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Cortisol and immune interferon can interact in the modulation of human natural killer cell activity[1]

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Summary. This paper reports that cortisol at physiological concentrations minimizes the enhancement of human natural killer (NK) cell activity in vitro by immune interferon (IFN-γ). This effect may be important for the regulation of NK cytotoxicity in vivo.
Key words. Natural cytotoxicity; cortisol; immune interferon.

Natural killer (NK) cells are a heterogeneous subpopulation of lymphocytes capable of lysing target cells without prior sensitization. Since NK cell activity is most evident against neoplastic and virus-infected targets, it is thought that NK cells in vivo play an important role in immunosurveillance[3,4]. Recently, a number of studies have been carried out in order to understand the physiological mechanisms by which NK cell activity is regulated. Evidence has been provided that interferon (IFN) preparations and IFN inducers enhance basal NK cell activity and counteract the effects of pharmacological agents which have been shown depress NK cell cytotoxicity[5,6]. Synthetic glucocorticoids, on the other hand, have been found to be inhibitors of rodent and human NK cell activity[7,8] and to suppress at high concentrations the enhancement induced in vitro by leukocyte type I interferon (IFN-α)[9].

On the basis of these reports, we thought it pertinent to investigate whether cortisol, the most representative endogenous glucocorticoid in man, and the immune interferon (IFN-γ), a glycoprotein produced by T-lymphocytes and more closely associated with immunosurveillance than other IFN preparations, can interact in the modulation of human NK cell activity.

Materials and methods. PBM cells were derived from heparinized venous blood samples obtained from healthy adult donors (males and females, aged 20–35 years), taking no medication, and fasting for 8 h before venipuncture. PBM cells were immediately separated by Ficoll-Hypaque density centrifugation[10]. The resultant preparations contained more than 98% mononuclear cells. PBM cells were washed and resuspended to the desired density in complete medium, which is the medium RPMI 1640 supplemented with 10% foetal calf serum (Eurobio, Paris, France), 1% L-glutamine and gentamicin (50 μg/ml).

Cortisol (Sigma Chemical Co., St. Louis, Missouri, USA) was initially dissolved in a little 95% ethanol and then diluted in distilled water to a 1×10^{-3} M stock solution. For use in experiments, this preparation was promptly diluted in distilled water to a final concentration ranging from 1×10^{-5} to 1×10^{-8} M. We have documented that NK cytotoxicity of PBM cells against K 562 targets was significantly reduced by cortisol at the above-reported range[11]. Highly purified human IFN-γ (generously provided by F. Dianzani, Institute of Virology, University of Rome, Italy), diluted in RPMI 1640 was used in the range from 5 to 30 IU/ml. Preliminary experiments on the dose-response curve within this range showed the 15 IU/ml concentration to give an optimal enhancement of NK cell activity. The effects on NK cell activity of treatment with either cortisol or IFN-γ require a relatively long period of time to become manifest. In the present series of experiments, PBM cells, resuspended in complete medium to a density of 3×10^6 cells/ml, were incubated for 20 h in the presence of cortisol and/or IFN-γ at 37°C in a humidified atmosphere of 95% air and 5% CO_2. We carried out the majority of experiments using cortisol at the concentration of 1×10^{-6} M and IFN-γ at the concentration of 15 IU/ml. No significant differences in viability were observed after in vitro incubation of PBM cells with both agents, in comparison with untreated PBM cell preparations.

NK cell activity was determined in a 4-h direct ^{51}Cr-release assay as previously described[12]. The human cell line K 562 was used as the source of target cells. Percentages of NK cell-mediated cytotoxicity were computed from the following formula:

$$\%\text{cytotoxicity} = \frac{\text{cpm (test)} - \text{cpm (spontaneous)}}{\text{cpm (total)} - \text{cpm (spontaneous)}} \times 100$$

Test cpm, total cpm and spontaneous cpm represent the radioactivity released in the supernatants from target cells incubated with effectors, in an equal aliquot of target cells in suspension and in supernatants from target cells incubated without effector cells, respectively. Spontaneous release never exceeded 10% of the maximum release. The variations among triplicates were less than 5%.

