Factors Underlying a Benign or Malignant Course of Infection Induced by Different Strains of *Pseudomonas aeruginosa*

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Generally speaking, different microbes cause different diseases due to a specific response of the macroorganism to the peculiar biological properties of the infectious agent. Knowledge of the concrete principle of this specificity sheds light on the pathogenesis of a disease and enables the physician to positively affect its course and outcome. Many scientists have directed their efforts to resolve this problem, and solid data have now been obtained on numerous infectious agents and the disorders induced by them. Nevertheless, the problem is far from being definitively solved with respect to many infectious processes and, in particular, the wound infection.

During the creation of an experimental model of surgical sepsis, it was found that the infectious processes induced by *Pseudomonas aeruginosa* strains №9 and №453 (obtained from the culture museum of the L. A. Tarasevich Research Institute for the Standardization and Monitoring of Medical and Biological Preparations) differ significantly from each other [3,4]. Bacteria of strain №9 induced only a local inflammatory process, there were no metastatic foci in organs, and the animals did not die. We defined such a course of an infection as benign. After inoculation with bacteria of strain №453 the lethality over a period of 3-14 days was 77%, and in 60% of the infected rats secondary pyemic foci were noted in the kidneys, i.e., the infectious process developed malignantly in the form of pyosepticemia. Subsequent studies of wound infection revealed a cause (probably not the only one) of the benign course of the process induced by bacteria of strain №9.

MATERIALS AND METHODS

Albino rats weighing 180-200 g were infected by an injection into the triceps surae muscle of 0.3 ml of suspension flushed from agar of a 24-h culture of *Pseudomonas aeruginosa* (strains №9 and №453) containing 8×10^9 cells/ml in a 10% solution of calcium chloride. The animals were sacrified 3, 4, and 7 days later (a minimum of 10 rats infected with every strain in each period). A bacteriological assay of blood and spleen was performed. The titer of antimicrobial antibodies was determined in the serum. For this purpose a series of test-tubes containing 0.45 ml of the flush of studied bacteria (1×10^9 cells per ml Hanks solution) and 0.05 ml of the studied serum in a progressive dilution 1/2 was prepared. The test-

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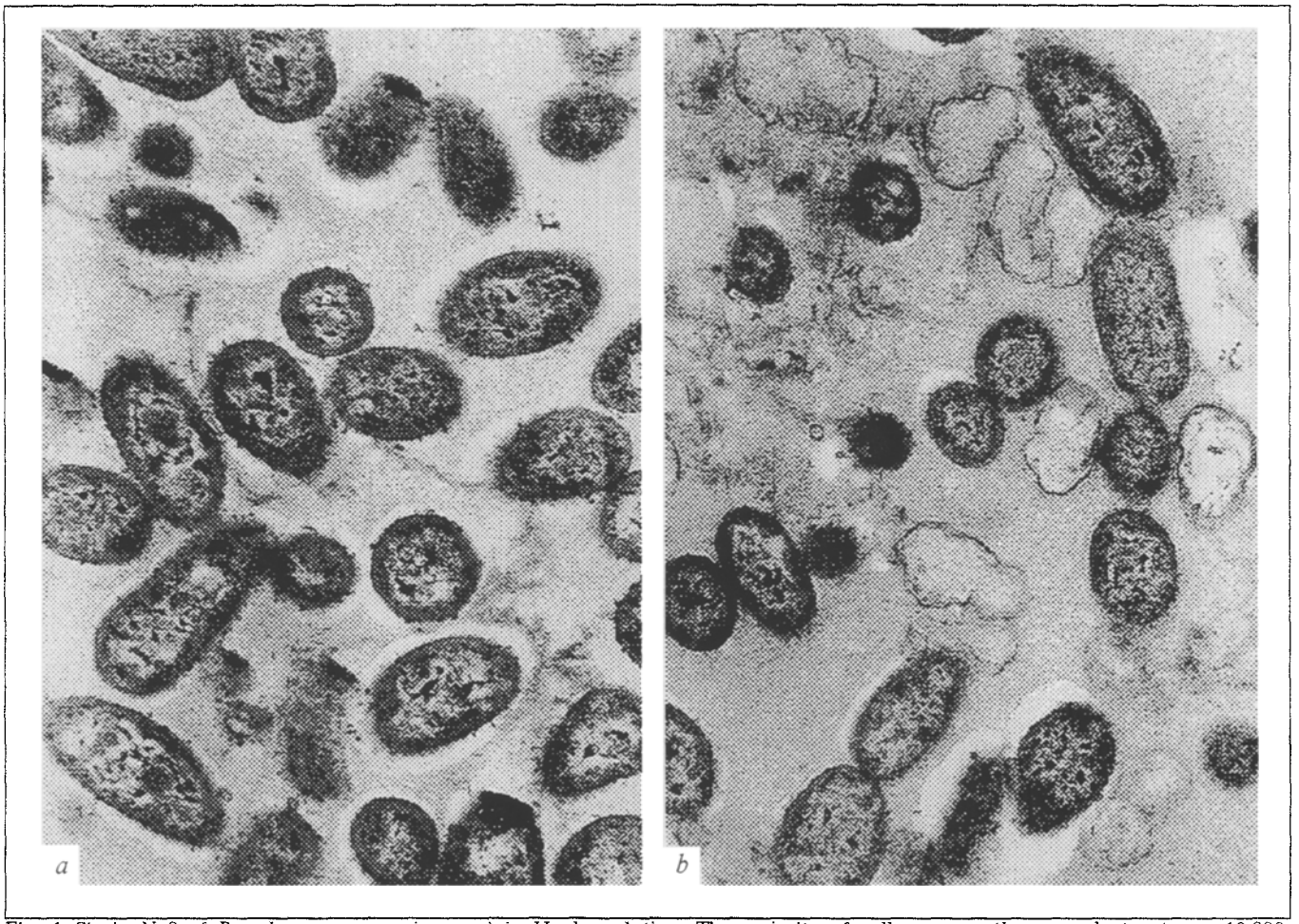

