Cellular distribution of protein kinase C isozymes in CD3-mediated stimulation of human T lymphocytes with aging

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Abstract Protein kinase C (PKC) is involved in a variety of cellular responses, such as the expression and secretion of IL-2, the regulation of cytotoxic killing and cell proliferation. It is known that these immune functions are altered with aging. Here, we show that anti-CD3-triggered T cell proliferation is significantly decreased with aging and that H7, an inhibitor of PKC, impairs the anti-CD3-induced T cell proliferation in a differential manner, lymphocytes of healthy young subjects being more sensitive to the PKC inhibitor than those of elderly subjects. We examined (Western blot) the presence and the cellular distribution of PKC isozymes in T lymphocytes of healthy young and elderly subjects in the resting state and after anti-CD3 mAb stimulation using antibodies directed against PKC α, β, δ, ε and ζ isoforms in the cytosol and the plasma membrane fractions. These five PKC isotypes were present in human T cells of young and elderly subjects. However, their distribution between the cytosolic and membrane fractions varied according to the isozymes and the age of the subjects. In resting lymphocytes of young subjects, all the PKC isozymes were found in the cytosolic fraction, except PKC-ζ. In resting lymphocytes of elderly subjects PKC-δ and -ε were almost equally distributed between the cytosolic and the membrane fractions, whereas PKC-α and -ζ were mainly found in the membrane fraction and PKC-β was almost exclusively located in the cytosolic fraction. The translocation of PKC-α, -β, -δ and -ε could be observed under anti-CD3 mAb stimulation in lymphocytes of young subjects, while in the case of elderly subjects only the PKC β isoform was translocated. Our results suggest that the decreased availability of cytosolic PKC may contribute to the diminished PKC-dependent responses to CD3-triggered stimulation of human T lymphocytes with aging.

Key words: Protein kinase C; T lymphocyte; Aging; Proliferation

1. Introduction

The specificity of the immune response is determined by the interaction between the T cell antigen receptor (TcR) and its cognate antigen. The TcR is coupled to the cytoplasmic non-receptor tyrosine-protein kinases by the cytoplasmic tails of the TcR-associated CD3 subunits. The signalling events downstream of protein-tyrosine phosphorylation following T cell receptor stimulation include the activation of the phosphatidylinositol pathway, $p21^{ras}$ and several serine/threonine protein kinases. The transmission of signals to the nucleus is proposed to involve a protein kinase cascade, including Raf-1, mitogen-activated (MAP) kinase and MAP kinase kinase. At least two intracellular pathways for regulation of this cytoplasmic protein kinase cascade coexists in T cells; one mediated by $p21^{ras}$ and the other by protein kinases C [1].

Stimulation of the TcR increases phospholipase C activity and that results in hydrolysis of PtdIns $(4,5)P_2$ yielding the second messengers $InsP_3$ and 1,2-diacylglycerol (DAG). DAG is responsible for the activation of some protein kinase C (PKC) isozymes. PKC has been implicated in the regulation of a variety of cellular processes, including proliferation, differentiation and release of hormones, lymphokines and neurotransmitters [2,3]. Recent studies have shown that several cytokine gene expression is strictly dependent on a T cell-specific protein complex, called nuclear factor of activated T cells (NF-AT) and the de novo synthesis of NF-ATn is absolutely dependent on the activation of PKC stimulated via T cell antigen receptor and/or phorbol esters [4–6].

Molecular cloning had so far revealed that PKC consists of a large family of at least 12 different members. They are divided in three major groups: the calcium-dependent or conventional protein kinases (cPKC) consisting of the α, β1, β2 and γ isoforms; the calcium-independent or novel protein kinases (nPKC) consisting of the δ, ε, η, θ and μ isoforms; and the atypical protein kinases (aPKC) consisting of the ζ, ι and λ isoforms [2,7]. The members of the PKC family are dependent on phosphatidylserine, but show clearly different requirements for other modulators. On the one hand, cPKC are activated by Ca^{2+} and DAG and this activation is further enhanced by *cis*-polyunsaturated fatty acids and lysophosphatidylcholine. On the other hand, nPKC respond to DAG and phorbol esters and the ε isoform may also be activated by *cis*-unsaturated fatty acids. The δ and ε PKC isozymes are the best-characterized member of this group and they exist in a phosphorylated form. In contrast, aPKC are not activated by DAG. PKC-ζ does not respond to PMA, but it requires phosphatidylserine and is activated by *cis*-unsaturated fatty acids [8,9]. In T lymphocytes, all subspecies of PKC, with the exception of PKC-γ, have been detected [2,10]. Recent results have shown that β PKC is the dominant isozyme in T cells [11,12].

