Best Available Copy

AD 675728

TRANSLATION NO. 2014

DATE: July 1, 1968

DDC AVAILABILITY NOTICE

Qualified requestors may obtain copies of this document from DDC.

This publication has been translated from the open literature and is available to the general public. Non-DOD agencies may purchase this publication from the Clearinghouse for Federal Scientific and Technical Information, U. S. Department of Commerce, Springfield, Va.

OCT 14 1968

A

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

20051006076

Reproduced by the
CLEARINGHOUSE
for Federal Scientific & Technical
Information Springfield Va. 22151

Best Available Copy

2014

UDC 576.851.513+576.851.511].097.3

STUDIES ON B. ANTHRACIS AND B. CEREUS ANTIGENS BY MEANS OF FLUORESCENT-SEROLOGICAL AND CYTOCHEMICAL TECHNIQUES

[Following is a translation of an article by Ye. N. Levina and L. N. Kats, Institute of Epidemiology and Microbiology imeni Gamaley, Academy of Medical Sciences USSR, in the Russian-language periodical Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii (Journal of Microbiology, Epidemiology, and Immunobiology), No 4, Moscow, April, 1966, pp 98-103.]

There is an extensive literature on the antigenic composition of microorganisms. The results of these studies were the basis of the diagnostic preparations used mainly for final identification of pure cultures of microorganisms (agglutination and precipitation reactions, etc.

In recent years new methods have been suggested for rapid identification of microorganisms based on the antigenic properties of bacteria revealed in a mixed culture - fluorescent-serological technique, agglomeration and indirect hemagglutination methods, etc. Study of these methods showed that in order to obtain significant results with them, highly specific diagnostic preparations must be developed. This can be done only after investigation of the antigenic characteristics of various microorganisms with modern research techniques.

Up to now serological methods of identifying the causative agent of anthrax were of limited value (Ascoli reaction). Some years ago the fluorescent-serological method was proposed (Meysel' et al., 1957; Levina, 1958; Pritulin and Kuz'min, 1959; Cherry and Freeman, 1959; others). Study of the specificity of this method showed that some representatives of the sporiferous aerobes, B. cereus, can be detected by means of fluorescing serums prepared from commercial antianthrax serums (Levina et al., 1962; Blagoveshchenskiy et al., 1962; Akhmerov, 1962).

The purpose of this work was to investigate the reasons for the cross serological reactions in B. anthracis and some strains of

Best Available Copy

B. cereus. The principal aim was to determine the antigenic compositio of these microorganisms. We used the technique of fluorescing antibodies to study the antigenic characteristics of the strains and various cytochemical methods to determine the chemical nature of the antigens. These methods enabled us to investigate the entire cell, i.e., the object normally used with the fluorescent-serological technique of identifying anthrax bacilli.

The vaccinal strain of B. anthracis (Tsenkovskiy's second vaccine) 71/12 and 4 B. cereus strains (104, 1312, 96, and 103) were used in the experiments. Cultures were grown from spores on meat-peptone agar at 37°. Three-hour cultures were investigated in the phase of logarithmic growth; 18-hour cultures, in the phase of stationary growth. Smears from bacterial suspensions were placed on slides, slightly dried, fixed with ethyl alcohol, and treated with fluorescent serums. The latter were prepared from commercial anthrax precipitating serums by combining the globulin fractions of the latter with fluorescein isothiocyanate in the usual fashion.

For cytological investigation, the fixed preparations were stained with Loeffler's blue (cell contents stained) and by Gutstein's method (cell membrane stained). Special histochemical techniques were used to treat the preparations: Schiff's periodic acid test for polysaccharides, Sudan black for lipids, alcian blue for acid mucopolysaccharides, and thionine for the presence of metachromasia.

To determine the chemical nature of the antigens, the preparations were treated with crystalline enzymes: pepsin, trypsin, chymotrypsin, ribonuclease, hyaluronidase, and lysozyme. A mixture of ether, methyl alcohol, and chloroform was used to extract the fats. After treatment with the enzymes and extraction of the fats, the fluorescent-serological and cytochemical investigations were carried out by the methods described above.

