Europ. J. Cancer Vol. 15, pp. 615–617.
© Pergamon Press Ltd. 1979. Printed in Great Britain

0014-2964/79/0401-0615 $02.00/0

Cyclic-AMP in Human Lymphocytes

Levels in Acute Leukemia and Infectious Mononucleosis

A. BEN-ZVI,* A. RUSSELL,* A. SHNEYOUR†§ and N. TRAININ†

*Department of Paediatrics, Hadassah University Hospital, Jerusalem, Israel
†Department of Cell Biology, The Weizmann Institute of Science, Rehovot, Israel

Abstract—*Alterations in cyclic-AMP levels before and after trypsin treatment as compared with cyclic-AMP levels of normal individuals are found in peripheral blood lymphocytes of patients suffering from various clinical conditions in which a defect in cell mediated immunity is suspected. Such conditions include infectious mononucleosis and acute lymphatic leukemia. It is concluded that cyclic-AMP levels in lymphocytes correlate with the clinical and immunological state of the patients and might be of diagnostic value.*

INTRODUCTION

CYCLIC nucleotides play a major role as intracellular mediators of the immune response [1, 2]. Adenosine 3′5′-cyclic-monophosphate (cAMP) levels in lymphocytes have also been implicated with acquisition of immunocompetence [3], and a correlation exists between the extent of the trypsin-induced increase in cAMP levels and the degree of maturity attained by the thymus-derived (T) lymphocytes [4].

Several studies of basal cAMP levels in lymphocytes isolated from peripheral blood of patients with different clinical states, report changes in cAMP levels, as compared with those found in lymphocytes of normal individuals [5–7]. Thus, it was of interest to study the levels and the effect of trypsin treatment on cAMP of lymphocytes isolated from clinical states, where impairment in cell mediated immunity is suspected.

MATERIALS AND METHODS

Lymphocytes

Lymphocytes were prepared from peripheral blood obtained by venipuncture and kept with heparin.

Accepted 28 September 1978.
§Correspondence: Dr. A. Shneyour, Department of Laboratory Medicine, Meir General Hospital Kfar-Saba, Israel.

Blood from children

Venous blood was obtained from children of various ages who were examined at the paediatric clinics of Beilinson Hospital, Petah-Tikva; Kaplan Hospital, Rehovot; and Hadassah Medical Center, Jerusalem. Seven of the children suffered from acute lymphoblastic leukemia (ALL) and were in acute state before treatment, 4 were in remission and 8 suffered from infectious mononucleosis.

Lymphocyte separation

Samples of 10 ml heparinized blood, kept at room temperature (24°C) were processed within 90 min after collection. The blood was diluted vol/vol with phosphate buffered saline (Dulbecco's modification) and then immediately layered over 15 ml of sodium metrizoate/Ficoll solution (lymphoprep, Nyegaard & Co., Oslo, Norway). Lymphocytes were separated as described by Bøyum [8].

Trypsin treatment

Trypsin treatment was performed in disposable plastic tubes 10 × 74 mm by the addition of 1 ml of trypsin 1-300 (Nutritional Biochemical Corp., Cleveland, Ohio) 0.3% in Puck's saline A to 2×10^6 lymphocytes suspended in 1 ml Eagle's medium (Dulbecco's modification, Gibco, Grand

Island, N.Y.), and incubation at room temperature (24°C) for 5 min. The cells were spun down at 450 *g* for 2 min and extracted for 2 min at 80°C by the addition of 0.1 ml 0.1 N HCl to each one of the pellets. The pH of the extracts was brought up to 4.0 by the addition of 1 M sodium acetate buffer at pH 4.6. Each blood sample was done in triplicate.

cAMP assay

cAMP was determined in the extracts after separating the cells according to Gilman's competition-binding method with a binding protein isolated from rabbit striated muscle. The preparation of rabbit binding protein and the assay conditions were identical to that described for bovine striated muscle binding protein [9]. cAMP was undetectable in extracts treated with beef heart cAMP phosphodiesterase (Sigma) 0.5 mg/ml at room temperature for 90 min after which the enzyme was inactivated by rapid heat treatment.

