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POTENTIAL THERAPEUTIC APPLICATIONS FOR INTERLEUKIN 1: ANTI-TUMOR AND HEMATOPOIETIC EFFECTS

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Interleukin 1 (IL 1), a major immunoregulatory monokine, was originally defined by its ability to act as a co-mitogenic factor for murine thymocytes (Gery et al., 1972). Since then IL 1 has been revealed to act as a mediator of host defenses that modulates inflammatory, immunological as well as hematological reactions (Oppenheim et al., 1986). In this report we will discuss two distinct effects of IL 1 with potential therapeutic applicability: First, antitumor cell activities and second, the bone marrow restorative activities that probably account for the radioprotective effects of IL 1 (Neta et al., 1986).

There is in vitro and in vivo evidence that IL 1 may play an important role in host defense against tumors by augmenting in vitro monocyte-mediated tumor cytotoxicity (Onozaki et al., 1985), by augmenting in vitro NK cell activity (Matsushima et al., 1985) by having direct in vitro antiproliferative and cytocidal effects for some tumor cells (Onozaki et al., 1985; Lovett et al., 1986) and by inducing in vivo tumor regression in mice (Nakamura et al., 1986).

In the course of investigations of the in vitro cytostatic effects of IL 1 (Onozaki et al., 1985), we established that IL 1 inhibited the incorporation of tritiated thymidine by the mouse myeloid leukemic cell line, M1 (Onozaki et al., 1986). Coincidentally, we observed that incubation of M1 cells with IL 1 in conjunction with lipopolysaccharide endotoxin (LPS) resulted in both synergistic growth inhibitory effects and the differentiation of M1 cells into macrophage-like cells (Onozaki et al., 1986). We therefore examined the effects of combinations of IL 1, with IFN-β or TNF (which could be produced by M1 cells in response to LPS), on the growth and differentiation of M1 cells (Onozaki et al., 1987).

We have also shown that administration of IL 1 protects mice from death (Neta et al., 1986). This increased survival was paralleled by the recovery of hematopoietic system from radiation damage. In the course of investigating the reasons for the marrow (BM) restorative effects of IL 1, we have observed that administration of IL 1 to normal mice markedly increases cycling of bone marrow cells. These observed properties of IL 1 induction of BM cell cycling, differentiation of leukemic cell and its cytostatic effect may have therapeutic utility.

MATERIALS AND METHODS

Reagents – RPMI 1640 was purchased from M. A. Bioproducts (Walkersville, MD). Fetal calf serum (FCS) was obtained from Hyclone Laboratories (Logan, UT). Lipopolysaccharide (LPS, E. coli 0127:B8, Westphal) and latex particles with a diameter of 0.81 μm were from Difco Laboratories (Detroit, MI). Ox erythrocytes were from Nippon Biotest Laboratory (Tokyo). Rabbit antibody, IgG, against ox erthrocytes was prepared from antiserum obtained by immunizing rabbits with ox erythrocytes. Concanavalin A (ConA) was from Pharmacia Fine Chemicals, Uppsala, Swede. [^{3}H] Thymidine ([^{3}H]TdR, 2 Ci/ml) was from New England Nuclear (Boston , MA). Preparation and purification of recombinant human TNF (pI 5.9, 17,000 dalton, 1.9 x 10^6 U/mg), production and purification of recombinant human IL 1α (pI 5.3, 18,000 dalton, 2 at 3 x 10^7 U/ml) and natural human IL 1-β (pI 7.0, 17,000 dalton, 2.3 x 10^7 U/mg) have been reported (Yamada et al., 1985; Furutani et al., 1986; Matsushima et al., 1985). Mouse recombinant IL 1-α was kindly provided by Dr. P. Lomedico of Hoffman-LaRoche (Lomedico et al., 1984). Mouse interferon-α (1.5 x 10^6 U/mg)-β (5.3 x 10^7 U/mg) and anti-mouse IFN-β immunoglobulin (rabbit) were from Lee Biomolecular Research Laboratories, Inc., San Diego, CA. Antiserum against human TNF was obtained by immunizing rabbits with purified TNF.

Purified human recombinant IL 1-α (specific activity 7.5 x 10^6 units/mg protein as assessed by the co-mitogenic effect in the thymocyte proliferation assay (Matsushima et al., 1985) and recombinant GM-CSF supplied in sucrose (Lot #344-061-47 specific activity of 4 x 10^7 units/mg protein) were a generous gift of Immunex Corp., Seattle, WA.

Cell cultures – The clone of M1 cells used was M2/436-7 that had been established by Dr. Ichikawa (Moore & Rouse, 1983) (Chest Disease research Institute, Kyoto University, Kyoto, Japan) and provided through Dr. K. Akagawa (Department of Cellular Immunology, NIH, Shinagawa, Tokyo, Japan). This clone is known to differentiate into macrophages but not into neutrophils. The cell line was recloned by limiting dilution, and was maintained in RPMI 1640, 100 μg/ml of Streptomycin and 10% heat activated FCS. To determine the effects of cytokines or LPS, one hundred μl of a cell suspension of M1 cells (2 x 10^5 cells/ml) in RPMI 1640 supplemented with antibiotics, 10% FCS, and test samples was cultured in wells of flat-bottomed microtiter plates (Sumitomo Bakelite, Tokyo) at 37°C in 5% CO_2 in air for varying periods. After culture, the viable cell number as judged by trypan blue dye exclusion was determined using a hemocytometer.

