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The Effect of Interleukin-2 on Rosette Formation and Its Dependence on the Level of Serotonin

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It is well known that the interaction between systems is realized by direct cell contacts and transmitters setting up a two-way stream of information. In the present work serotonin, an immunomodulator with a dose-dependent opposite action, was used as a regulator of physiological system. The large group of regulatory peptide factors called "interleukins" [14,16] has generated interest because of their effects on the immune system. Interleukin-2 (IL-2) was preferred in the present study due to its wide use in therapy, as well as the following considerations: 1) IL-2 is a growth factor for T lymphocytes of various functional orientation and may have a direct effect on quiescent T cells [17], B lymphocytes [4], and monocytes [11]; 2) some of the above-mentioned cells containing serotonin-sensitive structures of the receptive type are controlled by the serotoninergic system [7]; 3) there is a functional relationship between IL-2 and the neuroendocrine system, as is attested by its effect on the activity of pituitary cells [1], on nerve growth factor function [3], on the level of some neurotransmitters [13], and on the expression of the IL-2 receptors in the brain [12]. All these facts enable us to consider the possibility of a relationship between serotonin and IL-2 in the process of immunomodulation.

The aim of the present investigation was to explore the modulation of rosette formation (RF) by IL-2 and the interaction between serotonin and IL-2 at the earliest stage of development of the organism's reaction to antigen. A minimal action with practically no side effects was probably related to the simultaneous administration of the drugs and antigen, to the extremely rapid manifestation of the serotonin [4] and IL-2 effects, and to the use of doses, substantially lower than accepted. The models used simulate likely physiological situations and permit assessment of the time required for the engagement of non-specific regulators in the modulation of a specific immune process.

MATERIALS AND METHODS

Experiments were carried out on 320 CBA mice (no less than 7 animals in one group) 3-4.5 months old. Sheep erythrocytes (SE) were used as antigen in doses of 5×10^6 or 5×10^7 per mouse. The reaction to the antigen was assessed on the 5th day after immunization. For this purpose a

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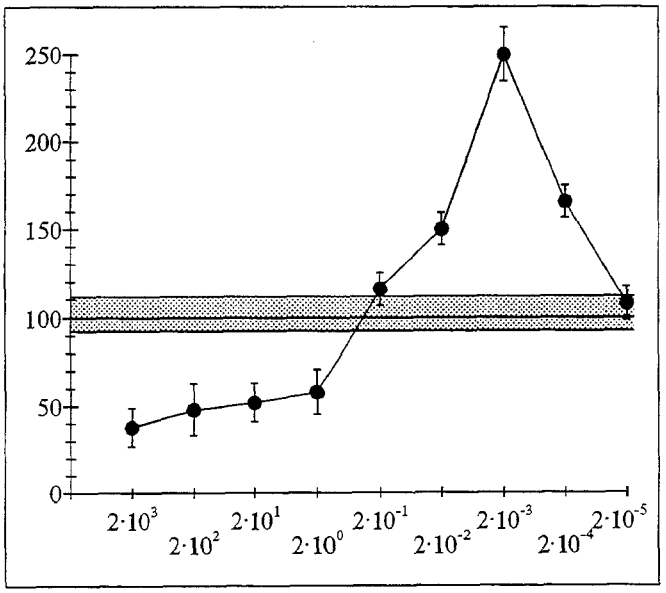

Fig. 1 "Dose–response" curve for administration of human recombinant IL–2 in mice. Results are presented in % vis–a–vis mice treated with SE only. IL–2 was injected once i.p. together with SE in the indicated doses (IU/mouse).

suspension of spleen cells was incubated in thermostat at 37°C together with an equal volume of 3% SE suspension (15-20 per cell) for 15 min, and, after vigourous stirring with a Pasteur pipette a mobile preparation (a coverglass resting on a ring of mineral oil with the suspension inside) was prepared. The number of rosette-forming cells (RFC) per 1000 cells viewed was counted using phase-contrast optics (×1000). The reliability of SE sorption was monitored according to the mobility of the preparation. Sulfuric serotonin-creatinine (Reanal, Hungary) and recombinant human IL-2 (Institute of Organic Synthesis, Latvian Academy of Sciences) were used in the experiments. All drugs were injected i.p. The injected components were mixed individually for each animal. The data were processed statistically using the Student *t* test. The figures present arithmetic means with the confidence intervals.

RESULTS

Since the IL-2 receptors express different affinities [2,10], a "dose-response" experiment was performed first. It was found that the level of IL-2 at the moment of organism-antigen contact affects the development of the subsequent reaction. Like serotonin [5], IL-2 in high doses inhibited and in low doses stimulated RF (Fig. 1); in other words, IL-2 displayed itself as a bimodal immunomodulator. In general, the "dose-response" curve obtained was the same as in a case where the opposite action is realized by receptors with different affinities to the ligand.

