

Identification of Fibronectin Receptors on T Lymphocytes

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Abstract. We report the identification of fibronectin receptors on thymocytes and T lymphoma cells. Affinity chromatography of extracts of the T cell lymphoma, WR16.1, on a fibronectin-Sepharose column combined with specific elution using a synthetic peptide containing the cell attachment-promoting sequence, arginine-glycine-aspartic acid, yielded two polypeptide components having apparent molecular masses of ~160 kD reduced and 175 and 150 kD non-reduced. Immunoprecipitations from surface-iodinated WR16.1 cells or fibronectin-adherent thymocytes using a rabbit antiserum raised against the fibronectin receptor that is present on human fibroblasts revealed, in each case, the same two radiolabeled components. In contrast, immunoprecipitation from fibronectin-nonadherent T lymphoma cells, designated WR2.3, revealed the presence of only the smaller subunit. Al-

though the lymphocyte receptor and the fibronectin receptor identified on fibroblasts share immunologic determinants, they differ in that the molecular mass of the lymphocyte protein is larger. Moreover, trypsinization of either thymocytes or the WR16.1 T lymphoma cells resulted in a subsequent loss of their ability to adhere to fibronectin-coated substrates and a reduction in the electrophoretic mobility of each of the polypeptide chains of the fibronectin receptor present on their surfaces. These changes, however, were not observed with normal rat kidney fibroblasts or mouse 3T3 fibroblasts in response to trypsinization. The data establish the existence on normal lymphocytes of fibronectin receptors that are quite similar to those found on fibroblasts. The possible function of this molecule on thymocytes is discussed.

FIBRONECTIN has emerged as the prototype of extracellular matrix molecules that promote the adhesion of cells (Klebe, 1974; Pearlstein, 1976; Rubin et al., 1979; Ruoslahti and Hayman, 1979; Grinnell, 1980). The primary site within the fibronectin molecule that is recognized by cells consists of the tripeptide sequence, L-arginyl-glycyl-L-aspartic acid (RGD)¹ (Pierschbacher and Ruoslahti, 1984a) for which there exists at least one cell surface receptor (Hasegawa et al., 1985; Horwitz et al., 1985; Pytela et al., 1985a).

We have previously identified a subset of thymocytes that bind to fibronectin but not to other extracellular matrix proteins such as laminin, collagen, or vitronectin (Cardarelli and Pierschbacher, 1986). The interaction between this group of thymocytes and the fibronectin molecule appears to be mediated by the same RGD sequence (Pierschbacher and Ruoslahti, 1984a; Hynes, 1987) recognized by the fibronectin receptor on fibroblastic cells (Pytela et al., 1985a), indicating that the receptor on thymocytes is the same as that on fibroblasts or is a member of the same family of RGD-directed receptors (Ruoslahti and Pierschbacher, 1986). The presence of fibronectin receptors on other hemopoietic cells has also been reported (Giancotti et al., 1986; Patel and

Lodish, 1986; Savagner et al., 1986). The possibility that distinct fibronectin receptors might exist, though, is suggested by the findings that platelets have a distinct fibronectin receptor that recognizes also other proteins containing the RGD sequence (Pytela et al., 1986), and that monocytes (Bevilacqua et al., 1981; Hosein and Bianco, 1985) and macrophages (Rourke et al., 1984) have fibronectin receptors that are functionally sensitive to trypsin whereas the fibroblast fibronectin receptor is resistant to trypsin in the presence of calcium (Oppenheimer-Marks and Grinnell, 1984). Furthermore, the monocyte receptor (Hosein and Bianco, 1985) appears to be immunologically distinguishable from the fibroblast receptor even though it, too, seems to recognize the RGD sequence in fibronectin (Wright and Meyer, 1985). To obtain more direct information on the similarities or differences of lymphocyte fibronectin receptors with receptors serving a similar function on other types of cells, we have isolated the RGD-directed fibronectin receptor from T lymphocytes.

Materials and Methods

Preparation of Cells

Murine thymocytes were isolated as described previously (Cardarelli and Pierschbacher, 1986). The fibronectin-adherent thymocyte population was obtained by selective attachment to fibronectin-coated substrates (10 µg/ml). The adherent lymphocytic cells were collected by treating them for 10 min

1. *Abbreviations used in this paper:* GRGDSP, glycine-arginine-glycine-aspartic acid-serine-proline; GRGESP, glycine-arginine-glycine-glutamic acid-serine-proline; RGD, L-arginyl-glycyl-L-aspartic acid.

with 1 mg/ml RGD peptide, resuspending in Dulbecco's modified Eagles media (DME) as described (Hayman et al., 1985). Contaminating nonlymphoid cells such as thymic macrophages and dendritic cells required an incubation period of 1 h for detachment and thus were not present in thymocytes prepared by this procedure. This was verified by replating the cells on fibronectin-coated substrates and visually inspecting the reattached cells.

Lymphoma cells WR16.1, WR2.3 (Raschke, 1980), and EL-4 (Gorer, 1950), normal rat kidney fibroblasts, or mouse 3T3 fibroblasts were cultured in DME supplemented with 10% heat-inactivated fetal calf serum, glutamine (2 mM), penicillin (100 U/ml), and streptomycin (100 µg/ml) (Irvine Scientific, Santa Ana, CA).

