Loss of Attachment to Fibronectin With Terminal Human Erythroid Differentiation


Human erythroblastic progenitors (colony-forming unit-erythroid [CFU-E] and burst-forming unit-erythroid [BFU-E]) have been shown to attach to fibronectin (Fn), a property that might be involved in the local regulation of erythropoiesis. In this study, we have investigated changes in cell attachment to Fn upon terminal erythroid differentiation. We first purified CFU-E from human marrow by avidin-biotin immune rosetting. This negative selection procedure yielded a cell population containing ~80% blasts that, after characterization by colony-assays and electron microscopy, appeared to consist of CFU-E (10% to 15%) and their immediate progeny (85% to 90%), here defined as "preproerythroblasts." In the presence of fibroblasts, purified cells differentiated into reticulocytes in 7 to 10 days. Cell attachment to Fn was inversely correlated to the stage of differentiation of the erythroid cell: more than 50% of the CFU-E population reproducibly adhered to Fn, whereas at most 30% of the preproerythroblasts had the same capacity. Adhesion was further lost at late maturation stages, and a constant finding was the inability of reticulocytes to adhere to Fn. Finally, CFU-E adhesion to Fn was blocked by polyclonal IgG raised against the Fn receptor and by a monoclonal antibody against VLA-5.

In the present study, we have investigated how adhesion to Fn is modulated during terminal erythroid differentiation. For this purpose, we developed a procedure for obtaining a highly enriched population of very immature erythroid cells, i.e., CFU-E and their immediate progeny. These purified cells were then induced to differentiate in vitro, and during this process, we investigated their changing ability to attach to Fn. We found that CFU-E attach strongly to Fn, and that this property is lost at specific stages of terminal erythroid maturation. Moreover, the binding of CFU-E to Fn is mediated by a receptor bearing some similarities to the integrin family.

MATERIALS AND METHODS

Bone marrow samples. Normal marrow samples were obtained after informed consent from hematologically normal patients undergoing hip surgery. Cells were flushed from the bone fragments with α medium containing 100 to 200 μg/mL DNAse (type I, Sigma Chemical Co, St Louis, MO). Cells were separated on Ficoll-Hypaque (1.077 g/mL) (Eurobio, Paris, France). Light-density mononuclear cells were washed twice, resuspended at a concentration of \(3 \times 10^6\) cells/mL in α-medium supplemented with 10% fetal

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calf serum (FCS) (Flow Laboratories, Paris, France), and incubated in 75 cm² flasks for 1 hour at 37°C in a 5% CO₂ atmosphere in air to remove plastic-adherent cells. The nonadherent mononuclear cells (NA-MNC) were subsequently fractionated by immune rosetting (see below).

**Antibodies.** Monoclonal antibodies (MoAbs) against human HLA-DR (OKIa1), human T cells (OKT3), and human CALLA (CD10; OK-B-cALLA) were purchased from Ortho Diagnostic Systems (Roussy, France). MoAb recognizing HPCA (CD34) was from Becton Dickinson (Mountain View, CA), B1 (anti-B cells) from Coulter Immunology (Hialeah, FL). MoAbs CH1 (IgM recognizing granulocytes and CFU-GM), CS4 (recognizing glycophorin A) and CK26 (binding to lymphoid and monocytic cells) have been previously described, and were generously provided by Dr Berthier (INSERM U.217, Grenoble, France). Anti-human HLA-DR K5 MoAb was a gift from Dr Fermand (INSERM U.108, Hôpital St Louis, France). FAB-152 MoAb, which recognizes an 88 Kd glycoprotein on platelets, monocytes, and erythrocyte cells and belongs to the CD36, was a gift from L. Edelman (Institut Pasteur, Paris, France). Anti-platelet GPIIIa C17 MoAb and anti-glycoporphin A (GPA) polyclonal antibody used in electron microscopy were kind gifts from Drs F. Tetteroo and L.C. Anderson.

