Fibronectin-independent Adhesion of Fibroblasts to the Extracellular Matrix: Mediation by a High Molecular Weight Membrane Glycoprotein

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ABSTRACT Fibroblastic CHO cells readily adhere to fibronectin (Fn) coated substrata. From the parental cell population we have recently selected a series of adhesion variants (AD^v cells) that cannot adhere to Fn substrata (Harper and Juliano. 1980. *J. Cell. Biol.* 87:755–763). However, AD^v cells readily adhere to substrata coated with extracellular matrix material (ECM) derived from human diploid fibroblasts by a mechanism that does not involve fibronectin (Harper and Juliano. 1981. *Nature (Lond.)*. 290:136–138). The Fn-dependent adhesion mechanism of parental cells (type I adhesion) and the ECM-dependent adhesion of AD^v cells (type II adhesion) can also be discriminated on the basis of their differential sensitivity to proteolysis, with the type II mechanism being far more sensitive. In this communication we report that parental CHO cells possess both type I and type II mechanisms whereas AD^v cells possess only the type II mechanism. We also identify a high molecular weight membrane glycoprotein (gp 265) that seems to play a role in type II adhesion. This component is detected by [^{125}I]lactoperoxidase or [^{3}H]borohydride-galactose oxidase labeling of surface proteins in WT and AD^v cells. Cleavage of gp 265 with low doses of proteases correlates completely with the loss of type II adhesion capacity. Thus CHO cells possess two functionally and biochemically distinct adhesion mechanisms, one involving exogenous Fn and the other mediated by the membrane component gp 265.

Fibroblasts can interact with each other and also with the components of the extracellular matrix (ECM) in which they are situated. These components are many and varied in nature and include glycosaminoglycans, collagens, and glycoproteins. The ability of a fibroblast to function within this environment depends on the ability of the cell to make and break adhesive connections with the matrix. Most studies of the mechanisms involved in fibroblast adhesion have stressed that role of fibronectin (Fn), a pericellular protein that appears to be localized at points of cell-cell or cell-substratum contact and which can interact with collagen, glycosaminoglycans, or fibrinogen (5, 18, 21, 30, 34). However, the question of the possible involvement of other membrane components in adhesion is only now being explored. Recently cell surface proteins other than fibronectin have been implicated in cell-substratum (32) and cell-cell (26, 29) adhesion in fibroblasts, whereas in epithelial cells, laminin rather than fibronectin seems to play the role of an adhesion promoting macromolecule (27). These studies indicate that several distinct macromolecules can be involved in adhesion in various cell types.

CHO cells are a line of transformed fibroblasts that have an absolute requirement for exogenous fibronectin to attach to a collagen substratum (6, 33). We have recently described a series of CHO cell variants (termed AD^v) that are completely defective in their ability to adhere to Fn-coated collagen or other Fn-coated substrata. However, AD^v cells will attach to substrata coated with multivalent ligands such as concanavalin A (Con A) or poly-L-lysine. The nonadherent phenotype does not appear to be the result of a cytoskeletal defect because the cells assume a normal CHO morphology when attached and spread on Con A, and moreover are capable of the complex cytoskeletal events involved in lectin induced patching and capping (6).

Subsequently, we have observed that AD^v cells readily adhere to the ECM material derived from cultures of human diploid fibroblasts (HDFs). This ECM consists of components

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adsorbed onto the substratum from the medium and of cellular material that remains attached to the substratum after the HDFs are removed by the chelation of divalent ions. Material of this type has also been termed SAM or substrate attached material; extensive biochemical analyses of SAM from mouse fibroblasts have been published by Culp and his colleagues (16, 24), who have shown the presence of glycosaminoglycans, fibronectin, actin, and lipid in SAM. In a previous communication (7) we have reported that AD^v cell adhesion to ECM does not appear to involve Fn. Thus, (*a*) relatively high concentrations of exogenous Fn will not promote attachment of the AD^v cells to ECM-coated substrata (nor to other substrata); (*b*) anti-Fn antiserum, which blocks parental cell adhesion to Fn-collagen, has no effect on AD^v adhesion to ECM; (*c*) adhesion of AD^v cells to the ECM is far more trypsin sensitive than the adhesion of parental cells to Fn-collagen. Based on these observations, we suggested that there are two mechanisms for CHO cell adhesion, one of which is mediated by Fn and is exemplified by WT adhesion to Fn-collagen (type I mechanism), the other of which is independent of Fn and is exemplified by AD^v cell adhesion to ECM (type II mechanism).

In this communication we further characterize the mechanism of AD^v cell adhesion to the ECM. We show that the type II adhesion mechanism is not unique to the AD^v cells, but is also expressed by the WT cells. In addition, using surface labeling techniques and selective proteolysis, we explore the membrane biochemistry of the cells relevant to ECM adhesion. We have found a high molecular weight cell surface glycoprotein (gp 265), clearly distinct from Fn, whose presence appears to correlate with the ability to adhere to the ECM. In this communication we focus on the role of cellular mechanisms in adhesion to ECM; in a forthcoming report we will deal with the characteristics of the ECM that are involved in type II adhesion (Harper and Juliano, manuscript in preparation). Cell interactions with the ECM may be important in cell growth control (3), in ontogeny (4), and in metastasis (13) and thus clearly deserve careful study.

