Immunology 1990 **70** 498–503

Priming the macrophage respiratory burst with IL-4: enhancement with TNF-α but inhibition by IFN-γ

W. A. PHILLIPS, M. CROATTO & J. A. HAMILTON *University of Melbourne, Department of Medicine, Royal Melbourne Hospital, Victoria, Australia*

Accepted for publication 4 May 1990

SUMMARY

Pre-exposure to bacterial lipopolysaccharide (LPS) or certain cytokines is known to enhance the ability of murine macrophages to generate a respiratory burst in response to subsequent stimulation, a phenomenon referred to as 'priming'. We report here that the cytokine interleukin-4 (IL-4) can prime murine macrophages. Pretreatment of murine bone marrow-derived macrophages (BMM) with 10 U/ml murine IL-4 for 48 hr was found to enhance the respiratory burst following subsequent stimulation with phorbol myristate acetate (PMA) (10^{-6} M) or zymosan (1 mg/ml). Human tumour necrosis factor-alpha (TNF-α) (10^{-9} M) can also prime BMM for an enhanced respiratory burst and the combination of TNF-α and IL-4 resulted in an enhanced (greater than additive) priming effect. In contrast, interferon-gamma (IFN-γ) (100 U/ml), although by itself capable of priming the BMM respiratory burst, was found to antagonize the priming effects of IL-4. These results demonstrate that IL-4 can be added to the growing list of cytokines capable of modulating the respiratory burst response of macrophages, suggesting a possible role for this cytokine in inflammation and in the host response to infection. The opposing effects of TNF-α and IFN-γ when interacting with IL-4 highlight the difficulties in predicting the effect of a given cytokine *in vivo*, where potential interactions with other cytokines must be considered.

INTRODUCTION

When macrophages interact with an appropriate stimulus, they respond by generating reduced oxygen species, such as superoxide and hydrogen peroxide, by a process referred to as the 'respiratory burst'. These oxygen-derived metabolites are thought to be important components of the macrophage microbicidal and tumouricidal mechanisms (Johnston, 1978; Badwey & Karnovsky, 1980; Nathan, 1983). Their inadvertent release into surrounding tissues may also have a role in the potentiation of the inflammatory response and associated tissue damage (Halliwell, 1982; Henson & Johnston, 1987).

In murine macrophages, the capacity to produce a respiratory burst is related to the stage of differentiation and activation of the cell (Nathan & Root, 1977; Johnston, Godzik & Cohn, 1978). Peritoneal macrophages from a previously infected host or collected following elicitation in response to an acute inflammatory challenge are able to generate greater quantities of oxygen radicals when stimulated than resident macrophages (Nathan & Root, 1977; Johnston *et al.*, 1978). This increased respiratory burst capacity can be reproduced *in vitro* by exposing the macrophages to certain agents, including bacterial lipopolysaccharide (LPS) and the cytokines interferon-gamma (IFN-γ), tumour necrosis factor-alpha (TNF-α) and granulocyte–macrophage colony-stimulating factor (GM-CSF) (Pabst & Johnston, 1980; Murray, Spitalny & Nathan, 1985; Reed *et al.*, 1987; Phillips & Hamilton, 1989). These agents do not trigger a respiratory burst but only 'prime' the macrophage for an enhanced response to subsequent stimulation.

Interleukin-4 (IL-4) was originally described as B-cell stimulating factor-1 and was believed to be primarily active on B lymphocytes. However, it is now known that IL-4 has effects on a variety of cell types, including both B and T lymphocytes, mast cells and macrophages (Paul & Ohara, 1987; O'Garra *et al.*, 1989). The treatment of murine macrophages with IL-4 stimulates functions associated with differentiation and activation: enhanced antigen-presenting ability (Zlotnik *et al.*, 1987), increased expression of Ia antigens (Crawford *et al.*, 1987; Stuart, Zlotnik & Woodward, 1988), and enhanced tumouricidal (Crawford *et al.*, 1987) and microbicidal (Wirth, Kierszenbaum & Zlotnik, 1989) capacities. IL-4 has also been reported to co-operate with IFN-γ in activating macrophage anti-microbial activity (Belosevic *et al.*, 1988).

In the study reported here, we have used murine bone marrow-derived macrophages (BMM) to investigate the influence of IL-4 on the macrophage respiratory burst. We demonstrate that IL-4 can prime such macrophages for

Correspondence: Dr W. A. Phillips, University of Melbourne, Dept. of Medicine, Royal Melbourne Hospital, Victoria 3050, Australia.

enhanced respiratory burst activity. When tested in combination with other known priming agents, IL-4 was found to co-operate with TNF-α for a greater than additive increase in priming. However, another priming agent IFN-γ, was found to inhibit the priming effects of IL-4.

