EFFECT OF ULTRASOUND ON CELLULAR AND HUMORAL FACTORS OF IMMUNITY

E. P. Moskalenko, L. P. Sizyakina,
and E. N. Sologub

UDC 612.017.1.014.45

KEY WORDS: ultrasound; immunity; stimulation.

The current importance of immunocorrection will be evident from the progressive increase in incidence of diseases such as cancer, septicemia, and chronic and indolent infections developing against a background of secondary immunodeficiency states. Among methods used to stimulate function of the immune system, the measured use of certain physical factors is promising [1, 8]. However, information on the action of ultrasound on the immune system is fragmentary and contradictory [4, 7], since investigators have used different conditions of ultrasonic treatment.

The object of this investigation was to study the biological effects of low-frequency ultrasound on the immune system.

EXPERIMENTAL METHOD

CBA mice (300 animals) were treated by a single exposure to ultrasound with a frequency of 65 kHz, amplitude of oscillations of 3 μ, duration 20, 40, and 60 sec, under continuous conditions. The region treated with ultrasound was the spleen and the contact medium was degassed mineral oil. Intact mice kept on the standard animal house diet served as the control. The animals were killed on the 1st, 3rd, 5th, 7th, 14th, 21st, 28th, and 35th days after ultrasonic treatment. Complement activity was determined by the 50% hemolysis test [2], lysozyme by the method in [3], properdin activity as in [5], and the number of T and B rosette-forming lymphocytes (T- and B-RFL) in the peripheral blood by the spontaneous rosette-formation method [6]. Morphological and functional changes in the lymphoid organs under the influence of ultrasound were studied by analysis of sections through the spleen, thymus, and lymph nodes, stained with hematoxylin and eosin and by Brachet's method. Mice treated with ultrasound also were vaccinated (1 I.U. of *Bordetella pertussis* strain 688 in a volume of 0.1 ml intraperitoneally). The state of antipertussis immunity was studied by determining antibodies in the passive hemagglutination test (the specificity of the reaction was confirmed by the passive hemagglutination inhibition test). The number of rosette-forming cells (RFC) was studied by Salberg's method. Antibody titers are given in logarithmic units. Statistical analysis of the data was carried out by Student's t test.

EXPERIMENTAL RESULTS

The experiments showed that the rise of serum lysozyme activity after treatment with ultrasound was fluctuating in character and reached peaks on the 3rd and 7th days, followed by a fall on the 14th day and subsequent return to normal (Fig. 1). The greatest difference compared with the initial level was observed in animals exposed for 40 and 60 sec ($P < 0.05$). Definite changes also were found in the activity of complement, which also followed a fluctuating course with the greatest rise on the 3rd and 14th days and normalization toward the 21st day ($P < 0.05$). These features of the time course of complement activity were found in animals exposed to ultrasound for all durations. The time course of properdin activity was characterized by two statistically significant increases ($P < 0.05$) on the 3rd and 14th days, followed by a return to normal. The changes mentioned above could be detected most clearly in the serum of mice exposed to ultrasound for 40 sec.

Rostov Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR N. V. Vasil'ev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 95, No. 5, pp. 75-78, May, 1983. Original article submitted January 19, 1982.

