IMMUNOLOGY

CHANGES IN THE PHAGOCYTIC ACTIVITY OF LEUCOCYTES DURING THE PROCESS OF INFECTION

G. A. Gurvich

Gamaleya Institute of Epidemiology and Microbiology (Dir. – Active Member of the Academy of the USSR Prof. G. V. Vygodchikov) of the Academy of Medical Sciences of the USSR, Moscow

(Received 15 February, 1955. Presented by Active Member of the Academy of Medical Sciences of the USSR, P. F. Zdrodovsky)

I. I. Mechnikov's famous rule regarding change in the phagocytic properties of leucocytes during the immunogenic process could not be used in daily laboratory practice because immunology has no method available which would afford regular determination of these changes.

Wright's method gave divergent results in the hands of different investigators.

In the first part of the present investigation we attempted to work out a method which would assure the regular determination of the changes in leucocytes in the course of the development of immunity.

On comparing the phagocytic activity of the leucocytes of normal and immune animals, it has been customary since Wright's time to wash the leucocytes thoroughly in physiological solution and to carry out the phagocytic reaction in normal serum. Since the comparison of the phagocytic properties of various leucocytes gives little perspective when phagocytosis is weakly evidenced (antibodies are absent), we rejected Wright's method and attempted to carry out the comparison of the phagocytic properties of the leucocytes of normal and immunized animals at the functioning level of activity, i.e., under conditions of "functional load", by introducing standard immune serum into the reaction. In the presence of standard immune serum, the leucocytes of normal animals develop a high phagocytic activity (control) which varies with the dilution of this serum. The difference of the phagocytic activity of the leucocytes of immune animals, if found, should be indicated by some kind of deviation by the phagocytic indicators from the indicators of the control. In order to establish the time when the deviation is most significant, multiple determinations of the phagocytic indicators were carried out under experimental conditions instead of the customary single-moment registration of the number of captured microbes. Seeking the most harmless liquid for washing leucocytes, we decided on fresh citrated guinea pig plasma, and washed 0.15 ml of leucocytes in 4 ml of plasma only once. Control experiments with washed leucocytes and normal serum showed that antibodies were not found after such washing.

Thus, the principal features of this method are, first, the replacement of normal serum by immune serum, which assures high functional activity of the cells under investigation; secondly, the evaluation of the results of the reaction not only by the absolute indications of phagocytosis, but also by the nature of these changes during the experiment; and, thirdly, the use of those methods of washing which ensure the maximum safety for the leucocytes. The methods were developed on guinea pigs with a typical experimental infection with Gärtner's bacillus. The actual experimental methods were as follows.

The standard immune serum was obtained from guinea pigs which were immunized three times with heated vaccine prepared from Gärtner's bacillus. The serum was inactivated and stored in dried form. The agglutination titer was 1:320. Immediately before the experiment, dissolved standard serum was diluted with fresh normal serum in the proportion of 1:20. In order to obtain leucocytes, heart blood of guinea pigs was mixed with an equal volume of a 2.5% solution of sodium citrate and centrifuged briefly to precipitate the cellular components. After the upper layer of citrated plasma was removed, the upper grayish layer of the precipitate, in which

leucocytes predominated, was siphoned off. The leucocytes were washed once by centrifuging in 25 volumes of normal citrated plasma. An 18-hour culture of Gärtner's bacillus in a 1 to 10 milliard suspension in physiological salt solution was used as the objective of the phagocytosis. The phagocytic mixture consisted of the three above-named ingredients in the following proportions.

	Test phagocytic mixture	Control phagocytic mixture
Leucocytes of immune animal	0.09	–
Leucocytes of normal animal	–	0.09
Diluted standard serum	0.09	0.09
Bacterial suspension	0.02	0.02
Total volume	0.2	0.2

The phagocytic mixture in paraffinned test tubes was placed in a water bath at 37° for 30-50 minutes. Samples were taken every 5-10 minutes during this time. Two smears were prepared from each sample, fixed with methyl alcohol, and stained with Manson's blue. On examination of the smears, 50 leucocytes were counted and the phagocytic index, representing the average number of bacteria in a single leucocyte, was computed.

Thus, the phagocytic capacity of the leucocytes was expressed by a number of phagocytic indexes which characterized, first, the level of the phagocytic activity of the leucocytes under consideration, and, secondly, the change in the number of bacteria ingested by leucocytes in the course of the experiment.

