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Age related impairment in phosphatidylinositol breakdown of polymorphonuclear granulocytes

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It is well known that with aging the immune response decreases. Most of the effector functions occur through specific receptors. Thus, we investigated the effects of various stimulants, acting through receptors or directly through the GTP-binding G_i protein, on phosphatidylinositol breakdown in PMNLs of young and elderly subjects and try to modulate it. A marked decrease in inositol phosphate (IP_1, IP_2, IP_3) formation in PMNLs of elderly was found under FMLP stimulation when compared to that of young subjects. Neither GTPγS, nor AlF_4^- could induce an increase of IP_3 in PMNLs of elderly comparable to that of young subjects. The results suggest that at least an alteration exists at the GTP-binding G_i protein level, as well as in the mechanism of linkage of the receptor to the G protein.

Aging; Inositol phosphate; GTPγS; (Granulocyte)

1. INTRODUCTION

It is well known that elderly subjects are prone to infections, cancer and autoimmune disorders [1–3]. The immune functions were extensively studied in aged humans and other animals and the results suggest that the B and T lymphocyte functions are altered [4–6]. In our previous studies we have demonstrated that phagocytic cell functions were also altered with aging [7,8]. It is thought that this decline contributes largely to the increased incidence of the above mentioned diseases. Most of the effector functions that occur during the immune response necessitate the stimulation of specific receptors [9–15].

In PMNLs, as in many cells, the turnover of phosphoinositides is controlled by guanine nucleotide-binding G proteins. G proteins are activated when GTP is bound and inactivated when bound GTP is hydrolyzed. A large amount of information is now available on the signal transduction mechanisms of various receptors linked to GTP-binding G_i proteins [16–18] in the case of cells from young subjects, whereas this is not yet completely elucidated in cells of the elderly. Thus the aim of our present work was to study the effects of various stimulants acting through receptors or directly through the GTP-binding G_i protein, on phosphatidylinositol breakdown in PMNLs of young and elderly subjects and try to modulate it.

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Abbreviations: IP_1, inositol 4-monophosphate; IP_2, inositol 1,4-bisphosphate; IP_3, inositol 1,4,5-trisphosphate; GTPγS, guanosine-5'-*O*-(3-thiotriphosphate); PIP_2, phosphatidylinositol 4,5-bisphosphate; DAG, diacylglycerol; PKC, protein kinase C; PLC, phospholipase C; PLA_2, phospholipase A_2; FMLP, *N*-formyl-methionyl-leucyl-phenylalanine; PMNLs, polymorphonuclear granulocytes; PT, pertussis toxin

2. MATERIALS AND METHODS

2.1. *Patients*

PMNLs were obtained from 10 healthy young (<30 yr) and 10 healthy old (>65 yr) subjects. All of them gave fully informed consent and were volunteers. The selection of these persons

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was based on the criteria of good physical and mental health confirmed by careful clinical and laboratory investigations [19].

2.2. *PMNL*

PMNLs were separated by Ficoll-Hypaque density-gradient centrifugation according to the method of Böyum [20]. The cells were viable at least 95% as judged by trypan blue exclusion.

2.3. *Materials*

N-Formyl-methionyl-leucyl-phenylalanine (FMLP, 10^{-8} M), neomycin, chloroquine, GTPγS (200 ng/ml) were from Sigma; pertussis toxin was from List Biological Laboratories; *myo*-[2-^{3}H]inositol was from Amersham (spec. act. 614 GBq/mmol).

2.4. *Experimental conditions*

The cells were resuspended in HBSS medium with or without 1 mM Ca^{2+}. The incubations were carried out in a CO_2 incubator (CO_2 5%, air 95%, humidity 95% at 37°C). The preincubation times with various inhibitors were the following: for 500 ng/ml PT, 2 h; for 10^{-4} M neomycin, for 10 min; 10^{-4} M chloroquine, 30 min. *myo*-[2-^{3}H]inositol loading: to 1×10^7 cells/ml, 20 μCi/ml *myo*-[2-^{3}H]inositol was added and incubated for 2 h at 37°C. After this incubation the cells were washed with warm HBSS and diluted to 5×10^6 cells/ml. After adding stimulators (10^{-8} M FMLP; 10 mM AlF_4) to the cell suspension the reaction was stopped with cold TCA at a final concentration of 6%. In experiments with GTPγS (200 ng/ml) the cells were saponified (15 μg/ml saponin, 5 min) in HBSS without Ca^{2+}. The preincubations with inhibitors were carried out during loading with *myo*-[2-^{3}H]inositol. The lipids were extracted with chloroform/methanol/HCl (100:50:1, v/v%) and the aqueous phase was used for IPs determination.

