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ENHANCED FIBROBLAST COLLAGEN PRODUCTION BY A MACROPHAGE-DERIVED FACTOR (CEMF)

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SUMMARY: A basic fraction with pI values of 10.0-10.4 was isolated from macrophage culture medium. This fraction stimulated the production of collagen into fibroblast culture medium but inhibited the production of other proteins. Both changes were strongly dependent on the concentration of the factor. As revealed by SDS-PAGE the production of type I collagen was especially stimulated. The specific enhancement of fibroblast collagen production was also observed in the presence of serum proteins. The collagen synthesis enhancing macrophage-derived factor preparation (CEMF) contained three proteins, one major (M_r 23 kD) and two minor (M_r 49 and 71 kD) fractions in SDS-PAGE. When prelabeled fibroblast medium was exposed to CEMF a protein with M_r of 350 kD or greater was converted to a smaller size. This change was inhibited by EDTA but not by serum proteinase inhibitors.

INTRODUCTION

Macrophages have been shown to interfere with fibroblast collagen synthesis during inflammatory reactions (1-3). Attempts to identify in macrophages chemical factors responsible for this interaction have been carried out, e.g. in silicosis (4). However, the putative agents responsible for the regulation of fibroblast collagen synthesis are poorly defined (5). In this work we present some characteristics for the collagen synthesis enhancing macrophage-derived factor (CEMF) (6,7).

MATERIALS AND METHODS

Rat peritoneal macrophages (MF) were cultured in Dulbecco's modification of Eagle's minimum essential medium (DMEM) in the presence of antibiotics as described before (6,7). Preparation of the collagen synhesis enhancing macrophage-derived factor (CEMF) from

Abbreviations used: CEMF = collagen synthesis enhancing macrophage-derived factor, MF = macrophage, SDS-PAGE = sodium dodecyl sulphate-polyacryl amide gel electrophoresis.

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MF-media was carried out by preparative isoelectric focusing (6,7). CEMF was radioiodinated by the chloramine T procedure (8).

Rat granuloma fibroblasts were induced into viscose cellulose sponges, harvested by collagenase-trypsin digestion (9) and cultured similar to macrophages except that the medium was supplemented with 10 % fetal calf serum (FCS). Cells of early subcultures (2-4) were used. When collagen synthesis was measured in these cells they were exposed to [^{3}H]proline (1-5 µCi/ml; 15.3 Ci/mmol, The Radiochemical Centre, Amersham, U.K.) for 24 h in the presence of fresh ascorbic acid (50 µg/ml) in the DMEM but without serum (if not otherwise stated). After incubations the amounts of protein bound radioactive proline and hydroxyproline in the media proteins were determined (10). Medium samples for SDS-PAGE were dialyzed, lyophilized and solubilized into SDS-buffer according to O'Farrel (11). Occasionally samples were digested with pepsin at +4 °C for 18 h. After electrophoresis in the gradient gel (5 to 22 %) the proteins were fixed and stained conventionally and the gel was prepared for the detection of radioactivity (12). Other experimental details are presented in the legends for the figures.

RESULTS

The preparation of CEMF has been presented before (6,7). The isoelectric point of CEMF varied in different runs between 10.0-10.4. When exposed to CEMF the fibroblasts produced slightly more collagenous proteins but clearly less non-collagenous proteins (Figure 1). The enhancement of collagen production and inhibition of total protein synthesis were both dependent on the concentration of added CEMF. As revealed by SDS-PAGE the synthesis of type I collagen

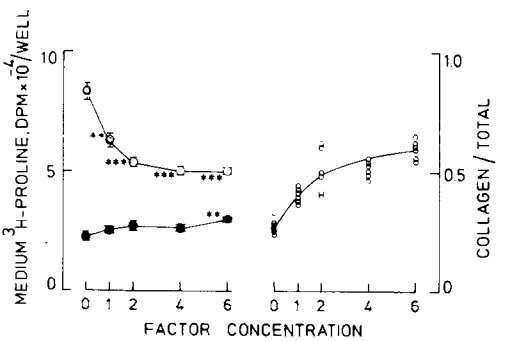


Figure 1. The effect of different CEMF concentrations on protein and collagen production by granuloma fibroblasts. CEMF concentrations on the fibroblasts growing in multidish plates (growth area 1.9 cm^2; Nunc Products) refer to the original concentration in the MF-medium assuming that no significant losses happened during the isolation. The figures on the left indicate means ± S.E.M. of six assays for each concentration. Open circles express the radioactivities in total proteins and closed circles radioactivities in collagen, based on the specific radioactivities of hydroxyproline (**; $p < 0.01$, ***; $p < 0.001$ compared to the plain DMEM). On the right the ratios of radioactivity of collagen to total protein bound radioactivity are expressed for each assay.