MATERIALS AND METHODS

Cytokines

Recombinant murine IL-4, purified from *Escherichia coli* (specific activity $\geq 7 \times 10^3$ U/μg, with 1 U/ml giving 50% maximum stimulation of the HT-2 cell line; Mosmann *et al.*, 1986), was a gift from DNAX Research Institute, Palo Alto, CA. Recombinant murine IFN-γ was obtained from Nippon La Roche, Tokyo, Japan, and recombinant human TNF-α was a gift from Dr G. R. Adolf, Ernst-Boehringer Institute, Vienna, Austria.

Other reagents

LPS purified from *E. coli* 0111 : B4 by the Westphal method was obtained from Difco, Detroit, MI. RPMI-1640 was purchased from Flow Laboratories, McLean, VA and fetal calf serum (FCS) was obtained from Commonwealth Serum Laboratories, Parkville, Victoria. Horse heart cytochrome c type III, phorbol myristate acetate (PMA) and zymosan A were obtained from Sigma Chemical Company, St Louis, MO. PMA was stored at −70° as a stock solution of 10^{-2} M in dimethylsulphoxide and was diluted in buffer immediately prior to use. Zymosan was washed in phosphate-buffered saline (PBS) and boiled for 30 min before use. All other chemicals were of analytical grade and were obtained from standard commercial sources.

Mice

Mice (6–12 weeks old) were obtained from the Walter and Eliza Hall Institute, Parkville. The LPS-hyporesponsive strain C3H/HeJ was used throughout.

BMM

Murine BMM were obtained from precursor cells in bone marrow as described before (Vairo & Hamilton, 1985). Femoral bone marrow cells were cultured in 175 cm^2 Lux tissue culture flasks at 2×10^5 cells/cm^2 for 3 days in 50 ml of culture medium (RPMI-1640 supplemented with 20 mM HEPES and 15% heat-inactivated FCS) containing 5×10^{-5} M 2-mercaptoethanol, 0·1 g/l neomycin sulphate and 20% L cell-conditioned medium (prepared as previously described; Vairo & Hamilton, 1985). The non-adherent population, containing primitive precursor cells, was collected and washed and aliquots were cryogenically preserved in liquid nitrogen in growth medium containing 10% dimethylsulphoxide but without L cell-conditioned medium. As required, aliquots were thawed at 37° and the cells washed, seeded into Linbro 24-well (1·7 cm diameter) dishes at 5×10^4 cells in 1 ml of culture medium containing 20% L cell-conditioned medium and grown to confluence (5 days at 37°, 5% CO_2, with an extra 10% L cell-conditioned medium added at Day 3). The cells were then washed three times with sterile PBS to remove non-adherent progenitor cells. The remaining adherent BMM were a relatively pure and homogeneous population with >95% of the adherent cells binding the macrophage-specific colony stimulating factor-1 (Tushinski *et al.*, 1982).

Respiratory burst

The respiratory burst of adherent BMM was assessed by the stimulus-induced reduction of cytochrome c. BMM were cultured for up to 48 hr in RPMI-1640 containing 10% heat-inactivated FCS and appropriate concentrations of cytokine. At the conclusion of this pretreatment the culture medium was removed and replaced with 1 ml of Krebs–Ringer phosphate buffer (121 mM NaCl, 5 mM KCl, 1·3 mM $CaCl_2$, 1·2 mM $MgSO_4$, 3·1 mM NaH_2PO_4, 12·5 mM Na_2HPO_4, and 11 mM glucose, pH 7·3) containing 80 μM cytochrome c and 10^{-6} M PMA or 1 mg/ml zymosan. The cells were incubated for 90 min at 37°, the supernatants collected and the absorbance read at 550 nm. When zymosan was used as a stimulus the supernatants were first clarified by brief centrifugation (30 seconds) in an Eppendorf microfuge. The amount of cytochrome c reduced was calculated by using a differential molar extinction coefficient of $2{\cdot}1 \times 10^4$/M/cm (Johnston *et al.*, 1978) and results expressed as nanomoles of cytochrome c reduced per milligram of cell protein. All experiments were carried out in triplicate.

