Immunology 1991 **72** 344–349 ADONIS 0019280591000591

IL-4 suppresses IL-1β, TNF-α and PGE_2 production by human peritoneal macrophages

P. H. HART, R. L. COOPER & J. J. FINLAY-JONES *Department of Microbiology and Infectious Diseases, School of Medicine, Flinders University of South Australia, Adelaide, Australia*

Accepted for publication 27 November 1990

SUMMARY

Human interleukin-4 (IL-4) down-regulates IL-1 and tumour necrosis factor-alpha (TNF-α) production by monocytes stimulated *in vitro*. In contrast, in studies of activation of murine macrophages, both stimulatory and inhibitory functions of murine IL-4 have been documented. To investigate whether opposing activities of IL-4 reflect a difference in the target cell studied, due either to cell maturation or the site from which the cells were isolated, we examined the effect of IL-4 on human peritoneal macrophage production of IL-1β, TNF-α and prostaglandin E_2 (PGE_2). Human peritoneal macrophages stimulated with lipopolysaccharide (LPS) produced levels of these mediators that were at least as great as those previously reported for monocytes. Similarly, IL-4 was inhibitory for peritoneal macrophage mediator production after *in vitro* stimulation. Thus, IL-4 has effects on human peritoneal macrophages similar to those on blood monocytes. In addition, as it down-regulates mediator production by cells that have left the circulation, it may be important in controlling the immune response in tissues.

INTRODUCTION

Interleukin-4 (IL-4) is a pleiotropic glycoprotein of approximately 20,000 molecular weight (MW) produced by T lymphocytes, mast cells and bone marrow stromal cells.[1] In addition to its role in B-cell proliferation and T-cell activation and proliferation (reviewed by reference 2), murine IL-4 has been described as a 'macrophage-activating factor'. For murine macrophages, IL-4 induces many cell surface markers similar to those induced on B cells, including class II antigens and CD23.[1,3,4] IL-4 also stimulates murine macrophage tumouricidal activity both *in vitro*[3] and *in vivo*[5] and enhances murine macrophage phagocytosis and killing of the parasite *Trypanosoma cruzi*.[6] IL-4 primes the respiratory burst of murine bone marrow-derived macrophages.[7] In support of this macrophage-activating activity, some studies have shown that IL-4 can increase murine peritoneal macrophage production of tumour necrosis factor (TNF)[8] and IL-1[9]. However, more recently, IL-4 down-regulated IL-2- and lipopolysaccharide (LPS)-induced TNF production by murine macrophages both *in vitro* and *in vivo*[10] and, in another study, IL-4 expression was associated with the progression of murine leishmaniasis.[11]

In contrast, human IL-4 suppresses superoxide generation by activated monocytes,[12] and does not stimulate monocyte tumouricidal activity.[13] We[14] and others[15 17] have reported that, in humans, IL-4 suppresses monocyte pro-inflammatory mediator production, with the suppression determined, at least in part, at the mRNA level.[14,15,17] There is now some evidence that the previously reported IL-4-induced HLA-DR expression on monocytes is due to interferon-gamma (IFN-γ).[18] Complementing its anti-inflammatory role, other studies have identified IL-4 as a differentiative signal for human monocytes.[16]

There may be several explanations for this apparent discrepancy between species in the stimulatory/suppressive activity of IL-4 on cells of the macrophage lineage. With few exceptions,[7] studies *in vitro* with murine macrophages have used resident or elicited peritoneal cells in contrast to studies in humans, which used circulating monocytes. The former may have peritoneal tissue- or differentiation-determined cell properties which influence the cells' response to IL-4. This possibility was suggested by Stuart *et al.*,[4] who detected differing effects of IL-4 on murine bone marrow-derived and thioglycollate-elicited macrophages. Alternatively, IL-4 may have contrasting effects *per se* on monocytes/macrophages from different species.

In this study, we have isolated human peritoneal macrophages from the overnight peritoneal effluents of continuous ambulatory peritoneal dialysis (CAPD) patients with bacterial peritonitis. On a per cell basis, these cells responded to LPS stimulation by production of levels of IL-1β, TNF-α and prostaglandin E_2 (PGE_2) not lower than those previously reported for human monocytes.[14] Increasing concentrations of recombinant human IL-4 suppressed, in a dose-dependent manner, macrophage production of IL-1β, TNF-α and PGE_2, and suggested that the anti-inflammatory properties of human

Correspondence: Dr P. Hart, Dept. of Microbiology and Infectious Diseases, School of Medicine, Flinders University of South Australia, G.P.O. Box 2100, Adelaide 5001, Australia.

