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Macrophage–Fibroblast Interactions in Collagenase Production and Cartilage Degradation

By Ghislaine HUYBRECHTS-GODIN, Pierre HAUSER and Gilbert VAES
Laboratoire de Chimie Physiologique, Université de Louvain and International Institute of Cellular and Molecular Pathology, Avenue Hippocrate, 75, B-1200 Bruxelles, Belgium

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Rabbit bone-marrow macrophages and fibroblasts were cultured, independently or together, with pieces of ^{35}S-labelled cartilage or at the surface of dried [^{14}C]collagen gels. Each type of cell, cultivated alone, rapidly degraded the proteoglycan of cartilage, but only the fibroblasts degraded collagen. The co-culture of both types of cell had no consistent effect on the rate of proteoglycan degradation, but it stimulated the rate of collagen degradation. In parallel, the accumulation of collagenase in the culture fluid was enhanced but not that of neutral proteinase. Conditioned media from macrophage cultures added to cultures of fibroblasts had the same effect as the living macrophages in stimulating the production of collagenase. Their action was itself enhanced when the macrophages had been activated by concanavalin A-stimulated spleen-cell factors. These data suggest that fibroblasts may act as effector cells in producing collagenase and degrading collagen in response to soluble factors released by macrophages under the control of lymphocyte factors.

Connective-tissue destruction, involving the removal of its main structural macromolecules collagen and proteoglycan, is often observed in chronic inflammatory processes. It is particularly evident in rheumatoid arthritis, where the articular cartilage is lysed under the advancing edge of the inflammatory synovial pannus (Krane, 1974). The association of macrophages and fibroblasts, together with T- and B-lymphocytes, within this chronic inflammatory tissue (Kobayashi & Ziff, 1975; Ishikawa & Ziff, 1976) suggests that either of these cells may somehow participate in the pathology of the cartilage erosion.

In previous studies, we have shown that rabbit macrophages or fibroblasts, when cultivated together with pieces of cartilage in a serum-free medium, are both independently capable of degrading the proteoglycan of cartilage, by secreting a metal-dependent neutral proteinase active on the peptide core of the proteoglycan subunits (Vaes *et al.*, 1977; Hauser & Vaes, 1978*a*; Huybrechts-Godin & Vaes, 1978). Moreover, soluble factors released by rabbit spleen cells upon stimulation with either mitogen or antigen markedly enhance the secretion of the proteinase by the macrophages (Hauser & Vaes, 1979). In the present paper, we report that macrophages interact with fibroblasts so as to stimulate their capacity to produce collagenase and to degrade the collagen of cartilage in cultures. Activation of the macrophages by mitogen-stimulated lymphocyte factors enhances this interaction.

Experimental

Animals and materials

The culture media, serum (heat-inactivated by a 30 min incubation at 56°C), the other special chemicals and the animals (male rabbits of the Termonde White Strain, aged 1–2 months) were from the same suppliers as mentioned in our previous studies (Hauser & Vaes, 1978*a*,*b*, 1979; Huybrechts-Godin & Vaes, 1978). Microtest II culture plates were from Falcon Plastics (Los Angeles, CA, U.S.A.), and Multiwell plates from Costar (Cambridge, MA, U.S.A.).

Cultures of macrophages and fibroblasts

Rabbit bone-marrow macrophages were isolated and cultured as described elsewhere (Hauser & Vaes, 1978*a*,*b*). The nucleated non-adherent cells, collected after the first 7 days of culture of the marrow cells, were washed and resuspended in our (Hauser & Vaes, 1978*a*) basal serum-free culture medium. When examined by optical microscopy, 80–100% of this cell population appeared to belong to the mononuclear phagocyte line, consisting of approx. 10% monoblasts, 25% promonocytes and 65% monocytes–macrophages. The contaminating cells were essentially degenerating granulocytes. Although lymphocytes were not identified at this stage, their occasional presence can, however, not be totally excluded. The absence of fibroblasts from this

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population was controlled by culturing 10^6 cells for 20h in 6cm-diameter Petri dishes containing 4ml of basal medium supplemented with 5–10% serum. Fibroblasts adhere under these conditions and can then be distinguished under phase-contrast microscopy from the cells of the monocyte–macrophage line.

Rabbit fibroblasts, growing out from skin explants, were collected after 3 weeks of culture, washed and resuspended in serum-free basal culture medium as previously described (Huybrechts-Godin & Vaes, 1978). Unless otherwise indicated, these experiments were done with fibroblasts collected after their first passage.

