Lymphoid Precursor Cells Adhere to Two Different Sites on Fibronectin

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Abstract. Several precursor lymphoid cell lines, blocked at specific stages of differentiation, adhere specifically to fibronectin in vitro. Whereas the Ba F3 cell line, which has both immunoglobulin heavy- and light-chain genes in germline configuration, interacts with the arg-gly-asp-containing cell-binding domain of fibronectin, the B-committed line PD 31, which is undergoing rearrangement of immunoglobulin light-chain genes, does not. Accordingly the Ba F3, but not the putative PD 31 surface fibronectin receptor, binds to an affinity matrix containing the 115-kD cell-binding domain of fibronectin. PD 31 cells recognize a different domain of the fibronectin molecule, which is contained within the carboxy terminal segment possessing a high-affinity binding site for heparin. A polyclonal antibody raised against the fibronectin receptor of mouse erythroleukemic cells inhibits adhesion of these lymphoid lines to fibronectin. It precipitates two major species of 140 and 70 kD from surface-radioiodinated Ba F3 cells and species of 140 and 120 kD from PD 31 cells. We propose that the two types of cells express different fibronectin receptors mediating substrate adhesion, and suggest that receptor(s) with different specificity might be expressed in the course of B cell maturation. Because we show that these adhesion properties are shared by normal bone marrow lymphoid precursors, we infer that these receptors may play a role in normal lymphopoiesis.

Whereas the molecular mechanisms underlying immunoglobulin gene rearrangements are now understood at least in outline (Tonegawa, 1983) and the complex process of mature lymphocyte recirculation and homing is being actively investigated (Gowans and Knight, 1964; reviewed by Gallatin et al., 1986), remarkably little is known about the processes of membrane remodeling involved in the maturation of lymphoid precursors and in their release into the circulation. A link between successful rearrangement of antigen receptor genes and plasma membrane remodeling is likely, in that only a fraction of the cells produced daily in the mature compartments survives and is released into the circulation (Gallatin et al., 1986).

In the normal adult mammal, hematolymphopoiesis is confined to the bone marrow, which provides a unique inductive microenvironment responsible for the ordered proliferation and differentiation of all the cell lineages whose mature progeny is found in the circulation (Zuckerman and Rhodes, 1985). Both stromal cells (Dexter et al., 1985) and the extracellular matrix (Zuckerman and Rhodes, 1985) play key roles in maintaining a balanced hemopoiesis, but the requirement for specific cellular and matrix factors is less understood.

Recent work has shown that both normal reticulocytes (Patel et al., 1985) and murine erythroleukemia cells (Patel and Lodish, 1984, 1986; Giancotti et al., 1986) possess a 140-kD fibronectin receptor recognizing the arg-gly-asp sequence within the cell-binding domain of fibronectin (Pierschbacher et al., 1982; Yamada and Kennedy, 1984). Loss or modification of this receptor protein is responsible for the loss of adhesion to fibronectin observed in differentiated cells (Patel and Lodish, 1986). This receptor is similar in binding specificity to those described in human osteosarcoma cells (Pytela et al., 1985), mouse fibroblasts (Giancotti et al., 1985), platelets (Gardner and Hynes, 1985; Pytela et al., 1986) and chick embryo fibroblasts (Horwitz et al., 1985; Akiyama et al., 1986).

Here we show that several pre-B lymphoid cell lines grown in culture attach specifically to fibronectin in vitro, and provide evidence suggesting that this process is mediated by receptors that exhibit immunologic cross-reactivity with the erythroid receptor. At variance from uncommitted B cell precursors, which interact with the arg-gly-asp fibronectin recognition sequence, pre-B cells undergoing the rearrangement of heavy- and/or light-chain genes recognize a different region of the fibronectin molecule which is part of the carboxy-terminal end containing the high-affinity binding site for heparin. We suggest that fibronectin receptors with different specificity are expressed in the course of pre-B cell maturation. Because we also show that a significant fraction of normal bone marrow pre-B cells specifically adheres to fibronectin and to its carboxy-terminal fragment containing the high-affinity site for heparin but not the arg-gly-asp recognition sequence, we infer that multiple receptors may play a role in normal lymphoid precursor cell adhesion to the extracellular matrix.
### Table I. Attachment of Lymphoid Cells Lines to Fibronectin In Vitro

