INVESTIGATION OF THE PHAGOCYTIC REACTION OF THE LEUKOCYTES AND ITS HUMORAL STIMULATION FOLLOWING INJECTION OF TUBERCULOSIS ANTIGEN*

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It has been shown experimentally that the phagocytic activity of the leukocytes is increased within a few days, h, or even min after the injection of various antigens into the organism [1-3, 6-8, 10-12]. Reports have appeared of the specificity of this phenomenon [2, 3, 7, 11], although some workers have found nonspecific changes in the phagocytic activity of the leukocytes during immunization [5, 6]. The cause of this early rise in phagocytosis during immunization has received little study. It has been attributed to specific stimulation of the nervous receptors at the site of injection of the vaccine [7], the specific reconstruction of the leukocytes themselves [4], and to the opsonizing influence of the disintegration products of leukocytes [6].

In this paper we describe the results of a study of the dynamics and specificity of the phagocytic reaction of the leukocytes and the part played by the humoral factor in the production of this reaction after injection of tuberculosis antigen.

EXPERIMENTAL METHOD

The investigation was conducted on 26 dogs. The animals were immunized by the intradermal injection of BCG vaccine in a dose of 0.01-10 mg, or of a living virulent culture of Mycobacterium tuberculosis, human type (strain $H_{37}RV$) in a dose of 1 mg. The antigen was made up in 0.2 ml physiological saline and injected into the tip of the ear.

Parallel studies were made of the phagocytic activity of the leukocytes in relation to BCG cells (in a series of experiments the phagocytosis of virulent mycobacteria of the human and bovine types was investigated simultaneously) and to a 24-h culture of Staphylococcus albus or to killed typhoid bacteria. The required concentration of mycobacteria for phagocytosis was obtained by centrifugation of a dense suspension of these organisms in physiological saline until the turbidity corresponded to a bacterial cell concentration of 50,000,000/ml. Suspensions of staphylococci and typhoid bacteria were prepared in relation to a standard containing 10,000,000 bacterial cells/ml.

Blood for investigation was taken from the external vein of the leg and stabilized with heparin. To study the phagocytic activity of the leukocytes, 0.1 ml of the test blood and 0.1 ml of the corresponding bacterial suspension were mixed together in a tube and placed on a water bath at 38° for 20 min. The tubes were shaken every 5 min. Films with BCG cells and mycobacteria were stained by Ziehl-Neelsen's method and counterstained with methylene blue. The remaining films were stained by the Romanowsky–Giemsa method. The phagocytic activity of the leukocytes was investigated immediately after immunization, 5 and 15 min and 1 and 3 h later, and on the following days–in some cases until 1 month after immunization. Parallel with the study of the phagocytic activity of the

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TABLE 1. Phagocytic Activity of Leukocytes in Relation to BCG Cells and Mycobacteria tuberculosis (according to phagocytic number)

Statistical index	Before injection of antigen	After injection of antigen				
		5 min	15 min	1 h	3 h	24 h
M	0,51	0,55	0,83	0,91	0,93	0,93
m_{diff}	—	0,06	0,06	0,08	0,18	0,15
n	28	28	28	28	28	21
P	—	0,6	0,001	0,001	0,05	0,02

TABLE 2. Phagocytic Activity of Leukocytes Against Staphylococci and Typhoid Bacteria

Statistical index	Before injection of antigen	After injection of antigen				
		5 min	15 min	1 h	3 h	24 h
M	1,05	0,53	0,52	0,73	0,49	0,70
m_{diff}	—	0,13	0,13	0,15	0,20	0,13
n	28	28	28	28	28	21
P	—	0,001	0,001	0,05	0,02	0,02

leukocytes, at the same times the effect of the blood plasma and serum of the immunized dogs on the activity of the leukocytes from the blood of intact animals was investigated. In a tube, 0.1 ml of plasma or serum was mixed with 0.1 ml of blood taken from an intact animal. After contact for 20 min in a water bath, 0.1 ml of a suspension of BCG cells (in some experiments, mycobacteria tuberculosis) was added to the mixture, and the tubes were kept in the bath for a further 20 min, after which films were made. To investigate the specificity, experiments were carried out at the same time with suspensions of staphylococci or typhoid bacteria. The degree of phagocytosis was assessed by the number of microorganisms ingested by 100 polymorphs and monocytes. Before and during the first 3 h after injection of the antigen the number of leukocytes in the peripheral blood was counted.

