

# PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

## ON THE REASONS FOR THE PROTECTIVE ACTION OF BACTERIAL ANTIGENS IN ACUTE RADIATION SICKNESS

M. I. Ravich-Shcherbo and L. G. Prokopenko

From the Department of Biochemistry of the Kursk Medical Institute

(Presented by Active Member of the AMN SSSR N. N. Zhukov-Verezhnikov)

Translated from *Byulleten' Éksperimental'noi Biologii i Meditsiny*, Vol. 56, No. 12, pp. 36-38, December, 1963

Original article submitted December 21, 1962

The works of a number of investigators [3,4,9], have noted a protective action of bacterial antigens, and also foreign sera, in acute radiation sickness. In our investigations [5,6], two- and threefold immunization with B. paratyphi B vaccine, carried out prior to irradiation of rabbits with roentgen rays in a dose of 600 or 1000 R, prevented the inhibiting action of penetrating radiation on the synthesis of antibodies in response to the proteins of human serum, injected after the irradiation.

The purpose of this work was to study the reasons for the protective action of bacterial antigens on the production of antibodies against human serum proteins, associated with irradiation of the animals in a dose of 1000 R.

### EXPERIMENTAL METHOD

The investigations were carried out on rabbits, irradiated with the  $\gamma$ -rays of  $\text{Co}^{60}$  in a dose of 1000 R.

In the first series of experiments, the animals were injected subcutaneously, in the right thigh, with 500 million microbial bodies of B. paracoli, 48 h prior to irradiation; then, after 24 h, immunization was begun with the proteins of human serum, carried out in two injections with a 10 day interval in between, the injections being given subcutaneously into the left thigh.

In the second series of experiments, immunization with both antigens was carried out according to the same schema, but using the same extremity for both.

The third series of experiments differed from the first by a double injection of the vaccine of B. paracoli, 12 and 2 days before the irradiation.

The control consisted of rabbits that were irradiated with a dose of 1000 R and, after 24 h, were twice immunized subcutaneously in the right thigh with the two antigens simultaneously.

The rabbits were sacrificed in the period when antibodies are being formed with maximum intensity, i.e., 48 h after the second immunization with human serum proteins.

We investigated the serum and extracts of remote and regional lymph nodes, the spleen, the lungs, and the liver. Extracts of the parenchymatous organs were prepared according to the method developed in the laboratory under the direction of S. Ya. Kaplanskii [2], and the extracts of the lymph nodes, by the method of Demling et al. [7]. Quantitative measurement of the antibodies was performed by using the serological reactions of agglutination, ring precipitation, and passive hemagglutination. Total protein in the serum and extracts was determined by the method of Lowry, the protein fractions, by the method of paper electrophoresis in veronal-medinal buffer (pH 8.6, ion strength of 0.1). The plasmocyte count was carried out in lymph node impressions, stained with azure and eosin and methyl green pyronine.

### EXPERIMENTAL RESULTS

The concentration of antibodies against Bact. paracoli that were formed up to the moment of irradiation did not change after  $\gamma$ -irradiation. Agglutination against B. paracoli, in the rabbits of the first and second series of experiments, was observed only in the lymph nodes that were regional to the site of immunization (Fig. 1A). Antibodies

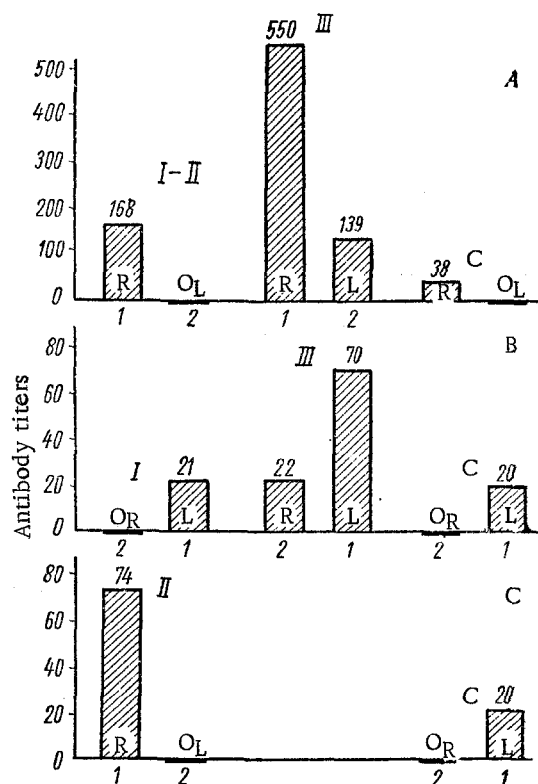


Fig. 1. The titers of antibodies against *B. paracoli* and human serum proteins in the lymph nodes (mean data). A) Antibody titers against *B. paracoli*; B and C) antibody titers against human serum proteins; I, II, and III) ordinal number of the series of experiments; C) control. 1) lymph node regional to the site of application of the given antigen; 2) remote lymph node; R) right extremity; L) left extremity.