Aging is accompanied by changes in the immune system, including impairment of delayed type hypersensitivity and other T cell functions [13,14]. Although the mechanisms underlying these age-dependent defects in T cell functions are not completely elucidated, alterations in signal transduction may

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contribute to the observed defects [15–18]. Several functions of T cells that either partially depend on PKC activity or are regulated by the activation of PKC exhibit abnormalities during aging [19,20]. For instance, T cells from elderly subjects stimulated through the TcR/CD3 complex show diminished production of IL2 and a decrease in IL2 receptor expression as well as reduced activation of the AP1 and NFκB transcription factors [21,22].

The effects of aging on the activation of PKC isozymes in human lymphocytes are largely unknown. One recent study has shown that the level of PKC-α was decreased in T lymphocytes of elderly subjects while that of PKC-β was unchanged [23]. The aim of the present study was to investigate the presence of PKC isozymes in human T lymphocytes of elderly subjects and to assess their distribution between the cytosol and the membrane fractions in the resting state and their translocating capacity after stimulation with an anti-CD3 mAb.

2. Materials and methods

2.1. Materials

Chemicals for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) were purchased from Bio-Rad (Richmond, CA). Protease inhibitors, phosphatase inhibitors, PMA, H7 and anti-CD3 (clone C-7048 derived from UCHT1) were obtained from Sigma (St Louis, MO). Polyclonal, affinity-purified antibodies against PKC isoforms (α, β, δ, ε and ζ) and RPMI 1640 culture medium were obtained from Gibco BRL (Grand Island, NY). The enhanced chemiluminescence kit (ECL) Western blot detection system, antirabbit Ig, HRP and [^{3}H]thymidine were purchased from Amersham (Arlington Heights, IL). DEAE cellulose (Whatman) was obtained from Mandel Scientific (Montreal, Canada).

2.2. Subjects

The 16 elderly subjects participating to this study ranged from 65 to 87 years old (mean 78 years) and included 5 women. All subjects were ambulatory and in good health, satisfying the inclusion criteria of the SENIEUR protocol for immunologic investigation of human elderly subjects. The 15 young subjects were healthy volunteers having a mean age of 35 years and included 5 women. All subjects gave their informed consent.

2.3. Lymphocyte separation

Heparinized blood was diluted 2-fold with RPMI 1640 medium containing 2% fetal bovine serum. Lymphocytes were isolated from peripheral blood samples by Ficoll-Hypaque density sedimentation using procedures previously described [24]. Monocytes and B cells were removed by adhesion to plastic dishes and to nylon wool. The resulting highly enriched T cell population consisted of greater than 97% OKT3 (CD3)-positive cells with less than 1.0% surface IgM, CD16 or OKM5-positive cells. Lymphocytes were phenotyped as CD3+ cells by incubating them for 30 min with anti-CD3 mAb (Sigma) and washed twice in PBS. Cells were then exposed to a fluorescein isothiocyanate (FITC)-conjugated goat antimouse IgG for 30 min on ice and washed twice with PBS. Fluorescence analysis was performed by flow cytometry on a FACScan instrument (Beckton Dickinson). At least 10,000 cells were analysed in each experiment [24].

2.4. Cell culture and proliferation assays

Peripheral blood lymphocytes (PBL) were cultured in a final volume of 200 μl of complete media in triplicates in 96-well flat-bottomed microculture plates (Corning, NY). Their proliferative response was measured by exposing the cells to the anti-CD3 mAb. Optimal culture conditions were as determined previously [25], 2×10^5cells/well, 3 days for [^{3}H]thymidine incorporation, 500 nM of PMA, 100 μM for H7 and finally 5 μg/ml for anti-CD3 mAb. When used, H7 was present during the 72-h culture. Cell proliferation assays were performed by addition of 0.25 μCi/well of [^{3}H]thymidine 4 h before harvest. The [^{3}H]thymidine uptake was determined in a standard liquid scintillation counter (Packard, USA).

