

## PDF hosted at the Radboud Repository of the Radboud University Nijmegen

This full text is a publisher's version.

For additional information about this publication click this link.

<http://hdl.handle.net/2066/14799>

Please be advised that this information was generated on 2014-11-11 and may be subject to change.

JIM 04672

## The influence of culture conditions and serum lipids on interleukin-1 production by human monocytes

Jos W.M. Van der Meer<sup>1</sup>, Joke S. Van de Gevel<sup>1</sup>, Victor W.M. Van Hinsbergh<sup>2</sup>  
and Peter C.J. Leijh<sup>1</sup>

<sup>1</sup> Department of Infectious Diseases, University Hospital Leiden, Leiden, The Netherlands,  
and <sup>2</sup> Gaubius Institute, Health Division TNO, Leiden, The Netherlands

(Received 17 August 1987, accepted 22 October 1987)

Reliable assessment of IL-1 production by human monocytes is critically dependent on the methods for isolation and culture of these cells. In the present study, the quality of pipettes and the preparation of Ficoll-Isopaque appear to be crucial for IL-1 production from both LPS-stimulated and unstimulated monocytes. Different brands and lots of polystyrene culture wells give rise to great variation in IL-1 production. When carefully prepared, hydrophobic teflon membranes, to which mononuclear phagocytes poorly adhere, are used as the culture substrate, stimulation of IL-1 production is observed.

The HLA DR3 haplotype of the monocyte donors did not influence IL-1 production. The addition of normal human AB serum to the cultures usually increases IL-1 production, although strong inhibition of both unstimulated and LPS-stimulated IL-1 production was also observed after addition of a diet-induced hyperlipemic AB serum. This inhibition was not due to cholesterol, chylomicrons, high- or low-density lipoproteins.

When monocytes were cultured at different temperatures, the only abnormality found was a decrease of cell-associated IL-1 at 41°C.

---

*Key words:* Interleukin-1; In vitro culture; Monocyte; Lipoprotein; HLA-DR

---

### Introduction

Interleukin-1 (IL-1), a group of closely related proteins with a molecular weight of 17 000, is considered the major endogenous pyrogen (Dinarello, 1984). Apart from its pyrogenic properties, IL-1 has a variety of other effects (Dinarello, 1984, 1986a). IL-1 is mainly produced and secreted by mononuclear phagocytes after these cells are triggered by a large variety of stimuli.

When we tried to develop a reliable assay to measure the production of IL-1 by human monocytes under normal and pathologic conditions, we encountered a number of methodological problems. In this paper we report the strict precautions that need to be taken for both the isolation and in vitro culture of these cells to measure IL-1 production in a reliable fashion.

### Materials and methods

#### *Preparation and culture of cells*

Venous blood samples from volunteers were collected in a sterile fashion in 50 ml polypro-

---

*Correspondence to:* J.W.M. Van der Meer, Department of Infectious Diseases, University Hospital Leiden, Leiden, The Netherlands.

pylene tubes (Falcon Plastics, Cockeysville, MD) containing 3 ml of preservative-free heparin (20 U/ml). In one series of experiments, healthy donors typed for HLA were used. The blood samples were transported on ice, immediately submitted to Ficoll-Isopaque density centrifugation as described elsewhere (Van der Meer et al., 1982). The cells in the monocyte-lymphocyte-rich interphase were washed with saline and counted, and, unless stated otherwise, incubated at a concentration of  $2.5 \times 10^6$ /ml in RPMI (Flow Laboratories, Irvine, Scotland) either with or without serum (see below) at 37°C in a water-saturated atmosphere of 5% CO<sub>2</sub> in air. Cultures were incubated without any addition, with 5 ng/ml *E. coli* 0111:B4 lipopolysaccharide (LPS, Difco Laboratories, Detroit, MI) or with 12.5 µg/ml polymyxin B (Pfizer, Rotterdam, The Netherlands).

After 24 h of incubation, the culture supernatants were harvested and the cells were lysed by repeated freezing and thawing in order to measure cell-associated IL-1. The samples were stored at -20°C until they were assayed for IL-1 activity. Since we did not find differences in IL-1 production whether or not the non-adherent cells were removed after 2 h of incubation, the latter cells were not removed in the experiments reported here.

In some experiments, resident peritoneal macrophages from male Swiss mice (20–30 g, Central Institute for the Breeding of Laboratory Animals, TNO, Bilthoven, The Netherlands), isolated as described elsewhere (Van Furth and Cohn, 1968) were used.

#### *Pipettes and culture substrates*

For the handling of media, reagents and cells, either glass pipettes that were sterilized by heating at 120°C for 12 h or disposable plastic pipettes (Greiner Nürtingen, F.R.G.) were used. The following culture plates were used: Nunclon and Nunc U well (Nunc, Kamstrup Roskilde, Denmark), Falcon U well and Falcon petri dish 35 mm (Falcon Plastics, Cockeysville, MD), Costar flat bottom and Costar U well (Costar Hamden, CT), Sterilin multiwell (Sterilin, Feltham, U.K.), Petriperm 55 mm dish (Heraeus Christ, Osterode, F.R.G.). Also, teflon culture bags were prepared as described elsewhere (Van der Meer et al., 1981),

using hydrophobic teflon film (Jansens M&L, St. Niklaas, Belgium); before usage the teflon film was cleansed with water and absolute ethanol and heated at 180°C for 4 h to destroy endotoxin.

#### *Sera and lipoproteins*

To study the effect of serum on the production of IL-1 by monocytes, 1% heat-inactivated (56°C, 30 min) AB serum from normal donors was added to the cultures. Furthermore heat-inactivated sera rich in either cholesterol, triglycerides or chylomicrons were tested. High density lipoproteins and low density lipoproteins were isolated by ultracentrifugation as described by Redgrave et al. (1975) and added to the cell cultures in various concentrations in the presence of 1% human serum albumin.

#### *Assay for IL-1 activity*

IL-1 activity in the samples was measured using the D10.G4.1 cell line, an IL-1-sensitive T cell line, maintained as described by Kaye et al. (1984). The cells were used between 7 and 14 days after each subculture and plated on 96 well flat bottom culture plates (Nunc). The supernatants and lysates to be tested for IL-1 activity were diluted 10- and 100-fold and assayed in triplicate in the presence of 1 µg/ml phytohemagglutinin. Incubation of the D10.G4.1 cells with 1 µg/ml phytohemagglutinin (Burroughs Wellcome Research, Triangle Park, NC) served as a control on all culture plates; as a positive control, 1 U/ml of highly purified human IL-1 (Dinarello et al., 1977) was included. The D10.G4.1 cells were incubated with the samples for 48 h at 37°C at 5% CO<sub>2</sub> in air. [<sup>3</sup>H]thymidine was added 24 h before the cultures were terminated; at the end of the culture period DNA was trapped on glassfilter paper and radioactivity determined by liquid scintillation. To prove that the lymphocyte-activating activity was due to IL-1, neutralization with an absorbed rabbit antibody against human IL-1 (Dinarello et al., 1977) was performed by incubating the cell supernatants with 1% of either the antiserum or normal rabbit serum before addition to the D10.G4.1 cells in a number of experiments.

