Macrophages stimulate the activation of plasminogen by fibroblasts

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1. INTRODUCTION

Cellular interactions between different cell types are likely to play a critical role in the local remodeling of connective tissue under many inflammatory situations. Macrophages and fibroblasts are among the main effector cells in these processes and they have been shown to be able to cooperate both in tissue growth and fibrogenesis [1,2] and in the degradation of connective tissue matrices [3]. We have shown previously that macrophages release a factor that stimulates the production of collagenase and of a proteoglycan-degrading neutral protease by rabbit skin fibroblasts or by adherent synovial cells and that this production can itself be modulated by lymphocyte products [4,5]. We now report here that macrophages may also stimulate skin fibroblasts to activate plasminogen.

2.MATERIALS AND METHODS

Fibroblasts from rabbit skin explants were isolated and cultured as previously described [6]. Peritoneal macrophages were obtained from male rabbits (3–4 kg, of the Termonde White strain) that had been injected intraperitoneally with 30 ml of sterile light mineral oil (Merck, Darmstadt, FRG) 4 days earlier. The peritoneum was washed with our 'basal' culture medium [7], supplemented with 5 mM-EDTA and 5% heat-inactivated (30 min at 56°C) FCS. The cells were collected by centrifugation, washed with phosphate-buffered saline and resuspended in basal medium supplemented with 10 mM-HEPES and 5% acid-treated [8] FCS. They were then plated either on round glass coverslips (14 mm-diameter) laid within the wells (16 mm-diameter) of Costar Multiwell culture plates (Cambridge, MA, USA), or in Falcon plastics (Los Angeles, CA, USA) culture Petri dishes. After incubation for 15 h in a water-saturated atmosphere of air/CO_2 (9:1), non-adherent cells were removed and the remaining cells were extensively washed with serum-free HEPES-supplemented basal medium. The absence of fibroblasts among the macrophages was verified by culturing the adherent cells for several days in basal medium supplemented with 5% FCS: under these conditions, fibroblasts proliferate actively and can then easily be identified in the plates at the end of the culture.

Macrophage-conditioned media were obtained by culturing [4,7] adherent macrophages for 6 days at 37°C in plastic Petri dishes in basal medium supplemented with 10 mM-HEPES and 5% acid-treated FCS at a density of 5×10^5 cells/ml (about 8×10^4 cells/cm^2). The conditioned medium was then centrifuged and stored at −20°C until use. Fibroblast-conditioned media were obtained by culturing fibroblasts at confluence under similar conditions for 6 days in plastic Petri dishes (9 cm-diameter) in 10 ml of culture medium.

Fibrin degradation was evaluated as done previously [9] by culturing the fibroblasts, the macrophage-coated coverslips and/or the cell-free con-

Abbreviations: FCS, fetal calf serum; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid

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ditioned media in the wells of [^{3}H]fibrin-coated Multiwell plates in 2 ml of HEPES-supplemented basal medium. To evaluate fibrinolysis was plasminogen-dependent or not, the cultures were done in medium supplemented with 1 or 2% acid-treated FCS depleted [10] or not of plasminogen. The efficiency of the plasminogen-depletion was verified by incubating fibrin plates under the conditions of the cell cultures with plasminogen-depleted serum and 10 U of urokinase (Sigma Chemical Co., St Louis, MO, USA) per ml: only insignificant amounts of ^{3}H were then released over several days of culture. Blank values, corresponding to the amount of ^{3}H-radioactivity spontaneously released from the plates in the absence of cells or enzyme (about 5% of the total radioactivity, after several days of culture), were subtracted from the percentage release achieved in their presence and the results were normalized by expressing that difference as a percentage of the difference obtained between the 100%-degradation value (as evaluated by incubating the plates with 50 μg of trypsin in 2 ml of basal medium/well) and the blank.

Unless mentioned above, the chemicals and the culture media were from our previous suppliers [4,9,11].

