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Heterogeneity of protein kinase C expression and regulation in T lymphocytes

S. Lucas, R. Marais*, J.D. Graves, D. Alexander+, P. Parker* and D.A. Cantrell

Lymphocyte Activation Laboratory and +*Cell Surface Biochemistry Laboratory, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX* and **Ludwig Institute for Cancer Research, Middlesex Hospital, London W1P 8BT, England*

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The purpose of the present study was to examine protein kinase C (PKC) isotype expression in T lymphoblasts derived from peripheral blood and the T leukaemic cell Jurkat. Using antisera reactive with PKC α, β_1, and β_2 and γ, it was observed that T cells expressed two PKC isotypes, PKC α and β_1. No PKC γ was detected in T lymphocytes. In lymphoblasts, high levels of PKC β compared to PKC α were found whereas Jurkat cells expressed high levels of α compared to PKC β. Differences in the calcium sensitivity of phorbol ester-induced phosphorylation were observed in Jurkat and T lymphoblasts which correlated with the relative levels of PKC α and β isotypes expressed by the cells.

Protein kinase C; Signal transduction; CD3 antigen

1. INTRODUCTION

Activation of T lymphocytes is associated with PKC-regulated phosphorylation of a number of T cell proteins including the CD4, CD8 and CD45 molecules, the γ subunit of the CD3 antigen and two cytosolic proteins of M_r 80000 and 19000 [1-6]. PKC also regulates the expression of cellular receptors for IL2 and the production of lymphokines such as IL2, and thus has an important role in the intracellular signalling pathways that control T cell activation [7,8].

The purification and gene cloning of PKC has led to identification of at least seven isotypes of PKC [9-12] that have different enzymological characteristics [14,15] and may control distinct cellular responses [13]. Most cells express more than one PKC isotype [15-17] and it appears that T lymphocytes express a minimum of two isotypes [18-20], type II or PKC β and type III or PKC α [18-19]. Recent studies have suggested that T lymphocytes may be heterogeneous in the ratio of PKC isotype expression [13,19]. In this respect, we have observed differences in the calcium sensitivity of PKC in two T cell populations used commonly in signal transduction studies; peripheral blood-derived T lymphoblasts and the T-leukaemic cell Jurkat (J6) [21]. Since different PKC isotypes exhibit differential calcium sensitivity in vitro [13], the object of the present study was to explore whether differences in PKC isotype expression correlated with differences in PKC regulation in Jurkat cells and peripheral blood lymphocytes.

Correspondence address: D.A. Cantrell, Lymphocyte Activation Laboratory, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, England

Abbreviations: PDBu, phorbol 12,13-dibutyrate; PKC, protein kinase C; IN2, interleukin 2; SDS, sodium dodecyl sulphate

2. MATERIALS AND METHODS

2.1. *Materials and cells*

Reduced streptolysin O was obtained from Wellcome diagnostics (Dartford, Kent, England). [γ-^{32}P]ATP (370 MBq/ml) and myo-[^{3}H]inositol (3.05 TBq/mmol) were obtained from Amersham International (Amersham, England). All nucleotides were from Boehringer Mannheim (Sussex, England). PDBu and other biochemicals were obtained from Sigma (Dorset, England). PKC isotype-specific antisera were raised in rabbits against peptides unique to the different PKC isotypes. The characterisation and specificity of these antisera have been described previously [14]. The J6 subclone of Jurkat cells and human peripheral blood-derived T lymphoblasts were prepared and maintained as described previously [2]. The purity of the T lymphoblast populations used was $\geq$99% as judged by flow cytofluorometric analysis with a CD3 antibody [2].

2.2 *Cell permeabilisation, phosphorylation and immunoprecipitation*

T cells were washed twice and resuspended at 5×10^7 cells/ml in permeabilisation medium containing 0.4 IU/ml streptolysin 0, 120 mM KCl, 30 mM NaCl, 10 mM Hepes (pH 7.2), 10 mM EGTA and 10 mM $MgCl_2$. The concentrations of $CaCl_2$ required to give free calcium in the range of nominal zero to 1 μM at 37°C and pH 7.2 were predicted by the computer program CHELATE using appropriate dissociation constants for Ca^{2+}, Mg^{2+} and H^+ [21]. Phosphorylation of proteins in permeabilised cells was carried out by permeabilising 2.5×10^7 cells in the presence of 100 μM [γ-^{32}P]ATP (1 μCi/pmol) and the specified stimulant. Following incubation at 37°C for 10 min, cells were pelleted at 8000 $\times$ *g* for 5 s and lysed in a buffer containing 150 mM NaCl, 10 M Tris/HCl (pH 7.4), 1% NP40, 1 mM EDTA, 10 mM Na pyrophosphate and 50 mM NaF. The