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Ig genes</th>
<th>Adherent cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HC</td>
<td>LC</td>
</tr>
<tr>
<td>Ba F3</td>
<td>G</td>
<td>G</td>
</tr>
<tr>
<td>38 B9</td>
<td>pR</td>
<td>G</td>
</tr>
<tr>
<td>PD 31</td>
<td>R</td>
<td>pR</td>
</tr>
<tr>
<td>70 Z/3</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>WEHI 231</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>MPC 11</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Circulating B lymphocytes*</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>MEL</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Cells were assayed for attachment to fibronectin (5 μg/cm²) or BSA (2%) on 35-mm diameter plastic petri dishes. The entries under Ig genes and Surface Ig refer to the configuration of genes for immunoglobulin heavy chains (HC), light chains (LC), and to the presence (+) or absence (−) of surface Ig, respectively. Abbreviations: G, germline; pR, partially rearranged; R, rearranged. The cells are listed in order of their level of commitment along the B cell lineage. MEL cells were included as a positive control. * Identified by immunofluorescence on a monocyte-depleted mononuclear fraction from peripheral blood.

### Materials and Methods

#### Materials

Histopaque 1077, Nonidet-P40, cyanogen bromide-activated Sepharose 4B, and phenylmethylsulphonyl fluoride (PMSF) were purchased from Sigma Chemical Co. (St. Louis, MO). Laminin was from Collaborative Research (Lexington, MA), Collagen type I from Flow Laboratories, Inc. (McLean, VA), N-acetyl-d-glucosamine from Pfistieh Laboratories (Waukegan, IL), wheat germ agglutinin-agarose from E. Y. Laboratories (San Mateo, CA), DEAE-Sepharose from Pharmacia Fine Chemicals (Piscatway, NJ), octyl-β-D-glucopyranoside from Calbiochem-Behring Corp. (San Diego, CA), and the synthetic peptides gly-arg-gly-asp-ser-pro-cys, gly-arg-gly-asp-ser-pro, and gly-arg-glu-asp-ser-pro from Peninsula Laboratories, Inc.

Bone marrow cells were prepared from 4-5-week-old BALB/c mice from Dr. R. Jaenisch's breeding colony at the Whitehead Institute. Femurs were isolated as described by Oliver and Goldstein (1978), and bone marrow cells were obtained by flushing the femurs with DME/5% FCS plus 5 mM NaEDTA, and washed once in DME/5% FCS. A mononuclear fraction was prepared by standard Ficoll gradient centrifugation using Histopaque 1077. Cells at the DME-Ficoll interface were collected, washed once in DME/5% FCS, and depleted of monocytes/macrophages by incubation for 1 h at 37°C in a tissue culture grade 60-mm diameter plastic dish (Falcon Labware, Oxnard, CA). Nonadherent cells were collected, and the dishes were flushed with a gentle stream of medium to detach loosely adherent cells which were pooled with the unattached fraction. This preparation contains 40–50% cells positive for the B lineage–restricted antigen B-220 (Coffman, 1982), as assayed by indirect immunofluorescence. Lymphocytes from mouse peripheral blood were isolated with the same procedure from 4-month mice of the same strain.

#### Attachment Assays

Adhesion assays of cells grown in suspension were carried out in either 35-mm diameter plastic petri dishes (Falcon Labware) or in 6-mm diameter, 96-well polystyrene plates (Linbro, Flow Laboratories, Inc.) exactly as described by Patel and Lodish (1984, 1986). Each experimental point is the average of triplicate (35-mm dishes) or quadruplicate determinations (96-well plates), and the standard deviation was within 10%. Adhesion assays of bone marrow cells and peripheral lymphocytes were carried out in 60-mm diameter petri dishes coated with the indicated amounts of fibronectin or its heparin-binding fragment, where 8 x 10⁶ cells per dish in 4 ml of DME/5% FCS were incubated at 37°C for 60 min. Nonadherent cells were collected and the dishes were rinsed twice with 2 ml of the same medium, which was pooled with the nonadherent cells. Adherent cells were detached by incubation in DME/5% FCS plus 5 mM EDTA at 4°C for 20 min. Both adherent and nonadherent cells were counted with a Coulter counter (Coulter Electronics Inc., Hialeah, FL), and further analyzed by indirect immunofluorescence.

