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Differences in the synthesis and kinetics of release of interleukin 1α, interleukin 1β and tumor necrosis factor from human mononuclear cells*

Cell-associated and secreted interleukin 1α (IL 1α), IL 1β and tumor necrosis factor α (TNF-α), produced by human mononuclear cells (MNC) *in vitro* in response to lipopolysaccharide, were measured by radioimmunoassay. After 18 h of incubation, total production of IL 1α in medium containing 1% heat-inactivated serum was two-to-three times higher than IL 1β. However, in the presence of 1% serum and 5% fresh plasma, IL 1α and IL 1β were produced in similar amounts. Independent of the culture conditions, 90% of the IL 1α remained cell associated whereas 80% of IL 1β was extracellular. The kinetics of production and release of IL 1α, β and TNF-α were also studied. IL 1α and TNF-α reached maximal levels within 6 h of stimulation, whereas IL 1β reached maximal levels between 12 and 16 h. IL 1α remained primarily cell associated (80%) for the first 24 h. After 48 h, extracellular IL 1α exceeded cell-associated levels. IL 1β was primarily secreted (80%), appearing in the extracellular fluid within 6 h. TNF-α appeared in the extracellular fluid within 1 h of incubation, with < 10% cell associated at any time during the 48 h of incubation. Although the three cytokines share many biological activities, this study provides evidence that MNC IL 1α is predominantly a cell-associated cytokine acting on a cell-cell basis, whereas IL 1β and TNF-α are secreted as paracrine mediators.

1 Introduction

Cytokines such as IL 1 and TNF are products of activated mononuclear cells (MNC) and important mediators in infection, inflammation and immunological challenge [1, 2]. Two IL 1 peptides, IL 1α [3] and IL 1β [4], as well as TNF-α [5] have been cloned and studies using recombinant molecules have confirmed the biological activities attributed to these cytokines [1, 2, 6]. Although IL 1α, IL 1β and TNF-α are different proteins transcribed from different genes, they share many activities including fever [6–8], induction of liver acute-phase protein synthesis [9], hemodynamic shock [10], and catabolic effects on bone and connective tissue [11, 12]. Further studies indicate that synergism can be demonstrated between TNF-α and the two forms of IL 1 [10, 13, 14], and that all three cytokines may induce each other *in vivo* as well as *in vitro* [8, 15, 16]. Because of these interactions, it is often difficult to discriminate between the effect of IL 1α, IL 1β or TNF-α in bioassays.

In the absence of exogenous stimulants, cytokines are not present in MNC. Activation of these cells leads to *de novo* synthesis of mRNA and new protein which is first detectable in the cytosol and subsequently secreted into the extracellular space. mRNA levels as measured by Northern blot analysis revealed that IL 1β is the predominant IL 1 form in human MNC [17]. On the protein level, intracellular production as well as release of biologically active IL 1 has been demon-

strated from lipopolysaccharide (LPS)-stimulated human MNC using the standard lymphocyte-activating factor assay [18].

Investigations using gel chromatography and IEF demonstrated differences in size as well as in pI between cell-associated and extracellular IL 1 [19]. However, the relative amount of IL 1α and IL 1β in the two compartments, as detected by specific immunoreactivity, or a comparison with TNF-α has not been determined.

In the present study, we used recently developed radioimmunoassays [20–22] in order to quantify the differences between cell-associated and extracellular IL 1α and IL 1β production from LPS-stimulated human MNC under various test conditions. Furthermore, we compared the kinetics of IL 1α, IL 1β and TNF-α production and determined their secretion from LPS-stimulated MNC.

2 Materials and methods

2.1 Preparation and incubation of human MNC

Heparinized human donor blood from healthy donors (10 U/ml heparin) was diluted 1 : 3 in 0.15 M saline and MNC were separated over Ficoll-Hypaque (Ficoll Trennlösung, Biochrom KG, Berlin, FRG). MNC were washed three times in saline and suspended in ultrafiltered [23] Eagle's MEM containing 2 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, 10 mM Hepes (all from Whittaker M. A. Bioproducts Inc., Walkersville, MD) and 2% heat-inactivated (56°C for 30 min) human AB serum from a single AB donor. Five hundred microliter aliquots of this MNC suspension (5×10^6 cells/ml) were added to flat-bottom 24-well multititer plates (A/S Nunc, Roskilde, Denmark). Equal volumes of LPS (*E. coli* B : 55, Sigma, St. Louis, MO) in either MEM or MEM containing 10% fresh autologous human plasma were added. Following incubation at 37°C in a humidified atmosphere with

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Abbreviation: MNC: Mononuclear cells

5% CO_2, 50 µl of plasma was added to the plasma-free incubations in order to adjust the plasma content in all samples for the subsequent cytokine measurements. Fifty microliters of MEM was added to the plasma-containing wells in order to compensate the dilution factor. MNC SN were separated and centrifuged for 1 min at 13 000 × g in 4 °C and the SN containing the extracellular IL 1 were stored at −70 °C until assayed. MNC pellets in the wells were resuspended in MEM containing 5% plasma and 1% heat-inactivated AB serum (1 ml each) and exposed to three freeze-thaw cycles in order to lyse the cells and obtain the cell-associated pool of cytokines. These samples were also frozen at −70 °C until assay. Control experiments in which known amounts of IL 1α and IL 1β were added to MNC lysates, then frozen and thawed three times, indicated that this process did not destroy the immunoreactivity of the cytokines.

