 0014-4754/95/100914-13$1.50 + 0.20 © Birkhäuser Verlag Basel, 1995

The extracellular matrix of the hematopoietic microenvironment

G. Klein

University Medical Clinic, Department of Internal Medicine II, Section for Transplantation Immunology and Immunohematology, Otfried-Müller-Strasse 10, D-72076 Tübingen (Germany), Fax +49 7071 29 44 64

Abstract. The bone marrow microenvironment plays an important role in promoting hematopoietic progenitor cell proliferation and differentiation and the controlled egress of these developing hematopoietic cells. The establishment of long-term bone marrow cultures, which are thought to mimic hematopoiesis in vitro, and various stromal cell lines has greatly facilitated the analysis of the functions of this microenvironment. Extracellular matrix (ECM) molecules of all three categories (collagens, proteoglycans and glycoproteins) have been identified as part of this microenvironment and have been shown to be involved in different biological functions such as cell adhesion and anti-adhesion, binding and presentation of various cytokines and regulation of cell growth. It is suggested that these matrix molecules in combination with cytokines are crucial for compartmentalization of the bone marrow. Although many cell adhesion molecules have been characterized on the surface of hematopoietic progenitor cells, the nature of cellular receptors for the ECM components is less well defined. During leukemia, many immature blood cells are released from bone marrow, but it is not yet known whether these abnormalities in hematopoiesis are also caused by an altered microenvironment or altered composition of its extracellular matrix. The elucidation of the involvement of specific ECM-isoforms and as yet not characterized ECM components and their receptors in the bone marrow will certainly help towards a better understanding of these phenomena.
Key words. Cell-matrix interactions; adhesion; cytokines; collagens; proteoglycans; tenascin; laminin; fibronectin; cellular receptors.

Introduction

Hematopoietic stem cells which are capable of self-renewal can differentiate into myeloid and lymphoid committed progenitor cells. After clonal expansion these progenitors terminally differentiate into mature blood cells including lymphocytes, granulocytes, monocytes, erythrocytes and megakaryocytes. In the adult organism these differentiation processes take place in the bone marrow and are critically dependent on interactions of the hematopoietic stem cells and progenitor cells with the surrounding microenvironment. This hematopoietic microenvironment consists of various stromal cell elements, secreted and membrane-bound cytokines, and a complex extracellular matrix (ECM) (for detailed reviews see refs 18, 25, 30, 32, 49, 67, 101). The heterogeneous population of stromal cells includes fibroblast-like cells, adipocytes, and macrophages, and several authors also consider endothelial cells as part of the stromal cell population. The stromal cells synthesize and secrete various cytokines including colony stimulating factors, interferons and interleukins. These growth factors may either directly interact with the developing hematopoietic cells, or they may be secreted and stored in the ECM which then presents the factors to the developing hemotopoietic cells in a biologically active form. The ECM of the bone marrow of different species has been characterized and has been shown to include members of all three categories of matrix molecules: collagens, glycoproteins and proteoglycans[15,26,43,71]. These matrix components do not form an inert scaffolding, but play an active role in control of cell adhesion and migration within the bone marrow, presentation of cytokines and, directly or indirectly, in proliferation processes. They are also believed to be involved in the compartmentalization of the bone marrow giving rise to specific sites of hematopoietic cell differentiation. This review is intended to summarize the present knowledge of extracellular matrix molecule expression in the bone marrow microenvironment and the functional role of the ECM during normal and leukemic hematopoiesis.

Specificity of the bone marrow microenvironment

During normal hematopoiesis the different myeloid and lymphoid blood cells develop within the cavities of flat bones and the trabecular regions of long bones and, with the exception of the early pro-T-progenitor cells, are not released into the peripheral blood until they are mature. Thus, egress of the developing hematopoietic cells is a highly regulated process governed by interactions with the microenvironment[86]. The specificity of the hematopoietic microenvironment is convincingly demonstrated by bone marrow transplantation into irradiated recipients. Intravenously injected hematopoietic stem cells can 'home' to the bone marrow and, lodging in the appropriate microenvironment, they can reconstitute hematopoiesis of the recipient. This complex process which involves several steps such as specific recognition of bone marrow endothelial cells (via specific addressins?), transmigration into the extrasinu-

soidal spaces, and specific localization within the bone marrow, is only poorly understood. It is likely, however, that mechanisms similar to bone marrow transplantation are involved in the migration of embryonic hematopoietic stem cells from the yolk sac to the liver, spleen and finally to the bone marrow.

The specific interactions of the hematopoietic cells with their microenvironment are at least partially destroyed in leukemic patients. In such cases, immature blast cells are not retained within the marrow and are found in high numbers in the peripheral circulation, suggesting a breakdown of specific adhesive mechanisms[44] but the molecular basis of this abnormality is not known.

Analyses of the bone marrow microenvironment

The bone marrow stroma in vivo is difficult to analyze because of the very dense cell packing in this tissue where cells seem to be in close contact with each other or the ECM components, but do not necessarily have direct interactions. Thus, the marrow stroma in vivo is one of the less well studied tissues in vertebrates. The introduction of long-term bone marrow cultures has greatly facilitated these analyses and has clearly established the role of the microenvironment in proliferation and differentiation of primitive hematopoietic progenitor cells. Depending on the culture conditions, myelopoiesis, as first described by Dexter and colleagues[28,29], or lymphopoiesis, as identified by Whitlock and Witte[120], can be preferentially induced in vitro. In both cases the formation of an adherent stromal cell layer which produces and deposites an extracellular matrix meshwork (a typical example is shown in fig. 1) is a prerequisite for the development of hematopoietic cells in these cultures. These adherent stromal cells are

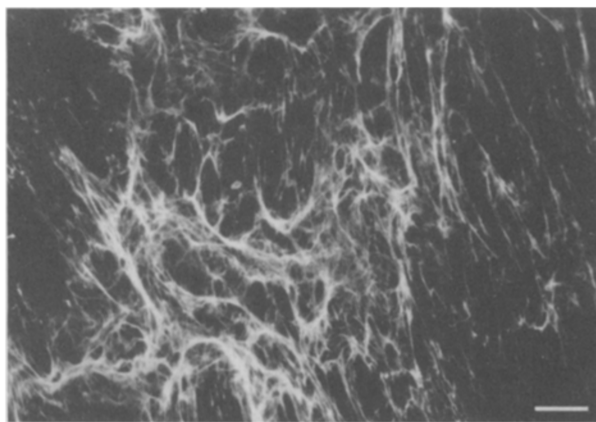

Figure 1. This immunostaining of a human long-term bone marrow culture shows a typical example of an extracellular meshwork laid down by extracellular matrix molecules. The network overlies the adherent stromal cell layer which cannot be seen in this immunohistochemical staining. The staining was performed with an antiserum against the heparan sulfate proteoglycan perlecan, but similar staining patterns could be seen for other ECM components such as various collagen types, and glycoproteins like fibronectin or tenascin. Bar = 50 μm.

generated from the light density bone marrow mononuclear fraction which is obtained after Percoll® or Ficoll® density gradient centrifugation of bone marrow aspirates. The origin of the bone marrow stromal cells is not clear, and the question of whether stromal and hematopoietic cells have a stem cell in common has not yet been resolved[53,54]. The adherent stromal layer is formed between day 5 and day 20 of the long-term culture and, in close contact with the hematopoietic progenitor cells, it can provide all the necessary stimuli for growth and differentiation of hematopoietic cells without addition of exogeneous cytokines. Thus, these cultures are thought to mirror hematopoiesis in vivo. Culture conditions for long-term bone marrow cultures have been defined for mouse[29] and modifications have been described for human cultures[40]. In the murine system hematopoiesis can be sustained for longer periods than in the human cultures. The reason for this difference is not clear.

The nature of the stromal cells in the long-term cultures has been defined by morphological criteria, antibody staining of the cytoskeleton and cell surface markers, and by enzyme reactions[30]. Most stromal cells identified in this way have their counterparts in the in vivo bone marrow microenvironment, but the recognition and characterization is much easier in the long-term cultures. This applies especially to studies concerning the composition of the extracellular matrix synthesized by bone marrow stromal cells. Various ECM components of the hematopoietic microenvironment were first identified in long-term cultures by biochemical, immunohistological or molecular biological methods (see below). Another way to dissect these complex interactions within the bone marrow are studies of simplified interactions between stromal cells and hematopoietic cells, isolated ECM components and hematopoietic cells or growth factors and hematopoietic cells. A number of stromal cell lines have been generated, mainly from murine bone marrow stromal cells but some human stromal cell lines are also available (for an extensive review see ref. 27). The morphological characterization of these cultured stromal cell lines has led to a number of descriptive names including fibroblastoid, endotheloid, epitheloid and smooth muscle-like cells or reticulum cells, blanket cells, adipocytes and preadipocytes. However, it has yet to be clarified whether morphological differences correlate with functionally different cell types, since it is known that different culture conditions may influence or change the morphology of these cells. A major challenge will be to prove whether these immortilized cell lines resemble or are identical to the cells found in vivo.

Nevertheless, stromal cell lines could be used in different assay systems to purify and characterize early hematopoietic progentior cells. These cell lines might also become clinically important, e.g. for the ex vivo mainte-

nance or expansion of hematopoietic stem or progenitor cells, or for improvements in gene transfer protocols.

Profiles of cytokine expression have been analyzed for most of these stromal cell lines (see ref. 27). Analyses of the extracellular matrix synthesized by the bone marrow stromal cell lines have been restricted to collagen types I–IV, fibronectin and one laminin isoform (see below). Since it is becoming more and more evident that the extracellular matrix is far more complex, a refined analysis of the extracellular matrix production by these stromal cell lines will be necessary.

