Characterization of NAD:arginine ADP-ribosyltransferases

Joel Moss, Enrico Balducci, Eleanor Cavanaugh, Hyun Ju Kim, Piotr Konczalik, Elena A. Lesma, Ian J. Okazaki, Maryann Park, Michael Shoemaker, Linda A. Stevens and Anna Zolkiewska
Pulmonary-Critical Care Medicine Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD, USA

Abstract

NAD:arginine mono-ADP-ribosyltransferases catalyze the transfer of ADP-ribose from NAD to the guanidino group of arginine on a target protein. Deduced amino acid sequences of one family (ART1) of mammalian ADP-ribosyltransferases, cloned from muscle and lymphocytes, show hydrophobic amino and carboxyl termini consistent with glycosylphosphatidylinositol (GPI)-anchored proteins. The proteins, overexpressed in mammalian cells transfected with the transferase cDNAs, are released from the cell surface with phosphatidylinositol-specific phospholipase C (PI-PLC), and display immunological and biochemical characteristics consistent with a cell surface, GPI-anchored protein. In contrast, the deduced amino acid sequence of a second family (ART5) of transferases, cloned from murine lymphoma cells and expressed in high abundance in testis, displays a hydrophobic amino terminus, consistent with a signal sequence, but lacks a hydrophobic signal sequence at its carboxyl terminus, suggesting that the protein is destined for export. Consistent with the surface localization of the GPI-linked transferases, multiple surface substrates have been identified in myotubes and activated lymphocytes, and, notably, include integrin α subunits. Similar to the bacterial toxin ADP-ribosyltransferases, the mammalian transferases contain the characteristic domains involved in NAD binding and ADP-ribose transfer, including a highly acidic region near the carboxy terminus, which, when disrupted by *in vitro* mutagenesis, results in a loss of enzymatic activity. The carboxyl half of the protein, synthesized as a fusion protein in *E. coli*, possessed NADase, but not ADP-ribosyltransferase activity. These findings are consistent with the existence at the carboxyl terminus of ART1 of a catalytically active domain, capable of hydrolyzing NAD, but not of transferring ADP-ribose to a guanidino acceptor. (Mol Cell Biochem **193**: 109–113, 1999)

Key words: ADP-ribosylation, bacterial toxins, glycosylphosphatidylinositol-linked proteins, NAD glycohydrolases

A family of vertebrate ADP-ribosyltransferases has been purified that, like cholera toxin, modify arginine and other simple guanidino compounds (NAD:arginine ADP-ribosyltransferases) [1]. Several vertebrate transferases of this type have been cloned and characterized including those from rabbit and human skeletal muscle, chicken heterophils and erythroblasts, and mouse lymphocytes (Fig. 1). Expression of NAD:arginine ADP-ribosyltransferases in mammalian tissues appears to be restricted thus far to muscle, hematopoietic cells (e.g., lymphocytes, polymorphonuclear leukocytes) and epithelial cells. Of the mammalian ADP-ribosyltransferases, those from rabbit and human skeletal muscle were first to be cloned [1, 2]. The nucleotide and deduced amino acid sequences of the human skeletal muscle enzyme were 80.8 and 81.3% identical, respectively, to those of the rabbit.

