Expression of alpha-smooth muscle actin in the stromal cells of bone marrow in fetuses in different stages of development, in multiple myeloma and monoclonal gammopathy of unknown significance

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Summary

Several disorders are associated with a monoclonal immunoglobulin detected by serum or urine electrophoresis, the most common being a monoclonal gammopathy of unknown significance, multiple myeloma, Waldenstrom's macroglobunemia, and amyloidosis.

Plasma cells, the immunoglobulin secretory cells of the immune system, are normal constituents of bone marrow (BM). Plasma cells are seen in small numbers in the stroma, surrounding blood vessels in the marrow. Their perivascular disposition is consistent with their secreting capacity.

The hematopoietic microenvironment has a crucial role homing and regulating precursor cell growth both in physiologic and pathologic conditions. Cellular components such as branched adventitial reticular cells, macrophages, endothelial cells and fat cells constitute the supporting framework (stroma) for hematopoiesis, which takes place in the extravascular compartment. The presence of myiod cells (MCs) in human bone marrow has been observed during hematopoiesis in embryonic life, whereas during adult life, it is strictly related to various pathologic conditions.

The aim of this study was to examine in the stroma of BM the presence, distribution and quantitation of cells expressing a-smooth muscle actin (MCs) from patients with monoclonal gammopathy of unknown significance, those with plasma cell myeloma and embryos (gestational age 15 to 25 weeks). For this reason, a series of 20 trephine bone marrow biopsies from adult patients and ten fetal specimens of the spine and femur were examined for the presence of stromal myoid cells using a monoclonal recognising alpha-smooth muscle actin, a contractile microfilament expressed solely by smooth muscle cells, myofibroblasts and related cells.

Our results suggest that the appearance of MCs and subsequent fibrosis is not a feature of malignant BM disorders such as MM but it is also seen to a lesser degree in the BM stroma of individuals with monoclonal gammopathy of unknown significance (MGUS). 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: Alpha-smooth Muscle Actin; Stromal cells; Bone marrow; Multiple Myeloma; Fetuses.

Introduction

Monoclonal gammopathy in the absense of evidence of plasma cell myeloma or lymphoproliferative disorder is not an uncommon finding and becomes more common with advanced age. Monoclonal gammopathies are found in approximately 5% of healthy individuals over the age of 80. These have been referred to as "benign monoclonal gammopathies"; however, because the natural history is unknown and some patients will eventually develop plasma cell myeloma or a lymphoproliferative disorder, the term "monoclonal gammopathy of unknown significance" (MGUS) is preferred (1). MGUS is usually of IgG type; a minority is of IgM or IgA type.

Plasma cell myeloma (multiple myeloma) is a systemic clonal proliferation of plasma cells associated with a serum or urine monoclonal protein abnormality and skeletal disease. Skeletal manifestations due to production of osteoclast-activating factor (OAF) by neoplastic plasma cells result in diffuse osteopenia or lytic bone lesions.

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It may not be possible to make a histological distinction between [1] the plasmacytosis of chronic inflammatory or other disease, [2] that of benign monoclonal gammopathy and [3] that of early or smoldering multiple myeloma. However, a monoclonal population may be identified by immunological investigation on smears of aspirates, on imprints of biopsies and on cryostat or paraffin sections. Though no single morphological feature of plasma cells is characteristic of a neoplastic clone, a high incidence of any of the following features within a population suggests malignancy: large nuclei, prominent nucleoli, cellular and nuclear pleomorphism, multinuclearity, crystaline or other inclusions and nucleocytoplasmic asynchronism. Bone marrow histology is similar in the three conditions listed above. Small groups of plasma cells are found near blood vessels and among the hematopoietic and fat cells. In early MM there are also small paratrabecular and periarterial clusters of plasma cells. The incidence of multiple myeloma has also increased considerably in the last two decades. In addition, MCs are normally present during the prenatal period, even before the seeding of the marrow stroma by

hematopoietic cells. This characteristic embryonic cell type reappears in adult bone marrow in mainly neoplastic conditions.

It is known that multiple myeloma is associated with fibrosis of bone marrow stroma. There are no relevant studies in the literature about the capacity of bone marrow stromal cells expressing α-smooth muscle actin, as well as the occurrence of fibrosis, in cases of monoclonal gammopathy of unknown significance.

