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Production of Inflammatory Mediators by Human Macrophages Obtained from Ascites

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ABSTRACT. **Ascites is a readily available source of human macrophages (Mø), which can be used to study Mø functions in vitro. We characterized the mediators of inflammation produced by human peritoneal Mø (hp-Mø) obtained from patients with portal hypertension and ascites.**

The production of the cytokines interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) was found to be lipopolysaccharide (LPS) concentration dependent (0–10 μg/ml) with a maximal production at 10 μg/ml and also dependent on the time of exposure to the stimulus (0–36 h). IL-1β, IL-6 and TNF-α production after LPS administration reached a plateau at 24 h.

In vitro stimulation for 24 h with LPS does not influence the eicosanoid production from endogenous arachidonate. 13 min of exposure of the cells to the calcium ionophore A23187 gives a significant increase in eicosanoid production from both exogenous and endogenous arachidonate. The main eicosanoids produced are the 5-lipoxgenase products LTB_4 and 5-hydroxyeicosatetraenoic acid (HETE). The increase in production of the other eicosanoids is not significant. The eicosanoid production depends on the stimulus concentration. The optimal A23187 concentration is 1 μM.

Oxygen radical production was measured in the Mø by a flowcytometric method. The fluorescence intensity of phorbol 12-myristate 13-acetate stimulated and dihydro-rhodamine 123 loaded hp-Mø increases significantly after 15 min.

We conclude that LPS stimulation of hp-Mø from liver disease results in similar production of IL-1β, IL-6 and TNF-α, but that the profile of the eicosanoid production of these Mø stimulated with LPS and A23187 differs from Mø of other origin and species.

INTRODUCTION

Macrophages (Mø) are of central importance in the initiation and regulation of non-specific and specific immune responses. They take part as active phagocytic cells, serve as antigen presenting cells (APC) for T-lymphocytes and are a major source of a wide range of inflammatory products including eicosanoids and cytokines in different types of immune responses (e.g. host defence against microorganism, antitumor and chronic inflammation). When monocytes or tissue Mø are stimulated, both develop into active Mø. Profound changes then occur in morphology and function. Major changes are: increase in size, more complex and numerous surface folds and an increase in the numbers of vacuoles, lysosomes, phagosomes and endoplasmatic reticular elements. The adhesiveness and expression of Ia antigens increases. The Mø become more sensitive for agents which trigger oxygen radicals and they secrete a large range of products including enzymes, coagulation factors, proteins of complement system, factors regulating cell activities and proliferation, bioactive lipids, nucleotide metabolites and reactive oxygen metabolites (1, 2).

In vivo Mø activation appears to be mediated and/or modulated by microbial pathogens and their products, lipid mediators and cytokines (3, 4). In vitro activation can be initiated by a variety of agents, including calcium ionophore A23187 (A23187) (5), lipopolysaccharide (LPS) (6, 7) or cytokines (8, 9). The cytokines interleukin-1 (IL-1) (10), interleukin-6 (IL-6) (11) and tumor necrosis factor (TNF-α) (12) are important Mø cytokine products when stimulated with LPS (7), which is one of the most potent stimuli of Mø (13).

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Arachidonic acid can be metabolized to biologically active eicosanoids. The cyclooxygenase pathway generates the prostanoids and the 5-lipoxygenase pathway produces leukotriene B_4 (LTB_4) and 5-hydroxyeicosatetraenoic acid (5-HETE) (14, 15). Eicosanoids exhibit distinct biological effects (16–19). Like the cytokines they have an important function in controlling the immune reaction. A23187 is one of the most potent stimuli of eicosanoid production (5, 20, 21).

The respiratory burst is a characteristic feature of phagocytes. The production and release of radical oxygen intermediates (ROI) by Mø plays a major role in several of the effector functions of these cells. Oxygen radicals are of critical importance for antibacterial defence. They are also involved in tumoricidal activities, in suppression of lymphocyte proliferation (22, 23) and have been implicated as important mediators of tissue damage in inflammation (24, 25). Oxygen radicals may be involved in a positive feedback mechanism of inflammation; superoxide anion (O_2^-) can induce IL-1 production in monocytes and polymorphonuclear leucocytes (PMNs) (26).

We have previously isolated human peritoneal Mø (hp-Mø) from ascitic fluid, obtained from patients with liver cirrhosis to investigate various aspects of their metabolism and the role of Mø in inflammation (27, 28). The aim of this study is to analyze the profile of eicosanoid production, the levels of cytokine production and the respiratory burst activity in this type of hp-Mø, regarding the potential influence of liver disease on these parameters, in comparison with other types of Mø.

MATERIALS AND METHODS

Subjects

The hp-Mø were obtained from ascitic fluid of 12 patients with liver cirrhosis, 2 patients with congestive heart failure, 1 patient with primary slcerosing cholangitis, 1 patient with malignant mesothelioma and 1 patient with the Budd-Chiari syndrome (Table 1). None of the patients were on drugs which have been reported to influence the lipoxygenase and cyclooxygenase enzymes.

Cell isolation

The hp-cells were isolated by centrifugation of the ascitic fluid at 400xg (4°C) and washed with phosphate buffered saline (PBS, pH 7.4, 4°C, Oxoid, UK). The concentrated hp-cell suspension was separated on Percoll (d = 1.064 g/ml, Kabi-Pharmacia, Sweden). A small sample of cells was stained by Hemacolor (Merck, Germany) and the different cell types were counted under a microscope (Zeiss, standard 25, Germany). The viability of the cells was tested by trypan blue exclusion.

