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EFFECTS OF PHORBOL MYRISTATE ACETATE ON INTERLEUKIN - 2 AND ACCOMPANYING INTERFERON PRODUCTION OF HUMAN LEUKOCYTES INDUCED BY HEAT-INACTIVATED *STAPHYLOCOCCUS AUREUS*

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Summary. — Interleukin-2 (IL-2) production induced by heat-inactivated *Staphylococcus aureus* (SAU) was enhanced by simultaneous addition of phorbol myristate acetate (PMA). The effect was optimal at a concentration of 10 ng/ml SAU; in the presence of 10 ng/ml PMA, the amount of SAU required for maximal IL-2 production was lower. The kinetics of SAU and of SAU plus PMA-induced IL-2 production were similar. Stimulated mononuclear cells produced interferon (IFN) in addition to IL-2. The titre of accompanying IFN was decreased in cultures stimulated with the SAU plus PMA combination. Plastic nonadherent sheep erythrocyte-positive cells were the most active in the SAU-induced IL-2 production. In contrast, the bulk of the IFN activity was produced by the nonadherent E rosette-nonforming cells. Neutralization of IFN with specific antibodies and pH 2 treatment indicated that SAU-induced IFN consisted mainly of alpha-IFN.

Key words: *interleukin-2*; *interferon*; *S. aureus*; *phorbol myristate acetate; human leukocytes*

Introduction

Heat-inactivated SAU induces IL-2 production similar to that induced by concanavalin A (CA) or staphylococcus enterotoxin A (SEA) (Rosztóczy *et al.*, 1986). It has been reported that tumour promoter phorbol esters enhance lymphokine production by phytohaemagglutinin M (PHA), CA, SEA, and calcium ionophore (Efrat *et al.*, 1982; Vilcek *et al.*, 1983; Yamamoto *et al.*, 1985; Croll *et al.*, 1987). With the enhancement of IL-2 production in mind, we have investigated the effect of PMA on SAU-induced IL-2 production. Since bacteria are effective inducers of IFN in cultures of human leukocytes (Baron *et al.*, 1981—82; Rönnblom *et al.*, 1983), we also looked for accompanying IFN activity in the culture media of SAU-stimulated

human mononuclear cells (MNC). Characterization of the effector cell population and the type of IFN produced in SAU-induced human MNC was also attempted.

Materials and Methods

Human peripheral blood mononuclear cells (MNC) were prepared by centrifugation on Ficoll-Uromiro (Böyum, 1968) from freshly drawn human venous blood kindly provided by the University Blood Bank. Plastic-adherent and nonadherent fractions of the MNC were separated by using foetal calf serum(FCS)-pretreated plastic Petri dish adherence. The plastic-nonadherent cells were further separated to E-rosette-positive and E rosette-negative fractions by the sheep red blood cell (SRBC) rosetting procedure (Jondal, 1974).

The *S. aureus* strain and the preparation of the heat-killed suspension were the same as described previously (Rosztóczy *et al.*, 1986).

Phorbol myristate acetate was a product of the Sigma Chemical Company, Illinois, U.S.A.

Induction of IL-2. The heat-killed SAU suspension was adjusted so as to contain 2×10 bacteria per milliliter of phosphate-buffered saline (PBS). A 50 μl aliquot of this suspension was added in a 1/20 vol to MNC cultures, to establish a 20 : 1 ratio between SAU and MNC; this ratio was found to be optimal in preliminary experiments. The MNC cultures were incubated for 14 hr with the inducer, then centrifuged, and the supernatants were assayed for their IL-2 activities on an IL-2 dependent murine cytotoxic T lymphocyte line (CTLL) and mouse thymocytes, as described previously (Rosztóczy *et al.*, 1986).

Assay of IFN. All titrations were carried out on human embryo fibroblasts (HEF) with a cytopathic micromethod, vesicular stomatitis virus (VSV) being used for challenge (Rosztóczy, 1976). Titres were calibrated against an international reference reagent of HuIFN-alpha (G-023-901-527) and expessed in international reference units (IU).

Characterization of IFN. The reference reagens sheep antiserum to human leukocyte IFN (G-026-502-568) and sheep antiserum to human fibroblast IFN (G-028-501-567) were obtained from the Antiviral Substance Program (National Institute of Health, Bethesda, Maryland, U.S.A.). For the neutralization of IFN activity with antiserum, 100 μl of the preparation was mixed with one-tenth vol of appropriate concentrations of reference antisera containing a 4-fold excess of neutralizing potency relative to the antiviral activity of the IFNs. After incubations for 1 hr at 37 °C, the samples were titrated for residual activity.

The pH 2 sensitivity of the IFN activity was investigated by means of dialysis against pH 2 glycine/HCl buffer for 24 hr at 4 °C. Samples were neutralized by further dialysis against PBS for an additional 12 hr.

