Interleukin-1-Glucocorticoid Hormone Interrelationship in the Regulation of the Immune Response

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Interleukin-1 (IL-1) is the main endogenous transmitter of both the acute-phase reaction and the immune response [8]. *In vivo* administration of IL-1 leads to the stimulation of nonspecific protective reactions and to the activation of immunocompetent cells. At the same time, an important component of the complex biological effect of IL-1 is its action upon the neuroendocrine system, resulting specifically in a rise of the glucocorticoid hormones in the blood plasma [3,6]. High concentrations of glucocorticoids are capable, in turn, of depressing immune reactions [10]. This is may be the pathway by which the immune response is regulated by negative feedback; however, the mechanism of its realization remains unclear.

This paper is a study of the IL-1-glucocorticoid interaction in the regulation of the immune response *in vitro* and *in vivo*.

MATERIALS AND METHODS

Purified human recombinant IL-1 was used in this study. The activity of the preparation evaluated in the test of costimulation of mouse thymocyte proliferation, was 10^8 IU/mg protein [1,9]. Heat-inactivated recombinant IL-1β (90°C for 1 h) was used as a control preparation in *in vivo* experiments. Heat-inactivated IL-1β lacked costimulating mitogenic activity in the cell culture.

CBA mice received in the morning a single intraperitoneal injection of IL-1β or hydrocortisone suspension (Gedeon Richter, Hungary). At various time after injection the animals were sacrificed by cervical dislocation, and peripheral blood, thymus, and spleen were taken. The serum was aspirated after blood clotting, centrifuged at 7000g, and stored at -20°C.

The serum concentration of corticosterone was measured by radioimmunoassay. Isolated thymocytes and spleen cells were cultured for 18 h in order to estimate the spontaneous proliferation or for 72 h in the concanavalin A (Con A)-induced proliferation assay, in 96-well microplates (Flow) in the presence of 5% CO_2, as described earlier [2]. The production of IL-2 by the spleen cells stimulated with Con A for 36 h, was evaluated using the IL-2-dependent CTLL-2 cell line [2]. The proliferation level was assessed by measuring ^{3}H-thymidine incorporation, for which purpose the cells were transferred to glass fiber filters (Flow) with a cell harvester (Titertech) and the radioactivity was measured in a scintillation β-counter.

In the course of immunization, mice were challenged intravenously with 2×10^7 sheep erythrocytes (SE). IL-1β was injected simultaneously with SE. On

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TABLE 1. Effect of Recombinant IL-1β and Hydrocortisone on the Proliferation Level (cpm/min) of Con A (0,5 mg/ml)-Stimulated Mouse Thymocytes ($M \pm m$)

IL-1β concentration (IU/ml)	Hydrocortisone concentration (μg/kg)			
	0	0.1	1	10
100	30015±1722*	14509±973*	5864±328*	2327±183
10	25536±2237*	12293±1031*	4143±451*	1408±117
1	17386±1498*	10722±720*	3530±286*	1807±212
0.1	9310±726	5693±739	2301±137	1934±126
Control	7342±692	4179±410	2109±176	1928±104

Note: Here and below an asterisk indicates values differing reliably from the control ($p<0.05$).

the 5th day the titer of SE-hemagglutinating antibodies in the serum and the number of antibody-producing cells (APC) in the spleen were evaluated [7].

RESULTS

The addition of hydrocortisone to the mitogenic lectin-stimulated lymphocytes *in vitro* drastically inhibited proliferation. IL-1β abrogated the glucocorticoid-induced inhibition (Table 1). The recombinant IL-1β preparation under study proved to stimulate T- and B-lymphocyte proliferation andto increase IL-2 production and IL-2 receptor expression [9], whereas glucocorticoids inhibit lymphocyte proliferation precisely due to the reduced production of specific growth factors (such as IL-2, etc.) by the cells. This is probasbly the mechanism of the IL-1 and glucocorticoid competitive interaction in their influence on lymphocyte proliferation *in vitro*. As may be judged from the results presented, IL-1 and glucocorticoids behave as antagonists in the regulation of lymphocyte proliferation *in vitro*.

On the other hand, two hours following recombinant IL-1β injection (10 μg/kg) injection into the mice, the serum corticosterone level rose in a dose-dependent manner, the maximum being reached with an IL-1β dose of 100 μg/kg. The detailed time-course analysis revealed that the serum corticosterone level rose significantly just 20 min after injection of 10 mg/kg IL-1β, reached the maximum one hour later, and slowly dropped thereafter. On the second day after IL-1β challenge no increase of the corticosterone level could be registered in the mouse sera. Heat-inactivated recombinant IL-1β failed to induce corticosterone secretion.

