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Calorimetry: a highly sensitive technique for evaluating the effect of IL-2, IFN-γ and IL-10 on the response of peripheral blood mononuclear cells

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Abstract

We report the application of a calorimetric method for evaluating the metabolic response of peripheral blood mononuclear cells (PBMC) either from *S. mansoni* infected patients or from normal subjects under stimulation with antigen or with cytokines. Our results demonstrate that the antigenic stimulation of the PBMC with soluble adult worm antigenic preparation (SWAP) was able to discriminate PBMC from infected and non-infected donors, based on calorimetric cell reactivity. The total heat release in the stimulation of PBMC with interleukin 2 (IL-2) or gamma interferon (IFN-γ) was greater for PBMC from infected patients than normal subjects. The activation of the release heat in PBMC induced by IFN-γ, IL-2 or antigen (SWAP) was inhibited by pretreatment of PBMC with interleukin 10 (IL-10). However, this inhibition mediated by IL-10 could be promptly reversed by pretreatment of IL-10 with its respective anti-IL-10 monoclonal antibody. Our present results extend the use of the calorimetric assay and suggest its application for studying cellular reactivity in basic and applied immunological systems.

Keywords: PBMC; Antigen; Cytokines; Schistosomiasis; Calorimetric assay

1. Introduction

Many of the regulator functions of peripheral blood mononuclear cells (PBMC) are mediated by soluble proteins whose expression and secretion are induced by antigen-stimulation. These proteins, designated as cytokines, act by binding to receptors on target cells and induce several biochemical responses [1]. There is evidence supporting the paradigm that inflammation is governed by a complex network of cytokines [2], which involve an intricate mechanism of regulation. It is well established that the interleukin 10 (IL-10) is an immunoregulatory cytokine produced in human system by CD4+/TH1 and CD4+/TH2 subsets of T lymphocytes [3,4]. Interleukin 10 strongly downregulates lymphocytes and macrophage production of several interleukins (IL-1, IL-2, IL-4, IL-5, IL-6 and IL-8) and gamma interferon (IFN-γ) [5–8]. Gamma interferon is a lymphokine produced by both naive (TH0) and TH1 $CD4^{+}/TH^{1}$ subset and by nearly all CD8+ T cells [9] and is a potent activator of the PBMC, phagocytes and vascular endothelial cells [10–12].

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The study of the cytokine network and cytokine effects on cellular immunorregulation (up or down) are complex. Thus, the development of a rapid and sensitive procedure for evaluating the cellular response to stimulation with cytokines or antigens would permit a better understanding of the regulation of cellular reactivity by several cytokines (cytokines network).

In the present communication we report the application of a calorimetric method for evaluating PBMC metabolic responses under stimulation with cytokines or antigen. In this study PBMC reactivity was studied using human schistosomiasis as a model.

2. Materials and methods

2.1. Reagents

Antigens were obtained from *S. mansoni* as a soluble protein derivative which is called soluble adult worm antigenic preparation (SWAP). The SWAP was prepared as described by Chensue and Boros [13] and was conjugated onto polyacrylamide beads as described by Doughty et al. [14].

Mouse monoclonal antibodies against interleukin 10 (DNAX-USA) and interleukin 10 (DNAX-USA) were a gift from Dr. Rodrigo Correa-Oliveira (FIO-CRUZ, Brazil).

2.2. Cell separation

Peripheral blood mononuclear cells (PBMC) from infected patients or from normal subjects were isolated by a Ficoll-Hypaque gradient according to Bicalho et al. [15]. Blood samples from chronically *S. mansoni*-infected patients and normal subjects were provided by Dr. Francisco Chagas de Lima e Silva and Dr. Télcia Vasconcelos Barros Magalhães, Santa Casa Hospital of Belo Horizonte.

2.3. Gamma interferon (IFN-γ) production

A gamma interferon-rich supernatant was produced according to Kiener and Spitalny [16]. Briefly, PBMC [1×10^7/ml RPMI-1640 (cell culture medium)] were stimulated with Concanavalin A (Con A) for 30 min at 37°C, in 5% CO_2. After incubation, the cells were transferred to tubes and centrifuged at 400× g for 30 min at room temperature. The pellet obtained was resuspended in RPMI-1640 medium and a new incubation was performed for 18 h at 37°C in 5% CO_2. After the last incubation the cells were centrifuged (400 g for 30 min) and the supernatant, cell-free and interferon gamma (IFN-(γ)-rich, was collected and tested in the presence of PBMC either from *S. mansoni* infected patients or from normal subjects.

