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Integral Membrane Glycoproteins Related to Cell-Substratum Adhesion in Mammalian Cells

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Broad spectrum antisera have been raised against surface membrane-derived material from baby hamster kidney cells and mouse mammary tumor epithelial cells. These antisera disrupt cell-substratum adhesion in their respective cell types. Using an antibody neutralization (blocking) assay, adhesion-related glycoproteins have been isolated from non-ionic detergent extracts of each cell type. The purified material in each case consisted of a restricted population of glycoproteins of approximately 120,000-160,000 M_r. Purified material from each system blocked the disruption of adhesion induced by the heterologous antiserum on either cell type. The antisera were capable of disrupting cell-substratum adhesion of a large number of cell types and species sources. In addition, antibody blocking activity could be detected from partially purified extracts of several adult hamster cell types and a variety of cultured cell types. Thus, in addition to having similar substratum-associated glycoproteins (eg, fibronectin) and cytoskeleton-associated proteins (eg, α-actinin and vinculin) cells from different species and tissue sources appear to have a relatively conserved class of integral membrane glycoproteins involved in cell substratum-adhesion.

Key words: cell-substratum adhesion, cell surface, integral membrane glycoproteins, conserved structure

Studies on cell-substratum interactions in many laboratories over the past decade have indicated that components in the substratum, the surface membrane, and the cytoskeleton all play a role in the maintenance and control of these adhesive contacts (reviewed or discussed recently in [1-6]). In addition to the many molecular components involved, one must also account for different kinds of adhesive mechanisms, such as those that involve calcium-dependent and calcium-independent factors [7,8], and different stages in the establishment of fully stabilized adhesive contacts [9]. Clearly, cell-substratum adhesion is a complicated, transmembrane phenomenon.

In attempting to identify and localize the molecular constituents involved in the tramsmembrane control of adhesion in fibroblasts, most investigators have concentrated on substrate-associated components that interact with the plasma membrane at its outer surface [10-13], and those cytoskeletal components that may interact with the plasma membrane at its inner surface [14-16]. Little is known,

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however, about the identity and role of integral membrane components with which these more peripheral adhesion-related molecules may interact.

Previous work in this laboratory has demonstrated the usefulness of broad spectrum antisera as probes for substrate adhesion-related integral membrane molecules in both fibroblastic and epithelial cells [17–19]. This approach is similar to that used previously to identify molecules involved in cell–cell interactions in a variety of systems [20–24]. In our laboratory, two broad spectrum antisera that disrupt cell–substratum adhesion in their respective systems have been produced. The first, anti-M, was raised against purified surface membranes of a transformed cell line (C13/B4) derived from the hamster fibroblast BHK21/C13. When applied to living cultures of BHK21/C13 in the absence of complement, the antiserum induces reversible rounding and detachment of cells from the substratum [17,18]. Monovalent Fab fragments of anti-M immunoglobulin were able to induce the same response [17]. The disruption of adhesion could be shown not to be the result of a nonspecific coating of the cell by antiserum since antisera rasied against other complex surface immunogens, which bound equally well to BHK21/C13 cells, were unable to induce the detachment response [18]. Similarly, a second antiserum, anti-SFM I, raised against material shed into serum-free tissue culture medium by a murine mammary tumor epithelial cell line (BALB/c MT2), was able to detach these cells from the substratum in a reversible and nontoxic manner [19].

These probes are too complex to be used directly in localizing adhesion-related antigens, but they can be used in an antibody neutralization assay to detect the presence of adhesion-related antigens in solubilized non-ionic detergent extracts of C13/B4 or BALB/c MT2 cells. Using such an antibody-blocking assay, a protocol was developed for the purification of the adhesion-related antigens being detected by our antisera [19,25]. In the work reported here, we demonstrate that the adhesion-related antigens isolated from the hamster fibroblast system and the murine mammary epithelial system are similar in their polypeptide composition and demonstrate cross-reactivity with respect to their antibody-blocking activity. This raises the possibility that the antigens from the two systems being detected by this approach are related. Although preliminary, these data suggest the possibility that the particular molecules identified by this approach represent a class of relatively conserved adhesion-related integral membrane glycoproteins relevant to cell–substratum adhesion.

MATERIALS AND METHODS

Cells

Large and small quantities of BHK21/C13 and C13/B4 hamster fibroblasts were grown as described previously [17]. The BALB/c MT2 mammary tumor epithelial cell line was cloned and grown as described previously [19]. The original cells were obtained from Dr. E. Lasfargues Medical Research Institute, Camden, New Jersey. Human melanoma cell lines (WM9, WM115, WM46) were established from human biopsy material and were obtained from Dr. Meenhard Herlyn (Wistar Institute). Continuous cell lines are checked regularly to assure absence of myloplasma. Rat primary mesothelial cells were derived from primary tissue by Dr. John Aronson (Wistar Institute). Cells of endothelial morphology were obtained

from rabbit aorta by established procedures [26] by Dr. Ray Dorio (University of Pennsylvania). Other tissue cells were derived from minced adult organs and were allowed to grow out for 7–10 days in Eagle's minimum essential medium and 10% fetal calf serum. Other established cell lines tested are grown routinely at the Wistar Institute.