## Results

### Isolation of cells

In our initial experiments, a highly variable IL-1 activity was found when monocytes were incubated without addition of LPS. Often the [<sup>3</sup>H]thymidine uptake of the D10.G4.1 cells incubated with a 1 in 10 or 1 in 100 dilution of such a monocyte supernatant was greater than 50 000 cpm, and would sometimes not increase significantly by the addition of LPS. Therefore, stimulation, for instance by microbial products (LPS, bacterial cellwalls, etc.) during the isolation and *in vitro* culture of the monocytes was suspected.

The Ficoll-Isopaque gradients appeared to contribute to the high IL-1 activity. Ficoll-Isopaque as prepared in large quantities by the hospital pharmacy appeared to stimulate the monocytes, whereas Ficoll-Isopaque, carefully prepared using pyrogen-free media, glassware and plastics, does not cause stimulation of monocytes (Fig. 1). We found that these carefully prepared batches of Ficoll-Isopaque still had some stimulatory capacity, when we used mouse resident peritoneal macrophages, which were centrifuged on Ficoll-Isopaque (Fig. 2). The cells respond well to an LPS stimulus. The experiments with polymyxin B (Fig. 2) suggest the stimulatory effect is not solely due to traces of endotoxin.

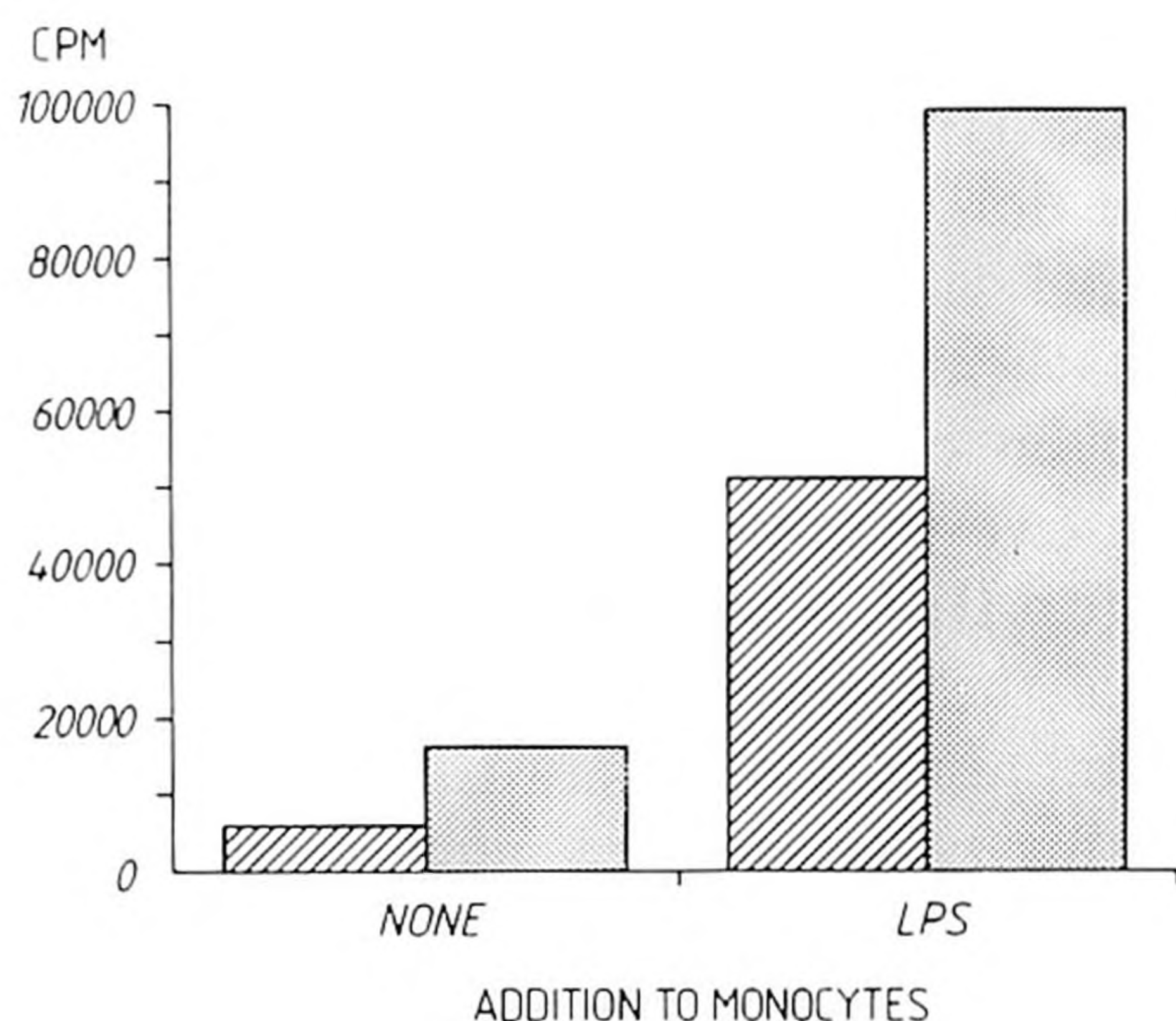


Fig. 1. The effect of different preparations of Ficoll-Isopaque on IL-1 production by non-stimulated and LPS-stimulated monocytes. One batch of Ficoll-Hypaque (hatched) was carefully prepared in the laboratory using pyrogen-free media, glassware and plastics, the other (stippled) was made in large quantity by the hospital pharmacy. The supernatants were tested on D10.G4.1 cells in a 1 in 10 dilution.

