Fig. 2c). These results indicate that the receptors for a lectin with affinity for lactose residues are not highly specific for the chicken lactose lectin.

In adult chicken kidney, endogenous CLL-I was found in the cytoplasm and on the luminal and basal surfaces of tubule cells (Fig. 3a). Glomeruli were unstained. Nuclei in some kidney sections also bound antibody. Whether this indicates the presence of lectin or an immunologically cross-reactive material in the nuclei remains to be determined. Exogenously added CLL-I bound predominantly to the luminal surfaces of tubules and to peritubular and glomerular material (Fig. 3b).

In adult chicken pancreas, staining for endogenous lectin gave bright fluorescence which was confined to the extracellular matrix surrounding exocrine lobules (Fig. 4a). Exogenously added CLL-I (Fig. 4b) showed little additional staining, suggesting that there are few receptor sites not occupied by endogenous lectin.

Adult chicken heart contains little endogenous CLL-I as measured by hemagglutination [4]. This result was confirmed by the absence of significant staining for endogenous CLL-I (Fig. 5a). The adult heart also contained few binding sites for exogenously added CLL-I (Fig. 5b), except those associated with blood vessels.

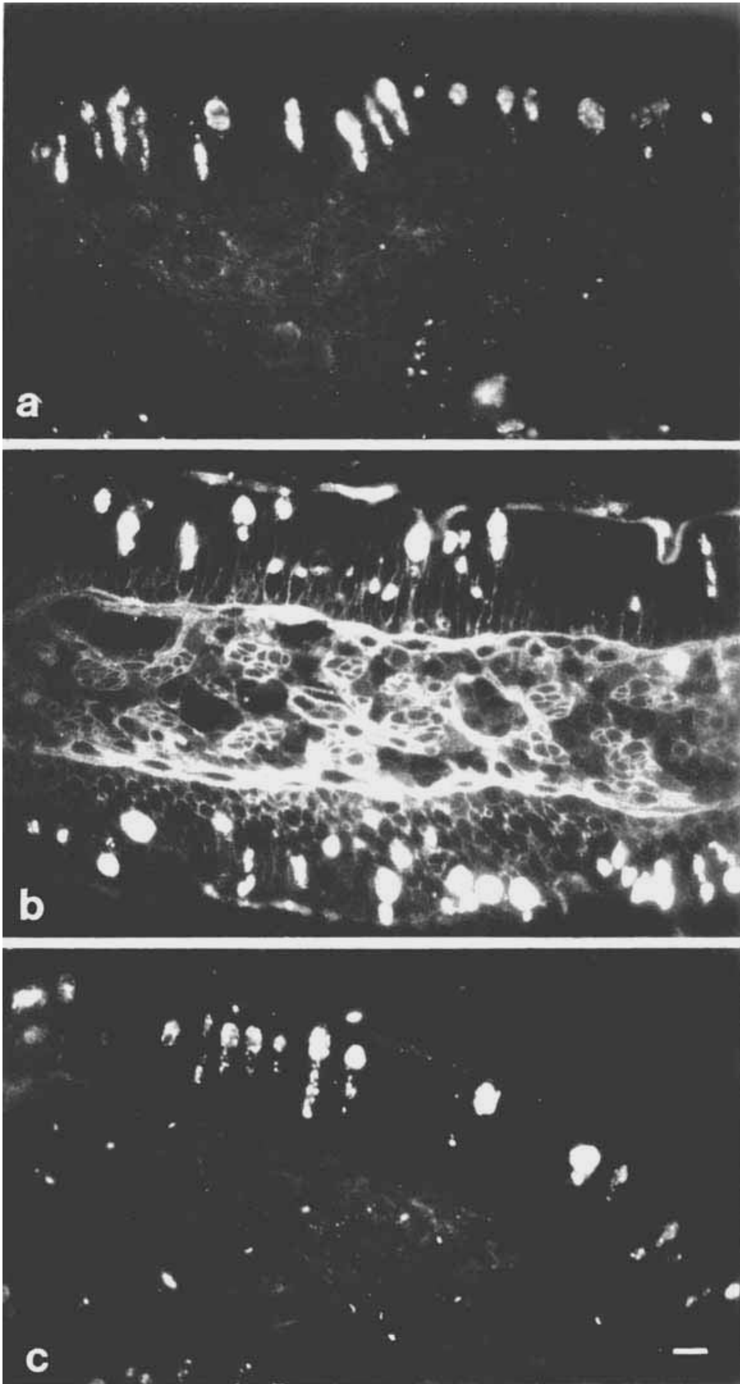

