Synergism between Membrane Gangliosides and Arg-Gly-Asp-directed Glycoprotein Receptors in Attachment to Matrix Proteins by Melanoma Cells

Gordon F. Burns,* Carolyn M. Lucas,* Geoffrey W. Krissansen,* Jerome A. Werkmeister,; Denis B. Scanlon,§ Richard J. Simpson,§ and Mathew A. Vadas*

*Division of Human Immunology, Institute of Medical and Veterinary Science, Adelaide, South Australia 5000, Australia; Commonwealth Scientific and Industrial Research Organization, Division of Protein Chemistry, Parkville, Victoria 3050, Australia; Joint Protein Structure Laboratory, Ludwig Institute for Cancer Research (Melbourne Branch) and the Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria 3050, Australia

Abstract. The identification of specific cell surface glycoprotein receptors for Arg-Gly-Asp-containing extracellular matrix proteins such as fibronectin has focused attention on the role of gangliosides in this process. Is their involvement dependent or independent of the protein receptors? In attachment assays with cells from a human melanoma cell line, titration experiments with an antibody (Mel 3) with specificity for the disialogangliosides GD2 and GD3, used together with a synthetic peptide containing the cell binding sequence Arg-Gly-Asp, show that their joint effect is synergistic. Both the Mel 3 antibody and the synthetic peptide individually cause rapid detachment of melanoma cells from fibronectin substrate but, when used together, much smaller concentrations of both are required to achieve the same effect. The Mel 3 antibody was not nonspecifically reducing receptor binding to the Arg-Gly-Asp sequence since, in binding assays with radiolabeled peptide performed with cells in suspension, very little peptide is bound by the melanoma cells under these conditions but addition of Mel 3, an antibody of IgM isotype, causes a two- to threefold increase in specific binding. The simplest interpretation of these data is that the Mel 3 antibody is causing sufficient clustering of membrane gangliosides in local areas and producing a favorably charged environment to facilitate peptide binding by specific glycoprotein receptors.

An understanding of cell attachment to extracellular matrix proteins will provide insights into such processes as cell migration and spreading, and tissue invasion by tumor cells. For this reason, the mechanisms by which different cells attach to fibronectin have been much studied and it is now clear that more than one interaction is involved. Gangliosides have been suggested as candidate receptors for fibronectin since their addition to cultures caused rounding up and detachment of cells from the fibronectin substrate (24, 29, 47). Also, the insertion of gangliosides into the membranes of ganglioside-deficient cells enabled them to bind fibronectin on the cell surface (40), and mAbs directed against determinants on the carbohydrates of the gangliosides GD2 and GD3 (41) prevented the attachment of melanoma cells to extracellular matrix proteins including fibronectin (12, 45).

In another approach, Pierschbacher and colleagues identified a peptide within the cell-binding domain of fibronectin that mediated the cell attachment activity of this molecule (31–33), and Hayman et al. (18) and Yamada and Kennedy (46) went on to demonstrate that peptides containing the sequence arginyl-glycyl-aspartic acid (Arg-Gly-Asp) caused detachment of cultured cells from their substratum and prevented baby hamster kidney and Chinese hamster ovary cells from spreading on fibronectin substrates. From this information, Pytela et al. (35) were able to identify a specific glycoprotein receptor of ~140 kD on osteosarcoma cells and rat fibroblasts. It is now apparent that the fibronectin receptor thus isolated is but one of a family of structurally similar proteins that bind to Arg-Gly-Asp sequences contained within a variety of extracellular matrix proteins (reviewed in references 21 and 37).

Within the fibronectin molecule there is an additional domain that also appears to be involved in cell attachment. This domain is located towards the carboxy-terminal end of the A chain, which contains heparin binding activity (27), and high levels of Arg-Gly-Asp in solution have little effect on cells adhering to fragments containing this heparin-binding domain (27). Binding of this fragment, however, did not promote melanoma cell motility (27), indicating that cell binding to different domains of the fibronectin molecule may result in different functions. The relationship between the ganglioside "receptor" and other receptors is uncertain. The negative charges on the sialic acid moieties of GD2 and GD3 could
bind fibronectin by interacting with the clusters of positively charged residues present in the type III homologies of the fibronectin molecule (30, 39). Alternatively, Chesh and Klier (10) have suggested that, for optimal cell adhesion, the active redistribution of gangliosides into discrete areas of cell attachment may cause synergy with cell surface receptors by creating an appropriate electrostatic environment.

