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Redistribution of Cell Surface Anionic Sites on Hepatoma Cells after Treatment with Concanavalin A*

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Abstract—*Chang rat hepatoma ascites cells were treated with Con A and subsequently labeled with cationized ferritin (CF). When ascites cells were treated with Con A, fixed with glutaraldehyde, then labeled with CF, the labeling on the surface of the microvilli and plasma membrane was clustered and irregular. The areas of the cell surface between CF clusters were either lightly labeled or completely free of label. Cells fixed before Con A and CF treatment or cells incubated with Con A and α-methyl-D-mannoside were labeled with a continuous, even layer of ferritin particles. Treatment of ascites cells with cetyl pyridinium chloride before CF labeling resulted in an almost complete absence of ferritin particles from the cell surface.*

INTRODUCTION

LECTINS are a group of proteins, isolated from plants and animals, that can bind to certain saccharide components of cell membranes. Since changes in the molecular organization of the carbohydrate portion of plasma membranes play an important role in the expression of altered growth behavior seen in transformed cells, lectins have been widely used as probes to study the membrane alterations that accompany cell transformation [1–3]. Concanavalin A (Con A) isolated from jack beans is probably one of the most commonly used lectins and has been used frequently in many agglutination and cell surface studies of normal and transformed cells [4–7].

Danon *et al.* [8] have developed a polycationic derivative of ferritin, cationized ferritin (CF) which permits visualization of negative charges on the surface of plasma membranes. CF has been used to study the distribution of cell surface anionic sites of transformed and normal cells [9–12].

The binding of Con A to the plasma membrane can induce a lateral redistribution of Con A receptors on the surfaces of normal and transformed cells [13, 14]. Recently, Robinson *et al.* [15] reported that in Chang hepatoma ascites cells [16, 17], surface sialoglycopeptide fractions, obtained via papain digestion, also possess Con A receptor activity. Because the surface of mammalian cells has an overall negative charge due mainly to the presence of sialic acid in cell surface coat glycoproteins, it seems likely that CF and Con A receptors could be on the same sialoglycopeptides. Thus the effect of Con A on the distribution of negatively charged saccharide containing surface components of Chang hepatoma ascites cells was examined.

MATERIALS AND METHODS

Under ether anesthesia, the ascites fluid containing the ascites tumor cells was withdrawn from rats and centrifuged at 250 *g* for 10 min [18]. The loosely packed cells were resuspended 3 × in Dulbecco's phosphate buffered saline (PBS) at 25°C to remove blood corpuscles.

The ascites cells were then incubated in Con A (Sigma, St. Louis, Mo.) solution (100 μg/ml PBS) at 25°C for 30 min. After rinsing in PBS, the cells were fixed in 2% glutaraldehyde in PBS at pH 7.2 for 30 min at 25°C. Following a rinse in PBS and veronal buffered saline (VBS) (0.085% sodium barbital in 0.9% NaCl (pH 7.2), the cells were exposed to CF (0.33 mg CF/ml VBS) (Miles-

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Yeda, Kankakee, Ill.) for 5 min at 25 C, then rinsed 3 × in VBS. After post-fixation with osmium, the cells were dehydrated in a graded series of ethanol and embedded in Spurr low viscosity embedding media [19].

Control cells were incubated for 30 min at 25°C in the Con A solution to which had been added 0.25 M α-methyl-D-mannoside (Sigma, St. Louis, Mo.). Additional controls consisted of treatment of fixed ascites cells for 20 min at 25°C before CF exposure with either poly-L-lysine (Miles-Yeda, Kankakee, Ill.) (10 μg/ml VBS) or with 0.05% cetyl pyridinium chloride (Sigma, St. Louis, Mo.) in 0.05 M Tris-maleate buffer at pH 7.4 to which had been added 8.72% sucrose. A portion of the ascites cells received no Con A treatment and were fixed in glutaraldehyde for 30 min at 25°C, rinsed and then incubated with CF. The remainder of the preparation for electron microscopy was carried out as above.

RESULTS

If fixed hepatoma cells were treated with CF, the cell surface labeling was distributed in an even and continuous, uniform layer of ferritin particles (Fig. 1). In spite of the many microvilli present on this ascites cell, the entire external surface of the plasma membrane is covered with ferritin particles (Fig. 1).

When ascites cells were treated with Con A at 25°C before glutaraldehyde fixation and exposure to CF, the ferritin labeling on the surface of the microvilli and smooth portions of the plasma membrane was clustered and irregular (Fig. 2). The CF clusters were not restricted to limited portions of the cell surface, but were distributed on the entire periphery of the ascites cells with the plasma membrane surface between the CF clusters either lightly labeled or completely free of label (Fig. 2).