Fig. 1. Indirect immunofluorescence studies of sections of adult chicken intestine. a) Fixed intestinal tissue demonstrating localization of endogenous CLL-I. b) Staining after incubation of section with purified CLL-I demonstrating available binding sites for the added lectin as well as the endogenous lectin. c) Staining after incubation of section with purified CLL-I in the presence of 300 mM lactose which blocks the binding of the exogenously added lectin. Only the endogenous CLL-I fixed to the tissue is now detected by the antibody. Bar, 10 μm.

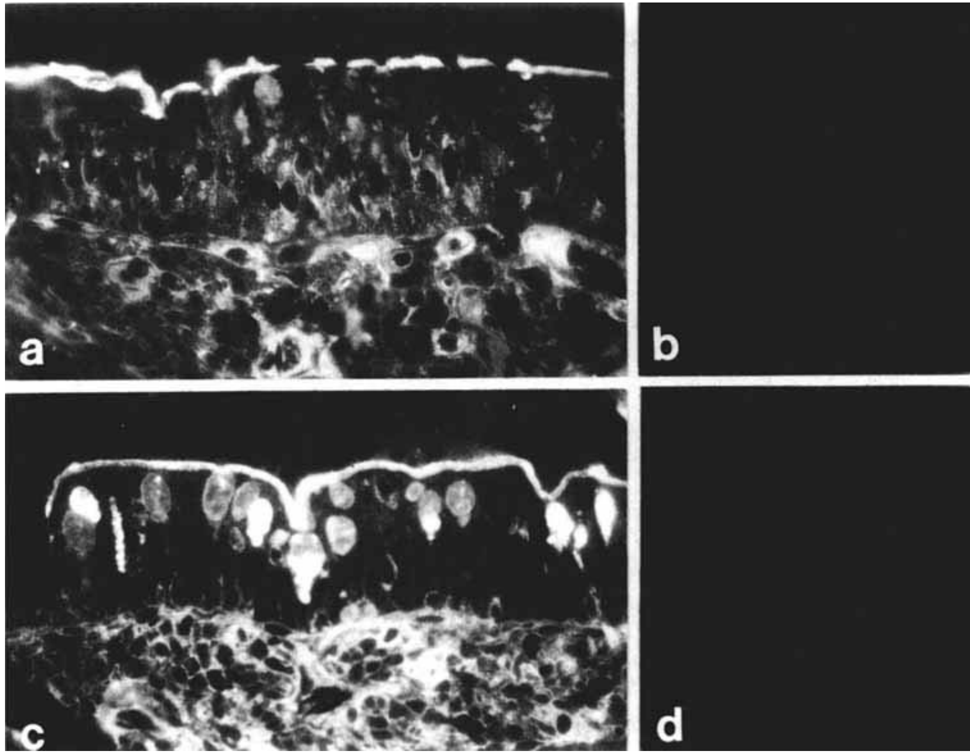

Fig. 2. Indirect immunofluorescence studies of sections of adult chicken intestine stained with different exogenous lectins. a) Fixed sections were incubated with 300 μg/ml of Concanavalin A, washed, then reacted with a rabbit immunoglobulin fraction containing antibodies directed against Concanavalin A followed by fluorescent goat anti-rabbit IgG. b) As in (a) but with incubation of Concanavalin A performed in the presence of 300 mM α-methyl mannoside. c) As in (a) but the exogenous lectin added was purpurin, a lactose-specific lectin from D purpureum and the first antibody was a rabbit immunoglobulin fraction raised against purpurin. d) As in (c) but purpurin was added to the sections in the presence of 300 mM lactose.

Lectin Binding to Cultured Cells

Chick embryo fibroblasts contain little or no endogenous CLL-I detectable by hemagglutination activity or radioimmunoassay [Beyer, Ceri, and Barondes, unpublished results]. When these cells were stained by indirect immunofluorescence, no surface lectin could be detected (Fig. 6a). However, fibroblast cell surfaces bound considerable exogenous CLL-I (Fig. 6b).