The methodology was tested finally in 52 phagocytic experiments on 30 guinea pigs at varying lengths of time after infection with Gärtner's bacillus. A change in the phagocytic activity of the leucocytes of infected animals was found in 44 of these experiments. In 8 cases, negative results were obtained. The clearest difference between the phagocytic activity of the leucocytes of normal and immune animals was demonstrated in guinea pigs which had first been infected with 25 million bacteria and then with a lethal dose (500 million bacteria). The results of one of these experiments are shown in Fig. 1.

The phagocytic activity of the leucocytes of a normal animal (control) was characterized by a high level of the phagocytic indexes throughout the experimental period. The phagocytic indexes of immune guinea pigs grew in the course of the first 30 minutes of the experiment, remaining lower than the control indexes, however; then they were observed to decrease toward the end of the experiment. The latter was not always evident. Cases when the phagocytic indexes of an immune animal changed little in the course of the entire experiment were not rare. However, it is important that in the vast majority of cases, the indexes were considerably lower than those of the control animals.

The experiments which were carried out require reappraisal of the phagocytic reaction in vitro. Apparently, this reaction does not ensure a sufficiently complete characterization of the leucocytic activity under customary conditions (Wright's method, or opsonocytophagic test). If the usual method is changed and immune serum is included in the reaction as a requisite component, thus putting the leucocytes of both the normal and immunized animals in conditions of maximum ingestion of bacteria, it will become clear that different leucocytes act differently under identical conditions and that, consequently, the substance of the phagocytic reaction is not exhausted by the action of the opsonins alone. In our experiments, the deviation of phagocytic activity from the normal level consisted of a decrease in the phagocytic indexes. The biological meaning of this fact remains as yet undiciphered, but we are forced to consider it incorrect to regard this decrease literally and to relate it to an actual decrease in functional activity. Rather, a change in the capacity of immune animals to digest the ingested bacteria should be considered.

In the second part of the work, we attempted to watch the dynamics of the indicated phagocytic shifts in the course of the infectious process and to determine the relation of these shifts to the practical immunity of the animal to the given infection.

Nature of the experimental infection. Guinea pigs infected intraabdominally with a suspension of an 18-hour culture of Gärtner's bacillus develop an infection of the nature of acute septicemia; in fatal cases, death occurs between the fifth and tenth day of infection. The blood and internal organs of the dead animals contain a profusion of Gärtner's bacilli. In nonfatal cases, the above-indicated period is critical, followed by

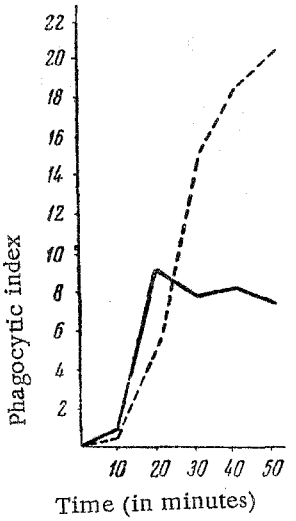

Fig. 1. Difference in the phagocytic activity of the leucocytes of normal (- - -) and immune (———) guinea pigs.

recuperation which is accompanied by a sterilization of the internal organs. In order to produce experimental infection and the consequent post-infection immunity, guinea pigs were infected with doses of 25-50 million bacteria, causing death in 50% of the animals. Such "harsh" experimental conditions were employed with the calculation that the immunological shifts of the surviving animals would prove to be expressed maximally and that the immunity obtained after a second infection would be as strong as possible. Cultures of the blood and internal organs (liver, spleen) during the development of experimental infection showed that the stimulant circulated in the blood and was present in the internal organs during the first 5 days of the disease; by the 10th day the surviving animals were freed of the stimulant and cultures made after longer intervals remained sterile.

Phagocytic shifts in experimentsl animals. The phagocytic activity of the leucocytes of 15 infected guinea pigs was investigated by the method described above 2, 5, 10, 15, 20 and 30 days after the administration of the live culture; at each of these times, from 4-6 infected guinea pigs and one control were investigated. The average experimental results are presented in Fig. 2. As is evident in Fig. 2, the phagocytic indexes of the control animals grew sharply during the course of the experiment and reached high figures [23]. With this as a basis, the deviation of the experimental animals from normal phagocytic activity is expressed by a lowering of the phagocytic indexes. If the divergence of the phagocytic indexes for normal and infected animals (the cross-hatched area in Fig. 2), is considered to be the measure of this change, the dynamics of the indicated property can be delineated in general outline under the conditions of a given experiment. It is found 5 days after infection, reaches a maximum on the 10-15th day and begins to die away on the 20th day.

