EXPERIMENTAL GENETICS

Increased Interleukin-2 Production by Human Peripheral Blood Lymphocytes Treated with Low Doses of Dexamethasone

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Glucocorticoids (GC) are physiological regulators of immunological functions. Hormone-activated GC receptors alter the transcription of many genes, resulting in modified immune responses. The direction in which GC act depends on their blood levels. Severe stress or moderate stress-inducing exercise, during which blood levels of GC rise, produce an immunodeficiency state or elicit a transient immunosuppressive reaction, with a drop in NK-cell activity [3,12] and the CD4/CD8 ratio [6], in levels of secretory IgA in the saliva [12], and in the intensity of *in vitro* proliferative responses to T- and B-cell mitogens and antigens [5]; nonspecific immunity is also depressed [9]. The suppression of cell proliferation correlates with elevated blood levels of cortisol [5]. GC have been shown to inhibit the synthesis of mRNA for interleukin-2 [2], interleukin-1 [8], and interleukin-3 [4] and to decrease the stability of mRNA for interleukin-1β [8] and interfere with the expression of the interleukin-6 gene by accessory cells [17]. In contrast, short-term stress factors (e.g., moderate sports activity) increase the body's immunological resistance [6].

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GC are therefore true immunomodulators that can depress as well as enhance immune reactions. Such properties of GC were also observed in the present study of dexamethasone (Dx) for its effects on concanavalin A (ConA)-induced interleukin-2 (IL-2) production by human peripheral blood lymphocytes (PBL).

MATERIALS AND METHODS

Mononuclear cells were isolated from the peripheral blood of healthy subjects by Ficoll-Verografin gradient centrifugation. The cells were washed twice in phosphate-buffered saline and resuspended in RPMI-1640 medium (Flow Laboratories, UK) supplemented with 20 μg/ml gentamicin, 2 mmol/liter HEPES, and 2 mmol/liter L-glutamine.

To obtain IL-2-containing supernatants, PBL were resuspended in serum culture medium at a concentration of 10^6 cells/ml (1.5 ml of cell suspension per well of a 24-well plate (Nunc, Denmark)), incubated in the presence of 20 μg/ml ConA (Calbiochem, USA) with or without added Dx for 4 h at 37°C in a humidified atmosphere containing 5% CO_2, and then washed twice in the same plate with serum-free medium and resuspended again in serum-free medium and incubated

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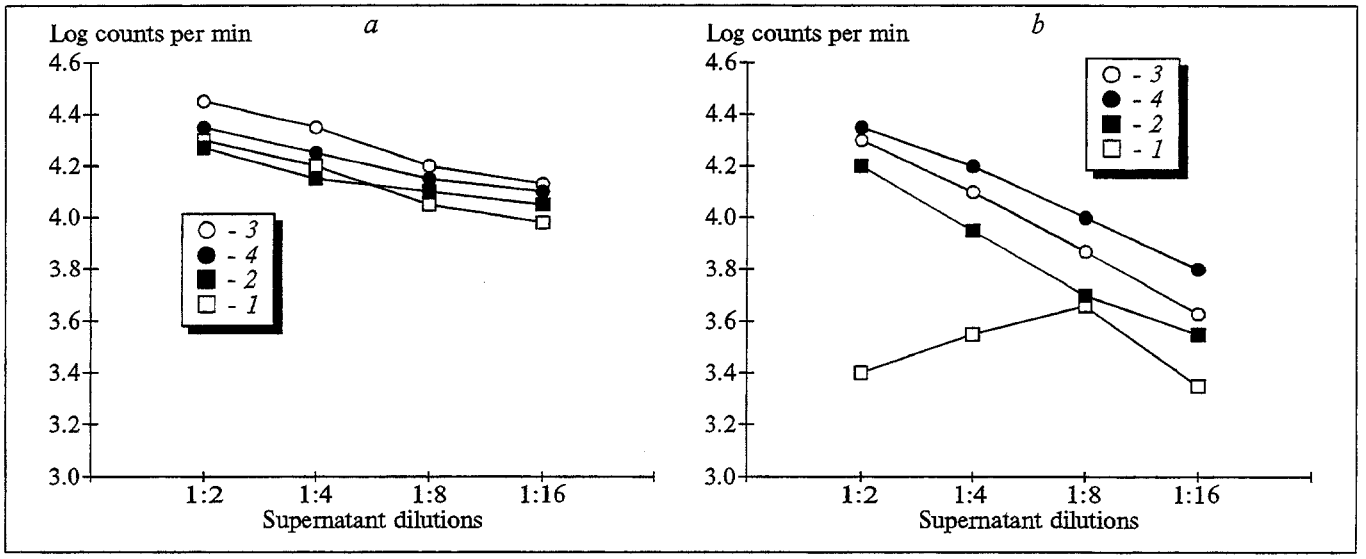