2.5. PKC isolation and immunoblot analysis

T lymphocytes (5×10^7 cells/ml) were exposed to anti-CD3 mAb (5 μg/ml) for 10 min and a purified $F(ab')_2$ fragments of sheep antimouse IgG was added (8 μg/ml) for 5 min. Cytosolic and particulate fractions were prepared in lysis buffer (containing Tris 20 mM, pH 7.5, sucrose 330 mM, EDTA 2 mM, EGTA 0.5 mM, PMSF 2 mM, DTT 10 mM, aprotinin 1 mg/ml, benzamidine 10 mM). The cells were disrupted by passage through a 26-gauge needle and were centrifuged at $100{,}000 \times g$. Cytosolic and NP-40 solubilized particulate PKC were partially purified on a DE52 ion-exchange column (Whatman) and eluted with 200 μl of NaCl (80 mM)-containing lysis buffer. For immunoblot analysis, PKC-containing fractions were concentrated using Centricon 30 microconcentrators (Amicon). The protein concentration was measured by the method of Lowry et al. [26]. Equal amounts of cytosol- and membrane-purified PKC were loaded in each lane (1 μg protein) and fractionated by SDS-PAGE on 10% acrylamide gels and electrophoretically transferred to nitrocellulose membranes. The membranes were incubated with the respective polyclonal anti-PKC isozymes antibodies (dilution 1:1000). The secondary antibody was an antirabbit HRP conjugate (dilution 1:1000). Immunoblots were developed by chemiluminescence as described previously [15]. The relative immunostaining pattern for individual isozymes was quantitated by scanning densitometry. The scanning was performed with a hand-held instrument (Lightning Scanner 256) and data analysed with the Scan Analysis software (Macintosh microcomputer).

2.6. Statistical analyses

Student's t test was used as routine statistical test with statistical significance set at $P < 0.05$.

3. Results

3.1. Proliferation assays

The functional activity of lymphocytes was assayed by their capacity to proliferate in response to an anti-CD3 mAb (Table 1). The basal proliferative activity of lymphocytes of elderly subjects did not differ significantly from that of young subjects with respect to the amount of [^{3}H]thymidine taken up by the cells. However, exposure of the cells to the anti-CD3 mAb resulted in a large increase in radioactive counts and a proliferative index of 46 ± 13 in the case of young subjects. In marked contrast, the proliferative index of lymphocytes from elderly subjects was approximately one-half of that found for young subjects ($P < 0.02$). Similar observations were made in the case of PMA-induced proliferation of lymphocytes ($P < 0.05$). In a second set of experiments the effect of H7, an inhibitor of PKC, was assessed on the basal proliferative response. Results show that H7 did not significantly influence the amount of [^{3}H]thy-

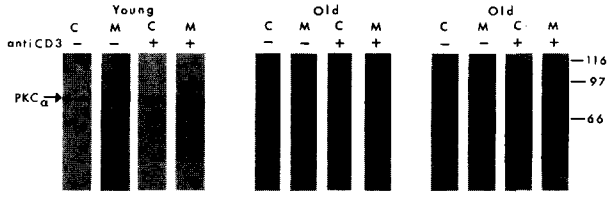

Fig. 1. PKC α isoform immunoreactivity (Western blots) in the cytosol (C) and membrane (M) fractions of human T lymphocytes obtained from healthy young ($n = 15$), in the resting state (young anti-CD3−) and after anti-CD3 mAb stimulation (young anti-CD3+) and from elderly subjects ($n = 16$) in resting state (old anti-CD3−) and after anti-CD3 mAb stimulation (old anti-CD3+). PKC, partially purified from cytosolic and solubilized membrane fractions, was subjected to SDS-PAGE (1 μg of protein/lane). Proteins were transferred to nitrocellulose sheets and immunoblotted with an antibody specific for PKC-α. The arrow indicate the position of PKC-α. The numbers at the right represent the relative size of protein standards (section 2). Data are representative of 15 (young) and 16 (elderly) experiments, respectively.

