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Human lymphocyte antigen CD38 catalyzes the production of cyclic ADP-ribose

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The human lymphocyte antigen CD38 has been shown to share sequence homology with ADP-ribosyl cyclase, the enzyme that catalyzes the conversion of NAD^+ to cyclic ADP-ribose (cADPR), a potent Ca^{2+}-mobilizing agent. In this study COS1 cells from African Green Monkey kidney were transiently transfected with CD38 cDNA, inducing expression of authentic CD38 on the cell surface. We demonstrate that CD38 expressed in this manner can convert NAD^+ to cADPR in the extracellular medium as assessed by Ca^{2+} release from sea-urchin egg microsomes.

Cyclic ADP-ribose; CD38; ADP-ribosyl cyclase; NAD^+ glycohydrolase; NADase

1. INTRODUCTION

Cyclic ADP-ribose (cADPR) is a naturally occurring metabolite of NAD^+ with potent Ca^{2+}-mobilizing activity. It has been implicated in regulation of the Ca^{2+}-induced Ca^{2+} release mechanism and possesses many characteristics of a novel second messenger acting on a ryanodine-sensitive Ca^{2+} release channel distinct from the IP_3 receptor [1,2]. ADP-ribosyl cyclase, the enzyme that synthesizes cADPR from NAD^+, is ubiquitous in mammalian and invertebrate tissues [3–5]. The cyclase from *Aplysia* ovotestis has been sequenced and shows considerable homology with the human lymphocyte antigen CD38 [6]. CD38 is a type II integral membrane glycoprotein of lymphoid and myeloid cells that displays an unusual pattern of exoression during development. For example, both immature and terminally differentiated lymphocytes within the thymus, spleen and lymph nodes are $CD38^{+ve}$ whereas resting peripheral blood lymphocytes are mostly $CD38^{-ve}$ [7]. The function of CD38 remains elusive although it has been implicated in terminal differentiation of HL-60 cells [8] and proliferation of B-lymphocytes [9].

This study demonstrates that CD38 expressed on the surface of COS 1 cells catalyzes the conversion of NAD^+ to cADPR and thus acts as an ADP-ribosyl cyclase, as suggested by homology with the *Aplysia* cyclase. Furthermore, the cyclase activity is present in the extracellular domain of CD38 and leads to the production of extracellular cADPR.

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2. MATERIALS AND METHODS

2.1. *Materials*

Sea urchins (Echinus) were obtained from St. Davids Oceanarium, UK. Fluo-3 was from purchased from Calbiochem and cADPR was from Amersham. All other reagents were purchased from Sigma.

2.2. *Transfection of COS 1 cells*

COS1 fibroblasts (6×10^6 cells in 1 ml serum-free RPMI) were transfected by electroporation (960 μF, 750 V/cm) with CD38 cDNA (10 μg) cloned in pCDM8 as described previously [7]. Transfected cells were maintained in RPMI-1640 medium supplemented with 10% foetal calf serum until maximal expression was obtained (approximately 3 days). Typically, 40% of the transfected cells were $CD38^{+ve}$ as determined by immunofluorescent staining/FACSCAN analysis with the CD38 monoclonal antibody HIT2 [10].

2.3. *NAD^+ incubation*

Flasks of adherent CD38-transfected COS cells were taken three days after transfection along with non-transfected control cells. Following washing with PBS (pH 7.4), an addition of 200 μM β-NAD^+ in 10 ml of PBS was made to each flask. The flasks were incubated at 37°C and samples of the incubation medium were taken at time intervals up to 1 hour. These samples were immediately frozen and stored at −70°C.

2.4. *cADPR bioassay*

Release of microsomal Ca^{2+} from sea-urchin egg homogenate was used as a bioassay for cADPR. 5% sea-urchin egg homogenate was prepared as described previously [11]. Additions of 20 μl of medium incubated with $CD38^{+ve}$ cells and control cells were made to 500 μl of 5% egg homogenate, and Ca^{2+} uptake and release was followed by monitoring extra-microsomal Ca^{2+} using fluo-3 (3 μM). Fluorescence intensity of fluo-3 was measured using a Perkin-Elmer LS50B fluorimeter at excitation and emission wavelengths of 490 nm and 535 nm, respectively. Ca^{2+} traces were calibrated by addition of a Ca^{2+} standard solution and related to a standard curve of authentic cADPR-induced Ca^{2+} release.

