EFFECT OF EXTRACTS OF THE SPLEEN ON THE PRIMARY IMMUNE RESPONSE IN MICE

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UDC 612.017.1-06:612.411.015.21

The possibility of enhancing the primary immune response in sexually mature mice by means of splenic extracts was demonstrated by Jerne's direct test in experiments on mice. The importance of the dose of the extracts and the time of their administration for the manifestation of their stimulating action was studied.

KEY WORDS: immune reaction; spleen; nonspecific stimulators.

It is generally considered that factors influencing lymphocyte proliferation in the immune response are not present in the spleen. Injection of splenic extract does not induce lymphocytosis [5], incorporation of ^{3}H-thymidine into peripheral lymph nodes is not increased [3], and nor is the weight of the spleen, but it improves the chances of survival of irradiated animals [2]. There are few data on the action of splenic extracts on immunity, and such as do exist are evidence mainly of the absence of general hyperplasia of the lymphoid organs.

The object of this investigation was to study the effect of splenic extracts on the primary immune response of syngeneic sexually mature mice.

EXPERIMENTAL METHOD

Experiments were carried out on 285 male CBA mice weighing 18-22 g.

Splenic extracts were prepared as follows. Mouse spleen cells were disintegrated by freezing and thawing three times and then homogenized in physiological saline, pH 7.4. The coarse cell debris was removed

*Deceased.

TABLE 1. Effect of Dose of Splenic Extracts when Injected 24 h after Immunization on Number of PFU (M ± m)

Protein content (in μg)	Number of PFU in spleen (per 10^6 karyocytes)	*P*
100	67,5±6,4	<0,01
50	256,6±15,6	<0,01
25	386,0±28,1	<0,01
13	168,3±31,2	<0,5
Control	136,9±12,0	

Legend. Each dose of splenic extract was tested on 11 animals.

Department for Synthesis and Testing of Labeled Compounds, Research Institute of Medical Radiology, Academy of Medical Sciences of the USSR, Obninsk. (Presented by Academician of the Academy of Medical Sciences of the USSR B. N. Klosovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 82, No. 10, pp. 1228-1230, October, 1976. Original article submitted April 7, 1976.

TABLE 2. Effect of Interval between Immunization and Injection of Splenic Extract on Number of PFU (per 10^6 karyocytes, M ± m)

Injection of extract	Time between immunization and testing, days			
	3	P	4	P
Control	21,2±1,1	—	183,8±16,6	—
Before immunization:				
2 days	62,7±7,3	<0,01	67,0±7,1	<0,01
1 day	24,8±3,2	>0,2	91,3±3,7	<0,01
Simultaneously with immunization	153,8±14,1	<0,01	170,0±12,1	0,5
After immunization:				
1 day	107,9±10,0	<0,01	353,8±30,5	<0,01
2 days	32,5±6,0	>0,1	148,0±10,1	>0,1

Legend. Ten animals were used for each interval.

