CHANGES IN THE PHAGOCYTIC ACTIVITY OF LEUKOCYTES IN THE PROCESS OF IMMUNOGENESIS FOLLOWING ANTIPLAGUE VACCINATION

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CHANGES IN THE PHAGOCYTIC ACTIVITY OF LEUKOCYTES IN THE PROCESS OF IMMUNOGENESIS FOLLOWING ANTIPLAGUE VACCINATION

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According to the findings of many researchers one of the main factors in the mechanism of recovery from the plague and in the development of postvaccinal immunity is the rearrangement of phagocytic reaction of the organism (Zabolotnyy, 1956; Pokrovskaya, 1947; Zhukov-Verezhnikov, 1940, 1945; Korobkova, 1956; Akimovich, 1960; Burrows and Bacon, 1956, et al.).

M. P. Pokrovskaya and L. S. Kaganova (1947) showed that while in nonimmunized animals is observed a peculiar "paralysis" of the phagocytic reaction with regard to virulent strains of plague bacilli, a high phagocytic capacity of RES is characteristic of immune organisms.

Burrows and Bacon (1956) have ascertained the presence in virulent plague bacilli of surface antigens of V and W which possess the capacity of impending the act of phagocytosis. These authors explain the resistance of phagocytosis by the peculiarities of the bacterial envelope-capsule. We must note that such a capsule, which impedes phagocytosis, is formed only after the plague bacillus had been for some time in the organism. The opinion of M. P. Pokrovskaya and L. S. Kaganova on the "paralysis" of phagocytic activity in plague infection is not shared by V. V. Akimovich (1960), who, like Burrows and Bacon explains this phenomenon by the peculiarities of the virulent plague bacillus and not by a damage of the act of phagocytosis itself, as it is assumed by M. P. Pokrovskaya.

V. V. Akimovich has observed high levels of phagocytic staphylococci in the organism of animals infected with plague during the entire period of infection.

According to the findings of M. P. Pokrovskaya the rearrangement of RES takes place under the effect of antiplague vaccination. The essence of this rearrangement consists in hyperplasia of the elements of RES and in so-called polyblastic reaction on the part of the elements of active mesenchyma.

In the literature are available many data concerning changes in the phagocytic activity of leukocytes in the process of immunogenesis after antiplague vaccination. S. G. Abramova (1944) and L. S. Kaganova (1946) point to the increase of phagocytic activity of leukocytes in immune animals. A. I. Yelfimova (1940) and I. I. Derteva (1959) obtained in immune animals under the effect of antiplague serum a decrease in phagocytic activity of the blood cells as compared with washed-off leukocytes.

N. I. Kolesinskaya (1959) showed that in guinea pigs and rabbits immunized with strain 17 there is observed a rise in the phagocytic number of leukocytes during a prolonged period, whereas in those immunized with strain EB after a comparatively short-lived rise of phagocytic activity its sharp fall occurs. We considered as necessary to study the process of changes in the phagocytic reaction of the organism in the dynamics of postvaccinal immunity in view of the fact that this question is insufficiently clarified in the literature. Many investigations give only a descritpion of the phagocytic reaction but not a factual evaluation of the degree of phagocytosis. We must also note that the majority of authors estimates the phagocytic reaction only by the number of the encompassed bacilli and does not take into account the degree of the completeness of the phagocytic reaction.

In the present investigation we tried to solve the problem of changes in the phagocytic activity of leukocytes in the process of antiplague immunization.

Changes in the Phagocytic Number and in the Number of Active Leukocytes in the Process of Antiplague Immunization

Technique of Experiments. The phagocytic activity of leukocytes was determined in nonimmunized white mice, as well as in those immunized with a live antiplague vaccine. The immunization of mice was performed by a subcutaneous injection of 100 million microbial cells of strain EB. To determine phagocytic activity of leukocytes the blood was taken from the mouse tail. 0.04 ml of blood was mixed with 0.02 ml of 1.5%

solution of sodium citrate and 0.02 ml of suspension of bacteria EB 229 containing 1 billion microbial cells in 1 ml. After 30 min of incubation at 37° smears were prepared, fixed and stained with Giemsa-Romanovsky's mixture. The phagoctyic number -- number of bacteria absorbed by a leukocyte (for this purpose 100 leukocytes, polymorphnuclear and mononuclear, were examined), and the percentage of active leukocytes served as indexes of phagocytic activity. The phagocytic activity of leukocytes was determined by the 7, 9, 10, 12, 14, 16, 17, 19, 21, 22 and 24th day after vaccination (five animals each time).