Assay for differentiation of M1 cells – One hundred μl of a suspension of M1 cell (2 x 10^5 cells/ml) in RPMI 1640 medium supplemented with antibiotics and 10% FCS was cultured in wells of flat-bottomed microtiter plates at 37°C in 5% air. Polymyxin-B (10 μg/ml) as indicated was added to cultures. Usually cells were cultured for 3 days, and their differentiation was determined by measuring development of FcR expression or phagocytic activity. The assay for FcR was performed by the modified method of (Bianco et al., 1970). Briefly, M1 cells were cultured with 0.4% EA (ox erythrocytes sensitized with IgG of rabbit anti-ox erythrocytes antiserum) at 37°C for 3hr. After cultures, the percentage of M1 cells with more than three erythrocytes attached was determined by counting at least 200 M2 cells in an hemocytometer. To assess phagocytic activity, M1 cells were cultured with 4% polystyrene latex particles for 8hr, and the percentage of cells ingesting more than 10 latex particles was determined in a hemocytometer by counting at least 200 cells.

Assay for IL 1 activity – IL 1 activity was determined by measuring the incorporation of [^{3}H]TdR by C3H/HeJ mouse thymocytes cultured for 3 days in the presence of 0.5μg/ml ConA and serially diluted IL 1 as described (Matsushima et al., 1985). One unit per milliliter was defined as the reciprocal of the dilution at which 50% of the maximum thymocyte proliferation response was obtained.

Assay for IFN activity – The antiviral activity of an IFN sample was assayed by inhibition of the cytopathic effect of vesicular stomatitis virus on mouse L929 fibroblast cells (provided by Dr. Y Ito of NIH, Tokyo). Antiviral activity expressed in IFN units was determined from the reciprocal of the highest dilution of the sample that reduced the viral cytopathic effect by 50%.

Assay for TNF activity – The activity of TNF was determined by a L929 fibroblast cell lytic assay. Briefly, one hundred μl of a suspension of TNF-sensitive mouse L929 fibroblast cells (4 x 10^5 cells/ml) were cultured with serially diluted test samples in wells of flat-bottomed microtiter plates at 37°C for 18 hr in 5% CO_2 in air in the presence of actinomycin D (1 μg/ml). After culture, the plates were washed, and cells lysis was determined by staining the plates with crystal violet (0.5%) in methanol/water (1:4 V/V). After solubilizing the dye-stained cells with 0.1 ml of 0.1% SDS, the dye uptake was calculated by an automatic micro ELISA autoreader (Immuno Reader NJ-2000, Inter-Med). One unit of TNF activity was defined as the reciprocal of the dilution of samples that lysed 50% of the test cells.

Preparation of conditioned medium of M1 cells – M1 cells suspended in RPMI 1640 medium supplemented with 10% FCS at a density of 1 x 10^6 cells/ml were cultured with or without cytokines or LPS for 2 days. The culture supernatants were dialysed against RPMI 1640 medium, and IL 1, IFN and TNF activities were measured.

Mice – Inbred strains of mice, C57BL/6J, C3H/HeJ, and B6D2F1 were obtained from Jackson Laboratories, Bar Harbor, ME. C3H/HeN mice were also used (Animal Genectics and Production Branch, NCI, Frederick, MD). The mice were housed in the Veterinary Department Facility at the Armed Forces Radiobiology Research Institute in cages of 10-12 mice with filter lids. Female mice, 8-12 weeks of age were used for all experiments. Standard lab chow and HCL acidified water (pH 2.4) were given ad libitum. All cage cleaning procedures and injections were carried out in a microisolator.

Administration of cytokines and hydroxyurea – IL 1 administered to three or four mice per experimental group which were sacrificed by cervical dislocation. Lymphokines were diluted in 0.5% bovine serum albumin in normal saline (sterile-filtered) to a concentration of 10 μg/ml and 100 μg/ml, respectively and stored at -20°C until just prior to intraperitoneal (IP) injection into mice. The lymphokines were diluted in pyrogen-free saline and administered at doses of 100ng/0.5 ml per mouse for IL 1-α and 5 μg/0.5 ml per mouse for GM-CSF. All preparations contained less than 0.06ng of LPS per injection as assessed by the Limulus amebocyte assay. In some experiments hydroxyurea (HU) was injected (900 mg/kg body weight IP) 2 hours prior to sacrifice of the mice.