The present study presents evidence supporting this concept. We show that an mAb that binds to GD2 and GD3 inhibits attachment and, when used in relatively high concentrations, causes detachment of melanoma cells from fibronectin substrates. At low concentrations the antibody acts synergistically with a synthetic peptide containing the Arg-Gly-Asp sequence such that concentrations of antibody or peptide, which individually had no effect on attachment, together caused rapid loss of adhesion by the melanoma cells. In addition, we used a synthetic peptide containing the Arg-Gly-Asp sequence and a radiolabeled tyrosine residue to demonstrate that melanoma cells in suspension bound only weakly to the peptide. However, when the cells were reacted with the IgM mAb with specificity for GD2/GD3, this binding was increased severalfold. We favor the interpretation that the IgM antibody against GD2/GD3 was causing localized clustering (patching) of the gangliosides to provide favorable areas for attachment of the peptide to specific glycoprotein receptors.

Materials and Methods

Cell Cultures

The melanoma cell lines used in this study have been maintained in our laboratory for several years and have been demonstrated to be free of mycoplasma contamination. The LiBr line was maintained in RPMI 1640 medium supplemented with 10% heat-inactivated FCS (Flow Laboratories, Irvine, Scotland), glutamine (2 mM), 2-mercaptoethanol (3 × 10⁻⁴ M), penicillin (100 μg/ml), and streptomycin (100 μg/ml). The cells were regularly passaged at subconfluence by detaching the adherent cells by vigorous treatment with 0.1 mg/ml for 2 h at 37°C and the wells were washed twice with PBS and twice with medium. LiBr melanoma cells from subconfluent cultures were resuspended in RPMI 1640 medium containing 0.2% BSA (Sigma Chemical Co.) to 10⁶ cells/ml. To each of the fibronectin-coated wells was added 100 μl of the cell suspension together with the antibody or peptide being tested (10 μl) and the plates were incubated for 2 h at 37°C in 5% CO₂ before assay. Detachment assays were performed in essentially the same way, but in these tests the cells were incubated alone for 2 h at 37°C to enable attachment, then the agents under test were added in a volume of 10 μl and the cells incubated for a further 30 min.

Preparation of Synthetic Peptides and Radiolabeled Peptides

Fibronectin was purified from human plasma by affinity chromatography on gelatin-Sepharose 4B (Pharmacia Fine Chemicals, Inc., Uppsala, Sweden) as described by Russolati et al. (30); the purity and yield were then determined by SDS-PAGE with Coomassie Blue staining and by a protein assay (Bio-Rad Laboratories). The fibronectin was added to the wells of flat-bottomed microtiter plates (Flow Laboratories, Inc., McLean, VA) at 0.1 mg/ml for 2 h at 25°C and the wells were washed twice with PBS and twice with medium. LiBr melanoma cells from subconfluent cultures were resuspended in RPMI 1640 medium containing 0.2% BSA (Sigma Chemical Co.) to 10⁶ cells/ml. To each of the fibronectin-coated wells was added 100 μl of the cell suspension together with the antibody or peptide being tested (10 μl) and the plates were incubated for 2 h at 37°C in 5% CO₂ before assay. Detachment assays were performed in essentially the same way, but in these tests the cells were incubated alone for 2 h at 37°C to enable attachment, then the agents under test were added in a volume of 10 μl and the cells incubated for a further 30 min.