#### Immunofluorescence

Cells were washed once in Hank's balanced salt solution supplemented with 20 mM Hepes and 1% bovine serum albumin (BSA), resuspended in 1 ml of the same solution, and reacted for 15 min on ice with (a) 1 mg/ml control IgG of the same species as that of the secondary fluorescent antibody, (b) RA3-6B2 rat monoclonal antibody (Coffman, 1982) or affinity-purified IgG fraction of goat anti–mouse Ig, and (c) fluorescein-conjugated F(ab)₂ fragments of either goat anti-rat or rabbit anti–goat IgG. Cells were washed with Hank's balanced salt solution/20 mM Hepes/1% BSA after each step, and finally resuspended in 0.15 M NaCl, 10 mM Tris-HCl, 0.2% NaN₃ (pH 8.0). Fluorescent cells were counted with a Zeiss III RS epifluorescence microscope (Carl Zeiss, Inc., Thornwood, NY). At least 200 cells were counted, and...
Attachment of Selected Lymphoid Lines to Fibronectin In Vitro

Six cell lines of B lymphoid lineage blocked at specific stages of differentiation were screened for their ability to attach to fibronectin-coated dishes (Table I). Ba F3 is an interleukin 3-dependent clone that is positive for the B lineage-restricted B220 antigen, and has the immunoglobulin genes in germine configuration. Clones with similar properties have been shown to undergo in vivo differentiation along the B lineage (Palacios and Steinmetz, 1985), and therefore Ba F3 likely represents a B stem cell. 38 B9 is an Abelson transformant derived from fetal mouse liver that has undergone heavy- but not light-chain gene rearrangement (Alt et al., 1981). PD 31 is an Abelson-transformed cell line derived from adult mouse bone marrow that in vitro actively undergoes light-chain gene rearrangement (Lewis et al., 1982). 70 Z/3 is a chemically transformed pre-B cell line that can be induced to express surface immunoglobulin in vitro (Paige et al., 1978), whereas WEHI 231 is a lymphoma line constitutively expressing high levels of surface IgM (Ralph, 1979). MPC II is an IgG-producing mouse myeloma line (Kuehl and Scharff, 1974). MEL is a Friend virus–transformed erythroleukemic line (Friend et al., 1971), which was used here as a positive control, in that it expresses a well-characterized receptor for fibronectin (Patel and Lodish, 1984, 1986).

The rationale for using these different lymphoid cell lines was to determine whether adhesion to fibronectin is developmentally regulated. Data in Table I and Fig. 1 suggest that adhesion to fibronectin decreases with differentiation. A greater extent of adhesion (40–70%) was observed with the pre-B lines, whereas the WEHI 231 and MPC II cells yielded the lowest values (20–30%) and B lymphocytes from peripheral blood did not adhere at all. Note that 70 Z/3 and WEHI 231 cells exhibited a relatively high attachment to BSA-coated dishes, which contributes to an overestimation of the degree of adhesion to fibronectin inasmuch as albumin is used to block unspecific protein adsorption sites after coating the dishes with fibronectin. Attachment to fibronectin is specific, because neither laminin nor type I collagen promoted comparable levels of adhesion (not shown). These findings suggest that the specific attachment to fibronectin is
Figure 2. Effect of antifibronectin antibodies and of a synthetic peptide on Ba F3 cell attachment to fibronectin. 96-well polystyrene plates were coated with 10 µg/cm² fibronectin. (a) N-296 (triangles) or N-294 (circles) monoclonal antibodies were added, and the plate incubated for 1 h at 4°C before addition of Ba F3 cells. (b) The indicated concentrations of the synthetic peptide gly-arg-gly-asp-ser-pro-cys were added immediately before the addition of cells. The indicated concentrations of the synthetic peptide gly-arg-gly-asp-ser-pro-cys inhibited cell attachment in a dose-dependent fashion (Fig. 2 b) whereas a control gly-arg-glu-asp-ser-pro peptide was without effect (not shown).