In selected experiments, the role of superoxide in mediating the stimulus-induced reduction of cytochrome c was confirmed by the inclusion of appropriate controls to which superoxide dismutase was added at a concentration of 30 μg/ml (Johnston *et al.*, 1978).

Protein determination

After removal of the cytochrome c reaction mixture, cell layers were washed with PBS and the total protein in each culture well determined by the Lowry method using bovine serum albumin as a standard. The Lowry procedure was performed directly in the culture plates.

Maintenance of LPS-free conditions

All laboratory ware, if not of a sterile plastic disposable nature, was thoroughly washed and heated to 240° for 3 hr. Media and buffers were filtered through Zetapor membranes (AMF Cuno, Meriden, CT). Reagents were routinely prepared in pyrogen-free water (Abbott Hospital Products, Sydney,) and LPS levels were regularly monitored in all cytokine preparations, media, buffers, and other reagents by using the Limulus lysate assay (Commonwealth Serum Laboratories) in which the minimal detectable level was 10 pg/ml.

RESULTS

Unprimed BMM did not generate a respiratory burst in response to stimulation with PMA. However, 48 hr pre-exposure of the BMM to certain cytokines, including TNF-α, primed the cells, allowing a respiratory burst response following subsequent stimulation (Phillips & Hamilton, 1989). As shown in Fig. 1, pre-exposure of BMM to IL-4 (10 U/ml) also primed BMM for a respiratory burst in response to PMA ($P < 0{\cdot}001$, paired *t*-test, $n = 23$).

Pretreatment of BMM with both IL-4 (10 U/ml) and TNF-α (10^{-9} M) together resulted in an enhancement of the PMA-stimulated respiratory burst, which was significantly greater ($P < 0{\cdot}02$, $n = 23$) than would be predicted on a simply additive basis (Fig. 1).

Figure 2 demonstrates the dose-dependency of priming with IL-4, alone and in combination with a constant level of TNF-α.

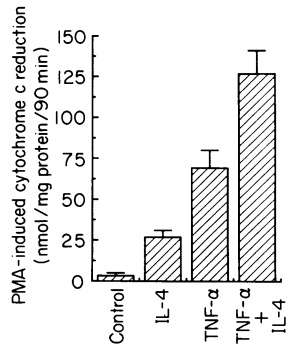

Figure 1. PMA-induced respiratory burst of BMM: priming with IL-4 and TNF-α. BMM were treated for 48 hr with medium alone (control), IL-4 (10 U/ml), TNF-α (10^{-9} M), or IL-4 and TNF-α together. Following this pretreatment period, the cells were washed and the respiratory burst of the adherent BMM assessed by cytochrome c reduction in response to PMA (10^{-6} M). Results shown are mean ± SEM from 23 independent experiments.

Table 1. Priming the respiratory burst of BMM: comparison of IL-4 to LPS

	PMA-induced cytochrome c reduction* (nmol/mg protein/90 min)	
Pretreatment†	–	+TNF-α (10^{-9} M)
Control	7 ± 2‡	32 ± 6
IL-4 (10 U/ml)	35 ± 3§	113 ± 8§
LPS (10 ng/ml)	11 ± 6¶	34 ± 11¶

* Respiratory burst was assessed by cytochrome c reduction in response to 10^{-6} M PMA.

† BMM from LPS-hyporesponsive C3H/HeJ mice were pretreated for 48 hr with medium alone (control), IL-4 (10 U/ml) or LPS (10 ng/ml) in the absence or presence of TNF-α (10^{-9} M).

‡ Shown are mean ± SEM from four independent experiments.

§ $P < 0.05$ compared to control.

¶ Not significantly different from control.

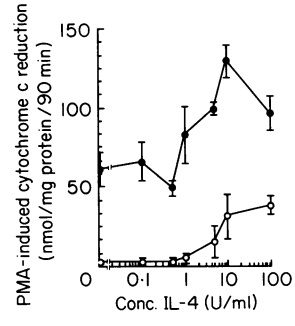

Figure 2. Dose-response of IL-4 effects on priming. BMM were pretreated for 48 hr with increasing concentrations of IL-4, alone (○) or in the presence of 10^{-9} M TNF-α (●). The respiratory burst was assessed in response to PMA (10^{-6} M). Results shown are mean ± SEM from five independent experiments.

