INDUCTION OF ANTIBODIES AGAINST NATIVE DNA BY COMPONENTS OF Bordetella pertussis

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Bordetella pertussis cells and some of their components, if injected into mice, cause an increase in the DNA-binding activity of their sera detectable at the ionic strength of physiological saline mainly on the 14th day. The inhibition method showed that interaction of the sera with native DNA is specific. The maximal increase in the content of serum protein reacting with DNA in solutions of low ionic strength (0.05 M NaCl) was observed on the seventh day. However, after injection of B. pertussis cells and their cytoplasmic membranes the increased content of DNA-binding proteins continued until the 14th day. KEY WORDS: antibodies against DNA; pertussis antigens; autoimmune processes.

Antibodies against native DNA discovered in the sera patients with systemic lupus erythematosus, according to existing views, are an indicator of serious changes in the system of immunologic recognition, which play an important role in the pathogenesis of this disease. Until recently all attempts to induce such antibodies experimentally have ended in failure [3, 10]. In 1974 a report was published [5] of the appearance of proteins interacting with native DNA in the sera of experimental animals after they had been injected with a lipopolysaccharide isolated from Escherichia coli. These results are particularly interesting in connection with existing views on the ability of endotoxin to perform the role of polyclonal stimulator of B-cells [6]. Components of Bordetella pertussis cells also interact actively with lymphocytes [8, 9]. In the present investigation an attempt was made to induce proteins reacting specifically with native DNA in animals receiving injections not only of lipopolysaccharides from E. coli, but also various components of B. pertussis.

EXPERIMENTAL METHOD

A suspension of B. pertussis strain 222, cytoplasmic membranes isolated from B. pertussis cells, soluble pertussis antigens obtained with the aid of sodium deoxycholate followed by precipitation with ammonium sulfate to 30% saturation – fraction 1Da [4], and after dialysis through a cellophane membrane – fraction D [1], and also lipopolysaccharides from E. coli (Difco, USA) were used.

The above-mentioned substances were injected intraperitoneally into C57BL/6 mice (females weighing 20 g) in a dose equivalent to 50 ED_{50}, namely 50 μg as protein for soluble pertussis antigens, $1.5 \cdot 10^9$ bacterial cells for B. pertussis, and the number of cytoplasmic membranes corresponding to that number of bacteria. The dose used by Fournie et al. [5], who showed that 50 μg of E. coli endotoxin induces the appearance of antibodies against native DNA in experimental animals, was chosen for the lipopolysaccharide. Blood for analysis was taken from the retro-orbital space before and 1, 7, and 14 days after injection of the above-mentioned substances. Animals of the control group received an injection of 0.15 M NaCl. Each group consisted of ten animals.

The DNA-binding activity was determined by a radioimmunologic method, described previously [2] in solutions of varied ionic strength. Each solution contained 0.015 M trisubstituted sodium citrate and 0.005 M Tris-HCl buffer, pH 8.0. Differences in ionic strength was produced by varying the NaCl concentration, which was 0.05 M (solution with low ionic strength) and 0.15 M (physiological saline). Tritiated DNA with specific activity $1.1 \cdot 10^4$ CPM/μg, isolated by Marmur's method [7] from E. coli cells and additionally purified with pronase, was used in the reaction in a dose of 0.1 μg. The test serum (2 μl) was added to samples containing the

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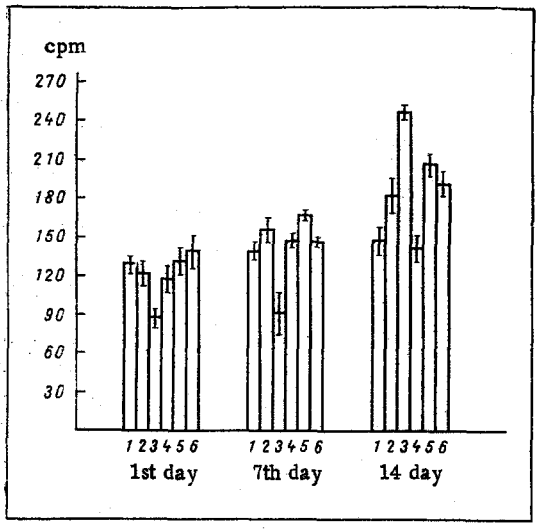