In the first set of experiments, MNC incubations with various LPS concentrations were performed for 18 h in the presence or absence of plasma. The LPS dose-response curve as well as the influence of added plasma were studied. In the second set of experiments, MNC were stimulated with a single concentration of LPS (500 ng/ml) in the presence of 1% serum and 5% plasma and the time-course of IL 1 and TNF production and release was determined. In order to study whether the kinetics of cytokine production and/or release are influenced by the presence of LPS, we compared the effect of constant LPS presence with that of a 2-h exposure to LPS stimulation on MNC IL 1 and TNF-α production. For this purpose, MNC in MEM containing 1% serum, 5% plasma and 500 ng/ml LPS were incubated at 37 °C in multititer plates. After 2 h, LPS-containing cell SN were removed from every second well and adhering cells washed by adding and removing fresh MEM containing 1% serum and 5% plasma three times. After this, incubation in fresh medium was continued for various time periods. In parallel MNC cultures, 500 ng/ml LPS was constantly present during incubation.

2.2 Cytokine measurement

Extracellular and cell-associated levels of immunoreactive IL 1α, IL 1β and TNF-α were measured using specific RIA [20–22]. All samples were diluted 1 : 5 in BSA buffer (0.25% BSA, Sigma) and assayed in duplicates.

3 Results

3.1 Dose response of LPS-induced MNC IL 1α and IL 1β production: the influence of 5% plasma

The total IL 1 production (cell-associated plus extracellular) from LPS-stimulated MNC in 1% serum is depicted in Fig. 1A. In response to LPS concentrations of 0.5–500 ng/ml, IL 1 production is dose dependent, and MNC produce two to three times more IL 1α than IL 1β during 18 h of incubation. By 5 µg/ml LPS, the maximum dose we used, equal amounts of IL 1α and IL 1β were induced, although the total IL 1 production was decreased compared to the incubations with 500 ng/ml LPS. This decrease may be due to cyclooxygenase products induced by high-dose LPS [24]. Cell-associated and extracellular levels of IL 1 are also depicted. Independent of the LPS dose, the cell-associated samples contained 5–10 times more IL 1α than IL 1β (Fig. 1B), whereas in the extracellular com-
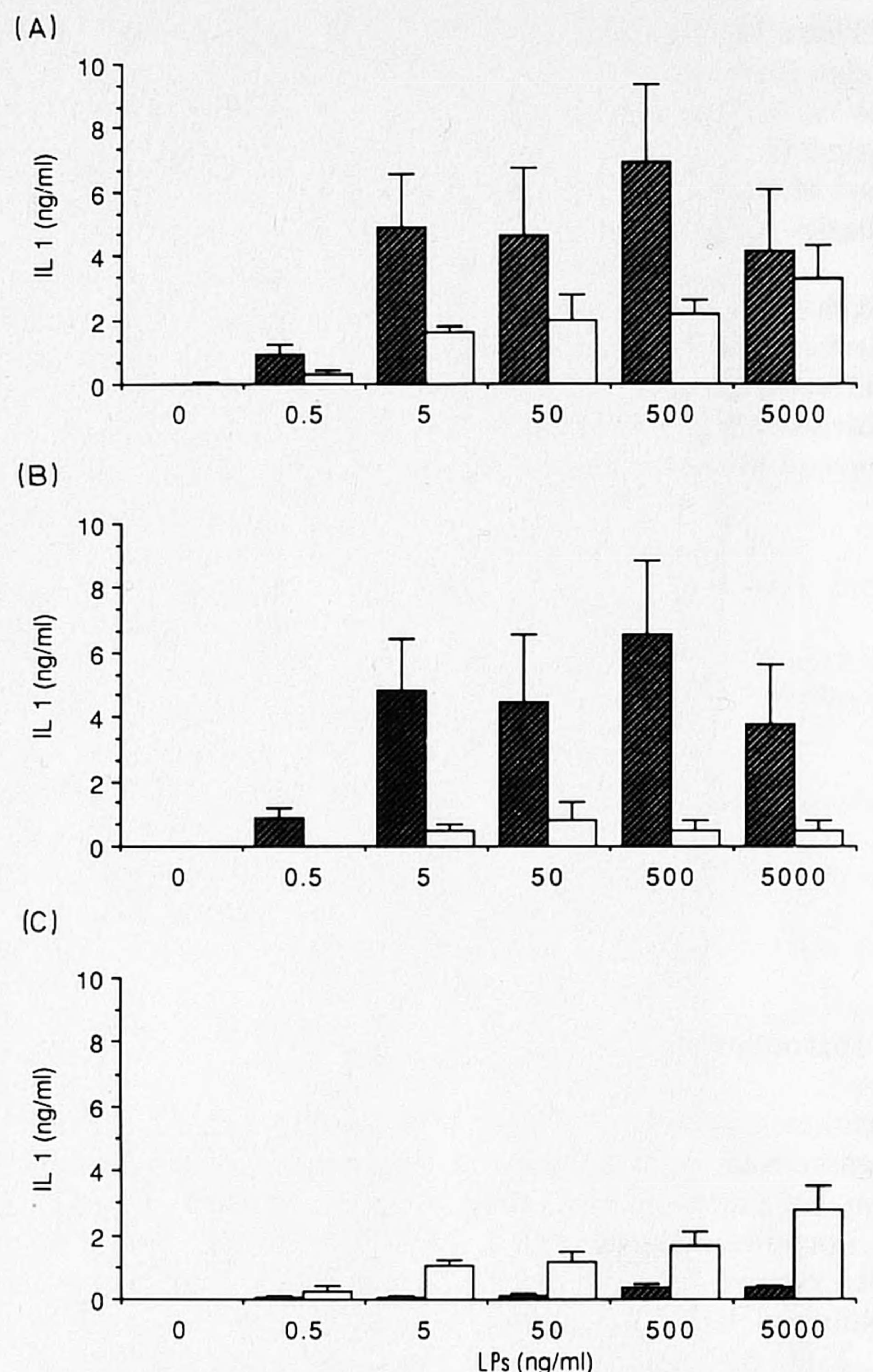