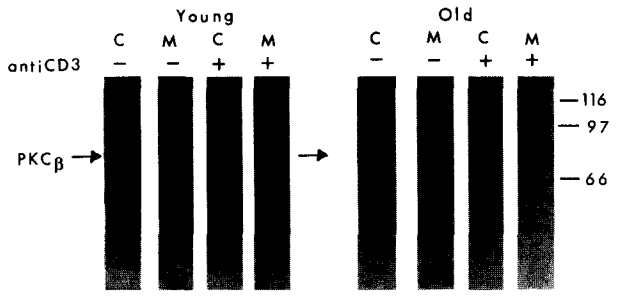

Fig. 2. PKC β isoform immunoreactivity (Western blots) in the cytosol (C) and membrane (M) fractions of human T lymphocytes obtained from healthy young, in the resting state (young anti-CD3−) and after anti-CD3 mAb stimulation (young anti-CD3+) and from elderly subjects in the resting state (old anti-CD3−) and after anti-CD3 mAb stimulation (old anti-CD3+). Experimental details are described in the legend of Fig. 1. Data are representative of 15 (young) and 16 (elderly) experiments, respectively.

midine uptake in T lymphocytes from young or elderly individuals. However, it inhibited lymphocyte proliferation when cells had been stimulated by exposure to the anti-CD3 mAb. For instance, the proliferative index of T lymphocytes from young and elderly subjects decreased to the same level and that corresponded to an approximately 4-fold decrease in the case of cells from the young individuals and approximately 2-fold for the elderly subjects.

We then investigated the presence and cellular distribution of various PKC isozymes in lymphocytes of young and elderly subjects at rest and after stimulation with an anti-CD3 mAb.

3.2. PKC-α

In the case of lymphocytes from young subjects, PKC-α was nearly exclusively found in the cytosol, although a small amount was associated with the plasma membrane fraction (Fig. 1, young anti-CD3−). After stimulation with anti-CD3 mAb almost the totality of the isozyme translocated to the membrane (Fig. 1, young anti-CD3+). In the case of lymphocytes of elderly subjects, two typical situations were found. In one series ($n = 7$), the totality of PKC-α was found associated with the membrane fraction in resting cells (Fig. 1, old anti-CD3−). In the other series ($n = 9$), the distribution of PKC-α was found in almost equal amounts in the cytosol and the plasma membrane fractions (Fig. 1, old anti-CD3−). Independently of the distribution of PKC-α in the resting state, no changes in PKC-α distribution could be obtained after stimulation, suggesting that PKC-α did not translocate (Fig. 1, old anti-CD3+) in the case of elderly subjects. The qualitative observations made on immunoblots were confirmed by densitometric scanning (Table 2).

3.3. PKC-β

In the resting state, PKC-β was mainly found in the cytosol of lymphocytes of young subjects, but a small quantity was detectable in the membrane fraction (Fig. 2, young anti-CD3−). In lymphocytes of elderly subjects, the isozyme was found almost exclusively in the cytosol (Fig. 2, old anti-CD3−). After stimulation, PKC-β translocated to the plasma membrane in the case of lymphocytes from young (Fig. 2, young anti-CD3+) and elderly subjects, although a small quantity was still detectable in the cytosol (Fig. 2, old anti-CD3+; Table 2).

3.4. PKC-δ

The major quantity of PKC-δ was found in the cytosol of resting lymphocytes of young subjects, but a small amount was also associated with the membrane fraction (Fig. 3, young anti-CD3−). An unidentified protein band of 50 kDa appeared in the cytosolic fraction of lymphocytes of young and elderly subjects, but this was not observed in the membrane fraction. After anti-CD3 mAb stimulation, most of the enzyme translocated to the membrane (Fig. 3, young anti-CD3+). PKC-δ was distributed in almost equal quantity in the cytosol and membrane fractions of resting lymphocytes of elderly subjects (Fig. 3, old anti-CD3−). No translocation could be observed after anti-CD3 mAb stimulation (Fig. 3, old anti-CD3+). These observations were confirmed by densitometric scanning (Table 2).