Affinity Chromatography

The 120-kD chymotryptic cell-binding fragment of fibronectin was prepared and coupled to cyanogen bromide-activated Sepharose (Sigma Chemical Co., St. Louis, MO) as described previously (Pierschbacher et al., 1981; Pytela et al., 1985a). The cells were surface labeled with 1 mCi of ¹²⁵I-sodium iodide and 0.2 mg/ml of lactoperoxidase. 1×10^9 WR16.1 cells (5.0 ml of packed cells) were solubilized in 100 mM octylglucoside containing 3 mM phenylmethylsulfonyl fluoride in PBS (extraction buffer) according to methods described by Pytela et al. (1985a). The extract was applied to a 3-ml column of 120-kD fragment-Sepharose and nonbound material was removed by washing with 30 ml of extraction buffer. Elution of bound material was accomplished, after washing with 6 ml of a 1 mg/ml solution of glycine arginine-glycine-glutamic acid-serine-proline (GRGESP) peptide, by eluting with 6 ml of 1 mg/ml glycine-arginine-glycine-aspartic acid-serine-proline (GRGDSP) peptide in extraction buffer.

Liposome-binding Assay

[³H]phosphatidylcholine liposomes containing ¹²⁵I-labeled purified fibronectin receptor were prepared by the method of Mimms et al., (1981) as described (Pytela et al., 1985a and b, 1987). The diluted liposomes were then added to microtiter wells which had been coated with 20 µg/ml fibronectin, vitronectin (Hayman et al., 1983), or BSA and incubated overnight at 4°C. Unbound material was removed, the bound liposomes were solubilized in 1% SDS, and the radioactivity was determined. To purify the liposomes,

they were suspended in 30% sucrose and centrifuged at 15°C for 5 h at 100,000 rpm. The liposomes were recovered at the top of the sucrose gradient and quantitated by both gamma and scintillation counting before analysis by SDS-PAGE.

Immunoprecipitation of Receptors

Cells ($5-10 \times 10^7$) were surface labeled with 1 mCi ¹²⁵I-sodium iodide and 0.2 mg/ml of lactoperoxidase. A cell extract was made by adding 400 µl of extraction buffer (see above), incubated for 20 min, and centrifuged, and the supernatant was collected. ~800 µl of immunoprecipitation buffer (0.1% SDS, 0.5% Triton X-100, 0.5% deoxycholic acid [sodium salt] in PBS) was then added to the supernatant. The extract was incubated at 4°C for 20 min and centrifuged at 12,500 g for an additional 20 min. The supernatant was collected and 50 µl of the respective antibodies (30 µg/ml) was added. This was incubated overnight at 4°C. Protein A-Sepharose was added to the extract-antibody suspension and incubated for 1 h at 24°C then washed three times with immunoprecipitation buffer containing 0.5 M NaCl, followed by one wash with RIPA alone and one wash with PBS. This was subsequently solubilized by boiling (100°C) in 1X sample buffer (3% SDS, 5% 2-mercaptoethanol, 10% glycerol, 200 mM Tris-HCl, pH 6.8, and 0.001% Bromophenol Blue) and analyzed by SDS-PAGE (Laemmli, 1970).

Trypsinization of Fibronectin Receptors

Mouse 3T3 fibroblasts or normal rat kidney fibroblasts were detached from the culture dishes with 100 µg/ml trypsin (Sigma Chemical Co.) and washed twice in PBS containing 1 mg/ml BSA. Thymocytes, lymphoma cells (WR16.1), mouse 3T3 fibroblasts, and normal rat kidney fibroblasts (NRK) were collected and 100 µg/ml trypsin (Sigma Chemical Co.) containing 1,300 U/ml in DME was added. This was incubated for 30 min at 37°C followed by three washes in PBS containing 500 µg/ml soy bean trypsin inhibitor (Sigma Chemical Co.). Both trypsinized and nontrypsinized cells were assessed for the presence of fibronectin receptor on their surface by immunoprecipitation and for their ability to bind to microwells which had been coated overnight with fibronectin (10 µg/ml in 100 µl per well). This yields wells coated at an approximate density of fibronectin of 0.3 µg/cm² (Engvall and Perlmann, 1972; Solonen and Vaheri, 1979). Attachment of tryp-

