

ADHESION AND DETACHMENT CHARACTERISTICS OF CHINESE HAMSTER CELL MEMBRANE MUTANTS

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ABSTRACT

We have investigated the adhesion and detachment properties of wild-type Chinese hamster cells and of variant lines which possess altered cell surface glycoproteins as detected by galactose oxidase- ^3H borohydride labeling. The wild-type and variant lines tested all adhered to protein-coated glass surfaces at the same rate; however, the variant cells differed from wild type and from each other in terms of the ease with which they were detached by trypsinization. Morphological differences between the various lines were also apparent. Our results suggest that the carbohydrate moieties of the terminal region of surface glycoproteins are not directly involved in the initial phase of cell-to-substratum attachment, but that they may modulate the proteolytic susceptibility of surface components which are involved in cell detachment.

KEY WORDS cell adhesion · surface glycoproteins · membrane mutants

The proteins and glycoproteins of the cell surface have been implicated in the phenomena of cell-to-cell recognition and cell-to-cell substratum adhesion (12, 23). However in most cases, the precise nature and functions of the macromolecules involved in adhesion processes have yet to be defined. Some investigators (16, 17) have obtained evidence that surface oligosaccharide moieties and glycosyltransferase enzymes are the mediators of cell-to-cell adhesion, while other workers have implicated particular glycoproteins (25) and glycosaminoglycans (2) in cell-to-substratum attachment. The processes of cell-to-cell and cell-to-substratum adhesion resemble each other in many respects (3, 7); thus it seems reasonable to study the easily manipulated process of cell-to-substratum attachment in hope of learning something of the basic characteristics of cell-to-cell attachment.

In this communication we have attempted to evaluate the role of surface carbohydrate moieties in attachment to the substratum by exploiting the availability of mutant clones of Chinese hamster cells (line CHO) which possess known alterations of their surface glycoproteins. Thus we have compared the adhesion and detachment characteristics of wild-type (WT) Chinese hamster cells with those of a clone of lectin resistant cells (C2P1) (19) whose glycoproteins lack terminal sugar residues (10), and with those of clones of drug-resistant cells (C4S4, C5S3) (11) which possess an extra high molecular weight surface glycoprotein (8, 9).

MATERIALS AND METHODS

Cells

Wild-type and mutant Chinese hamster cells were usually maintained in exponential growth in suspension culture in medium alpha plus 10% fetal calf serum (6). The phytohemagglutinin resistant variant C2P1 was ob-

tained from Dr. P. Stanley, while the drug resistant variants C4S4 and C5S3 were obtained from Dr. V. Ling. The variant lines were stored at -70°C until needed and were thawed and placed in culture for a maximum of 1 mo before the experiments described. All lines were tested for mycoplasma by the Bacteriology Department of The Hospital for Sick Children and were found to be negative.

Cell Adhesion Assay

The assay used was a modification of the technique of Walther et al. (22) and has been described in detail elsewhere (7). Basically we have measured the rate of attachment of radiolabeled suspension culture cells to scintillation vials pretreated with serum containing culture medium. The validity of this approach has been ascertained by comparing the radioactivity assay with direct cell counts; the two methods showed close agreement. The serum coated vials employed in this study provide an adequate support for normal cell growth in complete culture medium, thus the adhesion events measured here are likely to reflect normal cell processes.

Cell Detachment Assay

We have measured the susceptibility of wild-type and mutant cells to detachment by trypsin in the following way. Monolayer cultures of cells were radiolabeled overnight with tritiated amino acids ($1\ \mu\text{Ci}/\text{ml}$). The radioactive culture fluid was removed and the cells were suspended by trypsinization. About 1×10^6 cells were reseeded in 40-mm tissue culture dishes in nonradioactive medium. The cells were allowed approx. 16 h to attach and recover from the effects of reculture. The culture fluid was then removed the cells were gently washed three times in phosphate-buffered saline, and were then treated with dilute solutions of $2 \times$ crystallized trypsin, usually at room temperature. The number of cells detaching after different periods of exposure to trypsin was assessed by measuring the amount of released radioactivity via liquid scintillation counting. In preliminary experiments the number of cells as measured by the radiolabel technique closely corresponded with measurements made by direct cell counting (7).