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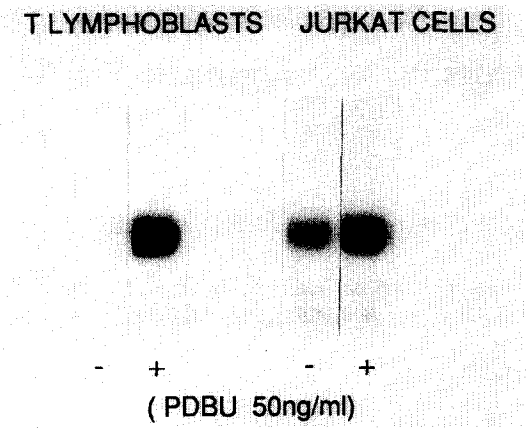

Fig.1. Ca^{2+} and PDBu stimulation of CD3 γ chain phosphorylation in T lymphoblasts and J6 cells. Permeabilised cells were incubated for 5 min in the presence of 500 nM Ca^{2+}, and [γ-^{32}P]ATP either with (+) or without (−) 20 ng/ml PDBu. The data show SDS-PAGE analyses of CD3 antigen immunoprecipitates.

CD3 antigen was immunoprecipitated using the CD3 antibody UCHT1 [2,22]. CD3 antigens were separated by 12% (w/v) SDS-PAGE under reducing conditions. Gel autoradiographs were scanned using an LKB Ultroscan XL densitometer.

2.3. *Western blotting*

Cells were lysed (2×10^7/ml) in SDS sample buffer (1% SDS, 10% (v/v) glycerol, 100 mM Tris-HCl buffer, pH 6.8, 0.2% Bromophenol blue, 0.1 M dithiothreitol) and analysed by SDS-PAGE. Proteins were electrophoretically transferred at 4°C from SDS gels to nitrocellulose filters and immunoblotted as described [4] using PKC isotype-specific antisera diluted 1000-fold with or without competing peptide. Bound antibody was visualised using ^{125}I-protein A (Amersham International) followed by autoradiography.

3. RESULTS

3.1. *Different signalling requirements for CD3 antigen phosphorylation in J6 and peripheral blood-derived T cells*

We have recently described a permabilished cell system that uses Ca^{2+}/EGTA buffers to control cytosolic [Ca^{2+}] levels, thus allowing the [Ca^{2+}] dependency of PKC activation to be monitored [21]. In T cells, the γ subunit of the CD3 antigen is phosphorylated by PKC-mediated events [2,21,23]. The effect of [Ca^{2+}] on PKC-mediated CD3 γ-chain phosphorylation in J6 cells and T lymphoblasts is shown in figs 1 and 2. In T lymphoblasts, 500 nM Ca^{2+} induced a weak phosphorylation of the M_r 26000 CD3 γ chain which was enhanced 5-fold by PDBu (fig.1). In contrast, in J6 cells, 500 nM Ca^{2+} alone induced strong CD3 antigen phosphorylation which was enhanced only 2-fold by PDBu (fig.1). A more detailed analysis of the [Ca^{2+}] dependency of CD3 γ chain phosphorylation in T lymphoblasts and J6 cells is shown in fig.2. In T lymphoblasts, PDBu-induced CD3 γ-subunit phosphorylation is relatively insensitive to [Ca^{2+}] and 60% maximal CD3 γ-chain phosphorylation was induced by PDBu at a nominal zero [Ca^{2+}] (fig.2A). Maximal PDBu-induced phosphorylation occurred at 500 nM and declined at 1 μM Ca^{2+}. In J6 cells, the effect of PDBu was markedly sensitive to [Ca^{2+}] with only a weak (16% of maximal) effect at nominal zero Ca^{2+} and a maximum synergy at 150 nM Ca^{2+} (fig.2B). Ca^{2+} alone induced significant CD3 antigen phosphorylation which was readily detectable at 150 nM Ca^{2+} and 500 nM Ca^{2+} (figs 1 and 2).