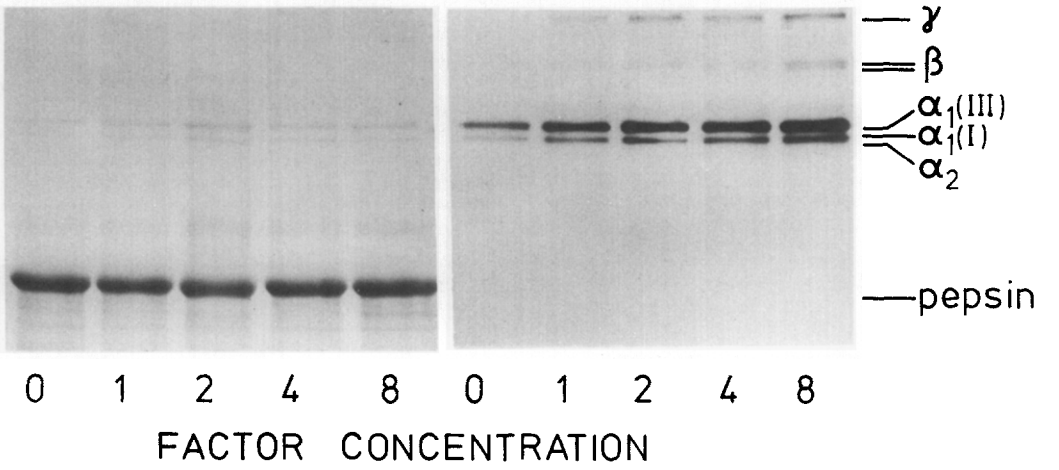
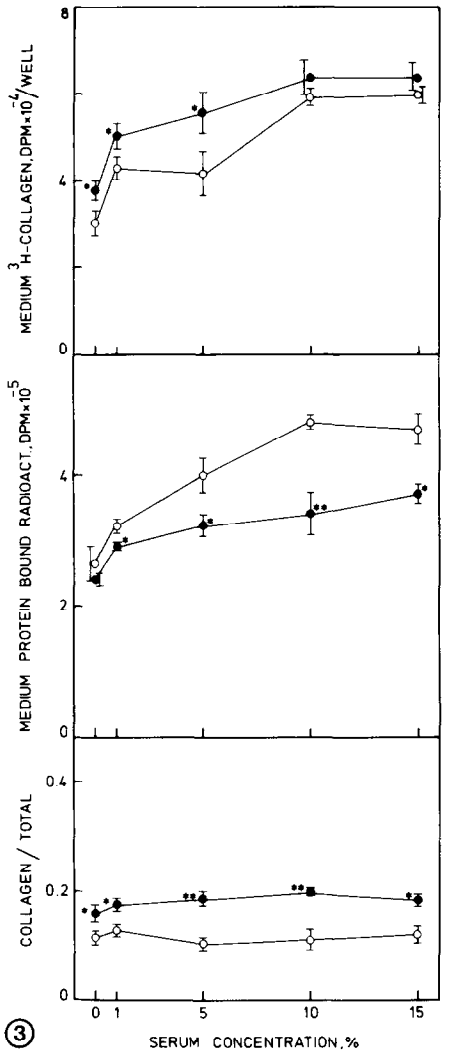


Figure 2. The effect of different CEMF concentrations on the production of different collagens by granuloma fibroblasts. Experimental details are similar to those of Figure 1, except that the cells were growing on petri dishes (57 cm^2). After labeling 10^5 cpm from each culture medium were digested with pepsin and analyzed with SDS-PAGE in the presence of mercaptoethanol. On the left side the gel is shown after protein staining and on the right after fluorography. Different collagen chains are indicated.

was especially increased (Figure 2). The presence of FCS in the fibroblast medium during CEMF exposure did not inhibit the increase in collagen/total protein ratios (Figure 3). At the highest serum concentrations (10-15 %) this was, however, for the most part due to the diminished total protein radioactivities, not to increased activities of hydroxyproline (Figure 3).

The CEMF was radioiodinated before electrophoresis to overcome difficulties in protein band detection caused by the background staining with Coomassie Blue of electrophoretograms containing ampholytes. The main radioactivity was found in a protein of 23 kD (Figure 4). Two minor bands with mol. wts of 49 and 71 kD were also observed.

During incubation of prelabeled fibroblast medium with CEMF a protein with M_r of 350 kD or greater was converted to a smaller size (Figure 5). Serum proteins (0.01 %) or alpha-1-antitrypsin (0.05 %) did not inhibit this change but 10 mM EDTA conserved the protein pattern similar to that produced in control incubation (Figure 5).