The hydropathy profiles of ART1 from these species demonstrated hydrophobic regions near the amino and carboxy termini as has been found with glycosylphosphatidylinositol (GPI)-linked proteins [1, 2]. Consistent with the hypothesis that the transferase is GPI-linked is the observation that transferase activity is released from the cell

```
hART1    MQMPAMMSLL LVSVGLHEAL QAQSHPITRR DLFSQEIQLD MALATFDDQY        50
Rt6-1    MPSNNFK--- -FFLTWWLTQ QVTGL----- ---AVPFMLD MAPNAFDDQY        38
RT6.2    MPSNICK--- -FFLTWWLIQ QVTGL----- ---TGPLMLD TAPNAFDDQY        38
hART3    MKTGHFE--- -IVTMLLATM ILVDI----- -FQVKAEVLD MADNAFDDEY        40
hART4    MQVA------ ---------- -IK------- ------IDFD FAPGSFDDQY        20
mART5    MILEDLL-MV LSCLSLHALW KVRAVPI--- ------LPLS LVPDTFDDAY        40

hART1    VGCAAAMTAA LPDLNHTEFQ ANQVYADSWT LASSQWQERQ ARWPEWSLSP       100
Rt6-1    EGCVEDMEKK -APQLLQEDF NMNEELKL-E WEK------- AE--IKWKEI        77
RT6.2    EGCVNKMEEK -APLLLQEDF NMNAKLKV-A WEE------- AK--KRWNNI        77
hART3    LKCTDRMEIK YVPQLLKEEK ASHQQLDT-V WEN------- AK--AKWAAR        80
hART4    QGCSKQVMEK LTQ---GDYF TKDIEAQKNY FRMW------ QK--AHLAWL        59
mART5    VGCSEEMEEK -AGLLLKEEM ARHALPAP-I LGSS------ TR--GLGTPA        80

hART1    TRPSPPPLGF RDEHGVALLA YTANS-PL-- -HKEFNAAVR EAGRSRAHYL       146
Rt6-1    KNCMSYPAGF HDFHGTALVA YTGNIHRS-- ----LNEATR EFKINPGN--       119
RT6.2    KPSRSYPKGF NDFHGTALVA YTGSIAVD-- ----FNRAVR EFKENPGQ--       119
hART3    KTQIFLPMNF KDNHGIALMA YISEAQEQTP FYHLFSEAVR MAGQSREDYI       130
hART4    NQGKVLPQNM STTHAVAILF YTLNS-NV-- -HSDFTRAMA SVARTPQQYE       105
mART5    SQATLPP-GF KAQHGVAIMV YTNSSNTL-- -YWELNQAVR TGGGSRELYM       126

hART1    HHFSFKTLHF LLTEALQLL- GSGQ-RPPR- CHQVFRGVHG LRFRPAGPRA       193
Rt6-1    --FHYKAFHY YLTRALQLL- --SDQGCRS- ---VYRGTN- VRFR-YTGKG       158
RT6.2    --FHYKAFHY YLTRALQLL- --SNGDCHS- ---VYRGTK- TRFH-YTGAG       158
hART3    YGFQFKAFHF YLTRALQLLR KPCEASSKTV ---VYRTSQG TSFT-FGGLN       176
hART4    RSFHFKYLHY YLTSAIQLLR KDSIMENGTL CYEVHYRTKD VHFNAYT-GA       154
mART5    RHFPFKALHF YLTRALQLLR GSGGCSRGP- GEVVFRGVGS LHFEPKRLGD       175

hART1    TVRLGGFASA SLKHVAAQQ- ---FGEDTFF GIWTCLGAPI KGYSFFPGEE       239
Rt6-1    SVRFGHFASS SLNRSVATSS PFFNGQGTLF IIKTCLGAHI KHCSYYTHEE       208
RT6.2    SVRFGQFTSS SLSKKVAQSQ EFFSDHGTLF IIKTCLGVYI KEFSFRPDQE       208
hART3    QARFGHFT-- -LAYSAKPQA A--NDQLTVL SIYTCLGVDI ENFLDKESER       221
hART4    TIRFGQFLST SLLKEEAQE- ---FGNQTLF TIFTCLGAPV QYFSLKKE--       198
mART5    SVRLGQFTSS SVDERVARR- ---FGNATFF NLRTCFGAPI QALSVFPEER       221

hART1    EVLIPPFETF QVINASRPAQ GPARIYLRAL GKHSTYNCEY IKD-------       282
Rt6-1    EVLIPGYEVF HKVKTQSVER YIQISLDSPK RKKSNFNCFY SGSTQAAN--       256
RT6.2    EVLIPGYEVY QKVRTQGYNE IFLDS---PK RKKSNYNCLY SSA-------       248
hART3    ITLIPLNEVF QVSQEGAGNN LILQS---IN KTCSHYECAF LGGLKTENCI       268
hART4    -VLIPPYELF KVINMSYHPR GD-WLQLRST GNLSTYNCQL LKA-------       239
mART5    EVLIPPHEVF LVTGFSQDGA QSIVTLSSYD QTCSHFNCAY LGG-------       264

hART1    ---------- ---------- ---------- ----KKYK-- ----------       286
Rt6-1    ---------- ---------- ---------- VSSLGSRE-- ----------       264
RT6.2    ---------- ---------- ---------- ----GARE-- ----------       252
hART3    ENLEYFQPIY VYNPGEKNQK LEDHSEKNWK LEDHGEKNQK LEDHGVKILE       318
hART4    ---------- ---------- ---------- ----SSKK-- ----------       243
mART5    ---------- ---------- ---------- ----EKRH-- ----------       268

hART1    ----SGPCHL DNSAMGQSP- ---LSAVWSL LLLLWFLVVR AFPDGPGLL        327
Rt6-1    ---------- -----SCVP- ----LFLVVL LGLLVQQLTL AEP------        287
RT6.2    ---------- -----SCVS- ----LFLVVL PSLLVQLLCL AE-----PE        276
hART3    PTQIPAPGPV PVPGPKCHPS ASSGKLLLPQ FGMVIILISV SAINLFVAL        367
hART4    -----CI--- ----PDPIA- ---IASLSFL TSVIIFSKSR V--------       268
mART5    ----GCVSSR AVGQPEAPS- ---TEALALQ SGKTLLLDPR KLQLSRAGP        309
```

