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Prostaglandin E_2 depresses natural cytotoxicity by inhibiting interleukin-1 production by large granular lymphocytes

JEAN HERMAN & A. R. RABSON *South African Medical Research Council Human Cellular Immunology Unit, School of Pathology, South African Institute for Medical Research and University of the Witwatersrand, Johannesburg, South Africa*

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SUMMARY

Enriched large granular lymphocytes treated with varying concentrations of prostaglandin E_2 (PGE_2) for varying time periods showed considerably reduced natural cytotoxicity against K-562 target cells. The same cells when activated by lipopolysaccharide, produced substantially less interleukin-1 (IL-1) as compared to cells not treated with PGE_2. It is concluded that the inhibition of natural killer (NK) cell activity produced by PGE_2 is due to inhibition of IL-1 production by these cells.

Keywords prostaglandin E_2 natural killer cells interleukin-1

INTRODUCTION

Recent evidence has suggested a role for prostaglandins (PG) in the regulation of tumour growth in mice and humans. For example, administration of indomethacin and other PG synthetase inhibitors, can slow the growth of certain tumours in mice (Lynch, Astoin & Salomon, 1978a; Lynch & Salomon, 1979). Furthermore, Bennett *et al.* (1975) reported a correlation between PGE_2 production by human breast cancer and metastasis, in that tumours with high PGE production were more likely to have metastasized to bone. PGE has been shown to inhibit a variety of lymphocyte functions in mice and humans. PGE_1 can inhibit PHA-induced lymphocyte proliferation (Smith, Steiner & Parker, 1971) and lymphokine production (Lomnitzer, Rabson & Koornhof, 1976). More recently PGE_2 has been shown to be a potent inhibitor of natural killer (NK) cell activity (Bankhurst, 1982). At concentrations of PGE_2 in the physiological range (10^{-8}M) significant suppression of NK cell activity could be observed employing different targets and a variety of effector:target ratios. Furthermore, the suppressive effect of PGE_2 was directed at the effector cell population but did not affect the target cell.

We have recently observed (Herman, Kew & Rabson, 1984) that purified natural killer (NK) cells (large granular lymphocytes; LGL) when activated either by lipopolysaccharide (LPS) or a variety of tumour cells including K-562 cells, release considerable amounts of interleukin-1 (IL-1) which is important in the effector:target interaction. As PGE_2 is a potent inhibitor of NK cell activity, the present study assessed whether various concentrations of this PG could inhibit IL-1 production by enriched LGL.

Correspondence: Dr Jean Herman, South African Institute of Medical Research, PO Box 1038, Johannesburg 2000, Republic of South Africa.

MATERIALS AND METHODS

Preparation and Identification of LGL. Mononuclear cells (MN) were obtained from heparinized peripheral blood by Ficoll-Hypaque density centrifugation. They were depleted of adherent cells by incubation on plastic Petri dishes for 1 h at 37°C, after which non-adherent cells were decanted and depleted of B cells on nylon wool columns. Cells passing through this column were placed on a Percoll density gradient (Pharmacia Fine Chemicals, Uppsala, Sweden) containing seven different concentrations of Percoll (from 40% to 60%) made up in RPMI 1640 with 10% heat-inactivated fetal calf serum (FCS), 2 mM L-glutamine and antibiotics. The gradient was centrifuged for 30 min at 550 *g* after which cells migrating to the 40% Percoll layer were collected and washed. 70–90% of cells in this fraction had the morphological characteristics of LGL and demonstrated fluorescence when treated with the fluoresceinated monoclonal antibody Leu 7 (Becton and Dickinson, Mountain View, California, USA).

K-562 target cells. The myeloid cell line K-562 was maintained as a mycoplasma free stationary suspension culture which was subcultured 24–48 h before use in the NK assay. K-562 cells were labelled by adding 100 μCi sodium chromate ($Na_2{}^{51}CrO_4$, specific activity 250–500 mCi/mg) to 5×10^6 cells, which were incubated at 37°C for 2 h with occasional shaking. The cells were then washed three times and resuspended at a concentration of 1×10^5 cells/ml in RPMI 1640 with 10% FCS, L-glutamine and antibiotics.

Cytotoxicity assay. The assay used was a standard short term (4 h) chromium release assay (Bennett *et al.*, 1975) employing an effector:target ratio of 20:1. Percentage cytotoxicity was calculated as follows:

$$\% \text{ Cytotoxicity} = 100 \times \frac{\text{test ct/min} - \text{spontaneous release}}{\text{maximal ct/min} - \text{spontaneous release}}$$

To assess the effect of PGE_2 on NK cell activity enriched LGL were treated with various concentrations of PGE_2 for various time periods. After washing the cells three times in RPMI 1640 they were set up in cytotoxicity assays.

