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Inflammatory mediators and activity of human peritoneal macrophages

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Abstract

Human peritoneal macrophages (hp-Mϕ) are a source of inflammatory mediators. After stimulation *in vitro* for 24 h with LPS there was a significant increase in cytokine production (IL-1, IL-6 and TNFα), but not in the production of eicosanoids from endogenous arachidonate. Leukotrienes are the predominant eicosanoids formed after stimulation with calcium ionophore for 15 min, while prostaglandin formation is insignificant. The fluorescence intensity of TPA-stimulated and DHR123 loaded hp-Mϕ (a measure of the respiratory burst) increases significantly in a short period of time. Hp-Mϕ will be useful as a model for testing the effects of anti-inflammatory drugs on eicosanoid and cytokine production and respiratory burst activity *in vitro*.

Introduction

In the absence of infection, macrophages (Mϕ) represent the predominant cell type in ascitic fluid. These cells are an important source of inflammatory mediators. We have isolated human peritoneal Mϕ (hp-Mϕ) from ascitic fluid, obtained from patients with liver cirrhosis, to investigate various aspects of their role in inflammation. In previous experiments we have shown with the novel 5-lipoxygenase inhibitor E6080 that the hp-Mϕ can be used as a model for testing anti-inflammatory drugs *in vitro* [1]. The aim of this study was to characterize other functions of hp-Mϕ in addition to eicosanoid production, including cytokine production and respiratory burst activity, to expand our anti-inflammatory drug testing model.

Materials and methods

Human peritoneal macrophages (hp-Mϕ) were obtained from ascitic fluid of 8 patients with liver cirrhosis, 1 patient with congestive heart failure and 1 patient with the Budd-chiari syndrome. None of the patients were on drugs which have been reported to influence lipoxygenase or cyclooxygenase enzymes. Cells were isolated by centrifugation of the ascitic fluid. The concentrated cell suspension was separated on Percoll ($d = 1.064$ g/ml). The hp-Mϕ purity was $95.7 \pm 1.2\%$ and viability was $86.8 \pm 1.9\%$.

For the measurement of cytokine production 1×10^6 leucocytes per ml RPMI were stimulated by incubation with 10 µg/ml lipopolysaccharide (LPS) for 24 h (37 °C, 5% CO_2). Interleukin-1 (IL-1) and

interleukin-6 (IL-6) production was measured in the medium by ELISA. Cytotoxicity of the medium on the tumor necrosis factor-α (TNF-α) sensitive cell line WEHI-164 [2] was used to determine levels of TNFα. In these samples the endogenous eicosanoid production (LTB_4, PGE_2, TXB_2 and $6kPGF_{1\alpha}$) was also measured by radioimmunoassay.

For the measurement of exogenous eicosanoid production, 1×10^6 leucocytes per ml phosphate buffered saline ($PBS + Ca^{2+}$) were labelled with ^{14}C-arachidonic acid for 2 min and triggered by 1 μM calcium ionophore A23187 for 13 min. The formation of eicosanoids was measured by RP-HPLC. In these stimulated and labelled samples the endogenous eicosanoid production was also measured. The exogenous eicosanoid formation was expressed as the mean percentage of total formation of the most common metabolites per patient.

The respiratory burst activity was measured with dihydrorhodamine 123 (DHR123). DHR123 is oxidized intracellularly to fluorescent rhodamine 123 (R123) by oxygen radicals. The fluorescence intensity was measured with a fluorescence activating cell scanner (FACS) and expressed as mean channel number (MCN). 0.25×10^6 leucocytes per ml were incubated in RPMI with 100 ng/ml phorbol 12-myristate 13-acetate (37 °C, 10 min) and 333 ng/ml DHR123 (10 min) or with 10 μg/ml LPS plus 333 ng/ml DHR123 for 24 h.

The data are expressed as the mean ± SEM. Data were analyzed with Student's *t*-test and considered significant at $p < 0.05$.

