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Age associated changes in intracellular cyclic adenosine monophosphate

A. BIRKENFELD & A. BEN-ZVI *Department of Obstetrics & Gynecology and Department of Pediatrics, Hadassah University Hospital, Ein-Karem, Jerusalem, Israel*

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SUMMARY

Cyclic adenosine monophosphate (cAMP) levels of isolated human peripheral blood lymphocytes show age associated changes. These changes are apparent in basal cAMP levels as well as in cAMP levels after trypsin treatment. Both cord blood lymphocytes and lymphocytes isolated from the blood of old people exhibit lower basal levels of cAMP and diminished increase in cAMP by trypsin treatment, in comparison to lymphocytes isolated from the other age groups. The results seem to indicate a low number of reactive cells and a low reactivity of the cells in the case of cord blood lymphocytes, and a decrease in number of the reactive cells without a decrease in specific reactivity of isolated lymphocytes of old people.

Keywords cyclic AMP lymphocytes

INTRODUCTION

Trypsin increases intracellular levels of cAMP in lymphocytes. The extent of the trypsin-induced increase in intracellular cAMP correlates with the type of the lymphocytes and with the state of maturity attained by them. Thus, hydrocortisone resistant thymocytes and peripheral blood lymphocytes are the best responders to trypsin while transformed lymphocytes do not react at all (Shneyour, Patt & Trainin, 1976).

Human peripheral blood lymphocytes are subjected to age associated changes in subpopulations. These changes are reflected in various immunological parameters like formation of spontaneous rosettes at different temperatures, response to the plant mitogens phytohaemagglutinin and concanavalin A (Ben-Zvi *et al.*, 1977), production of some lymphokines (Eife *et al.*, 1974) and cytotoxicity (Xanthou *et al.*, 1976). Thus it became of interest to learn if cAMP levels of human peripheral blood lymphocytes before and after trypsin treatment reflect such age associated changes.

MATERIALS AND METHODS

Source of lymphocytes. Lymphocytes were prepared from peripheral blood obtained by venipuncture and kept with heparin. (1) *Cord blood*: cord blood was collected from the umbilical cord of 18 normal newborns shortly after delivery at the maternity unit of the Kaplan Hospital, Rehovot. (2) *Blood from children and adolescents*: venous blood was obtained from 17 children and 16 adolescents of various ages, free of symptoms and disease, who were examined at the pediatric

Correspondence: Dr A. Ben-Zvi, Department of Pediatrics, Hadassah University Hospital, Ein Karem, Jerusalem, Israel.

clinics of Hadassah Medical Center, Jerusalem, Beilinson Hospital, Petah-Tikva and Kaplan Hospital, Rehovot. (3) *Blood from normal adults*: venous blood was obtained from 22 normal, healthy volunteers at ages ranging from 20 to 65 years. (4) *Blood from elderly adults*: blood from elderly, healthy volunteers was collected from 18 individuals at the Neve-Amit old age home, Rehovot. The elderly donors were either free of disease or suffered from degenerative cardiovascular disorders. Their age ranges from 66 to 96 years.

Lymphocyte separation. Ten millilitres of heparinized blood were diluted vol./vol. with phosphate-buffered saline (Dulbecco's modification) kept at room temperature (24°C) within 90 min after collection and then were immediately layered over 15 ml of sodium metrizoate/Ficoll solution ('lymphoprep', Nyegaard & Co., Oslo, Norway). Lymphocytes were separated as described by Böyum (1968).

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, USA) 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, New York, USA), 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 of 0·1 N HCl to each 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 by the use of a specific binding protein as described by Gilman & Murad (1974). Each cAMP determination was done in triplicate.

RESULTS

The basal levels of intracellular cAMP in separated human peripheral blood lymphocytes are shown in the lower part of Fig. 1. Elderly people lymphocytes (above 65 years) and newborn lymphocytes from cord blood contain the lowest levels of cAMP in comparison with the other age groups, while those isolated from children belonging to age group 1–11 years show age-dependent gradual decrease in cAMP levels of their peripheral blood lymphocytes.

The difference in cAMP content of isolated peripheral blood lymphocytes of different age groups after trypsin treatment (upper part of Fig. 1) are less prominent. Cord blood lymphocytes

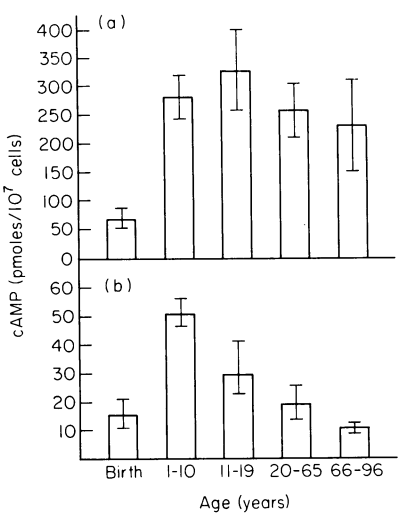

Fig. 1. cAMP levels in human lymphocytes of various age groups. (b) Shows the basal (untreated) values ± s.d. (a) cAMP levels after trypsin incubation ± s.d. ($P < 0.001$).

