ABILITY TO GROW in vitro AND CHARACTER OF TRANSFORMATION OF SYNOVIAL FLUID CELLS FROM PATIENTS WITH RHEUMATOID ARTHRITIS

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Growth of cells from the synovial fluid of patients with rheumatoid arthritis in vitro was demonstrated. In the process of transformation five types of cells were observed to appear: macrophages, polykaryocytes, fibroblast-like cells, histiocytes, and lymphocytes. The intensity of cell growth in culture was found to depend on the activity of the disease, and a significant decrease ($P < 0.01$) of this intensity occurred during treatment of the patients. Growth of fibroblast-like cells, with the formation of colonies, is evidence of their possible role in the genesis of inflammatory processes and sclerosis of the joints. The phenomenon of cytopathic interaction between lymphocytes and fibroblast-like cells observed in the experiments is an indicator of autoimmune conflict in the joint and a possible mechanism of self-maintenance of the autoimmune process.

KEY WORDS: rheumatoid arthritis; synovial fluid; cell growth.

The in vitro culture of cells and tissues is a method that has been widely used to study pathological processes [4-6, 13]. The opinion is held by some workers that the main site of immunologic conflict in rheumatoid arthritis is the joints [3, 10].

The object of this investigation was to study the character of transformation of cell populations arising during in vitro growth of cells from the synovial fluid of patients with rheumatoid arthritis, the character of intercellular interactions in culture, and the possibility of correlation between the ability of the cells to grow and the course of the pathological process.

EXPERIMENTAL METHOD

Synovial fluid was obtained by puncture of the knee joint from patients with rheumatoid arthritis and persons with traumatic injury of the joint. To prevent clotting heparin was added to a dose of 50 units to 10 ml fluid. The resulting fluid was filtered through capron and centrifuged; the supernatant was poured off and the residue was washed three times with medium No. 199. The cells were transferred in doses of $3 \cdot 10^6$ to flasks and grown on cover slips at 37°C in a medium of the following composition: 50% Eagle's medium, 30% medium No. 199, 20% human serum; 0.5 ml glucose, 0.2 ml vitamin C, 0.1 ml vitamin B_1, 0.2 ml vitamin B_6, and 0.1 ml vitamin B_{12} were added to each 100 ml of medium. The medium was changed every 3-4 days.

The cover slips were taken from the flasks after 1, 2, 3, 4, ..., and 20 days, washed with physiological saline, fixed with methyl alcohol for 10 min, and stained by Romanovsky's method and with hematoxylin–eosin. The cells in the preparations were counted in 25 fields of vision by Gorskii's method [1] and the mean

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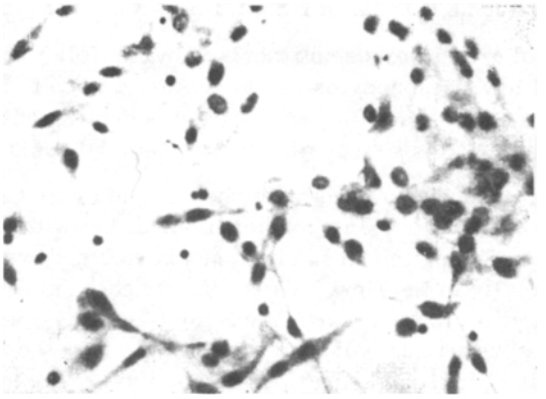

Fig. 1. Six-day culture of synovial fluid cells from patient with rheumatoid arthritis. Romanovsky, 200 ×).

