A STUDY OF THE POSTVACCINATION IMMUNITY OF GUINEA PIGS REPEATEDLY INOCULATED WITH A STRAIN OF BRUCELLA MELITENSIS

COMMUNICATION II.

Sh. Khodzhaev

From the Brucellosis Division (Head – Prof. P. A. Vershilova) of the N. F. Gamaleya Institute of Epidemiology and Microbiology (Director – Prof. S. N. Muromtsev) of the AMN SSSR, Moscow

(Received March 21, 1958. Presented by Active Member Acad. Med. Sci. USSR G. P. Rudnev)

Many years of research by P. F. Zdrodovskii, B. V. Voskresenskii, P. A. Vershilova, V. A. Shtriter, Kh. S. Kotlyarova and others [1-5] on the reproduction of experimental brucellosis in different species of animals and on studies of aspects of pathogenesis and immunity of this condition laid the foundations of the scientific development of methods of active prophylaxis of infections caused by these microorganisms.

Work by P. A. Vershilova proved that the immunological basis of the guinea pig could be modified by the subcutaneous injection of living vaccine from the strain of Br. abortus 19-BA, using a dose of 10^9 organisms.

A result of the use of living vaccine from strain BA, a marked decrease in the incidence of the disease has been achieved among persons working in areas of brucellosis among sheep. Moreover cases are known where persons vaccinated with living brucellosis vaccine have developed the disease after meeting large doses of the causative organism; under these circumstances the incidence of the disease among those vaccinated varied from 0.5 to 6-8%.

Every case of breakdown of immunity is known to be due, as a rule, to the relative nature or to the weak intensity of postvaccinal immunity. It may be considered that in both the phases of sterile and unsterile immunity, its intensity in reasonably identical conditions of vaccination may differ, depending on the individual susceptibility of the host on the one hand, and on the character and the frequency of super- and reinfection on the other. In this connection the study of the possible conditions for breakdown of immunity at different stages of vaccination becomes extremely important. To this end we carried out investigations to determine the reaction of immune guinea pigs to repeated inoculations of virulent organisms of the brucella group, in the phase both of unsterile [6] and of sterile (postvaccination) immunity.

EXPERIMENTAL METHOD

Guinea pigs, weighing 350-400 g (50 in number) were inoculated subcutaneously in the right inguinal region with a dose of 10^9 organisms of a vaccine strain of Br. abortus 19-BA, suspended in 1 ml of physiological saline. $7\frac{1}{2}$ months after vaccination, these guinea pigs, in a state of sterile immunity, were given three injections (at monthly intervals) of a virulent strain of Br. melitensis 548, in a dose of 20 organisms, subcutaneously in the right inguinal region. The intensity of the sterile (postvaccinal) immunity was studied in 3 subgroups of immunized guinea pigs.

The first subgroup of animals (10 in number) were autopsied 30 days after the first inoculation, and their organs (lymphatic glands, liver, spleen, brain, bone marrow, heart muscle, blood and urine) were used to inoculate culture media. The guinea pigs of the second and third subgroups were reinoculated at monthly intervals

after the primary reinoculation, with Br. melitensis 548 in a dose of 20 organisms, given subcutaneously in the right inguinal region.

One month after the second reinoculation, the 10 guinea pigs of the second group were autopsied, and bacteriological examination of the organs was carried out. The animals of the third subgroup were reinoculated three times (at intervals of one month), and 30 days after the third reinoculation with the same strain in a dose of 20 organisms these also were autopsied, and their organs used to inoculate culture media. As a control of each group, autopsy was carried out at the same times on guinea pigs which had simply been vaccinated as well as those subjected to one and two reinoculations.

