Immunology 1993 **80** 654–657

Regulation of CD3 expression in a protein kinase C isozyme-deficient T-cell line

A. LONG, M. O'CONNELL, R. M. J. LISKAMP* & D. KELLEHER
*Department of Clinical Medicine, Trinity College Dublin, Ireland and *Department of Organic Chemistry, Borlaeus Laboratories, University of Leiden, Leiden, The Netherlands*

Accepted for publication 13 September 1993

SUMMARY

Using the T-cell lymphoma line HuT 78, and a clone derived from HuT 78, designated K-4, the role of protein kinase C (PKC) isozymes in the expression of a variety of T-cell surface antigens was investigated. HuT 78 expresses PKC isozymes α and β while K-4 expresses only PKCα. Flow cytometric analysis revealed that incubation of HuT 78 cells with phorbol 12-myristate 13-acetate (PMA) results in significant down-regulation of surface expression of CD3. While K-4 cells expressed reduced amounts of CD3, a similar reduction in CD3 expression was not observed when these cells were stimulated with PMA. The regulation of expression of CD11a (LFA-1), CD44, CD45RA and CD45RO and of the class II molecules DR and DP in response to PMA, was similar in both cell lines.

Protein kinase C (PKC) is a serine/threonine kinase which participates in signal transduction in a wide variety of cell types (reviewed in ref. 1). In T cells, antigen binding to its receptor results in the hydrolysis of the membrane phospholipid, phosphatidylinositol 4,5-bisphosphate with the production of inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 releases intracellular calcium stores while DAG is a physiological activator of PKC. Activation of the enzyme results in its translocation from the cytosol to the membrane.

PKC consists of a family of closely related enzymes encoded by genes on different chromosomes.[2-6] This family may be further divided into three subgroups, one consisting of α, βI, βII and γ which are structurally related, another consisting of δ, ε, θ and η (novel or nPKC family) and the 'atypical PKC' which comprise ζ and λ. Differences in activation requirements, substrate specificities and intracellular distribution would suggest that the isozymes have multiple functions and substrate specificities.

PKC is the cellular receptor for phorbol esters and other tumour promoters which bind and directly activate the enzyme.[7,8] Phorbol 12-myristate 13-acetate (PMA) activation of T lymphocytes is associated with PKC-regulated phosphorylation of multiple T-cell proteins; these include the CD4, CD8 and CD45 molecules and the γ subunit of the CD3 complex (reviewed in ref. 9). Phosphorylation of CD3 and CD4, which are thought to be physically associated,[10] is accompanied by a decrease in their cell-surface expression.[11] Modulation of CD4 in response to PMA activation has also been shown to be regulated at the level of transcription and translation.[12]

We examined the down-regulation of CD3 expression in response to PMA in HuT 78 cells which expresses PKCα and β and a mutant cell line which we derived from HuT 78 which expresses PKCα but not β.[13] Both cell lines express PKCδ, ε and ζ while neither of the cell lines express PKCγ (data not shown). PKC activity was lower in K-4 cells relative to HuT 78 but these cells still possessed functional calcium- and phospholipid-dependent kinase activity, albeit at approximately 50% of the levels detected in HuT 78 (Fig. 1). Notably, calcium-independent kinase activity in the presence of diolein and phospholipid was significantly higher than activity in the presence of calcium, suggesting relatively high activity of calcium-independent nPKC isozymes. Ca^{2+}-independent activity was also reduced in K-4 but to a lesser extent.

As PKC can regulate the expression of various T-cell surface molecules, we examined the expression of several cell-surface markers on resting and PMA-stimulated HuT 78 and K-4 cells. When HuT 78 cells were stimulated with PMA, there was a significant decrease in CD3 expression (Fig. 2). This down-regulation of CD3 was partially inhibited by the PKC inhibitor staurosporine and by a specific PKC pseudosubstrate inhibitor derived from the sequence of PKCα and β.[14] This inhibitor is predicted to inhibit only the conventional PKC isoforms (α, β, γ) of which only α and β are expressed in lymphocytes.[15] These data suggest that PMA-induced CD3 down-regulation is mediated by either PKCα or β. K-4 cells are specifically deficient in PKCβ but contain normal amounts of PKCα. Resting K-4 cells expressed CD3 at a lower density than HuT 78 (Fig. 3B). However, when these cells were stimulated with PMA there was little decrease in CD3 surface expression (Figs 3B and 4). The changes in down-regulation of CD4 and of the T-cell receptor paralleled those of CD3 in HuT 78 and K-4 cells (72% versus 21% reduction CD4 mean fluorescence intensity (MFI), HuT 78 versus K-4: 53% versus 27% reduction T-cell receptor (TcR) MFI, HuT 78 versus K-4, means of six experiments).