Recovery of bone marrow cells – Femurs were removed and placed on ice in Hanks' Balanced Salt Solution (HBSS) containing 100 units/ml of penicillin and 100 μg/ml of streptomycin (Gibco, Grand Island, NY). Single cell suspensions were prepared by washing each cavity of the femur with 3 ml of HBSS with a sterile syringe and 26 gauge needle. Cell counts were obtained using a haemocytometer. Viability, as assessed by trypan blue exclusion was always >95%. Cytospin slides were prepared on a Shannon Cytospin II (250g., 6 minutes) using 10^5 cells and 0.1 fetal calf serum per slide. They were stained with a modified Wright's Giemsa stain (Diff-Quick, CMS, ILL), and evaluated by light microscopy.

In vitro bone marrow cell proliferation assay – GM-CSF was diluted in complete media containing RPMI 1640, 10% fetal calf serum (Hyclone), 100 units/ml penicillin, 100 μg/ml streptomycin, 10^{-5} M 2-mercaptoethanol, and 2 mM L-glutamine. GM-CSF was added to 96 well microtiter plates (Falcon) in 0.1 ml volumes to yield final concentrations of 10, 1, and 0.1 ng/ml. Next, the bone marrow cells were added in 0.1 ml volumes at concentrations of 1 x 10^5 and 5 x 10^4 cells/well. The cultures were incubated for 2 days at 37°C with 5% CO_2 in air. The cells were then pulsed with 1 μC1 [^{3}H] thymidine per well and harvested 18 hrs later (Skatron Cell harvester, Sterling, VA) onto glass fiber filters which were then counted in Betacount scintillation fluid on a Mark III scintillation counter.

Cell sizing procedure – The bone marrow cells were resuspended at a 1:100 dilution of the original cell concentration in Isoton II, an isotonic solution specific for the Coulter sizing system. Cell profiles were obtained at several cell concentration (ranging from 1:500 to 1:10), using different media and at different time points (1,2, or 4 hrs after recovery) with similar results.

The cells were collected by the Coulter sampling stand equipped with a manometer. Since the bone marrow cells range in diameter from 1μ-10μ a manometer with 70μ diameter aperture was utilized. The relevant Coulter ZM settings were amplification – 8 and current – 100. For the Coulter channelyzer C-1000, the base channel threshold was set at 15 to exclude red blood cells and the count range switch was set at 4K. (Similar results were obtained at 1K and 10K). These settings were determined after calibrations of the manometer with 10μ beads. Using a shape correction factor of 1.38 for lymphocytes, the size or the threshold factor of each channel was found to be 5.39 μm and the range of volumes analyzed was 80.87 to 620 μm.

After a sample of cells was sized, the frequency distribution, as determined by the channelyzer, was stored and analyzed using the Accucomp software package and an Apple III personal computer.

Flow cytometric analysis of DNA content and cell size – Flow cytometric analysis of DNA content was performed using the propidium iodine (PI) staining technique (Crissman & Stein kamp, 1982). Statistical analysis of the results were performed using the Mann Whitney and Wilcoxon Sign Ranks tests.

RESULTS AND DISCUSSION

In vitro regulation of M1 tumor cell growth and differentiation by cytokines – First it was established that incubation of M1 cells with LPS had cytostatic effects and induced the cell line to produce IL 1, IFN and TNF activities (Onozaki et al., 1987). All three cytokines by themselves (human recombinant IL 1-α at 10 U/ml, murine IFN-β at 10^3 U/ml and human recombinant TNF at 10^3 U/ml) also reduced the number of M1 cells recovered after 2 days and more markedly after 3 days of culture. Combinations of IL 1-α and IFN-β or TNF revealed that paired cytokines had more than additive growth inhibitory effects. Incubation of M1 cells with combinations of IL 1-α and IFN-β or IL 1-α and TNF almost completely suppressed cell replication (Onozaki et al., 1987). We established that all the cytokine preparations and culture

media did not contain LPS as assessed by limulus amoebocyte assay (sensitivity limit of 0.125 ng/ml).

We then investigated the hypothesis that the cytostatic effects of cytokines on M1 cells were accompanied by increased cell differentiation. The effect of cytokines on differentiation was evaluated by assaying FcR expression by M1 cells. Cells that attached more than 3 erythrocytes were considered FcR positive. IL 1-α (10 U/ml), IFN-β (10^3 U/ml) and TNF (10^3 U/ml) by themselves did not induce FcR expression. In contrast, FcR expression was induced by combinations of IL 1-α and IFN-β or IL 1-α and TNF (Onozaki et al., 1987). FcR expression became evident after 2 days of culture, and became more pronounced after 3 days of incubation. Combinations of cytokines increased not only the percentage of cells that bind 3-10 erythrocytes, but also the percentage of cells that bound more than 10 erythrocytes. Concentrations equal to or greater than 10 μ/ml IFN-β and >100 μ/ml TNF in combination with IL 1-α induced FcR expression in a dose dependent manner (Fig. 1). IL 1-α and IFN-β or TNF also synergistically stimulated another indicator of differentiation namely phagocytosis. Again,, although none of the cytokines by themselves enhanced the phagocytic activity of M1 cells, IL 1-α with IFN-β or IL 1-α with TNF increased phagocytic activity. The concentration of IL 1-α needed to induce FcR expression or phagocytic activity was determined by culturing cells with varying doses of IL 1-α with or without IFN-β or TNF. Concentrations equal to or greater than 1 U/ml IL 1-α synergized in inducing differentiation. By morphological criteria cells treated with IL 1-α and IFN-β or IL 1-α and TNF after 3 days of culture developed the characteristics of macrophages such as increased adherence and spreading, enlargement, increased vacuolization, and more nonspecific esterase activity.

