Identification and Characterization of CD39/Vascular ATP Diphosphohydrolase*

Elzbieta Kaczmarek‡, Katarzyna Koziat‡, Jean Sévigny§, Jonathan B. Siegel‡, Josef Anrather‡, Adrien R. Beaudoin§, Fritz H. Bach‡, and Simon C. Robson‡

From the §Sandoz Center for Immunobiology, New England Deaconess Hospital, Harvard Medical School, Boston, Massachusetts 02215 and §Département de Biologie, Université de Sherbrooke, Sherbrooke, Québec, J1K 2R1 Canada

Vascular ATP diphosphohydrolase (ATPDase) is a plasma membrane-bound enzyme that hydrolyses extracellular ATP and ADP to AMP. Analysis of amino acid sequences available from various mammalian and avian ATPDases revealed their close homology with CD39, a putative B-cell activation marker. We, therefore, isolated CD39 cDNA from human endothelial cells and expressed this in COS-7 cells. CD39 was found to have both immunological identity to, and functional characteristics of, the vascular ATPDase. We also demonstrated that ATPDase could inhibit platelet aggregation in response to ADP, collagen, and thrombin, and that this activity in transfected COS-7 cells was lost following exposure to oxidative stress. ATPDase mRNA was present in human placenta, lung, skeletal muscle, kidney, and heart and was not detected in brain. Multiple RNA bands were detected with the CD39 cDNA probe that most probably represent different splicing products. Finally, we identified an unique conserved motif, DLGLGASTQ, that could be crucial for nucleotide binding, activity, and/or structure of ATPDase. Because ATPDase activity is lost with endothelial cell activation, overexpression of the functional enzyme, or a truncated mutant thereof, may prevent platelet activation associated with vascular inflammation.

The quiescent vascular endothelium maintains blood fluidity by inhibiting blood clotting, by the regulation of platelet activation and adhesion, and by promoting fibrinolysis. In large part, the antithrombotic effects are mediated by surface molecules present on resting endothelial cells (EC), such as thrombomodulin and heparan sulfate (1). ECs also have the potential to regulate platelet activation by the synthesis of prostacyclin, nitric oxide and by the surface expression of ATP diphosphohydrolase (ATPDase). This ecto-enzyme (EC 3.6.1.5) has been also described as apyrase, ecto-ATPase, ecto-ADPase, nucleotide phosphohydrolase, or ATP pyrophosphohydrolase (2). ATPDases are generally low abundance proteins that have been difficult to purify, given their sensitivity to detergents and the propensity to co-isolate with other proteins. High levels of ATPDase activity are, however, associated with the vasculature (endothelium, smooth muscle, and cardiac cells), lymphocytes, and platelets (2).

Extracellular tri- and diphosphate nucleosides appear in tissue fluids and plasma as a consequence of lysis of blood cells and tissues and through secretion from platelet-dense granules (3). Vascular ATPDase expressed by quiescent ECs hydrolyzes extracellular ATP and ADP to AMP, which is further converted to adenosine by 5′-nucleotidase (3, 4). In addition, ADP, which interacts with purinergic P2R receptors, is a powerful agonist for platelet recruitment, adhesion, and aggregation, whereas adenosine is an antagonist of these processes (5–7). Thus, the function of ATPDase is critical for the inhibition of platelet aggregation following the hydrolysis of ADP to AMP with the ultimate generation of adenosine (4). We have recently provided evidence for the loss of ATPDase activity following EC activation, such as would occur in vascular inflammatory states or xenograft rejection, where platelet deposition is a consistent component (8, 9). Such changes in the level of expression and activity of the ATPDase may, therefore, be of pathogenetic significance and prompt our attempts to identify and study this vascular ecto-enzyme.

The biochemical characterization of ATPDase purified from various tissues and organisms has generally revealed a highly glycosylated protein of molecular mass 70–100 kDa. This ecto-enzyme is Ca2+- and Mg2+-dependent, is not sensitive to known inhibitors of the various other ATPases, and hydrolyzes nucleoside tri- and diphosphates but not monophosphates. These enzymatic characteristics have facilitated classification of these proteins into a subgrouping termed E-type ATPases (2).