 0007-4888/83/9505-0658$07.50

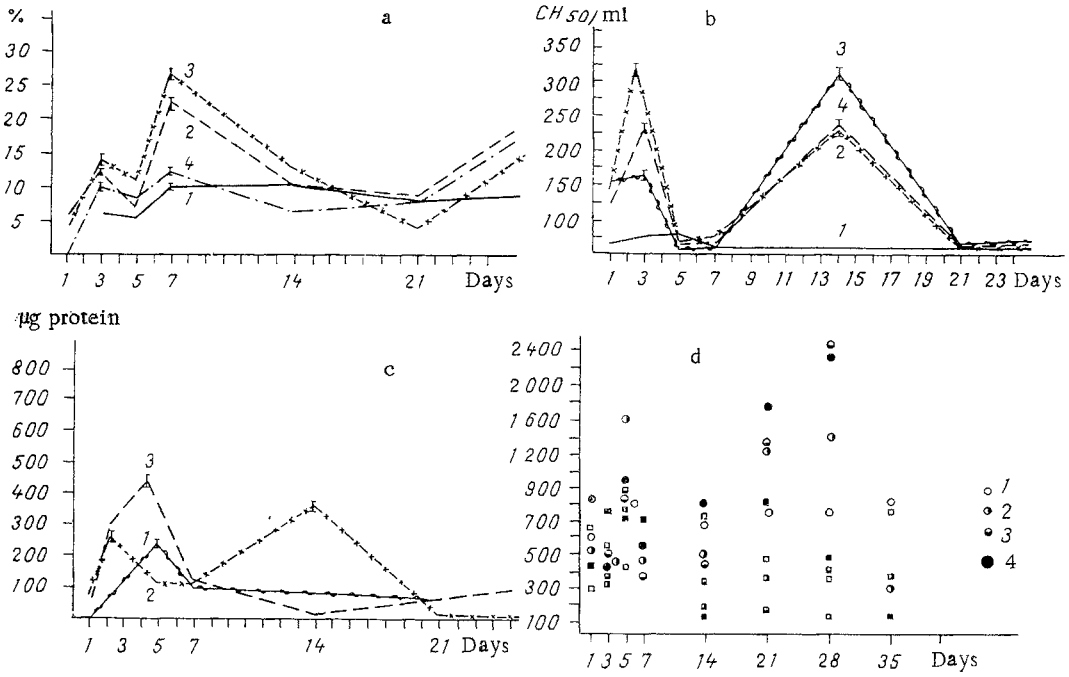

Fig. 1. Time course of lysozyme activity (a), complement titer (b), and properdin concentration (c) in blood serum and number of T- and B-RFL in peripheral blood of "sonicated" mice. a, b: 1) Control; 2) exposure 60 sec, 3) 40 sec, 4) 20 sec; c: 1) exposure 60 sec, 2) 40 sec, 3) 20 sec; d: circles — T-RFL, squares — B-RFL; 1) control, 2) exposure 60 sec, 3) 40 sec, 4) 20 sec.

To analyze the effect of low-intensity ultrasound on the ratio between populations of T and B cells, the time course of the number of T- and B-RFL in the peripheral blood was studied (Fig. 1).

In the early stages after sonication (until the 5th day) there was a statistically significant increase in the number of B lymphocytes, followed by a marked decrease toward the 14th day, and a second increase, smaller than the first, and found only after treatment with ultrasound for 60 sec. Meanwhile changes in the number of T-RFL in the early stages of the investigation were small (the only exceptions were animals exposed to ultrasound for 20 sec). However, on the 28th day after a single exposure to ultrasound a marked and statistically significant increase was observed in the number of T-RFL, changing to a decrease on the 35th day.

Analysis of the histological structure of the lymphoid organs revealed changes of similar type, namely dilatation of the arterial and venous vessels and a marked decrease in the number of lymphocytes in both T- and B-dependent zones during the first week of the investigation (Fig. 2).

Later marked hyperplasia of cells of the T-dependent zones was observed both in the spleen and the lymph nodes, together with signs of increased migration of lymphocytes, confirmed by changes in the endothelial lining of blood vessels in the paracortical zone of the lymph nodes; the structure of the remaining zones had no particular features. This effect still continued 1 month after sonication (Fig. 2).

After a single exposure to low-intensity ultrasound activation of nonspecific defensive factors and also structural changes in the cellular component of the immune system were thus discovered.