All IL 1 preparations tested including human recombinant IL 1-α, natural IL 1-β and mouse recombinant IL 1-α, behaved similarly and synergized with murine IFN-β or human recombinant TNF in inducing differentiation as assessed by either FcR expression or by phagocytic activity. Dose response studies revealed that at 1 U/ml all three preparations of IL 1 exhibited optimal activity.

The combination of IFN-β and TNF resulted in enhanced growth inhibition. However, mixtures of IFN-β and TNF failed to induce FcR expression or phagocytosis by M1 cells. Consequently, the cytostatic effects of these two cytokines could be dissociated from differentiative effects.

The cytostatic and differentiative effects of TNF and LPS are mediated by IFN-β – We previously observed that TNF as well as LPS, but not IL 1, stimulated M1 myelomonocytic cells to produce detectible supernatant IFN activity after 2 days of incubation (Onozaki et al., 1987). This IFN activity was almost completely neutralized by a rabbit anti-mouse IFN-β, but not by a control antiserum. In order to determine whether the elaborated IFN-β mediated the differentiation-inducing activity of TNF and LPS, the effect of anti-mouse IFN-β antibody on M1 cell differentiation was studied. The phagocytic activity induced by IFN-β, or TNF in conjunction with IL 1-α, or LPS by itself was inhibited by the anti IFN-β antibody, but not by control rabbit serum (Fig. 2). The antibody also inhibited FcR induction by IL 1-α and IFN-β, IL 1-α and TNF or by LPS by itself (data not shown). IFN-α alo induced phagocytic activity only if added with IL 1-α. Thus, IFN-α like IFN-β in conjuction with IL 1 can induce cell differentiation. The activity of IFN-α was not inhibited by anti-IFN-β antibody, suggesting that IFN-α is also capable of inducing M1 cell differentiation. The role of IFN-β in the cytostatic effects of the cytokines was also investigated. The results on Fig. 3 show that anti-IF-β partly blocked the growth inhibitory activity of TNF and LPS, but not that of IL 1, suggesting that the cytostatic effect of TNF on M1 cells also are mediated by IFN-β.

A number of tumor cell lines have been reported to have varying degrees of sensitivity to the antiproliferative effect of IL 1, including a human melanoma cell line, A375, a clone of mouse fibroblast cell line, L929, a human myeloid cell line, K562, and a mouse T lymphoma cell line, Eb (Onozaki et al., 1985; Lovett et al., 1986). The present study demonstrates enhanced in vitro cytostatic effects of IL 1 in combination with IFN or TNF on an immature myeloid M1 cell line. Our study suggests that even when the cytostatic effects of a cytokine such as IL 1 are modest, the growth inhibitory effect of cytokines can be strikingly accentuated by interactions with other cytokines. The in vitro synergistic tumoricidal effect of the combination of TNF and IFN-gamma has been well documented for several tumor cell lines (Williamson et al., 1983; Tsujimoto et al., 1986). It

