EFFECT OF T-ACTIVIN ON ANTIBODY-FORMING CELLS OF THE RAT SPLEEN

I. N. Bol'shakov, L. V. Khoroshikh, V. Ya. Arion, and Yu. M. Lopukhin

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Since the immunocorrective agent T-activin became available, many investigations have been undertaken to study the mechanism of its action on the T system of immunity [1, 2, 5-7, 9, 11, 12].

Investigations have demonstrated the heterogeneity of T-activin as regards both its physicochemical properties [3] and its biological effect [9].

Its effect on the end result of antibody production on involvement of the preparation with the inductive and productive phases of the primary immune response (PIR) is of definite interest. Additionally, activity of the preparation when administered in different ways has not been studied.

The aim of the investigation described below was to study the effect of T-activin, when infused in different ways, on the antibody-forming cells (AFC) of the rat spleen in response to injection of a thymus-dependent antigen, namely sheep's red blood cells (SRBC).

EXPERIMENTAL METHOD

Experiments were carried out on 193 male Wistar rats weighing 200 g. T-activin (batch 119) was obtained from the Laboratory of Molecular Immunology, Research Institute of Physicochemical Medicine, Ministry of Health of the RSFSR. The number of AFC in the spleen was determined by the method of local hemolysis in a liquid medium, as described by Cunningham [10]. The animals were immunized with SRBC in a volume of 0.5 ml of a 50% suspension. The spleen cells were collected and resuspended in one-tenth part of medium 199. Freeze-dried guinea pig serum was used as complement.

T-activin was injected in doses of 0.5 and 1 μg in 0.5 ml of bidistilled water per rat. Endolymphatic (EL) infusion was carried out into lymph nodes of the ileocecal angle of the peritoneal cavity by means of thin silicone-coated catheters 0.25 mm in diameter, after preliminary induction of ether anesthesia for 15 min, followed by laparotomy.

Groups of animals receiving SRBC intramuscularly (IM), intravenously (IV), and intraperitoneally (IP) in a 50% suspension and in a volume of 0.5 ml served as the controls for determination of the efficacy of the EL infusion into the mesenteric lymph nodes in the peritoneal cavity. Groups of animals receiving injections of 0.5 ml of physiological saline by the corresponding routes served as controls for determination of the efficacy of action of T-activin on AFC in the spleen when given by the IV, EL, IP, IM, and subcutaneous (SC) routes. To determine the effect of T-activin on AFC of the spleen in the inductive and productive phases of PIR, T-activin was given 24 and 72 h after IP immunization with SRBC. In order to observe the purity of the experiment, each experimental series was accompanied by a control, in which physiological saline was given instead of T-activin. Injections of the preparation and of 0.9% NaCl solution were carried out under sterile conditions. Since EL infusion of T-activin and of the solvent were carried out under anesthesia, and accompanied by laparotomy, these same manipulations also were undertaken when other modes of administration were used.

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TABLE 1. Number of AFC ($\times 10^6$ cells) in Spleen and Mesenteric Lymph Nodes of Rats in Response to IV, IM, EL, and IP Injection of SRBC

Mode of injection	AFC in spleen	*n*	AFC in lymph nodes	*n*
IP	857±100	4	496±89	4
IV	566±170	3	286±61	4
EL	1015±200	3	685±184	4
IM	665±120*	4	197±31*	4
Total		14		16

Legend. Asterisk indicates significance of difference compared with EL route of administration ($p < 0.05$).

TABLE 2. Number of AFC in Spleen ($\times 10^6$ cells) of Rats in Response to IV, IM, IP, EL, and SC Injection of T-activin in Dose of 1 μg 24 h after Immunization with SRBC

Mode of injection	Number of AFC	*n*	*p*
EL	850±145	10	
IP	474±87*	7	<0,05
IM	462±80*	7	<0,05
SC	315±73*	7	<0,05
	187±10*	7	<0,05

Legend. Asterisk indicates significance of difference compared with EL route of **administration.**

TABLE 3. Stimulating Effect of T-activin on AFC of Rat Spleen when Injected by Different Routes, 24 h after Immunization with SRBC

Anesthesia laparotomy	Dose of T-activin, μ g	Mode of injection	AFC, × 10^6 cells	*n*	Volume of solvent, ml	AFC, × 10^6 cells	*n*	*p*
+	0,5	EL	2217±157	6	0,5	542±55	5	<0,001
—	0,5	IV	1354±369	7	0,5	1303±109	3	>0,05
—	0,5	IM	826±139	6	0,5	844±14	3	>0,05
—	0,5	IP	980±201	5	0,5	991±36	3	>0,05
+	1,0	EL	850±145	10	0,5	542±55	5	>0,05
+	1,0	IV	315±73	7	0,5	205±46	4	>0,05
+	1,0	IM	462±80	7	0,5	844±14	3	<0,05
+	1,0	IP	474±87	7	0,5	901±312	3	>0,05

EXPERIMENTAL RESULTS

Injection of SRBC into rats revealed a tendency toward a higher PIR during EL infusion both in the spleen and in the mesenteric lymph nodes (Table 1).