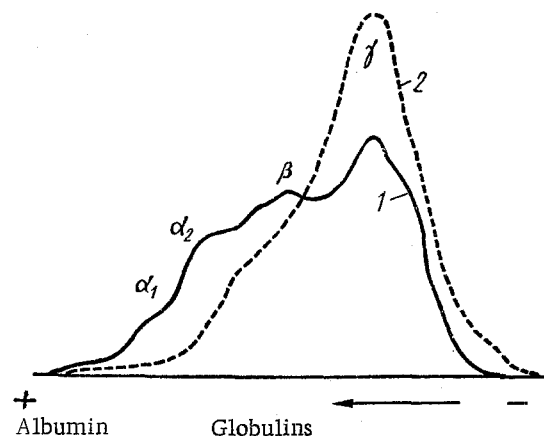


Fig. 2. Electrophoregrams of the proteins of lymph node extracts. 1) Before immunization; 2) after immunization.

against human serum proteins, in the animals of all the series of experiments, were noted only in the serum and the regional lymph nodes. The extracts from the remaining organs were serologically inactive. Synthesis of precipitins in the rabbits of the first series of experiments was as inhibited as in the control animals. Thus, the precipitin titers in the serum of the control rabbits were equal to an average of 1:4, and in the regional lymph nodes, 1:20; in the animals of the first series of experiments, the antibody titers were correspondingly 1:2 and 1:21 (Fig. 1B). The formation of antibodies against human serum proteins occurred much more intensively in the rabbits of the second series of experiments (the titers were 1:22 and 1:74 respectively) (Fig. 1C). The same concentration of precipitins was noted in the animals of the third series of experiments. Agglutinins were determined in them in both the regional and remote lymph nodes (see Fig. 1A).

The performed investigations show that the protective action of preliminary immunization is manifested locally in the lymph nodes, which are producing antibodies against the antigen injected prior to irradiation.

It is essential to note that the "electrophoretic homogenization" of the proteins in the extracts of regional and remote lymph nodes was manifested equally in all series of the experiments (Fig. 2).

Study of the plasmocyte reaction showed that the number of plasmocytes in the regional lymph nodes of the singly immunized rabbits was 4.4 times greater than the number in the remote lymphatic apparatus. After twofold vaccination with *B. paracoli* (third series of experiments), the number of plasmocytes rose both in the regional and in the remote lymph nodes. Immunization with the second antigen after irradiation did not show any essential influence on the plasmocyte reaction (Fig. 3).

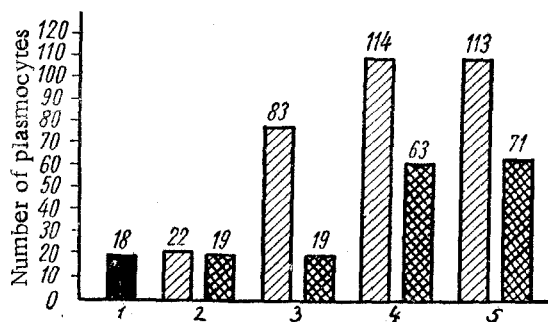


Fig. 3. Plasmocytic reaction in the lymph nodes (mean data). Light columns) regional lymph nodes; dark) remote lymph nodes; black column) lymph node of intact rabbits (1); 2) irradiated and immunized rabbits; 3) rabbits singly immunized before irradiation; 4) rabbits doubly immunized before irradiation; 5) rabbits doubly immunized before and after irradiation with different antigens.

The obtained data permit the assumption that the reason for the protective action of preliminary immunization is the stimulation of plasmocyte formation which it causes; as we know, these cells are at a high level of differentiation [1], and are more radioresistant than their precursors. Thus, they retain the capacity to produce antibodies in response to antigenic stimulation applied after irradiation. Our conclusions are in accord with the observations of other investigators [8], who noted the retention of plasmacytes during marked reduction in the amount of lymphoid elements within animals that were subjected to roentgen irradiation.

#### SUMMARY

By comparing the dynamics of antibody accumulation and plasmocytic reaction in the regional and remote lymph nodes of irradiated rabbits, immunized prior to the irradiation with Bact. paracoli vaccine a conclusion was drawn that the cause of protective effect of preliminary immunization lies in the increased number of plasmacytes in the lymph nodes caused by this immunization.

#### LITERATURE CITED

1. A. M. Igonin, Byull. éksp. biol., 8, 110 (1959).
2. S. Ya. Kaplanskii, O. B. Kuzovleva, and V. D. Uspenskaya, Biokhimiya, 4, 469 (1956).
3. N. N. Klemparskaya, Med. radiol., 3, 85 (1958).
4. V. G. Kulikova, N. V. Luchnik, N. V. Timofeev-Risovskii, et al., Works of the Institute of Biology, Ural Branch, Academy of Sciences, USSR [in Russian], 9, 107, Sverdlovsk (1957).
5. M. I. Ravich-Shcherbo and L. G. Prokopenko, Zh. mikrobiol., 12, 68 (1960).
6. M. I. Ravich-Shcherbo and L. G. Prokopenko, Trudy Kursk. med. in-ta, 15, 320 (1961).
7. L. Demling, H. Kinzleier, and N. Henning, Z. ges. exp. Med., 122, 416 (1954).
8. W. Ehrlich, D. Drabkin, and C. Forman, J. exp. Med., 90, 157 (1949).
9. J. B. Graham and R. M. Graham, Proc. nat. Acad. Sci., 35, 102, Wash. (1949); Cancer, 3, 709, Philad. (1950).

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. Some or all of this periodical literature may well be available in English translation. A complete list of the cover-to-cover English translations appears at the back of this issue.