To investigate the reorganization of the immune system under the influence of ultrasound the intensity of immunogenesis in response to injection of an antigen was studied. On the 7th day antibody titers in previously sonicated animals were found to be much higher than titers in control animals, namely 2.4 and 1.2 logarithmic units, respectively. In mice of both groups, a tendency then occurred for the titers to fall and on the 14th day the antibody titer of the experimental mice was 1.8 and of the control mice 0.9. Intensification of antibody formation in response to injection of T-dependent antigen in previously sonicated animals al-

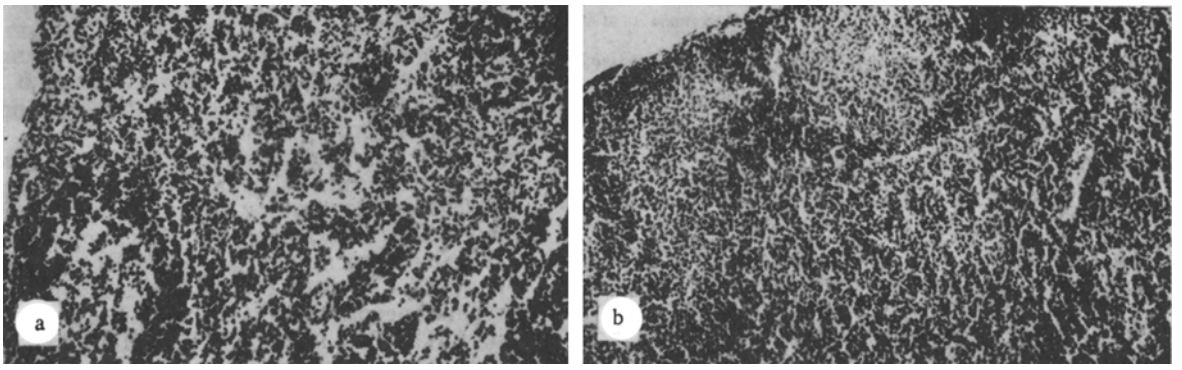

Fig. 2. Histological structure of lymph nodes of mice exposed to ultrasound: a) 7 days after sonication, 200 ×; b) 14 days after sonication, 100 ×. Hematoxylin—eosin.

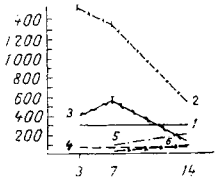

Fig. 3. Time course of T- and B-RFL in sonicated mice immunized with pertussis vaccine. 1) Control (T-RFL); 2) ultrasound + immunization (T-RFL); 3) immunization (T-RFL); 4) control (B-RFL); 5) ultrasound + immunization (B-RFL); 6) immunization (B-RFL). Abscissa, time (in days); ordinate, absolute number.

so was confirmed by the number of RFC in the experiment and control series on the 7th day ($102.5 \cdot 10^6$ and $49 \cdot 10^6$, respectively). Later during the investigation the difference between the numbers of RFC in the experimental and control mice disappeared. Analysis of the number of T- and B-RFL in the peripheral blood showed that in previously sonicated and immunized animals the number of T-RFL was 2.5 times higher than in immunized mice, whereas the number of B-RFL showed no significant change (Fig. 3).

Analysis of the data thus shows that low-intensity ultrasound gives rise to an immunostimulant effect. Evidence in support of this conclusion is given by changes in factors of natural resistance, the increase in the number of T-RFL in the peripheral blood, hypertrophy of the T-dependent zones in the lymph nodes and spleen, and the intensification of antibody formation which could arise in response to antigenic loading in sonicated animals. The less marked changes, which persisted for a long time (1 month after a single exposure to ultrasound), incidentally, affected the T system of immunity. Reorganization of the immune system in the early stages after sonication was probably due to its stressor effect, whereas the mechanisms of its influence on the immune system leading to a lasting effect in the later stages after sonication still require elucidation. Possibly ultrasound, as a stressor of nonantigenic nature, may not only change immunologic reactivity of the animal but may itself induce reorganization of the immunocompetent system in the same way as antigenic stimuli [1].

LITERATURE CITED

1. N. V. Vasil'ev and L. F. Roginich, The Effect of Magnetic Fields on Infection and Immunity [in Russian], Tomsk (1973).
2. A. B. Gabrilovich and S. V. Soboleva, in: Izvest. Rostov. Nauch.-Issled. Inst. Epidemiol., Mikrobiol., Gig., 23, 138 (1962).
3. V. G. Dorofeichuk, Lab. Delo, No. 1, 28 (1968).
4. V. N. Kuznetsov, in: Mechanisms of Injury, Resistance, Adaptation, and Compensation [in Russian], Vol. 2, Tashkent (1976), p. 252.
5. G. M. Larionov, in: Problems in the Infectious Pathology of Transbaikalia [in Russian], No. 3, Part 2, Chita (1972), p. 302.