Fig. 1. IL-2 production by ConA-stimulated PBL incubated without (*1*) or with Dx in different doses (mol/liter): 10^{-9} (*2*), 10^{-10} (*3*), or 10^{-11} (*4*). Cells were incubated in culture medium containing 2% AB serum (*a*) or in serum-free culture medium (*b*).

for 18-20 h as indicated above. Supernatants were collected and stored at -20°C until use.

IL-2 activity in the supernatants was assayed by their ability to maintain the growth of an IL-2-dependent cytotoxic T-cell line (CTLL-2). Their serial 2-fold dilutions in serum-free medium (1:2 to 1:8) were added in a volume of 100 μl to triplicate wells of a 96-well flat-bottomed plate (Nunc, Denmark) containing 4×10^3 cells in 100 μl of the medium supplemented with 20% fetal calf serum (Flow Laboratories, UK) and 2.8×10^{-5} mol/liter 2-mercaptoethanol, so that the final serum concentration in each well was 10%. Triplicate control cell wells contained 100 μl of culture medium instead of the diluted samples. As the standard, a recombinant IL-2 (Amersham, UK) was used. After a 20-h incubation at 37°C in the presence of 5% CO_2, all cultures were pulsed with 40 kBq of ^{3}H-thymidine per well. Four hours later cells were harvested on fiberglass filters, and ^{3}H-thymidine incorporation was measured in a Mark III liquid scintillation counter (Tracor Analytic, USA). The titration curves of the test and control samples were analyzed by a probit method [1,7]. The IL-2 content in the supernatants was expressed in international units (IU) or as a percentage of the control.

RESULTS

The influence of pulsing with Dx on IL-2 production was examined in a wide dose range (10^{-5} to 10^{-11} mol/liter). Serum-free culture medium was used to rule out the possible impact of serum factors and of the additional quantities of GC contained in the serum. Its use demonstrated the stimulating effect of low Dx doses more clearly, although less IL-2 was released in response to ConA than in the case of medium containing 2% human AB serum (Fig. 1).

PBL from a total of ten healthy donors were tested. The enhancing effect on IL-2 release was strongly marked with PBL from seven of them (Fig. 2) and much less so with PBL from two others (Fig. 2, graphs *4* and *7*). In one case no stimulation was noted at all (Fig. 2, graph *10*). The use of Fisher's exact test led us to reject the hypothesis of increased IL-2 release *in vitro* by human PBL treated with low Dx doses, even if the two cases of an ill-defined enhancing effect were considered to be negative too ($p<0.0045$).

It should be mentioned that ConA-stimulated PBL failed to proliferate in the serum-free medium under the experimental conditions used, even in the presence of Dx doses that stimulated IL-2 production.

Our results indicate that the effect of Dx on IL-2 production could vary from marked enhancement to complete suppression, depending on the dose. Also, the same dose could elicit different effects for different individuals. A switch from stimulation to suppression of IL-2 production by Dx was usually observed in the dose range of 10^{-9} to 10^{-8} M.

