Ectocellular CD38-catalyzed synthesis and intracellular Ca^{2+}-signalling activity of cyclic ADP-ribose in T-lymphocytes are not functionally related

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Abstract Cyclic ADP-ribose (cADPR) is a natural metabolite of β-NAD^+ with a potent Ca^{2+}-mobilizing activity in different cell types, including T-lymphocytes. We investigated (i) whether stimulation of T-lymphocytes with different agonists affects the intracellular concentration of cADPR, and (ii) whether the lymphocyte antigen CD38, through its ectocellular ADP-ribosyl cyclase and cADPR-hydrolase enzymatic activities, can account for the regulation of the intracellular levels of cADPR and the Ca^{2+}-mobilizing effects of this nucleotide in Jurkat and HPB.ALL T-lymphocytes. The anti-CD3 antibody OKT3, the sphingolipid sphingosine and lysophosphatidic acid induced an increase in intracellular cADPR with concomitant increases in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). In contrast, activation of an ectocellular ADP-ribosyl cyclase by preincubation of cells with β-NAD^+ led to a dose-dependent increase in cADPR, but no changes in $[Ca^{2+}]_i$ were observed. However, extensive washing of the cells following preincubation with NAD^+ demonstrated that the increases in cADPR were not intracellular but due to cell surface-associated nucleotide. Accordingly, measurements of ADP-ribosyl cyclase activity in intact T-cells showed ectocellular synthesis of cADPR, but no evidence was obtained for a shift of this activity into the cells which could account for intracellular accumulation of cADPR. Taken together, the results indicate no direct involvement of the ADP-ribosyl cyclase activity of CD38 on the regulation of the cADPR-mediated intracellular Ca^{2+}-signalling in T-lymphocytes.

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Key words: Cyclic adenosine diphosphate ribose; CD38; ADP-ribosyl cyclase; Intracellular calcium; NAD^+; Human T-lymphocyte; T-cell receptor

1. Introduction

Cyclic ADP-ribose (cADPR) is a natural metabolite of β-NAD^+ with a potent Ca^{2+}-mobilizing activity in different cell types, including hepatocytes, pancreatic and adrenal chromaffin cells, macrophages, PC12 cells and T-lymphocytes [1–4]. At present, it is still debatable whether cADPR functions as a second messenger in response to stimulation of membrane receptors and little is known about the mechanisms by which the intracellular concentration of this nucleotide is regulated. Identification and characterization of the enzymatic activities responsible for the synthesis and hydrolysis of cADPR are important for elucidating this question.

CD38 is a type II transmembrane glycoprotein predominantly expressed on the activated phenotypes of lymphoid and monocytic cells, but is also reported to be present in many other eukaryotic cell types [5–7]. Considerable interest on the CD38 glycoprotein has emerged from the fact that it is also a bifunctional ectoenzyme, catalyzing both the synthesis and the hydrolysis of cADPR [5–7]. The two enzyme activities are located at the ectocellular, C-terminal domain of CD38 and are definided as ADP-ribosyl cyclase, which catalyzes the synthesis of cADPR and nicotinamide from β-NAD^+, and cADPR hydrolase, which degrades cADPR to ADPR [8,9]. The possibility that the ADP-ribosyl cyclase activity of CD38 might be responsible for the synthesis of endogenous cADPR in various cell types has been considered by different groups. However, the apparent contradiction between ectocellular CD38-catalyzed synthesis and intracellular Ca^{2+}-mobilizing activity of cADPR is so far unresolved and conclusive evidence for a link between CD38 and intracellular cADPR is still lacking. In this study, we also addressed the question whether CD38 may be involved in the regulation of endogenous cADPR in human T-lymphocyte cell lines, and obtained data which clearly indicate no direct role for the ADP-ribosyl cyclase/cADPR hydrolase activities of CD38 in regulating intracellular cADPR and, consequently, cADPR-mediated Ca^{2+}-signalling in Jurkat and HPB.ALL T-lymphocytes.

2. Materials and methods

2.1. Materials

cADPR was either purchased from Amersham-Buchler (Braunschweig, Germany) or was prepared enzymatically from β-nicotinamide adenine dinucleotide (β-NAD^+), as previously reported [10]. ADP-ribose, β-NAD^+, α-NAD^+ and NGD^+ were obtained from Sigma (Deisenhofen, Germany). Fura-2/AM was purchased from Calbiochem (Bad Soden, Germany). The anti-CD3 monoclonal antibody OKT3 was purified from hybridoma supernatants on Protein G Sepharose (Pharmacia Biotech, Freiburg, Germany). Perchloric acid, methanol (LiChrosolv) and trifluoroacetic acid were purchased from Merck (Darmstadt, Germany) and tetrabutylammonium dihydrogen phosphate was obtained from Fluka (Neu-Ulm, Germany). All other chemicals were of the highest purity available. MilliQ water (Millipore Waters, Eschborn, Germany) was used throughout all experiments.