Materials and Methods

Bone marrow specimens: 1) Ten bone marrow biopsy specimens from patients diagnosed as having Plasma cell myeloma (MM) interstitial pattern on the basis of cytomorphological and immunohistochemical diagnosis. 2) Ten bone marrow biopsy specimens from individuals over the age of 80 and diagnosed as having benign monoclonal gammopathy. Follow-up of these patients did not show a transformation to MM or other malignant disorder. 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. Paraffin sections, 5 μm thick, were Giemsa and periodic acid Schiff (PAS) stained.

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 hours with anti-asm-1 hybridoma supernatant containing 5μg/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 perisinusoid zone adjacent to the abluminal layer of marrow sinusoidal walls, 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 (MCs) is shown in Table 1.

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 gestational week	3	0-1	+		
20th gestational week	4	1-2	+ to ++	+ to ++	+ to ++
25th gestational week	3	2	++	++	++
MGUS	10	1-2	++	+	+
MM	10	2-3	++ to +++	++ to +++	++ to +++

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 of the stromal cells and moderate amounts of fibrosis (grade 2) were observed during the 25th week of gestation.

Monoclonal gammopathy: In all examined cases of bone marrow individuals with monoclonal gammopathy of unknown significance, the number and distribution of the stromal cells expressing α-smooth muscle actin was variable. In the intermediate and peritrabecular zone, scattered positive cells were seen. At these sites minimal fibroplasia (grade 0-1) was observed. In the perisinusoidal zone focal accumulations of myoid cells were encountered surrounding the wall of the sinusoidal vessels (Figure 1). At these sites small to moderate

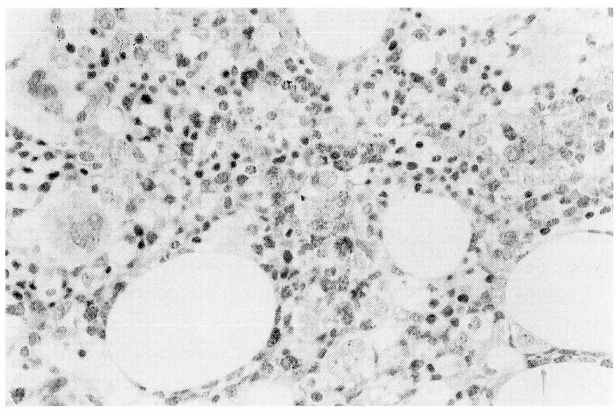

Figure 1. — Monoclonal gammopathy of unknown significance. Bone marrow biopsy showing focal accumulations of stromal cells expressing α-smooth muscle actin and accompanied by mild to moderate fibroplasia. High magnification (×250).

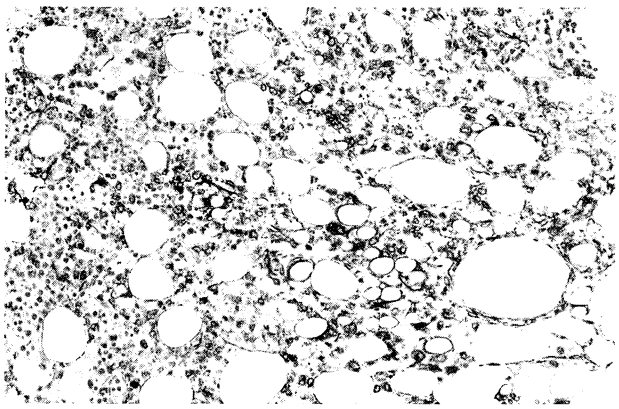

Figure 2. — Multiple myeloma. Bone marrow biopsy showing a moderate to marked number of positive cells expressing α-smooth muscle cells occurring amongst neoplastic plasma cells. Low magnification (×100).

amounts of reticulin fibers (grade 1-2) were observed.

Multiple Myeloma: In all cases of MM the bone marrow stroma in the perisinusoidal, intermediate and paratrabecular zone exhibited a moderate to marked number of positive myoid cells (MCs) expressing α-smooth muscle actin, occurring among the neoplastic plasma cells and accompanied by a moderate to marked fibrosis (grade 2-3) and presence of coarse collagen fibers (Figure 2).

Discussion

During fetal life many stromal cells containing α-smooth muscle actin (MCs) are connected with vascular sinusoids in the primitive architecture of bone marrow. In contrast, in the stromal cells of normal adult BM, these MCs do not exist. These cells reappear in adult bone marrow in myeloproliferative diseases, acute myeloid leukemia, Hodgkin's disease, metastatic carcinoma, multiple myeloma, non-Hodgkin's lymphoma and hairy cell leukemia. The appearance of stromal myoid cells is usually associated with an increase in the deposition of reticulin and collagen fibers.