Purification was performed by negative selection using avidin-biotin affinity-purified goat anti-mouse IgM (µspecific) and IgG (H + L) F(ab')2 fragments were obtained from Cappel-Cooper Biomedical (Malvern, PA). A rabbit antisera prepared against the purified human placental fibronectin receptor (HFnR-Ab) was purchased from Telios Pharmaceuticals (La Jolla, CA). This antibody recognizes mainly the 115 Kd β1 subunit of the receptor, and is less reactive with the 160 Kd subunit. A rat MoAb BIE5, which is directed against the α5 subunit of the human Fn receptor and which selectively inhibits cell attachment to Fn was kindly provided by C. Damsky (University of California, San Francisco, CA) and used as hybridoma supernatant.

**Enrichment of erythroblastic cells by immune rosetting.** Cell purification was performed by negative selection using avidin-biotin immune rosetting according to the original technique described by Fürfang and Thierfelder. Briefly, 1.2 mg avidin (Vector Laboratories Inc, Burlingame, CA) was covalently coupled to 2 x 10⁹ glutaraldehyde-fixed sheep red blood cells (SRBC-A) as described in detail elsewhere. SRBC-A can be kept at 4°C in sterile conditions for at least 4 to 6 months without loss of reactivity. The purification was performed in two steps: NA-MNC (usually 0.5 to 3 x 10⁹ cells) were first mixed with a 10-fold excess of SRBC-A. The mixture was centrifuged 6 minutes at 200g, and the pellet was incubated 45 minutes at 4°C. Cells were then gently resuspended at a concentration of 1 x 10¹⁰ cells/mL in alpha-medium supplemented with 5% FCS (α5%) and layered over Ficoll-Hypaque. Mature granulocytes that had phagocytized SRBC-A were eliminated in the pellet (fraction P-1). Cells harvested at the interface (fraction I-1) were washed twice, resuspended at a concentration of 4 x 10¹⁰ cells/mL, and incubated 45 minutes at 4°C with an excess of the following MoAbs: OKIa1, OKT3, OKB-cALLA, B1, anti-HPCA, K5, CH1, CS4, CK26. At the end of the incubation period, cells were washed twice, resuspended at a concentration of 4 x 10¹⁰ cells/mL in α5% and incubated with a 1/40 dilution of a mixture of biotin-conjugated goat anti-mouse IgM and IgG for 45 minutes at 4°C. Excess biotinylated immunoglobulin was removed by three washes in α5%. Finally, labeled cells were incubated with SRBC-A in the proportion of 1:10, as described above, and rosettes were allowed to form. Cells were then gently resuspended and adjusted to the initial concentration. Rosettes were removed by centrifugation over Ficoll-Hypaque (fraction P-2), and unlabeled cells were recovered at the interface layer (fraction I-2). Cells recovered in each fraction (I-1, P-1, I-2, P-2) were counted and the number of rosettes was determined. Purified cells (I-2) were characterized morphologically on May-Grünewald-Giemsa (MGG) stained cytocentrifuged slides, by electron microscopy (see below), and by their ability to form colonies in methylcellulose colony-assays.

**Electron microscopy.** The simultaneous detection of specific membrane markers (GPIIIa and GPA) by immunogold and intracellular peroxidase activities was determined on the purified erythroid cell population (I-2) as previously described in detail. Briefly, immunolabeling was first performed by incubating cells with specific antibodies recognizing either GPIIIa or GPA, and then with a second antibody coupled with 15 nm and 5 nm gold particles, respectively. Cells were then fixed in 1.25% glutaraldehyde in Gey’s buffer, washed, incubated in diaminobenzidine medium, post-fixed with osmium tetroxide, dehydrated, and embedded in Epon. Sections were examined with a Philips electron microscope CM 10 (Eindhoven, The Netherlands) without staining in order to easily identify ferritin molecules by their own contrast.