IL-4 described for peripheral blood monocytes are relevant to extravasated monocytes, i.e. peritoneal macrophages.

MATERIALS AND METHODS

Reagents

Reagents were obtained as gifts as indicated: recombinant human IL-4 (Dr F. Lee, DNAX, Palo Alto, CA), $2{\cdot}1 \times 10^7$ U/mg, of which 50 U IL-4/ml were functionally equivalent to 2·5 U/ml of the IL-4 preparation previously used;[14] recombinant human IFN-γ, 10^6 U/ml (Lot no. 302, Ro 23-4400; Hoffmann-La Roche, Nutley, NJ); recombinant human TNF-α, $2{\cdot}5 \times 10^7$ U/mg, and a monoclonal antibody (mAb) to TNF-α with a neutralization titre of 6000 units of TNF-α per μg mAb (Dr G. R. Adolf, Ernst-Boehringer Institut, Vienna, Austria); polyclonal rabbit anti-TNF-α for ELISA (Professor I.F.C. McKenzie, University of Melbourne, Australia); mAb to IL-1β (H6) and the biotinylated form of another anti-IL-1β mAb (H67) (Professor A. C. Allison, Syntex, Palo Alto, CA); and mAb (FMC32) to CD14 (Professor H. Zola, Flinders Medical Centre, South Australia).

Macrophage isolation

Overnight (approximately 8 hr) peritoneal effluents (1·4–1·9 litres) were obtained from seven CAPD patients with bacterial peritonitis. Three patients were diagnosed with *Staphylococcus epidermidis* infections, three with *Klebsiella* sp. and one with *Moraxella* sp. Effluents were centrifuged for 15 min at 400 ***g*** prior to pellet resuspension in RPMI-1640 medium (Flow Laboratories, McLean, VA) supplemented with 2 mM MOPS (Sigma, St Louis, MO), 13·3 mM $NaHCO_3$, 2 mM glutamine, 50 μM 2-mercaptoethanol, 100 U/ml penicillin and 100 μg/ml streptomycin (subsequently referred to as 'complete RPMI'), with an osmolality of 290 mmol/kg H_2O. Cell suspensions were layered over Lymphoprep gradients (Nycomed, Oslo, Norway) and centrifuged (30 min, 500 ***g***) following the manufacturer's instructions. Buffy coat cells were removed, pelleted and resuspended as before for a second passage over an identical Lymphoprep gradient. Buffy coat cells were washed twice with, and finally resuspended in, complete RPMI. Macrophage enrichment to $90 \pm 7\%$ (mean $\pm$ SD, $n = 7$) was determined by cell morphology on Giemsa-stained cytocentrifuge smears and by staining for CD14 with mAb FMC 32.[19] Isolated peritoneal macrophages were approximately 95% peroxidase positive as determined by the Sudan black method. Polymorphonuclear cells were the main contaminant ($9 \pm 6\%$, $n = 7$); contaminating lymphocytes were also present.

Maintenance of LPS-free conditions

During macrophage isolation and subsequent culture, extreme care was taken to limit LPS contamination of isolation fluids;[14,20] these precautions included use of disposable plasticware whenever possible, soaking of glassware in E-Toxaclean (Sigma), and filtering of buffers and culture medium through Zetapor filters (Cuno, Meriden, CT). LPS levels < 50 pg/ml in all reagents were confirmed in the Limulus lysate assay (Commonwealth Serum Laboratories, Melbourne, Australia).

Macrophage culture

As previously described for human monocytes,[14,20] 10^6 peritoneal macrophages were cultured in 1 ml complete RPMI medium supplemented with 1% (v/v) foetal calf serum in 2-cm^2 tissue culture plastic wells (Linbro, Flow). IL-4 was added from 0·5 to 250 U/ml; IFN-γ was added at 100 U/ml. Where indicated, LPS from *Escherichia coli* 0111:B4, purified by the Westphal method (Difco, Detroit, MI), was added to a final concentration of 100 ng/ml. Polymyxin-B sulphate, which inhibits LPS binding to cell membranes, was added at 1 μg/ml to cultures to which LPS was not deliberately added. Triplicate cultures for each test variable were incubated at 37° in 5% CO_2/95% air for 18 hr. To terminate, culture supernatants were removed and centrifuged to remove non-adherent cells.[14,20] Supernatants were stored frozen. All assays for mediators were performed within three freeze-thaw cycles of the samples.