The addition of α-methyl-D-mannoside to the room temperature Con A incubation medium resulted in an even, smooth and continuous layer of CF label being formed around the outer surface of the plasma membrane (Fig. 3). There was no apparent clustering of CF particles on the surface of cells treated with this monosaccharide lectin binding competitor.

When the ascites cells were incubated with poly-L-lysine before CF labeling, no detectable alterations in the CF binding pattern were seen. There were no discontinuities in the CF label on the cell surface and no apparent reduction of CF particles (Fig. 4). The binding pattern of the ferritin particles resembled the binding pattern of cells treated with Con A-methyl mannoside or cells that were fixed before CF treatment: a smooth and even layer of CF on the cell surface.

If cetyl pyridinium chloride is used as a blocking agent before CF exposure, the amount of CF binding to the surface of the hepatoma cells is greatly reduced (Fig. 5). The reduction of CF is the same on all parts of the cell surface with the microvilli and smooth portions of the plasma membrane displaying no apparent difference in the quantity of bound ferritin particles. Individual CF particles and clusters are arranged into a discontinuous layer and are separated by ferritin free gaps of varying widths (Fig. 5).

DISCUSSION

The lateral redistribution of Con A receptors on the surface of normal and transformed cells is well documented [13, 14, 20]. The goal of this study was to determine how the lateral movement of lectin receptors influences the distribution of cell surface CF receptors. When hepatoma cells were pretreated with Con A before fixation and labeling, the CF particles were arranged into a patchy and discontinuous layer on the cell surface (Fig. 2). Glutaraldehyde fixation after Con A treatment prevented any additional lateral migration of cell surface receptors so that the clustering of CF particles is, most likely, lectin induced. The distribution of ferritin particles on cells treated with Con A in the presence of α-methyl-D-mannoside is identical to that found on cells prefixed in glutaraldehyde before CF treatment and substantiates this observation.

Data presented by Shimizu and Yamada [20] also lend support to the concept of lectin induced receptor redistribution on hepatoma cells. They noted that treatment of unfixed AH7974F rat ascites hepatoma cells with Con A-ferritin led to a clustering of ferritin particles. If, however, the ascites cells were fixed with glutaraldehyde before lectin exposure, the distribution of ferritin particules was homogeneous, indicating that the receptors had been moved laterally by lectins [20].

Subjeck and Weiss [10] have reported that when fixed Ehrlich ascites tumor cells were treated with CF at pH 7.4, ferritin binding was significantly more dense on the surfaces of microvilli than on smooth regions of the cell surface. Thus it could be argued that the

