Co-operation between metastatic tumor cells and macrophages in the degradation of basement membrane (type IV) collagen

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The co-culture of mouse peritoneal macrophages and Lewis lung carcinoma cells induces the release of a metal-dependent type IV collagen-degrading proteinase which is not produced in detectable amounts by either cell type cultivated alone. Conditioned media of the co-cultures degrade both pepsin-extracted type IV collagen from human placenta and mouse type IV procollagen. Thus macrophages can interact with tumor cells to degrade basement membrane type IV collagen: this might be of importance to allow cancer invasion and metastasis.

Basement membrane degradation *Type IV collagenase* *Cancer cell* *Macrophage* *Cell interaction* *Metastasis*

1. INTRODUCTION

Invasive and metastasizing cancer cells have the ability of migrating through interstitial connective tissue matrices and through basement membranes. The mechanism of this migration is still unknown, but it is believed to involve generally, as a necessary step, a degradation of basement membrane type IV collagen [1]. Although some metastatic tumor cells are able to produce a type IV collagen-degrading enzyme [2], nothing is known about factors which regulate that production. Host–tumor cells interactions might participate in that regulation. Macrophages often infiltrate tumors [3] and they can influence their metastatic capacity [4]. We report here that an interaction occurs between macrophages and Lewis lung carcinoma (LLC) cells in co-culture, inducing the release of a metal-dependent type IV collagen-degrading enzyme activity which is not produced in detectable amounts by either cell type cultured alone.

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2. MATERIALS AND METHODS

Two clonal subpopulations of Lewis lung carcinoma (LLC) cells [5] were used, E34 and H61, having respectively low and high metastatic potential. The cells were grown as in [6], with slight modifications: the basal medium was supplemented with 10 mM Hepes and 10% (v/v) NCTC 135 medium (Gibco-Biocult, Glasgow). The cells were harvested from subconfluent cultures after dispersion with EDTA (2 mM) in phosphate-buffered saline (8 mM Na_2HPO_4: 1 mM KH_2PO_4: 140 mM NaCl, pH 7.4); they were then sedimented and resuspended in basal medium supplemented with 5% acid-treated [7] foetal calf serum (FCS) and washed twice in that medium by repeated sedimentation (about 1 300 × *g* min) and resuspension.

Resident peritoneal macrophages were obtained from male Swiss OF_1 mice (from Animalabo, Brussels) as in [8]. Prior to harvesting the cells, the cultures were washed twice with phosphate-buffered saline containing 2 mM EDTA. Adherent cells were then collected by gentle scraping with a rubber policeman, washed twice and resuspended

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in basal medium supplemented with 5% acid-treated FCS; 90–95% of the collected cells appeared viable, as established by trypan blue exclusion.

To prepare conditioned media from tumor cells and/or macrophages, 0.1 ml of a suspension containing 5×10^4 LLC cells and/or 5×10^4 macrophages was added to 2 ml serum-free basal medium in Multiwell culture plates. The plates were kept at 37°C in a humidified atmosphere of air and CO_2 (9:1). After 10 days of culture, the conditioned media were collected, centrifuged, dialysed extensively against a 1% (v/v) dilution of TNCN (0.05 M Tris–HCl, 0.15 M NaCl, 5 mM $CaCl_2$, 1 mg NaN_3/ml, pH 7.4) in water, lyophilized and kept dry at −20°C. For the enzyme assays, the lyophilized media were reconstituted in TNCN so as to obtain preparations that were 1–200-fold concentrated in comparison with the initial concentration of the conditioned culture media. Trypsin activation of latent proteases was done as in [9].

Type IV collagen was extracted from human placenta by pepsin treatment and purified by repeated salt fractionation essentially as in [10]. Type IV collagen-degrading activity was estimated