Production of IL-1 by LGL. One million enriched LGL per millilitre were cultured for 24 h in the presence and absence of 20 μg/ml lipopolysaccharide (LPS: *E. coli* 0127: B8, DIFCO Laboratories). Supernatants were harvested and assessed for IL-1 activity by measuring their ability to stimulate the proliferation of BALB/c mouse thymocytes activated by concanavalin A (Con A). Briefly, 0·1 ml of 10×10^6/ml thymocytes were incubated with 10 μg/ml Con A and 0·1 ml of the cell culture supernatants for 72 h in round bottom microtitre plates. ^{3}H-thymidine (24 Ci/mmol, Amersham, UK) was added 6 h prior to culture termination. Cultures were harvested and ^{3}H-thymidine incorporation assessed by liquid scintillation counting. To assess the effect of PGE_2 on IL-1 production LGL were pulsed for 4 h with concentrations of PGE_2 ranging from 10^{-3}–10^{-9}M. After washing the cells were stimulated with LPS for a further 18 h after which supernatants were collected and tested for IL-1 activity.

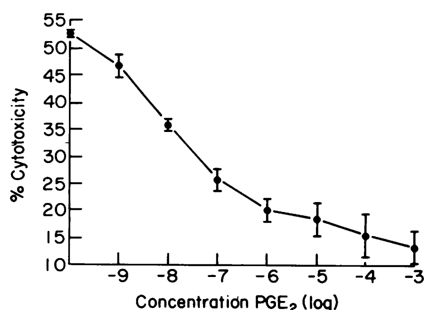

Fig. 1. The effect of varying concentrations of PGE_2 on the percentage cytotoxicity of purified LGL against K-562 target cells.

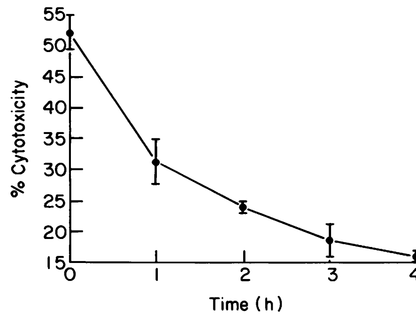

Fig. 2. The effect of PGE_2 incubated with LGL for varying time periods on their cytotoxic activity.

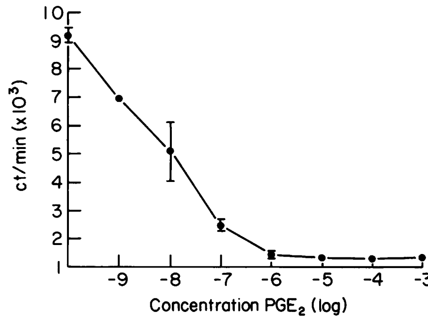

Fig. 3. The effect of varying concentrations of PGE_2 on the production of IL-1 by LPS stimulated LGL.

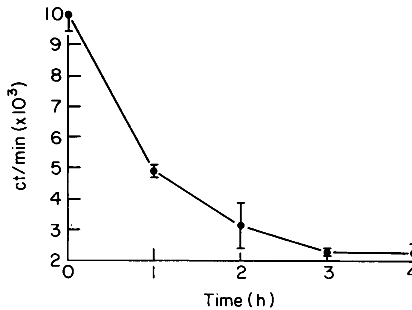

Fig. 4. IL-1 production by enriched LGL treated with 10^{-7}M PGE_2 for varying time periods.

RESULTS

The effect of PGE_2 on cytotoxic activity of purified LGL

The effect of various concentrations of PGE_2 on NK cell activity of enriched LGL against labelled K-562 cells is shown in Fig. 1. The levels of cytotoxicity exhibited by LGL pulsed for 4 h with PGE_2 are markedly reduced. Although very marked suppression of cytotoxicity was observed at concentrations of 10^{-6}–10^{-3}M, considerable inhibition of cytotoxicity could be observed at concentrations of 10^{-8}M. Furthermore, when LGL were pulsed with PGE_2 for even 1 h approximately 50% inhibition of cytotoxicity was observed. Maximum inhibition of cytotoxicity was observed, however, when the cells were treated with PGE_2 for a period of three or 4 h (Fig. 2).

The effect of PGE_2 on IL-1 production by LGL

As can be seen in Fig. 3 LGL pulsed for 4 h with varying concentrations of PGE_2 produced considerably less IL-1 when activated by LPS. Inhibition of IL-1 production was dose-dependent but even at concentrations of 10^{-9}–10^{-8}M significant suppresion of IL-1 production was observed. Although a 4 h pulse with PGE_2 produced the greatest suppression of IL-1 production, when cells were treated with 10^{-7}M PGE_2 for only 1 h, considerable inhibition of IL-1 production could be observed (Fig. 4).

