Regulation of Macrophage Tumoricidal Function: A Role for Prostaglandins of the E Series

Abstract. *Exogenously added prostaglandins E_1 and E_2, but not $F_{2\alpha}$, inhibited the tumoricidal activity of interferon-activated macrophages of mice. A role for adenosine 3',5'-monophosphate (cyclic AMP) in modulating macrophage functional activity was suggested because prostaglandins of the E series increase intracellular concentrations of cyclic AMP in macrophages and because treatment of interferon-activated macrophages with dibutyryl cyclic AMP consistently inhibits expression of cytotoxicity. Since the activated macrophage releases high concentrations of prostaglandin E_2, it is postulated that this prostaglandin could act locally in negative feedback inhibition to limit cell activities.*

Activated macrophages have been implicated as having an important surveillance role in differentiating normal from transformed cells and in selectively killing transformed cells by a nonimmunologic contact-mediated event (*1*). A number of agents, including synthetic polyanionic interferon inducers (*2*), bacterial endotoxins (*3*), virus (*4*), and products from antigen- and mitogen-stimulated lymphocytes (*5*) transform normal resting macrophages into these cytotoxic effector cells. Interferon has been suggested to be the ultimate inducer of macrophage activation because all of these preparations that render macrophages tumoricidal either induce or contain interferon, the lymphokine that activates macrophages has not been separated from type II interferon by physicochemical means (*5*), and antibody-purified fibroblast interferon activates macrophages directly (*6*). Once activated, macrophages display a number of other altered properties, including increased phagocytosis, degradative enzyme activities (*7*), ability to destroy facultative intracellular parasites (*8*), secretion of a number of biologically active products (*7*), and the ability to regulate cell proliferation (*9*).

An understanding of control mechanisms regulating physiologic changes in macrophages would be desirable. We have found that prostaglandins of the E series can reversibly inhibit the ability of interferon-activated macrophages to kill lymphoblastic leukemia cells in vitro. Previous reports have shown that the E prostaglandins exert a number of other inhibitory effects on macrophage functions including decreased random locomotion (*10*), lessened production by macrophages of plasminogen activator and colony-stimulating factors for macrophage stem cells (*11*), and prevention of lymphokine-induced inhibition of macrophage migration (*12*). Since the activated macrophage possesses a marked capacity for prostaglandin E_2 production (*13*), this released prostaglandin could act locally in negative feedback inhibition (Fig. 1) to limit cell activities and prevent repeated stimulation of macrophages.

A modification of our previous technique (*6*) was used to measure the ability of agents to interfere with macrophage activation by interferon preparations in vitro. Male $CD2F_1$ mice were obtained from the Mammalian Genetics and Animal Production Section of the National Institutes of Health, Bethesda, Maryland. Noninduced peritoneal cells were harvested with heparinized RPMI-1640 medium. An average yield consisted of 1.4×10^6 macrophages per mouse. Approximately 4×10^5 macrophages, as determined by morphologic criteria, were seeded into 16-mm wells in 1 ml of standard growth medium consisting of RPMI-1640 supplemented with 20 percent fetal calf serum. After 2 hours of incubation at 37°C in an atmosphere of 5 percent CO_2 in air, nonadherent peritoneal cells were removed by three cycles of aspiration with medium. The adherent cells (macrophages) were overlaid with 1 ml of growth medium. Partially purified mouse L-cell interferon (specific activity, 2×10^7 units per milligram of protein) that had been induced with Newcastle disease virus and purified by affinity chromatography on interferon antibody coupled to Sepharose was used (*14*). It was free of endotoxin contamination, as measured by the *Limulus* amoebocyte lysate assay. The interferon was added to the macrophage cultures at a final concentration of 1000 units per milliliter of culture medium. Agents tested for inhibitory activity on macrophage function were added in 100-μl portions to the macrophage cultures either for the 16-hour duration of exposure to interferon (induction phase) or for a 2-hour period after interferon treatment (expression phase). After the incubation period, macrophage cultures were washed three times with medium to remove interferon or inhibitor, or both, and the macrophages were overlaid with 4×10^4 MBL-2 lymphoblastic leukemia cells contained 2 ml of standard growth medium. All cultures were again incubated at 37°C in an atmosphere of 5 percent CO_2 in air, and viable leukemia cells were counted after 48 hours with a hemacytometer. The ratio of macrophages to target cells was approximately 10:1 at the beginning of each experiment. The percentage of growth inhibition of MBL-2 cells due to macrophage-interferon interaction was calculated by comparison to MBL-2 cells grown in the presence of normal resting macrophages alone.

Using the system described above, we found that resident macrophages from untreated mice did not influence MBL-2 proliferation. However, when macrophages were exposed to partially purified interferon for 16 hours and washed prior to addition to MBL-2 target cells, the macrophages suppressed the leukemia cell growth at 48 hours by 67.1 ± 3.4 percent (mean ± standard error obtained from eight separate experiments). Macrophage tumoricidal capacity was accompanied by increased spreading of macrophages on plastic and increased adherence of tumor cells on macrophage membranes. The cytotoxicity of interferon-activated macrophages was characterized previously as requiring cell-to-cell contact between macrophage and target cell, because conditioned media from treated macrophages were without effect on MBL-2 cell growth (*6*).

When prostaglandin E_1 or E_2 was added simultaneously with interferon to resting macrophages, the interferon induction of tumoricidal macrophages was inhibited—although the macrophages

Table 1. Agents that decreased macrophage tumoricidal function.