3.5. PKC-ε

PKC-ε appeared as two bands of M_r ~98 and ~72 kDa that were found mainly in the cytosol of lymphocytes of young subjects (Fig. 4, young anti-CD3−). Cell stimulation with anti-CD3 mAb resulted in a decrease of PKC-ε in the cytosol and an apparent translocation to the membrane fraction (Fig. 4, young anti-CD3+; Table 2). In the case of elderly subjects, PKC-ε was almost equally distributed between the cytosol and the membrane fractions of resting lymphocytes (Fig. 4, old anti-CD3−) and no apparent changes could be observed after stimulation with the anti-CD3 mAb (Fig. 4, old antiCD3+).

3.6. PKC-ζ

PKC-ζ was mainly found in the membrane fraction of resting

Table 1
[^{3}H]-Thymidine incorporation by lymphocytes of young and elderly subjects in 3-day cultures

	[^{3}H]thymidine incorporation (cpm)		Proliferation index		
	Young ($n = 15$)	Eldery ($n = 16$)	Young ($n = 15$)	Eldery ($n = 16$)	P value
Agents					
None	1921 ± 155	2375 ± 140	–	–	
Anti-CD3	88946 ± 28818	47507 ± 19089	46 ± 13	20 ± 8	0.02
PMA	28342 ± 4672	13896 ± 6679	14 ± 2	6 ± 3	0.05
H7	3813 ± 1425	8609 ± 4433	2 ± 0.8	4 ± 2	N.S.
H7 + anti-CD3	25686 ± 2136	24663 ± 1942	13 ± 2	10 ± 1	N.S.

Proliferation assays were set up as described in section 2. Results (cpm) are the mean ± S.D. Values of elderly are compared with that of young: N.S.: not significant. Limit of significance set at $P < 0.05$. The proliferation index corresponds to the ratio of label incorporation in stimulated cultures [cpm]/unstimulated (control) [cpm].

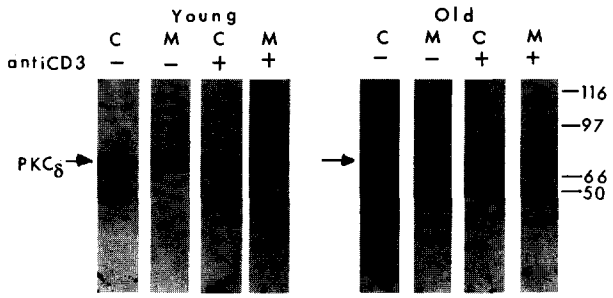

Fig. 3. PKC δ isoform immunoreactivity (Western blots) in the cytosol (C) and membrane (M) fractions of human T lymphocytes obtained from healthy young, in the resting state (young anti-CD3−) and after anti-CD3 mAb stimulation (young anti-CD3+) and from elderly subjects in the resting state (old anti-CD3−) and after anti-CD3 mAb stimulation (old anti-CD3+). Experimental details are described in the legend of Fig. 1. Data are representative of 15 (young) and 16 (elderly) experiments, respectively.

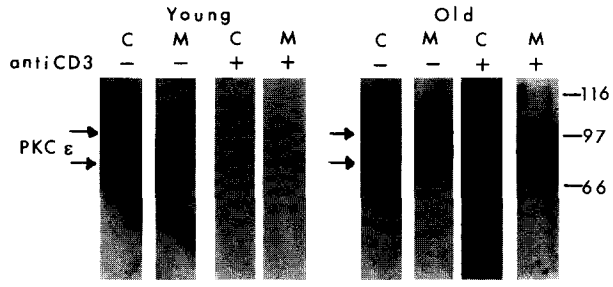

Fig. 4. PKC ε isoform immunoreactivity (Western blots) in the cytosol (C) and membrane (M) fractions of human T lymphocytes obtained from healthy young, in the resting state (young anti-CD3−) and after anti-CD3 mAb stimulation (young anti-CD3+) and from elderly subjects in the resting state (old anti-CD3−) and after anti-CD3 mAb stimulation (old anti-CD3+). Experimental details are described in the legend of Fig. 1. Data are representative of 15 (young) and 16 (elderly) experiments, respectively.

lymphocytes of young subjects, with a smaller amount detected in the cytosol fraction (Fig. 5, young anti-CD3−). After stimulation with anti-CD3 mAb, essentially no changes in its distribution or even a slight increase in the cytosol could be observed (Fig. 5, young anti-CD3+; Table 2). In the case of elderly subjects, nearly all PKC-ζ was also found in the membrane fraction in resting lymphocytes (Fig. 5, old anti-CD3−). Stimulation with anti-CD3 mAb induced an apparent equal distribution between the cytosol and membrane fractions of lymphocytes of elderly subjects (Fig. 5, old anti-CD3+). However, the scanning data indicate that after anti-CD3 mAb stimulation PKC-ζ decreased in the membrane and increased in the cytosol (Table 2).

