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COLLAGENASE-LIKE ACTIVITY ASSOCIATED TO THE LEUKEMIC WEHI-3B CELL LINE

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Abstract—The leukemic cell line, WEHI-3B presents a latent collagenase-like activity which is activated after trypsin treatment. The enzymatic activity is not released from cells and is specific for the degradation of interstitial collagens. After attachment of WEHI cells to a hemopoietic stroma derived from long-term bone marrow cultures, the organization of the stroma is disrupted. This effect was not observed after the attachment to stroma of normal progenitor cells. These results suggest that the collagenase-like activity in leukemic cells may contribute through collagen degradation, to the disorganization of the marrow stroma. The latter was confirmed by the use of a labeled-collagen containing stroma, which was degraded when cocultured with WEHI cells, but not with normal progenitor cells.

Key words: Collagenase, leukemia, bone marrow, hemopoietic stroma.

INTRODUCTION

IN-VITRO studies have shown that growth and differentiation of hemopoietic progenitors are determined, among other factors, by a complex network of interactions between the developing hemopoietic cells and the marrow microenvironment [1]. The latter includes stromal cells and their products, such as extracellular matrix (ECM) molecules and diffusible regulatory molecules [2]. In leukemogenesis, the original transformation of the immature precursors generates a blockade of the normal differentiation pattern and therefore distinct types of interactions with the microenvironment may occur [3]. During the transition from benign to invasive tumorogenesis, general and widespread changes occur in the organization of basement membranes and the extracellular matrix [4]. The latter is associated with the activation of release of hydrolytic enzymes which degrade various matrix components, like collagen and glyco-proteins [5–7].

In an attempt to understand the mechanisms by which leukemic cells cause abnormalities in the bone marrow microenvironment [8], we investigated whether hemopoietic leukemic cells contain ECM hydrolytic enzymes. Since collagen is the most abundant ECM component produced by bone marrow stromal cells [9], the presence of a collagenase activity in leukemic cells may be important in the onset of changes in the collagen-rich ECM associated to stromal cells [10, 11]. It is known that modifications in collagen metabolism by stromal cells affect progenitor survival and differentiation [12, 13].

In this communication we report the presence of a collagenase-like activity in the leukemic cell line, WEHI-3B. Results obtained suggest that this enzyme contributes to the structural disorganization of mouse hemopoietic stroma which occurs after the attachment of WEHI cells.

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MATERIALS AND METHODS

The Balb-c mice derived myelomonocytic leukemia cell line WEHI-3B [14] was kindly provided by Dr T. M. Dexter (Manchester, U.K.). Cells were grown in McCoy's medium containing 15% fetal calf serum (FCS, Gibco) at 37°C under 5% CO_2 in a humidified atmosphere. Collagenase-like activity in WEHI cells was studied in intact cells, in trypsin-treated cells, and in the trypsin extract thereafter produced. For the latter two cases, suspensions of WEHI cells were incubated with 0.05% trypsin for 20 min at 25°C, followed by the addition of soybean trypsin inhibitor [15]. After centrifugation, cells and the trypsin extract were saved.

Collagen degradation was studied by using as substrates, unlabeled or ^{3}H-labeled collagen, and estimated by SDS-PAGE [16] and by a collagenase-sensitive material assay [17], respectively.

^{3}H-proline labeled collagen type I was prepared as described [10]. Non-labeled collagen types I and III were prepared as described [18]. Collagen type IV was purchased from Sigma.

Marrow stroma and normal hemopoietic progenitor cells were obtained from long-term bone marrow cultures (LTBMC) prepared from Balb-c mice according to Dexter's method [19].

Cocultures were prepared by inoculating onto culture dishes, containing a confluent layer of unlabeled or labeled hemopoietic stroma (1.2×10^{-6} cells per dish), a suspension in LTBMC medium of 1×10^5 WEHI or normal hemopoietic progenitor cells. Cocultures were incubated at 33°C under 5% CO_2 for various periods of time.