Except where indicated, data were expressed as lytic units (LU)/10^7 effector cells. The LU values were derived from equation $y = A(1-e^{-kx})$ where y = fractional chromium release, A = a constant equal to the asymptote of the sigmoid curve, k = a constant proportional to effector cell activity for a given A and x = the E:T ratio. In these experiments 1 LU represents the number of cells required to give a 30% cytotoxicity.

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Statistical significance of differences in values of cytotoxicity produced under the different experimental conditions was determined by the paired Student t-test. $p < 0.05$ was considered significant.

Results and discussion. The effects of 20-h incubation of human PBM cells with cortisol at the concentration of 1×10^6 M and/or IFN-γ at the concentration of 15 IU/ml are shown in the table and figure 1. In vitro incubation of PBM cells with cortisol at the above-reported level resulted in a significant inhibition of NK cell activity ($p < 0.01$, NK activity expressed as LU/10^7 cells). NK cytotoxicity was reduced at all E:T ratios even though the magnitude of inhibition from the same donor varied as an inverse function of the ratio, with the greatest level of inhibition being observable at the lower ratios. Conversely, in vitro treatment of PBM cells with IFN-γ increased NK cell activity 3- to 10-fold in all examined cases and for all tested E:T ratios ($p < 0.01$, LU). Simultaneous treatment with the two agents resulted in a significant enhancement of NK cell activity when compared with cortisol treated ($p < 0.001$, LU) and with untreated PBM cell preparations ($p < 0.01$, LU). On the other hand, a significantly lower level of activation was observed in comparison with IFN-γ treatment alone ($p < 0.01$, LU).

Combined effects of cortisol and IFN-γ on the NK cell activity of human PBM cells

Subject No.	Treatment of PBM cells			
	Control	Cortisol	IFN-γ	Cortisol+IFN-γ
1	72	50	234	93
2	47	27	484	70.5
3	73.9	31.3	861	90.6
4	37.6	12.2	163	53.5
5	67.7	39.9	223	76.2
Mean	60	32.1	393.5	76.81
(± SE)	7.3	6.3	129.2	7.18
Significance	–	p<0.01	p<0.01	p<0.01

PBM cells were incubated for 20 h with $1x10^{-6}$ M cortisol and 15 IU/ml IFN-γ. NK cell activity, measured in a direct 4-h ^{51}Cr release assay against K 562 targets is expressed as LU/10^7 effector cells.

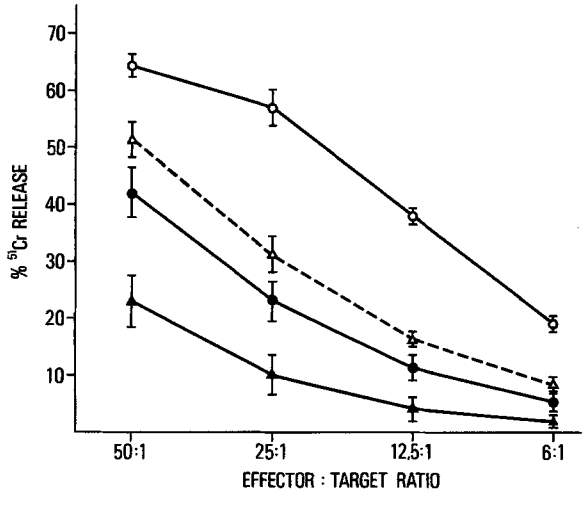

Figure 1. Effects of cortisol and/or IFN-γ on the NK cell activity of human PBM cell preparations (●—●, untreated PBM cells; ▲—▲, 1×10^{-6} M cortisol; ○—○ 15 IU/ml IFN-γ; △—△, cortisol + IFN-γ). PBM cells were incubated for 20 h with the two agents and NK activity was measured in a direct 4-h ^{51}Cr release assay against K 562 targets. Data are expressed as mean ± SE percent ^{51}Cr release of 5 separate experiments with PBM cell preparations from different donors.