Antisera

Anti-M serum, raised against surface membranes isolated from C13/B4 hamster fibroblasts, and anti-SFM I, raised against material shed into serum-free tissue culture medium by a mouse mammary tumor epithelial cell line (BALB/c MT2), were produced in goats as described [17,19]. Anti-M and anti-SFM I, round and detach cultures of adherent BHK21/C13 and BALB/c MT2 at dilutions of 1:50 and 1:256, respectively [25,19]. Anti-GP was raised in goats against lectin-purified material obtained from NP40 extracts of C13/B4 cells by the fractionation protocol in Figure 1 [25]. Anti-GP rounds and detaches adherent cultures of BHK21/C13 at dilutions up to 1:1,200–3,000 depending on the bleeding used. All antisera were heat-inactivated at 56°C 30 min before use to destroy complement.

Antibody Neutralization (Blocking) Assay

These assays test the ability of solubilized extracts of C13/B4 and BALB/c MT2 cells to block the rounding of their respective cell types induced by anti-M (or anti-GP) and anti-SFM I, respectively. The conditions for plating of target cells and for removing detergent from the extract before applying it to cells as well as all other details of the assay are described in Knudsen et al [25] and Damsky et al [19]. Typical blocking assays for anti-GP (or anti-M) and anti-SFM I are shown in Figures 2 and 3.

Purification of Adhesion-Related Glycoproteins

Purification of adhesion-related antigens from C13/B4 (or BHK21/C13) hamster fibroblasts and BALB/c MT2 mouse epithelial cells capable of blocking the rounding induced by anti-M or anti-SFM I was accomplished by a procedure modified from that of Knudsen et al [25] and Damsky et al [19]. Cells were extracted with 0.5% Nonidet NP40 (Particle Data Labs, Elmhurst, Illinois; NP40) in 0.01 M Tris, pH 8.0, containing 2 mM phenylmethylsulfonylfluoride (PMSF). The extract was acidified to pH 4 with 10 M acetic acid. Following a 30-min incubation at 4°C and 10 min at 37°C, the precipitate was removed by centrifugation at 4,000 rpm for 20 min in a PR 6000 centrifuge (Damon/IEC, Needham, Massachusetts). Following neutralization of the supernatant to pH 8 with 1 M Tris base, material was precipitated overnight by the addition of six volumes of acetone. The precipitate was washed with phosphate-buffered saline (PBS) before being extracted with 0.5% NP40 in buffer containing 0.10 Tris and 0.5 mM $Cacl_2$ (TNC buffer) in the presence of 2 mM PMSF and centrifuged at 15,000 rpm for 30 min in an SS34 Sorvall Rotor (Dupont-Sorvall, Wilmington, Delaware). Affi-gel chromatography was performed on the supernatant as described [25]. Material eluting between 0.03 and 0.08 M salt was made 0.1 in NaCl and applied to a wheat germ agglutinin (WGA) column. The WGA column was washed with TNC buffer

containing 0.1 M NaCl and then with TNC buffer alone. The material eluting in the absence of salt contained 80% of the anti-M or anti-SFM I blocking activity present in the applied sample. The remaining activity was present in the material eluted subsequently in the presence of 4% *n*-acetyl glucosamine. The peak containing 80% of the blocking activity was fractionated further by Lens culinaris (Lens) affinity chromatography as described [19,25]. The material bound by this lectin and eluted in the presence of 10% glucose contained the biologically active material used to compare the specific blocking activity and polypeptide composition of adhesion-releasing material from BHK21/C13 (or C13/B4) cells and BALB/c MT2 mammary tumor epithelial cells. Protein concentrations were determined by the method of Lowry et al [27]. In order to determine the protein content of lectin-purified samples, it was necessary to remove the sugar hapten present in the eluted material. This was accomplished by precipitating with acetone and resolubilizing the resulting pellet in the TNC buffer. Samples at various stages of purification were analyzed by electrophoresis in 7.5% polyacrylamide gels in the presence of sodium dodecyl sulfate (SDS-PAGE) by the method of Laemmli [28]. Purified material was iodinated with ^{125}I by the chloramine T method.

Determination of Cross-Reactivity of Antisera

To test anti-GP and anti-SFM I for their ability to induce rounding in cells of heterologous cell type and species source, target cells were plated at 5×10^3 cells/microtiter well in their respective growth media. After 24 h the medium was replaced with 2-fold serial dilutions of anti-SFM I or anti-GP, both in medium containing 10% heat-inactivated fetal calf serum. Rounding was scored after 16–24 h. End points were defined as the greatest dilution at which at least 90% of the target cells were rounded.

Detection of Antibody-Blocking Activity in Partially Purified Extracts of Heterologous Cell Types

Adult hamster liver, kidney, and heart as well as newborn hamster epidermis were extracted with NP40 and processed to the detergent-extracted acetone precipitate step in Figure 1. The protein concentration of each extract was measured and 2-fold serial dilutions of extracts were tested for their ability to block anti-GP induced or anti-SFM I induced rounding of either BHK21/C13 or BALB/c MT2 cells. Specific blocking activity of each extract was then calculated as the minimum micrograms of protein required to block the rounding induced by end-titer dilutions of anti-GP or anti-SFM I under the conditions of the blocking assay [19,25]. In addition, the specific blocking activity of the 120,000–160,000 M_r glycoproteins purified through the entire protocol from either hamster fibroblasts or mouse epithelial cells was measured for the ability to block rounding of either cell type by either antibody.