Results

The effect of PMA on SAU-induced IL-2 production was studied by simultaneous addition of increasing amounts of PMA to the culture media of MNC induced by the optimal dose of SAU. We observed a dose-response relationship in the range 1 to 10 ng/ml PMA. The effectivity of the latter concentration seemed to be maximal and resulted in a 3—4-fold enhancement of IL-2 activity (Fig. 1).

Since incorporation of ^{3}H-thymidine into the indicator cells did not follow a linear pattern with the dose of IL-2 applied, we quantified these semiquantitative results by assaying 3-fold dilutions of IL-2 preparations induced by SAU and by SAU plus PMA. The dose response relationship indicated a difference in activity of at least 20—30-fold in favour of the SAU plus PMA-induced IL-2 preparation (Fig. 2). The enhancing activity of PMA on the efficiency of IL-2 induction by SAU could also be demonstrated by decreasing the dose of SAU from 20 to 5 bacteria per cell in the presence of PMA, without any loss in the inducer activity (Fig. 3).

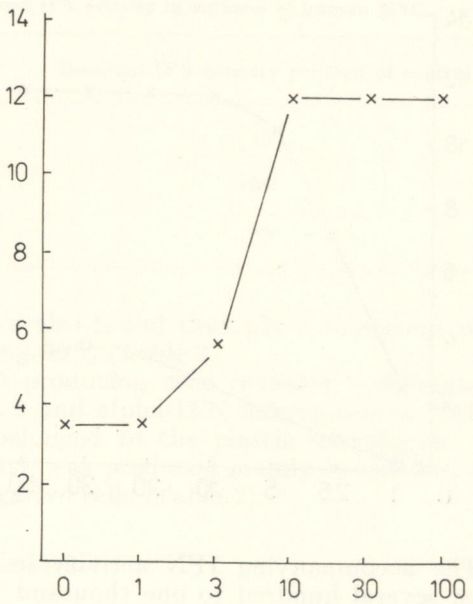

Fig. 1.
Effect of PMA concentration on SAU-induced IL-2 production
Ordinate: IL-2 activity cpm $\times 10^4$; abscissa: PMA dilution reciprocals (ng/ml).

Comparison of the kinetics of IL-2 production in cultures induced by SAU or by SAU plus indicated a similar time course, but the SAU plus PMA-treated cultures produced a markedly higher IL-2 activity in comparison with those induced by SAU alone (Fig. 4).

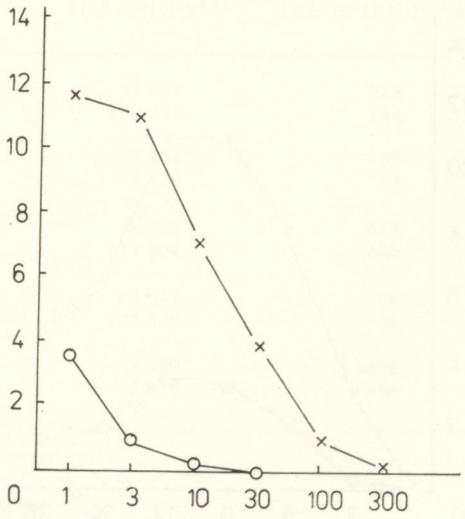

Fig. 2.
Endpoint titration of IL-2 preparations induced by SAU or SAU plus PMA
Ordinate: IL-2 activity cpm $\times 10^4$; abscissa: dilution reciprocals
○——○ SAU
×——× PMA + SAU

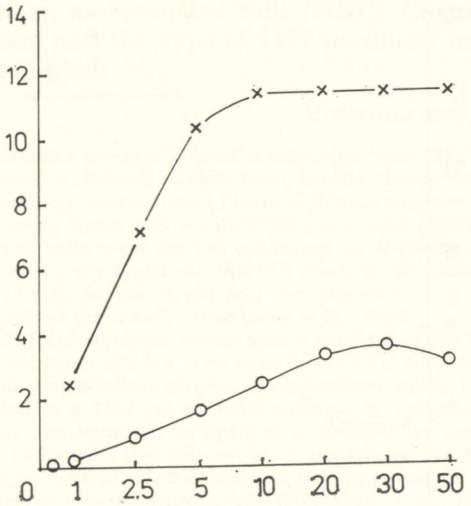

Fig. 3.
Effect of 10 ng/ml PMA on IL-2 production induced by different quantities of SAU
Ordinate: IL-2 activity cpm × 10^4; abscissa: *S. aureus*/mononuclear cell ratio.
○——○ SAU
×——× PMA + SAU

The accompanying IFN activity in the SAU-induced cultures ranged from several hundred to one thousand IU/ml. Simultaneously added PMA did not enhance IFN production; conversely, it exerted an inhibitory effect. Neutralization of SAU-induced IFN with HuIFN-alpha and HuIFN-beta antibodies indicated that alpha-IFN was the predominant type in these preparations. Polyclonal antiserum to poly I:C-induced human fibroblast IFN did not neutralize the SAU-induced IFN, while antiserum to Sendai virus-induced human alpha-IFN abolished approximately 85% of the anti-