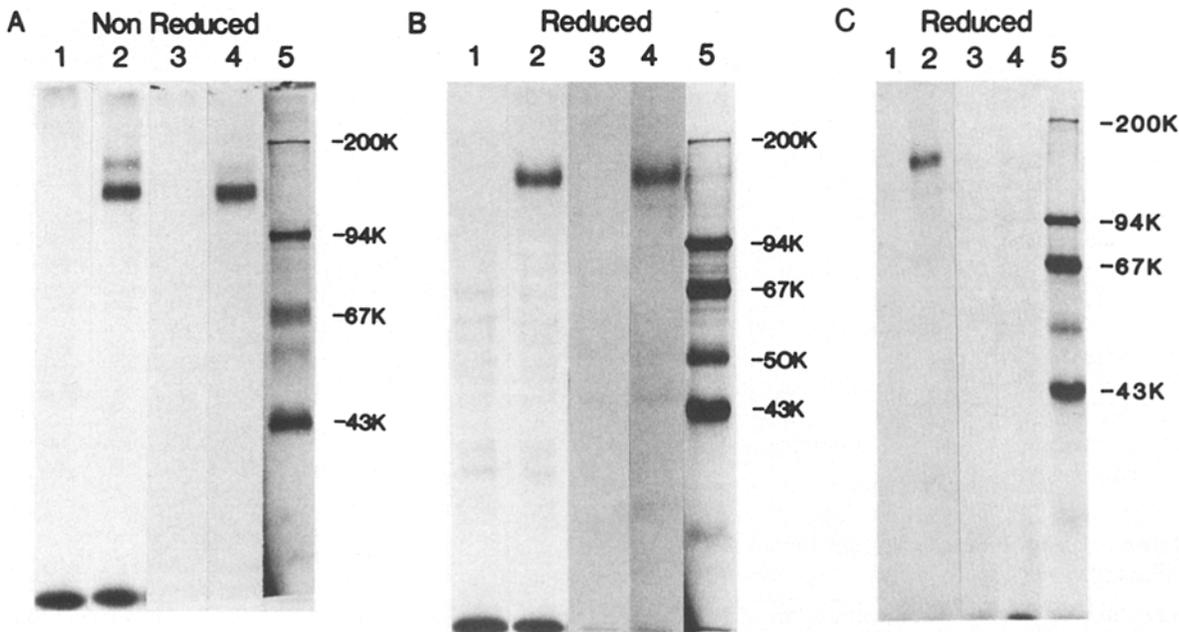


Figure 1. Immunoprecipitation of fibronectin receptors on T lymphoma cells and thymocytes. Thymic lymphoma cells (lanes 1 and 2) and fibronectin-adherent thymocytes (lanes 3 and 4) were surface labeled with ¹²⁵I and immunoprecipitated with rabbit anti-human fibroblast fibronectin receptor antibody (lanes 2 and 4) or preimmune IgG (lanes 1 and 3) under (A) nonreducing conditions or (B) reducing conditions. (C) Fibronectin-adherent (lanes 1 and 2) and fibronectin-nonadherent thymocytes (lanes 3 and 4), isolated by panning on fibronectin-coated substrates, labeled with ¹²⁵I and immunoprecipitated with rabbit anti-human fibroblast fibronectin receptor antibody (lanes 2 and 4), or preimmune IgG (lanes 1 and 3) under reducing conditions. Lane 5 depicts molecular mass standards, myosin (200 kD), phosphorylase B (94 kD), BSA (67 kD), and ovalbumin (43 kD). K, thousands.

sinized or nontrypsinized cells to fibronectin-coated wells, in the absence or presence of 500 µg/ml soy bean trypsin inhibitor, was determined after a 2-h incubation at 24°C as described (Cardarelli and Pierschbacher, 1986).

SDS-PAGE and Autoradiography

A 7.5% SDS-PAGE analysis of eluted material was carried out under reducing and nonreducing conditions (Laemmli, 1970). Autoradiography was performed by placing Kodak XAR X-ray film between the dried gel and a Cronex Lightning Plus intensifying screen (DuPont Instruments, Newtown, CT) at -70°C for 3-5 d. ¹⁴C-labeled standards used were myosin (200 kD), phosphorylase B (94 kD), BSA (67 kD), and ovalbumin (43 kD) (New England Nuclear, Boston, MA). For scanning the X-ray, an automatic computing densitometer ACD-18 (Gelman Sciences, Inc., Ann Arbor, MI) was used and read at 450 nm.

Results

Immunologic Relationship between the Fibronectin Receptors on T Lymphocytes and Those on Fibroblastic Cells

To begin to address the question of whether T lymphocytes can express a fibronectin receptor similar to that found on fibroblastic cells, a rabbit antiserum raised against the fibronectin receptor isolated from human placental tissue was used to immunoprecipitate labeled material from fibronectin-adherent murine thymic lymphoma cells (designated WR16.1) or from fibronectin-adherent mouse thymocytes each of which had been surface iodinated. In both cases, SDS-PAGE of the precipitate under nonreducing conditions revealed two radiolabeled components having approximate apparent molecular masses of 175 and 150 kD (Fig. 1 A). When reduced, these two bands migrated as a closely spaced doublet with a M_r of ~160 kD (Fig. 1 B). When immunoprecipitates from equal numbers of surface-iodinated, fibronectin-adherent and -nonadherent thymocytes were compared, smaller amounts of labeled receptor could be obtained from the preparation of nonadherent cells (Fig. 1 C), which was visible only after prolonged exposure.

To determine whether the structures recognized on the thymocyte by this antibody included a functional fibronectin receptor, we asked whether the antibody would inhibit the attachment of thymocytes to fibronectin. Indeed, when antibody to the human fibronectin receptor was included in an assay designed to detect the adhesion of thymocytes to fibronectin, it reduced the number of adherent cells to background levels (Fig. 2). This inhibition was dependent upon the concentration of antibody present. In contrast, no inhibition was observed when an antibody to the vitronectin receptor, a similar cell surface adhesion molecule, was included in the assay at concentrations capable of inhibiting attachment of vitronectin-adherent cells.

