Selective Cell Adhesion of Neuronal Cell Lines*

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Cloned neural cell lines derived from ethylnitrosourea-treated rat embryos (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) adhere preferentially to monolayers of cells obtained by dissociation of neural tissue of either chick or rat embryos. One such cloned line, B103, has been investigated in some detail. B103 cells will bind to cells obtained from any of the major regions of the embryonal nervous system. B103 cells will bind only poorly to chick embryo fibroblasts, Chinese hamster ovary cells, or embryonal liver cells from either the chick or the rat.

A plasma membrane-enriched fraction prepared from B103 cells shows the same relative binding characteristics to embryonal neural and non-neuronal cells as intact B103 cells. Treatment of the membranes with trypsin at low concentrations or treatment of the target cells with low concentrations of glutaraldehyde or formaldehyde also abolishes binding. Binding does not take place at 0°C.

A very similar binding pattern to that of B103 cells and plasma membranes is shown by B50 and B65 cells and plasma membranes in that both of these cell lines bind preferentially to monolayers prepared from cells from embryonal nervous tissue. The plasma membranes from these cells however show significant differences in binding to other cultured neural cell lines. It is suggested that only a part of the cells adhesive components is retained on the plasma membranes in that both of these cell lines bind specifically to neural cells from either chick or rat embryos. Plasma membrane-enriched fractions prepared from these cells retain at least in part these specific adhesive properties. To the best of our knowledge this is the first demonstration of the presence in cloned neural cell lines of "specific" cell-cell adhesion components.

We present evidence in this communication that some of these cell lines adhere preferentially to neural cells from either chick or rat embryos. Plasma membrane-enriched fractions prepared from these cells retain at least in part these specific adhesive properties. To the best of our knowledge this is the first demonstration of the presence in cloned neural cell lines of "specific" cell-cell adhesion components.

Further, examination of the adhesive specificity of several neural cell lines suggests that many of these cell lines have multiple adhesive determinants on their cell surface.

There are many reasons why a given cell type will adhere more rapidly to a monolayer prepared from one cell type as compared to a monolayer prepared from a different cell type. One such reason may be that the pair of cells which gives the fastest adhesion rates has a greater number of complementary sites of a given type than the pair that does not, or the pair of cells that gives the faster rate may have a different type of...
adhesive site than the pair of cells which adhere with a slower rate. Finally one can consider the possibility that difference in the rate of adhesion reflects steric factors on the complementary cell surfaces, and that the actual number and type of adhesive molecules are the same in the slow and fast combination. These and other alternatives are not easily distinguishable. Although differences in rates of adhesion between different cell pairs are usually described in the literature as differences in specificity, we will use the term preferential adhesion to describe this phenomenon. We will show, however, that in the case of the adhesion of plasma membranes to cells it is possible in some favorable circumstances to determine the minimum number of chemically distinct adhesive molecules which are involved in membrane to cell adhesion.

MATERIALS AND METHODS

Cell Culture—Cloned rat neural cell lines, the generous gift of Dr. D. Schubert (16) of the Salk Institute, were grown on Falcon tissue culture flasks in Dulbecco’s modified Eagle’s medium (with 10% fetal calf serum) buffered with 4.2-hydroxyethyl-1-piperazineethanesulfonic acid, pH 7.4, at 37°C by gentle washing with a Pasteur pipette. This technique is less likely to modify the cell surface than either trypsin or EDTA.

Membrane Preparation—The isolated tissue culture cells (approximatively 1 x 10^6) were trypsinized at 37°C for 20 min in CMF-CS with 1 mg/ml of collagenase (Worthington type III). Collagenase treatment suspensions, diluted to 8 ml with CMF-CS at 4°C, were allowed to settle at unit gravity for 3 min, sedimented clumps were discarded and the supernatant fluid was centrifuged at 150 x g for 5 min. The pellet was washed once with 4 ml of CMF-A, and resuspended in 4 ml of CMF-A at 4°C. Cell counts were determined with a hemocytometer.

