Suppression of Phagocytosis in Immunized Animals

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It is found that phagocytosis in animals immunized with *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* is suppressed in comparison with intact animals, and the degree of suppression rises with the antibody titer. Morphological study of the site of infection shows that antibodies markedly enhance bacteriopyknosis, i.e., convert bacteria into nonphagocytizable detritus. Focal suppression of phagocytosis in immunized animals is attributed to rapid bacteriopyknosis and, consequently, to a sharp decrease in the number of intact bacteria that can be digested by phagocytes.

Key Words: *infectious immunity; antibodies; phagocytosis; nonspecific organism's resistance*

Enhanced phagocytosis in immunized individuals has been considered as the main mechanism of antibacterial immunity. This enhancement is provided by antibodies [1,2,5,9]. Antibodies mediate bacterial binding to the phagocyte surface with subsequent formation of the antibody-bacterium complexes which trigger the classical pathway of complement activation. As a result, bacteria bind to phagocytes via the receptors for antibodies and complement [6,10]. Opsonization, i.e., the phagocyte-stimulating effect of antibodies, was confirmed by *in vitro* experiments: absorption and killing of bacteria by neutrophils and macrophages is considerably increased in the presence of antibodies. Systemic administration of antibodies often produces a protective effect and alleviates the disease. The protective effect of antibodies *in vivo* and the opsonizing effect *in vitro* provide the basis for the modern concept of infectious immunity. This theory has been confirmed by considerable experimental and clinical data accumulated during the last century; however, there is evidence contradicting it. It was found that despite the pronounced opsonizing effect, antibodies elicit no protective effect in some experimental infections [7,8,11]. These reports, however, did not disprove the classic Mechnikov theory, since the authors could not explain their findings and had no doubts concerning the facts that phagocytosis is the main antimicrobial mechanisms and the immunity is provided by enhanced phagocytosis.

Here we report a simple experiment demonstrating that phagocytosis *in vivo*, in contrast to that *in vitro*, is inhibited by antibodies and make an attempt to explain this phenomenon.

MATERIALS AND METHODS

Experiments were carried out on nonpedigree rats weighing 170-300 g. The animals were infected with one-day-old agar cultures of *Pseudomonas aeruginosa* (strain 453), *Staphylococcus aureus* (strains 3377 and 3384), and *Staphylococcus epidermidis* (strain 4017) isolated from burns. The bacteria were suspended in Hanks' solution, and 0.3 ml of this suspension was injected into the gastrocnemius muscle. The intensity of phagocytosis was assessed by the mass of exudate in the site of injection after 7 days. All necrotic detritus from the site of infection was collected and weighted. For active immunization the same cultures were injected into rats in a 10-fold lower dose 3 times at one-week intervals. Immunized and control (intact) rats were infected simultaneously with the same bacterial suspension one week after the last immunization. Some animals were sacrificed

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under ketamine narcosis 0.5 to 22 h after the injection, and the sites of infection were studied morphologically. The antibody titer was determined by the agglutination reaction [3].

The significance of differences was evaluated by the Wilcoxon test.

RESULTS

Differences between experimental and control animals in terms of pus weight were noted only for two of the four strains. Phagocytosis was maximally suppressed in immune animals with blue pus infection (Table 1). It was significantly lowered in animals infected with *S. aureus* 3377. In animals infected with *S. aureus* 3384 the difference between control and experimental groups was insignificant, while neither control nor experimental rats infected with *S. epidermidis* developed abscesses, i.e., macroscopic manifestations of phagocytosis.

Comparison of the weight of purulent foci with the antibody titer showed the maximum rise of the antibody titer (from 0 before immunization to 1/1280-1/5120 after it) in animals with blue pus infection characterized by the maximum drop of phagocytosis.

