REVIEWS

The Role of Agglutination in Bacterial Infection

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The process of concentration of microorganisms, which leads to inhibition of their growth and death, is a mechanism of nonspecific resistance and immunity in bacterial infection. It was suggested that inhibition and death of microorganisms are caused by deficiency of essential substances in the concentration site, which determines the significance of agglutination in the immunity. Agglutination of microbial bodies increases their concentration in tissues. By contrast, dispersion of bacteria in the tissues weakens the effect of the concentration factor and, consequently, increases the virulence of the bacteria.

Key Words: *antibodies; agglutination; mechanisms of nonspecific resistance and immunity; bacterial motility*

Agglutination of bacteria was discovered by A. Charrin and J. Roger in 1889 [14]. This discovery led to the conception of antibodies and their interaction with antigens.

Based on extensive studies of the mechanisms underlying this reaction, G. Wilson and A. Miles have suggested that agglutination prevents propagation of bacteria from the primary focus to the lymph and blood [20]. However, they attributed this effect not only to agglutination but also to opsonizing activity of antibodies. W. Clark claimed that "the ability of antibodies to agglutinate bacterial cells *in vitro* probably plays no role in the immunity *in vivo*" [8]. A similar idea was suggested by N. V. Medunitsyn in his recent review: "There are no sufficient proofs to believe that numerous interactions between antibodies and infectious agents observed *in vitro* occur *in vivo*. Presumably, most of these interactions are aimed at opsonization of the infectious agent and stimulation of phagocytosis, which is the major mechanism of antiinfectious immunity " [1].

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Our investigations have shown that the protective mechanism of agglutination consists in the concentration of considerable numbers of bacteria in a limited volume in the hosts' body. This inhibits growth and causes death of the bacteria. In electron microscopy studies we observed structural damage to bacteria — clarified cytoplasm separated from the cell wall — 2 h after intramuscular injection of rats with *Pseudomonas aeruginosa* strain 453 (L. A. Tarasevich State Institute of Standardization and Control of Medical Biological Preparations). At the same interval, the number of damaged bacteria in immunized rats was considerably greater. The amount of damaged microbial cells was high after injection of intact animals with bacterial suspension in 5% immune serum but not in Hanks' solution. The number of damaged microorganisms in the center was always greater than at the periphery of the accumulation focus.

Since this strain of *P. aeruginosa* is complement-resistant, the observed bacterial damage could not be inflicted by the complement. Damaged bacteria were observed at the site remote from the host's cells, for example, from phagocytes. Consequently, the damage cannot be attributed to direct or indirect (release of bactericidal substances in the extracellular space) ac-

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tivity of phagocytes. Thus, some bactericidal factor, other than complement and phagocytosis, operates in the focus of infection. This factor is stronger in immunized animals or in the presence of exogenous antibodies. The amount of damaged cells is greater in the center than at the periphery of the accumulation focus, suggesting that this is due to deficiency of vital substance in the immediate vicinity or excess of harmful substance secreted by the bacteria. Both the deficiency of vital and excess of harmful substances have the strongest effect in the sites of the highest concentration of microbial bodies.

To check up this hypothesis, we have infected two groups of rats with the same number of bacterial cells using diluted (dispersed focus of infection) and concentrated (dense focus of infection) suspension. The parameters characterizing the severity of pathological process 7 days after infection [3] are summarized in Table 1. Statistical analysis has shown that the motile microbe *P. aeruginosa* strain 453 injected in diluted and concentrated suspension caused disease of practically the same severity and similar development of abscesses in the primary focus. The severity of disease (by weight loss) and abscesses was smaller in rats injected with concentrated suspension of the immotile microbes *P. aeruginosa* strain 103 and *Staphylococcus aureus* strain 3377. The hypothesis that the density of microbial bodies is the major factor determining the severity of infection was confirmed by the above-mentioned experiments with the motile microbe *P. aeruginosa* strain 453: high density of this microbe in the primary focus (injection in concentrated suspension) rapidly decreases due to its proliferation into the extracellular spaces.