MATERIALS AND METHODS

Cells

The growth and maintenance of parental (WT) and nonadherent variant (AD^{v}F11), Chinese hamster ovary cells have been fully described elsewhere (6). HDFs were maintained in modified Eagle's medium (MEM) plus 15% fetal calf serum (FCS). Cells of the 5th–10th passage were used in experiments.

Adhesion Assay

The preparation of substrata and the adhesion assay have previously been described in detail (6). Briefly, aliquots of washed ^{3}H-labeled, suspension-adapted WT or AD^{v}F11 cells in α-MEM + 1 mg/cm^3 bovine albumin were added to tissue culture dishes coated with one of the various substrata and then usually incubated at 37°C (but sometimes at 4°C or 21°C). At various times, the dishes were removed from the incubator, washed three times with PBS (phosphate buffered saline pH 7.2), thereby removing the unattached cells, and the fraction of radioactivity remaining on the dish from the attached cells was determined. The substrata used in these studies included culture dishes coated with (*a*) CSP (cell surface protein), kindly donated by Dr. K. Yamada, National Institutes of Health, (*b*) CIG, (cold insoluble globulin) affinity purified by the method of Engvall and Ruoslahti (2), (*c*) serum (10% fetal calf serum in MEM-α-medium), or (*d*) ECM (substratum attached material derived from HDFs by the method of Rollins and Culp [24]). In some instances the cells or substrata were treated in various ways before the adhesion assay. For tests of proteolytic sensitivity the cells were incubated for 10 min at room temperature with various concentrations of trypsin (2 × crystallized, Sigma Chemical Co., St. Louis, Mo.) or thrombin (2000 U/mg, Sigma Chemical Co.). Proteolytic digestion was terminated by the addition of a large volume of cold PBS plus 10 mg/cm^3 bovine albumin followed by three washes in this buffer. For tests of sensitivity to cytoskeletal inhibitors the cells were preincubated for 10 min at room temperature with 25 μg/cm^3 cytochalasin D or 10^{-3}, 10^{-4} M tetracaine. In all cases of pretreatment, cell viability was >85% as determined by trypan blue exclusion. In some experiments the ECM substrata were incubated with rabbit antihuman CIG serum (courtesy Dr. R. Rajaraman, Dalhousie University, Nova Scotia, Canada) for 90 min at room temperature, followed by thorough washing with PBS. The pretreated cells or substrata were used in the adhesion assay in the usual manner. For studies of protease-treated cells (and their controls) adhesion assays were usually conducted for only 30 min so as to minimize the possibility of resynthesis or re-expression of cleaved surface components.

Cell-Surface Radiolabeling

The surface proteins of whole cells were radiolabeled via lactoperoxidase catalysed incorporation of ^{125}I into tyrosine residues or by [^{3}H]borohydride reduction of galactosyl residues following neuroaminidase and galactose oxidase treatment, as previously described (9). Appropriate controls, omitting any of the enzymes resulted in at least a tenfold decrease in the incorporation of radiolabel. These labeling procedures did not result in cell-cell aggregation or in cell death as determined by trypan blue exclusion. After radiolabeling, aliquots of the cells were sometimes further processed by treatment with various concentrations of trypsin or thrombin as described above. In some cases a plasma membrane enriched fraction was prepared using a two-phase separation procedure; the characteristics of CHO cell membranes prepared in this way have been fully documented elsewhere (10). Cells and membranes were solubilized with boiling 0.2% SDS and prepared for polyacrylamide gel electrophoresis.

Gel Electrophoresis

The distribution of radiolabeled proteins was determined by slab polyacrylamide gel electrophoresis (PAGE) in the presence of SDS according to Laemmli (14). Usually acrylamide gradients of 5–15% or 5–10% were used, but gels consisting of 3.0% to 4.0% acrylamide stabilized with 0.38% linear polyacrylamide were used to resolve the high molecular weight range. In all cases the samples were prepared in solubilizer and ~40–60 μg of protein per lane was loaded onto the gel; the gels were stained with Coomasie Brilliant Blue to visualize the proteins, the position of the molecular weight standards were marked with India ink, and the gels were prepared for autoradiography. Lactoperoxidase ^{125}I-labeled gels were dried and radioautographed directly in cassettes containing DuPont Cronex image enhancers (DuPont Instruments, Wilmington, Del.). The [^{3}H]borohydride-galactose oxidase labeled gels were destained, impregnated with diphenyloxazole (PPO), dried, and autoradiographed according to the method of Laskey and Mills (15). To prevent the gradient gels from cracking during the drying procedure, 20% glycerol was added during the final dehydration step. The molecular weight markers used included ribonuclease (RNA-12,000), chrymotrypsinogen (CTN-24,000), ovalbumin (OA-43,000), bovine albumin (BSA-68,000), phosphorylase A (PSA-95,000), β-RNA polymerase subunits (POL-155, 165,000), myosin (MYO-200,000), human or bovine cold insoluble globulin (CIG-220,000), chick cell surface fibronectin (CSP-235,000), and filamin (FIL-250,000) (obtained from Dr. P. J. A. Davies, The University of Texas Medical School).