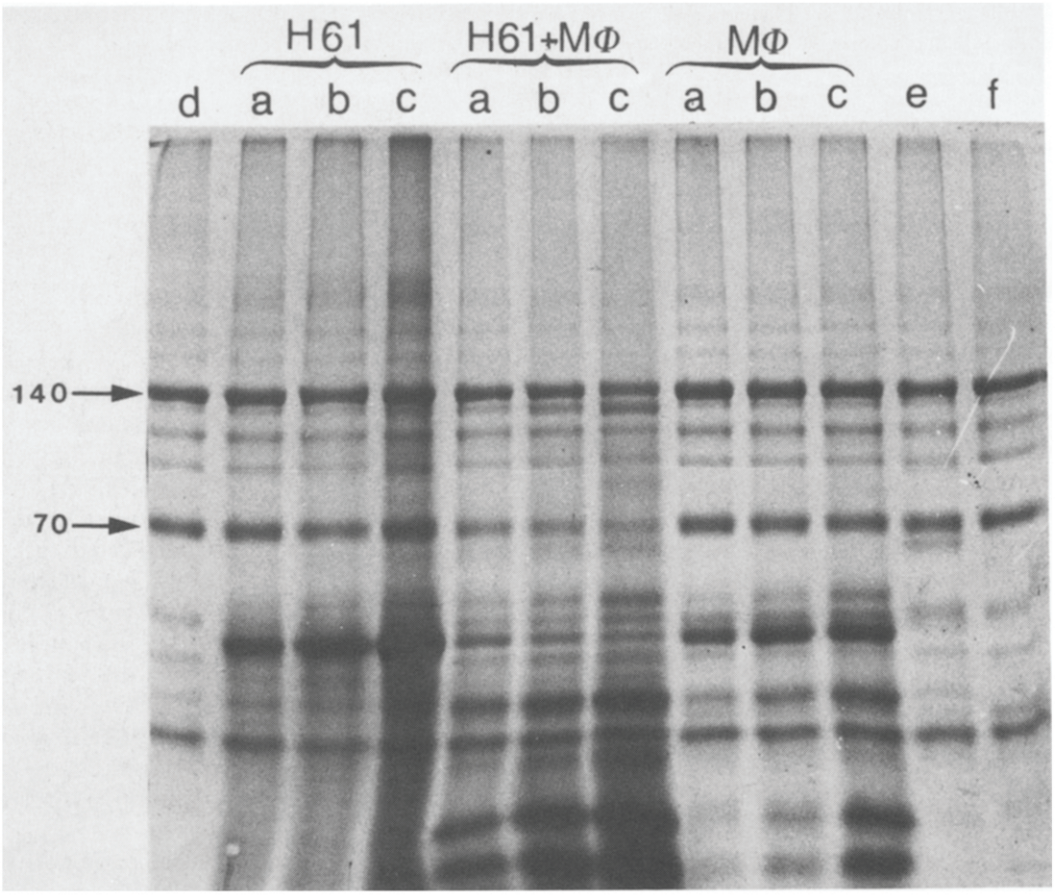

Fig.1. SDS–polyacrylamide gel electrophoresis of type IV collagen after its incubation for 24 h at 27°C (pH 7.4) with media conditioned by the culture of LLC cells (H61), macrophages (MΦ), or both cell types together (H61 + MΦ). Each medium was used at a 1-fold (a), 25-fold (b), or 100-fold concentration (c). Type IV collagen was also incubated with TNCN buffer (d), trypsin (e) or α-chymotrypsin (f) (both proteinases at an enzyme: substrate ratio of 1:50 (w/w). Arrows 140 and 70 indicate the position of the two principal chain fragments of type IV collagen, of M_r 140 000 and 70 000, respectively.

at pH 7.4 by incubating 10 μl substrate (2 mg/ml in TNCN) with 25 μl reconstituted culture media or control solutions at 27°C for the time indicated. The reaction was stopped by addition of 20 μl 0.2 M Tris–HCl, 6 mM EDTA, 1.5 M urea, 6% (w/v) sodium dodecylsulphate (SDS), 45% (v/v) glycerol and 50 mM dithiothreitol (pH 6.8). The samples were heated 2 min at 100°C and analyzed on 5.5–11% SDS–polyacrylamide gel gradient electrophoresis [11].

^{14}C-Labelled type IV procollagen from mouse EHS sarcoma [12] was generously given by Dr L. Liotta (National Cancer Institute, Bethesda, MD). α-Chymotrypsin (90 U/mg) was from Boehringer (Mannheim). Other materials were as in [5,6,9].

3. RESULTS AND DISCUSSION

Type IV collagen-degrading activity could not be detected in culture media wherein either E34 LLC cells, or H61 LLC cells, or resident peritoneal macrophages had been cultivated for 10 days, even after a 100-fold concentration of the media and/or activation by trypsin. However, concentrated, conditioned media of co-cultures of both LLC cells (either E34 or H61) and macrophages degraded significantly the two main components (M_r 140 000 and 70 000) of the type IV collagen substrate (fig.1,2). The degradation was dependent both on the concentration of conditioned medium (fig. 1) and on the incubation time (fig.2). It occurred at