Fig. 1. Alignment of vertebrate ADP-ribosyltransferases. Deduced amino acid sequences of mammalian ADP-ribosyltransferases were aligned using the protein alignment program of GeneWorks. Dashes were inserted to facilitate alignment. Amino acid position is indicated on the right. hART1, ART1 from human skeletal muscle; Rt6-1, mouse Rt6-1; RT6.2, rat RT6.2; hART3, ART3 from human testis; hART4, ART4 from human spleen; mART5, ART5 from mouse lymphoma cells.

surface with phosphatidylinositol-specific phospholipase C (PI-PLC). In addition, the protein released by PI-PLC reacted with anti-cross reacting determinant (anti-CRD) antibodies which recognize an epitope on the oligosaccharide portion of the GPI-anchor [3]. Further, the transferase released by PI-PLC from cells grown in the presence of [³H]ethanolamine, a constituent of the oligosaccharide, contained radiolabel [3].

Based on these studies it appears that ART1 is glycosyl-phosphatidylinositol (GPI)-anchored and conserved across species.

The muscle enzyme in C2C12 mouse myotubes modified integrin $\alpha 7$ [4, 5]. Integrin $\alpha 7$, as a dimer with integrin $\beta 1$, is a major integrin receptor for extracellular matrix protein laminin in skeletal muscle [6]. ADP-ribose appeared to be

incorporated in the extracellular domain of $\alpha7$, possibly in an arginine-rich region. The fact that an integrin is ADP-ribosylated suggests that the modification may affect cell interactions with the extracellular matrix. ADP-ribosylated integrin was processed by an extracellular phosphodiesterase, releasing 5′AMP, while ribose remains attached to the protein backbone [5].

The fact that the ART1 transferases are GPI-anchored is of interest in view of the prior finding that ADP-ribosylarginine hydrolase, the enzyme that cleaves the ADP-ribose-arginine bond, appears to be soluble and intracellular [7]. Since ADP-ribose is attached to extracellular domains of integrin, the modified proteins may not come in contact with the ADP-ribosylarginine hydrolase. The biochemical data suggest that the ADP-ribosylated integrins are processed by extracellular phosphodiesterases, releasing 5′AMP and leaving ribose covalently attached to the arginine. The presence of ribose would prevent further modification of the arginine residue.

Binding of integrin $\alpha7\beta1$ to laminin in solution did not seem to be influenced by ADP-ribosylation of the $\alpha7$ subunit ([8], data not shown). Interactions in solution, in the presence of detergent, differ, however, from the interactions at the cell surface, where binding of the two proteins may still be affected by ADP-ribosylation. Moreover, ADP-ribosylation could modulate transmembrane signalling by integrin $\alpha7\beta1$ triggered by ligand binding.

