THE EFFECT OF CELL DENSITY ON THE EXPRESSION OF CELL ADHESIVE PROPERTIES IN A CLONED RAT ASTROCYTOMA (C-6)[+]

Roger Santala[‡] and Luis Glaser

Department of Biological Chemistry, Division of Biology and Biomedical Sciences, Washington University, St. Louis, Missouri 63110

Received September 30,1977

SUMMARY: The cell-cell adhesion characteristic of C-6 astrocytoma cells changes as a function of cell density. Cell suspensions prepared from monolayers having a density lower than 1 x 10^5 cells/cm^2 show maximal affinity for plasma membranes and cells obtained from monolayers at densities greater than 1 x 10^6 cells/cm^2 shows minimal affinity for plasma membranes. The adhesive component retained on plasma membranes is present at essentially equal levels in membranes prepared from cells at different density. This modulation in cell surface affinity appears to be due to cell-cell contact and appears to represent a suitable model for the study of the modulation of cell-cell adhesion as a result of cell contact.

In a previous communication (1) we have examined the adhesive properties of plasma membranes prepared from a number of cloned neural cell lines. The adhesive properties of these membranes could be explained if the adhesion was mediated by two pairs of mutually complementary adhesive components Aa and Bb, where cells from each line can express from one to all four of these components, but plasma membranes only express the components designated a and b. Thus a membrane fraction which contains the ligand a will only bind to cells that express the ligand A, while membranes which contain the ligand b will only bind to cells that express the ligand B. Similar conclusions have been reached by Stallcup (2) who has examined cell to cell adhesion under a variety of conditions without the use of membranes. A number of changes in cell surface adhesive components have been detected during embryonal development (3), these changes are presumed to be the result of complex cellular interactions

[+]Supported by grants GM-18405 from NIH and BMS 75-22638 from NSF.

[‡]Supported by training grant GM-07157.

ISSN 0006-291X

during development. Cell heterogeneity in embryonal tissue makes it very difficult to elucidate the chemical basis of these changes in adhesive properties. In this communication we present evidence that the cell surface adhesive components of a rat astrocytoma (C-6) change as a function of cell density. This change may represent a useful simple model for the study of cell surface differentiation during development. Previous work in other laboratories with the same cell line has demonstrated that the rate of synthesis of the S-100 protein increases during confluency (4-6) and that the activity of the catecholamine sensitive adenyl cyclase increases at confluency (7,8).

MATERIALS AND METHODS

C-6 cells were obtained from Dr. S. Pfeiffer and were grown in Dulbeccos modified Eagle's medium (Grand Island Biological Co. (Gibco)) with 25 mM glucose and with 10% fetal calf serum buffered with $NaHCO_3$ (44 mM). A plasma membrane enriched fraction was prepared as described previously (1) from cells grown with the addition of 50 µCi of ^3H leucine to each T-75 flask for 48 hours. Binding of membranes to cells was measured by adding membranes (approximately 25 µg protein) to a suspension of 5 x 10^6 cells in 1 ml of calcium and magnesium free Hanks (Gibco) solution buffered with 0.02 M N-2-hydroxyethylpiperazine-N'-ethane sulfonic acid (Hepes), pH 7.4 and containing 5 mg/ml of lipid free serum albumin (CMF-A). The cells and membranes were incubated in a standard glass scintillation counting vial at 37° in a rotary shaker at 100 rpm. At the times indicated the mixture was gently pipetted into a 5 ml conical centrifuge tube containing 1 ml CMF-A at 0°. The cells and adhering membranes were sedimented at 150 x g for 5 min. The pellet was rinsed with 1 ml of CMF-A without stirring and dissolved in 1 ml of 1% Triton x 100 for counting. The background binding obtained either without incubation or in membrane samples taken through the whole procedure without cells is less than 10% of the total membrane counts. The procedure has been described in detail elsewhere (1).