6. R. V. Petrov, M. A. Stenina, and K. A. Lebedev, Byull. Éksp. Biol. Med., No. 2, 197 (1976).
7. D. W. Anderson and J. L. Barress, Clin. Immunol. Immunopath., 14, 18 (1979).
8. J. F. Fry, Trans. IEEE, 67, No. 4, 190 (1979).

EFFECT OF SPECIFIC ALLOANTISERUM AGAINST T SUPPRESSORS ON RESISTANCE OF MICE TO TUBERCULOSIS

A. S. Apt, Z. P. Abramova, A. M. Moroz, B. V. Nikonenko, and M. M. Averbakh

UDC 616-002.5-092:612.112.94.017.1]: 612.6.052

KEY WORDS: alloantiserum; sensitivity to tuberculosis.

The writers showed previously that mice of the inbred line I/St are highly susceptible to infection with tuberculosis, whereas those of inbred line A2G are resistant [2]. Parameters of immunity to tuberculosis, such as the level of the delayed-type hypersensitivity (DTH) reaction and of antibody synthesis to tuberculin, were opposite in mice of these lines at the same times after infection: a high level of DTH and a low level of antibodies in A2G mice and the opposite picture in I/St mice [1]. These data confirm the earlier concept [3] of immunological causes of sensitivity and resistance to tuberculosis and the protective role of cellular immunity in this disease.

Immunologic reactions to any antigens, including intracellular pathogenic agents, are under the control of interacting immunocompetent cells [9], among which a special place is occupied by the various subpopulations of T suppressors. After the surface marker of T suppressors had been established — a product of the I-J subregion of the H-2 complex [11], the use of alloantisera against allelic products of the I-J locus (loci) yielded interesting data on the part played by suppressor I-J-positive cells in antitumor immunity [6, 7]. However, this approach has not yet been used to study immunity against infection.

This paper describes a method of obtaining alloantisera against the product of the I-J locus and gives the results of experiments to study the effect of these antisera *in vivo* on survival of mice after infection with tuberculosis.

EXPERIMENTAL METHOD

Inbred and congeneic recombitant mouse lines A2G, A/Sn, A.TL, B10.A(5R), and B10.HTT were reared in the nursery of the authors' Institute. DBA/2 mice used to obtain hybrid recipients were obtained from Z. K. Blandova (Research Laboratory of Experimental Biological Models, Academy of Medical Sciences of the USSR, Moscow Province), and the B10.A(3R) mice were obtained from B. D. Brondz (All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow), to whom the writers are grateful.

Preparation of the alloantisera took place in two stages. First, blast cells were obtained from donors' thymus cells by stimulation with concanavalin A (con A) *in vitro*. By this procedure it is possible for I-J antigen to appear on the cell surface and, in addition, con A induces preferential proliferation of T suppressors [5]. Second, the recipients were immunized with the blast cells thus obtained. The stages are so described below.

Con A-blasts were obtained as follows. In B10.A(5R) and B10.A(3R) mice aged 8-10 weeks cells were isolated from the thymus by pressing the gland through a stainless steel sieve with pore diameter 100 mesh, by means of a Teflon pestle. This stage, and also subsequent rinsing of the cells twice, were carried out in medium 199 (Institute of Poliomyelitis and Virus Encephalitis, Academy of Medical Sciences of the USSR, Moscow), containing 10% heat-inactivated (56°C, 45 min) embryonic calf serum (Flow Laboratories, England) and 15 mM HEPES (from the same firm). After rinsing the cells were transferred to culture medium of the fol-

Central Research Institute of Tuberculosis, Ministry of Health of the USSR, Moscow.
(Presented by Academician of the Academy of Medical Sciences of the USSR L. K. Bogush.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 95, No. 5, pp. 78-81, May, 1983. Original article submitted June 23, 1982.

