E-Cadherin Is Functionally Involved in the Maturation of the Erythroid Lineage

Sorin Armeanu,* Hans-Jörg Bühring,‡ Monika Reuss-Borst,‡ Claudia A. Müller,* and Gerd Klein*

*University Medical Clinic, Section for Transplantation Immunology and Immunohematology and‡Department of Internal Medicine II, 72076 Tübingen, Federal Republic of Germany

Abstract. Differentiation and proliferation of hematopoietic progenitors take place in the bone marrow and is a tightly controlled process. Cell adhesion molecules of the integrin and immunoglobulin families have been shown to be involved in these processes, but almost nothing was known about the involvement of the cadherin family in the hematopoietic system. A PCR screening of RNA of human bone marrow mononuclear cells with specific primers for classical cadherins revealed that E-cadherin, which is mainly expressed by cells of epithelial origin, is also expressed by bone marrow cells. Western blot analysis and immunofluorescence staining of bone marrow sections confirmed this unexpected finding. A more detailed analysis using immunoaffinity columns and dual color flow cytometry showed that the expression of E-cadherin is restricted to defined maturation stages of the erythropoietic lineage. Erythroblasts and normoblasts express E-cadherin, mature erythrocytes do not. A functional role of E-cadherin in the differentiation process of the erythroid lineage was indicated by antibody-inhibition studies. The addition of anti-E-cadherin antibody to bone marrow mononuclear cultures containing exogeneous erythropoietin drastically diminished the formation of erythropoietic cells. These data suggest a non-anticipated expression and function of E-cadherin in one defined hematopoietic cell lineage.

ADHERINS are a family of structurally and functionally related molecules which mediate homophilic, calcium-dependent cell–cell interactions (10). The classical cadherins to which the E-, N-, and P-cadherins belong are highly conserved transmembrane glycoproteins with similar domains for adhesion sites, calcium binding and the interaction with the cytoskeleton (12). Recently, a second class of cadherins was identified by polymerase chain reaction which differ from the classical cadherins by unique structural features (32, 33). Cadherins have been demonstrated to play major roles during morphogenesis (29), the maintenance of epithelial tissues and also in malignant tumors (30). Most of these cadherin-dependent interactions are homophilic as well as homotypic, but two recent publications also showed that these adhesion molecules can also be involved in heterotypic and even in heterophilic interactions (5, 31). These two publications also demonstrated that bone marrow–derived, nonresident leukocytes can interact with epithelial tissues via an E-cadherin mediated pathway.

In the bone marrow hematopoietic progenitor cells proliferate and differentiate within a specific microenvironment which comprises resident nonhematopoietic stromal cells, a complex extracellular matrix and various secreted cytokines (7, 8, 15). Close interactions of the developing progenitor cells with this microenvironment are necessary for the controlled egress of the hematopoietic cells. Under normal circumstances only mature cells leave the bone marrow, immature cells, with the exception of the early pro-T-cells, are retained within the marrow (24). In contrast, during leukemia when a breakdown of adhesive mechanisms occurs, many immature blast cells are released into the periphery (11). Various adhesion molecules of different families including the integrins and members of the Ig superfamily (21, 26, 28, 34), but also adhesive and anti-adhesive molecules of the extracellular matrix (4, 15, 17–19, 23) have been implicated in the interactions of developing hematopoietic progenitors and their microenvironment. So far, the cadherin family did not seem to play a role in these highly specific interactions.

In an attempt to look for an involvement of cadherins in human hematopoiesis we screened messenger RNA of purified human bone marrow cells by reverse transcriptase–PCR (RT–PCR)[1] with specific primers for the classical cadherins. Here, we report that E-cadherin, which is also known as uvomorulin (35) and mainly present on cells of

Address correspondence to Dr. Gerd Klein, University Medical Clinic, Dept. II, Otfried–Müller-Straße 10, 72076 Tübingen, FRG. Ph.: 7071-294465. Fax: 7071-294464. E-mail: gerd.klein@uni–tuebingen.de.

1. *Abbreviation used in this paper:* RT-PCR, reverse transcriptase PCR.

epithelial origin, is expressed on a subset of human bone marrow cells. Using flow cytometric analysis and affinity column separation we identified this subset as distinct maturation stages of the erythroid lineage. Functional studies indicated that E-cadherin plays an important role in the maturation of erythrocytes within the bone marrow.

Material and Methods

Hematopoietic Cells and Cell Lines

Normal bone marrow aspirates were obtained after informed consent from healthy donors undergoing harvest for allogeneic bone marrow transplantation. The bone marrow mononuclear cells were separated from mature erythrocytes and granulocytes by Percoll density (1.077 g/ml) gradient centrifugation. Bone marrow cells from patients with myeloblastic acute leukemia (FAB classification AML M2; reference 2) were obtained at the time of initial diagnosis, and the mononuclear cells were also purified by density gradient centrifugation. The erythroleukemic cell line K562 and the myeloblastic cell line KG1a were those used in our previous studies (17–19). The human epidermoid carcinoma cell line A431, which is known to express high levels of E-cadherin, was used for control experiments. All these cell lines were grown in RPMI-1640 culture medium supplemented with 10% fetal calf serum.

Long-term Bone Marrow Cultures

5×10^6 purified bone marrow mononuclear cells, either from healthy donors or from patients with myeloblastic AML M2 leukemia, were grown in 25 cm^2 tissue culture flasks in RPMI-1640 medium containing 12.5% fetal calf serum, 12.5% horse serum, and 10^{-6} M hydrocortisone (Dexter conditions). The medium was changed completely every week. Under these conditions an adherent stromal cell layer consisting of elongated fibroblast-like cells and macrophages is formed during a period of two to three weeks. The stromal cell layer supports myelopoiesis in vitro for some weeks, but in the absence of erythropoietin erythropoiesis is not supported by these culture conditions. For RT–PCR analysis the stromal cell layer was harvested after 4 wk, when an almost confluent adherent stromal cell layer was present, after washing the long term culture twice with prewarmed PBS in order to remove most of the myeloid cells which adhere to the stromal cells. For immunofluorescence stainings the bone marrow cells were grown on glass cover slips under the same culture conditions as described before.

