The effect of polyazolidinammonium on the dynamics of the synthesis of pseudotuberculosis antibodies

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Abstract. The use of a 1% solution of polyazolidin-ammonium modified with iodine hydrate ions (PAAG) in combination with disintegrated Yersinia pseudotuberculosis membranes made it possible to obtain hyperimmune serum with a high content of antibodies and species specificity. By the number of antibodies and the growth dynamics of their titers, PAAG was not inferior to Freund's complete adjuvant. At the sites of injection of PAAG, connective tissue densification of the subcutaneous tissue was not observed.

1. Introduction

Pseudotuberculosis microbe causes disease in humans, and less often in animals [1, 2].

The use of diagnostic antibodies obtained from various antigens of Yersinia pseudotuberculosis is one of the most common methods for indicating pseudotuberculosis infection [3]. Hyperimmune diagnostic blood serum is obtained from donor laboratory animal with a high content of pseudotuberculosis antibodies requiring the joint use of antigens and adjuvants [4].

The cell wall of Yersinia pseudotuberculosis contains a large number of protein or polysaccharide antigens. They have different specificity [5-7]. However, the destruction of the cell wall of the pseudotuberculosis microbe by sodium dodecyl sulfate allowed us to obtain an antigenic preparation with species specificity [8]. Currently, a large number of adjuvants are used to enhance the action of antigens in the production of diagnostic hyperimmune sera. Among them, polyelectrolytes that are able to bind antigen molecules of various chemical nature into conjugates are becoming increasingly popular. Their bonds with proteins are especially strong [9].

From the group of polyelectrolytes, polyazolidin-ammonium modified with halogen hydrate ions (PAAG) is promising for use as an adjuvant in the preparation of pseudotuberculosis antibodies [10, 11]. However, the dynamics of the production of pseudotuberculosis antibodies during its use is not well understood, which determined the purpose of this study. The dynamics of the formation of antibodies caused by the action of PAAG were compared with a similar process proceeding in the presence of Freund's complete adjuvant (FCA). FCA is one of the most effective adjuvants for the production of hyperimmune serums. However, it contributes to the formation of significant foci of inflammation in the subcutaneous tissue of animals and is of high cost [4, 12].

2. Materials and methods

Bacterial mass of Yersinia pseudotuberculosis III O:3 serotype (Y. pseudotuberculosis O:3) were
grown on meat-peptone agar at 26 °C for three days and washed, two times with a saline solution.

To obtain disintegrated cell membranes, the bacterial suspension was treated with an ultrasonic disintegrator. Then, the destroyed cell membranes were separated from the cytoplasm and periplasm by centrifugation. The resulting cell membranes were destroyed with sodium dodecyl sulfate, which was subsequently released by dialysis in running water [8].

Hyperimmune sera were obtained by subcutaneous immunization of six-month-old male rabbits of the “Chinchilla” breed along the back at 4-5 points in a volume of 1 ml of the antigen-adjuvant mixture in a ratio of 1:1 with an interval between subsequent immunizations of 2 weeks. A total of 7 immunizations were carried out. Blood for the study was taken before each immunization.

Disintegrated membrane of (DM) Y. pseudotuberculosis O:3 was used as an antigen, preparation was made with a protein concentration of 2 mg / ml. In the control groups, the antigen was replaced with physiological saline (PS). As adjuvants, 1–1% solution of PAAG in PS and 2–FCA were used. In total, 4 groups of rabbits were immunized: 1 – DM + PAAG; 2 – DM + FCA; 3 – PS + PAAG; 4 – PS + FCA. In each group there were 3 animals.

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Sensitivity and specificity of hyperimmune sera were studied in indirect solid-phase enzyme-linked immunosorbent assay (ELISA) [13].

The specificity of the sera obtained was determined after the sixth immunization.

As antigens for ELISA, DM was used. Y. pseudotuberculosis O:3 at a concentration of 20 μg / ml and formalized cells of Yersinia pseudotuberculosis I O:1 serotype, Yersinia pseudotuberculosis III O:3 serotype, Yersinia pseudotuberculosis IV O:4 serotype, Yersinia pseudotuberculosis V O:5 serotype (Y. pseudotuberculosis O:1, O:3, O:4, O:5), Yersinia enterocolitica 66-82 O:3 serotype, Yersinia enterocolitica 383 O:9 serotype (Y. enterocolitica O:3, O:9), Enterobacter aerogenes ATCC-13048 (E. aerogenes), Escherichia coli 4295 (E. coli), Proteus vulgaris 19 (P. vulgaris), Salmonella typhimurium 1626 (S. typhimurium) (obtained from the state-owned collection of pathogenic microorganisms of the Federal state healthcare institution antiplague research Institute "Microbe"), as well as a single brucellosis antigen produced by JSC "Pokrovsky Plant of Biopreparations" on the basis of Brucella abortus (B. abortus ). Bacterial suspensions for ELISA were prepared at a concentration of 1 billion, cells / ml.