Figure 1. Dose response of endotoxin-induced IL 1α (■) and IL 1β (□) production from MNC during 18 h of incubation in tissue culture medium containing 1% serum. (A) Total (cell associated + extracellular); (B) cell-associated levels, and (C) extracellular levels. Each bar represents the mean of four experiments ± SEM.

partment we detected 5–10 times more IL 1β than IL 1α (Fig. 1C). Comparing the results of all these studies it is clear that after 18 h of incubation, 80% of the total IL 1β production is secreted whereas 90% of the IL 1α remains cell associated.

IL 1 production from LPS-stimulated MNC in the presence of 1% serum and 5% plasma is depicted in Fig. 2. Compared to results obtained in the absence of plasma, total IL 1α and IL 1β production is enhanced by a factor of 10 in response to low-dose LPS (0.5 ng/ml) and by a factor of 3–5 when 5–5000 ng/ml LPS are used as stimuli. In response to 0.5 ng/ml LPS, MNC produce three times more IL 1α than IL 1β (Fig. 2A). In contrast, using higher LPS concentrations (0.5 and 5 µg/ml) in the presence of 1% serum and 5% plasma during incubation, IL 1α and IL 1β are produced in similar amounts. Most of the IL 1α remains cell associated and the IL 1β is secreted, as observed previously in the absence of plasma. As depicted in Fig. 2B, the cell-associated samples contain 5–10 times more IL 1α than IL 1β and MNC secrete 5–10 times more IL 1β than IL 1α (Fig. 2C). Similar to the plasma-free incubations, 80%–90% of total IL 1α remains cell associated at all concentrations of LPS, whereas 80% of total IL 1β is found in the extracellular compartment after 18 h of incubation.
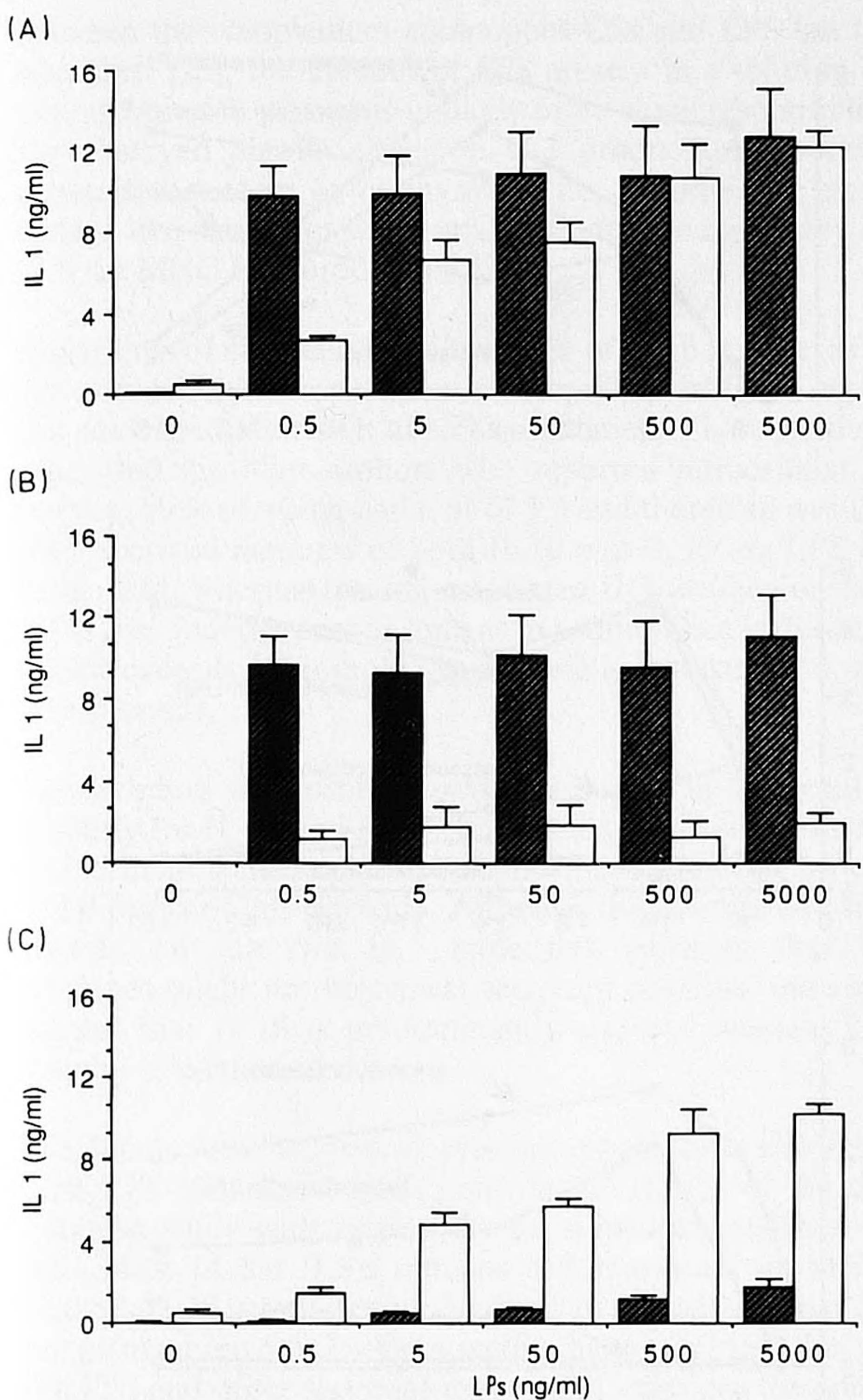
(A)

(B)

(C)

LPs (ng/ml)

Figure 2. Dose response of IL 1α and IL 1β production from MNC during 18 h of incubation in tissue culture medium containing 1% serum, 5% autologous plasma and various concentrations of LPS. (A) Total IL 1 (cell associated + extracellular); (B) cell-associated levels; (C) extracellular concentrations. Each bar represents the mean of four experiments ± SEM.