Surface Labeling

Cell surface oligosaccharidic moieties were tritiated using the galactose oxidase- ^3H borohydride technique as described elsewhere (6). Surface labeling patterns of isolated membranes were analyzed by polyacrylamide slab gel electrophoresis in the presence of sodium dodecyl sulfate and reducing reagent, followed by fluorescence-enhanced autoradiography as detailed in a previous communication (9).

Materials

Neuraminidase was obtained from Behring Werke AG (Marburg/Lahn, West Germany), while galactose

oxidase and $2 \times$ crystallized trypsin were purchased from Sigma Chemical Co. (St. Louis, Mo.). Tritiated borohydride $100\ \text{Ci}/\text{M}$ and ^3H -amino acid mixture ($1.0\ \text{mCi}/\text{ml}$) were obtained from New England Nuclear (Boston, Mass.). Tissue culture media were obtained from Grand Island Biological Co. (Grand Island, N. Y.) and the Ontario Cancer Institute (Toronto, Canada). Other chemicals were of reagent grade and used as purchased.

RESULTS

Adhesion and Detachment Characteristics

As seen in Fig. 1, wild-type cells and cells of two mutant Chinese hamster clones displayed very similar adhesion kinetics. At the experimental temperature of 33°C , cell attachment proceeded rapidly for the first 15 min and thereafter slowed with about 60% of the cells attaching in 2 h. Experiments at lower temperatures where cell adhesion proceeded less rapidly (7) also failed to reveal any significant differences between the

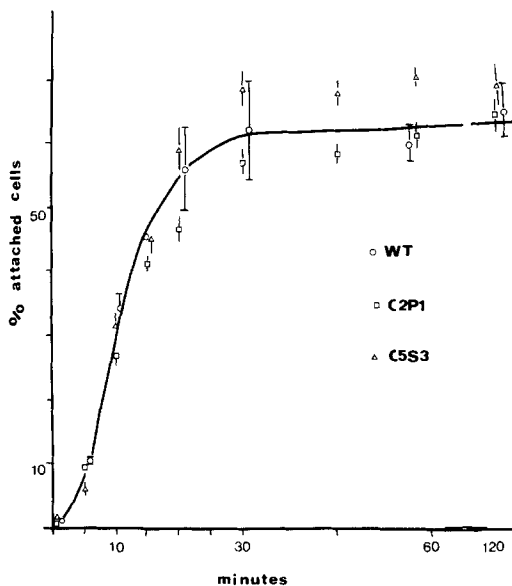


FIGURE 1 Attachment of WT and Mutant Cells. The adhesion kinetics of wild-type and mutant clones of exponentially growing Chinese hamster suspension culture cells was measured as described in Materials and Methods. The experimental temperature was 33°C . The data for clones C2P1 and C5S3 represent the means and standard errors of triplicate determinations and indicate intraexperimental error. The data for clone WT represents the mean and standard errors of three separate experiments and indicate interexperimental error. (\circ) Clone WT, (\square) clone C2P1, and (\triangle) clone C5S3.

adhesion kinetics of wild-type cells and of several mutant clones, namely C2P1, C5S3, and C4S4 (data for C4S4 is not shown). Although the fraction of cells which adhere during a 2-h incubation varied somewhat from experiment to experiment (60–80% attachment), no consistent differences between the clones tested was observed in this regard. In addition, the slopes of the rising phases of the attachment vs. time curves were similar in all instances for clones WT, C2P1, and C5S3.

By contrast to the adhesion studies, the rate with which cells were detached by trypsinization differed considerably among the clones tested. Thus, as seen in Fig. 2 and in Table I, the lectin resistant variant C2P1 was rather refractory to detachment with trypsin, while wild-type cells and drug resistant C4S4 and C5S3 cells were readily detached. The precise rates of release of cells by trypsin varied somewhat from one experiment to another, and release rates were markedly affected by the concentration of the enzyme and by temperature. Nonetheless, the order displayed in Fig.