RESULTS

In order to compare the adhesion-related antigens present in hamster fibroblasts (C13/B4 or BHK21/C13) and murine mammary tumor epithelial cells (BALB/c MT2), NP40 extracts of each cell type were subjected to the purification protocol shown in Figure 1 [see also 19,25]. The fractions at each purification step

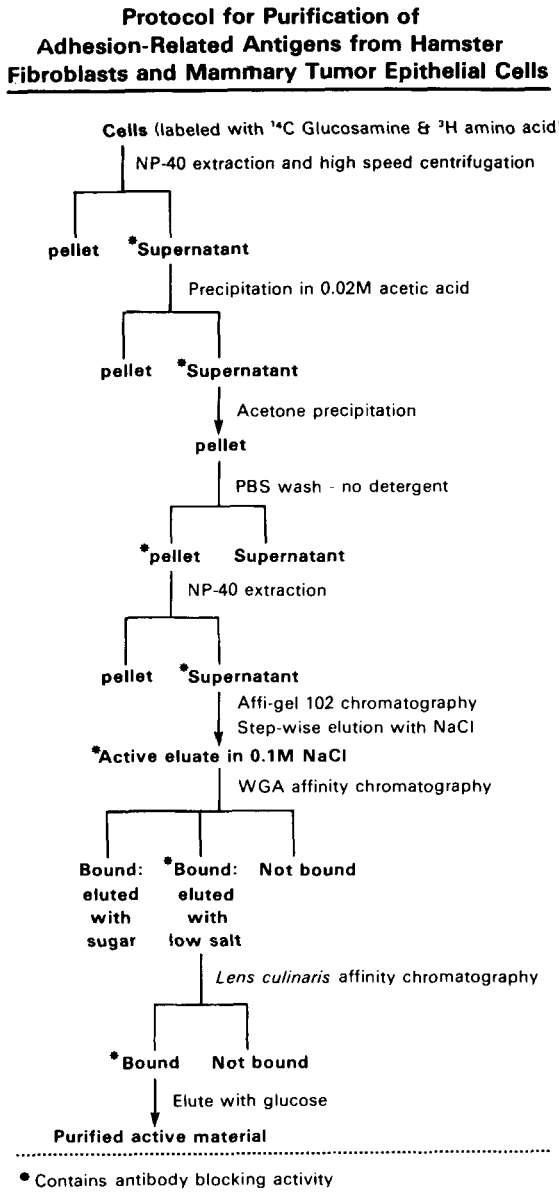

Fig. 1. Purification procedure for adhesion-related antigens in NP40 extracts of C13/B4 and BALB/c MT2 cells. Modified from [19,25].

were tested for their ability to block cell rounding induced by anti-M (raised against hamster fibroblast membranes) or anti-SFM I (raised against mouse epithelial cells). Results of typical blocking assays are displayed in Figures 2 and 3. SDS-PAGE

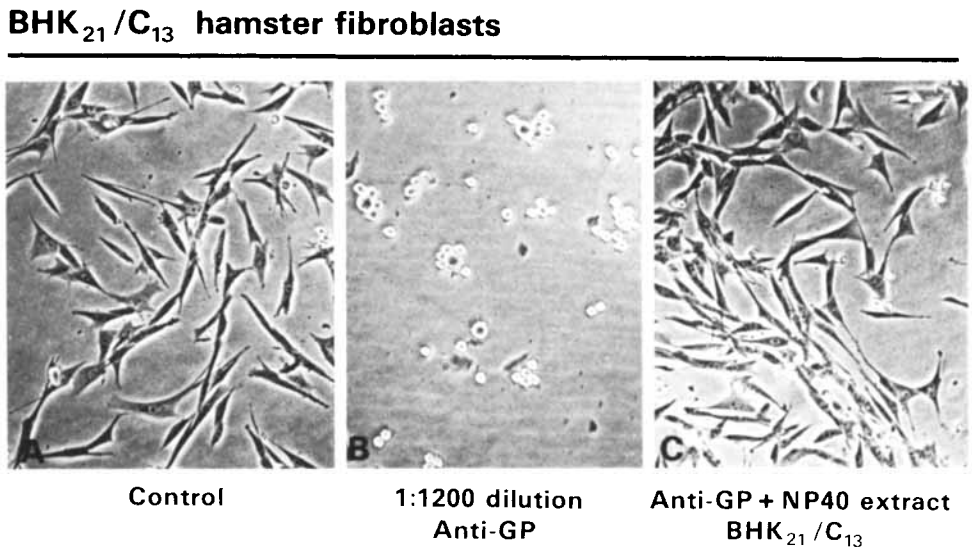

Fig. 2. Blocking assay for the detection of adhesion-related antigens in extracts of C13/B4 cells. NP40 extracts or fractions obtained during purification are depleted of detergent and mixed 1:1 with twice and end-titer dilution of anti-GP before being applied to cultures of adherent BHK21/C13 cells [25]. A) Untreated BHK21/C13 cells. B) BHK21/C13 plus 1:1200 dilution of anti-GP. C) BHK21/C13 plus a 1:1 mixture of 1:600 anti-GP and 30 μg NP40 extract of C13/B4 cells.