The results of experiments with *P. aeruginosa* strain 453 provide an insight into the biological significance of the motility of pathogenic bacteria. It has been generally accepted that the motility is a virulence-determining factor. This was confirmed by investigations of *P. aeruginosa* [17-19], *Vibrio cholerae* [12], and *Proteus mirabilis* [16]. Our experiments demonstrate how the motility of a microorganism increases its virulence. By moving away from each other, motile pathogenic microorganisms reduce the population density, thus weakening the effect of the concentration factor. Interestingly, monoclonal antibodies against the flagellar protein of *Proteus mirabilis* inhibit the motility of these bacteria *in vitro* and exhibit pronounced protective activity *in vivo* in a mouse model of burn sepsis after infection into the burn zone [13]. These antibodies have no protective effect after intraperitoneal infection. The different effects of these antibodies were attributed to specific functions of peritoneal phagocytes. We think that the concentration factor is a more simple and convincing explanation of this phenomenon. The antibodies to flagellar protein inhibit microbial motility irrespective of the route of administration. Blockade of the microbe in the wound does not allow it to weaken the effect of the concentration factor by proliferating, thus providing therapeutic effect. In the abdominal cavity even immotile bacteria are rapidly dispersed, which weakens the effect of the concentration factor and abolishes the therapeutic effect of the antibodies.

The importance of the concentration factor and of microbial motility as a counter-measure against this factor is illustrated by the results of our experiments. For quantitative evaluation of the effects of various media on the motility of bacteria we used the following method. Wells (6 mm in diameter) were cut in agar in a Petri dish. Pieces of filtering paper rolled in tubes were inserted into the wells. The height of the tube was 2-3 mm greater than the depth of the well. The same volume of microbial suspension in the studied medium with 3.3×10^9 cells/ml was poured in each tube and the dishes were left in a thermostat for 20-22 h, after which the diameter of bacterial growth around the well was measured.

By this method we studied the motility of *P. aeruginosa* strain 453 in several media. The following

TABLE 1. Body Weight of Animals and Weight of Exudate in the Primary Focus after Infection by Concentrated and Diluted Suspension of Bacteria

Microorganism	Volume of suspension, ml	Number of animals	Number of inoculated bacteria, $\times 10^8$	Mean weight loss 7 days after infection, g	Mean weight of exudate, mg
P. aeruginosa strain 453	0.5	10	1.6	9.2	339
	0.05	10	1.6	12.4	364
P. aeruginosa strain 103	0.5	15	5	15	941
	0.05	15	5	8.5	596**
S. aureus strain 3377	0.5	10	5	9.3	309
	0.05	10	5	0.3**	154*

Note. *$p<0.05$, **$p \leq 0.01$ between the results obtained after infection by concentrated or diluted suspension.

results were obtained. If the motility of the bacteria in Hanks' solution is taken as 100%, in Hanks' solution containing 5% nonimmune rat serum it is 120%, in Hanks' solution with 5% immune rat serum against *P. aeruginosa* strain 453 it is 80%, in a water-salt tissue extract (in Hanks' solution) predominantly from the muscles of intact animals it is 30%. Thus, a decrease in microbial motility is a factor of nonspecific resistance in the body, and restriction of bacterial motility potentiates the effect of the concentration factor.

The hypothesis on the role of the deficiency of a vital substance was checked up in experiments with iron [7,9-11,15]. All living organisms require iron because it is a component of respiratory enzymes. In the mammalian body practically all iron occurs in the bound form, predominantly in the form of hemoglobin. The concentration of free iron in the extracellular space is low. Moreover, a specific mechanism of extraction of free iron from the extracellular space has developed in the course of evolution. The plasma protein transferring binds free iron. The presence of transferring in blood plasma renders a huge number of bacteria nonpathogenic; these are the bacteria that cannot live under conditions of iron deficiency. Pathogenic and arbitrarily pathogenic bacteria have adapted to iron deficiency. They secrete siderophores, proteins that can compete with transferring for free iron. Iron bound by siderophores is internalized by microbial cell via special surface receptors [9].

We have compared the severity of pathological process by body weight and amount of exudate in the primary focus 7 days after infection in two groups of rats. Control rats were injected with *P. aeruginosa* strain 453 as suspension in Hanks' solution. Experimental rats were injected with the same suspension containing hemoglobin in the concentration similar to that in blood. Hemoglobin was obtained by cytolysis of erythrocytes from an intact rat.

On the 7th day of disease, the intensity of inflammation in the primary focus and weight loss in experimental group was significantly higher than in the control (16 g *vs.* 2 g). Thus, high concentration of iron in the primary focus is positive for the microorganisms and negative for the host, i.e., when iron is not injected in the focus of infection, the microorganism's functions are partially suppressed. Undoubtedly, the effect of the concentration factor is not associated only with iron deficiency. It should be stressed that irrespective of the number and nature of the substances which are necessary for the reproduction of the microorganisms in the host's body, the deficiency of these substances will have the most pronounced effect in the sites where the microorganisms competing for these substances are concentrated in the limited space.