DISCUSSION

Lectins from plant and invertebrate sources which specifically bind to various carbohydrate moieties have been extensively used to examine the distribution of oligosaccharide-containing materials in cells and tissues. By using labelled lectins as histological reagents it

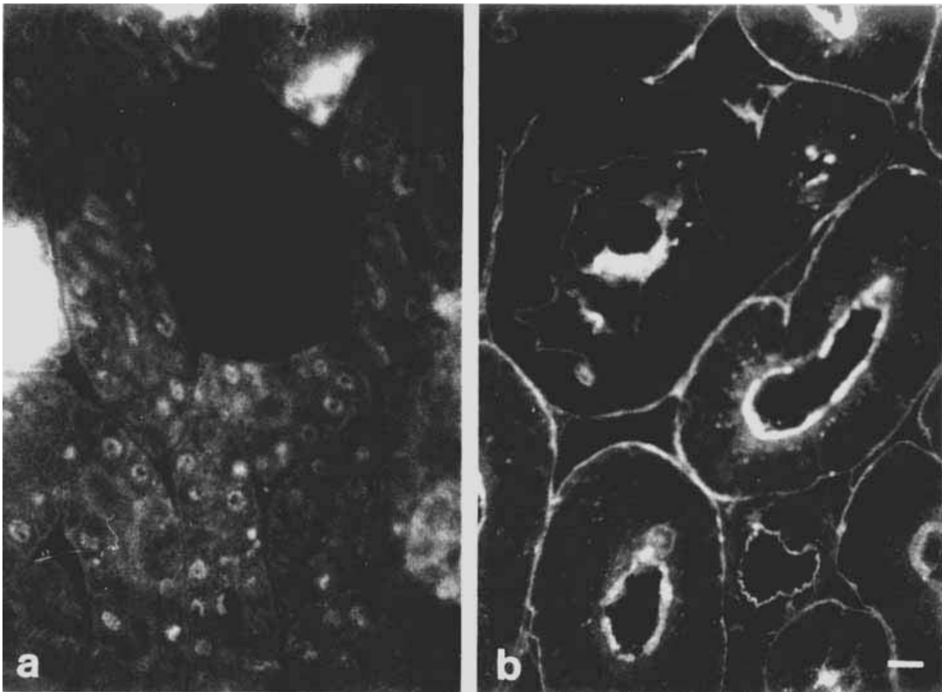

Fig. 3. Indirect immunofluorescence studies of sections of adult kidney cortex. a) Localization of endogenous CLL-I performed as in Figure 1a. b) Localization of both endogenous and added exogenous CLL-I as in Figure 1b. Controls in which the exogenous CLL-I was added in the presence of 300 mM lactose gave results indistinguishable from Figure 3a. Bar, 10 μm.

has been possible to show changes in embryonic [11] or tumor cell surfaces [12], differences in cell surface oligosaccharides of different cell types within an organ [13], and differential distribution of specific oligosaccharides within a tissue [14–16]. The present study describes the first attempt to localize binding sites for a vertebrate lectin within various tissues of the organism from which the lectin itself was derived.

In this report, we have shown that a lectin, CLL-I, detectable in a number of chicken tissues can bind to a variety of cellular and extracellular sites in the chicken. In some tissues, such as intestine, sites not normally occupied by the endogenous lectin can bind considerable exogenous lectin. These binding sites are found especially in intercellular spaces and basement membranes. The sites are not specific for the chicken lectin since another lactose-binding lectin, purpurin, also binds to them. In other chicken tissues such as pancreas, extracellular sites appear saturated by endogenous lectin; but similar sites surrounding kidney tubules are not saturated and bind considerable added CLL-I. Adult heart not only has little or no endogenous CLL-I but also few available binding sites.