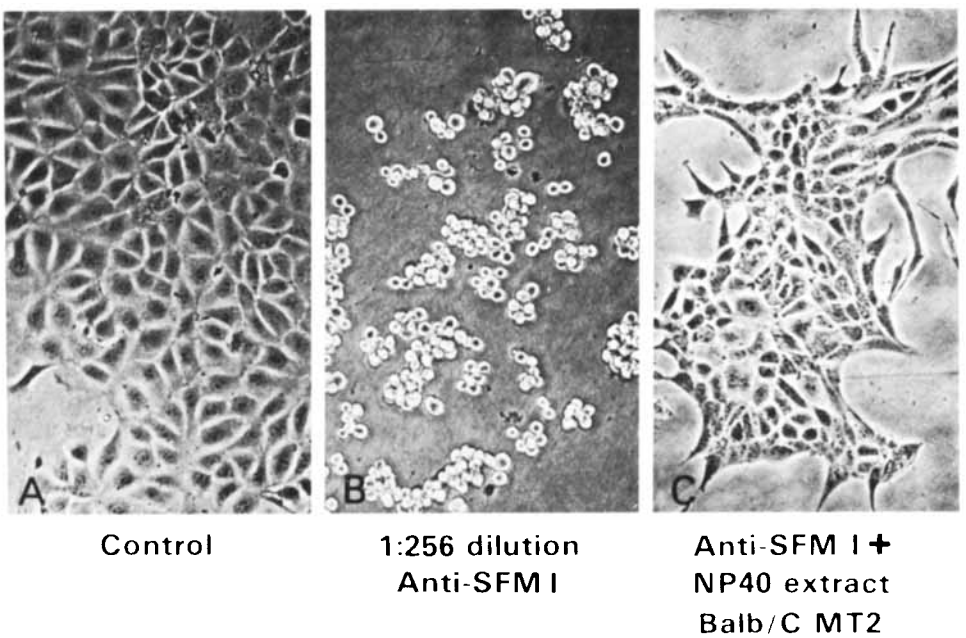

Fig. 3. Blocking assay for the detection of adhesion-related antigens in extracts of BALB/c MT2 [19]. Conditions are the same as for Figure 2, except that anti-SFM I is used. A) Untreated BALB/c MT2. B) BALB/c MT2 + 1:256 dilution of anti-SFM I. C) BALB/c MT2 plus a 1:1 mixture of anti-SFM I 1:128 and 30 μg BALB/c MT2 NP40 extract.

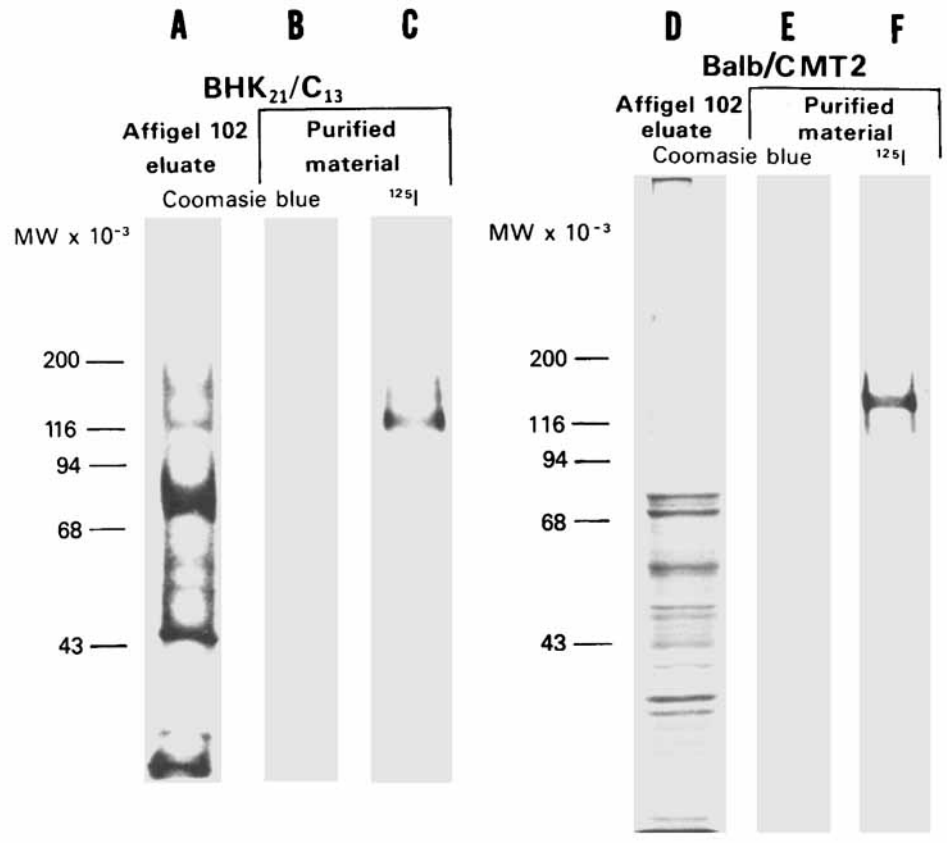

Fig. 4. Comparison of adhesion-related antigens from hamster fibroblasts (lanes A–C) and from mouse mammary tumor epithelial cells (lanes D–F) purified by the protocol in Figure 1. Lanes A, B, D, and E are stained with Coomassie Blue and represent the material following Affi gel chromatography (lanes A and D) and material following the final purification step (lanes B and E). Lanes C and F are autoradiographs of ^{125}I-labeled purified material.

