by weight) than any other VSG studied. Third, one experiment was performed in which the 121 C-terminal precipitation-blocking glycopeptide was treated with *Streptomyces griseus* endoglycosidase H (Miles). After treatment, the C-terminal fragment was repurified by passage through a column of concanavalin A–Sepharose, on which it was no longer retained. After removal of the oligosaccharide side chain, blocking activity was not eliminated but was reduced 10-fold. This reduction could be due to conformational changes arising during the several repurification steps which were used after glycosidase treatment. The possible role of carbohydrate in cross-reactivity needs further investigation.

From the evidence of its susceptibility to proteolytic cleavage[6], the VSG C-terminal polypeptide region may be rather loosely folded. We must therefore also consider that slight denaturation may expose antigenic determinants in this area which are normally masked by the native configuration of the polypeptide.

It might reasonably be assumed that VSGs have evolved by gene duplication and mutation or are made by some kind of gene-splicing mechanism. Therefore, crossreactivity is perhaps not surprising. Another argument for some conservation of structure is that all VSGs must presumably attach to the plasma membrane in a similar manner. The crossreacting determinants are apparently masked on living trypanosomes[8] and so is the C-terminal region of the VSG[10]. These facts do not greatly encourage us to believe that crossreacting determinants could provide a basis for immunisation, although this possibility will undoubtedly be explored. The significance of crossreactivity must be clarified by immunochemical and amino acid sequence studies of a range of VSGs.

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Dexamethasone-induced adhesion in hepatoma cells: the role of plasminogen activator

TRANSFORMED CELLS are frequently less adhesive than normal cells. This decrease in adhesion has been associated with the loss of specific cell surface proteins, changes in morphology, alterations in the underlying cytoskeleton and the increased activity of the serine protease, plasminogen activator[1–3]. Hepatoma tissue culture (HTC) cells exhibit increased levels of adhesion to glass[4,5] and decreased plasminogen activator activity[6,7] when incubated in the presence of the synthetic glucocorticoid, dexamethasone. Plasminogen activator converts plasminogen in serum to plasmin. It has been shown that plasmin causes the reversible dissociation of intracellular actin-containing cables in anchorage-dependent rat embryo cells[8] and may also be partially responsible for decreased adhesion of Rous sarcoma virus-transformed chicken embryo fibroblasts[3]. Cell–substrate adhesion presumably requires specific cell surface glycoproteins and intact cytoskeletal elements[9,10]. It is possible that plasmin acts either to modify 'adhesive' proteins on the cell surface or to disrupt linkages between these surface components and the underlying cytoskeleton. We have used variant HTC cells selectively resistant to the dexamethasone inhibition of plasminogen activator[6] to test the hypothesis that hormonal induction of cell adhesion is secondary to the inhibition of plasminogen activator which in turn allows the accumulation of the glycoproteins necessary for adhesion. We have modified cell adhesion assay techniques[5,11] to provide a sensitive measure of cell–substrate adhesion for both wild-type and variant HTC cells in the presence and absence of serum and plasminogen. We report here that plasminogen activator does not have an important role in the hormonal regulation of HTC cell adhesiveness.