By trypan blue staining, all cells were viable after the 18 hr culture period. We have previously shown with human monocytes that under these conditions, and using the lymphokines IL-4 and IFN-γ, there is no change in the number of cells recovered.[14] Thus, macrophage mediator activities have been expressed according to the number of cells at the beginning of the 18 hr culture.

Assays of IL-1β

The activity of IL-1 was measured by the murine thymocyte comitogenesis assay using a final concanavalin A concentration of 0·2 μg/ml.[14,20] One unit of IL-1 activity was defined as the amount that stimulated 50% maximal thymocyte proliferation; the units of IL-1 activity were expressed as the reciprocal of the dilution that induced half maximal [^{3}H]thymidine (TdR) incorporation. IL-1β (Boehringer, Mannheim, Germany, Cat No. 1 059 394) was used as the standard. None of the lymphokines or LPS used to stimulate the macrophages *in vitro* acted as a comitogen for thymocytes under the conditions described.

Immunoreactive IL-1β was assayed as previously described.[14,21] IL-1β concentrations were calculated from a standard curve prepared with an IL-1β standard from the National Institute for Biological Standards and Control, Hampstead, London, U.K.; the assay was sensitive to $\geqslant 0{\cdot}02$ ng IL-1β/ml.

Assays of TNF-α

TNF-α was measured functionally with actinomycin D-treated L929 target cells.[14,22] One unit of TNF-α activity was defined as that causing a 50% absorbance change of trypan blue-stained L929 cells; the units of TNF-α activity were expressed as the reciprocal of the dilution necessary to achieve this 50% absorbance change. One unit of TNF-α/ml approximated 0·4 pM. Confirmation of TNF-α as the L929 cytotoxic activity in macrophage supernatants was performed using an anti-TNF-α mAb.

Immunoreactive TNF-α was measured by a sandwich ELISA and a biotinylated rabbit anti-human TNF-α as previously described.[23] The assay was sensitive to levels of TNF-α of $\geqslant 0{\cdot}04$ ng/ml.

Assay of PGE_2

Levels of PGE_2 in macrophage culture supernatants ($\geqslant 0{\cdot}03$ ng/ml) were determined by immunoassay using competitive adsorption to dextran-coated charcoal[14,20] (^{3}H-PGE_2; Amersham, Amersham, Bucks, U.K. PGE_2 antiserum, Advanced Magnetics, Cambridge, MA).

Table 1. IL-1β, TNF-α and PGE_2 levels detected in the supernatants of unstimulated and LPS-treated peritoneal macrophages

	Macrophages*	
	Unstimulated	+LPS (100 ng/ml)
IL-1β		
U/10^6 cells	5·4±2·9 (7)	382±119 (7)
ng/10^6 cells	ND (7)	1·0±0·6 (7)
TNF-α		
U/10^6 cells	0·7±0·7 (7)	103±42 (7)
ng/10^6 cells	0·2±0·1 (5)	2·7±0·7 (7)
PGE_2		
ng/10^6 cells	1·6±0·7 (4)	44·9±8·8 (7)

* Peritoneal macrophages (10^6 in 1 ml) were incubated for 18 hr without and with LPS (100 ng/ml). IL-1, TNF-α and PGE_2 were measured in the culture supernatants. Data are mean±SEM (n); ND, not detected.

Expression of results

Unless otherwise indicated, mean values±SEM for measurements in supernatants from triplicate cultures have been presented. The significance of differences between donors, and between treatment groups for a single donor, was assessed by using a two-tailed Student's t-test. The results were considered significantly different if $P<0·05$.

