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Antigenic Spectrum Of Causative Agent Of Anthrax.

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ABSTRACT

The establishment of the taxonomic status of a large group of aerobic spore-forming microorganisms that are close in their basic properties to the anthrax pathogen will make it possible to more reliably carry out its differential diagnosis. The conducted studies are a scientific rationale for the development of highly active and specific antigens and antibodies to create diagnostic products. The research results indicate a high heterogeneity and close relationship of soluble antigens in different representatives of the genus Bacillus. In the extracts of spore cells of the anthrax pathogen and closely related bacteria, a significant amount of interspecific antigens was found, which in the animal's body induces the synthesis of antibodies, which react well when performing serological reactions. Interspecific antigens are mainly polypeptides that migrate rapidly in an electric field. At the same time, the anthrax pathogen has also identified species-specific antigens, a characteristic feature of which is minimal mobility in an electric field.

Keywords: anthrax, antigens, antibodies, SDS antigenextract, polypeptide spectrum, spores, vegetative cells, diagnostics

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INTRODUCTION

Sporadic outbreaks of anthrax, observed in the regions of Russia, cause great economic damage to livestock in connection with the death of farm animals, as well as the need for sanitary and quarantine measures that require large material and physical costs [1,2,3,4]. In turn, the emergence and spread of anthrax among animals creates a real threat of human infection. The above determines the relevance of work on the further in-depth study of the anthrax pathogen. First of all, it concerns the establishment of the taxonomic status of a large group of aerobic spore-forming microorganisms that are close in their basic properties to the anthrax pathogen, which will make it possible to more reliably carry out its differential diagnosis [5,6,7,8,9,10]. In the present work, the polypeptide spectra of SDS-spore antigenic extracts and vegetative cells of B. Anthracis and aerobic spore-forming bacteria were studied by electrophoresis in PAGE.

MATERIALS AND METHODS

The study of the polypeptide spectrum of SDS antigenextracts was performed using the following cultures: B. anthracis, piece 55 (VNIIVViM) and 71; B. cereus pieces 8035 and pieces 16; B. mesentericus pcs.66; B. megaterium, piece 182; B.subtilis pcs.433. Cultures were grown on MPA. Cultures that were in the logarithmic (9 hrs.) And stationary growth phases (24 hrs), as well as seven-day culture spores, were taken into the experiment.

Grown cultures from the agar surface were washed with a 0.85% sodium chloride solution, washed three times by centrifugation in the same solution. The precipitate was dissolved in 50 µl of distilled water with the addition of 50 µl of a lysis solution containing 10 mM Tris buffer pH 6.8, 3% sodium dodecyl sulfate (SDS), 0.1% mercaptoethanol, 0.02% bromophenol blue. A suspension of bacterial cells with a lysing mixture was boiled for 10 minutes and 10 µl each was applied on PAAG. The separation of the polypeptides in the gel was carried out in a vertical chamber at 80 V, 20 mA for 18 hours until the complete passage of the bacterial mixture with bromophenol blue. Polypeptide spectra in the gel were stained with silver.

Densimetry of PAG plates and the results of the immunoblot were performed in transmitted and reflected light, respectively, on a SHARP JX-330 scanner (“Pharmacia Biotech”). Molecular weights and relative content of protein fractions were determined using Image Master 1D Prime v 3/00 programs (“Pharmacia Biotech”) using the LMW ELECTROPHORESIS CALIBRATION KIT (“Pharmacia Biotech”).

RESULTS AND DISCUSSION

Research has shown that the overwhelming number of polypeptides of SDS antigens from spore-forming bacilli is observed in the molecular mass range (mM) from 92 kD to 13 kD. There are clear differences in the spectra in the quantitative content of the polypeptides of the SDS antigen-extracts of B.anthracis cultures that are in different growth phases and in spores. For example, in the case of B. anthracis, pieces 55 (VNIIVViM) during the logarithmic growth phase (9 hours), up to 60 clearly visible polypeptide fractions are observed in the 10–15% PAAG fractionation period, during the stationary phase (24 hours) up to 50 and in the period of sporulation - up to 34 fractions.

A similar picture of reducing the number and content of fractions of SDS-antigen extract is also observed in the case of B. anthracis pcs. 71: in the lagorifmic phase - up to 54 fractions; in the stationary phase - up to 45; in disputes - up to 34 polypeptide fractions. The process of growth and sporulation of the studied B.anthracis cultures is accompanied by a qualitative and quantitative redistribution of low molecular weight (mM) polypeptides into more “heavy” ones, which, apparently, is due to a change in metabolism during the period of cell growth and sporulation. In the SDS antigen extract of the vaccine strain 55 (VNIIVViM) of the anthrax microbe, the appearance of more pronounced polypeptides with mM from 40 kD to 90 kD and a decrease in clear fractions in the mM range from 13 kD to 40 kD in the stationary phase of cells compared to the logarithmic bacilli growth stage . In tab. 1 shows data on the content of individual groups of polypeptides of SDS-antigen extracts, differing in mM during the growth and sporulation of B.anthracis cultures.