RESULTS AND DISCUSSION

Fig. 1 shows a growth curve for C-6 cells, growth is essentially logarithmic until the cells reach a density of 2 x 10^6 cells/cm^2. The data in Fig. 2 show that the rate and extent adhesion of plasma membranes to cells is dependent on the cell density of the culture from which the cells were obtained. Fig. 3 summarizes data from five separate experiments similar to those in Fig. 2. Although the absolute values vary from experiment to experiment, it is clear that cell density affects the

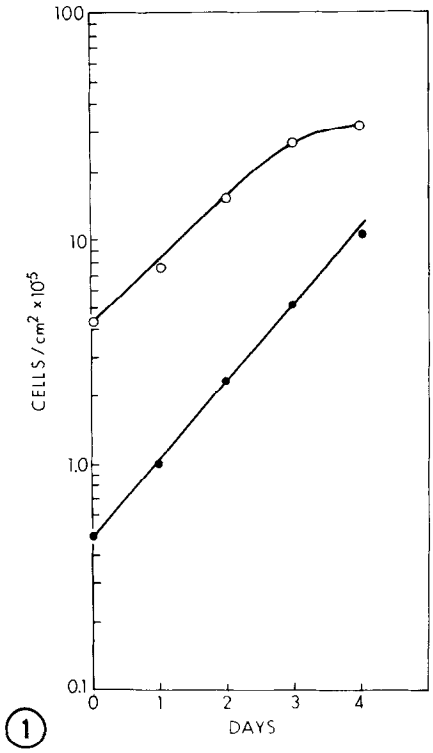


Fig. 1. Growth curve of C-6 cells. The closed and open circles represent two separate cultures innoculated at different initial cell densities. Cell numbers were determined in duplicate 35 mm culture dishes with a Coulter particle counter.

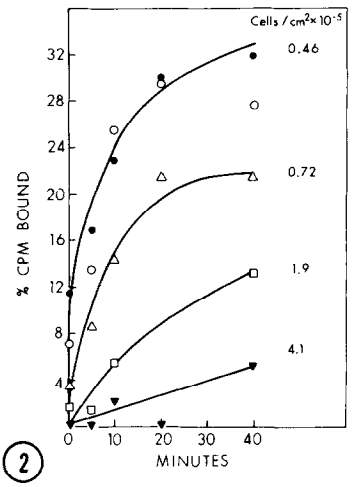


Fig. 2. Binding of plasma membranes to cells. A plasma membrane enriched fraction was prepared from cells grown to a density of 2×10^5 cells cm^2, and the binding measured to cell suspensions obtained from cultures at the cell densities indicated. The open and closed circles represent duplicate experiments.

ability of the cells to bind plasma membranes. Membranes prepared from cells grown to different densities are essentially indistinguishable (Fig. 4). In this experiment membranes were prepared from cells at densities of 0.67×10^5 cells/cm^2, 2.4×10^5 cells/cm^2 and 8×10^5 cells/cm^2. All these membrane preparations bind preferentially to cells grown to low density as compared to cells grown to high density cells, and show intermediate levels of binding to cells at intermediate densities. If high density cells are replated at low density, they regain the ability to bind membranes within 24 hours.

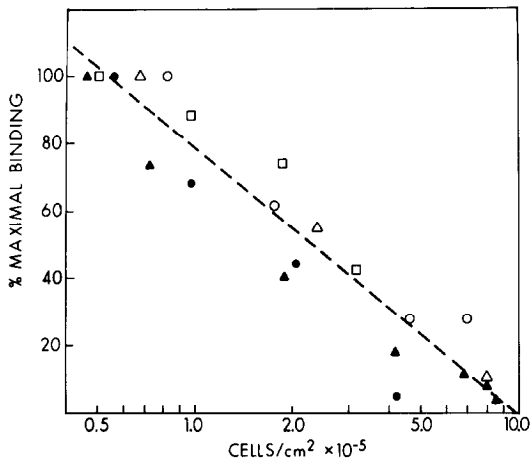


Fig. 3. Effect of cell density on binding characteristics of cells. The data are from five different experiments essentially like those in Fig. 2. 100% binding is taken as the highest binding observed in each experiment. Each symbol represents a different experiment.