RESULTS

Characteristics of WT and AD^{v}F11 Cell Adhesion

We have already described (6, 7) that AD^v cells such as clone F11 fail to adhere to Fn-coated substrata but will adhere to ECM-coated substrata. By contrast WT cells readily adhere to both types of substrata. This behavior is further documented in Table I; here we also show that neither the serum form (CIG) nor the cellular form (CSP) of Fn will promote F11 attachment. Although the adhesion processes of AD^{v}F11 and WT are dissimilar in terms of the role of Fn, they are very much alike in terms of sensitivity to temperature or to cytoskeletal inhibitors. Thus low temperature (4°C) or tetracaine (10^{-3} M) can effectively block the adhesion of either WT or AD^{v}F11 cells to ECM (Table II) just as they can block the adhesion of WT cells to Fn-coated substrata (11). As reported previously (7) adhesion of AD^{v}F11 cells to ECM substrata is exquisitely sensitive to proteolytic treatment of the cells, whereas WT adhesion to Fn substrata is quite insensitive to proteolysis. This is further documented in Fig. 1. Thus, there

TABLE I

Adhesion of WT and AD^v Cells to Different Substrata

Substratum	Attachment WT	AD^{v}F11
	%	
ECM	100	100
CSP (50 μg/ml)	100	5
Clg (50 μg/ml)	88	7
FCS (10%)	86	2

Adhesion of ^{3}H-labeled WT and AD^v F11 to tissue culture dishes coated with the above mentioned materials was performed for 60 min at 37°C as described in Materials and Methods. The results represent the means of triplicate determinations differing by not >10%.

TABLE II

Perturbation of Adhesion to ECM

Treatment of Cells		Attachment WT	AD^v F11
		%	
PBS at	37°C	100	100
	21°C	75.1 ± 6.2	39.9 ± 18.3
	4°C	4.0 ± 1.3	14.3 ± 8.2
PBS at	37°C		
plus Tetracaine	10^{-3} M	39.9 ± 3.2	15.6 ± 2.6
	10^{-4} M	50.2 ± 6.1	50.1 ± 11.6

Adhesion of WT and AD^v F11 cells to tissue culture dishes coated with ECM material was performed for 60 min as described in Materials and Methods. These results represent the means and standard errors of triplicate determinations.

is a 40% reduction in AD^{v}F11 adhesion to ECM after incubation of cells with 2 μg/cm^3 trypsin. By contrast 200 μg/cm^3 trypsin reduced WT adhesion to Fn by <20%. The effect of proteolysis on WT adhesion to ECM is similar to the effect on WT adhesion to Fn (Fig. 1). This may be a reflection of the heterogenous nature of the ECM which probably consists, in part, of Fn (CIG) derived from the serum present in culture medium.

These various observations, namely (*a*) the inability of the AD^{v}F11 cells to use Fn as an adhesive ligand, (*b*) the failure of attachment of F11 to the ECM to be blocked by an anti-Fn antiserum (7), and (*c*) the large difference in proteolytic sensitivity between ECM and Fn-mediated adhesion, suggest that AD^{v}F11 attachment to ECM and WT attachment to Fn are mediated by two distinct mechanisms. The membrane components involved in these two mechanisms should be readily distinguishable on the basis of their differential proteolytic sensitivity, with the cell surface components involved in type I adhesion (Fn dependent) being trypsin insensitive relative to the surface components involved in type II adhesion (ECM-dependent).

Presence of the Trypsin-sensitive (type II) Mechanism in WT Cells

The studies described above do not allow one to determine if the type II adhesion mechanism (ECM-mediated, trypsin-sensitive) pre-existed in the parental cell population but was more readily detected in the AD^v cells because of loss of the type I mechanism (Fn-mediated, trypsin-insensitive), or if the mutagenesis and selection procedure for the AD^v cells caused the de novo appearance of the type II mechanism in these cells. In an attempt to distinguish between these two possibilities, we reasoned that if the type II as well as the type I mechanism were expressed by WT cells, then WT cells could attach to the ECM substratum by either mechanism. Thus, treatment of WT with low concentrations of trypsin (<10 μg/ml) sufficient to abolish AD^{v}F11 attachment, would have no apparent effect on WT adhesion to the ECM because the type I mechanism would remain intact. If however, the Fn in the ECM was made unavailable, for example by masking with an anti-Fn antibody, then WT cells could attach only via non-Fn-mediated mechanisms (type II) and should exhibit a proteolytic sensitivity similar to that of AD^{v}F11 attachment. We have tested this hypothesis and results can be seen in Fig. 2. The adhesion of AD^{v}F11 cells to ECM is drastically reduced when the cells are treated with low doses of trypsin, whereas the adhesion of WT cells to ECM or Fn substrata is essentially unperturbed. If however, the ECM substrata are pretreated with anti-Fn antisera, the adhesion of WT cells becomes extremely sensitive to trypsin and, as in the case of AD^{v}F11 cells, 10 μg/ml of trypsin reduces the adhesion of WT by 90%. Pretreatment of the ECM substratum with an anti-Fn antisera has no effect on AD^{v}F11 adhesion; however, the antiserum will completely block WT adhesion to Fn-collagen substrata (7). These data support the concept that the highly trypsin-sensitive mechanism mediating AD^v cell attachment to ECM (type II) is also present in the WT cells, but has not been previously observed because of the simultaneous presence of the trypsin-insensitive (type I) mechanism that mediates attachment to Fn.