Co-cultures of macrophages and fibroblasts with ^{35}S-labelled cartilage pieces

Fibroblasts were distributed in the wells of Microtest plates and cultured for 20h in basal medium supplemented with 10% (v/v) foetal calf serum (40000 cells in 0.2ml of medium/well). The medium was then removed and the adherent fibroblasts were washed with phosphate-buffered saline (Hauser & Vaes, 1978*b*). The macrophage suspension (60000 cells in 0.2ml of basal medium) was added to each well, followed by a piece of rabbit ear, or articular cartilage, biosynthetically labelled with ^{35}S in its proteoglycan. The labelled cartilage was prepared and (unless otherwise indicated) heat-treated (30min at 60°C) as described by Hauser & Vaes (1978*b*). After 1–8 days of culture, the degradation of cartilage proteoglycan and collagen was evaluated by the amounts of ^{35}S-labelled fragments and hydroxyproline released from the cartilage pieces. It was expressed as a percentage of the total amount of ^{35}S or hydroxyproline initially present in the cartilage (Hauser & Vaes, 1978*b*). Degradation of native collagen was considered to occur only when the amount of hydroxyproline lost from the heat-treated cartilage during the culture was significantly larger than the maximum amount solubilized by trypsin (25μg/ml; 0.2ml/well) under conditions similar to those of the cell cultures.

Co-cultures of macrophages and fibroblasts on [^{14}C]-collagen-coated plates

Radioactively labelled salt-soluble collagen (20000–60000d.p.m./mg) was purified as described previously (Vaes, 1972) from the skin of guinea pigs that had received [^{14}C]glycine intraperitoneally. It was freeze-dried and stored at −20°C until use. A [^{14}C]collagen solution (2mg/ml) was prepared under gentle stirring at 4°C in sterile 0.01M-sodium phosphate buffer, pH7.4, containing 0.42M-NaCl and 0.01% (w/v) NaN_3. Just before coating the plates, 1 vol. of the solution was mixed at 0°C with 2 vol. of sterile 0.01M-sodium phosphate buffer, pH7.4, containing 0.01% NaN_3. Portions (0.2ml) of the mixture were pipetted into the wells (16mm in diameter) of Multiwell plates and spread over their surface. The plates were then incubated at 37°C for at least 48h to allow the collagen gels to reconstitute and dry. Before the addition of the cells, the plates were preincubated for 24–48h with basal medium (1ml/well) containing 10 times its normal concentration of antibiotics (10^6 i.u. of penicillin/litre and 1g of streptomycin/litre). This medium was then removed and the wells were washed with basal medium.

Macrophages were distributed in the wells [2×10^5 cells/well, in 1.9ml, or occasionally in 0.9ml, of basal medium, either containing or not 26.5% (v/v) concanavalin A-stimulated spleen-cell-conditioned medium] and cultured alone for 20h. Fibroblasts were then added (10^5 cells/well, in 0.1ml of basal medium). The degradation of collagen was followed over 1–8 days of culture by assaying in 50μl samples of culture medium the amounts of soluble ^{14}C-labelled material released from the gels at various time intervals. The results were expressed as a percentage of the total [^{14}C] collagen initially present in the wells; this total amount (100%) was determined for each plate by measuring the radioactivity released by a solution of crude bacterial collagenase (4mg in 2ml). Degradation of native collagen was considered to occur only when the amount of ^{14}C-labelled material solubilized from the plates was significantly larger than the maximum amount solubilized by trypsin (50μg in 2ml).

Preparation of conditioned media

Conditioned media from rabbit skin fibroblast cultures were prepared as described by Huybrechts-Godin & Vaes (1978), but in serum-free basal medium. They were used either immediately or after their storage at −20°C. To prepare macrophage-conditioned media, the non-adherent cells, collected after the first 7 days of culture of the rabbit bone-marrow cells (Hauser & Vaes, 1978*a*), were first cultured for 20h in 10cm-diameter Petri dishes (5×10^6 cells/dish, in 10ml of basal medium) to allow their adherence to the dish. This adherence, which develops progressively during the differentiation of the mononuclear phagocytes, was found indeed (Hauser & Vaes, 1978*a*) to be considerably accelerated by changing the initial culture medium, which contained 50% (v/v) of serum, for a medium with lower (0–15%) serum content. The supernatant medium was then removed and replaced by fresh basal culture medium, either containing or not 25% (v/v) of lymphocyte-conditioned medium. The adherent cells were further cultured for 4 days after which their conditioned culture media were collected,

centrifuged (about 1300*g*-min at 4°C) and used immediately for the experiments.

