Differential expression of α-smooth muscle actin molecule in a subset of bone marrow stromal cells, in b-cell chronic lymphocytic leukemia, autoimmune disorders and normal fetuses

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Summary

Lymphocytes are a constituent of normal marrow. Both B and T lymphocytes are derived from bone marrow stem cells. Lymphocytes are found in normal marrow as single cells and in lymphoid aggregates or follicles. Lymphocytes and precursors are particularly prominent in bone marrow from children in which they may account for up to 40% of the bone marrow cells.

The development of hematopoetic cells within the bone marrow (BM) occurs in intimate association with cells of the bone marrow microenvironment. This phenotypically diverse population of connective tissue-type cells includes fibroblasts, macrophages, adipocytes and endothelial cells and, collectively, represents the stromal tissue of the bone marrow. The presence of myoid cells in human bone marrow has been observed during hemopoiesis in embryonic life, whereas during adult life, it is strictly related to different pathologic conditions such as metastatic carcinoma, Hodgkin's disease, hairy cell leukemia and chronic myelo-proliferative diseases.

Under normal circumstances, lymphoid cells may constitute up to 20% of the population of nucleated cells in the bone marrow. However, there may be an absolute or a relative increase, the latter due to a reduction in hematopoietic tissue, as in some skeletal areas in advancing age, or in hypoplastic conditions.

The aim of this study was to examine the presence, distribution and quantitation of cells expressing α-smooth muscle actin in the stroma of the BM of patients with nodular type b-cell chronic lymphocytic leukemia (B-CLL), patients with autoimmune disorders and embryos (gestational age 15 to 25 weeks).

For this reason, we investigated the presence of myoid cells (MCs) in a series of 20 trephine bone marrow biopsies from adult patients and ten fetal specimens of the spine and femur, using a monoclonal antibody recognizing alpha-smooth muscle actin, a contractile microfilament expressed exclusively by smooth muscle cells, myofibroblasts and related cells.

The results of our study showed that: 1. BM stromal myoid cells represent a distinct subpopulation of reticular cells in the bone marrow, undergoing cytoskeletal remodeling in response to various stimuli (fetuses). 2. The appearance of BM stromal myoid cells is not only seen as a characteristic feature in B-CLL, but is also seen, to a lesser degree, in the stroma of bone marrow in patients with autoimmune disorders. 3. Stromal cells with phenotypic smooth muscle features appear in bone marrow during pathological situations in a manner reminiscent of what occurs during normal development.

Key words: B-CLL; Autoimmune disorders; Fetal bone marrow; Alpha-Smooth Muscle Actin.

Introduction

Lymphoid follicles (lymphoid aggregates) are a constituent of normal bone marrow and increase with age and in autoimmune and chronic inflammatory disorders [1, 2]. Lymphoid follicles are present in up to 47% of bone marrow specimens and are most prevalent in women and the elderly [1].

Reactive lymphoid follicles must be distinguished from marrow involvement by low-grade lymphoma, principally malignant lymphoma, small lymphocytic and malignant lymphoma, follicular, predominantly small-cleaved cell. Follicular center-like structures may be seen in follicular lymphoma involving the bone marrow as well as in reactive lymphoid follicles, In general, follicles that are well circumscribed, nonparatrabecular, few in number (two or fewer per biopsy), and contain a mixture of cell types (small and large lymphocytes, plasma cells, histiocytes) are not cytologically monotonous and are likely to be benign. In contrast, follicles that are numerous, usually large, paratrabecular, infiltrate the surrounding marrow or are cytologically monotonous, are more likely to represent involvement by malignant lymphoma. In doubtful cases, immunohistochemical studies to assess lymphocyte clonality may be helpful.

All types of blood forming cells derive from a small pool of immature not morphologically identified progenitor cells. Hemopoiesis is regulated and sustained by a complex cellular interaction of hemopoietic and stromal elements and a network of cytokine growth factors, including the interleukins and colony stimulating factors [3, 4].

The stroma provides the supporting framework for hematopoiesis, which takes place in the extravascular compartment. This is composed of reticulin fibres, which are produced by inconspicuous elongated fibroblasts and exist as a fine network, readily displayd by Gomori silver stain.

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There are also many macrophages containing haemosiderin attached to stromal fibres, some of which are embedded in the centres of hemopoietic cell clusters. Endothelial cells line the marrow sinusoids, a single layer of cells being supported by reticulin fibres on the basal layer. Endothelial cells are interconnected by tight junctions which appear to be effective barriers between vascular and extravascular spaces. Together, these components constitute the bone marrow hematopoietic microenvironment [5-7].