4. Discussion

PKC is involved in a variety of cellular responses, such as IL-2 expression, IL-2 secretion, regulation of cytotoxic killing and cellular proliferation [3,27]. These immune functions are altered with aging [13,19,20,28]. The role of various isoforms of PKC in cell activation and proliferation is under intense investigation [9], but there are only a limited number of reports concerning PKC isoforms expression in lymphocytes with aging [23,29] The goal of the present study was to examine PKC isozyme distribution in resting and in CD3-stimulated lymphocytes of healthy elderly and young subjects.

T cell proliferation in response to activation has been reported to decrease with aging in humans and in animal models [25,28]. The causes for this impairment of response have not been still completely identified. T cell activation is a complex phenomenon that involves a cascade of events that leads to gene activation and lymphocytes leaving the resting state to go through the cell cycle and to divide. A number of kinases are involved, including PKC [30]. When T cells from young and elderly subjects were exposed to an anti-CD3 mAb or PMA, both responded with a significant increase in label uptake with respect to basal level, although the stimulation index in the case of lymphocytes from the young subjects was approximately twice that of the elderly subjects. Of significance, the sensitivity of the proliferative response to H7, an inhibitor of PKC, that can also affect other protein kinases, was markedly different with aging. Whereas, lymphocytes from both groups of donors showed a decreased proliferative response, the inhibitor brought the value of the stimulation index to the same level. In this connection, a dose-related inhibitory effect of H7 on the proliferation of T lymphocytes of young subjects has already been reported [30]. Our data suggest that PKC plays a more determinant role in T cell response in the case of young subjects. Presently, there is no obvious explanation for this phenomenon, but it can be hypothesized that the diminished sensitivity of T cells from elderly subjects to H7 arises from the fact that these T cells might already undergo a persistent activation of the transduction cascade for some still unknown reason. In earlier studies, we have shown that IP_3 was increased in the

Table 2
Relative distribution in arbitrary densitometry units for PKC isozymes in T lymphocytes of young and elderly subjects at resting state and after anti-CD3 mAb stimulation in the cytosol and the membrane fractions

		PKC α		PKC β		PKC δ		PKC ε(p98)		PKC ε(p72)		PKC ζ	
		Young	Old	Young	Old	Young	Old	Young	Old	Young	Old	Young	Old
Cytosol	Resting	10	12	10	5	10	10	10	5	10	4	10	5
	Stimulated	1.5	24	2	1	3	14	1	7	1.5	8	17	15
Membrane	Resting	2.5	26	3	0.8	4	6	3	6	6	6	23	22
	Stimulated	17	21	10	4	14	7	4	7	5	9	24	16

The numbers indicate the density of immunostaining relative to each PKC isozyme determined in the cytosol of lymphocytes from young subjects at resting state. An arbitrarily value of 10 was assigned in each case and other values of densitometry were assigned in a relative manner. Immunostained gels (Figs. 1–5) were scanned with a hand-held instrument and data analysed with the Scan Analysis software (MacIntosh microcomputer).

