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AGE-RELATED EVENTS IN HUMAN ACTIVE T LYMPHOCYTES: CHANGES IN THE PHOSPHOINOSITIDASE C ACTIVITY

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Since PHA-stimulated active T lymphocytes from aged humans showed changes in the metabolic pattern of inositol lipids in comparison with young subjects, we studied the possible role of phosphoinositidase C (PIC) in the generation of this phenomenon. The breakdown of exogenous [^{3}H]phosphatidylinositol 4, 5-bisphosphate was found to be optimal at neutral pH and Ca^{++} concentrations close to millimolar levels. Under these conditions PIC activity of resting lymphocytes did not differ in aged and young subjects, while, after short periods of PHA stimulation (up to 4 hr) the substrate hydrolysis was lower and delayed in the elderly group in comparison with that of controls. Our findings support the hypothesis that the age-related default of this enzyme, responsible for the age-related changes in the inositol lipid pathway of this peculiar subpopulation, could be involved, as a primary event, in the mechanisms leading to the reduced proliferative response of aged active T lymphocytes.

Cell growth and differentiation are controlled by signal transduction systems involving cyclic nucleotides or the secondary messengers derived from polyphosphoinositide metabolism (1-3). The hydrolysis of phosphatidylinositol 4, 5-bisphosphate ($PtdInsP_2$) is a widespread receptor coupled signalling system at the plasma membrane of most eukarjotic cells. This reaction, catalysed by a specific phoshoinositidase C (PIC), yields two products, inositol 1,4,5-trisphosphate ($InsP_3$) and diacylglycerol (DAG), which serve as intracellular mediators and lead to cellular calcium mobilisation and protein kinase C (PKC) activation (2-5).

During ageing a number of changes in cyclic nucleotides and key regulatory enzymes have been described (6). Both an accumulation of enzymes with lowered activity and a lowered induction of certain enzymes in response to external stimuli have been described during ageing (7, 8). In a previous work, we found age-related differences in the metabolic pattern of inositol

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lipids mainly represented by an increase in the *in vitro* phosphorylation of $PtdInsP_2$ and a decrease in the in vitro phosphorylation of phosphatidylinositol 4-phosphate (PtdInsP) after short periods (up to 4 hr) of PHA stimulation (9). To address the question of whether phosphoinositidase C is involved in this phenomenon, we characterised this enzyme activity in whole cell homogenates from active T lymphocytes of aged humans prior to and after mitogenic induction.

MATERIALS AND METHODS

Subjects. This study included 10 elderly subjects (M) over 65 (72.0 ± 3.9) (mean$\pm$S.D.) considered normal on the basis of clinical and biochemical findings, according to the Senieur protocol (10). The control group consisted of 10 healthy donors (M) of 20-30 years of age (26.1 ± 2.9) (mean$\pm$S.D.). None of them was taking any drug which could affect the immune system.

Cell Preparations. Mononuclear cells were separated from heparinized peripheral blood on a density gradient (MSL, Eurobio, Paris) (11). PBL were grown in suspension in RPMI 1640 medium (Gibco, Paisley, Scotland) plus 50 units/ml penicillin, 50 μg/ml streptomycin, 2 mM L-glutamin and 15% foetal calf serum (Gibco, Paisley, Scotland) at 37°C in 5% CO_2 a a density of 10^6 cells/ml. Cells were stimulated with 10 μg/ml PHA (Wellcome Diagnostics, Dartford, England) up to 72 hr or left untreated as controls. The incubation was stopped by washing the cells three times with a PHA-deprived medium, the active E rosette test was performed as previously described and the purified active T subpopulation was harvested following a second density gradient (12).