Isolation of Functional Fibronectin Receptor from a Murine T Cell Lymphoma

By using affinity chromatography of cell extracts over a matrix of insolubilized fibronectin, a cell surface receptor can be isolated from osteosarcoma cells (Pytela et al., 1985a) or from tissue (Pytela et al., 1986). To determine whether the lymphocyte protein recognized by the anti-human fibronectin receptor antibody might itself be a functional fibronectin receptor, we applied the affinity chromatography procedure to fibronectin-adherent lymphocytic cells. Because it is

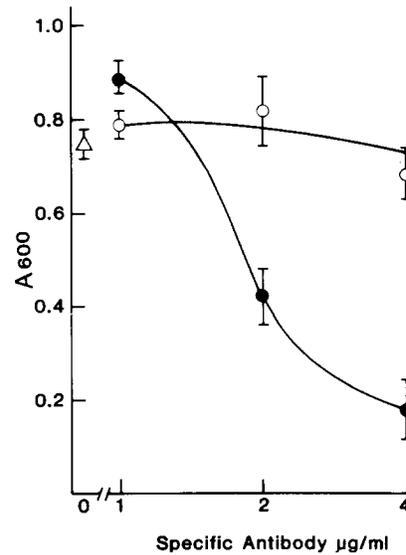


Figure 2. Inhibition of thymocyte attachment to fibronectin by antifibroblast fibronectin receptor antibody. Thymocytes (2×10^7 cells/ml) were preincubated in the (open triangle) absence or presence of (solid circles) protein A-purified anti-human fibroblast fibronectin receptor antibody or (open circles) anti-human vitronectin receptor antibody at the designated concentrations for 30 min at 24°C. Attachment of thymocytes to fibronectin was assessed as described in Materials and Methods. Each point represents the mean \pm SEM ($n = 6$).

difficult to obtain large numbers of fibronectin-adherent mouse thymocytes and because we had found the fibronectin receptor on WR16.1 cells to share the unique characteristics of the thymocyte receptor, we examined detergent extracts of the WR16.1 cells that had been surface iodinated for functional receptor. The material eluted from the fibronectin matrix with an Arg-Gly-Asp-containing synthetic peptide migrated on SDS-PAGE under nonreducing conditions as two components with apparent molecular masses of 175 and 150 kD (Fig. 3 A). Reduced, a closely spaced polypeptide doublet having molecular weights near 160 kD could be visualized (Fig. 3 B). Immunoprecipitation of the material specifically eluted from the fibronectin-Sepharose column with the anti-fibroblast fibronectin receptor antibody revealed two components with a similar molecular mass. When the same affinity chromatography experiment was done using cells that had been metabolically labeled with [³⁵S]methionine, the same two polypeptide chains were eluted from the column (not shown). No material was eluted with a control peptide containing the sequence Arg-Gly-Glu that does not have cell attachment-promoting activity (Pierschbacher and Ruoslahti, 1984b). A 70-kD protein continued to leach out of the column even after extensive washing. The amount of this protein diminished during the elution period and was not affected by the presence of peptide in the elution buffer. Also, the amount of this protein was not consistent from experiment to experiment (see Fig. 3), indicating that its interaction with the affinity matrix is nonspecific. Moreover, the 70-kD protein did not become incorporated into liposomes (see below), indicating that it is not necessary for the receptor to interact with fibronectin.