Cell and Membrane Binding Assays—Cell to cell binding was measured by the binding of dissociated labeled cells to a confluent cell monolayer (23) on derivatized (1.7 cm²) glass vials as described by Gullieb and Glaser (24) and Gottlieb et al. (15). Suspension of cloned neural cells obtained as described above could be substituted without alteration in technique for the dissociated embryonal cells in Gottlieb et al. (15). For most cell lines 1.5 to 2.0 x 10^6 cells were used to form the monolayer; 1.0 x 10^6 probe cells were used per assay which had between 0.5 to 5 x 10^4 cells of each type. For each experiment reported below, all assays were carried out with confluent monolayers at 37°C and 120 rpm except the data in Fig. 5 which were obtained at 90 rpm.

Membrane to cell binding was assayed by adding aliquots (approximately 95 µg of protein with 25,000 dpm of [3H]glucosamine) from a plasma membrane-enriched fraction of cultured neuronal cells to 5 x 10^4 target cells in 1 ml of CMF-A in acid-washed standard glass scintillation vials and incubating the mixture in a New Brunswick rotary shaker G76 at 100 rpm, 37°C, for the times indicated. The reaction mixture was then gently pipetted with Pasteur pipettes into a 5 ml conical centrifuge tube containing 1 ml of CMF-A at 0°C. The cells and adhering plasma membranes were sedimented at 150 x g for 5 min. The pellet, rinsed without stirring with 1 ml of CMF-A, was solubilized in 1 ml of 1% Triton X-100. The radioactivity was determined in a scintillation counter using 3a70 (Research Products International) as a counting fluid.

In all membrane to cell binding experiments, a control without cells was included and the counts sedimented in this control were subtracted from the experimental values obtained in the presence of the cells, such a control remained constant for all incubation times examined.

RESULTS

Binding Characteristics of Cloned Neuronal Cell Lines—As an assay for cell-cell adhesion we have used the binding of radioactive probe cells to a target cell monolayer (23, 24). Using this assay we have previously shown the presence of an adhesive gradient in the chick neural retina (15). The data in Fig. 1 show that B103 probe cells adhere rapidly to monolayers prepared from chick tectal, retinal, or telencephalic cells but show relatively low ability to bind to monolayers prepared from Chinese hamster ovary cells, chick embryo fibroblasts, or embryonic chick liver.

In the experiments in Fig. 1C, we used a monolayer of liver cells prepared from that fraction of chick embryo liver cells which are competent to aggregate. Such liver cell monolayers will bind liver cells but not retina cells. Thus the lower rate of binding by liver cells may indicate that some factors are required for strong binding. We have demonstrated that liver cells contain a factor which can enhance specific binding (28) and that this factor can be removed by a membrane fractionation procedure which results in a decrease in the binding capacity of liver cells (29). We have also observed that liver cells have a surface component which binds to a similar extent to retina and liver cells (30). These results tend to support the idea that some factor required for strong binding may be present in the liver cells. It is difficult to determine whether liver cells need to synthesize a factor to bind to retina cells or whether the factor is present in the medium in sufficient concentration to bind liver cells. The fact that this factor can be removed from liver cells by a membrane fractionation procedure suggests that it may be present in the liver cells and may be associated with the plasma membrane. However, it is also possible that the factor is present in the medium and is bound to the liver cells. This possibility cannot be ruled out.

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1. The abbreviations used are: CMF, calcium- and magnesium-free Hank’s solution (Grand Island Biological Co.) buffered with 0.02 M 4,2-hydroxyethyl-1-piperazineethanesulfonic acid, pH 7.4; CMF-A, the same solution containing 5 mg/ml of lipid-free serum albumin; CMF-CS, the same solution containing 10% chicken serum.

2. D. I. Gottlieb and C. Arington, manuscript in preparation.
of binding of B103 cells to liver monolayers is not simply an indication that the liver cells are nonadhesive cells, but is an indication of binding selectivity. It would appear from the data in Fig. 1 that B103 has one or more adhesive components on its surface which allow these cells to bind selectively to monolayers prepared from cells derived from the embryonal nervous system.

The data in Fig. 1 were obtained in a heterologous system where B103, a rat cell line, is binding to chick neural cells. In Fig. 2, we show that this cell line also binds selectively to a monolayer prepared from cells obtained from the rat cerebral cortex but not to a monolayer prepared from rat liver cells. Organ adhesive specificity across species barriers has previously been shown by Yaffe and Feldman (25), Roth (9), and Garber and Moscona (26). B103 cells bind to neural cells from both chick and rat, but in a number of experiments we have failed to see any indication that this binding shows either spatial or temporal specificity within the nervous system. The possible significance of these observations will be discussed below. We have also examined the binding characteristics of several other cell lines, B50 and B65, which were also isolated by Schubert et al. (16) and are classified as neuronal and C-6, a methyl nitrosourea-induced rat astrocytoma (19).