PIC Assay. PIC assay (100 μl) contained 100 mM MES or Tris buffer, 150 mM NaCl, 0.06% taurodeoxycholate, 3 nmoles [^{3}H]$PtdInsP_2$ (spec. act. 30.000 dpm/nmole, Amersham, UK), 10 μg cytoplasmic protein. Incubation was at 37°C for 30 min. Hydrolysis was stopped by adding chloroform-methanol-HCl as described in ref. 13, and inositol phosphates, recovered from the aqueous phase, were analysed by HPLC essentially as described in ref. 14. Samples were loaded onto a Partisil 10 SAX column and eluted with a linear gradient from distilled H_2O to 2 M ammonium formate (pH 3.7, adjusted with phosphoric acid) at 1.5 ml/min. As reference compounds [^{3}H]InsP and [^{3}H]$InsP_2$ (100.000 dpm) were used and eluted as above. 1 ml fractions were collected, dissolved in Hionic-fluor (Packard) and counted by liquid scintillation. Protein was measured as in ref. 15.

RESULTS AND DISCUSSION

Active T lymphocytes play a key role in cell-mediated responses and during ageing they undergo a number of biochemical and morphological changes (9, 12). Although new insight into the biochemical basis for the age-related decline in T lymphocyte responsiveness to mitogens has recently emerged from studies of calcium signal generation (7, 16, 17), activation of inositol phosphate-dependent pathways (7, 18) and PKC function (7, 19), it is not yet clear which of the enzymes related to the phosphoinositide cycle could be involved and in which way. Our data strongly suggest the involvement of phosphoinositidase C activity in the generation of the age-related delayed responsiveness. In human active T cells $PtdInsP_2$ specific phosphoinositidase C was tested for Ca^{++} and pH requirement. As shown in Fig. 1 cytoplasmic phosphoinositidase activity requires Ca^{++} since the addition of EGTA abolishes

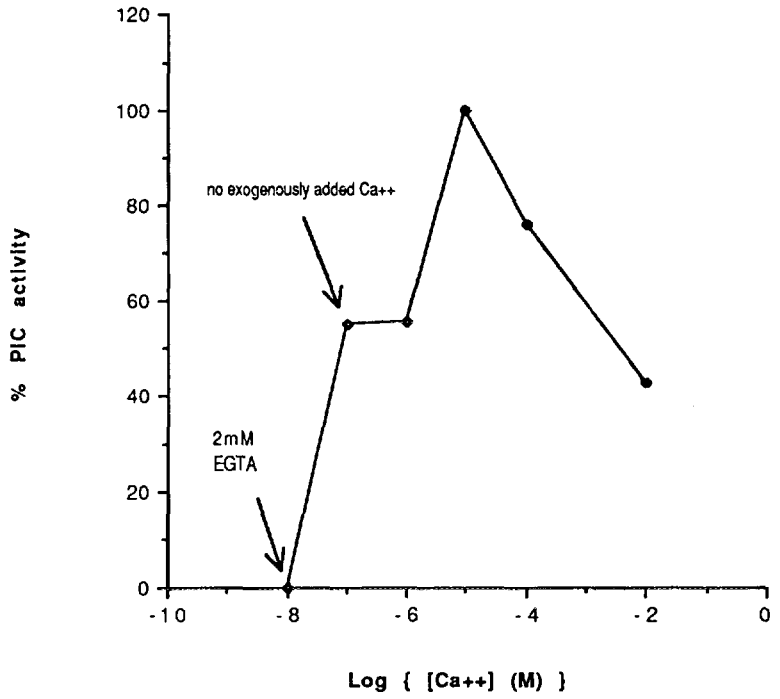

Fig.1. Ca^{++} dependency of PIC activity in human active T lymphocytes. The activity is expressed as percentage of the maximal $PtdInsP_2$ breakdown achieved.

