Antisera Inhibiting Mammalian Cell Spreading and Possible Cell Surface Antigens Involved

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ABSTRACT Antiserum against a rat neuronal tumor cell line (B103) has been prepared in rabbit by intravenous injection of live cells. This immune serum (anti-B103) precipitates a few cell surface proteins recognizable by two-dimensional gel electrophoresis as common radioiodinatable spots in 15 different rat neural cell lines and in mouse and rat fibroblast cell lines. The apparent molecular weight of one major common protein (II_4) is estimated by SDS gel electrophoresis to be somewhere between 80,000 and 90,000 and another protein (I_3) to be 120,000. These two proteins are consistently recognized in various cell lines by this antiserum. Furthermore, at a 1:20 dilution, this serum causes monolayer cells to round up usually within 0.5 h and detach from the plate within 3 h. It also inhibits spreading of freshly plated cells. These effects of the antiserum are reversible. Upon absorption of the antiserum with cells (e.g., absorbed with a glial cell line, B27), the serum no longer causes the rounding up of the monolayer cells, it does not inhibit cell spreading, and it does not immune-precipitate the two common proteins from the cell surface of various cell lines. Antisera against several other rat cell lines also precipitate the same common proteins (II_4 and I_3) from the cell surface and prevent cell spreading. These data suggest that the antibody acts first at the cell surface and then inhibits cell spreading or rounding up of spread cells. The consistent pattern of the immunoprecipitated cell surface proteins on the two-dimensional gel electrophoresis makes these two common surface proteins (II_4 or I_3 or both) possible candidates for target proteins to which the antibody binds. Thus, they may play a critical role in cell spreading.

Cell surface components have long been implicated in cellular interactions and differentiation. Involvement of specific plasma membrane components in cell-cell recognition, adhesion, embryonic development, and growth regulation has been recognized in a number of vertebrate systems (3, 7, 28, 32). Cell surface components are also involved in basic cellular functions, e.g., ion transport by Na^+/K^+ ATPase (22), glucose transport, and regulation of glycosylation (31). In addition, cell surface components may play an important role in basic in vitro processes of cellular morphogenesis, such as cell spreading.

Cell spreading has been studied in relation to other morphological and cellular changes. When freshly trypsinized fibroblasts are placed on a coverslip or petri dish, the cells attach and change from a spherical state to a radially spread state. Microvilli and filopodia on the spherical cell surface diminish as the cell spreads out (9, 39), and microfilament bundles and an actin network have been shown to reform rapidly, shortly after the cell starts to spread (12, 24). In the formation of myotubes, unless the myoblasts spread they never fuse into myotubes (4). Spreading may also be essential for growth, division, and migration of most cells in culture; for example, lymphocytes, which divide only in response to mitogens, seldom spread in culture. This leads to the question of why, in a suitable substratum, some cells spread while others do not. A possible answer is that the spreading cells may possess membrane proteins that are missing in those that do not spread.

During the course of our search for neural-specific surface antigens in a series of rat neural cell lines, we identified two cell surface antigens shared by neural fibroblastic cell lines. Antiserum containing activity against these two antigens inhibits cell spreading and causes monolayer cells to round up. In this report, we describe a correlation between the presence of these two common surface antigens and the cell-spreading

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phenomenon.

Independent of our observation, Wylie et al. (40) recently reported that an antiserum prepared against plasma membrane of baby hamster kidney (BHK) cells causes hamster cells to round up and detach. This antiserum precipitates glycoproteins of 50,000; 120,000; and 140,000 mol wt from the NP40 lysate of BHK cells, and it is hamster cell-specific in cell-rounding and detachment effects. This suggests that it recognizes a group of glycoproteins, different from II_4 and I_3, which are also involved in the spreading of cells.

MATERIALS AND METHODS

Cell Culture

Rat neuronal tumor cell lines B103, B27, B12, B35, B50, and B65 (34) were derived from neural tumors induced by ethylnitrosourea (ENU) and were a generous gift from Dr. D. Schubert. Cell lines established from the RT4 tumor (18) and a rat mammary tumor cell line 64-24 (19) were maintained in our laboratory. 3T3 cells were obtained from Dr. A. Levine. NRK is a rat fibroblast cell line derived from kidney explant (6) and was obtained from Dr. K. R. Porter. L6 and L8 are rat myogenic lines originally isolated by Yaffe (41): L6 was a gift from Dr. Schubert, and L8 was a gift from Dr. L. B. Chen. IMR-90 and Chinese hamster ovary (CHO) cell lines were obtained from Dr. D. Prescott, and mouse macrophage-like cell line PU5 from Dr. H. Grey. NB41A was obtained from Dr. J. Kates, and rat HTC and mouse L cells were obtained from Dr. T. Kano-Sueoka. All cell lines used for this study were cultured in Dulbecco's modified Eagle's (DME) medium supplemented with 10% fetal calf serum. Cells were subcultured by trypsinization.