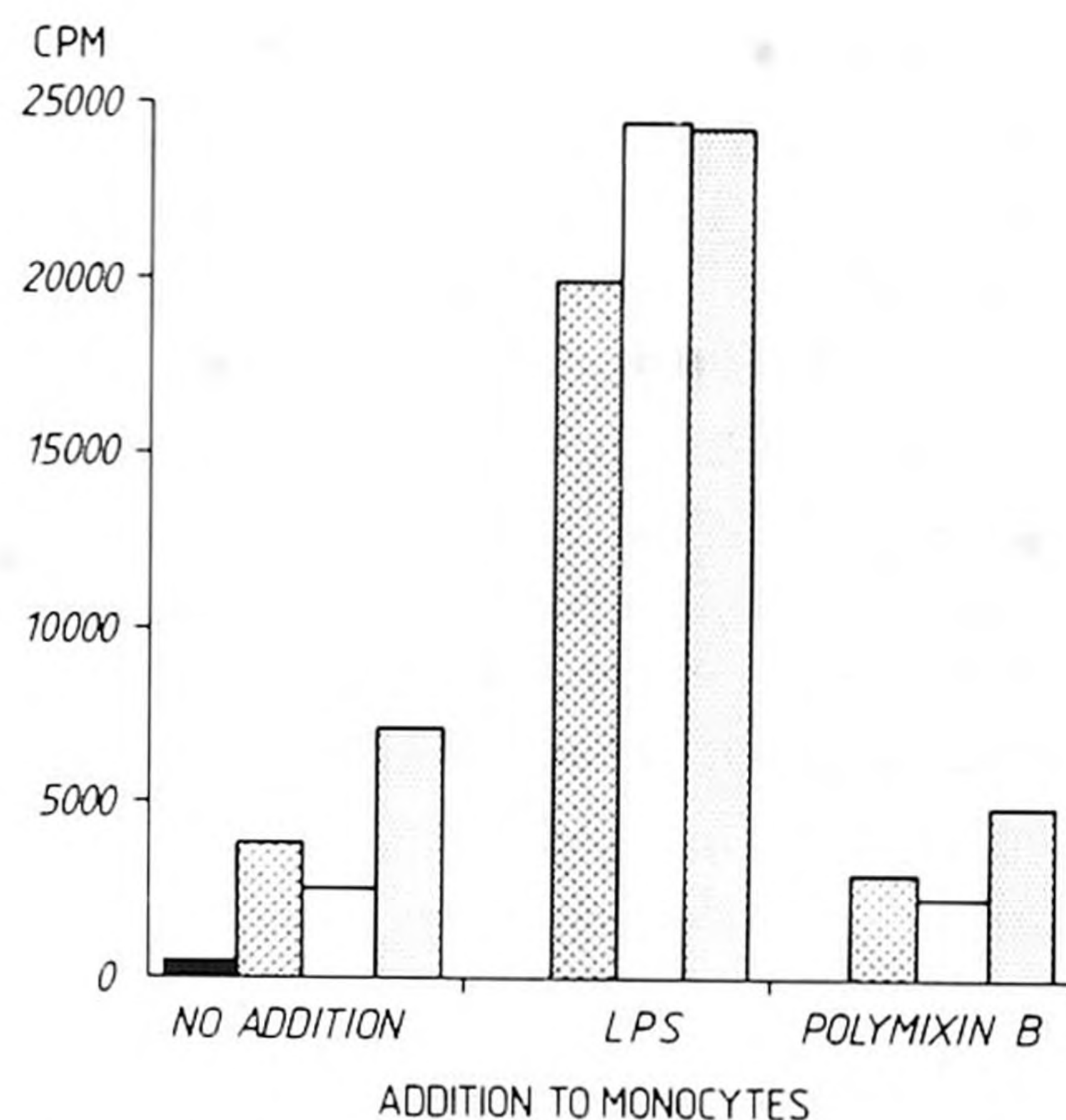


Fig. 2. The effect of carefully prepared Ficoll-Isopaque on mouse resident peritoneal macrophages. The cells were incubated at a concentration of  $5 \times 10^5$ , either directly (cross-hatched) after a single wash (open), or after Ficoll-Isopaque centrifugation (stippled). After 24 h of culture without any addition, with LPS, or with polymyxin B, the supernatants were harvested and tested on D10.G4.1 cells in a 1 in 10 dilution; PHA background (solid) is 630 cpm.

A second crucial problem appeared to be the usage of reusable heat-sterilized glass pipettes, which were also used in experiments with bacteria performed in our laboratory. These glass pipettes gave very high basal IL-1 concentrations whereas use of disposable plastic ones resulted in low unstimulated IL-1 activity and a high activity after incubation with LPS.

### Culture substrates

Next, the effect of different culture substrates was investigated. Considerable variation in stimulation of monocytes between the various brands of culture plates were found (Fig. 3). Different lot numbers also appear to produce marked differences. Although we have investigated several culture substrates, our investigations have not been so exhaustive that we are able to give strong recommendations. For that reason, in the representative experiment depicted in Fig. 3, the different brands of culture substrate are not indicated as such.

To investigate the role of adherence in the production and secretion of IL-1, cultures on teflon membranes, to which mononuclear phagocytes only loosely adhere (Van der Meer et al., 1981),