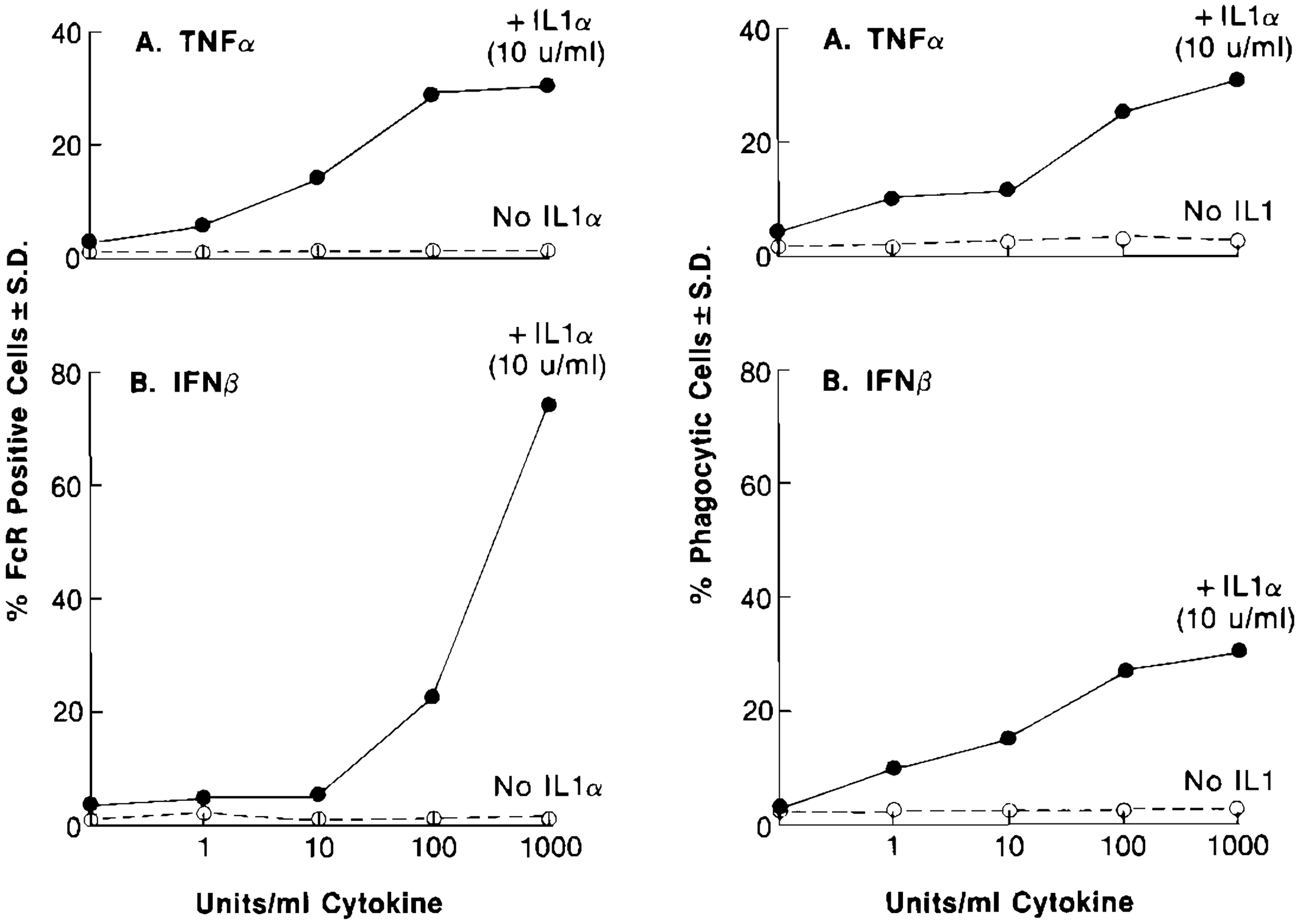

Fig. 1: Synergistic effect of IL 1α and IFNβ or TNFα on the induction of FcR expression and phagocytic activity of M1 cells. The % of FcR positive cells ± SD and the % phagocytic cells ± SD of triplicate cultures are shown. M1 cells were treated with varying doses of IFNβ or TNF with or without IL 1 for 3 days.

has been hypothesized that IFN-gamma may synergize with TNF by up-regulating the expression of receptors for TNF (Tsujimoto et al., 1986). Similarly, IL 1 may induce the expression of IFN and/or TNF receptors on M1 cells.

To effectively induce differentiation required the continuous presence of two cytokines, IL 1-α and IFN-β, or IL 1-α and TNF during the entire course of incubation. Therefore, cells probably require two distinct but concomitant signals provided by IL 1 and IFN-β or TNF. Although IL 1 by itself did not induce the production of IFN activity by M1 cells, both TNF and LPS induce the production of IFN activity. The differentiation inducing activity of TNF and LPS could be completely neutralized by specific antibody to IFN-β. IFN-β itself could induce differentiation of M1 cells in combination with IL 1. Consequently, IFN-β appears to mediate the induction of M1 cell differentiation into macrophages by TNF and LPS. Recently, Resnitsky et al. (1986) reported that IFN-β also mediates the differentiation of M1 cells induced by phorbol myristic acid or colony stimulating factor 1, since such differentiation can be blocked using specific antibody to IFN-β (Resnitzky et al., 1986). Therefore, IFN-β along with IL 1 may be essential mediators of M1 cell differentiation.

The relationship of differentiation to cytostasis is not clear cut. Although the induction of differentiation of M1 cells is always associated with growth inhibition, the reverse is not always the case. Based on our results we certainly cannot claim that antiproliferative effects of cytokines are necessarily associated with differentiative events. However, use of more sensitive assays of differentiation might establish a direct correlation between differentiative and cytostatic events.

In conclusion, combinations of cytokines act synergistically both to inhibit tumor growth and to enhance cell differentiation. Our study also shows that minimal non-toxic doses of IL 1 suffice for obtaining anti-proliferative effects of IL 1 provided it is used in conjunction with IFNβ or TNF. Thus, combinations of these cytokines may prove useful in treatment of some types of tumors in vivo.