The binding of the various cell lines is fastest to monolayers prepared from optic tectum, occurs more slowly to cultured neural cell lines, and is slowest to liver. Although the binding to liver shows the most variability, definite binding is observed with all probe cells from cultured cell lines. Fig. 3 shows that cells from lines B65 and B50 also bind preferentially to the chick optic tectum as compared to liver. As shown in Fig. 4, B65 and B103 cells also adhere to monolayers prepared from the other cell lines (similar data not shown have been obtained with B50 cells). The data in Fig. 5 show that B103 cells adhere poorly to a monolayer prepared from C-6 cells as compared to a monolayer of B103 or tectal cells. Under our assay conditions C-6 is a poorly adhesive line, it will bind preferentially to tectum as compared to liver, but

**Fig. 1.** Binding of B103 cells to neural and non-neural monolayers. A, binding of B103 cells to chick tectum and telencephalon. The binding of 5 x 10⁴ B103 cells labeled with [³H]leucine to monolayers prepared from 3 x 10⁶ 9-day-old tectal cells or 9-day-old telencephalic cells was measured. B, binding of B103 cells labeled with [³H]leucine to the indicated monolayers was measured using 4 x 10⁶ 9-day-old tectal cells, 1 x 10⁶ Chinese hamster ovary cells (CHO), and 1 x 10⁶ chick embryo fibroblasts. C, binding of B103 cells labeled with [³H]leucine to the indicated monolayers was measured. In this experiment, liver cells were allowed to aggregate in rotating culture. The aggregates were separated from the nonaggregating single cells and monolayers were prepared from the aggregates after mechanical dispersion of the cells. Monolayers used were prepared with 4 x 10⁶ cells obtained either from 9-day-old chick retina or liver.

will bind to a monolayer prepared from C-6 cells only slightly better than to a liver monolayer (data not shown).

The data presented so far would be compatible with the notion that the adhesion of all the cells tested is mediated by a single set of mutually complementary components present on the surface of these cells in different quantity. Alternatively the differences in the rate of adhesion could represent qualitative differences between the various cells such that certain adhesive components are not represented on all cell types. A distinction between these two interpretations is possible if conditions could be found where adhesion was selectively determined by only one of the several postulated adhesive components. We believe that this condition arises when we measure the adhesions of plasma membranes to cells as described below.

**Binding Characteristics of Plasma Membrane to Cells—** We prepared a plasma membrane-enriched fraction from B103 cells as described under "Materials and Methods." This fraction was enriched in plasma membrane markers and if the whole cells were labeled with ¹²⁵I by the use of lactoperoxidase this fraction was found to be heavily labeled with ¹²⁵I (Fig. 6). For convenience this fraction will be simply designated as
membrane fraction for the rest of this paper.

Freeze fracture electron microscopy of the B103 membrane fraction revealed apparently single compartment vesicles with both normal and inside-out orientation, as judged by the fracture plane that contained intramembranous particles, with an average vesicle size of 0.6 µm (range 0.3 to 1.5 µm).  

The B103 membrane fraction will bind to optic tectum, telencephalon, or neural retina equally well, thus reproducing one of the binding properties of whole cells (Fig. 7). Approximate the same extent of binding was observed if this membrane fraction was obtained from cells labeled with 125I by the lactoperoxidase method and the second from cells labeled with [3H]leucine. Since the membranes are prepared in the presence of serum albumin, the enrichment of membrane markers relative to the content of trichloroacetic acid-precipitable leucine counts is taken as a measure of relative specific activity of the fractions. The figure shows the percentage of recovery on the gradient of [3H]leucine counts, 125I counts, and phosphodiesterase (PDE). A is the top 1 ml of the gradient. Fraction I is the interface between 25 and 40% w/v sucrose layers, Fraction II the interface between 40 and 43% (w/v) sucrose layers, Fraction III the interface between 43 and 48% (w/v) sucrose layers, Fraction IV the interface between 43 and 48% (w/v) sucrose layers, and Fraction V is the pellet. The relative enrichment of trichloroacetic acid-precipitable 125I in Fraction I compared to homogenate is 5.7. The relative enrichment of phosphodiesterase is 11.0 and is maximal in Fraction I. In different experiments not shown, the enrichment of DPNH diaphorase and acid phosphatase in the same fraction was about 3 fold. The relative specific activity for DPNH diaphorase was maximal in Fraction III, and acid phosphatase was maximal in Fractions II and III.

