MORPHOLOGY AND PATHOMORPHOLOGY

ROLE OF BACTERIAL AGGLUTINATION IN WOUND INFECTION

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The ability of immune sera to sediment bacteria and to form precipitates with the supernatant of bacterial cultures has been known for about 100 years. The phenomena of agglutination and precipitation are highly specific, and they are therefore used for the diagnosis of infectious diseases, and for determining the species-specificity and other features of tissues. The role of these phenomena in the pathogenesis of infectious processes and, in particular, of wound infection has not yet been explained. In our studies of functional morphology of wound cells, we obtained evidence of an important role of bacterial agglutination in wound infection.

EXPERIMENTAL METHOD

The experimental material consisted of biopsy specimens from wounds and blood samples from patients with burns (four) and material from a discharging wound (one patient). The titer of antimicrobial antibodies was tested from five to 17 times in each patient. As the control we studied serum from seven healthy blood donors. Sufficient blood for determining the titer could be obtained by puncturing the skin of the finger.

The techniques of electron-autoradiographic analysis of cells of a wound and of the bacteria colonizing it, and for determination of the bactericidal activity and other parameters of function of the blood and wound neutrophils were described previously [1-5]. To determine the ability of the blood sera to aggregate (agglutinate) bacteria, a series of tubes was prepared containing 0.45 ml of a suspension of the test bacteria ($1 \cdot 10^9$ cells/ml medium 199) and 0.05 ml of the test serum, in stepwise increasing dilutions: 1/1, 1/2, 1/4, 1/8, and so on. The tubes were incubated at 37°C for 1 h. After incubation a drop of liquid from the tubes was introduced into a Goryaev counting chamber and examined under phase-contrast illumination and a magnification of 200×. The investigation began with maximal dilution at which the aggregating properties of the sera were not exhibited and the bacterial cells were distributed singly or in small (2-4 cells at a time) groups (Fig. 1). Agglutination was manifested as the joining of bacterial cells to form large or small collections (agglutinates), and by a sharp decrease in the number of single cells (Fig. 2). The ability of a given serum to agglutinate given bacteria was characterized by its titer, i.e., the minimal concentration in which agglutinates were found. After determination of the titer, experiments were carried out to determine the rate of growth of the bacteria in sera of different concentrations. Rows of tubes were incubated and then the color of the liquid in the tubes was compared after different time intervals. Medium 199 contains phenol red as pH indicator. During growth of the culture in the tube, acid products accumulate, the pH shifts, and the red color of the medium changes to yellow. The titer of the serum was determined in blood from seriously ill patients, in whom the development of wound sepsis was probable. Tests were carried out with microorganisms obtained from the wound, a secondary focus (if present), and blood (if bacteria were found in it) from each patient.

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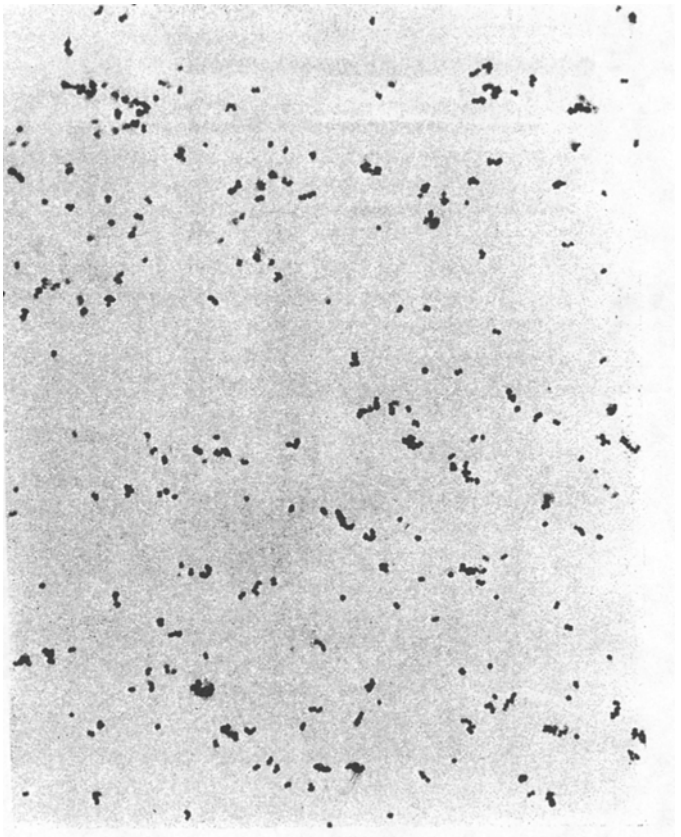

Fig. 1. Cells of *Staph. aureus* in Goryaev chamber in medium containing serum in the ratio of 1:640. No aggregation visible, bacteria arranged singly or in small (2-4 cells at a time) groups. 630×.

EXPERIMENTAL RESULTS

The study of agglutination revealed the presence of antibodies to *Pseudonomas pyocyanea* in a titer of 1:40-1:160 in all donors; antibodies to *Staphylococcus* were found in only one healthy blood donor in a titer of 1:40.

