Thermochimica Acta, 241 (1994) 87–94
Elsevier Science B.V., Amsterdam
SSDI 0040-6031(94)01677-9

Human schistosomiasis: evaluation of the reactivity of peripheral blood mononuclear cells by calorimetry

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(Received 28 May 1993; accepted 17 December 1993)

Abstract

Peripheral blood mononuclear cells (PBMN) from *Schistosoma mansoni*-infected patients were exposed in a calorimeter to soluble adult worm antigenic preparation (SWAP), phytohemaglutinin (PHA), 4-β-phorbol-12,13-dibutyrate (PDB) or interferon-γ (IFN-γ)-enriched supernatant obtained after calorimetric experiment. The total heat released by antigen-stimulated PBMN from infected patients was significantly higher than that produced by PBMN from normal subjects stimulated with the same antigen. Antigen-induced, cell-free supernatants obtained after calorimetric experiments were able to elicit in PBMN from normal subjects a heat release pattern similar to that seen with *S. mansoni*-infected cells. This activating effect, however, was fully removed by pre-incubation of the supernatant with monoclonal antibodies against IFN-γ.

The discrimination between PBMN from infected patients and normal subjects was also made possible by evaluation of the cell reactivity under stimulation with either PHA or PDB. Our results demonstrate that, due to its high sensitivity, calorimetry may become an important tool for basic immunological studies.

INTRODUCTION

In the last two decades, calorimetry has been shown to be a highly sensitive technique for assessing lymphocyte function. It is a convenient method for estimating the metabolic status of human blood cells and has often been used as an important tool in physiological and pathological studies of cell biology.

A significant increase in the heat production was noted in human leukocytes under stimulation with mitogen [1, 2], immune complex [3] or during phagocytosis [4]. In cells from diseased patients, alterations in the heat production by lymphocytes from non-Hodgkins lymphoma, chronic

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lymphocytic leukaemia, thyroid dysfunction and acromegalia have also been reported (for a review, see ref. 5).

In a previous report, we demonstrated that human peripheral blood mononuclear cells (PBMN) from non-infected or from *Schistosoma mansoni*-infected patients can be discriminated by microcalorimetry [6] because only PBMN from infected patients were reactive to SEA (soluble egg antigen) or to SWAP (soluble adult worm antigenic preparation), which are substances that specifically stimulate clonally restricted antigen-sensitive lymphocytes.

In the present paper, the reactivity of PBMN from *S. mansoni*-infected patients and from normal subjects were evaluated comparatively during activation with either (a) a mitogenic agent, phytohemaglutinin (PHA); (b) a protein kinase C activator, phorbol dibutyrate (PDB); (c) an antigen from *S. mansoni* (SWAP); or (d) a lymphokine, gamma interferon.

MATERIAL AND METHODS

Reagents

Soluble adult worm antigenic preparation (SWAP) was obtained as described by Smithers and Terry [7]. The conjugation of SWAP onto polyacrylamide beads was performed as described by Doughty et al. [8]. Mouse monoclonal antibodies against human gamma-interferon (IFN-γ) (American Type Cell Collection – ATCC – HB 8291 – IFGCP – F1BA10) were a gift from Dr. A.M. Goes and Dr. B. Doughty, A & M University, Texas. Phytohemaglutinin (PHA-P) was purchased from Difco-Lab. Detroit, MI, and 4-β-phorbol 12,13-dibutyrate (PDB) from Sigma.

Cell separation

Peripheral blood mononuclear cells (PBMN) were obtained either from patients with chronic schistosomiasis (viable eggs in their stools, positive intradermal test, positive LAI assay, or in some cases, positive ELISA test) or from normal subjects with no eggs in the stools, negative intradermal test and a negative LAI assay. The cells were isolated by a Ficoll-hypaque gradient according to Bicalho et al. [9], with slight modifications. The purity of the cell suspensions was determined by differential counting of Giemsa-stained smears.

Supernatant

Supernatants were obtained after calorimetric runs of PBMN from infected patients in the presence of beads coated with antigen (SWAP). The contents of the calorimetric cells were transferred to centrifuge tubes and

were spun at 400 g for 30 min. The supernatants (cell-free) were collected and tested against PBMN from normal subjects in the calorimeter.