Attachment and Detachment Assays

Fibronectin was purified from human plasma by affinity chromatography on gelatin-Sepharose 4B (Pharmacia Fine Chemicals, Inc., Uppsala, Sweden) as described by Russolati et al. (30); the purity and yield were then determined by SDS-PAGE with Coomassie Blue staining and by a protein assay (Bio-Rad Laboratories). The fibronectin was added to the wells of flat-bottomed microtiter plates (Flow Laboratories, Inc., McLean, VA) at 0.1 mg/ml for 2 h at 25°C and the wells were washed twice with PBS and twice with medium. LiBr melanoma cells from subconfluent cultures were resuspended in RPMI 1640 medium containing 0.2% BSA (Sigma Chemical Co.) to 10⁶ cells/ml. To each of the fibronectin-coated wells was added 100 μl of the cell suspension together with the antibody or peptide being tested (10 μl) and the plates were incubated for 2 h at 37°C in 5% CO₂ before assay. Detachment assays were performed in essentially the same way, but in these tests the cells were incubated alone for 2 h at 37°C to enable attachment, then the agents under test were added in a volume of 10 μl and the cells incubated for a further 30 min.

Radioligand Binding Assay

LiBr cells from subconfluent cultures were harvested, washed, and resuspended in cold incubation buffer (Hanks' balanced salt solution [HBSS], 1.6 mM CaCl₂, 10 mM Na₂HPO₄, 5 μg/ml cytochalasin B, 0.1% BSA), pH 7.2, to 4 × 10⁵ cells/100 μl. 100 μl suspended cells were incubated at 4°C, 2 h with 10 μM ¹²⁵I-LiBr (10 mM GRGDSPE; ± 1 mM GRGDSP) in the presence or absence of the mAb under test. In a series of preliminary experiments, various concentrations of Mel 3 or control antibodies were preincubated with the cells for various times on ice, at room temperature, or at 37°C before performing the binding assay (see Results). Bound ligand was separated from free by filtration through GF/C filters (Whatman, Inc., Clifton, NJ) presoaked for 18 h in 20% BSA (20% milk powder in PBS), using a filtration manifold (Millipore Corp., Bedford, MA). The filters were then washed extensively with HBSS and counted for cpm in a gamma counter (Packard Instrument Co., Downers Grove, IL). In every assay, each value was calculated as

Preparation of Synthetic Peptides and Radiolabeled Peptides

The peptides GRGDSP, KYGRGDSP, and GRGESP were synthesized on a peptide synthesizer (model 430A; Applied Biosystems, Inc., Foster City, CA) by a modified Merrifield Boc solid-phase peptide chemistry procedure (2). On completion of each synthesis, the crude peptide was cleaved from the resin support by treatment with hydrofluoric acid/95% liquid HF/95% anisole, 0°C for 30 min and purified by gel-permeation chromatography on a Bio-Gel P2 column (1 x 100 cm) in 0.5 M aqueous acetic acid.

The peptide KYGRGDSP (150 μg) was mixed with 1 μCi ¹²⁵I and incubated at room temperature for 12 min with Iodobeads as described by the manufacturer. The free ¹²⁵I was fractionated from bound ¹²⁵I on a 35-ml Bio-Gel P2 Column (Bio-Rad Laboratories, Richmond, CA) equilibrated with 25 mM NaOH. The fractions containing radiolabel were further purified by reverse-phase HPLC on a gradient system (model 334; Beckman Instruments, Inc., Fullerton, CA) coupled to a variable wavelength detector (model 163; Beckman Instruments, Inc.) set at 220 nm and a radioisotope detector (model 170; Beckman Instruments, Inc.) connected in series. Samples were chromatographed on an Ultrasphere C18 column (4.6 x 150 mm) with a gradient from 10 to 50% acetonitrile in phosphate buffer (10 mM, pH 7.0) over a duration of 10 min at a flow rate of 1 ml/min. The retention time of unlabeled peptide was 8.5 min, and that of the labeled peptide 12 min.
specific binding by subtracting the mean of duplicates performed in the presence of excess cold peptide from the mean of triplicates without cold; the SD of replicates was generally <10% of the specific binding. An excess of the control peptide GRGESP did not reduce the binding of 125I-GRGDSP.

