INHIBITION OF MACROPHAGE MIGRATION BY T CELLS AFTER BREAKDOWN OF NATURAL TOLERANCE TO ALPHA-FETOPROTEIN IN RATS

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The method of inhibition of macrophage migration (IMM) was used to study the state of cellular immunity after immunization of rats with cross-reacting mouse alpha-fetoprotein (MAFP). After a single injection of MAFP into the animals, peritoneal exudate cells became capable of inducing the IMM reaction on incubation both with MAFP and also with homologous rat AFP (RAFP). Repeated injection of MAFP weakens the IMM response to both proteins and leads to stimulation of macrophage migration, coupled with a further increase in the titer of antibodies against MAFP and RAFP. Fractionation of peritoneal cells showed that the IMM reaction to MAFP and RAFP is produced by T lymphocytes.
KEY WORDS: alpha-fetoprotein; natural tolerance; inhibition of macrophage migration reaction; antibody formation.

Natural tolerance to alpha-fetoprotein (AFP) in mammals can be overcome by immunizing the animals with heterologous [3, 4, 11] or chemically modified homologous [12] AFP. Immunization of rats with cross-reacting mouse AFP (MAFP) induces the formation of antibodies which react with some of the determination of homologous rat AFP (RAFP) [3, 4]. Antibody production against determinants of homologous protein is evidence that nontolerant B cells are present in the body.

The investigation described below was carried out to discover whether T lymphocytes are also involved in the immune response to homologous AFP.

EXPERIMENTAL METHOD

Peritoneal exudate cells (PEC) of rats immunized with MAFP were investigated by the inhibition of macrophage migration (IMM) test. For many immunological models it has been shown that the IMM reaction is a T-dependent phenomenon – a correlate of hypersensitivity of delayed type (HDT) in vivo [6, 9].

Wistar (males weighing 250-400 g) and August (males weighing 190-220 g) rats were used. The animals were immunized with a purified MAFP preparation by the method described previously [3, 4]. Antibodies were determined by double diffusion in agar [2] with specific test systems for MAFP and RAFP [3, 4].

To obtain PEC, on the day before the experiment the animals were given an intraperitoneal injection of 10 ml of a mixture of peptone (3%) and glycogen (0.25%) in physiological saline; the cells were taken by means of a syringe under anesthesia.

The IMM test was carried out by a modified micromethod [8]. The residue of washed PEC was suspended in the ratio of 1:1 in Hanks' solution with 10% bovine serum and antibiotics. Glass capillary tubes (Behringwerke, West Germany) were filled with them and then cut up into sections 1 cm long and sealed at one end with a mixture of wax and petrolatum (1:3). After centrifugation for 7 min at 650 rpm the capillary tubes were cut below the top level of the cell residue and placed on the floor of wells cut into plates (Falcon Plastics 3040),

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TABLE 1. IMM Reaction for Primary and Secondary Immune Response of Rats to Injection of MAFP

Days after immunization		No. of experiment											
		1		2		3		4		2		3	
		primary reaction to:								secondary reaction to:			
primary	secondary	MAFP	RAFP	MAFP	RAFP	MAFP	RAFP	MAFP	RAFP	MAFP	RAFP	MAFP	RAFP
4	4	—	—	0	0	—	—	—	—	—24 (1)	0	20 (3)	—12 (3)
12	11	—	—	27 (3)	31 (3)	24 (3)	24 (3)	—	—	0	0	20 (2)	0
18	20	82	49	25 (2)	25 (2)	70 (5)	30 (5)	32	25	0	25 (2).	0	—41 (3)
25	28	46	48	38 (2)	21 (3)	28 (2)	12 (1)	—	—	0	—66(3)*	—	—
32	35	34	26	—	—	—	—	—	—	—37 (2)	0	—	—
56	40	27	15	—	—	—	—	—	—	—28 (1)	—28 (3)	—	—
74	68	13	22	—	—	—	—	—	—	35 (2)	22 (2)	—	—
132	—	14	0	—	—	—	—	—	—				

Legend. In experiments Nos. 1 and 4 pooled PEC were used; in Nos. 1 and 2, pooled PEC from three rats in each case; in No. 4 from five rats; in experiments Nos. 2 and 3 PEC were obtained individually from five rats during the primary response and from four and three rats respectively during the secondary response; –) not tested; number of reacting rats shown in parentheses; a minus sign before the number implies index of stimulation of macrophage migration.