Recently, ATPDase from human placenta, porcine pancreas, bovine aorta, and chicken gizzard have been purified and partially sequenced (10–13). Our analysis of these sequence data has shown significant homology to human CD39 (Fig. 1) (14). CD39 is known to be an acidic glycoprotein with molecular mass 70–100 kDa that contains two potential transmembrane regions and six potential glycosylation sites. Interestingly, CD39 was originally described as a B-cell activation marker and has been shown to be expressed on the surface of other activated lymphocytes and quiescent vascular endothelium (14). Additionally, CD39 is considered to participate in the enhancement of cell-cell interactions; monoclonal antibodies (mAb) to certain epitopes of CD39 induce homotypic adhesion, probably through involvement of LFA-1 (CD11a/CD18) (15). Potato apyrase (a plant ATPDase) has been recently purified and was independently found to have sequence homology to...
certain newly identified nucleotide triphosphatases and to murine and human CD39 (16). Subsequent work by Wang and Guidotti has confirmed that B-cell CD39 had ecto-apyrase activity (17). Because the functional significance of these observations remains unclear, we have further evaluated the role of CD39/ATPase in modulating platelet reactivity and examined the distribution and nature of CD39 mRNA expression in human tissues.

MATERIALS AND METHODS

Purification and Sequencing of Mammalian ATPDase—Porcine pancreas and bovine aorta ATPDases were purified according to the methods described previously (11, 12). Purified proteins were sequenced at the Biotechnology Research Institute of Montreal. These sequence data have been placed in the EMBL, DDBJ, and GenBank databases.

Reverse Transcription-PCR—To amplify a cDNA fragment directly from mRNA, 0.1 μg of total RNA was prepared from human umbilical ECs (15) using oligo(dT)16 primer, and the first strand cDNA synthesis was carried out with Superscript Reverse Transcriptase (Life Technologies, Inc.). The product of this reaction served as a template for PCR amplification.

The following primers were designed based on the CD39 cDNA sequence (14) (GenBank accession no. S73813): forward primer, 5′-GAGAATCTAGACAGTAAAAGCCAAGGAAGC-3′; and reverse primer, 5′-GAAAGGATCCGAAAACAAAAGCTGCTACT-3′. The following primers were used for PCR amplification.

ATPDase Activity Measured by Hydrolysis of [14C]ATP and ADP—[14C]AMP was generated by transfection with pCDNA3 and pCDNA3-CD39. The resulting DNA fragment was cloned into the pcDNA vector (TA cloning kit; Invitrogen, San Diego, CA); subsequently, plasmid was multiplied in “One Shot” bacterial expression kit (Qiagen, Chatsworth, CA) and checked for the correct orientation of the insert by restriction mapping; this was then followed by confirmation of the correct sequence (Applied Biosystem, model 373A). All restriction enzymes were from New England Biolabs (Beverly, MA).

Transient Transfection—COS-7 cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum (FCS). Cells were seeded at 5 × 10⁵ cells per 30-mm well and transfected 20–24 h later with Lipofectamine (Life Technologies, Inc.) according to the manufacturer’s instructions. Briefly, the cells were exposed to 1 μg of DNA (pCDNA3 or pCDNA3-CD39) and 4 μl of Lipofectamine in Dulbecco’s modified Eagle’s medium without FCS for 5 h, followed by the addition of an equal volume of Dulbecco’s modified Eagle’s medium containing 20% FCS. Twenty-four h after transfection, the culture medium was changed (Dulbecco’s modified Eagle’s medium without 10% FCS), and −70 h posttransfection, COS-7 cells were used for analyses. Control COS-7 cells used for transfection were negative for CD39 as analyzed by Western blotting and FACScan with anti-CD39 mAb; ATPDase activity was negligible.

Immunocytochemistry—COS-7 cells were fixed with 0.05% glutaraldehyde for 15 min. The presence of CD39 antigen was revealed by anti-human CD39 mAb (Accurate, Westbury, NY) using biotinylated anti-human CD39 (IgG1) (Accurate) or an isotype-matched control mAb, anti-CD25/ACT-1 (IgG1) (Dako, Carpinteria, CA) as the first layer. Cells were washed twice and incubated with antiserum to human CD39 conjugated with fluorescein-5-isothiocyanate (Sigma) for 30 min on ice. Finally, cells were washed twice and analyzed by flow cytometry on a FACScan bench top model using CellQuest II software (Becton Dickinson, San Jose, CA). Data were collected from viable cells only, as determined by propidium iodide uptake.

Cell Lysate and Cell Membrane Preparation—Cells were washed three times with Tris-saline buffer, pH 8.0 at 4 °C, harvested by scraping in 20 mM Tris, pH 8.0, 50 mM NaCl, 0.1 mM phenylmethylsulfonyl fluoride containing aprotonin (0.02 KIU/ml) and centrifuged 800 × g for 15 min at 4 °C. Cells were resuspended in the harvesting buffer and then disrupted in a Potter homogenizer and centrifuged 800 × g for 15 min at 4 °C. Supernatants containing cell lysates were used for Western blot analysis. Cell membranes were used for ATPDase activity and platelet aggregation assays. These were prepared by ultracentrifugation of cell lysates at 47,000 rpm for 1 h at 4 °C, followed by second centrifugation (57,000 rpm for 50 min at 4 °C) of the resuspended pellet. Membranes were resuspended in 7.5% glycerol in 5 mM Tris-HCl, pH 8.0. Protein was measured according to the Bradford method (19).