For studies using labeled stroma, 7 day-old LTBMC were depleted of the non-adherent cells and the remaining stroma were incubated for 8 days in LTBMC medium containing ^{3}H-proline (2 μCi/ml, 20 Ci/mmol, Amersham) and ascorbic acid (50 μg/ml). Stroma were rinsed and further incubated in LTBMC medium for 24 h to assure complete elimination of free ^{3}H-proline. Under these labeling conditions, each stroma contained 37,000 cpm of collagen per 1×10^{-6} stromal cells, as judged by the collagenase-sensitive material assay.

Cell counts were performed by the use of an electronic counter (Coulter) and viability analysis determined by the trypan blue exclusion test.

RESULTS

As seen in Fig. 1, a suspension of intact WEHI cells incubated in culture medium containing unlabeled type I collagen was not able to degrade this substrate (lane 2). However, when cells were preincubated with trypsin and subsequently incubated with the substrate, collagen degradation was observed (lane 3). Trypsin-treated WEHI cells also degraded collagen type III (lane 6) but not collagen type IV (lane 8). The trypsin extract was not able to degrade either collagen type I (lane 4) or collagen types III and IV (data not shown).

These observations suggest that WEHI cells contain a membrane-associated collagenase. This was further confirmed by the use of an assay which utilizes labeled-collagen type I as substrate. As seen in Table 1, a chromatographically purified collagenase (Sigma Type VII) was able to degrade completely the labeled substrate. As compared to this standard, intact WEHI cells do not hydrolyze the radioactive substrate. However, trypsin-treated cells caused a 33% hydrolysis. A linear relationship between concentration of trypsin-treated WEHI cells and enzyme activity was observed. Based on the activity displayed by the standard collagenase, WEHI cells contain approximately five units of latent collagenase-like activity per 1×10^{-6} cells.

The trypsin-extract produced only a 7% hydrolysis of the substrate.

The hemopoietic stroma, which contains collagen as the main ECM component [9] is a highly organized structure that supports hemopoiesis *in vitro* [20]. Cocultures of bone marrow stroma and WEHI cells were used to investigate whether stroma–WEHI cells interactions may result in changes in the organization of the collagen-rich stroma attributable to the collagenase-like activity in WEHI cells.

TABLE 1. COLLAGENASE-LIKE ACTIVITY IN WEHI CELLS

	Hydrolysis	
	%	Units*
Collagenase type VII	100	32
Intact cells	0	0
Trypsin-treated cells	33	11
Trypsin extract	7	2

The rate of hydrolysis of labeled type I collagen by purified collagenase (32 U, type VII, Sigma) was set equal to 100%. Data are the average of four determinations; standard deviation was less than 5%.

* Units of collagenase per 2×10^6 cells.

When bone marrow stroma (Fig. 2a) was cocultured with normal progenitor cells, it looks uniform, well organized and contains several foci of hemopoietic cells (Fig. 2b).

When the stroma was cocultured with WEHI cells, approximately 60% of WEHI cells became strongly attached to the hemopoietic stroma. Later, the appearance of areas that have been cleared of stromal cells was observed. Subsequently, the holes were