The mechanism of the demonstrated modulation is not clear due to the polyfunctionality of IL-2, to the heterogeneity of its receptors not only in their affinity, but also within a broad spectrum of IL-2 receptors with the same affinity [8], and to the different sensitivities of the IL-2 target cells. The IL-2 effect may be related to a direct action on different types of cells, including cells which change the level of subsequent RF through interaction with the modulator. Moreover, IL-2 can interact both with the already existing and with just induced receptors [9,15]. The latter is possible due to the direct regulation by IL-2 of the transcription and expression of its own receptors and, therefore, of the corresponding IL-2-dependent processes. Another mechanism could be a short-term expression of IL-2 receptors induced by the antigen and depending on the presence of IL-2. As will be snown below, the second mechanism is more likely under the given experimental conditions.

Since serotonin and IL-2 are transmitters of different but functionally conjugated systems and act similarly, one would expect summated or synergistic effects for their combined use in doses producing a single-direction result. For the appropriate experiments IL-2 doses were chosen from those depicted in Fig.1, while the serotonin doses were determined by the previously obtained "dose-response" curve [5] in order to achieve the maximal opposite effects. The stimulatory dose was 1.5 μg/kg and the suppressive dose was 50 mg/kg. It was revealed that these transmitters, when used together in the optimal suppressive doses, produce not the usual but the opposite effect (Fig. 2, *1*). Treatment with the same dose of serotonin and a

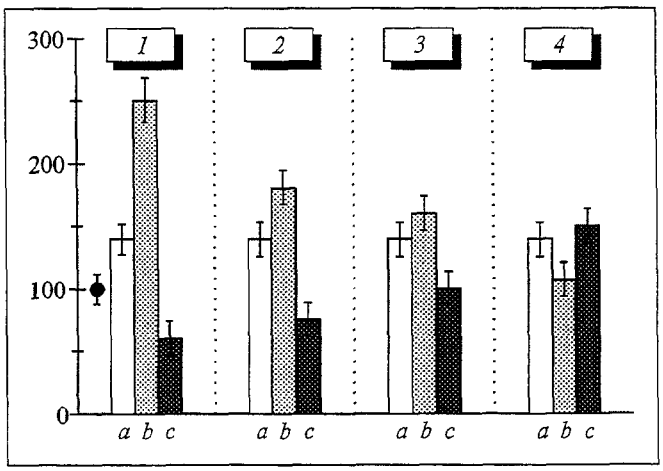

Fig. 2 Rosette formation in mice, treated with SE only (small black circle refers to control, 100%), SE+serotonin in a dose of 50 mg/kg (*a*), SE+IL–2 (*b*) in doses of 2×10^3 IU/mouse (*1*), 2×10^2 IU (*2*), 20 IU (*3*), and 2 IU (*4*); SE+serotonin (50 mg/kg)+IL–2 (*c*) in doses of 2×10^3 IU/mouse (*1*), 2×10^2 IU (*2*), 20 IU (*3*), or 2 IU (*4*).

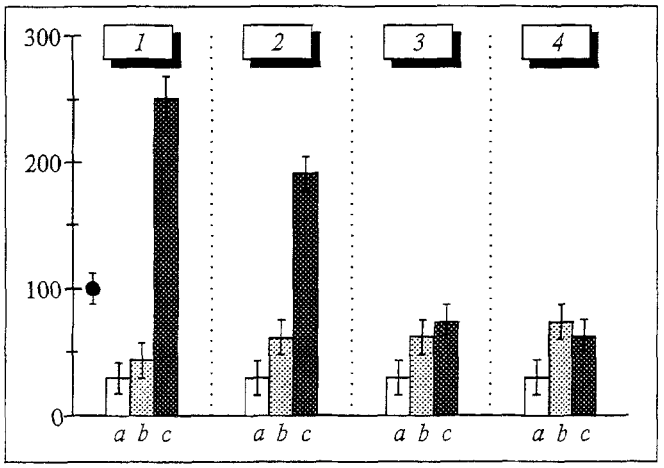

Fig. 3 Rosette formation in mice treated with SE only (small black circle refers to control, 100%), SE+serotonin in a dose of 15 μg/kg (*a*), SE+IL−2 (*b*) in doses of 2×10^{-3} IU/mouse (*1*), 10^{-3} IU (*2*), 2×10^{-4} IU (*3*), and 2×10^{-5} IU (*4*); SE+serotonin (1.5 μg/kg)+IL−2 (*c*) in doses of 2×10^{-3} UI/mouse (*1*), 10^{-3} IU (*2*), 2×10^{-4} IU (*3*), or 2×10^{-5} IU (*4*).

tenfold reduced dose of IL-2 resulted in a change of the direction of the effect, but the degree of this effect was less pronounced. A further decrease of the IL-2 doses gave rise to a predominance of the inhibitory effects of serotonin (Fig. 2, *2-4*). Combined administration of the transmitters in the optimal stimulatory doses also led not to a summation or synergism of their effects, but rather to a switch to the opposite effect (Fig. 3, *1*). As in the preceding experiment, a decrease of the IL-2 doses (with the same dose of serotonin) resulted initially in a decay of the combined effect, and then in a disappearance of the latter and in the predominance of the action of the optimal stimulatory dose of serotonin (Fig. 3, *2-4*).