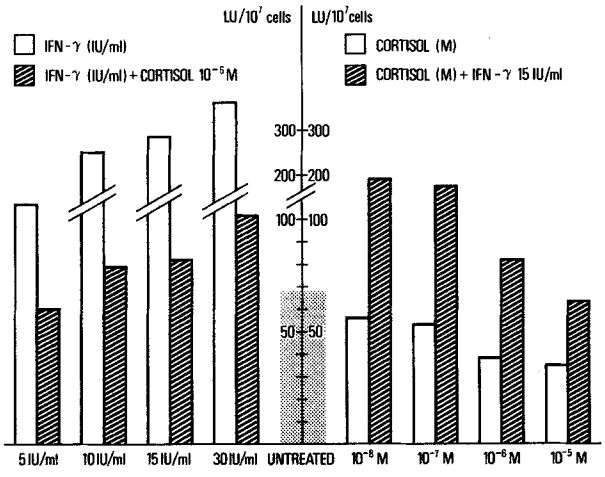

Figure 2. Dose-response relationships between cortisol (M) and IFN-γ (IU/ml). Left panel: PBM cells from an adult healthy donor were cultured for 20 h with different concentrations of IFN-γ in the presence or absence of cortisol 1×10^{-6} M. Right panel: PBM cells from an adult healthy donor were cultured for 20 h with different concentrations of cortisol in the presence or absence of IFN-γ 15 IU/ml. NK cell activity, measured in a direct 4-h ^{51}Cr release assay against K 562 targets, is expressed as LU/10^7 effector cells. These data are representative of a single experiment.

The dose-response relationships between cortisol and IFN-γ are summarized in figure 2. The effects of both agents were dose-dependent along the concentration range explored. Low-dose IFN-γ treatments were not able to revert the cortisol-induced inhibition of basal NK cell activity, whereas such an effect was evident using higher concentrations (> 10 IU/ml). On the other hand, physiological concentrations of cortisol were always effective in counterbalancing IFN-γ-induced boosting even if supraphysiological concentrations of the hormone (1×10^{-5} M) were required to give a complete suppression of IFN-γ enhancement.

The present study demonstrates that cortisol antagonizes IFN-γ-dependent augmentation of human peripheral NK cell activity against K 562 target cells. Furthermore, the body of these findings seems to us to be consistent with the view that endogenous glucocorticoids could play a role in the control of NK cell function in vivo, mainly in the course of stressful events. Under such circumstances, the unbound, biologically active fraction of the circulating cortisol may well be in the concentration range between 1×10^{-7} and 1×10^{-6} M; in fact, stress-mediated activation of the adrenocortical secretion leads to a rapid increase of several plasma steroid molecules potentially competing for the same binding sites of the corticosteroid-binding globulin (CBG), the specific carrier for cortisol.

In the present series of experiments, we employed a highly purified IFN-γ preparation which was proved to be effective at concentrations much lower than those recently reported by Robinson et al.[13] who used a commercially-available preparation. However, we did not test whether our preparation was free of T-cell growth factor (TCGF) or interleukin 2 (IL-2) and we cannot, therefore, exclude that under our experimental conditions IFN-γ could conceivably interact with exogenous IL-2 at the PBM cell level. IFN-γ could induce the expression of IL-2 receptors on NK effectors, in a way similar to that observed in T-lymphocytes[14,15] or synergize IL-2 via an augmentation of lysosomal enzyme production and release [16].