Tables 1 and 2 give statistically processed data of determinations.

TABLE 1

Changes in Phagocytic Number of Leukocytes in the Process of Immunization

День после иммунизации (1)	Среднеарифметическое (2)	Среднеквадратическое отклонение (3)	Средняя ошибка (4)
(5) Здоровые мыши . . .	0,880	0,044	0,022
7-й день (6) . . .	1,260	0,055	0,028
9-й	1,400	0,061	0,030
10-й	1,390	0,185	0,092
12-й	1,900	0,100	0,050
14-й	1,760	0,102	0,051
16-й	1,370	0,056	0,028
17-й	1,311	0,046	0,023
19-й	1,164	0,007	0,003
21-й	1,677	0,055	0,027
22 й	1,530	0,175	0,087
24-й	1,230	0,124	0,062

1 -- Day after immunization; 2 -- Arithmetic mean; 3 -- RMS deviation; 4 -- Mean error; 5 -- Healthy rats; 6 -- Day.

It may be seen from the tables that the phagocytic number of leukocytes increases beginning from the 9th day after immunization up to 12-14th day. During subsequent days there is observed some decrease of the phagocytic activity but toward the end of immunization period (22-24th day after vaccination) the phagocytic number continues to be higher than in nonimmunized animals.

TABLE 2

Changes of the Number of Active Leukocytes in the Process of Immunization

День после иммунизации (1)	Среднеарифметическое (2)	Среднеквадратическое отклонение (3)	Средняя ошибка (4)
7-й день (5)	60,50	3,65	1,92
9-й	76,00	5,00	2,50
10-й	76,75	4,50	2,25
12-й	77,25	1,66	0,83
14-й	82,25	1,60	0,80
16-й	81,00	1,75	0,87
17-й	82,50	2,27	1,13
19-й	83,00	1,80	0,90
21-й	87,00	1,31	0,65
22-й	85,75	1,31	0,65
24-й	79,65	1,65	0,83

1 -- Day after immunization; 2 -- Arithmetic mean; 3 -- RMS deviation; 4 -- Mean error; 5 -- Healthy rats; 6 -- Day.

Some researchers (Pokrovskaya, 1947; Korobkova, 1956) assert that the immunity upon vaccination with live antiplague vaccines is nonsterile during the initial period of its development and thereafter it becomes sterile.

Duration of the period of nonsterile immunity is determined by the time of stay of live bacteria of vaccinal strain in the animal organism. In the opinion of the majority of authors (M. P. Pokrovskaya and Ye. I. Korobkova) it is possible to isolate bacteria from the tissues of animals during 12-17 days after vaccination using normal bacteriological methods. During this period of the development of immunity can be observed, according to our data, the highest degree of rise of the phagocytic number. Pathophysiologically this stage of immunity is characterized, according to the available data (G. G. Korobkov, unpublished report), by endocrine shifts, in particular by an increase of the tonus of the sympathetic portion of autonomous nervous system. As is known, N. V. Puchkov and G. G. Golodets (1939, 1946) have ascertained the increase of the phagocytic activity of leukocytes under the effect of sympathicotropic agents.

We were unable to observe, as this was done by A. P. Yelfimova (1949) and I. I. Derteva (1960) in the experiments

on guinea pigs, any decrease of the phagocytic number in immune animals as compared with intact ones. But the phagocytic number in immune animals on the 12-14th day after inoculation is higher than on the 21-25th day.

As regards the data of S. G. Abramova and L. S. Kaganova, we must note that these authors determined the phagocytic number on the 15th day after immunization, i.e., during a period when according to our data the phagocytic number increases maximally.

The change in the number of active leukocytes in the process of immunization proceeds somewhat differently. The percentage of active leukocytes is recorded up to 12-14th day after inoculation, and thereafter it almost does not change. These data show that the change of phagocytic number does not proceed parallely with the change of the percentage of active leukocytes. Apparently, one of the ways of the change of phagocytic reaction in the process of antiplague immunization is a decrease of the number of bacteria captured by the leukocyte with a simultaneous increase of the percentage of active leukocytes.

