Brief Definitive Report

ACTIVATION OF HUMAN MACROPHAGES

Comparison of Other Cytokines with Interferon-γ

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We recently reported that partially purified native interferon-γ (nIFNγ) and pure recombinant interferon-γ (rIFNγ) induced human macrophages to secrete large amounts of reactive oxygen intermediates when appropriately triggered, and to kill the intracellular protozoal pathogens *Toxoplasma gondii* (1) and *Leishmania donovani* (2). Taking these properties to define macrophage activation, all detectable macrophage-activating factor (MAF) in the supernatant of mitogen- or antigen-stimulated blood mononuclear cells was eliminated by anti-IFNγ monoclonal antibody (1, 2). It thus appeared that IFNγ was necessary and sufficient for macrophage activation by these supernatants. However, additional studies are needed to learn whether other cytokines may also have MAF activity.

The proliferative, endocytic, secretory, locomotive, and cytotoxic properties of macrophages can be influenced by IFNα (3, 4), IFNβ (4–6), colony stimulating factor type 1 (CSF-1) (7–9), colony stimulating factor for granulocytes and macrophages (GM-CSF) (10, 11), pluripotent colony stimulating factor (p-CSF),[1] and migration inhibitory factor (MIF) (12). Also worthy of study is interleukin 2 (IL-2), the only secretory product of the T cell other than IFNγ currently available as the pure product of a cloned gene (13), and tumor necrosis factor (TNF) (14), whose potential for macrophage activation has not previously been examined. We studied highly purified preparations of each of the foregoing. Only IFNγ enhanced both the H_2O_2 secretion and antitoxoplasma activity of human macrophages.

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[1] K. Welte, E. Platzer, L. Lu, J. Gabrilove, R. Mertelsmann, and M. A. S. Moore. A CSF promoting clonal growth from early erythroid (BFU-E), multipotential (CFU-GEMM) and granulocyte-macrophage (CFU-GM) progenitors. Manuscript in preparation.

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TABLE I
Cytokines Used in This Study

Cytokine	M_r	Specific activity*	Purity	Source
rIFNγ	34,292	$2.6 \pm 0.4 \times 10^{6}$‡	Pure	Genentech, So. San Francisco, CA
nIFNα	17,800–20,000	1.4×10^{8}	Pure	Leukocytes induced with Sendai virus. Affinity purified§
rIFNαA	19,219	2×10^{8}	Pure	Hoffman-La Roche, Nutley, NJ (17)
rIFNαD	19,392	2×10^{8}	Pure	Hoffman-La Roche (17)
rIFNβ	19,987	2×10^{8}	Pure	Hoffman-La Roche (17)
CSF-1		4×10^{6}	~5%	Human urine, purified as in reference 20
GM-CSF		3.8×10^{5}	Partial	Culture supernatant of T5637 bladder carcinoma cell line (footnote 1)
p-CSF		1.5×10^{8}	Pure	As above, purified by DE52, AcA gel filtration, and reverse phase HPLC
MIF		Note‖	Note‖	T-T hybridoma concanavalin-stimulated supernate purified by salt fractionation, phenyl Sepharose chromatography, and ion exchange HPLC
TNF		10^{6}	Partial	Epstein-Barr virus–transformed B cell line supernate purified by sequential column chromatography (14)
nIL-2		7×10^{4}	Partial	Genzyme, Inc., Norwalk, CT
rIL-2	15,421	10^{6}	Pure	Biogen, Cambridge, MA

* U/mg protein. For IFN, international antiviral units. For other cytokines, units of bioactivity as defined in the cited references.
‡ Based on antiviral assays performed by BYR as described (1) and protein measurements by the method of Lowry et al. (15) using a bovine serum albumin standard. Mean ± SEM for 3 separate lots, including 1 from Biogen that was used in 1 experiment with equivalent results.
§ Using the monoclonal antibody described in reference 16. Contains a mixture of subtypes.
‖ Purified ~100-fold from the serum-free starting material. Negative for endotoxin by the limulus amebocyte lysate assay. IFNγ not detectable.

Materials and Methods

Human monocyte–derived macrophages were assayed for H_2O_2 release, adherent cell protein, and antitoxoplasma activity as described (1), except that the number of mononuclear cells plated ranged up to 2×10^6 and in many experiments, the volume of culture medium was increased to 0.5 ml/well after the first day. The test media described in Table I were added on day 4, 5, or 6 and H_2O_2 release tested 3 d later.

Results

H_2O_2-Releasing Capacity, Cell Shape, and Adherent Cell Protein. Fig. 1 compares the ability of 11 cytokines to enhance the oxidative metabolism of human monocyte–derived macrophages. rIFNγ, included as a positive control in each experiment, elevated macrophage H_2O_2-releasing capacity an average of eightfold above that of cells cultured in medium alone. 50% of the maximal stimulation required $\sim 1 \times 10^{-12}$ M rIFNγ (~0.1 U/ml). The non-γ IFNs were tested over a 10^5-fold range of concentrations, up to 10^4 antiviral U/ml. Pure nIFNα (a mixture of subtypes) did not enhance H_2O_2 release. In contrast to rIFNγ, nIFNα at ≥1,000 U/ml decreased cell spreading and the amount of protein adherent to the coverslips (Table II), suggesting toxicity. Results were similar with pure rIFNαA, rIFNαD, and rIFNβ, except that rIFNαD had less effect on cell protein and no effect on cell shape.

