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A microcalorimetric method to study the activation of murine peritoneal macrophages

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Abstract

A novel microcalorimetric method was used to continuously follow changes in the overall metabolic energy expenditure of rat peritoneal macrophages during PMA activation. Macrophages were isolated from: (1) healthy animals; (2) animals suffering from D-galactosamine induced acute liver injury (ALI); and (3) animals which sustained a glycerol induced renal failure (RF). Results from measurements on macrophages taken from healthy animals showed that microcalorimetric findings were reproducible and consistent with luminometric recordings of released reactive oxygen species. However, discrepancies were observed between results obtained with the two techniques in the ALI and RF groups, indicating differences in the mechanism by which these two conditions affect the macrophage system.

Keywords: Acute liver injury; Calorimeter; D-galactosamine; Luminometer; PMA; Peritoneal macrophages; Renal failure

1. Introduction

The ability of peritoneal macrophages and other phagocytic cells in the reticuloendothelial system (RES) to activate in response to immunological challenge is central to the innate body defence against external or internal invasions and insults [1]. Impaired function of the RES has been observed during different clinical and experimental conditions and may be correlated to high levels of septic and neoplastic complications [2].

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In the present study, RES function in animals suffering from (1) glycerol induced renal failure and (2) D-galactosamine induced acute liver injury has been assessed by measuring the ability of rat peritoneal macrophages to activate in response to stimulation by phorbol 12-myristate 13-acetate (PMA). Macrophage activity was studied using two different methods, luminometry and microcalorimetry. Luminometry is the commonly used method to measure the release of radical oxygen species during activation, whereas microcalorimetry provides a new, sensitive and continuous method to study both short- and long-term changes in metabolic activity [3,4]. Since macrophage activation is a complex process, we feel that incorporating a general method as microcalorimetry could provide new, and perhaps more comprehensive information about macrophage behaviour under different experimental and clinical conditions.

2. Experimental

2.1. Experimental design

Sprague-Dawley rats with an average weight of 255 g (212–325 g) (BK Universal AB, Sollentuna, Sweden) were divided into 3 groups. The first group received no treatment and was considered as normal control. Acute liver injury was induced in the second group (ALI) by intraperitoneal injection of D-galactosamine (1.1 g/kg of body weight, Sigma Chemical Co., St. Louis, USA). In the third group (RF), acute renal failure was induced by intramuscular injection of 50% (v/v) glycerol (10 ml/kg, Sigma Chemical Co.). After 24 h, peritoneal macrophages were harvested as described by Wang [5], using RPMI 1640 supplemented with 10% foetal calf serum, 0.3 g/l L-glutamine, 0.05 g/l gentamicin and 20 mM hepes-buffer (pH = 7.4). Macrophages were isolated (> 95%) by adhesion to cell culture plates. Blood samples were taken for determinations of serum levels of bilirubin and urea. Macrophage suspensions were prepared in supplemented RPMI 1640 prior to measurements. Viability, as determined by the trypan blue exclusion technique, was generally above 95% as experiments were initiated. At least four measurements were made in each experimental group.

2.2. Calorimetry

Heat production rates were measured using a 4-channel heat conduction calorimeter ("Thermal Activity Monitor" 2277, Thermometric AB, Järfälla, Sweden) equipped with 3 ml teflon coated titration/perfusion vessels [6, 7]. The sample compartment contained 2.7 ml of macrophage suspension in contact with air. All measurements were made at 37.0°C. Medium oxygen tension and pH were continuously monitored during some calorimetric experiments using miniaturised electrodes inserted into the vessel [8]. Macrophages were activated by the injection of 13.5 μl PMA-solution (0.1 μg/ml in 10% DMSO) to a final concentration of 0.5 ng/l.

2.3. Luminometry

Chemiluminescence was measured using an LKB Wallac 1250 luminometer (Bromma, Sweden). Cuvettes containing 2 ml of macrophage suspension (1×10^6 cells per ml, unless otherwise stated) were thermostated at 37.0°C. Macrophages were activated by the addition of 1 ml of incubation medium containing luminol (5-amino-2,3-dihydro-1,4-phthalazinedione, Sigma Chemical Co.) and PMA to final concentrations of 0.1 mg/l and 0.5 ng/l, respectively [9].

2.4. Oxygen consumption

The oxygen consumption rate was determined separately using a polarographic oxygen electrode with a 1.5 ml sample compartment, thermostated at 37.0°C (Han-