Western Blotting—SDS-polyacrylamide gel electrophoresis was performed according to Laemmli (20). Proteins (20 μg per lane) were separated on a 10% gel under denaturing conditions, transferred to polyvinylidene difluoride membrane (Immobilon P, Millipore, Bedford, MA) by semidy using electroblotting and probed with either a rabbit polyclonal antibody to porcine pancreas ATPDase or monoclonal antibody to CD39 (Accurate). Bands were visualized using horseradish peroxidase-conjugated goat antirabbit IgG or goat antimouse IgG (Pierce) and the Enhanced ChemiLuminescence assay (Amersham Life Science, Inc.) according to the manufacturer’s instructions.

Polyclonal Antibody to ATPDase—Polyclonal antibody to ATPDase was generated to a peptide sequence corresponding to the C terminus of the purified protein from porcine pancreas (KSTDQETYGALDGLGGA) (11). This antibody cross-reacted with ATPDase from bovine aorta, lung, and trachea, as determined by Western blot analysis.

ATPDase Activity Measured by Hydrolysis of ATP and ADP—[14C]AMP was generated by transfection with pCDNA3 and with pCDNA3-CD39. The resulting DNA fragment was cloned into the pcDNA vector (TA cloning kit; Invitrogen, San Diego, CA); subsequently, plasmid was multiplied in “One Shot” bacterial expression kit (Qiagen, Chatsworth, CA) and checked for the correct orientation of the insert by restriction mapping; this was then followed by confirmation of the correct sequence (Applied Biosystem, model 373A). All restriction enzymes were from New England Biolabs (Beverly, MA).

ATPDase Activity Measured by Hydrolysis of [14C]ADP to AMP—Monolayers of transiently transfected COS-7 cells and appropriate control cells were analyzed for the ability to hydrolyze extracellular [14C]ADP (50 μCi/reaction; DuPont NEN) and was carried out on thin layer chromatography (TLC) plates (Whatman Laboratory Division, Clifton, NJ). The solvent system comprised of isobutyl alcohol:1-phenyl-1,2-propanol:ethylene glycol monooethyl ether:NH₄OH at ratios 90:60:180:90:120. The separated compounds were scanned for radioactivity with a PhosphorImager (Molecular Dynamics, Sunnyvale, CA) and degradation of the [14C]ADP was determined by ImageQuant software according to the manufacturer’s instructions.

Platelet Aggregation Assays—Blood was taken after informed consent from apparently healthy human volunteers and anticoagulated with 0.1 volume 3.2% sodium citrate. Platelet rich plasma was prepared by centrifugation of whole blood at 280 × g for 15 min at 22 °C. The platelet aggregation assay used a two-sample, four-channel Whole Blood Lumi-Aggregometer, model 560 (Chronolog Corp., Havertown, PA). Platelet-rich plasma was preincubated with COS-7 cell membrane or cell lysate preparations for 10 min at 37 °C in a siliconized glass cuvette containing a stirring bar, followed by stimulation with either ADP (5 μM), collagen (5 μg/ml), or thrombin (0.1 unit/ml) (Chronolog Corp.). Platelet aggregation was recorded for at least 10 min. Data were expressed as the percentage of light transmission with platelet-poor plasma equal to 100%.

Effect of Reactive Oxygen Intermediates on ATPDase Activity—COS-7 cells (control, transfected with pCDNA3 and with pCDNA3-CD39) were exposed for 2 h to either H₂O₂ (100 μM; Sigma) or xanthine oxidase (100 units/ml; Boehringer Mannheim) and xanthine (200 μM; Sigma) to directly oxidize the oxidative stress observed to abrogate EC ATPDase function (8). These experiments were performed to determine the direct effect of reactive oxygen intermediates upon enzymatic function and immunoreactivity of the recombinant CD39/ATPase. Cells were harvested, and the lysates and membrane preparations were then used for biochemical analysis to determine their effect on stimulated platelet aggregation and were subjected to Western bloting.