After treatment with fluorescent serum of the noncapsulate forms of the vaccinal strain, the fluorescence was confined to the periphery of the microbial cell (Fig. 1, a). It follows, then, that certain antigens are concentrated in the cell membrane. In cultures of B. cereus 104, 96, and 1312 in the stationary phase, the fluorescence was similar to that of the vaccinal strain 71/12 - likewise in the cell membrane, an indication that it contains antigens related to the antigens of the anthrax bacillus. Cells of the vaccinal strain in the phase of logarithmic growth fluoresced like cells in the stationary phase. In only 2 of the 4 B. cereus strains studied (1312 and 96) was the fluorescence in the logarithmic phase of growth the same as in the vaccinal strain. This indicates that these strains and the anthrax bacilli have common antigens. B. cereus 103 cells did not fluoresce after treatment.

Best Available Copy

Cytochemical analysis of the cell membranes in all the strains in both the logarithmic and stationary phases showed a distinct Schiff reaction, weak staining with alcian blue and Sudan black (especially in B. anthracis), and absence of metachromasia. Thus, the cell membranes in these microorganisms contain polysaccharides as well as lipoproteins. However, no difference could be detected with the help of these methods in the composition of the cell membranes of the strains under study either in the logarithmic or stationary phase of growth.

The cells were then treated with enzymes. Pepsin did not destroy the cell membrane or have any noticeable effect on its antigens. The characteristic fluorescence of the membrane followed treatment with fluorescent serum.

Following treatment with trypsin and chymotrypsin, fluorescent-serological observation revealed differences in the degree of antigen injury in relation to the phase of development: in the logarithmic phase, fluorescence of the cell membrane after treatment with fluorescent serum decreased only in vaccinal strain 71/12 (Fig. 1, b, control 1, a). However, in all the strains cytological examination revealed sharp morphological changes in the form of disruption of the cell contours, deformation of the membranes, and loosening of the cell contents from the membrane (Fig. 1, e, control 1, d). In the stationary phase, all the cultures (especially B. cereus 104 and 96) showed a decrease in intensity of fluorescence of the membrane, but cell injury, as revealed by cytological examination, was less pronounced than in the logarithmic phase. These changes were manifested in partial destruction of the longitudinal cell walls of the streptobacilli and in sharply decreased capacity for staining. The shape of the cells was not affected.

Chymotrypsin had the same action as trypsin.

Upon the action of lysozyme on cultures in the logarithmic phase, the intensity of fluorescence following treatment with fluorescent serums did not diminish. Cytological investigation revealed only a slight change in the cell membranes, i.e., decreased capacity to adsorb gentian violet on their surface. B. cereus cultures in the stationary phase likewise showed no change in degree of fluorescence of the cell membranes. In the anthrax bacilli, the intensity of fluorescence of the cell membranes in this phase was considerably weakened. The cell body contained fluorescent formations of non-homogeneous, honey-comb character, an apparent indication of the presence of antigen complexes in these portions of the cell (Fig. 1, c, control 1, a on insert between pp 96-97 of this issue of the journal).

Ribonuclease and hyaluronidase reduced the intensity of fluorescence in all the strains in which the cell membranes fluoresced in the phase of logarithmic growth (71/12, 1312, 96). Cytological examination revealed in all the strains a decrease in basophilia of the cell walls