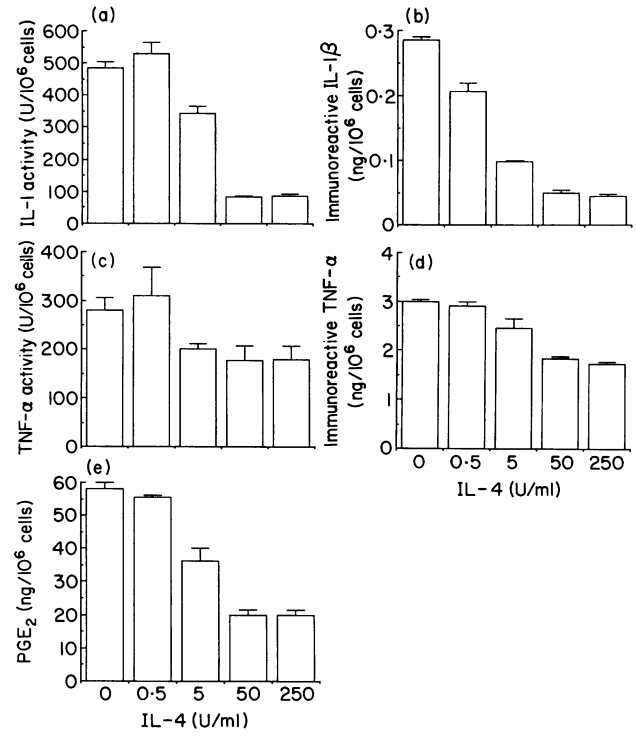

Figure 1. Mediator production by LPS-stimulated peritoneal macrophages from a representative donor. Effect of increasing concentrations of IL-4. Mean activites/levels (±SEM) for supernatants from triplicate cultures. (a) IL-1 activities (b) IL-1β levels. (c) TNF-α activities. (d) TNF-α levels. (e) PGE_2 levels.

RESULTS

LPS stimulation of human peritoneal macrophages for IL-1β, TNF-α and PGE_2 production

It was first necessary to determine whether peritoneal macrophages produced sufficient levels of mediators for up- and down-regulatory studies of their production by lymphokines. Table 1 demonstrates that there were low levels of IL-1β, TNF-α and PGE_2 secreted by human peritoneal macrophages when cultured *in vitro* under LPS-free conditions; in fact, for macrophages from several donors, these mediators were not detected. When incubated with LPS (100 ng/ml) for 18 hr, levels of IL-1β, TNF-α and PGE_2 were at least comparable to, and in many cases greater than, those produced by monocytes (Table 1).[14] Thus, human peritoneal macrophages, although isolated from an inflammatory environment, did not show significant residual stimulation for mediator production *in vitro*. Furthermore, LPS stimulated these cells *in vitro* for significant mediator production.

Effect of IL-4 on levels of macrophage IL-1

For four of the seven macrophage donors examined, IL-1 activity was not detected in the supernatants of unstimulated cells. For the other three donors, IL-4 concentrations of ⩾50 U/ml inhibited the detectable expression of a low level of endogenous activity. Figure 1 shows the response to IL-4 by

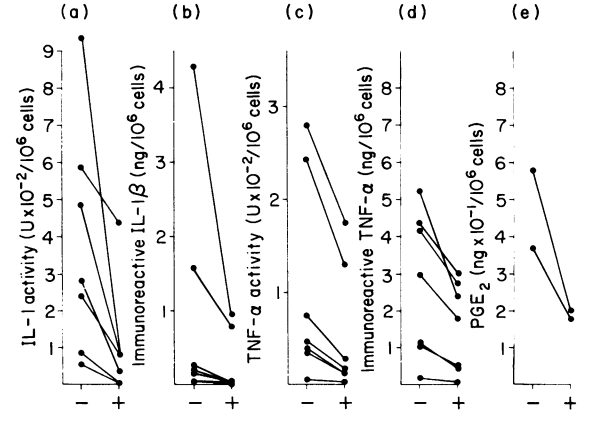

Figure 2. Production of mediators by human peritoneal macrophages exposed to LPS, and to LPS with IL-4. Macrophages from seven donors were incubated for 18 hr with LPS (100 ng/ml) together without (marked −) and with (marked +) IL-4 (50 U/ml). With the exception of PGE_2, mediators were measured in the culture supernatants of all seven donors. For PGE_2, levels were measured for only two donors. Each point represents the mean activity for triplicate cultures. (a) IL-1 activities. (b) IL-1β levels. (c) TNF-α activities. (d) TNF-α levels. (e) PGE_2 levels.

LPS-stimulated macrophages from a typical donor. Increasing concentrations of IL-4 suppressed the IL-1 activity produced in response to LPS (Fig. 1a). This was in contrast to the activities induced with IFN-γ and LPS, i.e. to 820 ± 70 U/10^6 cells (mean $\pm$ SEM) for triplicate cultures. Although the response to IL-4 varied from donor to donor, for six of seven donors significant suppression was seen in response to 5 U IL-4/ml ($P < 0{\cdot}05$). Maximal suppression by IL-4 was generally seen with exposure to 50 U IL-4/ml. When data from seven donors were considered, 50 U IL-4/ml reduced the mean ($\pm$SEM) activity induced by LPS from 382 ± 119 U/10^6 cells to 102 ± 57 U/10^6 cells ($P < 0{\cdot}05$, Fig. 2a). For macrophages from seven donors, IFN-γ (100 U/ml) enhanced LPS-induced activities to 520 ± 150 U IL-1/10^6 cells; this activity was reduced by 50 U IL-4/ml to 149 ± 64 U/10^6 cells ($P < 0{\cdot}05$).