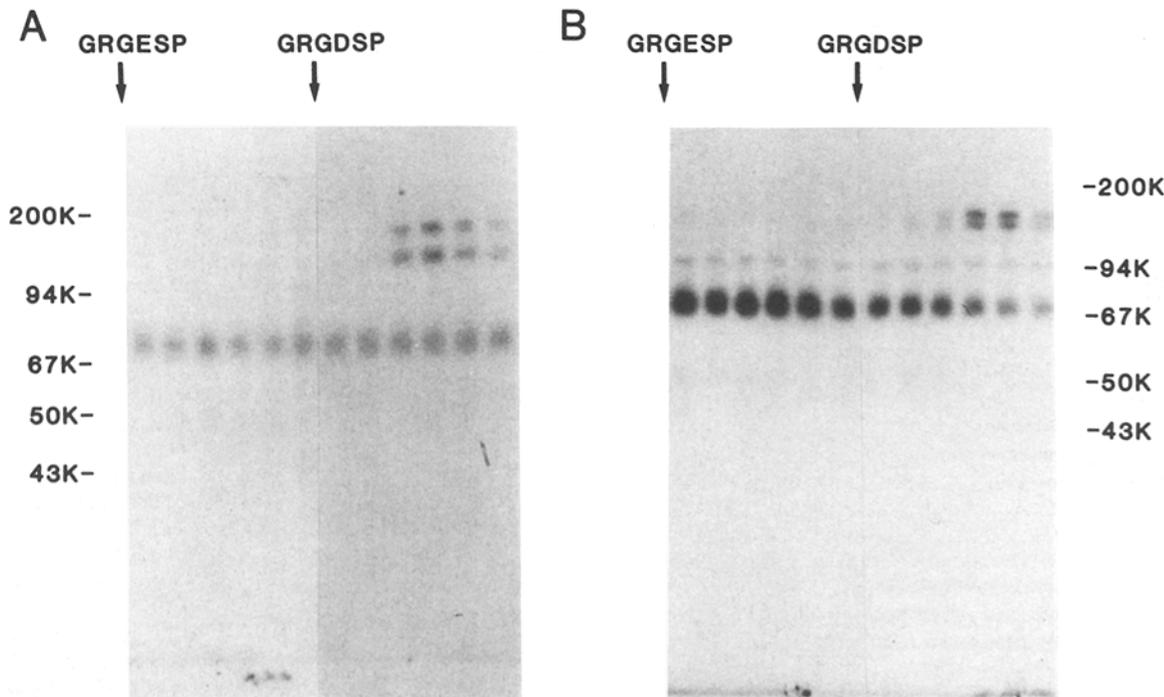


Figure 3. SDS-PAGE of fractions eluted from fibronectin-Sepharose column. ¹²⁵I-labeled T lymphoma cells were solubilized and applied to a fibronectin-Sepharose column as described in Materials and Methods. Fractions of 0.5 ml were collected and 75 μ l of each fraction was applied per lane and analyzed under (A) nonreducing conditions or (B) reducing conditions. The bound material was eluted from the column by adding 6 ml of 1 mg/ml GRGESP followed by 6 ml of 1 mg/ml GRGDSP. The samples analyzed under nonreducing and reducing conditions came from different experiments. Molecular mass markers which are shown are myosin (200 kD), phosphorylase B (94 kD), BSA (67 kD), and ovalbumin (43 kD). K, thousands.

Specificity of Binding of Fibronectin Receptor Containing Liposomes to Fibronectin

We next wanted to determine if the proteins eluted from the fibronectin-Sepharose column could be integrated into a liposome model membrane and retain their specificity of binding to fibronectin as the fibroblast receptor does (Pytela et al., 1985a). Liposomes containing the 165/155-kD pro-

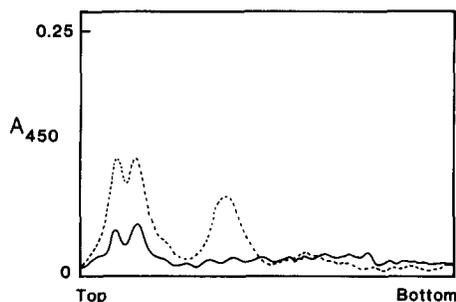


Figure 4. Gel scan of SDS-PAGE fractions obtained after sucrose gradient fractionation of 150/175-kD protein containing liposomes. Liposomes containing the 150/175-kD proteins were fractionated by sucrose gradient centrifugation as described in Materials and Methods. The top 200- μ l fraction of the gradient was analyzed by SDS-PAGE under nonreducing conditions (solid line) and compared with material containing the 150/175-kD protein before incorporation into liposomes (dashed line). The density of the gel bands was determined by using an automatic computing densitometer and read at an absorbance of 450 nm.

teins, purified by sucrose gradient centrifugation, showed that the receptor incorporated into liposomes was identical to the receptor which was purified by affinity chromatography (Fig. 4). Moreover, liposomes carrying these two polypeptides did attach to fibronectin-coated substrates but not to vitronectin or BSA coated surfaces. That the binding to fibronectin was specific, was further demonstrated by the fact that addition of 1 mg/ml of the Arg-Gly-Asp-containing peptide completely reduced the binding to background levels. In contrast, the control peptide did not inhibit the liposome binding to fibronectin (Fig. 5). The percentage of added counts which bound to fibronectin was found to be \sim 2%. This, however, does not represent all of the liposomes that have incorporated receptor, and is more probably a reflection of the capacity of the substrate because, when nonbound receptor liposomes were placed in a second microtiter well that had been coated with fibronectin, an additional 2% of the counts bound to the substrate in a specific manner.

Differences between the Lymphocyte Fibronectin Receptor and the Fibroblast Fibronectin Receptor

Immunoprecipitation of the material affinity purified from mouse T lymphoma cells with the antibody against the human fibroblast fibronectin receptor confirmed that the mouse protein was related to the human receptor, and we conclude that it serves as a receptor for fibronectin on the T lymphocyte. However, like the material immunoprecipitated from thymocytes, the protein affinity purified from the WR16.1 cells appeared slightly larger when compared with the

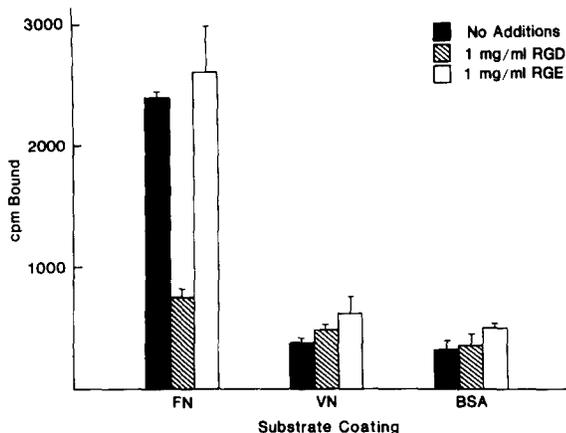


Figure 5. Binding of liposomes containing the 150/175-kD protein to fibronectin (FN)-, vitronectin (VN)-coated substrates. Liposomes were prepared as outlined in Materials and Methods. Dishes were coated with 20 μ g/ml of the indicated proteins. Attachment (counts per minute bound) of 3 H-labeled liposomes containing the 150/175-kD protein was assessed after an overnight incubation at 4°C. Where indicated, synthetic peptides (RGD, Gly-Arg-Gly-Asp-Ser-Pro; RGE, Gly-Arg-Gly-Glu-Ser-Pro) were added to the wells with the liposome suspension. The results from one experiment representative of six are shown with the mean of three replicate wells expressed \pm SD.

fibronectin receptor described on human osteogenic sarcoma cells (Pytela et al., 1985a) or compared with the receptor immunoprecipitated from normal rat kidney cells or murine 3T3 cells both of which are 140 kD reduced; 160 and 125 kD nonreduced (for simplicity we will refer to the smaller receptor as a "fibroblast fibronectin receptor"). The molecular mass differences of the fibronectin receptors on fibroblasts and lymphocytes does not appear to be due to variations in *N*-linked carbohydrates because the removal of *N*-linked carbohydrates by enzymatic treatment of either normal rat kidney cells, mouse 3T3 cells, or T lymphoma (WR16.1) cells with *N*-glycanase (Chu, 1986; Plummer et al., 1984), resulted in similar changes in electrophoretic mobilities of the fibronectin receptor from all cells tested.

