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STIMULATION AND SUPPRESSION OF CONTACT DERMATITIS IN MICE BY LOW-MOLECULAR-WEIGHT THYMUS HUMORAL FACTOR

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The effect of a low-molecular-weight lymphocytosis-stimulating substance (LSS) from the thymus on the development of contact sensitivity to picryl chloride was investigated in mice. Small doses of LSS were found to potentiate, whereas large doses suppressed this type of delayed hypersensitivity. Contact sensitivity can be transferred passively by means of lymph node and spleen cells isolated on the 6th day after immunization. The experiments showed that mice receiving large doses of LSS contain cells which suppress the passive transfer of contact sensitivity by immune cells. This suppression was absent after treatment of the cells with θ-antiserum and complement. It is concluded that the suppressor cells influence the effector phase of contact sensitivity.

KEY WORDS: *lymphocytosis-stimulating substance from the thymus; contact sensitivity; adoptive immunity; suppressor lymphocytes.*

It was shown previously that the lymphocytosis-stimulating substance (LSS) of the thymus can potentiate or depress reactions of the delayed hypersensitivity type. This action of the preparation depends on the dose used: A small dose of LSS stimulates, whereas a large dose inhibits this type of immunologic response [2, 3]. Since blockade is observed in the first case, and some increase in the strength of antigen-induced suppression of DNA synthesis in the spleen in the second case [3], it has been suggested that the dose-dependent effect of LSS on the development of hypersensitivity of delayed type (HDT) is connected with certain changes in the generation of suppressor T lymphocytes.

The object of this investigation was to examine this problem.

EXPERIMENTAL METHODS

Experiments were carried out on CBA and A/I mice aged 3 months. LSS was isolated from calf thymus by the method described previously [1].

Contact allergic dermatitis, induced in mice by picryl chloride [4], was used as the model of HDT. The mice were sensitized by a single application of 0.1 ml of a 5% solution of picryl chloride in ethanol to the abdominal region. LSS was injected intraperitoneally in a dose of 0.1 or 1 mg per mouse on the day after sensitization, next day, and 5 days after sensitization. Control animals received an injection of physiological saline at the same time. On the 6th day after application of picryl chloride, a 1% solution of the allergen was applied to the ear of some animals and the inflammatory reaction was read 24 h later, paying attention to thickening of the concha auriculae. Its thickness was measured by means of a micrometer with electromechanical transducer.

In most of the mice the provocation skin test was not carried out. They were used in the experiments to study passive transfer of contact sensitivity. The mice were decapitated on the 6th day after sensitization. A cell suspension was prepared from the spleen and lymph nodes (cervical and axillary) in Eagle's medium. A mixture of lymphocytes, in a dose of 40·

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10^6 living cells, was injected into the caudal vein of normal syngeneic recipients of the same age. To test the immunologic potential of the lymphocytes they were mixed with cells capable of inducing a positive allergic reaction. In this case $10 \cdot 10^6$ test cells were used. In some experiments the lymphoid suspension was treated with θ-antiserum and complement. The θ-antiserum was obtained from AKR mice immunized repeatedly with thymocytes from CBA mice. Before use the antiserum was inactivated and adsorbed with red blood cells and bone marrow cells of CBA mice. The antiserum was used in a concentration which caused 100% death of an adequate suspension of thymocytes. In the control, lymphocytes were treated with normal AKR mouse serum. An oily solution of picryl chloride was applied to the recipients' ear 2 h after adoptive cell transfer. The intensity of the dermatitis was assessed 24 h later.

EXPERIMENTAL RESULTS

The results of the study of the action of LSS on the course of contact dermatitis in the A/I mice are given in Table 1. They show that, depending on the dose of LSS used, the animals developed either an increased allergic reaction to picryl chloride or gave hardly any response at all. Similar results were observed in the CBA mice [3].

The passive transfer experiments showed that this effect of different doses of LSS was due to stimulation or inhibition of the specific immunologic activity of the lymphocytes (Table 2), for the same number of lymphocytes from animals of different groups differed in their ability to induce contact dermatitis in normal recipients. The addition of 10 million cells from donors receiving 0.1 mg LSS to lymphocytes of the control animals led to some increase in the response of the recipients to picryl chloride. In other words, in this case the response was directly dependent on the dose of cells injected. When lymphocytes from mice receiving 1 mg LSS were tested, sharp inhibition of the activity of cells isolated from control animals was observed. This depression of the immune response was not found when lymphocytes from intact unsensitized mice were tested.