Relationship of phagocytic shifts and immunity. The shifts in the phagocytic activity of the leucocytes of infected animals described above are of interest and deserve further study only if they actually are related, to some extent, to the immune state. Two variant experiments were set up to answer this question.

In the first variation, the time necessary for the development of immunity after the initial infection was measured. For this purpose, infected guinea pigs were infected a second time with massive doses (500 million bacteria, constituting 10-20 LD_{50}). The results are shown in Fig. 2B. It was found that two days after the first infection the animals not only had acquired no immunity to secondary infection, but even died sooner than the controls. However, already five days after the initial infection, the guinea pigs began to develop a resistance which was found consistently after this moment and at much later times (10th, 20th, 30th day). The results of the investigations on the immunity of the infected animals correspond fully with the results of cultures from these organs (see above). It is possible to isolate the stimulant from the blood and organs in the course of the first five days after infection. By the 10th day, i.e. by the moment the immunity is built up, all of the surviving animals are freed from the stimulant.

Correlating the time required for the development of immunity after infection of the guinea pigs with live culture with the dynamics of the phagocytic shifts, it is easy to observe a coincidence in the time that both indications appear. On the 5-10th day – at the critical time when the defensive forces begin to act – immunity developed and the prognosis of the disease was determined. This complicated process is reflected, apparently, in the functional condition of the leucocytes which undergo some kind of changes and which show the deviation from normal phagocytic activity described above under the conditions of the given experiment.

The second variant of experiments for the determination of the relationship between immunity and phagocytic shifts consisted in the comparison of the phagocytic reaction of immune and normal animals after infection with a known lethal dose of culture. This form of experimentation seemed worthwhile to us also because the previous experiments did not disclose the nature of that functional condition which the leucocytes

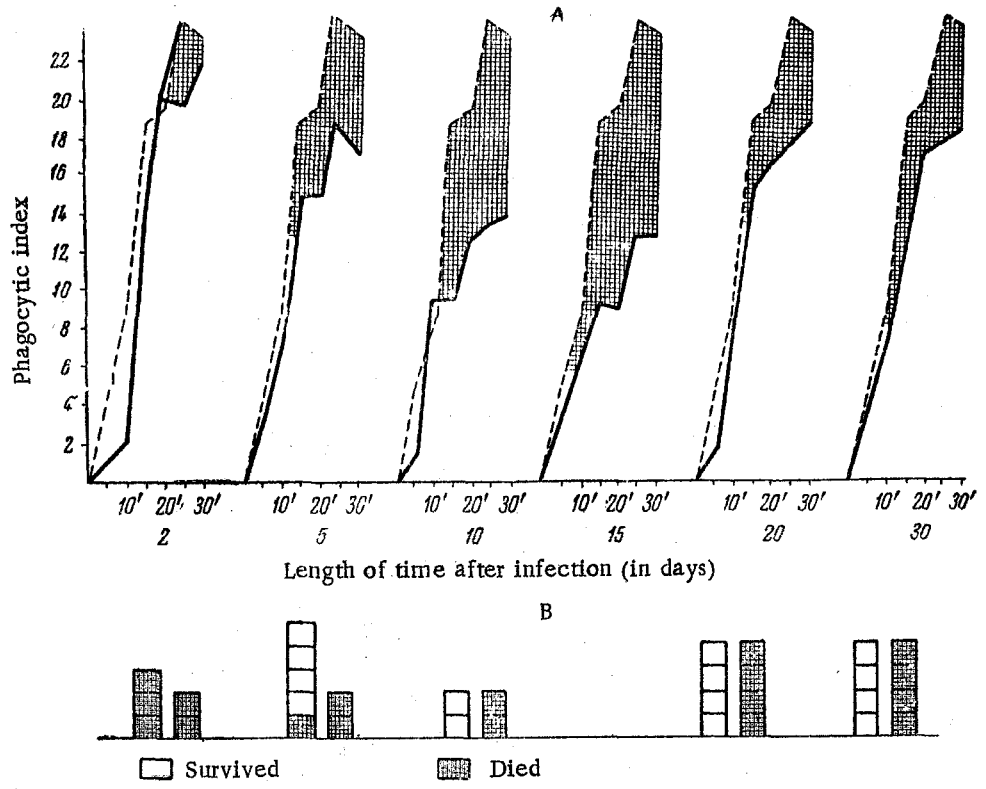

Fig. 2. Dynamics of changes in the phagocytic activity of leucocytes (A) and the survival of guinea pigs (B) after the first infection.
- - - - normal guinea pigs; —— infected guinea pigs. Left columns – experiment; right columns – control.