**Immunoprecipitation and Western Blot Analysis**

To analyze receptor proteins coprecipitated by the Mel 3 antibody that specifically binds gangliosides, 106 LiBr melanoma cells were lysed in Triton X-100 buffer and the clarified supernatant quantitated for protein content. A 50-μl volume containing 100 μg of protein was incubated overnight at 4°C with 200 μl Sepharose 4B beads covalently coupled to the Mel 3 antibody. The beads were washed three times in PBS and boiled in non-reducing buffer. The supernatant was then boiled in reducing buffer and subjected to electrophoresis in 7.5% SDS-polyacrylamide gels; 100 μg of total lysate was run in an adjacent track. At the completion of the run the immunoprecipitated proteins and total proteins were electrophoretically transferred to nitrocellulose paper, a strip containing the high molecular weight markers (Bio-Rad Laboratories) excised for Coomassie Blue staining, and the remaining tracks probed with rabbit antibodies (diluted 1:1,000) specific for the IIIα β chain of the vitronectin receptor on melanoma cells. Bound antibody was detected by an indirect enzymoassay using horseradish peroxidase as described previously (25).

**Results**

**The Effects of Anti-GD2/GD3 and of Synthetic Fibronectin Peptides Are Synergistic in Preventing Attachment and Inducing Detachment by Melanoma Cells**

The mAb Mel 3 behaved in a similar way to other anti-GD2 and anti-GD3 antibodies in preventing the attachment of melanoma cells to fibronectin-coated substrates, and also to dishes coated with laminin, fibronogen, or thrombospondin (Lucas, C. M., unpublished observations). In addition, it was found that Mel 3 caused rapid detachment of melanoma cells that were already attached to fibronectin (data not shown). It has been shown by others that the synthetic peptide GRGDSP, but not GRGESP, prevents spreading on and causes detachment from fibronectin substrate by a number of human and mouse cell types (18, 46). This was shown also to be the case for the human melanoma cell line LiBr. We found that GRGDSP at 200 μg/ml (300 μM) specifically prevented 50% of newly plated cells from attaching to fibronectin, and at 50 μg/ml (75 μM) specifically caused detachment of 50% of cells that had been allowed to attach to a fibronectin substratum for 2 h before addition of GRGDSP or the control peptide (Figs. 1 and 2). The above experiments present data from trials carried out with cells in serum-free medium supplemented with 2 mg/ml BSA. Essentially similar data were obtained in the presence of 5 or 10% FCS but higher concentrations of mAb or peptide were required and the majority of experiments were performed with defined serum-free medium.

Used alone, Mel 3 prevented attachment to a titer of 1:30 ascites and in separate experiments it was found that 100 μg/ml of purified antibody caused half-maximal inhibition (not shown). In the presence of GRGDSP at 100 μg/ml, a concentration which by itself caused no inhibition of attachment, half-maximal inhibition was obtained at a dilution of 1:160 of ascites (Fig. 1). The same synergistic effect between Mel 3 and GRGDSP was seen in detachment assays (Fig. 2).

**Ganglioside Codistributed with the Vitronectin Receptor Does Not Inhibit Binding of the Synthetic Peptide GRGDSP**

When melanoma cells are allowed to attach and spread onto plastic or fibronectin substrate, there is a rapid redistribution of the complex gangliosides, and GD2, at least, becomes preferentially localized into microprocesses that make direct contact with the fibronectin substrate (10). We have confirmed this observation at the level of light microscopy by staining with Mel 3, and have shown that this antigen is also left behind in focal attachment processes after removal of the cells from the coverslip (data not shown). In addition, Cheresh et al. (13) have shown that the vitronectin receptor on human melanoma cells exists as a functional complex with a GD2 ganglioside; thus, the antigens were demonstrated to colocalize in focal adhesion plaques as demonstrated by double-label transmission EM and indirect immunofluorescence, and purification of the vitronectin receptor on affinity columns caused copurification of GD2. We have found that the Mel 3 antigen is also physically associated with the vitronectin receptor on LiBr melanoma cells by immunoprecipitating LiBr lysates with Mel 3 and probing Western blots of the immunoprecipitates with rabbit antibodies to the vitronectin receptor. Moreover, such specific physi-