Preparation of Antiserum

B103 cells (1×10^7) were washed with saline and harvested with a rubber policeman. Cell pellets were suspended in 0.7 ml saline and injected into the ear marginal vein of an NZB rabbit. The rabbit was immunized on days 0, 7, 14, and 42, and bled on day 49. The serum was then heat-inactivated at 56°C for 30 min and stored at −20°C. For absorption, saline-washed B27 or NRK cells were harvested and pelleted. Heat-inactivated serum was diluted in saline (1:1) and absorbed with packed B27 or NRK cells (2:1, vol/vol) at 4°C for 30 min. Absorption was repeated four times, and after the last absorption cell debris was removed by centrifugation in a Beckman microfuge (Beckman Instruments Inc., Spinco Div., Palo Alto, Calif.). Rabbit antisera against other cell lines were prepared likewise by intravenous injection of live cells. The anti-B103 absorbed with rat liver (Tables II and IV) was prepared as follows: Liver from adult BDIX rats was homogenated gently with a tissue grinder. The particulate fractions were washed extensively until the supernate became clear. The anti-B103 serum was then absorbed three times with equal volumes of packed liver particulates at 4°C for 30 min. Anti-S100 protein was prepared by Dr. S. Silberstein using bovine S100, which was prepared according to H. R. Herschman et al. (University of California, Los Angeles, Calif., personal communication). Anti-β_2 serum was a gift from Dr. R. Kubo and was prepared against pure human β_2 microglobulin. Anti-rat brain serum was prepared by injecting finely minced young adult cerebral cortex with 0.2 ml saline (mixed with 0.3 ml of complete adjuvant) into a rabbit hind leg. The immunization and bleeding schedules were the same as those described for anti-B103. Anti-adult mouse thymus serum prepared by a similar procedure was a gift of Dr. R. Kubo.

Surface Labeling of Cells

Saline-washed cells (1×10^7) were harvested with a rubber policeman and surface labeled by lactoperoxidase-catalyzed iodination, according to Vitetta et al. (37), with a slight modification. Cells were suspended in 0.5 ml of phosphate-buffered saline (PBS) containing 30 μg of lactoperoxidase (Sigma Chemical Co., St. Louis, Mo.) and 0.5 mCi of carrier-free ^{125}I (~17 Ci/mg) (Amersham Corp., Arlington Heights, Ill.). The reaction mixture was initiated by the addition of 25 μl of 0.03% H_2O_2 at room temperature. Two additional 25-μl portions of H_2O_2 were added for the next two 3-min intervals. After 10 min, the reaction was terminated by adding 0.1 M KI in PBS. The cells were washed four times in PBS and lysed in 0.5% NP40 containing 250 U/ml of Trasylol (FBA Pharmaceuticals, New York). Particulate material was removed by centrifugation. Approximately 8% of the total radioactivity of the iodinated cells was found in the pellet. The TCA-precipitable radioactivity of the cell lysate accounted for 10–40% of the total radioactivity of the labeled cells.

Immunoprecipitation

Immunoprecipitation was carried out using the *Staphylococcus* system described by Kessler (21). 100-ml aliquots of NP40 lysate (~1×10^6 TCA-precipitable counts) were incubated with 10 μl of antiserum at 4°C for 2 h. Heat-inactivated *Staphylococcus* (Staph A) (200 μl of a 10%, vol/vol, solution) was then added and incubated for 15 min at 4°C. The adsorbent was washed four times and the antibody-antigen complex was extracted by 100 μl of SDS sample buffer (23). The antibody-antigen complex was removed from Staph A by centrifugation, and the radioactivity released was analyzed by a gamma counter. Immunoprecipitation using a second antibody (goat antirabbit IgG) gave a result similar to that of Staph A.

Two-dimensional Gel Electrophoresis

Immunoprecipitates were dissolved in the sample buffer of Laemmli (23), which contains mercaptoethanol, and analyzed by the double detergent, two-dimensional gel electrophoresis previously described (17). To accommodate a large sample volume (200 μl) and high protein concentrations (500 μg), first-dimension gels (SDS gel electrophoresis) were prepared in 19-cm glass tubes with an internal diameter of 4 mm. The separating gel is 12 cm long and the stacking gel, 4 cm long. The first-dimensional gel was run at 0.1 mA/gel for 12 h, and the current was boosted to 1 mA/gel until the dye front reached the bottom of the gel. Second-dimension gels are 1/16 inch thick. Electrophoresis was run at 2 mA for 10 h and then at 6 mA until the dye front completely ran off from the bottom of the gel. The gels removed from the glass plates were equilibrated in 35% ethanol and 10% acetic acid for 10 h and dried under vacuum on blotting paper. The dried gel was then exposed to x-ray film (Kodak X-mat XR-1) with an intensifying screen (Cronex, Lightning plus, DuPont Instruments, Wilmington, Del.) at −20°C.

Effect of Antiserum on Cultured Cells

To test the cell-rounding effect of anti-B103 and other immune and nonimmune sera, subconfluent (1 to 2×10^4 cells/cm^2) or confluent (5×10^4 cells/cm^2) cells were cultured for 48 h on 12 × 12 mm ethanol-sterilized coverslips in 35-mm culture dishes. The coverslips were washed twice with saline and then were transferred to multiwell tissue culture plates with a well diameter of 16 mm (Falcon Labware, Div. Becton, Dickinson & Co., Oxnard, Calif.). After this, 0.2 ml of complete medium which had been heat-inactivated at 56°C for 30 min (10% fetal calf serum, DME) was added to each well in addition to 10 μl of test serum. The cell morphology was examined every 20 min under an inverted microscope. In some cases, the coverslips were removed after a 3-h incubation with immune serum and returned to a well with fresh 10% fetal calf serum-DME medium. To test the antispreading effect of anti-B103 serum, confluent cells were subcultured by trypsinization, and 5×10^5 cells were plated on coverslips on 35-mm plates containing 0.5 ml of 10% fetal calf serum-DME medium plus 25 μl of test serum. Cell spreading was checked every 10 min after plating. For examination of the cell's ability to spread, some of the coverslips were removed and returned to wells with fresh medium without the antiserum.