Fig. 1. Strain № 9 of *Pseudomonas aeruginosa*. *a*) in Hanks solution. The majority of cells possess the normal structure, ×18,000; *b*) in Hanks solution containing 1/20 part of native serum against bacteria of strain № 9. Many cells look like small empty sacs, only the coats being preserved, ×14,000.

tubes were incubated at 37°C during one hour. After incubation a drop of the fluid from a test-tube was placed in a Goryaev chamber and examined under a phase-contrast microscope, ×200. The capacity of serum to agglutinate bacteria was characterized by the titer, i.e., the minimal concentration in which the bacterial cells were arranged not one by one, but united in groups, or agglutinates. After the study of agglutination, the optical density of the microbial suspension (transmission coefficient) was measured. For this, plastic cuvettes were filled with 0.4 ml of suspension and placed under a microscope between the field diaphragm and the condenser. The change of the light signal was recorded according to the exposure readings of the automatic device mounted on the microscope. The number of bacteria after the interaction between the microbial suspensions and sera was determined by measuring the number of colonies after seeding of the diluted suspensions. The electron microscopic examination of the bacteria was performed by a method described previously [1,2].

TABLE 1. Titer of Antimicrobial Antibodies to *Pseudomonas aeruginosa* Strains № 9 and № 453 at Different Times after Inoculation

Strain	Time after inoculation, days		
	3	4	7
№ 9	0–1/80	1/80–1/320	1/320–1/1280
№ 453	1/10–1/640	1/640–1/1280	1/1280–1/2560

RESULTS

One day after inoculation the state of the primary focus in animals infected by bacteria of strains №9 and №453 were not significantly different from each other. The shank and thigh of the damaged extremity were markedly swollen and limb function was impaired. The general state of the animals infected with the №9 strain was not affected; the inoculation of animals with strain №453 resulted in poor mobility, disorders in intestinal function, and nasal discharge. Considering that in our experiments the animals were sacrificed rela-

TABLE 2. Lysis and Agglutination of *Pseudomonas aeruginosa* strains No.9 and No.453 by Different Sera

Strain	Sera					
	Native of intact rat	Native against strain №9	Heated against strain №9	Native against strain №453	Heated against strain №9 + native of intact rat	Heated against strain №9 + native against strain №453
№ 9	L−	L+	L−	L−	L+	L+
	A−	A+	A+	A+	A+	A+
№ 453	L−	L−	L−	L−	L−	L−
	A−	A+	A+	A+	A+	A+

Note. L signifies lysis, A signifies agglutination; + means the presence of reaction, − means the absence of reaction.

tively soon after inoculation, it is not justified to conclude that lethality resulted from infection. But a distinction between strains appeared under these conditions, too. The lethality among the animals infected by strain №453 3-7 days after inoculation comprised 30%, whereas the animals infected by strain №9 did not die. The titer of antibodies is shown in Table 1.