Best Available Copy

GRAPHIC NOT REPRODUCIBLE

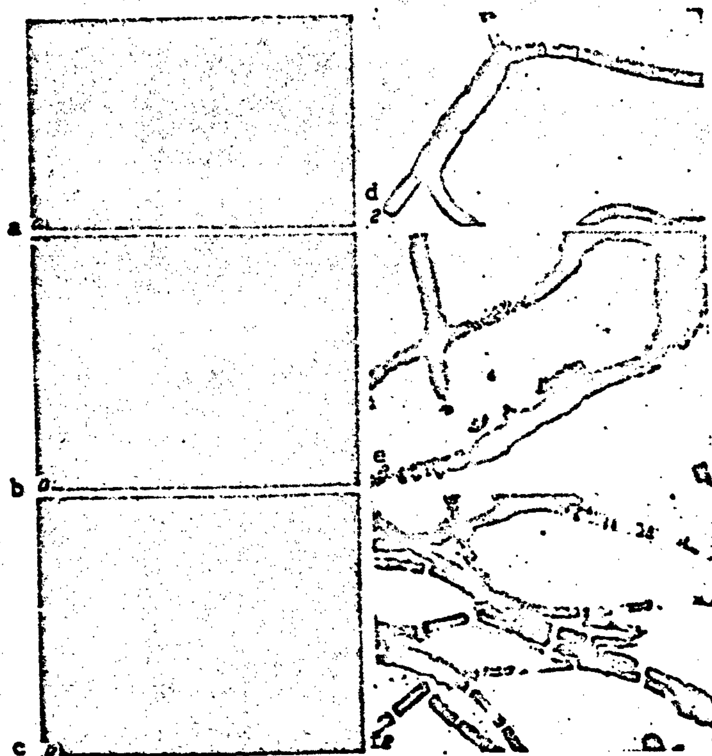


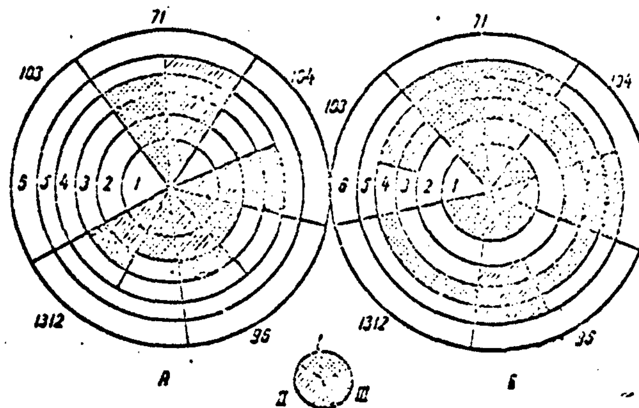
Fig. 1. Effect of enzymes on a B. anthracis (strain 71/12) cell.

a,b,c - st ned with luminescent serums (a - control; b - after trypsin; c - after lysozyme); d,e,f - stained by Gutstein's method (d - control; e - after trypsin; f - after extraction of lipids).

Best Available Copy

and no lysis of the cell walls, although they scarcely stained. Very slight deformation of the membranes was noted in only a few cells of B. anthracis 71/12 and B. cereus 104. In the phase of stationary growth these enzymes reduced the intensity of fluorescence (more so after ribonuclease than after hyaluronidase), especially in the B. anthracis vaccinal strain, which showed honey-comb fluorescence of the protoplasm. Cytological examination revealed a decreased capacity of the cell membranes for staining and slight deformation in some cells of all the strains under study.

Extraction of fats in the cultures in the phase of logarithmic growth had no effect on the fluorescence of the membranes, except in B. anthracis 71/12. Cytological examination revealed slight deformation of the membranes in only a few cells of strains 103, 104, and 96. The membranes frequently lost their capacity for staining. In most of the streptobacilli, the substance that unites individual cells into streptobacilli was destroyed (Fig. 1, f, control 1, d). B. anthracis 71/12 and B. cereus 104 in the stationary phase exhibited a marked decrease in intensity of fluorescence of the cell membranes and grainy fluorescence of some portions of the protoplasm. Cytological examination revealed that these strains experienced the same changes as in the logarithmic phase.



GRAPHIC NOT REPRODUCIBLE

Fig. 2. Effect of various enzymes on the antigenic composition and morphology of B. anthracis and B. cereus.
A - phase of logarithmic growth; B - phase of stationary growth. Injury to antigens (1) and morphology (II) slight; III - intense, after treatment with ribonuclease (1), hyaluronidase (2), extraction of fats (3), treatment with trypsin (4), lysozyme (5), pepsin (6)

Best Available Copy

The effects of the various enzymes on the antigen complexes are presented in Fig. 2, from which it is evident that the B. anthracis vaccinal strain has specific antigen complexes that differ from the antigen complexes of B. cereus in sensitivity to enzymes. In addition, there were antigen complexes common to B. cereus. The commonness and specificity of the antigens were determined, depending on the phase of development of the microorganisms, by complexes of different chemical composition. In the early phases of development, the antigenic specificity of the B. anthracis vaccinal strain was determined mainly by lipoproteins sensitive to trypsin, chymotrypsin, and fat solvents; in the later phases, by mucopeptides and mucoproteins sensitive to lysozyme and hyaluronidase. The commonness of the antigen components in the early phase was caused by substances sensitive to ribonuclease and hyaluronidase; in the mature cells, mainly by lipoproteins. Thus, the antigenic differences between the strains under study were more pronounced in the phase of logarithmic growth.

