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CD3 receptor modulation in Jurkat leukemic cell line

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Abstract: CD3 antigen is a crucial molecule in T cell signal transduction. Although its expression on cell surface is constitutive, dynamic regulation of TCR-CD3 level is probably the most important mechanism allowing T cells to calibrate their response to different levels of stimuli. In our study we examined the role of two main T cell signal transduction pathways in controlling the surface level of CD3 antigen, one based on protein kinase C activity and the other dependent on calcineurin. As an experimental model we used three clones derived from Jurkat cell line, expressing different levels of CD3 antigen surface expression: $CD3^{low}$ (217.6), $CD3^{+}$(217.9) or $CD3^{low/+}$ (217.7). The cells were stimulated with PMA or ionomycin, acting directly on PKC and calcineurin, respectively. Prior to the stimulation cells were incubated with PKC inhibitor - chelerythrine or calcineurin blocker - cyclosporine A. Changes in CD3 surface expression were measured by flow cytometry. Only PMA and chelerythrine were able to change CD3 expression suggesting important involvement of PKC in the regulation of its expression. To confirm these findings, PKC activity was estimated in Jurkat clones. Our data demonstrated that Jurkat clones with different CD3 expression showed also different PKC activities, so we conclude that PKC-dependent pathway is the main way of controlling CD3 level on Jurkat clones.

Key words: Jurkat cells - CD3 - Protein kinase C

Introduction

CD3 antigen is a multimeric protein, consisting of two heterodimers: CD3εγ, CD3εδ and one homodimer CD3ζζ, expressed exclusively on the surface of T cells, in combination with TCR chains [10]. Sophisticated and selective mechanisms allow only the expression of completely assembled and functional receptors [7]. A constitutive, but at the same time dynamic CD3 expression plays physiologically important role in the regulation of T cell function [8]. One of the mechanisms responsible for CD3 down-modulation, occurring physiologically after T cell stimulation, is dependent on the protein kinase C activity. PKC phosphorylates a serine residue after recognising a leucine based motif in the CD3γ chain, causing internalisation of the whole CD3-TCR complex [2]. As T cell activation is known to correlate with the degree of TCR down-modulation [8], this process appears to play an important role in the regulation of T cell function. Heterogeneity of CD3 surface level is a common feature of many pathologies including T cell lymphoma [11] and still not much is known about the principles of such aberrations as well as about the exact mechanism governing the expression of the investigated antigen. Therefore we addressed the question about the importance of two main T cell signalling pathways, one based on PKC and the other on calcineurin activity, in controlling CD3 surface level.

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Materials and methods

Jurkat human T cell line (E 6.1) heterogeneous in respect to CD3 surface expression was subcloned, using limited dilutions method, into 3 clones stably expressing different levels of CD3. Clone 217.6 expressed low levels of CD3 ($CD3^{low}$), clone 217 .9 showed high CD3 surface expression ($CD3^{+}$) and on clone 217.7 CD3 expression was bimodal, low and high ($CD3^{low/+}$). Cells were cultured in RPMI 1640 (Sigma, USA) with 10% FCS, penicillin, streptomycin and 2 mM glutamine (complete medium) at 37˚C in 5% CO_2.

Cells were suspended at concentration of 1×10^6 cells/ml in complete medium and incubated at 37˚C in 5% CO_2 for 6 h with and without PKC stimulator - PMA and calcineurin stimulator - ionomycin at a concentration of 0.05 μg/ml and 0.5 μM, respectively. Prior to stimulation, the cells were incubated for 1 h in the same conditions with PKC inhibitor - chelerythrine (0.1 μg/ml) or calcineurin inhibitor - cyclosporine A (1 μM). CD3 surface expression was measured by staining with anti-CD3ε monoclonal antibody conjugated with FITC (DAKO, Denmark). Cytometric analysis was performed on the FACSCalibur (Becton Dickinson) at the Department of Clinical Biochemistry, or Galaxy (DAKO) or FACScan (Becton Dickinson) at the Department of Pathophysiology,

