ACTION OF TUFTSIN ON FORMATION OF SUPPRESSOR MACROPHAGES IN VITRO AND IN VIVO

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Since 1970 the endogenous immunostimulator tuftsin (T) has been under intensive study [5]. A report has recently been published that under certain conditions T can also induce immunodepressor processes [3]. The aim of the present investigation was to discover the conditions for initiation of immunosuppressive activity of T.

EXPERIMENTAL METHOD

Experiments were carried out on male CBA/CaLacSto mice and female (C57BLC/6 × BALB/C)F_1-(B6C) mice; chemically synthesized T was generously provided by S. M. Adreev (Institute of Immunology, Ministry of Health of the USSR). Sheep's (SRBC) and human (HRBC) red blood cells were used as the antigen.

In the experiments of series I a suspension of spleen cells from intact mice in a concentration of 3×10^7/ml was incubated in the presence of T (concentration 10^{-4} M) in medium 199 for 15 min at 37°C. The volume of the incubation mixture was 5 ml. The cells were then washed 3 times with medium 199 and injected intravenously in a dose of 10^7 per mouse into syngeneic intact recipients. After 1 h, SRBC in a dose of 5×10^8 were injected intraperitoneally. The number of antibody-forming cells (AFC) was determined 4 days later by Jerne's method [4].

In the experiments of series II mice were immunized intraperitoneally with SRBC in a dose of 5×10^8, and on the 3rd day thereafter the spleens were removed and a cell suspension prepared in medium 199. The suspension was separated on glass into those cells which were adherent and those which were not [1]. Cells were obtained in the same way from intact animals. Adherent cells from sensitized mice were mixed with nonadherent cells from intact mice in the ratio of 1:30 (group 1), and conversely adherent cells from intact mice were mixed with nonadherent cells from immunized mice in the same ratio (group 2). The resulting suspension was incubated with T in a concentration of 10^{-4} M for 15 min at 37°C. The cells were then washed and injected intravenously into syngeneic intact recipients. In the control the cells were incubated in medium without T. Immunization and determination of AFC were carried out just as in the experiments of series I.

To detect possible suppression of the immune response under the influence of T in vivo (series III) mice were immunized intraperitoneally with HRBC in a dose of 5×10^8. Nonadherent spleen cells were isolated 3 days later as described above and injected intravenously into recipient mice in a dose of 10^7 per mouse. After 1 h T was injected intravenously in a dose of 0.3 mg/kg, and 1 h later, SRBC were injected intraperitoneally in a dose of 5×10^8. A group of mice receiving sterile physiological saline instead of T and a group of animals receiving SRBC alone were used in the control. The number of AFC was determined on the 4th day after immunization with SRBC. The results were subjected to statistical analysis by Student's t test. Differences were considered significant at the $p \leqslant 0.05$ level.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that spleen cells treated with T depressed the immune response of the recipient mice significantly (by 75%). In preliminary experiments with injection of lymphocytes isolated from the splenic suspension after incubation with T on a Ficoll—Verografin density gradient, no effect on the immune response could be observed (data not given).

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TABLE 1. Effect of Splenocytes Incubated with T on Immune Response of B6C Recipient Mice (mean values)

Group No.	Experimental conditions	Number of AFC per spleen	Number of NC per spleen, millions
1	Control ($5 \cdot 10^8$ SRBC)	16 218 (13 772—19 098)	210 (188—233)
2	Incubation of cells with T	3 890* (3 698—4 092)	93* (77—119)
3	Incubation of cells without T	16 982 (15 740—18 323)	219 (201—236)

Legend. Here and in Tables 2 and 3 NC denotes nucleated cells; confidence intervals shown in parentheses; *$p \leq 0.05$ compared with control; all groups consisted of 10 animals.