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Abbreviations: ADPR, adenosine diphosphate ribose; cADPR, cyclic adenosine diphosphate ribose; cGDPR, cyclic guanosine diphosphate ribose; $[Ca^{2+}]_i$, intracellular calcium concentration; FACS, fluorescence activated cell sorter; HPLC, high performance liquid chromatography; IB4, agonistic monoclonal antibody against CD38; LPA, lysophosphatidic acid; NGD^+, nicotinamide guanine dinucleotide; SPH, D-*erythro*-sphingosine

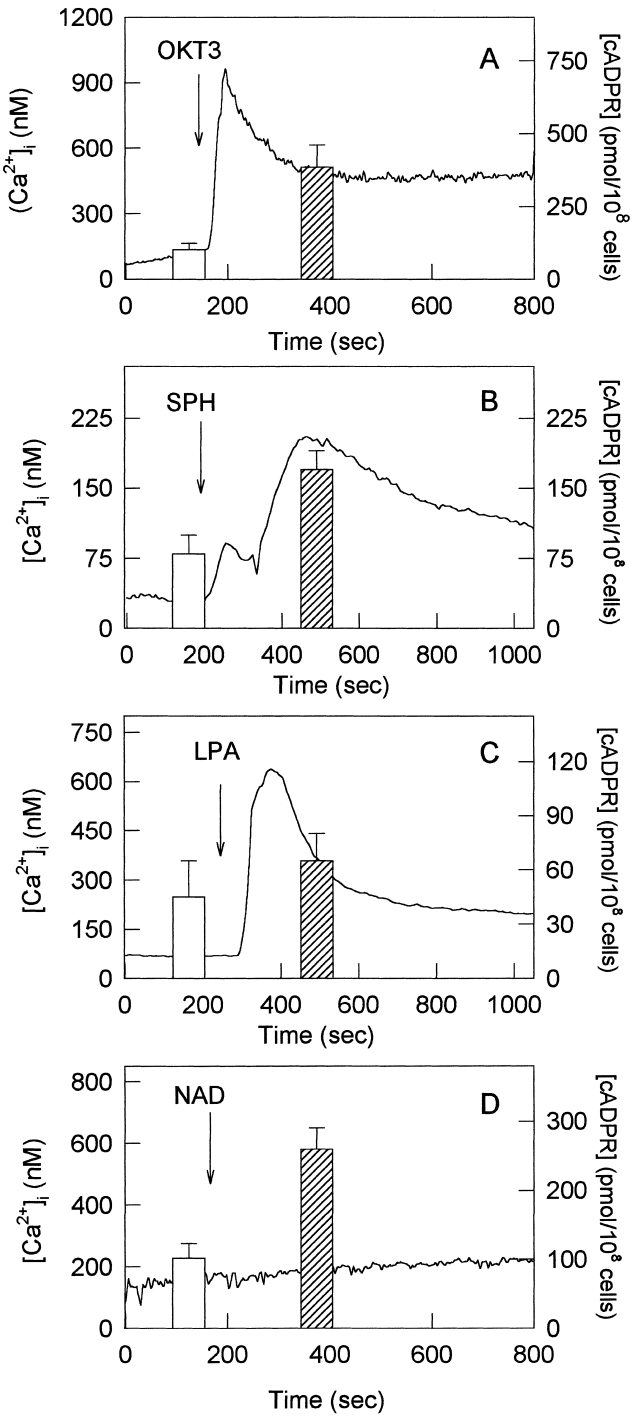
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2.2. Cell culture

The human T-lymphocyte cell lines Jurkat and HPB.ALL were cultured as described in earlier publications [11,12].

2.3. Measurement of $[Ca^{2+}]_i$

$[Ca^{2+}]_i$ was determined by spectrofluorometry, as previously described [11,12]. In brief, cells (1×10^6 cells) were loaded with Fura-2/AM (1 μM) in a medium containing (in mM): 140 NaCl, 5 KCl, 1 $MgSO_4$, 1 $CaCl_2$, 1 NaH_2PO_4, 5.5 glucose and 20 HEPES (pH 7.4). Measurements of fluorescence were subsequently performed in aliquots containing 2×10^5 cells using a Hitachi F-2000 fluorimeter with wavelength settings alternating between 340 ± 2.5 nm and 380 ± 2.5 nm (excitation ratio mode) and 510 ± 5 nm (emission) at room temperature.