3.2. *PKC isotype expression in J6 and peripheral blood-derived T cells*

To examine PKC expression in T lymphocytes, PKC isotype-specific antiserum were used in Western blot analyses of T cell lysates. As a positive control for each antiserum, PKC purified from bovine brain containing α, β and γ isotypes was analysed in parallel. The reactivity of the PKC α, β_1, β_2 and γ antisera with brain PKC is shown in fig.3A. However, no PKC γ was detected in J6 or normal T lymphoblasts (fig.3B). The reactivity of PKC α, β_1 and β_2 antisera with J6 and T lymphoblast PKC is shown in fig.3B. Both T cell populations examined expressed PKC α and β_1 iso-

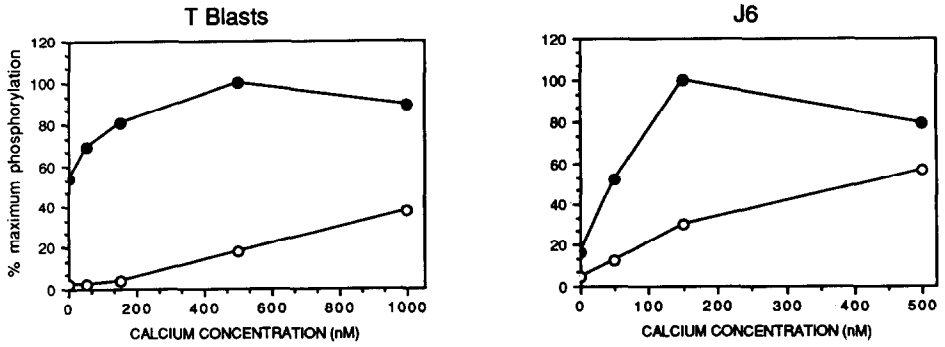

Fig.2. Ca^{2+}-dependence of CD3 γ-chain phosphorylation in permabilised J6 and T lymphoblasts. Permeabilised cells were incubated for 5 min with the indicated concentration of calcium either with (●) or without (○) 20 ng/ml PDBu. The CD3 antigen was immunoprecipitated, analysed by SDS-PAGE and ^{32}P-incorporation quantified by densitometric scans of gel autoradiographs. The data show the % maximum CD3 γ-chain phosphorylation in T lymphoblasts (A) and J6 cells (B).

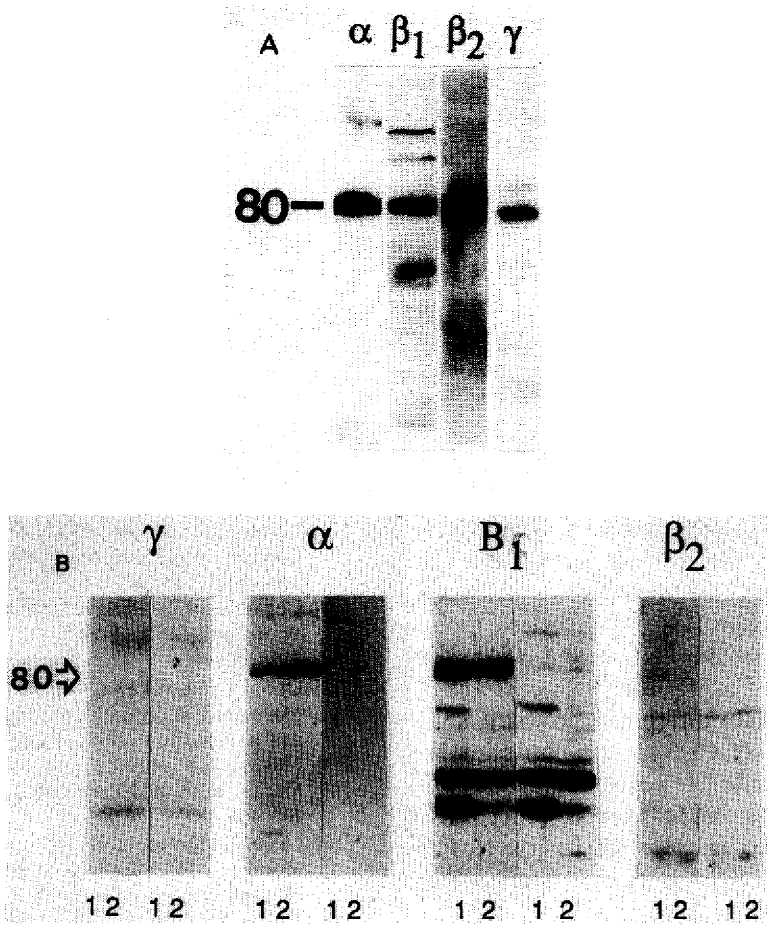

Fig.3. PKC isotype expression in brain, T lymphoblasts and J6 cells. Data show Western blot analyses of PKC γ, α, β_1 and β_2 isotypes. (A) Bovine brain; (B) T lymphoblasts and J6 cells (lanes 1 and 2 respectively).