When a leukocyte captures a great number of bacteria they secrete a great amount of toxin which paralyzes enzymes of the leukocyte (M. P. Pokrovskaya and L. S. Kaganova, 1947), and therefore the remaining bacteria can reproduce. Consequently a decrease of the phagocytic number at the expense of a decrease of the number of leukocytes capturing a great number of bacteria, is a factor which promotes the immunogenesis.

To study the role of the rearrangement of cellular and humoral factors in the process of the change of phagocytic activity in an immune animal we carried out a series of experiments. In these experiments, 25-27 days after their immunization with KB 229 the blood was taken from white mice and stabilized by the addition of 1.5% solution of sodium citrate. After centrifuging of the blood the plasma was sucked off and leukocytic film was removed. Leukocytes were thrice washed with physiological solution. The blood of healthy mice was analogously obtained and processed. The leukocytes and plasma thus obtained were used in the experiments for the determination of opsonic action of the "immune" plasma on the leukocytes obtained from healthy mice.

Under the effect of "immune" plasma the leukocytes obtained from intact animals phagocytize plague bacteria in the same degree as leukocytes obtained from immune animals and treated with autoplasma. On the other hand if the "immune" leukocytes are washed and treated with plasma obtained from intact animal, the phagocytic number decreases, reaching a level characteristic of a nonimmunized animal.

Therefore, changes of the phagocytic number in the process of immunization depend mainly on the humoral factors and not on the qualitative peculiarities of leukocytes.

Changes in the Phagocytic Activity of Leukocytes in Immunized Mice, Determined by the Method *in vivo*

Determination of the phagocytic activity by the method *in vitro* has several shortcomings. The most important is the fact that the conditions of the life of leukocytes in a test tube are very far from conditions of their life in the organism. To exclude these shortcomings, we have carried out experiments for the study of phagocytosis *in vivo* according to the technique of I. I. Mechnikov.

Sixteen to eighteen hours before the experiment white mice were injected intraperitoneally 2 ml of 5% peptone solution which caused accumulation in the abdominal cavity of sterile exudate containing a great number of leukocyte. Thirty minutes before the accumulation of exudate 0.5 ml of one-billion suspension of bacteria of the vaccinal strain was introduced into abdominal cavity.

From the obtained exudate smears were prepared which were then fixed and stained according to Giemsa-Romanovsky and the phagocytic number was calculated. The phagocytic activity of leukocytes of the exudate was determined by the 21-30th day after vaccination. Intact mice served as a control.

Upon determination of the phagocytosis in the white mice *in vivo* we obtained a very great variability of results, especially in immune animals. The results of determination of the phagocytic number *in vivo* may be influenced by a viscosity of the exudate, different content of leukocytes in it, the rate of absorption of the introduced peptone, the rate of exudate formation, etc.

In immune mice the variability of phagocytic number was more pronounced than in intact mice, which may be explained by the peculiarities of immunization process in different individuals.

It is known that in any group of animals there exist immunologically inert individuals whose number varies depending on the peculiarities of antigen and the multiplicity of its introduction, as well as on conditions of the maintenance of animals. It is therefore natural that in the experiments in question no immunity was developed in some mice (refractory individuals). However, despite the variability of obtained data, the use of strain 17 for immunization shows a statistically fully significant rise of the phagocytic number in the

immune mice. While using the strain 17 we observed the high phagocytic number in four out of five mice, and in the experiment with Past. pestis EB 229 a high phagocytic index was observed only in two animals which, apparently, is connected with a lower antigenicity of the subculture oi strain EB.

TABLE 3

Phagocytic Number in Intact and Immune Mice Determined in vivo

Наименование штамма (1)	M	e	m
(2) Контрольные мыши, штамм EB 229 . .	1,96	0,37	0,11
(3) Контрольные мыши, штамм 17	2,02	0,28	0,13
(4) Иммунные мыши, штамм EB 229 . . .	3,64	1,7	0,77
(5) Иммунные мыши, штамм 17	3,7	0,98	0,44

1 -- Designation of the strain; 2 -- Control mice, strain EB 229; 3 -- Control mice, strain 17; 4 -- Immune mice, strain EB 229; 5 -- Immune mice, strain 17.