RESULTS

Cell Surface Proteins Immune Precipitated by Anti-B103 Serum

Lactoperoxidase-catalyzed iodination (37) of B103 cell surfaces labels proteins of apparent mol wt of 70,000; 80,000–90,000; 110,000–120,000; and 150,000 (Fig. 1 *a*). Lane 1 in Fig. 1 *a* shows the separation of whole NP40 lysate, while lane 3 shows the separated surface proteins immunoprecipitated by anti-B103 serum from the NP40 lysate of radioiodinated B103 cells. It is apparent that the antiserum extracts major iodinatable proteins and that the immunoprecipitation is specific, in contrast to normal rabbit serum (lane *2*). Fig. 1 *b* shows the separation of anti-B103-immunoprecipitated B103 cell surface proteins by the double-detergent, two-dimensional gel electrophoresis system (17). The profile shown in Fig. 1 *b* is similar to that of cells first treated with 0.025% trypsin (Grand Island Biological Co., Grand Island, N. Y.) for 5 min at 37°C before labeling. Thus, these major iodinatable proteins are insensitive

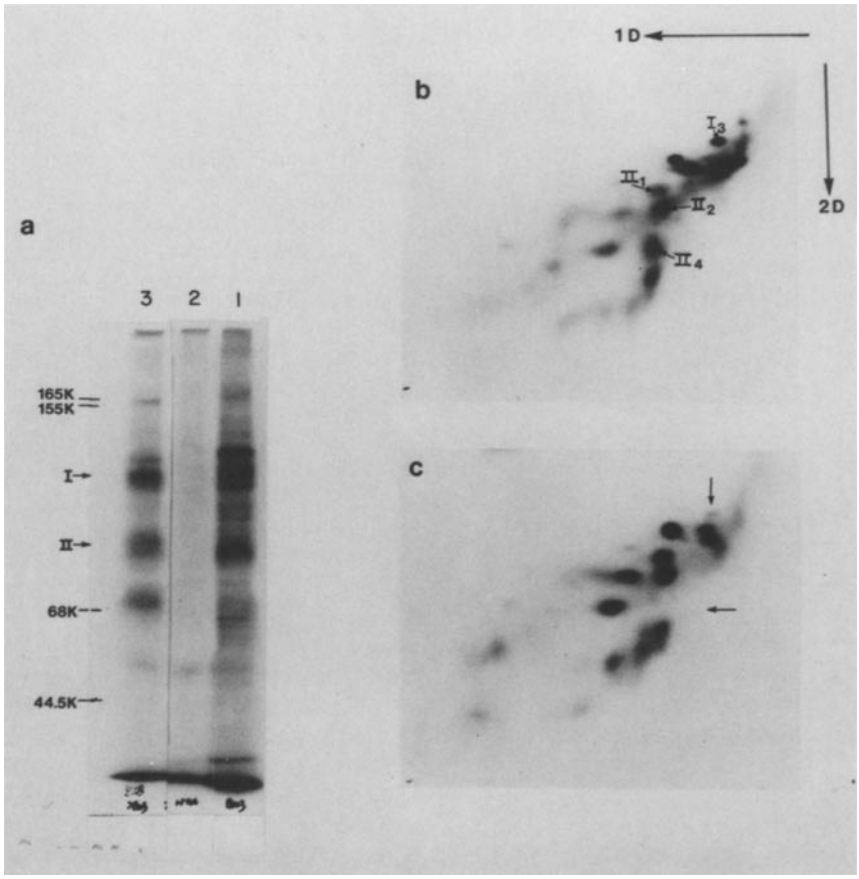

FIGURE 1 Single-dimension and two-dimension separation of B103 cell surface proteins. In Fig. 1 *a*, NP40 lysate of iodinated B103 cells was separated in a 7.5% polyacrylamide SDS gel (lane 1). Surface proteins extracted by anti-B103 serum (lane 3) and normal rabbit serum (lane 2) were analyzed on the same gel. The protein standards used for molecular weight calibration are ovine serum albumin, 44,500; bovine serum albumin (BSA), 68,000; and RNA polymerase, 155,000 and 165,000. *b* and *c* show the separation of surface proteins by a two-dimensional gel. The first dimension is 7.5% polyacrylamide gel. The direction of electrophoretic migration is shown by arrows and *1D* and *2D* for first- and second-dimension, respectively. Fig. 1 *b* shows surface proteins immunoprecipitated by anti-B103 serum from B103 cells. Fig. 1 *c* shows B103 cell surface proteins extracted by anti-B103 serum absorbed with B27 cells. Samples with ~40,000 and 20,000 cpm were analyzed in Fig. 1 *b* and *c*, respectively. Both gels were exposed to x-ray film with intensifying screen for 2 and 4 wk, respectively.

to this level of trypsin treatment, in spite of the fact that this trypsin treatment is sufficient to release B103 cells from the petri dish. All of these surface proteins can be immunoprecipitated by the antiserum from cells labeled with radioactive iodine either on tissue culture plates or labeled in suspension after harvest by scraping from the plate.