Antibodies

The monoclonal antibody HECD-1 specific for human E-cadherin (27) was purchased from Biermann (Bad Nauheim, FRG). For use in the CEPRATE LC biotin immunoaffinity column (see below) this antibody was biotinylated with a solution of 1 mg/ml N-thiosuccinimide biotin (Calbiochem-Behring Corp., La Jolla, CA) in dimethyl sulfoxide. Briefly, 10 μg of the biotin ester was added to 100 μg/100 μl antibody in sodium borate buffer, pH 9.3, and incubated for 2 h at room temperature. The reaction was stopped by the addition of 20 μl 1 M glycine. The biotinylated antibody was purified by gel filtration on a G-25 sephadex column (Pharmacia, Freiburg, FRG)

Polymerase Chain Reaction

Total RNA was isolated from bone marrow cells, from long term bone marrow culture–derived stromal cells or from hematopoietic cell lines by acid guanidinium isothiocyanate–phenol–chloroform extraction according to Chomczynski and Sacchi (6). E-cadherin and β-actin mRNAs were detected using RT–PCR methodology. Based on the published sequence for the human E-cadherin cDNA (3), specific sense (5'TCC ATT TCT TGG TCT ACG CC3') and anti-sense (5'CAC CTT CAG CCA ACC TGT TT3') primers corresponding to an extracellular portion of E-cadherin were designed with the aid of the HUSAR/GCG program from EMBL (Heidelberg, FRG), and were used to amplify a 362-bp PCR product from the positive control cell line A431. The following primers amplifying a 316-bp PCR product from human β-actin cDNA were used: sense (5'TCA GAA GGA TTC CTA TGT GGG C3') and anti-sense (5'CCA TCA CGA TGC CAG TGG TA3'). Reverse transcription was performed with

AMV–reverse transcriptase and buffers from Pharmacia at 42°C for 1 h with 3 pM anti-sense primers. PCR conditions with 2.5 U Taq-polymerase (Amersham-Buchler, Braunschweig, FRG) and 0.3 μM of each primer included denaturation at 94°C for 30 s, annealing at 60°C for 1 min and polymerisation at 72°C for 1 min, and a total of 33 cycles were run. PCR products were resolved by electrophoresis in 2.3% agarose gels (GIBCO-BRL, Eggenstein, FRG) and detected after ethidium bromide staining. Reactions were carried out in the presence or absence of 1 μg total RNA. RT-PCR products were not detected if the RNA was omitted.

FACS Analysis

For single color immunofluorescence studies, 5×10^5 cells were incubated with 20 μl human serum (RWD14 from Behring Werke, Marburg, FRG) for 15 min, washed with 0.1%BSA, 0.1% NaN$_3$ in PBS and incubated for 30 min with the first antibody. After two washing steps the cells were incubated for 20 min with FITC-conjugated goat anti–mouse IgG (Fab')$_2$ fragments (Dianova, Hamburg, FRG), washed, and analyzed for surface antigen expression using a FACScan© flow cytometer and FACScan Research software (Becton Dickinson GmbH, Heidelberg, FRG). As a positive control, the cells were labeled with the antibody W6/32.HL which recognizes the heavy chain of MHC class I antigens. An inactive variant of W6/32.HL, the antibody W6/32.HK, was used as a negative control (1). By excluding propidium iodide permeable cells only living cells were analyzed.

For two color FACS analysis the bone marrow mononuclear cells were labeled with the biotinylated HECD-1 antibody in combination with one of the following antibody conjugates: CD2-PE, CD3-PE, CD10-FITC, CD13-PE, CD19-FITC, CD33-PE, CD34-FITC, CD56-PE, and CD71-FITC which were all purchased from Dianova (Hamburg, FRG); and CD41-FITC and glycophorin A–FITC which were obtained from Immunotech (Marseille, France). After washing the biotinylated HECD-1 was stained with either streptavidin-phycoerythrin (SA–PE; Dianova) or with streptavidin–QuantumRed™ (SA–QR; Sigma, Munich, FRG) depending on the second antibody which was conjugated either with FITC or with phycoerythrin. The two color analysis was performed on a FACS Vantage (Becton Dickinson, Heidelberg, FRG). 25,000 events of each probe were aquired after compensation of the fluorescence signals and evaluated with the Lysis II software obtained from Becton-Dickinson.

Immunofluorescence Stainings

5-μm frozen sections of native bone marrow, taken from the sternum of hematopoietic healthy patients, were cut on a cryostat (Reichert & Jung, Heidelberg, FRG). The sections were allowed to air dry for one hour and could be stored at −20°C until use. The sections, as well as the long-term bone marrow cultures grown on glass cover slips, were fixed at −20°C in acetone for five minutes. The fixed sections or cultures were then incubated for one hour with HECD-1 in PBS containing 0.1% BSA. After washing, the bound antibodies were detected by a Cy3™-conjugated goat anti-mouse second antibody (Dianova) diluted 1:500. For localization of cell nuclei, the sections were counterstained with the dye 4',6-diamino-2-phenylindol-dihydrochloride (DAPI; Boehringer Mannheim, FRG) at 1 μg/ml. After another wash, the stained tissue or cells were mounted with elvanol embedding medium (16) and examined under a Zeiss axiophot photomicroscope equipped with epifluorescent optics. Control stainings were done by omitting the first antibodies. No staining above background could be observed.