The rise of phagocytic number during the reaction in vivo depends not only on the better physiological medium for leukocytes but also on the fact that a number of substances associated with inflammatory reaction is accumulated in the abdominal cavity. As is known many of these substances -- polypeptides, amino acids, ATP acid, sympathicotropic hormone -- are capable of enhancing phagocytosis to a great degree.

Several experiments, carried out to verify this assumption, have confirmed it. In these experiments, the phagocytic number was determined according to the method in vitro with the use of exudate. In this case, the phagocytic number was 2-3 times higher than in similar experiments with the blood.

In evaluating these experiments we can assume that in immune mice a greater number of substances which promote the capture of bacteria by leukocytes (ATP), adrenalin, leukotoxin, etc., is secreted during inflammatory process than in nonimmune mice. Therefore, in immune animals the phagocytic number, determined by the method in vivo, is considerably higher than in nonimmune mice. Hence it follows that in some cases the inflammatory reaction arising upon infection of animals and in man through the bites of plague-blocked fleas may stop the development of plague infection.

Changes in the Completeness of Phagocytic Reaction in Immunized Animals

The phagocytic reaction may be complete or incomplete if there is no lysis of the bacteria captured by the leukocytes, then such a phagocytosis cannot be considered as a protective reaction of the organism. The question of evaluation of the completeness of phagocytic reaction has not been as yet solved. In order to study this problem we carried out experiments using a technique proposed by V. M. Berman and Ye. M. Slavskaya (1959).

The blood, stabilized with a solution of sodium citrate and mixed with bacteria (in the same way as it was done in experiments in vitro), was placed in a thermostat at 37° for 30 min, and then two smears of incubated blood were applied to the agar in a Petri dish. After 2-3 min one smear was transferred on a heated slide and applied to the agar surface onto which the investigated blood was previously applied. A second smear was retransferred on the slide after two-hour incubation at 37°. During this time a part of bacteria was lysed under the effect of enzymes of leukocytes and stained poorly, whereas the surviving bacteria were sharply magnified and stained well. In the first smear were counted the phagocytic number and the percentage of active leukocytes, as in the preceding experiments; in the second smear only live bacteria (magnified and intensely stained) were counted in leukocytes. The evaluation of a degree of the completed phagocytosis was performed by way of determination of the difference between the phagocytic number of the first smear and second smear (retransferred from the agar in a Petri dish which was incubated in a thermostat during two hours). Then the percentage was determined of this difference in relation to the original phagocytic number (determined from a count in the first smear).

The experiments showed that both in immunized and in nonimmunized mice may exist both inert and active individuals with respect to phagocytosis.

Since an impression was created on the difference of antigenic properties in plague strain 17 and strain EB, we have carried out the experiments to verify the completeness of phagocytic reaction of these two strains. Table 4 gives data obtained in these experiments.

During determination of the completeness of phagocytic reaction of leukocytes with strain EB in nonimmunized (control) white mice we have not observed in all the ten experiments any completed phagocytosis according to phagocytic number. Upon determination of this index with strain 17 in control animals

the completeness of phagocytic reaction averaged 11.1%. In immunized animals, in the experiments both with strain 17 and with strain EB there is observed approximately equally completed phagocytosis with respect to phagocytic number -- 36-42%.

TABLE 4

Degree of the Completeness of Phagocytosis in Immunized and Control Animals in % (25-28th Day After Vaccination)

(1) Наименование эксперимента	M	σ	m
(2) Контрольные (неиммунизированные) белые мыши. Опыт с штаммом 17	11,1	4,8	1,8
(3) Иммунизированные белые мыши. Опыт с штаммом 17	35,8	11,5	3,8
(4) Контрольные неиммунизированные белые мыши. Опыт с штаммом EB	0	0	0
(5) Иммунизированные белые мыши . Опыт с штаммом EB	42,1	17,5	6,7

1 -- Designation of experiment; 2 -- Control (nonimmunized) white mice. Experiments with strain 17; 3 -- Immunized white mice. Experiments with strain 17; 4 -- Control nonimmunized white mice. Experiments with strain EB; 5 -- Immunized white mice. Experiments with strain EB.