Materials and Methods

Bone marrow specimens: 1) Ten marrow biopsy specimens from patients diagnosed as developing B-cell chronic lymphocytic leukemia (B-CLL) on the basis of cytomorphological and immunohistochemical diagnosis. 2) Ten marrow biopsy specimens from patients diagnosed as developing clinical autoimmune disorders (rheumatoid arthritis, Sjogren's syndrome). Follow-up of these patients did not show a transformation to B-CLL or other malignant disorders. 3) Fetal specimens of the spine and femur were derived from ten spontaneous miscarriages and fetal abortions (gestational age 15 to 25 weeks).

All the specimens were retrieved from the files of the Department of Pathology (Democritus University of Thrace, Alexandoupolis, Greece).

Light microscopy was done with paraffin sections, 5 μm thick, stained with Giemsa and periodic acid Schiff (PAS).

Immunohistochemistry: The presence of α-smooth muscle actin was examined in our samples by means of the avidin-biotin complex (ABC) peroxidase method using the monoclonal antibody anti-asm-1. Sections were pretreated with H_2O_2/methanol and subsequently with 0.1 M periodic acid, 0.005 M NaBH4 and normal horse serum. They were incubated for 20 h with anti-asm-1 hybridoma supernatant containing 5 μg/ml of IgG diluted 1:600. This first incubation was followed by ABC-peroxidase staining using the Vectastain Kit anti-mouse IgG (Vector Laboratories, Burlingame, Ca). Peroxidase activity was revealed with 30% DAB (3,3′-diaminobenzidine, Serva Heidelberg, FRG) in PBS containing 0.015% H_2O_2. Slides were weakly counterstained with Mayer's hematoxylin and mounted in Eukitt. Controls were performed by using a mouse IgG or by omitting the primary antibody.

The localization of anti-asm-1 immunoreactive cells was analysed in the following marrow compartments: the perisinusoidal zone adjacent to the surface of sinusoidal walls bordering the lumen (of the sinusoids), the intermediate zone of the hematopoietic marrow parenchyme and the peritrabecular zone bordering the bone surface.

Two observers using the following scale estimated the number of α-smooth muscle positive stromal cells independently:

\+ = staining of less than 20% of stromal cells,
++ = staining of between 20% and 50% of stromal cells,
+++ = staining of more than 50% of stromal cells.

The grade of fibrosis of the bone marrow was evaluated using the following scale:

0 = no reticulin increase,
1 = minimal focal increase in fine reticulin fibers,
2 = moderate multifocal or diffuse reticulin fibrosis,
3 = marked fibrosis with presence of course collagen fibers.

Results

The distribution of bone marrow stromal cells expressing α-smooth muscle actin (myoid cells) is shown in Table 1.

Fetal specimens: During the fetal life at the 15th week of gestation some immunoreactive stromal cells were seen along the network of thin-walled vessels penetrating into the marrow cavities of the spine and femur. At these sites, small amounts of reticulin fibers (grade 0-1) were observed. At the 20th week of gestation, peripheral (peritrabecular zone) and central (intermediate zone) vascular sinusoids were associated with stromal cells positive for α-smooth muscle actin. At these sites, small to moderate amounts of reticulin fibers (grade 1-2) were observed. A more intensive staining in the stromal cells and moderate amounts of fibrosis (grade 2) were observed during the 25th week of gestation.

Autoimmune disorders: In all cases examined from patients with autoimmune disorders (mainly rheumatoid arthritis and Sjogren's disease) the number and distribution of the stromal cells expressing α-smooth muscle actin was variable. In the perisinusoidal and peritrabecular zone, scattered positive cells were seen. At these sites minimal fibroplasia (grade 0-1) was observed. In the intermediate zone focal accumulations of myoid cells were encountered mainly around the benign lymphoid follicles (Figure 1). At these sites small to moderate amounts of reticulin fibers (grade 1-2) were observed. Moreover, scattered positive stromal cells expressing α-smooth muscle actin were found loosely arranged between the lymphocytes of the reactive follicles (Figure 2).

B-cell chronic lymphocytic leukemia: In all cases of B-CLL the bone marrow stroma in the perisinusoidal, intermediate and paratrabecular zone, exhibited a moderate to marked number of positive myoid cells occurring among the neoplastic lymphoid nodules, accompanied by a moderate to marked fibrosis (grade 2-3), presence of coarse collagen fibers and extensive depletion of hematopoietic cells (Figure 3). Positive stromal cells expressing α-smooth muscle actin were also found infiltrating the stroma of the neoplastic lymphoid nodules (Figure 4).

