

Effects of Dexamethasone on Human Natural Killer Cell Cytotoxicity, Interferon Production, and Interleukin-2 Receptor Expression Induced by Microbial Antigens

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Received 20 August 1985/Accepted 15 October 1985

Dexamethasone inhibits the expression of the interleukin-2 receptor, the synthesis of immune interferon, and the development of natural killer cells when added to peripheral blood mononuclear cells cultured with soluble microbial antigens (purified protein derivative and a polysaccharide extract from *Candida albicans* [MPPS]) or human recombinant interleukin-2.

The immunoregulatory effects of dexamethasone (Dex) have been recently investigated at the molecular level, showing that the impaired production of a series of interleukins (IL), such as IL-1, IL-2, and interferon (IFN) (4, 10, 17-19), is due to a block in RNA formation (1, 9). All these data have been obtained mainly in alloantigen and mitogen systems, although it is of interest to analyze the effects of Dex in antigen-induced immune responses.

We have developed a human system in which peripheral blood mononuclear cells (PBMC) specifically activated in vitro by a purified protein derivative (PPD) from *Mycobac-*

Dex on microbial antigen-induced IFN synthesis, IL-2 receptor expression, and NK cell activity development.

PBMC (10^6 /ml) were cultured for different amounts of time in RPMI 1640 medium (GIBCO Laboratories, Grand Island, N.Y.) containing 10% autologous heat-inactivated serum, 15 mM glutamine (Eurobio, Paris, France), and 40 μ g of gentamicin (Gentalyn; Schering Corp., Bloomfield, N.J.). Dex was added at the beginning of the cell culture period, and the concentration (10^{-7} M) used in these experiments has been chosen on the basis of a dose-response curve of the inhibition of lymphocyte proliferation (17) and on the lack of lytic

TABLE 1. Effect of Dex on proliferation, IL-2 receptor expression, and IFN synthesis of PBMC activated with PPD, MPPS, or rIL-2

Additions ^a	³ H]TdR incorporation (10^3 cpm \pm SD) ^b on day:			% Tac ⁺ cells ^c on day 5	IFN activity (U/ml) ^d on day:	
	5	7	9		3	5
None	1.2 \pm 0	1.3 \pm 1.0	3.3 \pm 0.9	9	0	0
PPD	16.8 \pm 0.8	25.5 \pm 1.2	19.3 \pm 0.9	17	400	700
PPD + Dex	5.9 \pm 1.1 (65) ^e	10.1 \pm 0.7 (60)	10.3 \pm 1.9 (47)	9 (47)	10 (97)	20 (97)
MPPS	15.3 \pm 0.7	23.8 \pm 0.5	21.2 \pm 1.2	20	300	500
MPPS + Dex	6.5 \pm 0.2 (57)	9.9 \pm 0.3 (58)	10.7 \pm 0.4 (49)	11 (45)	10 (96)	50 (90)
rIL-2	10.3 \pm 0	20.6 \pm 0.8	13.9 \pm 1.6	40	100	300
rIL-2 + Dex	2.4 \pm 0.3 (77)	8.7 \pm 1.9 (58)	12.9 \pm 1.7 (7)	21 (47)	0 (100)	30 (90)

^a PBMC (10^6 cells per ml) were cultured in the absence or presence of PPD, MPPS, and rIL2 (100 U/ml). Dex (10^{-7} M) was added at the beginning of cultures. Cultures were harvested at the time indicated.

^b [³H]thymidine ([³H]TdR) incorporation was determined in triplicate samples.

^c The percentage of Tac⁺ cells was determined by FACS IV analysis.

^d Supernatant fluids were tested for IFN activity.