To further characterize the fibronectin receptor on thymocytes, we asked whether the ability of thymocytes or the WR16.1 cells to attach to fibronectin substrates could be affected by pretreatment of the cells with trypsin. Fig. 6 demonstrates that both mouse thymocytes and the T lymphoma cells lose their capacity to recognize fibronectin after trypsinization even when the adhesion assays are carried out in the presence of 500 μ g/ml soybean trypsin inhibitor, whereas normal rat kidney cells or mouse 3T3 cells do not. These data suggested that differences may exist between the thymocyte fibronectin receptor and the fibronectin receptor on other types of cells. Immunoprecipitation of fibronectin receptor from trypsinized and nontrypsinized WR16.1 cells showed that the major effect of trypsin was on the high molecular mass component, changing its apparent molecular mass from 175 to \sim 163 kD (Fig. 7). However, the lower subunit also appeared slightly smaller after trypsinization (Fig. 7). SDS-PAGE of fibronectin receptor immunoprecipitated from NRK cells or 3T3 cells revealed two bands having apparent molecular masses of 160 and 125 kD. In agreement

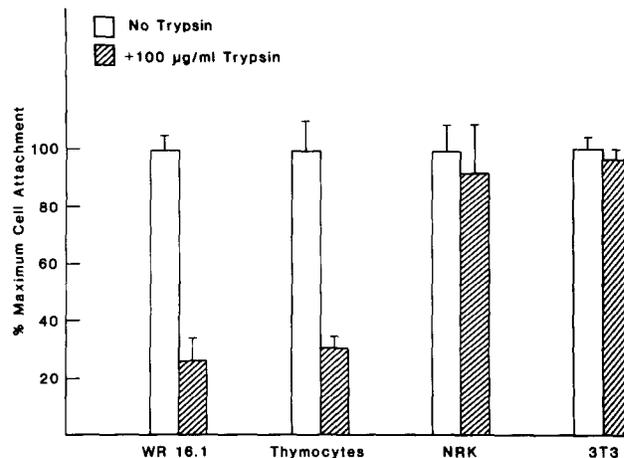


Figure 6. Effect of trypsin treatment on the adhesion of cells to fibronectin-coated substrates. Cells were preincubated in the presence or absence of 100 μ g/ml trypsin for 30 min at 37°C. After three washes with 500 μ g/ml soybean trypsin inhibitor, T lymphoma cells (WR16.1) (5×10^5 cells per well), thymocytes (2×10^6 cells per well), normal rat kidney fibroblast cells (NRK) (5×10^4 cells per well), or mouse 3T3 fibroblast cells (5×10^4 cells per well) were allowed to attach to microwells which had been coated with fibronectin (10 μ g/ml). The maximal attachment of each cell type to fibronectin in the absence of trypsinization was expressed as 100% and represents 80–90% of the cells plated in each case with the exception of thymocytes where only 10% are fibronectin adherent (Cardarelli and Pierschbacher, 1986) and the adhesion of trypsinized cells are expressed relative to those values. Mean \pm SEM, are expressed ($n = 8$).

with the functional resistance of the fibroblast receptor to trypsin, its electrophoretic mobility was also unaffected by pretreatment of the cells with trypsin (Fig. 7 A).

Fibronectin Receptor Epitopes on a Fibronectin-nonadherent T Lymphoma Cell

Table I demonstrates that whereas thymocytes, WR16.1 cells, and EL-4 cells bind to fibronectin, another T lymphoma cell line designated WR2.3 does not. Consistent with this functional lack of adhesion, immunoprecipitation with the anti-human fibroblast fibronectin receptor antibody revealed the presence of only the β subunit on WR2.3 cells (Fig. 8), suggesting that the α subunit of the receptor is required for function of the receptor.

Table I. Attachment of Thymocytes or T Cell Lines to Fibronectin[†]

Cells	Absorbance* 600 nm
Thymocytes	0.977 \pm 0.074
WR16.1	1.588 \pm 0.090
WR2.3	0.061 \pm 0.011
EL-4	1.496 \pm 0.087

* Absorbance (600 nm) of adherent cells stained with toluidine blue was measured using a Titertek ELISA plate reader. Mean \pm SEM are expressed ($n = 6$).
[†] Wells were coated with fibronectin at a concentration of 10 μ g/ml. Fibronectin was prepared in our laboratory from human plasma. The minimal absorbance due to trapping of cells in BSA-coated wells has been subtracted.