In order to rule out the possibility that IL-4 induced IL-1 inhibitors, IL-1β protein levels were measured by immunoassay. IL-1β antigen was regulated by IL-4 in an identical manner to that reported above for IL-1 activity (Fig. 1b). Immunoreactive IL-1β was not detected in the supernatants of control or IL-4-stimulated cells from any donor. For six of seven donors, significant inhibition of LPS-induced levels was again seen in response to 5 U IL-4/ml ($P < 0{\cdot}05$). IL-4 (50 U/ml) reduced LPS-induced IL-1β levels from $1{\cdot}0 \pm 0{\cdot}6$ to $0{\cdot}3 \pm 0{\cdot}2$ ng/10^6 cells (mean $\pm$ SEM, $n = 7$) (Fig. 2b). For the same seven donors, IFN-γ (100 U/ml) plus LPS stimulation resulted in the production of $1{\cdot}3 \pm 0{\cdot}8$ ng IL-1β/10^6 cells, and addition of IL-4 (50 U/ml) reduced levels to $0{\cdot}5 \pm 0{\cdot}2$ ng/10^6 cells ($P < 0{\cdot}05$). Loss of activity during freeze-thawing of samples may partially explain the relative differences between functional and immunoreactive levels of IL-1.

Effect of IL-4 on levels of macrophage TNF-α

We next determined whether IL-4 inhibited the production another macrophage pro-inflammatory mediator, TNF-α. Significant TNF-α activity was detected in the supernatants of unstimulated macrophages from two of the seven donors examined; for both donors, this activity was reduced to non-detectable levels by $\geqslant 50$ U IL-4/ml. When macrophages from seven donors were incubated with LPS, 103 ± 42 U TNF-α/10^6 cells (mean $\pm$ SEM) were measured. For five of seven donors, a significant decrease of LPS-induced activity was seen in response to 5 U IL-4/ml (Fig. 1c), while for the remaining two donors, 50 U IL-4/ml were necessary. Co-incubation with IL-4 (50 U/ml) resulted in a significant reduction of macrophage-secreted TNF-α activity to 55 ± 26 U/10^6 cells (mean $\pm$ SEM, $n = 7$, $P < 0{\cdot}05$, Fig. 2c). In response to IFN-γ (100 U/ml) with LPS, TNF-α levels increased to 177 ± 77 U/10^6 cells, and were suppressed by IL-4 (50 U/ml) to 122 ± 60 U/10^6 cells ($n = 7$).

Immunoreactive TNF-α production was also suppressed by IL-4 (Fig. 1d). When seven donors were considered, the levels detected for unstimulated cells ($0{\cdot}2 \pm 0{\cdot}1$) were reduced to $0{\cdot}1 \pm 0{\cdot}1$ by 50 U IL-4/ml. LPS stimulated supernatant TNF-α levels to $2{\cdot}7 \pm 0{\cdot}7$ ng/10^6 cells (mean $\pm$ SEM, $n = 7$); IL-4 (50 U/ml) reduced these levels to $1{\cdot}6 \pm 0{\cdot}5$ ng/10^6 cells ($P < 0{\cdot}02$, Fig. 2d). For seven donors, IFN-γ enhanced LPS-induced TNF-α production to $4{\cdot}7 \pm 1{\cdot}7$ ng/10^6 cells; this activity was reduced to $3{\cdot}11 \pm 0{\cdot}8$ ng/10^6 cells by 50 U IL-4/ml ($n = 7$).

Effect of IL-4 on levels of macrophage PGE_2

PGE_2 levels in the supernatants of LPS-stimulated peritoneal macrophages were approximately three-fold greater than those previously measured by us for human monocytes;[14] these levels were suppressed by increasing concentrations of IL-4 (Figs 1e and 2e). For the two donors for whom sufficient supernatants remained for PGE_2 assay, 50 U IL-4/ml maximally inhibited PGE_2 production, with LPS-stimulated levels of 58 and 37 ng/10^6 cells reduced to 20 and 17 ng/10^6 cells, respectively (Fig. 2e). As previously reported for human monocytes, IFN-γ (100 U/ml) did not have an effect on macrophage PGE_2 levels. For macrophages from three donors, LPS induced 34 ± 14 ng/10^6 cells (mean $\pm$ SEM), and LPS plus IFN-γ induced 31 ± 13 ng/10^6 cells ($P > 0{\cdot}05$).