Of the three patients in whom clinical wound sepsis was not established, the titer of antibodies to *Staphylococcus aureus* and *Ps. pyocyanea* was at the level of 1:80-1:160 at all times of observation (1.5 and 2 months). Antibodies to other bacteria (*Enterococcus, Escherichia coli, Acinetobacter,* nonfermenting bacillus) were not found. In the third patient, during the first 5 weeks after sustaining burns (45% of the body surface) his condition was assessed as severe, and during this time the titer against *Staphylococcus* and *Ps. pyocyanea* was low, namely from 0 to 1/20. When this patient was moved into a "Klinitron" bed for treatment of his burns by the open method, in a bacterium-free environment, this was accompanied by improvement of his condition and by a rise of titer to these microorganisms up to 1/80-1/320.

The study of agglutination in two patients with clinical signs of sepsis yielded the following results. First, the agglutination reaction is highly specific and characterizes not only the species, but also the strain of a microorganism. For example, in one patient two strains of *Ps. pyocyanea* obtained from the wound were indistinguishable in their sensitivity to antibiotics, but differed appreciably in titer: 1/80 and 1/1280. Second, antibody titers in these two patients against strains of *Staphylococcus* and *Ps. pyocyanea* isolated from them, changed in the course of the illness from 0 to 1/2560. Most important, four cases in which bacteria were isolated from the blood and two cases in which secondary suppurative foci were found coincided in time with absence of antibodies or with a low titer (not more than 1/20) of antibodies to the strain obtained from the blood or secondary focus. On the same day the titer of antibodies to a different strain of bacteria of the same species, namely *Staph. aureus,* obtained from the wound, could be 1/2560.

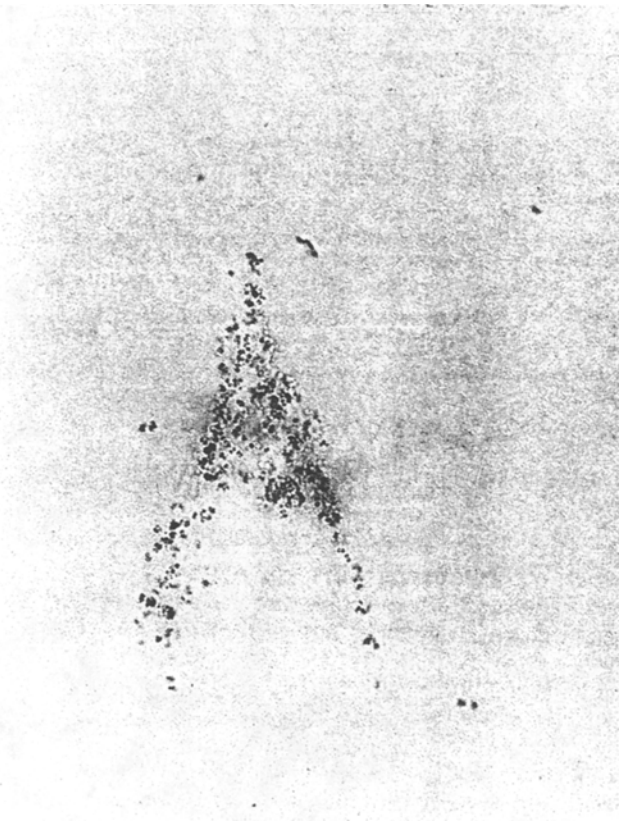

Fig. 2. Cells of *Staph. aureus* in Goryaev chamber in medium containing serum in the ratio 1:160. Aggregate of bacteria consisting of hundreds of cells; number of single cells sharply reduced. 630×.

Thus a serious general state of the patient accompanied by bacteriemia was observed in the absence of agglutination, or in the presence of very weak agglutination of bacteria penetrating into the blood stream. These data indicate that aggregation of bacteria localizes them in the primary focus and prevents the development of septicemia. At the same time, we found that immunologic deficiency, which determines the possibility of bacteriemia and septicemia, relates not to microorganisms in general, and not even to one particular species of microorganism, but only to a concrete strain, or in other words, it is distinguished by high specificity.

Reduction of the possibility of generalization of the process with an increase in the agglutinating power of the serum can be explained as follows. Penetration of infection into the lymph and blood stream takes place with tissue fluid when the permeability of the dilated lymphatics and blood vessels is increased. Together with fluid, only single bacteria can move freely. This is clear from an examination of the preparations in the Goryaev chamber. Bacterial aggregates lose their ability to move even in a Goryaev chamber, where movements are limited only by the walls of the chamber and there are fewer obstacles than in the tissues. Consequently, it is single bacteria which have the greatest chance of penetrating into the blood stream. Reduction of their number and an increase in the volume of the bacterial aggregates reduce the probability of generalization of infection.

Agglutination not only prevents bacteria from penetrating into the lymph and blood stream, but also depresses their viability. Proof of this was obtained by observing the rate of growth of cultures (on the basis of a change in color of the medium) in the presence of different concentrations of serum. Growth of the cultures was delayed when the concentration of serum was below maximal but higher than the titer. Retardation of growth of the cultures not at maximal, but at average concentrations of serum proved that this delay was unconnected with any bactericidal substance, but took place in accordance with the principle of equivalence of the components of the reaction, i.e., it is immunological in nature.