Figure 1. Inhibition of LiBr cell attachment to a fibronectin substratum in the presence of various dilutions of Mel 3 ascites fluid with increasing concentrations of GRGDSP peptide. Cells in serum-free medium were incubated for 2 h in the presence of Mel 3 antibody at the dilution shown or with an antibody to 2-microglobulin (None). Nonattached cells were washed away, and stained residual cells quantitated at an OD of 570 nm. Cell attachment quantitated by the measurement of OD was converted to percent inhibition calculated from controls.
The Journal of Cell Biology, Volume 107, 1988

3 antibody is causing clustering of the gangliosides, and that some such areas of high ganglioside concentrations would coincide with or be specifically associated with Arg-Gly-Asp-directed protein receptors and effectively mimic the redistribution of gangliosides into substrate-associated microprocesses seen upon cell attachment to fibronectin.

**Discussion**

Since the discovery of specific glycoprotein cell surface receptors for extracellular matrix proteins (6, 34, 35), the role of gangliosides has undergone a reappraisal. In the present study we present data substantiating the suggestion (10, 12) that gangliosides do not bind directly to fibronectin but facilitate specific receptor–ligand binding at points of attachment.

The anti–ganglioside antibody used, Mel 3, binds to both GD2 and GD3 (17) and might therefore be expected to be very effective in preventing melanoma cell attachment, since antibodies individually directed against GD2 or GD3 are additive in their effect (12). Nevertheless, when used together with the synthetic peptide GRGDSP in detachment assays, the effect on melanoma cell attachment was clearly synergistic. These data suggest that gangliosides and the specific receptors for the cell attachment sequence of fibronectin are operating at distinct but related levels in the attachment process. In this regard, it is of interest that, in similar assays performed with the CA2 antibody which identifies a glycoprotein molecule and also prevents cell attachment to fibronectin (5), the addition of synthetic peptide was additive whereas the mAbs CA2 and Mel 3 were synergistic in causing detachment (Burns, G. F., and C. M. Lucas, unpublished observations). Radiolabeled peptide binding assays carried out with melanoma cells in suspension indicated a possible mechanism of action for the gangliosides. We have shown previously (45) that melanoma cells maintained in suspension exhibit reduced levels of gangliosides on their surface membrane. As they reattach, the cells redistribute the gangliosides into the microprocesses that make direct contact with extracellular matrix proteins (10) and these are also laid down in the adhesion plaque (11). Treatment of melanoma cells with anti–ganglioside antibodies prevents this redistribution of gangliosides to the basal surface causing the micro-

---

**Figure 2.** Detachment of LiBr cells from a fibronectin substratum by Mel 3 and GRGDSP peptide. Cells were incubated for 2 h in serum-free medium to enable attachment and various concentrations of Mel 3 ascites and GRGDSP were added for a further 30-min incubation. Attached cells were quantitated as in Fig. 1 legend and percent detachment calculated from controls.

**Table I. Inhibition of Binding of Iodinated Synthetic Peptide KYRGDGP to Melanoma Cells in Suspension by Different Antibodies**

| Pretreatment of cells | Specific cpm* |
|-----------------------|---------------|
| Untreated             | 637 ± 330 (280–966) |
| Anti-β2 microglobulin | 494 ± 380 (244–934) |
| Mel 3                 | 2,661 ± 1,796 (1,104–5,452) |
| CA2                   | 69 ± 6 (62–74) |

Melanoma cells from the LiBr line were harvested from subconfluent cultures maintained in RPMI medium supplemented with 10% FCS. The harvested cells were washed and 4 × 10⁶ cells in 100 μl medium mixed with 10 μl of 1:10 ascites of the antibody under test in incubation buffer (BSS, 1.6 mM CaCl₂, 10 mM NaCl, 5 μg/ml cytochalasin B, 0.1% BSA). Cells with antibody were incubated at 37°C for 15 min, and cooled on ice; and 10 μM (~150,000 cpm) ³¹P-GRGDSP was added to each tube ± 1 nM unlabeled GRGDSP; and binding was allowed to occur for 180 min on ice. Specific cpm were determined as described in Materials and Methods. The results are tabulated from four independent experiments.