Radiolabeling of Cell Surface Proteins

To identify the cell surface components involved in type II adhesion we attempted to correlate the effect of proteolysis on

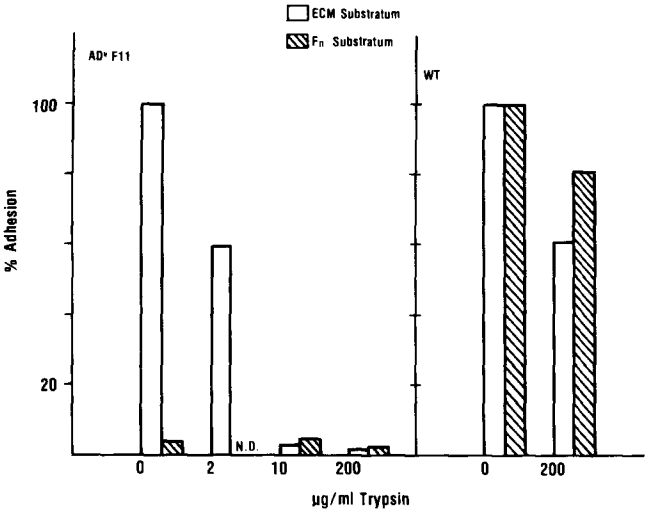

FIGURE 1 Proteolytic sensitivity of adhesion. *WT* or *AD^{v}F11* cells prelabeled with [^{3}H]leucine were treated with various doses (0–200 μg/ml) of trypsin for 10 min at room temperature; the proteolysis was stopped by the addition of a large excess of cold PBS plus 10 mg/ml BSA. After extensive washing, the treated cells were given the opportunity to attach to tissue culture dishes coated with ECM (derived from confluent HDFs) or Fn (from 10% FCS). The adhesion assay was performed for 30 min at 37°C. The results represent the means of triplicate or quadruplicate determinations and are normalized on the basis of adhesion of untreated WT or F11 to ECM being set equal to 100%. In absolute terms, WT adhesion to ECM or FN substrata usually ranges from 80–100% total cells, while F11 adhesion to ECM ranges from 40–70%, during 30 min in the absence of protease treatment.

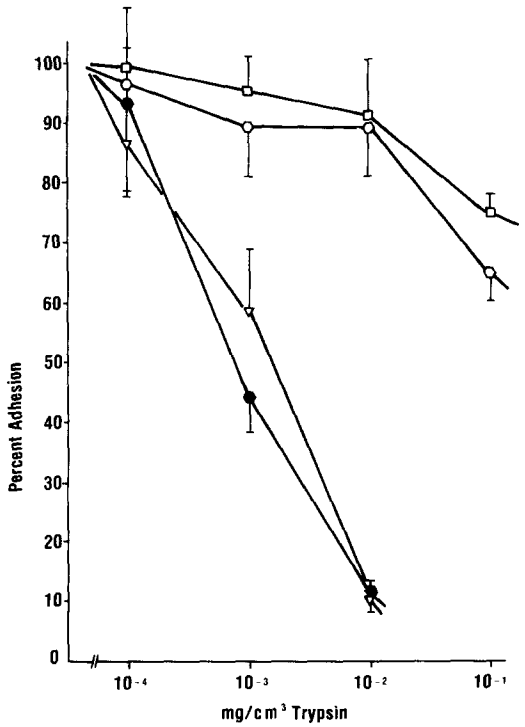

FIGURE 2 The Effect of anti-Fn antisera on the adhesion of trypsin treated WT or AD^{v}F11 cells to ECM or Fn substrata. Suspension adapted ^{3}H labeled WT and AD^{v}F11 cells were treated with PBS or with varying concentrations of trypsin for 10 min at room temperature. After extensive washing in PBS and BSA the cells were assayed for their ability to adhere to Fn or ECM substrata as described in Materials and Methods. In some instances the substratum was preincubated with anti FN-antisera before the adhesion assay as described (7). The adhesion assay was for 30 min. The results are the means ± SD of at least three determinations where the control value for adhesion of untreated WT or F11 cells was normalized to 100%. WT on FN (□), WT on ECM (○), WT on ECM pretreated with anti Fn (●), AD^{v}F11 on ECM (▽).

adhesion behavior with changes in the pattern of cell surface proteins. Because both cells lines express the trypsin-sensitive type II mechanism, any candidate components that are identified should be present in both WT and AD^{v}F11 cells and should demonstrate the same proteolytic sensitivity as the functional adhesion assay.