A lower significant suppression of phagocytosis was also noted in rats infected with *S. aureus* 3377 against the background of a less pronounced (compared with blue pus infection) rise of antibody titer from 1/20 (in the control) to 1/320-1/5120. The absence of statistically significant differences between intact rats and those infected with *S. aureus* 3384 in terms of the weight of purulent foci coincided with a relatively small rise of antibody titer in this infection (1/640-1/5120 *vs.* 1/320 in the control). Rats infected with *S. epidermidis* developed purulent foci and generated neither normal nor immune antibodies (agglutination reaction). Thus, the higher titer of antibodies, the more pronounced the suppression of phagocytosis. Consequently, our findings contradict the classical theory that phagocytosis is stimulated by antibodies. This contradiction was reconciled by morphological investigation of the infection foci.

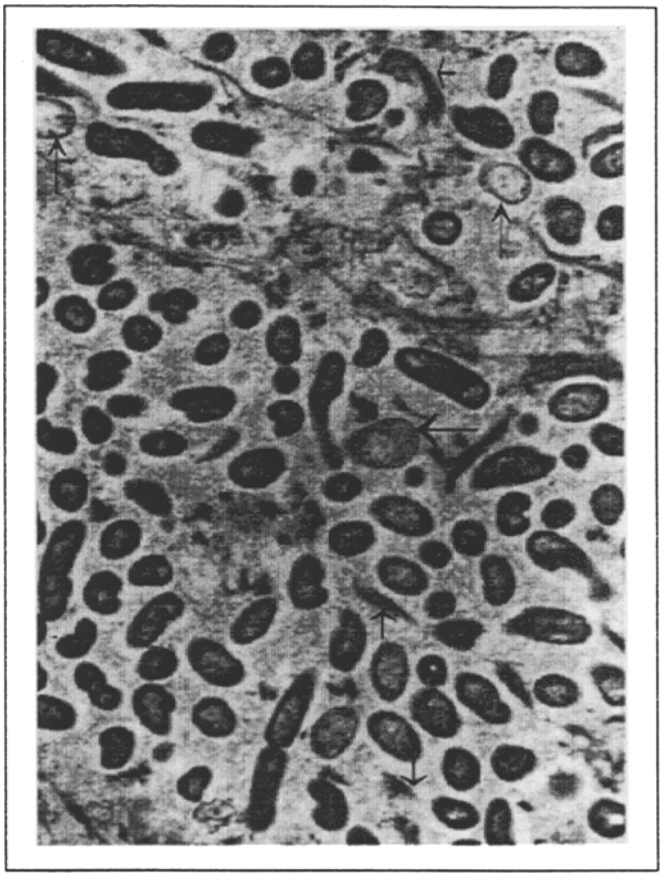

Fig. 1. The site of infection in control rat 0.5 h after inoculation of *P. aeruginosa*. Most bacteria preserve their normal structure. Long arrows indicate occasional cells with clarified cytoplasm and other structural alterations. Short arrows point at the detritus.

Morphological differences between control and experimental groups, as well as the differences in serological and phagocytosis parameters, were most pronounced in blue pus infection. The state of patho-

TABLE 1. Mass of Purulent Exudate in Infection Site in Control and Immunized Rats

Agent	Group	Number of animals	Number of injected bacteria	Titer of specific antibodies before infection	Mean mass of exudate, mg
P. aeruginosa (strain 453)	Control	7	1.5×10^8	0	404
	Immunization	7	1.5×10^8	1/1280-1/5120	38*
S. aureus (strain 3377)	Control	15	1×10^9	1/20	106
	Immunization	15	1×10^9	1/320-1/5120	35*
S. aureus (strain 3384)	Control	10	3×10^8	1/320	36
	Immunization	10	3×10^8	1/640-1/5120	30
S. epidermidis (strain 4017)	Control	5	3×10^9	0	No abscesses
	Immunization	5	3×10^9	0	No abscesses

Note. *$p<0.01$ in comparison with the control.

