ABILITY OF *Bordetella pertussis* VACCINE TO INDUCE DELAYED-TYPE HYPERSENSITIVITY TO DNA

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The writers showed previously [1, 2] that inoculation of pertussis antigens, in the form of a corpuscular suspension and its derivatives, leads to the appearance of DNA-binding proteins in the sera of immunized mice, with the characteristic time course of antibody formation. These findings pointed indirectly to the presence of an autoimmune component in this type of immunologic response arising during interaction of the immune system of the host with antigenic complexes of *Bordetella pertussis*.

However, in the light of modern views on levels of functioning of immunocompetent cells, serologic studies are insufficient to characterize the autoimmune processes completely, for antibodies against DNA could appear in the sera as a result of the polyconal stimulating action of the lipopolysaccharide fraction of the bacterial cells on the host's B lymphocyte population [7]. If the question of a possible autoimmune lesion in the postvaccinal period is to be finally settled there is no avoiding the problem of the role of the T system of immunity in this process. Results of clinical-immunologic studies published in the literature indicate that the pathogenesis of generalized autoimmune diseases of the systemic lupus erythematosus type is based on a disturbance of the regulatory function of the thymus-dependent lymphocyte population [7].

The object of the present investigation was to determine the probability of induction of delayed-type hypersensitivity to DNA over the whole range of immunologic reactions to injection of a corpuscular suspension of pertussis vaccine and its components.

EXPERIMENTAL METHODS

A suspension of *Bordetella pertussis* strain 688 cells, cytoplasmic membranes isolated from *B. pertussis* cells, and the soluble antigen obtained after dialysis through a cellophane membrane (fraction D), were used.

The above-mentioned substances were injected intraperitoneally into NIHHSFS/H mice (males, weight 16-18 g) in a dose equivalent to 50 ED_{50}, corresponding to 50 μg protein for soluble pertussis antigen, to $1.5 \cdot 10^9$ *B. pertussis* cells for the bacterial suspension, and the quantity of cytoplasmic membranes corresponding to this number of bacteria. The animals were decapitated in accordance with the "Rules for research with experimental animals." Material for investigation was obtained on the 3rd, 7th, 14th, and 28th days after immunization. Each group consisted of five or six mice, from whose spleens a mixed cell pool was prepared. Animals of the control group received an injection of 0.15 M NaCl solution.

The macrophage migration inhibition test was carried out by the method [6] in the modification developed in the writers' laboratory. The modification consisted essentially of replacing the glass capillary tubes by slit-like chambers cut in a transparent plastic plate. The cells migrated along the floor of the chambers after the plate had been tilted to a certain angle. The cellular component of specific antipertussis immunity was determined by addition of sonicated pertussis antigen to the culture medium. Autoimmune changes were re-

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TABLE 1. Inhibition of Migration of Splenocytes in Mice Immunized with Pertussis Antigen Complexes, in cm (M ± m)

Antigen for immunization	Days after immunization	No. of mice	Antigen		DNA		Dextran sulfate, 100 μg/ml
			sonicated, 100 μg/ml	pertussis, 10 μg/ml	100 μg/ml	10 μg/ml	
Corpuscular vaccine ($1,5 \cdot 10^9$)	3	5	—	2,9±0,2	—	2,1±0,2*	0,2±0,1
	7	6	5,7±0,2	4,7±0,3	2,7±0,6*	1,0±0,2*	0
	14	16	4,4±0,3	2,6±0,3	0,5±0,2	0,3±0,2	0,3±0,2
	28	5	2,0±0,3	2,0±0,3	0,2±0,1	0,3±0,2	0,2±0,1
Membrane preparation (30 μg by weight)	3	6	4,7±0,2	3,4±0,2	0,2±0,1	0	0,2±0,1
	7	5	0,8±0,2	0,5±0,1	0,4±0,2	0,2±0,1	0,2±0,1
	14	5	1,9±0,3	0,8±0,2	0,4±0,2	0,3±0,2	0
	28	6	1,1±0,3	1,2±0,4	0,3±0,2	0,2±0,1	0,1±0,1
Pertussis, 10 μg/ml	3	6	1,1±0,2	1,1±0,2	0	0	0,3±0,2
	7	6	2,1±0,2	1,6±0,3	0,6±0,4	0,4±0,2	0
	14	5	2,5±0,4	1,9±0,3	0,3±0,2	0	0
	28	5	1,5±0,4	0,9±0,2	0,2±0,2	0	0
Control	—	6	0,4±0,2	0,3±0,2	0	0	0,1±0,1

Legend. * Indicates values of delayed-type hypersensitivity to DNA which differ statistically significantly.

corded as the response of the cells to addition of native DNA, obtained from *E. coli* cells and additionally purified with pronase [8], to the culture medium. The final concentration of the test antigenic preparations in the culture medium was 100 and 10 μg/ml. As control for the nonspecific response of the immunocompetent cells to substances of polyanionic type, in each experiment in parallel tests a preparation of dextran sulfate (from Serva, West Germany), with a molecular weight of 500,000 daltons, was used. The results of the test were recorded under the microscrope with a magnification of ×16. Inhibition of migration was expressed as the number of centimeters by which the migration path of the cell layer was reduced in chambers after addition of antigens to the culture medium compared with the control. Student's t-test was used for the statistical analysis of the results.

EXPERIMENTAL RESULTS

The presence of the test antigen preparations in the chosen doses in the culture medium of splenocytes from intact animals, and of dextran sulfate, caused no appreciable inhibition of migration in any of the experiments, or the indices of inhibition varied within the limits of error of the zero value. This suggested that results obtained during culture of cells from immunized mice were an adequate reflection of the immunologic response to the corresponding antigen.

To judge from the values of inhibition of migration in Table 1 the cellular component of specific antipertussis immunity was regularly formed during immunization with both corpuscular pertussis vaccine and purified preparations of bacterial cell components. The highest degree of inhibition of migration was found in the group of animals immunized with whole bacterial cells, with values reaching a peak on the 7th day after injection of the vaccine. Splenocytes of animals immunized with membrane preparations and with dialysate gave a weaker response. However, inhibition of migration in these groups also differed significantly from the control at all times of observation until the 28th day after immunization, irrespective of the dose of sonicated pertussis antigen added to the culture medium. These results, in agreement with those obtained by other workers [3, 4], show that the formation of pertussis immunity is thymus-dependent in character and is accompaied by specific delayed-type hypersensitivity. Different results were obtained when a DNA preparation was used as inducer of secretion of migration-inhibiting factor. Statistically significant differences in inhibition of migration were observed only in the group of mice immunized with whole bacterial cells, on the 3rd and 7th days after inoculation, with extinction of the reaction at later stages of the investigation.

The results thus indicate that *B. pertussis* cells, so far as their action on the T system of immunity is concerned, are a multicomponent and heterogeneous system. Corpuscular vaccine induces not only specific immunity of cellular type, but also the appearance of hyper-

ergic reactions to DNA, a possible indication of the onset of an autoimmune process in the recipient.

Purified preparations of *B. pertussis* and, in particular, the dialysate, retain sufficient substances responsible for changes in specific immunoreactivity with the participation of the cellular components, but they do not induce delayed-type hypersensitivity to DNA, which could be an indication of a developing autoimmune process.

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