Separation of inositol phosphates: IPs were separated on a Dowex 1 × 8 ion exchanger (formate form) with an increasing concentration of ammonium-formate [21]. The activity of samples was measured in a Hitachi liquid scintillation counter.

3. RESULTS

At first we investigated the well-known phosphoinositide mobilizing effect of FMLP on PMNLs of young and elderly subjects. We found a marked decrease in inositol phosphate (IP_1, IP_2, IP_3) formation in PMNLs of elderly under FMLP stimulation when compared to that of young subjects (fig.1). All inositol phosphates were decreased but the most marked decrease was observed in IP_3 levels. Next the effects of GTPγS, a well-known direct stimulator of GTP-binding G_i protein, on phosphatidylinositol breakdown were investigated. GTPγS induced IP_3 formation in PMNLs of young subjects whilst its effect was very small in PMNLs of elderly (fig.2). The other widely used stimulator for phosphatidylinositol breakdown is AlF_4^-. A marked IP_3 formation could be observed in the case of young subjects (fig.3). The effects of AlF_4^- on IP_3 formation in PMNLs of elderly was similar to that of GTPγS (figs 2,3). The effects of different inhibitors on

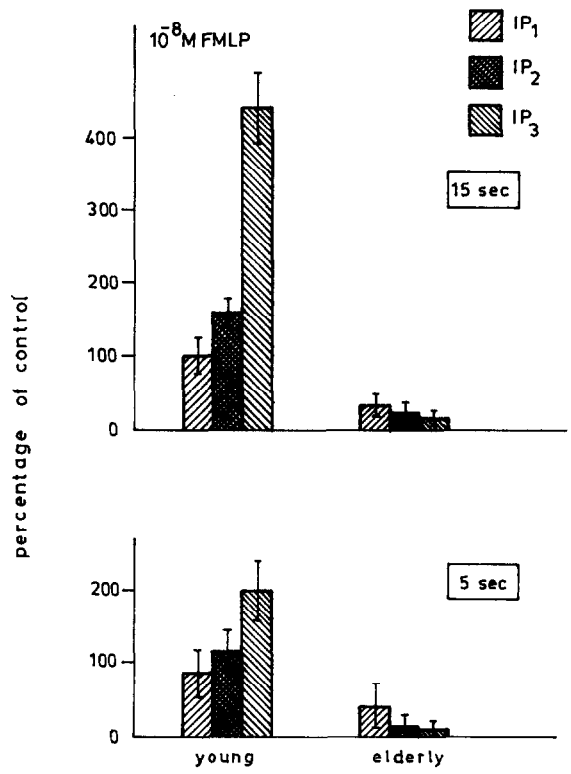

Fig.1. FMLP-stimulated inositol phosphate (▨ IP_1; ▩ IP_2; ▧ IP_3) mobilization in PMNLs of young and elderly subjects.

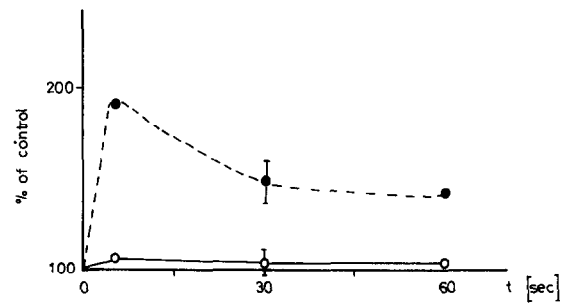

Fig.2. GTPγS (200 ng/ml)-stimulated inositol 1,4,5-trisphosphate formation in saponified PMNLs of young (●) and elderly (○) subjects.

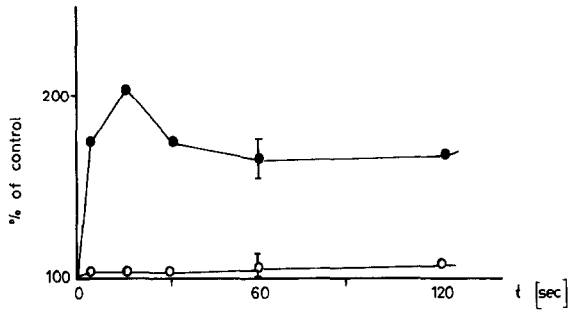

Fig.3. AlF_4^- (10 mM)-stimulated inositol 1,4,5-trisphosphate formation in PMNLs of young (●) and elderly (○) subjects.