Western Blot Analysis

Isolated bone marrow mononuclear cells or stromal cells of long-term bone marrow cultures were centrifuged at 150 g for 5 min and washed with PBS. Then, 0.5 ml ice-cold detergent extraction buffer (1% NP-40, 1% Triton X-100, 1 mM CaCl$_2$, 1 mM PMSF and 1 μg/ml Aprotinin in 50 mM Tris, pH 7.6) was added to the cell pellets. After an incubation of 30 min the crude protein extracts were centrifuged at 10,000 g for 10 min. The supernatants were mixed with 2× Laemmli's SDS sample buffer containing 1% β-mercaptoethanol and boiled for 5 min. Proteins were separated on a 10% polyacrylamide gel followed by transfer to nitrocellulose filter in a semidry transfer unit. Unspecific protein binding sites of the filters were blocked with 2% Blocking Reagent (Boehringer-Mannheim) in TBS for 1 h at 37°C. The filters were then washed with 0.05% Tween-20 in TBS, and incubated with HECD-1 antibody for 1 h. Then the filters were washed again for 30 min (the wash solution was changed every 5 min), incubated with HRP-conjugated anti–mouse immunoglobulins (Dako A/S, Glostrup, Denmark) for 30 min, and washed as described before. Bound

HRP was visualized by using the ECL system from Amersham-Buchler (Braunschweig, FRG).

Bone Marrow Cell Separation (Cell Pro™ Column)

10^7 bone marrow mononuclear cells isolated by density gradient centrifugation were washed twice with 1% BSA in PBS (washing buffer) and incubated for 30 min with 2 µg/ml biotinylated HECD-1 antibody. Then, unbound antibodies were removed with washing buffer and the cells were loaded onto a CEPRATE LC biotin immunoaffinity column preequilibrated with 5% BSA in PBS (Cell Pro™ column; Cell Pro, Wezembeck-Oppem, Belgium). Non-labeled cells were rinsed off with PBS from the column. By gravity flow the biotin-labeled E-cadherin positive cells were retained on the avidin-coated beads of the column. They could be released by gently squeezing the column according to the manufacturer's instruction.

Functional Assay

In a 24-well plate 10^6 cells/well/ml of purified bone marrow mononuclear cells from healthy donors were cultured in the absence or presence of 5 U/ml erythropoietin (Terry Fox Laboratory, Vancouver, Canada) in RPMI-1640 culture medium containing 12.5% horse serum, 12.5% fetal calf serum, and 10^{-6} M hydrocortisone. To analyze a functional role of E-cadherin, the mAb HECD-1 was added to the erythropoietin containing cultures at final concentrations of 0.5, 1.0, and 5.0 µg/ml, respectively. After 16 d of culture a defined aliquot of the non-adherent cells was harvested and transferred by cytospin centrifugation to a slide and stained for 3 min with May-Grünwald staining-solution and 7 min with Giemsa-solution. The total number of the cells on the slide as well as the number of erythropoietic cells were evaluated under a Zeiss axiovert microscope. All the experiments with cells of each donor were carried out in duplicate.

Results

Expression of E-Cadherin by Bone Marrow Mononuclear Cells

For an analysis of the expression of E-cadherin in the human bone marrow, a specific reverse transcriptase polymerase chain reaction with primers corresponding to bp 381–400 and bp 723–742 of the human E-cadherin sequence (this sequence data is available from Genbank/EMBL/DDBJ under accession number Z13009) was established using RNA of the epithelial cell line A431 as a positive control. The expected 362-bp cDNA fragment was amplified from RNA of bone marrow mononuclear cells, but not from RNA of normal bone marrow derived
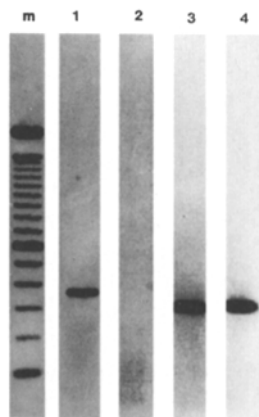
stromal cells after four weeks of culture (Fig. 1). Sequencing of the PCR product from the bone marrow mononuclear cells confirmed the identity to the published E-cadherin sequence. In control RT–PCR experiments using specific primers for human β-actin, comparably strong specific amplification products were obtained from RNA of the bone marrow mononuclear cells and the bone marrow derived stromal cells indicating that both RNA preparations were intact (Fig. 1). RT–PCR analyses of RNA from the erythroleukemic cell line K562 and the myeloblastic cell line KG1a, as well as from stromal cells derived from an AML-M2 myeloblastic leukemia patient also did not show any positive signal for E-cadherin expression. Thus, it seemed likely that a subpopulation of the non-adherent bone marrow cell fraction, which was lost during the 4-wk culture period, was the source of E-cadherin expression.

Western blot analysis with a human E-cadherin specific antibody further strengthened this observation. Protein extracts were obtained from isolated bone marrow mononuclear cells and from the stromal cell layer of a normal long term bone marrow culture after 4 wk of incubation. The stromal cell layer mainly consisted of fibroblast-like cells and macrophages. After immunoblotting, an E-cadherin specific band of 120 kD could be detected in the bone marrow mononuclear cell extract, but not in the extract derived from bone marrow stromal cells (Fig. 2). As a control, an extract of the E-cadherin expressing epithelial cell line A431 was run in parallel resulting in a very strong signal at 120 kD (Fig. 2, lane c).