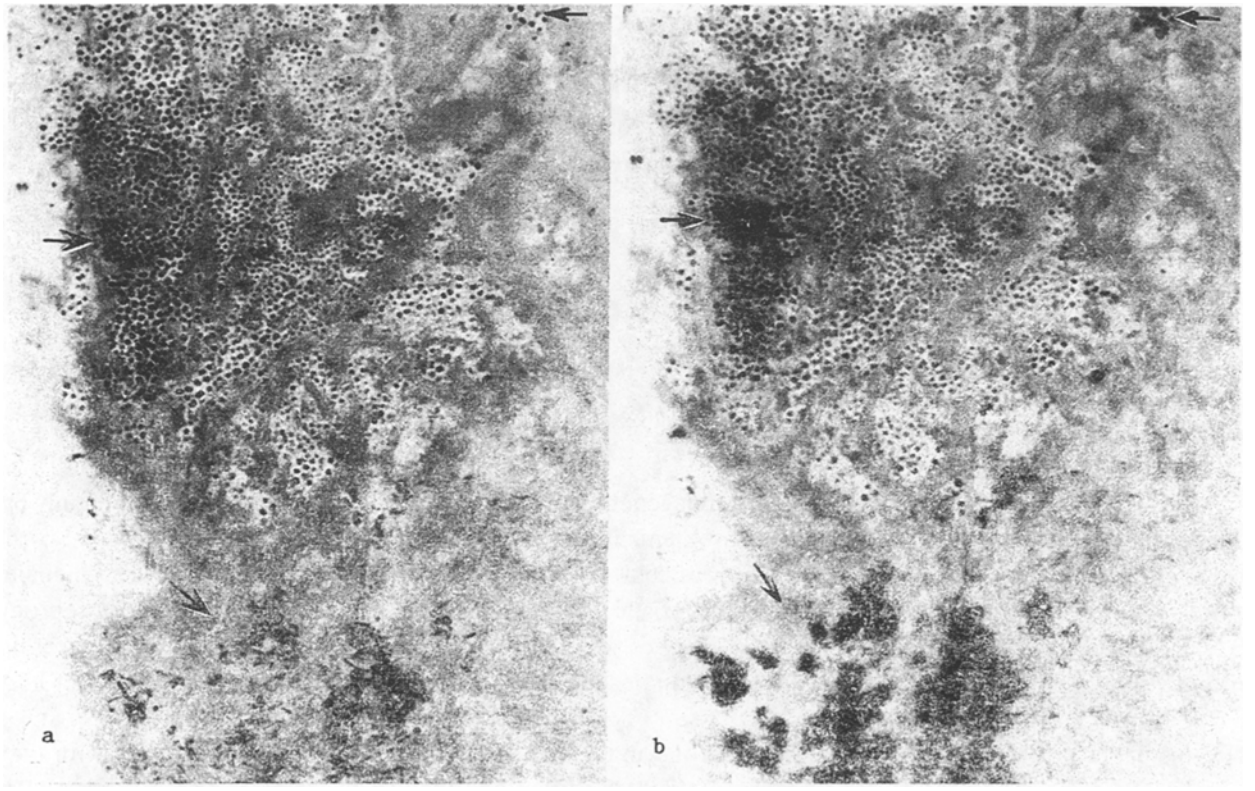

Fig. 3. Inhibition of activity of bacteria located in center of agglutinate. Region of wound incubated with uridine-^{3}H. a) Semithin section through two concentrations of bacteria: below – bacilli (long arrow), above – extensive concentration of cocci, peripheral regions of this concentration indicated by short arrows. 630×. b) Autoradiograph of same section. Bacilli (to which no antibodies were present) intensively labeled irrespective of their location in the concentration. Large proportion of cocci not labeled. Weak labeling present only above certain cocci located at periphery of concentration (short arrows). 630×.

The autoradiographic study of single bacteria located between aggregates showed that their viability is not less than that of bacteria incubated with nonagglutinating serum or without serum. In other words, antibodies with no appreciable action on single bacteria, somehow or other inhibit the vital activity of aggregated microorganisms. The cause of inhibition may be simply impairment of diffusion of substances between the bacterial bodies, when joined together quite tightly to form an agglutinate Under these conditions bacterial cells located in the inner zone of the agglutinate are deprived of the free access of nutrients or removal of metabolic products, and the vital activity of these bacteria is depressed. Direct proof of these arguments was obtained by autoradiographic investigation of the wounds. In a patient (with antibodies to *Staphylococcus* in a titer of 1/160 but with no antibodies to nonfermenting bacillus), concentrations of cocci and bacilli in the wound were distinguished by the distribution of the label. In concentrations of cocci, the label was incorporated only by cells located at the periphery, and bacteria in the center of the concentration were not labeled. Bacilli not connected together by antibodies and consequently more loosely arranged, were intensively and uniformly labeled irrespective of their location at the edge of in the center of the concentration (Fig. 3a, b).

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