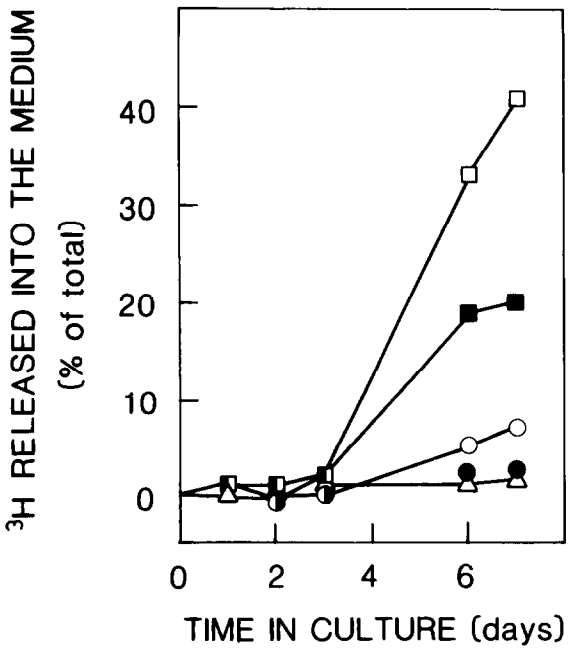

Fig.1. Plasminogen-dependent degradation of fibrin by macrophages and fibroblasts in co-cultures. The [^{3}H]fibrin-coated wells contained 20 000 (•) or 40 000 (○) fibroblasts, or 200 000 peritoneal macrophages (△) or 200 000 peritoneal macrophages together with either 20 000 (▪) or 40 000 (□) fibroblasts. The plasminogen-dependent release of ^{3}H-labelled soluble products is presented after deduction of the plasminogen-independent fibrinolysis (that was here insignificant). Each point is the mean of 2 cultures.

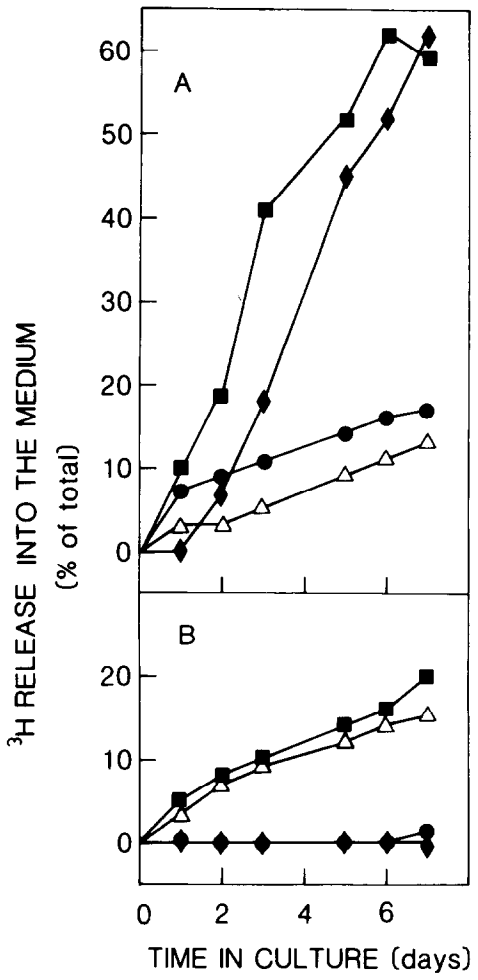

Fig.2. Stimulation by macrophage-conditioned culture medium of the plasminogen-dependent fibrinolysis achieved by fibroblasts. Plasminogen-dependent (A), presented as in fig.1, and plasminogen-independent (B) degradation of fibrin is shown under the action of fibroblasts (20 000/well: •), of macrophage-conditioned medium (20%, vol/vol: △). or both (▪). Also shown if the action of urokinase, 20 U/well (♦). Each point is the mean of 2 cultures.