Table 1. — *Reactivity of α-smooth muscle actin with BM stromal cells in different conditions*

Condition	No of cases	Grade of fibrosis	Immunohistochemical localization of positive cells		
			Perisinusoidal zone	Intermediate zone	Peritrabecular zone
Fetal specimens					
15th week of gestation	3	0-1	+		
20th week of gestation	4	1-2	+ to ++	+ to ++	+ to ++
25th week of gestation	3	2	++	++	++
Autoimmune Disorders	10	1-2	+	+ to ++	+
B-cell chronic lymphocytic leukemia (B-CLL) nodular type	10	2-3	++ to +++	++ to +++	++ to +++

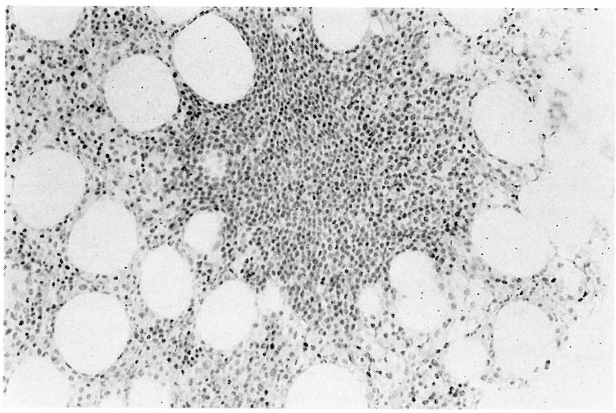

Figure 1. — Intermediate zone of bone marrow biopsy from a patient with an autoimmune disorder, showing focal accumulations of MCs around the benign lymphoid follicle. Low magnification (x100). Immunoperoxidase staining of bone marrow with anti-α-smooth muscle actin-1.

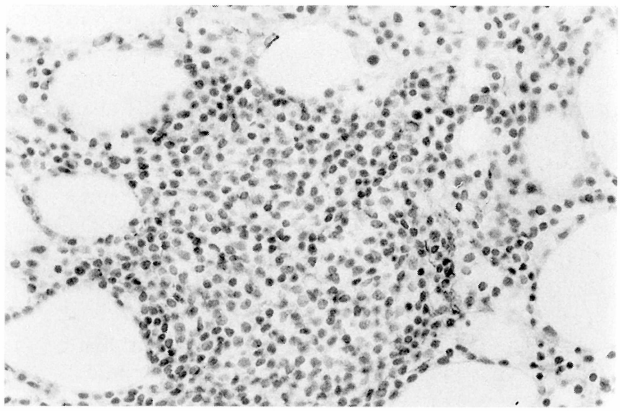

Figure 2. — Bone marrow biopsy in a patient with an autoimmune disorder, higher magnification, showing scattered positive MCs loosely arranged among the lymphocytes of the reactive follicle (x250). Immunoperoxidase staining of bone marrow with anti-α-smooth muscle actin-1.

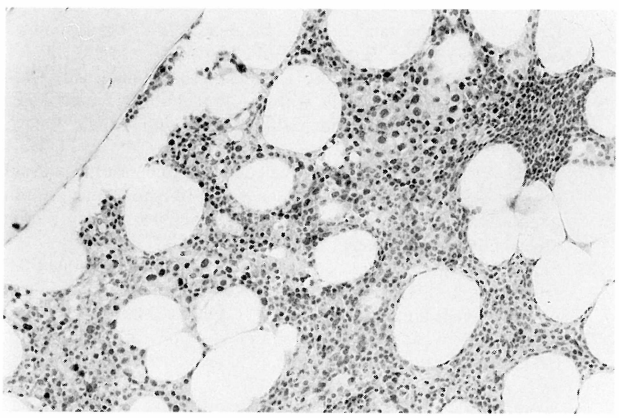

Figure 3. — Intermediate zone of bone marrow biopsy from a patient with BCLL, showing a moderate to marked number of positive MCs among the neoplastic lymphoid nodules, accompanied by fibrosis and depletion of hematopoietic cells. Low magnification (x100). Immunoperoxidase staining of bone marrow with anti-α-smooth muscle actin-1.