2.4. Assay of ADP ribosyl cyclase activity

ADP ribosyl cyclase activity was measured fluorimetrically either in intact cells or in subcellular fractions obtained after centrifugation of cell homogenates at $100\,000\times g$ (the resulting membrane and cytosolic fractions were designated P100 and S100, respectively). The assays were performed essentially as described in [13,14]. The cells or protein fractions were incubated in extracellular buffer (as indicated above for $[Ca^{2+}]_i$ measurements) at 37°C under continuous stirring. Enzymatic production of cGDPR from NGD^+ (0.1 mM) was monitored continuously at 410 nm (emission wavelength) with an excitation wavelength set at 300 nm, using a Hitachi F-2000 fluorimeter.

2.5. HPLC analysis of cADPR

Endogenous cADPR was extracted from unstimulated and stimulated cells using the perchloric acid procedure described in [15]. Quantification of cADPR in cell extracts was performed with a recently developed two-step HPLC method, which consists of a strong anion-exchange chromatography followed by a second, ion-pair reversed phase HPLC [15]. The first HPLC step was carried out on a PRPX 100 matrix (10 μm, Hamilton, Switzerland) packed into a 250×4.6 mm I.D. PEEK column (CS Chromatographie Services, Germany). A gradient from 1 mM Tris, pH 8.0, to 150 mM TFA was used at a flow rate of 1.5 ml/min. The gradient was (in % of eluent B): 0 min, 5%; 5 min, 5%; 12 min, 21%; 13 min, 21%; 25 min, 50%; 26 min, 5%; 29.5 min, 5%. Material co-eluting with standard cADPR was collected, lyophilized and redissolved in 200 μl of reversed-phase ion-pair HPLC buffer (20 mM KH_2PO_4, 5 mM tetrabutylammonium hydrogen phosphate, pH 6.0). The reversed-phase HPLC column used was a Hypersil BDS C18 column (5 μm; 250×4.6 mm I.D.; Hypersil, Cheshire, UK). A gradient from reversed-phase ion-pair buffer (see above) to methanol was used at a flow rate of 1.0 ml/min. The gradient was (in % of methanol): 0 min, 5%; 3 min, 15%; 8 min, 50%; 16 min, 50%; 18 min, 5%; 21 min, 5%.

The UV-absorbance detector (model 432, Kontron Instruments) was autozeroed at the start of each chromatogram and absorbance was measured at 270 nm. Data was processed by the MT2 data acquisition system from Kontron Instruments. Identification of the cADPR peak in cell samples was confirmed by co-chromatography with the standard compound.

2.6. Flow cytometry

Cells (1×10^5 cells) were resuspended in PBS buffer containing 5% FCS and 0.05% NaN_3 and incubated for 10 min at 4°C with human γ-immunoglobulin in order to block Fc-receptors. Then the cells were incubated with an anti-CD38 monoclonal antibody (5 μg/ml) for 1 h, at 4°C. After two washes with PBS, cells were further incubated with FITC-conjugated goat anti-mouse IgG (45 min, 4°C). The analysis of fluorescence was performed on a FACSorter (Becton Dickinson, Heidelberg, Germany) with an excitation wavelength set at 430 nm.

2.7. Preparation of subcellular fractions and Western blot

Jurkat cells (10^9 cells) were disrupted in 5 ml of buffer (20 mM HEPES, pH 7.5, 110 mM NaCl, containing the protease inhibitors antipain 5 μg/ml, leupeptin 5 μg/ml, pepstatin 6.9 pg/ml and Pefablock SC 13.9 μg/ml) using a Potter-Elvehjem homogenizer at 4°C. The homogenate was centrifuged at $500\times g$ for 10 min, the supernatant

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Fig. 1. Increases in endogenous cADPR and $[Ca^{2+}]_i$ in Jurkat T-lymphocytes, in response to stimulation with different agonists. Jurkat T-lymphocytes (10^6–10^8 cells) were collected by centrifugation and incubated in an extracellular buffer, for 15 min at 37°C. The cells were then stimulated with (A) the anti-CD3 antibody OKT3 (10 μg/ml), (B) sphingosine, SPH (10 μM), (C) LPA (40 μM) or (D) β-NAD (1 mM). As indicated by the bars, endogenous cADPR was extracted before (open bars) and after stimulation with each compound (filled bars) and subsequently measured by a 2-step HPLC method, as described [15]. $[Ca^{2+}]_i$ was continuously monitored in a suspension of Fura-2 loaded cells, before and after stimulation with each of the compounds indicated. Data are presented as mean ± S.D. ($n=4$–14). The basal cADPR values shown in B and C are from different series of experiments than in A and D. The $[Ca^{2+}]_i$ tracings are representative of, at least, 10 independent measurements.