Inhibition of supernatant activity by monoclonal antibodies

Inhibition of the ability of the IFN-γ-enriched supernatant to activate PBMN was carried out by pre-incubation for 30 min at 37°C of 1000 μl of the supernatant with 100 μl of mouse monoclonal antibody against human INF-γ (used as culture fluid preparations). After this treatment, the supernatant was tested in the calorimetric assay.

Calorimetric assay

Calorimetry was performed in a heat conduction batch-mixing calorimeter similar to Wadsö's [10], built in our laboratory and in the USA [11]. The Seebeck thermopiles have a conversion factor ("figure of merit") of 7 $\mu W\ \mu V^{-1}$, as indicated by the manufacturer. The calorimeter consists of two reaction vessels or cells, sandwiched between thermopiles and enclosed in an aluminum block that acts as a heat sink. The calorimeter mixing vessels (two), made of gold, each have a small and a large compartment holding up to 1.0 and 2.0 ml, respectively, in a total volume of 5.0 ml.

In a typical test, 2×10^6 PBMN from *S. mansoni*-infected patients or from non-infected donors in 1.0 ml, were added to the large compartment and the reagents (a) 200–300 polyacrylamide beads conjugated with antigens (SWAP), or (b) supernatants obtained after a calorimetric assay pre-treated or not with monoclonal antibodies, or (c) PDB (5 μl of 10^{-6} M solution), or (d) PHA (25 μl), were added to the smaller compartment according to experimental design. In all experiments, cells and reagents were diluted with MEM and each compartment contained a final volume of 1.0 ml. In the control vessel, cells were added to the large compartment and either MEM, polyacrylamide beads unattached to antigens (200–300) or 100 μl of monoclonal antibodies against IFN-γ were added to the smaller one. Heat flow measurements were performed at 25°C in a temperature-controlled room and each experiment was followed for 1–2 h (PDB). Mixing of the vessels for aeration was done at 5 or 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 $\times 500$ were used. Calibration was performed by neutralization of Tris by HCl based on $\Delta H^{\circ} = -47\ 267$ J/mol of 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 they were registered in an electronic recorder coupled to the microcalorimeter. After each calorimetric assay, cell viability was evaluated by the Trypan blue exclusion test or by MTT-metabolization [12].

RESULTS AND DISCUSSION

The method now presented for assessing the metabolic status of PBMN from diseased patients and normal subjects is based on the determination of the heat produced by stimulated PBMN using a highly sensitive calorimeter. The major advantage of this technique is that it allows a rapid and precise detection of PBMN reactivity. Calorimetry has previously been applied to the study of microbial metabolism [13], fermentation [14], the metabolic activity of red cells, granulocytes, platelets and lymphocytes [15], as well as in investigations of enzyme–ligand interaction [16], denaturation [17], Leishmania metabolism [18] and cellular evaluation in cancer research [19].

In our study, PBMN from *S. mansoni*-infected patients were activated by exposure either to antigen, to PHA, to a lymphokine (IFN-γ) or to a protein Kinase C activator (PDB); the metabolic response was measured by calorimetry. The results were expressed as total heat production in a given time interval, expressed as mJ or as the heat production rate, pW per cell.

The values obtained with SWAP-stimulated PBMN from infected patients were notably larger than those from normal subjects (Fig. 1A and Table 1, lines 1 and 4). However, the heat production rate of the control, unstimulated PBMN was smaller than that reported by other authors (1.21 pW per cell). For experiments performed at 37°C, the heat production rates reported by Gorski and Levine [1], Bandmann et al. [20] and Ikomi-Kumm et al. [21] were from 3 to 10, 2.2 ± 1.4, and 2.7 ± 0.5 pW per cell, respectively. A lower value (1.8 ± 0.7) was also reported by Ikomi-Kumm et al. [21] using heparinized blood lymphocytes. The values found by us can be basically explained by the temperature used in the experiments (25°C). Indeed, when the integrated form of the Arrhenius equation, $\ln k_1/k_2 = -E/R(1/T_2 - 1/T_1)$, was applied to our data at 25°C to calculate heat production rates at 37°C, using an activation energy value of 62.9 kJ mol^{-1} as given by Ikomi-Kumm et al. [21], rates in the range of 3.2 pW per cell were found, in agreement with the literature values [1, 20, 21].