Fig. 3 *a* depicts the pattern obtained after Coomassie Brilliant Blue staining of the total cell protein, run on a 5–15% acrylamide gradient. There are no apparent differences between WT and AD^{v}F11 and incubation with graded concentrations of trypsin (WT, lanes *1*, *6*, *9*, AD^{v}F11, lanes *2–5*) does not result in any change in pattern. This is not unexpected because the bulk of the Coomassie Blue stained components would derive from intracellular material. Figure 3 *b* however, shows the pattern obtained from the autoradiogram of a similar gel where the cell surface glycoproteins were labeled via the [^{3}H]borohydride-galactose oxidase technique. The most striking feature of the gel is the degradation and eventual disappearance of a high mol wt (265,000) glycoprotein component (gp 265). This component appears to be unaffected when the cells are incubated with control buffer or 0.1 μg/ml trypsin (WT, lanes *1*, *6*, *7*; AD^{v}F11, lanes *2* and *3*) but is degraded to a slightly lower (250,000) mol wt (WT, lane *8*; AD^{v}F11, lane *4*) with 1.0 μg/ml trypsin and is absent at 10 μg/ml trypsin (WT, lane *9*; AD^{v}F11, lane *5*). The trypsin concentrations that appear to drastically affect the appearance of this high molecular weight glycoprotein correlate well with loss of adhesion to ECM in response to trypsin (see Fig. 2). There do not appear to be any other major changes in the pattern of [^{3}H]borohydride-galactose oxidase labeled material due to trypsinization in the 0–10 μg/ml range.

We also examined the effects of proteolytic treatment on the [^{125}I]lactoperoxidase surface labeling patterns. An autoradiogram of a 5–15% gel of lactoperoxidase-labeled cells is seen in Fig. 4 *a*. The 1-D gel labeling patterns for WT and AD^{v}F11 cells are very similar and both contain a high mol wt (265,000) component corresponding to the one visualized by galactose oxidase labeling; however, the 265,000 mol wt component labels less well with lactoperoxidase than with galactose oxidase. As in the case of the galactose oxidase pattern (Fig. 3) the ^{125}I-labeled 265,000 mol wt component is cleaved to a slightly lower molecular weight by 1 μg/ml trypsin (lanes *2*, *4* for WT and *7*, *9* for AD^{v}F11) and is eliminated by 10 μg/ml trypsin (lanes *5* for WT, *10* for AD^{v}F11). Treatment with 10 μg/ml trypsin also results in loss of components with apparent mol wt of 145,000 (this is better visualized on a 3.5% gel) and 12,000, and a general reduction in labeling intensity. Plasma-membrane-enriched preparations from WT and AD^{v}F11 cells (Fig. 4 *b*) also displayed the 265,000 mol wt radioiodinated surface component; however the 250,000 mol wt component was also present, perhaps as a result of endogenous protease action during membrane preparation. The high molecular weight trypsin-sensitive component migrated slightly more slowly than filamin and considerably more slowly than CSP or CIG on gradient gels or on 3.5% polyacrylamide-linear polyacrylamide gels (not shown).

Thrombin Effects on Adhesion and Surface Labeling Patterns

In a previous report we briefly noted that treatment with 10 U/ml of purified thrombin could impair the ability of AD^{v}F11 cells to adhere to ECM material (7). We have now investigated the effect of different doses of thrombin on both cell attachment and on surface labeling patterns. As seen in Fig. 5 *a* treatment with 10 U/cm^{3} of thrombin results in an ~40% decrease in the adhesion of AD^{v}F11 cells to ECM, and that increasing the dose up to 100 U results only in a small further decrease. By contrast, thrombin treatment at 10 or 100 U/cm^{3} had absolutely no effect on WT cell adhesion to ECM or to Fn coated substrata. Fig. 5 *b* displays an autoradiogram of a 4% gel of [^{3}H]borohydride-galactose oxidase surface labeled AD^{v}F11 cells. Controls clearly reveal the 265,000 mol wt component (gp 265); cells treated with 100 U/cm^{3} thrombin show that the 265,000 mol wt component has been cleaved to 250,000 and that the amount of label in the 250,000 mol wt component is somewhat reduced relative to the 265,000 mol wt component in controls; otherwise no other changes are apparent in the gel patterns. Thus thrombin is highly selective in affecting the 265,000–250,000 mol wt components.