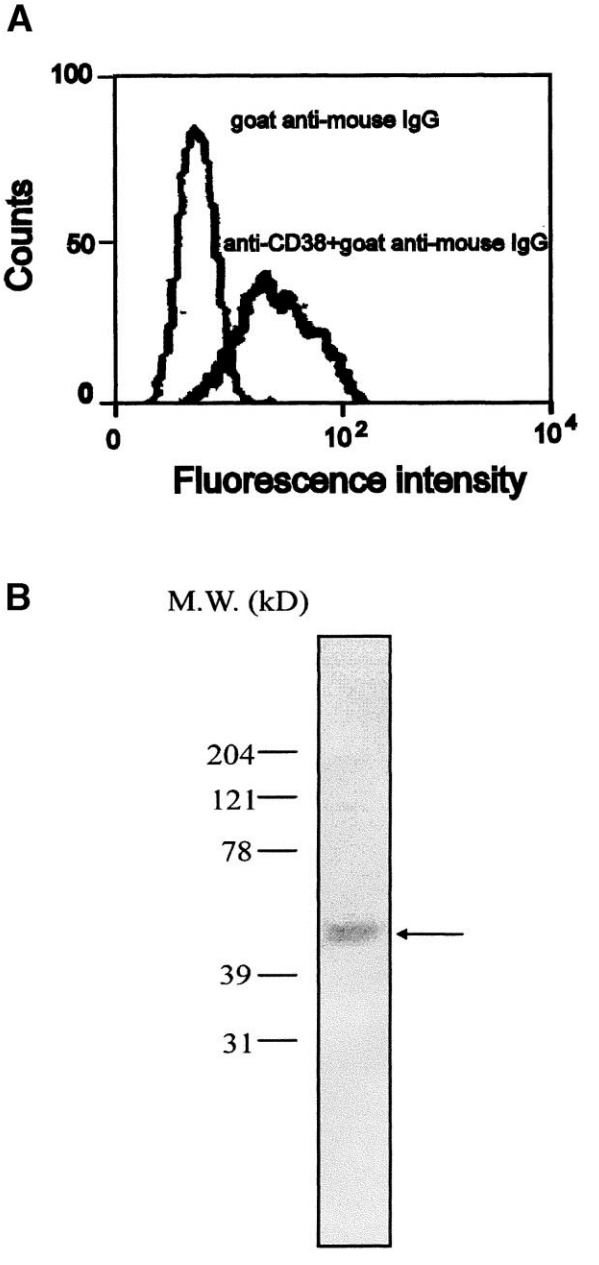


Fig. 2. Expression of CD38 in Jurkat T-lymphocytes. A: FACS analysis of cell surface expression of CD38 in Jurkat cells was performed as described in Section 2, using a mouse anti-CD38 monoclonal antibody (5 μg/ml). B: Western blot of CD38 in P100 membrane fractions from Jurkat cells was performed as described in Section 2. The arrow shows a band of approximately 45 kDa immunostained with the anti-CD38 antibody and corresponding to membrane-bound CD38. The position and molecular weight of marker proteins are also shown.

collected and further centrifuged at 10 000 × *g* (20 min, 4°C). The pellet (P10 fraction) was discarded and the supernatant centrifuged at 100 000 × *g* for 2 h at 4°C. The pellet thus obtained (P100 fraction) was harvested and used for Western blot of CD38 or for ADP-ribosyl cyclase assay (see Section 2.4).

Protein (100 μg) was boiled at 95°C for 5 min and subjected to SDS-PAGE in a 12.5% gel (3% stacking gel) under reducing conditions. Proteins were subsequently transferred onto nitrocellulose sheets by tank blotting (1.5 h, 660 mA constant, 4°C). The nitrocellulose sheets were immunostained with an anti-CD38 antibody (4 μg/ml) and, after thorough washing by a complex procedure, the blots were developed using the ECL-kit (Amersham), according to the manufacturer's instructions.

3. Results and discussion

We have previously shown that cADPR induced Ca^{2+}-mobilization from intracellular stores in Jurkat and HPB.ALL T-lymphocytes [1]. More recently we have analyzed the effects of stimulation of T-lymphocytes with different Ca^{2+}-mobilizing agonists, e.g. anti-CD3 monoclonal antibodies, sphingosine and lysophosphatidic acid (LPA), on the intracellular levels of cADPR. Upon stimulation of Jurkat T-cells with these agonists, parallel increases in the endogenous levels of cADPR and in $[Ca^{2+}]_i$ were observed (Fig. 1A–C). In contrast, incubation of Jurkat T-cells with high concentrations of NAD resulted in a highly increased cell-associated cADPR concentration, but in an almost non-detectable stimulation of intracellular Ca^{2+}-signals (Fig. 1D). Similar effects of NAD were obtained when using HPB.ALL T-cells (data not shown).

