QUANTITATIVE EFFECTS OF LYMPHOCYTES AND SERUM OF PATIENTS WITH RHEUMATOID ARTHRITIS ON HUMAN EMBRYONIC FIBROBLASTS IN VITRO

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Data concerning the cytotoxic action in vitro of circulating lymphocytes of patients with rheumatoid arthritis on homologous fibroblasts are confirmed and supplemented. The patients' blood serum did not cause destruction of fibroblasts.

Although the main morphological manifestations of rheumatoid arthritis are located in the synovial membranes and joints, lesions of the connective tissues in this disease are frequently systemic in character. At the height of the disease, infiltration of histiocytes and lymphocytes takes place not only into the synovial membranes of joints and the periarticular tissues, but also into connective-tissue septa in muscle tissue, the lungs, and liver, and in the walls of peripheral and coronary vessels. As a rule this inflammatory infiltration is accompanied by fibrinoid swelling, frequently terminating in degeneration and necrosis of connective-tissue fibers and cells infiltrated with lymphocytes [4]. The histogenesis, character, and dynamics of connective-tissue destruction in rheumatoid arthritis are very similar to the well-known cellular immunologic responses which develop after homografting, and to hypersensitivity of delayed (or tuberculin) type [2, 3]. It is thus reasonable to suggest that degeneration of connective tissue in rheumatoid arthritis is due, to some extent, to immune or, more precisely, autoimmune responses of delayed type directed against its components. Such responses are known to be effected by cells of the lymphoid series.

In a previous investigation [5], the cytotoxic action of circulating lymphocytes from patients with rheumatoid arthritis on homologous fibroblast in vitro was demonstrated histomorphologically and by means of a microfilming technique. Degeneration took place in medium No. 199 in the absence of complement.

The object of the investigation described below was to investigate, by a quantitative method, the cytotoxic action of immune lymphocytes from patients with rheumatoid arthritis and to study the possible role of serum antibodies in this effect.

EXPERIMENTAL METHOD

To obtain the required number of lymphocytes and volume of serum, 25 ml blood was taken from the cubital vein of each patient and control donor. The manipulations to isolate the lymphocytes and free them from erythrocytes were carried out in accordance with the recommendations of Walker and Palmer [14] and Pegg [11]. The resulting lymphocytes were suspended in medium No. 199 in a final concentration of $2 \cdot 10^6$ cells/ml.

Human embryonic fibroblasts, cultivated by the usual monolayer method, were taken from flasks 2-3 days after primary explantation by means of 0.02% versene solution. The resulting cell suspension ($2 \cdot 10^5$ cells/ml) in nutrient medium was distributed among flasks (0.5 ml in each). After incubation at 37° for 24 h

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TABLE 1. Effect of Lymphocytes and Blood Serum of Patients with Rheumatoid Arthritis on Homologous Fibroblasts in Vitro

Expt. No.	Number* of intact, living fibroblast ($\times 10^3$) after incubation for 48 h (M±m)							
	in medium No. 199	with lymphocytes of control donors	with lymphocytes of patients with rheumatoid arthritis	destruction of fibroblasts (in %)	with serum of control donors	with serum of patients with rheumatoid arthritis	destruction of fibroblast (in %)
1	27,5±0,7	22,3±2,0	15,6±2,5	31,1†	Not tested	Not tested	—
			1,6±0,7	92,9	"	"	—
2	25,7±1,5	21,0±1,4	7,7±1,1	63,4	23,7±1,1	18,7±1,3	21,1†
			11,2±0,9	46,7		19,2±1,0	19,0†
3	37,2±4,2	20,5±1,2	15,0±1,4	26,9†	23,2±1,4	24,2±4,3	0
4	36,4±3,7	33,1±2,9	27,3±3,1	17,5†	31,4±3,0	34,9±4,3	0
			21,7±1,7	34,5		28,1±2,9	13,4†
5	49,0±3,6	31,7±1,2	19,2±3,0	39,4	39,7±3,6	33,5±1,6	15,7†
			20,2±3,4	36,3		36,2±2,3	8,8†
6	19,8±2,7	15,0±0,8	7,7±1,2	48,7	18,7±1,3	16,2±1,3	13,4†
			15,2±1,4	0,0		19,0±1,7	0
7‡	55,1±6,9	47,1±6,2	27,4±2,9	41,8	Not tested	Not tested	—
8‡	29,5±4,0	31,5±2,5	21,8±1,9	30,8	"	"	—