**Suspension culture of purified erythroblastic cells.** Purified cells from the I-2 fraction were cultured at a concentration of 1 x 10⁹ cells/mL in α-medium containing 20% FCS and 3 U/mL human urinary erythropoietin (Ep). The batch of FCS used during the study contained less than 10 µg of Fn per milliliter as measured by an enzyme-linked immunosorbent assay (ELISA). Cultures were initiated in 96-flat bottom microtiter plate wells (Falcon, Becton-Dickinson, France, No. 3072; 200 µL per well) and incubated at 37°C in a water saturated air atmosphere with 5% CO₂. Cells were harvested regularly, and their number, viability (dye exclusion), morphology, plating efficiency, and ability to adhere to Fn were determined. Three stages of maturation were defined after MGG staining: immature (proerythroblasts and early basophilic erythroblasts), intermediate (late basophilic and polychromatophilic), and mature (acidophilic and enucleated). Reticulocytes were counted separately.

**Hematopoietic progenitor cell colony-assays.** Cells from the P-1, I-1, P-2, and I-2 fractions were plated in α-medium containing 0.8% methylcellulose, 30% FCS, 1% deionized bovine serum albumin (BSA), 10⁻⁴ mol/L β-mercaptoethanol, 3 U/mL Ep and 10% agar leukocyte conditioned medium. Final concentration of cells per dish was 1.5 x 10⁵ for NA-MNC and P-1 and 1 to 3 x 10⁴ for I-1, I-2, and P-2. Erythroid colonies were scored as previously described in detail.

**Adhesion assay.** The capacity of erythroid cells to adhere onto Fn was assessed in two different ways: purified I-2 cells and cells grown during various periods of time in suspension (see above) were incubated on Fn-coated 2.5 cm² tissue culture wells (2 to 4 x 10⁴ cells per well) in α5% for 2 hours at 37°C in a 5% CO₂ atmosphere. Nonadherent cells were then harvested, weakly adherent cells were removed by two mild washings, and strongly adherent cells were detached by vigorous pipetting or short trypsinization. Adherent and nonadherent cells were counted, stained with MGG for morphologic examination, and plated in methylcellulose colony-assays at 1.5 x 10⁴ cells per dish to assess the number of CFU-E in each fraction. In the second method, cells were grown as described above for suspension culture, except that they were incubated from the start on Fn-coated 96-flat bottom microtiter plate wells (1 x 10⁶ cells/mL, 200 µL per well) and kept on that substrate for the whole culture period. Every 2 days during the 6 to 10 day-culture period, one well was sacrificed and nonadherent and adherent cells were harvested separately, counted, and stained with MGG. Cell viability of both fractions was checked by dye exclusion (Nigrosin).

In both methods, tissue-culture wells were precoated with 50 to 80 µg/mL of Fn purified from human plasma by gelatin affinity chromatography, or human collagens I, III, and IV in the monoclonal form or laminin. Nonspecific binding sites were blocked by 1-hour incubation in 10 mg/mL BSA.
ERYTHROBLAST ADHESION TO FIBRONECTIN

In some experiments, I-2 cells were first incubated (45 minutes at 4°C) with a 1/10 dilution of the HFnR-Ab or undiluted B1E5 hybridoma supernatant, and then incubated as described above onto Fn-coated wells for 2 hours in the continuous presence of the antibody. Negative controls included incubation of cells either with nonimmune rabbit IgG or with anti-CD36 (FA6-152) or anti-GPA hybridoma supernatant (CS4).

Indirect immunofluorescence labeling. Purified erythroblasts were stained with the HFnR-Ab by indirect immunofluorescence. For that purpose, 0.5 to 1 x 10⁶ cells in 100 μL were incubated 30 minutes at 4°C with a 1/10 dilution of the HFnR-Ab or control nonimmune rabbit IgG. Cells were subsequently washed twice and resuspended in a 1/50 dilution of fluorescein-labeled goat anti-rabbit IgG F(ab')2. Incubation was continued for 30 minutes at 4°C. Cells were washed twice, resuspended at a final concentration of 1 x 10⁶ cells/mL in phosphate buffered saline (PBS) containing 1% BSA and examined under a Zeiss microscope equipped for epifluorescence.