To address the question of the role of ADP-ribosylation in intact cells, cultured C2C12 myotubes were used in laminin binding assays. The formation of laminin-integrin complex was followed by immunoprecipitation with either anti-laminin or anti-integrin $\alpha7$ antibodies. As evidenced by laminin dose-response experiments, by dependence of the co-immunoprecipitation of the two proteins on divalent cations, and by chemical cross-linking, laminin did not bind to integrin $\alpha7\beta1$ in intact cells. In addition, it was demonstrated that endogenous laminin, which was produced by C2C12 cells and deposited outside the cells, was unable to bind to integrin $\alpha7\beta1$, either at the cell surface, or in solution. The lack of integrin binding was paralleled by the lack of reactivity with 5D3 monoclonal antibody, which was shown to recognize the γ subunit in the heterotrimeric laminin molecule.

To investigate the role of ADP-ribosylation of the extra-cellular domain of integrin $\alpha7$ on the affinity of $\alpha7\beta1$ dimer for laminin or intracellular signalling, the mechanisms leading to integrin activation in cultured cells will be explored. These mechanisms may be similar to those described for several integrins in leukocytes, where a specific cellular activation is required in order to induce an active conformation of integrins in a process known as 'inside-out' signalling [9]. Once integrin $\alpha7\beta1$ is activated, its interactions with exo-genous laminin can be studied, with little interference from the endogenous laminin.

ADP-ribosyltransferase activity was also investigated in lymphocytes and related cells. It was detected in mouse lymphoma and thymoma cells [10] and cytotoxic T lympho-cytes (CTL) [11]; the murine ART1 cDNA was cloned from lymphoma (Yac-1) cells [12]. The deduced amino acid sequence of the GPI-anchored mouse ART1 transferase was 77 and 75% identical, to those from the human and rabbit skeletal muscle enzymes, respectively. Incubation of mouse CTL in the presence of NAD resulted in the ADP-ribosylation of membrane proteins, inhibition of CTL proliferation, and, to a lesser extent, cytotoxicity [11]. The suppressive effects of NAD on CTL were prevented by treatment of the cells with phosphatidylinositol-specific phospholipase C (PI-PLC), which releases most GPI-linked proteins from the cell surface, before incubation with NAD, consistent with the conclusion that a GPI-linked ADP-ribosyltransferase was responsible for modulating CTL function. In addition, ADP-ribosylation of a 40-kDa CTL membrane protein (p40) resulted in inhibition of p56[lck], a tyrosine kinase that exists in a complex with the 40-kDa protein [13]. Release of the membrane-bound transferase(s) following treatment with PI-PLC prevented the NAD-induced suppression of kinase activity. The relationship between the inhibition of p56[lck], following ADP-ribosylation of p40, and the inhibition of CTL proliferation has not been established. In other experiments, CTL transferases modified arginines in the extracellular domain of the lymphocyte function-associated molecule-1 (LFA-1), an adhesion molecule of the integrin family [14]. ADP-ribosylation inhibited LFA-1-mediated generation of inositol phosphates and suppressed homotypic cell adhesion. As in C2C12 cells, 5′-AMP was removed from the modified LFA-1 by extracellular phosphodiesterases. These data are consistent with a role for ADP-ribosylation in lymphocyte function.

A second lymphocyte ADP-ribosyltransferase ART5, was cloned from Yac-1 cells [15]. The nucleotide and deduced amino acid sequences of the ART5 transferase are 58 and 33% identical, respectively, to those of mouse ART 1. Further, the ART 1 and ART5 enzymes were only ~28% identical to the rodent RT6 family of proteins which are NAD glycohydrolases and, in the case of murine Rt6, NAD:arginine ADP-ribosyltransferases. Whereas the ART1 protein possessed hydrophobic amino- and carboxy-terminal signal sequences characteristic of GPI-anchored proteins, ART5 contained an amino-, but not carboxy-terminal hydro-phobic signal sequence. The amino-terminal signal sequence should direct export of proteins into the endoplasmic reticulum; in the absence of a carboxy-terminal signal sequence, the protein would not be retained by the GPI anchor. Indeed, when rat mammary adenocarcinoma (NMU) cells were transformed with the ART5 cDNA, arginine-specific ADP-ribosyltransferase activity was not released from the membranes following incubation with PI-PLC.