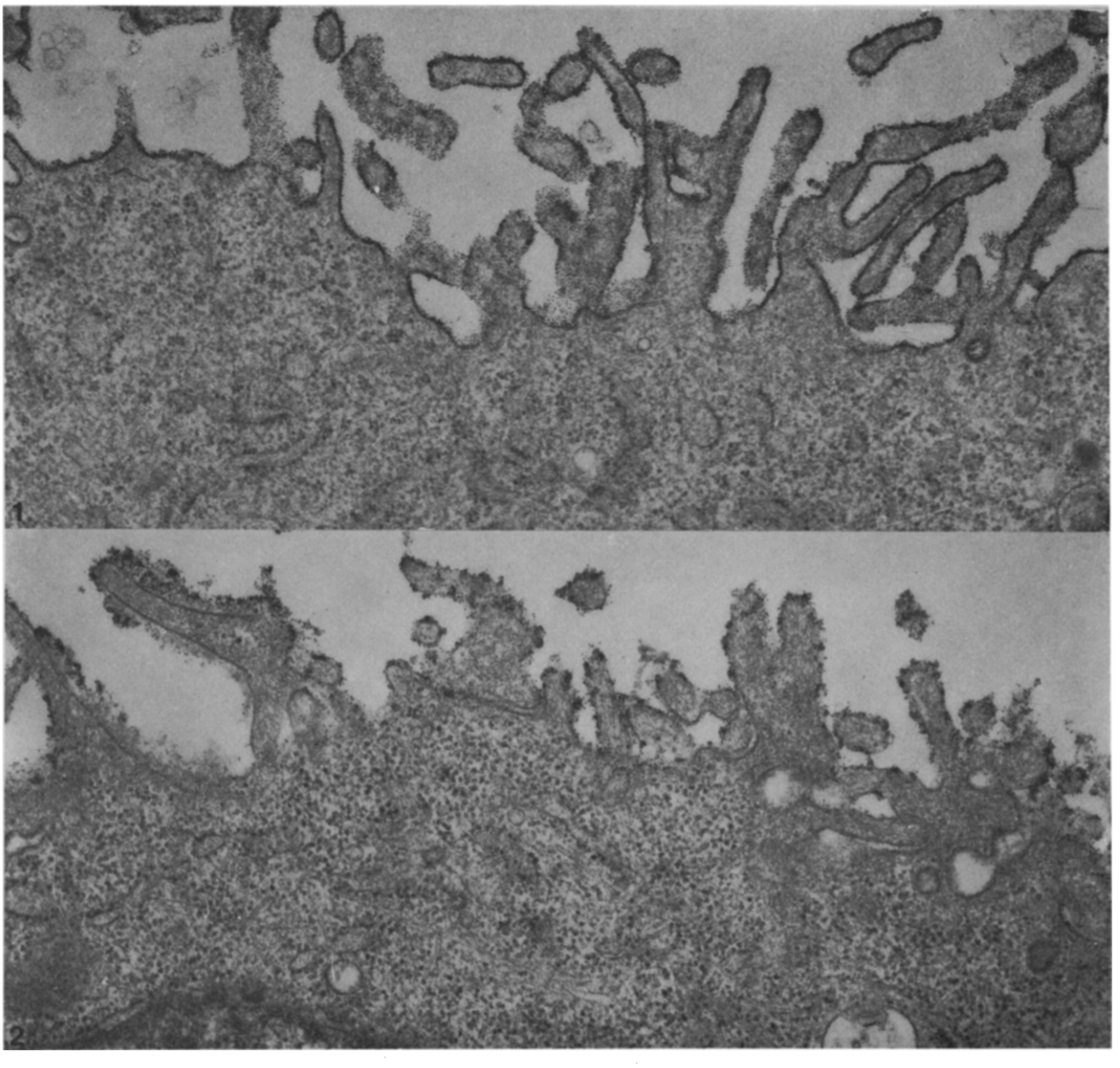

Fig. 1. *Chang hepatoma ascites cell, no Con A treatment and fixed with glutaraldehyde before CF labeling. Even, continuous layer of ferritin particles on smooth areas of the plasma membrane and microvilli. Lead citrate stain.* ×40,000.

Fig. 2. *Chang hepatoma ascites cell after* 30 min *Con A and* 5 min *CF treatment at RT. The ferritin label is clustered and discontinuous over the cell surface. Lead citrate strain.* ×40,000.