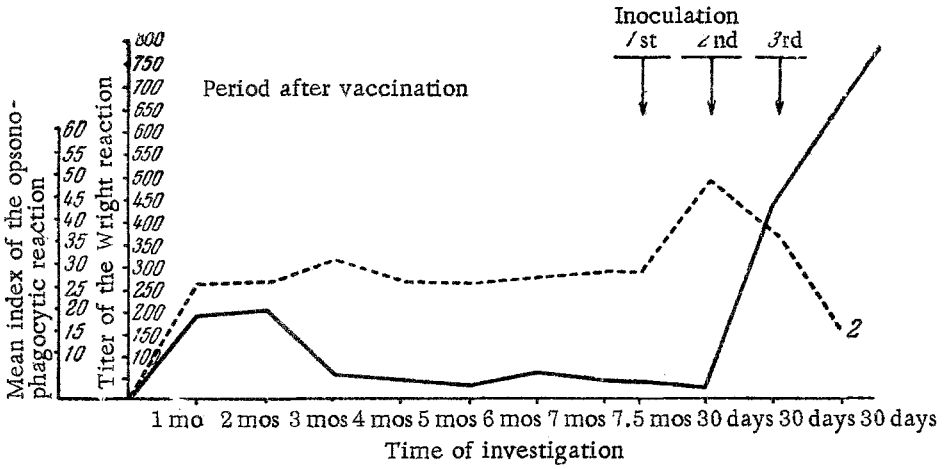

Fig. 1. Course of the Wright and opsono-phagocytic reactions in vaccinated guinea pigs during repeated reinoculation with a strain of Br. melitensis. 1) Wright reaction; 2) index of the opsono-phagocytic reaction.

Time since vaccin. at the time of inoc.	Number of times innocula-ted	Results of autopsy 30 days after inoculation
$7\frac{1}{2}$ months	Once	◪ ◪ □ □ □ □ □ □ □ □
	Twice, at monthly intervals	■ ■ ■ ■ ◧ ◧ ◪ □ □ □
	Three times, at monthly intervls.	■ ■ ■ ■ ■ ◧ ◧ ◧ ◧ ◪
Control of vaccine strain		□ □
Control of inoculation with Br. melitensis		■ ■

■ 1 ◧ 2 ◪ 3 □ 4

Fig. 2. Results of bacteriological investigations of immunized guinea pigs, reinoculated once, twice and three times with a strain of Br. melitensis. 1) Generalized infection; 2) infection of lymphatic glands; 3) regional infection; 4) culture from excreta.

Furthermore, besides bacteriological examinations, the agglutinin content of the animals was determined by Wright's reaction, together with the opsono-phagocytic reaction at the end of each month during vaccination (1 to 7 months) and also 30 days after each reinoculation, i.e. before autopsy of the animals. The mean values of the results obtained at each period of time in 20-25 immunized guinea pigs were calculated. Burnet's intradermal allergic test was carried out by the usual method at an interval of 30 days after vaccination and after each reinoculation.

EXPERIMENTAL RESULTS

The investigations during vaccination and repeated reinoculation with one, two or three doses of the organism in the phase of postinfective immunity gave the following results.

As can be seen in Fig. 1, in the guinea pigs immunized by inoculation of vaccine strain BA in a dose of 10^9 organisms showed characteristic immunological changes. The accumulation of agglutinins in the blood and the increase in the index of the opsono-phagocytic reaction began to take place on the 15th day after vaccination and reached their maximum after 2 months. After the third month, the trend of the Wright reaction and the opsono-phagocytic reaction in the vaccinated groups of guinea pigs showed a tendency to fall. At the moment of reinfection, i.e. $7\frac{1}{2}$ months after vaccination, the average titer of the Wright reaction and the opsono-phagocytic index reached their lowest values; 2 months after vaccination the Burnet intradermal allergic reaction was positive in the overwhelming majority of guinea pigs (25 of the 45 examined).

The Burnet Intradermal Allergic Reaction in Guinea Pigs at Different Intervals after Vaccination

Time after vaccination, months	Number of guinea pigs tested	Degree of the Burnet reaction					
		–	±	+	++	+++	++++
1	40	19	4	15	2	–	–
2	45	20	8	10	6	1	–
3	13	10	–	2	1	–	–
4	26	7	4	10	3	2	–
5	28	3	0	11	9	3	2
6	29	7	2	4	11	5	–
7	14	4	2	4	1	3	–

Note: Negative –; doubtful ±; weakly positive +; positive ++; markedly positive +++; strongly positive ++++.