Since stromal cell lines produce different ECM components the interactions seen with hematopoietic cells are still difficult to interpret because the effects may either be mediated by stromal cells and/or by their extracellular matrix. Experiments with defined purified matrix components will help to elucidate these interactions.

Cell-matrix interactions in the bone marrow

Collagens

The collagen family which shares triple helical domains as a common structural motif contains nineteen different characterized collagen types[76,115]. These collagens can be subgrouped according to their supramolecular structures: collagen types I–III and V form large fibrils, types VI and VII form microfibrils and anchoring fibrils, respectively. Networks are built up by types IV and VIII, and collagen types IX, XII and XIV represent the FACITs, fibril-associated collagens with interrupted helices[98]. In the bone marrow microenvironment, the fibrillar collagen types I, III and V and the basement membrane associated collagen type IV have been identified, mainly by immunofluorescence staining[5,125]. A more recent study analyzed the expression of the genes encoding for the mRNA of these collagen types[19]. All these studies demonstrated that collagen types I–V are abundantly synthesized and deposited in the bone marrow stroma. In addition, we have recently shown that the microfibrillar collagen type VI is also a major constituent of hematopoietic stroma[63].

Inhibition of collagen synthesis by the proline analogue *cis*-hydroxyproline in murine long-term bone marrow cultures leads to a reduction of stem and committed progenitor cell proliferation, suggesting a role of the collagens in hematopoiesis[124]. Koenigsmann et al.[64] identified collagen type I as an adhesive substrate for erythroid (BFU-E) and myeloid (CFU-GM) progenitor cells, and the specificity of these interactions was demonstrated by digestion with collagenase. But since collagen type I is ubiquitously distributed in many tissues of the body it seems unlikely that this collagen molecule could mediate specificity of early progenitor binding to bone marrow. Rather, these adhesive interactions could help to strengthen the overall binding to the stroma. The nature of the receptor for collagen type I on erythroid or myeloid progenitors was not identified in this study, since antibodies against the β1-integrin subunit and RGD-containing peptides were without any inhibitory effects. On B-lymphoid and myeloma plasma cells, which can also adhere to collagen type I, syndecan-1 has been identified as the counter-receptor[90,97] suggesting that this transmembrane heparan sulfate proteoglycan could also serve as a collagen receptor on other hematopoietic cells.

Functional analyses with the microfibrillar collagen type VI have shown that this collagen type is an even stronger adhesive substrate than collagen type I for various hematopoietic cells, and that these interactions are also not integrin-mediated. Again, the inhibition of hematopoietic cell attachment to collagen VI by heparin suggested a role for transmembrane proteoglycans[63].

Collagen type IV, which is mainly found in basement membranes of blood vessel endothelial cells and around fat depositions within the bone marrow, does not show an adhesive capacity for normal hematopoietic progenitor cells. However, this nonadhesive reaction is altered in chronic myelogeneous leukemic (CML) cells. CML progenitors showed an increased adhesion to type IV collagen, and it has been suggested that this alteration correlates with the facilitated egress of these cells from the bone marrow to the peripheral blood circulation[117].

In general, the turnover of the collagen matrix could be regulated by collagenase, but this enzyme does not seem to be transcribed by bone marrow fibroblast cells under normal conditions or after interleukin-1 treatment. This is in contrast to dermal fibroblasts which can be stimulated by interleukin-1 to express collagenase. Yet, both types of fibroblasts constitutively express mRNA for collagen type I[110]. It is not yet known if other cytokines could induce the expression of collagenase in bone marrow stromal cells, but the obvious difference between the dermal and the bone marrow stromal fibroblasts suggests that an undesired degradation of collagens in the bone marrow by at least some cytokines is avoided by the suppression of collagenase expression.

Proteoglycans

Compared to the collagen family, much more information exists on the functional role of proteoglycans in hematopoietic cell development. The proteoglycans consist of core proteins with covalently linked glycosaminoglycan (GAG) side chains. These GAG chains are usually very long, negatively charged, unbranched, and consist of different repeating disaccharide units. GAG display considerable sequence heterogeneity and this heterogeneity can be found within or between different GAG chains. With the exception of hyaluronic acid, which also does not contain a core protein, all these GAG chains are sulfated. According to their different disaccharide units four major proteoglycan classes can be distinguished: heparan sulfate, chondroitin sulfate,

dermatan sulfate and keratan sulfate proteoglycans (for reviews see refs 60, 65). With the exception of keratan sulfate proteoglycan, all of these proteoglycans and the GAG hyaluronic acid are found in the bone marrow microenvironment, either in a membrane-bound form or extracellularly. The proportions of the different forms of proteoglycans vary depending on the species[39,79,121].

Most of the studies on the role of proteoglycans have concentrated on the nature and function of the GAG chains; the involvement of the various core proteins is less well documented. Both hematopoietic cells and bone marrow stromal cells produce different heparan sulfate, dermatan and chondroitin sulfate proteoglycans[6,39,59,77,100,106,121]. Myelopoiesis in long-term cultures is only sustained in the presence of hydrocortisone and this hormone is known to influence the sulfation pattern of GAGs[99]. A decreased adhesion of hematopoietic progenitor cells to the adherent stromal layer is observed in hydrocortisone-deficient cultures which may be correlated to the modulated proteoglycan pattern. Studies by Gordon and colleagues[47,99] demonstrated that heparan sulfates, which are shed into the long-term culture medium, are involved in the adhesive interactions with hematopoietic progenitor cells, but these analyses did not distinguish between individual heparan sulfate proteoglycans.

A recent study analyzed the function of perlecan, a defined member of the heparan sulfate proteoglycans[62]. Perlecan consists of a large modular core protein of 467 kDa to which three GAG side chains are attached[56,113]. This molecule is synthesized by bone marrow stromal cells and is deposited in an extracellular network overlying the adherent stromal layers[62]. Purified perlecan, although adhesive for fibroblasts and endothelial cells[4,52], did not show an adhesive effect on hematopoietic cells. On the contrary, cells were repelled from the area coated with perlecan (fig. 2). This anti-adhesive effect seems to be located in the core-protein of perlecan, and not in the negatively charged heparan sulfate side chains, since heparitinase-treated perlecan still exhibited an anti-adhesive effect. In combination with adhesive substrates (e.g. fibronectin, see below), perlecan is able to modulate the strength of cell binding/adhesion in a concentration-dependent manner. Thus, individual proteoglycans may have different properties to those seen in whole proteoglycan preparations where only the overall effect can be analyzed. A likely explanation for the difference between the anti-adhesive effect of perlecan and the adhesive character of the proteoglycan fraction analyzed by Siczkowski et al.[99] is that perlecan is deposited in the extracellular matrix and is not present in the heparan sulfate fraction isolated from culture supernatants. Nevertheless, this example highlights the necessity of functional studies with purified matrix components.

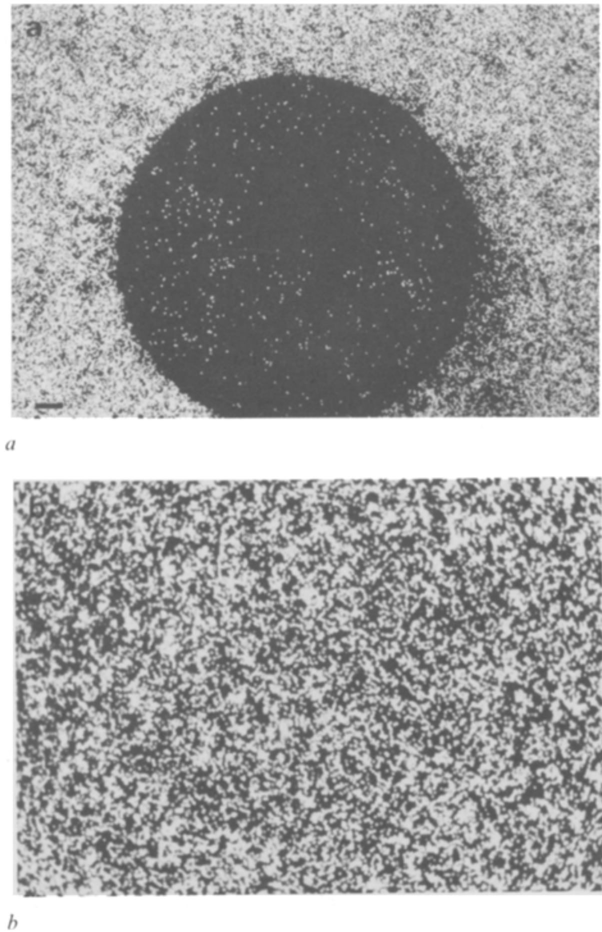

Figure 2. The anti-adhesive effect of the heparan sulfate proteoglycan perlecan on the human K562 hematopoietic cell line. The micrographs show two different attachment assays. 2 µg of perlecan, an anti-adhesive substrate, and 2 µg of collagen type III, which is a nonadhesive substrate, were spotted onto plastic and allowed to air-dry. After blocking unspecific plastic binding sites, the cells were added. After 1 hour of incubation at 37 °C, the round area coated with perlecan is exactly outlined by the K562 cells (represented by the white dots) which are repelled from this area and cover only the non-coated area (*a*). In contrast, using a nonadhesive substrate like collagen type III, the K562 cells cover the coated and the non-coated areas equally (*b*). Both micrographs show the cultures after 1 hour of incubation without any further manipulations (e.g. washing). Bar = 100 µm.