Northern Analysis—For tissue mRNA analysis, a Multiple Tissue Northern blot, a charge-modified nylon membrane to which poly(A)⁺ RNA (2 μg per lane) from multiple human tissues had been transferred, was used (Clontech Laboratories, San Diego, CA). For the probe, our

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(1) Robson, S. C., Caccamarche, E., Siegel, J. B., Candinas, D., Kozik, K., Millan, M., Hancock, W. W., and Bach, F. H. (1997) J. Exp. Med., in press.
pCDNA3-CD39 construct was digested with BamHI and XhoI, and CD39 cDNA fragment (1714 base pairs) was purified with the Gene-Clean kit (Bio101) and labeled with \(^{32}\)PdATP using Ready-To-Go labeling kit (Pharmacia Biotech Inc.). Prehybridization, hybridization, washes, and stripping of the membrane were carried out with the rapid hybridization protocol from Stratagene (La Jolla, CA). Final washes were at 60°C in 0.2 × sodium saline citrate/0.1% SDS. The blots were exposed to Kodak Biomax Mr film (Eastman Kodak Co.) with intensifying screens at 280°C for 1 day.

**RESULTS**

**ATPDase Sequence Analysis**—We purified ATPDase from pig pancreas and bovine aorta according to the method described earlier and sequenced these proteins (11, 12). The obtained sequences originated from the N terminus of the pig pancreas ATPDase and five internal fragments of bovine aorta ATPDase, and all showed remarkable homology to human CD39 (Fig. 1). Fig. 1 also presents previously published partial amino acid sequences of ATPDases purified from human placenta (10) and chicken gizzard (13), which confirm previously unrecognized homology with CD39. The molecular mass of both CD39 and vascular ATPDase is 78 kDa and becomes 54 kDa after removal of Asp-linked oligosaccharides by N-glycosidase (12, 15); these data suggest that CD39 and ATPDase may represent the same protein.

**Isolation of CD39 cDNA and Its Expression in a Mammalian System**—To test the hypothesis that CD39 has ATPDase activity, we isolated CD39 cDNA by reverse transcription-PCR reaction using RNA prepared from human umbilical EC as a template for the first cDNA strand. Primers homologous to CD39 generated PCR product of the expected size, viz. 1714 base pairs. The PCR product was subjected to restriction mapping and then sequenced (data not shown). This confirmed the identity of the PCR fragment with the published sequence of human CD39 (14). The PCR fragment was subcloned into the pRI vector, excised with NotI and SpeI, and subsequently cloned into a mammalian expression vector, pCDNA3. COS-7 cells were then transiently transfected with pCDNA3-CD39 and used for further analyses. In each experiment, we used nontransfected COS-7 cells and cells transfected with pCDNA3 alone as the controls.

The expression of CD39 on the surface of COS-7 cells was confirmed by flow cytometry and immunocytochemistry. Cells used for flow cytometry analysis were incubated with either mAb to CD39 or to CD25 of the same isotype. Cytofluorometric analysis confirmed the presence of CD39 on the surface of transfected COS-7 cells (Fig. 2). Immunocyto-staining confirmed that only cell populations transfected with pCDNA3-CD39 and not the control cells, were stained by the mAb to human CD39 (data not shown).

**Immunological Identity between CD39 and ATPDase**—To evaluate the identity of CD39 with ATPDase, cell lysates from control and transfected COS-7 cells were analyzed by Western blotting (Fig. 3). Both the mAb to CD39 and a polyclonal antibody to ATPDase recognized a band of the same mobility, with the molecular mass ~80 kDa (Fig. 3, A and B, respectively), a finding strongly supporting our hypothesis of identity between CD39 and ATPDase. In addition, the polyclonal antibody recognized a second band around 53 kDa, which could represent a truncated form of the ATPDase nonreactive with the
monoclonal antibody to CD39. Consistently, specific CD39 and ATPDase immunoreactivity was detected only in pCDNA3-CD39 transfected cell populations.

ATPDase Activity of CD39—On the basis of apparent molecular and antigenic similarities between CD39 and ATPDase, we next evaluated selected biochemical functional characteristics of CD39. We incubated COS-7 cell membrane preparations with exogenous ADP or ATP and determined ATPDase activity by measuring divalent cation-dependent phosphate release by standard biochemical techniques. The control COS-7 cells essentially did not hydrolyze ADP or ATP, whereas pCDNA3-CD39 transfected cells exhibited very high levels of ATPDase activity for both substrates (Fig. 4). The ATPDase expressed by CD39 transfected cells did not act on exogenous AMP. In addition, ADP hydrolysis was totally inhibited by 5 mM EDTA, reduced by 60% with 20 mM sodium azide and unaffected by 3 mM ouabain. These inhibitory data are largely consistent with the classification of the vascular ATPDase as an E-type ATPase (2).