RESULTS

Purification to homogeneity of very immature human marrow erythroblasts. The purification procedure described under Materials and Methods yielded in 20 experiments a population (I-2) containing 79% ± 3% of blasts that were HLA-DR, CD34, and GPA negative by indirect immunofluorescence (Fig 1). Positive labeling with the FA6-152 MoAb whose expression is restricted to platelets, monocytes, and erythroid cells suggested the erythroid origin of these cells.²⁹

The erythroid origin of purified I-2 cells was clearly demonstrated by the I-2 cells' ultrastructural characteristics and immunophenotype. Eighty percent of I-2 cells were classified as erythroid and included only two maturation stages (Fig 2): the most immature phenotype represented 10% to 15% of this cell fraction. Despite the absence of GPA and rhophocytosis, the population was recognized as erythroid by the presence of Theta granules (θ-Gr) and of a few ferritin molecules located in the θ-Gr and scattered through the cytoplasm (Fig 2A, B). These cells were presumably CFU-E, since the I-2 cell fraction contained the same proportion of progenitors giving rise to I-2 hemoglobinized clusters (see below). Other erythroid cells in the I-2 fraction were more mature, as shown by the presence of GPA (Fig

Fig 1. Photomicrographs of MGG-stained I-2 cells before culture (A), and after 7 days (B), and 10 days (C), in suspension culture in the presence of Ep. There was no difference in the morphology of cells grown in suspension or on an Fn substrate. (Original magnification, x 400)

Fig 5. Photomicrographs of MGG-stained adherent (A) and nonadherent (B) erythroblasts observed in a typical experiment where purified I-2 cells were cultured in Fn-coated wells for 7 days.
2B). However, GPA density was below the threshold of sensitivity expected for conventional immunofluorescence or avidin-biotin labeling, which explains why these cells were not eliminated by immune rosetting. One or 2 sites of rhopheocytosis were observed (Fig 2B), and the concentration of ferritin in the θ-Gr and in the cytoplasm was increased in comparison with the more immature cells (Fig 2C). Hemoglobin was absent, as judged by the negative dense peroxidase reaction. These properties, together with the low expression of rhopheocytosis and GPA, indicated that this population was less differentiated than the classical proerythroblast, and we have therefore designated these cells "preproerythroblasts" as they appear to represent a stage intermediate between CFU-E and proerythroblasts. The I-2 fraction contained on average 65% preproerythroblasts. Twenty to twenty-five percent of I-2 cells were nonerythroid, as recognized by electron microscopy. Most of these belong to the megakaryocytic lineage, and a minority were mast cells, basophil myelocytes, and promyelocytes (data not shown).

Clonogenic properties of purified I-2 cells were assessed in methylcellulose colony-assays (Table 1). If we define a CFU-E as a progenitor giving rise to a cluster of at least 10 to 15 hemoglobinized cells at day 9 to 10, 11% ± 1% of I-2 cells were CFU-E (n = 20), whereas preproerythroblasts variably gave rise to clusters of less than 10 cells that usually had lysed by day 9. The proportion of CFU-E matched the proportion of very early GPA negative erythroid cells recognized by electron microscopy. CFU-E enrichment was 36 ± 4-fold relative to NA-MNC and over 50 to 60-fold compared with native marrow. The enrichment of preproerythroblasts could not be calculated, as these cells cannot be accurately quantitated in unpurified marrow cells either by morphologic or colony-assays criteria. Recovery of CFU-E in the I-2
fraction was 41% ± 3%. In seven experiments where the different fractions obtained during the purification were cloned in methylcellulose, 10% CFU-E were found in the P-2 fraction (Table 1). The latter may represent a subset of HLA-DR+, CD34+ CFU-E. Our results also showed that contamination by other progenitor cells was negligible since in 20 experiments, BFU-E and CFU-GM represented 0.7% and 0.6% of I-2 cells, respectively (Table 1).