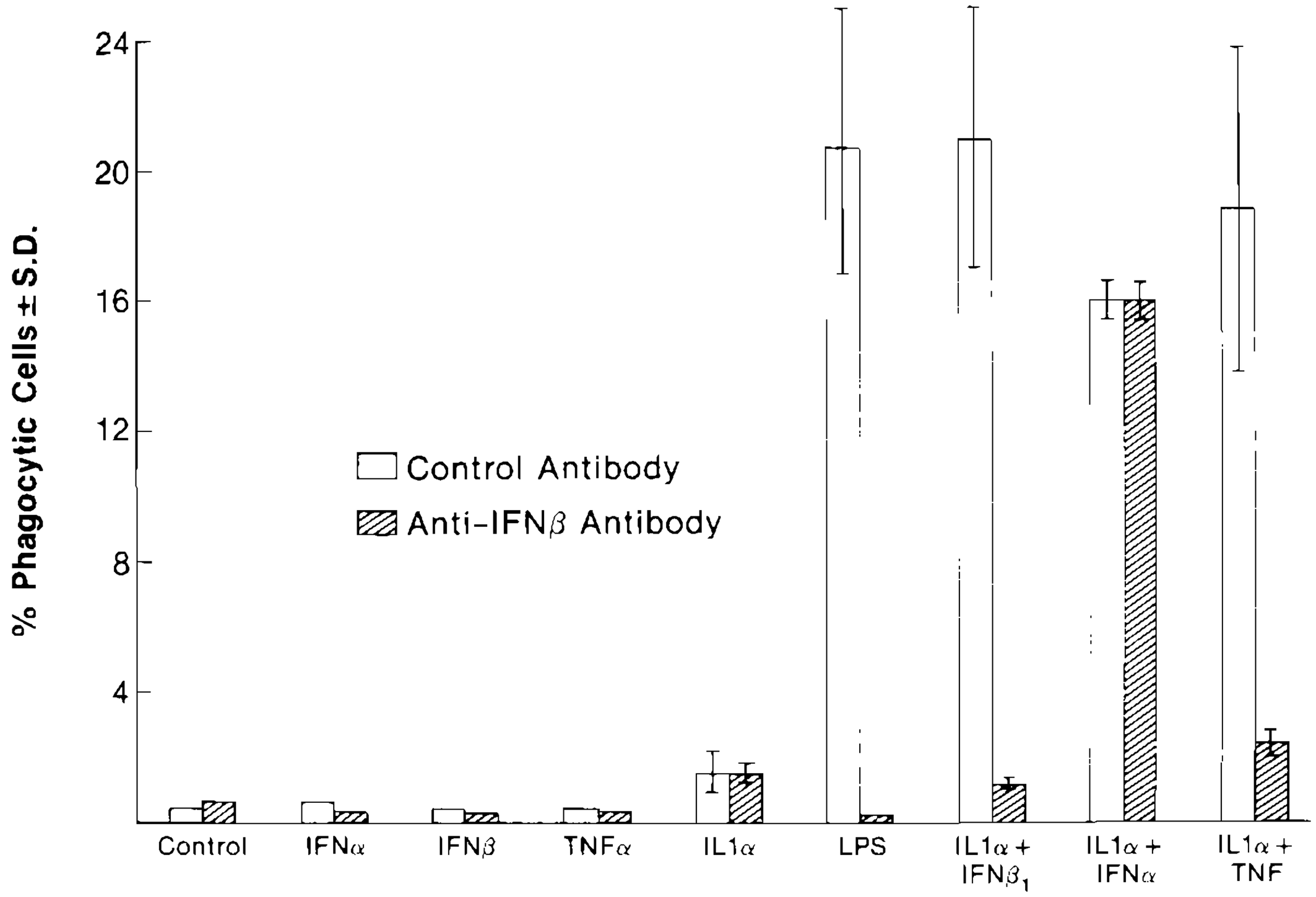

Fig. 2: Effect of anti-mouse IFNβ antibody on induction of phagocytic activity of Ml cells by cytokines and LPS. Ml cells were treated with cytokines or LPS in the presence of control rabbit antibody or anti-IFNβ antibody for 3 days, and the % of phagocytic cells determined. All the cultures except for those with LPS were incubated in the presence of polymyxin-B (10 μ/ml). IFNα, β and TNF were used at 10^3 U/ml, and IL lα at 10 U/ml. The % phagocytic Ml cells ± SD of triplicate cultures induced by cytokines or LPS in presence or absence of anti-IFNβ is shown.

Bone marrow restorative effects of IL 1 – Administration of LPS to mice 1 day prior to lethal doses of irradiation reduces the suppression of hematopoiesis (Smith et al., 1958). Intraperitoneal (IP) administration of IL 1, 20 hrs before a lethal dose of radiation, increases the survival of mice in association with hematopoietic recovery as indicated by the production of increased numbers of nucleated bone marrow cells by 5 – 13 days following irradiation (Neta et al., 1986). As observed with LPS, the number of endogenous splenic colonies (EFUs) was also greatly enhanced in IL 1 treated irradiated mice (Neta et al., 1986).

To evaluate further the myelopoietic consequences of IL 1, the effect of IL 1 on normal murine bone marrow was evaluated (Neta et al., 1987). Although 20 hrs after an IP injection of 100 ng IL 1 there was no increase in the number of bone marrow cells, the numbers of enlarged bone marrow cells were increased by 25.3 ± 4.1% as determined by Flow cytometry.

The cause of the bone marrow cell enlargement was investigated by giving IL 1 treated mice hydroxyurea (HU); an agent that arrests cells at the G1/S interphase of the cell cycle and is toxic to cells in the S phase. Treatment with HU eliminated the number of large bone marrow cells in IL 1 treated mice, but had no effect on the size distribution of cells from control mice (Table I). This result suggests that IL 1 induces bone marrow cells to enter the cell cycle. This hypothesis was further supported by data showing increases from 26.7 to 39.2% of large BM cells in the S+G1+M phases of the cell cycle in IL 1 treated over control mice as determined by flow cytometric analysis of cellular DNA content (data not shown).

