THE EFFECT OF BRUCELLOSIS ANTIGENS ON THE LIENAL CELL CULTURE OF SENSITIZED GUINEA PIGS

(UDC 576.851.42 : 578.085.23)

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 59, No. 3, pp. 68-71, March, 1965
Original article submitted July 12, 1964

During the recent years tissue culture has been used extensively in studying the problems of pathology and immunology of infections. Specific changes in reactivity of in-vitro grown cells from a sensitized organism in the course of many infections have been established by numerous investigations [2, 3, 5-7]. Our studies [1] demonstrated that brucellosis in guinea pigs previously sensitized with homologous as well as with heterologous antigens (E. coli, human γ-globulin) follows a more severe course and is characterized by a more rapid development of severe structural destruction of organs, than during initial infection. Because of the above, the study of the problem in in-vitro experiments, eliminating the effect of the complex correlations within an organism, is of definite significance. Elucidation of the capacity of splenic cells to react to the action of specific antigens may prove of significance in the problem of preservation by the tissue cells of their properties when grown outside the body.

The present study dealt with the investigation of action of brucellosis antigens on splenic cells in tissue cultures of animals sensitized with a killed brucella culture, and with the determination of action of brucellosis antigens on splenic cells during heterologous sensitization.

MATERIALS AND METHODS

Experiments were carried out on 24 male guinea pigs, weighing 300-400 g. The animals were divided into 4 groups: 1st group (6 guinea pigs) was sensitized with heat killed culture of caprine brucella, the 2nd group (5 animals)—with human γ-globulin, the 3rd group (5 animals)—with heat killed culture of intestinal rod, the 4th group (8 guinea pigs) was used as a "healthy" control.

The animals were sacrificed by decapitation at the height of sensitization. From each group, spleens of 2-3 guinea pigs were removed aseptically, cut into small pieces with scissors, washed three times in buffered Hanks' solution and trypsinized. The cells were grown in test tubes with pieces of mica. Each test tube received $5 \cdot 10^6$ splenic cells in 1 ml. The medium was changed after 48 h. 10^6 to 10^7 cells of heat killed culture of brucella were added to the splenic cell cultures of experimental and control animals. The splenic cells were subjected to antigenic action at the start of cultivation and during the change of nutrient medium (on the 3rd day of cultivation). Twenty-four and 48 h after contact with the antigens, i.e., 24, 48, 72 and 96 hours after cultivation, the mica slides were fixed in Bouin's fluid and stained with hematoxylin eosin for histological examinations. Cells on the test tubes walls were removed with trypsin and were counted with a hemocytometer. The cytotoxic index was determined by dividing the average number of cells in test tubes with the antigen by the average number of cells in control tubes, according to the previously described method [2]. 1000 cells in different fields were counted in the microscopic study of the preparations, cytograms were prepared and the percent of the damaged cells was determined. Agglutinated cells were counted in 100 fields. Analysis of variance was used to evaluate the significance of the obtained data.

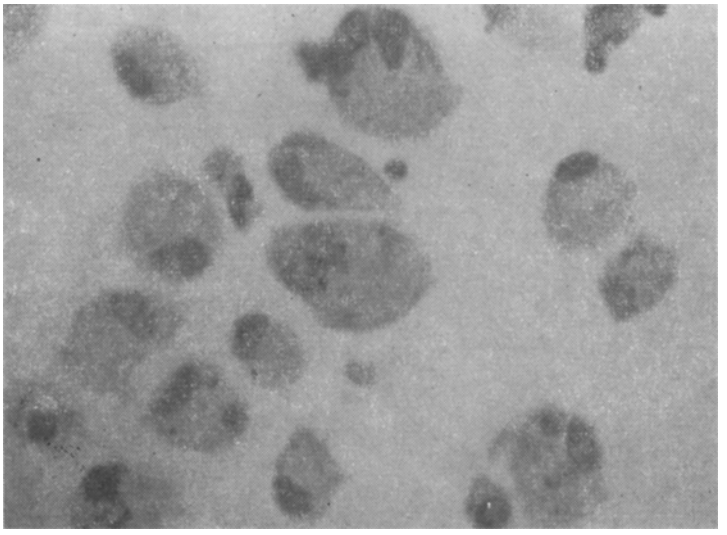

Fig. 1. Macrophages in lienal culture of a guinea pig, sensitized with γ-globulin 48 h after action of brucella antigen. Hematoxylin-eosin stain. Objective 40, ocular 7.