In order to assess the proliferative and differentiative behavior of the purified I-2 cells in vitro, we cultured them in suspension in the presence of 3 U/mL Ep (but no other hematopoietic growth factors). For the first 2 to 3 days, there was no net change in the number of cells, whereas during the next 7 to 9 days, there was a four- to ninefold increase over the initial number of cells, reflecting the proliferation of the maturing erythroblasts (Fig 3). Morphologic examination showed that I-2 cells differentiated synchronously up to the enucleation stage, with the production of many reticulocytes (Fig 1). In the absence of Ep, I-2 cells did not survive beyond 48 hours. Noneyroid cells were not maintained in these cultures.

Adhesion of purified erythroblasts to Fn during their differentiation. CFU-E and preproerythroblasts adhesion to Fn was evaluated in 19 separate experiments using I-2 cells. The proportion of Fn-adherent CFU-E was precisely quantitated by colony-assays of adherent and nonadherent cells and that of Fn-adherent preproerythroblasts was estimated from the proportion of total Fn-adherent I-2 cells. The mean (±SEM) proportion of Fn-adherent CFU-E was 58% ± 5%. It was over 65% in 10 of 19 experiments, and below 30% in only three experiments. In contrast to the high proportion of adherent CFU-E, only 31% ± 3% of total nucleated I-2 cells were Fn-adherent. As CFU-E represents 10% to 15% of the I-2 population, and preproerythroblasts 65%, this gives a proportion of Fn-adherent preproerythroblasts below 25%. This difference in the ability of CFU-E and preproerythroblasts to adhere to Fn was seen in each experiment, and suggests loss of adhesion to Fn as CFU-E matures in preproerythroblasts. No significant attachment of CFU-E and preproerythroblasts occurred onto either human collagen I, III, IV or laminin.

The effect of differentiation on the adherence to Fn was next investigated: seven experiments were performed using cells removed after varying periods of time from 7 to 10 day-cultures of highly CFU-E-enriched population, and six experiments were performed by culturing cells 7 to 10 days on an Fn substrate. Results from these 11 experiments have been pooled in Fig 4. In each experiment, adhesion to Fn decreased with maturation and was completely lost at late maturation stages. Even though the rate of decline varied between experiments, the pattern observed was similar in each experiment, ie, in the purified I-2 population before culture (day 0), the proportion of adherent CFU-E was higher (58%) than that of total purified cells (30%) and thus preproerythroblasts, and slowly declined over the next 8 days in culture to reach 20% at day 8 to 9 (polychromatophilic stage). At that stage, the capacity to adhere to Fn appeared to rapidly decline and was completely lost at the enucleation stage.
stage. Reticulocytes were always found exclusively in the nonadherent fraction. This was best appreciated in experiments in which purified 1-2 cells were cultured on Fn during several days in the presence of Ep. In a representative experiment, shown in Fig 5, 20% to 30% of the day 7 cells were still strongly adherent to Fn. Most of these adherent cells had a phenotype of polychromatophilic erythroblasts, and no reticulocytes were seen (Fig 5). However, some reticulocytes were already present in this culture, but they had been released from the Fn substrate and represented 25% of the nonadherent population (Fig 5). The same partitioning was seen for enucleating cells. Failure of enucleating erythroblasts and reticulocytes produced in culture (±Fn) to attach to Fn was not attributable to any change in their viability as assessed by dye exclusion. Furthermore, assessment of enriched reticulocytes, freshly isolated from the peripheral blood from three patients with hemolytic anemia showed that these also did not attach to Fn in our experiment, shown in Fig 5, 20% to 30% of the day 7 cells had matured into reticulocytes.