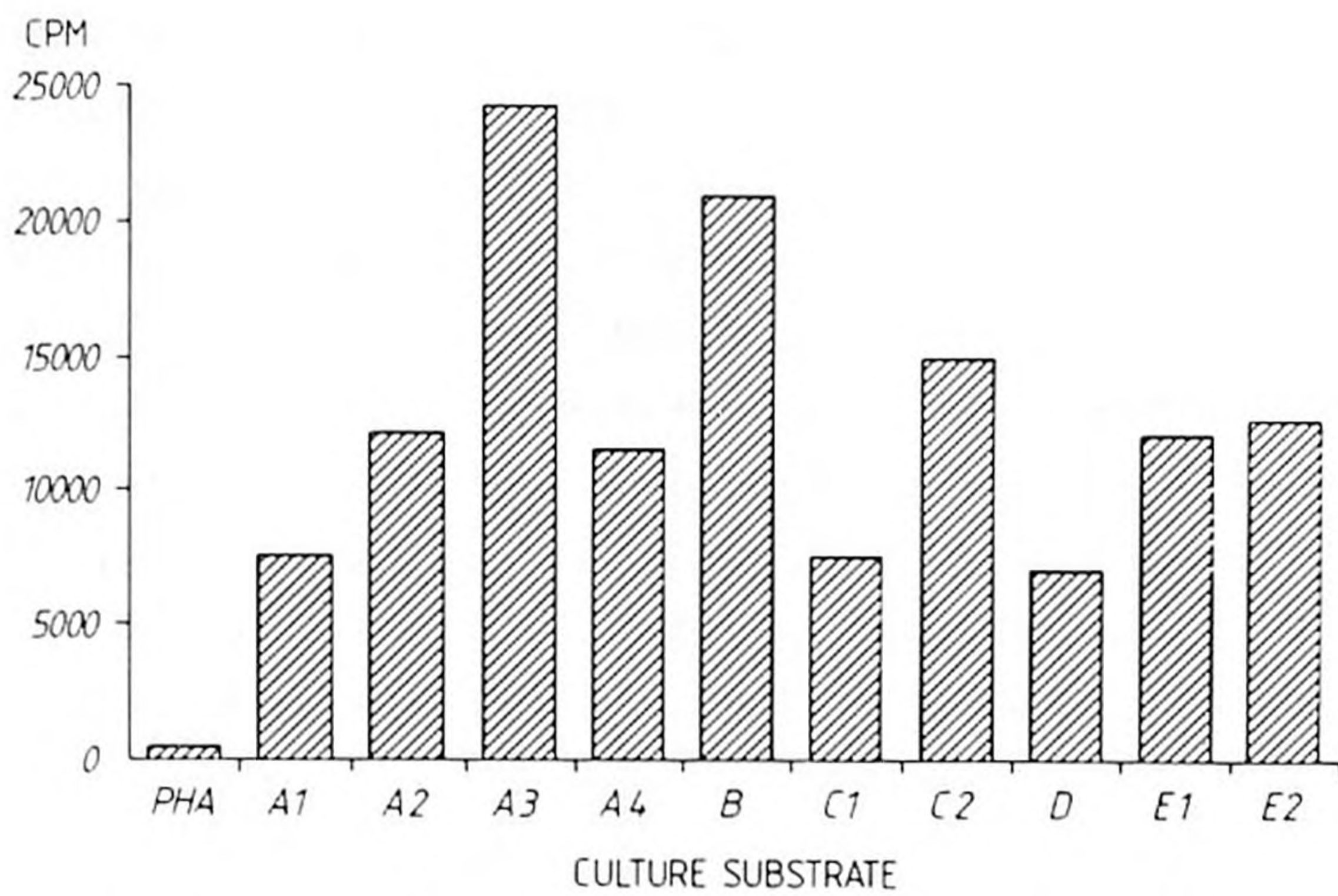


Fig. 3. The role of five different brands (A through E) of polystyrene culture substrates on basal IL-1 production. There is considerable variability of different lots (A1 through 4; C1, C2 and E1, E2) of one brand. The supernatants were tested in a 1 in 10 dilution.

were compared with those on polystyrene (Fig. 4). Contrary to our expectations, more cell-associated IL-1 was found in the teflon culture systems (especially the commercially available Petriperm dishes) than in plastic dishes. To rule out that these findings were due to adherence of IL-1 to plastic or to inactivation of IL-1 by this material, 0.1

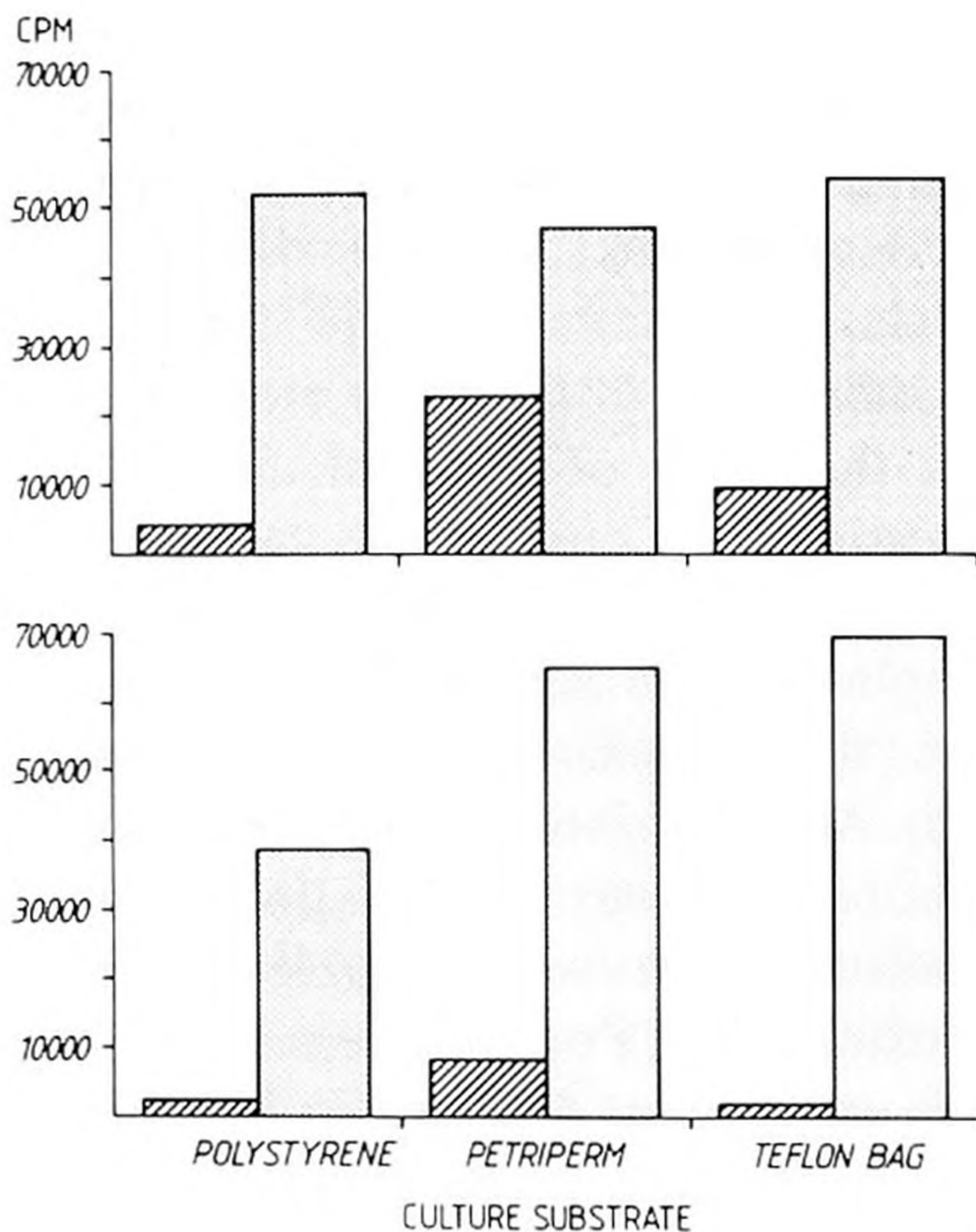


Fig. 4. Comparison of cell-associated (upper panel) and secreted (lower panel) IL-1 on polystyrene and hydrophobic teflon culture substrates. Monocytes are incubated without (hatched) or with (stippled) LPS. Results of both cell lysates and supernatants were tested in a 1 in 10 dilution.