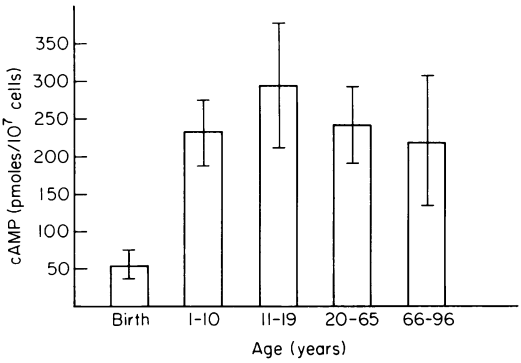

Fig. 2. Net increase in lymphocyte intracellular cAMP levels by age distribution.

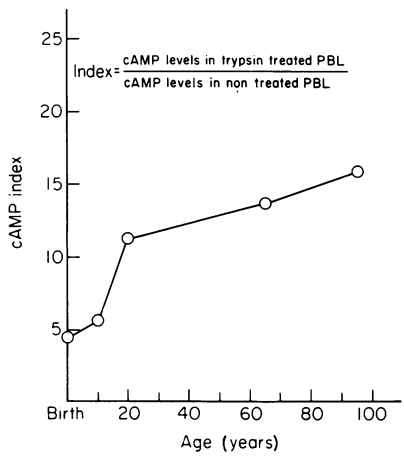

Fig. 3. The 'ageing index': ratio of cAMP level of treated lymphocytes to basal cAMP level.

$$\text{Index} = \frac{\text{cAMP levels in trypsin treated PBL}}{\text{cAMP levels in untreated PBL}}.$$

still contain considerably lower amounts of cAMP in comparison to the other groups, but the age dependent decrease in cAMP is small and old people lymphocytes (more than 65 years) contain cAMP levels similar to that of the adults. It should be emphasized that individuals belonging to the age group between 66–96 years show considerable variations as to their basal lymphocytes' cAMP content or the extent of its increase after trypsin treatment (see also Fig. 3).

Age distribution of the difference in cAMP content of lymphocytes before and after trypsin treatment shows the same pattern as that of cAMP levels after trypsin treatment and again cord blood lymphocytes show the smallest difference (Fig. 2). The age dependence of the ratios of cAMP levels after trypsin activations to that of the basal levels is shown in Fig. 3. This ratio is expressed as the cAMP ratio. cAMP levels of lymphocytes isolated from cord blood exhibit the lowest ratio. This ratio tends to increase with age rather than to increase and then to decrease again with advanced age, as do cAMP levels before and after trypsin treatment.

DISCUSSION

The number of T lymphocytes in peripheral blood, as well as their immunological reactivity, are subjected to age associated changes. Thus it is known that the number of spontaneous rosette

forming cells with serum red blood cells (E rosettes) is lower in cord blood and peripheral blood lymphocytes of old people than in blood of adults (Ben-Zvi *et al.*, 1977; Smith, Evans & Steel, 1974). Not only is the number of rosette forming cells different in the age groups at the two extremes, but the immunological properties of the cells also are different. Cord blood lymphocytes have been reported to produce diminished amounts of lymphotoxin, and to show poor phytohaemagglutinin-induced cytotoxicity, as compared with lymphocytes of older individuals (Böyum, 1968; Xanthou *et al.*, 1976). Roberts-Thomson *et al.* (1974) report a significant reduction in the reactivity of lymphocytes of old people when tested by three different systems. In the aged (above 60 years), as compared to the young (below 25 years), the number of positive delayed type hypersensitivity reactions to five ubiquitous antigens was significantly lower and so were the lymphocyte response to the mitogen phytohaemagglutinin and the presumed T cell-dependent late IgG response to monomeric flagellin.

Intracellular cAMP plays a major role in the regulation of the immune response (Bourne *et al.*, 1974; Watson, 1975). It has been shown that trypsin increases cAMP levels mainly in mature immunocompetent T lymphocytes (Shneyour *et al.*, 1976). In the present study we measured the levels of intracellular cAMP of peripheral blood lymphocytes before and after trypsin treatment and correlated them with the specific age group of the blood donor.

Our results show that cord blood lymphocytes and lymphocytes of old people contain less cAMP than lymphocytes of children and adults. Low values of cAMP are compatible with an early stage of maturation and low degree of immunocompetence (Shneyour *et al.*, 1976). Parallel results were obtained after trypsin treatment indicating that cord blood lymphocytes are relatively unresponsive to trypsin.

This low responsiveness cannot be explained only by a decreased number of cells responding to trypsin, since the low cAMP index of cord blood lymphocytes indicates low reactivity of the responding cells. The fact that the ratio of cAMP levels of old people remains as in adults or is actually increased while the absolute levels of cAMP before and after trypsin treatment are reduced seems to indicate that the specific reactivity remained elevated in cells obtained from this age group. As a matter of fact, old people lymphocytes are more reactive than any other age group lymphocytes, although the variations between different individuals in this group are very large.

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