The emergence of antibodies — proteins with two identical sites for the binding with a microbial cell — can be regarded as a useful mechanism increasing the adaptive potential of the higher animals to microflora. Agglutinates (conglomerates of microbial bodies) form as a result of the interaction between these antibodies and microbial cells.

We believe that the concentration factor accounts for the role of agglutination in the immunity *in vivo*. The binding of bacteria with each other creates high concentration of microbial bodies in tissues, thus potentiating the inhibiting effect of the deficiency of iron and other vital substances. By limiting the dispersion of bacteria in the tissue from the primary focus, agglutination reduces the volume of the tissues exposed to bacterial exo- and endotoxins. Blockade of receptors on cell surface potentiates the effect of the concentration factor. For example, special receptors for siderophores and other metabolites possess antigenic activity and stimulate the synthesis of antibodies to them. Antibodies bind to these receptors and block the entry of a vital metabolite to the microbial cell. Obviously, the receptor blockade is most efficient at the sites of the highest concentration of microbial cells

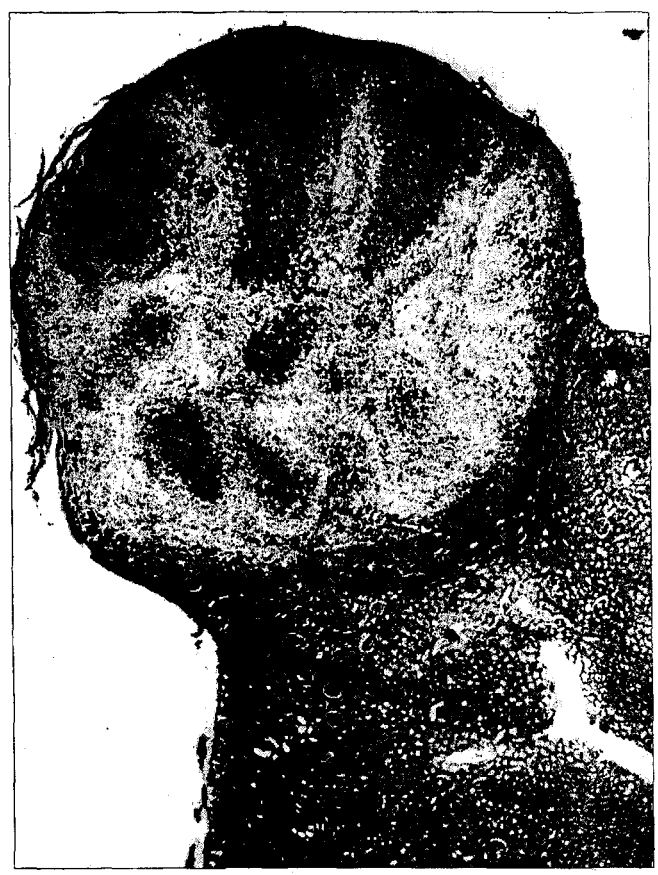

Fig. 1. Metastatic abscess under the kidney capsule in a control animal, ×25.

(deficiency due to receptor blockade is aggravated by deficiency due to competition).

The concept of the concentration factor explains not only the role of agglutination and bivalent antibodies in immunity, but also the biological expediency of the fact of that the immune response both in phylo- and ontogeny begins with the synthesis of IgM [6]. There are no receptors for these antibodies on the surface of phagocytes, i.e., without the complement these antibodies cannot act as opsonins and stimulate phagocytosis. However, these ten-valent antibodies are the most potent agglutinating immunoglobulin. Consequently, the most ancient and the earliest reaction of humoral immunity is not aimed at stimulating phagocytosis but at potentiating the effect of the concentration factor.

