ACTION OF RAT LYMPHOCYTES TREATED WITH FREUND'S ADJUVANT ON TISSUE CULTURES OF FIBROBLASTS

V. V. Sura and V. I. Vasil'ev

UDC 616-018.085.23:611-018.21-06:612.112.94

The cytopathic effect of immune lymphocytes in tissue culture has been demonstrated by a number of investigations. The possibility that this effect is organ-specific has not been ruled out. Lymphocytes usually destroy those tissue cultures from which antigens were used to immunize the donors of the lymphocytes. For instance, the lymphocytes of rats immunized with brain tissue together with Freund's adjuvant have a cytopathic action on the glial cells of puppies' brain tissue [8], and the lymphocytes of rats immunized with kidney tissue destroy cultures of rat kidney tissue [7], while lymphocytes of rats immunized with thyroid tissue have a cytopathic action on tissue cultures of the thyroid gland [5].

Lymphocytes of recipients of skin and kidney homografts have a cytopathic action on cultures of the donor's tissue cells [6, 11, 15]. The lymphocytes of animals receiving the adjuvant without tissue antigens have no cytopathic action, even on fibroblasts growing in the culture together with the glial cells, following intradermal injection of the adjuvant into the donors of the lymphocytes [8]. If the adjuvant is injected intraperitoneally without kidney antigen, the lymphocytes have no cytopathic action, or only a very slight action, on kidney tissue cultures [7]. Meanwhile, the immunomorphological changes [9], the phenomena of autoerythrophagocytosis [1, 3], and the possibility of obtaining "adjuvant" arthritis and of transferring it to a healthy recipient by the lymphocytes of an affected animal [10, 14], suggest the development of an autoagressive process as a result of the action of adjuvant on the animal without tissue antigens, and also that lymphocytes participate in this process.

The object of the present investigation was to examine the role of the lymphocytes in "adjuvant" diseases.

EXPERIMENTAL METHOD

Experiments were carried out on 36 noninbred albino rats, 17 of which received intraperitoneal injections of Freund's adjuvant by the method described previously [3]. All the animals received 4 injections of adjuvant. On the 8th-12th day after the last injection the animals were sacrificed by bleeding from the femoral vessels and the inguinal, axillary, and cervical lymph glands were removed in sterile conditions. These times were probably the most suitable for removing the lymph glands [7]. In the control series, and to determine the effect of lymphocytes on the tissue culture of fibroblasts, lymphocytes obtained from the lymph glands of 19 healthy intact animals were used. The lymph glands were freed from fatty cellular tissue and capsule, and ground in a homogenizer with the addition of medium No. 199. The resulting homogenate was filtered through four layers of gauze, the lymphocytes were washed off once in Earle's solution, suspended in medium No. 199, stained with 0.1% trypan blue solution, and the relative proportion of living cells was determined. Next, in doses of between 2 and 6 million living lymphocytes, the cells were transferred into medium-sized (25 mm in diameter) and larger (50 mm

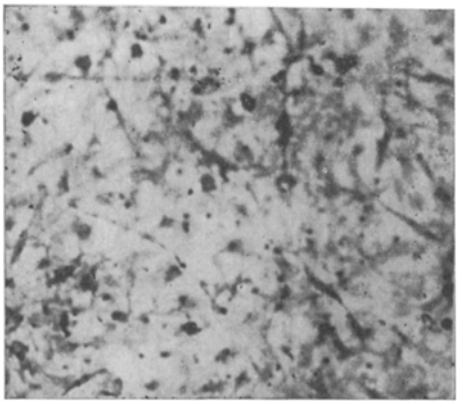

Fig. 1. Lymphocytes of control rat in a culture of fibroblasts. No cytopathic effect present. Stained with hematoxylin. Magnification: objective 9 ×, ocular 6 ×.

Group of Active Members of the Academy of Medical Sciences of the USSR E. M. Tareev, Laboratory of Experimental Biology, Number 24 City Hospital; Laboratory of Virology, Institute of Experimental and Clinical Oncology, Academy of Medical Sciences of the USSR, Moscow (Presented by Active Member of the Academy of Medical Sciences of the USSR E. M. Tareev). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 64, No. 10, pp. 70-73, October 1967. Original article submitted October 20, 1966.