Table 1 List of patients with ascites (n = 17)

Number	m/f	age	Diagnosis
1.	male	68	biliary liver cirrhosis
2.	male	65	congestive heart failure
3.	female	50	alcoholic hepatitis and liver cirrhosis
4.	female	72	alcoholic liver cirrhosis
5.	male	69	alcoholic liver cirrhosis
6.	female	14	alcoholic liver cirrhosis
7.	female	65	alcoholic liver cirrhosis
8.	male	69	congestive heart failure
9.	female	38	Budd- Chiari syndrome
10.	male	46	alcoholic liver cirrhosis
11.	male	65	cryptogenic liver cirrhosis
12.	male	59	alcoholic liver cirrhosis
13.	male	60	alcoholic liver cirrhosis
14.	male	39	primary sclerosing cholangitis
15.	female	42	alcoholic liver cirrhosis
16.	male	39	alcoholic liver cirrhosis
17.	male	82	malignant mesothelioma

Cytokine production

For the measurement of the cytokine production one million leucocytes per ml RPMI (RPMI-1640 + HEPES (GIBCO, UK) + penicillin/streptomycin (5×10^4 μ/l / 50 mg/l, Flow Lab, UK) + foetal calf serum (FCS) (10% FCS, GIBCO, UK) + L-glutamine (600 mg/l Flow Lab, UK)) were plated on plastic culture dishes (Costar, UK). The cells were stimulated with the addition of increasing concentrations of lipopolysaccharide (0–10 μg/ml, 36 h, LPS from *E. coli* 0111:B4 in PBS, Sigma, USA) or incubated in the absence or presence of LPS (10 μg/ml) for an increasing time period (0–36 h, 37°C, 7.5% CO_2). As blanc PBS was added. The net cytokine production is the production with LPS minus blanc of cells incubated for the same period of time. At the end of the incubation the supernatant was filtered (0.22 μm, Millipore, France) and kept at –70°C till analyzing. IL-1β and IL-6 production in the supernatant of the samples were measured by ELISA (IL-1β: Medgenix, Belgium, IL-6: Hycult, Holland). Tumour necrosis factor-α (TNF-α) production of the samples was determined with a bioassay and partly by ELISA (Cistron, USA). For this bioassay the TNF-α sensitive cell line WEHI-164 was used. The WEHI-164 cells were plated out in 96-wells plates (2×10^4 cells/50 μl/well, Costar, UK) and the samples (50 μl/well) or the human recombinant TNF-α (hr-TNF-α) standards (0.1–1000 μ/ml hr-TNF-α, 50 μl/well) were added. After 24 h of incubation (37°C, 7.5% CO_2), MTT (Tetrazolium salt, Sigma, USA) was added (0.125 mg/well) and after an incubation of 3 h the cells were lysed with buffer (20% sodium dodecyl sulphate (SDS) in 50% N,N-dimethylformamide (DMF), pH 4.7, 100 μl/well) during 18 h. The absorbance was measured at 595 nm with an ELISA-reader (BIO RAD, model 3550, UK). The TNF-α production by hp-Mø is expressed as the cytotoxicity against WEHI-164 cells compared to blanc (RPMI complete) (29).

Eicosanoid production

For the measurement of eicosanoid production from exogenous arachidonate, 1 × 10^6 leucocytes per ml PBS (+ Ca^{2+}, pH 7.4, Oxoid, UK) in polypropylene tubes (Greiner, The Netherlands) were labelled with ^{14}C-arachidonic acid (^{14}C-AA) (0.125 µCi, 55.7 MCi/mmol, Amersham, UK) for 2 min and triggered by A23187 (0–5 µM final concentration in dimethylsulfoxide (DMSO, Sigma, USA) for 13 min at 37°C. Controls were incubated with DMSO. ^3H-labelled standards consisting of $6kPGF_{1\alpha}$, prostaglandin E_2 (PGE_2), leukotriene B_4 (LTB_4), 12-HETE and 15-HETE (approximately 0.01 µCi of each eicosanoid, Amersham, UK) were added for recovery calculations and as retention time markers. The supernatants were then passed through Sep Pak C_{18} cartridges (Waters Ass., USA) and eluted with methanol, dried and dissolved in 200 µl methanol. Eicosanoid formation was measured on RP-HPLC using a Nucleosil $5C_{18}$ column (3 × 200 mm), Chrompak, The Netherlands). 100 µl sample was injected onto the column. As eluens a gradient of acetonitrile and 0.2% triethylamine/0.132% trifluoracetic acid/water (pH > > 3) was used. The eicosanoid formation is expressed as the mean % of total formation of the most common metabolites per patient. In these samples, both A23187 stimulated and non-stimulated, eicosanoid production from endogenous arachidonate was measured (LTB4, PGE_2, thromboxane B_2 (TXB_2) and $6kPGF_{1\alpha}$). In the cytokine samples (24 h, 10 µg/ml LPS In RPMI) also the eicosanoid production from endogenous arachidonate was measured by radio immuno assays (antibodies were obtained from Advanced Magnetics, USA and standards from Sigma, USA). The exogenous eicosanoid production is given as net production. This is the production of the cells with A23187 minus control values.