Two Major Surface Antigens of B103 Cells Shared by Other Mammalian Cell Lines

There are at least four proteins (II_1, II_2, II_3, and II_4) with apparent mol wt in the range of 80,000–90,000 that can be separated by electrophoresis in the second dimension (Fig. 1 *b*). Protein II_4 and the protein designated as I_3 (120,000 mol wt) were first found by precipitation with anti-B103 serum in all the ENU-induced neural tumor cell lines listed in Table I. Eight of the cell lines examined were derived from the rat central nervous system (B series; 34), and nine cell lines were from a tumor of the rat peripheral nervous system (RT4 family; 18). Proteins II_4 and I_3 are also immunoprecipitated anti-B103 serum from the surface of the other cell lines listed in Table I.

Fig. 1 *c* shows the B103 cell surface proteins extracted by anti-B103 serum that had been adsorbed with B27 cells (a rat glial cell line). Proteins II_4 and I_3 are missing in the gel profile, which indicates that there are II_4- and I_3-cross-reacting antigens on B27 cells. Thus, absorption of anti-B103 serum with B27 cells removed the antibody activities against II_4 and I_3. Table II shows a typical experiment in which the surface-labeled B103 proteins were immunoprecipitated with absorbed and unabsorbed anti-B103 sera. Proteins II_4 and I_3 can also be labeled with [^{35}S]methionine and [^{14}C]glucosamine when B103 cells are cultured in the media containing either of these radioactive compounds (data not shown). These results make it unlikely that they are serum derived or contaminants of lactoperoxidase.

A rat fibroblastic cell line, NRK, also possesses proteins II_4 and I_3 on its cell surface; the proteins can be precipitated by anti-B103 serum (data not shown). An antiserum raised against NRK cells can extract II_4 and I_3 from B103 cells (Fig. 2 *a*) but not the other seemingly neural-specific proteins, II_1, II_2, and II_3. The presence of II_4 and I_3 on both NRK and B103 cells is further demonstrated by sequential immunoprecipitation of B103 cell surface proteins first with anti-NRK serum and then with anti-B103 serum. When B103 cell surface proteins are first precipitated with anti-NRK serum and the supernate and then precipitated with anti-B103 serum, II_4 and I_3 are not present in

TABLE I

Cell Lines That Shared the II_4 (90,000) and I_3 (120,000) Proteins As Determined by Two-dimensional Gel Electrophoresis

Cell lines	Source	Reference
Neural cell lines (derived from ethylnitrosourea-induced neurotumors)		
Neuronal		
B11, B35, B50, B65, B103	Rat	Schubert et al. (34)
RT4-32-B1, RT4-32-B2, RT4-B6, RT4-B7, RT4-B8, RT4-73-E1, RT4-E3	Rat	Imada and Sueoka (18)
Glial		
B12, B27, B82	Rat	Schubert et al. (34)
RT4-52-D1, RT4-71-D2	Rat	Imada and Sueoka (18)
Fibroblastic cell lines		
NRK	Rat	Duc-Nguyen et al. (6)
3T3	Mouse	Todaro and Green (36)
IMR90	Human	Nichols et al. (29)
CHO	Chinese hamster	Kao and Puck (20)
Muscle cell lines		
L6	Rat	Yaffe (41)
L8	Rat	Yaffe (41)
Others		
HTC	Rat	Gelehrter and Tomkins (11)
L	Mouse	Sanford et al. (33)
PU5	Mouse	H. Grey (unpublished observations)
B103 tumor cells	Rat	Schubert et al. (34).

TABLE II

Precipitation of ^{125}I-labeled B103 Cell Surface Proteins with Unabsorbed and Absorbed Anti-B103 Sera

Serum*	Input counts‡	Counts precipitated	Counts precipitated§
	cpm	*cpm*	*%*
Anti-B103	2.10×10^6	49,490	12.7
Anti-B103 absorbed with liver	2.08×10^6	45,300	11.7
Anti-B103 absorbed with B27	2.15×10^6	17,800	4.4
Anti-B103 absorbed with B103	2.14×10^6	8,850	2.2
Normal rabbit serum	1.55×10^6	6,110	2.1

* Immunoprecipitation and absorption of antisera were performed as described in Materials and Methods. 10 μl of each serum were used.

‡ The input counts indicated here are gross counts of the ^{125}I-labeled B103 cell lysate. The percentage of TCA-precipitable counts in this experiment is 18.6%.

§ (Counts precipitated by the antiserum)/(TCA-precipitable input counts of cell lysate) × 100%.

the second immunoprecipitate (Fig. 2*b*). As expected, II_4 and I_3 are present in the first precipitate (Fig. 2*a*). After the anti-B103 serum has been absorbed with NRK cells, the II_4 and I_3 proteins can no longer be precipitated from B103 cell surface using this absorbed serum (Fig. 2*c*). Thus, these two proteins are common to fibroblasts as well as to several neural cell lines. In similar experiments with anti-B103 antiserum, we found that proteins II_4 and I_3 are also present in mouse 3T3 cells, rat myoblast lines L6 and L8, human fetal lung IMR90 cells, and the primary cells of tumors derived from B103 cells (Table I). That these two proteins are common to the cell surfaces of all these cell lines suggests that they play a role in mediating a basic cellular function.