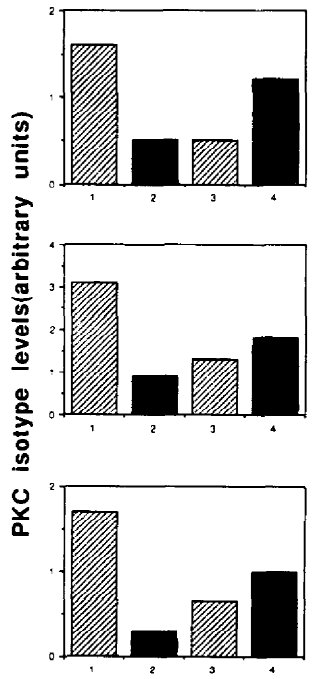

Fig.4. Data show 3 experiments in which relative levels (arbitrary units) of PKC α (columns 1,2) and PKC β_1 (columns 3,4) in Jurkat cells (▨) and T lymphoblasts (■) were quantitated by densitometric scans of PKC Western blots.

types. It has been described previously that the T leukaemic HPBALL cell expresses a high ratio of PKC α compared to PKC β [19]. Western blot analyses of J6 cells (fig.3) demonstrate that they also express high levels of PKC α compared to PKC β. Conversely, T lymphoblasts express higher levels of PKC β than J6 cells.

Quantitation of PKC α and β levels by densitometric analysis of PKC Western blots indicates that Jurkat cells express 4-fold higher levels of PKC α than T lymphoblasts. T lymphoblasts express 2-fold higher levels of PKC β than Jurkat cells (fig.4). Accordingly, the ratio of PKC β to PKC α is higher in T lymphoblasts, whereas the ratio of PKC α to β is higher in Jurkat cells.

4. DISCUSSION

The present study demonstrates that peripheral blood-derived T lymphocytes and J6 cells express PKC α and β but not λ isotypes. J6 cells express high levels of PKC α but low levels of the β isotype, whereas peripheral blood-derived T lymphoblasts contain high levels of PKC β but relatively low levels of the α isotype. High levels of PKC α have also been observed in the T leukaemic HPBALL cells. J6 and HPBALL cells are

leukaemic cells and it is possible that a high ratio of PKC α compared to PKC β is a characteristic of transformed T lymphocytes. J6 and HPBALL cells are also representative of an earlier, thymic stage of T cell ontogeny than peripheral blood-derived T lymphoblasts. The data suggest therefore that PKC isotype expression may be regulated during T lymphocyte differentiation.

J6 cells and T lymphoblasts differ in the $[Ca^{2+}]$ sensitivity of the phosphorylation of CD3 γ chains in permeabilised cells. One major difference is that at a nominal zero $[Ca^{2+}]$, PDBu induces 60% maximal CD3 antigen phosphorylation in T lymphoblasts but only 16% maximal CD3 antigen phosphorylation in J6 cells. PKC α and β isotypes are differentially sensitive to Ca^{2+} [13] since, using histone as a substrate, PKC α and β can be stimulated to 18% and 65% of maximal activity, respectively, in the absence of Ca^{2+}. The calcium sensitivity of PKC isotypes may be in part a substrate-based phenomenon [14]. Nevertheless, the in vitro calcium sensitivity of the PKC α and β isotypes and their relative levels of expression in J6 cells and T lymphoblasts, could explain differences in the $[Ca^{2+}]$ sensitivity of PDBu-induced CD3 antigen phosphorylation in permeabilised T cells. However, it must be considered also that T cells might express other PKC isotypes that vary in their calcium sensitivity. In this regard the expression of PKC δ and ϵ in T lymphocytes has not been examined.

In the absence of phorbol esters, Ca^{2+} induces CD3 antigen phosphorylation in J6 cells but not T lymphoblasts. It is not known whether this phenomenon is related to the pattern of PKC isotype expression in the cells. However, we have observed that inositol phospholipid turnover is stimulated by low levels of calcium (50 nM) in permeabilised J6 cells but not in T lymphoblasts (unpublished observation). This suggests that endogenous levels of diacylglycerol will be higher in permeabilised J6 cells compared to T lymphoblasts which would explain the higher basal PKC activation in the cells.

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