The data in Fig. 9 show the dependence of the extent of membrane binding on cell concentration. In the experiments shown, each assay contained membranes prepared from 5 × 10^6 target cells which would bind approximately 25 µg of membrane protein. If we assume that the membrane fraction is 50% protein by weight and consists of vesicles of average diameter 0.6 µm then between 500 and 1000 vesicles will be bound per cell. This is clearly a very rough estimate of the binding capacity of cells and membranes but indicates that the binding capacity occurs at a ratio of cells to membranes such that each cell must have multiple membrane binding sites on its surface, and that one cell can bind approximately one cell equivalent of membranes. Any losses in membranes

5 We are grateful to Mr. L. A. Andrews for these measurements.

fractions prepared from 3T3 cells or Chinese hamster ovary cells did not bind to any of the neural cells (data not shown).

The data in Fig. 9 show the dependence of the extent of membrane binding on cell concentration. In the experiments shown, each assay contained membranes prepared from 5 × 10^6 cells. Extrapolation of the initial slope from Fig. 9, suggests that these membranes would be bound by 6 × 10^9 target cells which would bind approximately 25 µg of membrane protein. If we assume that the membrane fraction is 50% protein by weight and consists of vesicles of average diameter 0.6 µm then between 500 and 1000 vesicles will be bound per cell. This is clearly a very rough estimate of the binding capacity of cells and membranes but indicates that the binding capacity occurs at a ratio of cells to membranes such that each cell must have multiple membrane binding sites on its surface, and that one cell can bind approximately one cell equivalent of membranes. Any losses in membranes
during preparation will decrease this number. The apparent binding capacity of the cells decreases with increasing cell concentration probably because the cells aggregate with each other at high concentration and become unavailable for binding to the membranes.

In a number of experiments we observe that the binding of B103 membranes to B103 cells is always slightly less than the binding of the same membranes to tectal cells (Fig. 10), although the volume of B103 cells is much greater than the volume of tectal cells. We therefore considered the possibility that different membrane vesicle fractions were binding to each of these cells. We have carried out a number of experiments in which the membrane fraction was first adsorbed with tectal cells or B103 cells and then the binding of residual membranes was assayed with both tectal cells and B103 cells. There was no indication that the residual membranes were enriched in binding capacity for either one or the other of the cell types examined, therefore, the same membrane vesicles appear to bind to B103 cells as to the chick optic tectum cells. In these adsorption experiments, a maximum of 60 to 70% of the membrane vesicle can be adsorbed to cells, the remaining 30 to 40% may represent inverted plasma membrane vesicles, damaged vesicles, or contaminating intracellular membranes.

Alteration of Membrane Binding Characteristics—As a possible guide for the isolation of adhesive components from the membranes, the effects of various changes in the cell or membrane structure on cell adhesion were examined. Specific binding of B103 membranes to tectal cells was abolished at 0° (data not shown), and also by gentle fixation of the cells with formaldehyde or glutaraldehyde (Fig. 11).

Incubation of cells or membranes with trypsin reduced binding of membranes to the cells. Isolated membranes were highly trypsin-sensitive (Fig. 12A). No binding activity was recovered in the supernatant from which trypsinized membranes had been removed using either [3H]lucine- or [3H]glucosamine-labeled membranes. Similarly a 20-fold concentrated supernatant fraction from trypsinized membranes did not inhibit membrane to cell binding.