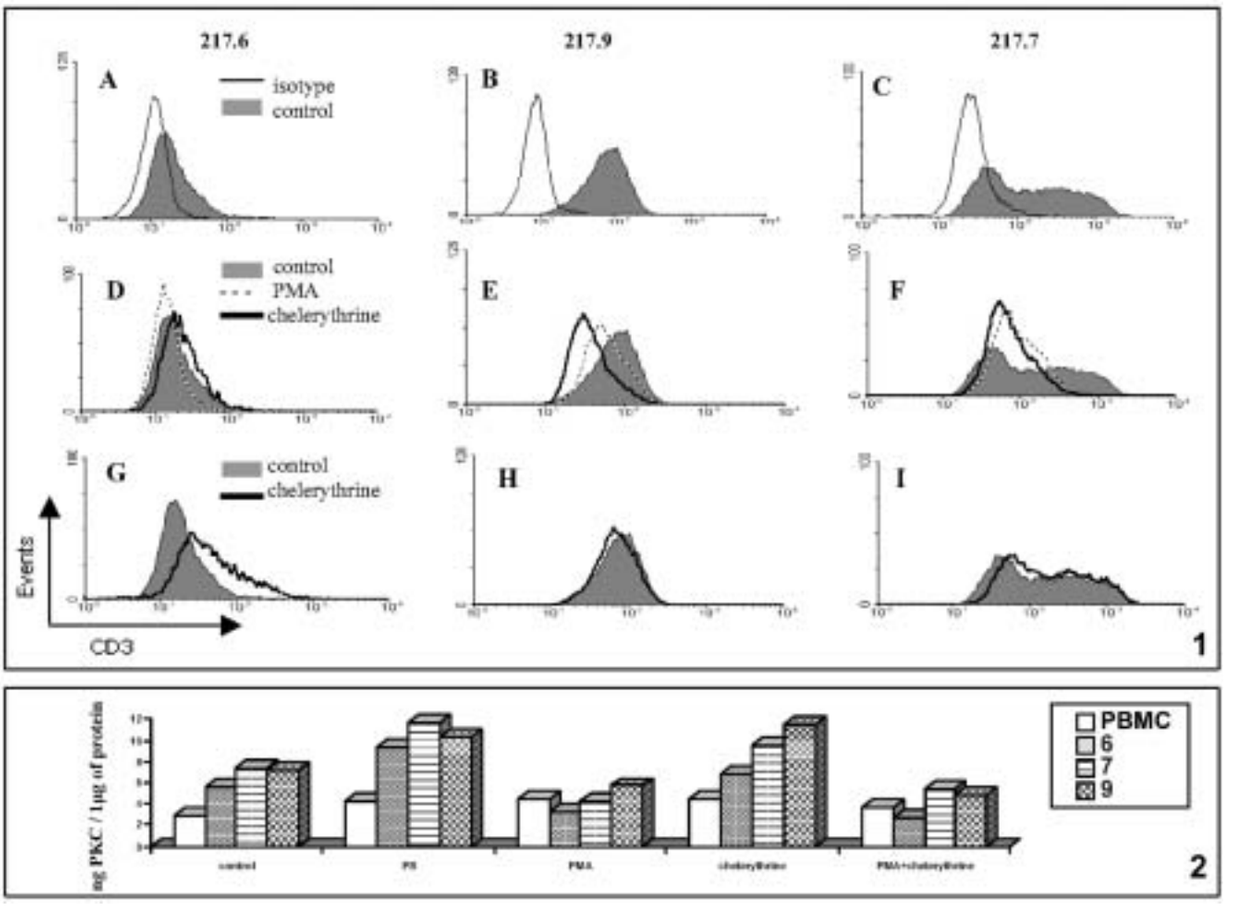

Fig. 1. Diversified effect of chelerythrine on CD3 expession depends on the initial receptor level. Jurkat clones express different levels of CD3 antigen: low on 217.6 clone (A), high on 217.9 (B) and ranging from low to high levels on clone 217.7 (C). PMA down-modulated CD3 expression on all clones (D, E, F), but effect of chelerythrine with PMA on CD3 expression was different for clones 217.6 (D) and 217.9 (E). Chelerythrine alone increased CD3 expression on clone 217.6 (G), and on low CD3 expressors of clone 217.7 $CD3^+$ (I). **Fig. 2.** Varied protein kinase C activity in three Jurkat clones. PKC activity in the Jurkat clones is much higher than in PBMC. The lowest activity was observed in 217.6 clone and high in 217.9 and 217.7 clones. Phosphatidyl serine (PS) increased PKC activity in all investigated cells. PMA decreased PKC activity in 3 clones, while increasing its activity in PBMC. Chelerythrine acted as PKC stimulator for all cells. PBMC treated with the combination of PMA and chelerythrine showed increased PKC activity, in contrast to Jurkat clones.

Medical University of Gdańsk. Data were analysed with WinMDI, version 2.9 (J. Trotter, The Scripps Research Institute).