These observations prompted us to investigate which enzyme(s) are involved in the regulation of endogenous cADPR in human T-cells and, in particular, whether the lymphocyte antigen CD38 might play a role in this process.

FACS analysis and Western blotting of cell surface expression of CD38 in Jurkat and HPB.ALL T-lymphocytes demonstrated the presence of the antigen in both cell lines,

Table 1
NAD does not induce internalization of the ADP-ribosyl cyclase/CD38 in Jurkat T-lymphocytes

	Surface expression of CD38 (FI, mean ± S.D.)[a]	cGDPR fluorescence[b]		Cyclase activity (%)[c]
		% pelleted	% extracellular	
Control	28.0 ± 5.0	–	–	100.0
NAD (1 mM)	30.5 ± 3.5	–	–	64.2
NGD (0.1 mM)	–	3.0	97.0	–
Nicotinamide (1 mM)	–	–	–	60.2

[a]Surface expression of CD38 was measured by FACS analysis in Jurkat T-cells, before and after treatment with NAD (1 mM) for 1–2 h. FI, fluorescence intensity in arbitrary units.
[b]Using the NGD assay, the fluorescence due to cyclase-synthesized cGDPR was measured after centrifugation of the reaction mixture both in the cell pellets (taken as an indication of intracellularly localized compound) and in the extracellular medium. Results are given as % of total fluorescence ($n = 6$).
[c]The effects of preincubation of Jurkat cells with NAD (1 mM, 30 min) or nicotinamide (1 mM, 30 min) on the ADP-ribosyl cyclase activity were measured using the NGD assay. Results are expressed as % of control values and are representative of 6 experiments carried out.

although relatively higher levels of expression of this molecule were observed in HPB.ALL cells, as compared to Jurkat cells (see Fig. 2 for data obtained on CD38 expression in Jurkat cells).

The high amount of cADPR formed after incubation of the cells with NAD and the obvious lack of stimulation of Ca^{2+}-signalling suggested that the cADPR produced did not reach its intracellular target site, the ryanodine receptor. In order to check this hypothesis, cells were subjected to thorough and extensive washing steps, following incubations with NAD and before extraction of cADPR. The results shown in Fig. 3A indicate that, in fact, upon extensive washing of the cells the levels of cADPR measured were not significantly different from endogenous control values. These results, therefore, demonstrate that the increased amounts of cADPR synthesized by the cyclase in the presence of high extracellular concentrations of NAD remain outside the cells, essentially membrane-associated, instead of being either produced intracellularly, by potentially internalized CD38 molecules or transported, by an unknown mechanism, into the cells. It should be noted that such an extensive washing of the cells before extraction of cADPR was not necessary when the cells were stimulated with the other agonists (Fig. 1A–C), since the extracellular buffer did not contain any NAD. Moreover, a potential overestimation of the cADPR values measured by HPLC due to co-chromatography with other compounds can also be excluded, based on our previous demonstration that the cADPR peak is completely converted to ADPR upon heat inactivation [15].

As shown in Fig. 3B, a dose-dependent increase in cell-associated cADPR was detected when cells were pre-incubated with β-NAD^+. In the presence of 1 mM β-NAD^+, approximately 3- and 10-fold increases in cell-associated cADPR, as compared to control levels, were measured in Jurkat and HPB.ALL cells, respectively. In contrast, α-NAD^+, which is not a substrate for the ADP-ribosyl cyclase CD38, induced no significant changes in cell-associated cADPR levels, in comparison to control values (Fig. 3C). The specificity for β-NAD^+ provides evidence for the involvement of the ADP-ribosyl cyclase CD38 on the NAD-induced increases of cell-associated cADPR.

In spite of the numerous investigations which have been conducted on the CD38-cADPR system [5–7,16–20], a still unresolved question is the relationship between the CD38-catalyzed production of cADPR which takes place in the extracellular space and the intracellular calcium-mobilizing activity of the nucleotide. As has been suggested from experiments with neuronal and lymphoid cells [16–18], a possible mechanism underlying an NAD-induced increase in intracellular cADPR would be an NAD-dependent internalization of CD38 with a concomitant shifting of cADPR metabolism to the intracellular environment. Previous studies have, in fact, shown that internalization of CD38 into cytoplasmic vesicles can occur in Namalwa B lymphoid cells [16,18]. These observations, however, raise further questions on the accessibility of the luminally-localized catalytic site of the CD38 molecule to cytosolic NAD^+ and, on the other hand, of intravesicular-generated cADPR to its cytosolic target.

