# MORPHOLOGY AND PATHOMORPHOLOGY

# Inhibiting Effect of Antibacterial Antibodies on Wound Infection Generalization

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Antibody tests in patients with wound infections are as a rule carried out in such a way that the data do not reflect the specific host-pathogen relationships. For example, the commonly carried out analysis of serum immunoglobulins of various classes [5] helps just to outline the patients' immunological status. Antibacterial antibody tests are almost always aimed at the detection of antibodies to a certain bacterial species. Such information is also unspecific with respect to the strain that caused the disease. Some authors report [1] that the titers of such antibodies in local and generalized infections do not differ substantially.

Research carried out to elucidate the pathogenesis of sepsis has revealed that analysis of antibodies to an autostrain rather than to a species is necessary to detect the agents of wound infection. Such antibodies may be areactive or react weakly with bacteria of a different strain of the same species. Antibodies specific for the autostrain have been found to influence bacterial propagation in the body.

## MATERIALS AND METHODS

The agglutinability of sera obtained from nine burn and three wound patients towards bacterial autostrains isolated from wounds, secondary foci, and blood was studied. The agglutination of the same strains by sera of nine healthy donors was studied for control.

For assessment of the capacity of the serum for agglutination of bacteria a number of tubes were prepared containing 0.45 ml of test bacteria suspension ($1\times10^9$ bacterial bodies per ml of medium 199) and 0.05 ml of test serum in dilutions starting from 1:2. The tubes were incubated at 37°C for one hour. After incubation a drop of liquid from each tube was placed in a Goryaev chamber and examined at a phase contrast illumination under ×200 magnification.

Examinations were started from the maximal dilution at which serum aggregability was not manifested and the bacterial cells were arranged singly or in small groups (2-4 cells each) (Fig. 1). Agglutination manifested itself in bacterial cells joining larger or smaller aggregates (agglutinates) and by a drastic reduction of the number of solitary cells (Fig. 2). If moving bacteria were examined, the number of moving cells was sharply reduced in marked agglutination.

The ability of serum to agglutinate certain bacteria was characterized by the titer, that is, the minimal concentration at which agglutinates formed. Strain similarity was assessed from culture properties, sensitivity to antibiotics, antigenic characteristics, and the titers of agglutination of the compared strains by the same sera.

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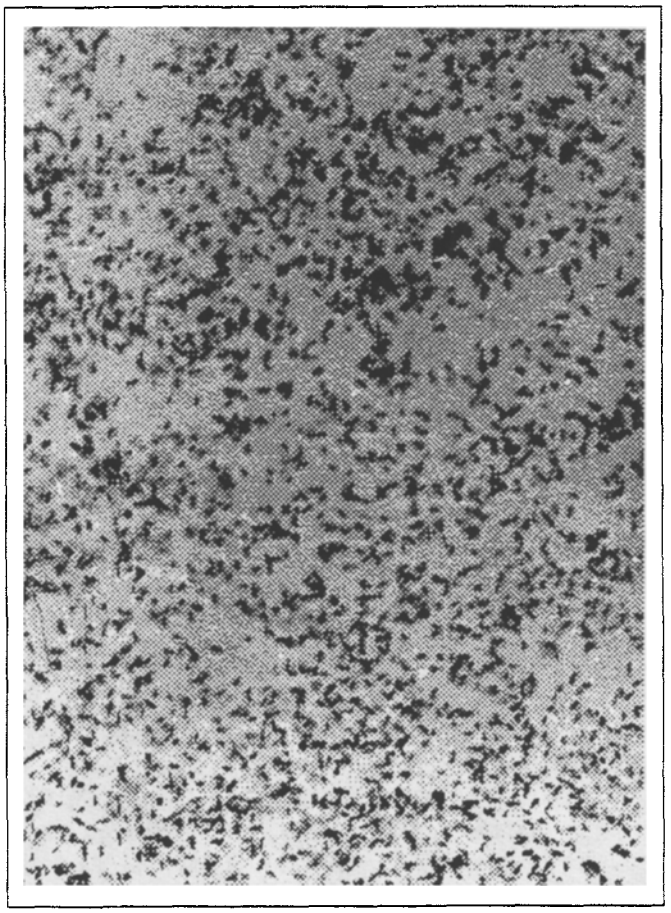

Fig. 1. *P.aeruginosa* in medium containing serum in 1/2560 dilution. Agglutination not detected, bacteria distributed singly. ×630.