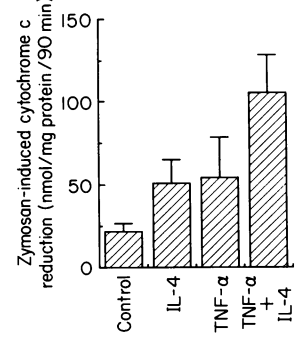

Figure 3. Zymosan-induced respiratory burst of BMM: priming with IL-4 and TNF-α. Experimental protocol and data presentation as for Fig. 1 except that respiratory burst was assessed in response to 1 mg/ml zymosan ($n = 5$).

In the presence of TNF-α (10^{-9} M), concentrations of IL-4 ≥ 5 U/ml resulted in a significantly greater response than TNF-α alone. This combined response was greater than additive at IL-4 concentrations of 5 and 10 U/ml ($P < 0.05$, $n = 3$, and $P < 0.01$, $n = 5$, respectively) but reduced to additive at 100 U/ml IL-4 (Fig. 2). The priming effects of IL-4 alone were apparent at 5 and 10 U/ml, although, in this particular set of experiments, statistical significance ($P < 0.05$) was not achieved until a concentration of 100 U/ml. (It should be noted that in other sets of experiments the priming effect of IL-4 alone at 10 U/ml was significant, see Figs 1 and 4 and Table 1).

In contrast to stimulation with PMA, unprimed BMM did generate a respiratory burst in response to zymosan (Phillips & Hamilton, 1989, 1990). Pre-exposure to IL-4 primed BMM for an increased respiratory burst following stimulation with zymosan ($P < 0.05$, $n = 4$). Again, pretreatment with a combination of both IL-4 (10 U/ml) and TNF-α (10^{-9} M) yielded results that were significantly greater ($P < 0.05$, $n = 4$) than the additive effects of IL-4 and TNF-α alone (Fig. 3).

To eliminate the possibility that undetectable levels of LPS could influence our results, we used cells from LPS-hyporesponsive C3H/HeJ mice in all experiments. Table 1 provides data confirming that these cells are unresponsive to LPS (10 ng/ml) under our experimental conditions, while IL-4 retains its priming effects. In addition, boiling the IL-4 (100°, 20 min) eliminated its priming effects, both alone and in combination with TNF-α ($n = 5$). Also, an ammonium sulphate precipitate of the supernate from the rat hybridoma cell line 11B11, which secretes an anti-murine IL-4 antibody (Ohara & Paul, 1985), was also found to block the priming effects of IL-4 in our system ($n = 3$, data not shown).

As IL-4 was found to co-operate with the priming effects of TNF-α, it was of interest to see if IL-4 would also co-operate with other known priming agents. Figure 4 demonstrates that IFN-γ can prime BMM, although to a lesser extent than IL-4. Combining IL-4 and IFN-γ during the pretreatment period resulted in a stimulated respiratory burst that was considerably

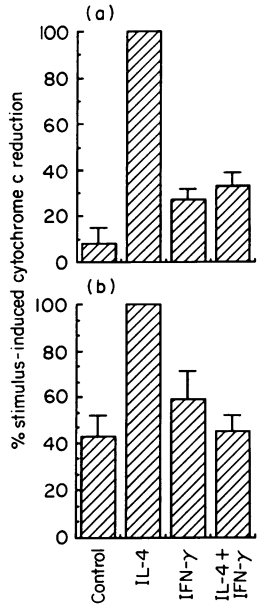

Figure 4. Antagonistic effect of IFN-γ on priming with IL-4. BMM were pretreated for 48 hr with medium alone (control), IL-4 (10 U/ml), IFN-γ (100 U/ml), or IL-4 and IFN-γ together. Data are expressed as a percentage of the response of BMM treated with IL-4 alone. Shown are mean $\pm$ SEM for (a) PMA-induced cytochrome c reduction (100% = 441 $\pm$ 5 nmol/mg protein/90 min, $n = 8$) and (b) zymosan-induced cytochrome c reduction (100% = 92 $\pm$ 26 nmol/mg protein/90 min, $n = 8$).

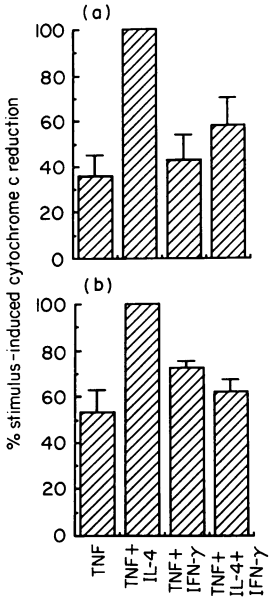

Figure 5. Antagonistic effect of IFN-γ on priming with IL-4 in the presence of TNF-α. Experimental protocol as for Fig. 4 except that all pretreatments were carried out in the presence of 10^{-9} M TNF-α. (a) Data are expressed as a percentage of the response of BMM treated with TNF-α and IL-4. Shown are mean $\pm$ SEM for PMA-induced cytochrome c reduction (100% = 206 $\pm$ 26 nmol/mg protein/90 min, $n = 3$) (b) Zymosan-induced cytochrome c reduction (100% = 155 $\pm$ 17 nmol/mg protein/90 min, $n = 3$).