PKC activity was examined with the use of PepTag® Assay for Non-Radioactive Detection of Protein Kinase C (Promega, USA). Cells were initially treated with chelerythrine and later incubated with/without PMA for 15 min; a portion of cells was left unstimulated as a control of spontaneous PKC expression. Cells were lysed after being adjusted to 5×10^6 in 0.5 ml, in the extraction buffer (25 mM Tris-HCL (pH 7.4), 0.5 EDTA, 0.5 EGTA, 0.05% Triton®1 X-100, 10 mM mercaptoethanol, 1 µg/ml leupeptin, 1 µg/ml aprotinin, 0.2 mM sodium vanadate, 1% PMSF in 100% ethanol) and then sonicated for 30 sec using Sonoplus 2070 (Bandelin Electronic, Germany). Cell extracts were centrifugated at 10000 × g for 20 min, and the membrane and cytoplasmic fraction was collected. The amount of proteins in lysates was measured by Bradford method. One µg of protein was used for further analysis. During PKC assay, PKC activator - phosphatidyl serine (PS), provided by the producer was used alternatively for the enzyme stimulation. Probes prepared in order to estimate PKC activity included 5 µl reaction buffer, 5 µl C1 peptide, 1 µl peptide protection solution, 9 µl PKC probe, and 5 µl activator solution (optional). Probes without and with 10 µg of pure PKC were used as negative and positive controls, respectively. Final reaction mixture of all probes was 25 µl. Cell extracts containing PKC were added just before incubation which was performed for 30 min at 30˚C. Reaction was stopped by boiling in water bath for 10 min. Probes with 1 µl of 80% glycerol were loaded onto 0.8% agarose gel and separated at 100 V for 40 min. Fluorescent bands of different electrophoretic mobility were visualized by UV transillumination and the pictures were stored for densitometric analysis using GDS-8000 Gel Documentation System and Labworks 4.0 (UVP, UK).

Results

Three Jurkat clones with different CD3 expression were used as an experimental model, allowing to investigate modulation of CD3 surface expression depending on two signalling pathways in T cells. PMA stimulation decreased CD3 expression on all Jurkat clones (Fig.

1D-F). Neither ionomycin nor calcineurin inhibitor cyclosporine A exerted any effect on CD3 expression on Jurkat cells (data not shown). Chelerythrine exhibited diverse effect on the CD3 antigen expression, dependent on its initial level. It increased CD3 surface expression on 217.6 clone when used alone, as well as in combination with PMA, inverting the effect of the stimulator (Fig. 1G). On 217.9 clone ($CD3^+$), chelerythrine not only did not invert PMA effect, but even increased CD3 down-modulating caused by PMA (Fig. 1E). Effects on the 217.7 clone, exhibiting CD3 expression ranging from low to high, confirmed previous observations as chelerythrine increased the CD3 level in the population with low level of the receptor and enhanced the PMA down-modulating effect on the population with high antigen level (Fig. 1F, I) . These results suggested a very important role of PKC in the regulation of CD3 expression.

To confirm that role, PKC activity was measured. The initial enzyme activity in all Jurkat clones was much higher than in normal PBMC (Fig. 2). Different PKC activity was determined in the investigated Jurkat clones. Clone 217.6 exhibited the lowest PKC activity, while clones 217.9 and 217.7 showed similar enzyme activity. Phosphatydyl serine increased PKC activity in all three Jurkat clones as well as in PBMC. Surprisingly, PMA appeared to inhibit PKC activity in Jurkat clones, but still was acting as an activator in PBMC. Paradoxically, chelerythrine increased PKC activity in all investigated cell types. In combination with PMA it decreased PKC expression on clone 217.6 to a much greater extent than on two other Jurkat clones (Fig. 2).

Discussion

Our experimental model based on three Jurkat clones with different CD3 expression, allowed us to examine whether signalling pathway dependent on protein kinase C and the one dependent on calcineurin, have any influence on CD3 expression regulation, in relation to its initial level of expression. Results obtained from stimulation and inhibition experiments measuring CD3 expression by flow cytometry suggested that calcineurin does not play a significant role in CD3 modulation, while PKC is a crucial enzyme in this process. PKC has been previously reported to participate in the proliferation and survival of cancer cells [6]. We showed that in all Jurkat clones PKC activity was much higher than in PBMC from healthy donors. Additionally, Jurkat cells reacted differently than normal lymphocytes to PMA treatment, by lowering PKC activity. This difference can be explained by the fact that part of PKC pool in Jurkat clones is initially activated, so PMA initiated its degradation since it is known to exert such effect on active kinase form [3]. Chelerythrine considered in some reports as a specific PKC inhibitor [5], appeared to act as a stimulator used at the concentration of 0.1 μg/ml, as suggested previously [9]. Additionally, it exerted diverse effect on CD3 expression in the clones depending on the initial level of receptors. Jurkat cells are known to express all isoforms of PKC [1], but as leukemic cells they can have some specific isoforms in bigger proportion [4] which can be the reason of CD3 initial level-dependent antigen regulation mechanism.

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