EFFECT OF SECRETION PRODUCTS OF MONONUCLEAR PHAGOCYTES ON CONNECTIVE-TISSUE CELL PROLIFERATION

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Secretion products of monocytes and macrophages take part in the regulation of granulo- and monocytopoiesis [5], lymphopoiesis [12], and connective-tissue cell proliferation [4, 8, 9, 11, 13]. Whereas the effect of monocytic products on hematopoietic and immunocompetent cells has been studied sufficiently completely, and in some cases monokines acting on target cells have been characterized physicochemically [12], there have been only isolated studies of the role of monocytes in the regulation of connective-tissue cell proliferation. The study of this problem is urgent because changes in proliferative activity of connective-tissue cells lie at the basis of such pathological processes as formation of the atherosclerotic plaque, development of keloid scars, and fibrosis of bone marrow.

Accordingly the aim of this investigation was to study the effect of secretion products of human blood monocytes and mouse macrophages on connective-tissue cell proliferation.

EXPERIMENTAL METHOD

Peritoneal macrophages obtained by flushingout the peritoneal cavity of intact noninbred mice were cultured as a monolayer on glass for 3 days in medium 199 with the addition of 10% bovine serum in a concentration of $4 \cdot 10^6$ cells/ml. Monocytes were obtained from venous blood of healthy donors by centrifugation in a Ficoll–Verografin density gradient, followed by removal of nonadherent cells [2]. Later the monocytes were cultured as a monolayer on glass in medium 199 containing 10% AB (IV) blood serum, in a concentration of $1 \cdot 10^6$ to $2 \cdot 10^6$ cells/ml for 3 days. To stimulate secretory activity of the mononuclear phagocytes latex particles (1 μ in diameter), at the rate of 10 particles per cell, lipopolysaccharide (LPS) of *Salmonella typhimurium* (15 or 10 μg/ml), or rhodexman — a yeast polysaccharide produced by the fungus *Rhodotorula rubra* (100 μg/ml), obtained from Leningrad Pharmaceutical Chemical Institute, were added to the cell cultures. After the end of the culture time, the culture media, conditioned by monocytes or macrophages, were centrifuged and kept in the frozen state (–20°C) until use. Altogether 24 samples of media conditioned by macrophages isolated from 30 mice and 60 media conditioned by monocytes isolated from 15 blood donors were tested. Medium 199 with the addition of the corresponding quantity of serum and stimulators, subjected to the same treatment as the conditioned media, served as the control.

Smooth-muscle cells, bone marrow fibroblasts, and cells of the transplantable 3T3 line were used as target cells. Smooth muscle cells were obtained from the umbilical vein by rinsing it with 0.125% solution of trypsin in versene followed by explantation of the cells into Carrel's flasks containing medium 199 and 20% AB (IV) serum [7]. Bone marrow fibroblasts were obtained in monolayer culture [1]. The donors were patients with somatic diseases in the stage of remission or compensation without any marked changes in the blood system. Cultures at the second or third passage were used for investigation. Fibroblastoid cells of line 3T3 were obtained from the Institute of Cytology and subcultured in Eagle's medium with the addition of 10% embryonic calf serum. Proliferative activity of the cells was estimated by determining the intensity of incorporation of ^{3}H-thymidine into DNA [11]. Radioactivity was measured on a "Mark 3" scintillation counter.

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To estimate the effect of media conditioned by mononuclear phagocytes a proliferation index was introduced, and was determined by the formula (A/B)•100%, where A is the intensity of incorporation of [^{3}H]thymidine into fibroblast DNA under the influence of media conditioned by mononuclear phagocytes, B the intensity of incorporation of [^{3}H]thymidine into fibroblast DNA under the influence of control medium with the addition of serum only. To estimate the effect of stimulation of mononuclear phagocytes on inhibitory activity of media conditioned by them, an index of stimulator effect was determined by the equation: $X = (M - N) - (K - 100)$, where X is the index of stimulator effect (in %), and M, N, and K are proliferation indices (in %) of target cells under the influence of medium conditioned by stimulated phagocytes (M), medium conditioned by unstimulated phagocytes (N), and control medium with the addition of the stimulator (K), respectively. The proliferation index of target cells under the influence of medium containing serum only was taken as 100. A negative value of the stimulator effect index is evidence of potentiation of the inhibitory action of conditioned media during stimulation of phagocytes, whereas a positive value indicates a decrease in this action. The experimental results were subjected to statistical analysis by nonparametric methods.