Effect of Anti-B103 Serum on Cell Spreading and on Monolayer Cells

When spindlelike B103 and flat RT4 cells (RT4-73-E1 and RT4-E3) are subcultured by trypsinization and replating, they rapidly attach to the plate (in ~30 min) and begin to spread. However, when these cells are plated in the presence of B103 antiserum (1:20 to 1:40 dilution), they attach but do not spread. The RT4-73-E1 cells are still round 2.5 h after plating in the presence of anti-B103 serum. Anti-B103 serum absorbed with B27 cells, however, does not have antispreading activity on RT4-E cells. Fig. 3 shows the phase-microscope images of spindlelike RT4-52-D1 cells 2 h after being plated in the presence of anti-B103 serum (Fig. 3*a*), anti-B103 serum absorbed with B27 cells (Fig. 3*b*), and without any immune serum (Fig. 3*c*). Only unabsorbed anti-B103 serum prevents spreading of B103, RT4-73-E1, RT4-E3, RT4-52-D1, NRK, 3T3, HTC, L, 64-24, and IMR90 cells (Table III).

When anti-B103 serum is added to monolayer cells (B103, RT4-52-D1, RT4-73-E1, RT4-32-B1, NRK, and 3T3) at 1:20 dilution, the cells round up and eventually detach from the petri dish within 3 h. At 1:10 dilution, the effect is accelerated and cells detach within 30 min. Fig. 4*b*, *d*, and *f* show the rounding up of 3T3 cells in the presence of anti-B103 serum. Both anti-B103 serum absorbed with B27 cells (Fig. 4*c* and *e*) and normal rabbit serum have no effect. The anti-B103-treated 3T3 cells regain their spread-out state 6 h after the anti-B103 serum has been withdrawn and the culture has been replenished with fresh regular medium (Fig. 4*h*).

To see whether the inhibition of cell spreading and cell rounding-up are limited to anti-B103 serum, we tested antisera against other cell lines and found that they also show similar effects (Table IV). However, antisera against cell surface antigens other than a few common surface proteins do not produce these effects. Even though anti-B103 serum absorbed with B27 cells still contains apparent antibodies against numerous B103 cell surface antigens (Fig. 1*c*), it does not have the antispreading activity. Although anti-S100 protein serum and anti-large external transformation-sensitive (LETS) serum clearly stain RT4-E1 cells (14), these two antisera have no effect on the rounding up of RT4-73-E1 cells. We have also found β_2-microglobulin (associated with HLA) to be present on human IMR90 cells (immunofluorescent staining with anti-β_2 serum); nevertheless, anti-β_2 serum has no effect on the rounding up of monolayer IMR90 cells or in the inhibition of their spreading. Anti-B103 serum, on the other hand, inhibits IMR90 cells from spreading. These results indicate that there are specific surface proteins critically involved in cell spreading, and that anti-

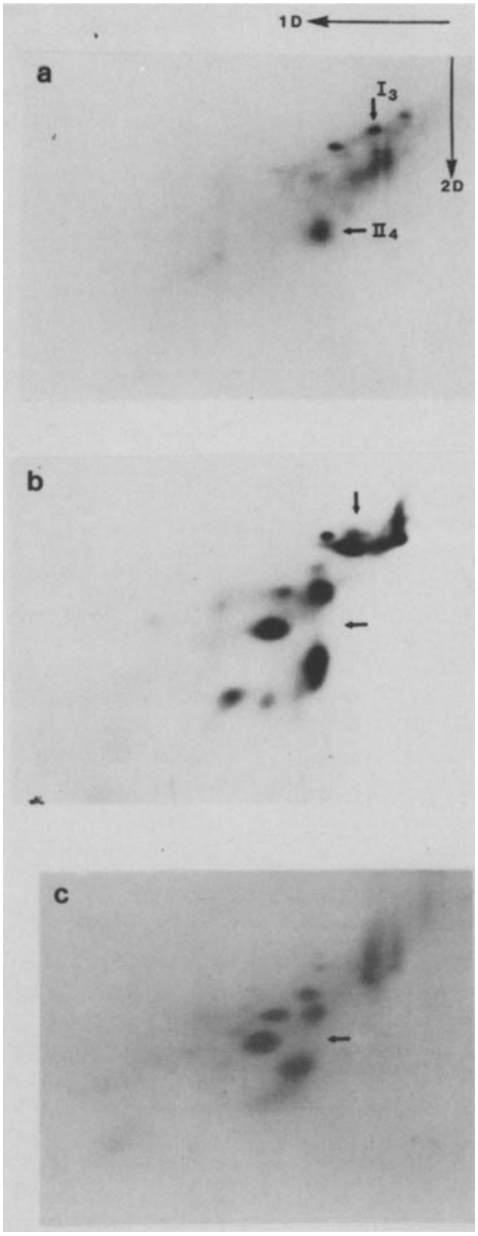

FIGURE 2 Two-dimensional gel electrophoresis of B103 cell surface proteins. (*a*) A lysate of radioiodinated B103 cells was immunoprecipitated by anti-NRK serum by using Staph A precipitating method, and the antigen-antibody complex was subjected to electrophoresis. (*b*) The supernate of *a* was then immunoprecipitated likewise with anti-B103 serum, and the complex was analyzed. (*c*) A lysate of radioiodinated B103 cells was immunoprecipitated by anti-B103 serum absorbed with NRK cells, and the complex was analyzed.