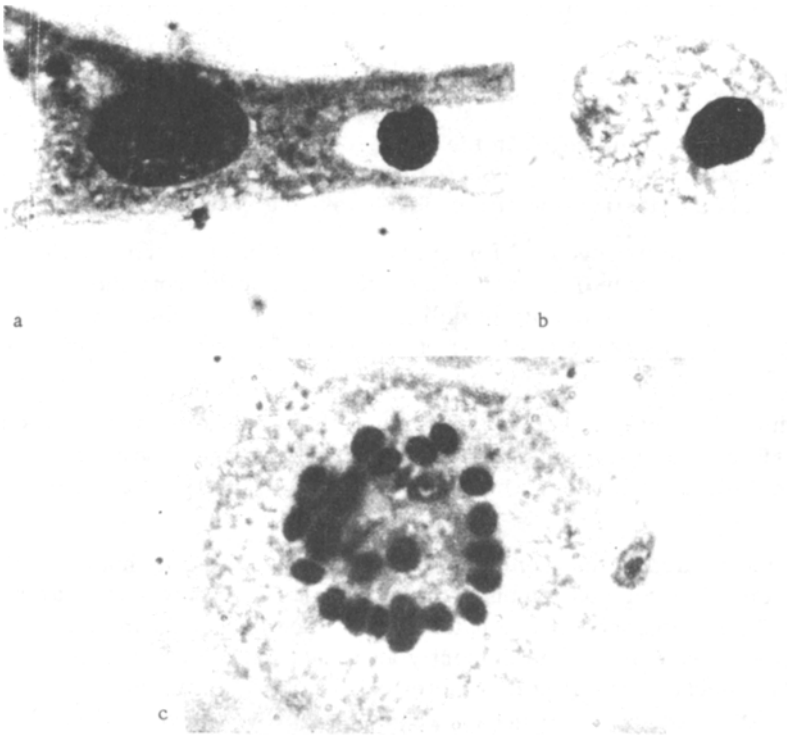

Fig. 2. Various types of transformed cells from synovial fluid of patient with rheumatoid arthritis in culture: a) fibroblast-like cell, phenomenon of incorporation of autolymphocyte; b) cells with vacuolated, frothy cytoplasm and eccentric nucleus; c) giant multinuclear cell. Romanovsky, 900 ×, immersion.

values calculated. The cultures were conventionally divided into three groups: good growth – from 100 cells or more, average growth – from 50 to 100 cells, poor growth – under 50 cells in a field of vision.

Cells from the synovial fluid were cultured from 91 samples taken from 33 patients with rheumatoid arthritis, i.e., synovial fluid was taken twice or three times from the same patient in the course of treatment. As a control, synovial fluid cells were cultured from 30 samples taken from 30 persons with traumatic injury of the joint.

EXPERIMENTAL RESULTS AND DISCUSSION

Most cultures of the control group were characterized by the following dynamics of growth in vitro: Neutrophils died during the first day, lymphocytes remained attached to the surface of the cover slip for 3-5 days, and then also died. The appearance of a few histiocytes and fibroblast-like cells was observed in solitary cases of persons with the diagnosis of posttraumatic hemarthrosis.

The original synovial fluid from the patients with rheumatoid arthritis contained tissue synovial cells and all forms of cells usually present in human peripheral blood, but with an increased number of leukocytes. Neutrophils in culture died during the first 24 h. Monocytes underwent marked transformation. After the first few hours and especially in the first 5 days, disconnected elongated cells with strongly basophilic cytoplasm – cells of the histiocyte type – appeared. Solitary large, elongated or flattened cells with a large oval nucleus, containing several nucleoli, and with palely stained cytoplasm, not giving off any processes, were found. On the basis of these observations and analysis of data in the literature [9] these cells were described as fibroblast-like. They multiplied intensively and formed colonies by the 6th-7th day [9] (Fig. 1). Well marked growth of histiocytes and fibroblast-like cells was observed in the culture growing without change of medium at pH 6.5. If a sufficiently well developed monolayer of histiocytes and fibroblast-like cells was obtained, similar cells of the second generation could be grown by subculture.

Later, large round cells with strongly vacuolated, frothy cytoplasm, not giving off processes, and with an eccentrically situated nucleus, often binuclear or even polynuclear, appeared in the primary culture. The patterns of "fusion" of these mononuclear cells into binuclear and polynuclear were observed. The fraction of polynuclear cells in the population increased appreciably with an increase in the duration of culture. The cultures could be kept viable without subculture for 20-25 days.

In the first days of culture examination in the phase-contrast microscope of native and also of stained preparations showed interaction between lymphocytes and fibroblast-like cells. This took the form of a phenomenon of adhesion and also destruction of the fibroblast-like cells by the lymphocytes (Fig. 2).

A criterion of the normal state, deduced for growth of cells in vitro from patients with traumatic injury to the joint, enabled the efficiency of growth of synovial fluid cells from the patients with rheumatoid arthritis to be assessed at 82%. The intensity of cell growth in culture was shown to depend on the activity of the disease, and a significant decrease ($P < 0.01$) of intensity of growth took place during treatment of the patients.

The presence of intensive growth of fibroblast-like cells, with the formation of colonies in some cases, is evidence of their possible role in the genesis of inflammatory changes and sclerosis of joints. The origin of the fibroblast-like cells is still uncertain at this stage but a solution to this problem must be urgently sought.

The cytopathic action of monocytes from synovial fluid on homologous human embryonic fibroblasts was described in 1964. Peripheral blood lymphocytes of patients with rheumatoid arthritis also possess cytopathic activity [7]. The phenomenon of cytopathic interaction between lymphocytes and fibroblast-like cells observed in the present experiments is evidently an indication of autoimmune conflict in the joint and may perhaps be one of the mechanisms of self-maintenance of the autoimmune process. Our previous findings [8] and the results of the present investigation regarding ability of synovial fluid cells of patients with rheumatoid arthritis to grow in vitro, together with the description of the character of transformation of cell populations in culture have been confirmed in a recently published paper by Swedish workers [12].

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