* Mean ± SD. Numbers in parentheses represent the range of the results.
processes to detach and the cells to cluster (11). Cheresh et al. (12) have recently demonstrated that anti–ganglioside antibodies effectively prevented the attachment of melanoma cells to solid substrates of synthetic hexapeptides containing the Arg-Gly-Asp sequence, and that such peptides were not bound directly by melanoma gangliosides separated on thin layer chromatograms. The same group (12) also found that mAbs directed to gangliosides on tumor cells failed to induce significant capping of the antigen. We have observed, however, that certain antigens are rapidly synthesized as they are capped, and that capping is masked by the constant redistribution of newly produced antigen unless cyclohexamide is included during the capping process (Burns, G. F., unpublished observations), and we have found that the Mel 3 antibody caused prominent clustering of the surface gangliosides. Hence, the simplest interpretation of our binding data is that the anti–ganglioside antibody, which is of IgM iso-
type, causes sufficient clustering of gangliosides in localized areas to produce artificial processes with an electrostatic environment conducive to ligand binding. Because such binding is not localized to microprocesses on the basal surface of the cell, attachment as such does not ensue, but we would predict that other events consequent upon specific ligand binding (42) would be observed. We are currently attempting to isotype switch the Mel 3 hybridoma to determine whether IgG antibodies with the same specificity require further cross-linking to produce the same effect.

The physical relationship between the ganglioside GD2 and Arg-Gly-Asp–directed receptors on the surface of melanoma cells has recently been the subject of study by Cheresh et al. (13). Immunostaining with antibodies to the vitronectin receptor and to GD2 revealed association of the two antigens, and affinity purification of the vitronectin receptor in the presence of Ca++ enabled copurification of GD2. We have confirmed these results by demonstrating biochemical coas-
sociation after immunopurification in the opposite direction, whereby immunoprecipitation of GD2 and GD3 by the Mel 3 antibody coprecipitated vitronectin chain antigen as detected by Western blotting (data not shown). That the ganglioside–receptor complex is stable on the cell membrane was illustrated in two-color fluorescent staining studies, where it was shown that ganglioside clustering induced by the Mel 3 antibody also caused colocalization of the vitronectin receptor. Hence, both components of the complex appear to migrate together within the membrane, perhaps suggesting that the fluidity of the membrane gangliosides may provide a mechanism for the rapid redistribution of receptor proteins during attachment. The formation of such ganglioside–receptor clusters induced by Mel 3 would also explain the increased attachment of synthetic peptide seen with melanoma cells maintained in suspension.

The role of gangliosides is clearly of more general significance than the binding of extracellular matrix proteins. The mAb, Mel 3, inhibited cytolysis of melanoma cells by cytotoxic lymphocytes by preventing conjugate formation (44), perhaps indicating a role for gangliosides in cell–cell adhesive processes; in support of this rat macrophages have a membrane receptor for gangliosides (36). Also, while the Mel 3 antibody alone had no effect on cell viability, it inhibited by 50% colony formation in agar by anchorage-de-
pendent melanoma cells (Burns, G. F., and J. A. Werkmeis-
ter, unpublished observations), an event which is unaffected by Arg-Gly-Asp–containing peptides (20). Gangliosides have also been implicated in a number of cell types in such diverse receptor-mediated processes as the binding of various growth factors (4, 23) and the binding of lipopolysaccharide (8), vari-
ous bacterial toxins (15, 43), viruses (26), thyroid stimula-
ting hormone (1), transferrin (28), and interferon (3). With the identification of specific glycoprotein receptors for many of these factors, it is now becoming apparent that the role of gangliosides is that of an accessory molecule to facilitate binding or to modulate receptor function rather than func-
tioning themselves as specific receptors. Whether or not the gangliosides simply confer a charged environment conducive to ligand binding or are involved in the regulation of bio-
chemical events such as receptor phosphorylation (9) awaits further study.

The present report in describing synergy between an mAb to gangliosides and synthetic peptides containing the cell-binding region of fibronectin in preventing melanoma cell at-
tachment has immediate practical implications. It has been

known for some time that preventing cells from adhering and spreading can interfere with their growth in vitro (16).