To analyze the expression of E-cadherin in the native bone marrow, immunofluorescence stainings of cryostat sections of this tissue were performed with the E-cadherin specific antibody HECD-1. Strong staining signals of individual hematopoietic cells, mainly at their cell surfaces, indicated a non-uniform distribution of E-cadherin in the human bone marrow (Fig. 3) which could not be attributed to morphologically identifiable specific cell types. An im-

Figure 1. RT–PCR analysis of E-cadherin expression of human bone marrow derived cells. RNA of isolated bone marrow mononuclear cells without being cultured (lanes *1* and *3*) and of bone marrow derived stromal cells after four weeks of culture (lanes *2* and *4*) were reverse-transcribed and amplified with human E-cadherin specific primers (lanes *1* and *2*) and human β-actin specific primers (lanes *3* and *4*). After separation on a 2.3% agarose gel, a strong 362-bp signal could be detected in lane *1* (bone marrow mononuclear cells), but no E-cadherin specific signal could be detected with RNA from bone marrow stromal cells (lane *2*). Strong 316-bp β-actin–specific signals could be detected in both RNA preparations (lanes *3* and *4*). Lane *m* represents the 100-bp ladder.

Figure 2. Western blot analysis of E-cadherin expression of human bone marrow cells. Protein extracts of isolated bone marrow mononuclear cells (lane *a*), of bone marrow stromal cells after 4 wk of culture (lane *b*) and of the epithelial cell line A431 (lane *c*) were separated on a 10% linear SDS-PAGE gel under reducing conditions. After transfer to nitrocellulose, the filter was incubated with the human E-cadherin specific antibody HECD-1. Positive signals were visualized by the ECL method. Bone marrow stromal cells did not express E-cadherin (lane *b*), but in the protein extract of isolated bone marrow cells a distinct band of 120 kD could be detected (lane *a*). A very strong signal was found in the control experiment with A431 cells which are known to express high levels of E-cadherin (lane *c*).
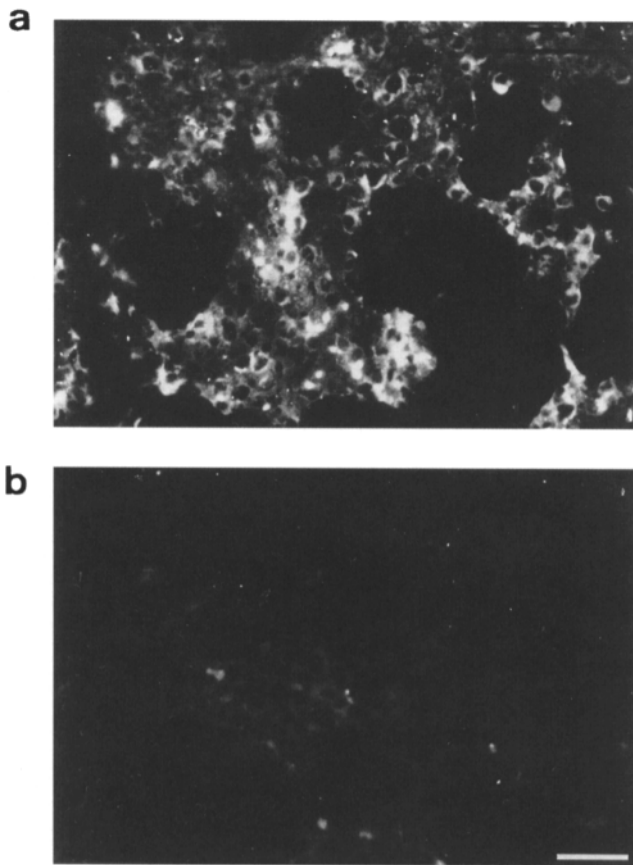
Figure 4. Isolation of E-cadherin expressing bone marrow cells. Bone marrow mononuclear cells isolated by density gradient centrifugation were labeled with the biotinylated HECD-1 antibody and applied to a Cell Pro™ column. Unlabeled cells were rinsed off the column, and bound cells were released by gently squeezing the column. After cytospin preparation the E-cadherin positive cells were stained according to Pappenheim and identified mainly as normoblasts and occasionally as erythroblasts. The photograph shows four typical normoblasts eluted from the column. These cells are characterized by their dense chromatin structure and the homogeneous cytoplasm. Bar, 5 μm.

Figure 3. Identification of E-cadherin–positive cells in the human bone marrow. The photographs show indirect immunofluorescence stainings of human bone marrow cryostat sections. In *a*, the section is stained with the human E-cadherin specific antibody HECD-1. Distinct stainings of individual cells, mainly at their cell surfaces, could be detected. *b* represents the control staining with the second antibody, the first antibody was omitted. Bar, 70 μm.

munofluorescence staining of an adherent bone marrow stromal cell layer after 4 wk of culture did not show any positive staining signals (not shown) indicating again that these cells do not express E-cadherin.

E-Cadherin Expression of Bone Marrow Cells Is Restricted to the Erythroid Lineage