2.4. Interleukin 2 (IL-2) production

The supernatant of Jurkat cells (10^7 cells/ml RPMI-1614) (TIB 152, American Type Culture Collection – ATCC, Rockville, MD) rich in IL-2 was produced by incubating these cells in the presence of 1% of phytohemoaglutinin (PHA-P) (Sigma, St Louis, MO) and 10 ng/ml PDB (phorbol 12,13-dibutyrate, Sigma) diluted in a cell culture medium, commercially called RPMI-1640 containing (i) 4.75 g/l of HEPES and 2.3 g/l of $NaHCO_3$ – (GIBCO-BRL, Gaithersburg, MD) and (ii) 5% inactivated fetal calf serum (Sigma). The incubation was performed at 37°C – 5% CO_2 for 4–6 h. After three washes, with RPMI-1640, the cells (5×10^6/ml) were reincubated for 20 h in RPMI-1640 containing 1% Nutridoma-HU (Boheringer-Mannheim, Germany). The cells were centrifuged at 400×g for 30 min and the IL-2-rich supernatant from the cell culture was collected and assayed in a calorimetric assay.

2.5. Calorimetric assay

The calorimetric assay was performed as described by Chaves et al. [17]. Briefly, a heat-conduction batch-mixing calorimeter similar to Wadso [18], built in our laboratory and in the US in collaboration with Dr. Rex Lovrien of the Department of Biochemistry, University of Minnesota, was used in our studies. This calorimeter is comparable to the one described by Lovrien et al. [19]. The calorimeter consists of two reaction vessel or cells sandwiched between thermopiles with a conversion factor (figure of merit) of $7\ \mu\ W\mu V^{-1}$, as indicated by the manufacturer. In a typical assay, PBMC (2×10^6/100 μl) from *S. mansoni*-infected patients or from normal subjects was added to the large compartment of both control and test vessels. In the small compartments were added

RPMI-1640/100 μl to the vessel control and the following reagents to the vessel test: (a) 50 μl of polyacrylamide beads (200–300) conjugated with antigens (SWAP) suspension, (b) 200 μl of supernatant of Jurkat cells (IL-2-rich), (c) 200 μl of supernatant IFN-(-rich or d) 10 U/10 μl of IL-10. In some experiments, PBMC were pretreated with 10 U/10 μl of IL-10 at 37°C for 30 min before the calorimetric assay. In the reversal test, 10 U/10 μl of IL-10 or IFN-(-rich supernatant (200 μl) were pretreated with monoclonal antibodies against IL-10 (10 μg/10 μl) or against human IFN-γ (100 μl of the cell culture supernatant) for 30 min at 37°C. Heat flow measurements were performed at 25°C in a temperature-controlled room and each experiment lasted 1 h. Mixing of the vessels for aeration was done at 10 min intervals, according to the need, but this was constant for each type of experiment. Thermal equilibrium in the cells was reached after 10–25 min of pre-incubation. Amplifier gains of ×1000 were used. Calibration was performed by neutralization of Tris–HCl based on ΔH^0=-47 267 J/mol H^+ neutralized. Standard calibration plots were described by $A=-92.52+299.46q$, where A is the integrated area under the output envelope and q the calculated amount of heat released. All heat production values were exothermic quantities and were recorded with an electronic recorder coupled to the microcalorimeter. Cell viability was evaluated before and after each test by the Trypan blue exclusion test and/or by MTT metabolization [20] and the percentage of dead cells was always less than 5%.

3. Results and discussion

Calorimetry has previously been applied to studies of microbial and Leishmania metabolism [21,22], metabolic activity of red cells, granulocytes, platelets and lymphocytes [23], cancer research [24], phagocytosis [25,26] and antigen–antibody reactions [27,28]. Rialdi and Eftimiadi [29] reported the correlation between oxygen consumption and release of heat in human neutrophils activated with PMA.