Fig. 1

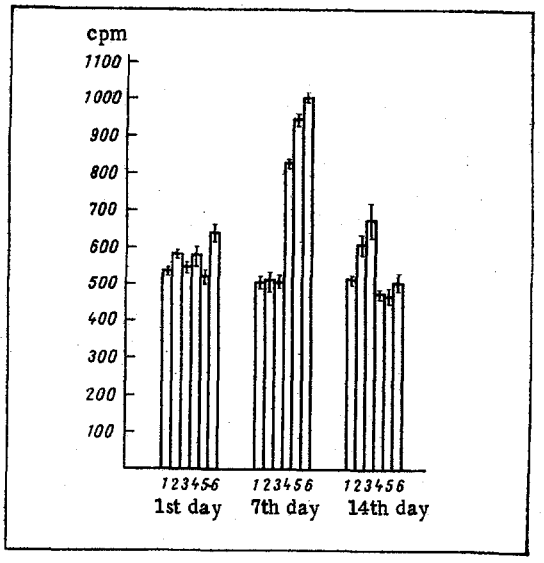

Fig. 2

Fig. 1. Binding of native DNA by animals' sera in solutions with ionic strength of physiological saline (0.15 M NaCl). Abscissa: 1) 0.15 M NaCl; 2) B. pertussis cells; 3) cytoplasmic membranes; 4) fraction D; 5) fraction 1Da; 6) lipopolysaccharides. Ordinate: radioactivity of adsorbed DNA-protein complex (in cpm).

Fig. 2. Binding of native DNA by sera of immunized animals in solutions with low ionic strength (0.05 M NaCl). Legend as in Fig. 1.

TABLE 1. Inhibition of Interaction of Native DNA and Serum Proteins with Animals Immunized with E. coli Lipopolysaccharides and Component 1Da of B. pertussis in Solutions of Different Ionic Strengths

Preparation	Inhibition of binding of native DNA-^{3}H by sera (in percent, mean value)			
	0,05 M NaCl		0,15 M NaCl	
	DNA	dextran sulfate	DNA	dextran sulfate
Lipopolysaccharide	89	28	96	10
Fraction 1Da	100	3	98,8	1

solution with low ionic strength and radioactivity was measured on an SL30 liquid scintillation counter (Intertechnique, France). In the test of inhibition of interaction between the antibodies and radioactive DNA, native DNA from calf thymus and dextran sulfate (Ferak, West Germany) with mol. wt. 500,000 were used. The inhibitor was added in a dose of 6 μg. The experimental results were subjected to statistical analysis by Student's method.

EXPERIMENTAL RESULTS

The results of determination of the binding of DNA by the sera of these animals at the ionic strength of physiological saline at various times after administration of the test preparation are given in Fig. 1. They show that 24 h after administration of the preparation the DNA-binding activity of the sera was not increased, but after injection of cytoplasmic membranes it was reduced compared with the control ($P < 0.05$). On the seventh day the DNA-binding activity of the sera was increased in mice receiving fraction 1Da of pertussis antigen ($P < 0.05$) and it remained low in mice receiving cytoplasmic membranes ($P < 0.05$). On the 14th day the level of DNA binding was increased in all groups ($P < 0.05$) except the animals receiving fraction D ($P > 0.05$). Interaction between the sera and native DNA under these circumstances was specific in character, as was confirmed by the results of inhibition of the direct reaction (Table 1).

It was shown previously that the sera of intact animals contain proteins which react with DNA [2]. Interaction of these proteins with DNA can be increased by reducing the ionic strength of the reaction mixture. To show whether the increase in DNA binding may not be due to the appearance of a large quantity of these proteins under the influence of the test preparation, DNA-binding activity was investigated in a solution of low ionic strength.

The results of the determination are given in Fig. 2. They show that the maximal increase in the quantity of protein reacting with DNA at a low ionic strength occured on the seventh day after injection of fractions D, 1Da, and lipopolysaccharides, but not until the 14th day after injection of the cytoplasmic membranes and B. pertussis cells ($P < 0.05$). These results, together with those of inhibition at low and normal ionic strength (Table 1) confirm that specific antibodies against native DNA may appear after injection of B. pertussis cells and their structural components. The explanation of this fact may be that B. pertussis cells contain a substance which, like the lipopolysaccharide of E. coli, can activate B-cells nonspecifically, so that tolerance is overcome and autoantibodies appear against native DNA. However, the possibility cannot be ruled out that injection of the components of B. pertussis cells may lead to an increase in the formation of alkaline proteins of non-immunoglobulin nature, capable of reacting with DNA.

To detect this action of bacterial preparations in the course of their production, the possibility that they may contain biologically active substances should be taken into account. A simple system for the detection of these substances would probably be to use their ability to induce the appearance of DNA-binding proteins in sera.

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