Significance of Bacterial Agglutination in the Development of Experimental Sepsis

A. A. Pal'tsyn, E. G. Kolokol'chikova, I. A. Grishina, N. V. Chervonskaya, A. K. Badikova, and E. P. Popova

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The development of signs of generalized infection were studied in rats infected with a suspension of *Pseudomonas aeruginosa,* with dissociated (controls) or agglutinated (experiment) immune serum. All signs of the generalization of the process were reliably lower in the experiment than in the control. A favorable course of the infection after the administration of agglutinated agent indicated that the infection was generalized and secondary foci were initiated during the first days postinoculation, when antibodies to the agent had not yet been produced.

Key Words: *antibodies; agglutination; bacteremia; sepsis*

Previous clinical studies [4-6] brought us to the conclusion that the entry of bacteria into the blood and the generalization of infection occur during the first few days after the appearance of bacteria in the primary focus, when antibodies to these bacteria have not yet been produced. When antibodies do appear, the bacteria in the primary focus become agglutinated. Agglutinates are less able to move through the intercellular spaces and to cross the histohematic barrier than are solitary bacterial cells. Hence, the possibility that an infectious process will become generalized lessens after the production of antibodies starts. If this assumption is true, agglutination of bacteria at the early stage of the disease, starting from the moment of their entry into the body till the onset of antibody production, will promote a more benign course of the infectious process, suppressing its generalization, among other things. The present research was undertaken to verify this hypothesis.

Pathoanatomy Department, Laboratory for the Prevention and Treatment of Bacterial Infections, A. V. Vishnevskii Institute of Surgery, Russian Academy of Medical Sciences, Moscow. (Presented by V. D. Fedorov, Member of the Russian Academy of Medical Sciences)

MATERIALS AND METHODS

Experiments were carried out with rats weighing 180 to 200 g, which were divided into 3 groups. Group 1 (controls) were infected by injection of 0.3 ml of a suspension of a 1-day culture of *P. aeruginosa,* strain 453, washed from agar, containing 8×10^9 cells/ml of a 10% $CaCl_2$ solution, into the right gastrocnemius [7]. Group 2 animals were infected in the same manner as group 1, but, in addition, 0.3 ml of immune rat serum heated for 5 min at 56°C was injected into the left gastrocnemius during the first 3 days postinfection. In group 3 all the conditions of inoculation were the same as in group 1, but the bacteria injected were not in a dissociated, but in an agglutinated (by adding 2-5% heated immune serum) suspension.

The rats were immunized by 3 injections at 7-day intervals of 0.3 ml of a washed-from-agar suspension of a 1-day culture of *P. aeruginosa,* strain 453, containing 2×10^9 cells/ml of isotonic NaCl solution. Blood for the preparation of serum was collected 7 to 60 days after the third injection of bacteria.

The animals of all groups were sacrificed 15 days after inoculation; bacteriological examination

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TABLE 1. Results of Observations and Bacteriological Analysis of Sacrificed and Dying Animals (Bacteriological Examination Could Not Be Performed in Some Animals)

Group	Number of animals surviving to day 15	Number of animals dying before day 15	Results of bacteriological analysis of spleen			Results of bacteriological analysis of kidneys			Number of animals with foci in the kidneys and results of bacteriological analysis of these kidneys		
			A	B	C	A	B	C	A	B	C
1. Infection with dissociated bacteria (control)	24	9	10	8	9	14	11	4	12	3	2
2. Infection with dissociated bacteria + injection of immune serum	13	5	4	9	3	6	7	3	3	1	0
3. Infection with agglutinated bacteria	30	0	5	15	10	3	11	16	1	1	3

Note. A) *P. aeruginosa*; B) other microflora; C) sterile. Reliability of difference between groups 1 and 3 by χ^2 test: death before day 15, $p<0.01$; incidence of isolation of *P. aeruginosa* from the kidneys, $p<0.01$; incidence of detection of metastatic foci containing *P. aeruginosa* in the kidneys, $p<0.01$; incidence of absence of microflora in the kidneys, $p<0.01$.

of the spleen and kidneys was carried out. Serum was obtained from the blood and the titer of antibodies to bacteria isolated from the spleen and kidneys was determined. Rats which died before day 15 postinfection were examined in the same way.

In order to determine the titer of agglutinating antibodies, a series of tubes with 0.05 ml of tested serum in ascending 1:2 dilutions and 0.45 ml of a *P. aeruginosa* (strain 453) suspension (1×10^9 cells/ml of Hanks solution) were prepared. The tubes were incubated at 37°C for 1 h. After incubation a drop of liquid from the tube was placed into a Goryaev chamber and examined at phase-contrast illumination and 200× magnification. The capacity of the serum to agglutinate bacteria was characterized by the titer, that is, the minimal concentration at which bacterial cells were not solitary but united in groups (agglutinates).