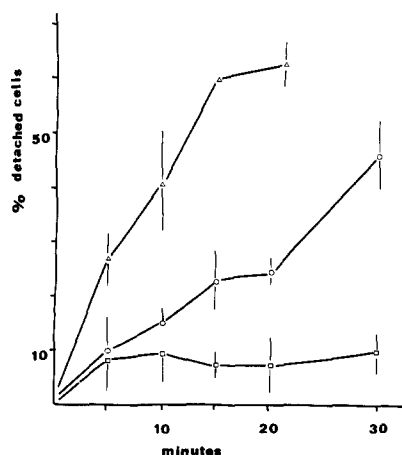


FIGURE 2 Detachment of WT and Mutant Cells by Trypsin. Subconfluent cell monolayers were treated with 100 $\mu\text{g}/\text{ml}$ trypsin at 25°C, and the detachment of cells as a function of time was measured as described in Materials and Methods. The ordinate (% detached cells) equals $\frac{N_t - N_0}{NT}$, where N_t is the number of cells detached at time t , N_0 is the number of cells detached by a rinse with buffer at $t = 0$, and NT is the total number of cells on the dish (this is determined by release of the attached cells through a 45-min incubation with 2.5 mg/ml trypsin). The data represent the means and standard errors of triplicate determinations within one experiment. (Δ — Δ) C5S3, (\circ — \circ) WT, and (\square — \square) C2P1.

TABLE I
Detachment of Chinese Hamster Cells by Trypsin

Clone	Experiments		
	1	2	3
	% Cells detached		
C5S3	60.0	67.9	67.0
C4S4	65.0	—	—
WT	25.0	22.6	41.0
C2P1	12.5	18.5	21.0

The detachment of cells by treatment with 100 $\mu\text{g}/\text{ml}$ crystalline trypsin for 20 min at 25°C was measured as described in Materials and Methods. The results represent the means of triplicate determinations.

2 always prevailed: that is, the drug resistant cells (C4S4, C5S3) detached more quickly than did wild-type cells, while the lectin resistant (C1P1) cells detached more slowly.

