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Brief Reports

Connective Tissue Elements in Rat Bone Marrow:

Immunofluorescent Visualization of the Hematopoietic Microenvironment

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Immunofluorescent staining of frozen sections of rat bone marrow for collagen types I and III revealed the presence of a distinctive, collagen-producing cell type. Morphologically, these cells closely resembled reticular cells. They were large, with branching cytoplasm and were closely related to an extensive intercellular matrix of collagenous material that surrounded the hematopoietic cells of the marrow. Biochemical studies demonstrated synthesis of collagen types I and III, in a ratio of 4:1, by fresh rat bone marrow cells.
KEY WORDS: Collagen; Connective tissue; Bone marrow; Hematopoietic microenvironment.

Introduction

There is a considerable volume of evidence indicating that connective tissue elements within the bone marrow provide functional, as well as structural, support for hematopoiesis. Thus interactions between hematopoietic elements and stromal, connective tissue elements play an essential role in the regulation of hematopoietic activity (Bentley, 1982a).

The presence of collagen-producing cells in normal hematopoietic tissue has recently been demonstrated by immunoperoxidase staining of paraffin sectins of human bone marrow, obtained at post-mortem (Bentley et al., 1981). These preparations did not, however, enable detailed visualization of the bone marrow connective tissue matrix. Technical modifications have now resulted in immunofluorescent preparations of greatly improved quality.

Materials and Methods

Adult, female Sprague–Dawley rats were killed by ether inhalation and the femurs dissected out. The rat femurs were frozen over dry ice and the bony cortices removed using a dental drill. The frozen cores of bone marrow were then sectioned in a cryostat at the thickness of 5 μm. The sections were stained by immunofluorescence for collagen types I and III, as described previously (Bentley and Foidart, 1980).

Negative control was achieved by substitution of preimmune guinea pig serum for the primary antibodies and by absorption of the primary antibodies with the specific target antigens. Positive control was achieved by parallel processing of tissues of known specificity.

Collagen synthesis by fresh rat bone marrow was confirmed biochemically. Rat bone marrow cells were incubated in vitro with ^{14}C proline, as described previously (Bentley, 1982b). Total collagen synthesis was estimated by collagenase digestion (Peterkovsky and Diegelmann, 1971). The type I:type III collagen synthesis ratio was estimated by sodium dodecyl sulfate–polacrylamide gel electrophoresis of labeled material (Laemmli, 1970), followed by fluorography (Bonner and Laskey, 1974; Laskey and Mills, 1975) and densitometric scanning.

Results

Sections stained for collagen types I and III were essentially similar in appearance. Positive cytoplasmic immunofluorescence for the respective procollagens was observed in the cytoplasm of a distinctive cell type, scattered throughout the hematopoietic compartment of the bone marrow (Figures 1, 2).

These cells were large, with branching cytoplasm, the cytoplasmic branches extending in the form of sheet-like processes between the hematopoietic cells. The fluorescent cytoplasmic extensions, in turn, appeared to merge into a generalized matrix of intercellular fluorescent material, distributed throughout the marrow. Intercellular collagen immunofluorescence was thus visible around each hematopoietic cell. Since the majority of the bone marrow cells did not fluoresce, the preparations revealed the cellular structure of the marrow in negative relief.

Collagen synthesis in labeled cultures of rat bone marrow cells was found to comprise 0.3% of the total protein synthesized in the media and 0.7% of the total protein synthesized in the cell pellets. The type I:type III collagen synthesis ratio was approximately 4:1. This is similar

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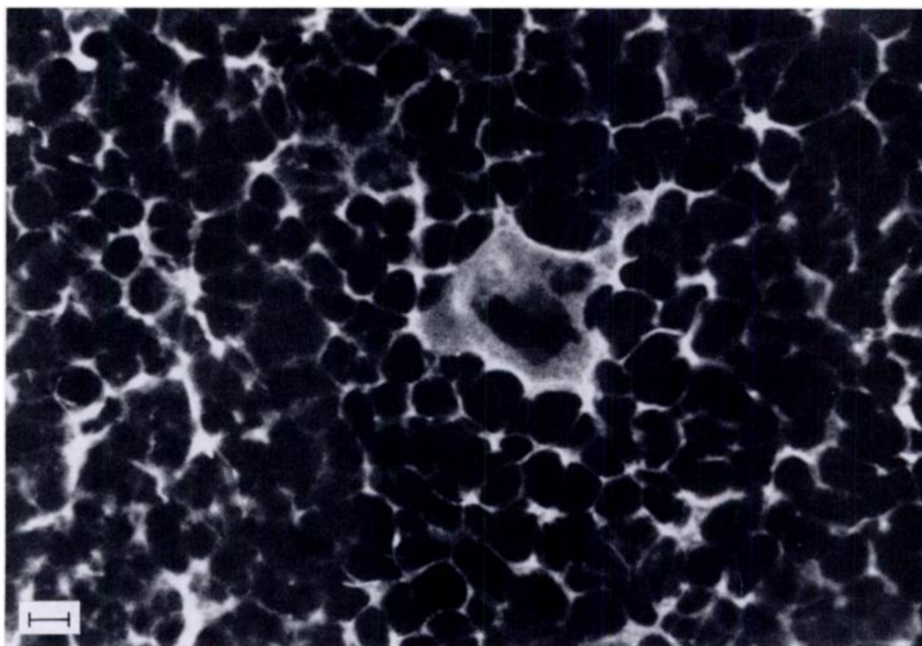