DISCUSSION

The data presented above, together with our previous observations (6, 7) clearly demonstrate that CHO cells possess at least two distinct mechanisms for adhesion to the substratum. The mechanism we have designated type I requires exogenous fibronectin to be present on the substratum, is relatively insensitive to proteolytic treatment of the CHO cell surface, and is

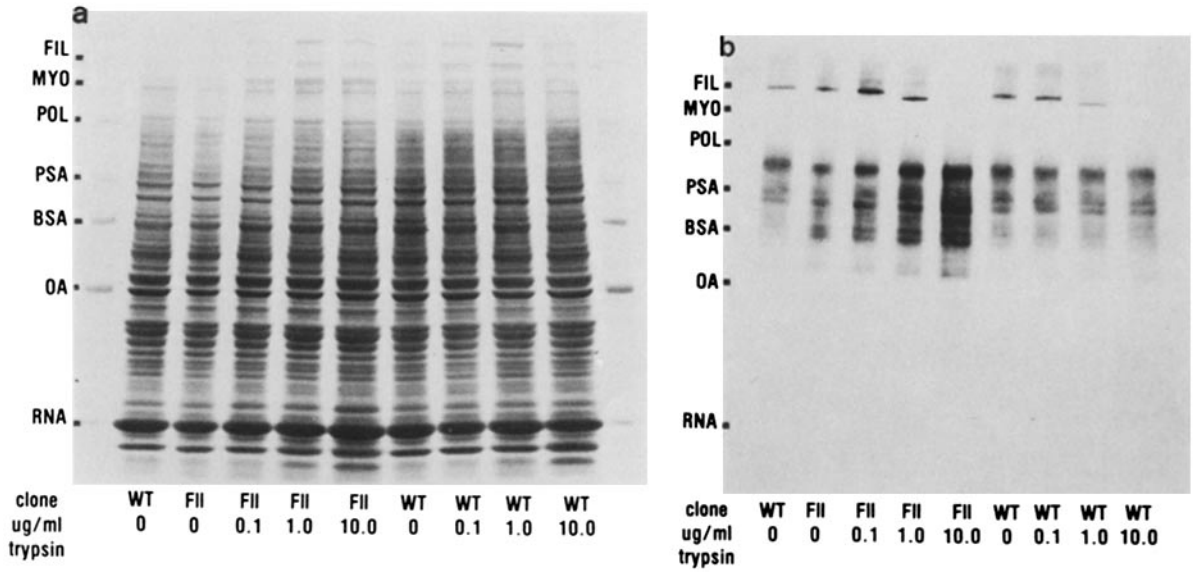

FIGURE 3 Polyacrylamide gel electrophoresis of control and trypsin treated ^{3}H-surface labeled WT and AD^{v} cells. Aliquots of [^{3}H]borohydride-galactose oxidase labeled WT and AD^{v}F11 cells (~60 μg protein and 24,000 cpm) were applied to 5–15% gradient polyacrylamide gels. Before solubilization both WT and AD^{v}F11 cells were treated with PBS or with graded amounts of trypsin for 10 min at room temperature as described in Materials and Methods. The gels were run and stained for protein with Coomassie Brilliant Blue, the position of the standards marked, and then further processed for autoradiography. (*a*) Coomassie Brilliant Blue patterns from WT and AD^{v}F11 cells. (*b*) Autoradiograms of ^{3}H-labeled glycoproteins of AD^{v} or WT cells. WT treated with PBS, lanes *1* and *6*; 0.1 μg/ml trypsin, lane *7*; 1.0 μg/ml trypsin, lane *8*; 10.0 μg/ml trypsin, lane *9*; AD^{v}F11 cells treated with PBS lane *2*; 0.1 μg/ml trypsin lane *3*; 1.0 μg/ml and trypsin lane *4*; 10.0 μg/ml trypsin, lane *5*.