Agglutination was attended by a slight increase of the optical density of the suspension. Thus, while the transmission coefficient of the solvent (Hanks solution) is accepted as 100%, the transmission coefficient of the bacterial suspension without serum was 40-50%, and the transmission coefficient of the agglutinated microbes was 30-38%. The bacteriological study of the number of bacteria showed at least a tenfold drop of the count in the samples of agglutinated suspensions compared to the samples without pronounced agglutination. Of course, the results of the bacteriological study must not be considered as evidence of a real drop of the number of bacteria in the suspension. More likely they indicate the degree of agglutination, since under these conditions colonies are formed not by single bacterial cells, but by agglutinates.

A difference in the interaction of strains №9 and №453 with the serum was noted in suspensions with a relatively high content of serum (1/10-1/40). Bacteria of strain №453 were conjugated in agglutinates of varying size, while solitary bacteria between the agglutinates preserved their mobility. Bacteria of strain №9 lost their mobility for serum concentrations of 1/40 and higher, and there was an abrupt drop of the number of bacteria in 1/20 and 1/10 dilutions in comparison with the samples of higher dilutions. Bacteria became less visible and acquired the appearance of shadows of bacterial cells due to their lysis. Lysis of bacteria was also detected under the electron microscope (Fig. 1). Determination of the optical density confirmed the visual data, namely, the transmission coefficient increased to 68-74%. Heating serum at 56°C for 5 min eliminated its ability to lyse bacteria. This attested to the participation of complement in microbe lysis [5,6]. The bacteriological assay showed that the amount of microbes in the 1/10-1/20 dilutions of native serum is 10-100 times lower than in the same dilutions of heated serum. As is shown in Table 1, the sera of some rats 3 days after inoculation did not possess an agglutinating activity (the titer is zero). However, even these sera were able to lyse bacteria of strain №9. Hence, the lysis test proved to be more sensitive than the agglutination reaction.

The interaction between bacteria of strain №9 and antiserum to strain №453 was studied in order to check whether the lysis of cells of strain №9 is related to activation of complement via an alternative pathway (without the formation of antigen-antibody complexes) or to enhanced sensitivity of the bacteria to the complement. It was found that these sera agglutinate bacteria of strain №9 only in a somewhat lower titer than bacteria of strain №453. However, there was no lysis of bacteria of strain №9 during agglutination by antiserum to strain №453. Bacteria of strain №9 were lysed in the mixture of heated anti-№9 strain-serum (as a source of antibodies) and native anti-№453-serum or native serum of an intact rat (as a source of complement) (Table 2).

As is shown in Table 2, the assumption that complement is activated via an alternative pathway has been not confirmed because lysis took place only in those samples where antibodies were homologous to strain №9. The result obtained does not support the idea of a high sensitivity of the cells of strain №9 to complement, because these cells were agglutinated but not lysed by anti-№453-serum. For the time being we can only posit a hypothetical interpretation of the described phenomenon. Immunization with bacteria of both strains resulted in the synthesis of different antibodies by the organism: in the case of №9 they can, and in the case of №453 they cannot, activate complement binding with antigen. An important role has been ascribed to complement in

opsonization of bacteria entering the circulation and their subsequent removal from the blood by liver and spleen phagocytes [7]. An another effect of complement, namely, the lysis of microbial cells, has been found in the present investigation. This effect, like opsonization, promoted purging of the blood of the infectious agent. The bacteriological assay of 22 rats 3 days after inoculation with bacteria of strain №9 revealed *Pseudomonas aeruginosa* microbes in the blood of 1 and in the spleen of 4 animals; microbes were present in the blood of 7 and in the spleen of 13 rats among the same group of 22 animals infected by strain №453.

Thus, one cause of the benign course of infection induced by *Pseudomonas aeruginosa* strain №9 was complement-dependent lysis of the infectious agent.

The results demonstrate the artificiality of discussing whether the macro- or microorganism has the leading or more important role in the infectious process. It would be equally wrong to assert that the benign course of infection induced by bacteria of strain №9 is governed by the microbe (its antigen specificity responsible for the production of special antibodies) or by the macroorganism (its capacity to activate complement). The peculiarity of the infection process in question derives from the interaction of the two partners involved.

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