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Table 1 - The relative content of polypeptide fractions of SDS-antigen extracts during the period of growth and sporulation of B. Anthracis cultures

№	Crop growth phasesB. anthracis	The relative content of fractions (%)			
		92-62 kD	60-42 kD	40-23 kD	22-13 kD
1	Strain 55 (VNIIVViM) Logarithmic growth phase	14,99	20,17	23,53	39,3
	Stationary growth phase	26,71	31,05	21,6	20,61
	Controversy	43,07	21,29	27,10	8,51
2	Strain 71 Logarithmic growth phase	20,71	28,31	23,41	26,23
	Stationary growth phase	16,71	28,31	23,41	31,58
	Controversy	35,75	20,49	24,94	18,70

On the basis of the presented data, it is possible to conclude about the highest content of “heavy” polypeptide fractions in the range of 92–62 kD mM observed in the SDS antigen-extracting spores in relation to the vegetative cells of B. anthracis. The most permanent components on PAGE electrophoregrams of SDS spore antigen extractant and B.Anthracis vegetative cells are polypeptide fractions with mM 90 kD (92-90 kD) and 79 kD, whose content increases during growth and sporulation (Table 2).

Table 2 - The relative content of polypeptides with mM 90 KD and 78 KD in SDS-antigen extract at different stages of B. Anthracis growth

Strain B. antracis	The relative content of protein fractions (%), growth stage					
	Protein fraction with mM 90 kD			Protein fraction with mM 78 kD		
	Logarithmic growth phase	Stationary growth phase	Controversy	Logarithmic growth phase	Stationary growth phase	Controversy
Strain 55 (VNIIVViM)	6,03	9,68	14,03	1,29	3,03	6,29
Strain 71	3,02	3,97	15,19	2,08	2,27	5,68

In the spore form of the anthrax pathogen, the pattern of distribution of the main fractions of SDS antigen extracts does not change as compared with vegetative cells. The formation of a polypeptide with mM 50 kD and other fractions is noted against the background of a decrease in the number of minor fractions in these zones. The marked changes in the polypeptide spectra of the B. anthracis SDS antigen extracts during the period of growth and sporulation ultimately translate into the formation of up to 8 major (major) spore polypeptides. The main faction spores B.anthracis pieces. 55 (VNIIVViM) with electrophoresis in PAG (10-15%) were: 13 KD (5.40%); 29 kD (13.13%); 40 kD (4.81%); 47 kD (5.65%); 50 kD (10.85%); 62-63 kD (2.75% and 4.58%, respectively); 78 kD (6.29%); 90 kD (14.03%). On electrophoregrams of SDS-antigen extract B.anthracis pcs. 71 marked main polypeptides with mm: 18 KD (5.67%); 29 kD (6.02%); 33 kD (5.38%); 47 kD (5.53%); 62 kD (4.81%); 78 kD (5.68%); 90 kD (15.19%). In total, the relative content of the marked main polypeptides in SDS antigen extracts for B. anthracis spores. 55 (VNIIVViM) is 67.49%, and for the disputes B. anthracis pieces. 71 - 53.29%.

The appearance of “basic” polypeptides is observed in the cases of C.cereus; B. mesentericus starting from the stationary growth stage and spore forms, which have mM 13 kD, 15 kD, 42 kD, 68 kD, 78 kD, 87 kD and 21 kD, 63 kD, 68-74 kD, 87 kD, respectively, interesting In our opinion, there was a presence of a “major”

polypeptide fraction with mM 85–87 kD in B. megaterium at all growth stages by analogy with the main polypeptide mM 90 kD in B. anthracis and the formation in the sporulation stage of polypeptide fractions with mM 13 kD- 18 kD, 38 kD, 63 kD, 75 kD. In the case of B.cereus pieces. 8035 The process of formation of the "main" fractions is less pronounced than that of other aerobic bacilli.

We have previously shown (Galiullin, AK and others, 1996) that antisera against B. anthracis pieces. 55, obtained on a subunit antigen mM 90 kD spore in the immunoblot, revealed a number of seroactive SDS antigen extract of the spores of this strain, which indicates that the antigenic epitopes in the polypeptides of the pathogen are conservative. This is evidenced by the data of immunoblot for the detection of seroactive fractions of SDS-spore antigen extract, vegetative cells of B. anthracis, pcs. 55 (VNIIVViM) using hyperimmune sera obtained by hyperimmunization of rabbits with formalinized spores and MM subunit 90 kD antigen, purified by disc electrophoresis in PAAG. In the immunoblot, hyperimmune sera detected more than 25 seroactive fractions of SDS-antigen extracts of spores and vegetative cells, which had qualitative and quantitative differences.

It was also revealed that the hyperimmune antiserum obtained for the discrete polypeptide fraction of B. anthracis spores. 55 (VNIIVViM), reveals in the immunoblot a large range of antigenic structures both in disputes and in the vegetative cells of this microorganism in the range of mM from 16 to 12 kD. This indicates that this fraction is a polypeptide precursor of bacterial proteins of the S-layer, which is present at all phases of the development of B.anthracis cells, including the maximum concentration in spores (Table 2).

CONCLUSION

1. It has been established that the soluble antigens are closely related to different members of the genus Bacillus. In the extracts of spore cells of the anthrax pathogen and closely related bacilli a significant amount of interspecific antigens was found.
2. Interspecific antigens of closely related bacilli to B. anthracis are mainly polypeptides that migrate rapidly in an electric field.
3. Soluble antigens of the anthrax pathogen also contain species-specific antigens, a characteristic feature of which is minimal mobility in an electric field.

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