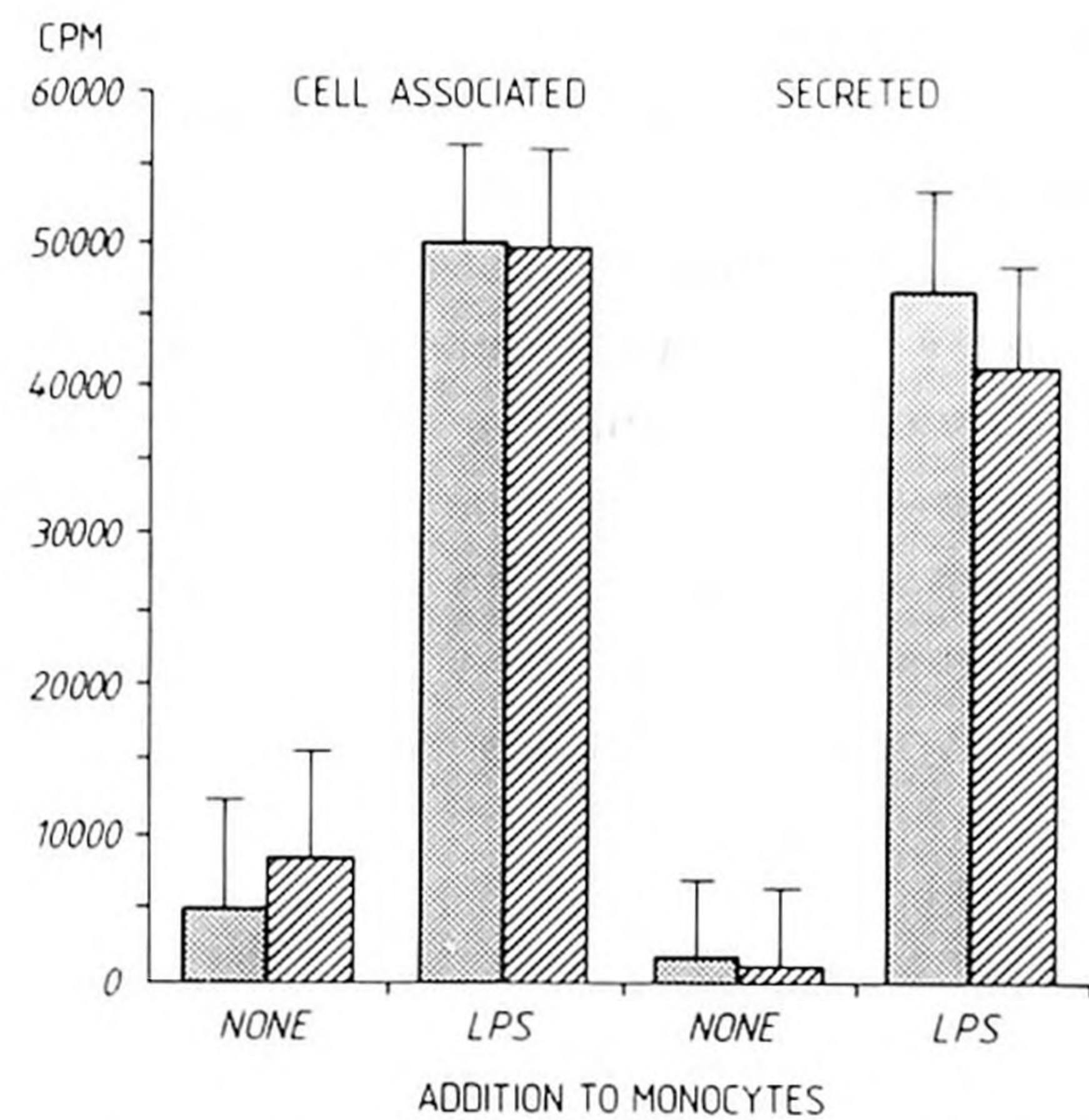


Fig. 5. Cell-associated and secreted IL-1 in monocyte cultures from five HLA DR3-positive (stippled) and five HLA non-DR3 (hatched) donors. Bars represent standard errors of the mean. The differences between the two groups of donors are not significant.

U/ml of purified IL-1 was incubated in polystyrene culture wells for 24 h; no loss of activity was found.

#### HLA-DR type of monocytes

Next, we investigated whether the DR locus of the blood donor had an important influence on either unstimulated or stimulated IL-1 production by monocytes. Since HLA DR3-positive donors are considered to be immunological high-responders (Lawley et al., 1981), monocyte preparations of 5 HLA DR3-positive and 5 HLA DR3-negative donors were cultured. To minimize experimental variation, the monocytes of five donors (three DR-positive and two DR-negative) were cultured on one day and the cultures of the remaining five were performed on another day. All assays for IL-1 were performed on 1 day. The results of these experiments (Fig. 5) do not show significant differences between these monocytes, neither for cell-associated nor for secreted IL-1 (Wilcoxon test).

#### Effects of normal and hyperlipemic serum

Addition of normal AB serum, inactivated at 56°C for 30 min, to the monocyte cultures led to a slight increase of IL-1 activity compared to serum-free culture; in contrast, addition of diet-induced turbid ('lipemic') heat-inactivated serum of

one of our donors with blood group AB, containing 7.85 mmol/l cholesterol (normal < 7.3 mmol/l) and 4.27 mmol/l of triglycerides (normal < 1.94), did profoundly inhibit IL-1 production and secretion in a reproducible fashion. The inhibition was dose-dependent, ranging from a 93% inhibition of IL-1 secretion at a serum concentration of 1% to a 68% inhibition at a serum concentration of 0.01%. Not only IL-1 production by non-stimulated monocytes, but that of LPS-stimulated cells was inhibited to a similar degree (data not shown).