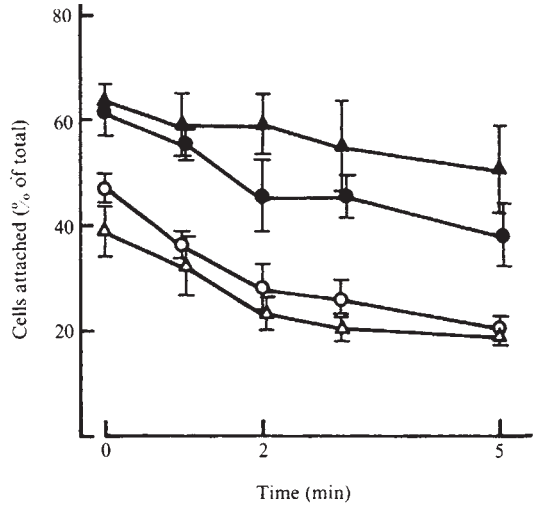

Fig. 1 Wild-type HTC cells were incubated overnight in radioactive medium containing 3% plasminogen-depleted fetal calf serum in the presence (●) or absence (○) of 0.1 μM dexamethasone, or in the same medium reconstituted with 4.8 μg ml^{-1} plasminogen in the presence (▲) or absence (△) of 0.1 μM dexamethasone. The assay conditions were the same as for cells incubated in serum-containing medium (see legend to Table 1). Plasminogen was obtained from outdated human plasma by affinity chromatography using lysine–Sepharose 4B (Pharmacia) followed by elution with ε-aminocaproic acid as described by Deutsch and Mertz[16]. Plasminogen-depleted serum was prepared by passing fetal calf serum through a lysine-Sepharose column and collecting the non-adsorbed fraction. This was acid treated to reduce the activity of protease inhibitors[17] and filter sterilised (0.45 μm pore size).

HTC cells were routinely grown in spinner culture without antibiotics in Eagle's minimal essential medium, modified to contain 2 mM glutamine, 5% each heat-inactivated fetal calf and calf serum (Grand Island Biological), 5.8 mM $NaHCO_3$ and 50 mM Tricine (Sigma). Variant HTC cell lines fully resistant to the dexamethasone inhibition of plasminogen activator were isolated using an agar–fibrin overlay technique to detect plasminogen-activator production by individual colonies of cells[6]. Glucocorticoid-receptor function was determined to be normal in variant cell lines on the basis of normal tyrosine aminotransferase induction by dexamethasone, assayed as described by Spencer and Gelehrter[12].

Wild-type HTC cells incubated for 18 h in serum-containing medium in the presence of 0.1 μM dexamethasone typically show a twofold increase in the per cent of cells remaining attached to a glass surface after 5 min of shaking, when compared to untreated cells (Table 1). After cells have been allowed to settle and attach, differences in adhesion between dexamethasone-treated and untreated cells are evident. In the presence of dexamethasone there are fewer loosely adherent cells initially, and those which remain after settling are more adherent than those in the untreated cell population. In the conditions of the assay, maximal detachment occurs within 2–5 min. We find that dexamethasone also induces adhesion in variant HTC cells which are resistant to the dexamethasone

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inhibition of plasminogen activator (Table 1). Although variant C cells are less adhesive than the wild-type HTC cells, there is a twofold increase in the adhesion of dexamethasone-treated cells incubated in the presence of serum. Two other HTC cell variants, A and B, which are also fully resistant to the dexamethasone inhibition of plasminogen activator, have similar dexamethasone-induced adhesion (data not shown). These results suggest that the mechanism by which dexamethasone increases cell-substrate adhesion does not depend on the inhibition of plasminogen activator.

When incubated in serum-free medium, wild-type HTC cells again show a twofold increase in adhesion in the presence of dexamethasone (Table 1). Variant C cells are less adhesive than the wild-type HTC cells in these conditions as well, but also show a twofold increase in adhesion when incubated in the presence of dexamethasone (Table 1). Likewise, variants A and B consistently exhibit dexamethasone-induced adhesion in the absence of serum. In both wild-type and variant HTC cells, the induction of adhesion by dexamethasone occurs to a similar extent in the presence and absence of serum.

We have also directly tested the effects of plasminogen, the substrate for plasminogen activator, on the dexamethasone induction of adhesion in wild-type and variant HTC cells in serum-free conditions. If the formation of plasmin from plasminogen prevents the accumulation of cell surface proteins required for adhesion, then the addition of plasminogen to serum-free medium should decrease or prevent dexamethasone induction of adhesion in the variant cell lines. Instead, we find that dexamethasone induces increased levels of adhesion in both wild-type and variant cells which have been incubated for 18 h in medium containing plasminogen (data not shown). This also suggests that plasminogen-activator activity does not modulate the adhesive response to dexamethasone.