Respiratory burst activity

ROI production was measured with dihydrorhodamine 123 (DHR 123, Molecular probes, USA). The stock solution was 1 mg/ml in N,N-dimethylformamide (Sigma, USA); further dilution was performed with PBS. DHR 123 is oxidized intracellularly to fluorescent rhodamine 123 (R123) by hydrogen peroxide.

Leucocytes (0.25 × 10^6/ml) were incubated in RPMI in polypropylene tubes with increasing concentrations of phorbol 12-myristate 13-acetate (0 – 1000 ng/ml, TPA diluted in DMSO: PBS = 1:10, Sigma, USA, 10 min, 37°C, blanc is 10% DMSO) directly followed by incubations with increasing concentrations of DHR 123 (0–100 ng/ml, 15 min, 37°C). In the time experiments TPA and DHR 123 were added to the cells at the same time and incubated for an increasing time period (0–1 h, 100 ng/ml TPA, 333 ng/ml DHR 123, 37°C). Samples were put on ice straight after the incubations. The fluorescence intensity (mean channel number (MCN) was measured with a fluorescence activating cell scanner (FACS, Becton Dickinson Immunocytometry Systems, USA). R 123 is detected as Fluorescence 1 (green; 500–530 nm) (30, 31). 5000 cells per sample were measured. The data were evaluated with the FACS SCAN program system (Becton Dickinson Immunocytometry Systems, USA). The net ROI production is the fluorescence intensity (in MCN) of the TPA stimulated and DHR 123 loaded cells, minus the fluorescence intensity (in MCN) of the non-stimulated cells which were also loaded with DHR 123 and incubated for the same period of time.

Statistical analysis

The data are expressed as the mean ± SEM. Data were statistically analyzed with Anova followed by the Student's t-test or the Student's t-test only; data were considered significant when $p < 0.05$.

RESULTS

Cell differentiation

The Percoll separated cell suspension was microscopically examined and the following composition of cells was found: 94.6 ± 1.2% Mø, 4.0 ± 1.1% lymphocytes and 1.2 ± 0.4% granulocytes (n = 17). The viability of the cells was 86 ± 1.3% (range: 94.2–75.8).

Cytokine production

To determine LPS concentration for optimal IL-1β, IL-6 and TNF-α production, hp-Mø were stimulated with increasing concentrations of LPS (0–10 µg/ml) for 36 h as shown in Figure 1. The highest production was with the highest LPS concentration used. All subsequent experiments were performed with 10 µg/ml LPS.

The time dependent net IL-1β, IL-6 and TNF-α production from 0–36 h with LPS is shown in Figure 2. The maximal net cytokine production for all three cytokines was reached at 24 h.

The IL-1β (n = 16), IL-6 (n = 10) and TNF-α (n = 10/17) production without or after stimulation with 10 µg/ml LPS for 24 h is shown in Table 2. LPS stimulation resulted in a significant increase in the production of each cytokine. Mø from 6 out of 16 patients had no IL-1β production without LPS, all patients had IL-6 production without LPS stimulation and 6 out of 10 patients had no TNF-α production without LPS.

Eicosanoid production

First the A23187 concentration was determined (0–5 µM) which yielded the most eicosanoid production. Figure 3 (top and middle) shows the LTB_4 and 5-HETE production from added ^{14}C-AA (exogenous production) and

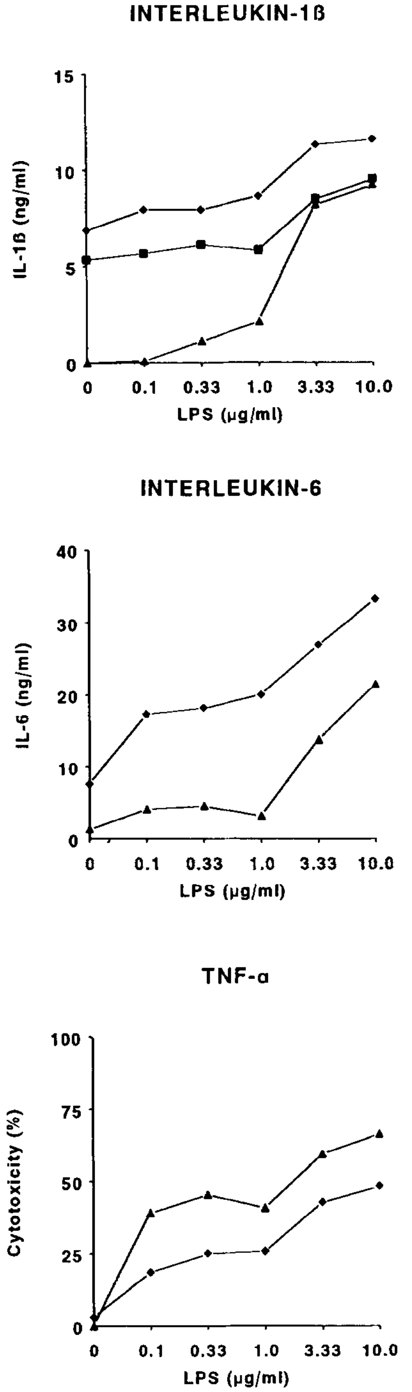

Fig. 1 Interleukin-1β (n = 3, ng/ml, top Fig) interleukin-6 (n = 2, ng/ml, middle Fig) and tumor necrosis factor (n = 2, % killing, bottom Fig) production by hp-Mø (10^6/ml) stimulated with an increasing concentration of lipopolysaccharide (LPS): 0–10 µg/ml. The results are shown separately per patient (■, ◆, ▲).