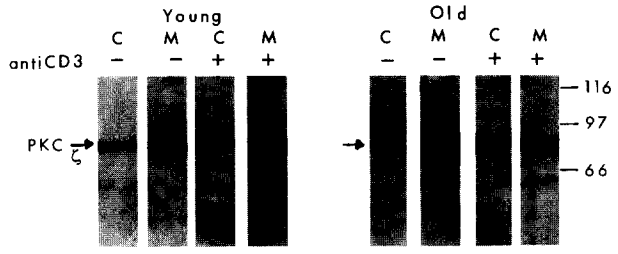

Fig. 5. PKC-ζ isoform immunoreactivity (Western blots) in the cytosol (C) and membrane (M) fractions of human T lymphocytes obtained from healthy young, in the resting state (young anti-CD3−) and after anti-CD3 mAb stimulation (young anti-CD3+) and from elderly subjects in the resting state (old anti-CD3−) and after anti-CD3 mAb stimulation (old anti-CD3+). Experimental details are described in the legend of Fig. 1. Data are representative of 15 (young) and 16 (elderly) experiments, respectively.

resting lymphocytes of elderly subjects, while its formation under stimulation was decreased [24]. Furthermore, the levels of DAG with aging are controversial, because some groups have demonstrated an increase [31], while others did not find any changes [18]. Thus, the increase of IP_3 as well as of DAG in the resting state with aging seem to corroborate this assumption.

We assessed cell distribution and T cell stimulation-induced translocation of five PKC isozymes (α, β, δ, ε and ζ) by Western blots. All five PKC isotypes were present in T cells of young and elderly subjects. However, their distribution between the cytosolic and membrane fractions varied with the isozyme studied and the age of the subjects. Of interest was the finding that in lymphocytes of elderly subjects PKC-δ and -ε were distributed almost equally between the cytosolic and the membrane fractions whereas PKC-α and -ζ were mainly found in the membrane fraction. The only exception was PKC-β which was almost exclusively located in the cytosolic fraction of resting T lymphocytes of elderly subjects. In the case of young subjects, PKC-α was detected in the cytosol fraction of lymphocytes. However, the novel PKC δ and ε isoforms partitioned to some extent to the cytosol fraction in the absence of stimulation by agonists. It is noteworthy to mention that in the case of PKC-ε of young subjects there is a decrease in the cytosol after stimulation but most of the isozyme does not translocate to the membrane (Table 2). Whether a degradation process occurs already during translocation or rapidly in the membrane needs further investigations. Cell distribution of PKC-ζ which was found to be important in the particulate fraction of lymphocytes of young subjects without appreciable redistribution after stimulation. Of interest PKC-ζ of elderly subjects apparently dissociated from the particulate fraction and moved to the cytosol. PKC-ζ has been already demonstrated in the membrane of other cell types in the resting state [32] and that raises the possibility that this 'reverse translocation' may occur in other cells. We can not offer a conclusive explanation at this time.

A redistribution of PKC to the membrane has been demonstrated in human platelets and in the soleus muscle of rats with aging [33,34]. Similar shifts in PKC isozymes distribution with development and aging have been demonstrated in cultured neonatal and adult ventricular myocytes [35]. However, our study is the first report showing an altered distribution of PKC isozymes in lymphocytes of elderly subjects, associated with an altered translocation after anti-CD3 stimulation, except in the case of PKC-β. These findings may be related to a chronic basal activation state of some PKC isozymes in elderly subjects. As a consequence an increased binding to the intracellular receptor for protein kinase C (RACK) [36] may occur maintaining this translocation state. In addition, the calpain (Ca^{2+}-dependent proteinases) activity, which may be responsible for membrane-associated downregulation of PKCs [37], could be decreased with aging. Further investigations are obviously needed to assess these possibilities.

It is widely accepted that the activation of cellular PKC results from the receptor-mediated translocation of inactive cytosolic enzyme to the lipid environment of the membrane. In vitro studies on the association of purified PKC with phospholipid vesicles and inside-out erythrocytes have led to the suggestion that PKC may associate with the plasma membrane in an inactive, but primed state [38]. Thus, in principle, membrane PKC activity may be stimulated in cells by activating the inactive form of the enzyme associated with membranes, a process not requiring the translocation of PKC from the cytosol. Recently, it has been demonstrated [39] that PKC indeed exists on the membrane of a variety of cells and tissues in both an active and an inactive state and that activation of this pool of inactive membrane PKC may be an important step in many PKC-mediated cellular events [40,41]. Such a phenomenon may also occur in lymphocytes of elderly subjects. Whatever is the exact cause, the decreased availability of cytosolic PKC as reported here may contribute to the diminished PKC-dependent responses after anti-CD3 stimulation of human T lymphocytes with aging.

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