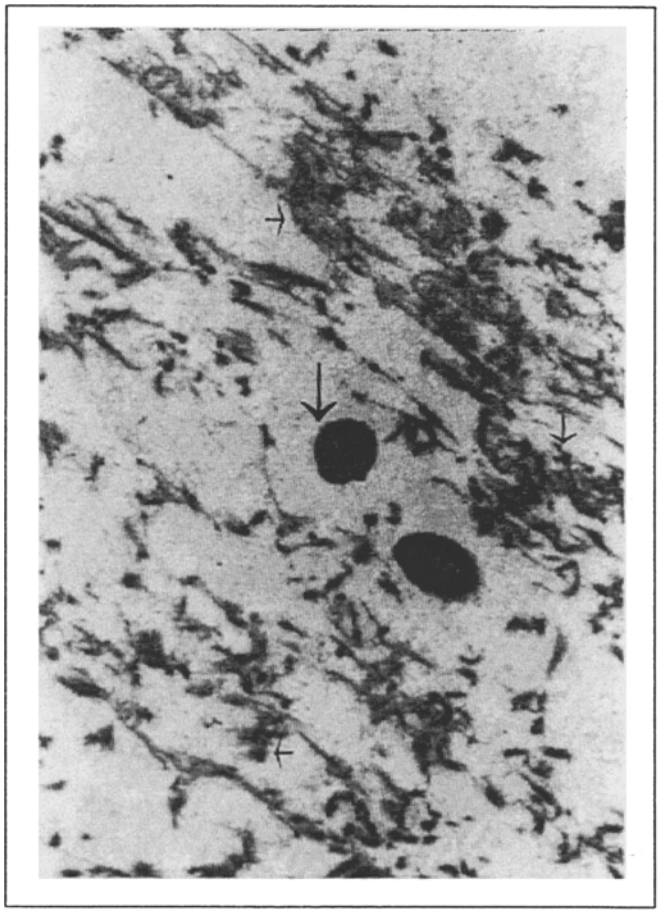

Fig. 2. The site of infection in immunized rat 0.5 h after inoculation of *P. aeruginosa*. Short arrows indicate occasional bacteria with preserved structure among bacteriopyknotic detritus. Long arrow indicates bacteria with homogenous cytoplasm.

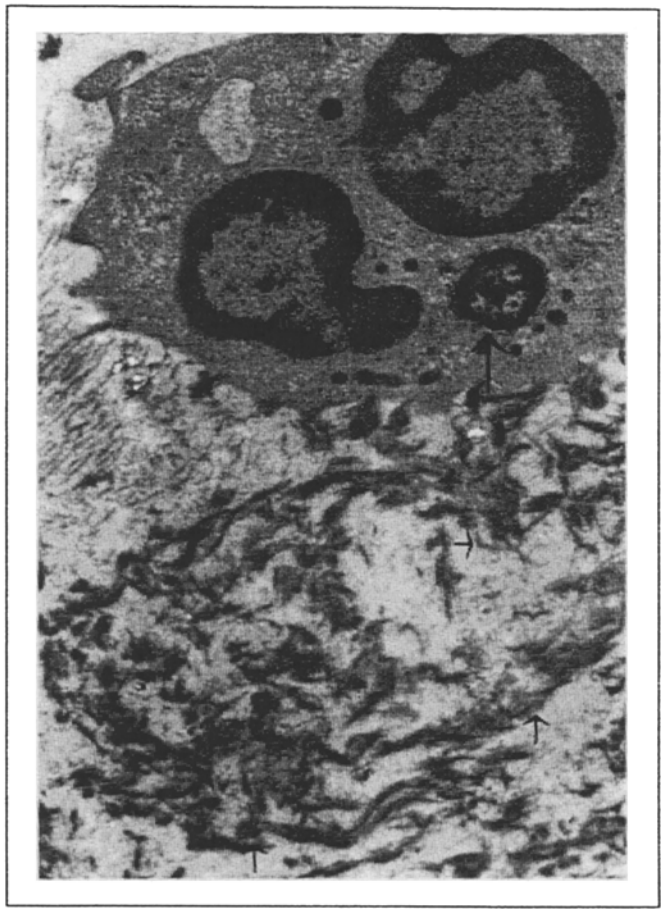

Fig. 3. The site of infection in immunized rat 2 h after inoculation of *P. aeruginosa*. Short arrows indicate considerable accumulation of pyknotic detritus in the intercellular space. Long arrow indicates a bacteria with preserved structure within a neutrophil.

