EFFECT OF IL-1 AND TNF-α ON GROWTH OF STROMAL FIBROBLASTS IN VITRO

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In a previous communication we described the results of the action of a number of growth factors, namely IL-1, TNF-α, and IL-3, on the formation of colonies of stromal fibroblasts (CFC-f colonies) in adhesive and complete cultures of primary explanted mouse bone marrow cells. It must be recalled that even in cultures of adhesive bone marrow cells, besides CFC-f other nonadherent cells also are present, as well as, evidently, a certain number of (not many) nonadhesive hematopoietic cells, which nevertheless remain in the cultures, however thoroughly they are washed. In this connection the problem of whether the growth factors studied act directly on CFC-f or whether their action is mediated through cells of other categories, present in the culture, and in particular, macrophages, requires further study.

The aim of the present investigation was to discover how IL-1 and TNF-α act on medullary stromal fibroblasts in subcultures free from contamination by other types of cells.

EXPERIMENTAL METHOD

Fibroblasts from subcultured strains of human medullary fibroblasts and skin fibroblasts, and also from Chinchilla rabbit bone marrow cells, were used. Fibroblasts taken from the surface of the plastic in the usual way with the aid of 0.25% trypsin solution [1], were transferred after 3-10 passages to 24-well plates, up to a total of 100-500 cells per well in medium MEM-α with 15% fetal calf serum. Recombinant human TNF-α (Institute of Molecular Biology, Academy of Sciences of the USSR) and purified mouse IL-1 (N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR) were added to the cultures 1-24 h after explantation of the fibroblasts at all times of culture. After 7-10 days the cultures were fixed with ethanol and stained with azureeosin; either the total number of fibroblasts (cultures of human bone marrow and skin) or the number of colonies containing not less than 50 fibroblasts (rabbit bone marrow cultures) was counted. In the latter case the cloning efficiency per 10^3 explanted cells (ECF-f) was determined.

EXPERIMENTAL RESULTS

On the 7th-10th day discrete colonies of fibroblasts, numbering from tens to hundreds of cells, were found in cultures of rabbits fibroblasts, whereas in cultures of human bone marrow and skin fibroblasts the cells grew in a layer and did not form colonies. The presence of IL-1 in the culture medium (Fig. 1) in concentrations up to 5 U/ml had virtually no effect on growth of rabbit bone marrow fibroblasts, but higher doses of this factor suppressed colony formation virtually completely. TNF-α (Fig. 1), in concentrations of about 0.05 U/ml, led to an increase by about 1.5 times in the number of fibroblasts in rabbit bone marrow cultures, and a further increase in the TNF-α concentration caused inhibition of colony formation (most marked with a concentration of $2.5 \cdot 10^4$ U/ml).

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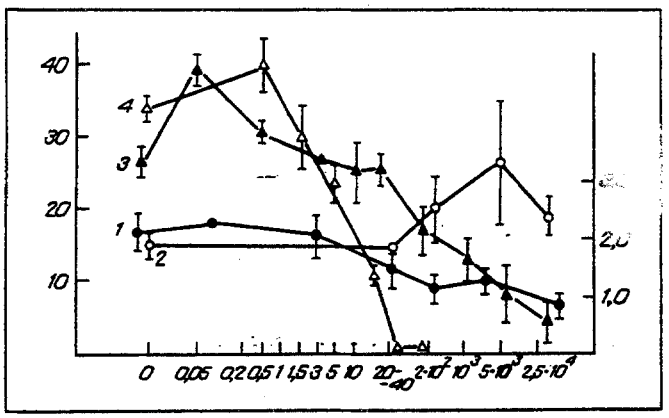

Fig. 1. Growth of rabbit and human bone marrow fibroblasts and human skin fibroblasts in the presence of TNF-α and IL-1. Abscissa, concentration of TNF-α and IL-1 (in U/ml); ordinate: scale on right – number of human bone marrow fibroblasts in the presence of TNF-α ($\cdot 10^3$) (1). Scale on left – number of human skin fibroblasts in the presence of TNF-α ($\cdot 10^3$) – (2), ECF-f of rabbit bone marrow fibroblasts in the presence of TNF-α ($\cdot 10^{-3}$) – in the presence of TNF-α ($\cdot 10^{-3}$) – (3), ECF-f of rabbit bone marrow fibroblasts in the presence of IL-1 ($\cdot 10^{-3}$) – (4).

Doses of TNF-α from 0.05 to 3 U/ml had virtually no effect on growth of the cells in human bone marrow fibroblast cultures, but higher concentrations of TNF-α also suppressed proliferation of stromal fibroblasts, although not to such a considerable degree as in cultures of rabbit bone marrow fibroblasts. Thus the action of IL-1 and TNF-α on subcultured stromal fibroblasts is virtually indistinguishable from the action of these growth factors on the formation of CFC-f colonies in cultures of primary explanted bone marrow cells. Hence it follows that the action of IL-1 and TNF-CG on primary explanted CFU-f is evidently aimed directly at the stromal cells and is not mediated by cells of other categories.

So far as human skin fibroblasts are concerned, high doses of TNF-α (more than $2 \cdot 10^2$ U/ml) clearly increased the number of fibroblasts in the cultures (by 1.7 times at a TNF-α concentration of $5 \cdot 10^3$ U/ml).

IL-1 and TNF-α are known to inhibit growth of several lines of tumor cells [2, 3]. At the same time, nonmalignant cells are resistant to the cytotoxic action of these factors, which behave as mitogens for diploid fibroblasts of nonstromal origin [3]. In the present experiments growth of human skin diploid fibroblasts also was stimulated by TNF (Fig. 1). Meanwhile TNF-α had an inhibitory action on growth of stromal medullary fibroblasts. The question accordingly arises, what are the differences between stromal and nonstromal fibroblasts that determine the different response to the action of TNF-α and of IL-1.

On the whole the results are evidence that factors secreted by activated macrophages and lymphocytes may exert a considerable influence on proliferation of stromal clonogenic cells and their cultural offspring. It is possible that exposure to these factors may reflect the mechanisms of regulation of the proliferative activity of stromal cells in vivo.

LITERATURE CITED

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