*Obtained by counting 4 or more flasks.
†Difference not significant ($P > 0.05$).
‡Lymphocytes used from patients with rheumatoid arthritis combined with Sjögren's syndrome.

and attachment of the fibroblasts to the glass, the subculture was washed with Hank's solution, and then the suspension of lymphocytes in medium No. 199 ($2 \cdot 10^6$ cells/flask) or the blood serum (0.2 ml + 0.8 ml medium No. 199 per flask) was added to it. Besides the four control flasks containing fibroblasts in medium No. 199, for each test sample of lymphocytes or serum from patient and donor there were at least four flasks with the same strain of fibroblasts. After incubation for 48 h with lymphocytes or serum the surviving fibroblasts were carefully rinsed with Hanks' solution and then removed from the glass with 0.25% trypsin solution. The number of living cells was counted in a Goryaev's chamber under a phase-contrast microscope after addition of trypan blue in a final concentration of 0.05%.

EXPERIMENTAL RESULTS

Altogether 13 patients with rheumatoid arthritis and evidence of a systemic character of the disease were investigated. Rheumatoid nodules were present in three cases, and the disease followed a severe clinical course in all patients. In two cases rheumatoid arthritis was combined with Sjögren's syndrome. In 9 cases a mixed visceral and articular form of the disease was present. As a rule, in each experiment lymphocytes and serum from 1 control donor and 2 patients were tested. No corticosteroids had been given to the patients for at least 6 days preceding testing.

Destruction of target cells, expressed as a percentage, was determined from the difference between the number of intact, living fibroblasts cultivated together with lymphocytes (or serum) of the control donors, and the number of the same fibroblasts cultivated together with lymphocytes (or serum) of patients with rheumatoid arthritis. The results are summarized in Table 1.

Circulating lymphocytes from patients with rheumatoid arthritis caused statistically significant destruction of fibroblasts in 10 of 13 cases. By contrast, the blood serum of these patients had no significant cytopathic action. Observations under the phase-contrast microscope showed that death of the fibroblasts was accompanied by the simultaneous death of the lymphocytes in contact with them. The more active the disease and the more severe its course, the higher the percentage of fibroblasts killed by the lymphocytes. No clear correlation could be established between the clinical and laboratory findings reflecting individual manifestations of the disease and the degree of cytopathic effect of lymphocytes on fibroblasts.

Morphological studies showed that "contact agglutination" of active lymphocytes around the fibroblasts or precise destruction of fibroblasts did not occur if

the lymphocytes were first frozen (to −40°) and then thawed four times, a procedure which is lethal for them. This method ruled out any possible cytotoxic action of virus-like particles on the culture of fibroblasts.

In investigations [7, 9, 10] of the action of mononuclear cells from the synovial fluid of affected joints and of lymphocytes from the lymph glands of patients with rheumatoid arthritis on homologous human connective-tissue cells in vitro, a cytotoxic effect was observed in fewer cases than in the experiments now described. This difference may be due to differences in the severity of the disease among the group of patients, and also to differences in the number of lymphocytes per fibroblast. In the present experiments the ratio was high (20 : 1). Probably the fact than in these experiments circulating lymphocytes were used is important, because they evidently consist predominantly of small lymphocytes, which are immunologically more active than monocytes from synovial fluid or lymph glands [8].

Comparison between the cytotoxic effect of immune lymphocytes on homologous target cells, as revealed by experiments in vitro on mice [1, 12, 13], and the cytotoxic activity of circulating lymphocytes of some patients with rheumatoid arthritis relative to target fibroblasts suggests that the patients investigated had lymphocytes which were hypersensitive toward tissue-specific antigens of connective-tissue cells. Nevertheless, the cytotoxic effect of cytophilic antibodies, for which the blood lymphocytes can act as passive carriers [6], cannot be ruled out completely.

The results thus suggest that an important role in immunopathological reactions causing destruction of connective tissue in rheumatoid arthritis is played by cellular hypersensitivity, probably brought about by cells of the lymphoid series. It is evident that only living, sensitized lymphocytes possess specific activity relative to connective-tissue cells.

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