An important observation was that supernatants of calorimetric experiments in which PBMN (schistosomiasis) were stimulated by antigen were able to stimulate PBMN from normal subjects, indicating the release of a cytokine to the medium. This could be demonstrated in experiments in which monoclonal antibodies against IFN-γ fully inhibited the heat release (Table 2, lines 3 and 4) elicited by those supernatants. Thus, the calorimetric burst observed derives not only from the antigen–receptor interaction on the cell surface of antigen-sensitive lymphocytes, but is due also to a metabolic enhancement that originates, at least in part, from propagation by a soluble mediator that our data indicate to be IFN-γ. In addition, we also demonstrated that the calorimetric assay is able to detect IFN-γ within a few minutes after cell activation with antigens.

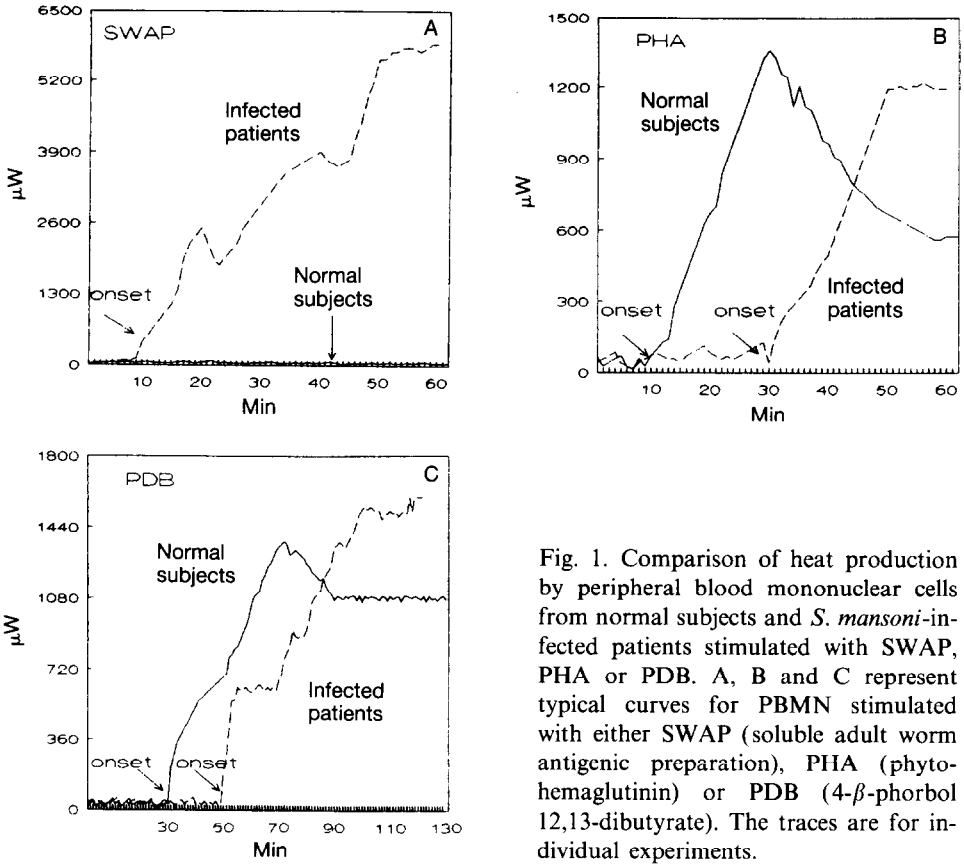

Fig. 1. Comparison of heat production by peripheral blood mononuclear cells from normal subjects and *S. mansoni*-infected patients stimulated with SWAP, PHA or PDB. A, B and C represent typical curves for PBMN stimulated with either SWAP (soluble adult worm antigenic preparation), PHA (phytohemaglutinin) or PDB (4-β-phorbol 12,13-dibutyrate). The traces are for individual experiments.

In another type of approach, PBMN were unspecifically stimulated with a mitogenic agent (PHA) or with a metabolic activator (PDB). In the experiments with PHA-stimulated PBMN, the values of the total heat produced by the cells from infected patients or normal subjects were similar, i.e. 5020 ± 349 and 5061 ± 374 mJ, respectively ($p > 0.05$) (Table 1). However, PHA-stimulated PBMN from infected patients and normal subjects could be discriminated by analysis of the shape of the thermograms and by the time of onset of the reaction (Fig. 1B). The delayed calorimetric burst and decreased heat production rate observed with cells from infected patients may reflect metabolic adaptations in these cells, as previously reported using other methods such as cytotoxicity, blastogenesis and quantification of inositolpolyphosphates (IPx) contents [22, 23].