However, Zocchi and collaborators [16,18] reported extensive decreases in ectocellular ADP-ribosyl cyclase activity in intact Namalwa B-lymphoma cells, following incubation with

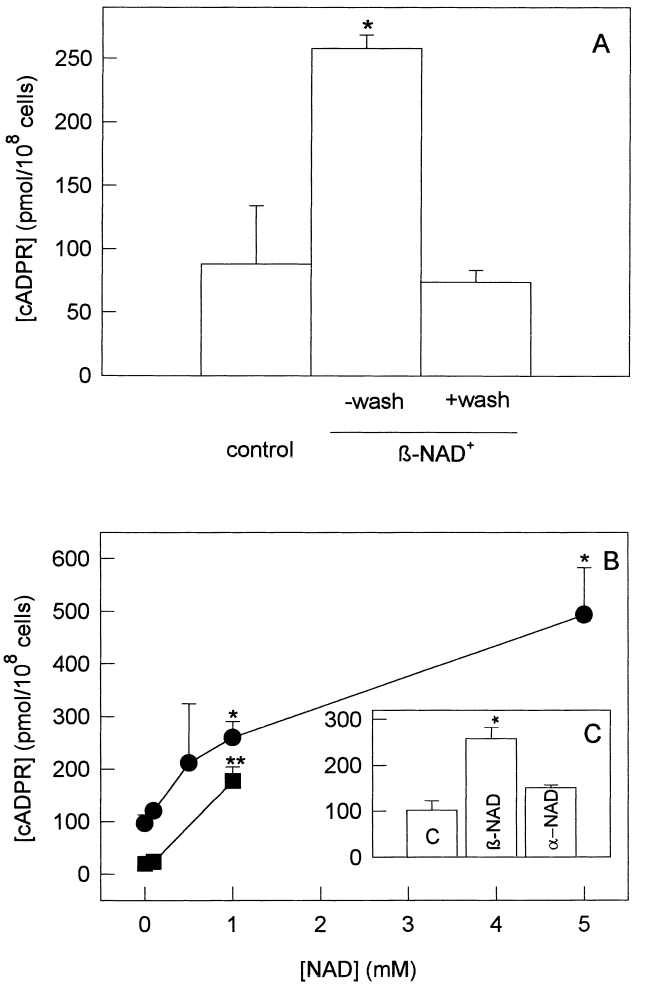


Fig. 3. Effect of exogenous NAD on the production of cADPR by an ADP-ribosyl cyclase in T-lymphocytes. A: Jurkat cells (1×10^8 cells/5 ml of extracellular buffer) were incubated for 15 min, at 37°C, either in the absence (control) or in the presence of β-NAD^+ (1 mM). NAD-treated cells were subsequently centrifuged and washed 3 times with 10 ml of extracellular buffer. cADPR was then extracted and quantified by HPLC. B: Cells (10^7–10^8 cells/sample) were preincubated with β-NAD^+, in the concentrations indicated, for 15 min, at 37°C. cADPR was subsequently extracted from Jurkat (●) and HPB.ALL cells (■) and analyzed by HPLC, as described in [15]. C: Jurkat T-cells were incubated in the absence (control) or in the presence of either β-NAD^+ or α-NAD^+ (1 mM), for 15 min at 37°C, before extraction and analysis of cADPR. Results are the mean ± S.D. of 3–26 independent experiments. $*P>0.99$, $**P>0.98$.

Table 2
Effects of β-NAD^+ and anti-CD38 on $[Ca^{2+}]_i$ in Jurkat T-lymphocytes

Compound added	$[Ca^{2+}]_i$ (nM)
–	116 ± 16.8
OKT3 (10 μg/ml)	533 ± 68*
IB4 (10 μg/ml)	112 ± 18
β-NAD (1 mM)	130 ± 30
β-NAD (1 mM)+OKT3 (10 μg/ml)	470 ± 44*

$[Ca^{2+}]_i$ was measured fluorimetrically in suspensions of Fura-2 loaded Jurkat T-cells, before and after addition of the compounds indicated. Increased levels of $[Ca^{2+}]_i$ refer to plateau values. Results are the mean ± S.D. of 4–10 independent measurements. Asterisks denote statistically significant differences from basal values, according to the Student's *t*-test ($P>0.99$).