DISCUSSION

The data presented in this study confirm the work of others that PGE_2 has a marked suppressive effect on NK activity (Droller, Schneider & Perlmann, 1978; Brunda, Herberman & Holden, 1980). Of interest, however, was the finding that the dose and time kinetics of the suppression of cytotoxicity were almost identical to the inhibition of IL-1 production produced by PGE_2 treated NK cells. These findings suggest that the inhibition of cytotoxicity was mediated via inhibition of IL-1 production. This finding is compatible with studies performed in our laboratory which indicate the important role of IL-1 in LGL:target interactions. Target cells treated with IL-1 bind considerably greater numbers of LGLs resulting in enhanced cytotoxicity (results unpublished). It could be expected, therefore, that agents which inhibit LGL IL-1 production will also depress NK cell activity. Although the cause of the depressed IL-1 production is not known, PGE_2 has also been shown to inhibit IL-1 production by monocytes (Oppenheim *et al.*, 1980).

The finding that PGE_2 inhibits IL-1 production by NK cells may partly explain the inhibitory effect of PGE_2 on a variety of lymphocyte functions in man. The findings are of even greater significance when one considers that PG, especially those of the E series have been shown to be elevated in many human and animal tumours (Bennett *et al.*, 1977a, 1977b). Furthermore, mice bearing a number of malignancies have been shown to produce elevated levels of PGE_2 (Lynch *et al.*, 1978a, 1978b). The results are interesting in light of the observations that administration of indomethacin or aspirin could inhibit tumour growth perhaps through restoration of NK cell reactivity (Lynch *et al.*, 1978a).

The effectiveness of NK activity is under the control of a complex regulatory network. Interferons have a major augmenting effect on NK cell activity and are probably important in maintaining this activity (Trinchieri *et al.*, 1981). This could be explained by our previous findings that IL-1 production by LPS stimulated LGL is increased by interferon treatment (Herman *et al.*, 1984). The finding that PGE_2 also inhibits NK cells by suppressing IL-1 production indicates a central role for IL-1 in the augmentation and inhibition of NK cell activity.

REFERENCES

BANKHURST, A.D. (1982) The modulation of human natural killer cell activity by prostaglandins. *J. clin. lab. Immunol.* **7,** 85.

BENNETT, A., CHARLIER, E.M., MCDONALD, A.M., SIMPSON, J.S., STAMFORD, I.F. & ZEBRO, T. (1977a) Prostaglandins and breast cancer. *Lancet,* **ii,** 624.

BENNETT, A., DEL TACCA, M., STAMFORD, I.F. & SEBRO, T. (1977b) Prostaglandins from tumours of human large bowel. *Br. J. Cancer,* **35,** 881.

BENNETT, A., MCDONALD, A.M., SIMPSON, J.S. & STAMFORD, I.F. (1975) Breast cancer prostaglandins and bone metastases. *Lancet,* **i,** 1218.

BRUNDA, M.J., HERBERMAN, R.B. & HOLDEN, H.T. (1980) Inhibition of murine natural killer cell activity by prostaglandins. *J. Immunol.* **124,** 2682.

DROLLER, M.J., SCHNEIDER, M.U. & PERLMANN, P. (1978) A possible role of prostaglandins in the inhibition of natural and antibody-dependent cell-mediated cytotoxicity against tumour cells. *Cell. Immunol.* **39,** 165,

HERMAN, J., KEW, M.C. & RABSON, A.R. (1984) Defective interleukin-1 production by natural killer cells of patients with cancer. *J. Immunol.* (Submitted).

LOMNITZER, R., RABSON, A.R., & KOORNHOF, H.J. (1976) The Effects of cyclic AMP on leukocyte inhibitory factor (LIF) production and on inhibition of leukocyte migration. *Clin. exp. Immunol.* **24,** 42.

LYNCH, N.R., ASTOIN, M. & SALOMON, J.C. (1978a) Mechanism of inhibition of tumour growth by aspirin and indomethacin. *Br. J. Cancer,* **38,** 503.

LYNCH, N.R., CASTES, M., ASTOIN, M. & SALOMON, J.C. (1978b) Mechanism of inhibition of tumour growth by aspirin and indomethacin. *Br. J. Cancer,* **38,** 503.

LYNCH, N.R. & SALOMON, J. (1979) Tumour growth inhibition and potentiation of immunotherapy by indomethacin in mice. *J. Natl. Cancer Inst.* **62,** 117.

OPPENHEIM, J.J., KOOPMAN, W.J., WAHL, L.M. & DOUGHERTY, S.F. (1980) Prostaglandin E_2 rather than lymphocyte-activating factor produced by activated human mononuclear cells stimulates increases in murine thymocyte cAMP. *Cell. Immunol.* **49,** 64.

SMITH, J.W., STEINER, A.I. & PARKER, C.W. (1971) Human lymphocytic metabolism. Effects of cyclic and noncyclic nucleotides on stimulation by phytohemagglutinin. *J. clin. Invest.* **50,** 442.

TRINCHIERI, G., SANTOLI, D., GRANATO, D. & PERUSSIA, B. (1981) Antagonistic effects of interferons on the cytotoxicity mediated by natural killer cells. *Fed. Proc.* **40,** 2705.