3.2 Kinetics of LPS-induced MNC IL 1α, IL 1β and TNF-α production and release

MNC from two donors were stimulated with 500 ng/ml LPS in the presence of 1% heated AB serum and 5% autologous plasma and incubated for various time periods at 37 °C. MNC SN as well as cell lysates were assayed for extracellular and cell-associated levels of IL 1α, IL 1β and TNF-α after 1, 2, 6, 16, 20, 24 and 48 h of incubation (Figs. 3 and 4). In both experiments, cell-associated IL 1α is detectable within 2 h, increases with time and reaches a plateau at maximal levels of 10 ng/ml within 6 h (Figs. 3A and 4A). After 24 h of MNC incubation, cell-associated concentrations of IL 1α start to decrease and are lower (2–4 ng/ml) in the 48-h samples. Extracellular concentrations of IL 1α are detectable within 6 h and increase to exceed cell-associated levels after 48 h of incubation (Figs. 3A and 4A).

The time-course of IL 1β production and release is shown in Figs. 3B and 4B. Like IL 1α, cell-associated IL 1β concentrations are maximal (3–5 ng/ml) within 6 h and start to decrease

after 20–24 h of incubation. In contrast to the IL 1α measurements, extracellular amounts of IL 1β exceed cell-associated levels of the 6-h samples and reach maximal concentrations (10–15 ng/ml) within 16 h of MNC incubation.

Secreted TNF exceeds cell-associated levels in the first sample taken after 1 h of incubation (Figs. 3C and 4C). Cell-associated TNF-α is less than 10% of total at all time points. Extracellular TNF-α increases with time, reaches maximal levels within 6–16 h (7–10 ng/ml) and decreases thereafter to concentrations of 3–5 ng/ml during the following time period.

We next compared the effect of a 2-h LPS stimulation *vs.* constant LPS stimulation on cytokine production and release (Fig. 5). Once again the culture medium contained 1% serum plus 5% autologous plasma and the LPS concentration we used was 500 ng/ml. MNC incubation was performed for 2, 4, 6, 12, 24 and 48 h, and cell-associated as well as extracellular concentrations of IL 1α, IL 1β and TNF-α were measured.

(A)

(B)

(C)

time of incubation (hours)

Figure 3. Time course of IL 1α, IL 1β and TNF-α production from human MNC incubated with 500 ng/ml LPS in MEM containing 1% serum and 5% autologous plasma. Cell-associated and extracellular cytokine levels were measured after 1, 2, 6, 16, 20, 24 and 48 h. The results depict a single donor. (A) IL 1α; (B) IL 1β; (C) TNF-α.

(A)

(B)

(C)