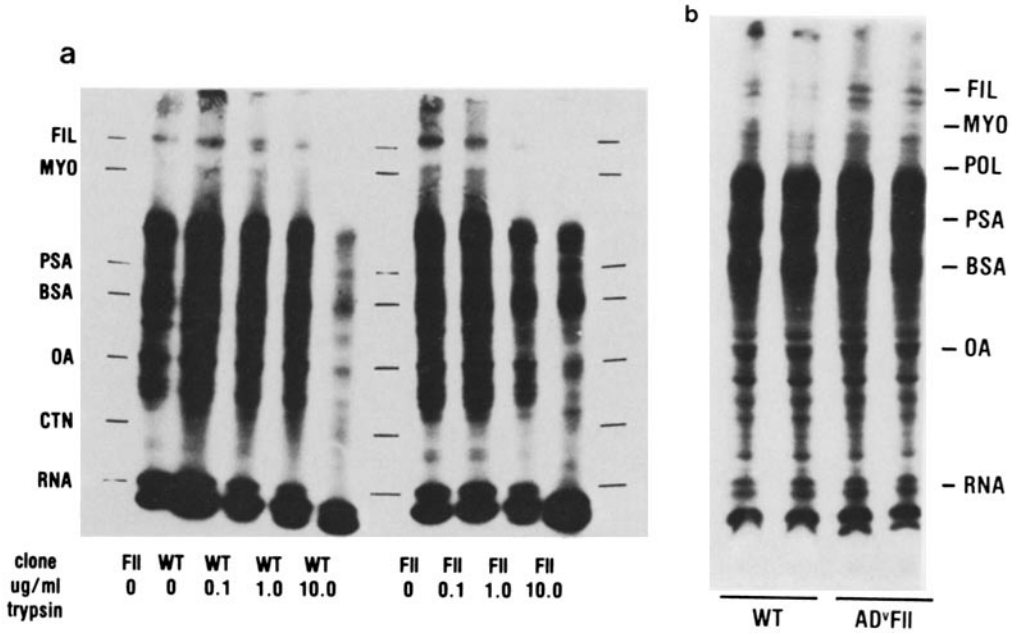

FIGURE 4 Polyacrylamide gel electrophoresis of control and trypsin treated ^{125}I lactoperoxidase labeled WT and AD^{v} cells. WT and AD^{v}F11 were surface labeled via the ^{125}I-lactoperoxidase method were, in some cases, further treated with trypsin as described in Materials and Methods and then solubilized, analyzed on gels and the gels developed for autoradiography. ~30 μg protein and 50,000 cpm were used per lane. (*a*) Effects of trypsin treatment on the ^{125}I autoradiogram. The clone and dose of trypsin used are indicated on the figure. This is an autoradiogram of a 5–15% gradient gel. As reported previously (9) the major ^{125}I-lactoperoxidase labeled proteins are in the 50–130,000 dalton range; this region of the autoradiogram is over exposed so as to bring out the higher molecular weight region. Shorter exposures of the same gel did not reveal changes in the labeling pattern in the 50–130,000 dalton range for cells exposed to 0.1 or 1.0 μg/cm^{3} trypsin. (*b*) ^{125}I autoradiogram of plasma membrane material. A membrane enriched fraction was prepared from ^{125}I surface labeled WT and F11 cells as described in Materials and Methods. The membrane fractions were analyzed on a 5–10% gel and prepared for autoradiography as above.

present in WT cells but not in AD^{v} variants. The mechanism we have designated type II requires as yet unidentified components of ECM material, is exquisitely sensitive to proteolysis, and is present in both WT and AD^{v} cells. Both mechanisms of adhesion are similar with respect to inhibition caused by low temperature or by cytoskeletal blocking agents. The presence of the type II mechanism in WT cells can be confirmed by treatment of the ECM substratum with anti-Fn serum that

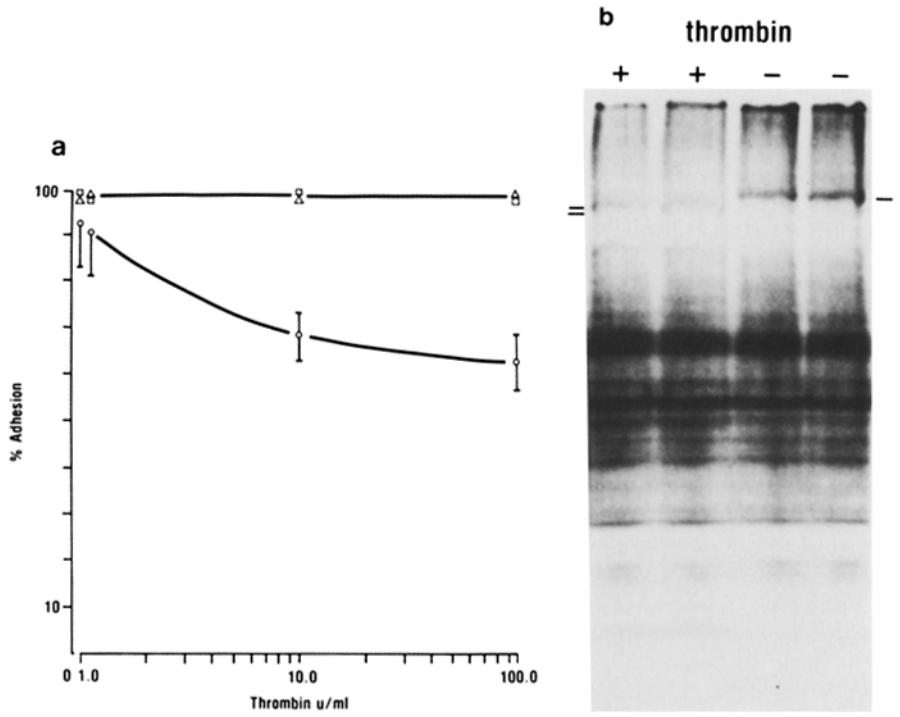

FIGURE 5 Effects of thrombin on the adhesion and ^3H surface labeling patterns of WT and AD^{v}F11 cells. (*a*) [^3H]leucine labeled WT or F11 cells were treated with various doses of thrombin as described in Materials and Methods. Adhesion to ECM or Fn coated substrata during 60 min at 37°C was determined as described. F11 adhesion to ECM (○). WT adhesion to ECM (△). WT adhesion to Fn (□). (*b*) Galactose oxidase [^3H]borohydride labeled AD^{v}F11 cells were treated with 100 U thrombin and analyzed by gel electrophoresis and autoradiographed as described in Materials and Methods. The position of gp 265 is indicated by a single marking and the position of gp 250 by a double marking. The autoradiogram shown is from a 5–10% gradient gel.

effectively blocks adhesion mediated via the type I mechanism.