## RESULTS

The analysis of donor sera revealed antibodies to *Pseudomonas aeruginosa* in titers 1/40 to 1/160 in all cases and to *Staphylococcus aureus* in one donor in 1/40 titer. In the patients the agglutination of bacteria isolated from the wound and from the blood was different. Sometimes the titers of antibodies to wound bacteria were low for several days after isolation (1/20-1/40) or were not detected at all. If the strain proved to be a permanent inhabitant of the wound, its titers grew to 1/80 and higher and remained at a level from 1/80 to 1/1280 until the patients were discharged. Still more frequently such titers (1/80 and higher) were found during the first examination and remained high later.

One or several positive inoculations were made from the blood of each patient. A total of twenty bacterial strains were isolated. Antibodies to 18 of these strains were not detected after their inoculation from the blood or were detected in low titers (1/20 and 1/40) (2 strains). In probing the causes of this phenomenon, one should first of all consider a possible technical error in some cases, involving a microbe which was thought to be inoculated from the blood actually having contaminated the sample during the collection of the material. Naturally, no antibodies to the contaminant can be detected. Many workers who have researched the problem of contamination [3,4,6-9] have failed to define the criteria permitting an absolutely precise differentiation between bacterial contamination and true bacteremia. We referred to bacteremia the cases characterized by the following features: 1) strains isolated first from the blood and then from secondary foci; 2) strains isolated from the blood two or more times; 3) strains to which antibodies were detected several days later but which were not detected in the wound at the moment of their isolation from the blood. Assessment according to these criteria referred ten of the discussed 18 cases to true bacteremia and eight were considered doubtful.

Since in the majority of bacteremia cases antibodies to bacteria found in the blood are present in low concentrations or not at all, we may come to the conclusion that the absence of bacterial aggregation induced by antibodies facilitates bacterial movement with tissue fluid flow and entry into the bloodstream