The inhibition appeared to be an effect on the monocyte, since incubation of purified IL-1 with this serum did not lead to an inhibition of activity. Likewise, no effect of this serum on the D10 cells was found. These findings led us to investigate the effect of different classes of lipoproteins. Sera of five patients with greatly elevated serum cholesterol concentrations (ranging between 8.74 and 19.82 mmol/l) and normal to greatly elevated serum triglyceride concentrations (ranging between 0.85 and 6.01 mmol/l) were investigated. No inhibitory effect of any of these sera was

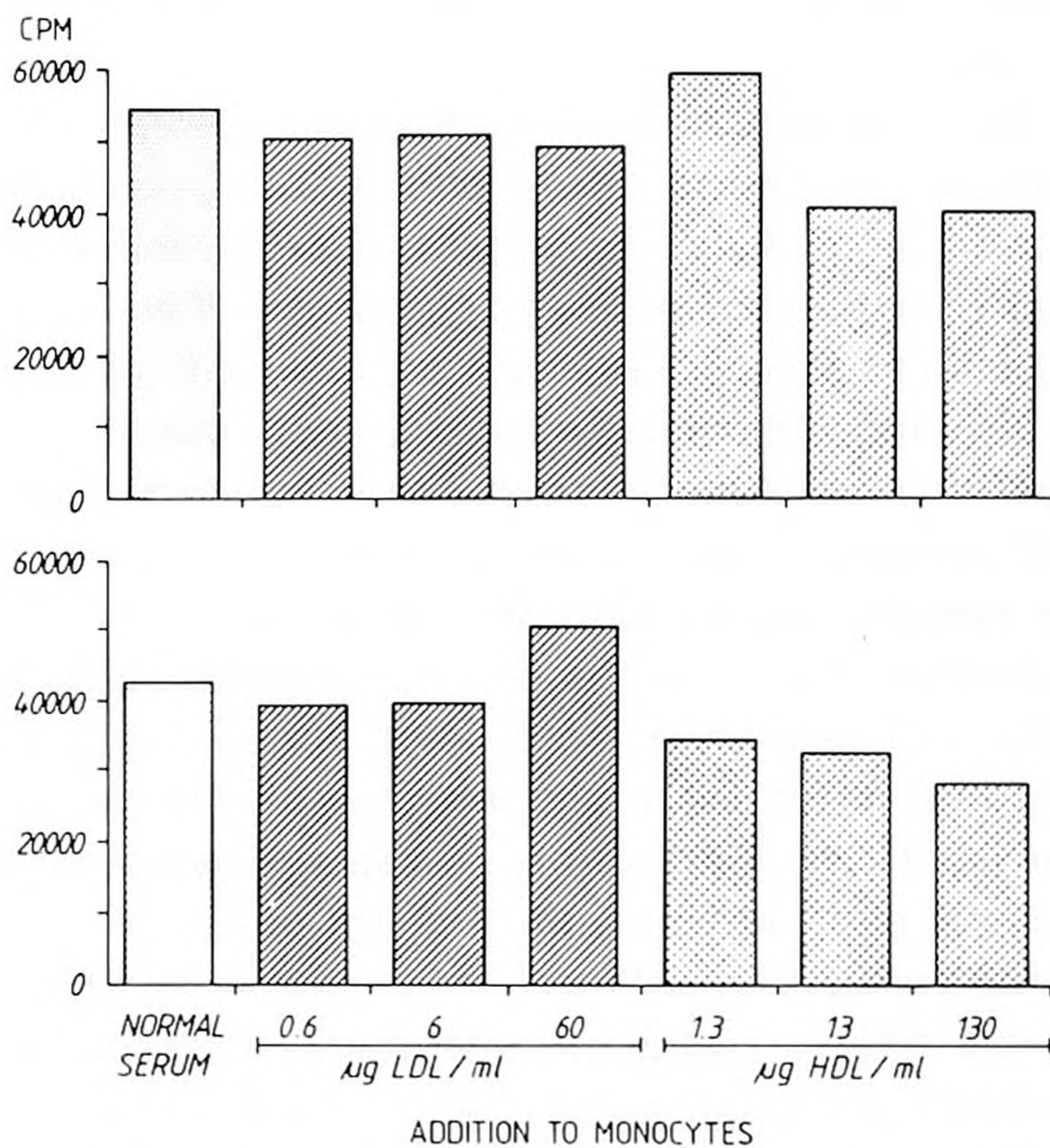


Fig. 6. The effect of low density and high density lipoproteins on IL-1 production of LPS stimulated monocytes. The cell lysates (upper panel) and the supernatants (lower panel) were tested in a 1 in 10 dilution.

found on IL-1 production or secretion of normal monocytes. Similarly no effect was seen of various concentrations serum taken before and during chylomicronemia, induced by the ingestion of 250 ml of fresh cream by a volunteer (data not shown). When isolated low-density lipoproteins and high-density lipoproteins were added to monocytes in the presence of LPS, no inhibitory effect of low density lipoproteins was found (Fig. 6). High-density lipoproteins had a slightly inhibitory effect, which did not reach statistical significance (Student's *t*-test). This inhibitory effect is minor compared to that of lipemic serum, which contained a ten-fold lower concentration of HDL.

#### Role of temperature

Cell-associated IL-1 was significantly lower at 41°C than at 31 and 36°C ( $P < 0.01$ , Student's *t*-test), whereas IL-1 secretion was not significantly influenced when monocytes were incubated