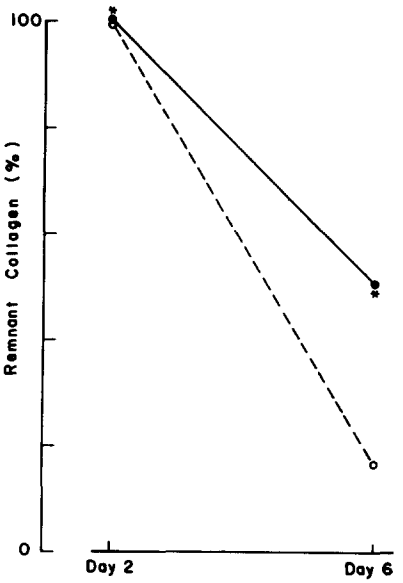

FIG. 3. Time-course of collagen degradation from labeled collagen-containing stroma, after cocultures with normal hemopoietic progenitors or WEHI cells. Culture dishes of labeled collagen-containing stroma were cocultured with culture medium (*—*), hemopoietic progenitors (●—●) and WEHI cells (○—○). At the indicated intervals, the amount of remnant labeled collagen in each stroma was measured. Radioactivity in control cultures at day 2 was set to 100% (15,000 cpm of collagen per dish). Each value is the mean of three separate experiments. S.D. was in all cases, less than 10%.

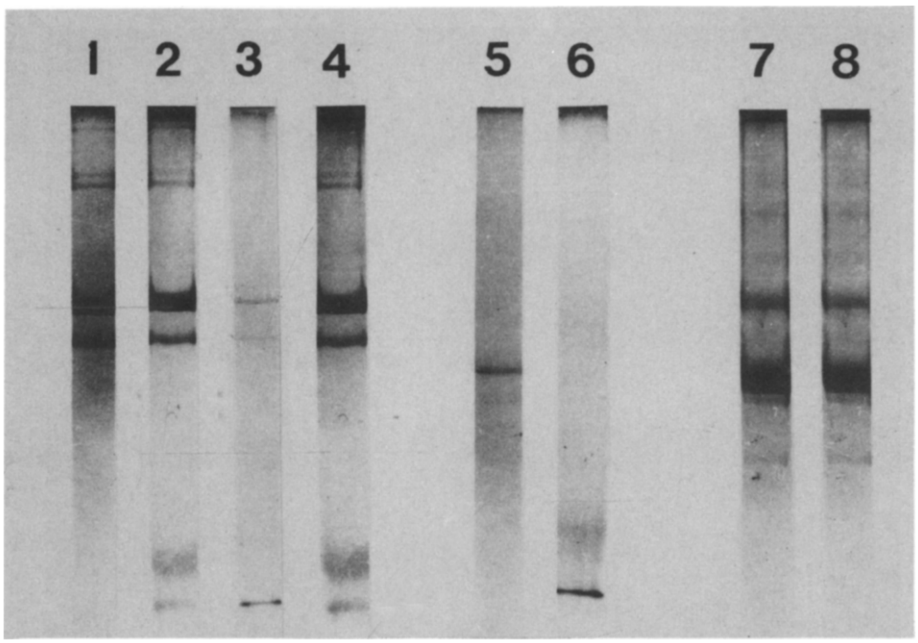

FIG. 1. SDS-PAGE patterns of collagen after incubation with intact, trypsin-treated WEHI cells and trypsin extract. Lanes 1, 5 and 7: standards of collagen types I, III and IV, respectively. Lanes 2 and 3: collagen type I after incubation with intact and trypsin-treated WEHI cells, respectively. Lane 4: collagen type I after incubation with trypsin extract. Lanes 6 and 8: collagen types III and IV after incubation with trypsin-treated WEHI cells, respectively.