On Northern analysis, an ART1 cDNA probe hybridized with a 1.6 kb band in poly(A)$^+$RNA from mouse cardiac and skeletal muscle. An ART5 cDNA probe, on the other hand, hybridized with 1.6 and 2.0 kb bands in mouse testis mRNA [15]. There was also weak hybridization of the ART5 probe with a 1.6 kb band in cardiac and skeletal muscle mRNA. ART5-specific oligonucleotide probes hybridized with poly(A)$^+$RNA from mouse skeletal muscle and testis, consistent with the fact that both enzymes ART1 and ART5 are expressed in muscle. Expression in rat testis was examined during development; the ART5 mRNA appeared preferentially at 30–60 days and was localized primarily to the seminiferous duct epithelial cells.

ART1 and ART5 enzymes expressed in *E. coli* as GST-fusion proteins were used to compare ADP-ribosyltransferase and NAD glycohydrolase activities of the purified enzymes [15]. The ADP-ribosyltransferase activity of ART1 was twice that of ART5 while the NAD glycohydrolase activity of ART1 was substantially less than its transferase activity. The ADP-ribosyltransferase and NAD glycohydrolase activities of the ART5 protein, however, were approximately equal. The K_m values for NAD with 20 mM agmatine as ADP-ribose acceptor in the ADP-ribosyltransferase assay were 118 and 142 μM for ART1 and ART5, respectively; the values for agmatine in the presence of 0. 1 mM NAD were 9 and 15 mM respectively.

Based on three-dimensional structure, photoaffinity labeling and site-directed mutagenesis, several of the bacterial toxin ADP-ribosyltransferases possess regions of similarity which together form, in part, the catalytic site [16]. The NAD-binding cleft is composed of an α-helix bent over a β-strand. An arginine or histidine and an active site glutamate, which are critical for enzyme activity, are located on two β-strands flanking the active site (Fig. 2). In the group of toxins, of which cholera toxin is a member, region I contains an arginine located on a β-strand which is essential for positioning NAD in the catalytic cleft, and also to form a hydrogen bond with the backbone carbonyl of serine 61 which is located in region II. In region III, the glutamate-X-glutamate sequence is essential for function. The second glutamate is essential for transferase and NAD glycohydrolase activities. Replacing the first glutamate with glutamine abolishes transferase activity but preserves NAD glycohydrolase activity. To date, all these toxins and their eukaryotic homologues possess NAD:arginine ADP-ribosyltransferase activity. In the second group of toxins exemplified by diphtheria toxin, a histidine in region I is
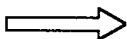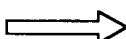
		Region I		Region II		Region III
		β		β/α		β
LT	1	NGDKLYR*ADS	58	GYVS*TSLSLR	107	HPYEQ*EVSAL
CT	1	NDDKLYRADS	58	GYVSTSISLR	107	HPDEQEVSAL
PT	3	PPATVYRYDS	49	AFVSTSSSRR	124	ATYQSEYLAH
iota	289	SNLIVYRRSG	335	NFISTSIGSV	375	YAGEYEVLLN
rART1	173	RCRQVFRGVH	199	GFASASLKNV	235	FPGEEEVLIP
mRt6-1	140	GCRSVYRGTN	164	HFASSSLNRS	204	YTHEEEVLIP
RT6.2	140	DCHSVYRGTK	164	QFTSSSLSKK	204	RPDQEEVLIP
hART4	136	CYEVHYRTKD	160	QFLSTSLLKE	193	FSLKKEVLIP
mART5	155	PGEVVFRGVG	181	QFTSSSVDER	217	FPEEREVLIP
chAT1	158	RCYYVYRGVR	183	QFTSTSLRKE	219	FPSEDEVLIP
DT	15	ENFSSYH*GTK	50	WKGFY*STDNKYDAAGY*	145	SSVEY*INNWE
ETA	434	YVFVGYHGTF	466	WRGFYIAGDPALAYGY	550	GRLETILGWP
hART3	88	MNFKDNHGIA	126	REDYIYGFQFKAFHFY	242	KESERITLIP
hPARP	856	NRRLLWHGSR	89	GKGIYFADMVSKSANY	985	LYNEYIVYDI