Results of Action of Rat Lymphocytes on Tissue Culture of Fibroblasts

Rat no.	Lymphocytes in culture, injection date	No. of flasks (exper.)	No. of lymphocytes in flask (millions)	Cytopathic effect 1st day	2nd day	3rd day	4th day
6	7/V	8	2—5	+—	++	+++	++++
1		8	2—5	+—	++	+++	++++
Control		8	2—5	—	—	—	+—
5	30/V	3	3—4	+—	++	+++	++++
Control		4	3—4	—	—	—	—
4	6/VI	9	3—5	+—	++	+++	+++
Control		5	3—5	—	—	—	—
9	11/VI	4	3—5	+—	++	+++	+++
Control		5	3—5	—	—	—	—
7	17/V	4	3—6	—	—	—	+—
10		4	3—6	—	—	—	+—
12		4	3—6	+	+	+	++
Control		4	3—6	—	—	—	—
18	18/VI	4	3—6	—	—	—	—
Control		4	3—6	—	—	—	—
17	24/VI	5	6—10	—	—	—	—
Control		4	6—10	—	—	—	—
19	25/VI	4	6—10	—	—	—	—
Control		4	6—10	—	—	—	—
13	29/VI	4	8—10	+	++	+++	++++
14		4	8—10	+	++	+++	++++
21		4	8—10	+	++	+++	++++
23		4	8—10	+	++	+++	++++
25		4	8—10	+	++	+++	++++
2		4	8—10	+	++	+++	++++

Legend: — cytopathic effect absent, + slight effect, ++ moderate effect, +++ considerable effect, ++++ strong effect present.
Note. The experiments were carried out in 1966.

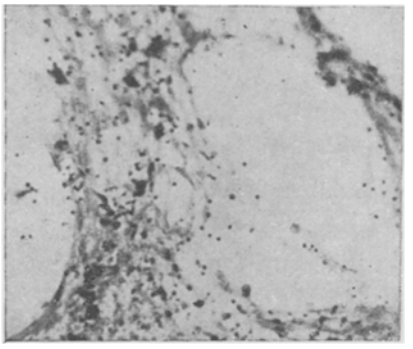

Fig. 2. Culture of fibroblast tissue with addition of lymphocytes from experimental rats. Distinct breakdown of layer appears — "honeycomb" culture. Hematoxylin stain. Magnification: obj. 6 ×, oc. 6 ×.

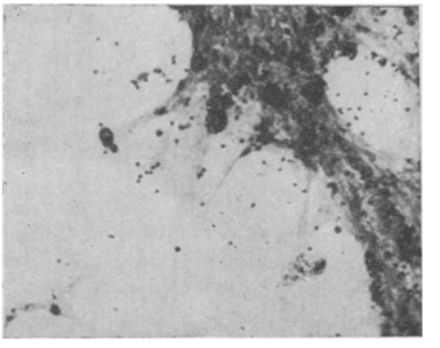

Fig. 3. Lymphocytes from experimental rats in fibroblast culture. Further disintegration of layer. Fourth day of observation. Partial retention of cells is shown. Hematoxylin stain. Magnification: obj. 6 ×, oc. 6 ×.

in diameter) Carrel's flasks with tissue cultures of fibroblasts obtained from albino rat embryos aged 8-12 days was used. The cells, obtained by trypsinization, were suspended in medium No. 199 with the addition of 20% inactivated bovine serum, and the resulting suspension was poured into the Carrel's flasks. After the formation of a monolayer, lymphocytes in medium No. 199 were introduced into the flasks along with the replacement medium.

EXPERIMENTAL RESULTS

Two series of observations were made. In series I (control, 11 animals) the action of lymphocytes from healthy intact animals on the tissue cultures was investigated. The behavior of the fibroblast culture

with and without addition of lymphocytes was compared. At the same time, during this experiment the optimal number of lymphocytes to be added to the tissue culture was determined. Altogether 66 investigations were made in the control series. It was found that the addition of lymphocytes from rats of the control group to a tissue culture of fibroblasts produces no changes in the culture. The lymphocytes freely flowed from the cells of the monolayer; no sign of contact agglutination or cytopathic effect was observed (Fig. 1). Addition of large numbers of lymphocytes (more than 6 million) into the middle-sized and more than 10 million into the large Carrel's flasks made it difficult to check the culture visually.