Statistical treatment of results

The degree of significance (P) of the difference in mean cAMP levels between age matched groups of 7 normal individuals and the patients was determined by the Student's t-test.

RESULTS

Peripheral blood lymphocytes (PBL) of children with infectious mononucleosis and of children with ALL in remission show intermediate basal cAMP levels which are about 40% of those of normal children (Fig. 1, lower part). On the other hand, basal cAMP level in PBL of children suffering from ALL at an active state of disease is much lower. The increased levels of cAMP after trypsin treatment reflect the same differences which exist in the basal levels. Lymphocytes of patients with ALL before treatment show a comparatively small increase in cAMP after trypsin treatment while those of patients with ALL in remission show an increase of about 40% of that of the control (Fig. 1, upper part). Lymphocytes of patients suffering from infectious mononucleosis show a trypsin-induced difference in cAMP level, which is about 60% of that shown by lymphocytes of normal patients (Fig. 1, upper part).

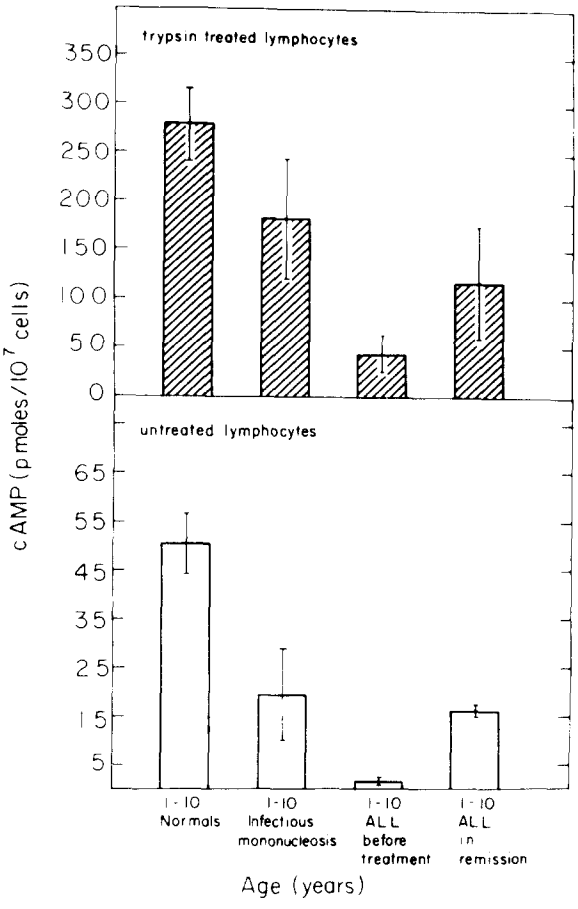

Fig. 1. cAMP levels in PBL of patients with infectious mononucleosis and ALL. The number of patients in each of the groups was the following: Normals, 7; Patients with infectious mononucleosis, 8; ALL patients before treatment, 7; ALL patients in remission, 4. Patients with infectious mononucleosis were at the acute phase of the disease with marked lymphocytosis. The data represent the mean+1 standard deviation. The lower part shows basal levels of cAMP. The upper part shows cAMP levels after trypsin treatment. P of the differences in mean cAMP levels before trypsin treatment is as follows: infectious mononucleosis, $P<0.001$*; ALL before treatment,* $P<0.001$*; ALL in remission,* $P<0.001$*; P after trypsin treatment: infectious mononucleosis,* $P<0.001$*; ALL before treatment,* $P<0.001$*; ALL in remission,* $P<0.001$.

DISCUSSION

Intracellular cAMP plays a major role in the regulation of the immune response [1–3]. It was shown also that trypsin increases cAMP levels mainly in mature immunocompetent T lymphocytes [4]. In the present study we tried to follow the levels of intracellular cAMP of PBL before and after trypsin treatment in patients with ALL and infectious mononucleosis.

The most prominent feature of both diseases is proliferation of lymphocytes of thymic origin [10, 11]. Some authors indicate a close association between the two diseases [12]. While there is clear evidence for impairment of humoral and cell mediated immunity in infectious mononucleosis [13], lymphocytes of ALL do not usually manifest such impairment [14, 15].