Concanavalin A-stimulated lymphocyte media were prepared from spleen-cell cutures in serum-free basal medium as described by Hauser & Vaes (1979) immediately before their use.

Enzyme assays

Assays and units of activity of neutral proteinase, with [^{3}H]acetylated casein as substrate, and of neutral collagenase were as previously described; the latent forms of both enzymes were first completely activated by trypsin (Vaes, 1972; Vaes *et al.*, 1978).

Results

Degradation of cartilage by macrophages and fibroblasts in co-cultures

Rabbit macrophages or fibroblasts were cultivated in a serum-free medium together with heat-treated or native ^{35}S-labelled rabbit ear or articular cartilage (Fig. 1). In confirmation of our previous reports (Vaes *et al.*, 1977; Hauser & Vaes, 1978*a*), both types of cell, cultivated alone, degraded the ^{35}S-labelled proteoglycan, but not the collagen, of the cartilage almost completely within a few days. The addition of macrophages to cultures of adherent fibroblasts had no consistent effect on the rate of degradation of proteoglycan. However, in these co-cultures, the release of the ^{35}S-labelled soluble material into the culture fluid was rapidly followed by the solubilization of almost all of the hydroxyproline of the cartilage. This solubilization was well above that achieved by trypsin, indicating extensive degradation of native collagen.

Cultures of macrophages and/or fibroblasts on [^{14}C]-collagen-coated plates

Macrophages or fibroblasts were first cultivated alone on the [^{14}C]collagen-coated plates. After a few days, the fibroblasts, but not the macrophages, induced the release of soluble ^{14}C-labelled degrada-

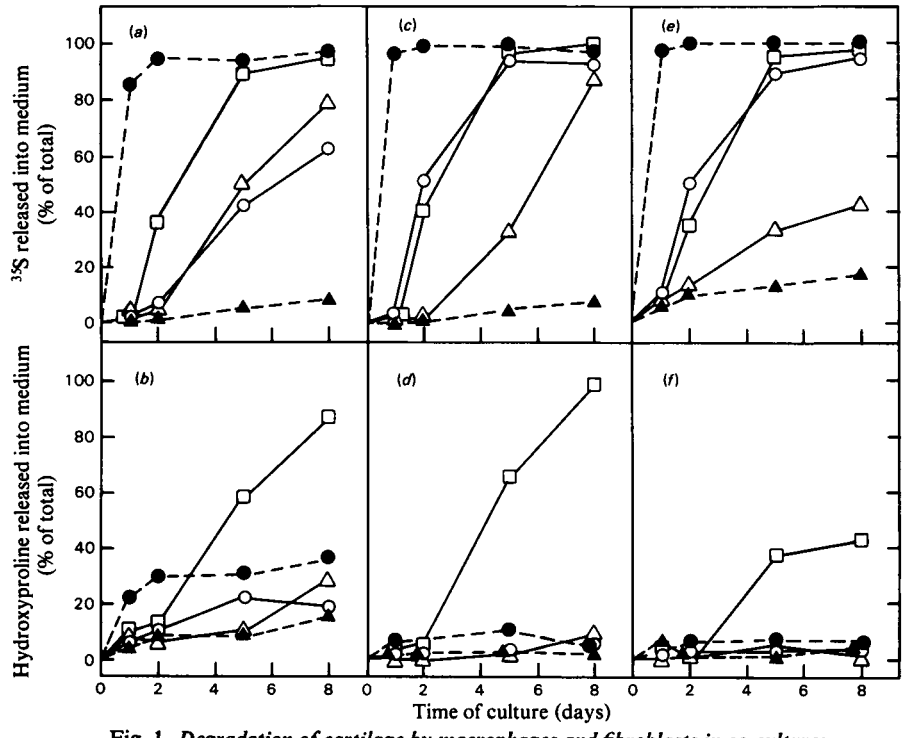

Fig. 1. *Degradation of cartilage by macrophages and fibroblasts in co-cultures*

Pieces of ^{35}S-labelled ear (*a*, *b*) or articular (*c*, *d*, *e*, *f*) cartilage, either heat-treated (*a*, *b*, *c*, *d*) or native (*e*, *f*), were cultivated for the time indicated in 0.2ml of medium under five conditions: with 6×10^4 macrophages (○), with 4×10^4 fibroblasts (△), with both types of cell (□), without cells (▲) or, as a further control, in the presence of 5μg of trypsin (●). The release of soluble ^{35}S-labelled material (*a*, *c*, *e*) or of hydroxyproline (*b*, *d*, *f*) from the cartilage is expressed as a percentage of the total ^{35}S or hydroxyproline present in the cartilage at the beginning of the culture. Each point is the mean of three cultures.