Figure 3. Adherence of selected cell lines to intact fibronectin or to its 115-kD chymotryptic fragment. 96-well polystyrene plates (Linbro) were coated with the indicated amounts of fibronectin (circles) or its 115-kD chymotryptic fragment (squares), and a cell-binding assay was carried out with (a) Ba F3, (b) PD 31, or (c) MEL cells.
Figure 4. Adherence of PD 31 cells to a heparin-binding fragment of fibronectin. Lack of inhibition by heparin and by antifibronectin antibodies. 96-well polystyrene plates were coated with (a) the indicated amounts or (b and c) with 4 μg/cm² of a heparin-binding chymotryptic fragment of fibronectin. A cell-binding assay was carried out (a) with PD 31 cells without further additions or in the presence of (b) the indicated concentrations of heparin or (c) antifibronectin monoclonal antibody N-296 (triangles) or N-294 (circles). The inset to panel a shows a Coomassie Blue-stained SDS-PAGE analysis of the heparin-binding preparation on a 5–15% gradient gel under (lane 1) reducing or (lane 2) nonreducing conditions. K, Mr standards in kilodaltons.

doublet is part of the high-affinity heparin-binding site of fibronectin, and is located between the carboxy-terminal fibrin-binding site and the cell-binding site.

Fig. 4 also shows that adhesion of PD 31 cells to microtiter wells coated with this heparin-binding preparation was not inhibited by either heparin (b) or by the N-294 or N-296 antifibronectin antibodies (c). Thus, it seems unlikely that adhesion is mediated by surface heparan proteoglycans or by trace contaminants that derive from the classical “arg-gly-asp” cell-binding domain. Rather, these experiments suggest that PD 31 cells express a fibronectin receptor with a different specificity.

To test this hypothesis at the molecular level, we subjected extracts from surface-radioiodinated Ba F3 and PD 31 cells to affinity chromatography on a matrix obtained by coupling the 115-kD chymotryptic fibronectin fragment to Sepharose (Pytel et al., 1985). Fig. 5 shows that the synthetic gly-arg-gly-asp-ser-pro peptide eluted a band of ~140 kD from Ba

Figure 5. Autoradiograms of SDS-PAGE analyses of fractions obtained by affinity chromatography of cell extracts from surface radioiodinated cells on the 115-kD chymotryptic fragment of fibronectin. Extracts from 125I surface-labeled Ba F3 and PD 31 cells (1 ml) were prepared, and chromatographed on 1-ml bed volume columns obtained by coupling a 115-kD chymotryptic fragment of fibronectin to Sepharose 4B. After washing, the columns were sequentially eluted (arrows) with 2 ml of a 1 mg/ml solution of gly-arg-gly-glu-ser-pro peptide (GRGESP), 2.5 ml of a 1 mg/ml solution of gly-arg-gly-asp-ser-pro (GRGDSP) peptide, and 2 ml of 6 M urea. Fractions of 0.5 ml were collected, and 40 μl of each analyzed on 7.5% Laemmli gels under reducing conditions. (A) Ba F3 cells; (B) PD 31 cells. In each panel: lane 1, flowthrough; lane 2, last wash fraction before elution; lanes 3–6, elution with GRGESP peptide; lanes 7–11, elution with GRGDSP peptide; lanes 12–15, elution with urea. The dried gels were exposed to Kodak XAR-5 film at -70°C with an intensifying screen. Exposure time was 8 h for lane 1 (in each panel) and 4 d for lanes 2–15. Specific activity of the extracts was (trichloroacetic acid-precipitable counts × 10⁶ μl⁻¹): Ba F3, 94,400; PD 31, 85,100. K, M, standards in kilodaltons.
F3 cells (A) but not from PD 31 cells (B). This finding is in good agreement with the cell adhesion experiments, and strongly suggests that the putative fibronectin receptor of the committed lymphoid line PD 31 does not recognize the arg-gly-asp cell recognition sequence on fibronectin.