Proteoglycans also seem to be involved in the regulation of proliferation and differentiation of hematopoietic cells. Early work by Spooncer and colleagues[106] demonstrated that the treatment of long-term bone marrow cultures with β-D-xyloside, which uncouples GAG synthesis from proteoglycan synthesis, results in a significant increase in hematopoietic cell numbers. In β-D-xyloside-treated cultures an increase in chondroitin/dermatan sulfate GAG could be observed in the supernatant, while cell-associated heparan sulfates were not altered. Yet, the exact role of the increased soluble GAG chains has not been elucidated. A further hint of the involvement of proteoglycans in hematopoietic differentiation was provided by the work of Luikart et

al.[75] who demonstrated that heparan sulfate proteoglycans from the bone marrow stroma could induce maturation of the myelomonocytic cell line HL60. Only ECM isolated from the bone marrow and not skin-derived ECM showed this inducitve capacity[74], implying tissue-specific effects of ECM. Further work showed that heparan sulfate was the responsible agent in the bone marrow ECM[75]. Tissue-specificity was also shown for adhesion of Bl-CFC (blast colony forming cells) which only bound to marrow-derived heparan sulfate, but not to heparan sulfate isolated from bovine kidney[47].

Another important role of marrow proteoglycans seems to be the binding and presentation of cytokines to hematopoietic cells. Gordon et al.[45] showed that GAGs isolated from bone marrow were able of binding exogenous granulocyte-macrophage colony stimulating factor (GM-CSF). Further work by Roberts et al.[91] identified heparan sulfates as the components responsible for binding of GM-CSF and interleukin-3 and their biologically active presentation of hematopoietic progenitors. Perlecan, although anti-adhesive for hematopoietic cells, is also able of binding GM-CSF. In a semi-solid culture medium where cell migration is at least impaired, if not inhibited, perlecan can present the bound cytokine to myeloid progenitor cells[62]. Thus, proteoglycans could serve to compartmentalize various growth factors in the bone marrow and, in combination with their adhesive or anti-adhesive capacities, they may provide sites of intermittent stimulating contacts for either induction or inhibition of hematopoietic cell growth or differentiation.

Specific receptors for the interaction with heparan or chondroitin sulfate proteoglycans on hematopoietic cells have not yet been identified. Although the characterization of such receptors is to be expected, one has to keep in mind that many other extracellular matrix molecules (e.g. fibronectin, collagens, laminin) have GAG binding sites. Some of the interactions observed for proteoglycans may, therefore, involve indirect binding of hematopoietic cells to macromolecular structures of the bone marrow stroma which are in intimate contact with the proteoglycans.

Fibronectin

Fibronectin, a ubiquitous ECM molecule, consists of two similar subunits joined by disulfide bonds. Due to alternative splicing this matrix component exists in a variety of isoforms[55]. Fibronectin is found in the native bone marrow and is also synthesized and deposited in an extracellular network by bone marrow stromal cells[7,125]. Early work of Patel, Lodish and coworkers showed that fibronectin is involved in adhesion and maturation of the erythroid lineage[81-84]. Using the murine erythroleukemic cell line (MEL) which can be induced to differentiate by dimethyl sulfoxide, it was shown in these studies that adhesion to fibronectin is developmentally regulated. The loss of adhesion to fibronectin during differentiation could be correlated with the loss of a 140 kDa receptor[82]. Fibronectin is also required for the differentiation of induced MEL cells into reticulocytes, since the induced cells are arrested in the late erythroblast stage in its absence. When fibronectin is present these cells enucleate and differentiate into reticulocytes and erythrocytes[83]. In addition to the erythroid lineage, lymphoid precursor cells (as indicated by lymphoid cell lines) were also shown to adhere to fibronectin[8].

More recent work has demonstrated that multipotent hematopoietic progenitor cells adhere to fibronectin, and the regions within the fibronectin subunits responsible for these interactions were characterized[116,122]. The modular fibronectin molecule contains several attachment sites. The central, 75 kDa RGD-containing domain can interact with cells via the $\alpha5\beta1$ integrin. An RGD-independent cell adhesion site is located in the COOH-terminal 33/66 kDa fragment which is also known as the 'heparin-binding domain'. This fragment harbours a sequence known as III-CS. A peptide derived from the III-CS sequence and designated CS-1 contains the LDV sequence which is known to interact with the $\alpha4\beta1$ integrin[50]. Two flanking regions of the CS-1 peptide termed FN-C/H I and FN-C/H II can interact with either the $\alpha4\beta1$ integrin or with membrane-bound proteoglycans[51]. Primitive murine hematopoietic cells (CFU-S12) can adhere to the CS-1 sequence in an $\alpha4\beta1$ dependent fashion[122]. Human multipotent progenitors also adhere to the 33/66 kDa fragment and, to a lesser extent, to the 75 kDa RGD-containing domain. In contrast, more differentiated clonogenic progenitors adhered equally well to both fragments[116]. Further work by Kerst et al.[58] indicated that the $\alpha4\beta1$ and $\alpha5\beta1$ integrin receptors are differentially expressed during myeloid maturation. The gradual loss of these receptors might be important in the controlled egress of myeloid cells from the bone marrow.

In addition to the adhesion mediating effect, the interaction of fibronectin with the $\alpha5\beta1$ integrin can also contribute, at least in part, to negative regulation of hematopoiesis. This was indicated by a recent study of Sugahara et al.[109] who showed a striking inhibition of GM-CSF- or stem cell factor (SCF)-induced proliferation of a myeloid cell line (MO7E) by fibronectin. The inhibition of cell growth by the addition of fibronectin was mediated by the $\alpha5\beta1$ integrin and not by the $\alpha4\beta1$ integrin, and induced signs of apoptosis (programmed cell death) in this cell line. Thus, fibronectin also seems to be involved in the growth control of hematopoietic cells.

Another interesting observation of fibronectin action on hematopoietic cells was recently reported by Moritz et al.[78]. These authors showed that retroviral-mediated

gene transfer into committed progenitor cells, and to a lesser degree in earlier progenitor cells, was significantly increased when these cells adhered to a fibronectin fragment containing the alternatively spliced CS-1 cell attachment domain. The soluble fragment, however, did not show this effect. These results indicate that hematopoietic progenitor cell adhesion to specific ECM molecules can alter retroviral infection efficiency and this may aid to develop improved gene transfer protocols for somatic gene therapy of hematopoietic progenitor cells.

Tenascin

The tenascin family consists of three members designated tenascin-C, which was the first described and the most intensively studied member, tenascin-R (restrictin), and tenascin-X[23,36]. Tenascins are large multidomain glycoproteins arranged as oligomers. Each subunit consists of EGF-like domains, fibronectin type III domains and a fibrinogen-like terminal knob (see fig. 3). Tenascin-C which is synthesized as a hexamer (synonym: hexabrachion) exists in different isoforms due to alternative splicing of various fibronectin type III repeats. During embryogenesis tenascin-C is widely expressed, but in the adult organism it shows a restricted expression pattern being synthesized mainly in regenerative organs like skin and gut, in healing wounds and during tumorogenesis[37].

In long-term bone marrow cultures only tenascin-C expression has been analyzed. Two splice variants of 6 and 8 kb are synthesized by both murine and human stromal cells[33,61], the larger isoform being more prominently expressed. Tenascin-C is deposited in an extracellular meshwork overlying the stromal cells. In the native bone marrow it can be detected by immunohistochemistry between the developing hematopoietic cells. There is evidence that tenascin-C can act as an anti-adhesive molecule for various cell types[21,95], and anti-adhesive domains have been identified in the epidermal growth factor (EGF)-like domains and the fibronectin type III repeats 7–8[20,87,104]. However, tenascin-C is also an adhesive substrate, for example for endothelial cells[57,107] and fibroblasts[3], and various receptors interacting with different domains of tenascin-C (see fig. 3) have been identified[3,24,57,107,123]. In the hematopoietic system, various human myeloid cell lines have been shown to adhere to tenascin-C[61]. A strong cell binding site could be mapped to the fibrinogen-like terminal knob (fig. 4; Klein and Erickson, unpubl. observations), but the nature of the responsible receptors on hematopoietic cells is still unclear. The question of which lineage-committed progenitor cells adhere to tenascin-C has also not yet been elucidated.