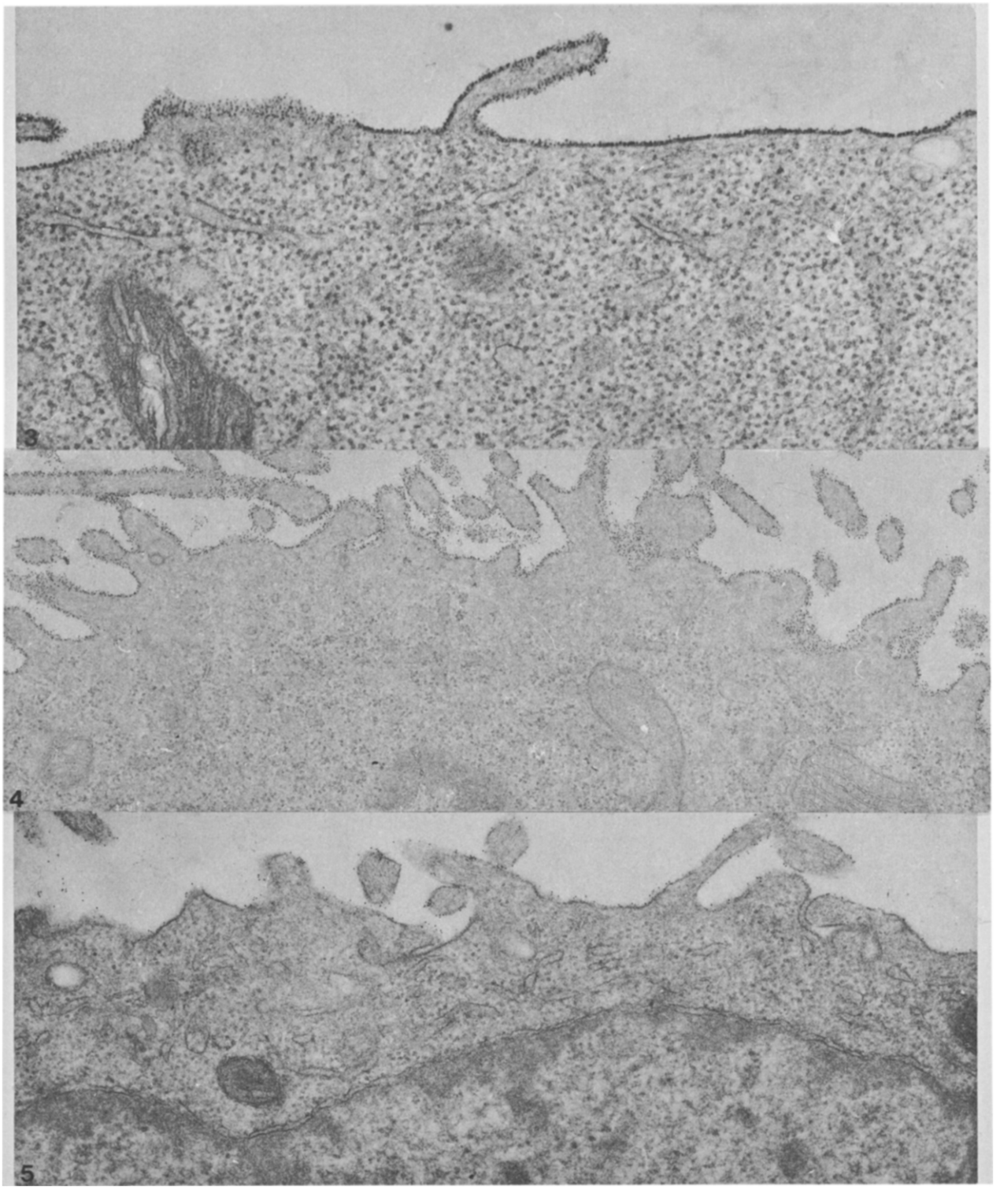

Fig. 3. Chang hepatoma ascites cell incubated as in Fig. 2 except for the addition of 0.25 M *α-methyl-D-mannoside to the Con A solution. Note the smooth and continuous layer of CF particles. Lead citrate stain.* × 50,000.

Fig. 4. Chang hepatoma ascites cell, 30 min *Con A incubation fixed in glutaraldehyde and treated with poly-L-lysine before CF exposure. Ferritin particles on surface of plasma membrane arranged into a continuous layer. Lead citrate stain.* × 40,000.

Fig. 5. Chang hepatoma ascites cell, 30 min *Con A incubation, fixed in glutaraldehyde and treated with cetyl pyridinium chloride before CF labeling. CF binding is greatly reduced with only a few particles of ferritin scattered over the cell surface. Lead citrate stain.* × 40,000.

clustering of ferritin particles seen in Chang hepatoma ascites cells after Con A exposure occurred independently of lectin treatment and represents a normal variation of CF binding behavior. However, examination of ascites cells that were fixed before exposure to Con A or the addition of α-methyl-D-mannoside to the lectin incubation solution consistently resulted in the formation of an even and continuous layer of CF over the entire periphery of the cell and not just the microvillar surface (Figs. 1 and 2). These observations suggest that the clustering of ferritin particles seen after Con A treatment was actually lectin induced and not necessarily an intrinsic property of CF binding.

Cell surface oligosaccharides are very complex [1] and it is reasonable to assume that receptors for Con A and CF could be on the same molecule. Indeed this has proven to be the case with Novikoff tumor cells [21–23]. Robinson *et al.* [15] have also reported the isolation of low molecular weight glycopeptides from the surface of these same hepatoma cells after papain digestion. Additional digestion of these glycopeptides with pronase resulted in the isolation of two sialoglycopeptide fractions which also possessed Con A receptor activity [15]. Since the negative cells surface charge is partly due to sialic acid residues of membrane oligosaccharides, data from this study argues for the presence of Con A and CF receptors on the same molecule.

The small amount of residual CF binding on those portions of the plasma membrane between the ferritin clusters may be accounted for by assuming that some of the components contributing to the overall negative charge on the cell surface are not affected by lectin treatment. Shimizu and Yamada [20] have found that lectin treatment of AH7974F rat ascites hepatoma cells does not alter the pattern of cell surface dialyzed iron staining. Since dialyzed iron interacts chiefly with sialic acid residues on the cell surface [24], the reduced CF binding seen between CF clusters probably represents non-lectin receptor associated sialic acid residues. The residual CF binding may also be due to phosphate and sulphate groups often found associated with the cell surface [25].

The blocking of CF binding by poly-L-lysine has been reported for mouse peritoneal macrophages [12] and guinea pig aortic endothelium [11]. By virtue of its polycationic properties, poly-L-lysine can interact with cell surface anions and prevent their binding with CF. However, repeated attempts to block CF binding on the surface of hepatoma ascites cells with poly-L-lysine resulted in no detectable reduction of ferritin particles around the cell periphery. On the other hand, when cetyl pyridinium chloride is used as a blocking agent, CF binding is greatly reduced [26]. Cetyl pyridinium chloride is a long chain aliphatic ammonium salt that has been used to block ruthenium red staining of heparin granules in mast cells [27]. Although the reasons for the inability of poly-L-lysine to block CF staining of ascites cell surface are uncertain, cetyl pyridinium chloride is presumably effective because it can precipitate saccharide polyanions [28] thus diminishing CF receptor sites.

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