Inhibitor	Concentration of inhibitor (*M*)	Incubation period after interferon stimulus (hours)	Inhibition of cytotoxic activity* (%)
Prostaglandin E_1	10^{-5}	2	72 ± 8
Prostaglandin E_2	10^{-5}	2	70 ± 3
Hydrocortisone	10^{-7}	2	67 ± 3
Dibutyryl cyclic AMP	10^{-5}	1/2	54 ± 6

*Values represent the means ± standard errors obtained from three or more separate experiments. All values were significant at $P < .001$ level.

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were observed to be activated as defined by morphological criteria—and remained fully viable. Both prostaglandins of the E series showed similar kinetics of inhibiting cytotoxic function (Fig. 2), with suppression of macrophage function requiring a concentration of more than $10^{-7}M$. In contrast, prostaglandin $F_{2\alpha}$ was ineffective at all concentrations tested. The prostaglandins or the prostaglandin synthetase inhibitor, indomethacin, were without effect on the tumoricidal function of resting macrophages in the absence of interferon.

To determine whether prostaglandin E could inhibit expression of tumoricidal function after the macrophages had reached their cytotoxic potential, we incubated macrophages for 2 hours with prostaglandin E_1 or E_2 after the 16-hour interferon treatment. Their tumoricidal function was markedly diminished (Table 1), indicating that the modulation observed probably involved events already initiated by the interferon stimulus. Since the E prostaglandins have been demonstrated to activate membrane adenylate cyclase and to increase intracellular concentrations of adenosine 3′,5′-monophosphate (cyclic AMP) in macrophages (*15*), dibutyryl cyclic AMP was tested in a similar manner to the E prostaglandins found to reproduce the effect of these prostaglandins on decreasing macrophage cytotoxicity (Table 1). Hydrocortisone was similarly active, and it has been suggested to diminish macrophage-mediated cytotoxicity by virtue of its membrane-stabilizing ability (*16*). Subsequent treatment of interferon-treated macrophages with a number of other agents, including dibutyryl guanosine 3′,5′-monophosphate (cyclic GMP), prostaglandin $F_{2\alpha}$, indomethacin, and colchicine did not significantly influence macrophage tumoricidal function.

Both prostaglandins and interferons appear to act as local mediators at their site of synthesis or in nearby tissues, since both are characterized by a high catabolic rate and short biologic half-life in serum (*17*). The dualistic regulation of macrophage function by interferons and prostaglandin E may be envisioned to occur through a balance in cyclic AMP– and cyclic GMP–associated signals, since such is characteristic of the modulation of both the proliferation and secretory functions of a number of cells of the hemopoietic system (*18*). However, direct evidence for this conclusion awaits corroboration of levels of cyclic nucleotides in the macrophages with the observed effects of the agents utilized in this study.

The ability of prostaglandin E to act as a biologic "resistor" that controls the expression of activated macrophage tumor killing may have considerable importance in understanding why certain tumors progress that contain a high macrophage content (*19*). It is of interest that cells transformed by chemical carcinogens or virus produce severalfold more prostaglandins than their untransformed counterparts (*20*). These released prostaglandins could subvert macrophage function at a critical time when the tumor burden is low. That such inhibitors exist has been suggested by observations that macrophages from animals implanted with syngeneic tumors develop depressed migratory ability in vivo and chemotactic responsiveness in vitro (*21*), and that surgical removal of tumors results in a rapid enhancement of monocyte chemotaxis (*22*).

A more comprehensive knowledge of the stimuli and conditions under which the tumoricidal activity of macrophages occurs should clarify their role in tumor resistance.

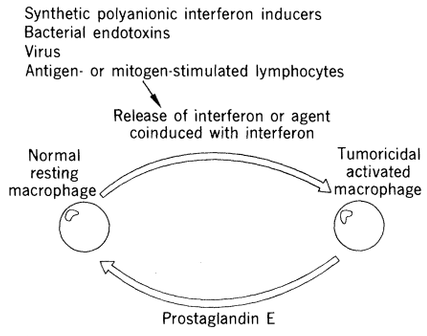

Fig. 1. Pathway for reversible inhibition of nonspecifically activated macrophages by prostaglandins of the E series. Since activated macrophages release prostaglandin E_2, such prostaglandin could allow for negative feedback inhibition of the activated state.

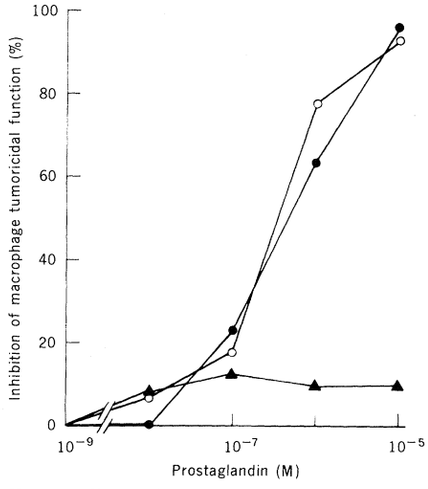

Fig. 2. Effects of prostaglandins on tumoricidal function of interferon-activated macrophages. Prostaglandins E_1 (●), E_2 (○), and $F_{2\alpha}$ (▲) were added simultaneously with interferon (1000 units per milliliter) to monolayers of resting macrophages for 16 hours at 37°C. The cultures were then washed and MBL-2 leukemia target cells were added. Cytotoxicity was determined after 48 hours of incubation with target cells at 37°C. Each point represents the mean obtained from triplicate determinations.

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