The function of lectins in the tissues in which they are normally found is not presently understood. In the cellular slime molds where this problem has been studied extensively, lectins have been implicated in specific cell interactions including cell–cell adhesion [17]. Evidence that plant lectins play a role in cellular interactions has also been presented [18]. Although CLL-I is abundant at intracellular sites [5], its presence on cell surfaces [5, 8] and at extracellular sites [4] suggest that it may sometimes also function in this

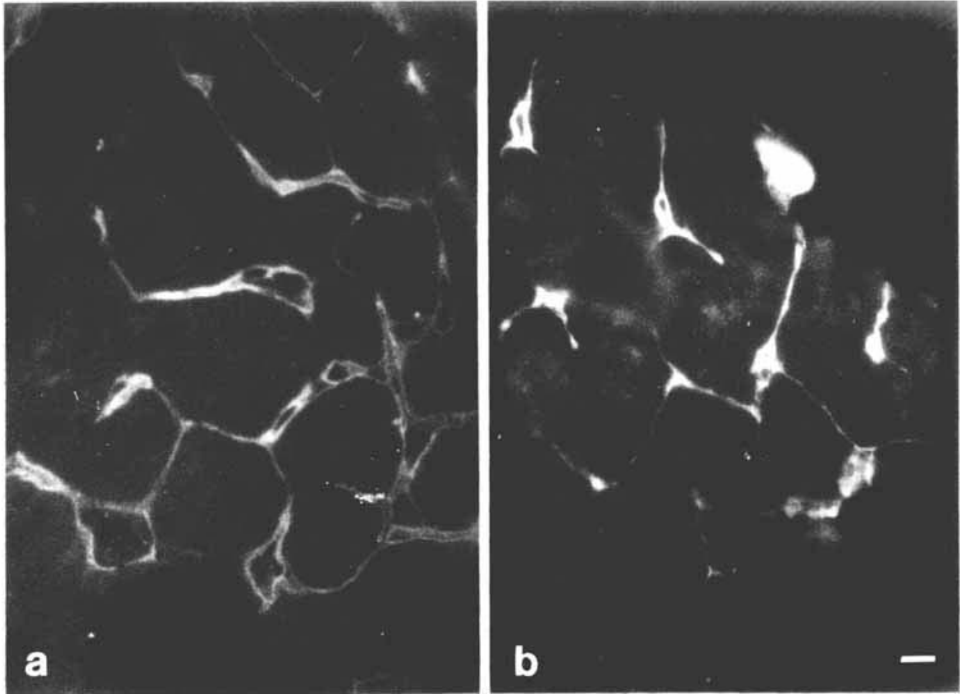

Fig. 4. Indirect immunofluorescence studies of sections of chicken pancreas. a) Localization of endogenous CLL-I performed as in Figure 1a. b) Localization of both endogenous and exogenously added CLL-I determined as in Figure 1b. Note that there was not much binding of exogenous CLL-I suggesting that there are few, if any, receptors for this lectin in this tissue or that they are already largely saturated by the endogenous lectin. Bar, 10 μm.

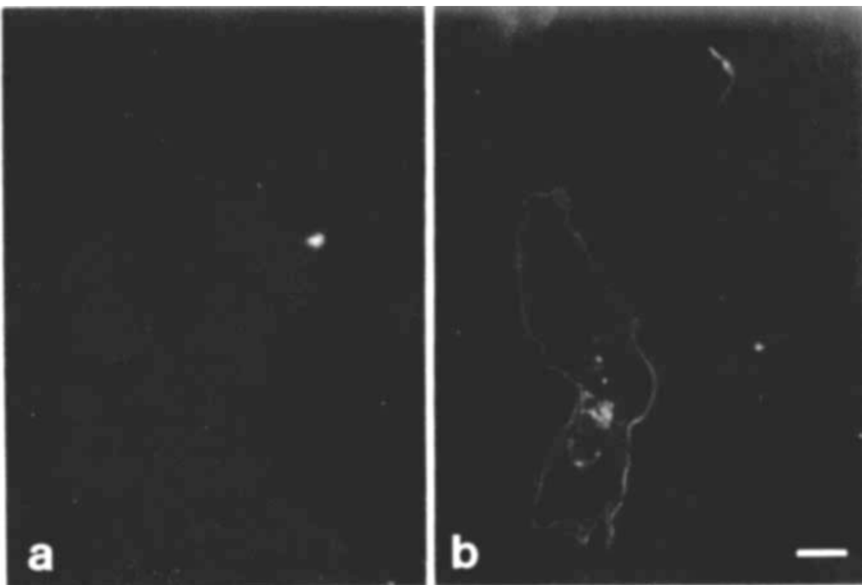

Fig. 5. Indirect immunofluorescence studies of adult heart. a) Localization of endogenous CLL-I as in Figure 1a showing very faint diffuse staining. b) Localization of exogenously added CLL-I determined as in Figure 1b. Note that there is no significant binding of the exogenous lectin to heart muscle or intercellular material. Bar, 10 μm.