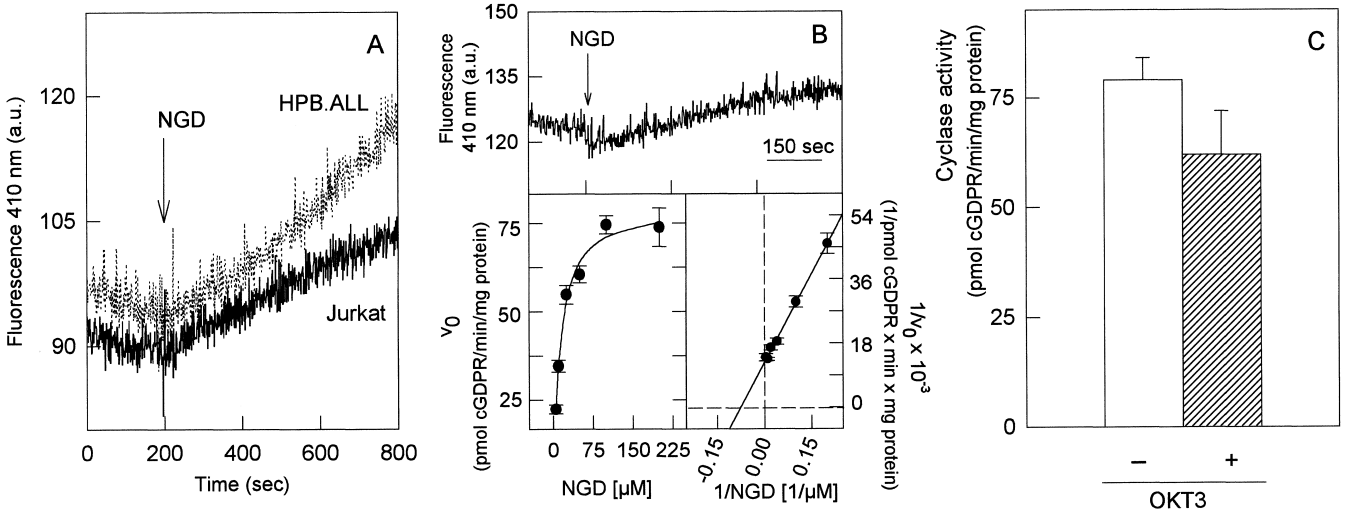


Fig. 4. Fluorimetric assays of ADP-ribosyl cyclase activity in Jurkat and HPB.ALL T cells. Cells (3×10^5 cells/600 μl of extracellular buffer) were incubated in a cuvette at 37°C, under continuous stirring. After 3 min, NGD (100 μM) was added and the subsequent increases in cGDPR fluorescence were continuously monitored for an additional 10 min. Two representative tracings from each T-cell line are shown (Jurkat cells, continuous line; HPB.ALL cells, broken line). B: Cyclase activity was measured in the P100 fraction from Jurkat cells (1 mg protein/ml of extracellular buffer), as described in A. The enzymatic reaction showed a typical Michaelis-Menten kinetic and from the Lineweaver-Burk plot of the experimental data, the kinetic constants K_m and V_{max} were determined. The results represent the mean ± S.D. of 7–11 independent measurements, performed with 4 different preparations of Jurkat cell membranes. C: P100 fractions were prepared from unstimulated and OKT3-stimulated (10 μg/ml, 10 min) Jurkat lymphocytes and cyclase activity was subsequently measured, under the same conditions, in both membrane fractions.

NAD. According to those authors, such observations were taken as an indication of NAD-induced internalization of CD38. In the present work, we also analyzed the effects of preincubation of Jurkat and HPB.ALL T-cells with NAD on the ectocellular cyclase activity. The following evidence against NAD-induced internalization of CD38 was obtained in our cell systems (Table 1): (i) FACS analysis showed no decrease in the cell surface expression of CD38 in Jurkat and HPB.ALL lymphocytes, after 1 h of exposure to NAD (1 mM); (ii) using the NGD-assay of ADP-ribosyl cyclase activity employing intact cells we observed that, after centrifugation of the cells, almost all the fluorescence due to enzymatically-synthesized cGDPR was found in the extracellular medium and not in the cell pellet; and (iii) preincubation with nicotinamide (which, together with cADPR, is also a product of the cyclase-catalyzed conversion of NAD) as well as with NAD inhibited the ADP-ribosyl cyclase activity, suggesting that rather a product-inhibition of the enzyme instead of internalization may be the reason for the observed decreases in ectocellular cyclase induced by NAD.