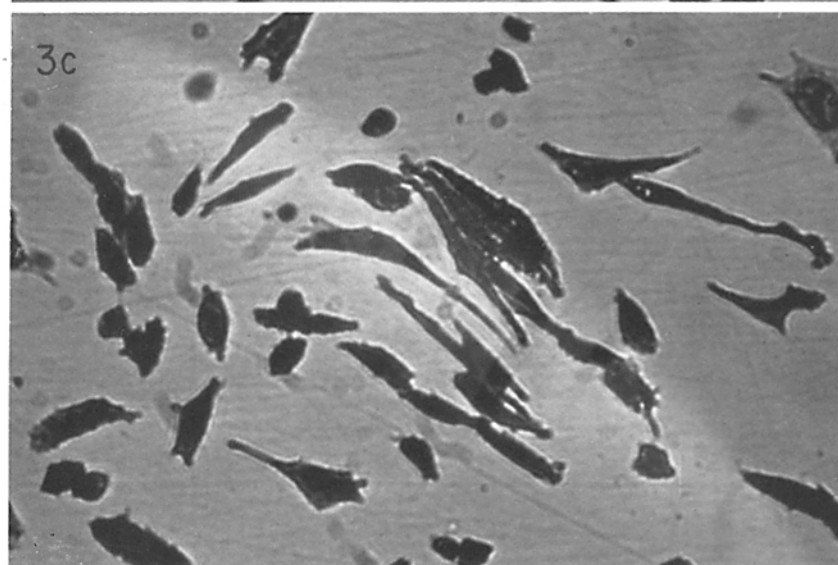
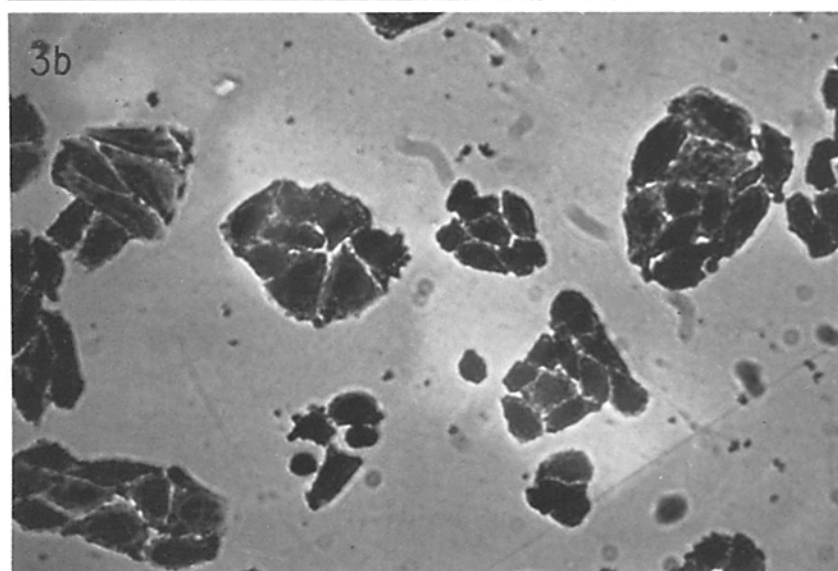
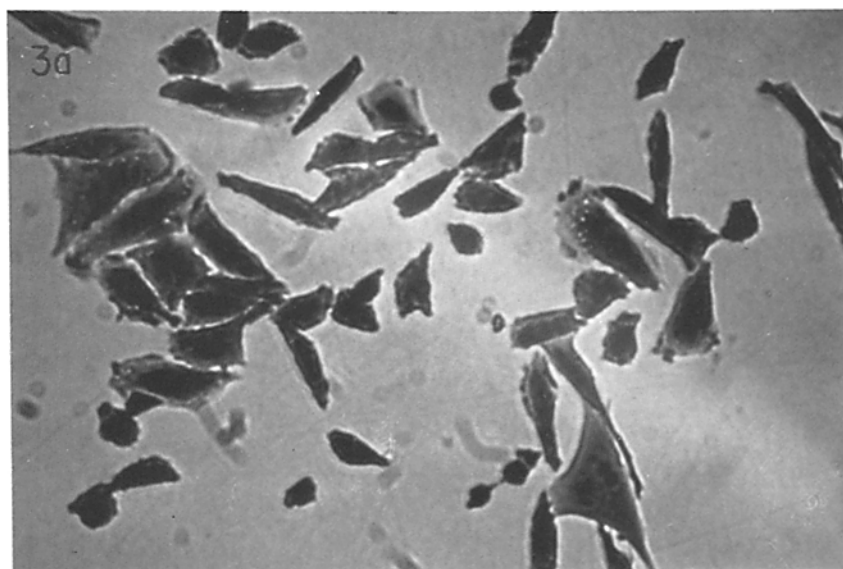
Cell Morphology

When wild-type Chinese hamster cells are plated at low density, they usually take on a bipolar or tripolar shape and appear to be fairly well spread on the substratum (Fig. 3). The drug resistant cells (C4S4 and C5S3) are usually more elongated and bipolar in shape than their wild-type counterparts and seem to resemble untransformed fibroblasts (note, however, that these cells still grow in suspension culture and are thus presumably “transformed”). The phytohemagglutinin resistant cells C2P1, on the other hand, have a less pronounced polar aspect, seem more rounded, and tend to form clusters.

Surface Labeling Patterns

As described previously (6) and shown in Fig. 4, the galactose oxidase- ^3H borohydride surface label technique coupled with polyacrylamide gel electrophoresis demonstrates the existence of three to four major classes of membrane glycoproteins in wild-type Chinese hamster cells, with the peaks of apparent mol wt 130,000, 100,000, and 60–65,000 being especially distinct. Drug resistant C4S4 and C5S3 cells display a prominent additional labeled peak in the molecular weight range of 165–175,000 (8) and have been shown to have an extra cell surface glycoprotein not found in wild-type cells (9).¹ Phytohemagglutinin resistant

¹ The prominent peak at 100,000 daltons and the very small peak at >200,000 are also somewhat more intensely labeled in C5S3 cells.