The results of the experiments of series II are given in the table. They show that a marked cytopathic effect was present in 11 animals, a moderate or doubtful effect in 3, and no effect whatever in 3 animals. In the control series a doubtful cytopathic effect was observed in 1 animal and absence of such effect in the other 7. In cases of a positive cytopathic effect, by the end of the 1st day an intensive contact agglutination of lymphocytes was observed on the cells of the culture, followed by destruction of the monolayer, usually in the form of "honeycombing" (Fig. 2), and by the end of the 4th day, in cases where the cytopathic effect was marked, destruction of the whole cell layer was observed; only single fibroblasts remained (Fig. 3).

The results obtained show that as a result of prolonged immunization with Fruend's adjuvant, lymphocytes acquire the ability to produce a cytopathic effect in a tissue culture of fibroblasts. The absence of cytopathic effect in 3 animals and its slight severity in another 3 can hardly be attributed to the experimental conditions, which were standard, although it is impossible to rule out completely the possibility of elusive technical errors. At the same time, it must be stressed that the reaction of the animals to intraperitoneal injection of the adjuvant may sometimes be very individual [3], as the results of the authors' previous observations showed, and this is probably reflected in the differences between the cytopathic action of lymphocytes from individual rats. The cytopathic action of the lymphocytes in these experiments was evidently due to autoimmunization processes arising as a result of absorption of products of inflammation and tissue destruction from the abdominal cavity against the background of repeated injections of the adjuvant, although a nonspecific cytotoxic effect cannot be ruled out. It may be postulated that the difference between these results and those obtained by other authors [8], who observed no cytopathic action of rat lymphocytes on fibroblasts after intradermal injections of the adjuvant, may be attributed to the mode of injection, because if the adjuvant is given by intradermal injection, the conditions for development of autosensitization are probably more limited. So far as the differences between the present results and those obtained by injecting the adjuvant without tissue antigens intraperitoneally, in which case no cytopathic action of the lymphocytes was obtained [7], are concerned they may perhaps be explained by the fact that the target cells consisted of a culture of kidney tissue and not of fibroblasts, as in the present experiments. This hypothesis requires experimental verification.

It is interesting to compare some phenomena observed following repeated intraperitoneal injections of adjuvant—the lupus-cell phenomenon, the appearance of antibodies against DNA, and of vasculitis, the "wire loop" sign in the kidneys [2-4], and finally, the cytopathic effect of the lymphocytes in the fibroblast culture—with the changes observed in systemic lupus, including the cytopathic action of the lymphocytes of such patients on tissue cultures of fibroblasts [13]. Possibly these phenomena may be based on similar pathogenetic mechanisms.

LITERATURE CITED

1. G. V. Kovalevskii, Zh. Mikrobiol., No. 8, 117 (1965).
2. V. V. Sura and A. M. Vikhert, In the book: Problems of Immunopathology in Clinical and Experimental Conditions [in Russian], Moscow (1966), p. 216.
3. V. V. Sura, V. A. Kolaev, and I. V. Konstantinova, Byull. Éksp. Biol., No. 9, 97 (1964).
4. V. V. Sura and Yu. V. Postnov, Byull. Éksp. Biol., No. 8, 118 (1965).
5. T. G. Trayanova, V. V. Sura, and G. J. Svet-Moldavsky, Lancet, Vol. 1 (1966), p. 452.
6. A. Börklund, Lab. Invest., Vol. 13 (1964), p. 120.
7. A. Govaerts, J. Immunol., Vol. 85, p. 514.
8. G. Holm, Clin. Exp. Immunol., Vol. 1 (1966), p. 45.
9. H. Koprowski and M. V. Fernandes, J. Exp. Med., Vol. 116 (1962), p. 467.
10. A. Laufer, C. Tal, and A. J. Behar, Brit. J. Exp. Path., Vol. 40 (1959), p. 1.
11. C. M. Pearson and F. D. Wood, J. Exp. Med., Vol. 120 (1964), p. 547.
12. J. A. Sharp and R. G. Burwell, J. Anat., Vol. 96 (1962), p. 457.
13. J. W. Steiner, B. Lancer, and D. L. Schatz, Arch. Path., Vol. 70 (1960), p. 424.
14. B. H. Waksman and C. Wenersten, Int. Arch. Allergy, Vol. 23 (1963), p. 129.
15. D. B. Wilson, J. Cell A. Compar. Physiol., Vol. 62 (1963), p. 273.