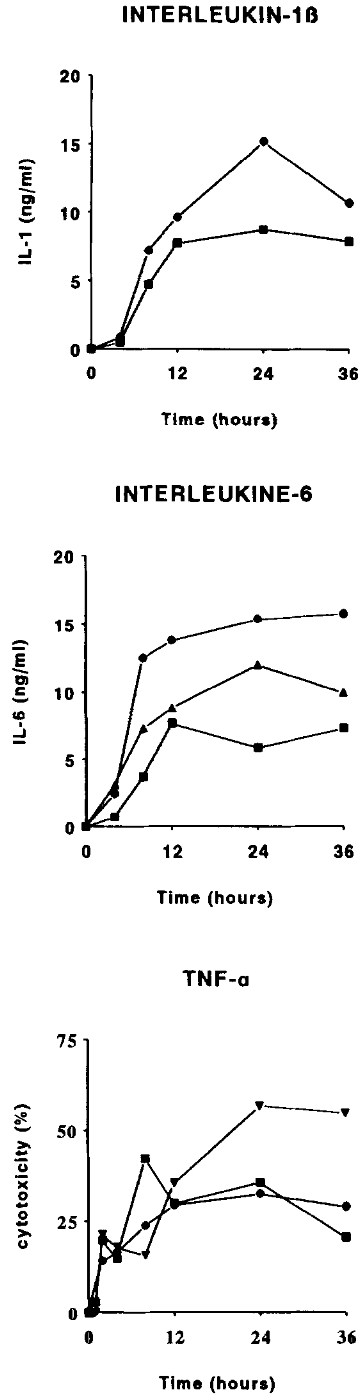

Fig. 2 The time dependency of net interleukin-1β (n = 2, ng/ml, top Fig), interleukin-6 (n = 3, ng/ml, middle Fig) and tumor necrosis factor (% killing, n = 3, bottom Fig) production of hp-Mø (10^6/ml) stimulated with 10 µg/ml lipopolysaccharide (LPS). The cells were stimulated for 0–36 h. The results are shown separately per patient (■, ●, ▲).

Figure 3 (bottom) the endogenous LTB_4 production as measured by radioimmunoassay (RIA) of 3 patients. The optimal concentration is 1–2 µM. Consequently all following experiments were performed with 1 µM A23187.

All eicosanoids formed from exogenous arachidonate by hp-Mø after A23187 stimulation are listed in Table 3A. The main net eicosanoids were the 5-lipoxygenase products LTB_4 and 5-HETE, the minor net eicosanoids were the cyclooxygenase products HHT and TXB_2. Other eicosanoids were less then 1.5% of ^{14}C-AA which was converted per product.

To investigate if eicosanoid production from exogenous and endogenous arachidonate were related to each

Table 2 Cytokine production by hp-Mø. IL-1β, IL-6 and TNF-α generation by 10^6 hp-Mø/ml stimulated for 24 h with 10 μg/ml LPS. Data are expressed as mean ± SEM and statistically analyzed by Student's t-test

Product	Control	LPS	n
IL-1β (ng/ml)	3.0 ± 1.2	8.7 ± 1.5*	16
IL-6 (ng/ml)	6.1 ± 1.4	32.6 ± 7.1*	10
TNF-α (ng/ml)	0.1 ± 0.1	2.7 ± 1.1*	10
(% killing)	21.6 ± 5.1	47.7 ± 3.9*	17

*$p < 0.05$ (increase vs controls). n = number of patients.

Table 3(A) Eicosanoid production from exogenous arachidonate by hp-Mø. Profile of eicosanoid production from exogenous ^{14}C-arachidonic acid in 10^6 hp-Mø/ml after A23187 stimulation (1 μM, 13 min, expressed as % of total formation per patient)

Net exogenous lipoxygenase products A23187 (%)		Net exogenous cyclooxygenase products A23187 (%)		n
LTB_4	32.1 ± 3.9*	6kPGF$_{1\alpha}$	0.5 ± 0.3	5
5-HETE	57.2 ± 3.9*	TXB_2	2.6 ± 1.3	5
di-HETE	1.4 ± 0.4	HHT	4.3 ± 1.6	5
12-HETE	0.3 ± 0.2	PGF$_{2\alpha}$	0.9 ± 0.5	5
15-HETE	0.7 ± 0.3	PGE_2	0.1 ± 0.04	5
		PGD_2	0.1 ± 0.1	5

*$p < 0.05$ (increase vs controls).

