CHEMICAL AND IMMUNOBIOLOGICAL CHARACTERISTICS OF BRUCELLA PROTECTIVE ANTIGEN

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The results of a study of a nontoxic brucella protective antigen, free from sensitizing properties, isolated from the cell wall of brucellas are described. The antigen has a well-marked protective action against experimental infection for 3-4 months in guinea pigs after a single immunization. Molecular heterogeneity of the antigen was established, and it consists mainly of a protein–polysaccharide complex. High- and low-molecular-weight fractions separated by gel filtration differed in their chemical and immunobiological characteristics. The high-molecular-weight fraction of the antigen was highly immunogenic and serologically active.

KEY WORDS: *brucellosis; protective antigen.*

An urgent problem in the immunoprophylaxis of brucellosis is the obtaining of nontoxic, nonsensitizing protective antigens (chemical vaccines) from brucellas.

This paper describes the study of the properties of a brucella protective antigen (BPA) obtained in the brucellosis laboratory of the N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR.

EXPERIMENTAL METHOD AND RESULTS

The antigen was isolated from a dried microbial mass of brucellas in the S-form by hydrolysis with acetic acid; it was immunologically effective, and it was free from toxic and sensitizing properties [1, 2].

A single injection of BPA into guinea pigs in a dose of 600 μg gave the same protective effect as living brucellosis vaccine, and after infection with different doses (40-200 bacterial cells of a virulent strain of *Brucella melitensis*) the percentage of animals remaining uninfected was 90 and 62.5 respectively.

Tests of the duration of the immunogenic action of BPA showed that in animals immunized with a single dose the antigen gave a high level of immunity for 3-4 months to infection with a virulent strain of *B. melitensis* in a dose of 40 microbial cells (60-90% of immune animals). Four months after immunization, the immunity "collapsed" in 70% of the animals, and the index of infection was 32.2. The low level of immunity in the guinea pigs 4 months after immunization was found to correlate with a drop in preventive activity of the serum from these animals: The 50% preventive dose (PD_{50}) during this period was 0.12 ml, compared with 0.024-0.041 ml 1-3 months after immunization.

A study of the antigenic properties of BPA showed that a single injection of it stimulated synthesis of complete and incomplete antibodies. Agglutinins and hemagglutinins of the IgM type appeared as early as after 6 days and reached a maximum 15-30 days after immunization, but disappeared completely after 2-4 months; IgG agglutinins appeared later than IgM antibodies and remained at the same level for 1-2 months, but disappeared from the

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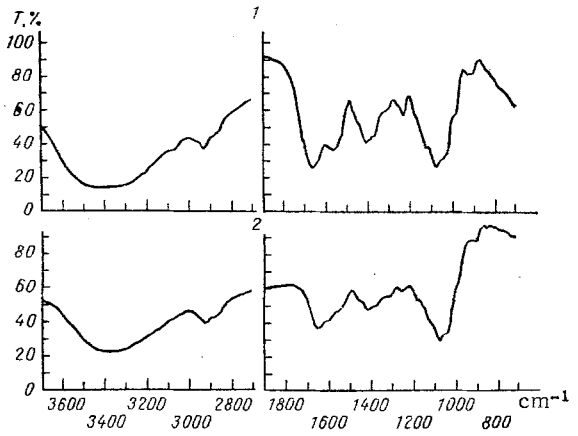

Fig. 1

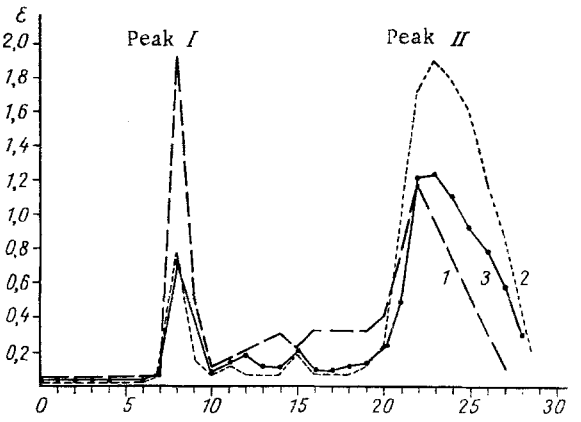

Fig. 2

Fig. 1. Infrared spectra of BPA: 1) original preparation; 2) BPA preparation after γ-ray irradiation in a dose of 3 Mrad.