analysis of the purified material on 7.5% acrylamide from each system reveals great similarity in the molecular composition of the purified material. In the mouse epithelial system Coomassie Blue staining revealed a single diffuse band of material in the 140,000 M_r region of the gel (Fig. 4, lane E). In the hamster fibroblast system, Coomassie Blue staining was almost undetectable even though 5 μg protein was loaded onto the gel (Fig. 4, lane B). The purified material from each system was then iodinated with ^{125}I in order to determine more effectively the components present. In the hamster fibroblast system three discrete bands of 120,000–160,000 M_r were resolved (Fig. 4, lane C). In the mouse epithelial system, a band with 110,000 M_r was revealed in addition to the band at about 140,000 M_r (Fig. 4, lane F). No components in any other region of the gel from either system were detected by the ^{125}I labelling. A similar molecular composition was evident when iodinated samples were run on 6% acrylamide gel. For the remainder of this paper, the purified material will be referred to as the 120,000–160,000 M_r glycoproteins to denote the general region of the gel to which these components migrate. In order to raise an antiserum of higher specificity for the hamster fibroblast system, 120,000–160,000 M_r material purified from hamster fibroblasts was used as an immunogen in goats. The resulting anti-GP rounds and detaches BHK21/C13 cells at dilutions up to 1:1,200–1:3,000 (depending on the bleeding used) compared with on-

ly 1:50 for the original anti-M serum. The anti-GP serum was used along with the anti-SFM I serum in all subsequent experiments.

The adhesion-related antigens from these two systems were similar not only with respect to the consumption of the purified material but also the behavior of the blocking activity during purification. Thus, both resisted precipitation by acetic acid at pH 4, eluted at similar salt concentrations from affi-gel-102, and bound to Lens culinaris. These results suggested that our antisera were detecting similar antigens. In order to determine whether the purified antigens from the two sources were similar in function as well as in biochemical composition, we determined the specific antibody-blocking activity of the purified material from each system for each antiserum on both cell types (Table I). Purified material from hamster fibroblasts is clearly able to block rounding of BHK21/C13 cells or BALB/c MT2 cells induced by either anti-GP or anti-M, although more protein was required to block the heterologous antiserum. Similar results were obtained using material purified from the mouse epithelial line.

Having found both biochemical and functional similarity between the adhesion-related glycoproteins from BHK and BALB/c MT2 cells, we predicted 1) that the two antisera should be able to round and detach a variety of cell types, and 2) that antibody-blocking activity would be detectable in partially purified extracts of a variety of tissue sources. The results summarized in Table II demonstrate that both antisera are able to detach cultured cells of epithelial and fibroblast origin from several mammalian species. Anti-GP, the more specific and higher titre antiserum of the two, was even able to detach secondary chick embryo fibroblasts.

TABLE I. Cross-Reactivity in Blocking Activity Purified From Extracts of Hamster Fibroblasts and Mouse Mammary Tumor Epithelial Cells

SOURCE OF EXTRACT	CELL LINE TESTED	Micrograms of extract protein to block rounding by end point dilution of: ANTI-GP	ANTI-SFM I
BHK_{21}/C_{13}	BHK_{21}/C_{13}	0.8	6.6
	Balb/C MT2	27.5	6.6
Balb/C MT2	BHK_{21}/C_{13}	20.2	10.2
	Balb/C MT2	20.2	2.0

A comparison of the amount of extract protein purified from BHK21/C13 and BALB/c MT2 cells required to block the homologous or heterologous antiserum on either cell type. The least amount of protein is required in the completely homologous systems (highlighted in black)—ie, BHK21/C13 extract blocking anti-GP-induced rounding of BHK21/C13 cells, but clearly, extracts from either cell source are able to block rounding induced by either antiserum on either cell type.

Higher concentrations of antibody were required to detach cells of heterologous tissue or species origin. Anti-GP and anti-SFM I were also tested on primary cultures derived by incubating minced adult hamster organs in tissue culture for 7–10 days. The particular cell types growing out of each organ were not characterized. However, cells of more than one morphology were present in all outgrowths. Both antisera rounded all cells growing out of minced liver, kidney, and heart. In addition, both were effective in rounding cells of endothelial morphology derived from rabbit aorta. Examples of anti-SFM I induced rounding of hamster liver derived cells and anti-GP rounding of rabbit aorta endothelial cells are shown in Figure 5. One interesting exception was noted in the widespread ability of anti-GP to round cells. Anti-GP was unable at any dilution either to detach adherent hamster peritoneal macrophages or to prevent their attachment and spreading.