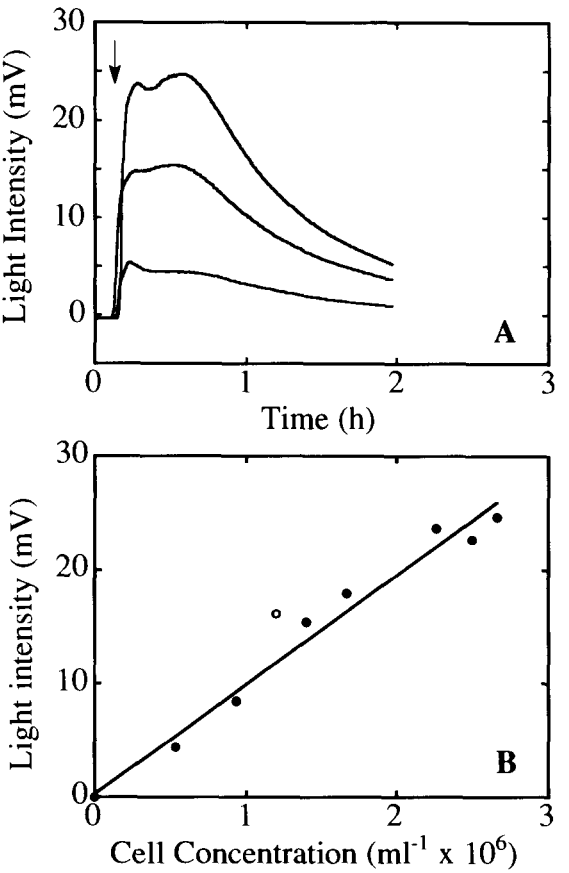

Fig. 1. **A** Chemiluminescence resulting from the release of reactive oxygen species by PMA-activated (0.5 ng/l) peritoneal macrophages. Records show the characteristic burst in chemiluminescence at three different cell concentrations (0.5, 1.4 and 2.7×10^6 cells per ml). The arrow indicates the time of PMA injection. **B** Chemiluminescence (peak values) as a function of cell concentration.

satech Ltd., Norfolk, UK). Oxygen uptake rates were calculated based on an oxygen solubility of 0.20 mmol/l in aerated medium ($pO_2 = 0.21$ bar) at 37.0°C.

2.5. Data presentation

Values are reported as mean ± SEM. Unpaired two tailed Student's t-test was used for statistical comparison of different data sets. Probability levels less than 5% were considered significant ($p < 0.05$).

3. Results and discussion

Macrophages taken from healthy animals responded to PMA stimulation with reproducible biphasic increases in chemiluminescence and heat output (P) (Figs. 1A

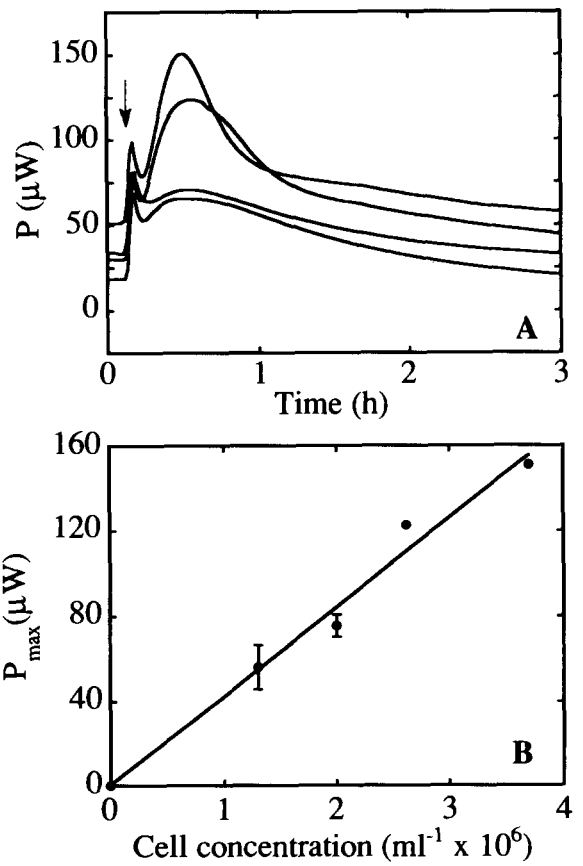

Fig. 2. **A** Thermal output (P) from peritoneal macrophages following activation with PMA (0.5 ng/l) as indicated by the arrow. Measurements are shown at cell concentrations of 1.3, 2.0, 2.6 and 3.7 × 10^6 cells per ml. **B** Thermal output (peak values) as a function of cell concentration.

and 2A). Peak values of both parameters were found to be linearly correlated to cell concentration, as shown in Figs. 1B and 2B, respectively. The energy expenditure of resting macrophages corresponded to an average heat output of 5.9 ± 0.5 pW per cell, a value similar to that reported by Ding et al. [10] (4.5 ± 0.5 pW per cell). During the secondary peak, cellular heat output increased up to 15 pW.

Records from miniaturised oxygen and pH electrodes mounted inside the calorimeter show that medium pO_2 transiently decreases during the burst, whereas medium pH remains relatively stable (Fig. 3). The decrease in oxygen tension is caused by a dramatic increase in cellular oxygen consumption, from a value of 7 ± 0.2 amol/s for resting macrophages up to 24 amol/s for PMA activated macrophages. The difference (17 amol/s) corresponds to an increased heat flow of about 8 pW per cell (calculated assuming the heat flow to be −475 kJ/mol of oxygen [8]), a value close to the 9 pW per cell measured calorimetrically. Experimental data thus support the conclusion that the biphasic nature of heat output observed for macrophages taken from healthy animals (cf. Fig. 2A), is caused by a rapid initial release of reactive oxygen species, followed by a selective up-regulation of oxidative metabolism.