3. RESULTS AND DISCUSSION

Incubation of CD38 transfected cells in medium containing NAD^+ resulted in the extracellular production of a Ca^{2+}-releasing factor which we identified as cADPR (see below). The production of cADPR (measured indirectly as the initial rate of Ca^{2+} release after incubation with sea-urchin microsomes) reached a maximum after 15 min and then decayed to zero within 1 h (Fig. 1). This corresponds with a maximum concentration of cADPR in the extracellular medium of 500 nM as determined from a standard curve of cADPR concentration vs. Ca^{2+} release (data not shown). Ca^{2+}-releasing activity was expressed as initial rate of release rather than maximal $[Ca^{2+}]$ attained during release which is influenced by rates of both Ca^{2+} release and resequestration. Measurement of initial rate of release proved to be a more reproducible indication of Ca^{2+}-releasing activity. No Ca^{2+} releasing activity was detected in medium incubated with non-expressing control cells. In addition, 200 μM NAD^+ alone was not sufficient to induce Ca^{2+} release.

To confirm the identity of the Ca^{2+}-releasing agent in the CD38 incubation medium as cADPR, the medium was assayed in the presence of authentic cADPR and IP_3 (Fig. 2). The sea-urchin egg homogenate system has two Ca^{2+}-releasing pathways, the cADPR-sensitive pathway and the IP_3 sensitive pathway [12]. Both of these pathways demonstrate the phenomenon of desensitization to sequential additions of agonists. However, the pathways are independent and cross-desensitization between the pathways is not seen even though release appears to occur from a common Ca^{2+} pool [1,12]. Fig. 2a and b demonstrate desensitization of the cADPR-sensitive pathway of Ca^{2+} release following subsequent additions of medium from $CD38^{+ve}$ cells incubated with NAD^+ for 15 min. Pre-treatment with authentic cADPR also abolishes the response to medium incubated with $CD38^{+ve}$ cells (instantaneous increases in fluorescence on additions represent contaminating Ca^{2+} rather than

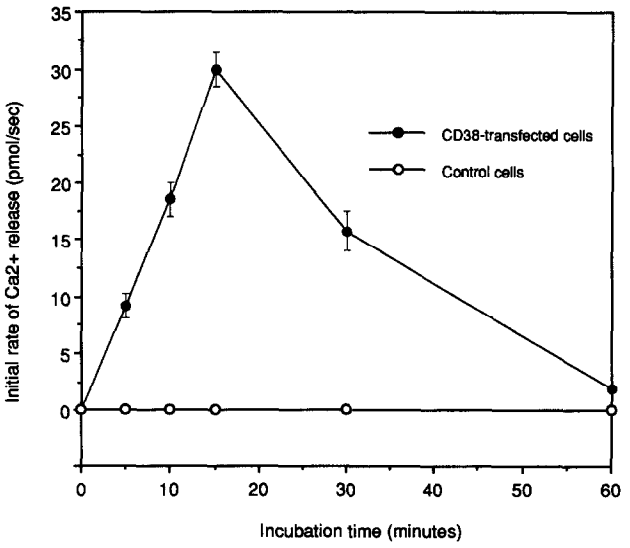

Fig. 1. Time course for production of Ca^{2+} releasing factor (cADPR) in the medium by CD38 transfected cells (filled circles) or control cells (open circles) incubated with NAD^+. The sea-urchin bioassay was performed as described in section 2. Initial rates of Ca^{2+} release were determined from calibrated traces of fluorescence intensity vs. time. The data is plotted as mean rate ± S.E.M. (n = 5) and is representative of 3 incubations.