*The index of stimulation for one rat was –133%.

resting with their sealed end against the walls of the wells, which were filled with 0.3 ml of Eagle's medium with L-glutamine (2 mM), 15% adult rat serum, and antibiotics. To the medium in the experimental tests 5% neonatal mouse or rat serum was added (the AFP concentration in the samples was about 60 μg/ml), whereas in the control tests 5% adult mouse or rat serum was added correspondingly. After incubation for 18 h at 37°C in an atmosphere with 5% CO_2 the capillary tubes were removed from the wells and the migration zones were drawn on paper with the aid of a projector, cut out, and weighed. The index of inhibition of migration (IIM) was calculated by the equation

$$\text{IIM} = \left[1 - \frac{\text{mean weight of migration zone in experiment}}{\text{mean weight of migration zone in control}}\right] \cdot 100,$$

using four to six parallel capillary tubes for each sample of cells. The significance of the results was determined by Student's t test.

To fractionate the T and B lymphocytes [10], PEC freed from macrophages by adsorption on plastic flasks (Falcon Plastics 3027) were applied to a monolayer of sheep's red cells covered with hemolytic serum, and after incubation for 30 min at 37°C and centrifugation for 5 min at 700 rpm, the "free" T lymphocytes were separated from the adherent B lymphocytes. The appropriateness of this method for separating mouse T and B lymphocytes has recently been verified [1].

EXPERIMENTAL RESULTS

The IMM test was carried out at different times after primary and secondary injection of MAFP and, in parallel experiments, antibodies against MAFP and RAFP in the rat sera were determined. Before immunization IIM of PEC of five rats was 8 ± 2% in the presence of MAFP and 2 ± 5% in the presence of RAFP. The mean values of significant (less than 30%) and highly significant (> 30%) IIM of PEC of immune rats of four experimental groups are given in Table 1. As Table 1 shows, the PEC became reactive to both MAFP alone and also to MAFP on the 12th day after primary injection of RAFP. This reactivity continued, diminishing gradually, until the 74th day, when antibodies against MAFP could still be found in the animals' sera, but antibodies against RAFP were no longer detectable. The strongest reaction was found on the 18th-25th day, when IIM reached 70-82% for incubation with MAFP and 30-49% for incubation with RAFP. Peritoneal exudate cells from individual animals showed reactivity to both antigens simultaneously and also preferential reactivity to one of the two antigens (see experiments No. 2 and No. 3 on the 25th day). The intensity of the IMM reaction correlated with the intensity of the humoral response: On the 18th day after immunization high values of IIM were obtained after incubation both with MAFP (82 and 70%) and with RAFP (49 and 30%) in experiments Nos. 1 and 3 for rats in whom antibodies against RAFP had already been determined, whereas antibodies against MAFP reached a titer of 1:4-1:16. In experiments Nos. 2 and 4, antibodies against RAFP were absent, and against

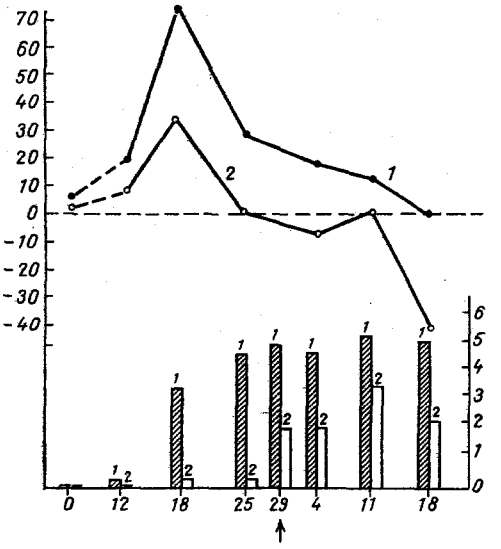

Fig. 1

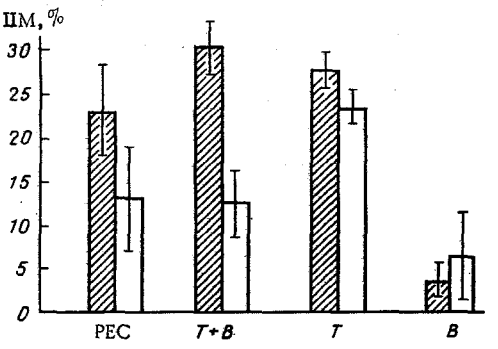

Fig. 2

Fig. 1. Kinetics of IMM reactions and antibody formation after immunization of rats with MAFP: 1) response to MAFP; 2) response to RAFP. Arrow indicates second injection of MAFP. Abscissa, days after injection of MAFP; ordinate: left) IIM (in %), right) $\log_2$ of antibody titer.