The results of binding studies after treatment of cells with trypsin were complex (Fig. 12B). The cells treated with trypsin never attained the same level of binding as the control cells which had been exposed to trypsin pretreated with trypsin inhibitor, and the rate of membrane binding to trypsinized cells was considerably slower. Similar results were observed whether the assay was carried out with 5 × 10⁶ cells (cell excess) or 5 × 10⁵ cells (membrane excess) (data not shown). These observations suggest either that there may be two components present on the surface of B103 cells responsible for cell adhesion, only one of which is trypsin sensitive or that a fraction of the total cell adhesive sites is protected from trypsin in intact cells. Plasma membranes prepared from trypsinized cells show reduced ability to bind to target cells (Fig. 13). Since all of the binding capacity of membranes is sensitive to low concentration of trypsin, this would suggest that trypsin-sensitive proteins present in the isolated membrane fraction are at least in part trypsin-resistant in whole cells, and that during membrane preparations their surface exposure has been altered.

Binding Characteristics of Membranes from Different Neuronal Cell Lines—The data in Fig. 14 compare the membrane binding properties of membranes prepared from cell lines B103, B50, and B65. The membranes from each of these cell lines bind preferentially to tectal cells as compared to liver cells, and each of these membrane preparations binds well to homologous neuronal cell line. Membranes prepared from B65 cells do not bind well to B103 cells but membrane

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\text{FIG. 7 (left). Binding of B103 membranes to neural and liver cells. The binding of } ^{3} \text{H}-\text{labeled B103 plasma membranes to } 5 \times 10^6 \text{ cells of the indicated neural origin obtained from 9-day-old chick embryos was measured. In different experiments the binding of B103 membranes to neural cells was consistently between 20 and 40% of the membrane input, while the binding to non-neural cells, such as liver, rarely exceeded 6%. Binding of membranes to cells from 7- and 8-day-old embryos were indistinguishable. The B103 membranes had 8600 cpm and the background in the absence of cells was 1200 cpm, and independent of time of incubation.}
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\text{FIG. 8 (center). Binding of B103 membranes to cells from rat and chick embryos. B103 membrane prepared as described from cells labeled metabolically with } [3H] \text{lucine were used. The cells used in the binding experiment were obtained from: 8-day-old chick embryo tectum, } \Delta; 9 \text{-day-old chick embryo liver, } \Delta; 14 \text{-day-old rat embryo cerebral cortex, } \bullet; \text{ and 14-day-old rat embryo liver, } \circ. \text{ Embryonic rat tissues were dissociated by methods described for the homologous chick embryo tissues. Studies of binding with cells from 15-day-old rat embryos produced similar results. Attempts to use older rat}
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\text{embryos produced a large percentage of damaged cells as determined by trypan blue exclusion. 100% = } 1.5 \times 10^9 \text{ dpm and the background in the absence of cells was } 8 \times 10^8 \text{ dpm.}
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\text{FIG. 9 (right). Dependence of membrane binding on cell concentration. B103 plasma membranes derived from approximately } 5 \times 10^6 \text{ cells were incubated under standard assay conditions with tectal cells at the indicated concentration. At the end of the experiment (30 min), the incubation mixture was added to CMF-A at 0° containing } 5 \times 10^6 \text{ B103 cells as carriers, centrifuged, and counted as described under "Materials and Methods." The carrier cells have no effect on cell binding, but help produce a visible pellet of cells during centrifugation and thereby minimize handling losses. The data represent the average of the binding observed in four separate experiments. The bars represent the observed binding range. The data shown at zero cells is the binding observed with } 2 \times 10^6 \text{ liver cells. At 30 min incubation the binding of membranes to cells had essentially reached a plateau at all cell concentrations. } 100% = 2.4 \times 10^8 \text{ dpm and control with no cells } = 2.2 \times 10^8 \text{ dpm.}
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The cloned neural lines exhibit many neuronal characteristics (16) and these cells have also been shown by Stallcup and Cohn (17) to have neuronal-specific cell antigens on their surface. The binding properties of B103 cells are analogous to the binding properties of dissociated embryonal neural cells in that they show selectivity for neural cells. B103 cells bind preferentially to neural cells from heterologous as well as homologous species but cannot distinguish among different neural cell populations with respect to region or embryonic age.

The selective binding observed in cell to cell adhesion experiments identical with those illustrated in Fig. 14, and similar experiments carried out with membranes prepared from C-6 cells, which adhere preferentially to tectal and B65 cells and very poorly to B103, C-6 cells, or liver.

The membranes prepared from each of these cell lines have distinct binding properties which are significantly different from the binding properties shown by intact cells. The data in Fig. 15 illustrate the results of membrane adhesive experiments with all possible combinations of these four cell lines.