tion products from the collagen. The addition of fibroblasts to cultures of macrophages initiated 20h earlier resulted in a more rapid and more extensive degradation of collagen (Fig. 2). This interaction between the two types of cell was observed in co-cultures containing various ratios of macrophages to fibroblasts. The effect was usually easily seen when 10^5 fibroblasts were added to culture wells already containing 2×10^5 macrophages; this ratio was selected for the studies further reported in this paper. Subsequently, however, it appeared that equally evident interactions between the two cell types were observed with an almost identical time sequence when the number of cells in the co-cultures was reduced to 50000 macrophages and 25000 fibroblasts (see Fig. 1 in Vaes *et al.*, 1980).

The rates of collagen degradation achieved by fibroblasts when they were cultivated alone varied from one cell line to another. The capacity to degrade collagen was retained over at least seven passages. As a rule, the less efficient the fibroblasts were at collagen degradation when they were cultivated alone, the more they appeared to benefit from their co-culture with macrophages (results not shown). Co-cultures of the same line of fibroblasts with preparations of macrophages obtained from different rabbits differed also in their rates of collagen degradation (Figs. 3*a* and 3*b*).

Controls with conditioned media

As a control, macrophage- and fibroblast-conditioned media (serum-free) were incubated for several days under the conditions of the cell cultures, independently or together, either with ^{35}S-labelled cartilage or on [^{14}C]collagen-coated plates. The

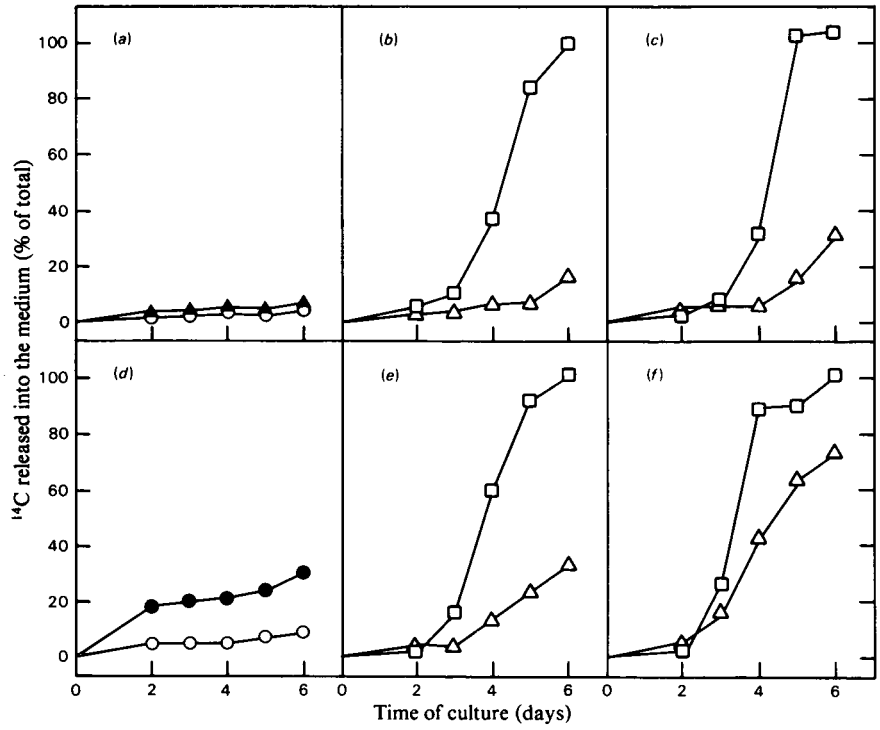

Fig. 2. *Co-cultures of various amounts of macrophages and fibroblasts on collagen-coated plates*
Macrophages (○), fibroblasts from the first passage (△) or both types of cell (□) were cultivated for the time indicated in 2ml of medium in Multiwell plates coated with a dried reconstituted [^{14}C]collagen gel. When present, the fibroblasts were added to the plates after the first 20h of culture. As controls, cultures were prepared without cells, either as such (▲) or in the presence of 50μg of trypsin (●). The release of ^{14}C-labelled material from collagen is expressed as a percentage of the total amount of collagen (^{14}C) initially present in the wells. Each point is the mean of two cultures, containing per well: (*a*) 10^5 macrophages or controls without cells; (*b*) 2×10^5 fibroblasts (from rabbit P37) with or without 10^5 macrophages; (*c*) 3×10^5 fibroblasts (from rabbit P37) with or without 10^5 macrophages; (*d*) 2×10^5 macrophages or controls without cells; (*e*) 10^5 fibroblasts (from rabbit P36) with or without 2×10^5 macrophages; (*f*) 2×10^5 fibroblasts (from rabbit P36) with or without 2×10^5 macrophages.