Our results with PDB showed that the total heat produced and the heat production rate were significantly ($p < 0.05$) decreased in PBMN from infected patients (Table 1, lines 3 and 6). The shapes of the curves and the time of onset of the reaction were distinct when PBMN from infected patients and normal subjects were compared (Fig. 1C).

TABLE 1

Heat released by stimulated peripheral blood mononuclear cells (PBMN) from *S. mansoni*-infected patients and from normal subjects

Cells from	Activation by	Heat production rate in pW/cell [a]	Total heat released after Δt/s by 2×10^6 cells in mJ [b]
Normal subjects [c]	SWAP	0.093 ± 0.0006	123 ± 10
	PHA	1.97 ± 0.21	5020 ± 349 [d]
	PDB	1.28 ± 0.15	4246 ± 835
Infected patients	SWAP	1.45 ± 0.13	10039 ± 826
	PHA	1.62 ± 0.18	5061 ± 374 [d]
	PDB	0.87 ± 0.02	3262 ± 460

[a] The steady state heat production rate was measured at the maximum peak of the power–time curve, at zero slope, for SWAP, PDB and PHA. In experiments with PBMN from infected patients stimulated by PDB, the first peak of the power–time curve, at zero slope, was considered as indicated in Fig. 1C. [b] The total heat released was measured after 3600 s for SWAP and PHA; the power–time curve for PDB was monitored for 7200 s. [c] The controls containing PBMN and either MEM or polyacrylamide beads unattached to antigen (SWAP) showed a total heat released of 8.61 and 8.28 mJ, respectively. [d] Not significant ($p > 0.05$) by the Student "t" test.

Stimulation of protein Kinase C has been accepted as an important step in cell activation [24] and alterations in PKC translocation from cytosol to membrane, or altered PKC reactivity to PDB stimulation in cells from infected patients, may be suggested as explanations for the differences

TABLE 2

Effect of supernatant rich in gamma interferon on the heat production by peripheral blood cells (PBMN) from normal subjects

Experiments	Heat production rate in pW per cell [a]	Total heat released after Δt/s by 2×10^6 cells in mJ [b]
(1) PBMN + medium	0.0010 ± 0.0002	16.3 ± 1.7
(2) PBMN + MoAB against IFN-γ [c]	0.0139 ± 0.0001	14.6 ± 1.3
(3) PBMN + MCS [d]	0.719 ± 0.0035	2399.7 ± 18.7 [f]
(4) PBMN + (MCS–MoAb) [e]	0.026 ± 0.0004	17.6 ± 4.6

[a] The steady state heat production rate was measured at the maximum peak of the power–time curve, at zero slope, in the expanded scale. [b] Total heat released was measured after 1800 s. [c] MoAb, monoclonal antibodies against gamma interferon (IFN-γ). [d] MCS, mononuclear cell supernatant IFN-γ-enriched. [e] (MCS–MoAb), MCS pre-incubated with MoAb for 30 min at 37°C. [f] Significant $p < 0.05$ by the Student "t" test when compared to the controls, (1) and (2), or to the test, (4). (1), (2) and (4) are not significant ($p > 0.05$).

found. These altered PBMN functions in vitro, detected during activation with PHA or PDB, may be a consequence of the complex host–parasite relationship induced in vivo by schistosome and/or egg antigens.

Thus, calorimetry was used to successfully discriminate between PBMN from non-infected and *S. mansoni*-infected patients under stimulation of antigen, PHA and PDB (Fig. 1 and Table 1). The participation of IFN-γ as a mediator in the propagation of cell stimulation could also be established by calorimetic assay. As a consequence, these results suggest that the highly sensitive calorimetric assay may become an important tool in the study of basic immunological mechanisms. Its value as a diagnostic tool, not an important point in the present case of schistosomiasis, may be significant in systems where long-lasting biological tests are required.

ACKNOWLEDGMENTS

We are grateful to Professor Rex Lovrien, Department of Biochemistry, University of Minnesota, for help with construction and operation of the calorimeters, and to FAPEMIG.

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