**Effect of anti-HFnR antibodies on the adhesion of purified erythroblasts to Fn.** In seven separate experiments, CFU-E and preproerythroblasts (1-2 cells) were first preincubated with either a 1/10 dilution of polyclonal IgG against HFnR or with undiluted B1E5 hybridoma supernatant and then incubated in Fn-coated wells. As shown in Fig 6, both antibodies specifically inhibited CFU-E and I-2 cells (ie, preproerythroblast) adhesion to Fn, as nonimmune rabbit IgG, anti-CD 36 (FA6-152), and anti-GPA had no effect.

HFnR-Ab labeled 100% of erythroid I-2 cells by indirect immunofluorescence, whereas enucleating cells and reticulocytes stained negatively, suggesting that the disappearance of the functional capacity of differentiating erythroblasts to adhere to Fn was paralleled by the disappearance of a cell-surface receptor (data not shown).

**DISCUSSION**

One of the major questions regarding hematopoiesis is the understanding of the mechanisms whereby progenitor cells interact with their marrow environment. Our recent observation\(^3\) that human marrow CFU-E and mature BFU-E selectively attach to ECM prepared from marrow fibroblasts and to purified Fn suggests that progenitor cell-matrix interactions might represent one such mechanism. In the present study, we have demonstrated that attachment to Fn is a property of most CFU-E and early erythroblasts in normal human marrow, and that this property is subsequently lost upon terminal erythroid differentiation.

We have approached this question by designing an in vitro model that reproduces the successive steps followed by a CFU-E when it differentiates into reticulocytes. Our strategy was first to purify human CFU-E directly from fresh marrow samples. Negative selection by avidin-biotin immune rosetting reproducibly yielded a homogenous population of very immature cells identified as erythroid by their positive labeling with FA6-152,\(^2\) and by ultrastructural criteria. The procedure described was nontoxic, and more powerful and reliable in our hands than panning.\(^3\)\(^,\)\(^2\)\(^1\) Moreover, large numbers of marrow cells could be easily processed within a few hours with minimal cell loss, in contrast to cell sorting.\(^2\)

The availability of a highly enriched population of very immature normal erythroblasts gave us the opportunity to delineate the ultrastructural changes that are associated with human erythroid cell differentiation. Previous information has relied on analyses of cells from patients with erythroblastic leukemia or of partially purified populations of normal CFU-E.\(^1\) The earliest markers detected in 1-2 cells were β-Gr and ferritin molecules (Fig 2). Most likely, cells that exhibited these two features only represent CFU-E. This
conclusion is based on our finding that this morphology identified the same proportion of cells in the purified population as were detected by colony-assay as CFU-E. Surprisingly, these cells did not express platelet-peroxidase-like activity previously described in presumptive CFU-E. However, after a few hours in culture, this activity was recovered (data not shown), suggesting its possible inactivation by the purification procedure. Cell-surface GPA and images of rhopheocytosis were seen on the other I-2 erythroid cells, but at a much lower density than what has been described for proerythroblasts. These cells, or "preproerythroblasts," therefore appear to represent an intermediate stage between CFU-E and proerythroblasts.

Human CFU-E have been purified only indirectly through the proliferation of partially purified blood or marrow BFU-E cultured 7 to 9 days either in liquid suspension or in semisolid assay in the presence of Epo. This approach also yielded 80% of very immature erythroblasts containing up to 50% BFU-E. However, in contrast to cells directly purified from marrow by avidin-biotin immune rosetting, which appear homogeneous with respect to their stage of maturation, BFU-E-derived cells are more heterogeneous and include many hemoglobinized proerythroblasts. CFU-E and early erythroblasts have also been purified from either thiophenicol-treated or Friend virus-infected mice spleen cells, but it is difficult to compare them with human cells.