Figure 1. Frozen section of rat bone marrow stained by immunofluorescence for type I collagen. Fluorescence is seen in the cytoplasm of a large cell in the center of the field. The cell has branching cytoplasm extending into the intercellular space which shows extensive positive immunofluorescence for collagen. The hematopoietic cells of the marrow do not fluoresce and are seen in dark relief, surrounded by fluorescent, collagenous material in the intercellular matrix. Original magnification ×600. Bar = 10 μm.

to the ratio observed in fresh murine bone marrow and in continuous murine bone marrow cultures (Bentley, 1982b).

It was noted that rat bone marrow cells synthesized far more type I than type III collagen, but displayed similar intensities of immunofluorescence for these proteins in stained preparations. Rates of synthesis, however, do not necessarily translate into absolute quantities of collagen present, as the latter are also dependent upon differential rates of catabolism. The anti-collagen antibodies may also differ in their binding affinities, both to collagen and to the fluorescein-labeled anti-immunoglobulin reagent.

Discussion

There are no previous studies in which the collagenous matrix of the bone marrow has been clearly visualized using a specific staining technique.

Immunofluorescent staining of frozen sections of rat bone marrow for collagen types I and III revealed the presence of a distinctive species of collagen-producing cells. The similarities observed between preparations stained for type I and type III would suggest that individual cells produce both types of collagen.

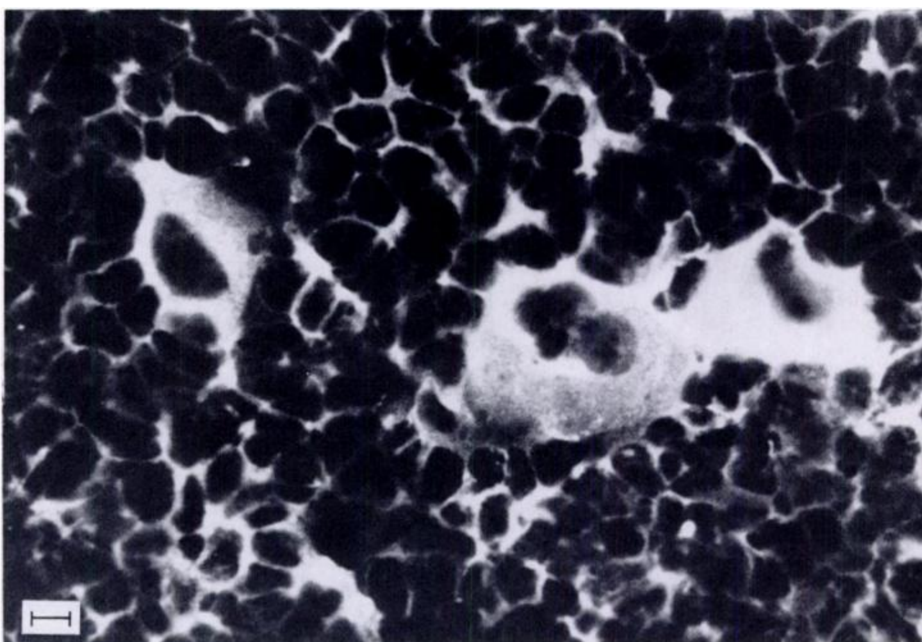

Figure 2. Frozen section of rat bone marrow stained by immunofluorescence for type III collagen. Three large collagen-producing cells are visible. Their appearance is essentially similar to that observed in Figure 1. Original magnification ×600. Bar = 10 μm.

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Reticular cells are generally considered to be the major cellular elements in bone marrow connective tissue. Weiss (1975) described reticular cells, observed by transmission electron microscopy of bone marrow, as "large, dendritic elements with broad, sheet-like cytoplasmic processes . . . which branch into the surrounding hematopoietic spaces, forming a spongework." This description would be perfectly applicable to the collagen-producing cells visualized by immunofluorescent examination of bone marrow sections, and it is thus tempting to identify these collagen-producing cells as reticular cells.

It is dangerous, however, to base such identification on morphological criteria alone, as cellular appearances in frozen sections stained by immunofluorescence may be very different from those observed in electron microscopic preparations. In the absence of a specific marker for reticular cells, the precise identity of the collagen-producing cells in bone marrow must remain unresolved. Studies of immunoperoxidase-stained bone marrow preparations, however, do not reveal the presence of collagen in hematopoietic cells (Bentley et al., 1981).

Immunofluorescent staining further revealed a collagenous matrix within the bone marrow that appeared far more extensive than that revealed by conventional, silver impregation techniques, or by ultrastructural examination. Silver impregation is not a specific staining technique for collagen, being dependent largely upon the physical structure of connective tissue fibers (Puchtler and Waldrop, 1978). Electron microscopic identification of collagen is dependent upon the visualization of typical cross-banded fibrils in tissue sections. It does not permit identification of collagens or procollagens at the molecular level. The observed disparities are not, therefore, surprising.

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