We have studied the effect of cytokines and antigen on PBMC reactivity by measuring the cellular heat release. Our results are expressed either as total heat production rate (mJ h^{-1}), as specific heat production rate (pW $cell^{-1}$) or as millivolts. The experiments were performed at 25°C using PBMC from heparinized blood. Our results expressed as heat production rate for unstimulated PBMC were smaller than those performed at 37°C by other authors [30,31]. However, when the integrated form of the Arrhenius equation, in $K_1/K_2=-E/R(1/T_2-/T_1)$, was applied to our data at 25°C for calculating heat production rates at 37°C, using an activation energy value of 62.9 kJ mol^{-1} as

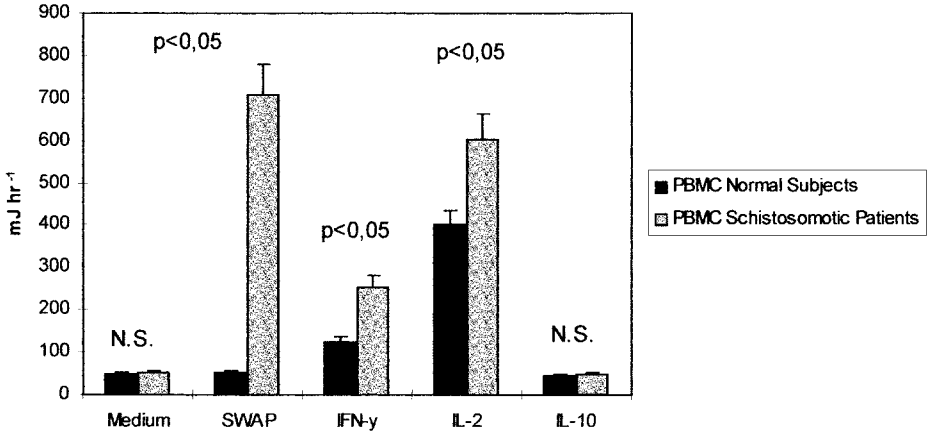

Fig. 1. Effect of antigen (SWAP), IFN-γ, IL-2 and IL-10 on total released heat by PBMC (2×10^6 cells) from *S. mansoni*-infected patients and normal subjects. PBMC: peripheral blood mononuclear cells; SWAP: soluble adult worm antigenic preparation; IFN-γ: interferon gamma; IL-2: interleukin 2; and IL-10: interleukin 10. The values represent the mean of seven experiments. $^*p<0.05$ significant; and N.S not significant when compared to normal subjects by Student's "*t*" test.

Table 1
Effect of interleukin 10 (IL-10) on released heat by PBMC under stimulation with antigen (SWAP), IFN-γ, or IL-2

Experiments groups	Heat production rate (pW cell^{-1})	Total released heat[a] (mJ h^{-1})	% Inhibition mediated by IL-10	*p<0.05
PBMC+MEDIUM	0.10±0.02	141±25^{A}		
PBMC+IL-10	0.09±0.01	132±30^{B}	–	–
PBMC+SWAP	0.53±0.04	708±67^{C}		
[PBMC+IL-10]+SWAP	0.26±0.03	352±21^{D}	64.2	C/D; C/A
[IL-10+MoAb anti-IL-10]+PBMC+SWAP	0.47±0.05	624±75^{E}		
PBMC+IFN-γ	0.19±0.01	254±23^{F}		
[IFN-γ+MoAb anti-FN-γ]+PBMC	0.12±0.01	152±13^{G}		
[PBMC+IL-10]+IFN-γ	0.06±0.004	75±7^{H}	70.4	F/H; F/A
PBMC+IL-2	0.45±0.05	601±80^{I}		
[PBMC+IL-10]+IL-2	0.11±0.02	142±14^{J}	76.3	I/J; I/A

The controls containing peripheral blood mononuclear cells (PBMC) in the presence of either polyacrylamide beads unattached to antigen (SWAP) or monoclonal antibody against IFN-γ showed a total released heat of 119.82±10.6, 149±28.3 respectively.
SWAP: soluble adult worm antigenic preparation; IFN-γ: interferon gamma; IL-2=Interleukin 2; IL-10: interleukin 10; MoAb anti-IL-10: monoclonal antibodies against interleukin 10; MoAb anti-IFN-γ: monoclonal antibodies against interferon gamma; the values represent the mean ±SEM of five experiments.
*p<0.05 is significant when compared to the controls by the Student's "t" test. Percent inhibition was calculated by the formula $(1-E/C)\times 100$ and refers to experiments in the presence (E) or in the absence (C) of IL-10.
[a]per 2×10^6 cells.