Finally, we compared the capacity of control and IL 1 treated BM cells to proliferate in

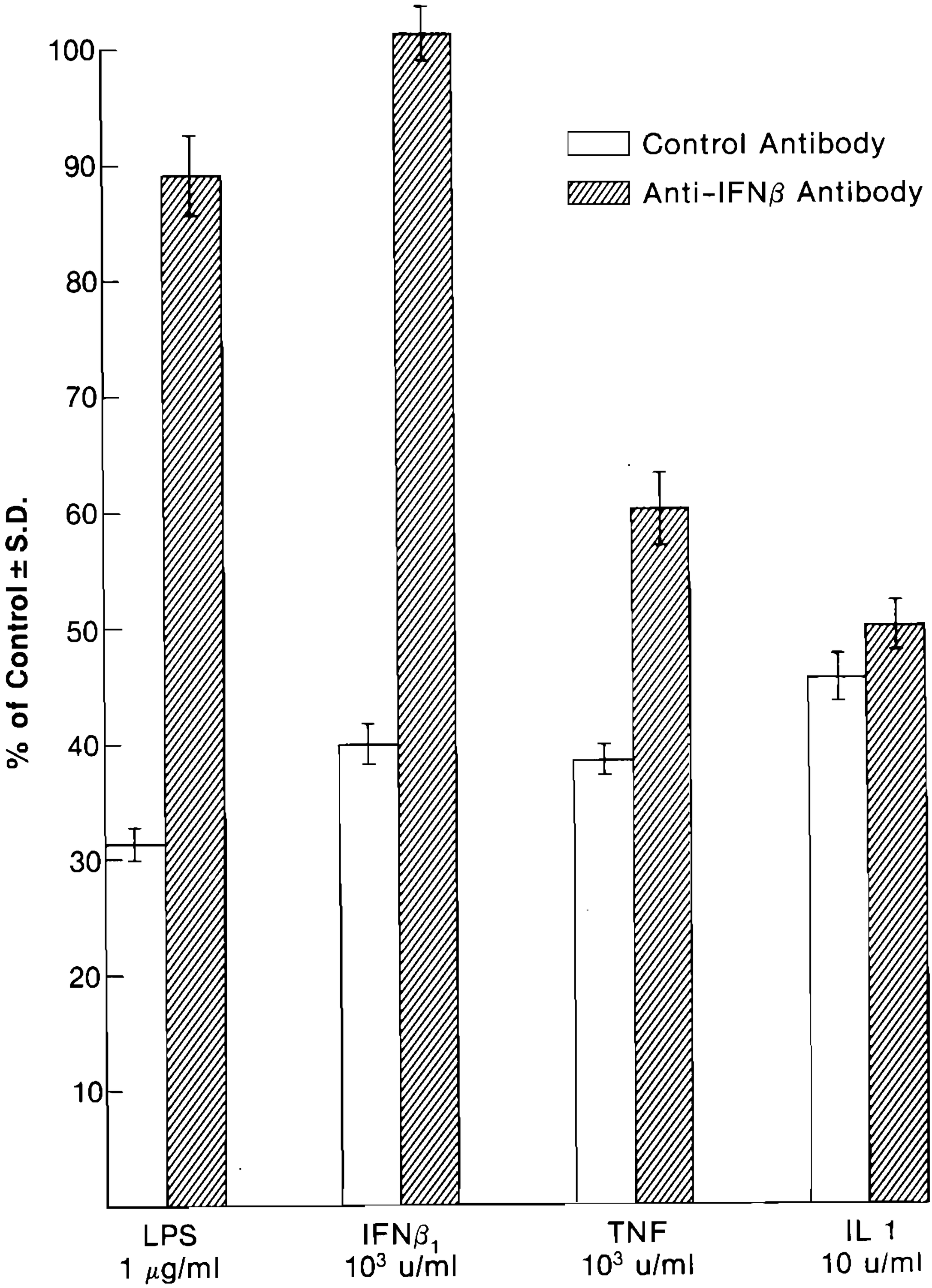

Fig. 3: Effect of anti-mouse IFNβ antibody on the growth inhibitory effects of cytokines and LPS for MI cells. MI cells were treated with varying doses of cytokines and LPS in the presence of control rabbit antibody or anti-IFNβ antibody for 3 days, and then the viable cell number determined. All the cultures except for LPS were conducted in the presence of polymyxin-B (10 μg/ml). Addition of polymyxin-B did not influence cell growth. The mean % of control ± SD of triplicate cultures is shown.

TABLE I

Effect of administration of Hydroxyurea (HU) on the size of bone marrow cells from IL 1 treated and control mice

		Cell Volume Distribution		
		Overall	Medium	Large
In vivo treatment IL 1	HU	(80-620)*	(215-323)	(324-620)
–	+	97 ± 5*	94 ± 3	88 ± 8
+	+	90 ± 6	79 ± 10**	74 ± 4**

*Range of cell volume in MM^3.