genic bacteria in the foci differed considerably as early as 0.5 h postinfection. The majority of bacterial cells in the control remained unchanged. The early signs of bacteriopyknosis, i.e., conversion of bacteria into detritus [3,4], were seen. There were intermediate forms between bacteria and detritus, i.e., bacterial cells with pronounced deformations (Fig. 1). Bacteriopyknosis predominated in the foci of immunized animals 0.5 hour postinfection. Most bacteria were turned into detritus. Occasional microbial cells with preserved contours and altered internal structure were seen (Fig. 2). One and two hours after infection, there were practically no morphologically unaltered bacteria in the intercellular space. Morphologically changed bacteria were seen in phagocytes. Thirty minutes after infection, occasional phagocytized bacteria were present only in macrophages, while 1 h postinfection more bacteria were internalized by both macrophages and neutrophils. After two hours, only phagocytized bacteria (primarily by neutrophils) remained morphologically unchanged, while in the intercellular space practically all bacteria underwent bacteriopyknosis (Fig. 3). The detritus was rarely scavenged by phagocytes. In the control, bacteriopyknosis was slower, and most bacteria preserved normal structure for 2 h postinfection. At 1 and 2 h postinfection, the number of phagocytized bacteria in the control was greater than in the experiment.

The differences between control and experimental animals infected with *S. aureus* and *S. epidermidis* were less pronounced than in blue pus infection. Pronounced bacteriopyknosis was observed in both groups (Fig. 4). Massive deposits of bacterial detritus were formed in rats infected with *S. epidermidis*. In these animals, bacteria phagocytized by macrophages were always seen (Fig. 5). However, as a result of intense bacteriopyknosis the number of phagocytes containing bacteria did not reach the threshold after which formation of an abscess starts.

The abscesses contained primarily neutrophils at various stages of degradation and occasional macrophages and lymphocytes. In all abscesses thorough examination revealed phagocytized and free (prob-