Table 1 Dexamethasone induction of adhesion in HTC cells

		Per cent cells attached		
		0	2 min	5 min
+Serum (n)				
Wild type (4)	Dexamethasone	65±3	58±3	52±6
	Control	36±4	25±3	25±4
Variant C(4)	Dexamethasone	37±3	30±5	23±2
	Control	22±3	11±1	10±1
−Serum (n)				
Wild type (9)	Dexamethasone	79±1	72±2	67±3
	Control	60±5	38±4	31±4
Variant C (4)	Dexamethasone	65±5	54±6	46±6
	Control	47±10	23±6	20±4

Wild-type or variant HTC cells were collected from spinner culture during the logarithmic stage of growth. Cells were resuspended in medium containing 3% fetal calf serum, 0.1 μCi ml^{-1}, ^{3}H-amino acid mixture (NEN) and 50 μg ml^{-1} neomycin, in the presence or absence of 0.1 μM dexamethasone (Merck). After an 18-h incubation at 37 °C in a gyrotory shaker-waterbath at 80 r.p.m., the cells were centrifuged, and the cell pellet washed twice in nonradioactive medium and resuspended to a final density of 10^6 cells ml^{-1} in nonradioactive medium. Cell viability, as measured by trypan blue exclusion, was always greater than 90%. One-ml aliquots of the cell suspension were distributed into untreated borosilicate glass scintillation vials and incubated without shaking for 2 h at 37 °C. At the end of the incubation period, the cells completely covered the surface of the vial with little or no piling up of cells. The medium was removed and 1 ml calcium- and magnesium-free phosphate-buffered saline (PBS), pH 7.3, added carefully down the side of each vial. The vials were then subjected to a shearing force of 100 strokes min^{-1} in a Dubnoff reciprocating shaker-waterbath (5-cm stroke) at 37 °C. At 0, 2 and 5-min exposure to this reciprocating force, duplicate vials were removed and the PBS aspirated. Cells adhering to the glass scintillation vials were lysed with 0.1 ml 1 M KOH and the lysate neutralised with 0.1 ml 1 M HCl. Radioactivity was assayed in a scintillation spectrometer after addition of 5 ml ACS scintillation fluid (Amersham–Searle). In control experiments not shown, cells were removed from the surface of vials by trypsin following the 5-min shaking period. These cells were viable by the criterion of trypan blue exclusion and the cell counts correlated with direct measurements of radioactivity in each vial. Radioactivity of the initial cell inoculum was determined from 1-ml aliquots of the cell suspension which were centrifuged, the cell pellets lysed in 0.1 ml 1 M KOH and the lysate neutralised in 0.1 ml 1 M HCl. The per cent of initial labelled cell inoculum remaining after shaking (average of duplicate vials) has been used as a measure of cell adhesiveness. The data presented represent the mean ± s.e.m. calculated from several experiments. In experiments carried out in serum-free medium (supplemented with 0.1% BSA), the assay was modified to include a 1-h incubation period for cell attachment and a reciprocating force of 140 strokes min^{-1}.

In conditions in which plasminogen is the only serum factor missing, wild-type cells exhibit a twofold increase in adhesion in the presence of dexamethasone (Fig. 1). When cells are incubated for 18 h in medium containing depleted serum which has been reconstituted with plasminogen, a similar, although somewhat greater, increase in adhesion is measured in dexamethasone-treated cells. In the absence of dexamethasone, cell adhesion is the same whether the medium contains plasminogen-depleted or reconstituted serum. These results also suggest that plasminogen-activator production does not have a major role in cell–substrate adhesion.

Note that the optimal assay conditions differ in the presence and absence of serum. When serum is present, cell–substrate adhesion may depend on interactions between the cell surface and serum proteins which are adsorbed to the glass[13]. This might be relevant to our observation that HTC cells incubated in serum-containing medium require a longer time to form stable adhesive bonds to a substrate than do cells in serum-free medium containing bovine serum albumin (BSA). It has been proposed that cell surface proteases on BHK cells modify substrate-adsorbed proteins during the process of cell adhesion[14]. Although other cell surface proteases may be involved in cell–substrate adhesion, the nearly identical adhesiveness of untreated HTC cells in fetal calf serum, plasminogen-depleted serum and reconstituted serum provides additional evidence which tends to rule out any important role for plasminogen activator or plasmin in this process.

Recently, a class of dexamethasone-induced proteins, tentatively described as sialogylcoprotein on the basis of electrophoretic properties in a two-dimensional gel system, has been localised on the surface of HTC cells[15]. It is possible that such proteins could confer increased adhesive properties on hormone-treated cells. Our data suggest that dexamethasone regulation of cell adhesion in HTC cells probably operates primarily through the synthesis of surface glycoproteins and that this process is not, in turn, regulated by the inhibition of plasminogen-activator activity.

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