**Mean % change in cell number in different cell size compartment in saline or IL 1 treated mice w/wo subsequently administrated HU. Mean ± SEM of 20 experiments is shown utilizing 3-4 mice in each group. BM from 4 different inbred mouse strains was assessed 20 hrs following IL treatment and 2 hrs following HU or saline administration, using a Coulter channelyzer.

**$p = < 0.01$

culture for 72 hr in response to GM-CSF; a growth factor for macrophage and granulocyte progenitor cell (Moore & Rouse, 1983). The results show that the proportion of GM-CSF responsive progenitor cells present in BM of IL 1 treated mice approximately doubled (Table II). Consequently, prior in vivo treatment with IL 1 does enrich the BM in progenitor cells with the capacity to subsequently proliferate in vitro in response to GM-CSF. This latter response to IL 1 as could be expected, was blocked by concomitant treatment of mice with HU. This indicates that IP administration of IL 1 induces an increased proportion of GM-CSF responsive BM cells to enter the S phase of the cell cycle.

We have demonstrated that administration of IL 1 to mice therefore has several stimulatory effects on bone marrow cells. IL 1 stimulates an increase in GM-CSF responsive macrophage and granulocyte progenitor cells in the bone marrow, increases the proportion of large bone marrow cells as measured by Coulter Volume channelyzer and by light scattering, increases the sensitivity to HU treatment and increases the proportion of cells in S and $G_2 + M$ phases of cell cycle. Since, the late S phase of the cell cycle was reported in numerous studies to be the most radioresistant phase of the cell cycle (Denenkamp, 1986), the radioprotective effect of this cytokine may be related to the induction of larger number of bone marrow cells into the radioresistant late S phase.

However, based on this in vivo data we cannot conclude whether the effects of IL 1 on BM cell cycling are direct or indirect. For example, IL 1 has been documented to indirectly induce T cells to progress into the S phase of the cell cycle by stimulating the production of IL 2 (Smith et al., 1980). Our results show that IL 1 by itself is nct a direct in vitro growth stimulant of bone marrow cells or of GM-CFU (Vogel et al., 1987). IL 1 presumably is acting indirectly as a co-stimulant. In fact, one of us has observed that following intraperitoneal administration of a single dose of recombinant IL 1, high titers, 1-2 x 10^3 units per ml of CSF, appeared in the circulation within 2 hr and persisted for up to 6 hr. (Vogel et al., 1987). Furthemore, there are reports that IL 1 can stimulate CSF production by stromal cells (Adamson, 1986). Similarly, fibroblasts stimulated with IL 1 release supernatant factors that support CFU-GM, BFU-E and CFU-GEMM colony formation (Zucali et al., 1987). It is therefore probable that in addition to CSF, IL 1 may stimulate the release of other hematopoietic growth factors.

As an endogenous pyrogen and mediator of the inflammatory response, IL 1 has been considered a noxious rather than a beneficial cytokine. However, IL 1 has been implicated as a differentiation and maturation-inducing agent for a variety of cells (Oppenheim et al., 1986). IL 1 has been proposed to participate in wound healing (Oppenheim, 1986) and to increase protection from infections (Kampschmidt & Pulliam, 1975). Our own studies demonstrated IL 1 to be a radioprotector (Neta et al., 1986). In addition, the enhanced levels of IL 1 detected the placenta and amniotic fluid (Flynn et al., 1985), in the circulation after exercise (Cannon & Kluger, 1983), and post ovulation (Cannon & Dinarello, 1985), suggest that this cytokine may play a constructive role in normal function, as well as in the recovery and repair of damaged BM cells.

IL 1 in synergy with CSF promotes the growth and differentiation of bone marrow progenitor cells into myeloid cells. IL 1 in conjunction with IFNβ also has cytostatic differentiative effects on MI cells. The differentiative effect of IL 1 on hematopoeitic cells resembles its effect on MI cells. Although the antitumor and restorative effects of IL 1 are apparently disparate, they may both be based on the capacity of IL 1 to synergistically enhance the effects of other induced cytokine signals. Consequently, IL 1, by initiating a number of cytokine cascades may have divergent in vivo beneficial effects.

TABLE II

Effect of IL 1 administration on subsequent in vitro GM-CSF induced proliferation of murine BM cells

In vitro GM-CSF (ng/ml)	Number of cells per well	In vivo IL 1 treatment –	+
None	5×10^4	594 ± 83	556 ± 70
None	1×10^5	662 ± 98	869 ± 109
10.0	5×10^4	13,611 ± 1,145	20,456 ± 1,195
10.0	1×10^5	19,852 ± 1,911	33,360 ± 2,131

*Mean cpm ± SD of ^{3}HTdR incorporated by triplicate cultures of incubated for 72 hr. The experiment is representative of the 15 experiments performed using 4 different inbred mouse strains. BM was obtained 25 hrs following IP administration of 100 ng IL 1 or saline.

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