Tenascin-C has been identified as a component with immunomodulatory properties[92] acting on mature

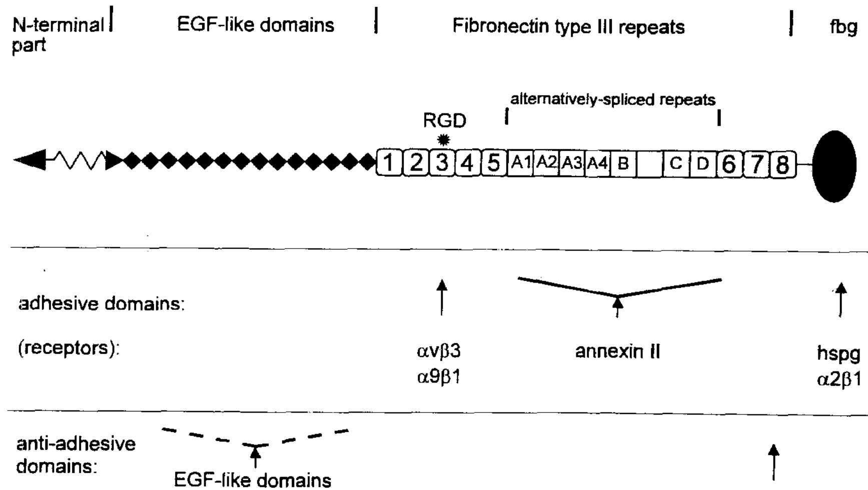

Figure 3. Structural model of one subunit of human tenascin-C and localization of adhesive cell binding sites.
The subunits consist of a C-terminal fibrinogen-like domain (fbg), various fibronectin type III repeats (rectangles), followed by 14 1/2 epidermal growth factor (EGF)-like domains (filled diamonds) and an N-terminal part which is involved in oligomerization of the subunits. The fibronectin type III repeats 1–5 (TNfn 1–5) and 6–8 (TNfn 6–8) are common to all tenascin-Cs, the alternatively spliced repeats are letter coded (TNfn A–D). The human TNfn3 repeat contains an RGD-sequence (indicated by the star) which can be used as a recognition site by the integrin $\alpha v\beta 3$[57,107]. Binding of the novel $\alpha 9\beta 1$ integrin has also been mapped to the TNfn3 repeat, but this interaction seems to be RGD-independent[123]. The second major cell adhesion site is located in the C-terminal fibrinogen-like domain. Cell surface proteoglycans and the $\alpha 2\beta 1$ integrin are possible receptors interacting with this domain[3,57,107]. A third cell adhesion site has recently been mapped to the alternatively spliced region TNfn A–D, the responsible receptor was identified as annexin II[24]. Domains responsible for the anti-cell adhesion activity of tenascin-C were found to be located in the EGF-like domains and the fibronectin type III repeats 7–8[20,87,104]. The model shown here was drawn according to Chiquet-Ehrismann[22] and Erickson[36].

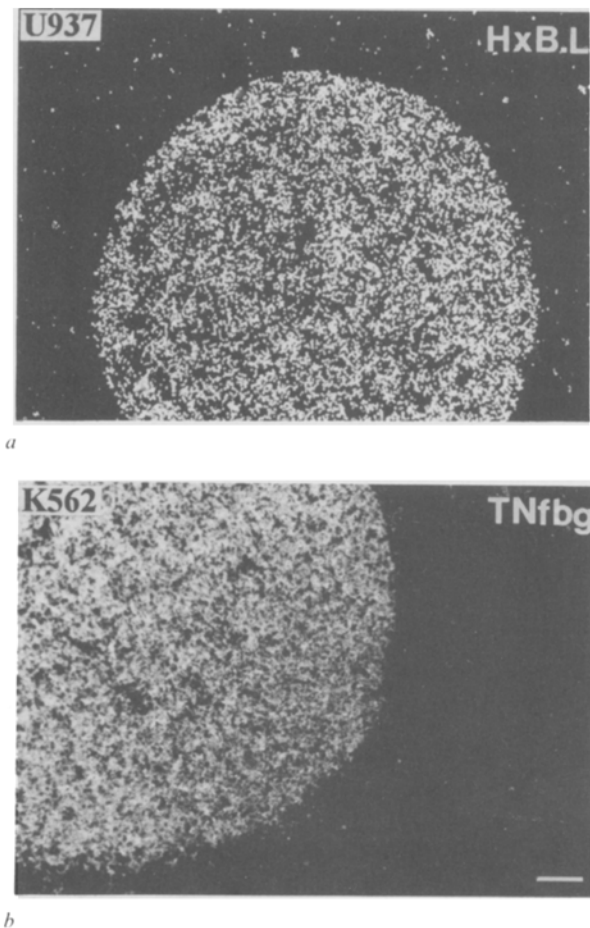

Figure 4. Adhesion of hematopoietic cell lines to tenascin-C and the fibrinogen-like terminal fragment.
2 μg of the large isoform of tenascin-C (rec. HxB.L) and of the fragment containing the fibrinogen-like terminal domain (TNfbg, see ref. 3) were spotted onto plastic and allowed to air-dry. After blocking the unspecific plastic binding sites, the promonocytic cell line U937 (HxB.L) (*a*) and the erythroleukemic cell line K562 (TNfbg) (*b*) were added. After 1 hour of incubation, the cultures were washed with prewarmed PBS. The areas coated with HxB.L or TNfbg were densely covered with cells (represented by the white dots) indicating adhesion of these cell lines to tenascin-C. Outside the coated areas only background binding could be observed. Bar = 100 μm.

blood cells. In addition it has been shown to stimulate cell proliferation of various non-hematopoietic cell lines[35]. Using bone marrow mononuclear cells we could recently show that tenascin-C also profoundly stimulates cell growth of these cells in a concentration-dependent manner (Schermutzki and Klein, unpub. observations). Thus, tenascin-C seems to influence both cell adhesion and cell proliferation of developing hematopoietic cells.
Tenascin-C synthesis of murine bone marrow stromal cells is strongly influenced by the presence of glucocorticoids. Addition of glucocorticoids to long-term bone marrow cultures at the outset, or to established long-term cultures, drastically downregulated tenascin-C expression[33]. Since these hormones are known to be important for myelopoiesis in vitro it is suggested that glucocorticoids can influence hematopoiesis in vitro by modifying the composition of the extracellular matrix.

Thrombospondin

Thrombospondin, a multifunctional homotrimeric glycoprotein of 450 kDa, consists of identical subunits which are covalently cross-linked at their NH_2-terminal ends by disulfide bonds. This molecule is synthesized and secreted by a wide variety of cells including platelets, fibroblasts, smooth muscle and endothelial cells and is involved in cell adhesion and binding to other extracellular components like GAGs and fibrinogen (for reviews see refs 11, 38, 66). Thrombospondin is abundantly present in the bone marrow microenvironment. It is synthesized and secreted by normal hematopoietic cells, as well as by leukemic cell lines and can act as an adhesive ligand for the developing hematopoietic cells. Pluripotent human progenitor cells (CFU-GEMM) and committed progenitors (BFU-E, CFU-GM) adhere to thrombospondin[72]. During differentiation, maturing granulocytes show a decreased adhesion to thrombospondin, while developing erythroid cells lose their adhesive capacity indicating that the receptors for thrombospondin are downregulated during hematopoietic development[72].
Thrombospondin contains an RGD sequence in the COOH-terminus of each subunit, but this sequence may be located in a cryptic region since binding of hematopoietic progenitor cells could not be inhibited even by high concentrations of RGD-containing peptides. Membrane-bound proteoglycans which can mediate adhesion to NH_2-terminal ends of the thrombospondin subunits have been described[2], but antibodies directed against the NH_2-terminal regions of thrombospondin also failed to inhibit progenitor cell adhesion[72].
Using HL60 cell maturation as a differentiation model, Suchard et al.[108] demonstrated that thrombospondin receptors are differentially regulated during differentiation. Although the nature of the receptors was not identified in these study, they could show that maturation of HL60 into macrophage-like cells increased thrombospondin receptor expression. Binding to these receptors was inhibitable by heparin. Induction into PMN (polymorphonuclear)-like cells also increased receptor expression, but these receptors were only partially inhibited by heparin suggesting two different receptor types.
A further study by Long et al.[73] showed that thrombospondin in combination with a cytokine, the stem cell factor, can act synergistically on hematopoietic progenitor cell adhesion, which then undergo colony formation. These data suggest that a distinct cytokine together with an extracellular matrix component can act as a common signal complex for hematopoietic progenitor development.

Laminin

Eight distinct laminin chains have been identified over the last few years. These molecules form cross-shaped heterotrimers and seven different assembly forms have been detected (for review see ref. 114). Due to the growing complexity a new nomenclature has been adopted for the laminin family[12]. However, in the bone marrow microenvironment laminin composition has not been analyzed in great detail. Only the prototype of the laminins, the murine EHS (Engelbreth-Holm-Swarm) tumor-derived laminin-1 consisting of $\alpha 1$, $\beta 1$ and $\lambda 1$ chains, has been studied. Using antibodies against this laminin type, its presence was shown by immunohistochemistry in long-term bone marrow cultures and in bone marrow tissue sections[33,125]. Interestingly, in bone marrow stromal cells signals for laminin expression were found predominantly within the stromal cells, perinuclearly. Western blot analysis of murine stromal cell extracts[33] and immunoprecipitation of human stromal cell extracts (fig. 5, unpublished) with antibodies against laminin-1 revealed the presence of the $\beta 1$ and $\gamma 1$ chains, but no $\alpha 1$ chain could be detected. Thus, the nature of the α chain of laminin within the bone marrow is still unclear.

Analyzing the effects of glucocorticoids on the laminin $\beta 1$ chain expression in long-term bone marrow cultures, Ekblom et al.[33] could demonstrate that this chain is downregulated by the presence of glucocorticoids but only when added at the onset of the cultures. In established long-term cultures, $\beta 1$ chain expression was not altered by the addition of glucocorticoids, in contrast to tenascin expression.

EHS-derived laminin did not show an adhesive capacity of various leukemic cell lines tested[42]. Only a slight adhesion to EHS-derived laminin has been observed with chronic myelogeneous leukemic committed progenitor cells, whereas normal progenitors (BFU-E and CFU-GM) showed negligible adhesion[117].

Thus, no specific roles in cell adhesion, migration or differentiation have yet been defined for the laminins in the bone marrow. A major challenge for the future will be to analyze the nature and functions of the laminin family in the bone marrow microenvironment.