Figure 4. The experimental design is the same as that shown in Fig. 3 but using the MNC of another donor.
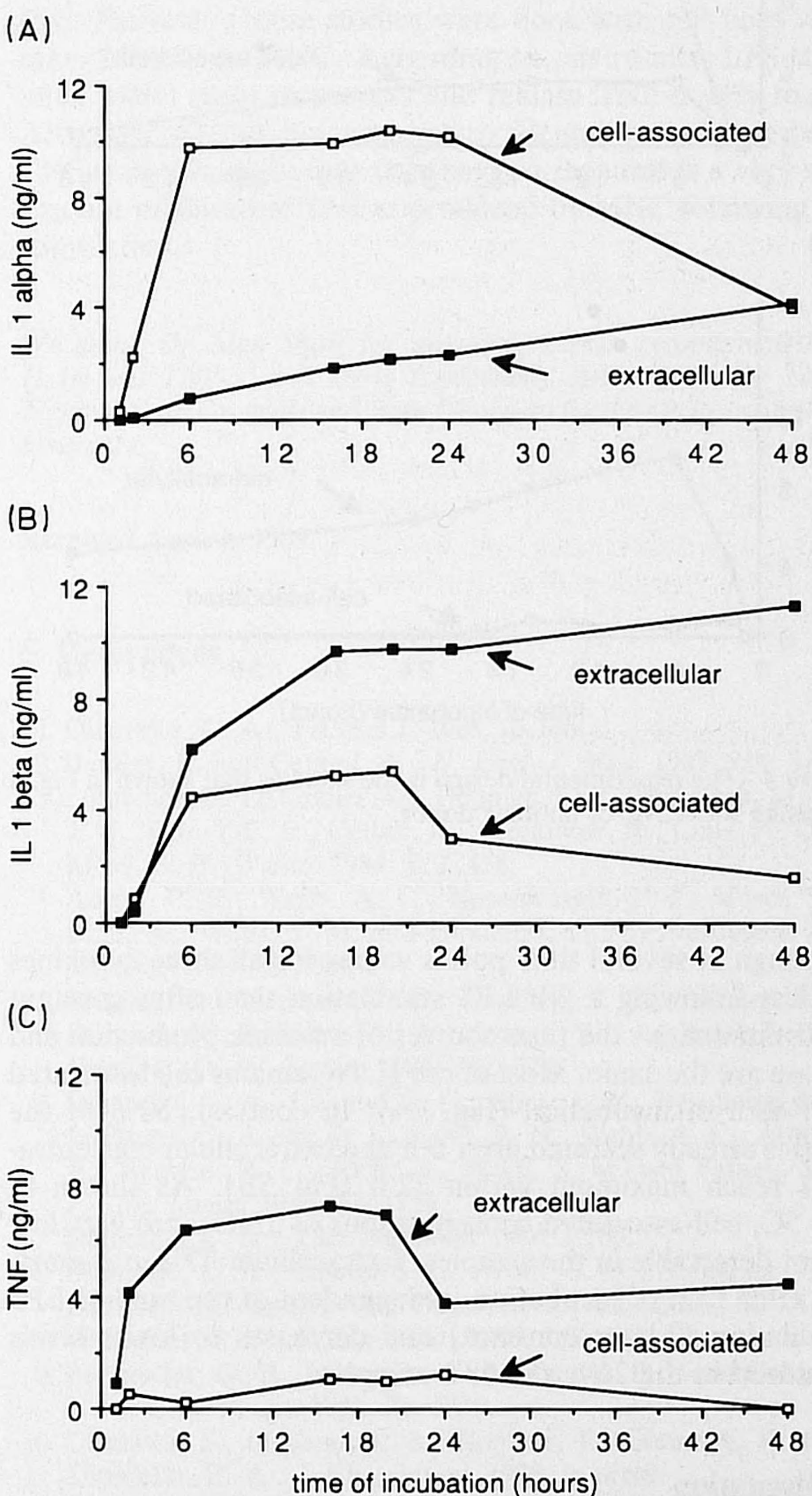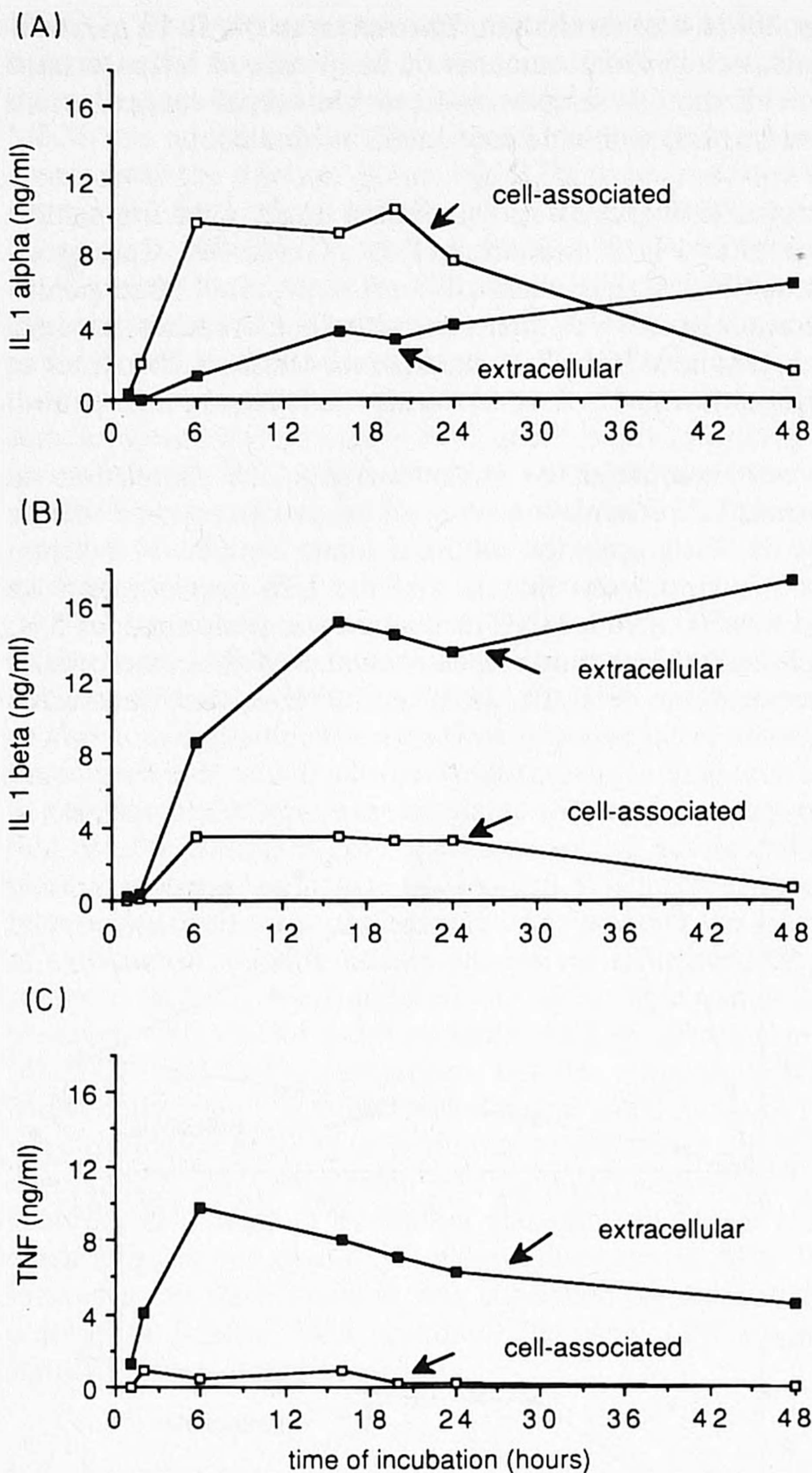
(A)

(B)

(C)

Figure 5. Time course of IL 1α, IL 1β and TNF-α production from human MNC incubated with 500 ng/ml LPS in MEM containing 1% serum and 5% autologous plasma. MNC incubated continually in LPS is compared to LPS stimulation for 2 h. Cell-associated and extracellular cytokine levels were measured after 2, 4, 6, 12, 24 and 48 h. (A) IL 1α; (B) IL 1β; (C) TNF-α.