Adult hamster kidney, heart, and liver as well as newborn hamster epidermis were extracted with NP40 and processed to the detergent-extracted acetone precipitate stage (Fig. 1). Extracts were tested for their ability to block anti-GP-induced rounding of BHK21/C13 cells and anti-SFMI-induced rounding of BALB/c

TABLE II. Titration of Antiserum-Induced Rounding of Heterologous Cell Types by Anti-GP and Anti-SFM I

Species	Tissue/Cell Source	Cell Line	MINIMUM DILUTION	
			Anti-GP	Anti-SFM I
Hamster	Fibroblast	BHK_{21}/C_{13}	1:1200	1:16
		C_{13}/B_4	1:1200	1:16
	Melanoma	CS473	1:1200	NT
Mouse	Mammary epithelium	Balb/C MT2	1:40	1:256
	Fibroblast	10T ½	1:160	1:32
Rat	Mesothelium	Wistar strain	1:160	1:16
Human	Fibroblast	WI-38	1:50	1:4
	Melanoma	WM 115	1:40	1:8
		WM 9	1:40	NT
		WM 46	1:256	NT
	Mammary tumor	MCF-7	1:80	1:8
Chick	Fibroblast	2° CEF	1:8	NE

The minimum dilution of anti-GP and anti-SFM I required to round cells derived from different tissue and species sources are given. In each case, the cells used to produce the original antigens are the most sensitive to their antisera (highlighted in black). However, cells from other tissue and species sources are also sensitive. Anti-GP is a more specific antiserum than anti-SFM I and therefore has a higher titre for all cell lines tested (except for the BALB/c MT2 cell line) than does anti-SFM I. Nonetheless, the qualitative reactivity of cell lines to the two antisera is similar.

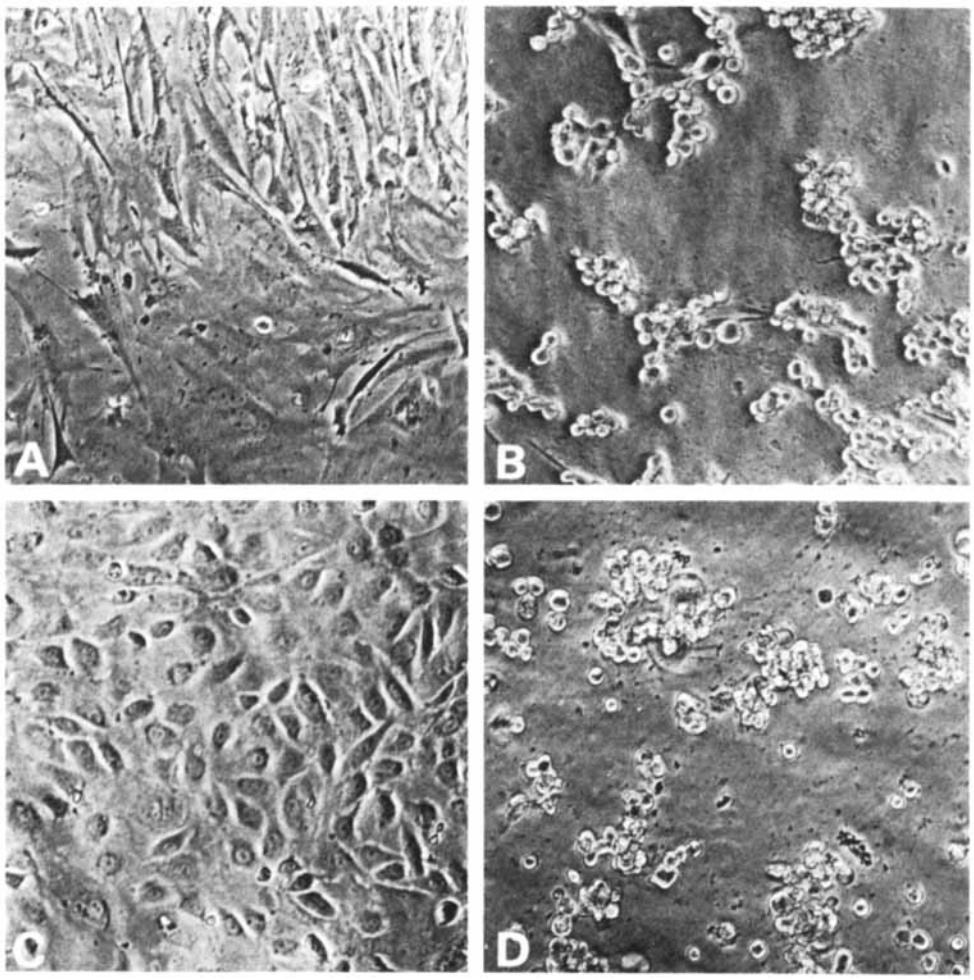

Fig. 5. A) Cells growing out of minced hamster liver: untreated. B) These cells after 6 hours treatment with a 1:10 dilution of anti-SFM I. C) Cells of endothelial morphology from rabbit aorta: untreated. D) These cells after 16 hours treatment with 1:20 dilution of anti-GP. In both cases, cells returned to their flattened morphology following removal of antisera.