In approximately nine percent of cases of MM the bone marrow lesion show reticulin fibrosis [2, 3]. In many of these the fibrosis is extensive. A disproportionate number of fibrotic myelomas produce monoclonal light chains only. Coarse fibrosis is strongly correlated with extensive diffuse marrow involvement and aggressive disease [4].

The presence of MCs suggests that these cells result from the differentiation of fibroblastic reticular stromal cells to malignant cells irrespective of their location. In the same way, the presence of numerous MCs in the BM of patients with myeloproliferative and other malignant conditions supports the possibility that the clonal proliferation of hematopoetic cells stimulates the appearance of MCs [5]. This expression is modulated by various stimuli including growth factors [6, 7]. In multiple myeloma, myeloma stem cells proliferate and circulate in the blood. Upon return to the bone marrow the premyeloma cells attach to the cytokine-rich stroma and differentiate to plasma cells. Complex interactions between myeloma and stromal cells induce a range of cytokines (IL-6), which determine tumor growth as well as an osseous and hematopoietic reaction [8].

In analogy to what has been proposed for the lymph nodes and spleen [9], the presence in MCs of an actin isoform specific for contractile cells suggests that these cells have a contractile function, helping in directing the migration of blood cells. Since MCs are not present in normal bone marrow, this possible function appears important only in cases where there is increased blood cell traffic, such as occurs in various myeloproliferative diseases and in blood loss anemia. Support for this hypothesis comes from experiments showing that the number of microfilament bundles in adventitial reticular cells increases in rabbits after blood-letting [10]. Bone marrow stromal cells appear to have an essential role in the regulation of hematopoiesis through the synthesis of extracellular matrix components, growth and/or differentiation factors [11, 12, 13, 14]. In vivo experiments suggest that the presence of bone marrow cells decorated by an antibody recognizing muscular actins is important for the growth of hematopoietic cells (15). The fact that during development MCs are present before the seeding of the bone marrow by hematopoietic stem cells suggest that MCs are involved in the formation of an appropriate microenvironment for homing and proliferation of hematopoietic stem cells. Subsequently, in our cases of MGUS it seems that a relative factor, to a lesser degree, triggers the reappearance of MCs expressing α-smooth muscle actin mainly in the perisinusoidal zone of the examined BM. The occurrence of these cells is associated with increased deposition of reticulin and collagen fibers (grade 1-2).

Under the influence of myeloma cells the stromal cells produce large quantities of adhension and extracellular matrix molecules which together with the cytokines influence the rate and type of tumor growth in MM, as well as the blood vessels, fibroblasts and fibers. Development of coarse fibrosis (collagen type III) accompanied by an inflammatory reaction proved to be an unfavorable prognostic sign.

Monoclonality may be demostrated by means of antibodies to heavy or light chains. Moreover, a ratio of 16 or more of one of the two light chains to the other – kappa to lambda or vice versa – indicates MM. Nevertheless, even with immunohistology it is not always possible to distinguish between MM and MGUS and follow-up studies are required. Monoclonal peripheral blood lymphocytes at a late stage of B cell differentiation are found in patients with MM, but usually not in MGUS or smoldering MM.

MGUS may occur in patients with many long-standing disorders, including infections, inflammations, tumors and cardiovascular, neurologic or renal disorders, and may regress completely with successful treatment of the underlying condition. Patients with MGUS who later developed Waldenstrom's macroglobulinemia have reported, heavy chain disease, CLL, 1° amyloidosis and

other lymphoproliferative disorders. Time from demonstration of MGUS to overt disease has varied from six months to 22 years. Development of MM after MGUS has been reported after intervals of 10-25 years. Myeloma cells have a characteristic pattern of antigen expression: CD38++, CD56+, CD54 and clg+; they have a variable expression of CD40 (which possibly suppresses apoptosis) and lack CD19, CD20, CD45 and membrane Ig.

In conclusion, the results of our work showed: 1) The appearance of stromal myoid cells (MCs) and the associated fibrosis is not a hall mark of malignant disorders of bone marrow including MM, but is also seen, to a lesser degree, in the BM stroma in individuals with MGUS. In the latter (MGUS), growth factors or other non-specific immune reactions from long-standing disorders, including infections, tumors or systemic diseases might stimulate the appearance of these cells with myoid phenotypic features in the stroma of BM. 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 the activity of these cells expressing α-smooth muscle actin may increase our understanding of the physiology and pathology of bone marrow.

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