Houghton et al. (19) reported some success in treating pa-
tients with malignant melanoma with an antibody to the gan-
glioside GD3. Attempts at treatment with other anti–mela-
noma mAbs of different specificity have been less successful, and it was suggested that the peculiar success of this antibody could be attributable to the fact that it was of IgG3 isotype and particularly effective in mediating complement- and cell-
mediated cytotoxicity (19). This interpretation was made less tenable by a recent report (22) of treatment with a human mAb to GD2 inducing regression of cutaneous malignant melanoma since the antibody used was of IgM isotype and would not mediate cell-mediated cytotoxicity. A comple-
ment-mediated effect for these antibodies remains a possibil-
ity but it is probably significant that the only recorded suc-
cesses in this area are with anti–ganglioside antibodies. In support of alterations in the adhesive properties of melanoma cells being responsible for successful treatment is the finding that injection of the synthetic peptide GRGDS together with murine melanoma cells dramatically inhibited the formation of experimental lung metastasis in mice (20). Our findings reported here suggest that future strategies should incor-
porate treatment with anti–ganglioside mAb and peptide given together.

The authors wish to thank Dr. Michael C. Berndt for providing the rabbit antiserum to platelet IIb–IIIa, and Mrs. Mari Walker for excellent secretar-
ial assistance in preparing the manuscript.

This work was supported by grants from the National Health and Medical Research Council (Australia) and the Anti-Cancer Foundation of South Australia.

Received for publication 18 August 1987, and in revised form 19 May 1988.

References
1. Aloj, S. M., G. Lee, E. Consiglio, S. Formisano, A. P. Minton, and L. D. Kohn. 1979. Dansylated thyrotropin as a probe of hormone-receptor in-

teractions. J. Biol. Chem. 254:9030–9039.
2. Barany, G., and R. B. Merrifield. 1980. Special methods in peptide synthe-
sis, part A. In The Peptides: Analysis, Synthesis, and Biology, Vol. 2. E. Gross and J. Meienhofer, editors. Academic Press, Inc., New York.

3. Besancon, F., and H. Ancel. 1974. Binding of interferon to gangliosides. Nature ( Lond.). 245:478–479.
4. Bremer, E. G., S. Hakomori, D. F. Bowen-Pope, E. Raines, and R. Ross. 1984. Ganglioside-mediated modulation of cell growth, growth factor

binding, and receptor phosphorylation. J. Biol. Chem. 259:6818–6825.
24. Kleinman, H. K., G. R. Martin, and P. H. Fishman. 1979. Ganglioside inhibition of fibronectin-mediated cell adhesion to collagen. **Proc. Natl. Acad. Sci. U.S.A.** 76:3367-3371.

25. Krissansen, G. W., M. J. Owen, W. Verbi, and M. J. Crumpton. 1986. Primary structure of the T3 subunit of the T3/T cell antigen receptor complex deduced from cDNA sequences: evolution of the T3 γ and σ subunits. **EMBO (Europ. Mol. Biol. Organ.)** J. 5:1799-1808.

26. Markwell, M. A. K., L. Svennerholm, and J. C. Paulson. 1981. Specific gangliosides function as host receptors for Sendai virus. **Proc. Natl. Acad. Sci. U.S.A.** 78:6048-6053.

27. McCarthy, J. B., S. T. Hagen, and L. T. Furcht. 1986. Human fibronectin cell-surface adhesive function: promoting domains for metastatic melanoma cells. **J. Cell Biol.** 102:179-188.

28. Okada, Y., H. Matsuura, and S. Hakomori. 1985. Inhibition of tumor cell growth by aggregation of a tumor-associated glycolipid antigen: a close functional association between gangliotriososido ceramide and transferrin receptor in mouse lymphoma L1210. **Cancer Res.** 45:2793-2801.

29. Perkins, R. M., S. Kellie, B. Patel, and D. R. Critchley. 1982. Gangliosides as receptors for fibronectin. **Exp. Cell Res.** 141:231-243.