Isolated bone marrow mononuclear cells were labeled with the biotinylated mAb HECD-1 and applied to the biotin immunoaffinity Cell Pro™ column. Through the biotin–avidin interaction labeled cells were retained in the column and unlabeled cells could be washed off the column. By gently squeezing the column the E-cadherin–positive cells were released. A morphological analysis of these cells revealed that after a single run the majority consisted of precursors of the erythropoietic lineage, in particular a few erythroblasts and mainly normoblasts (Fig. 4). FACS analysis of normal bone marrow mononuclear cells showed that 10–15% of the cells (depending on the donor) expressed E-cadherin (Figs. 5 and 6 *a*). By two color flow cytometry analysis no coexpression of E-cadherin with lymphoid (CD2, CD3, CD10, CD19, CD56, CD62L) or
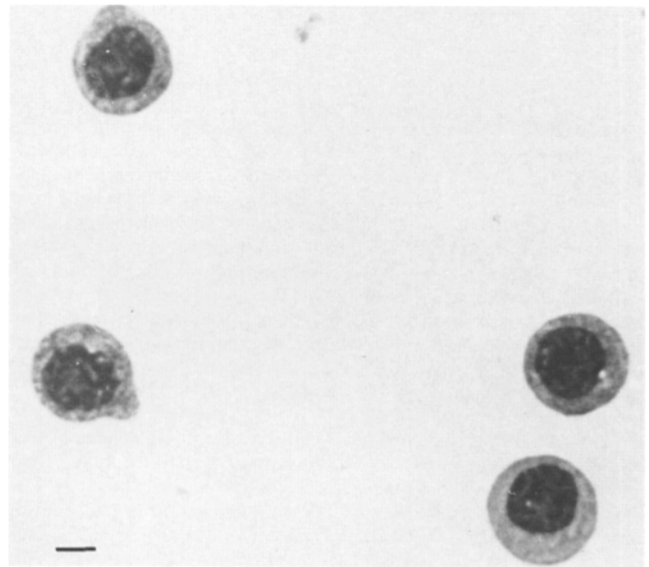
myeloid (CD13, CD33, CD41) cell surface marker molecules could be detected (Fig. 5). Similarly, CD34 which characterizes early differentiation stages of hematopoietic cells was absent on E-cadherin expressing cells. Co-expression of E-cadherin was found for glycophorin A and the transferrin receptor (CD71) which are marker molecules of the erythroid lineage in the normal bone marrow (Fig. 5). These results indicated that only cells of the erythropoietic origin carry the E-cadherin molecule in the hematopoietic system. E-cadherin appeared to be strongly expressed on erythroblasts which are normally included in the larger sized cell population gated in Fig. 6 *c* and to decrease during maturation of the erythroid precursors into normoblasts which are usually found in the smaller sized cell population gated in Fig. 6 *b* (22). Mature erythrocytes did not express E-cadherin. FACS analysis of long-term bone marrow cultures also did not show any positive signals for E-cadherin expression (not shown). Without the addition of exogeneous erythropoietin the differentiation process of erythroblasts is not supported by these culture conditions, only myeloid and stromal cell types are present in these cultures. The failure to detect any E-cadherin expressing cells in these cultures after a 4-wk incubation period confirmed our PCR and Western blot data (see above, Figs. 1 and 2).

Inhibition of Erythropoietic Differentiation

To support erythropoietic differentiation in bone marrow short term cultures 5 U/ml of erythropoietin was added to the culture medium. Without the addition of erythropoi-
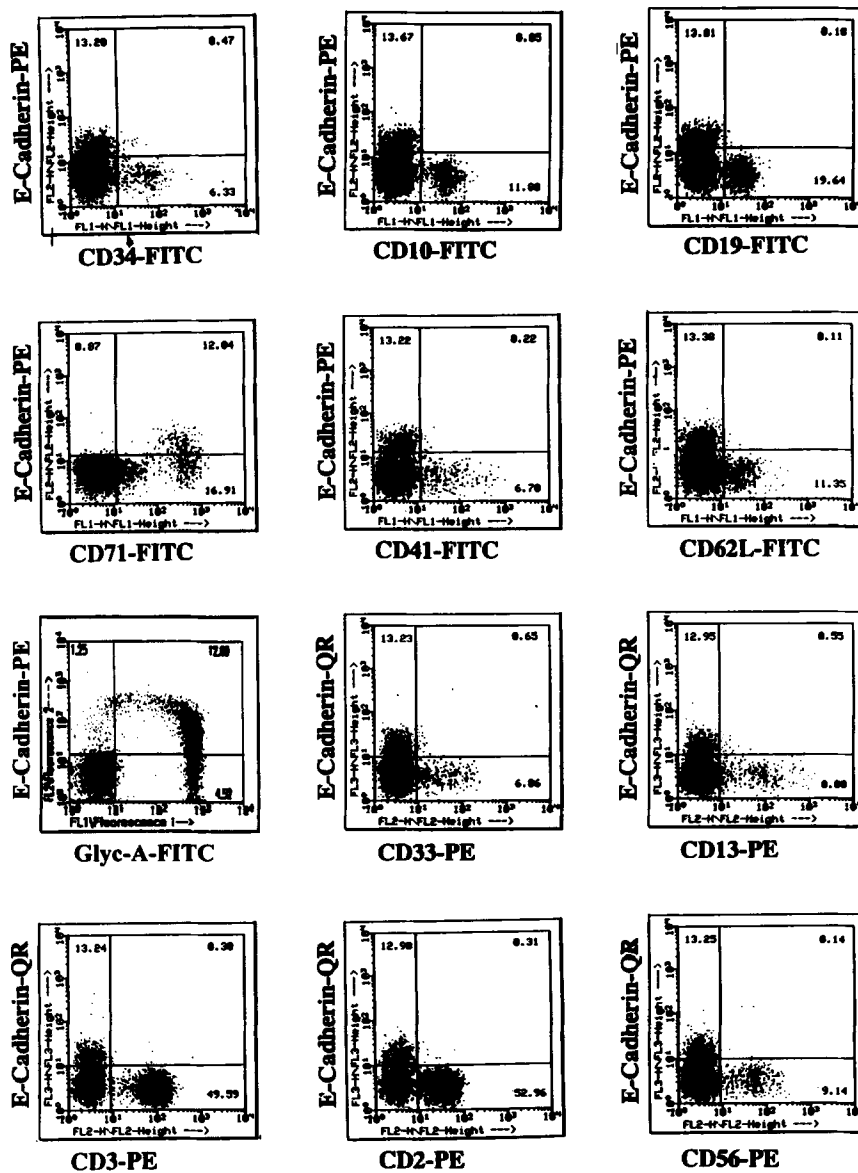
Figure 5. Two color FACS analysis of bone marrow mononuclear cells. The biotinylated HECD-1 antibody and the directly FITC- or PE-conjugated second antibodies against the different lymphoid, myeloid, early progenitors and erythroid cell surface marker molecule were used to label the isolated bone marrow mononuclear cells. The biotinylated anti-E-cadherin antibody was detected either by streptavidin–phycoerythrin (*PE*) or by streptavidin–QuantumRed™ (*QR*). Double-positive cells could be found in the upper right quadrant.