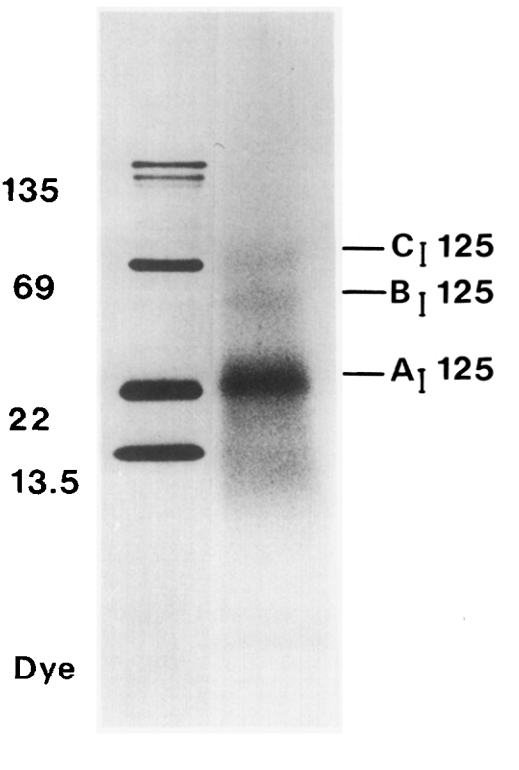


Figure 3. The effect of serum concentrations on the activity of CEMF on fibroblast collagen and total protein synthesis. Experimental details similar to those of Figure 1. The figures express means ± S.E.M. of six assays in serum concentrations indicated in the presence (closed circles) or absence (open circles) of CEMF, which was used in a concentration 2x higher than that in the original MF-culture medium.

Figure 4. Analysis of CEMF by SDS-PAGE. CEMF was radioiodinated before electrophoresis. On the right are mol. wt. markers: [^{3}H]acetic anhydride labeled (24) rat lathyritic collagen (pepsin digested, apparent M_r 135 kD), bovine serum albumin (69 kD), trypsin inhibitor (22 kD) and cytochrome C (13.5 kD) from the top of the gel, respectively. On the right [^{125}I]CEMF with one major (23 kD) and two minor (49 and 71 kD) bands.

DISCUSSION

The specific increase in medium collagens by fibroblasts exposed to CEMF can be explained by a combination of stimulated secretion of

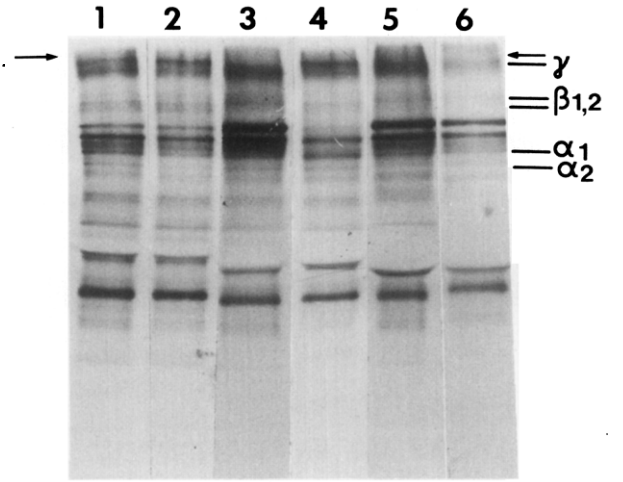


Figure 5. The effect of CEMF on prelabeled fibroblast medium proteins. Medium aliquots from [^{3}H]proline labeled fibroblast cultures were incubated for 6 h in the following conditions and analyzed by SDS-PAGE without mercaptoethanol: Lane 1; control incubation at 0 °C. Lane 2; control incubation at 37 °C. Lane 3; CEMF (equal to the original MF-medium) at 37 °C. Lane 4; CEMF + 10 mM EDTA at 37 °C. Lane 5; CEMF + 0.01 % serum at 37 °C and Lane 6; CEMF + 0.05 % alpha-1-antitrypsin at 37 °C. Arrows indicate a protein band, which is degraded by CEMF treatment. Pepsin digested collagens were used as mol. wt. markers.

collagen and decreased production of other proteins. The latter effect could be due to a generally diminished secretion and/or a specific increase in the degradation of non-collagenous proteins during the incubation. The mechanism of enhanced collagen production by CEMF is yet unclear. Recent results have revealed, that the aminoterminal extension peptide of procollagen or part of it might play an important role in the feedback regulation of further collagen synthesis (13-18). Moreover, the collagen fibril growth in vivo can be regulated by extracellular procollagen processing via the removal of aminopropeptides (19). The results in Figure 5 suggest that the synthetic changes in fibroblasts during CEMF exposure may also be connected to the extracellular processing of proteins, including procollagens which are the principal collagen forms in fibroblast culture media (20).

The molecular parameters of the main protein of CEMF resemble those described for the mouse macrophage elastase, a M_r 21-26 kD metalloprotease, resistant to the common serum proteinase inhibitors (21,22). This enzyme can degrade elastin and many extracellular glycoproteins of connective tissue matrix except collagen (23). It has been presented that this enzyme may play a role in the physiological and pathological remodeling of connective tissues (21,22).

The effect of CEMF on fibroblast cultures might be a combination of two different activities, one degrading non-collagenous proteins and the other promoting the production of collagenous proteins. Whether these effects are completely different activities or result from proteolysis of non-collagen proteins and non-helical parts of procollagens remains to be studied.

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