We believe that the reviewed data are useful in the light of the most important problem of infectious pathology: pathogenesis of sepsis and, specifically, role of agglutination in its development. Previously, we reported that in immune animals generalization of infection does not occur or is less pronounced and the severity of disease is much lower [2]. These data are not surprising, because the positive effect of immunization on pathological process has been known since the Pasteur's times. The mechanism of this phenomenon remains the subject of extensive investigations. Without analyzing the complex multicomponent effect of immunization on the organism (proliferation and maturation of immunocompetent cells, synthesis of antibodies, production of cytokines, etc.), it should be noted that we have observed an effect similar to that of immunization when 2-5% immune serum was added to microbial suspension before infection [4,5]. In this case the infecting agent was influenced only by antibodies. This influence was quite strong — generalization did not occur or its parameters were markedly reduced: none of the animals infected with agglutinated microbes died, while in the control group (injection with nonagglutinated microbes) lethality was 80%, the occurrence of pyemic foci with the causative agent in the kidneys (Fig. 1) was zero percent in experiment and 60% in the control, and the occurrence of the microbe in the spleen was 16% *vs.* 66% (control).

Thus, the following mechanisms are responsible for the suppression of infection generalization by antibodies. First, agglutination of the causative agent, which inhibits its functions and causes death under the influence of the concentration factor and hampers the crossing of the blood-tissue barrier and entry into the blood and lymph. Second, opsonization of the causative agent, which stimulates the phagocytizing activity of neutrophils and macrophages.

Agglutination of antibodies concentrates bacteria in the body, which inhibits their growth and leads to their death. This is caused by deficiency of vital substances, for example, of iron. The process of concentration potentiates the effect of the competition between the bacteria for the vital substance. This is confirmed by the fact that morphological damage to bacterial cells is more pronounced in the center of the primary focus, where the deficiency of vital substance(s) is greater than at the periphery. Experimental evidence that agglutination affects the immunity via the concentration factor could be helpful in the investigation of sepsis.

REFERENCES

1. N. V. Medunitsyn, in: *Immunology of Infectious Process,* V. I. Pokrovskii, S. P. Gordienko, and B. I. Litvinov (Eds.) [in Russian], Moscow (1994), pp. 122-147.
2. A. A. Pal'tsyn, I. A. Grishina, E. G. Kolokol'chikova, *et al., Byull. Eksp. Biol. Med.,* **117**, No. 5, 545-546 (1994).
3. A. A. Pal'tsyn, E. G. Kolokol'chikova, A. K. Badikova, *et al., Ibid.,* **123**, No. 3, 349-352 (1997).
4. A. A. Pal'tsyn, E. G. Kolokol'chikova, I. A. Grishina, *et al., Arkh. Pat.,* No. 5, 15-19 (1994).
5. A. A. Pal'tsyn, E. G. Kolokol'chikova, I. A. Grishina, *et al., Byull. Eksp. Biol. Med.,* **118,** No. 9, 292-294 (1994).
6. R. V. Petrov, *Immunology* [in Russian], Moscow (1987).
7. M. Brown and P. Williams, *Annu. Rev. Microbiol.,* **39**, 527-556 (1985).
8. W. R. Clark, *The Experimental Foundations of Modern Immunology,* New York (1980).
9. P. Cornelis, D. Hohnadel, J. M. Meyer, *Infect. Immun.,* 57, No. 11, 3491-3497 (1989).
10. E. Griffiths, in: *Medical Microbiology,* Vol. 3. London - New York, (1983) pp. 153-177.
11. E. Griffiths, H. Chart, and P. Stevenson, in: *Virulence Mechanisms of Bacterial Pathogens,* J. A. Roth (Ed.), Washington (1988) pp. 121-137.
12. M. N. Guentrel and L. J. Berry, *Infect. Immun.,* **11**, 890-897 (1975).
13. R. C. Harmon, R. L. Rutherford, W. Hsin-Mei, and M. S. Collins, *Ibid.,* **57**, No. 7, 1936-1941 (1989).
14. R. J. Jones, in: *Medical Microbiology,* C. S. Easman and J. Jeljaszewicz (Eds), Vol. 2, London - New York (1983), pp. 177-205.
15. D. Law and J. Kelly, *Infect. Immun.,* **63**, No. 2, 700-702 (1995).
16. A. T. McManus, C. G. McLeod, and A. D. Mason, *Arch. Surg.,* **117**, 187-191 (1982).
17. A. T. McManus, E. E. Moody, A. D. Mason, *Burns,* **6,** 235-239 (1980).
18. T. C. Montie, D. Doyle-Huntzinger, R. C. Craven, and L. A. Holder, *Infect. Immun.,* **38**, 1296-1298 (1982).
19. M. Rosok, M. Stebbins, K. Connelly, *et al., Ibid.,* **58,** 3819-3828 (1990).
20. G. Wilson and A. Miles, *Topley and Wilson's Principles of Bacteriology, Virology and Immunity,* Vol. 2, London, (1975).