etin only very few erythroblasts and normoblasts could be detected after a culture period of 16 d (Fig. 7). However, in the presence of exogeneous erythropoietin up to 50% of the cells differentiated into erythroblasts and normoblasts during this incubation time as judged by morphological criteria. The addition of the anti-E-cadherin antibody HECD-1 to the erythropoietin-containing cultures reduced the numbers of erythropoietic cells in a dose-dependent manner: 5 μg/ml of the antibody decreased the formation of erythropoietic cells almost to the level of the cultures grown in the absence of erythropoietin (Fig. 7). Toxicity of the antibody seemed very unlikely since the total cell numbers did not significantly differ in the presence or absence of the antibody and almost no dead cells were found in the antibody-containing cultures after 16 d of incubation.

Discussion

Members of the cadherin family are known to function as morphoregulatory proteins during embryonic develop-

ment, and are important in the maintenance of tissue integrity and in tumorogenesis (12, 25, 29, 30). Thus far, no involvement of cadherins in the hematopoietic system with its high turnover of cells has been reported. In the present study we demonstrated that E-cadherin, which is known to be synthesized mainly by polar epithelial cells, is transiently expressed on defined stages of the erythropoietic cell lineage. This was observed despite the obviously nonpolar structure of the erythropoietic cells. E-cadherin seems to be involved in regulating erythroid differentiation processes, because antibodies against E-cadherin inhibited erythropoietic differentiation in vitro.

A first hint of the involvement of the classical cadherins (E-, P-, N-cadherin) in the hematopoietic system was found by PCR analysis of the heterogeneous bone marrow cell population. By this method all three cadherins were found to be present in human bone marrow aspirates (Armeanu, S., and G. Klein, unpublished observations), but only for E-cadherin we were able so far to define exactly the hematopoietic cell types in the bone marrow which express this molecule.
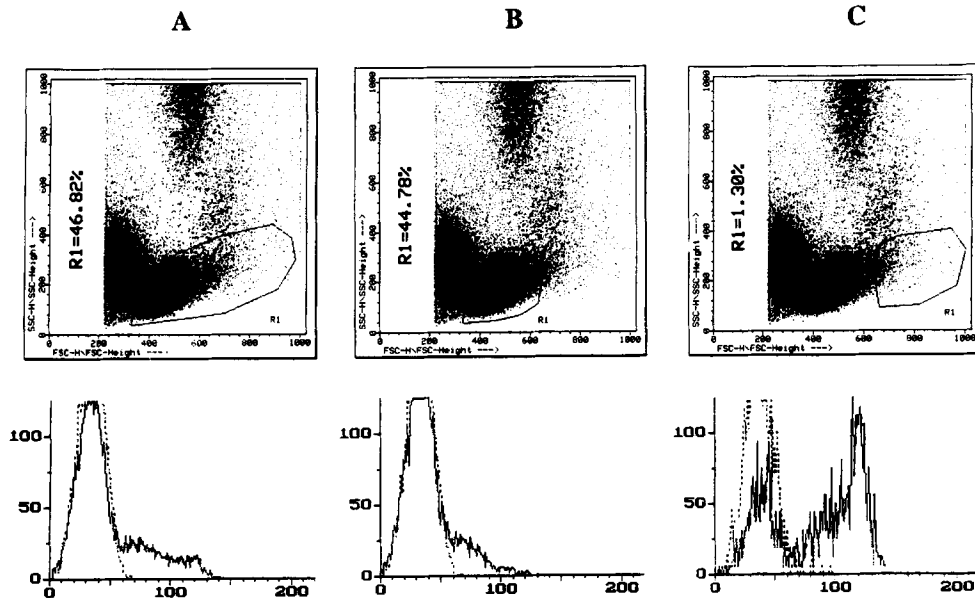
A B C

Figure 6. Single color flow cytometry analysis of bone marrow mononuclear cells. The figures show three different gatings of bone marrow cells labeled with HECD-1 in the FSC/SSC dot blot displays and the corresponding FACS histograms demonstrating the intensity of E-cadherin expression. The largest gate set in *A* contained about 15% of cells of the heterogeneous cell population which expressed E-cadherin with different intensities. Gating on the larger cell types (1.3%) revealed that these cells expressed E-cadherin with a much higher intensity (*C*) as compared to the smaller cell types analyzed in *B*. The dashed line in the histogram represents the negative control with the W6/32.HK antibody, the continuous line represents the labeling with the HECD-1 antibody.

Expression of E-cadherin was shown to be restricted to the erythroid lineage by immunoaffinity separation of bone marrow cells with the mAb HECD-1 which lead to a selective enrichment of erythroblasts and normoblasts. These progenitor cell types were comprised in the gates containing also the E-cadherin positive cells in the flow cytometry
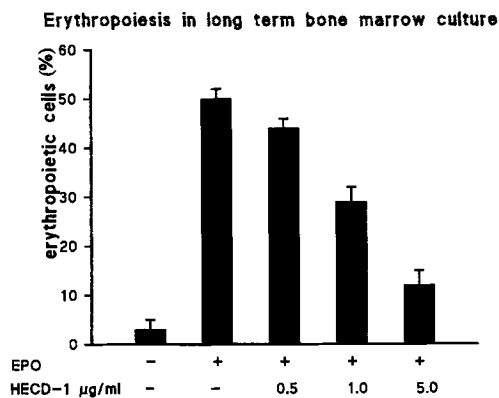
Erythropoiesis in long term bone marrow culture

