ANTIGENIC ACTIVITY OF STRUCTURALLY DEFECTIVE LIPOPOLYSACCHARIDE OF A STREPTOMYCIN-DEPENDENT MUTANT OF Salmonella enteritidis

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The lipopolysaccharide (LPS) of the streptomycin-dependent mutant of Salmonella enteritidis does not contain sugars from which the determinant groups responsible for specificity of receptors 9 and 12 of the O-antigen are built. This LPS consists principally of the basic structure and it stimulates the formation principally of IgG-antibodies in BALB/c mice. The LPS of the original strain of S. enteritidis, which contains terminal sugars of determinant groups, stimulates the synthesis of IgM- and IgG-antibodies in mice of this line.

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The specificity of the lipopolysaccharide (LPS) of the enterobacteria is determined by the structure of the side chains (determinant groups), which consist of simple sugars and are attached to a basis consisting of a branched or unbranched polymer. This basis is built of molecules of galactose, glucose, heptose, and hexosamines. The specificity of the O-antigen is largely determined by the structure of the terminal sugars of the side chain, especially the 3,6-didesoxy-sugars [6]. However, the chemical nature of the antigenic activity of LPS of the enterobacteria has not been completely elucidated.

The object of this investigation was to study the antigenic properties of the LPS of a streptomycin-dependent mutant, defective as regards the synthesis of its determinant groups.

EXPERIMENTAL METHOD

The LPS was extracted with a mixture of phenol and water [7] from the original normal strain of S. enteritidis (01, 9, 12) and its streptomycin-dependent mutant obtained by V. V. Sergeev. The isolated antigens were subjected to acid hydrolysis (0.2 M acetic acid, 12-17 h) and the resulting sugars were studied by paper chromatography. As reference substances for the 3,6-didesoxy-sugars, sugars obtained from Professor Staub (Institute Pasteur, Paris) were used, and the writers acknowledge their gratitude.

Male BALB/c mice were immunized by triple intravenous injection with heated vaccine prepared from the original strain (group 1) and a living culture of the mutant (group 2): $3 \cdot 10^8$, $5 \cdot 10^8$, and $5 \cdot 10^8$ bacterial cells. Two other groups of mice were immunized in the same way with cell walls (60, 100, and 100 μg) isolated from bacteria of the two strains [3]. Each group consists of 50 animals, and the experiment was repeated 3 times. The doses of corpuscular antigen and cell walls were equalized in their content of polysaccharide. Streptomycin-dependent mutants of salmonellas are avirulent to mice [2], so that the animals were injected with a living culture of the mutant.

Sera from ten animals of each group, obtained at different times after 1, 2, and 3 immunizations, were tested for the presence of O-antibodies. The activity of IgM- and IgG-antibodies in the sera was determined by treatment with 2-mercaptoethanol (2-ME) [4] and also in the fractions obtained by gel-filtration of the sera [5]. The columns used measured 3×90 cm and were packed with Sephadex G-200. Elution was carried out with tris-buffer (0.1 M, pH 8.0) with the addition of 0.2 M NaCl solution. The speed of elution was 20-25 ml/h. Samples were taken in a volume of 3-5 ml and tested in the passive hemagglutination reaction [1].

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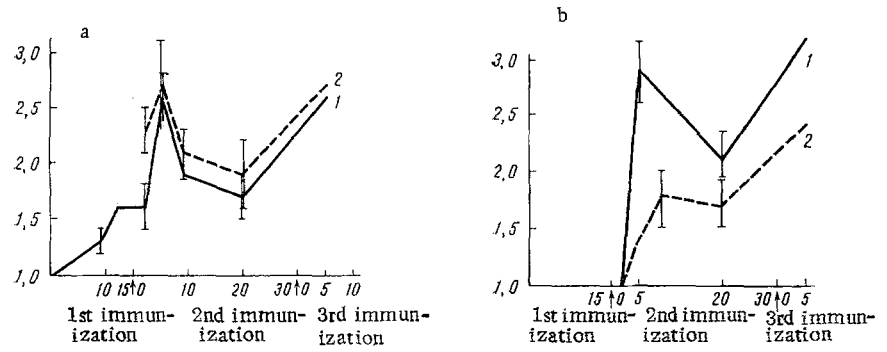

Fig. 1. Dynamics of antibody formation in mice after immunization with LPS of mutant (a) and original (b) strains: 1) before treatment with 2-ME; 2) after treatment with 2-ME. Abscissa, days after immunization; ordinate, log of titers.