In agreement with Shiratori et al. [11], peritoneal macrophages taken from animals suffering from D-galactosamine induced acute liver injury (ALI) demonstrated higher ability to rapidly release reactive oxygen species in response to PMA stimulation than macrophages taken from healthy animals (Fig. 4A). However, macrophages from the ALI group showed no increased secondary metabolic response to PMA, as could be seen from the calorimetric records in Fig. 4B. The induced liver injury resulted in significantly elevated levels of serum bilirubin in the ALI group as compared to the levels observed in the control group (23.4 ± 5.2 mmol/l and 4.6 ± 2.4 mmol/l, respectively; $p < 0.01$). In contrast to the ALI group, the luminometric response of macrophages taken from animals suffering from glycerol induced renal failure (RF) was

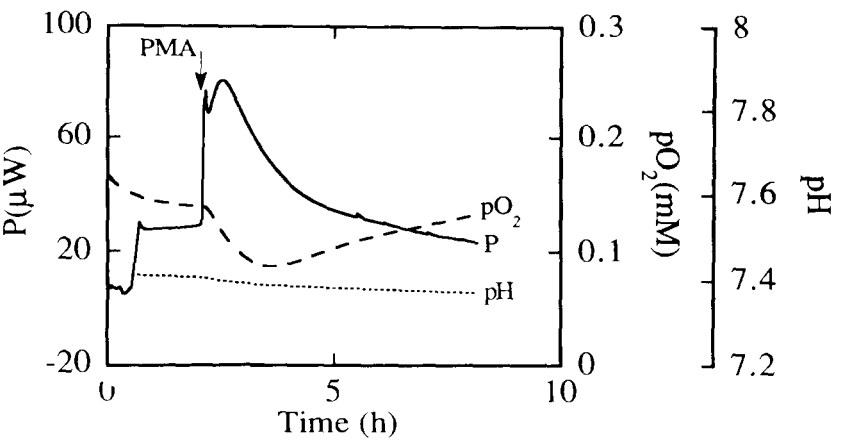

Fig. 3. Thermal output from PMA activated macrophages in addition to records showing the pH and pO_2 of the incubation medium. The decrease in medium oxygen tension during the burst is due to non-equilibrium distribution of oxygen over the gas-phase boundary.

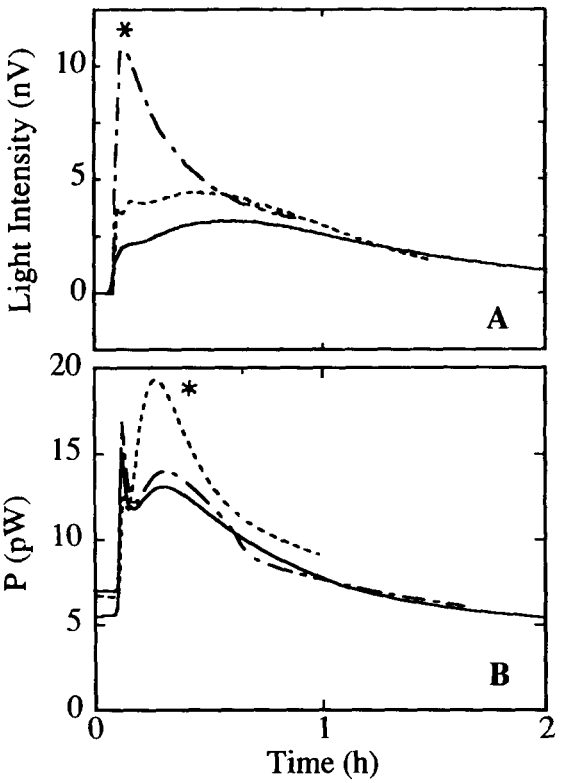

Fig. 4. Chemiluminescence or light intensity per cell (**A**) and the thermal power per cell (**B**) recorded from PMA stimulated macrophages taken from healthy animals (——), animals suffering from D-galactosamine induced acute liver damage (— · —) and from animals which have sustained a glycerol induced renal failure (— — —). Measurements were made at cell concentrations between 0.7 and 2.3 × 10^6 cells per ml (*$P < 0.05$ compared to control).

within the normal range, whereas the secondary metabolic response was highly elevated in this group (cf. Figs. 4A and B, respectively). Serum levels of urea in the RF animals were more than ten times higher than those observed for rats in the control group (48.3 ± 7.7 mmol/l as compared to 3.9 ± 0.5 mmol/l, $p < 0.01$).

4. Conclusions

Although experimental data is consistent and reproducible in the control group, calorimetric and luminometric results differ from each other in the RF and ALI groups. The observed differences are likely to be related to the mechanism by which these insults affect the peritoneal macrophages, and thus to their effect on the innate immune system. The increased secondary metabolic response observed in the RF group may be related to the increased production of cytokines by these cells under renal failure

conditions. In conclusion, we feel that further investigations combining calorimetry with present conventional techniques could provide a better understanding of these processes.

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