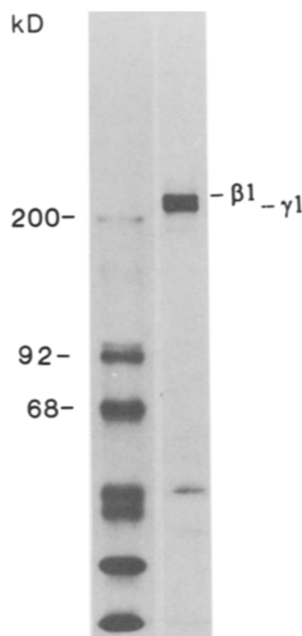

Figure 5. Identification of laminin chains synthesized by human bone marrow stromal cells. An adherent stromal cell layer of a human bone marrow long-term culture was labelled with (^{35}S) methionine overnight. The proteins were extracted and immunoprecipitated with an anti-laminin antiserum recognizing the $\alpha 1$, $\beta 1$ and $\lambda 1$ chains of laminin. Two strong signals from the $\beta 1$ and the $\lambda 1$ chains could be detected, but the $\alpha 1$ chain was never observed, not even after prolonged exposure time (right lane). A ^{14}C-methylated molecular weight marker which was run on the same gel is shown in the left lane. A similar result (identification of the $\beta 1$ and $\lambda 1$ chains, but not the $\alpha 1$ chain) was obtained by Ekblom[33], using immunoblotting of mouse long-term bone marrow culture extracts.

Hemonectin

A bone marrow specific extracellular matrix protein termed hemonectin was identified in rabbit bone extracts in 1987[16]. This 60 kDa component showed a particular adhesiveness for granulocytic precursor cells, but not for other cell lineages (for review see ref. 14). Antibodies against hemonectin localized this molecule in fetal liver, fetal bone marrow and adult bone marrow, but not in other adult organs like spleen, kidney or mammary gland[16,17,85]. In Steel/Steel-Dickie heterozygous mice, deficient at the stem cell factor locus (Sl/Sl^d mice), hemonectin was shown to be absent by immunohistochemistry. Addition of hemonectin to Sl/Sl^d cell lines could restore some of the hematopoietic deficiencies, as shown by increased colony formation. The molecular structure of hemonectin has not yet been identified, but a partial amino acid sequence analysis has revealed that this molecule is closely related to the plasma glycoprotein fetuin[119]. Further work will be needed to clarify whether these closely related molecules are products of the same gene and if they are functionally different.

Cellular receptors

Immature hematopoietic cells express a large variety of membrane-bound cell adhesion receptors. According to structural and functional similarities these adhesion molecules can be grouped into various families, including the integrins, cadherins, selectins, the immunoglobulin superfamily, and syndecans, and several non-classified adhesion receptors also exist (for review see refs 9, 41, 93, 105, 111, 118). These cellular adhesion molecules are involved in cell-cell and cell-matrix interactions, but in this paper we will focus our attention on extracellular matrix receptors. The most intensively studied matrix receptors are members of the integrin family. Integrins are divalent cation-dependent,

non-covalently linked heterodimers consisting of an α and a β subunit. Fifteen different α chains and at least seven different β chains have been characterized so far. Many of the α chains associate with a specific β chain, but subunit promiscuity also exists. The integrins are subclassified according to the presence of the specific β chain. The $\beta 1$ and the $\beta 3$ subfamily mainly serve in cell-matrix interactions, whereas the $\beta 2$ subfamily (the leukocyte integrins) is only involved in cell-cell interactions (and is not discussed here). Some of the $\beta 1$ integrins show ligand specificity, but others interact with several matrix molecules showing ligand promiscuity (see the table).

By flow cytometric analysis it has been shown that enriched human progenitor cells, characterized by the CD34 antigen, express $\alpha 4\beta 1$ and $\alpha 5\beta 1$ integrins[112]. Whereas the $\alpha 5\beta 1$ integrins interact with the central RGD-containing domain, the $\alpha 4\beta 1$ integrin recognizes an RGD-independent cell adhesion site at the COOH-terminal end, as shown by adhesion-blocking antibodies. In addition, $\alpha 4\beta 1$ is also involved in cell-cell interactions using VCAM-1 as the cellular ligand. VCAM-1 is expressed on stromal cells and its expression can be influenced by cytokines[34,80,94]. Both $\alpha 4\beta 1$/VCAM (and to a lesser extent $\alpha 4\beta 1$/fibronectin) and $\alpha 5\beta 1$/fibronectin interactions are used by the CD34 + cells[112]. The other members of the $\beta 1$ and $\beta 3$ integrin family ($\alpha 1\beta 1$, $\alpha 2\beta 1$, $\alpha 3\beta 1$, $\alpha 6\beta 1$, and $\alpha v\beta 3$) were not detected by FACS analysis on normal human progenitor cells. Similar data were obtained by other groups[70,94a,103].

On non-hematopoietic cell types interactions with collagen types I and VI, tenascin and thrombospondin are mediated by various members of $\beta 1$ and $\beta 3$ integrins (see the table). The absence of these integrins on hematopoietic progenitors (as indicated by FACS analysis), together with the fact that $\beta 1$ adhesion-blocking antibodies or RGD-containing peptides did not show any inhibitory effects on various hematopoietic cell lines[63,64,72], indicates that the observed adhesion of hematopoietic cell types of these ECM molecules are mediated by other cell surface components. Since the addition of heparin often shows at least partial inhibition in these cases the involvement of membrane-bound heparan sulfate receptors is suggested.

The family of syndecans, including four defined members[9], could be involved in these processes but up to now only syndecan-1 has been identified at certain stages of B-cell development and on myeloma cells[90,96]. Here, syndecan-1 can mediate binding to collagen type I[90,97]. The involvement of the other syndecan members has so far not been tested for hematopoietic cell interactions. Taking these data together it is obvious that the characterization of ECM receptors on hematopoietic cells is still in its infancy.

Functionally, the cellular adhesion receptors may serve for localization of hematopoietic progenitors within the bone marrow, but they may also mediate various intracellular signal transduction pathways leading to a regulated cell growth and differentiation. Abnormal function or expression of these receptors may be the cause of disordered hematopoiesis such as leukemia.

Table. Interactions of $\beta 1$ and $\beta 3$ integrins with extracellular ligands

Integrin	Ligand
$\alpha 1\beta 1$ (VLA-1)	collagen types I, IV, VI, laminin
$\alpha 2\beta 1$ (VLA-2)	collagen types I, IV, VI, (laminin), fibronectin, tenascin
$\alpha 3\beta 1$ (VLA-3)	collagen type I, laminin, fibronectin, epiligrin
$\alpha 4\beta 1$ (VLA-4)	fibronectin, [VCAM-1 as cellular ligand]
$\alpha 5\beta 1$ (VLA-5)	fibronectin
$\alpha 6\beta 1$ (VLA-6)	laminin
$\alpha 7\beta 1$	laminin
$\alpha 8\beta 1$	?
$\alpha 9\beta 1$	tenascin
$\alpha v\beta 3$ (VNR)	vitronectin, fibronectin, fibrinogen, von Willebrand factor, thrombospondin, tenascin

Pathological conditions

Abnormal interaction between the developing hematopoietic cells and their microenvironment may, at least partially, cause the premature egress of hematopoietic cells in leukemia, but it is not yet clear whether a 'leukemic' bone marrow microenvironment exists[48]. Several studies have focussed on the expression of cell adhesion receptors on leukemic cells[68–70,88,89]. An altered expression pattern was observed on leukemic CD34 + cells, which express $\alpha 1$, $\alpha 2$, $\alpha 3$, $\alpha 4$, $\alpha 5$, and $\alpha 6$ integrins in various amounts[88], whereas normal CD34 + progenitor cells synthesize $\alpha 4$ and $\alpha 5$ subunits[112]. However, because it is known that integrins can occur in a functionally inactive state, the expression of the integrin on the cell surface is, by itself, not adequate for cell adhesion[31]. Thus, the increased integrin expression pattern on leukemic CD34 + cells may only reflect malignant transformation.

Progenitor cells of chronic myeloid leukemia (CML) have been demonstrated to have a diminished adhesion capacity for binding to normal bone marrow stroma as compared to normal progenitor cells[46]. A recent study indicated that the defect is based on impaired $\beta 1$ integrin function, which could be restored by treatment with interferon α[10]. Addition of the cytokine did not alter the expression pattern of $\alpha 4\beta 1$ and $\alpha 5\beta 1$ integrins on CML cells, but could regulate normal $\beta 1$ integrin function.

Interactions of CML progenitor cells with isolated matrix molecules revealed that, in contrast to normal progenitor cells, adhesion to fibronectin is decreased, whereas adhesion to collagen type IV and laminin is increased[117]. Whether the expression of $\alpha 2\beta 1$ and $\alpha 6\beta 1$

on the malignant cells is responsible for these interactions has to be clarified.

Concerning modifications of the extracellular matrix composition in the leukemic microenvironment only very little is known. Advanced CML disease stages are often correlated with increased fibrosis. Here, an increased tenascin expression has been observed[102]. In hairy cell leukemia an increased insoluble fibronectin deposition is suggested to be responsible for the characteristic fibrosis[13]. It is not known if these altered expression patterns are directly involved in the different diseases, but these two examples suggest that more effort should be undertaken to analyze possible abnormalities of the extracellular matrix in malignant hematological disorders.

Conclusions and outlook

During the last years, a large body of evidence has accumulated that extracellular matrix components play crucial roles in hematopoiesis. The regulation of cell adhesion may be a fundamental process for cell growth and differentiation within the bone marrow. Cell binding activities of several ECM components for hematopoeitic progenitor cells have been identified, and recently, the first anti-adhesive substrate for hematopoietic cells was defined. However, the cellular receptors on hematopoietic cells responsible for the interactions with the ECM components are still ill-defined.