To determine whether this was a selective effect limited to CD3 and CD4, the effect of PMA activation on the expression of a range of other T-cell surface antigens was investigated. The

Correspondence: Dr A. Long, Dept. of Clinical Medicine, Trinity College Dublin Medical School, St. James's Hospital, Dublin 8, Ireland.

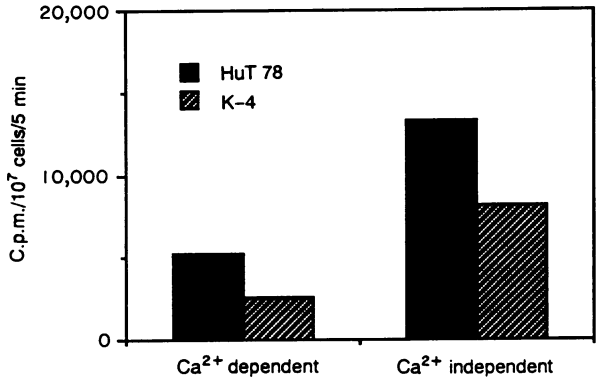

Figure 1. PKC activity of detergent extracts of HuT 78 and K-4 cells expressed as calcium dependent (Ca present) and calcium independent (in the presence of 2 mM EDTA and 2 mM EGTA).[16] Results are expressed as mean c.p.m. of ^{32}P incorporated from ATP into histone in the presence of phosphatidylserine (10 μg/ml) and diolein (60 μg/ml) in assay buffer containing 20 mM Tris, 5 mM $MgCl_2$ and 100 μg/ml Histone III-S. Incorporation in the absence of diolein is subtracted. Results are means of triplicate experiments.

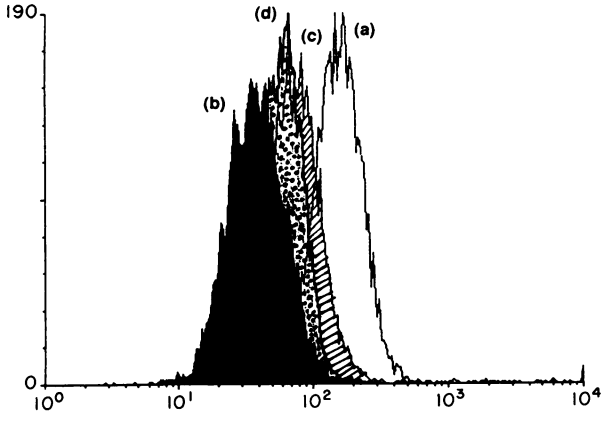

Figure 2. FACScan analysis of cell surface expression of CD3 in resting (a) and PMA-stimulated HuT 78 (b) cells in the presence of staurosporine 0·1 μM (c) or myristoylated PKC pseudosubstrate inhibitor 200 μM (d). Cells were activated with 25 ng/ml PMA in RPMI-1640 supplemented with 10% heat-inactivated fetal calf serum (FCS), 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin (Gibco BRL, Paisley, U.K.) and 5×10^{-5} M 2-mercaptoethanol for 24 hr at 37° in an atmosphere of 5% CO_2.