**DISCUSSION**

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**DISCUSSION**

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The selective binding observed in cell to cell adhesion
FIG. 13. Effect of trypsin treatment of cells on membrane-binding activity. Leucine-labeled B103 cells were treated for 10 min at 37°C with 2 mg/ml of trypsin in 5 ml of CMF/T75 flask. Trypsin inhibitor was then added and membranes were prepared by standard method. As control membranes were prepared from duplicate flasks incubated with trypsin-trypsin inhibitor. A, binding of control membranes to cells; O, binding of membranes prepared from trypsin-treated cells. A shows the binding to tectal cells and B to B103 cells. Different membrane preparations are shown in A and B. For control membranes in A, 100% = 8.4 x 10^4 dpm and the control in absence of cells is 1.2 x 10^3 dpm for membranes from trypsinized cells. 100% = 3.5 x 10^4 dpm and the control in absence of cells = 1.4 x 10^3.

In B for normal membranes 100% = 6.3 x 10^4 dpm and control in absence of cells 6.6 x 10^3 dpm. For the membranes from trypsin-treated cells, 100% = 2.3 x 10^4 dpm and the control in absence of cells = 1.8 x 10^3 dpm.

Fig. 14. Binding of B103, B50, and B65 membranes. [3H]Leucine-labeled membranes are prepared from R103, B50, and B65 cells. "M" indicates the membrane source and "C" the cell used in the experiment. The experiments were carried out under standard assay conditions. Thus, for example, M-103, C-65 indicates that the binding of B103 membranes to B65 cells was measured. We show the result of single experiments, but essentially the same data have been obtained in three independent experiments. L = 9-day-old liver cells and T = 8-day-old tectum cells. In various experiments 100% varied between 1.5 to 2.5 x 10^4 dpm and controls in absence of membranes were all in the range of 1.5 to 2.5 x 10^3 dpm.
Cell to cell adhesion and cell to membrane adhesion is measured in two different systems. Cell to cell binding is measured in monolayers, while membrane to cell binding uses cell suspensions. While it would be desirable to make these measurements under identical conditions, we have been unable to measure membrane to cell adhesion in a monolayer assay, and are unaware of any other research that has succeeded in carrying out such an assay. This may reflect the poor frequency of collision between suspended membranes and the cells in the monolayer or our inability to wash the monolayers gently enough so that the membranes remain attached to the cells.

The high sensitivity of the membranes to trypsin strongly suggests that the adhesive components retained in the plasma membrane fraction are proteins. The trypsin sensitivity of whole B103 cells suggests that at least a portion of the cellular adhesive components are also proteins. The inability of B103 membrane vesicles to bind to cells at low temperature, as well as the inability of membrane vesicles to bind to fixed cells could be explained if multiple adhesive components were involved in forming stable membrane to cell adhesion and if such a cluster of adhesive sites were not formed either at low temperature or with fixed cells because of restriction of mobility of the adhesive components in the plane of the membrane (31). Alternatively as suggested by Umbreit and Roseman (28) energy may be required for the formation of stable linkages and this might not be available at low temperature or in fixed cells. The effect of temperature on cell adhesion may vary with different cell lines, thus preliminary experiments suggest that the adhesion of B65 cells or B65 membranes to B65 cells is much less temperature-sensitive than similar experiments with B103 cells and suggest that different adhesive components may differ in their temperature sensitivity.

The data obtained by measuring the binding of B103, B65, B50, and C-6 cells and their plasma membranes to embryonic cells and cultured neuronal cell lines are clearly complex. One possible explanation of these observations is based on a model that assumes that the cell surface contains pairs of adhesive components designated Aa, Bb, etc. With this model we can explain our observations by postulating that only one of the components of each pair remains as the major functional adhesive component in the isolated plasma membrane as illustrated in Table I. Work is in progress using a much more extensive set of neuronal cell lines to expand this scheme. Preliminary data with five additional cell lines from the

| Cells | Plasma membranes |
|-------|------------------|
| B103  | B103, ab         |
|       | B65, b           |
| B50   | B50, ab          |
|       | C-6, b           |