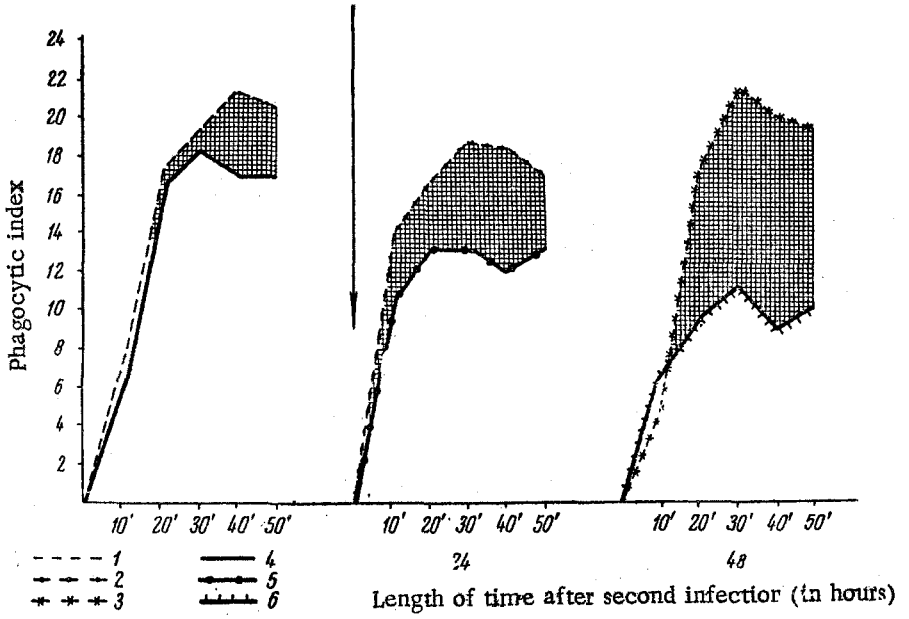

Fig. 3. Dynamics of the changes in the phagocytic activity of leucocytes after the second infection (↓) of guinea pigs.
1) Normal guinea pigs; 4) survivors of the infection; 2-3) initially infected; 5-6) secondarily infected.

reach after the shifts described above (20th, 30th day in Fig. 2). The question arises whether the leucocytes on the 30th day after infection are equivalent to the leucocytes of a normal animal and whether the evolution which the leucocytes undergo in the course of the infectious process leaves any mark on the latter.

In order to solve this problem 4 normal guinea pigs and 4 guinea pigs at the 30th day after the initial infection were injected with a known lethal dose of the culture (500 million bacteria). 24 and 48 hours later, blood was taken from the animals for the determination of the phagocytic activity of the leucocytes. The average experimental results are shown in Fig. 3. The data obtained indicate that in normal guinea pigs, initially infected with a large dose of the culture, the phagocytic activity of the leucocytes does not change in the course of the first 2 days. All the guinea pigs died between the 5th and 10th day.

In contrast to these control guinea pigs, the animals infected for the second time successfully survived infection which reflected a quick change in the phagocytic activity of the leucocytes, becoming less marked after 24 hours and more pronounced after 48 hours.

Thus, the evolution undergone by leucocytes in the course of infection did not occur without leaving any trace. During secondary infection, the entire process described above was repeated again with the difference that now the maximum changes occurred after only 2 days, instead of after 2 weeks as was the case with the initially infected animals.

The experiments carried out by us show, first, that the application of the phagocytic reaction according to the proposed method permits solution of the problem of the changes in the leucotytes themselves during the immunological process in a positive sense. Secondly, they show a number of new, regular occurrences in the course of phagocytic shifts and their connection with the acquired resistance of an animal to fatal infection.