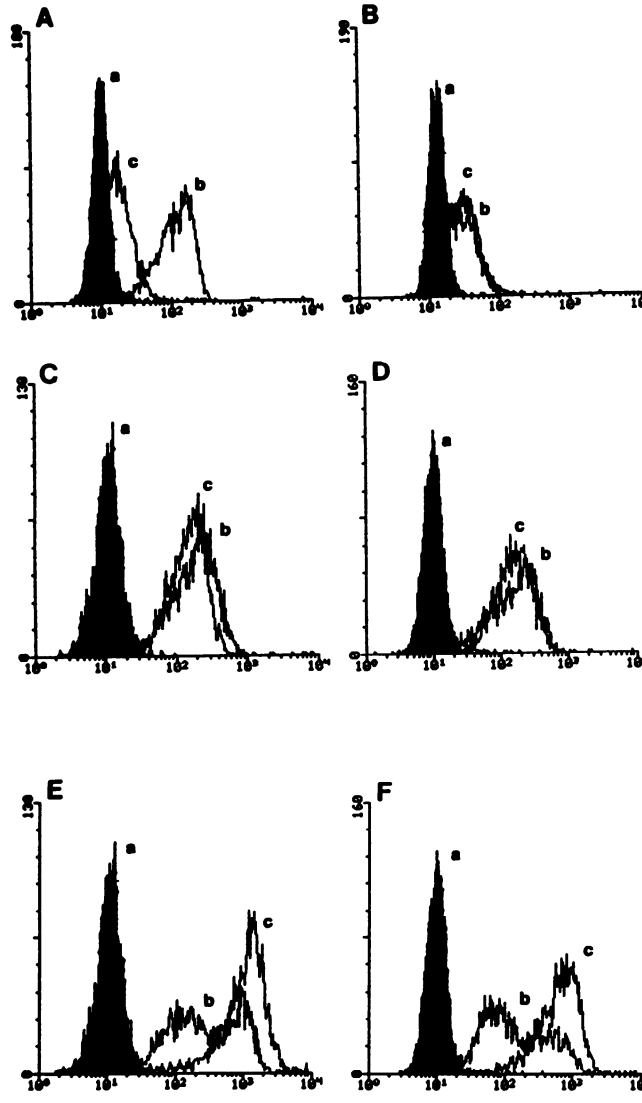

Figure 3. FACScan analysis of cell-surface expression of CD3, LFA-1 and CD44 in PMA-stimulated and resting HuT 78 and K-4 cells. Cells were activated with 25 ng/ml PMA in RPMI-1640 supplemented with 10% heat-inactivated FCS, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin (Gibco BRL) and 5×10^{-5} M 2-mercaptoethanol for 72 hr at 37° in an atmosphere of 5% CO_2. Resting (b) and PMA-stimulated (c) HuT 78 cells were labelled with antibody to CD3 (A), LFA-1 (C) and CD44 (E) followed by fluoresceinated rabbit anti-mouse antibody. Resting (b) and PMA-stimulated (c) K-4 cells were similarly labelled with antibody to CD3 (B), LFA-1 (D) and CD44 (F). Control is shown as shaded histogram (a). Fluorescence intensity is on a log scale.

results are presented in Fig. 3C–F. There was no significant difference in the pattern of expression of the adhesion molecules leucocyte function-associated antigen-1 (LFA-1) and CD44 in response to PMA in the two cell lines. LFA-1 expression decreased slightly in these lines while CD44 expression increased in both lines. HLA-DR expression increased by a mean of 3 and 5% in K-4 and HuT 78 cells, respectively, while HLA-DP increased by 16 and 10%, respectively (mean of six experiments). The expression of the leucocyte common antigen isoforms CD45RA and CD45RO all increased in response to PMA stimulation in both cell lines. It is notable that two-colour FACS analysis revealed substantial double-negative ($CD45RO^-CD45RA^-$) populations in unstimulated HuT 78 and K-4 cells. These double-negative populations decreased on stimulation with PMA with a corresponding increase in double- and single-positive populations. CD45RO increased by 109% and 183% in HuT 78 and K-4, respectively, while CD45RA increased by 44 and 39% over a 72-hr period (mean of six experiments).

While it has been known for a number of years that PKC consists of a family of related enzymes, attempts to elucidate functions of individual members of this family have been unsuccessful. In this study, we have utilized the HuT 78 cell line and a clone derived from this which, to our knowledge, differ only in the non-expression of PKCβ in K-4 cells. PKCβ is