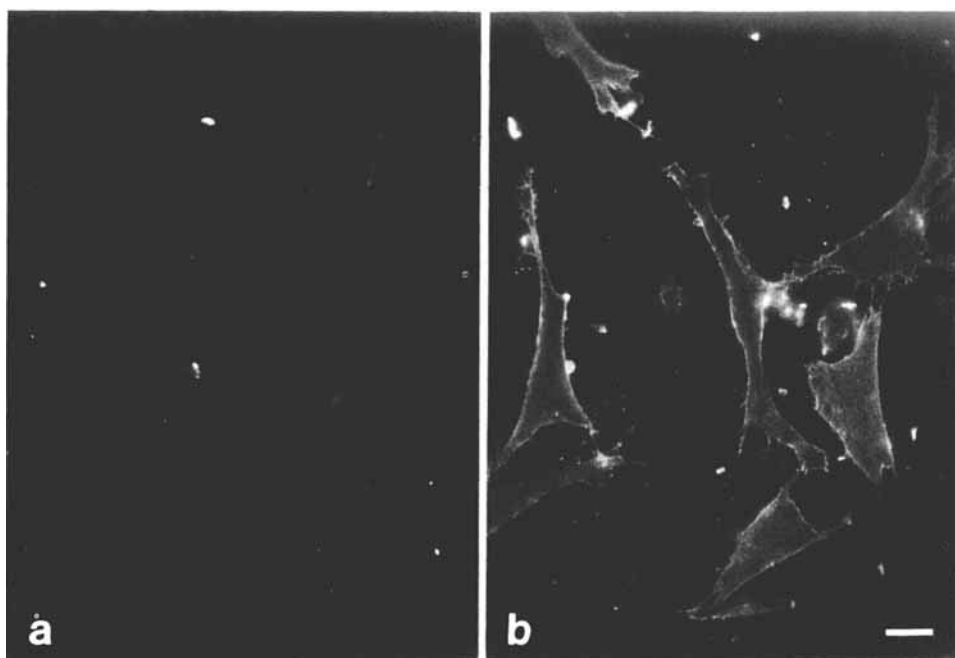

Fig. 6. Indirect immunofluorescence studies of fixed cultured chick embryo fibroblasts. a) Staining in the absence of exogenous CLL-I, as in Figure 1a. b) Staining after addition of exogenous CLL-I as in Figure 1b. Note that the added CLL-I binds to the surface of the fibroblasts. This binding was completely blocked by 300 mM lactose. Bar, 10 μm.

manner. Indeed, some evidence has been presented suggesting a role for this lectin in the fusion of vertebrate muscle cells [19, 20]. The present finding that receptors for added CLL-I are concentrated extracellularly as well as on fibroblast cell surfaces supports a possible role for this lectin in interactions between cells or between cells and extracellular materials.

The significance of the unoccupied binding sites for exogenous CLL-I in various chicken tissues is also not known. These sites are clearly not highly specific for CLL-I since purpurin, another lactose-binding lectin, will occupy them also. Specificity of receptors is also argued against by the finding that a mammalian lactose-binding lectin which is very similar to CLL-I binds to a large variety of substances with terminal β-galactose residues [21, 22] which are common in many cellular oligosaccharides. However, the finding that CLL-I, like other known lectins, does not have a very narrow range of binding specificities in no way precludes their possible role in biologically significant interactions with oligosaccharide receptors in the tissue sites where they are found. Rather, the specific role of lectins may be determined by the factors that determine their sites of deposition within and around cells and by the factors determining the deposition of the various oligosaccharide substances to which they can bind. The fact that specific binding sites for CLL-I are localized to certain regions of certain tissues indicates the potentiality for the interaction of such sites with cells containing CLL-I on their surface or with CLL-I secreted from cells.

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