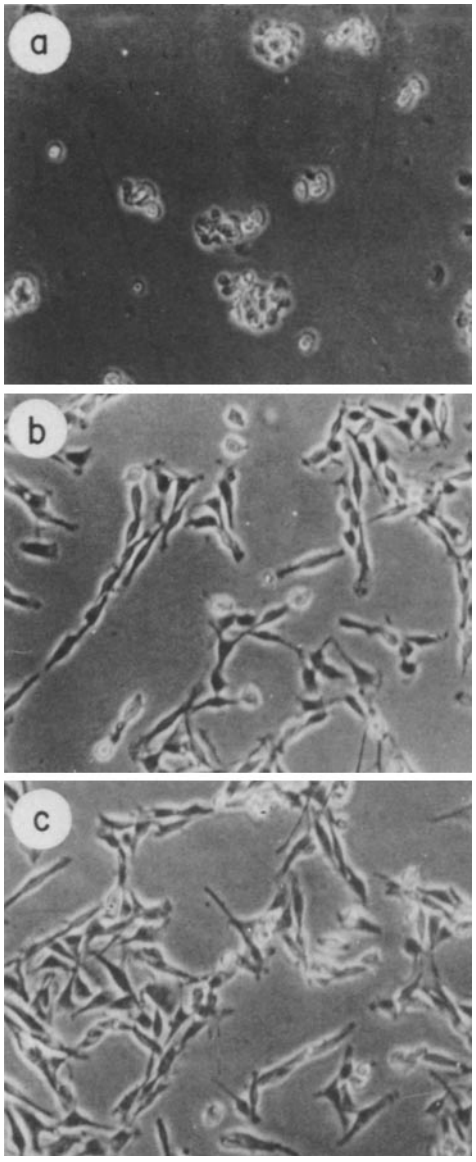

FIGURE 3 Effect of anti-B103 serum on RT4-52-D1 cells. Trypsinized RT4-D1 cells were plated in the presence of anti-B103 serum (1:20) (*a*), in anti-B103 serum absorbed with B27 (1:20) (*b*), and no immune serum (*c*). Pictures were taken 2 h after plating.

B103 serum contains antibody molecules against them. Sera that do not inhibit cell spreading and do not round up cells include normal rabbit serum, rabbit anti-mouse thymus, and rabbit anti-rat brain sera.

Other Anti-Cell Sera

Antiserum raised in rabbit against B103 cells by intramuscular injection in complete Freund adjuvant also has antibody activity against II_4 and I_3 and exhibits anti-cell spreading activity. Rabbit antisera against other cell lines have been prepared by intravenous injection of live cells; these include anti-NRK fibroblast, anti-RT4-51-AC14, anti-RT4-52-D1, and anti-RT4-B6 (Table IV). Each of these antisera immunoprecipitates the common proteins (II_4 and I_3) from the cells listed in Table I and can prevent cell spreading.

DISCUSSION

Anti-B103 serum inhibits freshly plated cells from spreading and causes monolayer cells to round up, whereas anti-B103 serum absorbed with B27 or NRK cells has neither of these effects. When B103 cell surface proteins precipitated by the absorbed antisera are analyzed by two-dimensional gel electrophoresis, two distinct proteins (II_4 and I_3) are absent from the gel profile (Fig. 1*c*). This indicates that the absorbed serum lacks antibodies against II_4 and I_3. Thus, it is likely that proteins II_4 and I_3 are responsible for cell-spreading activity.

The antispreading effect of the antiserum is obviously not cell-line specific (Table III). Freshly plated cells from the 10

TABLE III

Summary of Anti-B103 Activity on Different Cell Lines

	Antispreading	Rounding up cells
Neural lines		
B103	+	+
B12	+	+
RT4-52-D1	+	+
RT4-73-E1	+	+
RT4-E3	+	NT
RT4-32-B1	NT	+
Fibroblastic lines		
NRK	+	+
3T3	+	+
IMR90	+	+
L	+	NT
Others		
64-24	+	NT
HTC	+	+
CEF	−	−

NT, not tested.

This is a composite result of several experiments with anti-B103 antiserum. For detailed method, see text. For columns 2 and 3, serum is at 1:20 dilution. For antispreading, + indicates that freshly trypsinized cells were inhibited from spreading up to 24 h, as in Fig. 5d. For rounding up cells, + indicates that >80% of the cells round up after a 3-h incubation with the serum. − Indicates that there is no apparent effect on cell morphology in 30 min to 1 h with the presence of the serum in 1:20 dilution.

