Macrophage Cytokines Render WEHI-3B Tumor Cells Susceptible to Cytostasis by Prostaglandins

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ABSTRACT. The growth of the murine myelomonocytic leukemia tumor, WEHI-3B, has been shown to be inhibited by a two-step treatment: first, incubation for one hour with either interleukin-1 (human recombinant IL-1α or tumor necrosis factor (human recombinant TNF-α); second, subsequent exposure to prostaglandins. Preincubation with IL-1 rendered the tumor cells more susceptible to subsequent treatment with either prostaglandin E2 or to the stable synthetic analogue of prostacyclin DC-PG12. Preincubation with TNF-α rendered the tumor cells more susceptible to further treatment with PGE2 but not with DC-PG12. Preconditioning of the tumour cells with either IL-1α or TNFα did not affect cytostasis by subsequent culture of tumor cells in presence of either one of the cytokines. It is concluded that the interactions between macrophage cytokines and prostaglandins in enhancement of antitumor activity might imply first binding or induction of certain modifications in the tumor cells by the cytokines which render the cells more susceptible to exposure to prostaglandins.

INTRODUCTION

Interleukin-1 (IL-1; for review see (1)) and Tumor Necrosis Factor (TNF-α; for review see (2)), are cytokine products of macrophages expressing a wide array of biological activities, including antitumor activity (3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13).

Occurrence of interactions between macrophage cytokines and products of arachidonic acid (AA), is well documented. Thus, inhibitors of the cyclooxygenase pathway of AA as indomethacin, augment LPS-induced IL-1 response (14). Addition of exogenous prostaglandins suppress production of IL-1 by macrophages (14). IL-1 induction of IL-2 requires participation of metabolic intermediates governed by 5-lipoxygenase activity (15). Release of TNFα from macrophages was found to be regulated by PGE2 (16). The interaction between AA products (eicosanoids; prostaglandins and leukotrienes), was also expressed in respect to antitumor activity. Thus, indomethacin inhibited partially the effect of IL-1 on monocyte-mediated antitumor cytotoxicity, whereas pretreatment of monocytes with prostaglandin resulted in increase of monocyte cytotoxicity (7). We reported (3), that exogenous PGE2 enhances and leukotriene C4 inhibits IL-1 activity against WEHI-3B tumor cells. Interaction between the two cytokines, IL-1 and TNFα was also reported (6) as expressed by an additive effect in destruction of tumor cells.

The synergistic effect of IL-1 and PGE2 in inhibition of WEHI-3B cell growth was demonstrated by us when IL-1 and PGE2 were added simultaneously to tumor-cell cultures at the beginning of the incubation time (3). The aim of the present work was to determine if the synergistic antitumor cytostatic effect of a cytokine-prostaglandin combination is also exhibited when tumor cells are first treated with the macrophage cytokine and subsequently exposed to exogenous prostaglandin.

MATERIALS AND METHODS

Materials

Recombinant human interleukin-1 (HRIL-1), was a gift from Dr. S. Gillis, IMMUNEX (Seattle, Washington, USA). Recombinant Human Tumor Necrosis Factor (HRNF-α) was a gift from Dr. W. Fiers, University of Ghent, Belgium. Prostaglandin E2 (PGE2) and the stable synthetic analogue of prostacyclin (DC-PG12; 5E-13,14-didehydro-carboxyprostacyclin), were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). HRIL-1α and HRNFα concentrations are given in arbitrary units; 100 U IL-1α/ml=1 ng/ml; 1 U TNF-
Effect of cytokines and prostaglandins on WEHI-3B growth

WEHI-3B tumor cells were cultured (37°C, 7.5% CO₂) in 25-cm² as previously described (17). Briefly, WEHI-3B (10⁵ cells/100 μl complete medium), were incubated in 96-well multiwell dishes for 24 h. ³H-thymidire (³HdT) was than added and the multiwell dishes were incubated for further 18 h period. The cells were harvested and the incorporated radioactivity counted in a scintillation counter. The cytostatic effect of cytokines and prosta-glandins on WEHI-3B growth (inhibition of ³HdT incorporation) was determined under two experimental conditions; first, pretreatment with cytokine for one hour at 37°C, washing and subsequent addition of prostaglandin or cytokine at the beginning of 42 h incubation time; second, simultaneous addition at the beginning of incubation time of cytokine and prostaglandin either alone or in various combinations. Each prostaglandin and cytokine was added in a volume of 25 μl/well.

Fig. 1 Cytostatic effect on WEHI-3B cells of simultaneous addition of IL₁₀ and PG₁2 at the beginning of incubation time.

Fig. 2 Cytostatic effect on WEHI-3B of simultaneous addition of IL₁₀ and TNFα at the beginning of incubation time.

Fig. 3 Cytostatic effect on WEHI-3B cells of simultaneous addition of TNFα and PG₁2 at the beginning of incubation time.

Controls of WEHI-3B cells alone were supplemented with an appropriate volume of medium only. Each kind of treatment and controls were done in 5 parallel samples and each experiment was repeated at least 3 times with similar results. Stock solutions of PGF₂α and DC-PG₁2 in ethanol, were diluted to final concentrations in complete medium. The final concentration of ethanol in each well was proven to have no effect on WEHI-3B growth.

RESULTS

Effect of simultaneous addition of cytokines and prostaglandins on WEHI-3B growth

We confirmed previous results (3) showing synergistic effect of IL-1 and PG₁₂ in inhibition of WEHI-3B cell growth (results not shown here). A similar synergistic effect was now found between IL₁₀ and PG₁₂ (Fig. 1).