EXPERIMENTAL RESULTS

The study of the the effect of media conditioned by peritoneal macrophages on proliferation of smooth-muscle cells from the umbilical vein and of bone marrow fibroblasts revealed their inhibitory action on target cells (Fig. 1). During phagocytosis of latex by macrophages, potentiation of this action was observed (Fig. 2). The addition of LPS (15 μg/ml) or rhodexman to the macrophage culture had no significant effect on secretion by the macrophages of products with an inhibitory action on proliferation of smooth muscle cells and bone marrow fibroblasts. Media conditioned by monocytes also had an inhibitory action on proliferation of smooth muscle cells, bone marrow fibroblasts, and cells of line 3T3 (Fig. 1). By contrast with experiments with mouse peritoneal macrophages, addition of stimulators to cultures of human monocytes was not followed by any increase in the inhibitory ability of the media conditioned by them. Conversely, stimulation of monocytes by LPS (10 μg/ml) reduced the inhibitory action of conditioned media against proliferation of smooth-muscle cells but had no effect on the action of these media on other connective-tissue cells (Fig. 2). Neither latex nor rhodexman had any significant influence on the inhibitory effect of monocytes.

The inhibitory effects of conditioned media depended not only on the source of the cells of the mononuclear phagocyte system and the activators used, but also on the type of target cells: The greatest effect was discovered when smooth-muscle cells from the umbilical vein were used, the least effect when 3T3 cells were used. Differences in the sensitivity of the target cells were found when media conditioned by both unstimulated and stimulated macrophages and monocytes were used. Incidentally, the differences were quantitative in character, whereas the direction of the effect was always the same.

These results on the inhibitory effect of products of monocytes and macrophages on proliferation of smooth-muscle cells and 3T3 cells contradict the results of investigations by a number of workers [9, 11, 13], who showed that cells of the mononuclear phagocyte system can stimulate proliferative activity of connective-tissue cells. The reason is evidently that monocytes and macrophages were cultured for secretion products in medium containing serum, which is a powerful stimulator of proliferation on account of the platelet factor which it contains [14]. Under these conditions the inhibitory action of monocyte products is demonstrated better. The possibility of a similar effect of products of mononuclear phagocytes on fibroblasts has been described by several workers [4, 8]. This effect may be mediated by prostaglandin E, which is produced by monocytes and can inhibit connective-tissue cell proliferation [8]. Other probable candidates for the role of inhibitors are the lysosomal proteases of monocytes, which can exert a cytotoxic action on tumor cells [3]. The difference in character of the effect of the various stimulators on secretion by macrophages and monocytes of products inhibiting proliferation of target cells may be attributable either to species differences in the mononuclear phagocytes used or to differences in secretory activity of blood monocytes and tissue macrophages. This was described previously for certain other monokines [6, 10].

The results are thus evidence that secretion products of monocytes and macrophages can inhibit proliferative activity of connective-tissue cells, and this must be taken into account

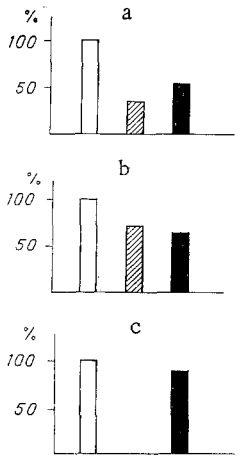

Fig. 1

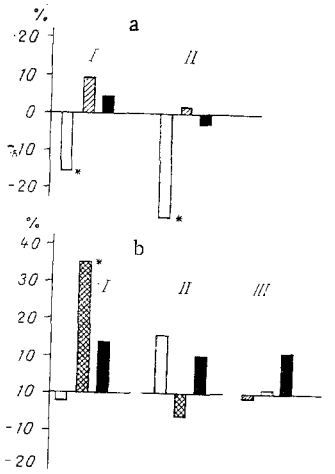

Fig. 2

Fig. 1. Effect of media conditioned by mononuclear phagocytes on proliferation of smooth-muscle cells (a), bone marrow fibroblasts (b), and 3T3 fibroblasts (c). Abscissa, cell proliferation under the influence of control medium, taken as 100 (unshaded columns), of media conditioned by unstimulated macrophages (obliquely shaded columns), and monocytes (black columns); ordinate, indices of target cell proliferation (in %). All indices of cell proliferation under the influence of conditioned media given above differ statistically significantly ($P < 0.05$) from one another (comparison of proliferation of different target cells under the influence of similar media) and from the proliferation index of the cells under the influence of control medium (comparison of the action of different media on the same target cells).

Fig. 2. Effect of stimulation of macrophages (a) and monocytes (b) on inhibitory activity of media conditioned by them directed against smooth-muscle cells (I), bone marrow fibroblasts (II), and 3T3 fibroblasts (III). Abscissa, change in degree of inhibitory action of media conditioned by phagocytes when latex (unshaded columns), LPS in concentrations of 15 μg/ml (obliquely shaded columns) and 10 μg/ml (cross-hatched columns), and rhodexman (black columns) were used as stimulators; ordinate, indices of stimulator effect (in %). Asterisk indicates that difference from zero is significant ($P < 0.05$).

when immunomodulating preparations affecting secretory activity of cells of the mononuclear phagocyte system are used.

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