TABLE IV

Effect of Different Antisera on Tissue Culture Cell Spreading *

Antiserum	Antispreading
Anti-B103	+
Anti-RT4-52-D1	+
Anti-RT4-B6	+
Anti-RT4-51-AC14	+
Anti-NRK	+
Anti-B103 absorbed with liver homogenate‡	+
Anti-B103 absorbed with NB41A	+
Anti-B103 absorbed with B27	−
Anti-B103 absorbed with NRK	−
Anti-RT4-52-D1 absorbed with B27	−
Anti-RT4-52-D1 absorbed with NRK	−
Anti-β_2 serum§	−
Anti-rat brain serum‖	−
Anti-mouse thymus serum‖	−
Normal rabbit serum	−
Anti-LETS serum¶	−
Anti-S100 serum‖	−
Con A**	+

+ Indicates that the serum at 1:20 dilution inhibits freshly plated cells from spreading. More than 90% of the cells do not spread out in a 24-h test period. − Indicates that practically all cells spread out in 30 min in the presence of the serum at 1:20 dilution listed in the left column.

* These antisera have been tested individually on the following cell lines: B103, RT4-52-D1, NRK, 3T3, and HTC.

‡ Rabbit anti-B103 serum was absorbed with the particulate fraction of adult liver homogenate.

§ Tested on human IMR90 cells only.

‖ See Experimental Procedures.

¶ A gift of L. B. Chen (4).

** Trypsinized cells were plated in the medium containing 50 μg/ml of concanavalin A.

different cell lines we have examined have responded to the antispreading activity of anti-B103 serum. In nine of these cell lines, proteins II_4 and I_3 have been detected, with rat mammary tumor cell line 64-24 being the exception. Even though anti-B103 serum inhibits the spreading of 64-24 cells and is absorbed on their cell surface, neither protein II_4 nor I_3 has been detected on two-dimensional gels. We have also observed that iodination of 64-24 cell surface proteins is generally inefficient. It may be that in the 64-24 cell line, these proteins are not so accessible to lactoperoxidase iodination or that they are in such a small quantity that they cannot be detected by our present technique. We have also found that NB41A cells, a subline of neuroblastoma C1300 (2), lacks II_4 but not I_3. NB41A cells usually do not spread out extensively in vitro. Absorption of anti-B103 serum with NB41A cells does not remove the antispreading activity or the anti-II_4 activity, but it does remove anti-I_3 activity. These data may suggest that it is only protein II_4 that is involved in spreading, not protein I_3. Splenocyte and leukemia cell lines, which do not spread in culture, lack both II_4 and I_3, whereas mouse macrophage line PU5 has proteins similar if not identical to II_4 and I_3. PU5 cells grow as monolayers in vitro and have normal spreading processes, whereas neither splenocytes nor leukemia cells grow in monolayer.

Although proteins II_4 and I_3 are present in most mammalian cell lines, they are not found in chicken embryonic fibroblast (CEF). Anti-B103 serum is unable to extract proteins equivalent to II_4 and I_3 from chick fibroblast cell surfaces, and anti-B103 serum does not prevent CEF cells from spreading. Anti-B103 serum therefore does not seem to cross-react with surface proteins of chicken cells which are responsible for cell spreading.

Antiserum raised against a glucose-regulated protein has been reported to stain the 3T3 cell periphery; however, the antiserum does not have an antispreading effect similar to that of anti-B103 serum on 3T3 cells (31). There are a few reports of the inhibitory effect of antiplasma membrane protein and antiglycolipid on cell growth (5, 15, 26), but the effect of these sera on monolayer cell spreading has not been tested. Stallcup and Cohn (35) have prepared antiserum against B103 cells, but they did not report any effect of the antiserum on B103 cells after the cells had been treated with the serum (approximately 1:10 dilution) for 30 min. Cell surface components, with a mol wt of >150,000, released to the culture medium or extracted from the cell surface can promote human diploid fibroblasts to attach and spread (27). This phenomenon may be similar to the flattening effect of LETS protein (42). An adhesion and spreading factor has been isolated from fetal calf serum. Absorption of this factor on substratum is required for the adhesion and spreading activity, and antiserum against the serum factor inhibits cell spreading (13). Recently, Thy-1 antigen has been reported to be common to neurons and fibroblasts in primary cultures of rat dorsal ganglion cells (8). Even though proteins II_4 and I_3 are common to both neural and fibroblastic cells, it is unlikely that they are related to Thy-1, as Thy-1 has a mol wt of 27,000 (38) and II_4 and I_3 are 80,000–90,000 and 120,000, respectively.

The major surface protein on 3T3 cells has been reported to be a "glucose-regulated protein" with a mol wt of 92,000 (31). Hubbard and Cohn (16) reported a 80,000–90,000 protein to be a major surface protein in HeLa cells. Even though in all the rat neural cell lines listed in Table I, protein II_4 (mol wt 80,000–90,000) is one of the major iodinatable cell surface proteins, it has not been determined whether protein II_4 is related to any of those proteins previously reported. It should be noted that there are several proteins with 90,000 mol wt, as revealed in this reported by two-dimensional gel electrophoresis.