TABLE 2. Effect of Immune and Intact Spleen Cells Treated with T on Immune Response of B6C Recipients (mean values)

Experimental conditions	Number of AFC per spleen	Number of NC per spleen, millions
Control ($5 \cdot 10^8$ SRBC)	10 965 (9 989—12 035)	292 (251—333)
Group 1 + T	4 560* (3 950—5 265)	149* (131—166)
Group 1 + medium	10 965 (10 416—11 542)	253 (224—283)
Group 2 + T	11 749 (10 175—13 566)	240 (219—261)
Group 2 + medium	10 914 (9 976—11 941)	234 (204—265)

TABLE 3. Action of T in Vivo on Immune Response of CBA Recipients Receiving Syngeneic Nonadherent Spleen Cells (mean values)

Group No.	Experimental conditions	Number of AFC per spleen	Number of NC per spleen, millions
1	Control ($5 \cdot 10^8$ SRBC)	144 544 (130 437—160 177)	226 (208—244)
2	T, 03 mg/kg	56 234* (50 746—62 316)	180* (164—194)
3	Physiological saline	125 314 (107 644—145 884)	220 (204—236)

In the experiments of series II (Table 2) significant suppression of the immune response compared with the control was observed only in group 1 + T. Thus the suppressive effect of T is realized only in the case of combined incubation of adherent and nonadherent cells. It is interesting to note that immune macrophages, under similar conditions did not acquire the ability to inhibit the immune response. A suppressor action of T likewise was not found on separate incubation of adherent and nonadherent cells with this preparation (data not given).

The experiments of the final series III (Table 3) showed that intravenous injection of T 1 h after transplantation of syngeneic spleen cells from donors immunized beforehand with HRBC significantly reduced the immune response of the recipients to SRBC.

We know from the literature that lymphoblasts obtained with the aid of conA secrete a factor under whose influence macrophages acquire the properties of suppressor cells [2]. On the basis of that communication and also on the results of the present investigation, it can be

postulated that T can exhibit immunosuppressor activity, potentiating the response of suppressor macrophage formation.

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SELECTIVE ROLE OF THE NUCLEUS MAGNUS RAPHE IN MECHANISMS OF ANALGESIA DURING ELECTRODERMAL NOCICEPTIVE STIMULATION, COLD STRESS, AND ACTION OF MORPHINE

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Analgesia during the action of morphine and of electrical stimulation of various parts of the brain is mediated through the nucleus magnus raphe [5, 13], whose serotoninergic (5-HT) neurons are the source of descending 5-HT pathways which reach the posterior horns of the spinal cord in the funiculus posterolateralis [6]. Division of this funiculus suppresses analgesia following systemic intracerebral injection of morphine, electrical stimulation of the central periaqueductal gray matter, the nucleus magnus raphe, and locus coeruleus, as well as certain types of pain-induced stress [5, 15]. Meanwhile there is evidence that suppression of 5-HT synthesis in the nucleus magnus raphe [1-3, 8] has no significant effect on the formation of antinociceptive responses during auricular electroacupuncture, certain types of unavoidable painful electrical stimulation, electrical stimulation of the central gray matter, and injections of morphine (M) into it. These facts suggest that the role of the nucleus magnus raphe differs in character in different types of analgesia.

It was accordingly decided to compare the role of the nucleus magnus raphe in the mechanisms of different types of analgesia.

EXPERIMENTAL METHOD

Experiments were carried out on 40 male albino rats weighing 200-250 g. Rats of the experimental group were anesthetized with chloral hydrate and a platinum–iridium electrode was inserted at coordinates AP −2.4, VD −6.2, L ± 0.5 mm [9], through which electrical stimulation of the nucleus magnus raphe with a current of 5 mA was applied for 20 sec. Animals of the control group (19 rats) underwent a mock operation without destruction of the nucleus magnus raphe. The experiments were carried out 12-15 days after the operation. Nociceptive sensitivity was estimated by measuring the latent periods (LP) of the tail withdrawal reaction (TWR) before and after the action of the analgesics. Analgesia was induced by intraperitoneal injection of M in a dose of 5 mg/kg, by electrodermal stimulation of the paws (2.5 mA, 60 Hz, 5 min), and by cold stress (CS; swimming in water at a temperature of 4°C for 2.5 min). After the experiments the animals of the experimental group were killed and sections 60 μ thick were cut from pieces of the brain tissue after fixation in neutral formalin, and

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