3. RESULTS

Macrophages of fibroblasts, cultivated alone on the fibrinplates, caused only a limited amount of plasminogen-dependent fibrin degradation (fig.1). However the activation of plasminogen was strongly enhanced when both types of cells were simultaneously cultured on the plates (fig.1). The plasminogen-dependent fibrinolytic activity of macrophages was not modified by the addition of fibroblast-conditioned medium to their cultures (not shown). On the contrary, the addition of macrophage-conditioned medium to cultures of fibroblasts enhanced their rate of plasminogen-dependent fibrinolysis (fig.2). This effect was also apparent when the macrophage medium had been first extensively dialysed (fig.3). It was reduced by heating the macrophage medium for 20 min at 80°C and completely abolished by heating it for 10 min at 100°C (fig.3). The factor responsible for the stimulation of fibroblasts was precipitated from macrophage media by $(NH_4)_2SO_4$ between 30% and 80% saturation (fig.3).

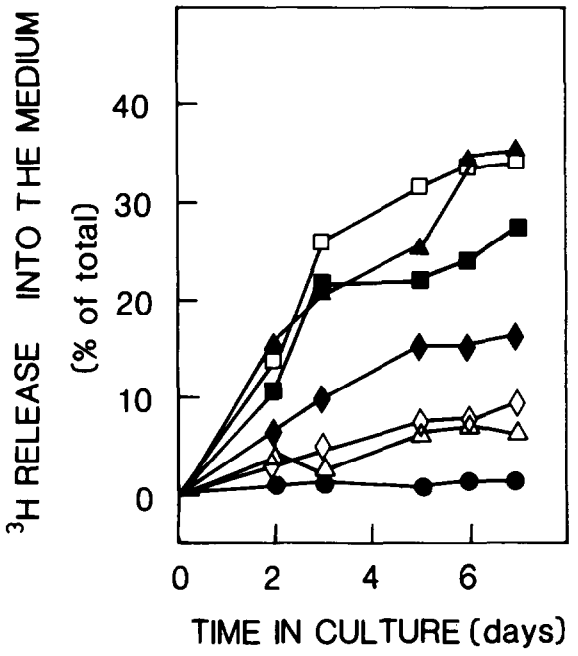

Fig.3. Properties of the macrophage-factor that stimulates fibroblast production of plasminogen activator. The plasminogen-dependent fibrinolysis (presented as in fig.1) is shown under the action of 20 000 fibroblasts/well (•), 25% vol/vol of macrophage-conditioned medium (△), or both, either as such (▪), or after dialysis of the macrophage medium through 3500 M_r cut-off membranes (□; dialysed against 4 × 200 vol of water over 36 h at 3–4°C, then reconstituted to isotonicity with concentrated Hank's balanced salt solution) or after its heating for 20 min at 80°C (◆) or for 10 min at 100°C (◇). In (▲), the macrophage medium has been replaced by its $(NH_4)_2SO_4$ precipitate (between 30 and 80% saturation) redissolved to its initial volume in basal culture medium. Each point is the mean of 2 cultures.

4. DISCUSSION

These results indicate that the activation of plasminogen by rabbit skin fibroblasts, low under basal conditions, can be strongly stimulated under the action of a thermolabile, non-dialysable, ammonium sulfate-precipitable, soluble factor released by rabbit peritoneal macrophages. Rabbit alveolar or bone marrow-derived macrophages apparently release a similar factor, but its demonstration is rendered more difficult by the high levels of fibrinolysis, largely plasminogen-independent, achieved by these cells or by their conditioned media (data not shown).

Among many other functions, plasmin can activate latent collagenase and neutral metalloproteases [12–14]. Thus rabbit macrophage factor(s) or monokine(s) appear to be able to stimulate the release or the activity of several neutral proteases active in collagen and proteoglycan degradation by rabbit fibroblasts [4,5], chondrocytes [15] or synovial cells [5]. Similarly, a human mononuclear cell factor has been shown to stimulate collagenase [16] or plasminogen activator [17] production by human synovial cells and a human synovial cell factor (possibly released by the macrophage-like, type-A synovial lining cells, as discussed in [3]) stimulates the activation of plasminogen by human chondrocytes [18]. It becomes thus more and more evident that macrophages and related mononuclear cells might play part of their role in tissue degradation by orienting through the secretion of monokines the metabolism of nearby fibroblasts towards the degradation of several extracellular macromolecules, including collagen, proteoglycan and fibrin.

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