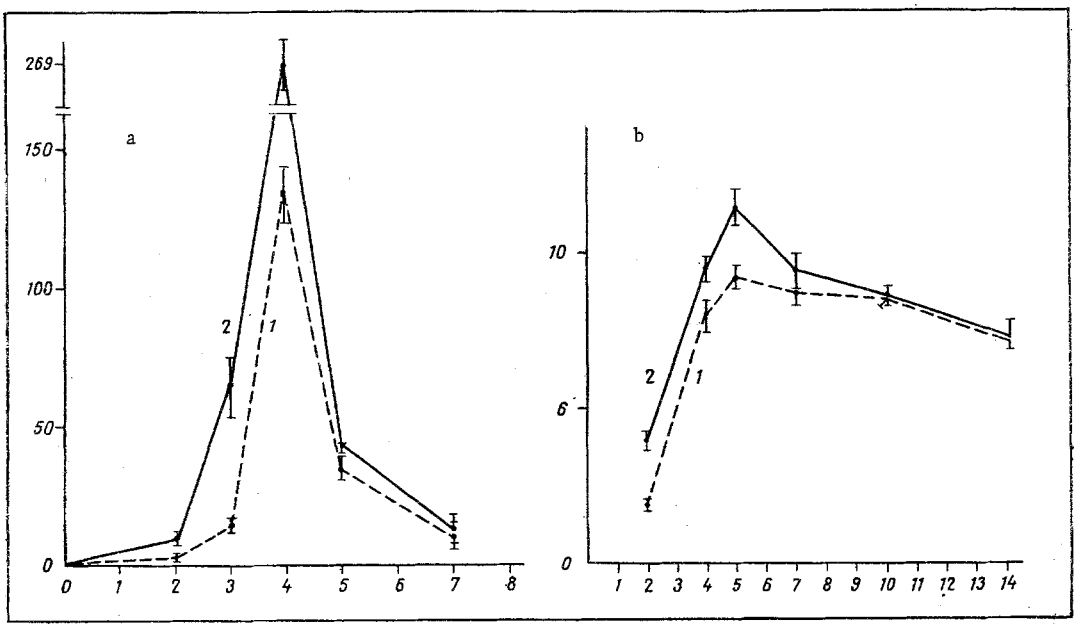

Fig. 1. Dynamics of PFU (a) and antibody titers (b) in spleen of CBA mice after injection of breakdown products of cells followed, 24 h later, by sheep's red cells: 1) control (sheep's red cells); 2) experiment (extract + sheep's red cells). Abscissa, days after immunization; ordinate: a) number of PFU (per 10^6 karyocytes); b) titers of antibodies ($\log_2$) with confidence intervals at P = 0.05.

after centrifugation for 10 min at 40,000g. The supernatant was diluted with physiological saline to a protein concentration (by Lowry's method [4]) of 0.26 mg/ml. The extract was injected intraperitoneally in a dose of 0.1 ml per mouse.

The mice were immunized with sheep's red cells ($7.5 \cdot 10^8$ cells per mouse). The immune response (number of plaque-forming cells – PFU) was estimated by Jerne's direct test [1] and the hemagglutination test (HT).

EXPERIMENTAL RESULTS

In the experiments of series I the effect of dose of splenic extracts on the immune response was studied (Table 1). Splenic extract containing different doses of protein was injected intraperitoneally into the animals of the experimental group 24 h after injection of the antigen. The control mice received a simultaneous injection of physiological saline. The experimental results were assessed on the fourth day after immunization. Injection of the extract in a dose of 100 μg inhibited plaque formation in the spleens of the mice, calculated per million karyocytes. Extract with a protein content of 50 μg increased the number of PFU compared with the control. The action of extract with a protein content of 13 μg did not differ significantly from the control. An

optimal stimulation effect was obtained after injections of extract with a protein content of 25 μg and this extract was used in the subsequent experiments.

To determine the optimal interval between immunization and injection of the extract on the number of PFU, splenic extracts were injected intraperitoneally into the animals 2 days before, at the same time as, or 1 and 2 days after immunization. The number of PFU was determined 3 to 4 days after immunization. As Table 2 shows, true stimulation was exhibited when the splenic extracts were injected 24 h after the antigen. If the mice received the extracts at the same time as the sheep's red cells, only some acceleration of the immune response was observed. With an increase in the interval between injections of the extract and sheep's red cells the effect disappeared. However, injection of extract 2 days before immunization first accelerated the immune response and then depressed it.

The dynamics of the number of PFU in the spleen in the control and in mice receiving extracts 24 h after immunization, when the stimulating effect of the extract was at its greatest, is illustrated in Fig. 1. The number of PFU, calculated per million karyocytes, was three times greater than the control on the second day, five times greater on the third, and twice as great on the fourth day. Only on the fifth day was the difference between the control and experimental animals not statistically significant. The results of determination of the number of PFU in the spleen of the experimental and control mice agreed with the results of the serological investigation (Fig. 1). Stimulation of antibody formation in the mice after injections of the extract was not accompanied by any corresponding increase in the number of nucleated cells.

Splenic extracts thus have a stimulating action on antibody production without causing generalized hyperplasia of lymphoid tissue. The effect of splenic factor depends on dose; stimulation is exhibited within a relatively narrow range of doses (from 25 to 50 μg) and it is determined by the interval between injection of the extract and immunization.

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