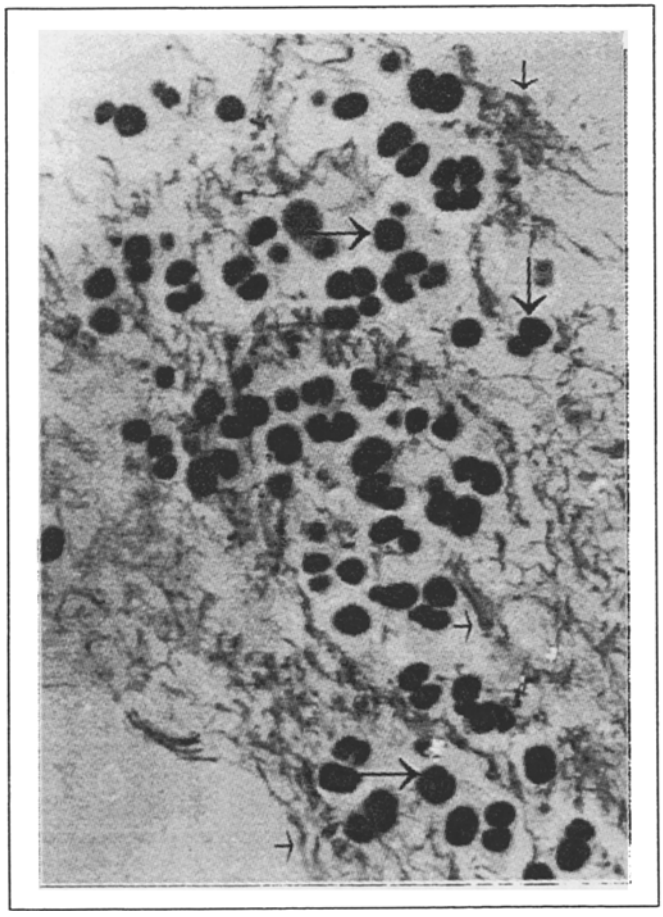

Fig. 4. The site of infection in control rat 2 h after inoculation of *S. epidermidis*. Long arrows indicate bacteria with preserved structure among bacteriopyknotic detritus (indicated by short arrows).

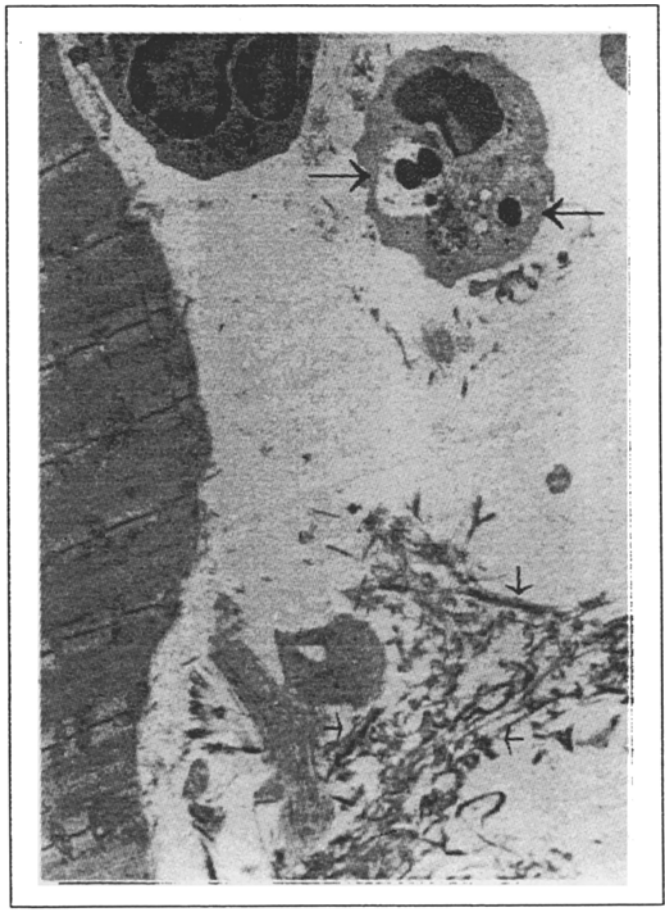

Fig. 5. The site of infection in control rat 2 h after inoculation of *S. epidermidis*. Long arrows indicate bacteria with preserved structure phagocytized by a neutrophil. Short arrows indicate an accumulation of bacterial detritus.

ably due to neutrophil degradation) bacteria. The occurrence of such bacteria was not high. Thus, microscopic study confirms the fact that the contents of an abscess consists predominantly of cells involved in phagocytosis: bacteria, phagocytes, and lymphocytes that produce lymphokines. Consequently, the weight of an abscess reflects the development of this process.

This morphological study provides an insight into why antibodies suppress phagocytosis in immunized animals but not stimulate it by opsonization. Undoubtedly, antibodies exert a potent opsonizing effect, but it is lower than stimulation of bacteriopyknosis. Under these conditions, the objects of phagocytosis are destroyed within several hours of infection. Formation of detritus at the site of infection in control animals implies that both phagocytosis and bacteriopyknosis are the manifestations of nonspecific resistance. Antibodies stimulate these processes. However, at least in the studied infections, bacteriopyknosis and phagocytosis compete for microbial cells. Since the number of bacteria is limited, bacteriopyknosis, being a more rapid process aimed at the whole mass of bacteria, sharply reduces the number of intact bacteria that can be engulfed by phagocytes.

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