Consequently, as a result of immunization there is observed a considerable increase of the phagocytic activity of leukocytes of the blood, which definitely affects the sensitivity of the animal to the infection with the plague and represents one of the most important factors which determine immune state of the organism.

Conclusions

1. During the first 12 days after subcutaneous injection to animals of the antiplague live vaccine the phagocytic number in leukocytes increases by 50-100%, whereas the number of active leukocytes increases by 10-15%.

Subsequently the phagocytic number slightly decreases but remains higher than in control animals. The percentage of active leukocytes increases on the 19-21st day after immunization by 20-25%.

2. In the experiments with exudate in vivo the phagocytic number in immune animals is considerably higher than in the experiments with the blood in vitro.

3. A completed phagocytic reaction is observed in white mice immunized with live antiplague vaccine EB, whereas in control (intact) mice there is no completed phagocytosis, as a rule.

Bibliography

Abramova S.G., Opsonophagocytic Test in the Immunization Against Plague, Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii (ZhMEI)(Journal of Microbiology, Epidemiology and Immunobiology), 1944, No 10 and 11

Akimovich V.V., Dobrotsvetova T.Ya., Phagocytic Reactions in Experimental Plague Infection, Tr. in-ta "Mikrob" (Transactions of the Institute "Microbe"), No 4, Saratov, 1960

Berman V.M., Slavskaya Ye.M., The Reaction of Completed Phagocytosis, in the book: Sovremennyye problemy immunologii (Present-day Problems of Immunology), Leningrad, 1959

id., The Completed Phagocytosis. Communication I. A New Methodical Principle of the Study of Completed Phagocytosis, ZhMEI, 1958, No 3

Golodets G.G., The Influence of Mediators on the Phagocytic Function of Leukocytes, Byull. eksper. biologii i meditsiny (Bulletin of Experimental Biology and Medicine), 1943, No 11, issue 1

Golodets G.G. and Puchkov N.V., The Effect of Products of [illegible]ous Stimulation on the Phagocytosis, Byull. exper. biologii i meditsiny, 1939, No 7, issue 5

Derteva I.I., The Effect of Antiplague Serum on Past. pestis in the Test Tube and in the Organism of an Animal. Communication II, Tr. in-ta "Mikrob," No 3, Saratov, 1959

Zhukov-Verezhnikov N.N., Immunologiya chumy (The Immunology of the Plague), Moscow, Medgiz, 1940

Yelfimova A.P., The Role of Phagocytosis in the Mechanism of Immunity in Plague, Author's Report of Candidate Dissertation, Saratov, 1949

Zabolotnyy D.K., Issledovaniye po chume (Studies on Plague), Selected Works, Vol 1, Kiev, 1956

Kaganova L.S., New Methods of the Preparation and Study of Antiplague Serums, Izvestiya Irkutskogo gos. n.-i. protivochumnogo in-ta Sibiri i DV (News of the Irkutsk State Scientific Research Atiplague Institute of Siberia and the Far East), Vol 6, Irkutsk, 1946

Korobkova Ye.I., The Phagocytosis in the Acquired Immunity to Plague, Tr. in-ta "Mikrob," No 4 Saratov, 1960

id., Zhivaya protivochumnaya vaktsina (The Live Antiplague Vaccine), Moscow, Medgiz, 1956

Kolesinskaya N.I., Opsonophagocytic Index of the Leukocytes of Guinea Pigs and Rabbits' Blood, Immunized by Live Antiplague Vaccines (EB, 17, EB - 17), Izvestiya Irkutskogo gos. n.-i. protivochumnogo in-ta Sibiri i DV, Vol 20, Ulan-Ude, 1959

Pokrovskaya M.P., Kaganova L.S., Tsitologicheskiy metod izucheniya mekhanizma immuniteta (Cytological Method of the Study of the Immunity Mechanism), Sverdlovsk, 1947

Burrows T.W, Bacon G.A., The Basis of Virulence in Past. pestis: the Development of Resistance to Phagocytosis in vitro. Brit. J. Exper. Path., Vol 37, No 3, 1956

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