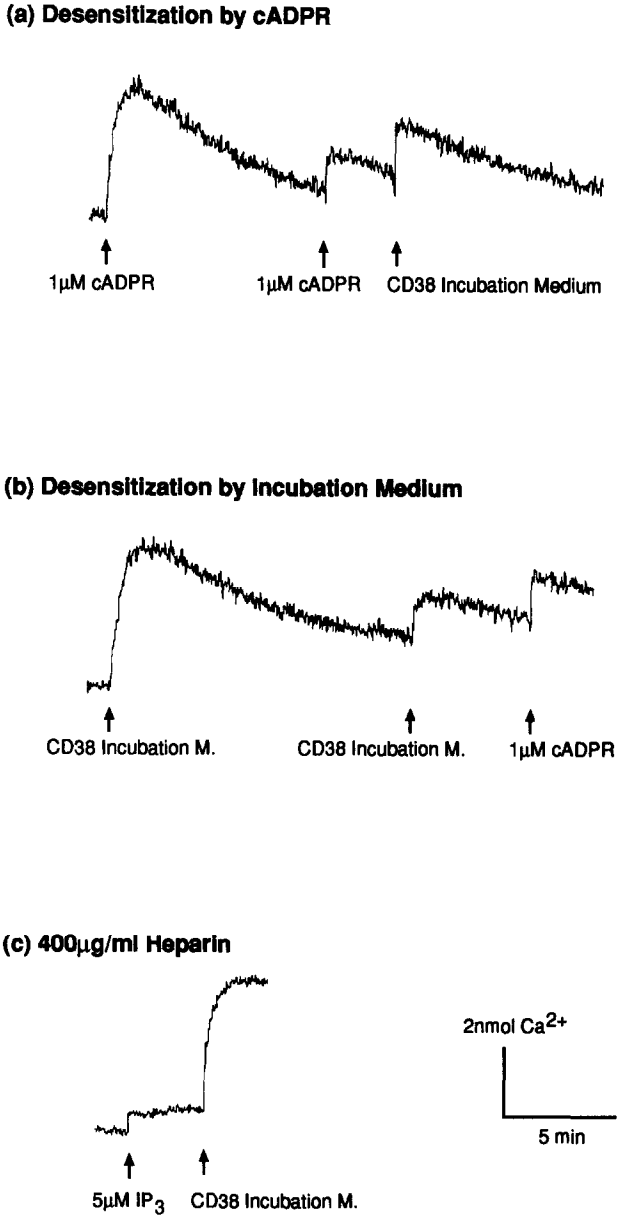

Fig. 2. Ca^{2+}-releasing activity of CD38 incubation medium in the presence of authentic cADPR and IP_3. Traces are of fluorescence intensity vs. time with an upward deflection representing Ca^{2+} release. (a) Desensitization to incubation medium following repeated addition of authentic cADPR. (b) Desensitization to authentic cADPR following repeated addition of incubation medium. (c) Effect of heparin on IP_3-induced release and incubation medium-induced release.

Ca^{2+} release). However, pre-treatment with 400 μg/ml heparin, an IP_3 antagonist, had no effect on the Ca^{2+} releasing activity of the NAD^+ incubation medium while completely blocking IP_3-induced Ca^{2+} release.

The results of this study demonstrate the production of cADPR in the extracellular medium by CD38 after the addition of NAD^+ and thus confirm the predicted ADP-ribosyl cyclase activity of CD38. Fig. 1 also demonstrates a decline in the extracellular concentration of cADPR after a period of about 15 min suggesting the existence of a cADPR hydrolase activity on the cell surface. This co-occurrence of ADP-ribosyl cyclase activity and hydrolase activity has been demonstrated in a variety of tissues [3], and in particular on the surface of human erythrocytes [13]. It remains a possibility that CD38 itself is responsible for hydrolysis of cADPR to ADP-ribose (ADPR). This would place CD38 in a growing family of bifunctional membrane-bound enzymes that possess both cyclase and hydrolase activity catalyzing the net conversion of NAD^+ to ADPR via cADPR [13,14]. These enzymes have previously been classified as NAD glycohydrolases (NADase), a group of enzymes catalyzing the hydrolysis of NAD^+ to ADPR whose function has remained elusive [15]. Indeed, a previous study has attributed a retinoic acid-induced NADase activity on the surface of HL-60 cells to the CD38 antigen [8].

The extracellular location of the CD38 catalytic domain is particularly intriguing. A role for extracellular cADPR may be envisaged or alternatively, activation may lead to internalization of CD38 and intracellular production of cADPR. In either case, this study presents the possibility that CD38 functions through regulating the production and hydrolysis of cADPR. Association of cyclase and hydrolase activity on a single enzyme would provide a mechanism for tight regulation of [cADPR], a prerequisite for what is perhaps a novel second messenger. The precedent for concerted regulation of a bifunctional enzyme has been set by 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFK/FBP), an enzyme catalyzing both the synthesis and hydrolysis of fructose-2,6-bisphophate that is reciprocally regulated by cAMP-dependent phosphorylation [16]. Interestingly, it has recently been suggested that ADP-ribosyl cyclase from sea-urchin eggs is regulated by cGMP-dependent phophorylation [17]. In addition, the catalytic activity of cell-surface CD38 may potentially be regulated by binding of extracellular factors. We are currently exploring this possibility.

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