STIMULATION OF IMMUNOGENESIS WITH THE LIPOPOLYSACCHARIDE PYROGENAL BY THE SCHWARTZ–BRAUN METHOD

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Experiments on rabbits using the method of determining antibody-forming cells suggested by Schwartz and Braun (Olitzki's modification) showed that the bacterial lipopolysaccharide pyrogenal stimulated proliferative processes taking place during immunization. This leads to an increase in the total number of antibody-forming cells in the spleen of rabbits immunized intraperitoneally with corpuscular typhoid vaccine and to an increase in the titers of O-antibodies.

In earlier investigations the writers discovered and studied the stimulant action of the bacterial lipopolysaccharide pyrogenal on antibody production in rabbits immunized parenterally with corpuscular typhoid vaccine [1, 2]. The object of the present investigation was to study the action of pyrogenal on antibody-forming cells in the spleen of rabbits immunized intraperitoneally with corpuscular vaccine obtained from Salmonella typhi strain Ty-4446.

EXPERIMENTAL METHOD AND RESULTS

Experiments were carried out on 81 male chinchilla rabbits weighing 2.2-3 kg. Each group contained on the average 4-5 animals. The rabbits were immunized with a dose of vaccine equal to 4-5 billion bacterial cells/kg and pyrogenal was given in a dose of 40-50 μg/kg. The rabbits were sacrificed 1, 3, 5, 7, 10, 15, 20, and 30 days after immunization. The spleen was removed and blood taken at the same time by cardiac puncture for determination of O-antibodies in the serum by the hemaglutination inhibition test. Antibody-forming cells (AFC) in the spleen were determined by the method of Schwartz and Braun in Olitzki's modification, based on counting the number of cells producing antibodies against Salmonella typhi by counting the zones of lysis appearing in agar seeded with this microorganism [7, 8].

These zones were formed as a result of the action of antibodies liberated by the corresponding lymphoid tissue cells and of exogenous guinea-pig complement. The total number of spleen cells was counted in a Goryaev's chamber. The titers of the O-antibodies were determined in the serum and also in homogenates of spleen tissue diluted 1:10. For the calculations all the titers were expressed as reciprocals. All indices except the weight of the spleen were calculated as logarithms to base 10 and subjected to statistical analysis.

The following results were obtained: the number of AFC, calculated per million spleen cells of the control group of animals,increased gradually to reach a maximum of 15 cells on the 20th day. The number of AFC in the whole spleen had two maxima on the 3rd-5th (60,000-77,000 cells) and 15th days (129,000 cells) after immunization. The largest total number of cells in the spleen of the control group of rabbits, namely 16×10^9 cells, was found on the 4th day after injection of the antigen. By the 10th day these figures had returned to their orginal level. The titers of O-antibodies determined both in the serum and in the homogenates also had two maxima (on the 10th and 20th days). Injection of pyrogenal did not increase the maximal percentage of AFC in the rabbit spleen. However, in the experimental group the maximum was ob-

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served on the 4th day, after which the level fell until it rose again to another maximum. Similar changes were observed when the total number of AFC was determined in the spleen of this group of rabbits. On the 4th day after immunization the total number of cells producing antibodies in the spleen of the experimental rabbits was 725,000, compared with only 62,000 in the control. The level of the second maximum of AFC in the stimulated group, it will be noted, was only one-fifth as high as the first and no difference from the control group could be detected at this period.

On the 30th day injection of antigen together with the lipopolysaccharide led to a slower decrease in both the relative percentage and total number of AFC in the spleen of the rabbits. The titers of O-hemaglutinins in the blood serum of the experimental group were 6-40 times higher than the corresponding control values on the 3rd-7th day after immunization.

The stimulant action of pyrogenal was not limited to the AFC, but the initial increase in number of these cells was accompanied by a general increase in the number of cells (up to 100×10^9) and in the weight of the spleen (by 0.7 g). Injection of pyrogenal alone, without vaccine, had no effect either on the number of AFC or on the total number of spleen cells.

It can be concluded from these results that the bacterial lipopolysaccharide pyrogenal intensifies the proliferative processes taking place during immunization, and can thus lead to an increase both in the number of AFC and in the total number of cells in the spleen. Injection of the stimulator also led to a slower decrease in the number of AFC in the rabbit spleen. Similar results have been obtained by Western workers who immunized mice and guinea-pigs with another antigen – sheep's red cells [3-6].

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