TABLE 2. IL-1β- and Hydrocortisone-Induced Changes in Mouse Thymus: Spontaneous Cell Proliferation *in Vitro* and Cell Count of Thymus from Mice Having Received Either or Both Preparations 24 Hours Previously ($M \pm m$)

Dose of agent	Number of cells per thymus, $\times 10^{-6}$	Level of spontaneous proliferation, cpm $\times 10^{-3}$
IL-1β,		
100 μg/kg	11±2*	4654±307*
10 μg/kg	19±2*	10184±683*
1 μg/kg	42±4	16159±1128
0.1 μg/kg	48±3	19273±1075
Hydrocortisone, 1 mg/kg	6±1*	2983±207*
Hydrocortisone + IL-1β, (10 μg/kg)	5±1*	1526±123*
Control	51±6	18970±1028

IL-1 challenge induced considerable alterations in the thymus. Twenty-four hours after a single injection of IL-1, a significant decrease in the thymus cell count was observed, as well as a reduced level of spontaneous proliferation of thymocytes *in vitro*. These indexes resembled those obtained in the hydrocortisone-treated animals. The combined administration of both agents resulted in an additive effect (Table 2). The observed changes persisted for 2 to 3 days following IL-1 injection; complete thymus repopulation was accomplished only on the 5th-7th day.

This biological action of IL-1 is apparently mediated via the induction of endogenous corticosterone synthesis and is related to the high level of proliferative activity in the thymus. Glucocorticoids drastically diminish the proliferating immature thymocyte population due to their marked antiproliferative activity and as a result lead to thymus depletion. However, unlike glucocorticoids, which induce accelerated lymphocyte death in the thymus, IL-1 can prevent apoptosis development in thymocytes [11]. Therefore, despite its corticosterone-inducing properties, IL-1 may promote increased longevity of the T-lymphocytes and contribute to their recruitment for carrying out protective functions.

Analysis of the influence of IL-1 and hydrocortisone on mature lymphocytes of mouse spleen revealed no changes similar to those in the thymus. Neither hydrocortisone nor IL-1 treatment affected the spleen cell count. On the contrary, mice having received IL-1 manifested spleen lymphocyte activation and stimulation of the immune response. Twenty-four hours after a single IL-1β injection the polyclonal mitogen-induced proliferative response and IL-2 production by the spleen lymphocytes were enhanced (Ta-

ble 3). As is shown in Table 4, IL-1 administration simultaneously with the antigen (SE) markedly increased the number of APC per spleen and raised the titer of serum hemagglutinin antibodies. These effects of IL-1 were dose-dependent and were registered precisely in the dose range which caused a marked rise of the serum corticosterone level.

The immunosuppressive properties of glucocorticoid hormones are well established [10]. One might assume that the IL-1-induced corticosterone could act as a negative regulator of the immune response, since the serum corticosterone level of 800 ng/ml achieved bytreatment with high doses of IL-1 is similar to the hormone concentrations sufficient to effectively inhibit lymphocyte proliferation *in vitro* (see Table 1). However, this is not what takes place

TABLE 3. Recombinant IL-1β-Induced Changes in Mouse Spleen Cells: Con A-Induced Proliferation and IL-2 Production *in Vitro* by Spleen Cells from Mice Having Received IL-1 24 Hours Previously ($M \pm m$)

IL-1β dose (μg/kg)	Proliferation level, cpm	IL-2 output, IU/ml
100	35119±2843*	34.2±4.3*
10	30421±2970*	27.4±3.8*
1	21183±1562*	16.7±1.1*
0.1	14274±1239*	6.8±2.9
Control	9084±2647	3.8±1.3

in vivo. Despite the induction of endogenous corticosterone and the inhibition of thymic T-lymphocyte proliferation, IL-1 used in the very same doses causes the activation of mature spleen lymphocytes and enhances the immune response, that is, the response to T-dependentantigen (SE).

It should be pointed out that a single injection of IL-1 in a dose range up to 10 mg/kg induced no signs of acute toxicity. The sera of treated mice did not contain detectable amounts of tumor necrosis factor (data not presented), the appearance of which serves as evidence of the development of high-dose IL-1-induced toxic shock [12]. Evidently, both the induction of corticosterone and immunostimulation are normal manifestations of the biological effect of IL-1 administered within the physiological dose range. The IL-1-induced corticosterone rise is transient; it accompanies the development of acute-phase reactions but does not interfere with the capacity of IL-1 to activate the immunocompetent cells.

The IL-1-caused sharp rise of the corticosterone level which occurs during the development of defense

TABLE 4. Effect of Recombinant IL-1β on the Number of Antibody-Producing Cells and Level of Antibody in Mice Immunized with SE

IL-1 dose (μg/kg)	Number of APC per 10^6 spleen cells	Titer of hemagglutinating antibodies ($-\log_2$)
100	428±3*	10.7±0.3*
10	241±11*	10±0
1	238±8*	10±0
0.1	214±5	10±0
0.01	205±13	9.3±0.3
Control	197±12	9.7±0.3

reactions is probably more necessary for the limitation of further endogenous IL-1 synthesis before toxic concentrations are reached than for the realization of a direct immunosuppressive effect. For instance, it has been shown that adrenalectomized mice are more sensitive to the toxic effect of IL-1, and, conversely, lipopolysaccharide- and/or IL-1-induced lethality can be overcome by treatment with glucocorticoid hormones [5]. Moreover, the slight increase of the glucocorticoid level observed for several hours following the phase of a markedly increased serum concentration may have an immunostimulatory effect, as low concentrations of glucocorticoids have been shown to induce IL-1 receptor expression by lymphocytes [4].

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