Characterization of an Antiserum against the Fibronectin Receptor of Mouse Erythroleukemia Cells: Immunologic Cross-reactivity with the Putative Lymphoid Receptor

To further characterize the fibronectin receptor(s) of mouse erythroleukemia cells (Patel and Lodish, 1984, 1986) and, we hoped, of B cells, an antiserum against the affinity-purified erythroid fibronectin receptor was raised in rabbits. Fig. 6 shows that a purified Ig fraction of this antiserum inhibited attachment of MEL cells (a), Ba F3 cells (b), and PD 31 cells (c) to fibronectin, whereas preimmune Ig were without effect. Even though in the case of PD 31 cells the degree of inhibition was lower and a higher concentration of antibody was required, these results were reproducible.

To identify cell surface components recognized by this antiserum, a detergent extract of 125I surface-labeled MEL cells was prepared; part was immunoprecipitated with either preimmune or with antifibronectin receptor serum, while the remainder was subjected to an affinity chromatography purification procedure as detailed in Materials and Methods (Patel and Lodish, 1986). As shown in Fig. 7, the specific immunoprecipitates from the total cell extract (lane 3) and the affinity-purified receptor (lane 5) contained the 140-kD protein characterized previously as the erythroid fibronectin receptor (lane 4) (Patel and Lodish, 1986). Note that the immunoprecipitate from the total cell extract (lane 3) appears to migrate slightly faster than the affinity-purified receptor (lanes 4 and 5). This might be due to limited proteolysis of the labeled protein during incubation of the total cell extract with antibody. Alternatively, the radioactive iodine might have labeled a lower molecular mass precursor form of the receptor in a small number of permeable cells. Since the precursor does not bind to the 115-kD Sepharose affinity matrix but is recognized by this antibody (V. P. Patel, unpublished), the immunoprecipitate from the total cell extract could be expected to appear broader. A protein of ~140 kD was also immunoprecipitated with immune serum from a detergent extract from 125I surface-labeled Ba F3 cells (lane 8) and from affinity-purified Ba F3 fibronectin receptor (not shown), suggesting that the adhesion of Ba F3 cells to fibronectin was mediated by a similar receptor. In addition to the 140-kD protein, a second major protein of 70 kD was...
immunoprecipitated from the Ba F3 cell extract. This protein was not present in the RGD eluate from the fibronectin affinity column (Fig. 5a, lanes 7-11). Whether these proteins are functionally related, or whether the lower molecular mass form is a proteolytic product is unclear at present. In any case, the availability of the antifibronectin receptor antisera allowed us to screen a large number of lymphoid lines for the presence of related surface molecules.

**Immunoprecipitation of 125I Surface-labeled Lymphoid Cell Extracts with Antifibronectin Receptor Serum**

To test whether cells of B lineage express proteins immunologically related to the erythroid fibronectin receptor, we immunoprecipitated from the Ba F3 cell extract. This protein glycosylation, since digestion of Ba F3 and PD 31 immunoprecipitates with N-glycanase (but not O-glycanase) produced a shift of the 140- and 120-kD proteins to a single band of ~100 kD (not shown). The absence of reactive material in MPC 11 cells was not due to differences in the specific activity of the radioiodinated extracts, because in this particular experiment the specific activity of the Ba F3 and MPC 11 extracts was the same, and about twice that of 38 B9 and PD 31 cells (compare also lanes 1-4). Thus, this myeloma line did not attach to fibronectin and did not express proteins specifically recognized by the antisera against the erythroid fibronectin receptor. On the other hand, the Ba F3, 38 B9, and PD 31 lines, which adhered to fibronectin, expressed species that are immunologically cross-reactive with the erythroid receptor molecule.