Although at several time points values for all three cytokines are less following a 2-h LPS stimulation than after constant LPS stimulation, the time courses of cytokine production and release are the same. Most of the IL 1α remains cell associated until 48 h of incubation (Fig. 5A). In contrast, 50% of the IL 1β is already secreted after 6 h and extracellular concentrations reach maximum within 12 h (Fig. 5B). As shown in Fig. 5C, cell-associated concentrations of TNF-α are very low or not detectable in the samples. Extracellular TNF-α is maximal after 12 h of incubation, independent of the time of LPS stimulation (2 h or constant) and decreases to lower levels measured in the 24-h and 48-h samples.

4 Discussion

We have demonstrated differences in the amount of cell-associated and extracellular IL 1α, IL 1β and TNF-α produced *in vitro* by human MNC stimulated with LPS. First we compared cell-associated and extracellular IL 1α and IL 1β productio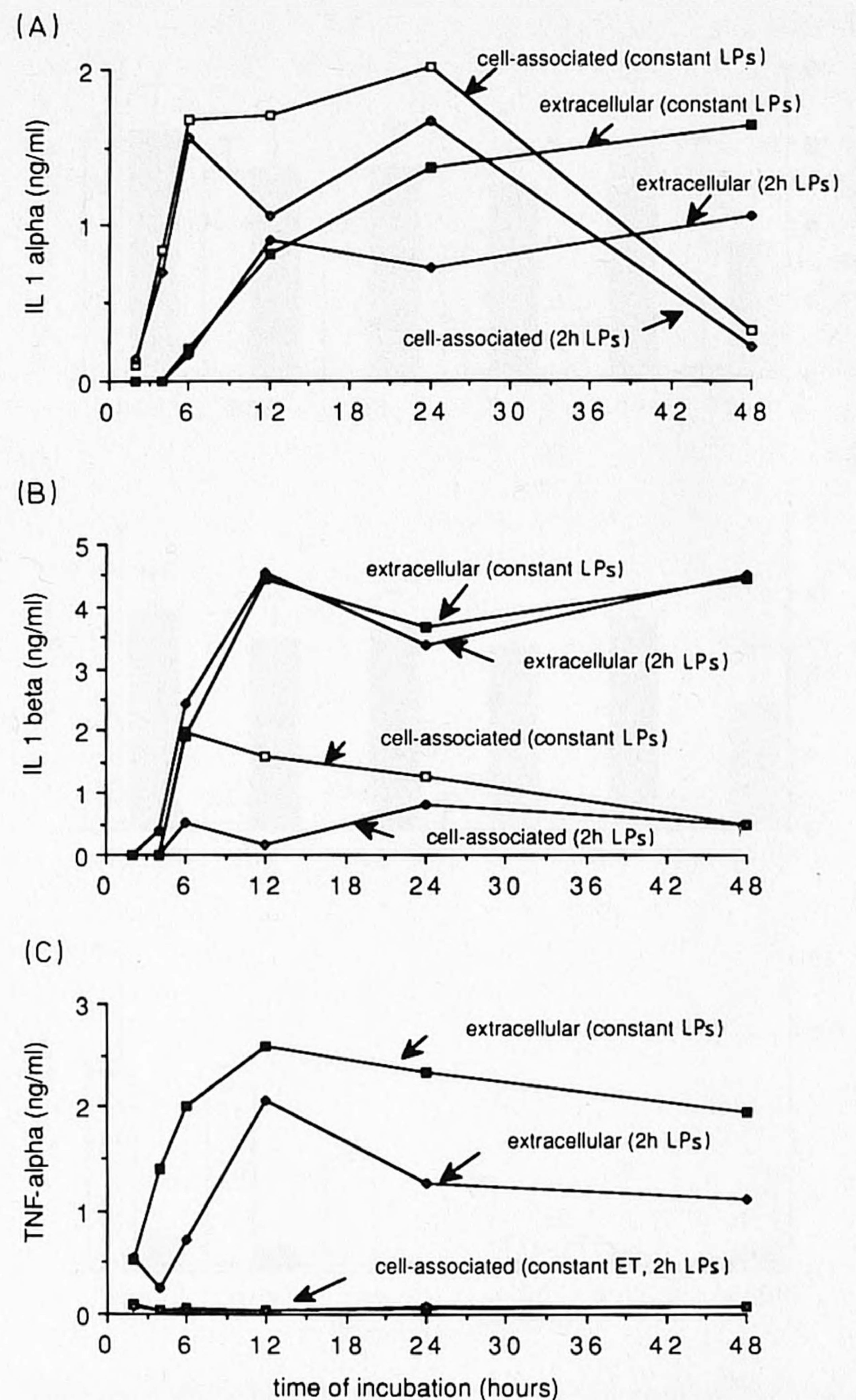n in response to various concentrations of LPS and tested the influence of 5% autologous plasma present during 18 h of incubation. Second, we investigated the kinetics of production and release of IL 1α, IL 1β and TNF-α from human MNC stimulated with 500 ng/ml LPS in the presence of 1% AB serum and 5% plasma.