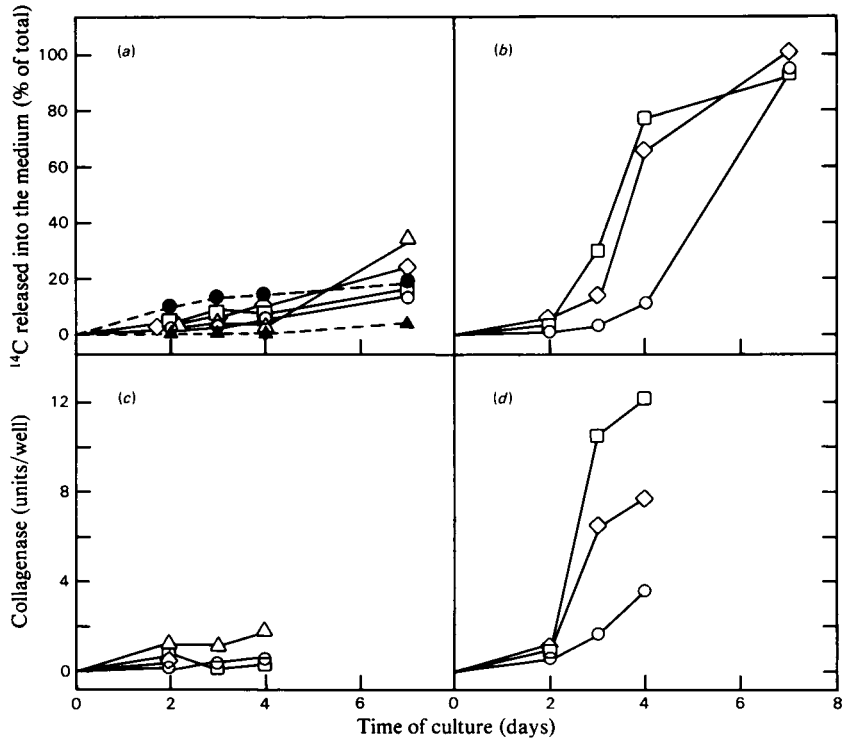

Fig. 3. *Secretion of collagenase by macrophages and fibroblasts in co-cultures*

The same line of fibroblasts (△) was cultivated in 1 ml of medium with preparations of macrophages obtained from three different rabbits (○, □ and ◇). The cultures were prepared either on [^{14}C]collagen-coated plates (*a*, *b*) or on non-coated plates (*c*, *d*). The release of soluble ^{14}C-labelled material from collagen (*a*, *b*) is expressed as a percentage of the total collagen ^{14}C initially present in the wells. The secretion of collagenase (*c*, *d*) is expressed in units/well and presented in a cumulative manner. (*a*) and (*c*) refer to the cultures of either 10^5 fibroblasts or 2×10^5 macrophages prepared independently of each other; (*b*) and (*d*) refer to the co-cultures of the fibroblasts with each of the three macrophage strains. Controls without cells (▲) or with trypsin (●) are as in Fig. 2. Each point is the mean for two cultures.

macrophage-conditioned media contained directly active neutral proteinase (1.5–3.0units/ml) but insignificant collagenase activity. The fibroblast-conditioned media contained neutral proteinase (9–13units/ml) and collagenase (57–70units/ml), both completely latent in these particular media. Four experiments were done: in no case did mixtures (1:1) of macrophage medium and fibroblast medium provide more extensive degradation of either substrate than could be accounted for by the sum of the activities displayed independently by each medium.