Fig. 9 illustrates the results we obtained with Ba F3, 38 B9, and PD 31 immunoprecipitates from selected, 125I surface-labeled cell lines. This may be due to differences in protein glycosylation, since digestion of Ba F3 and PD 31 immunoprecipitates with N-glycanase (but not O-glycanase) produced a shift of the 140- and 120-kD proteins to a single band of ~100 kD (not shown). The absence of reactive material in MPC 11 cells was not due to differences in the specific activity of the radioiodinated extracts, because in this particular experiment the specific activity of the Ba F3 and MPC 11 extracts was the same, and about twice that of 38 B9 and PD 31 cells (compare also lanes 1-4). Thus, this myeloma line did not attach to fibronectin and did not express proteins specifically recognized by the antisera against the erythroid fibronectin receptor. On the other hand, the Ba F3, 38 B9, and PD 31 lines, which adhered to fibronectin, expressed species that are immunologically cross-reactive with the erythroid receptor molecule.

Fig. 8 illustrates the results we obtained with Ba F3, 38 B9, and PD 31 cells expressing the receptor molecules when the transformation event(s) occurred, we have extended our analysis to cells from normal bone marrow, which is the site of maturation (Osmond and Nossal, 1974; Osmond, 1975). We prepared a fraction enriched in mononuclear cells by density gradient centrifugation, and
this fraction was depleted of monocytes and macrophages by adherence to tissue culture grade plastic dishes. This preparation is enriched in cells of B lineage, in that 40-50% of the cells were positive for the antigen B220, which is a B-restricted antigen expressed throughout the life cycle of these cells (Coffman, 1982). Table II shows that 55% of the B220-positive cells and 26% of the surface Ig-positive cells adhered to intact fibronectin, whereas 94% of the B220-positive cells and 92% of the surface Ig-positive cells adhered to the heparin-binding fragment of fibronectin. Why a population of bone marrow cells adheres to the heparin-binding fragment but not to intact fibronectin is unclear at present, but even with PD 31 cells we have routinely observed that a larger number of cells adhere to the heparin-binding fragment. Attachment of bone marrow pre-B cells to fibronectin was specific, in that they did not adhere to either laminin or collagen type I, nor did they show an increased attachment on a combination of fibronectin and collagen (not shown). Because circulating B lymphocytes do not adhere to fibronectin (Table I), although a fraction of pre-B and B cells from the bone marrow do (Table II), we conclude that differentiation of bone marrow pre-B cells into B lymphocytes is accompanied by a loss of adhesion to fibronectin. Whether the adhesion of normal bone marrow cells to fibronectin is mediated by the same molecule(s), we characterized in the transformed lymphoid cell lines remains to be established.

Discussion

We have shown that several B lymphoid cell precursor lines, blocked at specific stages of differentiation, specifically adhere to fibronectin in an in vitro assay. Whereas Ba F3 cells, presumably a B stem cell (Palacios and Steinmetz, 1985), adhere to the classical cell attachment domain of fibronectin, the B-committed line PD 31 does not. Rather, adhesion of PD 31 cells is mediated by a different region of the fibronectin molecule, which is part of the carboxy-terminal region containing the high-affinity binding site for heparin. An antibody raised against the fibronectin receptor of mouse erythroleukemic cells inhibits lymphoid cell adhesion to fibronectin, and precipitates surface proteins of 140 and 70 kD from surface-radioiodinated Ba F3 cells and of 140 and 120 kD from B-committed cells. Based on this evidence, we propose that these cell lines express different fibronectin receptors that mediate substrate adhesion, and suggest that a receptor with different specificity might be expressed in the course of B cells maturation. Because a large proportion of mouse bone marrow cells of B lineage specifically adhere to fibronectin and to this heparin-binding fragment in vitro, we infer that these proteins might play a role in lymphoid precursor cell adhesion to the extracellular matrix.

