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Cyclic AMP Metabolism in Peripheral Blood Lymphocytes from Patients with Hodgkin's Disease

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Abstract—*Intracellular levels of cyclic AMP and cAMP phospodiesterase activity of peripheral blood mononuclear cells of patients with Hodgkin's disease (HD) were studied. The cAMP levels were elevated by 137% in HD patients as compared to normal subjects. The levels were reduced during clinical remission but remained significantly higher than normal controls. These high levels of cAMP in HD patients may be due to reduced degradation of cAMP as the cAMP phosphodiesterase activity was reduced to 50% of normal. This observed altered metabolism still persisted even after depletion of adherent monocytes, indicating that the defect was in the lymphocytes.*

INTRODUCTION

CYCLIC adenosine 3',5'-monophosphate (cAMP) has been shown to be involved in a variety of cellular functions [1]. Intracellular levels of cAMP have been shown to play an important role in cellular functions of immune response. Increased levels of intracellular cAMP, either by exogenous addition or by metabolic increase through increased production and/or decreased degradation, have been shown to inhibit cellular cytotoxicity [2, 3], E-rosette formation by T-lymphocytes [4] and response to mitogen [5–8]. Increased cAMP levels have also been shown to result in increased suppressor cell activity [9–13].

The suppression of cellular immune response in Hodgkin's disease (HD) patients has been attributed to humoral factors in the sera of HD patients [14, 15] as well as suppressor cells of both monocytes and T-lymphocytes [16–19]. Intrinsic defects of the T-lymphocytes have also been shown to be responsible for defective immune response of HD patients [20, 21]. The present studies were therefore undertaken to investigate the cAMP metabolism in peripheral blood lymphocytes of HD patients.

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MATERIALS AND METHODS

Chemicals

[8-^{3}H]cAMP was obtained from Amersham and was diluted with 50% ethanol to get a concentration of 3.8 pmol (0.0625 μCi)/ml. All non-radioactive chemicals were of analytical grade. Fetal calf serum (FCS) was obtained from Difco. After reconstitution the serum was inactivated by heating at 56°C for 1 hr and stored in aliquots at -20°C.

Normal controls

Seventeen members of the institute staff volunteered to serve as normal controls.

Patients

Twenty-five Hodgkin's disease patients diagnosed at the Tata Memorial Hospital, Bombay, were studied before start of treatment. Eight patients in clinical remission who were off the treatment for 2–8 yr were also studied.

Preparation of mononuclear cells

The peripheral blood mononuclear cells (PBMNC) were isolated using the method described by Boyum [22], washed with saline and suspended in 10 mM Tris-HCl, pH 7.5, at a concentration of 10×10^6 cells/ml.

For monocyte depletion, plastic adherence was used [23]. The mononuclear cell preparations were plated on plastic Petri dishes precoated with

FCS and incubated at 37°C in 5% CO_2 atmosphere for 90 min in Eagle's minimum essential medium supplemented with 10% FCS, glutamine and 25 mM HEPES. The non-adherent cells were collected, washed thrice with saline and suspended in 10 mM Tris–HCl, pH 7.5, at 10×10^6 cells/ml.

The cells were disrupted by two cycles of freezing and thawing.

Estimation of cAMP

One hundred milliliters of the cell extract was treated with 100 μl of 10% trichloroacetic acid (TCA) and the mixture was centrifuged at 1000 *g* for 15 min at 4°C. The supernatant was collected and washed with diethyl ether to remove TCA. The TCA-free supernatant was dried over phosphorus pentoxide under vacuum and cAMP was estimated by competitive binding assay using bovine adrenal protein by the method of Tsang *et al.* [24]. The dried pellet was dissolved in 100 μl of buffer (6.7 mM Tris, 0.45 mM EDTA, 1 mM theophylline, pH 7.7). To this 100 μl [^{3}H]cAMP and 100 μl bovine adrenal protein (5 mg/ml) were added. The tubes were incubated for 90 min at 4°C. The unbound cAMP was removed by adsorption on activated charcoal coated with dextran (60,000–90,000 mol. wt). For this 0.5 ml of a 2.5% suspension of charcoal containing 0.025% dextran in saline was added. The tubes were centrifuged at 2000 *g* for 15 min at 4°C. The radioactivity of bound cAMP (0.5 ml) was determined in an LKB LS counter using Bray's scintillation fluid. A standard cAMP curve of 1–8 pmol was always run simultaneously. The cAMP content was determined from the inhibition of binding and expressed as pmol/10^6 cells. All the samples were run in triplicate.