The tissue-specific composition of the extracellular matrix within the bone marrow still needs a better understanding. This applies especially to the characterization of specific isoforms, since it is becoming more and more evident that, due to alternative splicing or the existence of different distinct subunits, ECM molecules can occur in different isoforms often correlated with distinct functions. The involvement of the core proteins of the proteoglycans in the hematopoietic process also has to be better defined. It is likely that new members of the collagen family will prove to be present in the marrow, serving different functions.

Another interesting area of research will be to test the influences of cytokines on the extracellular matrix expression of the bone marrow. Therapeutically, several protocols exist which use cytokines to mobilize bone marrow stem/progenitor cells for autologous transplantations. The molecular mechanism of this mobilization is not understood, but certainly changes in the adhesion interactions within the bone marrow microenvironment have to occur. Whether ECM components are affected by the cytokine treatments and, as a result of that, change their adhesive properties will have to be studied in great detail. Progress in the understanding of this process is of great clinical importance – and the patients will certainly benefit from that.

Acknowledgements. I wish to thank Dr. Lydia Sorokin (Max-Planck-Arbeitsgruppe, Erlangen, Germany) and Martina Seiffert (University Tübingen, Germany) for their critical review and Franka Schermutzki for her help with the preparation of the manuscript. This work was supported by grants from the Deutsche Forschungsgemeinschaft and from the Deutsche Krebshilfe/Dr. Mildred Scheel Stiftung.

1 Anklesaria, P., Greenberger, J. S., Fitzgerald, T. J., Sullenberger, B., Wicha, M., and Campbell, A., Hemonectin mediates adhesion of engrafted murine progenitors to a clonal bone marrow stromal cell line from Sl/Sl^d mice. Blood *77* (1991) 1691–1698.

2 Asch, A. S., Tepler, J., Silbiger, S., and Nachman, R. L., Cellular attachment to thrombospondin molecule: cooperative interactions between receptor systems. J. biol. Chem. *266* (1990) 1740–1745.

3 Aukhil, I., Joshi, P., Yan, Y., and Erickson, H. P., Cell- and heparin-binding domains of the hexabrachion arm identified by tenascin expression proteins. J. biol. Chem. *268* (1993) 2542–2553.

4 Battaglia, C., Aumailley, M., Mann, K., Mayer, U., and Timpl, R., Structural basis of β1 integrin-mediated cell adhesion to a large heparan sulfate proteoglycan from basement membranes. Eur. J. Cell Biol. *61* (1993) 92–99.

5 Bentley, S. A., Collagen synthesis by bone marrow stromal cells: a quantitative study. Br. J. Haemat. *50* (1982) 491–497.

6 Bentley, S. A., Kirby, S. L., Anklesaria, P., and Greenberger, J. S., Biochemical and functional characterization of proteoglycans produced by Sl/Sld murine bone marrow stromal cell lines. J. cell. Physiol. *145* (1990) 53–59.

7 Bentley, S. A., and Tralka, T. S., Fibronectin-mediated attachment of hematopoietic cells to stromal elements in continuous bone marrow cultures. Expl Hemat. *11* (1983) 129–138.

8 Bernardi, P., Patel, V. P., and Lodish, H. F., Lymphoid precursor cells adhere to two different sites on fibronectin. J. Cell Biol. *105* (1987) 489–498.

9 Bernfield, M., Kokenyesi, R., Kato, M., Hinkes, M. T., Spring, J., Gallo, R. L., and Lose, E. J., Biology of the syndecans: a family of transmembrane heparan sulfate proteoglycans. A. Rev. Cell Biol. *8* (1992) 365–393.

10 Bhatia, R., Wayner, E. A., McGlave, P. B., and Verfaillie, C. M., Interferon-α restores normal adhesion of chronic myelogenous leukemia hematopoietic progenitors to bone marrow stroma by correcting impaired β1 integrin receptor function. J. clin. Invest. *94* (1994) 384–391.

11 Bornstein, P., Thrombospondins: structure and regulation of expression. FASEB J. *6* (1992) 3290–3299.

12 Burgeson, R. E., Chiquet, M., Deutzmann, R., Ekblom, P., Engel, J., Kleinmann, H., Martin, G. R., Meneguzzi, G., Paulsson, M., Sanes, J., Timpl, R., Tryggvason, K., and Yurchenco, P. D., A new nomenclature for laminins. Matrix Biol. *5* (1994) 209–212.

13 Burthem, J., and Cawley, J. C., The bone marrow fibrosis of hairy-cell leukemia is caused by the synthesis and assembly of a fibronectin matrix by the hairy cells. Blood *83* (1994) 497–504.

14 Campbell, A. D., The role of hemonectin in the cell adhesion mechanisms of bone marrow. Hemat. Path. *6* (1992) 51–60.

15 Campbell, A. D., and Wicha, M. S., Extracellular matrix and the hematopoietic microenvironment, J. Lab. clin. Med. *112* (1988) 140–146.

16 Campbell, A. D., Long, M. W., and Wicha, M. S., Haemonectin, a bone marrow adhesion protein specific for cells of the granulocytic lineage. Nature *329* (1987) 744–746.

17 Campbell, A. D., Long, M. W., and Wicha, M. S., Developmental regulation of granulocytic cell binding to hemonectin. Blood *76* (1990) 1758–1764.

18 Chabannon, C., and Torok-Storb, B., Stem cell-stromal cell interactions. Curr. Topics Microbiol. Immun. *177* (1992) 123–136.