The fibronectin receptor of Ba F3 cells belongs to the family of receptors that recognize the arg-gly-asp sequence on the cell-binding domain of fibronectin (reviewed by Ruoslahti and Pierschbacher, 1986). Indeed, Ba F3 cells adhere to both intact fibronectin and to its 115-kD chymotryptic fragment that contains the cell binding site (Fig. 3a). Attachment to fibronectin is inhibited by both a monoclonal antibody (N-294) directed against the cell-binding domain of fibronectin and by a heptapeptide containing the arg-gly-asp cell attachment recognition sequence of fibronectin (Fig. 2). They also possess a 140-kD cell surface protein that is specifically bound by the affinity matrix containing the 115 kD cell-binding fragment of fibronectin, and is specifically

Table II. Adhesion of Bone Marrow Cells to Intact Fibronectin and to its Heparin-binding Fragment

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Adherent cells</th>
<th>Fibronection</th>
<th>Heparin-binding fragment</th>
<th>BSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total population</td>
<td>48</td>
<td>78</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>B 220 positive cells</td>
<td>55</td>
<td>94</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Surface Ig positive cells</td>
<td>26</td>
<td>92</td>
<td>ND</td>
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</table>

As detailed in Materials and Methods, we prepared a population of mononuclear bone marrow cells depleted of monocytes/macrophages. These were assayed for adhesion to fibronectin (20 µg/cm²), to its carboxy-terminal heparin-binding fragment (5 µg/cm²), or to BSA (2%) on 60-mm diameter plastic petri dishes. The attached and unattached populations were then stained with either anti-B220 or anti-Ig antibodies. Of the cells used in this assay 49% were B220 positive and 20% were positive for surface Ig. ND, not determined.
eluted by a synthetic peptide containing the classical arg-gly-asp sequence (Fig. 5 a). This protein has, therefore, all of the properties of a cell surface fibronectin receptor.

At variance from MEL and Ba F3 cells, the B lineage-committed PD 31 cells adhere to intact fibronectin but not to its 115-kD chymotryptic fragment that contains the classical cell-binding site of fibronectin (Fig. 3 b). Accordingly, they have no specific surface protein that could be eluted from an affinity matrix containing the 115-kD chymotryptic fragment of fibronectin (Fig. 5 b). PD 31 cells do adhere to chymotryptic fragments of fibronectin purified by affinity chromatography on heparin-Sepharose (Fig. 4 a); adhesion is not inhibited either by a 20-fold excess of heparin (Fig. 4 b) or the fibronectin monoclonal antibodies N-294 or N-296 (Fig. 4 c). Although the heparin-binding preparation is not purified to homogeneity, we are confident that adhesion of PD 31 cells to these peptides does not involve interactions with the classical arg-gly-asp sequence, and is probably not mediated by cell surface-associated heparan sulphate proteoglycans. Thus, we conclude that one or more surface molecules on PD 31 cells are receptors that bind to a different region of fibronectin.

Adhesion to the heparin-binding domain of fibronectin is not restricted to PD 31 cells, in that these heparin-binding fragments of fibronectin also promote adhesion of Ba F3, MEL cells, and normal rat kidney fibroblasts (data not shown). Thus, even cells with a classical fibronectin receptor recognize, in addition, a different region of the fibronectin molecule. These data are consistent with recent evidence that the heparin-binding region of the fibronectin molecule mediates important biologic effects both in fibroblasts (Woods et al., 1986) and in melanoma cells (McCarthy et al., 1986).