^e Numbers in parentheses represent percent inhibition.

terium tuberculosis and an antigenic extract from *Candida albicans* (MPPS) proliferate, release IL-1, IL-2, and immune IFN, and exert natural killer (NK) cell activity (12, 15, 16, 23). Furthermore, analyzing the effect of Dex on the proliferative response to MPPS, Piccolella et al. (17) found that production of both IL-1 and IL-2 was inhibited. Since a relationship among IL-2, IFN production, and NK cell activity has been extensively reported (3, 6, 8, 13, 14, 20, 22), we have investigated in this paper the inhibitory effect of

effect on PBMC, as shown by evaluating cell count and viability in 3-day cultures in the presence or absence of Dex (2; unpublished observations). Cultures were stimulated with PPD (10 μ g/ml; Statens Serum Institut, Copenhagen, Denmark), a purified polysaccharide from *C. albicans* (MPPS; 10 μ g/ml [15]), or human recombinant IL-2 (rIL-2; 100 U/ml, kindly provided by Sandoz A.G., Basel, Switzerland). PBMC proliferation (10^5 cells) was evaluated as [³H]thymidine incorporation after 20 h of pulsing with 0.5 μ Ci of [³H]thymidine (Amersham Corp., Arlington Heights, Ill.). The expression of Tac antigen was evaluated by an

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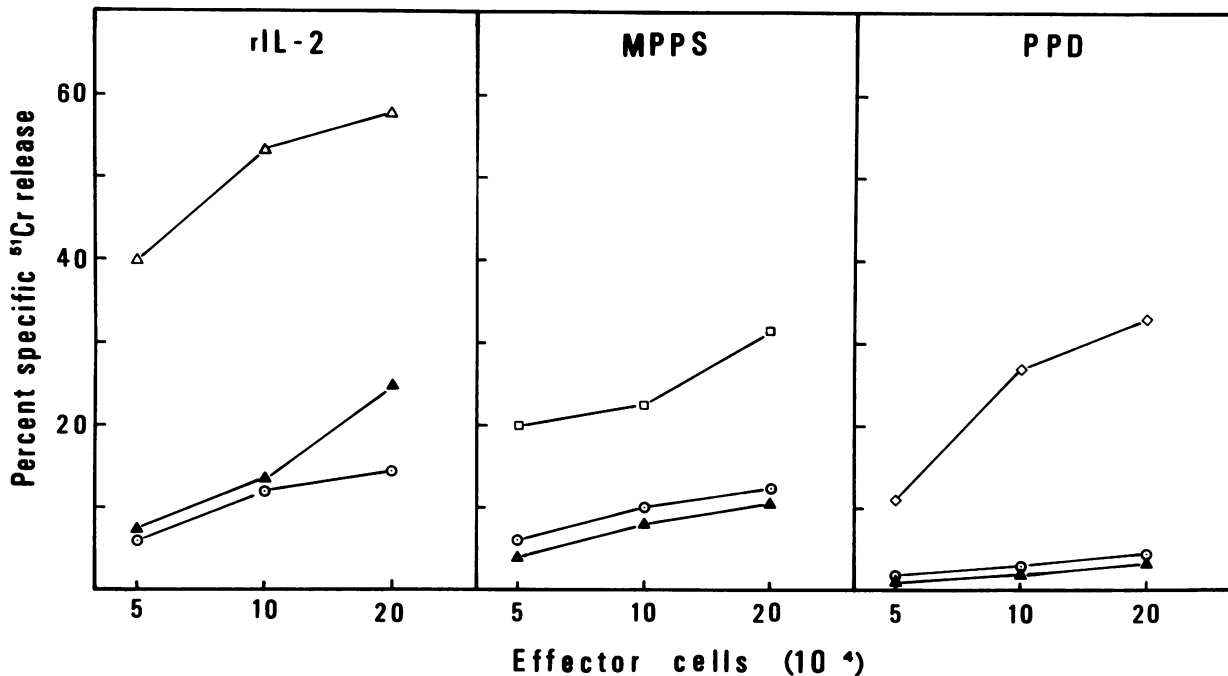


FIG. 1. Effect of Dex on the development of NK cell activity in PBMC cultures stimulated with rIL-2, MPPS, or PPD. PBMC (10^6 cells per ml) from healthy donors were cultured for 7 days with 100 U of rIL-2 per ml (Δ), 10 μ g of MPPS per ml (\square), or 10 μ g of PPD per ml (\diamond) or with culture medium only (\circ). Dex at 10^{-7} M (\blacktriangle) was added at the beginning of the cell culture periods. PBMC were then depleted of macrophages, rosetted, and tested as effector cells against 10^4 K562 target cells in a 4-h ^{51}Cr release cytotoxicity assay (7). The percentage of specific ^{51}Cr release was determined by the following formula: % specific release = [(experimental counts per minute - spontaneous counts per minute)/(maximal counts per minute - spontaneous counts per minute)] \times 100. Standard deviation of triplicate wells was usually $<10\%$.