It should be pointed out that in the control group of vaccinated guinea pigs, subjected to bacteriological examination, the vaccine strain was found up to 3 months after vaccination; on and after the fourth months after vaccination in no case were we able to isolate the vaccine strain from the animal's organs. Being satisfied in this manner that the vaccinated guinea pigs had passed both the phase of unsterile and the phase of sterile immunity, we began to study the role of repeated inoculation on the breakdown of postinfective immunity.

The results of the bacteriological examination of the organs of guinea pigs reinoculated on one, two or three occasions are illustrated by the data shown in Fig. 2, from which it can be seen that these results differed according to the number of reinoculations.

In the first group of vaccinated animals, for instance (10 in number), which received only one infecting dose, a strain of Br. melitensis was isolated from the regional lymphatic gland of only 2 guinea pigs, whereas in the remaining 8 animals (80%) no disturbance of the sterile immunity was observed. In contrast to this, in the experiments in which reinoculation was repeated twice at intervals of one month, the results of bacteriological examination revealed a breakdown of immunity in the majority of the experimental animals, i.e. Br. melitensis was isolated from 7 of the 10 guinea pigs, and moreover in 5 animals a generalization of the infection was found; in one a strain of Br. melitensis was isolated from many of the lymphatic glands, in the other – only from the regional lymphatic gland. Despite the two reinoculations, three guinea pigs of this subgroup preserved their insusceptibility.

The guinea pigs of the third subgroup, exposed to three infecting doses (at monthly intervals), were all found to be infected on bacteriological examination. Of the 10 guinea pigs in this subgroup, in 5 a generalized infection was demonstrated, in four Br. melitensis was isolated from the lymphatic gland, and in one guinea pig only the infection was regional in character. Further, at autopsy on 2 guinea pigs inoculated 3 times, the inguinal and cervical lymphatic glands were found to be enlarged to the size of walnuts, and contained pus. Subcultures taken from the pus from these guinea pigs also gave positive results of isolation of Br. melitensis.

The immunological reactions both during vaccination and, more especially, after repeated infection, showed very characteristic changes. In particular, as seen in Fig. 1, the mean titer in the Wright reaction and the mean index of the opsono-phagocytic reaction were characterized during the course of vaccination by a gradual rise, reaching a maximum at the end of the second month. From the third month after vaccination a gradual fall was observed in the blood agglutinin titer, and 7-8 months after vaccination the mean titer of the Wright reaction was 1:33, and the mean index of the opsono-phagocytic reaction throughout the process of vaccination, if we disregard slight variations, remained on the whole at roughly the same level (20-28). Of special interest were the fluctuations of the mean agglutinin titer and of the index of the opsono-phagocytic reaction in response to repeated infection. Thus it may be seen from Fig. 1 that whereas $7\frac{1}{2}$ months after vaccination and 30 days after the first inoculation the mean titer of the Wright reaction was 1:20 and the agglutinin titer was not increased, despite the inoculation of a virulent strain, soon after the second inoculation the trend of the Wright reaction altered sharply, on account of a rapid accumulation of agglutinins; on the 30th day after the second inoculation the mean titer (which was 1:20 after the first inoculation) reached 1:450.

Moreover, in guinea pigs receiving three inoculations, the accumulation of agglutinins on the 30th day after the third inoculation reached on the average the value of 1:750, i.e. almost 40 times greater than the original figure.

Analysis of the results obtained on the accumulation of agglutinins in the blood, by comparison with the results of bacteriological investigations of each guinea pig taken individually, revealed a characteristic picture: a close connection was observed between the accumulation of agglutinins and the degree of dissemination of organisms throughout the organs of the guinea pig. In the animals of the first subgroup, for instance, receiving one inoculation, the titer of the Wright reaction stayed at the same level both before and after infection, whereas during a generalized infection the mean titer of the agglutinins reached 1:1000. On the other hand, in guinea pigs from which strains could be isolated only from the lymphatic glands, the mean titer was 1:550, and in the presence of regional infection – 1:30.