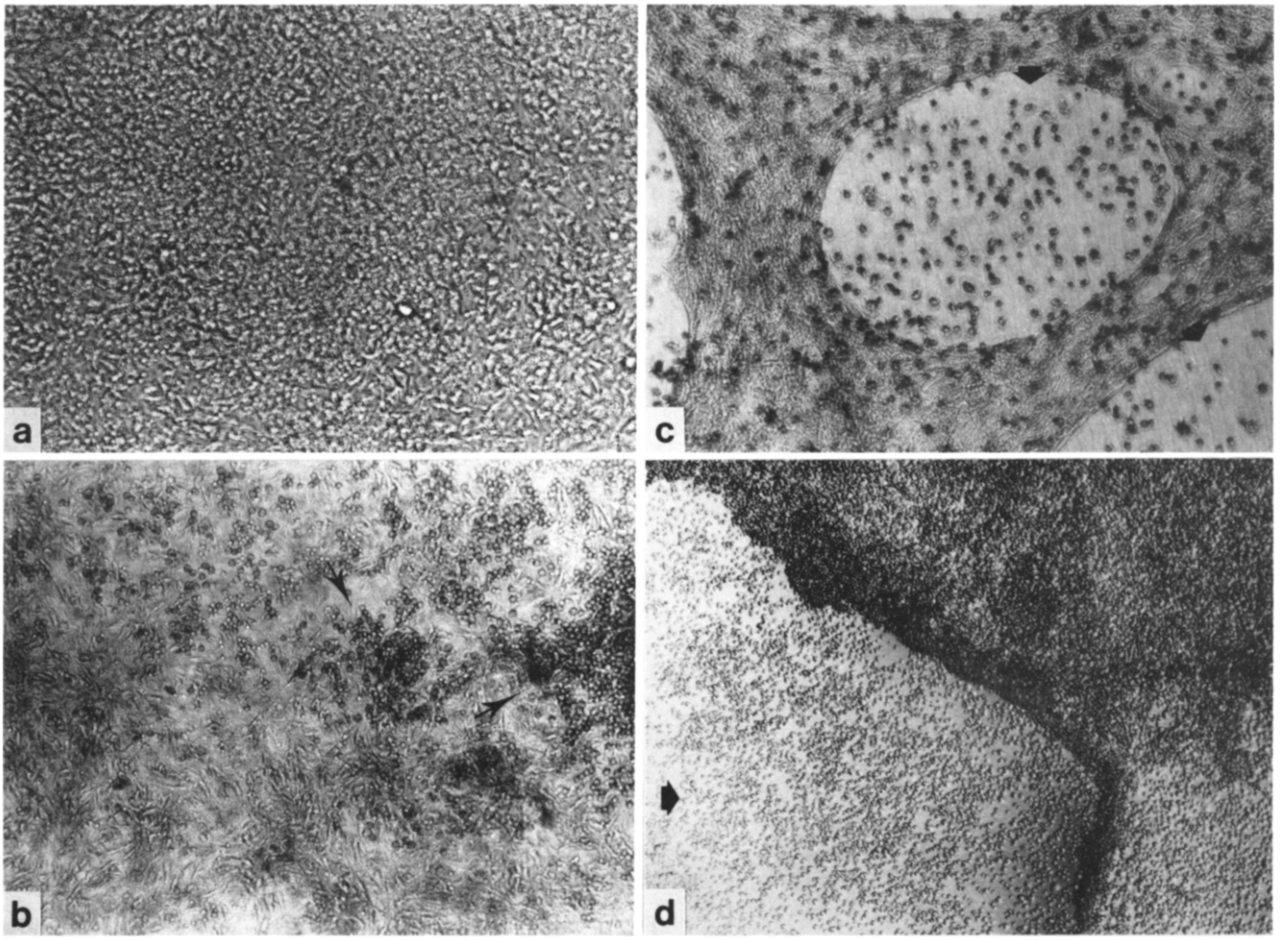

FIG. 2. Photomicrographs of cocultures of bone marrow stroma with normal hemopoietic progenitors or WEHI cells. Bone marrow stroma was cocultured for 3 days at 33°C, with: (a) culture medium; (b) normal progenitor cells; (c) and (d) intact WEHI cells. Magnification: 100× in (a), (b) and (c); 250× in (d). Cells in (c) have been stained with May–Grünwald–Giemsa. Long arrows in (b) indicate hemopoietic foci. Arrow heads in (c) and (d) indicate stroma disruption.

gradually enlarged with concomitant disorganization of the stroma, which led to the complete retraction of the remnant monolayer of cells (Fig. 2c and d). Note that at this stage of the coculture, hemopoietic foci are not present. Released stromal cells under this condition were all alive, as judged by cell viability higher than 90%.