30. Petersen, T. E., H. C. Hogers, K. Skorstengaard, K. Vibe-Pedersen, P. Sahl, L. Sottrup-Jensen, and S. Magnunson. 1983. Partial primary structure of bovine plasma fibronectin: three types of internal homology. **Proc. Natl. Acad. Sci. U.S.A.** 80:137-141.

31. Pierschbacher, M. D., and E. Ruoslahti. 1984. Cell attachment activity of fibronectin can be duplicated by small synthetic fragments of the molecule. **Nature (Lond.)** 309:30-33.

32. Pierschbacher, M. D., E. G. Hayman, and E. Ruoslahti. 1981. Location of the cell attachment site in fibronectin with monoclonal antibodies and proteolytic fragments of the molecule. **Cell.** 26:259-267.

33. Pierschbacher, M. D., E. G. Hayman, and E. Ruoslahti. 1981. Synthetic peptides with cell attachment activity of fibronectin. **Proc. Natl. Acad. Sci. U.S.A.** 80:1224-1227.

34. Pytel, R., M. D. Pierschbacher, and E. Ruoslahti. 1985. A 125/115 kDa cell surface receptor specific for vitronectin interacts with the arginine-glycine-aspartic acid adhesion sequence derived from fibroblasts. **Proc. Natl. Acad. Sci. U.S.A.** 82:5766-5770.

35. Pytel, R., M. D. Pierschbacher, and E. Ruoslahti. 1985. Identification and isolation of a 140 kilodalton cell surface glycoprotein with properties expected of a fibronectin receptor. **Cell.** 40:191-196.

36. Reidl, M., O. Forster, H. Rumpold, and H. Bernheimer. 1982. A ganglioside-dependent cellular binding mechanism in rat macrophages. **J. Immunol.** 128:1205-1210.

37. Ruoslahti, E., and M. D. Pierschbacher. 1987. New perspectives in cell adhesion. **Science (Wash. DC).** 238:491-497.

38. Ruoslahti, E., E. G. Hayman, M. D. Pierschbacher, and E. Engvall. 1982. Fibronectin: purification, immunocherchemical properties and biological activities. **Methods Enzymol.** 82:803-831.

39. Schwarzbauren, J. E., J. W. Tamkun, I. R. Lemischka, and R. O. Hynes. 1983. Three different fibronectin mRNA’s arise by alternative splicing within the coding region. **Cell.** 35:421-431.

40. Spiegal, S., K. M. Yamada, B. E. Horn, J. Moss, and P. H. Fishman. 1983. Incorporation of fluorescent gangliosides into human fibroblasts: motility, fate, and interaction with fibronectin. **J. Cell Biol.** 99:699-704.

41. Svennerholm, L. 1963. Chromatographic separation of human brain gangliosides. **Neurochemistry.** 10:613-623.

42. Thorens, B. J. J., Merrot, and F. Vassali. 1987. Phagocytosis and inflammatory stimuli induce GM-CSF mRNA in macrophages through post-transcriptional regulation. **Cell.** 48:671-679.

43. Van Heiningen, W. E. 1974. Gangliosides as membrane receptors for tetanus toxin, cholera toxin, and serotonin. **Nature (Lond.).** 249:415-417.

44. Werkmeister, J. A., T. Triglia, P. Andrews, and G. F. Burns. 1985. Identification of a structure on human melanoma cells recognized by CTL exhibiting anomalous killer cell function. **J. Immunol.** 135:689-695.

45. Werkmeister, J. A., T. Triglia, I. R. Mackay, J. P. Dowling, G. A. Vargas, G. Morstyn, and G. F. Burns. 1987. Identification of a glycolipid antigen associated with differentiation of melanoma cells monitored by a monoclonal antibody Leo Mel 3. **Cancer Res.** 47:225-230.

46. Yamada, K. M., and D. W. Kennedy. 1984. Dualistic nature of adhesion protein function: fibronectin and its biologically active peptide fragment can autoinhibit fibronectin function. **J. Cell Biol.** 99:26-36.

47. Yamada, K. M., D. W. Kennedy, G. R. Grotendorst, and T. Momoi. 1981. Gangliosides: receptors for fibronectin? **J. Cell. Physiol.** 109:343-351.