By comparison of the results of the Wright reaction and the mean index of the opsono-phagocytic reaction in all 3 subgroups of guinea pigs it was shown that after repeated infection the titer of the Wright reaction rose and, on the contrary, the index of the opsono-phagocytic reaction fell. In guinea pigs with a low agglutinin titer and in case of regional infection the index of the opsono-phagocytic reaction was high (50-60), but in guinea pigs with an average Wright reaction titer of 1:1000, in the presence of generalized infection the index of the opsono-phagocytic reaction fell to 15 units.

A study of the trends of the Burnet intradermal allergic test also showed a reaction characteristic of the immunological reorganization of the animals during the process of vaccination and after each repeated inoculation. As may be seen from the table, in the fifth month after vaccination, in the overwhelming majority of the guinea pigs (in 25 of 28) the intradermal allergic reaction was positive. After the sixth month the intensity of the Burnet reaction gradually fell and in 7 of 29 guinea pigs at this time it was negative. The character of the intradermal allergic test in the vaccinated guinea pigs $7\frac{1}{2}$ months after vaccination, i.e. before the first inoculation, was shown by total extinction in 4 guinea pigs, by a weakly positive reaction in 2 and by a positive reaction in only 4 guinea pigs.

In the guinea pigs of the same subgroup, even a single superimposition of the infective agent, i.e. infection with Br. melitensis $7\frac{1}{2}$ months after vaccination had a strongly sensitizing effect. Only 30 days after the single inoculation the Burnet reaction became positive in all 10 guinea pigs. In the majority of the animals (in 6 of 10), 30 minutes after the single inoculation a positive Burnet intradermal reaction was noted, with an area of edema measuring 4 × 5 cm, and with tissue necrosis. In the remaining 4 guinea pigs the reaction was positive. Subsequently, in the guinea pigs subjected to two and three inoculations, an increase in the intradermal allergic reaction was observed also, with the development of tissue necrosis.

It may thus be seen from the results described that frequent superimposition of infection in the form of single, double or triple inoculation led to a severe allergic reorganization of the animal.

As a general conclusion from the results it may be said that the intensity of immunity in guinea pigs vaccinated with strain 19-BA of Br. melitensis is high in relation to a single reinfection during the period of sterile immunity.

The results obtained during double and triple reinfection indicate a comparatively rapid breakdown of the resistance of the vaccinated animal. This breakdown of immunity in 70% of guinea pigs after double reinfection and in 100% after triple (in contrast to the first subgroup of guinea pigs, in which 80% of the animals were insusceptible after a single reinfection) justifies the provisional hypothesis that such a breakdown of immunity is possible in vaccinated animals not only as a result of an encounter with a large dose of Br. melitensis, but also as a result of repeated encounters with small doses of infection.

SUMMARY

The "break" of vaccinal immunity both in the steril and nonsterile phases led the authors to perform the following experiments: the vaccinal process was experimentally induced in guinea pigs by administration of the 19-BA, vaccine strain. The intensity of the sterile (postvaccinal) immunity was studied by repeated infection with a virulent Br. melitensis culture. It has been established that 17½ months after immunization in 80% of cases the guinea pigs were still immune to a single infection with Br. melitensis. In reiterated infection with one month interval broken immunity was recorded in 70% of the cases, while a three-fold succession of infection with the same time interval – showed disrupted immunity in nearly 100% of the cases and a subsequent development of a generalized infection.

LITERATURE CITED

[1] P. A. Vershilova, Byull. Éksptl. Biol. i Med., 23, 6, 429-433 (1947).

[2] B. V. Voskresenskii, P. A. Vershilova and V. A. Shtriter, Brucellosis, 366-367, (Moscow, 1937). [In Russian].

[3] P. F. Zdrodovskii, Brucellosis (Moscow, 1953). [In Russian].

[4] Kh. S. Kotlyarova, Recovery, Immunity and Active Immunization in Brucellosis. Doctorate Dissertation, (Moscow, 1940). [In Russian].

[5] V. A. Shtriter, The Immunology of Brucellosis, Doctorate Dissertation, (Moscow, 1940). [In Russian].

[6] Sh. Khodzhaev, Zhur. Mikrobiol., Épidemiol. i Immunobiol., 9, 97-99 (1958).