Fig. 2. Regions of similarity among ADP-ribosyltransferases. Bacterial toxin ADP ribosyltransferases were grouped according to 3-dimensional structure [16]. In the group exemplified by CT, region I contains a critical arginine (asterisk) on a β-strand, region II contains an aromatic amino acid-hydrophobic amino acid-Ser(asterisk)-Thr-Ser-hydrophobic amino acid motif, and region III contains the active site glutamate (asterisk) on a β-strand. In the group exemplified by DT, region I contains a histidine (asterisk) on a β-strand, region II contains a Tyr-X$_{10}$-Tyr motif within a hydrophobic rich segment of amino acids, and region III possesses the catalytic glutamate (asterisk) on a β-strand. Amino acid position is indicated to the right of each amino acid segment. LT – heat-labile enterotoxin of *Escherichia coli;* CT – cholera toxin; PT – pertussis toxin; iota – *Clostridium perfringens* iota toxin; DT – diphtheria toxin; ETA – *Pseudomonas aeruginosa* exotoxin A; rART1 – rabbit ART1; mRt6-1 – mouse Rt6-1; RT6.2 – rat RT6.2; hART3 – human ART3; hART4 – human ART4; mART5 – mouse ART5; hPARP – human poly(ADP-ribose) polymerase; chAT1 – AT1 from chicken heterophils.

involved in NAD binding, and several closely-spaced aromatic and hydrophobic amino acids in region II are hypothesized to be involved in hydrophobic interactions with the nicotinamide and adenine rings of NAD. Similar to the other group of bacterial toxin transferases, there is an active site glutamate located on a β-strand in region III. The active site glutamate is equivalent to that found in the third position in the glutamate-X-glutamate sequence in the NAD:arginine ADP-ribosyltransferases.

Comparison of the deduced amino acid sequence of the mammalian NAD:arginine ADP-ribosyltransferases and cholera toxin is consistent with the conclusion that the active site amino acid triad is found in the carboxy half of the protein. Further work was done to analyze the catalytic site. Characterization of the active site of ADP-ribosyltransferases was performed by truncations at the amino terminus of ART1. Removal of the amino terminal region resulted in a loss of transferase activity. The enzyme, however, retained NAD glycohydrolase activity. These data support the hypothesis that a catalytic site is present in the carboxy-terminal domain of the transferase [17]

The regions of sequence similarity among bacterial toxin transferases are also apparent in the alignment of the mammalian ADP-ribosyltransferases [18]. Further, site-directed mutagenesis of the rabbit muscle ART1 [18] and computer modeling of mouse ART2 [19] support the notion of a catalytic cleft, similar to that found in cholera toxin, that contains the critical arginine, serine and glutamate-X-glutamate motif in regions I, II, and III, respectively (Fig. 2). The conserved structure of the active site among bacterial toxin and mammalian ADP-ribosyltransferases is consistent with the hypothesis that these enzymes possess a common mechanism of NAD binding and ADP-ribose transfer.