A Comparison of the antigen components of B. anthracis and B. cereus showed that the strains with residual virulence such as the B. anthracis vaccinal strain 71/12 had a fuller set of antigens than did the representatives of the sporiferous aerobes studied.

Thus, there is a definite relationship between the immunochemical and cytological findings. For example, after enzymatic digestion or extraction of substances responsible for the antigenic properties at the particular stage of development, the microbial cell exhibited both alteration of the morphology and injury to the antigen complexes as shown by change in fluorescence of the cell after treatment with fluorescent serums. This relationship was most pronounced after the action of the vaccinal anthrax strain in the phase of logarithmic growth. In cases where the action of the enzymes did not affect the intensity of fluorescence but merely resulted in morphological changes, these substances may be said to have no determining influence on the antigenic structure of the given strain, e. g., the action of lysozyme on B. cereus 104 and 96 in the stationary phase of growth.

We conclude from our study of the antigenic characteristics of noncapsulate sporiferous bacilli that antigens are concentrated in the cell wall and cytoplasmatic membrane. The antigens in the wall consist of a great variety of mucopeptides and lipoproteins sensitive to lysozyme, trypsin, and, possibly, to hyaluronidase and ribonuclease and fats extracted from the cell with solvents.

Conclusions

1. Study of the antigenic characteristics of noncapsulate forms of the B. anthracis vaccinal strain and sporiferous aerobes by means of immunochemical and cytochemical methods showed that antigen complexes are concentrated mainly in the surface structures of the bacterial cell - in the wall and cytoplasmatic membrane. Depending on the species

Best Available Copy

and age-related characteristics of the strain, these antigens consist of a wide variety of mucopeptides and lipoproteins sensitive to lysozyme, trypsin, hyaluronidase, ribonuclease, and fat solvants.

2. Comparison of the antigenic characteristics of the noncapsulate forms of B. anthracis and sporiferous aerobes showed that the former have antigens in common with the latter as well as specific ones. The commonness and specificity of the antigens are caused by complexes with different chemical composition, depending on the phase of growth of the microorganisms. The differences are most pronounced in the phase of logarithmic growth. The pathogenic microorganisms have a fuller set of substances determining their antigenic properties than do the nonpathogenic microorganisms.

3. The serological methods based on determining the antigenic characteristics of B. anthracis can be safely used to detect vegetative noncapsulate B. anthracis cells only in the initial phase of logarithmic growth.

Bibliography

Akhmerov, D. Sh. et al. Uchen. zapiski Kazansk. vet. in-ta (Scientific Notes of the Kazan' Veterinary Institute), Vol 81, 1962, p 35.

Blagoveshchenskiy, V. A., Kul'berg, A. Ya., Bulatova, T. I., et al. Zh. mikrobiol. (Journal of Microbiology), No 3, 1962, p 18.

Levina, Ye. N. Ibid., No 1, 1958, p 9.

Levina, Ye. N., Ogiyevetskaya, M. M., and Kachelkina, A. I. In the book: Lyuminestsiruyushchiye antitela v mikrobiologii (Fluorescent Antibodies in Microbiology), Moscow, 1962, p 117.

Meysel, M. N., Kabanova, Ye. A., Levina, Ye. N., et al. Izv. AN SSSR. Seriya biol. (News of the Academy of Sciences USSR. Biology Series), No 6, 1957, p 718.

Pritulin, P. I. and Kuz'min, N. A. Veterinariya (Veterinary Medicine), No 7, 1959, p 69.

Cherry, W. B. and Freeman, E. M. Zbl. Bakt., I Abt. Orig., Vol 175, 1959, p 582.

- END -

CSO: 12362-D

Best Available Copy