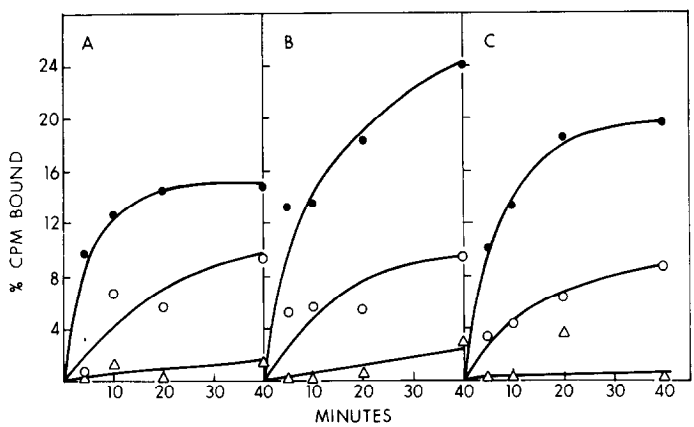
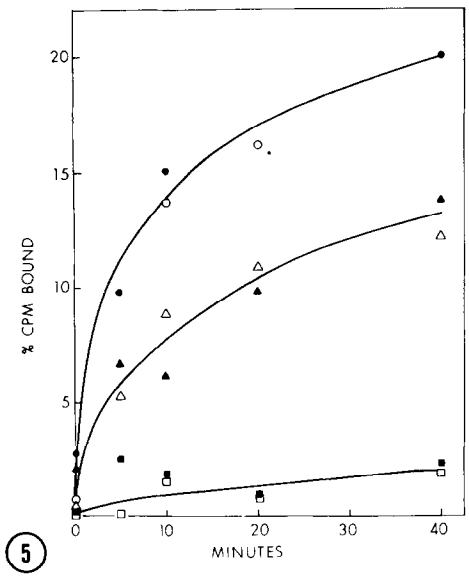


Fig. 4. Effect of cell density on membrane binding characteristics. A plasma membrane enriched fraction was prepared from cells at densities of 0.67 x 10^5 (A), 2.4 x 10^5 (B) and 8 x 10^5 (C) cells/cm^2. The binding of these membranes to cells grown to the same three densities is shown. ● cells at 0.67 x 10^5 cells/cm^2; ○, cells at 2.4 x 10^5 cells/cm^2 and △, cells at 8 x 10^5 cells/cm^2.

There are two possible explanations for the observation that membranes bind at intermediate levels to cells grown to densities between 1 x 10^5/ cm^2 to 1 x 10^6/ cm^2. The first is that the number of sites per cell to which the membranes can bind decreases with increasing cell density.



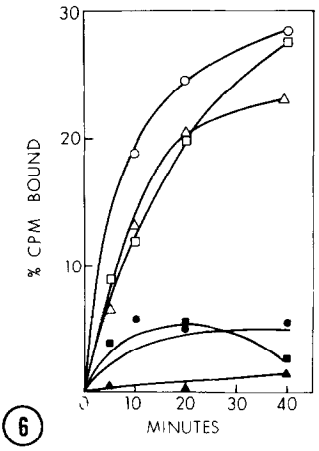


Fig. 5. Effect of membrane concentration on binding. A plasma membrane enriched fraction was prepared from cells grown to a density of 7.5×10^5 cells/cm^2. The binding of these membranes was measured to cells grown to densities of 0.6×10^5 cells/cm^2 (●); 1.0×10^5 cells/cm^2 (▲); and 4×10^5 cells/cm^2 (■). In the experiments with the open symbols the membrane concentration was 1/4 of that used in the standard assay.

Fig. 6. Binding of B65 and B103 plasma membranes to C-6 cells. The binding of plasma membranes prepared from B103 (●), B65 (▲), and C-6 (■) to C-6 cells is shown. Open symbols C-6 cells grown to density of 0.26×10^5 cells/cm^2. Closed symbols C-6 cells grown to density of 3×10^5 cells/cm^2.

The second explanation is that the affinity of the binding sites on the cells decreases with increasing cell density. These two possibilities can be distinguished by carrying out binding experiments at different membrane concentrations. If the number of sites has decreased then lowering the membrane concentration will result in an increased fraction of the membranes being bound. If the affinity of the sites has changed, the percentage of the membranes bound will be the same. This is actually found to be the case as shown in Fig. 5, and is in agreement with the observation made previously with other cell lines that under these assay conditions the cells are present in excess (1).