In the presence of 1% AB serum, LPS induces more total IL 1α than IL 1β. This is a surprising new finding since other authors studying mRNA levels found more mRNA for IL 1β than for IL 1α in LPS-stimulated MNC [17]. Therefore, translation following transcription seems to be regulated or at least influenced differently for the two forms of IL 1. Compared to plasma-free incubations, in the presence of 5% plasma, both IL 1α and IL 1β production as well as release are increased. The enhancing effect of plasma is more effective on IL 1β than on IL 1α synthesis, so that the addition of plasma to the MNC incubation leads to the production of equal amounts of IL 1α and IL 1β. The mechanism by which plasma enhances LPS-induced IL 1 production is unknown. Although a synergism

between the complement component C5a and LPS has been described [25], the amount of C5a present in a solution containing only 5% plasma is unlikely to be alone responsible for the observed plasma effect on IL 1 production. Therefore, other factors seem to be involved, *i.e.* thrombin or platelet factors like serotonin which may also act synergistically with LPS on MNC IL 1 production [26].

Regardless of culture conditions, 80% of IL 1β is secreted into the extracellular compartment whereas 90% of IL 1α remains cell associated after 18 h of LPS stimulation. These results are supported by other authors who reported intracellular IL 1 activity, 90% of which had a pI of 5.5 and therefore was IL 1α [18]. Secreted amounts of both IL 1α and IL 1β are LPS dose dependent, whereas the cell-associated IL 1 production seems to be dose independent as long as the stimulating LPS concentration exceeds a threshold (5 ng/ml without plasma, 0.5 ng/ml with plasma).

Transcription and protein synthesis seems to be regulated similarly for IL 1α and IL 1β. In contrast, IL 1 release into the extracellular space seems to be controlled differently for the α and β forms of this cytokine. Although the absence of a signal peptide on the two IL 1 molecules indicates that both cytokines might not be typical secretory proteins, our results suggest that IL 1β is predominantly secreted whereas IL 1α remains cell associated.

The time-course studies of cytokine production and release from LPS-stimulated MNC confirm the results of the dose-response study with respect to the compartmentalization of IL 1. Most of the IL 1α remains cell associated up to 24 h, whereas IL 1β is found predominantly in the extracellular compartment within 6 h. Previous studies have suggested that plasmin [27] and other serine proteases [28] influence the amount of IL 1β secreted into the extracellular fluid and the present results are consistent with this finding, since the fresh plasma present during incubation probably contains proteases. A recent study suggests that the phosphorylation of the intracellular precursor of IL 1α may facilitate the processing and release of IL 1α and that this phosphorylation does not take place on IL 1β [29]. However, our results demonstrate IL 1β as the predominantly secreted form of IL 1 whereas IL 1α remains cell associated. Therefore, in the mechanism of secretion, phosphorylation seems to be less important for IL 1β than processing of the precursor by other factors like proteases [28].

We also measured TNF-α levels in the time course experiments. In contrast to IL 1β, the majority of IL 1α is present in the extracellular fluid already after 1 h of incubation. Transcription and translation on the one hand and transport into the extracellular space on the other hand seem to be closely linked for TNF-α. The differences in secretion kinetics for TNF-α compared to the IL 1 peptides could be explained by the different molecular structure of this cytokine. In contrast to both forms of IL 1, TNF-α possesses a typical signal peptide for cleavage and transport [5], suggesting that TNF-α is a secretory peptide. Recent studies, however, claim that TNF-α is also active as a cell-associated molecule [30].

We did not differentiate between cytosolic and membrane-bound IL 1 or TNF in this study. Since no ultracentrifugation was performed before measurement of cell-associated cytokines after the three freeze-thaw cycles, these samples may contain both cytosolic and membrane-bound IL 1 and/or TNF-α.

Several studies provide evidence that both forms of IL 1 exist as cytosolic as well as membrane-bound molecules. Conlon et al. demonstrated membrane-bound IL 1α on human MNC after 18 h of LPS stimulation [31]. Studies using anti-IL 1β antibodies identified cell surface IL 1 as the IL 1β molecule [32]. Cytosolic IL 1 has been specifically identified as IL 1α by IEF [18] and also as IL 1β by immunoprecipitation [28]. Sisson et al. [33], who fractionated the cell-associated compartments of immunoreactive cytokines in MNC stimulated with GM-CSF, found most of the cell-associated IL 1α and IL 1β in the cytosolic compartment.

Like LPS, GM-CSF also induced TNF-α in MNC, 90% of which was secreted into the extracellular compartment. Most of the low amount of cell-associated TNF-α was in the cytosolic compartment [33]. In contrast, a recent study described TNF acting as a cytotoxic membrane-bound protein [34]. However, these studies were done with cell lines which may differ from MNC. According to our results, LPS-stimulated MNC seem to process and release TNF-α very readily. Although we did not attempt to identify membrane-bound TNF-α in this study, our data suggest that this is a very small amount of the total TNF-α produced by MNC following LPS stimulation.

5 References

1 Dinarello, C. A., *FASEB J.* 1988. *2:* 108.

2 Beutler, B. and Cerami, A., *N. Engl. J. Med.* 1987. *316:* 379.

3 Lomedico, P. T., Gubler, U., Hellman, C. P., Dukovich, M., Giri, J. G., Pan, Y-C. E., Collier, K., Semionow, R., Chua, A. O. and Mizel, S. B., *Nature* 1984. *312:* 458.

4 Auron, P. E., Webb, A. C., Rosenwasser, L. J., Mucci, S. F., Rich, A., Wolff, S. M. and Dinarello, C. A., *Proc. Natl. Acad. Sci. USA* 1984. *81:* 7907.