FMLP- and GTPγS-stimulated inositol phosphate formation were also studied (fig.4) to gain further understanding of the underlying mechanism of this age-related phosphatidylinositol breakdown alteration. It was found that PT, an inhibitor of GTP-binding G_i protein, was without any effect on PMNLs of the elderly, while neomycin, an inhibitor of PLC, inhibited the IP formation in PMNLs of elderly, too. The effect of chloroquine, an inhibitor of PLA_2, on the FMLP- and GTPγS-induced phosphatidylinositol breakdown was very surprising. After the preincubation with chloroquine a marked increase of inositol phosphates was found. The various inhibitors used did not change the basic IP levels (not shown).

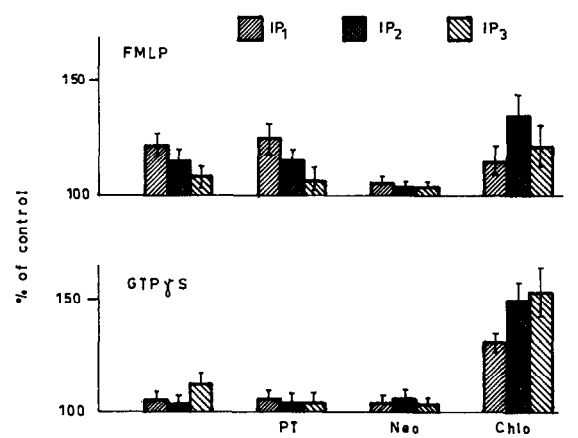

Fig.4. Effects of various inhibitors on FMLP- and GTPγS-induced inositol phosphate (▨ IP_1; ▩ IP_2; ▧ IP_3) formation in PMNLs of elderly subjects. PT, pertussis toxin for GTP binding G_i protein; Neo, neomycin for phospholipase C; Chlo, chloroquine for phospholipase A_2.

4. DISCUSSION

The age-related changes in receptor-mediated effector functions were extensively studied during these last years. It seems evident that in most cases the receptor number is unaffected by aging [22] and this disturbed responsiveness may be connected to the alteration of the receptor signal transduction mechanism. Based on our previous results and the findings of others [23] it can be assumed that with aging an alteration of the post-receptorial signal transduction mechanism occurs.

Our results show that the PIP_2 hydrolysis i.e. inositol phosphate formation practically could not be induced by FMLP in PMNLs of elderly. Thus, the question arises as to where the exact alteration is situated on the signal transduction pathway. The alteration could occur at various levels such as the receptor, the GTP-binding G_i protein, the PLC and finally the PIP_2 availability. To get some more information about the alteration of PIP_2 hydrolysis, we studied the effects of direct stimulation of GTP-binding G_i protein on PIP_2 hydrolysis and modulate them by various inhibitors. Non-hydrolysable analogues of GTP can retain G proteins in a functional state and allow an assessment of the role of G proteins. Our results show that neither GTPγS nor AlF_4 could induce a substantial IP formation in PMNLs of elderly. The irresponsiveness of G protein to GTPγS stimulation provides indirect evidence of the alteration of this G protein with aging. This is compatible with the results obtained by aluminium fluoride complexes, which are thought to interact with G protein. Thus these results suggest that at least an alteration exists at the GTP-binding G_i protein level as well as in the mechanism of linkage of the receptor to the GTP-binding G protein.

The fact that chloroquine, an inhibitor of PLA_2 enzyme, stimulates IP formation in PMNLs of elderly, suggests that (i) the elevated intracellular free Ca^{2+} level, measured in PMNLs of elderly [24], which could be decreased by chloroquine, inhibits the PIP_2 hydrolysis in PMNLs of elderly, or that (ii) the PLA_2 pathway might exert an inhibitory role on PIP_2 hydrolysis in the case of elderly. This latter was already suggested during the study of the respiratory burst with aging [25], but other studies also seem to suggest some sort of control by one pathway on the other [26]. The

study of this phenomenon and the elucidation of the exact nature of this post-receptorial signal transduction alteration with aging need further investigations leading to the development of new strategies in immunorestoration.

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