MT2 cells. Blocking activity was detectable in all cases. About 20-fold more extract protein from the hamster tissues was required to block rounding in the heterologous (mouse) than in the homologous (hamster) system. These results and those in Tables I and II suggest that the adhesion-related glycoproteins being detected by our antisera are widespread in mammalian cells and occur in animal tissues in vivo as well as in cells adapted to tissue culture conditions.

DISCUSSION

The results presented here suggest the existence of a class of integral membrane glycoproteins that is relevant to cell–substratum adhesion and which is detec-

table in a wide variety of mammalian cell and tissue types. These adhesion-related antigens were detected initially in NP40 extracts of hamster fibroblasts (BHK21/C13 or C13/B4) and in BALB/c MT2 mouse mammary tumor cells by their ability to block both cell rounding and detachment caused by anti-M and anti-SFM I antisera, respectively [17,19,25]. An antibody-blocking assay in conjunction with the purification scheme in Figure 1 was used to isolate a restricted population of integral membrane adhesion-related glycoproteins from hamster fibroblasts and mouse epithelial cells. The polypeptide composition, as analyzed by one-dimensional SDA-PAGE, of the fraction containing the adhesion-related material from both systems was similar, in each case, consisting of 2–3 resolvable components within the same restricted region of the gel (Fig. 4), one or more of which is clearly involved in some aspect of cell–substratum adhesion. The adhesion-related components in these fractions are considered to be integral membrane antigens because attempts to release blocking activity with high salt, low salt, EDTA, and butanol have all been unsuccessful, whereas non-ionic detergents do release the blocking activity. The adhesion-related components are protein in nature since the blocking activity is sensitive to heat, trypsin, and urea [19,25]. Finally, these proteins are glycosylated since they incorporate the ^{14}C glucosamine included in the growth medium and since the blocking activity binds to Lens culinaris lectin and is eluted in the appropriate sugar hapten. However, the glycosylated portion appears not to be critical for the antibody-blocking activity since incubation of purified material with mixed glycosidases under conditions that remove 40% of bound ^{14}C glucosamine do not affect blocking activity [19]. This reduces the possibility that the presence of immunological cross reactivity is due to closely related, highly antigenic sugar moities bound to unrelated polypeptide backbones.

The relevance of the purified material to substrate adhesion was substantiated by using the 120,000–160,000 M_r glycoproteins from C13/B4 hamster fibroblasts as an immunogen. The resulting anti-GP had a greatly enhanced rounding titer when compared to the anti-M [25]. Furthermore, the 120,000–160,000 M_r region as well as the blocking activity are removed from purified extracts of C13/B4 and BALB/c MT2 when they are passed over columns of immobilized anti-GP or anti-SFM I immunoglobulin, respectively. Both blocking activity and the 120,000–160,000 M_r glycoproteins are present in the material eluted from these columns [19,25]. Finally, analysis by the purification scheme in Figure 1 of a nonadherent variant of an adherent hamster melanoma cell line indicates that it contains a greatly reduced amount of the 120,000–160,000 M_r glycoproteins when compared with the adherent parent melanoma cell line. This is accompanied in the nonadherent line by a greatly reduced expression of anti-GP blocking activity (Knudsen et al, this volume). Despite these data, we still cannot rule out the participation of undetected components in other regions of the gel or determine which of the bands in the 120,000–160,000 M_r region are responsible for the blocking activity until further purification is achieved. Nevertheless, the fact remains that blocking activity from the hamster fibroblast and the mouse mammary epithelial systems behave similarly throughout a complex purification scheme and that bands in the molecular weight region around 120,000–160,000 M_r are the components most readily detectable by SDS-PAGE in each system.

The similarity in the biological activity of the adhesion-related material isolated from the hamster fibroblast and mouse epithelial cells is demonstrated in

Tables I and II. Table I demonstrates that there is considerable cross-reactivity in the blocking activity of the material isolated from the two systems, suggesting that the adhesion-related molecules being identified by anti-GP and anti-SFM are similar, although not identical. This is confirmed by the results in Table II and by the fact that antibody-blocking activity is detectable in a variety of adult tissues.

It will be of considerable interest to follow up the observation that anti-GP is unable to detach adherent macrophages. This could mean either that macrophages adhere by mechanisms that do not include the antigens detected by our antisera, or that macrophages employ mechanisms in addition to those being perturbed by our antisera so that they are impervious to treatment with even high concentrations of anti-GP. The fact that intact hamster macrophages are unable to absorb out detectable rounding activity from anti-GP under conditions in which BHK21/C13 cells remove all rounding activity suggests that macrophages do not express these antigens on their surface (Buck, unpublished experiment). Experiments are also in progress to determine whether these antigens are present on cells such as lymphocytes, neutrophils, and monocytes, which adhere in vivo only under certain circumstances.