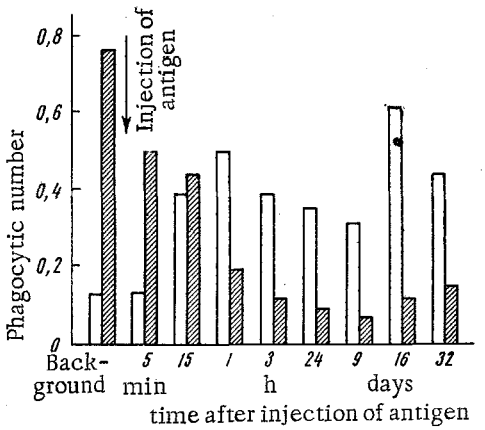
EXPERIMENTAL RESULTS

During the first h after injection of the antigen an increase in the phagocytic activity of the leukocytes in relation to BCG cells and M. tuberculosis was observed. In individual dogs it was recorded 5 min after injection of the antigen, but on the whole this increase after 5 min was not statistically significant. A regular, statistically significant increase in phagocytosis was observed after 15 min. During the following h and days the level of phagocytosis remained high. The phagocytic number rose to 1.5-8 times its original value. The dynamics of phagocytosis during the subsequent days showed two distinct patterns: either the phagocytic activity of the leukocytes continued high or, more frequently, 2-6 days after injection of the antigen a decrease in phagocytosis took place, followed by a new rise after the 2nd week, so that in this case the reaction could be described as biphasic. These patterns were identical whether the dogs were immunized with BCG or Myobacteria tuberculosis. Data showing the dynamics of phagocytosis during the first 24 h after injection of the antigen are given in Table 1.

The increase in phagocytosis was specific. In relation to staphylococci and typhoid bacteria the phagocytic activity of the leukocytes was depressed from the first few min after injection of the antigen, and it continued low thereafter (Table 2).

Some idea of the most typical relationships between specific and nonspecific phagocytosis may be obtained from the figure. The phagocytic reaction of the leukocytes was accompanied by a statistically significant increase in their number in the blood 5 min and 1 and 3 h after, and by a near-significant decrease in their number 15 min after injection of the antigen.

Plasma and serum obtained from the blood of the immunized dogs acquired the property of increasing the phagocytic activity of the leukocytes of intact animals in relation to BCG and M. tuberculosis cells. This property



Phagocytic activity of the leukocytes against BCG cells and staphylococci after injection of tuberculosos antigen. White columns—phagocytic activtiy of leukocytes against BCG cells; shaded columns—against staphylococci.

was clearly demonstrable 5 min after, and reached its maximum 15 min after injection of the antigen. The plasma and serum obtained at these times increased the phagocytic activity of the intact leukocytes to 2-6 times its value in the corresponding leukocytes treated with plasma or serum obtained before injection of the antigen. At the end of the first h the stimulating effect became weaker, and later disappeared. It is clear from Table 3 that a statistically significant increase in the ability to stimulate phagocytosis was found in the liquid portion of the blood only during the first h after injection of the antigen.

The stimulating effect of plasma and serum on phatocytosis was specific. Only in individual experiments did an increase in nonspecific phagocytosis appear after 5 min. As a rule the phagocytic activity of the leukocytes of an intact animal, treated with plasma or serum obtained 5 min after injection of the antigen, was considerably depressed in relation to staphylococci and typhoid bacteria. A particularly intensive inhibitory effect on nonspecific phagocytosis was observed in the liquid portion of the blood 15 min after injection of the antigen; this subsequently grew weaker, and 3 h after the injection this effect was very slight or completely absent (Table 4).

A statistically significant relationship was found between the stimulating effect of the liquid part of the blood on specific phagocytosis [the coefficient of correlation (r) was 0.48, m_{diff} 0.11, n 68, and $P < 0.001$]. The relationship between these indices was particularly close during the first 15 min after injection of the antigen.

TABLE 3. Effect of Serum and Plasma on Phagocytic Activity of Intact Leukocytes in Relation to Cells of BCG and M. tuberculosis

Statistical index	Before injection of antigen	After injection of antigen			
		5 min	15 min	1 h	3 h
M	0,38	0,64	0,84	0,51	0,34
m_{diff}	—	0,05	0,07	0,04	0,06
n	34	34	34	34	34
P	—	0,001	0,001	0,001	0,5

TABLE 4. Effect of Serum and Plasma on Phagocytic Activity of Intact Leukocytes in Relation to Staphylococci and Typhoid Bacteria

Statistical index	Before injection of antigen	After injection of antigen			
		5min	15 min	1 h	3 h
M	0,62	0,43	0,45	0,52	0,59
m_{diff}	—	0,09	0,05	0,04	0,05
n	25	25	25	25	25
P	—	0,05	0,01	0,02	0,6

The ability of the serum and plasma to stimulate phagocytosis lasted for a considerable time in vitro: at 2-5° the serum and plasma retained this property when stored in the liquid form for not less than 1 week, and in dried form for 1 month.

Two factors have a bearing on the understanding of the mechanism of the increase in the phagocytic activity of the leukocytes. Firstly, the appearance of ability to stimulate specific phagocytosis in the liquid part of the blood precedes the increase in the phagocytic activity of the immunized animal's own leukocytes. Secondly, the demon-

strated effect of stimulation of phagocytosis was transient, whereas the phagocytic activity of the leukocytes after immunization remained at a high level for a long time. Hence, it follows that the increase in early phagocytosis after injection of the antigen in our experiments was associated, not with an opsonizing action, but with stimulation of the activity of the leukocytes. It appears from the facts described that the stimulators of specific phagocytosis found in the liquid part of the blood are fixed by the leukocytes, which become modified, as is manifested by their acquisition of a presistently increased phagocytic activity. Hence, our findings confirm Mechnikov's old idea [9] of the existence of "stimulins" of phagocytosis, the action of which is directed, not towards the object of phagocytosis, but towards the phagocytes.

The problem of the origin of the stimulators of specific phagocytosis and of their role in the formation of immunity requires special study.

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