Although II_4 and I_3 are the proteins that correlate remarkably

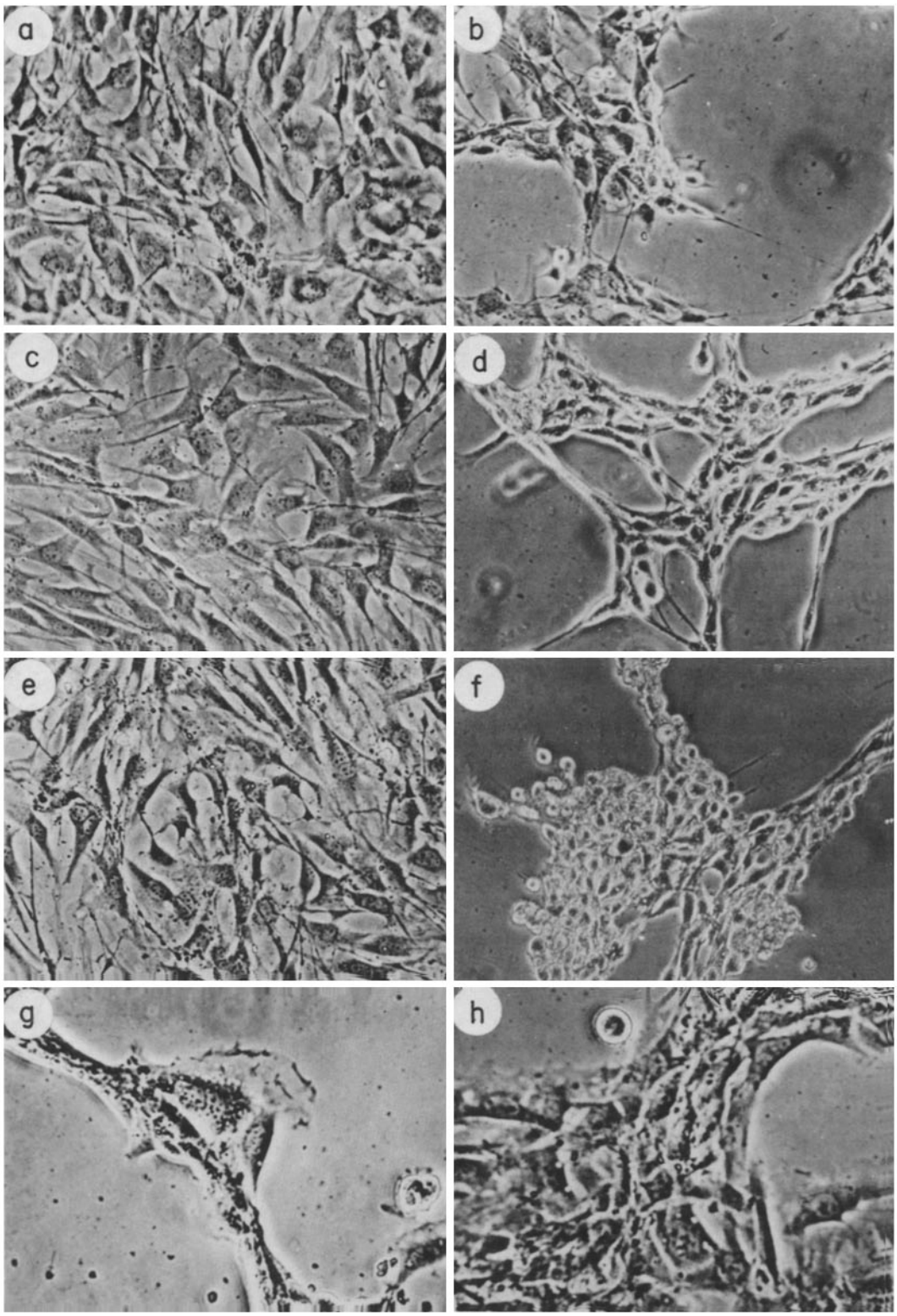

FIGURE 4 Effect of anti-B103 serum on monolayer 3T3 cells. (*a*) Time 0; (*b*) 30 min in 5% anti-B103 serum; (*c*) 1 h in anti-B103 serum absorbed with B27; (*d*) 1 h in anti-B103 serum; (*e*) 2 h in anti-B103 serum absorbed with B27 cells; (*f*) 2 h in anti-B103 serum; (*g*) 3 h after returning to normal medium after antiserum treatment (5% anti-B103 serum for 2 h); (*h*) 6 h after returning to normal medium after antiserum treatment (5% anti-B103 serum for 2 h).

well with cell-spreading activity, we cannot yet rule out the possibility that other membrane proteins, either beyond the molecular weight separation range of the present gel system (10,000–150,000) or in quantities too small to be detected by this system, are involved in the spreading phenomenon. Definitive proof for the involvement of proteins II_4 and I_3 or both in cell spreading should be obtained when a monospecific antibody to each of them becomes available.

The anti-cell spreading activity of anti-B103 serum cannot be absorbed by adult liver homogenate; neither can antiserum

prepared against adult rat brain homogenate cause antispreading of various cell lines (Table IV). However, proteins II_4 and I_3 exist in primary cultures of fetal brain and fetal liver (data not shown) and in tumors originated from B103 cells (Table I). These results suggest to us that these cell surface proteins may have in vivo counterparts. Spreading proteins may not be present in most adult tissues, but they are likely to be expressed when cell migration and growth are essential, for example, during early embryogenesis, organogenesis, regeneration, and wound healing. On the other hand, mobile cells like macrophages may have these proteins expressed most of the time on their cell surface. Trypsin and chelating agents of divalent cations such as EDTA and EGTA can also cause spread cells to round up (10, 25, 30). It remains to be determined whether or not these agents and the anti-B103 antiserum described here use a similar route to transmit the external signal to the cytoskeleton, for example, through transmembrane proteins, as proposed by Ash et al. (1).

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