DISCUSSION

Murine peritoneal macrophages have been used much more frequently than human peritoneal macrophages in cytokine studies, presumably because of the comparatively ready availability of human peripheral blood. A small number of studies have used peritoneal macrophages obtained from healthy women undergoing laparoscopy,[24] or from CAPD patients who have been peritonitis-free for at least a month.[25] In this study, we report the use of human macrophages from peritoneal effluents obtained from patients with bacterial peritonitis. It is unknown to what extent these cells were activated *in vivo*. However, after several washings and purification *in vitro*, these cells secreted minimal, if any, of the examined products of activation (IL-1, TNF-α and PGE_2) (Table 1). It has also been suggested that inflammatory peritoneal macrophages may be old, exhausted and dying. However, several studies have shown that, when incubated *in vitro* with opsonins, inflammatory macrophages from CAPD patients have phagocytic and bactericidal properties equivalent to those of freshly isolated phagocytic cells (for example reference 26). Of cells isolated from the peritoneal cavity of CAPD patients without peritonitis ($4\text{–}8 \times 10^6$ cells from an overnight 'dwell' of 2 litres), macrophages tend to be the predominant type.[25,27] These cells produce cytokines in response to LPS (data not shown). However, insufficient cells from the same donor were obtainable for purification of macrophages and analysis of their response to multiple agents/concentrations.

The production of cytokines by peritoneal macrophages as measured in this study compares favourably with those reported for alveolar macrophages. Several studies have documented an approximately three-fold greater production of TNF-α by alveolar macrophages than by monocytes in response to LPS.[28,29] In contrast, alveolar macrophages secrete into the supernatant 25–100% of the IL-1β activity measured for monocytes.[28–30] We have found that peritoneal macrophages, when stimulated by LPS, secrete levels of IL-1, TNF-α and PGE_2 approximately equal, three- to five-times and three-times, respectively, greater than those reported for monocytes.[14] Alveolar macrophages also secrete greater amounts of endogenous and LPS-stimulated PGE_2 than monocytes.[28,30] Thus, by the levels of TNF-α, IL-1β and PGE_2 produced by peritoneal macrophages, this cell can be compared favourably with the alveolar macrophage, one of the most studied human examples

of a tissue-associated monocyte-derived macrophage. However, peritoneal macrophages are easier to obtain, and the number of cells which can be isolated is greater.

In this study, and as previously reported by us[14] and others[15] for monocytes, IL-4 inhibited IL-1β production more efficiently than TNF-α production (Figs 1 and 2). Several studies have reported differential control of the production by monocytes of these two inflammatory mediators.[20] Several groups have reported that IL-4 acts, at least in part, at the mRNA level.[14,15,17] We speculate that the relative difference in the inhibitory activity of IL-4 may reflect that monocyte IL-1β production is controlled predominantly at the transcriptional level, while TNF-α production is under much post-transcriptional regulation.[31]

From this study, we conclude that the anti-inflammatory activity of IL-4 is not unique for circulating peripheral blood monocytes, but is also seen for peritoneal macrophages, which result from extravasation of monocytes into the peritoneal cavity. The extent of priming or differentiation of peritoneal macrophages, as determined by expressed antigens, is the subject of ongoing studies. However, this study has provided a direct comparative experiment to the murine peritoneal macrophage studies. The results reported here do not support any suggestion, therefore, that the differing activities documented for murine IL-4 relate to the source or age of the target cells. It remains possible that the biological actions of IL-4 may differ between species. That the actions of IL-4 may vary between species has been suggested not only for macrophages, but also for induction of lymphokine-activated killer cells,[32,33] and also for the control of Ig isotype from B cells (reviewed by reference 2). For other interleukins, inter-species differences in biological activities have also been reported; murine IL-5 has been confirmed as a B-cell activation signal while its human counterpart was not active in a series of B-cell activation assays (reviewed by reference 2).

