Vital Activity of Bacteria Is Directly Inhibited *In Vitro* by Antibodies

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The level of vital activity of *Pseudomonas aeruginosa* bacteria was determined according to the rate of pH decline in the culture medium *in vitro*. The addition of immune serum to this medium initiated bacterial agglutination and lowered the level of vital activity of the culture. The aggregation of bacteria by centrifugation suppressed their vital activity in the same way as agglutination. Inhibition of microbial vital activity during agglutination and aggregation due to the centrifugal force may be attributed to a slowing down of the rate of diffusion of nutrients and metabolites through the aggregates.

Key Words: *antibodies; agglutination; bacteria in culture*

It is known that antibodies, as one of the elements in the host protective mechanism, can inhibit the vital activity of bacteria, leading to their destruction. Direct damage to microbial cells is accomplished by either complement or phagocytes, whereas antibodies act as a trigger or booster activating the complement or opsonizing microorganisms [1,3,5,6]. It is thought that antibodies do not exert any adverse effect directly on bacterial vitality [4]. In the present study we showed that antibodies can suppress the vital activity of bacteria in the absence of complement and phagocytes.

MATERIALS AND METHODS

A 24-h culture of *Pseudomonas aeruginosa* (strain 453, L. A. Tarasevish State Research Institute of Standardization and Control of Biomedical Preparations) washed off agar was cultured in Hottinger broth containing 0.5 ml 0.3% neutral red per 20 ml. Two initial concentrations of microorganisms in the broth were used 2×10^9cells/ml and 8×10^9 cells/ml.

For the preparation of immune serum rats were injected with 0.3 ml of *P. aeruginosa* (strain 453) suspension in Hanks' solution in a concentration of 1×10^9 cells/ml intramuscularly twice with a 7-day interval. The serum was obtained 7 days after the second injection. The titer of the serum was from 1/640 to 1/5120 in the agglutination test for live microorganisms. The technique of determining agglutination was described previously [2]. Normal serum was obtained from intact rats. The complement was destroyed by heating the immune and normal sera at 56°C for 5 min before use.

Four groups of preparations were studied.

1. Immune serum was added to bacterial suspension in Hottinger broth in amounts of 10% of the suspension volume at 8×10^9 cells/ml bacterial concentration and of 2.5% at 2×10^9 cells/ml. The addition of immune serum caused agglutination of bacteria.

2. Normal serum was added to bacterial suspension in volumes equal to those in the first group. This serum did not induce agglutination.

3. A mixture of normal serum and bacterial suspension prepared as mentioned above was doped

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TABLE 1. Mean Value of Light Transmission by Culture Medium in Percent (Transmission at the Beginning of Culturing is Taken as 100%)

Concentration of bacteria	Temperature of culturing	Number of experiments	Groups			
			1	2	3	4
2×10^9	20°C	5	206±61	220±79	126±41	202±85
2×10^9	37°C	5	554±368	490±284	68±9	354±188
8×10^9	20°C	9	199±53	213±85	133±30	193±49
8×10^9	37°C	9	690±606	1031±886*	93±22	651±572

Note. *$p<0.01$ in comparison with groups 1 and 4 in a bacterial concentration of 8×10^9 cells/ml and at incubation temperature 37°C (sign test).

with ethanol (10% of suspension volume) to inhibit microbial activity.

4. As in the second group, the bacterial suspension was added to normal serum and immediately thereafter microbes were sedimented by centrifugation (1500 g, 7 min). Centrifugation resulted in bacterial agglutination and this allowed incubation to be started when the nonuniform bacterial suspension mimicked the agglutination effect in group 1.

For assessment of bacterial vitality in the experimental series the accumulation of acid metabolites was studied in culture medium according to the change of color of the medium from red to yellow at a low pH. The color change was recorded photometrically. For this purpose the light transmission of the culture medium was measured immediately after the addition of all components to the test tube according to the protocol for each group and then again 20 h after culturing at 20°C and 20 h after incubation at 37°C. Prior to photometry the culture was centrifuged for 20 min at 1500 g, after which the supernatant was poured into plastic cuvettes, and light transmission was measured with an Axiophot microscope (Opton) at wavelength 520 nm. The cuvettes were placed between the field diaphragm and the condenser. Illumination was determined as a reading indicated by an automatic light exposure meter.