Table 3(B) Eicosanoid production from endogenous eicosanoid by hp-Mø. Eicosanoid production from endogenous arachidonate in 10^6 hp-Mø/ml with and without A23187 stimulation (1 μM, 13 min, expressed as ng/ml)

	Control (ng/ml)	A23187 (ng/ml)	n
Lipoxygenase product			
LTB_4	1.0 ± 0.3	7.6 ± 1.7*	11
Cyclooxygenase products			
PGE_2	0.1 ± 0.1	0.24 ± 0.1	11
TXB_2	1.8 ± 0.2	1.8 ± 0.3	5
6kPGF$_{1\alpha}$	0.4 ± 0.4	0.4 ± 0.3	5

* $p < 0.05$ (increase vs controls).

other, four eicosanoids were measured in the same samples by RIA, as in which ^{14}C-AA conversion was determined by HPLC (Table 3b). The main eicosanoid was also a 5-lipoxygenase product (LTB_4). Cyclooxgenase production did not increase significantly with A23187.

Furthermore eicosanoid production from endogenous arachidonate was measured in the same samples as in which the cytokine production was measured (24 h LPS). LPS did not significantly influence eicosanoid production from endogenous arachidonate over 24 h as measured by RIA (Table 4).

Respiratory burst

To measure the respiratory burst activity of the hp-Mø, the method published by Emmendorffer et al using dihydrorhodamine DHR 123 for human neutrophils (31) was modified for peritoneal Mø. The optimal net for-

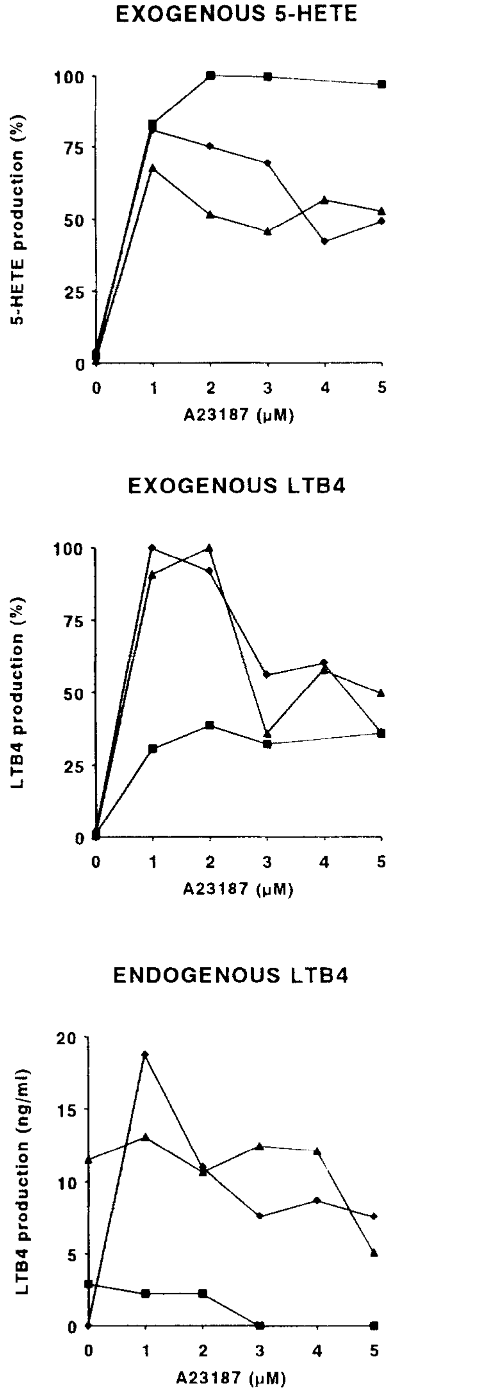

Fig. 3 Eicosanoid formation from exogenous (top Fig; 5-HETE, middle Fig; LTB_4, % of total formation per patient) and endogenous arachidonate (bottom Fig; LTB_4, ng/ml) production of hp-Mø (10^6/ml). Cells were prelabeled with ^{14}C-AA (2 min), triggered with 1 μM A23187 for 13 min and ^{3}H labelled standards were added afterwards. The results of three patients are shown separately: (■, ◆, ▲).

mation of ROI loaded with (DHR 123) (0–1000 ng/ml, 10 min) and stimulated with phorbol 12-myristate 13-acetate (TPA) (0–1000 ng/ml, 15 min) is given in Figure 4 (top left and top right). All subsequent experiments were performed with 100 ng/ml TPA and 333 ng/ml DHR 123. To check whether this method depended on the cell activity, the ROI production was also measured in

Table 4 Eicosanoid production from endogenous arachidonate by hp-Mø. Eicosanoid production from endogenous arachidonate in 10^6 hp-Mø/ml with LPS (10 μ/ml, 24 h, expressed as ng/ml). LPS stimulation does not significantly (P < 0.05) stimulate eicosanoid production (vs controls)

	Control (ng/ml)	LPS (ng/ml)	n
Lipoxygenase products			
LTB_4	1.0 ± 0.2	1.2 ± 0.3	16
Cyclooxygenase products			
PGE_2	1.7 ± 0.3	2.1 ± 0.2	16
TXB_2	3.4 ± 0.3	3.2 ± 0.2	5
$6kPGF_{1\alpha}$	2.7 ± 0.3	3.1 ± 0.3	5

cells (n = 4) which were kept on ice during the stimulation and loading (10 + 15 mins.). In Figure 4 (left bottom) the temperature dependency for the ROI production at 4°C (n = 4) and 37°C (n = 10) is shown. There was no significant ROI production increase at 4°C, but TPA increased the net ROI production significantly at 37°C.

The respiratory burst activity dependence on the time of stimulus is shown in Figure 4 (bottom right). The ROI production was significantly different after 15 min of stimulation. DHR 123 and TPA were given at the same time now.