Indirect evidence for a receptor-mediated process in the interaction of lymphoid precursor cells with fibronectin comes from the studies with an antiserum raised against the erythroid fibronectin receptor. Immunoglobulins in this antiserum, but not in preimmune serum, block adhesion of MEL and Ba F3 cells to fibronectin-coated wells (Fig. 6, a and b). The same antibody immunoprecipitates a major protein of ∼140 kD from both a total cell extract or an affinity-purified fibronectin receptor preparation from surface radiolabeled MEL cells (Fig. 7), proving that it specifically recognizes the fibronectin receptor. A similar experiment with Ba F3 cells gave identical results (not shown), suggesting that the surface molecules recognized by this antibody in cells of B lineage define their receptor for fibronectin. Inasmuch as the antiserum against the erythroid fibronectin receptor also inhibits attachment of PD 31 cells to intact fibronectin (Fig. 6 c) and immunoprecipitates two major polypeptides of 140 and 120 kD from surface-labeled PD 31 and 38 B9 cells (Figs. 8 and 9), we suspect that these polypeptides are the fibronectin receptor(s) of these B-committed lymphoid lines and that they are immunologically cross-reactive with but not identical to the erythroid and Ba F3 cell receptors. Some support for our hypothesis comes from the results obtained with the mouse plasmacytoma line MPC 11. These cells do not adhere to fibronectin (Table I and Fig. 1), and bear little, if any, surface proteins that are specifically immunoprecipitated by the antiserum against the fibronectin receptor (Fig. 8). Whether our inability to detect a species cross-reacting with a fibronectin receptor in MPC 11 cells reflects a property of the normal plasma cell or is rather linked to neoplastic transformation remains to be established.

Increasing evidence suggests that the "fibronectin receptors" are members of a large family of related but different proteins mediating cell interactions with several extracellular components. Thus, the platelet IIb/IIIa glycoprotein complex, which mediates platelet interaction with fibronectin (Ginsberg et al., 1985), vitronectin (Pytel et al., 1986), fibrinogen (Bennett and Vilaire, 1982), and von Willebrand factor (Ruggeri et al., 1982), shares antigenic determinants with the fibronectin receptor of neutrophils and monocytes (Burns et al., 1986) and with endothelial cell membrane proteins (Fitzgerald et al., 1985; Thiagarajan et al., 1985). These homologies have recently been found to extend to two leukocyte surface glycoproteins, Mac-1 (Springer et al., 1979) and lymphocyte function-associated antigen-1 (LFA-1) (Davignon et al., 1981), which are encoded by the same 20-kb genomic clone encoding platelet IIb/IIIa (Cosgrove et al., 1986). These surface proteins consist of at least two subunits that undergo characteristic changes of mobility on SDS-PAGE, depending on whether reducing or nonreducing conditions are used. Under nonreducing conditions, indeed, the larger subunit migrates at a slower rate while the smaller subunit migrates faster (see, e.g., Pytel et al., 1985, 1986; Burns et al., 1986; Patel and Lodish, 1986). This is most likely due to the presence of intrachain disulfide bonding in cysteine-rich regions of the molecule, as convincingly deduced from the cDNA sequence of the smaller subunit of the chicken fibroblast protein complex, integrin (Tamkun et al., 1986). In the case of the lymphoid surface molecules characterized here, under nonreducing conditions we could not detect an increased mobility on SDS-PAGE analysis (Fig. 9). Whether this finding is due to a structural difference of the lymphoid receptor(s) remains to be established.

B220 is an early B lineage-restricted antigen defined by the monoclonal antibody RA3-6B2 (Coffman, 1982). About 50% of the B220 positive cells of a mouse bone marrow preparation, enriched in lymphoid precursors, exhibit specific attachment to fibronectin and a even higher percentage adheres to a heparin-binding fragment of fibronectin in vitro (Table II). This is also the case with surface Ig-positive cells of the bone marrow (Table II). We do not know whether increased adhesion of lymphoid cells to the heparin-binding fragments of fibronectin reflects an altered conformation of the fibronectin molecule caused by the chymotrypsin treatment or a specific, developmentally regulated event. This question can be addressed experimentally. The function of multiple fibronectin receptors with different specificities, which are apparently expressed at different stages of B cell differentiation, might be involved in the migration of cells within the bone marrow. Expression of fibronectin receptors on lymphoid cells might also serve to anchor these cells to the fibronectin matrix of the bone marrow and prevent their premature release into the bloodstream. Because, however, peripheral blood lymphocytes do not adhere to fibronectin (Table I), surface fibronectin receptors of pre-B cells must be lost or modified at the terminal stages of development. It will be of interest to determine whether the released cells reacquire their ability to adhere to fibronectin in the process of homing into the peripheral lymphoid organs.
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