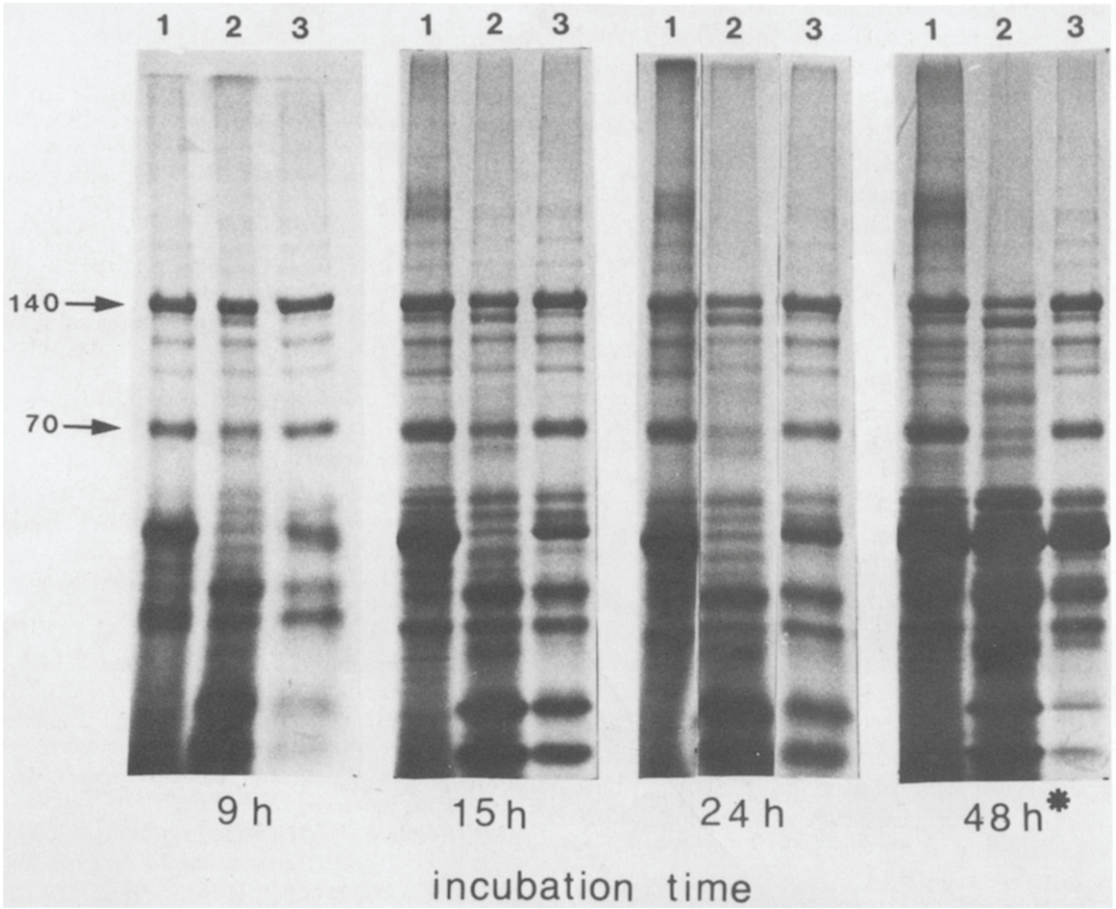

Fig.2. SDS–polyacrylamide gel electrophoresis of type IV collagen after its incubation for various periods of time at 27°C (pH 7.4) with 100-fold concentrated media (same as in fig.1) conditioned by the culture of H61 LLC cells (lane 1), macrophages (lane 3) or both cell types together (lane 2). In (*), an additional aliquot (25 μl) of conditioned medium was added after the first 24 h incubation.

a limited number of cleaving sites at <30°C. Under the same conditions and at an enzyme:substrate ratio of 1:50 (w/w), chymotrypsin exerted no detectable effect and trypsin degraded only about 10% of the M_r 70000 fragment during a 24 h incubation (fig.1). This type IV collagen-degrading activity was not activated by trypsin and was not dialyzable. It was completely abolished by heat-treatment (10 min at 100°C) and inhibited by EDTA (20 mM) but not by inhibitors of serine- or cysteine-proteinases (either 5 mM-phenylmethanesulphonyl fluoride, or 5 μg leupeptin/ml, or 0.5 mM 4-hydroxymercuribenzoate). It was thus presumably due to a metal-dependent proteinase. Conditioned media of co-cultures, but not of cultures in which LLC cells or macrophages had been cultivated separately, did also degrade type IV ^{14}C-labelled procollagen (not shown) and type I collagen [13]. Type I collagen was degraded into typical 1/4 and 3/4 fragments at a rate at least 100-times greater (as judged on polyacrylamide-gel electrophoresis) than the rate of degradation of type IV collagen by the same conditioned media.

Mixtures of equal volumes of 200-fold concentrated LLC cells- and macrophage-conditioned media did not degrade type IV collagen nor type IV procollagen (not shown). This indicates that these degradations are not due to the activation of a latent proteinase, present in one medium, by an activator, present in the other. It seems thus that the co-culture of both cell types induced the release of a metalloproteinase acting upon type IV collagen. No difference in this respect was observed between the two LLC cell lines used, although their metastatic behaviours differed widely [5].

Preliminary experiments indicate that macrophage-conditioned media can be substituted for the macrophages to induce LLC cells to secrete type IV collagenase. However, we have not yet excluded the possibility of a reciprocal interaction between the two cell types, in which the LLC cells could also induce the production of such an enzyme by the macrophages. Nevertheless, whatever its mechanism might be, the interaction observed here in vitro between macrophages and tumor cells in the degradation of type IV collagen, if it occurs also in vivo, might play a critical role in the destruction of basement membranes by cancer cells, thus opening the way for tumor invasion and metastasis [1].

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