Partially purified CSF-1 and GM-CSF did not enhance H_2O_2 release. However, these preparations did interact with the macrophages, as evidenced by marked spreading of the cells and an increase in the adherent cell protein by a factor of 1.25 ± 0.48 (n = 3) for CSF-1 (8) and 2.23 ± 0.29 (n = 5) for GM-CSF. When

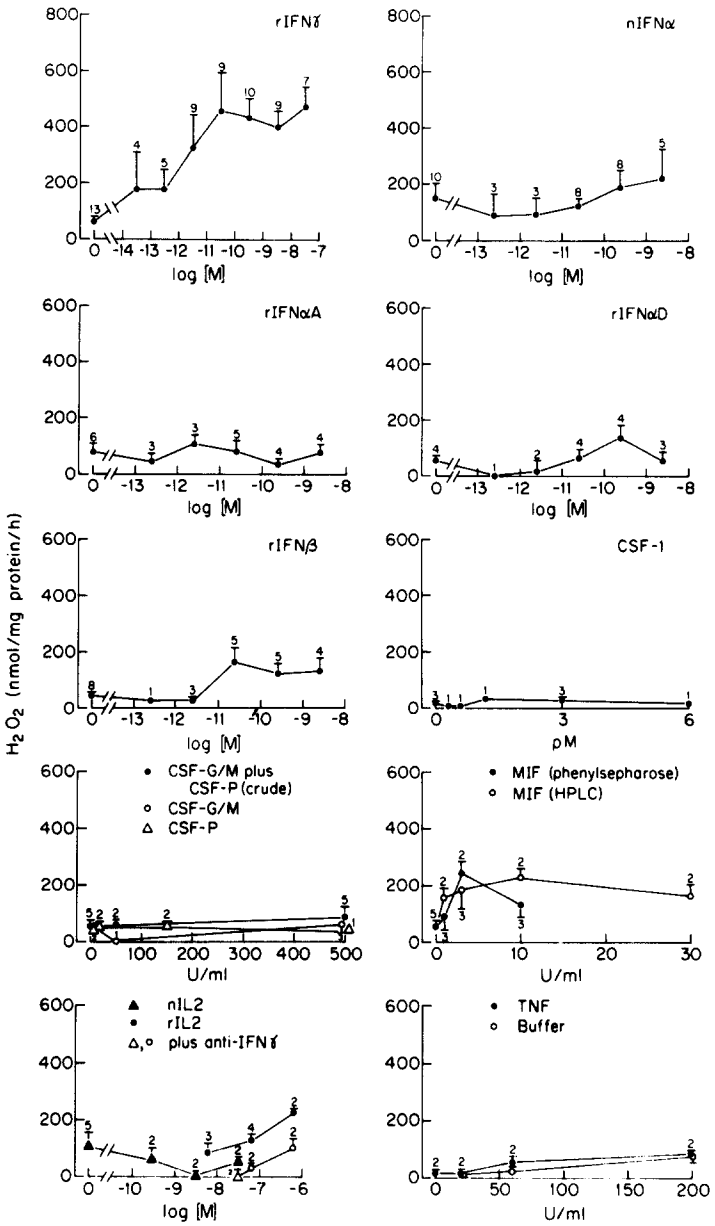

FIGURE 1. H_2O_2 release by phorbol myristate acetate-challenged human macrophages after 3 d in the indicated cytokines. Means ± SEM for the number of experiments noted above each point, each in triplicate with cells from a different donor. A unit of MIF is the amount in the active chromatographic fractions derived from 1 ml of starting material.

added from day 5 or 6 of culture, pure p-CSF affected neither H_2O_2 release, cell shape, nor adherent cell protein (the latter was 0.97 ± 0.11 times the control, n = 3). TNF was similarly without effect. MIF-rich fractions increased H_2O_2-releasing capacity approximately fourfold above the control. Spreading and adherent cell protein also tended to increase, the latter by a factor of 1.28 ± 0.20 (n = 5). rIL-2 enhanced H_2O_2-releasing capacity slightly at 10^4 U/ml, but

TABLE II

Effect of IFNγ, IFNα, IFNβ, and IL-2 on Adherent Cell Protein

Test agent	Adherent cell protein (% of control)*		
	100‡	1,000‡	10,000‡
rIFNγ	125 ± 26 (8)§	110 ± 20 (8)	Not done
nIFNα	116 ± 33 (8)	84 ± 12 (8)	49 ± 5 (5)
rIFNαA	77 ± 22 (4)	83 ± 21 (3)	63 ± 14 (4)
rIFNαD	64 ± 3 (3)	96 ± 36 (3)	75 ± 30 (3)
rIFNβ	86 ± 22 (5)	56 ± 18 (5)	46 ± 7 (5)
rIL-2	88 ± 9 (3)	99 ± 13 (4)	85 ± 13 (2)

* Control values (cell protein on saline-rinsed coverslips after incubation in medium alone) averaged 23.9 ± 6.6 μg (13 experiments).
‡ U/ml.
§ Mean ± SEM for the number of experiments in parentheses, each in triplicate.

