CHARACTER OF THE IMMUNE RESPONSE IN RATS AND MICE WITH ADJUVANT DISEASE

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Phasic changes in the immune response were observed in rats and mice with adjuvant disease: stimulation of antibody formation on the seventh day after injection of Freund's complete adjuvant (FCA) and inhibition on the 21st day. Inhibition of production of normal antibodies against O- and Vi-typhoid antigens also was demonstrated.

KEY WORDS: adjuvant disease; immune response; antibodies.

The mechanism of development of adjuvant disease — an experimental model of rheumatoid arthritis — has not yet been explained. It has been suggested that the changes observed in adjuvant polyarthritis are based on an allergic reaction of delayed type due to T-lymphocytes, by means of which it can be transferred [4]. However, according to recently published reports adjuvant disease can be reproduced after elimination of T-lymphocytes [1, 2]. The problem of the changes in the humoral immune response in adjuvant polyarthritis has received less study.

For the reasons given above, in the investigation described below the pattern of antibody formation after injection of various antigens and the level of normal antibodies were studied in animals with adjuvant disease.

EXPERIMENTAL METHOD

Noninbred male albino rats weighing 200-220 g and noninbred male albino mice weighing 14-16 g were used. The Freund's complete adjuvant (FCA) consisted of a culture of BCG (2 mg/ml), inactivated by autoclaving, in an oily medium (1 part lanolin and 30 parts mineral oil), tetanus toxoid of batch 227 obtained from the I. I. Mechnikov Institute of Vaccines and Sera, and tetanus toxin of batch 21, obtained from the N. F. Gamaleya Institute of Epidemiology and Microbiology. The animals received a single intradermal injection of FCA in a dose of 0.1 ml per rat and 0.05 ml per mouse, into the footpads of the hind limbs. The animals were immunized by a single intraperitoneal injection of a 10% suspension of sheep's red blood cells (SRBC) in a dose of 2.5 ml per rat and 0.2 ml per mouse on the 7th, 14th, and 21st days after injection of the FCA. The hemagglutinin titers in the blood serum of the rats were tested on the 7th, 14th, and 21st days after immunization by the usual method. The mice were killed on the 5th day after immunization, a suspension of spleen cells was prepared in medium 199, and the number of antibody-forming cells (AFC) was determined by Jerne and Nordin's local hemolysis in agar method [3]. The mice of group 2 were immunized with tetanus toxoid in a dose of 4 fixation units given as a single injection on the 21st day after the FCA. The intensity of immunity was studied on the 12th day after immunization by intramuscular injection of 0.2 ml of toxin in dilutions of 1:1000, 1:500, and 1:200. The level of normal antibodies and of agglutinins against typhoid O- and Vi-antigens was investigated in the rats of group 2 by the usual method. The severity of the pathological process was estimated from the intensity of the inflammatory changes [5] and the change in the animals' body weight. Student's t-test was used for statistical analysis of the results.

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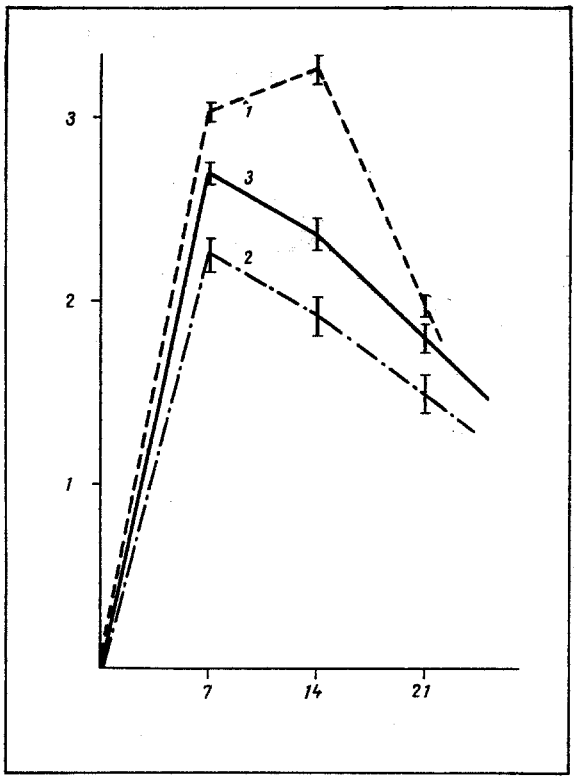

Fig. 1. Changes in hemagglutinin titer of rats with adjuvant disease. Abscissa, time of investigation (in days); ordinate, log of hemagglutinin titer. 1) Immunization on 7th day after injection of FCA; 2) immunization on 21st day after injection of FCA; 3) control.