The high sensitivity of the EL mode of injection of T-activin was confirmed in a series of experiments in which the preparation was given in a dose of 1 μg per rat in the inductive phase of PIR (Table 2).

If the dose of the preparation was reduced by half (0.5 μg per rat) and if the EL method was used, the number of AFC in the spleen was increased by 2.6 times (2217 ± 157 compared with 850 ± 145, $p < 0.001$) respectively. The stimulating effect compared with the control series, in which the solvent was injected by the corresponding methods, was clearly visible only as a result of EL infusion of the preparation. A more than fourfold increase in the number of AFC was found

TABLE 4. Effect of Ether Anesthesia and Laparotomy on AFC in Rat Spleen in Response to IP Injection of SRBC

Anesthesia + laparotomy	AFC, $\times 10^6$ cells	n	p
—	857±100	4	0,05
Anesthesia	507±99*	3	
Anesthesia + laparotomy	1271±512	4	>0,05

Legend. Asterisk indicates significance of differences compared with anesthetized animals.

TABLE 5. Stimulation of AFC in Spleen of Rats by T-activin in Inductive and Productive Phases of PIR

Anestheisa + laparotomy	Dose of T-activin, μg	Mode of injection	Number of AFC in spleen, × 10^6 cells				p
			inductive phase of PIR	n	productive phase of PIR	n	
+	0,5	EL	2217±157	6	837±17	3	<0,001
—	0,5	IV	1354±369	7	1220±80	6	<0,05
—	0,5	IM	826±139	6	575±34	5	>0,05
—	0,5	IP	980±201	5	1831±255	7	<0,05
+	1,0	EL	850±145	10	109±24	7	<0,001
+	1,0	IV	315±73	7	421±138	8	>0,05
+	1,0	IM	462±80	7	141±37	8	<0,01
+	1,0	IP	474±87	7	229±58	6	<0,05

in the spleen compared with the control (solvent). If the preparation was injected in a dose of 1 μg, no stimulating effect appeared compared with the controls (Table 3).

Analysis of Table 3 shows that operative trauma and ether anesthesia are of definite importance (for example, injection of the solvent by the same route during induction of anesthesia, laparotomy, or in their absence).

To study the effect of laparotomy and anesthesia on AFC in the spleen three series of experiments were carried out with IP injection of SRBC only (Table 4).

Thus ether anesthesia for 15 min significantly reduced the number of AFC in the spleen in response to injection of thymus-dependent antigen. Laparotomy did not significantly increase the number of AFC, but created conditions for it to increase. Laparotomy and anesthesia had opposite actions on AFC, and together gave only a very weak effect. For instance, IP and IM infusions of physiological saline given 24 h after immunization with SRBC, in conjunction with ether anesthesia and laparotomy, resulted in a stable number of AFC in the spleen compared with series of experiments conducted without anesthesia and laparotomy (Table 3).

Involvement of EL perfusion of T-activin in a dose of 0.5 μg in the inductive and productive phases of PIR showed that the preparation stimulated AFC in the spleen 2.6 times more effectively 24 h, than 72 h after immunization. Injection of the same dose of the preparation IP, but without mock laparotomy or anesthesia, gave the opposite result: stimulation of AFC was 1.9 times higher 72 h after immunization than in the inductive period of immunogenesis. Injection of T-activin IM or IV, 24 or 72 h after immunization, was not reflected in the number of AFC.

With an increase in the dose of T-activin (1 μg) the same rule was maintained that stimulation of AFC in the inductive phase of the immune response was preserved, especially after EL infusion (Table 5).

Thus analysis of the results showed that T-activin can exert a stimulating action in response to immunization with thymus-dependent antigen, increasing the number of AFC in the spleen, especially if the preparation was given by EL infusion. T-activin exerted the maximal stimulating action on AFC in the spleen in the inductive phase of PIR virtually independently of the mode of its administration.

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