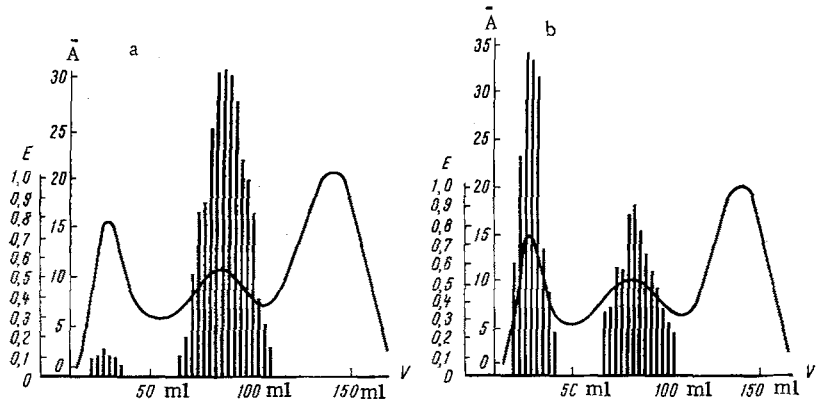

Fig. 2. Gel-filtration of mouse sera after double immunization with LPS of mutant (a) and original (b) strains. Vertical lines denote activity of antibodies (A) of fractions, 1st peak of curve represents IgM, 2nd peak represents IgG, and 3rd peak represents albumin.

Activity of the antibodies in the samples (fraction) was estimated from the initial activity (A) expressed per unit of protein concentration (E, in mg/ml). After a few transformations the following formula was deduced:

$$A = \frac{2n}{E_{in}},$$

where n is the number of twofold stepwise dilutions; E_{in} the initial concentration of protein.

EXPERIMENTAL RESULTS

Investigations of the polysaccharides of O-antigens of both strains of S. enteritidis by paper chromatography revealed significant differences in their structure. Neither tyvelose, rhamnose, nor mannose, which are constituents of the determinant groups of the O-antigen of the original strain, were found in the polysaccharide fraction of the mutant strain. Serologic tests confirmed the results of chromatographic analysis, i.e., no receptors 9 and 12 were found in the LPS of the mutant. The polysaccharide of the streptomycin-dependent mutant is thus built principally of the basic structure and it can be used as a model for studying the antigenic activity of the LPS basis. The polysaccharide of the original strain has been used as a model to study the antigenic activity of determinant groups.

As a result of immunization of the mice with antigens from both strains, the dynamics of antibody formation in the two cases followed a qualitatively different pattern (Fig. 1). Antibodies against LPS of the mutant were found in the blood after the first immunization, and against LPS of the original strain after the 2nd immunization. Antibodies against LPS of the mutant that were detected were mainly resistant to 2-ME,

while those against LPS of the original strain were both sensitive and resistant to 2-ME. A difference in the immune response was found after the 1st and 2nd injections of antigen, but is disappeared after the 3rd injection.

Fractionation of the antisera on a Sephadex column (Fig. 2) showed that humoral antibodies in mice immunized with two doses of the mutant culture were localized mainly in the 2nd (i.e., IgG) fraction, while antibodies in mice immunized in the same way with heated vaccine were concentrated in both fractions, although the activity of the first (IgM) fraction was much higher than that of the second.

Crossed serologic tests showed that the LPS of the mutant reacted with antiserum against LPS of the original strain, but antiserum against LPS of the mutant was inactive when tested with LPS of the original strain.

It can thus be concluded that mainly IgG-antibodies are synthesized against the basic structure of the LPS of salmonellas, while IgM- and IgG-antibodies are synthesized against their determinant groups. The basic and the determinant groups of the LPS stimulate the formation of antibodies of different specificity in mice.

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