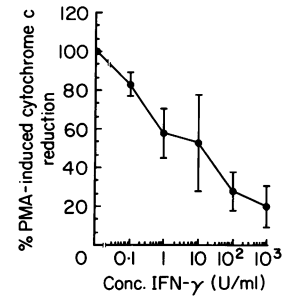

Figure 6. Dose-dependence of IFN-γ antagonism of priming with IL-4. BMM were pretreated for 48 hr with IL-4 (10 U/ml) in the presence of increasing concentrations of IFN-γ. Respiratory burst was assessed in response to 10^{-6} M PMA and results are expressed as a percentage of the response of cells pretreated with IL-4 alone. Shown are mean $\pm$ SEM for four independent experiments (100% = 38 $\pm$ 12 nmol/mg protein/90 min).

less than with IL-4 alone ($P < 0.01$ and $P < 0.02$ when stimulated with PMA and zymosan, respectively) and not statistically different to the result obtained with IFN-γ alone (Fig. 4). The co-operative interaction of IL-4 with TNF-α was also antagonized by IFN-γ ($P < 0.05$) (Fig. 5). The presence of IFN-γ did not, however, significantly influence the priming effects of TNF-α. The dose-dependence of the inhibitory effect of IFN-γ on the priming of the respiratory burst by IL-4 is shown in Fig. 6. Boiling the IFN-γ completely eliminated its ability to antagonize the priming effects of IL-4 ($n = 4$, data not shown).

DISCUSSION

We have previously demonstrated that murine BMM fail to produce a respiratory burst in response to stimulation with PMA unless they have been primed by prior exposure to certain cytokines, including TNF-α, GM-CSF and IFN-γ (Phillips & Hamilton, 1989, 1990). Unprimed BMM do generate a respiratory burst in response to zymosan, and this can be further enhanced by pretreatment with TNF-α or GM-CSF (Phillips & Hamilton, 1990). We report here that, like TNF-α and GM-CSF, IL-4 can also prime murine BMM for an enhanced respiratory burst in response to both PMA and zymosan. Furthermore, low concentrations (5–10 U/ml) of IL-4 together with TNF-α were found to give a greater than additive priming effect (Figs 1–3).

Because bacterial LPS has previously been shown to prime murine macrophages (Pabst & Johnston, 1980; Phillips & Hamilton, 1989), it was of some concern that our IL-4 preparation may contain low (undetectable) levels of LPS. However, a number of points indicate that the effect observed can be attributed to IL-4 and not contaminating LPS. LPS was undetectable (< 10 pg/ml) at culture concentrations of IL-4. All experiments were performed using macrophages from the LPS-hyporesponsive mouse strain C3H/HeJ. Concentrations of LPS up to 100 ng/ml were shown to have no effect on the respiratory burst of these cells and did not synergize with TNF-α (Table 1). In contrast to the co-operativity observed between IL-4 and TNF-α (Figs 1 and 2), in LPS-responsive macrophages LPS has been reported to inhibit the priming effects of TNF-α (Ding &

Nathan, 1987), a result that we have also observed in our laboratory (W. A. Phillips, M. Croatto and J. A. Hamilton, unpublished data). Also, boiling the IL-4 was found to eliminate its effects, a result that would not be expected if the effects were due to contaminating LPS, and a monoclonal antibody against murine IL-4 was found to block the action of IL-4 in our system. We therefore conclude that the effects observed can indeed be attributed to IL-4. Thus IL-4 joins the cytokines such as TNF-α, GM-CSF and IFN-γ, which are able to prime the respiratory burst of murine macrophages.

Given the proposed role for oxygen radicals produced during the respiratory burst in the macrophage microbicidal mechanisms (Johnston, 1978; Badwey & Karnovsky, 1980; Nathan, 1983), the increased respiratory burst response induced by IL-4 could be expected to be translated into increased microbicidal capacity. Consistent with this, Wirth *et al.* (1989) have reported that IL-4 activates murine resident peritoneal macrophages for increased killing of the protozoan parasite *Trypanosoma cruzi* and that this IL-4-mediated increase in parasite killing is reduced by the presence of oxygen radical scavengers. However, using another parasite, *Leishmania major*, others have been unable to demonstrate any increased microbicidal activity in response to IL-4 alone, but observed that IL-4 could co-operate with IFN-γ for a synergistic increase in killing (Belosevic *et al.*, 1988).