Fig. 2. Fractionation of BPA on column with Sephadex G-100. Abscissa, No. of sample; ordinate, coefficient of extinction (E) in ultraviolet region at wavelengths of: 1) 230 nm, 2) 260 nm, 3) 280 nm.

blood serum after 4 months. However, incomplete antibodies of both classes of immunoglobulins could still be found at that time.

The dynamics of antibody formation in animals immunized with BPA thus correlated with the state of immunity of the vaccinated animals.

The results described above are evidence of the well-marked protective properties of BPA under experimental conditions (absence of toxicity, of sensitizing properties, and of pyrogenic action of the preparation), so meeting the requirements for its use as a prophylactic agent for the immunization of man against brucellosis.

A study of the chemical composition of this antigen showed that it is complex in nature, and consists mainly of a protein–polysaccharide component: It contained on average 45% total sugars, 30% protein, 7% lipids, 1% phosphorus, and 3% nucleic acids.

Infrared spectral analysis of BPA (Fig. 1) confirmed the results of biochemical analysis and its mainly protein–polysaccharide nature. Smoothing out of the absorption bands in the 3000-2800 cm^{-1} region and absence of absorption at 1740 cm^{-1} were found, in connection with the spectrum of the lipid component. There is reason to suppose that the protective active of brucella immunogens is dependent on the integrity of their protein–lipid structure, as shown by the sharp changes in the absorption spectra of the 1660 and 1560 cm^{-1} bands (amide I and amide II), disappearance of the 1230 cm^{-1} band (amide III), and also the appearance of an absorption band at 1610 cm^{-1} in the antigen which had lost its immunogenic activity after γ-ray irradiation in a dose of 3 Mrad (Fig. 1). Weakening of absorption of the amide bands suggests rupture of the polypeptide chain and indicates that the lowering of immunogenicity of BPA correlates with disturbance of the integrity of the molecular structure of the antigen.

An immunoelectrophoretic analysis showed that the preparation obtained consists mainly of two antigenic components differing in their electrophoretic mobility in the cathodal zone, and that it differs from the toxic antigenic complexes of brucellas, which have a precipitation line of lipopolysaccharide nature located at the starting point around the well containing antigen. All antigens of brucellas isolated from the cell wall by different chemical methods are characterized by the formation of a precipitation line located along the gutter containing serum during immunoelectrophoresis [3].

The protective antigen was separated by gel filtration on a column with Sephadex G-100 into two main fractions containing substances of different molecular weights (Fig. 2). Analysis of the absorption spectrum in the UV region showed that during elution of the

second peak, the ratio E_{230}^{260} increased. For the separate fractions of the second peak its value was about 2, evidence that it contains substances of nucleic acid nature.

The macromolecular component of the BPA (peak I) accounted for only 13% of the original antigen, whereas peak II, including the low-molecular-weight fractions, accounted for 41.7% of the yield of dry substance.

The predominant monosaccharide in BPA was shown by paper chromatography to be glucose; much smaller amounts of mannose, ribose, and glycosamines also were found. Large quantities of a fast-migrating sugar, xylose, were found in the polysaccharide of the macromolecular component of BPA, together with ribose. The low-molecular-weight fractions contained large quantities of glucose and mannose and traces of a fast-migrating sugar. The total content of sugars in the dry preparations of peaks I and II was identical (about 50%). The content of total protein in peak I was about 8.2%, whereas low-molecular-weight fractions contained up to 24% of protein. Nucleic acids contained in the original BPA (3.6%) were found almost entirely in peak II (3.48%), whereas only traces of nucleic acids were found in peak I. The substances of peaks I and II of the antigen in turn were nonhomogeneous on rechromatography on Sephadex G-100, especially in the case of peak II.

The study of the immunobiological activity of the high- and low-molecular-weight fragments of BPA gave especially interesting results. The serological and immunogenic activity of the brucella antigen was shown to be associated with the macromolecular component (peak I). The titer of peak I in the ring-precipitation test was 1:128,000, whereas peak II was virtually without serological activity (titer 1:2000). Tests of immunogenic activity showed that the macromolecular fraction had high protective action against brucellosis infection (80% of immune animals), even higher than the original BPA (70%), much higher than the immunogenicity of the low-molecular-weight fraction of the antigen (33%). The results indicate that prospects are good for obtaining purified vaccines on the basis of the macromolecular fractions of the brucella antigenic complex.

Further more detailed investigations are required to obtain a fuller understanding of the chemical basis of the toxicity and immunogenicity of complex brucella antigens; these are justified not only by theoretical considerations but also by the need for having purified nontoxic preparations for the prevention of brucellosis.

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