Cyclic AMP phosphodiesterase activity determination

cAMP phosphodiesterase activity was assayed by the method of Bucher [25] with the modification that cobra venom was used in place of *Crotalus atrox* venom as a source of 5′-nucleotidase. The liberated inorganic phosphorus (P_i) was estimated by the method of Ames [26] and the activity was expressed as nmol of P_i formed/10^6 cells in 30 min.

The statistical analyses were carried out by Student's *t* test.

RESULTS

Cyclic AMP levels in PBMNC of normal subjects, untreated HD patients and treated HD patients who were in clinical remission and off the treatment for 2–8 yr have been determined (Table 1). The cAMP levels were significantly elevated in HD patients (2.396 pmol in HD patients vs 1.008 pmol/10^6 cells in normal subjects). The levels remained elevated even in patients who were in clinical remission. When cAMP phosphodiesterase activity was determined normal PBMNC showed significantly higher activity than PBMNC from both untreated and treated patients.

As the depressed immune response in HD patients has been reported to be due to an intrinsic T-cell defect, the cAMP metabolism was studied in a PBMNC population which was depleted of plastic adherent monocytes. The data in Table 2 show that the cAMP content of adherent-monocyte-depleted PBMNC from HD patients was still significantly higher than normal. The cAMP phosphodiesterase activity was, on the other hand, significantly reduced in HD patients compared to normal. These data suggest that the increased levels of cAMP were not due to an increased percentage of monocytes in PBMNC of HD patients but due to changes in lymphocyte metabolism of cAMP.

Table 1. Cyclic AMP contents and phosphodiesterase activity in peripheral blood mononuclear cells of patients with Hodgkin's disease

	cAMP content (pmol/10^6 cells)	Phosphodiesterase activity (nmol P_i formed/10^6 cells)
Normal (17)*	1.00 ± 0.480† (0.40–2.25)	4.28 ± 1.259 (1.79–6.81)
HD patients		
Untreated (25)	2.40 ± 1.242‡ (0.90–5.10)	2.18 ± 0.815‡ (1.08–3.20)
Treated (8)	1.87 ± 0.604§ (0.50–2.90)	2.15 ± 0.475‡ (1.67–2.91)

*Values in parentheses are number of samples studied.
†Values are mean ± S.D.; range in parentheses.
‡Significantly different from normal; $P < 0.001$.
§Significantly different from normal; $P < 0.01$.

Table 2. Cyclic AMP content and phosphodiesterase activity of monocyte-depleted peripheral blood mononuclear cells of patients with Hodgkin's disease

	cAMP content (pmol/10^6 cells)	Phosphodiesterase activity (nmol P_i formed/10^6 cells)
Normal (6)*	0.86 ± 0.406† (0.40–1.55)	3.46 ± 0.724 (2.84–4.90)
HD patients	1.93 ± 0.525‡ (0.95–2.95)	1.79 ± 0.437§ (1.29–2.45)

*Values in parentheses are number of samples studied.
†Values are mean ± S.D.; range in parentheses.
‡Significantly different from normal; $P < 0.001$.
§Significantly different from normal; $P < 0.01$.

DISCUSSION

Impairment of cell-mediated immune response in HD patients has been well documented [14–19]. The factors reported to be responsible for this impairment include intrinsic defect of the lymphocytes [20, 21]. The present studies were carried out to investigate the cAMP metabolism of PBMNC of HD patients. The intracellular levels of cAMP in PBMNC were elevated by 137% in HD patients as compared to normal subjects. Though there was a fall in the levels of cAMP in PBMNC of the patients who had attained clinical remission it was still higher than in normal PBMNC. This indicates that the lymphocyte defects still persist after the patients have attained clinical remission.

The phospodiesterase activity in the same preparations were reduced by 50%, indicating that the elevated levels of cAMP in PBMNC of HD patients may be due to reduced degradation of cAMP.

The elevated levels of cAMP were observed even in a monocyte-depleted preparation of PBMNC of HD patients. This suggests that the defect is in the lymphocytes of HD patients. As B-lymphocyte function has been reported to be normal in HD patients [27] the abnormal cAMP metabolism observed in the present study may be due to a defect in T-lymphocytes.

The involvement of elevated levels of cAMP in increased suppressor cell activity has been reported [9–13]. The suppressor activity of T-lymphocytes has been attributed to the subpopulation bearing Fc receptor for IgG (Tγ-cells). Tγ-cells have been shown to have elevated levels of cAMP as compared to non-Tγ-cells [28, 29]. Elevated levels of Tγ-cells in HD patients have been reported [21, 30]. The impairment of cAMP metabolism observed in the present study may in part be responsible for the impaired cell-mediated immune response observed in HD patients.

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