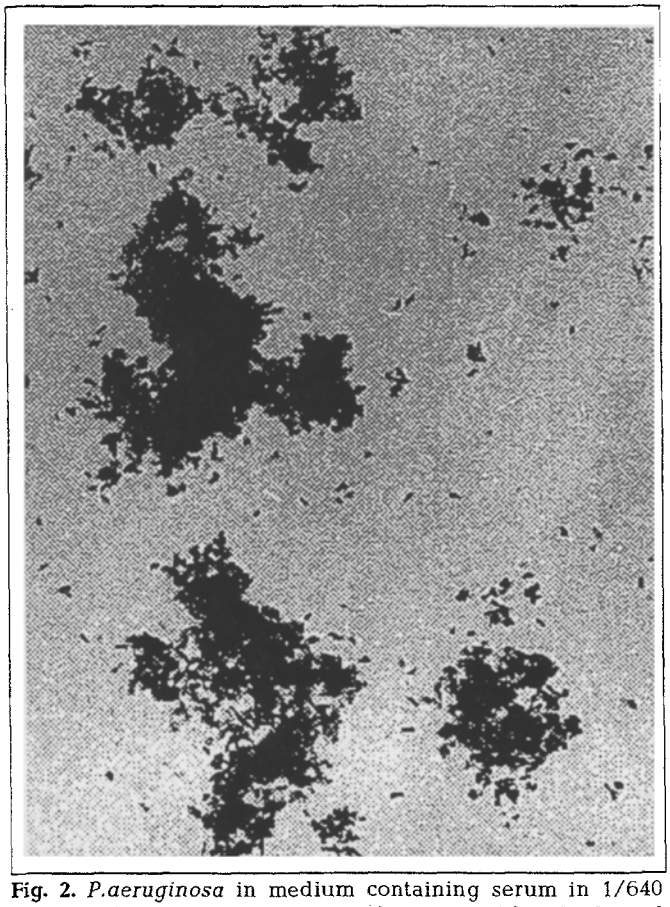

Fig. 2. *P.aeruginosa* in medium containing serum in 1/640 dilution. Bacteria grouped in agglutinates of hundreds and thousands of cells. Number of solitary cells sharply reduced. ×630.

through dilated vascular wall intercellular spaces at the inflammation focus. Antibody binding of bacteria in aggregates impedes their movement through the intercellular spaces and localizes bacteria in the primary focus, thus preventing generalization of the infectious process. Antibody-bacterial interactions are so specific that strains of a single bacterial species, often indistinguishable in terms of their antibiotic sensitivity, may differ greatly in the degree of agglutination with the same serum.

Let us follow the course of sepsis development in patient K. One month after burn, *S.aureus* (we will designate it strain A) was isolated from her blood; no antibodies to this strain were detected at that time. Two days later a similar strain was isolated from the wound. One week after isolation from the wound antibodies to strain A were detected, though in a very low titer, 1/20. Two weeks later the titer was as high as 1/5120 and remained high, though with slight fluctuations, for 4.5 months until the patient died of septicopyemia. Strain A was no longer from the blood, although it persisted in the wound until it healed. Blood samples were either sterile or contained (4 times) *S.epidermidis* (all the cultures were similar in terms of the tested characteristics). *S.epidermidis* was not isolated from the wound. Antibodies to this staphylococcus were either not detected or detected in low titers. Their maximal titer, 1/40, was observed twice and both times coincided with the maximal titer to strain A. Most likely, this coincidence was due to the presence of strain A and *S.epidermidis* cross-antigens. Autopsy showed purulent meningitis, sternal osteomyelitis, pulmonary abscess, and small abscess in the liver. Strain A was isolated from the brain, sternum, lung, liver, and kidney, but not from the cadaveric blood. These data suggest the following scheme of sepsis development in the patient. Strain A that had entered the wound but had not yet induced antibody production penetrated into the blood through dilated vessels of the inflamed bottom of the wound and disseminated in the body, forming secondary foci in the tissues. Hence, generalization of the infectious process and the onset of secondary foci formation occurred approximately two months before septicopyemia was clinically diagnosed. After strain A had spread from the wound through the body, immunization with this strain was intensified and the antibody titer became so high that a new penetration of strain A into the blood become impossible. The presence of this strain in the secondary foci and the high titer of antibodies to it indicate that it was the cause of sepsis and death. It is noteworthy that during the clinical manifestation of sepsis, *S.epidermidis* was isolated from the blood but was not the causative agent of sepsis in this patient, in contrast to the widely held opinion that the culture obtained from the blood in clinically manifest sepsis is the etiological agent of the disease [2].

The results prompt the following scheme of sepsis development. The possibility of bacteria entering the blood is highest during the first few days after their appearance in the wound, when no antibodies to them are produced. The level of bacteria in the wound being still low at this time, sepsis cannot clinically manifest itself, although bacteremia can initiate the development of secondary foci in the body. The prolonged presence and multiplication of bacteria in the wound immunize the body; formed antibodies bind bacterial cells in aggregates, thus impeding their movement through the intercellular spaces and their penetration through the vascular wall into the bloodstream. When massive multiplication of bacteria in the wound alone or both in the wound and in the secondary foci results in sepsis, the antibody concentration increases so that the strain which caused sepsis cannot appear in the blood. Still, some strains may occasionally be detected in the blood and mistaken for the cause of the disease, leading to erroneous treatment policy.

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