IFN-γ enhanced IL-1β and TNF-α production by LPS-stimulated macrophages. In contrast, IL-4 suppressed mediator production induced by LPS. Similar opposing activities for IL-4 and IFN-γ were noted with monocytes.[14,17] This result might provide further impetus to the search for human analogues of the murine helper T-cell subsets,[34] clones of which produce IFN-γ or IL-4. With opposing activities of IL-4 and IFN-γ on macrophages, the balance of the production of these lymphokines may determine the suppression or development, respectively, of inflammation.

We have previously reported an augmentation by IL-4 of the suppressive activity of glucocorticoids on monocytes.[35] We have suggested that steroids, supplemented with low doses of IL-4, may provide an improved treatment, for example, for the chronically inflamed rheumatoid arthritic joint. We feel that the peritoneal macrophage is more similar to the macrophage of joint tissue and synovial fluid because it has also moved from the bloodstream, most probably by chemotaxis, to the inflamed tissue. That tissue macrophages, and not only blood monocytes, can respond to IL-4 by inhibition of the production of IL-1, TNF-α and PGE_2, strengthens the importance of this finding.

ACKNOWLEDGMENTS

We would like to thank all those who supplied biological reagents for this study, particularly Dr F. Lee (DNAX, Palo Alto, CA). This work was supported by grants from the National Health and Medical Research Council of Australia.

REFERENCES

1. Paul W.E. & Ohara J. (1987) B-cell stimulatory factor-1/interleukin-4. *Ann. Rev. Immunol.* **131,** 429.
2. Mizel S.B. (1989) The interleukins. *FASEB J.* **3,** 2379.
3. Crawford R.M., Finbloom D.S., Ohara J., Paul W.E. & Meltzer M.S. (1987) B cell stimulatory factor-1 (interleukin-4) activated macrophages for increased tumoricidal activity and expression of Ia antigens. *J. Immunol.* **139,** 135.
4. 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.
5. Tepper R.I., Pattengale P.K. & Leder P. (1989) Murine interleukin-4 displays potent anti-tumor activity *in vivo*. *Cell,* **57,** 503.
6. 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.
7. Phillips W.A., Croatto M. & Hamilton J.A. (1990) Priming of the macrophage respiratory burst with IL-4: synergy with TNF-α but inhibition by IFN-γ. *Immunology,* **70,** 498.
8. Somers S.D. & Eriksen K.L. (1988) Comparison of macrophage activation with interleukin-4 produced by a T-cell hybridoma to gamma interferon. *Proc. AACR,* **29,** 368 (Abs).
9. Wolpe S.D., Davatelis G., Luedke C. & Cerami A. (1987) Differential regulation of cachectin/TNF and IL-1 by IL-4 (BSF-1). *J. Leuk. Biol.* **42,** 359 (Abs.).
10. McBride W.H., Economou J.S., Nayersina R., Comora S. & Essner R. (1990) Influences of interleukins 2 and 4 on tumor necrosis factor production by murine mononuclear phagocytes. *Cancer Res.* **50,** 2949.
11. Heinzel F.P., Sadick M.D., Holaday B.J., Coffman R.L. & Locksley R.M. (1989) Reciprocal expression of interferon γ or interleukin 4 during the resolution or progression of murine leishmaniasis. Evidence for expansion of distinct helper T cell subsets. *J. exp. Med.* **169,** 59.
12. Abramson S.L. & Gallin J.I. (1990) IL-4 inhibits superoxide production by human mononuclear phagocytes. *J. Immunol.* **144,** 625.
13. Hudson M.M., Markowitz A.B., Gutterman J.U., Knowles R.D., Snyder J.S. & Kleinerman E.S. (1990) Effect of recombinant human interleukin 4 on human monocyte activity. *Cancer Res.* **50,** 3154.
14. Hart P.H., Vitti G.F., Burgess D.R., Whitty G.A., Piccoli D.S. & Hamilton J.A. (1989) Potential antinflammatory effects of interleukin 4: Suppression of human monocyte tumor necrosis factor α, interleukin 1, and prostaglandin E_2. *Proc. natl. Acad. Sci. U.S.A.* **86,** 3803.
15. Essner R., Rhoades K., McBride W.H., Morton D.L. & Economou J.S. (1989) IL-4 down-regulates IL-1 and TNF gene expression in human monocytes. *J. Immunol.* **142,** 3857.
16. Te Velde A.A., Klomp J.P.G., Yard B.A., deVries J.E. & Figdor C.G. (1988) Modulation of phenotypic and functional properties of human peripheral blood monocytes by IL-4. *J. Immunol.* **140,** 1548.
17. Donnelly R.P., Fenton M.J., Finbloom D.S. & Gerrard T.L. (1990) Differential regulation of IL-1 production in human monocytes by IFN-γ and IL-4. *J. Immunol.* **145,** 569.
18. 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.
19. Hancock W.W., Zola H. & Atkin R.C. (1983) Antigenic heterogeneity of human mononuclear phagocytes: immunohistologic analysis using monoclonal antibodies. *Blood,* **62,** 1271.
20. Hart P.H., Whitty G.A., Piccoli D.S. & Hamilton J.A. (1988) Synergistic activation of human monocytes by granulocyte-macrophage colony stimulating factor and interferon-γ. Increased tumor necrosis factor α but not IL-1 activity. *J. Immunol.* **141,** 1516.