Secretion of collagenase and of neutral proteinase in the co-cultures

In parallel to co-cultures on [^{14}C]collagen-coated plates, co-cultures of the same line of fibroblasts with preparations of macrophages obtained from three different rabbits were prepared in non-coated Multiwell plates, in order to assay the amounts of collagenase and neutral proteinase secreted by the cells in the culture medium. The usual collaboration was seen between the two types of cells in the degradation of collagen (Figs. 3*a* and 3*b*). A parallel stimulation of the secretion of collagenase was observed that correlated well with the rate of degradation of the collagen fibres in the coated plates (Figs. 3*c* and 3*d*). The stimulation was observed when either the total collagenase activity, displayed after trypsin treatment of the culture media (Fig. 3), or the directly active collagenase were assayed (results not shown). The amounts of directly active enzyme varied from culture to culture; in the present experiments, they represented 30–60% of the total collagenase of the media. There was no effect of the co-cultures on the secretion of neutral proteinase (results not shown).

Effects of conditioned medium of each cell type on the secretion of collagenase and neutral proteinase by the other

The addition of macrophage-conditioned medium to cultures of fibroblasts stimulated their production of collagenase (Fig. 4), but not that of neutral proteinase (results not shown). The effect was observed after a latent period of 1–2 days. It was optimal with 50% (v/v) macrophage-conditioned medium. However, it was abolished when the macrophage-conditioned medium had been either heat-treated (15min at 100°C) or dialysed (at 3–4°C, against 3×4 vol. of basal culture medium over 48h) before its addition to the cultures of fibroblasts. Indeed when control fibroblasts produced, over 5 and 7 days of culture, 803 ± 100 and 1172 ± 42 units of collagenase/10^6 cells (mean ± S.D. for three cultures) respectively, fibroblasts supplemented with 50% (v/v) macrophage-conditioned medium produced 1138 ± 70 ($P<0.01$ by Student's t test) and 1546 ± 48 units ($P<0.001$) respectively, but their collagenase production was unchanged compared with that of the controls when the macrophage medium had been either heated (750 ± 87 and 1249 ± 49 units respectively) or dialysed (717 ± 45 and 1178 ± 110 units respectively).

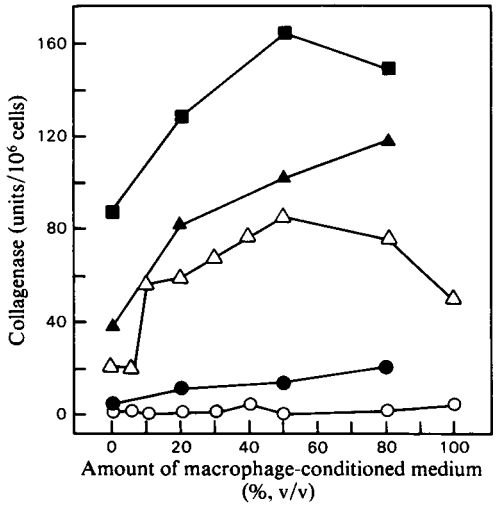

Fig. 4. *Stimulation by macrophage-conditioned culture medium of the production of collagenase by fibroblasts*
Fibroblasts (10^5 cells) were cultured in Multiwell plates in basal medium containing the indicated amounts of macrophage-conditioned media. At various time intervals, samples of the media were taken for collagenase assays; these were done after complete activation of the latent enzyme by trypsin. Each point is the mean for two to three cultures. In a first experiment, the fibroblasts were cultured in 1ml of medium, with a macrophage-conditioned medium that contained 0.34 unit of neutral proteinase/ml; samples (400μl) of the medium were taken for the assays after 2 days (○) and 6 days (△). In a second experiment, the fibroblasts were cultured in 2ml of a conditioned medium obtained from cultures of macrophages prepared in the presence of 25% of concanavalin A-stimulated lymphocyte medium and containing 2.54 units of neutral proteinase/ml; samples (500μl) of the medium were taken for the assays after 1 day (●), 3 days (▲) or 7 days (■). Both macrophage-conditioned media contained insignificant amounts of collagenase (less than 0.4unit/ml) compared with the amounts produced by the fibroblasts.

Conditioned media obtained from different cultures of macrophages (Fig. 5) varied in their ability to stimulate the production of collagenase by the same fibroblast line. Greater stimulation and more constant effects were observed within the same experiment when the conditioned media were obtained from cultures of macrophages in the presence of 25% concanavalin A-stimulated lymphocyte media. There was, however, no correlation between the activity of neutral proteinase within any individual macrophage-conditioned medium and the stimulatory effect exerted by that medium on the production of collagenase by the fibroblasts (Fig. 5). Direct treatment of the macrophages by concanavalin A had no effect on that interaction (results not shown). Also the addition of concanavalin A-stimulated lymphocyte media directly to cultures of fibroblasts (at a 12.5%, v/v, concentration) had no effect on the secretion of collagenase by these cells nor did it modify their rate of collagen degradation when they were cultivated on collagen-coated plates (results not shown).