Figure 7. Anti–human E-cadherin specific antibodies inhibit differentiation of erythropoietic progenitors into erythroblasts and normoblasts. 10^6 isolated bone marrow mononuclear cells/well were cultured in the presence (*Epo* +) or absence (*Epo* -) of 5 U/ml erythropoietin. Without this cytokine, only very few erythropoietic cells could be detected after 16 d of culture. The presence of erythropoietin stimulated the differentiation of erythropoietic cells drastically, up to 50% of the cells could be identified as erythroblasts and normoblasts. The addition of the mAb HECD-1 decreased the formation of these erythropoietin-induced erythropoietic cells in a concentration-dependent manner, only 12% (+/-3%) of the cells were of erythropoietic origin in the presence of 5 μg/ml anti-E-cadherin antibody. The experiments were carried out in duplicate.

analysis (22). A higher labeling of E-cadherin appeared to be present on the larger erythroblasts than on the erythroid precursors of the smaller normoblast stage suggesting gradual loss of its expression during maturation. Double labeling experiments with the anti-E-cadherin antibody and various myeloid and lymphoid cell surface markers only revealed coexpression with the molecules glycophorin A and CD71, the transferrin receptor, as two typical erythroid marker antigens. Bone marrow derived stromal cells encompassing fibroblast-like cells and macrophages did not express E-cadherin as shown by RT–PCR, Western blot analysis, and immunofluorescence staining of adherent stromal layers. Thus, E-cadherin seemed only to be synthesized by cells of the erythroid origin within the human bone marrow.

As another type of bone marrow–derived cells expressing E-cadherin the murine Langerhans cells of the epidermis have been identified and shown to interact with keratinocytes in a homotypic fashion via E-cadherin (30). A similar homotypic interaction of erythropoietic progenitor cells with another cell type in the human bone marrow seems unlikely since we could not detect another E-cadherin positive bone marrow cell. Normally, erythroblasts differentiate in close association with a central macrophage in a structure called the erythroblastic islands (13, 22). Macrophages of bone marrow aspirates of in vitro long term cultures did not show any E-cadherin expression. Therefore, interaction of erythroblasts with the central macrophage via E-cadherin seems to be unlikely although we cannot exclude with certainty that the single central macrophage of the erythroblastic island represents a specialized bone marrow cell type with specific differentiation characteristics and surface adhesion molecules.

A heterotypic heterophilic interaction involving E-cad-

herin has been recently reported for epithelial cells and intraepithelial lymphocytes with $\alpha^E\beta7$ as the counter receptor (5). Up to now no $\alpha^E\beta7$ expressing cells have been demonstrated in the human bone marrow still restricting expression of this integrin type to intraepithelial lymphocytes (9). Clearly, macrophages present in the bone marrow have to be analyzed for $\alpha^E\beta7$ integrin expression.

By addition of the mAb HECD-1 to bone marrow mononuclear cultures in the presence of erythropoietin we could demonstrate a strong inhibitory effect of this antibody on erythroid differentiation. Addition of the antibody to the cell cultures did not lead to an induction of the differentiation process indicating that binding of the antibody could not serve as a substitute for the ligand of E-cadherin on erythropoietic cells. However, it is not clear whether differentiation of the erythroblasts is a molecular event triggered by cell–cell adhesion. Members of the cadherin family are mainly thought to be involved in mediating cell adhesive interactions, but through their interactions with the cytoplasmic catenins (14) these molecules could also be involved in major signal transduction events. Thus, it might be possible that E-cadherin does not function as an adhesion-mediating molecule and is therefore not important for the retention of erythropoietic cells in the bone marrow. Instead, E-cadherin might have a major role as a signal transduction molecule in this differentiation process.

To our knowledge we have shown here for the first time an involvement of one member of the cadherin family during hematopoietic lineage maturation. The expression and function of E-cadherin was certainly not anticipated and a major task for the future will be to define the ligand of E-cadherin on erythropoietic cells. This ligand may either be E-cadherin itself or it could be a heterophilic interaction. Other members of the cadherin family can also be functionally involved in hematopoiesis and the present study shows that the search for other cadherin molecules in the hematopoietic system is certainly justified.