Our results, on the other hand, were obtained from PBM cells and not from NK-enriched preparations. It could, therefore, be suggested that the incubation of PBM cells with cortisol and/or IFN-γ could promote the activity of cells other than NK effectors able to produce a number of soluble factors poten-

tially affecting cytotoxicity. A generation of IL-2 from PBM cells could conceivably take place and could play a role in mediating the response of NK activity to IFN-γ and cortisol. It is known that glucocorticoids are able to suppress IL-2 production and via this effect to interfere profoundly with T-lymphocyte proliferation in rodents and man[17]. Most recently, the potent synthetic glucocorticoid dexamethasone was demonstrated to strongly inhibit synthesis of TCGF mRNA in human normal PBM cells stimulated in culture with phytohemoagglutinin, and concomitantly to inhibit the accumulation of IFN-γ mRNA in these cells[18]. We are not aware of data on glucocorticoid effects on IL-2 receptor interaction, which is thought to represent a crucial event which mediates IL-2 promoted cell-cycle progression. Glucocorticoids, on the other hand, have been shown to reduce dramatically the activity of plasminogen activator, a serine proteinase produced by NK cells and postulated to play an important role in the cytolytic process[19]. In human fibroblasts, inhibition by glucocorticoids of plasminogen activator has been shown to be due to induction of a cellular inhibition[20].

However, regardless of what mechanisms are involved, data obtained with the present study coupled with previous observations lead us to suggest that endogenous cortisol participates in the control of human NK cell activity and is capable under stressful conditions of intruding into the complex interplay between IFN, IL-2 or other soluble factors which enhance NK activity when an IFN-inducing stimulus exists within a tissue (e.g., virus infection or neoplastic cells).

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Immigration of lymphoid precursor cells into the thymic rudiment in *Xenopus*[1]

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Summary. The origin of thymic lymphocytes was investigated, using a new reliable method to mark cells in *Xenopus*. It was easily observed that extraneous cells immigrated into the thymic rudiment 4 days after fertilization and differentiated into a cell population identified as thymic lymphocytes in a fully developed thymus. Clearly, lymphoid precursor cells are of extrinsic origin.
Key words. Xenopus thymus; thymic rudiment; lymphoid precursor cell; cell immigration.

Two theories as to the origin of thymic lymphocytes have been offered: the transformation and the immigration theories. The former is supported by Turpen et al.'s[2] experiments. Using diploid-triploid chimera embryos of *Rana pipiens* produced by orthotopic transplantation of the gill arch region, they observed that thymic lymphocytes arose from the elements in the primary rudiment of the thymus. On the other hand, Le Douarin and Jotereau[3] proposed a contrary view that thymic lymphocytes originate from circulating embryonic stem cells from heteroplastic transplantation of chick and quail thymic rudiments. Further, in *Xenopus laevis*, Tochinai[4] grafted diploid thymic rudiments of larvae into triploid tadpoles and emphasized that thymic lymphocytes are of extrinsic origin.

Recently, in *Xenopus* species, an excellent new genetic cell marker was found[5]. The purpose of this study is to re-examine the origin of thymic lymphocytes with the use of this cell marker.

Materials and methods. Fertilized eggs of *Xenopus laevis* and *Xenopus borealis*[6] were obtained by injecting a gonadotrophic hormone into their dorsal lymph sac. The eggs were dejellied with 2.5% sodium thioglycollate, sterilized by placing them in Steinberg's solution containing 0.05% Chloramin-T (Wako Pure Chemical Ind., Osaka) for 1 min, and then cultivated at 23 °C for 22 h. For chimerae preparation, 22-h-old embryos (neural tube stage; st. 21 by Nieuwkoop and Faber[7], 1975) of *X. laevis* and *X. borealis* were used. Approximately in the middle of its anteroposterior length each embryo was cut manually into two parts. Then chimera embryos were produced by joining the anterior half of an embryo of *X. laevis* and the posterior half of an embryo of *X. borealis*, and were then cultivated in Steinberg's solution for 3, 4 and 35 days. The operation was performed on a clean bench.

The investigation was carried out using paraffin and epon-embedding. Chimera larvae cultivated for 3 and 4 days were fixed in Carnoy's solution for 30 min and serial sections 4 μm in thickness made using the ordinary paraffin method. After removing the paraffin, the sections were stained in a 0.5% solution of quinacrine dihydrochloride (Wako Pure Chemical Ind., Osaka) in McIlvain's buffer (pH 7.0) according to Thiébaud[5], and