The addition of fibroblast-conditioned medium to cultures of macrophages (up to 50%, v/v) had no consistent effect on the rate of secretion of either neutral proteinase or collagenase by the macrophages (results not shown).

Discussion

Rabbit bone-marrow macrophages (Hauser & Vaes, 1978*a*) and rabbit fibroblasts in culture (Huybrechts-Godin & Vaes, 1978; Vaes *et al.*, 1977) produce and secrete a metal-dependent neutral proteinase that degrades cartilage proteoglycan into soluble fragments. Their degradative action was observed by culturing the cells together with pieces of cartilage biosynthetically labelled with ^{35}S in their proteoglycan moieties. The action of non-activated macrophages on cartilage appeared, however, to be limited to the degradation of proteoglycan. As a rule, the collagenous component of the cartilage or of the [^{14}C]collagen-coated plates was unaffected over 7–8 days of culture. This is probably related to the low amount of collagenase (less than 0.5unit/10^6 cells over 4 days) produced by the macrophages during

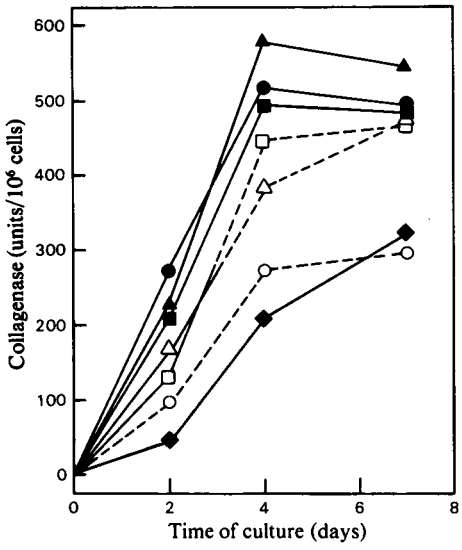

Fig. 5. *Stimulation of the fibroblast production of collagenase by conditioned media obtained from macrophage cultures stimulated or not by concanavalin A-stimulated lymphocyte factors*

Fibroblasts (10^5 cells/well) were cultured in Multiwell plates in 2ml of medium. Samples (250μl) of the media were taken at the indicated times for collagenase assay (done after complete activation of the latent enzyme by trypsin); the amounts of collagenase released are presented in a cumulative manner. Each point is the mean for two cultures. The fibroblasts were cultivated in basal medium either without (♦) or with 50% (v/v) macrophage-conditioned medium. The macrophages were obtained from three different rabbits and their conditioned media were collected after their culture with (●, ▲, ■) or without (○, △, □) 25% (v/v) concanavalin A-stimulated lymphocyte media. The following activities of neutral proteinase were found in these conditioned media: 2.5 (○) and 3.6 (●) units/ml for macrophage strain no. 1; 0.6 (△) and 2.1(▲) units/ml for macrophage strain no. 2; 0.5 (□) and 4.0 (■) units/ml for macrophage strain no. 3. All these media contained only insignificant amounts of collagenase (less than 0.5unit/ml) compared with the amount produced by the fibroblasts. The differences observed on day 2 between the effects of macrophage-conditioned media that were or were not stimulated by lymphocyte factors are significant at $P<0.05$ (Student's t test).

the cultures, as observed also by Werb & Gordon (1975). On the occasional times when early degradation of collagen occurred, we could often relate it to a certain degree of contamination of the cultures by fibroblasts.

On the other hand, and in confirmation of the work of others (see Woolley & Evanson, 1979), fibroblasts produced large amounts of collagenase in the early stage of culture (from the second or third day onwards). The enzyme was recovered from the culture medium mostly as a latent trypsin-activatable form. Part of it was, however, sometimes directly active but, for unknown reasons, the proportion of directly active to latent enzyme was highly variable from one culture to another. We have not established whether all the active enzyme was present as such in the medium during the cultures or whether all or part of it originated after the cultures from spontaneous 'autoactivation' (Eeckhout & Vaes, 1977) of latent collagenase. Such 'autoactivation' could also occur in the medium during the culture and could be responsible for degradation of collagen. Indeed, after 8–10 days of culture with cartilage, the fibroblasts slowly degraded its collagen, provided that some foetal calf serum (usually 0.5%) was added to the culture medium (G. Huybrechts-Godin & G. Vaes, unpublished work). However, they degraded the collagen of the [^{14}C]collagen-coated plates more rapidly, even in the absence of serum. The difference between the two substrates with respect to their rates of collagen degradation may possibly be due to greater accessibility of the collagen fibres to the enzyme on the plates or to a lower degree of cross-linking between the molecules within the dried reconstituted fibres on the plates, favouring faster collagenolysis; it may possibly also be due to a difference in the type of collagen present on the plates (type I, from guinea-pig skin) or in the tissue (presumably mostly type II). Whenever it occurred in cartilage, the degradation of collagen was always subsequent to the removal of proteoglycan.