TABLE III

Comparison of IFNγ with Other Cytokines for Induction of Antitoxoplasma Activity in Human Macrophages

Agent*	U/ml	% Toxoplasmas killed at 4–6 h	No. toxoplasmas/ vacuole at 20 h
Medium	—	3.3 ± 1.6 (7)‡	6.1 ± 0.3 (9)
rIFNγ	100–300	42.9 ± 4.3 (7)	1.9 ± 0.1 (10)
nIFNα	300–500§	0.0 ± 0.0 (2)	5.2 ± 0.2 (2)
rIFNαA	300–500§	1.0 ± 0.7 (2)	5.8 ± 0.2 (2)
rIFNαD	300–500§	0.8 ± 0.7 (4)	5.1 ± 0.3 (5)
rIFNβ	300–500§	0.0 ± 0.0 (3)	5.0 ± 0.4 (4)
CSF-1	500	9.0 ± 4.2 (3)	5.4 ± 0.5 (3)
GM-CSF	500	2.8 ± 1.9 (4)	6.1 ± 0.3 (4)
MIF	1–30‖	7.2 ± 3.0 (6)	5.9 ± 0.6 (6)
nIL-2	10–100	5.0 ± 5.0 (2)	4.6 ± 0.4 (2)
rIL-2	10–100	5.5 ± 3.3 (4)	4.8 ± 0.3 (4)

* Macrophages were incubated with the indicated cytokines for 3 d before being washed and challenged with toxoplasmas. The course of infection was followed in control medium for the next 20 h.
‡ Mean ± SEM for the number of experiments in parentheses, each in duplicate.
§ Higher concentrations were not tested because rounding up of the macrophages interfered with counting intracellular organisms.
‖ As defined in the legend to Fig. 1.

this effect was eliminated by the addition of monoclonal antibody against IFNγ.

Antitoxoplasma Activity. Under the conditions tested, none of the agents except rIFNγ conferred on macrophages the ability to kill *T. gondii* or to inhibit its intracellular replication (Table III).

Discussion

Therapy is now feasible with purified products of cloned genes. This heightens interest in the identification of cytokines that augment the antimicrobial activity of host cells. Recent studies establish that IFNγ promotes host cell–mediated inhibition of a variety of nonviral pathogens, such as toxoplasma (1, 19), leishmania (2), chlamydia (20), rickettsia (21), and malaria.[2] However, it is not clear whether other cytokines can also enhance the nonviral antimicrobial activity of human macrophages or its biochemical correlate, their capacity to secrete reactive oxygen intermediates (22). In the present work we tested individually many of

[2] C. F. Ockenhouse, S. Schulman, and H. L. Shear. Induction of crisis forms in the human malaria parasite, *Plasmodium falciparum*, by gamma-interferon activated, monocyte-derived macrophages. Submitted for publication.

the cytokines that affect macrophages, with special emphasis on non-γ IFNs. Our findings are consistent with earlier studies in which all detectable MAF activity was depleted from unfractionated, polyclonally activated mononuclear cell supernatants by monoclonal antibody to IFNγ (1, 2, 20). Thus, when tested in vitro in partially or highly purified form and over a wide range of concentrations, the following affected neither the antitoxoplasma activity of human macrophages nor, with one exception, their secretion of H_2O_2: nIFNα, rIFNαA, rIFNαD, rIFNβ, CSF-1, GM-CSF, p-CSF, MIF, nIL-2, rIL-2, and TNF.

MIF-rich preparations increased macrophage H_2O_2 secretion above control approximately threefold less than rIFNγ. It will be of interest to test the ability of MIF-treated macrophages to kill parasites more susceptible to oxidative injury than toxoplasmas (22). We have not yet studied the effect of pure MIF or of monoclonal anti-MIF antibodies, and thus have not proven that MIF was the active factor from the T-T hybridoma used here. It also remains to be seen if MAF is associated with the MIF produced by uncloned, nontransformed lymphocytes. Further study of MIF is underway.

Thus far, IFNγ appears to be the only known host-derived substance with the ability to activate both the oxidative metabolism and antitoxoplasma activity of human macrophages.

Summary

Cytokines affecting mononuclear phagocytes were screened for activation of human macrophages to secrete H_2O_2 and kill toxoplasmas. In contrast to recombinant interferon-γ (rIFNγ), the following factors, tested in partially or highly purified form and over a wide range of concentrations, did not augment these functions: native interferon-α (nIFNα), rIFNαA, rIFNαD, rIFNβ, colony stimulating factor (type 1) (CSF-1), CSF for granulocytes and macrophages (GM-CSF), pluripotent CSF (p-CSF), tumor necrosis factor (TNF), native interleukin 2 (nIL-2), and rIL-2. Partially purified migration inhibitory factor (MIF) enhanced H_2O_2-releasing capacity submaximally without inducing antitoxoplasma activity, and warrants further study.

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