Results

In Table 1 the results are given of both the cytokine and endogenous eicosanoid production and the respiratory burst activity of the hp-Mϕ stimulated for 24 h with LPS. The main exogenous eicosanoids produced after 15 min stimulation with A23187 were LTB_4 (32.1 ± 3.9%) and 5-HETE (57.2 ± 3.9%). The minor products were HHT (4.3 ± 1%) and TXB_2 (2.6 ± 1.3%). The production of the other exogenous eicosanoids was less than 1.5% per product ($n = 9$).

The net endogenous eicosanoid production with A23187 after 15 min was 5.7 ± 3.8 ng/ml LTB_4, 0.2 ± 0.2 ng/ml PGE_2, 0 ± 0.3 ng/ml TXB_2 and 0 ± 0.4 ng/ml $6kPGF1\alpha$ ($n = 5$). As shown in the table, the net endogenous eicosanoid production with LPS after 24 h did not increase significantly.

Table 1
The mean cytokine and endogenous eicosanoid production and the respiratory burst activity of human peritoneal macrophages stimulated with LPS for 24 h.

Product	Control	LPS	*n*
IL-1 (ng/ml)	4.0 ± 1.8	11.4 ± 1.6*	10
IL-6 (ng/ml)	8.1 ± 2.0	19.5 ± 1.8*	4
TNF_α(% killing)	11.2 ± 3.1	53.7 ± 4.6*	9
Endogenous (ng/ml)			
LTB_4	1.3 ± 0.5	1.8 ± 0.3	10
PGE_2	2.2 ± 0.2	2.6 ± 0.2	10
TXB_2	3.4 ± 0.3	3.2 ± 0.2	10
$6kPGF_{1\alpha}$	2.7 ± 0.3	3.1 ± 0.3	10
Resp. burst (mcn)	495 ± 52	484 ± 37	2

* $p < 0.05$ (increase).

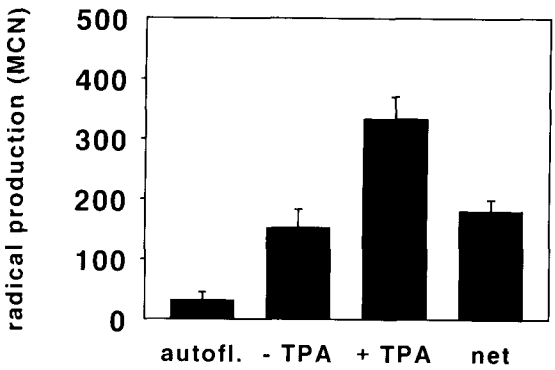

Figure 1
Spontaneous and TPA-stimulated (10 min) respiratory burst activity of DHR123 loaded (15 min) human peritoneal macrophages. Cellular autofluorescence is shown as control ($n = 10$).

Figure 1 shows the significant ($p < 0.05$) increase in oxygen radical production of the hp-Mϕ stimulated with TPA for 10 min and loaded with DHR123 (15 min) compared to the control, whereas the net fluorescence intensity with LPS at 24 h was zero.

Discussion/conclusion

Ascitic fluid is a readily available source of human peritoneal macrophages, which can be used to characterize the cell functions of the hp-Mϕ and to investigate various aspects of the role of macrophages in inflammation.

The results reported here give an outline of some cell activities of the hp-Mϕ. IL-1, IL-6 and TNFα production in these hp-Mϕ increase significantly following exposure to LPS, whereas eicosanoid production from endogenous arachidonate was not increased. After stimulation with calcium ionophore A23187 for 15 min the leukotrienes were the predominant eicosanoids formed, while prostaglandin formation was insignificant.

Measuring the respiratory burst of hp-Mϕ with dihydrorhodamine123 and a FACS is an easy, fast and specific method [3, 4]. The fluorescence intensity of TPA-stimulated hp-Mϕ loaded with DHR123 increase significantly in a short period of time. With LPS, the net fluorescence intensity was zero after 24 h.

The results show that ascites is a promising source of inflammatory cells. By measuring their eicosanoid and cytokine production and respiratory burst activity, hp-Mϕ will be useful as a model for testing a wide range of anti-inflammatory drugs *in vitro*.

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