Another hypothesis recently proposed by Prasad and collaborators is based on a structural model for CD38 consisting of a dimer possessing a central cavity formed by head-to-head association of two monomers. According to this model, the catalytic domain of CD38 is located in the central cavity of the pore-forming dimeric structure, which could then form a channel to allow cADPR generated from extracellular NAD to enter the cell [19]. While such a model was proposed on the basis of elucidation of the tridimensional structure of the soluble ADP-ribosyl cylase from *Aplysia californica*, direct evidence for the existence of a similar structure for the CD38 molecule in mammalian cells has, so far, not been obtained.

None of the models mentioned above is, however, compatible with data obtained in this work on the effects of NAD on $[Ca^{2+}]_i$ in the T-cell lines studied. In fact, according to such models, if a direct coupling between NAD-induced ectocellular production of cADPR and intracellular activity of the cyclic nucleotide would exist, it would be expected that NAD, per se, would also induce an increase in $[Ca^{2+}]_i$. In contrast, as shown in Fig. 1, we observed no significant changes in $[Ca^{2+}]_i$ in T-cells, following incubation with β-NAD^+. Also ligation of CD38 with the agonistic monoclonal antibody IB4 had no effect on $[Ca^{2+}]_i$ in our Jurkat T-cell clone (Table 2). Moreover, preincubation of Jurkat cells with β-NAD^+ also had no influence on subsequent OKT3-stimulated increases in endogenous cADPR (Table 2).

Our conclusion that CD38/ADP-ribosyl cyclase is not involved in synthesis of intracellular cADPR was further confirmed by direct fluorimetric measurements of the CD38/ADP-ribosyl cyclase activity in intact Jurkat and HPB.ALL cells, as well as in subcellular fractions.

The results represented in Fig. 4A were performed with intact cells and show that both cell lines contained an ectoenzymatic cyclase activity, although HPB.ALL cells present relatively higher activity levels than Jurkat lymphocytes. The relative magnitudes of the ectocellular cyclase activities (130 ± 20 pmol cGDPR/min/10^6 cells for HPB.ALL and 58 ± 14 pmol cGDPR/min/10^6 cells for Jurkat lymphocytes) were in contrast with the relative basal levels of intracellular cADPR measured in the absence of extracellular NAD^+ in these cell lines (22.4 ± 6.2 and 101.7 ± 21.6 pmol of cADPR/10^8 cells, in HPB.ALL and in Jurkat cells, respectively). Although the effective endogenous concentration of cADPR is the result of a balance between cyclase and hydrolase activities, the lack of correlation between the two parameters mentioned above also suggests no direct coupling of the CD38/ADP-ribosyl cyclase to the synthesis of intracellular cADPR.

Further characterization of the membrane-bound cyclase activity, most likely CD38, was also performed in P100 frac-

tions prepared from Jurkat cells. Fig. 4B shows a typical fluorimetric tracing of cyclase activity measurement in the P100 membrane fraction and the correspondent data of kinetic analysis. The cyclase-catalyzed reaction showed a typical Michaelis-Menten kinetics and from the Lineweaver-Burk plot of the experimental data the kinetic constants $K_m = 13$ μM and $V_{max} = 79$ pmol cGDPR/min/mg protein were determined.

Along these lines, another observation in support of our view was that no significant changes in the membrane-bound cyclase activity were observed in P100 fractions prepared from Jurkat cells which had been pre-stimulated with the anti-CD3 monoclonal antibody OKT3 (Fig. 4C). If CD38 would be involved in TCR/CD3 complex-mediated cADPR formation, the opposite result might be expected.

However, CD38 may have a role in the long term regulation of basal levels of cADPR. In fact, Zocchi et al. [21] have shown that transfection of a cDNA for CD38, followed by expression of CD38 in $CD38^-$ HeLa and 3T3 cells resulted in increased intracellular cADPR, accompanied by a modest increase in basal $[Ca^{2+}]_i$. In addition, a reduction in cell doubling time in close correlation with the increased expression of ectocellular cyclase/CD38 activity was observed [21]. Takahashi and collaborators [22] also showed that differentiation of HL-60 cellls with retinoic acid was accompanied by an elevation of CD38 expression and a concomitant increase in basal intracellular cADPR, but the mechanism involved in these effects is not known.

In conclusion, the results obtained in this work demonstrate that the ADP-ribosyl cyclase activity of the CD38 glycoprotein is not responsible for the agonist-induced increases in cADPR in human T-cells and, consequently, does not play a major role in the regulation of cADPR-mediated Ca^{2+}-signalling in T-lymphocytes. However, a modulatory long term effect of CD38 on the basal level of cADPR in eukaryotic cells cannot be ruled out completely, at present.

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