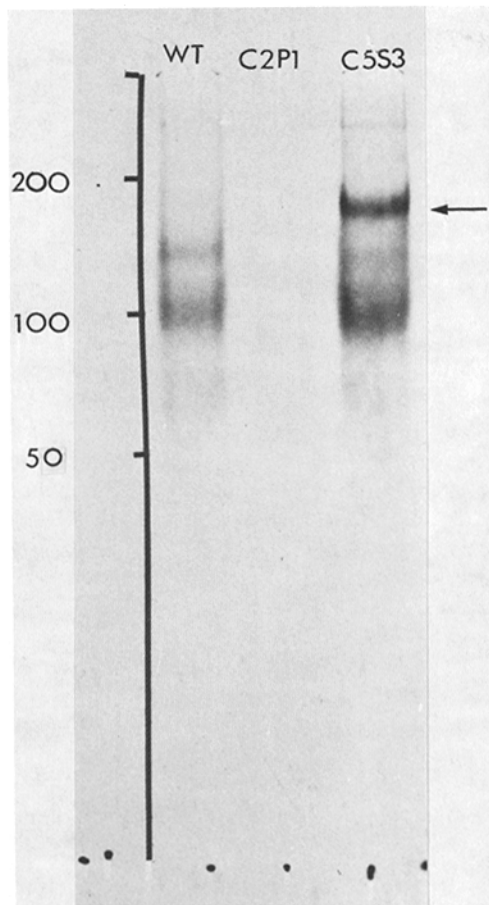


FIGURE 4 Surface Labeling Patterns of WT and Mutant Cells. Wild type WT, C2P1, and C5S3 cells were surface labeled with the galactose oxidase [^3H]borohydride technique as described in Materials and Methods. Plasma membranes from the labeled cells were analyzed by polyacrylamide slab gel electrophoresis in the presence of SDS followed by autoradiography. The patterns resulted from the application of equal amounts ($100\ \mu\text{g}$) of membrane protein for each sample. The extra surface glycoprotein in C5S3 is indicated by an arrow. (a) WT, $123\ \text{cpm}/\mu\text{g}$ protein, (b) C2P1, $44\ \text{cpm}/\mu\text{g}$ protein, and (c) C5S2, $198\ \text{cpm}/\mu\text{g}$ protein. The scale at left indicates the apparent mol wt $\times 10^{-3}$.

cells are labeled far less effectively than are wild-type cells (on the basis of incorporated radiolabel per milligram membrane protein, C2P1 cells are labeled about 10–30% as effectively as wild-type cells under the same experimental conditions), and C2P1 cells also show a strikingly altered gel pattern with little label corresponding to the major peaks seen in wild-type cells. The labeling pattern of C2P1 is typical for lectin resistant lines of this type and has been ascribed to the presence of truncated oligosaccharide side chains, deficient in galactose residues, on the cell-surface glycoproteins (10).

CONCLUSIONS

Our findings show that the clones of Chinese hamster cells which adhere to the substratum at almost identical rates, show distinct differences in the rates at which they are detached from the substratum by trypsin. These results provide additional support for the concept first formulated by Weiss, that cell attachment and detachment are distinct processes and should not be confused under the loose designation of cell adhesion (23). Our results also demonstrate that the correlation which is frequently made between cell shape and cell adhesiveness may not be valid. The extent to which cells spread out and flatten has been used as a rough index of their adhesiveness (4, 21). Thus the elongated bipolar appearance of untransformed fibroblasts is often contrasted with the rounded, less orientated appearance of their virally transformed counterparts, and frequently it is assumed that the transformed cells are less adhesive and that this may be related to loss of anchorage dependence of growth (18). Our results show that “adhesiveness,” at least as defined in terms of resistance to detachment with trypsin, is not directly correlated with the degree of elongation, but rather may depend on the chemical nature of the cell surface. Thus C5S3 and C4S4 cells which are quite elongated and fibroblastic in shape are detached by trypsin far more readily than C2P1 cells, even though these latter cells are

FIGURE 3 Morphology of WT and Mutant Cells. Wild-type and mutant (C2P1, C5S3) Chinese hamster cells were plated into tissue culture dishes (1×10^5 cells/40-mm dish) and cultured for 48 h. The cells were rinsed in PBS, fixed in PBS plus 1% glutaraldehyde, followed by fixation in methanol and staining with Giemsa. The cells were photographed using a Reichert photomicroscope. (a) WT, (b) C2P1, and (c) C5S3.

more rounded and not as well spread on the substratum.