References

1. Okazaki IJ, Moss J: Structure and function of eukaryotic mono-ADP-ribosyltransferases. In: M.P. Blaustein, H. Grunicke, D. Pette, G. Schultz, M. Schweiger, (eds). Reviews of Physiology, Biochemistry and Pharmacology, Springer-Verlag, New York, NY, 1996, pp. 51–104
2. Zolkiewska A, Nightingale MS, Moss J: Molecular characterization of NAD:arginine ADP-ribosyltransferase from rabbit skeletal muscle. Proc Natl Acad Sci USA 89: 11352–11356, 1992
3. Okazaki IJ, Zolkiewska A, Nightingale MS, Moss J: Immunological and structural conservation of mammalian skeletal muscle glycosyl-phosphatidylinositol-linked ADP-ribosyltransferases. Biochemistry 33: 12828–12836, 1994
4. Zolkiewska A, Moss J: Integrin α7 as substrate for a glycosyl-phosphatidylinositol-anchored ADP-ribosyltransferase on the surface of skeletal muscle cells. J Biol Chem 268: 25273–25276, 1993
5. Zolkiewska A, Moss J: Processing of ADP-ribosylated integrin α7 in skeletal muscle myotubes. J Biol Chem 270: 9227–9233, 1995
6. von der Mark H, Durr J, Sonnenberg A, von der Mark K, Deutzmann R, Goodman SL: Skeletal myoblasts utilize a novel β1 -series integrin and not α6β1 for binding to the E8 and T8 fragments of laminin. J Biol Chem 266: 23593–23601, 1991
7. Moss J, Stanley SJ, Nightingale MS, Murtagh JJ Jr., Monaco L, Mishima K, Chen H-C, Williamson KC, Tsai SC: Molecular and immunological characterization of ADP-ribosylarginine hydrolases. J Biol Chem 267: 10481–10488, 1992
8. Zolkiewska A, Thompson WC, Moss J: Interaction of integrin α7β1 in C2C12 myotubes and in solution with laminin. Exp Cell Res 240: 86–94, 1998
9. Ginsberg MH, Du Y, Plow EF: Inside-out integrin signalling. Curr Opin Cell Biol 4: 766–771, 1992
10. Soman G, Haregewoin A, Rom RC, Finberg R: Guanidine group specific ADP-ribosyltransferase in murine cells. Biochem Biophys Res Commun 176: 301–308, 1991
11. Wang J, Nemoto E, Kots AY, Kaslow HR, Dennert G: Regulation of cytotoxic T cells by ecto-nicotinamide adenine dinucleotide (NAD) correlates with cell surface GPI anchored/arginine ADP-ribosyl-transferase. J Immunol 153: 4048–4058, 1994
12. Okazaki IJ, Kim H-J, Lesma E, McElvaney G, Moss J: Molecular characterization of a glycosylphosphatidylinositol-linked ADP-ribosyltransferase from lymphocytes. Blood 88: 915–921, 1996
13. Wang J, Nemoto E, Dennert G: Regulation of CTL by ecto-nicotinamide adenine dinucleotide (NAD) involves ADP-ribosylation of a p56[lck]-associated protein. J Immunol 156: 2819–2827, 1996
14. Nemoto E, Yu Y, Dennert G: Cell surface ADP-ribosyltransferase regulates lymphocyte function-associated molecule-1 (LFA-1) function in T-cells. J Immunol 157: 3341–3349, 1996
15. Okazaki IJ, Kim H-J, Moss J: A novel membrane-bound lymphocyte ADP-ribosyltransferase cloned from Yac-1 cells. J Biol Chem 271: 22052–22057, 1996
16. Domenighini M, Rappuoli R: Three conserved consensus sequences identify the NAD-binding site of ADP-ribosylating enzymes, expressed by eukaryotes, bacteria and T-even bacteriophages. Mol Microbiol 21: 667–674, 1996
17. Kim H-J, Okazaki IJ, Takada T, Moss J: An 18-kDa domain of a glycosylphosphatidylinositol-linked NAD:arginine ADP-ribosyl-transferase possesses NAD glycohydrolase activity. J Biol Chem 272: 8918–8923, 1997
18. Takada T, Iida K, Moss J: Conservation of a common motif in enzymes catalyzing ADP-ribose transfer. J Biol Chem 270: 541–544, 1995
19. Koch-Nolte F, Petersen D, Balasubramanian S, Haag F, Kahlke D, Willer T, Kastelein R, Bazan F, Thiele H-G: Mouse T cell membrane proteins Rt6-1 and Rt6-2 are arginine/protein mono(ADPribosyl)-transferases and share structure motifs with ADP-ribosylation toxins. J Biol Chem 271: 7686–7693, 1996