References

1. Barnstable, C. J., F. Bodmer, G. Brown, G. Galfre, C. Milstein, A. F. William, and A. Ziegler. 1978. Production of monoclonal Ab to group A erythrocytes, HLA for genetic analysis. *Cell.* 14:9–20.
2. Bennett, J. M., D. Catovsky, M. T. Daniel, G. Flandrin, D. A. G. Galton, H. R. Gralnick, and C. Sultan. 1985. Proposed revised criteria for the classification of acute myeloid leukemia. *Ann. Intern. Med.* 103:620–625.
3. Bussemakers, M. J. G, S. G. M. Mees, A. Van Bokhoven, F. M. J. Debruyne, and J. A. Shalken. 1993. Molecular cloning and characterization of human E-cadherin cDNA. *Mol. Biol. Rep.* 17:123–128.
4. Campbell, A. D., and M. S. Wicha. 1988. Extracellular matrix and the hematopoietic microenvironment. *J. Lab. Clinic. Med.* 112:140–146.
5. Cepek, K. L., S. K. Shaw, C. M. Parker, G. J. Russell, J. S. Morrow, D. L. Rimm, and M. B. Brenner. 1994. Adhesion between epithelial cells and T lymphocytes mediated by E-cadherin and the $\alpha^E\beta_7$ integrin. *Nature (Lond.).* 372:190–193.
6. Chomczynski, P., and N. Sacchi. 1987. Single step method of RNA isolation by acidic guanidinium thiocyanate phenol-chloroform extraction. *Anal. Biochem.* 162:156–159.
7. Dexter, T. M., L. H. Coutinho, E. Spooncer, C. M. Heyworth, C. P. Daniel, R. Schiro, J. Chang, and T. D. Allen. 1990. Stromal cells in haemopoiesis. *In* Molecular Control of Haemopoiesis. John Wiley & Sons Ltd., Chichester, UK. *Ciba Found. Symp.*148:76–95.
8. Dorshkind, K. 1990. Regulation of hemopoiesis by bone marrow stromal cells and their products. *Annu. Rev. Immunol.* 8:111–137.
9. Erle, D. J. 1995. Scratching the surface. *Curr. Biol.* 5:252–254.
10. Geiger, B., and O. Ayalon. 1992. Cadherins. *Ann. Rev. Cell Biol.* 8:307–332.
11. Gordon, M. Y. 1988. Adhesive properties of haematopoietic stem cells. *Br. J. Haemat.* 68:149–151.
12. Grunwald, G. B. 1993. The structural and functional analysis of cadherin calcium–dependent cell adhesion molecules. *Curr. Opin. Cell Biol.* 5:797–805.
13. Hanspal, M., and J. Hanspal. 1994. The association of erythroblasts with macrophages promotes erythroid proliferation and maturation: a 30 kD heparin-binding protein is involved in this contact. *Blood.* 84:3494–3504.
14. Kemler, R. 1993. From cadherins to catenins: cytoplasmic protein interactions and regulation of cell adhesion. *Trends Genet.* 9:317–321.
15. Klein, G. 1995. The extracellular matrix of the hematopoietic microenvironment. *Experientia.* In press.
16. Klein, G., M. Langegger, C. Goridis, and P. Ekblom. 1988. Neural cell adhesion molecule during embryonic induction and development of the kidney. *Development.* 102:749–761.
17. Klein, G., S. Beck, and C. A. Müller. 1993. Tenascin is a cytoadhesive extracellular matrix component of the human hematopoietic microenvironment. *J. Cell Biol.* 123:1027–1035.
18. Klein, G., S. Conzelmann, S. Beck, R. Timpl, and C. A. Müller. 1994. Perlecan in human bone marrow: a growth-factor-presenting, but anti-adhesive, extracellular matrix component for hematopoietic cells. *Matrix Biol.* 14:457–465.
19. Klein, G., C. A. Müller, E. Tillet, M.-L. Chu, and R. Timpl. 1995. Collagen type VI in the human bone marrow microenvironment: a strong cytoadhesive component. *Blood.* In press.
20. Lichtman, M. A. 1981. The ultrastructure of hemopoietic environment of the marrow: a review. *Exp. Hematol.* 9:391–410.
21. Liesveld, J. L., J. M. Winslow, K. E. Frediani, D. H. Ryan, and C. N. Abboud. 1993. Expression of integrins and examination of their adhesive function in normal and leukemic hematopoietic cells. *Blood.* 81:112–121.
22. Loken, M. R., V. O. Shah, K. L. Dattilio, and C. I. Civin. 1987. Flow cytometric analysis of human bone marrow: I. Normal erythroid development. *Blood.* 69:255–263.
23. Long, M. W. 1992. Blood cell adhesion molecules. *Exp. Hematol.* 20:288–301.
24. Petrides, P. E., and K. H. Dittmann. 1990. How do normal and leukemic white blood cells egress from the bone marrow? *Blut.* 61:3–13.
25. Ranscht, B. 1994. Cadherins and catenins: interactions and functions in embryonic development. *Curr. Opin. Cell Biol.* 6:740–746.
26. Ruoslahti, E., N. A. Noble, S. Kagami, and W. A. Border. 1994. Integrins. *Kidney Intl.* 45(Suppl. 44):S-17–S-22.
27. Shimoyama, Y., S. Hirohashi, S. Hirano, M. Noguchi, Y. Shimosato, M. Takeichi, and O. Abe. 1989. Cadherin cell–adhesion molecules in human epithelial tissues and carcinomas. *Cancer Res.* 49:2128–2133.
28. Springer, T. A. 1990. Adhesion receptors of the immune system. *Nature (Lond.).* 346:425–434.
29. Takeichi, M. 1991. Cadherin cell adhesion receptors as a morphogenetic regulator. *Science (Wash. DC).* 251:1451–1455.
30. Takeichi, M. 1993. Cadherins in cancer: implications for invasion and metastasis. *Curr. Opin. Cell Biol.* 5:806–811.
31. Tang, A., M. Amagai, L. G. Granger, J. R. Stanley, and M. C. Udey. 1993. Adhesion of epidermal Langerhans cells to keratinocytes mediated by E-cadherin. *Nature (Lond.).* 361:82–85.
32. Tanihara, H., K. Sano, R. L. Heimark, T. St. John, and S. Suzuki. 1994. Cloning of five human cadherins clarifies characteristic features of cadherin extracellular domain and provides further evidence for two structurally different types of cadherin. *Cell Adhes. Commun.* 2:15–26.
33. Tanihara, H., M. Kido, S. Obata, R. L. Heimark, M. Davidson, T. St. John, and S. Suzuki. 1994. Characterization of cadherin-4 and cadherin-5 reveals new aspects of cadherins. *J. Cell Sci.* 107:1697–1704.
34. Teixidó, J., M. E. Hemler, J. S. Greenberger, and P. Anklesaria. 1992. Role of beta 1 and beta 2 integrins in the adhesion of human CD34hi stem cells to the bone marrow. *J. Clin. Invest.* 90:358–367.
35. Vestweber, D., and R. Kemler. 1985. Identification of a putative cell adhesion domain of uvomorulin. *EMBO J.* 4:3393–3398.