The ability of IFN-γ to prime the respiratory burst of murine macrophages is well established (Murray *et al.*, 1985; Reed *et al.*, 1987; Phillips & Hamilton, 1989). However, in contrast to the positive co-operativity observed between IL-4 and TNF-α, IFN-γ was found to negate the primary effects of IL-4 (Figs 4–6). This effect was specific for IL-4, as IFN-γ did not appear to inhibit the priming effect of TNF-α (Fig. 5). Preliminary experiments indicate that IL-4 can also prime murine resident peritoneal macrophages. Although in these cells, in contrast to BMM, IFN-γ (100 U/ml) induces a stronger priming effect than IL-4 (10 U/ml), the combination of both IFN-γ and IL-4 together was not significantly different from IFN-γ alone (data not shown).

The antagonistic effect of IFN-γ on IL-4-induced responses is not restricted to macrophages. IFN-γ has been reported to inhibit IL-4-stimulated B-cell proliferation (Mond *et al.*, 1985), IgE production (Pène *et al.*, 1988) and expression of the IgE receptor (CD23) and class II major histocompatibility antigens (Galizzi *et al.*, 1988; Rousset *et al.*, 1988). However, there are also some reports of positive co-operativity between IL-4 and IFN-γ on macrophage function (Belosevic *et al.*, 1988; Bieber *et al.*, 1989; Littman *et al.*, 1989). The mechanism of IFN-γ antagonism of IL-4-mediated events is not clear although, in lymphocytes at least, it would appear that the inhibitory effect of IFN-γ does not involve changes in the binding of IL-4 to its receptor (Paul & Ohara, 1987; Galizzi *et al.*, 1988).

Oxygen radicals produced by the macrophage respiratory burst are thought to have a role in inflammatory processes (Halliwell, 1982; Henson & Johnston, 1987). IL-4 produced at sites of inflammation may therefore contribute to the pathology of this disease by enhancing the respiratory burst of macrophages, leading to increased inflammatory activity and tissue damage. Similarly, IFN-γ, by virtue of its *in vitro* macrophage priming activity, could also be expected to have a pro-inflammatory effect. However, our results suggest the possible scenario where IFN-γ, due to its inhibitory action on the priming effects of IL-4, could actually have an anti-inflammatory effect by reducing, or restricting, the potential for IL-4-mediated enhancement of the macrophage respiratory burst. Such a situation is consistent with the finding that IFN-γ appears to have a therapeutic value in the treatment of rheumatoid arthritis, despite its *in vitro* macrophage activating (pro-inflammatory) properties (Browning, 1987).

Considering the positive co-operativity observed between IL-4 and TNF-α, it is interesting that IL-4 has been found to down-regulate TNF-α production in human monocytes (Essner *et al.*, 1989; Hart *et al.*, 1989). However, a preliminary report suggesting that in elicited murine macrophages IL-4 can enhance TNF-α release (Somer & Erickson, 1988) raises the possibility that substantial differences may exist in the IL-4-induced responses of different species or different macrophage populations. Studies to clarify this are planned.

We have demonstrated that IL-4 can prime the respiratory burst of murine macrophages for enhanced activity in response to stimulation. This action of IL-4 can be further enhanced by TNF-α or inhibited by IFN-γ. Such co-operative and antagonistic interactions between cytokines highlights the inherent difficulties in extrapolating *in vitro* data with a single cytokine into the *in vivo* situation where multiple cytokines may be present.

Note added in proof

We have recently become aware of a paper (Abramson & Gallin (1990) *J. Immunol.* **144,** 625) reporting that IL-4 inhibits the respiratory burst of human mononuclear phagocytes, a result which appears to be contradictory to our findings. Since recent experiments in our laboratory have also demonstrated a similar inhibitory effect of human IL-4 on human peripheral blood monocytes (unpublished data), it is likely that these opposing results indicate species-dependent effects of IL-4 on the respiratory burst of macrophages as alluded to above.

ACKNOWLEDGMENTS

We wish to thank Dr F. Lee, DNAX Research Institute, for kindly providing IL-4, Dr E. R. Adolf, Ernst-Boehringer Institute, for providing TNF-α, and Dr A. Kelso, Walter and Eliza Hall Institute, for providing anti-murine IL-4 antibody.