To investigate if the collagenase-like activity in WEHI cells contributes to the observed disorganization of the stroma (collagen degradation?), coculture studies were done with labeled collagen-containing stroma.

As seen in Fig. 3, after 2 days of coculture with either hemopoietic progenitors or WEHI cells, no differences were observed in the amount of remnant labeled collagen associated to the stroma. However, after 6 days, the amount of stromal remnant labeled collagen in cocultures with WEHI cells was 32% of that measured in cocultures with normal progenitor cells.

DISCUSSION

Bone marrow stroma contains several cell phenotypes which produce and organize extracellular matrix components. Among these components, collagens type I and III play an important role in the structure of the matrix, mainly via its binding to fibronectin [21].

Collagen production by marrow stroma has been involved in the regulation of hemopoiesis, since inhibition of collagen synthesis correlates closely with a decreased hemopoiesis [12].

The content of collagen in the stroma of a normal functioning bone marrow is the result of the balance between the rates of synthesis by stromal cells and the degradation by specific collagenases produced by normal hemopoietic cells [22, 23]. In stromal cells derived from leukemic marrow, we have observed a decrease in collagen synthesis as compared to normal marrow [24]. However, there is no information concerning the production of collagenase activities by leukemic cells. It seems important to study the latter in order to understand the relationships between leukemic cells and the marrow stroma, via interactions with ECM components [8]. It has been reported that cancer cells can modulate the interactions with ECM or basement membrane components in such a way that a proteolytic cascade is established, causing tissue degradation and tumor cell invasion [25].

The results reported here show that the leukemic cell line WEHI-3B, contains a collagenase-like activity. This activity is present as a latent enzyme, which is activated by a proteolytic process. The enzyme is highly specific for interstitial collagens (types I and III), but not for basement membrane collagen (type IV).

The disruption of a bone marrow stroma that occurs after the attachment of WEHI cells seems to be the consequence of changes in the organization of the ECM, rather than a toxic process at the level of the stromal cells. Thus, activation of leukemic latent-collagenase *in situ* may result in collagen degradation with the concomitant disorganization of the marrow stroma. This conclusion is supported by the observation that after coculture of hemopoietic precursors or WEHI cells with a labeled collagen-containing stroma, collagen is degraded more rapidly in the presence of the leukemic than the progenitor cells.

The observation that cocultures of stroma with normal hemopoietic progenitors do not result in any gross damage to the stroma, suggests that in normal hemopoiesis, matrix degradation (proteolysis?) occurs as a regulated process. Normal progenitor–stroma interactions probably play a role in this regulation through the release of endogenous protease inhibitors [23]. The attachment of the leukemic cell to the marrow stroma seems to preclude such regulation occurring. The latter may result in an impaired regulation of matrix degradation with a loss of the capacity of the stroma to support normal hemopoiesis.

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REFERENCES

1. Wolf N. S. (1979) The haemopoietic microenvironment. *Clin. Hemat.* **8,** 469.
2. Dexter, T. M., Spooncer E., Toksoz D. & Lajtha L. G. (1980) The role of cells and their products in the regulation of in vitro stem cell proliferation and granulocytes development. *J. Supramol. Struct.* **13,** 513.
3. Sachs L. (1985) Regulators of growth, differentiation and the reversion of malignancy: normal hematopoietic and leukemia. In *Molecular Biology of Tumor Cells* (Wahren B. *et al.*, Eds), p. 257. Raven Press, New York.
4. Nicolson G. L. (1988) Cancer metastasis: tumor cells and host organ properties important in metastasis to specific secondary sites. *Biochim. biophys. Acta* **948,** 175.
5. Wirl G. & Frick J. (1979) Collagenase: a marker enzyme in human bladder cancer. *Urol. Res.* **7,** 103.
6. Liotta L. A. & Rao C. N. (1985) Role of the extracellular matrix in cancer. *Ann. N.Y. Acad. Sci.* **460,** 333.
7. Bejarano P. A., Noelken M. E., Suzuki K., Hudson B. G. & Nogase H. (1988) Degradation of basement membranes by human matrix metalloproteinase 3(stromelisin). *Biochem J.* **256,** 413.