RESULTS

All the animals had 1/640 to 1/5120 titers of antibodies to *P. aeruginosa* isolated from the spleen and kidneys. No antibodies to other bacterial species isolated from these organs were detected. The absence of antibodies indicates that the isolation of any bacteria other than *P. aeruginosa* from the spleen and kidneys reflects transitory bacteremia in the sick animals, most likely caused by microflora of the intestine or upper respiratory tract. The strains to which no antibodies are found are not a cause of wound infection, but are accidental for the disease of a certain animal. Isolation of such strains from the blood and viscera does not indicate the agent of the infection, although it may be used as an indicator of the disease severity (Table 1).

The following parameters were used to assess the generalization of the infectious process: the number of animals dying before day 15 postinfection; the incidence of *P. aeruginosa* detection in bacteriological analysis of the spleen; the incidence of *P. aeruginosa* detection in bacteriological analysis of the kidneys; abscesses in the kidneys caused by *P. aeruginosa*. The spleen was selected as an object of bacteriological investigation because it is in this organ that the bacteria circulating in the blood are concentrated and phagocytized [9,11,13]. It is well known that the state of bacteremia is not permanent [2,8,10,14]. Bacteria may rapidly disappear from the blood, particularly at the stage of the disease when antibodies appear, speeding up blood purification due to opsonization. Our previous experiments demonstrated that bacteria stay in the spleen longer than in the blood and, hence, analysis of the spleen permits a more reliable detection of bacterial transmission in the blood than analysis of the blood itself. Examination of the kidneys was prompted by the findings of V. G. Teplyakov *et al.* [7], who revealed that the disease caused by *P. aeruginosa* strain 453 is characterized by predominant localization of secondary foci in the kidneys.

The above indicators of generalization of the infectious process were the most pronounced in the control (group 1). Group 2 (passively immunized animals) did not differ much from the control according to all the parameters. In an attempt to find out why passive immunization was ineffective, we titered antibodies in animals of the 2nd group 1 day after inoculation and injection of the immune serum. The titer of the serum used for immunization was 1/5120; the titer of the serum

of passively immunized animals did not exceed 1/10. Such a low titer does not prevent bacteremia, as was shown by clinical observations [6]. Comparison of the results in groups 1 and 2 confirms these clinical data and indicates that in the case of a 1/10 titer, generalization of the infectious process is virtually not suppressed.

In group 3 animals, to which the infective dose was injected in an agglutinated form, the infection ran a more benign course than in controls, all the indexes of the disease severity and generalization of the process being lower. The parameters directly indicating the generalization of the infectious process, such as animal mortality and formation of secondary foci (incidence of isolation of the agent from the kidneys upon bacteriological analysis and incidence of detection of macroscopically visible abscesses containing *P. aeruginosa*), were reliably lower than in the control ($p<0.01$).

Secondary or metastatic foci are considered to be a comparatively late sign of a septic process already in full bloom. The presence of this sign permits a final diagnosis of septicopyemia. Cases of sepsis where secondary foci are detected only at autopsy are common. Thus, inoculation with an agent in an agglutinated form affects the late manifestations and outcome of sepsis. At the same time, inoculation of agglutinated, rather than a dissociated, agent is significant only during the first 3-4 days postinfection. In the selected model of the pathological process, antibodies in titers 1/10 to 1/640 appear as soon as on day 3, the titers increasing to 1/640-1/1280 on day 4. With such titers, the bacteria in the primary focus are agglutinated by endogenous antibodies, and it is apparently no longer important for further (after day 4) resorption of the agent and the products of its vital activity from the primary focus, in which form, dissociated or agglutinated, the bacterium was initially introduced. The decline in the level of generalization of the process after injection of agglutinated agent and the fact that this measure may be significant only till day 4 of the disease prove that contamination with the bacteria initiating secondary foci occurs at an early stage of the process, before the production of antibodies to the agent of the primary focus is begun. This assumption is in line with our previous clinical findings [4-6].

The results of numerous attempts at immunotherapy of wound infection are contradictory [1,3,12]. Positive and negative observations have not been clearly explained, and the method is not yet universally accepted. The results of this research permit us to formulate the three conditions conducive to effective passive immunotherapy.

1. The antibodies for inoculation should be specific to the strain causing the disease so as to provide for agglutination in titers of at least 1/80-1/160.

2. The dose of injected serum should be sufficient to create such a titer.

3. Immunotherapy may be effective only during the first few days after infection, when the production of antibodies has not yet started, the agent is disseminating in the body, and secondary foci are being initiated. In patients with depressed immunological function and delayed production of their own antibodies immunotherapy may be effective in later periods as well.

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