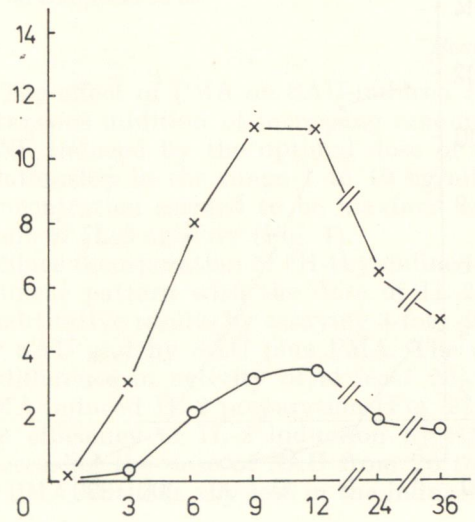

Fig. 4.
Effect of PMA on the kinetics of SAU-induced IL-2 production
Ordinate: IL-2 activity cpm × 10^4; abscissa: hours after induction.
○——○ SAU
×——× PMA + SAU

Table 1. Characterization of SAU-induced IFN activity in cultures of human MNC

Treatment	Residual IFN activity per cent of control
Antiserum to HuIFN-alpha	15
Antiserum to HuIFN-beta	>98
pH 2	58

viral activity of this preparation. We also found that pH 2 treatment decreased the antiviral activity by nearly 50% (Table 1).

Characterization of IL-2 and IFN-producing cells revealed a difference between the cell types producing IL-2 and alpha-IFN in response to SAU. The IL-2 producing human MNC belonged to the plastic-nonadherent E rosette-forming cells. The IFN activity was produced mainly in cultures of the plastic-nonadherent E rosette-negative cells (Table 2).

Discussion

The tumour-promoting agent PMA exerts multiple biological activities, including a synergism with mitogens for T cell activation (Ashman, 1984). Its enhancing effects on gamma-IFN and IL-2 production have also been reported (Efrat *et al.*, 1982; Vilcek *et al.*, 1983). Since heat-killed SAU

Table 2. Effect of PMA on SAU-induced IL-2 and IFN production by different fractions of MNC

MNC fraction*	PMA**	IL-2 activity***	Titre of IFN IU/ml***
Unseparated	−	31 431	512
	+	106 838	144
Adherent	−	2 144	32
	+	5 658	24
Nonadherent	−	38 572	512
	+	117 304	160
E-rosette positive	−	42 017	64
	+	128 330	64
E-rosette negative	−	3 799	1536
	+	7 916	384

* cells were cultured at a concentration of 5×10^6/ml
** 10 ng/ml
*** mean values of three independent experiments

proved to be an effective inducer that can easily be removed from the crude IL-2 preparation, investigation of the possible stimulatory effect of PMA on SAU-induced IL-2 production seemed of interest. Our results indicate an approximately 30-fold increase in IL-2 activity in the case of the combined application. Besides IL-2, SAU-induced IFN too, however, the production of this accompanying IFN was decreased when SAU and PMA were applied together. Investigation of the effector cells indicated that different cell types were involved in IL-2 and IFN production. The majority of the IL-2 was produced by nonadherent, E-rosette-forming cells, while the nonadherent, E-rosette-nonforming cells were most active in the production of IFN which was mainly the alpha type. The enhancement of SAU-induced IL-2 production by PMA may be based on the protein kinase C-activating effect of PMA in the target cells (Niedel *et al.*, 1983; Nishizuka, 1984). This mechanism of action might have an inhibitory effect on IFN production in SAU-induced B lymphocytes, either because of differences between the regulation of IL-2 and alpha-IFN genes, or because T and B cell react oppositely to PMA treatment. It is possible that in B cells the protein kinase C may have lower affinity and fewer binding sites for PMA as it was observed in BALB/c nude mice (Kumar *et al.*, 1987). We thought that some gamma-IFN was also present in the culture media of the SAU-induced MNC since approximately 15% of the IFN was resistant even to an excess amount of alpha-IFN antiserum. It should be considered, however, that the HuIFN--alpha antiserum used was prepared against Sendai virus-induced human leukocyte IFN, and alpha-IFN preparations induced by different inducers might comprise different subspecies of alpha-IFN. The almost 50% of the IFN activity which was inactivated by pH 2 treatment exceeded the IFN activity which did not react with antiserum to HuIFN-alpha. This seems to indicate presence of an acid-labile alpha-IFN, which has also been described by other authors in different human leukocyte IFN preparations (Fischer and Rubinstein, 1983; Matsuoka *et al.*, 1985).

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