indirect immunofluorescence assay on FACS IV (Becton Dickinson and Co., Paramus, N.J.) with anti-Tac monoclonal antibodies, kindly provided by T. A. Waldmann (11), and affinity-purified goat anti-mouse immunoglobulin G and F(ab')₂-fluorescein isothiocyanate (Cooper Biomedical, Inc., West Chester, Pa.) as the second reagent (22). The presence of IFN in culture supernatants was quantitated biologically, and the activity was expressed in laboratory units as previously reported (21). The IFN activity was characterized as gamma type by its sensitivity to pH 2.0 and anti-IFN γ monospecific antiserum, kindly provided by M. P. Langford (University of Texas Medical Branch at Galveston), and by its resistance to neutralization by anti-IFN α antibodies (Schering Corp.). Table 1 shows the inhibitory effects of Dex on cell proliferation, IL-2 receptor expression, and IFN production. The presence of Dex significantly reduced all three parameters studied in both microbial antigen- and rIL-2-stimulated cultures.

The effect of Dex on the development of NK cell activity was then investigated. The cytotoxic activity of stimulated PBMC was tested in a ^{51}Cr release assay (7) with K562 cells as targets. The presence of rIL-2, MPPS, or PPD in the culture induces the development of significant NK cell activity at all effector-to-target cell ratios tested (Fig. 1). When Dex was added at the beginning of the cell culture period, full inhibition of cytotoxicity was observed in both microbial antigen- and rIL-2-stimulated cultures.

Piccolella et al. (17) have already described a suppressive effect of Dex on MPPS-driven cell proliferation and IL-2 production. This has been confirmed in the present paper with another recall microbial antigen, such as PPD, indicating a general inhibitory effect of Dex on the immune response to microbial antigens. Furthermore, the present

results also demonstrate a strong inhibitory effect of Dex on rIL-2-driven proliferation of resting PBMC. This effect seems to be due to the significant decrease of IL-2 receptor expression, indicating that Dex inhibits both IL-2 production and action. However, other authors have reported that activated cells, pretreated with Dex and then cultured in the absence of the drug, respond to exogenous IL-2, implying that the expression of IL-2 receptor was unmodified by Dex treatment (4, 10). This apparent contrast with our findings can be explained by considering that the inhibitory effect of Dex is transient and that exogenous IL-2 induces the expression of new synthesized IL-2 receptors (18). In fact, Larsen and Grabstein (9) recently reported that the synthesis of mRNA for IL-2 receptors and for IFN in phytohemagglutinin-induced T cells is inhibited by Dex but can be restored by adding rIL-2.

The block of the lymphokine cascade (IL-1, IL-2, and IFN) caused by Dex in the PPD- or MPPS-stimulated cultures is accompanied by a significant impairment of NK cell activity. In fact, NK cells respond to IL-2 (20, 22), and it is known that immune IFN, released by rIL-2-activated cells, participates in NK cell activation (6, 8, 14). However, the failure of rIL-2 to induce NK cell activity in Dex-treated cultures suggests that the effect of Dex on NK cell activation is related more to the expression of IL-2 receptors and IFN levels than to a lack of IL-2.

We conclude that Dex modulates the activation of NK cells by interfering with the lymphokine cascade. Since NK cells seem to be involved in defenses against infectious diseases (5), patients treated with a glucocorticoid, such as Dex, would risk infections not only due to the lack of IFN-mediated bactericidal effects of macrophages but also due to the decreased NK cell activity.

We thank R. Morelli for preparation of the MPPS antigen, E. Mannella for the FACS analysis, C. Ramoni for his advice to perform the cytotoxic assay, M. Piantelli for criticism and suggestions, and D. Guerritore for his continuous encouragement during this study.

This work was supported by Italian National Research Council grants no. 85.00438 (CNR-MRC Joint Projects), 85.00875.52, 85.00844.52, and 85.02144 and 85.02199.44 (Progetti Finalizzati Controllo Malattie da Infezione ed Oncologia).

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