TABLE 1. Changes in Number of AFC in Mice with Adjuvant Disease

Day of immunization after injection of FCA	Group of animals	Number of animals	Body weight of animals, g	Spleen, mg	Number of AFC in spleen
7th	E	11	15,7±0,39	80,8±5,34	103 520±10 830
			$P<0,001$	$P<0,01$	$P<0,01$
	C	10	18,6±0,54	102,8±5,4	66 712±7 344
14th	E	5	18,8±1,07	109,2±19,9	68 080±18 111
			$P<0,001$	$P>0,05$	$P<0,01$
	C	4	24,5±1,12	157,0±53,9	125 750±21 123
21st	E	6	22±1,8	212,8±37,4	33 450±9 148
			$P<0,001$	$P<0,01$	$P<0,001$
	C	5	28,4±0,85	328,8±60,5	101 600±20 257
	E	11	18,9±0,99	175,8±27,5	3 709±1 177
			$P<0,001$	$P<0,001$	$P<0,001$
	C	9	27,3±2,38	268,2±48,9	17 020±2797,6

Legend. E) Experimental group, C) control.

EXPERIMENTAL RESULTS

All the animals developed the basic feature of the disease (arthritis), which appeared on the 6th-8th day after injection of FCA, in the hind limbs initially, and later in the forelimbs and tail.

The results of investigation of the primary immune response to SRBC in the rats and mice at different periods of development of polyarthritis are shown in Fig. 1 and Table 1. In rats immunized with SRBC on the 7th day after injection of FCA antibody formation was increased compared with the control at all times of observation. In the case of immunization on the 14th day the antibody titers were significantly higher than the controls only on the 7th day of observation. When SRBC were injected on the 21st day the hemagglutinin titers of the experimental animals were lower than the controls. Similar results were obtained when the num-

TABLE 2. Resistance of Mice with Adjuvant Disease to Tetanus Toxin after Immunization with Tetanus Toxoid

Dilution of toxin	Injection of tetanus toxoid on 21st day	Group of animals	Number of animals	Number of animals developing tetanus by 3rd day	Number of dying animals
1:1000 ($9LD_{50}$)	+	F	25	13 $P<0,001$	—
		C′	28	3	—
	—	C″	10	10	10
1:500 ($18LD_{50}$)	+	E	38	22 $P<0,025$	8 $P>0,05$
		C′	40	13	3
	—	C″	15	15	15
1:200 ($45LD_{50}$)	+	E	27	19 $P<0,001$	8 $P>0,05$
		C′	24	5	2
	—	C″	20	20	20

Legend. E) Experimental mice, C') immunized intact mice, C") nonimmunized mice with adjuvant disease.

ber of AFC was studied in the mouse spleen. For instance, in mice immunized with SRBC on the 7th day after injection of FCA the immune response was enhanced (Table 1). The number of AFC was significantly reduced when SRBC were injected on the 14th day after the adjuvant. Inhibition of the immune response was greatest in animals immunized on the 21st day after injection of FCA (Table 1).

The experiments to study antitoxic immunity showed that when tetanus toxoid was injected on the 21st day after FCA the resistance of the experimental mice to the toxin was significantly lower than that of the controls (Table 2). Consequently, after immunization of mice with tetanus toxoid on the 21st day after injection of FCA antitoxin production was inhibited.

The results thus indicate that during the development of adjuvant disease in rats and mice identical phasic changes take place in the immune response: On the 7th day after injection of FCA antibody formation is enhanced, but on the 21st day it is inhibited.

In all animals with adjuvant arthritis a significant fall was observed in the titer of normal antibodies against typhoid O- and Vi-antigens. For instance, the titers of agglutinins against typhoid O-antigen, expressed in $\log_{10}$ units, was 2.2 ± 0.05 on the 7th day after injection of FCA and 2.15 ± 0.027 on the 21st day, whereas in the control animals it was 2.95 ± 0.055 ($P < 0.01$). The titers of agglutinins against typhoid Vi-antigens were 1.04 ± 0.027 on the 7th day after injection of FCA, 1.0 ± 0.027 on the 21st day, and 1.82 ± 0.03 in intact animals ($P < 0.01$).

LITERATURE CITED

1. G. Bolebara et al., Quad. Sclavo Diagn., 9, 636 (1973).
2. J. Hollingworth et al., Proc. Soc. Exp. Biol. (New York), 152, 183 (1976)
3. N. K. Jerne and A. A. Nordin, Science, 140, 405 (1963).
4. C. Pearson and F. Wood, J. Exp. Med., 120, 547 (1964).
5. K. Sacki, K. Wake, and H. Bamasaki, Arch. Int. Pharmacodyn., 222, 132 (1976).