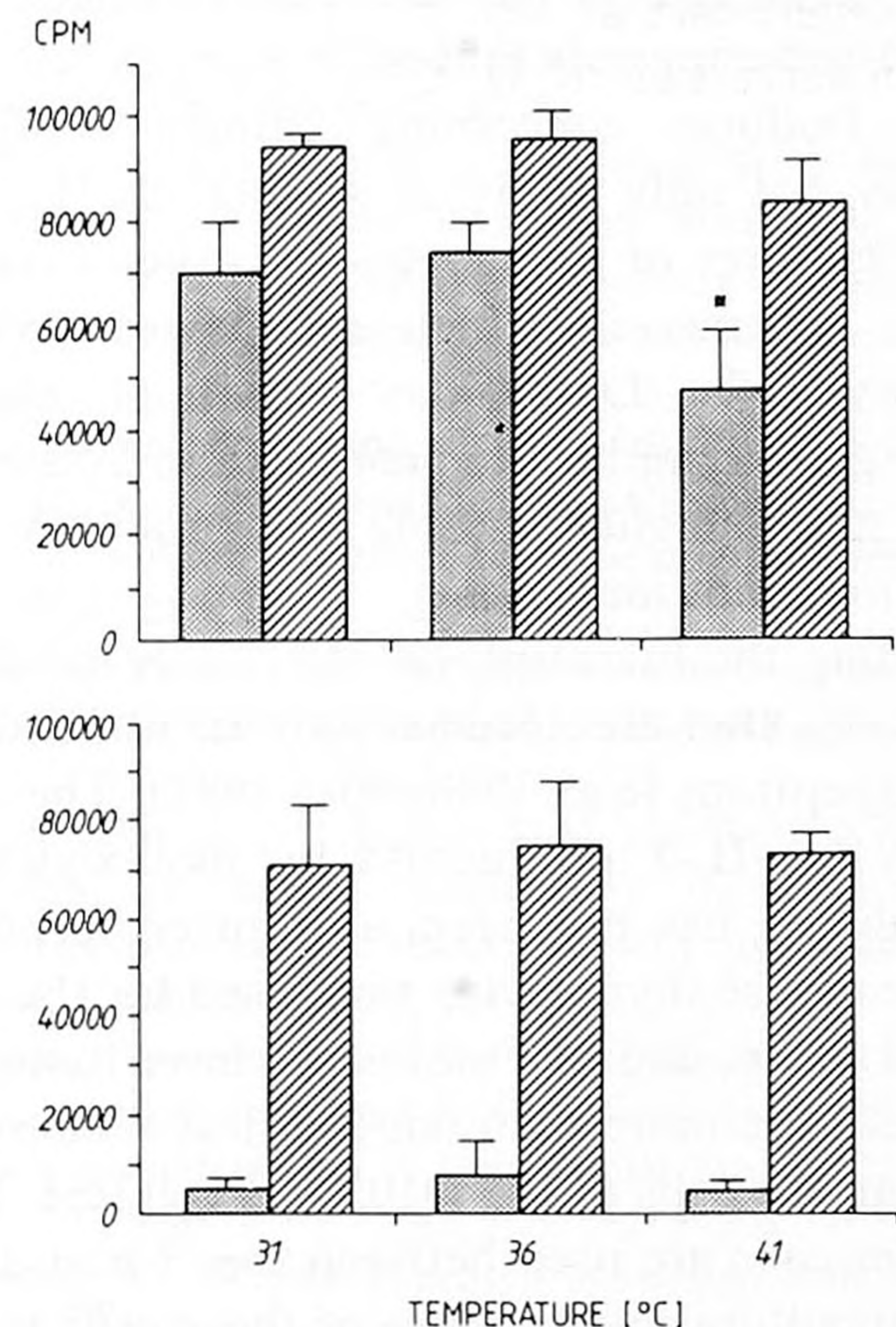


Fig. 7. The effect of incubation temperature on IL-1 production by human monocytes of four different donors. Cell-associated (upper panel) and secreted IL-1 (lower panel) are shown. The bars represent standard errors of the mean. Cell-associated IL-1 at 41°C is significantly ( $P < 0.01$ ) lower than that at 36°C and 31°C.

either in the presence or in the absence of LPS (Fig. 7).

## Discussion

IL-1 production by monocytes *in vitro* appears to be critically dependent on culture conditions, since picograms of endotoxin (Duff and Atkins, 1982), as well as other microbial products (Oppenheim et al., 1980; Dinarello, 1981; Ikejima et al., 1984) are strong inducers of IL-1. Therefore, if one wants to estimate the state of IL-1 production by monocytes under normal or pathological conditions, strict precautions for the isolation and culture of the cells are necessary. High quality of each of the ingredients is needed in these experiments, as we demonstrated for pipettes, Ficoll-Isoopaque preparations, sera and culture substrates. Since we did not encounter problems with culture media as such, we have not investigated these in detail. However, if such problems are encountered, ultrafiltration devices as described by Dinarello et al. (1987) can be used to reject interleukin-1-inducing substances.

Our findings concerning culture conditions probably not only apply to studies on IL-1, but also to cultures of monocytes for other purposes, and to systems, in which monocytes serve as accessory cells. Day-to-day variations encountered, e.g., in lymphocyte proliferation assays may well be due to highly variable IL-1 production due to culture conditions alone.

Reading the literature on IL-1, only rarely the precautions that are necessary are mentioned with a few exceptions (e.g., Dinarello, 1981). The methodology for IL-1 production by monocytes and macrophages has not been scrutinized, probably, because mouse thymocytes were used for the interleukin-1 assay, and like others, we have found that these cells are more than ten-fold less sensitive for IL-1 than the cells of the D10.G4.1 cell line. When the latter cells are used between day 7 and day 10 after subculture the response of these cells to IL-1 is remarkably constant (unpublished observations).

Apart from these methodological considerations, our findings indicate that testing for IL-1 is probably the most sensitive way to detect stimulation of these cells *in vitro*. Although we have a

relatively long experience with the culture of mononuclear phagocytes (Van der Meer, 1980; Van der Meer et al., 1982), not until we performed the experiments described here did we recognize the extent that culture methods have on cell stimulation. For instance, using hydrophobic teflon film as a culture substrate to prevent adherence of mononuclear phagocytes in culture (Van der Meer et al., 1981), we would simply autoclave the teflon membrane and use it. This report demonstrates that large amounts of IL-1 are produced if these membranes are used without meticulous preparation. Despite these precautions, more IL-1 is produced when cells are cultured in either of both types of teflon culture system used than in polystyrene culture dishes. Since adherence to a surface is considered one of the stimuli for IL-1 mRNA synthesis and translation (Auron et al., 1984; Lepe-Zuniga et al., 1985), these findings are somewhat unexpected. More studies are needed to establish which mechanisms are responsible for the stimulation of IL-1 production. In this respect it is of interest that hemodialysis membranes, such as cuprophane, to which monocytes also poorly adhere (Van Ginkel et al., 1977), are considered to play a role in the elevation of plasma IL-1 in patients undergoing hemodialysis (Lonnemann et al., 1987).

We have investigated whether the DR3 haplotype of the major histocompatibility complex, which is considered to represent the high immunological responder state in the human (Lawley et al., 1981), is associated with an intrinsic capacity to produce more IL-1. In a limited number of carefully controlled experiments we could not demonstrate a significant difference. In view of the sample size, we cannot exclude that there is a difference, but if so the difference between HLA DR3 and non-DR3 is small and unlikely to be responsible for the immunological differences reported. These observations are in agreement with those in mice, in which the amount of IL-1 release in the presence of LPS did not appear to be linked to the MHC haplotype (Abehsira-Amar et al., 1985).

We were intrigued by the inhibitory effect of the hyperlipemic serum of one of our serum donors. However, we could not reproduce the findings either with other sera with high con-

centrations of the various lipoproteins or with isolated lipoproteins. Quite a number of reports have dealt with both inhibitory and stimulatory effects of lipoproteins on lymphocyte proliferation (e.g., Curtiss and Edgington, 1978; Cuthbert and Lipsky, 1986). Most of these studies point to the lymphocyte as the target. The serum of our donor, however, did not interfere with the lymphocytes (D10.G4.1 cells), used for detection of IL-1. Although we were not yet able to reproduce the phenomenon with other sera, the lesson from these experiments is that the serum used in these culture systems should be checked for either stimulatory or inhibitory activities.