19 Chichester, C. O., Fernandez, M., and Minguell, J. J., Extracellular matrix gene expression by human bone marrow stroma and by marrow fibroblasts. Cell Adhes. Commun. *1* (1993) 93–99.
20 Chiquet, M., Vrucinic-Filipi, N., Schenk, S., Beck, K., and Chiquet-Ehrismann, R., Isolation of chick tenascin variants and fragments. Eur. J. Biochem. *199* (1991) 379–388.
21 Chiquet-Ehrismann, R., Anti-adhesive molecules of the extracellular matrix. Curr. Opin. Cell Biol. *3* (1991) 800–804.
22 Chiquet-Ehrismann, R., Tenascin and other adhesion-modulating proteins in cancer. Cancer Biol. *4* (1993) 301–310.
23 Chiquet-Ehrismann, R., Hagios, C., and Matsumoto, K., The tenascin gene family. Perspect. Dev. Neurobiol. *2* (1994) 3–7.
24 Chung, C. Y., and Erickson, H. P., Cell surface annexin II is a high affinity receptor for the alternatively spliced segment of tenascin-C. J. Cell Biol. *126* (1994) 539–548.
25 Clark, B. R., Gallagher, J. T., and Dexter, T. M., Cell adhesion in the stromal regulation of haemopoiesis. Baillieres clin. Haemat. *5* (1992) 619–652.
26 Coulombel, L., Rosemblatt, M., Gaugler, M. H., Leroy, C., and Vainchenker, W., Cell-cell matrix and cell-cell interactions during hematopoietic differentiation. Bone Marrow Transplant. *9* Suppl. 1 (1992) 19–22.
27 Deryugina, E. J., and Müller-Sieburg, C. E., Stromal cells in long-term cultures: keys to the elucidation of hematopoietic development? Crit. Rev. Immun. *13* (1993) 115–150.
28 Dexter, T. M., Allen, T. D., and Lajtha, L. G., Conditions controlling the proliferation of haematopoietic stem cells in vitro. J. cell. Physiol. *91* (1977) 335–344.
29 Dexter, T. M., Spooncer, E., Simmons, P., and Allen, T. D., Long-term marrow culture: an overview of techniques and experience, in: Long-term Bone Marrow Culture, pp. 57–96, Eds D. C. Wright and J. S. Greenberger. Alan R. Liss, Inc., New York 1984.
30 Dexter, T. M., Coutinho, L. H., Spooncer, E., Heyworth, C. M., Daniel, C. P., Schiro, R., Chang, J., and Allen, T. D., Stromal cells in haemopoiesis, in: Molecular Control of Haemopoiesis, Ciba Found. Symp., vol. 148, pp. 76–95, Wiley, Chichester 1990.
31 Diamond, M. S., and Springer, T. A., The dynamic regulation of integrin adhesiveness. Curr. Biol. *4* (1994) 506–517.
32 Dorshkind, K., Regulation of hemopoiesis by bone marrow stromal cells and their products. A. Rev. Immun. *8* (1990) 111–137.
33 Ekblom, M., Fässler, R., Tomasini-Johansson, B., Nilsson, K., and Ekblom, P., Downregulation of tenascin expression by glucocorticoids in bone marrow stromal cells and in fibroblasts. J. Cell Biol. *123* (1993) 1037–1045.
34 Elices, M. J., Osborn, L., Takada, Y., Crouse, C., Luhowskyj, S., Hemler, M. E., and Lobb, R. R., VCAM-1 on activated endothelium interacts with the leukocyte integrin VLA-4 at a site distinct form the VLA-4/fibronectin binding site. Cell *60* (1990) 577–584.
35 End, P., Panayotou, G., Entwistle, A., Waterfield, M. D., and Chiquet, M., Tenascin: a modulator of cell growth. Eur. J. Biochem. *209* (1992) 1041–1051.
36 Erickson, H. P., Tenascin-C, tenascin-R, and tenascin-X – a family of talented proteins in search of functions. Curr. Opin. Cell Biol. *5* (1993) 869–876.
37 Erickson, H. P., and Bourdon, M. A., Tenascin: an extracellular matrix protein prominent in specialized embryonic tissues and tumors. A. Rev. Cell Biol. *5* (1989) 71–92.
38 Frazier, W. A., Thrombospondin: a modular adhesive glycoprotein of platelets and nucleated cells. J. Cell Biol. *105* (1987) 625–632.
39 Gallagher, J. T., Spooncer, E., and Dexter, T. M., Role of the cellular matrix in haematopoiesis. I. Synthesis of glycosaminoglycans by mouse bone marrow cell cultures. J. Cell Sci. *63* (1983) 155–171.
40 Gartner, S., and Kaplan, H. S., Long-term culture of human bone marrow cells. Proc. natl Acad. Sci. USA *77* (1980) 4756–4759.
41 Geiger, B., and Ayalon, O., Cadherins. A. Rev. Cell Biol. *8* (1992) 307–332.
42 Giancotti, F. G., Comoglio, P. M., and Tarone, G., Fibronectin-plasma membrane interaction in the adhesion of hemopoietic cells. J. Cell Biol. *103* (1986) 429–437.
43 Gordon, M. Y., Extracellular matrix of the marrow micorenvironment. Br. J. Haemat. *70* (1988) 1–4.
44 Gordon, M. Y., Adhesive properties of haematopoiestic stem cells. Br. J. Haemat. *68* (1988) 149–151.
45 Gordon, M. Y., Riley, G. P., Watt, S. M., and Greaves, M. F., Compartmentalization of a haematopoietic growth factor (GM-CSF) by glycosaminoglycans in the bone marrow microenvironment. Nature *326* (1987) 403–405.
46 Gordon, M. Y., Dowding, C. R., Riley, G. P., Goldman, J. M., and Greaves, M. F., Altered adhesive interactions with marrow stoma of hematopoietic progenitor cells in chronic myelogenous leukemia. Nature *328* (1987) 342–344.
47 Gordon, M. Y., Riley, G. P., and Clarke, D., Heparan sulfate is necessary for adhesive interactions between human early hemopoietic progenitor cells and the extracellular matrix of the marrow microenvironment. Leukemia *2* (1988) 804–809.
48 Greenberger, J. S., Is the marrow stroma of AML patients a "leukemic" stroma? Expl Hemat. *20* (1992) 1041–1042.
49 Greenberger, J. S., The hematopoietic microenvironment. Crit. Rev. Oncol. Hemat. *11* (1991) 65–84.
50 Guan, J. L., and Hynes, R. O., Lymphoid cells recognize an alternatively spliced segment of fibronectin via the integrin receptor $\alpha 4\beta 1$. Cell *60* (1990) 53–61.
51 Haugen, P. K., McCarthy, J. B., Skubitz, A. P. N., Furcht, L. T., and Letourneau, P. C., Recognition of the A-chain carboxy-terminal heparin binding region of fibronectin involves multiple sites: two recognition sequences act independently to promote neural cell adhesion. J. Cell Biol. *111* (1990) 2733–2742.
52 Hayashi, K., Madri, J. A., and Yurchenco, P. D., Endothelial cells interact with the core protein of basement membrane perlecan through $\beta 1$ and β integrins: an adhesion modulated by glycosaminoglycan. J. Cell Biol. *119* (1992) 945–959.
53 Huang, S., and Terstappen, L. W. M. M., Formation of haematopoietic microenvironment and haematopoietic stem cells from single human bone marrow stem cells. Nature *360* (1992) 745–749.
54 Huang, S., and Terstappen, L. W. M. M., Formation of haematopoietic micorenvironment and haematopoietic stem cells from single human bone marrow stem cells. Correction Nature *368* (1994) 664.
55 Hynes, R. O., Fibronectins. Springer Verlag Inc., New York 1990.
56 Iozzo, R. V., Perlecan: a gem of a proteoglycan. Matrix Biol. *14* (1994) 203–208.
57 Joshi, P., Chung, C. Y., Aukhil, I., and Erickson, H. P., Endothelial cells adhere to the RGD domain and the fibrinogen-like terminal knob of tenascin. J. Cell Sci. *106* (1993) 389–400.
58 Kerst, J. M., Sanders, J. B., Slaper-Cortenbach, I. C., Doorakkers, M. C., Hooibrink, B., van Oers, R. H., von dem Borne, A. E., and van der Schoot, C. E., Alpha 4 beta 1 and alpha 5 beta 1 are differentially expressed during myelopoiesis and mediate the adherence of human CD34+ cells to fibronectin in an activation-dependent way. Blood *81* (1993) 344–351.
59 Kirby, S. L., and Bentley, S. A., Proteoglycan synthesis in two murine bone marrow stromal cell lines. Blood *70* (1987) 1333–1341.
60 Kjellén, L., and Lindahl, U., Proteoglycans: structures and interactions. A. Rev. Biochem. *60* (1991) 443–475.
61 Klein, G., Beck, S., and Müller, C. A., Tenascin is a cytoadhesive extracellular matrix component of the human hematopoietic microenvironment. J. Cell Biol. *123* (1993) 1027–1035.
62 Klein, G., Conzelmann, S., Beck, S., Timpl, R., and Müller, C. A., Perlecan in the human bone marrow: a growth factor presenting, but repelling extracellular matrix component for hematopoietic cells. Matrix Biol. *14* (1994) 457–465.

63 Klein, G., Müller, C. A., Tillet, E., Chu, M.-L., and Timpl, R., Collagen type VI in the human bone marrow microenvironment: a strong cytoadhesive component. Blood (1995) in press.
64 Koenigsmann, M., Griffin, J. D., DiCarlo, J., and Cannistra, S. A., Myeloid and erythroid progenitor cells from normal bone marrow adhere to collagen type I. Blood *79* (1992) 657–665.
65 Kolset, S. O., and Gallagher, J. T., Proteoglycans in haemopoietic cells. Biochim. biophys. Acta *1032* (1990) 191–211.
66 Lawler, J., and Hynes, R. O., Structural organization of the thrombospondin molecule. Semin. Thromb. Hemost. *13* (1987) 245–254.
67 Lichtman, M. D., The relationship of stromal cells to hematopoietic cells in marrow, in: Long-term Bone Marrow Culture, pp. 57–96, Eds D. C. Wright and J. S. Greenberger. Alan R. Liss, Inc., New York 1984.
68 Liesveld, J. L., Winslow, J. M., Kempski, M. C., Ryan, D. H., Brennan, J. K., and Abboud, C. N., Adhesive interactions of normal and leukemic human CD34 + myeloid progenitors: role of marrow stromal, fibroblast, and cytomatrix components. Expl Hemat. *19* (1991) 63–70.
69 Liesveld, J. L., Winslow, J. M., Frediani, K. E., Ryan, D. H., and Abboud, C. N., Expression of integrins and examination of their adhesive function in normal and leukemic hematopoietic cells. Blood *81* (1993) 112–121.
70 Liesveld, J. L., Dispersio, J. F., and Abboud, C. N., Integrins and adhesive receptors in normal and leukemic CD34+ progenitor cells: potential regulatory checkpoints for cellular traffic. Leuk. Lymph. *14* (1994) 19–28.
71 Long, M. W., Blood cell adhesion molecules. Expl Hemat. *20* (1992) 288–301.
72 Long, M. W., and Dixit, V. M., Thrombospondin functions as a cytoadhesion molecule for human hematopoietic progenitor cells. Blood *75* (1990) 2311–2318.
73 Long, M. W., Briddell, R., Walter, A. W., Bruno, E., and Hoffman, R., Human hematopoietic stem cell adherence to cytokines and matrix molecules. J. clin. Invest. *90* (1992) 251–255.
74 Luikart, S. D., Sackrison, J. L., and Maninglia, C. A., Bone marrow modulation of HL60 phenotype. Blood *70* (1988) 1119–1123.
75 Luikart, S. D., Maninglia, C. A., Furcht, L. T., McCarthy, J. B., and Oegema, T. R. Jr., A heparan sulfate-containing fraction of bone marrow stroma induces maturation of HL-60 cells in vitro. Cancer Res. 50 (1990) 3781–3785.
76 Mayne, R., and Brewton, R. G., New members of the collagen superfamily. Curr. Opin. Cell Biol. *5* (1993) 883–890.
77 Minguell, J. J., and Tavassoli, M., Proteoglycan synthesis by hemopoietic progenitor cells. Blood *73* (1989) 1821–1827.
78 Moritz, T., Patel, V. P., and Williams, D. A., Bone marrow extracellular matrix molecules improve gene transfer into human hematopoietic cells via retroviral viruses. J. clin. Invest. *93* (1994) 1451–1457.
79 Oguri, K., Okayama, E., Caterson, B., and Okayama, M., Isolation, characterization and localization of glycosaminoglycans in rabbit bone marrow. Blood *70* (1987) 501–510.
80 Osborn, L., Hession, C., Tizard, R., Vassallo, C., Luhowskyj, S., Chi-Rosso, G., and Lobb, R., Direct cloning of vascular cell adhesion molecule-1 (VCAM1), a cytokine-induced endothelial protein that binds to lymphocytes. Cell *59* (1989) 1203–1211.
81 Patel, V. P., and Lodish, H. F., Loss of adhesion of murine erythroleukemic cells to fibronectin during erythroid differentiation. Science *224* (1984) 996–998.
82 Patel, V. P., and Lodish, H. F., The fibronectin receptor on mammalian erythroid cells: characterization and developmental regulation. J. Cell Biol. *102* (1986) 449–456.
83 Patel, V. P., and Lodish, H. F., Fibronectin matrix is required for differentiation of murine erythroleukemia cells into reticulocytes. J. Cell Biol. *105* (1987) 3105–3118.
84 Patel, V. P., Ciechanover, A., Platt, O., and Lodish, H. F., Mammalian reticulocytes lose adhesion to fibronectin during maturation to erythrocytes. Proc. natl Acad. Sci. USA *82* (1985) 440–444.
85 Peters, C., O'Shea, K. S., Campbell, A. D., Wicha, M. S., and Long, M. W., Fetal expression of hemonectin: an extracellular matrix hematopoietic cytoadhesion molecule. Blood *75* (1990) 357–364.
86 Petrides, P. E., and Dittmann, K. H., How do normal and leukemic white blood cells egress from the bone marrow? Blut *61* (1990) 3–13.
87 Prieto, A. L., Andersson-Fisone, C., and Crossin, K. L., Characterization of multiple adhesive and counteradhesive domains in the extracellular matrix protein cytotactin. J. Cell Biol. *119* (1992) 663–678.
88 Reuss-Borst, M. A., Klein, G., Waller, H. D., and Müller, C. A., Differential expression of adhesion molecules in acute leukemia. Leukemia *9* (1995) 869–874.
89 Reuss-Borst, M. A., Bühring, H. J., Klein, G., and Müller, C. A., Adhesion molecules on CD34 + hematopoietic cells in normal human bone marrow and leukemia. Ann. Hemat. *65* (1992) 169–174.
90 Ridley, R. C., Xiao, H., Hata, H., Woodliff, J., Epstein, J., and Sanderson, R. D., Expression of syndecan regulates human myeloma plasma cell adhesion to collagen type I. Blood *81* (1993) 767–774.
91 Roberts, R., Gallagher, J., Spooncer, E., Allen, T. D., Bloomfield, F., and Dexter, T. M., Heparan sulfate bound growth factors: a mechanism for stromal cell mediated haemopoiesis. Nature *332* (1988) 376–378.
92 Rüegg, C. R., Chiquet-Ehrismann, R., and Alkan, Ş. Ş., Tenascin, an extracellular matrix protein, exerts immunomodulatory activities. Proc. natl Acad. Sci. USA *86* (1989) 7437–7441.
93 Ruoslahti, E., Noble, N. A., Kagami, S., and Border, W. A., Integrins. Kidney Int. *45* Suppl. 44 (1994) S-17–S-22.
94 Ryan, D. H., Nuccie, B. L., Abboud, C. N., and Winslow, J. M., Vascular cell adhesion molecule-1 and integrin VLA-4 mediate adhesion of human B cell precursors to cultured bone marrow adherent cells. J. clin. Invest. *88* (1991) 995–1004.
94a Saeland, S., Duvert, V., Caux, C., Pandrau, D., Favre, C., Vallé, A., Durand, J., Charbord, P., de Vries, J., and Banchereau, J., Distribution of surface-membrane molecules on bone marrow and cord blood $CD34^+$ hematopoietic cells. Expl. Hemat. *20* (1992) 24–33.
95 Sage, H., and Bornstein, P., Extracellular proteins that modulate cell-matrix interactions. SPARC, tenascin, and thrombospondin. J. biol. Chem. *266* (1991) 14831–14834.
96 Sanderson, R. D., Lalor, P., and Bernfield, M., B lymphocytes express and lose syndecan at specific stages of differentiation. Cell Reg. *1* (1989) 27–35.
97 Sanderson, R. D., Sneed, T. B., Young, L. A., Sullivan, G. L., and Lander, A. D., Adhesion of B-lymphoid (MPC-11) cells to type I collagen is mediated by the integral membrane proteoglycan, syndecan. J. Immun. *148* (1992) 3902–3911.
98 Shaw, L. M., and Olson, B. R., FACIT collagens: diverse molecular bridges in extracellular matrices. Trends Biochem. Sci. *16* (1991) 191–194.
99 Siczkowski, M., Clarke, D., and Gordon, M. Y., Binding of primitive hematopoietic progenitor cells to marrow stromal cells involves heparan sulfate. Blood *80* (1992) 912–919.
100 Siczkowski, M., Robertson, D., and Gordon, M. Y., Synthesis and deposition of glycosaminoglycans in the murine hemopoietic stromal line S17: modulators of the hemopoietic microenvironment. Expl Hemat. *20* (1992) 1285–1290.
101 Singer, J. W., Keating, A., and Wight, T. N., The human hematopoietic microenvironment, in: Recent Advances in Haematology, pp. 1–24. Ed. A. V. Hofmann. Churchill-Livingstone, Inc., New York 1985.
102 Soini, Y., Kamel, D., Apaja-Sarkkinen, M., Virtanen, I., and Lehto, V.-P., Tenascin immunoreactivity in normal and pathological bone marrow. J. clin. Path. *46* (1993) 218–221.
103 Soligo, D., Schiro, R., Luksch, R., Manara, G., Quirici, N., Parravicini, C., and Lambertenghi-Deliliers, G., Expression of integrins in human bone marrow. Br. J. Haemat. *76* (1990) 323–332.