In previous experiments (1) we have shown that plasma membranes from a neural cell line designated B65 (9) express an adhesive ligand designated as "b" while membranes prepared from a cell line designated B103 (9) cells express the ligands "a" and "b". The data in Fig. 6 show that low density C-6 cells can bind both B65 and B103 membranes and most therefore at a minimum express the complementary ligand B on their surface. This characteristic also disappears from high density cells. Since we have previously shown that C-6 membranes express "b" as an adhesive component, we conclude that the simplest explanation for the observed changes in membrane to cell adhesion is a decrease in the affinity with which B located on the cells can bind "b" located on the membrane. Membrane to cell binding thus is a very sensitive parameter of the functional state of cell surface adhesive components. By contrast, cell to cell adhesion measurements involve not only the initial binding but are rapidly followed by irreversible steps (10). It is therefore not surprising that in cell to cell binding assays (11), low and high density cells behave very similarly since these assays primarily measure the irreversible steps that follow the initial recognition events (F. Moya, personal communication).

Since the change in the cell surface adhesive components occurs at C-6 densities where the cells are still in logarithmic growth, it seems unlikely that the change is due to a limitation in medium growth factors. Daily changes in the medium of high density cells has no effect on the ability of these cells to bind membranes. Growth of low density cells for 48 hours in 1% serum, only resulted in a partial decrease (30 to 40%) of their ability to bind membranes. Growth of the cells for 24 hours in 0.5 mM dibutyryl cyclic AMP and 1 mM theophylline, brought about drastic morphological changes in the cells, but again only partially decreased their ability to bind plasma membranes.

It appears likely that the changes in cell adhesion are the result

of cell-cell contact as has been extensively documented for the synthesis of S-100 protein by these same cells (4-6). The changes in affinity reported in this communication are in many ways comparable to the down shift in hormone receptor concentrations or affinity at high occupancy (12). These changes may be of regulatory importance to the cell especially if one assumes that occupancy of these adhesive sites is a first step in the induction of S-100 synthesis and in changes in the catecholamine sensitive adenyl cyclase. An effect of cell density in cell adhesion in 3T3 cells has been noted by Dorsey and Roth (13).

REFERENCES

1. Santala, R., Gottlieb, D.I., Littman, D. and Glaser, L. (1977) J. Biol. Chem., in press.
2. Stallcup, W.B. (1977) Brain Research 126, 475-486.
3. Glaser, L., Merrell, R., Gottlieb, D.I., Littman, D., Pulliam, M.W. and Bradshaw, R.A. (1976) in Surface Membrane Receptors, edited by R.A. Bradshaw et al., Plenum Press, New York, pp. 109-133.
4. Pfeiffer, S.E., Hirschman, H.R., Lightbody, J. and Sato, G. (1971) J. Cell Physiol. 75, 329-340.
5. Hirschman, H.R., Grauling, B.P. and Lerner, M.P. (1973) in Tissue Culture of the Nervous System, edited by G. Sato, Plenum Press, New York, pp. 187-245.
6. Labourdette, G. and Marks, A. (1975) Eur. J. Biochem. 58, 73-79.
7. Schwartz, J.P., Morris, N.R. and Breckenridge, B.M. (1973) J. Biol. Chem. 218, 2699-2709.
8. Morris, S.A. and Makman, M.H. (1976) Molecular Pharmacology 12, 362-372.
9. Schubert, D., Heinemann, S., Carlisle, W., Tarikas, H., Kimes, B., Patrick, J., Steinbach, J.H., Culp, W. and Brandt, B.L. (1974) Nature 249, 224-227.
10. Umbreit, J. and Roseman, S. (1975) J. Biol. Chem. 250, 9360-9368.
11. Gottlieb, D.I. and Glaser, L. (1975) Biochem. Biophys. Res. Commun. 63, 815-821.
12. Lesniak, M.A. and Roth, J. (1976) J. Biol. Chem. 251, 3720-3729.
13. Dorsey, J.K. and Roth, S. (1973) Devel. Biol. 33, 249-256.