The co-cultivation of macrophages and fibroblasts in the presence of either cartilage or [^{14}C]collagen-coated plates had no effect on the rate of degradation of the proteoglycan nor on the secretion of neutral proteinase by the cells. However, it caused a more rapid and more extensive degradation of collagen. This was most obvious in serum-free cultures containing added cartilage pieces, where neither type of cell alone exerted any effect on the collagen, or in plate cultures involving lines of fibroblasts that were particularly slow in degrading collagen when they were cultivated alone. Parallel to this effect, an increased accumulation of collagenase was found in the culture medium. The fibroblasts obviously produced the collagenase, as conditioned medium from macrophage cultures added to cultures of fibroblasts had the same effect as living macrophages in stimulating the production of collagenase.

The mechanism of this synergism is so far unknown. It is unlikely to be due to a mere depletion by the macrophages of some nutritional factor present in the culture medium of the fibroblasts, as it is observed equally well, and with about the same time sequence, with 2×10^5 macrophages+10^5 (or 2×10^5) fibroblasts as with 50000 macrophages+25000 fibroblasts per

well. Moreover, conditioned media from macrophage cultures no longer stimulate fibroblasts after their dialysis against fresh culture medium or after heat-treatment. Thus the action of the macrophages is likely to be due to the addition to the culture medium of a heat-labile dialysable factor that stimulates the fibroblast production of collagenase.

The co-operation between the two types of cell is independent of any special treatment of the cells, in contrast with the collaboration observed in the production of collagenase between rabbit epithelial and stromal corneal cells, which required the presence of cytochalasin B (Johnson-Muller & Gross, 1978), or between rabbit peritoneal macrophages and chondrocytes, which required treatment of the macrophages with endotoxin (Deshmukh-Phadke *et al.*, 1978). Activation of the latent collagenase of fibroblasts by a proteinase secreted by the macrophages appears unlikely in view of the absence of interaction observed for the digestion of collagen by a mixture of macrophage- and fibroblast-conditioned media. A proteinase secreted by the macrophages could, however, act as a signal to the fibroblasts to stimulate their production of collagenase, as has been shown for other proteinases (Werb & Aggeler, 1978). However, we did not observe any correlation between the activity of neutral proteinase assayed (on casein) within various individual macrophage-conditioned media and the stimulatory effect exerted by these media on the production of collagenase by the fibroblasts. It is also unlikely that the macrophages act in our system by stimulating the proliferation of fibroblasts through the production of a 'macrophage-dependent fibroblast-stimulating activity' similar to that demonstrated in a mouse system by Leibovich (1978). Indeed the activity in the mouse was not produced in the absence of serum and, moreover, in our serum-free co-cultures, the fibroblasts did not proliferate. Further work is obviously required to characterize the nature and the mode of action of the factor involved in our experiments. This factor may be similar to the monocyte factor reported by Dayer *et al.* (1978) to stimulate the production of collagenase by human rheumatoid-synovial cells in culture.

Of particular interest are the observations that indicate that the interaction between macrophages and fibroblasts could itself be enhanced by the addition of concanavalin A-stimulated spleen-cell factors (but not of concanavalin A alone) to the cultures of macrophages. These lymphocyte factors had, however, no direct effect on the fibroblasts themselves. These observations raise the possibility that the rate of secretion of collagenase by fibroblasts can be modulated by interactions with components of the humoral or cellular immune systems. A chemotactic attraction of fibroblasts to a lymphocyte-derived factor has already been described (Postlethwaite *et al.*, 1976), as well as lymphocyte-mediated activation of fibroblast proliferation (Wahl *et al.*, 1978) and collagen accumulation (Johnson & Ziff, 1976; Wahl *et al.*, 1978). If all these events also occur *in vivo* in response to antigenic stimulation, then the fibroblast should be considered as a potential effector cell of immune reactions, and its activation by immunological events may be responsible for some of the deleterious effects observed on cartilage and other connective-tissue structures in chronic inflammatory situations.

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