104 Spring, J., Beck, K., and Chiquet-Ehrismann, R., Two contrary functions of tenascin: dissection of the active sites by recombinant tenascin fragment. Cell *59* (1989) 325–334.

105 Springer, T. A., Adhesion receptors of the immune system. Nature *346* (1990) 425–434.

106 Spooncer, E., Gallagher, J. T., Krizsa, F., and Dexter, T. M., Regulation of haemopoiesis in long-term bone marrow cultures. IV. Glycosaminoglycan synthesis and the stimulation of haemopoiesis by beta-D-xylosides. J. Cell Biol. *96* (1983) 510–514.

107 Sriramarao, P., Mendler, M., and Bourdon, M. A., Endothelial cell attachment and spreading on human tenascin is mediated by $\alpha_2\beta_1$ and $\alpha_v\beta_3$ integrins. J. Cell Sci. *105* (1993) 1001–1012.

108 Suchard, S. J., Mansfield, P. J., and Dixit, V. M., Modulation of thrombospondin receptor expression during HL60 cell differentiation. J. Immun. *152* (1994) 877–888.

109 Sugahara, H., Kanakura, Y., Furitsu, T., Ishihara, K., Oritani, K., Ikeda, H., Kitayama, H., Ishikawa, J., Hashimoto, K., Kanayama, Y., and Matsuzawa, Y., Induction of programmed cell death in human hematopoietic cell lines by fibronectin via its interaction with very late antigen 5. J. expl Med. *179* (1994) 1757–1766.

110 Takahashi, G. W., Moran, D., Andrews, D. F. III, and Singer, J. W., Differential expression of collagenase by human fibroblasts and bone marrow stromal cells. Leukemia *8* (1994) 305–308.

111 Takeichi, M., Cadherin cell adhesion receptors as a morphogenetic regulator. Science *251* (1991) 1451–1455.

112 Teixidó, J., Hemler, M. E., Greenberger, J. S., and Anklesaria, P., Role of beta 1 and beta 2 integrins in the adhesion of human $CD34^{hi}$ stem cells to the bone marrow. J. clin. Invest. *90* (1992) 358–367.

113 Timpl, R., Proteoglycans of basement membranes. Experientia *49* (1993) 417–428.

114 Timpl, R., and Brown, J. C., The laminins. Matrix Biol. *14* (1994) 275–281.

115 van der Rest, M., and Garrone, R., Collagen family of proteins. FASEB J. *5* (1991) 2814–2823.

116 Verfaillie, C. M., McCarthy, J. B., and McGlave, P. B., Differentiation of primitive human multipotent hematopoietic progenitors into single lineage clonogenic progenitors is accompanied by alterations in their interaction with fibronectin. J. expl Med. *174* (1991) 693–703.

117 Verfaillie, C. M., McCarthy, J. B., and McGlave, P. B., Mechanisms underlying abnormal trafficking of malignant progenitors in chronic myelogeneous leukemia. Decreased adhesion to stroma and fibronectin but increased adhesion to the basement membrane components laminin and type IV collagen. J. clin. Invest. *90* (1992) 1232–1241.

118 Vestweber, D., Selectins: cell surface lectins which mediate the binding of leukocytes to endothelial cells. Semin. Cell Biol. *3* (1992) 211–220.

119 White, H., Totty, N., and Panayotou, G., Haemonectin, a granulocytic-cell-binding protein, is related to the plasma glycoprotein fetuin. Eur. J. Biochem. *213* (1993) 523–528.

120 Whitlock, C. A., and Witte, O. N., Long-term culture of B lymphocytes and their precursors from murine bone marrow. Proc. natl Acad. Sci. USA *79* (1982) 3608–3612.

121 Wight, T. N., Kinsella, M. G., Keafing, A., and Singer, J. W., Proteoglycans in human long-term bone marrow cultures: biochemical and ultrastructural analyses. Blood *67* (1986) 1333–1343.

122 Williams, D. A., Rios, M., Stephens, C., and Patel, V. P., Fibronectin and VLA-4 in haematopoietic stem cell-microenvironment interactions. Nature *352* (1991) 438–441.

123 Yokosaki, Y., Palmer, E. L., Prieto, A. L., Crossin, K. L., Bourdon, M. A., Pytela, R., and Sheppard, D., The integrin $\alpha9\beta1$ mediates cell attachment to a non-RGD site in the third fibronectin type III repeat of tenascin. J. biol. Chem. *269* (1994) 26691–26696.

124 Zuckerman, K. S., Rhodes, R. K., Goodrum, D. D., Patel, V. R., Sparks, B., Wells, J., Wicha, M. S., and Mayo, L. A., Inhibition of collagen deposition in the extracellular matrix prevents the establishment of a stroma supportive of hematopoiesis in long term murine bone marrow cultures. J. clin. Invest. *75* (1985) 970–975.

125 Zuckerman, K. S., and Wicha, M. S., Extracellular matrix production by the adherent cells of long-term murine bone marrow cultures. Blood *61* (1983) 540–547.