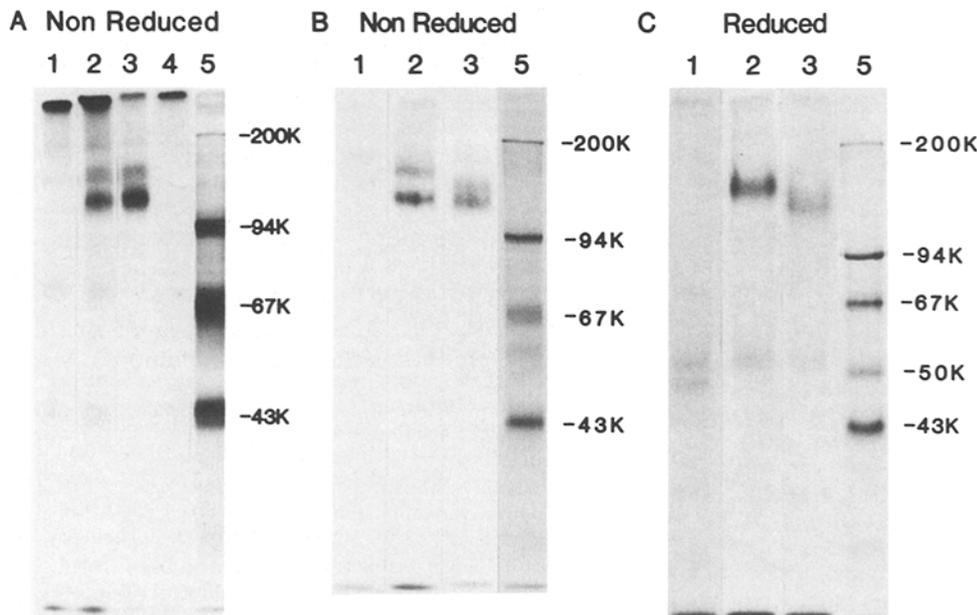


Figure 7. Immunoprecipitation of fibronectin receptors on trypsinized cells. (A) Normal rat kidney cells were treated with trypsin (lanes 3 and 4) or buffer without trypsin (lanes 1 and 2), radiolabeled with ^{125}I , and immunoprecipitated with rabbit anti-human fibroblast fibronectin receptor antibody (lanes 2 and 3) or preimmune IgG (lanes 1 and 4) under nonreducing conditions. (B, C) T lymphoma cells were treated with trypsin (lanes 1 and 3) or buffer without trypsin (lane 2) and immunoprecipitated with rabbit anti-human fibroblast fibronectin receptor antibody (lanes 2 and 3) or preimmune IgG (lane 1). Lane 5 represents molecular mass markers, myosin (200 kD), phosphorylase B (94 kD), BSA (67 kD), and ovalbumin (43 kD). K, thousands.

Discussion

We have identified a glycoprotein on the surface of fibronectin-adherent murine thymocytes (Cardarelli and Pierschbacher, 1986) as well as on mouse T lymphoma cells that is immunologically and functionally related to the fibronectin receptor isolated from human osteogenic sarcoma cells or normal human placental tissue (Pytela et al., 1985a; Pytela et al., 1987). The lymphocyte molecule is similar to the fibroblast fibronectin receptor in that it binds from detergent extracts of the cells to a matrix of insoluble fibronectin and can be eluted from that affinity matrix with soluble synthetic peptides containing the cell attachment determinant of fibronectin (RGD). However, this molecule differs from the fibroblast receptor, in its apparent size and its sensitivity to proteolysis by trypsin. Despite these differences, based on its presence on fibronectin-adherent thymocytes on its behavior on SDS-PAGE, which is characteristic of the known RGD-directed receptors (Pytela et al., 1986), and on its ability to confer a fibronectin-binding capacity to phosphatidylcholine liposomes, we conclude that this lymphocyte protein serves as a receptor for fibronectin on these cells.

The physiological function of the fibronectin receptor on thymocytes and T lymphoma cells is currently unknown. Most of the events involved in thymocyte differentiation appear to take place in the thymus (Rothenberg and Lugo, 1985; Stutman, 1978; Metcalf, 1966). Because of this, we have speculated that the presence of fibronectin receptors on thymic lymphocytes may be important for their differentiation. It has been suggested that attachment of myeloid cells to fibronectin-coated surfaces triggers the cell to phagocytize C_3b - and C_3bi -coated particles (Wright et al., 1983; Pommier et al., 1983). Complement receptor activation is a rapid and reversible process that can be regulated by the RGD-binding site of fibronectin (Wright and Meyer, 1985). Ac-

cordingly, fibronectin receptors may play an important role in the differentiation of monocytes into a more active phagocytic cell. Conversely, homotypic lymphocyte aggregation is stimulated by phorbol esters, and this aggregation can be blocked by monoclonal antibodies to lymphocyte function-associated antigen-1 (LFA-1) (Rothlein and Springer, 1986). It is therefore conceivable that like the complement receptor or lymphocyte function-associated antigen-1 (Springer et al., 1982), fibronectin receptor activity on lymphocytes may be coupled to cell activation either as an initiator of activation or as a response to activating signals. Perhaps, as in other systems (Gospodarowicz et al., 1978), the extracellular matrix may provide the developing T lymphocyte with a competence-inducing signal that would allow it to respond to growth- and differentiation-inducing factors within the thymus. Alternatively, Savagner et al., 1986, found that chick pre-T lymphocytes could be induced by a factor from the thymus to penetrate a basement membrane in vitro. Thus, it is possible that chemotactic factors, antigen activation, or hormone type stimuli could induce the expression of fibronectin receptors on circulating lymphocytes, enabling them to traverse connective tissue and enter lymphoid organs or sites of inflammation. Indeed, deposits of fibronectin have been found in lymph nodes (D'Ardenne et al., 1983) and in buccal mucosa associated with T lymphocytes (Matthews et al., 1986), and it has been proposed that the connective tissue of the thymic interlobular septae could be the major pathway for the traffic of lymphocytes both into and out of the thymus (Kendall, 1981). We and others (Berrih et al., 1985) have found by immunofluorescence abundant fibronectin in this region.