21. KENNEY J.S., MASADA M.P., EUGUI E.M., DELUSTRO B.M., MULKINS M.A. & ALLISON A.C. (1987) Monoclonal antibodies to human recombinant interleukin 1 (IL-1)β: quantitation of IL 1β and inhibition of biological activity. *J. Immunol.* **138,** 4236.
22. ROBBINS D.S., SHIRAZI Y., DRYSDALE B.-E., LIEBERMAN A., SHIN H.S. & SHIN M.L. (1987) Production of cytotoxic factor for oligodendrocytes by stimulated astrocytes. *J. Immunol.* **139,** 2539.
23. MCLAUGHLIN J.P., ELWOOD N.J., RAMADI L.T., PICA M.R. & MCKENZIE I.F.C. (1990) Improvement in sensitivity of enzyme-linked immunosorbent assay for tumour necrosis factor. *Immunol. Cell Biol.* **68,** 51.
24. BECKER S., JOHNSON C., HALME J. & HASKILL S. (1986) Interleukin-1 production and antigen presentation by normal human peritoneal macrophages. *Cell. Immunol.* **98,** 467.
25. DAVIES S.J., SUASSUNA J., OGG C.S. & CAMERON J.S. (1989) Activation of immunocompetent cells in the peritoneum of patients treated with CAPD. *Kidney Int.* **36,** 661.
26. VERBRUGH H.A., KEANE W.F., HOIDAL J.R., FREIBERG M.R., ELLIOTT G.R. & PETERSON P.K. (1983) Peritoneal macrophages and opsonins: Antibacterial defense in patients undergoing chronic peritoneal dialysis. *J. infect. Dis.* **147,** 1018.
27. CICHOCKI T., HANICKI Z., SULOWICZ W., SMOLENSKI O., KOPEC J. & ZEMBLA M. (1983) Output of peritoneal cells into peritoneal dialysate. *Nephron,* **35,** 175.
28. WEWERS M.D. & HERZYK D.J. (1989) Alveolar macrophages differ from blood monocytes in human IL-1β release. *J. Immunol.* **143,** 1635.
29. THOMASSEN M.J., BARNA B.P., RANKIN D., WIEDEMANN H.P. & AHMAD M. (1989) Differential effect of recombinant granulocyte macrophage colony-stimulating factor on human monocytes and alveolar macrophages. *Cancer Res.* **49,** 4086.
30. IWAMOTO G.K., MONICK M.M., BURMEISTER L.F. & HUNNINGHAKE G.W. (1989) Interleukin 1 release by human alveolar macrophages and blood monocytes. Am. J. Physiol. **256,** C1012.
31. HAN J., BROWN T. & BEUTLER B. (1990) Endotoxin-responsive sequences control cachectin/tumor necrosis factor biosynthesis at the translational level. *J. exp. Med.* **171,** 465.
32. MULE J.J., SMITH C.A. & ROSENBERG S.A. (1987) Interleukin 4 (B cell stimulatory factor 1) can mediate the induction of lymphokine-activated killer cell activity directed against fresh tumor cells. *J. exp. Med.* **166,** 792.
33. WIDMER M.B., ACRES R.B., SASSENFELD H.M. & GRABSTEIN K.H. (1987) Regulation of cytolytic cell populations from human peripheral blood by B cell stimulatory factor 1 (interleukin 4). *J. exp. Med.* **166,** 1447.
34. MOSMANN T.R. & COFFMAN R.L. (1987) Two types of mouse helper T-cell clone. Implications for immune regulation. *Immunol. Today,* **8,** 223.
35. HART P.H., WHITTY G.A., BURGESS D.R., CROATTO M. & HAMILTON J.A. (1990) Augmentation of glucocorticoid action on human monocytes by interleukin-4. *Lymphokine Res.* **9,** 147.