3. Results

Antibody activity of blood sera of rabbits immunized with DM was studied in ELISA with DM Y. pseudotuberculosis O:3, table 1.

**Table 1.** The results of determining the antibody activity of blood sera of rabbits immunized.

| Serum Collection Time | Antibody titer of the obtained sera in ELISA with DM 20 μg / ml |
|-----------------------|---------------------------------------------------------------|
|                       | DM + PAAG          | DM + FCA          | PAAG + PS       | FCA + PS       |
| Before immunization   | 1:800              | 1:800             | 1:800           | 1:800           |
| After 1st immunization| 1:6400             | 1:6400            | –               | –              |
| After 2nd immunization| 1:25600            | 1:51200           | 1:1600          | 1:1600          |
| After 3rd immunization| 1:51200            | 1:102400          | –               | –              |
| After 4th immunization| 1:102400           | 1:204800          | 1:1600          | 1:3200          |
| After 5th immunization| 1:204800           | 1:204800          | –               | –              |
| After 6th immunization| 1:204800           | 1:409600          | 1:6400          | 1:6400          |
| After 7th immunization| 1:409600           | 1:409600          | –               | –              |

Table 1 shows that the maximum value of titers of specific antibodies in the blood of animals immunized with PAAG reached after 5 immunizations (after 2.5 months) and was 1:409600. By the number of antibodies and the growth dynamics of their titers, the PAAG was not inferior to FCA.
In the control groups of high titers, no specific antibodies were detected, which indicates the stimulating effect of adjuvants on antigen genesis only in combination with the antigen, table 1.

In the process of immunization, there was also a lack of connective tissue compactions of the subcutaneous tissue at the sites of administration of the PAAG and the presence of these formations when using the FCA, figure 1.

Figure 1. Connective tissue compactions of the subcutaneous tissue at the sites of administration of the FCA.

The specificity of the obtained blood sera was studied in ELISA with whole bacterial cells, table 2.

Table 2. The results of determining the specificity of the sera obtained in the experimental groups.

| Bacterial cells        | Antibody titers of the obtained sera in ELISA with bacterial cells diluted to 10^9 cells / ml |
|------------------------|-----------------------------------------------------------------------------------------------|
|                        | DM + PAAG                                                                                     | DM + FCA                                                                 |
| Y. pseudotuberculosis O:1 | 1:51200                                                                                     | 1:51200                                                                 |
| Y. pseudotuberculosis O:3 | 1:25600                                                                                     | 1:51200                                                                 |
| Y. pseudotuberculosis O:4 | 1:25600                                                                                     | 1:25600                                                                 |
| Y. pseudotuberculosis O:5 | 1:25600                                                                                     | 1:51200                                                                 |
| Y. enterocolitica O:3   | 1:200                                                                                         | 1:200                                                                 |
| E. coli                | 1:400                                                                                         | 1:400                                                                 |
| S. typhimurium          | 1:100                                                                                         | 1:100                                                                 |
| P. vulgaris            | 1:400                                                                                         | 1:400                                                                 |
| E. aerogenes           | 1:200                                                                                         | 1:200                                                                 |
| B. abortus             | 1:400                                                                                         | 1:400                                                                 |

The specificity of hyperimmune sera was similar when both adjuvants were used. Hyperimmune sera were characterized by species specificity, table 2.

Minor nonspecific reactions were noted with representatives of the Enterobacteriaceae family and B. abortus in the titers 1:200-1:800. However, positive titers of specific antibodies of Y. pseudotuberculosis are 500 times higher than positive antibody titers to other types of bacteria, which allows the use of the obtained hyperimmune sera when creating immune-enzyme test systems for the diagnosis of pseudotuberculosis, table 2.

4. Conclusion
Based on the work done, the following conclusions can be drawn:

- Use of a 1% solution of PAAG in combination with DM Y. pseudotuberculosis, allows to obtain hyperimmune serum with a high content of antibodies.
- The obtained hyperimmune serum has specific specificity.
By the number of antibodies and the growth dynamics of their titers, PAAG is not inferior to FCA.

At the injection sites of PAAG, connective tissue seals of subcutaneous tissue are not observed.

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