The extreme proteolytic sensitivity of the type II mechanism has allowed us to tentatively identify a cell surface component involved in this mechanism. The high molecular weight glycoprotein we have designated gp 265 seems the most promising candidate as a mediator of type II adhesion. Upon treatment of WT or AD^v cells with trypsin in the range of 0.1 → 1 $\mu g/cm^3$, gp 265 is cleaved to a form with slightly lower apparent molecular weight (gp 250); this change is correlated with a 40–50% reduction in type II adhesion to ECM. Treatment with 1–10 $\mu g/cm^3$ trypsin results in the complete loss of gp 265 and gp 250; in addition, other nongalactoprotein surface components of apparent mol wt 145,000 and 12,000 are cleaved, and a general reduction in labeling intensity is observed; these changes in surface components are paralleled by a total inhibition of type II adhesion to ECM. Treatment of the cells with 10–100 U of thrombin results in the complete cleavage of gp 265 to gp 250 and in a 40% inhibition of type II adhesion to ECM. The observed changes in type II adhesion and surface labeling patterns are consistent with the concept that gp 265 mediates type II adhesion, that its effectiveness is diminished upon cleavage to gp 250, and that total loss of these components abolishes type II adhesion.

At the point we cannot completely rule out a role for other surface components in type II adhesion. Proteolytic cleavage of the 145,000 or 12,000 mol wt components seems to require doses of trypsin larger than those needed to produce substantial (50%) impairment of the type II mechanism, but the possible role of these components cannot be dismissed. It is interesting to note that other investigators have identified a 150,000 mol wt trypsin-sensitive surface component that seems to play a role in cell-cell adhesion of hamster fibroblasts (26), and may correspond to our 145,000 mol wt component. Another consideration is that protease sensitive surface components may be embedded in dense regions of the autoradiograms where their loss upon trypsinization would not be readily detected, although shorter exposure of the autoradiograms, producing lighter intensities in the 40,000–180,000 mol wt range do not reveal marked changes in this region in response to trypsin doses <10.0 $\mu g/cm^3$. With these cautionary notes in mind, we feel that the gp 265 component is an excellent candidate for a role in type II adhesion.

We had observed gp 265 in previous studies of the CHO cell surface (12) but had always assumed that it was a form of cellular fibronectin. It now seems likely that gp 265 is distinct from fibronectin for the following reasons; (*a*) gp 265 does not comigrate with fibronectin from diverse sources including human or bovine plasma (CIG) and chick cell membranes (CSP); (*b*) it seems unlikely that CHO cells have a substantial amount of surface Fn because several workers have reported that it is not possible to visualize surface fibronectin in untreated CHO cells by immunofluorescence (19), and complement mediated lysis of CHO cells does not occur in the presence of anti-Fn antibody (17); (*c*) CHO cells are completely dependent upon exogenously added Fn for promotion of adhesion to collagen and thus seem to lack functional endogenous fibronectin (6, 33). In a similar vein, it is unlikely that gp 265 is laminin, another high molecular weight pericellular protein (8, 28), because (*a*) the gp 265 band does not correspond to the 200,000 or 400,000 mol wt subunits of laminin, and (*b*) laminin has been reported to promote the adhesion of epithelial cells but not of CHO cells ((27). Thus, gp 265 seems to be distinct from previously described macromolecules having adhesion-promoting activity. It remains to be seen whether molecules such

as Fn, laminin, and gp 265 comprise a class of cellular effector molecules with different but related structures and functions, such as is the case with the immunoglobulins. Recently Noonan and his colleagues (25) have described a trypsin sensitive, Con A binding protein of mol wt 265,000 in the CHO cell membrane; this component is distinct from fibronectin. We are currently exploring the possibility that the 265,000 mol wt component described by Noonan's group and our gp 265 are the same, and serve as the cell membrane mediator of type II adhesion.

The existence, in a single cell type, of more than one mechanism for adhesion should not be surprising. Fibroblasts may need to interact with a number of components of the extracellular matrix and may have several mechanisms for doing so. Thus, multiple adhesion mechanisms may be more the rule than the exception, and different mechanisms may prevail in different circumstances. For example, multiple mechanisms of adhesion have recently been identified in mammalian hepatocytes (20) and in avian embryonic cells (1). The relative physiological importance of the type I and type II adhesion mechanisms is difficult to evaluate at this point. Clearly the type II process is weaker because the kinetics of adhesion are somewhat slower and the degree of cell spreading is less (7). However the type II process is certainly sufficient to promote firm attachment of most of the F11 cells (60–90%) during 1 h, and may play an important role in circumstances or environments where type I (Fn dependent) adhesion is impaired. It has recently become apparent that many different types of macromolecules can serve as adhesion promoting factors. Thus, cell to substratum adhesion can be promoted by exogenous lectins (6, 22), cellular lectins (20, 31), exogenous or endogenous glycosyl transferases (22, 23), as well as by Fn or laminin (34, 27). The common elements relating these various macromolecular ligands seem to be their capacity to form multivalent bonds with the cell surface and their ability to mobilize the cytoskeleton. Thus there are probably a number of macromolecular ligands capable of mediating different adhesion processes in vitro. The identification of the physiologically relevant adhesion processes, their biochemical bases, and their roles in tissue organization, ontogeny, and neoplasia promises to be an exciting field of study.

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