DISCUSSION

Aim

In previous experiments we have shown that ascites is a good source of hp-Mø which can be used to study eicosanoid production by human Mø (27, 28, 32). These Mø can be used as a model for testing the effects of anti-inflammatory drugs on the eicosanoid production in vitro, as we have shown previously with drugs such as malotilate (33) and E6080 (34). By gaining more information on the inter-relationships between the inflammatory mediators, such as eicosanoids, cytokines and oxygen radicals of the hp-Mø we expect to expand our ex vivo anti-inflammatory drug testing model with human inflammatory cells.

The aim of this study was to characterize in addition to the eicosanoid production other cell functions of hp-Mø including cytokine production and respiratory burst activity.

Results

We investigated the cytokine production of hp-Mø

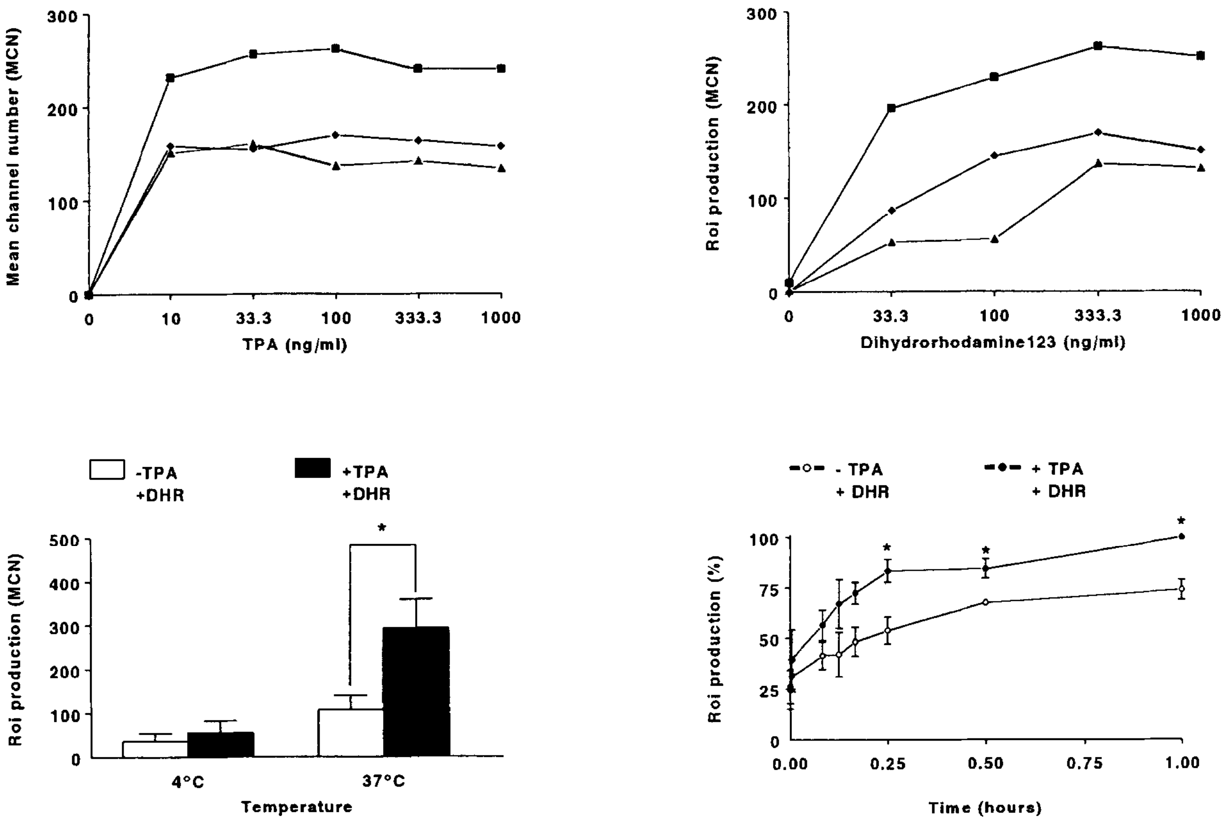

Fig. 4 The radical oxygen intermediates (ROI) of hp-Mø (0.25 x 10^6/ml) was measured with dihydrorhodamine 123 (DHR 123). Left top Figure shows the dependency of net ROI production on phorbol 12-myristate 13-acetate (TPA) concentration (0–1000 ng/ml, 10 min TPA + 15 min 333 ng/ml DHR 123, 37°C, n = 3). Right top Figure shows the dependency of net ROI production on DHR 123 concentration (0–1000 ng/ml, 10 min 100 ng/ml TPA + 15 min DHR 123, 37°C, n = 3). The results of three patients are shown separately; (■, ◆, ▲). Left bottom Figure shows the spontaneous (open bar) and TPA-stimulated (closed bar) respiratory burst activity at 4°C (n = 4) versus 37°C (n = 10) (10 min blanc or 100 ng/ml TPA + 15 min 333 ng/ml DHR 123). In these 3 graphs the ROI production is given in mean channel number (MCN). Data are expressed as mean ± SEM and statistically significant (*) when p < 0.05 vs controls (spontaneous respiratory burst). Right bottom Figure shows the stimulus time dependency (n = 3). Blanc (○) or TPA (●) and DHR 123 were given at the same time (t = 0–1 h, 100 ng/ml TPA, 333 ng/ml DHR 123, 37°C, n = 3). The ROI production is expressed as % increase of maximal increase (=100%) per patient with TPA. Data are expressed as mean ± SEM and statistically significant (*) when p < 0.05 vs controls (t = Oh).

stimulated with LPS as this is an important stimulus for Mø in vivo (7, 13). The eicosanoid production was determined both with LPS and A23187 as this stimulus is known to be a potent trigger for lipoxygenase products derived from Mø (20, 21, 5).