Thus, IL-2 and serotonin can not only modulate similarly the same antigen-induced reaction, but also enter into interaction, which, given a specific balance of these modulators, results in fundamental reorganizations in the modulatory process.

To avoid the possibility of interaction among IL-2, serotonin, and SE in the mixture the following experiment was performed before treatment. We checked to see if there were any differences between the concurrent administration of the components with three syringes and the administration of a rapidly prepared "cocktail", as described above. Just as in the previous experiment, the stimulation induced by the drugs injected singly switched to inhibition the degree of which was the same for combined injection with three syringes and with the cocktail (Fig. 4, *1-4*). Therefore, in further experiments the cocktail was used.

The next experimental series was performed to reveal if the order of the signals coming from the antigen, IL-2, and serotonin is important for the manifestation of the obtained regularities. The components were injected with two syringes in a different order two times with an interval of 2 sec (the needles were inserted simultaneously). The drugs were used in the optimal low doses (stimulatory). The findings attested to the following: 1) IL-2 injected immediately after the SE+serotonin mixture resulted successfully in a suppressive effect (Fig. 4, *5*, *6*), which appeared for the synchronous action of both modulators. 2) In contrast to this serotonin injection immediately after the SE+IL-2 mixture failed to cause an inhibitory effect, and the stimulation typically induced by IL-2 in low doses appeared owing to a 2-sec lead of its signal in relation to that of serotonin. Thus, the preeminence of the transmitter of the immune system was probably due to blocking of the immunocyte capacity to react to the transmitter of another system. 3) When the IL-2+serotonin mixture was administered after SE there was no modulatory effect at all (Fig. 4, *8*). It may be concluded that sertain events which determine the course of the immune process of interest take place within a period lasting less than 2 sec from the entry of the antigen in the organism. Hence, for the exchange of information (in our case) by means of intersystemic messengers a very short period of interaction is sufficient. It is evident that the 2-

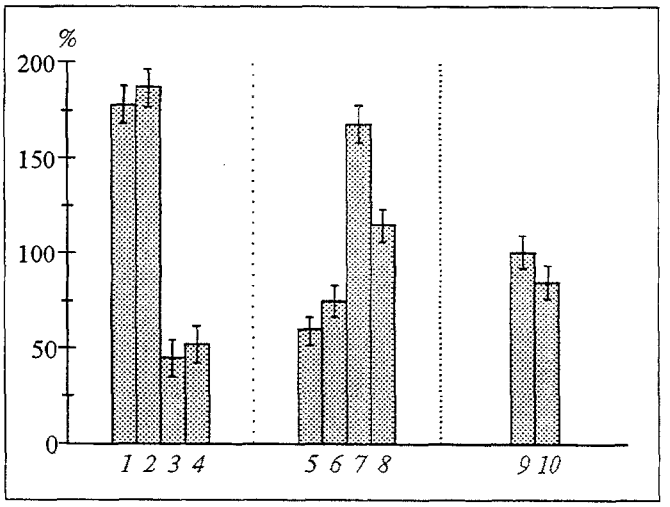

Fig. 4. Influence of the sequence of combined administration of serotonin and IL−2 in low doses (1.5 μg/kg and 2×10^{-3} IU/mouse) on their effect. First experiment: mice, treated with SE only (small black circle refers to control, 100%), SE+serotonin (*1*), SE+IL−2 (*2*), SE+serotonin+IL−2 synchronously with 3 syringes (*3*), and the same with 1 syringe (*4*); second experiment: mice treated with SE+serotonin+IL−2 using 3 syringes (*5*), SE+serotonin and IL−2 2 sec later (*6*), SE+IL−2 and serotonin 2 sec later (*7*), and SE and serotonin+IL−2 2 sec later (*8*). Control groups (small black circles): treated with SE (*9*), or with SE and saline 2 sec later (the injections were synchronous) (*10*).

sec interval is not the limit for IL-2, because its action on the cell membrane is noted in a split second. The same is also true for serotonin. The effect of the sequence of administration perhaps relates to a different rate of activation of the corresponding systems by the biologically active substances under investigation. The findings attest that there are common mechanisms in the regulation of the immune system by serotonin and IL-2, because the succeeding immune response may be radically changed not only due to the concentrations of each of the modulators, but also owing to their balance at the moment of perception of the antigen information. It is to be noted that some failures in therapy are probably related to the discovered effect of the combined action of serotonin and IL-2 when their concentrations attain the corresponding ratio due to pathological processes and drug therapy.

Since the events occurring in the first moments after immunization are not clearly understood, the findings cannot be completely explained. But the importance of this period is obvious.

Thus, IL-2 is a bimodal immunomodulator with a substantial activity (even in very low doses) when it is present in a specific concentration at the moment of reception of antigen information. The revealed functional conjugation between IL-2 and serotonin allows us to assume that IL-2, just like serotonin, may be an intersystemic messenger mediating the interaction between the immune and serotoninergic systems.

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