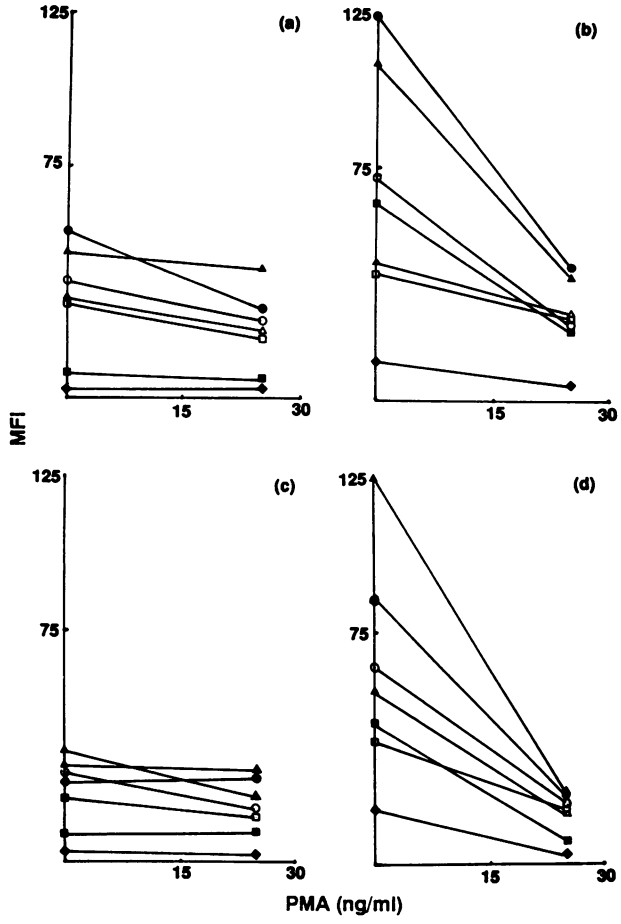

Figure 4. The effect of PMA stimulation on CD3 expression on HuT 78 and K-4 cells at 24 and 72 hr. Cells were incubated in the presence or absence of PMA (25 ng/ml) for 24 or 72 hr. Following incubation, cells were indirectly labelled with FITC-conjugated CD3 and the MFI of the cells plotted against PMA concentration. (a) and (c) CD3 expression on K-4 cells at 24 and 72 hr, respectively. (b) and (d) CD3 expression on HuT 78 cells at 24 and 72 hr, respectively. Results are expressed as seven distinct experiments.

expressed strongly in cells of the immune system and T lymphoblasts, neutrophils and B cells have been shown to express higher levels of PKCβ than α.[15,17-19] This may reflect a dominant role for PKCβ in the immune system. We have previously shown that PKCβ deficiency is also associated with a deficiency in PMA-driven interleukin-2 (IL-2) secretion.[13] In this study, we demonstrate using the PKC inhibitor, staurosporine, that PMA-induced CD3 down-regulation is mediated by PKC. Furthermore, as the myristoylated pseudosubstrate inhibitor is predicted to affect only the conventional PKC isoforms α and β,[14] this further suggests a role for either or both of these specific isoforms in CD3 down-regulation. CD3 down-regulation in response to PKC activation was significantly reduced in PKCβ-deficient cells which strongly express PKCα. While total PKC activity was reduced by approximately 50% in K-4 cells relative to HuT 78, modulation of molecules other than CD3 was unaffected by this lower kinase activity. These data thus suggest that PKCβ plays a selective role in CD3 down-regulation in response to PMA. However, definitive confirmation of this would require transfection of K-4 cells with the PKC-β gene. The mechanism by which PKCβ deficiency results in defective CD3 down-regulation may relate to the association of this isozyme with the cytoskeleton. The process of CD3 receptor internalization requires effective microfilament assembly. Inhibition of microfilament assembly with cytochalasin D effectively blocks T-cell activation through the T-cell receptor.[20] PKCβ has previously been shown to associate physically with spectrin, a membrane protein that cross-links actin at the plasma membrane.[21] The finding that both CD3 down-regulation and cytokine production are deficient in a PKCβ-deficient cell line suggests the possibility that both processes may be regulated by PKCβ interaction with cytoskeletal elements. K-4 cells have lower surface CD3 expression suggesting that such a pathway may also play a role in the re-expression of CD3. This may explain why PKC inhibitors did not fully reverse CD3 down-regulation. K-4 cells are also defective in spontaneous shape changing suggesting that PKCβ does have a significant role in cytoskeletal assembly (manuscript submitted). It is unclear as yet whether CD3 internalization plays a role in signalling for cytokine release or whether the defective cytokine production seen in PKCβ-deficient cells is regulated through alternative pathways. However, the K-4 cell line should provide a useful tool for further studies into the mechanism of CD3 down-regulation and its involvement in cellular signalling.

ACKNOWLEDGMENTS

D.K. is a Wellcome Senior Fellow in Clinical Science. This work was supported by a grant from the Cancer Research Advancement Board of the Irish Cancer Society. We would like to thank John Kruÿtzer for his assistance in the preparation of the pseudosubstrate peptide.

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