Work from many other laboratories on the identity of substrate adhesion-related proteins has concentrated on matrix-associated fibronectin and the cytoskeleton-associated proteins, α-actinin and vinculin. Antisera to these molecules have extensive cross-reactivity in different cell and species types. For example, antiserum to vinculin isolated from chicken gizzard detects vinculin in chick fibroblasts, chick intestine [14,15], human mammary epithelial cells (Damsky and Burridge, unpublished data), and many other cell types. This suggests that the homologous proteins in different cell types share some related or identical antigenic determinants. Our results with the adhesion-related integral membrane glycoproteins detected by anti-GP and anti-SFM I, although preliminary, suggest a similar relationship among these molecules in different cell types. That the molecules are not identical is suggested by the fact that higher concentrations of antiserum are required to detach cells from heterologous tissue or species sources and that more extracted protein from a tissue from a heterologous species is required to block the antisera. A more precise comparison of these integral membrane glycoproteins will require further purification of the components in the 120,000–160,000 M_r region from each cell type and the production of monospecific and monoclonal antibodies. The utility of the monoclonal antibody approach is suggested by the results of Noonan et al [29]. They have produced monoclonal antibodies to different proteolytic fragments of chick fibroblast fibronectin. Some of these antibodies are highly specific for chick cells, whereas others react across species lines. This suggests that the species specificity lies in only some of the antigenic domains. Some degree of species variability would be expected in the integral membrane antigens identified here as well. Furthermore, if these molecules function by interacting directly with substrate-associated material, variability in different tissues of the same species would also be expected since the substrates of epithelial cells and fibroblasts, for instance, differ in several respects.

In conclusion, a class of integral surface membrane glycoproteins containing components relevant to the transmembrane control of cell–substratum adhesion has been identified and isolated from hamster fibroblast and mouse epithelial cell sources. The glycoproteins from the two sources have similar molecular weights and

display some immunological cross-reactivity. This suggests that they may be related. Using a blocking assay together with antisera that disrupt substratum adhesion, we have demonstrated the presence of components with similar biological activity in a wide variety of adherent cell types from several species. This class of molecules appears to be relevant to adhesion in vivo since extracts of several adult tissues also block the antisera. Thus the possibility exists that, in addition to containing similar extracellular matrix glycoproteins and cytoskeletal elements relevant to cell-substratum adhesion, cells maintain a conserved class of integral membrane glycoproteins important to the control of cell-substratum adhesion.

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REFERENCES

1. Culp L: Curr Top Membranes Transport 11:327, 1978.
2. Grinnell F: Int Rev Cytol 53:65, 1978.
3. Hynes RD, Destree A: Cell 15:875, 1978.
4. Yamada K, Olden K: Nature (London) 275:179, 1978.
5. Chen W-T, Singer SJ: Proc Natl Acad Sci USA 77:7318, 1980.
6. Kleinman HK, Klebe RJ, Martin GR: J Cell Biol 88:473, 1981.
7. Urishihara H, Takeichi M: Cell 20:363, 1980.
8. Brackenbury R, Rutishauser U, Edelman G: Proc Natl Acad Sci USA 78:387, 1981.
9. McClay DR, Wessel GM, Marchase RB: Proc Natl Acad Sci USA 78:4975, 1981.
10. Perkins ME, Ji TH, Hynes RO: Cell 16:941, 1979.
11. Kleinman HK, Martin GR, Fischman PH: Proc Natl Acad Sci USA 76:3367, 1979.
12. Wagner D, Hynes RO: J Biol Chem 255:4304, 1980.
13. Yamada K, Kennedy D, Kimata K, Pratt R: J Biol Chem 255:6055, 1980.
14. Geiger B, Tokuyasu K, Dutton A, Singer SJ: Proc Natl Acad Sci USA 77:4127, 1980.
15. Burridge K, Fermisco J: Cell 19:587, 1980.
16. Bennett V: J Biol Chem 255:6424, 1980.
17. Wylie D, Damsky CH, Buck CA: J Cell Biol 80:385, 1979.
18. Damsky CH, Wylie D, Buck CA: J Cell Biol 80:403, 1979.
19. Damsky CH, Knudsen KA, Dorio RJ, Buck CA: J Cell Biol 89:173, 1981.
20. Gerisch G: In Brinkley BR, Porter KR (eds): "International Cell Biology." New York: Rockefeller University Press, 1977, p 36.
21. Brackenbury R, Thiery J-P, Rutishauser U, Edleman G: J Biol Chem 252:6835, 1977.
22. Thiery J-P, Brackenbury R, Rutishauser U, Edelman G: J Biol Chem 252:6841, 1977.
23. Bertolotti R, Rutishauser U, Edelman G: Proc Natl Acad Sci USA 77:4831, 1980.
24. Dunia I, Nicolas J, Jacobs H, Bennedetti EL, Jacob F: Proc Natl Acad Sci USA 76:3887, 1979.
25. Knudsen KA, Rao P, Damsky CH, Buck CA: Proc Natl Acad Sci USA 78:6071, 1981.
26. Habliston DL, Whitaker C, Hart MA, Ryan US, Ryan JW: Am Rev Respir Dis 119:853, 1979.
27. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: J Biol Chem 193:265, 1951.
28. Laemmli U: Nature (London) 227:680, 1970.
29. Noonan K, Noonan N, Yamada K: J Supramol Struct Cell Biochem Suppl 5:301, 1981.