The results of the experiments in which monocytes were incubated at different temperatures are somewhat divergent from those of Dinarello et al. (1986b), who published decreasing IL-1 secretion when incubation temperature was raised from 34°C to 37°C to 39°C. The differences became especially apparent when the cells were incubated with indomethacin. In our experiments secretion of IL-1 was unaffected by temperature, whereas decreased cell-associated IL-1 was found at 41°C. One explanation for these different observations may be the temperatures chosen (31°C, 36°C and 41°C in our experiments). It is also possible that, apart from the addition of a prostaglandin inhibitor, slightly different culture conditions account for differences in the relative amounts of IL-1 that are cell-associated or secreted.

From the foregoing it will be clear that a number of precautions are extremely important when IL-1 production by human monocytes under normal, as well as under pathological conditions, has to be measured. Only when these measures are taken, it is possible to measure IL-1 production in a reliable fashion.

### Acknowledgements

We thank Dr. C.A. Dinarello for the reagents, Dr. R.P. De Vries for providing us with HLA DR-typed donors, Dr. H.J. Kempen and Dr. J. Gevers Leuven for their help with the lipoprotein studies, and Dr. A. Blussé van Oud Alblas and Professor R. Van Furth for their support.

### References

- Abehsira-Amar, O., Damais, C., Parant, M. and Chedid, L. (1985) Strain dependence of muramyl dipeptide-induced LAF (IL 1) release by murine adherent peritoneal cells. *J. Immunol.* 134, 365.
- Auron, P.A., Webb, A.C., Rosenwasser, L.J., Mucci, S.F., Rich, A., Wolff, S.M. and Dinarello, C.A. (1984) Nucleotide sequence of human monocyte interleukin-1 precursor cDNA. *Proc. Natl. Acad. Sci. U.S.A.* 81, 7907.
- Curtiss, L.K. and Edgington, T.S. (1978) Identification of a lymphocyte surface receptor for low density lipoprotein inhibitor, an immunoregulatory species of normal human serum low density lipoprotein. *J. Clin. Invest.* 61, 1298.
- Cuthbert, J.A. and Lipsky, P.E. (1986) Promotion of human T lymphocyte activation and proliferation by fatty acids in low density and high density lipoprotein. *J. Biol. Chem.* 261, 3620.
- Dinarello, C.A. (1981) Endogenous pyrogen. In: P.J. Edelson, H. Koren and D.O. Adams (Eds.), *Methods for Studying Mononuclear Phagocytes*. Academic Press, New York.
- Dinarello, C.A. (1984) Interleukin-1. *Rev. Infect. Dis.* 6, 51.
- Dinarello, C.A., Renfer, L. and Wolff, S.M. (1977) Human leucocytic pyrogen: purification and development of a radioimmunoassay. *Proc. Natl. Acad. Sci. U.S.A.* 74, 4626.
- Dinarello, C.A., Cannon, J.C., Mier, J.W., Bernheim, H.A., LoPreste, G., Lynn, D.L., Webb, A.C., Auron, P.E., Reuben, R.C., Rich, A., Wolff, S.M. and Putney, S.D. (1986a) Multiple biological activities of human recombinant interleukin-1. *J. Clin. Invest.* 77, 1734.
- Dinarello, C.A., Dempsey, R.A., Allegretta, M., LoPreste, G., Dainiak, N., Parkinson, D.R. and Mier, J.W. (1986b) Inhibitory effects of elevated temperature on human cytokine production and natural killer activity. *Cancer Res.* 46, 6236.
- Dinarello, C.A., Lonnemann, G., Maxwell, R. and Shaldon, S. (1987) Use of ultrafiltration to reject human interleukin-1-inducing substances derived from bacterial cultures. *J. Clin. Microbiol.* 25, 1233.
- Duff, G. and Atkins, E. (1982) The inhibitory effect of polymyxin B on endotoxin-induced endogenous pyrogen production. *J. Immunol. Methods* 52, 323.
- Ikejima, T., Dinarello, C.A., Gill, D.M. and Wolff, S.M. (1984) Induction of human interleukin-1 by a product of *Staphylococcus aureus* associated with toxic shock syndrome. *J. Clin. Invest.* 73, 1312.
- Kaye, J. and Janeway, Jr., C.A. (1984) Induction for receptors for interleukin 2 requires T cell antigen: Ia receptor cross-linking and interleukin 1. *Lymphokine Res.* 3, 175.
- Lawley, T.J., Hall, R.P., Fauci, A.S. and Frank, M.M. (1981) Defective Fc receptor functions associated with the HLA B8DW3 haplotype. *New Engl. J. Med.* 304, 185.
- Lepe-Zuniga, J.L. and Gery, I. (1984) Production of intracellular and extracellular interleukin-1 (IL-1) by human monocytes. *Clin. Immunol. Immunopathol.* 31, 222.
- Lonnemann, G., Bingel, M., Koch, K.M., Shaldon, S. and Dinarello, C.A. (1987) Plasma IL-1 activity in humans un-



- dergoing hemodialysis with regenerated cellulosic membranes. *Lymphokine Res.* 6, 63.
- Oppenheim, J.J., Togawa, A., Chedid, L. and Mizel, S. (1980) Components of mycobacteria and muramyl dipeptide with adjuvant activity induce lymphocyte activating factor. *Cell. Immunol.* 50, 71.
- Redgrave, T.G., Roberts, D.C.K. and West, C.E. (1975) Separation of plasma lipoproteins by density gradient ultracentrifugation. *Anal. Biochem.* 65, 42.
- Van der Meer, J.W.M. (1980) Characteristics of mononuclear phagocytes in culture. In: I. Carr and W.T. Daems (Eds.), *The Reticuloendothelial System. A Comprehensive Treatise. I. Morphology.* Plenum Press, New York, p. 735.
- Van der Meer, J.W.M., Van de Gevel, J.S. and Van Furth, R. (1981) Teflon film as a substrate for the culture of mononuclear phagocytes. In: P.J. Edelson, H. Koren and D.O. Adams (Eds.), *Methods for Academic Press, New York*, p. 121.
- Van der Meer, J.W.M., Van de Gevel, J.S., Blussé van Oud Alblas, A., Kramps, J.A., Van Zwet, T.L., Leijh, P.C.J. and Van Furth, R. (1982) Characteristics of human monocytes cultured in the Teflon culture bag. *Immunology* 47, 617.
- Van Furth, R. and Cohn, Z.A. (1968) Origin and kinetics of mononuclear phagocytes. *J. Exp. Med.* 128, 415.
- Van Ginkel C., Van Aken, W.G., Oh, J.I.H. and Vreeken, J. (1977) Stimulation of monocyte procoagulant activity by adherence to different surfaces. *Br. J. Haematol.* 37, 35.