Although there is no doubt that IL-2 production can be stimulated by low Dx doses, the curve profiles should also be considered, for it is not clear why IL-2 production by PBL of some donors was again suppressed by Dx in a lower concentration, while its higher concentration was stimulatory (Fig. 2, graphs *3*, *4*, and *5*). In light

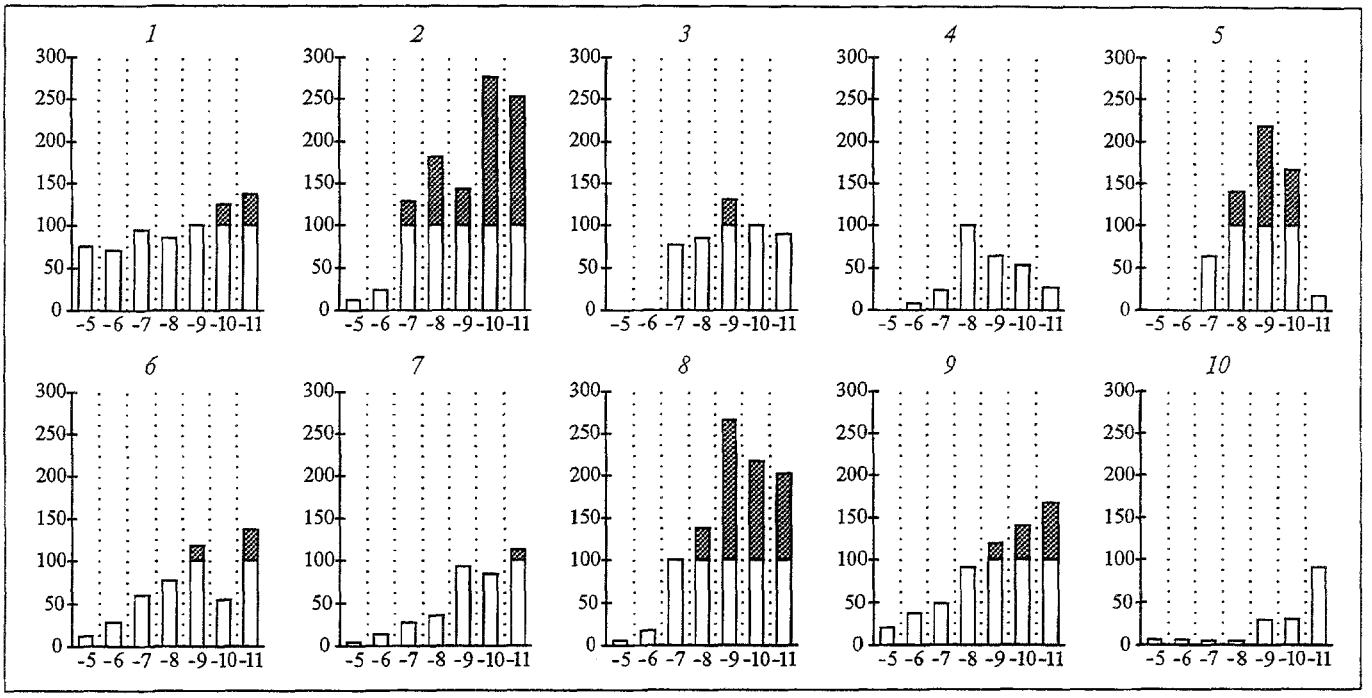

Fig. 2. IL−2 production by ConA−stimulated PBL from different subjects after incubation with Dx. Abscissa, log Dx doses; ordinate, % of control values as calculated by the formula: (IL−2 level without Dx/IL−2 level with Dx)×100%

of what is currently known about the impact of GC on lymphoid cells *in vitro*, it seems plausible that the direct effect of Dx on IL-2 gene expression can be modified by an indirect dose-dependent influence of GC-induced proteins; thus, the observed effects may be attributable to GC-induced modification of lipocortin synthesis [15]. Increased lipocortin synthesis is known to result in decreased levels of prostaglandins whose suppressive effect on lymphocytes may be due in part to the induction of suppressive factors [14]. Interference of other antiproliferative proteins is also possible [11,13]. The foregoing suggests that Dx inhibits IL-2 production (to different degrees in cells from different donors) while simultaneously inhibiting the elaboration of suppressor factors that depress IL-2 production, so that more IL-2 is released than in control samples. Since the ratio between the degrees to which IL-2 production and suppressor factor production are inhibited may vary from one individual to another, so, too, may the shapes of the curves describing IL-2 release.

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