It has also been postulated that the interaction of erythroid and myeloid progenitor cells with fibronectin may represent a mechanism whereby these cells are anchored in the bone marrow until they receive the proper signals for differentia-

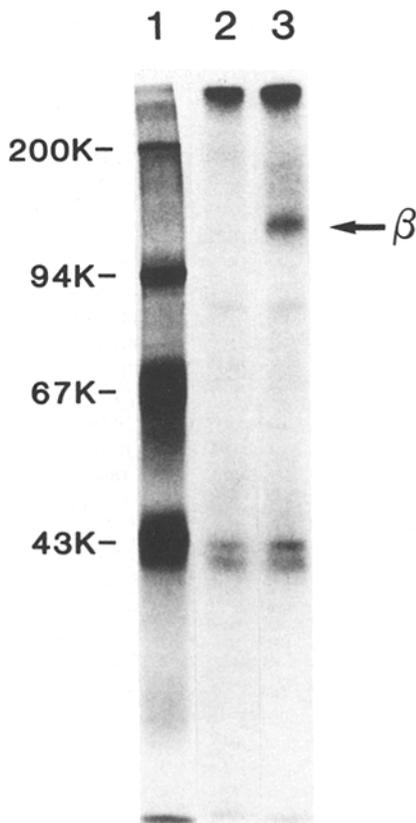


Figure 8. Identification of the β subunit of the fibronectin receptor on a fibronectin-nonadherent T lymphoma cell. Thymic lymphoma cells, WR2.3, were surface labeled with ^{125}I and immunoprecipitated with rabbit anti-human fibronectin receptor antibody (lane 3) or preimmune IgG (lane 2) under nonreducing conditions. Lane 1 depicts molecular mass standards, myosin (200 kD), phosphorylase B (94 kD), BSA (67 kD), and ovalbumin (43 kD).

tion and migration (Giancotti et al., 1986; Patel and Lodish, 1986). Indeed, reticulocytes or uninduced murine erythro-leukemia cells attach to fibronectin and this adhesion is lost upon differentiation (Patel and Lodish, 1984; Patel et al., 1985). Perhaps the fibronectin receptor serves to hold the developing thymocyte within the thymus until it has matured and is ready to emigrate to the periphery. The distinction between these and other possible functions of the fibronectin receptor on T lymphocytes awaits further study.

We have shown here that there are differences in sizes between the fibronectin receptor on thymocytes and the fibroblast receptor. The possibility that these differences could be accounted for by carbohydrate differences seems unlikely because treatment of receptors from either thymocytes or fibroblasts with *N*-glycanase (Chu, 1986; Plummer et al., 1984) resulted in equal reductions in the molecular weights of each of these receptors (Cardarelli and Pierschbacher, unpublished results). It is conceivable that these size differences as well as immunologic differences among fibronectin receptors on different cell types detected by others (Hosein and Bianco, 1985) could be due to differences in posttranslational modifications or alternative mRNA splicing, or it could be that distinct fibronectin receptors exist on different cell types as is the case with fibroblasts and platelets (Pytela et al., 1986).

The sensitivity of the lymphocyte fibronectin receptor to proteolysis by trypsin also suggests that differences exist in the receptors from the different sources. Only 7% of the mass of the larger subunit of the lymphocyte receptor and 2% of the lower subunit was removed by treatment with trypsin, indicating that the majority of the molecule is quite resistant to this enzyme. The fact that the majority of the molecule remained associated with the cell surface after trypsinization indicates that peptide bonds near the amino termini of the two polypeptides were cleaved in that the carboxy termini appear to be cytoplasmic (Argaves et al., 1986 and unpublished). That trypsinized lymphocytes no longer bind to fibronectin suggests that treatment with this enzyme destroys the binding site on the receptor. However, it is not clear whether the actual binding site is removed or whether the functional conformation of the receptor is destroyed by this procedure.

Burns et al. (1986) have presented evidence that the interaction of several types of leukocytes with fibronectin is dependent on a surface molecule which shares an immunologic determinant with the platelet protein IIb/IIIa. Our results indicate that the fibronectin receptor expressed by thymocytes is similar to that of fibroblasts but differs in some respects. Therefore, because the extent of the family of adhesion receptors is not yet known and because minor differences in the receptors from the different cell types do exist, one must exercise caution in drawing conclusions about the absolute identity of functionally similar receptors from different sources until their complete structure is known.

Taken together, the data presented here establish the existence of adhesion receptors on both thymocytes and T lymphoma cells that recognize fibronectin via its RGD sequence. These receptors differ from those found on fibroblasts yet they are similar in many respects. The presence of these receptors on thymocytes indicates that the interaction of thymocytes with the extracellular matrix may be important in their differentiation or migration during development. Antibodies to these murine receptors are currently being produced so that their precise localization in lymphoid tissues can be determined.

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