This work was supported by a D. W. Keir Fellowship from the Royal Melbourne Hospital (W. A. Phillips) and a Program Grant from the National Health and Medical Research Council of Australia.

REFERENCES

BADWEY J.A. & KARNOVSKY M.L. (1980) Active oxygen species and the functions of phagocytic leukocytes. *Ann. Rev. Biochem.* **49,** 695.

BELOSEVIC M., DAVIS C.E., MELTZER M.S. & NACY C.A. (1988) Regulation of activated macrophage antimicrobial activities. Identification of lymphokines that cooperate with IFN-γ for induction of resistance to infection. *J. Immunol.* **141,** 890.

BIEBER T., RIEGER A., NEUCHRIST C., PRINZ J.C., RIEBER E.P., BOLTZ-NITULESCU G., SCHEINER O., KRAFT D., RING J. & STINGL G. (1989) Induction of FcεR2/CD23 on human epidermal Langerhans cells by human recombinant interleukins 4 and γ interferon. *J. exp. Med.* **170,** 309.

BROWNING J. (1987) Interferons and rheumatoid arthritis: insight into interferon biology? *Immunol. Today,* **8,** 372.

CRAWFORD R.M., FINBLOOM D.S., OHARA J., PAUL W.E. & MELTZER M.S. (1987) B cell stimulatory factor-1 (interleukin 4) activates

macrophages for increased tumoricidal activity and expression of Ia antigens. *J. Immunol.* **139,** 135.

DING A.H. & NATHAN C.F. (1987) Trace levels of bacterial lipopolysaccharide prevent interferon-γ or tumor necrosis factor-α from enhancing mouse peritoneal macrophage respiratory burst capacity. *J. Immunol.* **139,** 1971.

ESSNER R., RHOADES K., MCBRIDE W.H., MORTON D.L. & ECONOMOU J.S. (1987) IL-4 down-regulates IL-1 and TNF gene expression in human monocytes. *J. Immunol.* **142,** 3857.

GALIZZI J.-P., CABRILLAT H., ROUSSET F., MÈNÈTRIER C., DE VRIES J.E. & BANCHEREAU J. (1988) IFN-γ and prostaglandin E_2 inhibit IL-4-induced expression of FcεR2/CD23 on B lymphocytes through different mechanisms without altering binding of IL-4 to its receptor. *J. Immunol.* **141,** 1982.

HALLIWELL B. (1982) Production of superoxide, hydrogen peroxide and hydroxyl radicals by phagocytic cells: a cause of chronic inflammatory disease? *Cell Biol. Internat. Reports*, **6,** 529.

HART P.H., VITTI G.F., BURGESS D.R., WHITTY G.A., PICCOLI D.S. & HAMILTON J.A. (1989) Potential anti-inflammatory effects of interleukin-4. Suppression of human monocyte TNFα, IL-1 and PGE_2 levels. *Proc. natl. Acad. Sci. U.S.A.* **86,** 3803.

HENSON P.M. & JOHNSTON R.B. JR. (1987) Tissue injury in inflammation. Oxidants, proteinases, and cationic proteins. *J. clin. Invest.* **79,** 669.

JOHNSTON R.B. (1978) Oxygen metabolism and the microbicidal activity of macrophages. *Fed. Proc.* **37,** 2759.

JOHNSTON R.B., GODZIK C.A. & COHN Z.A. (1978) Increased superoxide anion production by immunologically activated and chemically elicited macrophages. *J. exp. Med.* **148,** 115.

LITTMAN B.H., DASTVAN F.F., CARLSON P.L. & SANDERS K.M. (1989) Regulation of monocyte/macrophage C2 production and HLA-DR expression by IL-4 (BSF-1) and IFN-γ. *J. Immunol.* **142,** 520.

MOND J.J., FINKELMAN F.D., SARMA C., OHARA J. & SERRATE S. (1985) Recombinant interferon-γ inhibits the B cell proliferative response stimulated by soluble but not by sepharose-bound anti-immunoglobulin antibody. *J. Immunol.* **135,** 2513.

MOSMANN T.R., CHERWINSKI H., BOND M.W., GIEDLIN M.A. & COFFMAN R.L. (1986) Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J. Immunol.* **136,** 2348.

MURRAY M.J., SPITALNY G.L. & NATHAN C.F. (1985) Activation of mouse peritoneal macrophages *in vitro* and *in vivo* by interferon. *J. Immunol.* **134,** 1619.