The suppressor activity of the lymphocytes from animals receiving the large dose of LSS disappeared completely if the cells were first treated with θ-antiserum and complement (Table 3).

The results of these experiments thus indicate that the dose-dependent effect of LSS on the development of contact sensitivity discovered in mice was associated with the presence or absence of cells possessing suppressor activity. Since the suppressive effect of the lymphocytes was abolished after their treatment with θ-antiserum and complement, this suggests that these cells belong to the T suppressor class. The action of suppressor cells extends to the effector phase of the immune response because they block the reaction of lymphocytes already sensitized.

Such suppressor cells are generated also during ordinary immunization [9]. As a rule they appear on the 6th day after sensitization and their activity rises sharply with time.

The biological role of the suppressor cells is considered to be to regulate the intensity of the immune response, i.e., they perform a homeostatic function [6]. From this point of view, the immunologic reaction is the result of interaction between effector and suppressor cells. If effectors predominate, a positive immune response arises. If, however, the suppressor cells are stimulated more strongly, the response is inhibited or indeed tolerance may arise. It is suggested that the same mechanism of regulation is exhibited during the development of contact sensitivity [7].

TABLE 1. Effect of Different Doses of LSS on Allergic Dermatitis Induced by Picryl Chloride in A/I Mice

Experimental conditions	Number of mice	Difference in thickness of concha auriculae before and 24 h after application of picryl chloride, 10^{-3} cm (M±m)	P
Physiological saline (control)	8	14.3±0,33	—
LSS, mg			
0,1	10	17.4±1,0	<0,01
1	10	2,3±0,04	<0,001

TABLE 2. Effect of Different Doses of LSS on Passive Transfer of Contact Dermatitis

Mice	Treatment of mice donating cells used for passive transfer in dose of 40×10^6	Treatment of mice donating cells tested for immunologic potential in dose of 10×10^6	Number of recipients	Thickening of concha auriculae, 10^{-3} cm (M ± m)
A/I	Injection of physiological saline (control)	—	4	9,5±1,8
	LSS, 0.1 mg	—	5	10,9±1,9
	LSS, 1.0 mg	—	4	4.25±0,52
	Control	LSS, 0.1 mg	5	13.8±0,93
	»	LSS, 1.0 mg	6	4,5±1,3
CBA	Injection of physiological saline (control)	—	6	10,0±1,52
	LSS, 0.1 mg	—	4	13,0±2,70
	LSS, 1.0 mg	—	6	2,8±0,20
	Control	LSS 0.1 mg	5	16.2±1,75
	»	LSS, 1.0 mg	5	7,4±0,10
	»	Intact	5	9,8±1,41

TABLE 3. Effect of θ-Antiserum on Suppressor Activity of Lymphocytes from CBA Mice Receiving Large Dose of LSS during Passive Transfer of Contact Dermatitis

Treatment of mice donating cells used for passive transfer in dose of 40×10^6	Treatment of mice donating cells used for suppression of content dermatitis in dose of 10×10^6	Number of mice	Thickening of concha auriculae, 10^{-3} cm M ± m	P
Injection of physiological saline (control)	—	5	11,4±1,4	—
LSS, 1 mg	—	4	4,0±1.3	<0,05
Control	LSS, 1 mg	7	3,6±0,4	<0,001
Control	LSS, 1 mg, treated with θ-antiserum	5	14.2±1,7	>0.05
Control	LSS, 1 mg, treated with normal AKR mouse serum	5	4,4±0,8	<0.05

It can accordingly be postulated that small doses of LSS somehow stimulate the activity of effector cells whereas large doses stimulate suppressor cells. Since the cells responsible for the development of HDT and suppressor cells belong to different populations of T lymphocytes [8], the mechanism of the dose-dependent action of LSS may be different. It has been shown that the behavior of regulatory T cells is determined by the activity of effector cells. If the latter is low, the regulator lymphocytes exhibit a stimulating action and, conversely, if the activity of the effectors is high, they have a suppressor effect on the development of the immune response [5]. There is every reason to suppose that the dose-dependent effect of LSS reflects different degrees of activation of the effector cells only.

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