We have previously shown that the primitive BFU-E, which give rise to multiclustered colonies, do not attach to Fn, whereas 30% of CFU-E, which represents the most mature type of progenitor, express this property. In this study, we assessed the attachment to Fn of purified CFU-E, proerythroblasts, and their progeny, generated in cultures that support their terminal erythroid differentiation. Interestingly, the majority of CFU-E recovered in the purified population selected for these studies showed a higher proportion of Fn-adhering members. Although a direct comparison of Fn-adherence for purified and unpurified CFU-E was not undertaken, it is possible that cells defined as CFU-E by colony-assays are heterogeneous with respect to their Fn-adherence properties, and that our purification procedure isolated a subset of CFU-E with higher Fn-adherence potential. Alternatively, it is possible that the adhesion assay may give different absolute results according to the composition of the population being tested. What the present studies have revealed is that adherence to Fn also clearly changes during the later stages of erythroid cell differentiation: (1) CFU-E attached to Fn in the highest proportion, close to 60% (Fig 4), (2) the proportion of adherent proerythroblasts was consistently half that observed with the copurified CFU-E, and (3) enucleating erythroblasts and reticulocytes derived from these immature erythroblasts were unable to attach to Fn. These data demonstrate that the Fn-adhesion property is lost in differentiating erythroblasts in two sequential stages: an initial decline during the transition from CFU-E to proerythroblasts, with a subsequent loss of all remaining adhesive properties at the time of enucleation.

Expression of Fn-adhesion during human erythroid differentiation appears to be strikingly correlated to the migratory capacity of these cells. In semisolid culture media, all BFU-E exhibit an extensive migratory capacity, which is abruptly lost at the CFU-E level. In vivo, marrow cell egress occurs at the reticulocyte stage, and young reticulocytes are motile, whereas very few CFU-E circulate. It is thus tempting to speculate that these changes in erythroid cell motility reflect changes in cell attachment to Fn and possibly other adhesive glycoproteins of the marrow ECM. This is strengthened by the recent observation that glycoprotein IV, an 88 Kd glycoprotein identified as a thrombospondin receptor, is present on mature BFU-E, CFU-E and erythroblasts, and is similarly lost during terminal erythroid differentiation.

Loss of attachment to Fn with terminal differentiation has been previously observed in DMSO-induced murine erythroleukemic cells, and hemin-induced human K 562 cells and ascribed to the loss of the 140 Kd Fn receptor molecule identified as VLA-5 on uninduced K562. Results obtained with human reticulocytes isolated from patients with hemolytic anemia or recently splenectomized have shown that these cells can attach to Fn, which appears to contradict the results presented in this study. However, reticulocytes produced in response to hemolysis are not identical to those produced during steady-state erythropoiesis, and their adhesion properties might be altered by the underlying disorder. Furthermore, the marked susceptibility of cell adhesion to the conditions in which the functional attachment assay is performed might explain quantitatively different results.

Both the anti-α5β1 polyclonal IgG and the anti-α5 MoAb inhibited CFU-E and proerythroblast adhesion to Fn. This strongly suggests that VLA-5 (α5β1) functions as an Fn receptor on normal human erythroblastic cells. The Fn receptor was undetectable from reticulocytes by indirect immunofluorescence. However, changes in the expression of the Fn receptor during in vitro erythroblastic differentiation could not be analyzed in more detail by immunofluorescence: the polyclonal antibody recognizes not only VLA-5, but all integrins sharing the β1 subunit, and some of these molecules may be expressed by erythroblasts, and the B1E5 MoAb is not suitable for fluorescence labeling (C. Damsky, personal communication, January 1989).

The above results demonstrate that adhesion to Fn is precisely regulated during normal human erythroid differentiation and may influence cell migration in the bone marrow. A challenging question will be to determine if stage-specific expression of integrins will influence cell behavior by triggering early events associated with erythroid cell proliferation and differentiation.

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