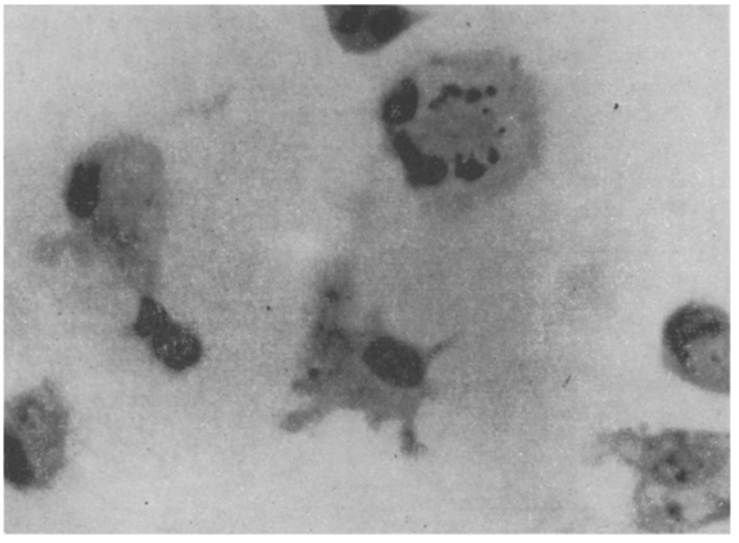

Fig. 2. Damaged macrophages in lienal culture of a guinea pig, sensitized with brucella, 48 h after action of brucellosis antigen. Hematoxylin-eosin stain. Objective 40, ocular 7.

RESULTS

Addition of brucellosis antigens to the splenic cells of normal and sensitized with γ-globulin and E. coli guinea pigs brought about in some cases an insignificant (statistically not reliable) decrease in the number of cells ($P > 0.01$). Index of cell damage was equal to 0.84-1.04. Increase in cytotoxicity with increase in the concentration of brucellosis antigens from 10^6 to 10^7 cells was also not established statistically.

Upon addition of 10^6 cells of the antigen to the culture of spleens of guinea pigs sensitized with brucella, a marked decrease in cell concentration was observed (365.2 ± 5.4 in control, 271.6 ± 6.8 in experimental), as well as considerably high indicators of the index of their damage, of the order of 0.67-0.77. Using the antigen concentration of 10^7 cells led to even a more drastic reduction in cell number (to 193.1 ± 7.8) and to higher indices of damage – 0.47-0.56, which confirms the increase in cytotoxicity ($P < 0.01$).

The number of cells decreased even after 24 h of incubation, and after 48 h this phenomenon became more evident. The cytotoxic index was higher after addition of antigen during the 1st day of cultivation. Upon addition of antigen after 48 h of cultivation the cytotoxic effect was somewhat lower. This allows to assume that the cultured cells retain the ability to react to the action of the antigen only during a particular time.

A day after the beginning of cultivation of spleens of normal animals the lymphoidal cells numbered 47.0-54.8% of the cell population, macrophages—43.1-47.7%, granulocytes—0.5-2.4%, fibroblasts—1.6-3.1%. Upon cultivation of guinea pig cells sensitized with different antigens, there was observed almost the same distribution of cellular elements with some increase in percent of macrophages. A part of macrophages (2.9-5.1% in healthy and 2.9-5.2% in sensitized pigs) was damaged .

Upon addition of antigen to the culture of cells of healthy guinea pigs, the damaged macrophages, as well as in control culture, made up 2.7-4.1% (Fig. 1). Similar results have been obtained also in the course of action of brucellosis antigens on cells after heterologous sensitization (3.0-5.1%).

Upon addition of antigen in concentration of 10^6 to the splenic cells of a homologous sensitized guinea pig, the number of damaged macrophages increased noticeably (8.4-18.7%). Increasing the dose of antigen to 10^7, the number of damaged macrophages increased (17.6-38.2%). The damage to the cell structure was expressed in the loss of normal contours, swelling of the cell membrane, break-up of the nucleus and appearance of cytolysis (Fig. 2).

Decrease in percent of lymphoidal cells and the presence of agglutinated cells in the cultures, obtained from guinea pigs sensitized with brucella, was observed under the influence of antigens.

Therefore, in the course of action of brucellosis antigen on splenic cells of animals sensitized with brucella, there was noted a clearly expressed cytotoxic effect, which was evident by the decrease of the total number of cells by the damaged macrophages and by the decrease in percent of the lymphoidal cells. Also characteristic was the formation of agglutinates, composed of lymphoidal cells. Brucellosis antigens did not bring about evident cytotoxic effect in healthy guinea pigs, as was the case after heterologous sensitization with γ-globulin and E. coli. Evidently, the cytotoxic action of the indicated antigens on splenic cells is determined by specific factors and is associated with the antibodies found in the sensitive cells. This point of view is confirmed by the presence of agglutinates, and also by decrease in the cytotoxic effect using specific immune sera [4].

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