The degree of bacterial agglutination under conditions of agglutination (group 1) and of centrifugation (group 4) was compared by measuring the concentration of microbial cells in the upper layer of liquid in test tubes of the 1st and 4th series 2 and 20 h after the beginning of culturing at 20°C. The concentration was estimated by the magnitude of light transmission. Hottinger broth was used for these experiments without indicator dye.

RESULTS

Photometric data are listed in Table 1. During culturing the color changed from red to yellow in all experimental series except the third. The magnitude of transmission for light with wavelength 520 nm was increased in accordance with the acidity change as a result of bacterial vital activity. This was attested, first, by the absence of marked changes of transmission in group 3, where microbial vitality was suppressed by the addition of ethanol. Second, the value of transmission for the medium after incubation at 37°C was higher than after culturing at 20°C in all tests in series 1, 2, and 4. Third, the differences between the results for incubation at 20°C and 37°C were more sizable at a high bacterial concentration, i.e., they depended on the number of cells altering the composition of the medium.

Since the magnitude of transmission directly reflects the vigor of bacterial vital activity, the effect of antibodies on microbial cell vitality may be assessed. The preparations of the 1st group with antibodies reliably differed from those of the 2nd one (without antibodies) in terms of microbial vital activity only in one case when the bacterial concentration was 8×10^9cells/ml and incubation was performed at 37°C. Under the same experimental conditions the vitality of bacteria aggregated prior to the test by centrifugation (the 4th group) was reliably lower. Aggregation of microbes either under the influence of antibodies (agglutination) or by the action of centrifugal force may promote movement of the bacteria in the test tube from the upper layer of liquid to the lower one near the bottom. Therefore, the degree of aggregation was estimated by the bacterial concentration in the upper layer 2 and 20 h after the start of the experiment.

If the concentration of microbes in the upper layer of culture medium is taken as 1 for the 1st group, the same value for the 4th group will be 0.7 and for the 2nd 4.4. In other words, the degree of bacterial aggregation in groups 1 and 4 was very similar, but differed significantly from that in group 2.

Thus, in our experiments microbial vital activity decreased in the presence of antibodies. One manifestation of antibody action was agglutination

of bacteria. The size of conglomerates depended on the bacterial concentration, larger aggregations being formed at higher concentrations. A reliable decrease of the vital activity of microorganisms was observed only in experiments with large aggregations. Such a result suggests that the mechanism of suppression of bacterial vitality involves the disturbance of diffusion of nutrients and metabolites for cells situated in the center of the bacterial conglomerates. The more massive the agglutination, the greater number of microbial cells that are separated from the surface of the conglomerate by a layer of bacteria bound with antibodies. The medium moves more slowly between bacterial cell bodies situated close together inside the conglomerate that in the upper layer. This influences the rate of nutrient influx and metabolite outflow, the effect being the more pronounced the thicker the layer impeding the diffusion and the higher the level of metabolism. Therefore, the decrease of bacterial vital activity under conditions of antibody action was marked only in the case of large conglomerates and incubation at 37°C. In small agglutinations the layer hampering diffusion is not thick and consequently only a small portion of microbial cells suffer. At a low temperature the rate of metabolism is also low.

The data obtained for the 4th group testify to the suppressive role of diffusion for bacterial vitality *in vitro*. Increased microbial aggregation was the only effect observed in this group as compared with the 2nd group. Nevertheless, the vital activity of microorganisms reliably declined for the 4th group for a concentration of 8×10^9cells/ml and incubation at 37°C as compared to the 2nd series. The value of this decrease was nearly the same as in group 1, where bacteria were bound with antibodies.

Thus, the inhibition of bacterial vital activity by antibodies *in vitro* is insignificant. It becomes evident only at high concentrations of microbes (in large conglomerates) and at an optimal temperature of incubation. The mechanism of suppression, consisting in impeded diffusion of substances in conglomerates, undoubtedly also operates *in vivo*. Besides diffusion of nutrients and metabolites, the diffusion of bactericidal substances of the host becomes important *in vivo*. Thus, a diffusion factor is to be reckoned among other factors involved in the antibacterial effect of antibodies.

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