Fig. 2. IMM reaction with fractionated immune T and B lymphocytes. Peritoneal exudate lymphocytes mixed with normal PEC in ratio of 1:4. PEC) Original immune peritoneal exudate cells; T + B) mixture of normal PEC with immune lymphocytes, purified from macrophages; T) mixture of normal PEC with T fraction of immune lymphocytes; B) mixture of normal PEC with B fraction of immune lymphocytes. Shaded columns show reaction to MAFP, unshaded columns, reaction to RAFP.

MAFP they were discovered only in the original sera; the values of IIM were correspondingly lower than in experiments Nos. 1 and 3: 32-38% for MAFP and 21-25% for RAFP.

During the secondary immune response the reactivity of the PEC differed from the primary in the following respects (Table 1): 1) at different periods of the secondary response the value of IIM did not exceed 25% and in many cases it was 0; 2) IMM was replaced by a phenomenon of stimulation of macrophage migration, induced by both antigens at different periods of the secondary response; 3) a response of stimulation of macrophage migration developed as a rule to one of the two antigens, whereas this reaction was absent to the other antigen at the same period. This dissociation between the reactivity of PEC to MAFP and RAFP was observed during 40 days of the secondary response, but by the 68th day both antigens induced the IMM reaction. This phenomenon of stimulation of macrophage migration in the presence of specific antigen has been observed more than once [5, 14], but its nature has not yet been studied.

The kinetics of the IMM reactions and of antibody formation was compared (Fig. 1) in three August rats, in which the cellular and humoral responses were most uniform. The reactivity of PEC both to the immunogen and to the homologous protein began to be detectable simultaneously with the appearance of antibodies against MAFP in the animals' sera on the 12th day of the primary response. The maximum of the IMM reaction was observed on the 18th day, but the peak of antibody formation occurred on the 30th day after immunization, when the IMM reaction was much weaker. Repeated injection of MAFP induced an increase in antibody formation both to MAFP and to RAFP, whereas the IMM reaction was inhibited.

Fractionation of the immune lymphocytes obtained on the 26th day of the primary response from three August rats showed that the IMM reaction to MAFP and RAFP is T-dependent (Fig. 2). In the presence of MAFP migration of the normal macrophages mixed with the fraction of immune T lymphocytes was depressed just as it was in the mixture with unfractionated immune lymphocytes (T + B), and by an amount equal to the depression of migration of the original PEC. As regards RAFP, the activity of the T fraction of immune lymphocytes was considerably increased compared with the original PEC. The response of the B fraction of immune lymphocytes did not differ significantly from that of normal cells.

During immunization of rats with cross-reacting MAFP reactivity thus develops to homologous AFP, at least in those T lymphocytes which produce IMM factor. Similar results have recently been obtained with a model of natural tolerance to thyroglobulin in rabbits [13]. It remains unclear whether MAFP and RAFP react with the same or different populations of T lymphocytes, and also what role is played by the T lymphocytes discovered in these experiments, in cooperation between T and B cells during antibody formation against homologous protein. The dissociation observed in this investigation between the secondary response of PEC to the two antigens, and also in the dynamics of IMM and of antibody formation, may indicate a "switching" of differentiation of the two subpopulations of T cells: the effectors of HDT and the helpers [7]. The possibility cannot be ruled out that the appearance of reactivity in rats to RAFP after immunization with MAFP is the result not of the breakdown of natural tolerance to RAFP, but of an increase in the affinity of the receptors of T lymphocytes directed toward MAFP with respect to its common determinant with RAFP. Whatever the case, the development of the reaction of T cells which behave as effectors of HDT to homologous AFP can provide a basis for specific immunotherapy during the growth of AFP-producing tumors.

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