NATHAN C.F. (1983) Mechanisms of macrophage antimicrobial activity. *Trans. R. Soc. Trop. Med. Hyg.* **77,** 620.

NATHAN C.F. & ROOT R.K. (1977) Hydrogen peroxide release from mouse peritoneal macrophages: dependence on sequential activation and triggering. *J. exp. Med.* **146,** 1648.

O'GARRA A., UMLAND S., DEFRANCE T. & CHRISTIANSEN J. (1989) 'B-cell factors' are pleiotropic. *Immunol. Today*, **9,** 45.

OHARA J. & PAUL W.E. (1985) Production of a monoclonal antibody to and molecular characterization of B-cell stimulatory factor-1. *Nature (Lond.)*, **315,** 333.

PABST M.J. & JOHNSTON R.B. (1980) Increased production of superoxide anion by macrophages exposed *in vitro* to muramyl dipeptide or lipopolysaccharide. *J. exp. Med.* **151,** 101.

PAUL W.E. & OHARA J. (1987) B-cell stimulatory factor-1/interleukin 4. *Ann. Rev. Immunol.* **5,** 429.

PÈNE J., ROUSSET F., BRIÈRE F., CHRÈTIEN I., BONNEFOY J.-Y., SPITS H., YOKOTA T., ARAI N., ARAI K., BANCHEREAU J. & DE VRIES J.E. (1988) IgE production by normal lymphocytes is induced by interleukin 4 and suppressed by interferons γ and α and prostaglandin E_2. *Proc. natl. Acad. Sci. U.S.A.* **85,** 6880.

PHILLIPS W.A. & HAMILTON J.A. (1989) Phorbol ester-stimulated superoxide production by murine bone marrow-derived macrophages requires preexposure to cytokines. *J. Immunol.* **142,** 2445.

PHILLIPS W.A. & HAMILTON J.A. (1990) Colony stimulating factor-1 is a negative regulator of the macrophage respiratory burst. *J. Cell. Physiol.* (in press).

REED S.G., NATHAN C.F., PIHL D.L., RODDRICKS P., SHANEBECK K., CONLAN P.J. & GRABSTEIN K.H. (1987) Recombinant granulocyte/macrophage colony-stimulating factor activates macrophages to inhibit *Trypanosoma cruzi* and release hydrogen peroxide: comparison with interferon. *J. exp. Med.* **166,** 1734.

ROUSSET F., DE WAAL MALEFIJT R., SLIERENDREGT B., AUBRY J.-P., BONNEFOY J.-Y., DEFRANCE T., BANCHEREAU J. & DE VRIES J.E. (1988) Regulation of Fc receptor for IgE (CD23) and class II MHC antigen expression on Burkitt's lymphoma cell line by human IL-4 and IFN-γ. *J. Immunol.* **140,** 2625.

SOMER S.D. & ERICKSON K.L. (1988) Comparison of macrophage activation with interleukin-4 produced by a T-cell hybridoma to gamma interferon. *Proc. AACR*, **29,** 368 (Abstr.).

STUART P.M., ZLOTNIK A. & WOODWARD J.G. (1988) Induction of class I and class II MHC antigen expression on murine bone marrow-derived macrophages by IL-4 (B cell stimulatory factor 1). *J. Immunol.* **140,** 1542.

TUSHINSKI R.J., OLIVER I.T., GUILBERT L.J., TYNAN P.W., WARNER J.R. & STANLEY E.R. (1982) Survival of mononuclear phagocytes depends on a lineage-specific growth factor that the differentiated cells destroy. *Cell*, **28,** 71.

VAIRO G. & HAMILTON J.A. (1985) CSF-1 stimulates Na^+, K^+-ATPase mediated $^{86}Rb^+$ uptake in mouse bone marrow-derived macrophages. *Biochem. Biophys. Res. Commun.* **132,** 430.

WIRTH J.J., KIERSZENBAUM F. & ZLOTNIK A. (1989) Effects of IL-4 on macrophage functions: increased uptake and killing of a protozoan parasite (*Trypanosoma cruzi*). *Immunology*, **66,** 296.

ZLOTNIK A., FISCHER M., ROEHM N. & ZIPORI D. (1987) Evidence for effects of interleukin 4 (B cell stimulatory factor 1) on macrophages: enhancement of antigen presenting ability of bone marrow-derived macrophages. *J. Immunol.* **138,** 4275.