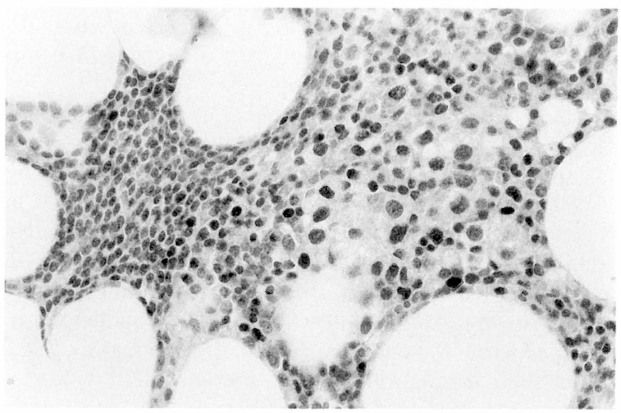

Figure 4. — Intermediate zone of bone marrow biopsy from a patient with BCLL, showing positive stromal cells expressing α-smooth muscle actin infiltrating the stroma of the neoplastic lymphoid nodule (x250). Immunoperoxidase staining of bone marrow with anti-α-smooth muscle actin-1.

Discussion

Approximately 10% of bone marrow cells in the normal adult are small lymphocytes. These lymphocytes are diffusely scattered throughout the interstitium. Lymphocytic aggregates are present in approximately 20% of marrow biopsies performed for a variety of reasons other than for malignant lymphoma or chronic lymphocytic leukemia [8]. The incidence of this finding increases with age, and is higher in females than in males. These structures may result from a nonspecific immune response and whether they represent a normal finding is questionable.

Different hematopoietic disorders are commonly associated with fibrotic changes in bone marrow stroma [9]. The contribution of fibrosis to hematopoietic failure and histogenesis, and to properties of cells that constitute the fibrotic bone marrow stroma are not clear. Some of these cells display ultrastructural similarities to smooth muscle cells [10]. The expression of alpha-smooth muscle actin, a differentiation marker of smooth muscle cells, has been observed in the BM stroma of different hematopoietic disorders such as BM involvement by metastatic carcinoma, Hodgkin's disease, multiple myeloma, chronic myelomonocytic leukemia, and primary myelofibrosis [10, 11]. In all those cases the incidence of cells expressing alpha-smooth muscle actin was correlated with the degree of fibrotic change in the stroma. Alpha-smooth muscle actin-positive stromal cells have been observed during hematopoiesis in embryonic life and early infant stages of BM development, but whether they represent a normal feature in adult normal BM stroma is arbitrary [11, 23-26]. These observations show that fibrotic BM stroma, like other fibrotic sites, contains cells displaying mixed fibroblastic and smooth muscle characteristics [12, 13]; these cells were referred to by Gabbiani et al as "myofibroblasts" [14].

Cytoskeletal proteins have been shown to represent specific differentiation markers during development and pathologic conditions. Some of these proteins (e.g. desmin) are differentiation markers of all muscle cell types, while others (e.g. α-smooth muscle actin and smooth muscle myosin) are differentiation markers specific for smooth muscle cells. These latter proteins have been found to be expressed by a distinct subset of stromal cells in various lymphatic organs including the spleen [15, 16], lymph nodes [15, 16], tonsil and thymus [17]; for such cells the name myoid cells (MCs) has been proposed [15, 16]. There are many articles about the presence of MCs in the stroma of primary carcinoma as well as in the stroma of carcinoma metastases [18, 19].

The presence of MCs suggests that these cells are the result of differentiation of fibroblastic stromal cells to malignant cells irrespective of their location. In the same way, the presence in the bone marrow of numerous MCs in myeloproliferative, myelodysplastic, and other malignant conditions supports the possibility that the clonal proliferation of hematopoietic cells stimulates the appearance of MCs [10, 22]. This expession is regulated by various stimuli including cytokines and growth factors [20, 21].

The results of our study showed: 1) The appearance of stromal MCs and fibrosis is not only a characteristic feature of malignant disorders of bone marrow including B-cell chronic lymphocytic leukemia, but is also seen in reactive conditions such as benign lymphoid follicles found in the stroma of bone marrow in patients with autoimmune disorders. In the latter (autoimmune disorders), growth factors or other non-specific immune responses, play a role to in a lesser degree, in bone marrow MCs differentiation and proliferation. 2) Stromal cells with phenotypic smooth muscle features appear in bone marrow during pathological situations in a manner reminiscent of what occurs during normal development. Further studies on stromal cells may increase our understanding of the physiology and pathology of bone marrow.

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