The results seen in Fig. 4 and elsewhere (8, 9, 10) demonstrate that the Chinese hamster clones used in this study varied markedly in the patterns of their cell-surface glycoproteins. In spite of this, they attach to the substratum at the same rate suggesting that the terminal surface oligosaccharide moieties detected by galactose oxidase labeling do not play a direct role in the initial events of cell-to-substratum attachment. The case of C2P1 is particularly interesting since previous studies (20) have shown that variants of this type lack particular glycosyltransferases, and thus most, if not all, of their glycoproteins may be abnormal in that they lack *N*-acetyl-glucosamine, galactose, and sialic acid residues in the terminal positions of their oligosaccharide side chains. Weiss (24) has previously demonstrated that sialic acid does not play a role in substratum attachment, and current results suggest that galactose and possibly glucosamine are also not involved in the initial phase of cell attachment.

Although they do not differ in adhesion rates, the variant lines used in this study do display differences in morphology and in the rate at which they are detached by trypsin. Atherley et al. (1) have also studied a variant Chinese hamster line which is resistant to detachment by trypsin and have found that these cells are deficient, relative to wild type, in the amount of cellular hyaluronic acid and other complex carbohydrates. These results seem analogous to our findings with C2P1 which is deficient in terminal sugar residues and is resistant to detachment. However, the morphology of the detachment variants studied by Atherley et al. did not differ from wild type, whereas C2P1 clearly does differ. Another set of detachment variants (in BALB/C 3T3) have been studied by Pouysseur and Pastan (13). These lines were selected for rapid detachment by trypsin and the variant cells were shown, by the lactoperoxidase labeling technique, to lack certain high molecular weight surface proteins. The variant 3T3 cells were morphologically distinct from wild type, and in contrast to the Chinese hamster variants used in the present study, the variant 3T3 cells differed from wild type in their rate of reattachment to the substratum after dispersal by trypsinization. Recently it has been shown that these variant 3T3 cells also have a defect in the synthesis of surface glycoproteins (14).

Present findings taken with those from previous communications (1, 13) indicate that the ease of proteolytic detachment from the substratum can be affected by several types of cell surface alterations and thus seems a rather nonspecific aspect of cell behavior. It is interesting to note that in no case has any correlation been found between susceptibility to trypsin detachment and growth control properties (1, 13). Our results taken with those of other workers (1, 14) suggest that cell surface oligosaccharide moieties may modulate the susceptibility to proteolysis of membrane components involved in cell detachment. Thus cell lines rich in surface oligosaccharides such as C5S3 and C4S4 seem more susceptible to proteolytic detachment than WT cells, while C2P1, a line which is deficient in terminal saccharides, is more difficult to detach. There is considerable evidence that mild proteolysis does not degrade the cell-substratum junction per se, but rather that it involves an alteration of the cytoskeleton which leads to cell rounding and to the formation of retraction fibrils which eventually rupture and release the cell (15). It seems unlikely, however, the cell-surface carbohydrate moieties, whether in glycoproteins or on glycosaminoglycans, are directly involved in the initial bond formation with the substratum, since drastic differences in the amount and composition of the surface oligosaccharides are not reflected by differences in adhesion rates.

In apparent contrast to our results, another group studying ricin-resistant BHK cells, which may be similar in phenotype to C2P1, has recently found that the ricin-resistant lines adhere less well than wild-type cells (5). However, these adhesion studies, as well as those of Pouysseur and Pastan (13) in 3T3, were carried out on cells initially dispersed by trypsinization, while our studies employed suspension culture cells whose membranes were unperturbed. It is well known that proteolytic treatment can have marked effects on readhesion phenomena (3), perhaps creating adventitious differences between cell types.

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