Table 1

Model of adhesive specificities in cells and plasma membranes

The letters Aa and Bb are used to designate pairs of adhesive components. As described in the text, the only the components designated a, b appear to be expressed on the isolated plasma membranes. The table summarizes the binding data shown in Figs. 13 and 14, and indicates for each cell or membrane preparation the hypothetical binding components on its surface required to explain these observations.
Schubert collection (16) could all be explained using the same two sets of complementary molecules designated Aa and Bb as used in Table I. At least one additional adhesive component must be present in the cells to account for the binding of intact cells to liver, or the binding of C-6 cells to C-6 cells. This component also is not detected during the membrane binding assay. It should be clear that if an adhesive component is not expressed in the plasma membrane under our assay conditions, this could be a kinetic phenomenon and the relevant molecules may actually be present in the membrane preparation.

We had previously suggested that one of the complementary adhesive components was not functional in membranes prepared from embryonal cells in order to account for the inhibition by these membranes of cell aggregation (12, 14).

To explain our observation of asymmetry in membrane to cell binding with various cell lines, we have postulated that B103 cells contain one adhesive component "b" but not its complementary structure "B." These observations strongly suggest that specific binding in this system results from the recognition of two dissimilar molecules and not from dimerization of a single molecule as has been suggested in other systems (32). The presence of multiple adhesive components on the surface of the neuronal cell lines, which may also be present on the surface of embryonal cells, is of interest because it suggests the possibility that a large variety of adhesive specificities could be generated with only a few adhesive components. For example, 16 different cell surface specificities can arise if a cell can have on its surface one or more of the components designated as A, a, B, b, in general 2n different cell surfaces can be generated in this way from "n" cell surface components. It should be clear that membranes prepared from B65 cells could contain small quantities of the ligand "a," all that our assays indicate is that this ligand is not present in large enough quantity to allow B65 membranes to bind to B103 cells.

There are major differences between the membrane binding experiments and the cell to cell binding experiments. In cell to cell binding experiments, all cell lines show a significant rate of binding to liver, and the rate of binding to monolayers prepared from the cultured neuronal cell lines is always less than the rate of binding to tectal monolayers. In membrane to cell binding experiments many of the cell lines bind as well or better to suspensions of cultured neuronal cells as to optic tectum, and do not bind well to liver. This discrepancy is most notable with B65 cells, but is also true with B50 cells and in part with B103 cells. These differences serve to emphasize two points, the first is that the binding of cells to liver and to neuronal cells must be due to different adhesive components. The second is that the rate-limiting step in the cell to cell adhesion assay and in the membrane to cell adhesion assay may be different. In addition, it is possible as we have emphasized previously (15), that under the conditions used to measure cell to cell adhesion many of the adhesive sites on the cells in the monolayer have already been occupied by interaction with neighboring cells. This would not be true in a membrane to cell adhesion assay.

In adsorption experiments we have not been able to separate the plasma membrane fraction from B103 cells that bind to tectal cells from that which binds to B103 cells. However, the preferential binding shown by the neuronal cell lines and membranes to cells obtained from the embryonal nervous system may be determined by different adhesive molecules than those involved in the binding of the same cell and membranes to neuronal cell lines since in the absorption experiments these sets of adhesive specificities would only be separated in the unlikely event that they segregated into different populations of membrane vesicles.

The apparent loss of surface adhesive components from the plasma membrane fractions indicates that the binding of membranes to cells can only be used to prove the presence of certain adhesive components on the surface of the cell from which the membranes were prepared, but cannot be used to prove the absence of an adhesive component in the surface of the original cell.

The observations presented in this paper should be useful as a guide to the isolation and characterization of cell surface adhesive components. Our demonstration of the selective adhesion of neural cell lines to the cells derived from the nervous tissue and our evidence for multiple adhesive components on a single cell surface have potential implications for the way in which tumor cells grow in the animal as well as for developmental organization.

Since the original submission of this manuscript, two papers have appeared which are relevant to our work. Stallcup has independently reached the conclusion that adhesion between different neural cell lines reflects the presence of multiple pairs of adhesive components upon the cell surface of these cells. This conclusion is based on the differential effects of trypsin, temperature, and antibodies on the binding of B50 cells to various cell monolayers (33). Obrink et al. (34) have shown that plasma membranes prepared from chick and rat liver cells retain the adhesive specificities of the parental cells, thus demonstrating that membranes will also be useful for the study of cell adhesive specificity in non-neural systems.

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