Our results demonstrate a clear difference in basal cAMP levels as well as in cAMP levels after trypsin treatment between PBL in infectious mononucleosis and in ALL before the induction of remission (Fig. 1). We confirmed that human lymphoblasts contain low basal levels of cAMP, which can be accounted for by their higher phosphodiesterase activity [5] and they also lack the ability to respond to trypsin. The slight response to trypsin seen in PBL of untreated ALL patients can be attributed to a small minority of normal lymphocytes present in the peripheral blood of these patients [16]. PBL of ALL patients in remission are composed mainly of lymphocytes with normal appearance. These cells exhibit higher basal levels of cAMP and increased reactivity toward trypsin (Fig. 1). The fact that PBL of infectious-mononucleosis patients are actively dividing cells [17] may explain their relatively low cAMP levels, as compared to the controls only.

REFERENCES

1. H. R. Bourne, L. M. Lichtenstein, K. L. Melmon, Y. Weinstein and G. M. Shearer, Modulation of inflammation and immunity by cyclic-AMP. *Science* **184,** 19 (1974).
2. J. Watson, Cyclic nucleotides as intracellular mediators of B cell activation. *Transplant. Rev.* **23,** 223 (1975).
3. A. I. Kook and N. Trainin, Intracellular events involved in the induction of immune competence in lymphoid cells by a thymus humoral factor. *J. Immunol.* **114,** 151 (1975).
4. A. Shneyour, A. Patt and N. Trainin, Trypsin-induced increase in intracellular cyclic AMP of lymphocytes. *J. Immunol.* **117,** 2143 (1976).
5. W. N. Hait and B. Weiss, Increased cyclic nucleotide phosphodiesterase activity in leukaemic lymphocytes. *Nature (Lond.)* **259,** 325 (1976).
6. N. Lavin, G. S. Rachelefsky and S. A. Kaplan, An action of disodium cromoglycate: inhibition of cyclic-3,5'-AMP phosphodiesterase. *J. Allergy clin. Immunol.* **57,** 80 (1976).
7. C. W. Parker and J. W. Smith, Alterations in cyclic adenosine monophosphate metabolism in human bronchial asthma. *J. clin. Invest.* **52,** 48 (1973).
8. A. Bøyum, Separation of leucocytes from blood and bone marrow. *Scand. J. clin. Lab. Invest.* **21,** Suppl. 97 (1968).
9. A. G. Gilman and F. Murad, Assay of cyclic nucleotides by receptor protein binding displacement. In *Methods in Enzymology.* (Edited by S. P. Colowich and N. O. Kaplan) Vol. 38, p. 49. Academic Press, New York (1974).
10. P. J. Sheldon, M. Papamichail, E. H. Hemsted and E. J. Holborow, Thymic origin of atypical lymphoid cells in infectious mononucleosis. *Lancet* **i,** 1153 (1973).
11. M. A. Smith, J. Evans and C. M. Steel, Age-related variation in proportion of circulating T cells. *Lancet* **ii,** 922 (1974).
12. M. H. Freedman, G. E. Gilchrist and G. D. Hammond, Concurrent infectious mononucleosis and acute leukemia. *J. Amer. med. Ass.* **214,** 1677 (1970).
13. B. Shohat, H. Joshua and J. Grinblat, Impaired cellular immunocompetence in infectious mononucleosis as assessed by a local Graft-vs-Host reaction in rats and restoration of the immunocompetence by trypsin. *Cell Immunol.* **25,** 116 (1976).
14. J. F. Harris and R. C. Bagai, Immune deficiency states associated with malignant disease in man. *Med. Clin. N. Amer.* **56,** 501 (1972).
15. J. M. Dupuy, F. M. Kourilsky, D. Fradelizzi, N. Feingold, C. Jacquillat, J. Bernard and J. Dausset, Depression of immunologic reactivity of patients with acute leukemia. *Cancer (Philad.)* **27,** 323 (1971).
16. F. M. Kourilsky, L. Lovric and A. Levacher, Phytoheamagglutinin and lymphocytes from acute leukaemia. *Lancet* **ii,** 856 (1966).
17. H. Bowcock, Mitotic leukoblasts in the peripheral blood in infectious mononucleosis. *Amer. J. Med. Sci.* **198,** 384 (1939).