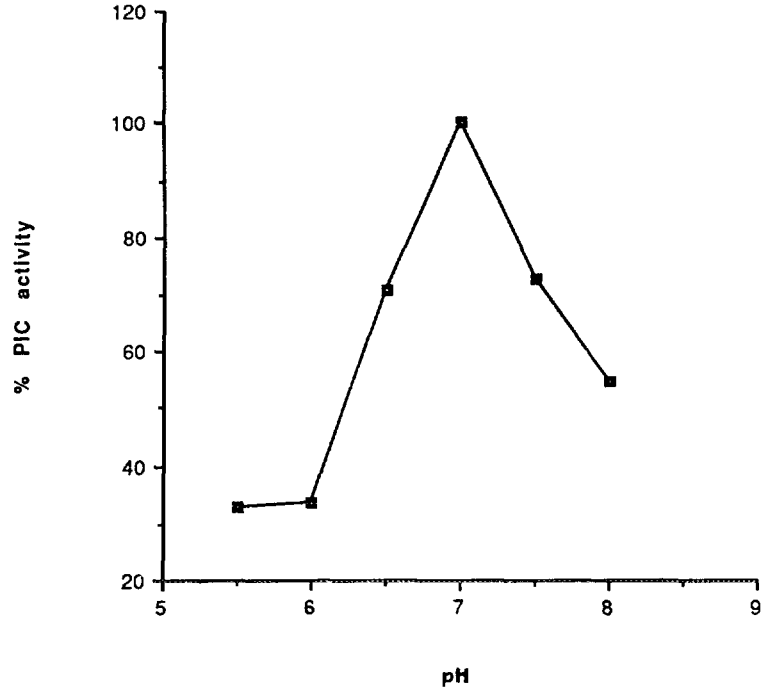

Fig.2. pH dependency of PIC activity in human active T lymphocytes. The activity is expressed as percentage of the maximal $PtdInsP_2$ breakdown achieved.

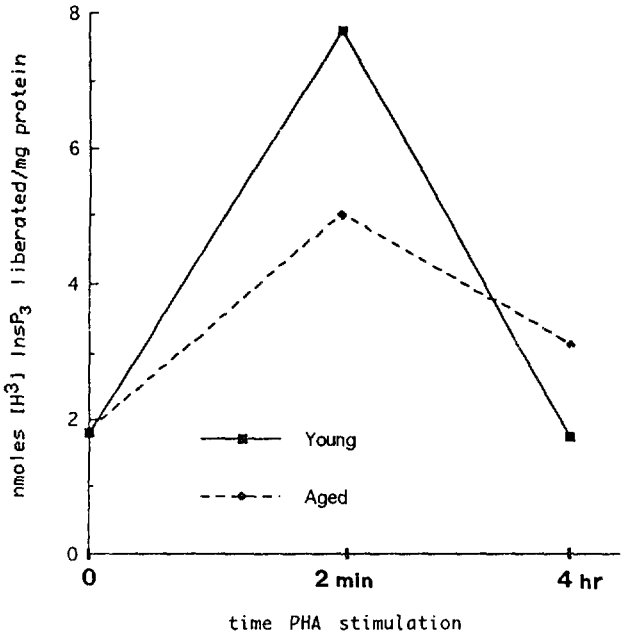

Fig. 3. PIC activity in active T lymphocytes from both young and aged subjects at different time intervals of PHA stimulation.

$PtdInsP_2$ breakdown. Moreover, the hydrolytic activity of the enzyme is dependent upon extracellular Ca^{++}, being improved at millimolar levels of exogenous calcium. This argues against the presence of the α form of PIC which seems to be insensitive to calcium with PtdInsP and $PtdInsP_2$ as substrates (20). Regarding pH, although the enzyme acts over a broad range, the maximal hydrolysis occurs at pH 7.0 (Fig.2). In these conditions there are no differences between resting active T lymphocytes from young and aged donors. In contrast, within 2 min of PHA treatment there is a transient and approximately 100% increase in cytoplasmic PIC activity in both aged and young individuals, but with an elderly : young ratio of almost 1:2. After 4 hr, in the young group PIC activity returns to initial levels while in the elderly group $PtdInsP_2$ enzymatic hydrolysis is still greater than prior to PHA stimulation and increased almost two-fold compared to that of the control group (Fig.3). Therefore this delayed activity is supposed to be closely related to the substantial delay of the *in vitro* cell proliferation occurring in active T cells of aged humans (Rana R. et al., manuscript in preparation) An open question remains which PIC isoforms are affected by ageing and where they are distributed, being already demonstrated the existence of the inositol lipids cycle besides at the plasma membrane (21-23) also at the cell nucleus (24-29).

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