8. Gordon M. Y., Dowding C. R., Riley G. P., Goldman J. M. & Greaves M. F. (1987) Altered adhesive interactions with marrow stroma of haemopoietic progenitor cells in chronic myeloid leukemia. *Nature* **328,** 342.
9. Bentley S. A. (1982) Collagen synthesis by bone marrow stromal cells: a quantitative study. *Br. J. Haemat.* **50,** 491.
10. Fernández M. & Minguell J. J. (1987) Collagen synthesis by human bone marrow fibroblasts. *Experientia* **43,** 1223.
11. Kirby S. L. & Bentley S. A. (1987) Proteoglycans synthesis in two murine bone marrow stromal cell lines. *Blood* **70,** 1777.
12. Zuckerman K. D., Rhodes R. K., Goodrum D. D., Patel V. R., Sparks B., Wells J., Wicha M. S. & Mayo L. A. (1985) Inhibition of collagen deposition in the extracellular matrix prevents the establishment of stromal supportive of hematopoiesis in long-term murine bone marrow cultures. *J. clin. Invest.* **75,** 970.
13. Spooncer E., Gallagher J. T., Krizsa F. & Dexter T. M. (1983) Regulation of haemopoiesis in long-term bone marrow cultures. IV Glycosaminoglycans synthesis and the stimulation of haemopoiesis by β-D-xylosides. *J. Cell Biol.* **96,** 510.
14. Metcalf D. (1979) Clonal analysis of the action of GM-CSF and differentiation of myelomonocytic leukemic cells. *Int. J. Cancer* **24,** 616.
15. Bauer E. A., Stricklin G. P., Jeffrey J. J. & Eisen A. Z. (1975) Collagenase production by human skin fibroblast. *Biochem. biophys. Res. Comm.* **64,** 232.
16. Laemmli U. K. (1970) Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. *Nature* **227,** 680.
17. Peterkofsky B. & Diegelman R. (1971) Use of a mixture of proteinase-free collagenase for the specific assay of radioactive collagen in the presence of other proteins. *Biochemistry* **10,** 988.
18. Niyibizi C., Fietzek P. P. & van der Rest M. (1984) Human placenta type V collagens. *J. biol. Chem.* **259,** 14170.
19. Dexter T. M., Allen T. D. & Lajtha L. G. (1977) Conditions controlling the proliferation of haemopoietic stem cells *in vitro*. *J. cell. Physiol.* **91,** 335.
20. Campbell A., Wicha M. S. & Long M. (1985) Extracellular matrix promotes the growth and differentiation of murine hematopoietic cells *in vitro*. *J. clin. Invest.* **75,** 2085.
21. Hynes R. O. (1985) Molecular biology of fibronectin. *Ann. Rev. Cell Biol.* **1,** 67.
22. Horowitz A. L., Hance A. J. & Crystal R. G. (1977) Granulocyte collagenase: selective digestion of type I relative to type III collagen. *Proc. natn. Acad. Sci. U.S.A.* **74,** 897.
23. Welgus H. G., Campbell E. J., Bar-Shavit Z., Senior R. M. & Teitelbaum S. L. (1985) Human alveolar macrophages produces a fibroblast-like collagenase and collagenase inhibitor. *J. clin. Invest.* **76,** 219.
24. Fernández M., Barahona C., Martínez J. & Minguell J. J. (1990) Changes in collagen synthesis by human bone marrow fibroblasts with progressive subcultivation. In press.
25. Pourreau-Schneider N., Delori P., Boutiere B., Arnoux D., George F., Sampol J. & Martin P. M. (1989) Modulation of plasminogen activator systems by matrix components in two breast cancer cell lines: MCF-7 and MDA-MB-231. *J. natn. Cancer Inst.* **81,** 259.