The experiments showed that the production of the cytokines IL-1β, IL-6 and TNF-α was LPS concentration dependent and also dependent on the time of exposure to the stimulus. LPS stimulation influenced the net IL-1β, IL-6 and TNF-α production significantly, but did not have an appreciable effect on the eicosanoid production from endogenous arachidonate. A short stimulation with A23187 gave a significant increase in lipoxygenase products from exogenous and endogenous arachidonate. The eicosanoid production from exogenous and endogenous arachidonate depended on the A23187 concentration.

Production of measurable amounts of ROI in vitro of the hp-Mø can be triggered by TPA. With the lowest concentration of the stimulating agent the ROI production was maximal, higher concentrations of TPA did not change the level of production. The measurement of ROI production was DHR 123 concentration dependent. Beyond 333 ng/ml the ROI production slightly decreased, the DHR 123 concentration was no longer a limiting factor. With optimal concentrations of TPA and DHR 123 the net respiratory burst activity increased significantly. When stimulus and DHR 123 were given at the same time, the increase was significant after 15 min.

Comparison of cytokine and eicosanoid production in Mø from different species and/or origin

Similar kinetics as we had found for LPS induced cytokine production have been previously reported for human alveolar Mø (35–37) hp-Mø obtained from continuous ambulatory peritoneal dialysis (CAPD) patients (38, 39), a murine macrophage cell line (40) and murine peritoneal Mø (41, 43). In all these observations the cytokine production increased with increasing LPS concentration with a maximun in the range 1–10 µg/ml LPS. Human alveolar Mø had with LPS a maximal IL-1β (0–42 h and IL-6 (0–72 h) production by 24 h (a slight increase at 72 h for IL-6) (35, 36).

The IL-1 production of the murine macrophage cell line P388D1 (0–18 h) and murine peritoneal Mø (Alderey Park, 0–24 h) increased in time with LPS (40, 42). Peritoneal Mø from C3H/HEN and C3H/HEJ mice had with LPS (0–72 h) a maximal IL-1 production at 24 h (4). The IL-6 production was maximal between 8–12 h for the murine macrophage cell line (40).

TNF-α production of Mø of other origin or species usually reached a maximal level at an earlier time than our hp-Mø. TNF-α (0–42 h) production of human alveolar Mø peaked between 4–8 h (35). The murine macrophage cell line stimulated with LPS reached a plateau at 2 h (40). Rat peritoneal Mø reached a maximum at 6 h (44) and guinea pig peritoneal Mø around 8 h (45).

In our experiments LTB_4 and 5-HETE were the main eicosanoids formed (89%), when the Mø were stimulated with A23187. In normal bovine alveolar Mø A23187 stimulated the release of both the cyclooxygenase and lipoxygenase products (21), also in resident mouse peritoneal Mø (46, 47). Rat peritoneal Mø and rat Kupffer cells mainly synthesized cyclooxygenase products after A23187 stimulation (32).

In alveolar Mø from healthy volunteers, exogenous AA was predominantly metabolized via the 5-lipoxygenase pathway (20, 48, 49), but also cyclooxygenase products were formed, when stimulated with A23187 (20, 49).

Leukotrienes are known to mediate inflammatory reactions. LTB_4 is an important chemotactic agent for leucocytes (16), generates superoxide anion in neutrophils (17), whereas the sulfidopeptide LTs (LTC_4, LTD_4 and LTE_4) primarily affect smooth muscle contraction, increase vascular permeability (18) and promotes lysosomal enzymes (19). Specific inhibition of the enzyme 5-lipoxygenase has therapeutical potential in a variety of inflammatory conditions shown by different animal models and clinical trials (50–52) and as mentioned before we have tested the drugs malotilate (33) and E6080 (34) on the eicosanoid production in our in vitro model.

The pattern of eicosanoid metabolism depends on the stimulus applied (20, 21, 48), as well as the maturity of the cell (49), site of origin of macrophage and the species (32). In relation to this, in vivo priming might be another important point for influencing the eicosanoid and cytokine profile when the cells are stimulated in vitro. Cytokines and eicosanoids also regulate their own and each other's release in vivo and in vitro (53–55). The exact interaction of these mediators involved in human macrophage inflammation still has to be investigated.

Comparison of cytokine and eicosanoid production in hp-Mø from liver cirrhosis and CAPD patients

The high cytokine production of our hp-Mø with LPS was probably due to priming of the cells in vivo. The patients, where the cells were isolated from, have a chronic inflammation. Peritoneal Mø obtained from CAPD patients during peritonitis and compared to Mø obtained from the same type of patients during an infection free period, stimulated with LPS in vitro, released significantly more IL-1β and TNF-α (39, 56) whereas their prostacyclin release (measured as $6kPGF_{1\alpha}$, without and with LPS) had declined sharply (38).