5 Pennica, D., Nedwin, G. E., Hayflick, J. S., Seeburg, P. H., Derynck, R., Palladino, M. A., Kowr, J. W., Aggarwal, B. B. and Goeddel, D. V., *Nature* 1984. *312:* 724.

6 Dinarello, C. A., Cannon, J. G., Mier, J. W., Bernheim, H. A., LoPreste, G., Lynn, D. L., Love, R. N., Webb, A. C., Auron, P. E., Reuben, R. C., Rich, A., Wolff, S. M. and Putney, S. D., *J. Clin. Invest.* 1986. *77:* 1734.

7 Dinarello, C. A., *Year Immunol.* 1986. *2:* 68.

8 Dinarello, C. A., Cannon J. G., Wolff, S. M., Bernheim, H. A., Beutler, B., Cerami, A., Figari, I. S., Palladino, M. A. and O'Connor, J. V., *J. Exp. Med.* 1986. *163:* 1433.

9 Perlmutter, D. H., Dinarello, C. A., Punsal, P. I. and Colton, H. R., *J. Clin. Invest.* 1986. *78:* 1349.

10 Okusawa, S., Gelfand, J. A., Ikejima, T., Connolly, R. J. and Dinarello, C. A., *J. Clin. Invest.* 1988, in press.

11 Dewhirst, F. E., Stashenko, P. P., Mole, J. E. and Tsurumachi, T., *J. Immunol.* 1985. *135:* 2562.

12 Krane, S. M., Dayer, J. M., Simon, L. S. and Byrne, S., *Collagen Relat. Res.* 1985. *5:* 99.

13 Mandrup-Poulsen, T., Bendtzen, K., Dinarello, C. A. and Nerup, J., *J. Immunol.* 1987. *139:* 4077.

14 Elias, J. A., Gustillo, M., Baeder, W. and Freundlich, B., *J. Immunol.* 1987. *138:* 3812.

15 Dinarello, C. A., Ikejima, T., Warner, S. J. C., Orencole, S. F., Lonnemann, G., Cannon, J. G. and Libby, P., *J. Immunol.* 1987. *139:* 1902.

16 Libby, P., Ordovas, J. M., Auger, K. R., Robbins, A. H., Birinyi, L. K. and Dinarello, C. A., *Am. J. Pathol.* 1986. *124:* 179.

17 Demczuk, S., Baumberger, C., Mach, B. and Dayer, J. M., *J. Mol. Cell. Immunol.* 1987. *3:* 255.

18 Lasfargues, A., Ledur, A., Charon, D., Szabo, L. and Chaby, R., *J. Immunol.* 1987. *139:* 429.

19 Lepe-Zuniga, J. L., Zigler, J. S., Jr., Zimmerman, M. L. and Gery, I., *Mol. Immunol.* 1985. *22:* 1387.

20 Lisi, P. J., Chu, C. W., Koch, G. A., Endres, S., Lonnemann, G. and Dinarello, C. A., *Lymphokine Res.* 1987. *6:* 229.

21 Lonnemann, G., Endres, S., Van der Meer, J. W. M., Ikejima, T., Cannon, J. G. and Dinarello, C. A., *Lymphokine Res.* 1988. *7:* 75.

22 Van der Meer, J. W. M., Endres, S., Lonnemann, G., Cannon, J. G., Ikejima, T., Okusawa, S., Gelfand, J. A. and Dinarello, C. A., *J. Leukocyte Biol.* 1988. *43:* 216.

23 Dinarello, C. A., Lonnemann, G., Maxwell, R. and Shaldon, S., *J. Clin. Microbiol.* 1987. *25:* 1233.

24 Knudsen, P. J., Dinarello, C. A. and Strom, T. B., *J. Immunol.* 1988. *137:* 3189.

25 Okusawa, S., Dinarello, C. A., Yancey, K. B., Endres, S., Lawley, T. J., Frank, M. M., Burke, J. F. and Gelfand, J. A., *J. Immunol.* 1987. *139:* 2635.

26 LoPreste, G. and Dinarello, C. A., *Lymphokine Res.* 1987. *6:* 72 (Abstract).

27 Matsushima, K., Taguchi, M., Kovacs, E. J., Young, H. A. and Oppenheim, J. J., *J. Immunol.* 1986. *136:* 2883.

28 Auron, P. E., Warner, S. J. C., Webb, A. C., Cannon, J. G., Bernheim, H. A., McAdam, K. J. P. W., Rosenwasser, L. J., LoPreste, G., Mucci, S. F. and Dinarello, C. A., *J. Immunol.* 1987. *138:* 1447.

29 Kobayashi, Y., Appella, E., Yamada, M., Copeland, T. D., Oppenheim, J. J. and Matsushima, K., *J. Immunol.* 1988. *140:* 2279.

30 Decker, T., Lohmann-Mathes, M. L. and Gifford, G. E., *J. Immunol.* 1987. *138:* 957.

31 Conlon, P. J., Grabstein, K. H., Alpert, A., Prickett, K. S., Hopp, T. P. and Gillis, S., *J. Immunol.* 1987. *139:* 98.

32 Bayne, E. K., Rupp, E. A., Limjuco, G., Chin, J. and Schmidt, J. A., *J. Exp. Med.* 1986. *163:* 1267.

33 Sisson, S. D. and Dinarello, C. A., *Blood* 1988. *72:* 1368.

34 Kriegler, M., Perez, C., DeFay, K., Albert, I. and Lu, S. D., *Cell* 1988. *53:* 45.