TNFα alone was cytostatic at a high dose of 1000 U/ml and this effect was additively reinforced by simultaneous addition of IL₁₀ (100 U/ml) (Fig. 2). A synergistic cytostatic effect was shown to occur between TNFα (1000 U/ml) and 100 ng/ml PG₁₂ (Fig. 3). On the other hand, no such syner-

gistic effect was observed in a combination of TNFα (1000 U/ml) and PG₁₂ (1.25 or 12.5 ng/ml; Fig. 4). Quantities of PG₁₂ higher than 12.5 ng/ml were markedly cytostatic; 75% and 91% inhibition of ³HdT incorporation for PG₁₂ 125 and 1250 ng/ml, respectively).

Effect of pretreatment with IL₁₀ or TNFα on subsequent exposure to prostaglandins

Pretreatment with IL₁₀ (100 U/ml) increased cytostasis by subsequent exposure of WEHI-3B cells to
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PGE₂ (10–1000 ng/ml). The effect was also evident by pretreatment with 10 U/ml IL₁a (Fig. 5). Pretreatment with IL₁a (100 U/ml) increased also cytotasis by subsequent exposure of WEHI-3B cells to PGE₂, 12.5 ng/ml (Fig. 6).

Prior treatment with TNFα (1000 U/ml), rendered the WEHI-3B cells susceptible to subsequent treatment with PGE₂ but not to subsequent treatment with PGE₁ (Fig. 7).

**Effect of pretreatment with IL-1 or TNF on subsequent exposure to the same or to the unrelated cytokine**

Pretreatment with IL₁a (100 U/ml), had no effect on subsequent exposure to either ILα itself or to TNFα (Fig. 8). Pretreatment with TNFα did also not affect cytojasis toward WEHI-3B cells by subsequent exposure to either TNFα or to IL₁α (Fig. 8). Prior treatment with either one of the cytokines without subsequent exposure did not affect appreciably thymidine incorporation by WEHI-3B tumor cells (Fig. 8).

All the experiments performed with IL₁α were repeated with IL₁β with similar results (Data not shown here).
We have reported (3), that the cytostatic effect of HrIL-1 toward WEHI-3B tumor cells was enhanced by simultaneous addition of PGE2 to tumor-cell cultures. A similar synergistic effect was now found by simultaneous addition of IL-1 and PGI2. Another macrophage cytokine, HrTNFα, was also synergistic with PGE2 in respect to cytostatic activity against WEHI-3B tumor cells. However, unlike IL-1, the cytostatic effect of TNFα was not enhanced by the prostacyclin PGI2. It should be noted that TNFα on a dose comparable basis, was less cytostatic for WEHI-3B cells than IL-1. It is also possible that the difference found now between the two cytokines in respect to their interaction with PGE2 or PGI2, could be attributed to differences in the mode of action between the two prostaglandins. Differences between PGE2 and PGI2, in their activity were described previously. Thus, while mouse resident peritoneal macrophages stimulated in vitro by LPS, produced both PGE2 and PGI2, only PGE2 had a negative regulatory effect on macrophage activation for tumor-cell killing (18). It was also reported that PGE2 was more effective than PGI2 for inhibition of granulomatous inflammation (19).

It has been reported (2, 11, 20, 21, 22) that a prerequisite of antitumor activity of cytokines is their binding to cell-surface receptors. In view of this assumption, we sought to determine if pretreatment of WEHI-3B tumor cells with macrophage cytokines will render them more susceptible to subsequent exposure to prostaglandins. We found now that the synergy in cytostatic effect toward WEHI-3B cells between IL-1 and PGE2 or PGI2, and between TNFα and PGE2, was also evident when WEHI-3B were first put in contact with the cytokine, washed and then cultured in presence of the prostaglandin. As in the case of simultaneous addition, pretreatment with TNFα did not render the WEHI-3B tumor cells more susceptible to subsequent exposure to PGI2. It has been reported (3) that WEHI-3B tumor cells, unlike other WEHI lines (23, 24), do not synthesize eicosanoids when stimulated with the calcium ionophore A23187. Accordingly, release of eicosanoids by WEHI-3B cells do not play a role in the enhancement of cytostatic effect of cytokines by prostaglandins.

The interrelationship between macrophage cytokines and arachidonic acid metabolites is well documented in respect to involvement of AA metabolites in induction of cytokine production (14, 16, 25, 26), and to modulation of cytokine activities by AA metabolites (15, 27). However, the mechanism of synergy between cytokines and AA products in their antitumor activity, is not yet clear. Some possibilities can be considered; a) pretreatment with either IL-1 or TNFα might induce increase in the total number of cell surface receptors for prostaglandins. Such a possibility was considered in respect to regulation of binding of TNFα by treatment of cells with γ-interferon (20), b) pretreatment with a macrophage cytokine of WEHI-3B tumor cells might induce some damage which render the cells more susceptible to subsequent contact with prostaglandins.

It should be noted that pretreatment with either one of the cytokines did not render the WEHI-3B tumor cells more susceptible to a second exposure to cytokines. It could be that prior binding of cytokines to cell receptors prevents binding to the tumor cells of subsequently added cytokines. It has been also claimed (28) that pretreatment with IL-1 down regulates cellular response to TNFα by inducing decrease in the number of receptors for TNFα.

In conclusion, our present results indicate that the interaction between macrophage cytokines and prostaglandins in enhancement of antitumor activity, might imply first binding or induction of certain modifications in the tumor cells by the macrophage cytokine resulting in increased susceptibility to subsequent exposure to prostaglandins.

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