The TNF-α bioactivity of the liver cirrhosis Mø seems lower then Mø of CAPD patients with peritonitis, however TNF-α was measured by different bioassays. Although the methods are comparable the % killing is higher with the ^{3}H-thymidine incorporation cytostatic assay compared to the MTT tetrazolium cytotoxic assay (29). Therefore the TNF-α bioactivity of hp-Mø from liver cirrhosis patients compared to Mø from CAPD patients with peritonitis could be in the same range. The concentrations of TNF-α measured by ELISA in supernatants of liver cirrhosis patients however showed a TNF-α production comparable to Møs from CAPD patients without peritonitis.

A pronounced difference between the Mø from liver cirrhosis and the Mø of CAPD patients with peritonitis was observed in the PGE_2 production. The PGE_2 release of human peritonitis Mø without LPS was initially much higher than the hp-Mø in our observations. Furthermore a significant increase in PGE_2 production in response to LPS was seen (no difference compared to infection-free Mø of CAPD patients). In conclusion the Møs of liver cirrhosis patients have striking features with Møs from CAPD patients with peritonitis (Table 5).

Oxygen radical production

In response to TPA the ability to generate oxygen radicals was enhanced. TPA stimulates the intracellularly and extracellularly respiratory burst of phagocytes (57, 58). The flow cytometric method we have used here reflects the total production of H_2O_2 inside single, stimulated, cells. DHR 123 is oxidized by a hydrogen peroxide and peroxidase dependent system. This method with DHR 123 seemed to be a highly sensitive indicator for the respiratory burst (59–61). In comparison with human monocytes and neutrophils the respiratory burst of monocyte-derived Mø was very low when measured with the luminol-amplified chemiluminescence assay (61), which measures intra- and extracellular H_2O_2 and O^{2-} (58). We were able to measure with DHR 123 a clear increase in the respiratory burst activity, when the Mø were stimulated with TPA. Our results were similar to Banati (30) whom measured the respiratory burst of rat peritoneal Mø with DHR 123. The Mø exhibit spontaneous respiratory burst activity before stimulation and after stimulation the fluorescence intensity had increased about a factor 3, our ROI production (in MCN) increased a factor 2.4.

Table 5 Comparison of cytokine and PG synthesis by human peritoneal macrophages from liver cirrhosis and CAPD patients without and with peritonitis. Compared are the eicosanoid and cytokine synthesis without stimulus (24 h PBS = blancs) and with stimulus (24 h LPS = LPS), LPS = 5 μg/ml CAPD hp-Mø (10^6 cells/ml) and 10μg/ml with liver cirrhosis hp-Mø (10^6 cells/ml). n = number of patients

Liver cirrhosis (this study)			n	CAPD (38, 39, 56)			
				Control	n	Peritonitis	n
IL-1β (ng/ml)	blancs	3.0 ± 1.2	16	0.3 ± 0.1	9	0.2 ± 0.1	8
	LPS	8.7 ± 1.5		1.0 ± 0.2		6.6 ± 2.8	
TNF-α (% killing)	blancs	21.6 ± 5.1	17	4.3 ± 5.1	21	12.9 ± 4.1	12
	LPS	47.7 ± 3.9		15.8 ± 6.5		61.9 ± 7.7	
TNF-α (ng/ml)	blancs	0.1 ± 0.1	10	1.0 ± 0.3	12	1.0 ± 0.3	12
	LPS	2.7 ± 1.1		2.6 ± 0.4		9.3 ± 4.3	
$6kPGF_{1\alpha}$ (ng/ml)	blancs	2.7 ± 0.3	5	19.1 ± 3.6	10	3.4 ± 1.4	10
	LPS	3.1 ± 0.3		21.4 ± 4.3		4.0 ± 1.2	
PGE_2	blancs	1.7 ± 0.3	5	15.1 ± 2.7	10	9.4 ± 2.6	10
	LPS	2.1 ± 0.2		27.4 ± 4.5		31.0 ± 4.4	

Conclusion

Ascitic fluid is an easily available source of large quantities of hp-Mø. In this study we have characterized the eicosanoid and cytokine production and respiratory burst activity of human Mø isolated from peritoneal ascitic fluid. The IL-1β, IL-6 and TNF-α kinetics of hp-Mø stimulated with LPS is similar to that of human lung Mø, peritoneal Mø of CAPD patients and peritoneal Mø of other species. The cytokine production is LPS concentration dependent and dependent on the time of exposure to LPS. The profile of eicosanoid production depends on both the species origin, location and the stimulus applied. LPS did not effect the eicosanoid production from endogenous arachidonate of the hp-Mø, while a stimulation with A23187 both increased the lipoxygenase production from exogenous and endogenous arachidonate. The Mø of liver cirrhosis patients have striking features with Mø from CAPD patients with peritonitis, probably due to priming of the Mø in vivo.

With optimal concentrations of TPA and DHR 123 the net respiratory burst activity in the hp-Mø increased significantly. Whether a relation exists between formation of cytokines and eicosanoids and the respiratory burst activity of the hp-Mø still has to be examined. Characterization of hp-Mø function show that ascites may be a promising source of Mø to set up a model for testing the effects of anti-inflammatory drugs in vitro on different types of inflammatory mediators produced by human Mø.

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