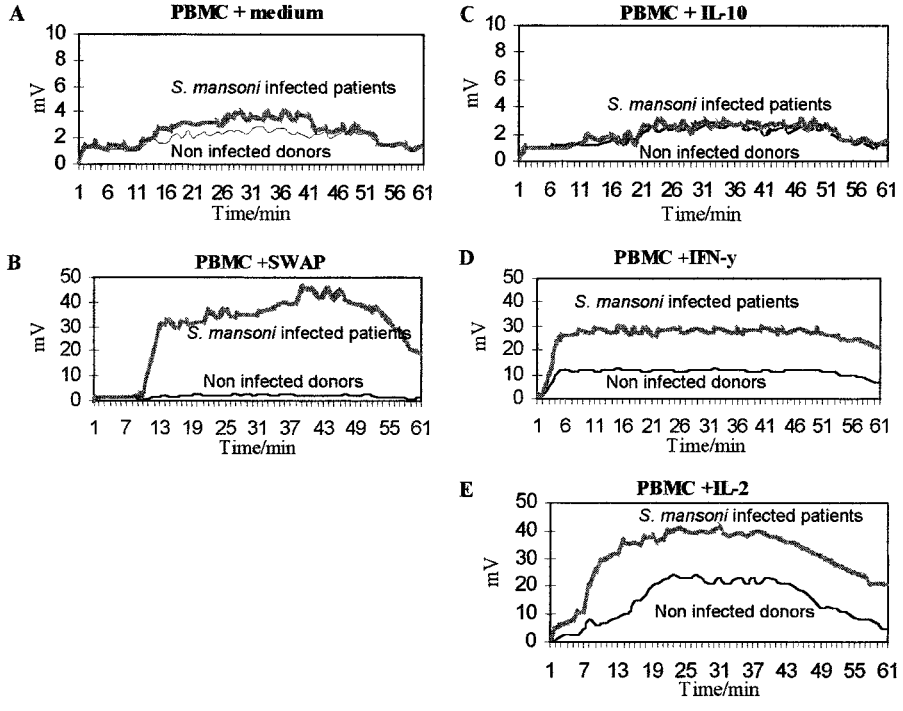

Fig. 2. Typical curves of released heat by PBMC from *S. mansoni*-infected patients and normal subjects under stimulation with medium (control A); antigen (SWAP B); interleukin 10 (IL-10 C); gamma interferon (IFN-γ D) and interleukin 2 (IL-2 E). PBMC: peripheral blood mononuclear cells; SWAP: soluble adult worm antigenic preparation; IFN-γ: interferon gamma; IL-2: Interleukin 2; and IL-10: Interleukin 10.

given by Ikomi-Kumm et al. [32], the rates range from 1.0 to 1.9 pW per cell which are in agreement to Ikomi-Kumm et al.

Our data with cytokines showed that gamma interferon (IFN-γ) and interleukin 2 (IL-2) were able to induce a significant increase in the heat release in PBMC either from normal subjects or from *S. mansoni* infected patients (Fig. 1). Interleukin 10 (IL-10), however, was inert for inducing changes in the heat production in cells from either infected or non-infected donors (Fig. 1 and Table 1). On the other hand, our experiments with antigen (SWAP) clearly permitted discrimination between cells from infected and non-infected donors (Table 1, Figs. 1 and 2 panel B). We have previously demonstrated that the released heat by PBMC from infected patients induced by antigen is mediated by gamma interferon [33]. Our present results showed that IL-10 inhibited the released heat by PBMC from *S. mansoni* infected patients under stimulation with antigen (SWAP), IFN-γ or IL-2 (Table 1 groups 2–4), suggesting that IL-10 was able to modulate the PBMC response. This inhibitory or modulatory effect mediated by IL-10 was abolished by the pretreatment of IL-10 with its respective anti-IL-10 monoclonal antibody (Table 1 group 2). These data reinforce the role of IL-10 as a cytokine suppressor of IL-2 and IFN-γ effects on PBMC. The present results are analogous to that of Chaves et al. [34] using other different methodologies (chemiluminescence and MTT dye reduction).

These results extend the application of the calorimetric assay and clearly demonstrate that this test is a reliable methodology for studying the network cytokines and their effects on cellular functions.

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