We also investigated the activity of expressed enzyme by studying the capacity of intact transfected COS-7 cells to hydrolyze radiolabeled ADP, assayed by thin layer chromatography (TLC). In a representative experiment, transiently transfected cells converted 68% of the 50 $\mu$Ci of $[^{14}C]ADP$ to $[^{14}C]AMP$ over 20 min, as compared to 11 and 9% for cells transfected with the pCDNA3-only and nontransfected COS-7 cells, respectively, under the identical experimental conditions.

Additionally, protein from crude preparations of human umbilical cord vein immunoprecipitated with anti-CD39 also had all of the required characteristics of E-type ATPase (2).

Effect of CD39/ATPDase on Platelet Aggregation—Because our interest in the vascular ATPDase has concerned the regulation of platelet activation by adenosine nucleotides in the setting of vascular inflammatory disorders, we next investigated the effect of COS-7 cells transfected with pCDNA3-CD39 on platelet aggregation in vitro. Membrane preparations from cells expressing CD39 consistently inhibited platelet aggregation in response to exogenous ADP, whereas membranes from the control cells transfected with pCDNA3-only had no significant effect (Fig. 5A). Comparable specific inhibitory effects were observed when platelets were stimulated with collagen (5 $\mu$g/ml) or by low levels of thrombin (0.1 unit/ml) in the presence of the transfected COS-7 membranes. Thus, platelet aggregation in response to agonists other than ADP was inhibited only by COS-7 cells expressing CD39 (Fig. 5, B and C). These latter observations also confirm the established dependence of platelet aggregation following collagen and thrombin stimulation upon platelet-released ADP in vitro.
Effect of Oxidative Stress on ATPDases Activity—To generate oxidative stress and to evaluate the effect of reactive oxygen intermediates upon the ATPDase function of CD39, COS-7 cells transfected with pCDNA3-CD39 were exposed for 2 h to either H₂O₂ or xanthine oxidase and xanthine. Cells were harvested, and cell lysates were used for analysis of their effect on platelet aggregation (Fig. 6A). A striking effect of oxidation was observed for the interaction of transfected cells with platelets. After incubation with the xanthine oxidase/xanthine combinations, pCDNA3-CD39 transfected cells almost completely lost their ability to inhibit platelet aggregation, whereas the prior incubations with H₂O₂ completely abolished the antiaggregatory effect of ATPDase expressed by the transfected COS-7 cells. These results were concordant with the observed loss of ATPDase biochemical activity following oxidative interaction (Fig. 6B) and the reduced immunoreactivity with anti-CD39 monoclonal antibodies (Fig. 6C).

Determination of a Potentially Important Motif in CD39/ATPDase—Once we had determined identity between CD39 and the ATPDases, the subsequent analysis of available amino acid sequences of the ATPDases purified from different mammalian sources led us to the conclusion that there was only one shared sequence that was common to human placental and the porcine pancreatic ATPDase and also present within human CD39, viz. DLGGASTQ, contained within the N-terminal sequence of the porcine pancreatic ATPDase (Fig. 1). This sequence was also identified as the putative apyrase conserved region 4 by Handa and Guidotti (16). However, there are some modifications of this sequence for other ATPDases; therefore, this motif may also be presented as (D/E/L/M/V)GG(A/G)S(T/A/V)Q.

Northern Analysis—To determine tissue-specific CD39/ATPDase mRNA distribution, we analyzed RNA purified from various human tissues by hybridization with the CD39 cDNA (1714-base pair fragment). We found high levels of CD39/Vascular ATPDase...
mRNA in human placenta, lung, skeletal muscle, kidney, and heart but no detectable signal in brain and very little in liver (Fig. 7). We were able to detect as many as five different mobility mRNA transcripts reacting with the same probe, from two up to four of them in the individual tissues. To confirm that these bands were specific for CD39, we hybridized the same blot with 5′ and 3′ fragments of CD39 cDNA, 427 and 342 base pairs, respectively. The resulting patterns of hybridization were identical with that one with the full-length probe (data not shown).

DISCUSSION

Our data demonstrate in a persuasive manner that CD39 encodes the vascular ATPDase. This conclusion was originally based on the discovery of sequence homologies between CD39 and human placental ATPDase and bovine aortic ATPDase (Fig. 1). Our hypothesis was further substantiated by other recognized sequence homologies that we noted in mammalian and avian nonvascular ecto-enzymes and the report that the potato soluble apyrase contains certain “apyrase conserved regions” also found in garden pea nucleoside triphosphatase, Saccharomyces cerevisiae golgi guanosine diphosphatase (GDPase), Toxoplasma gondii isoforms of an nucleoside triphosphatase, NTP1 and NTP3, a yeast hypothetical 71.9-kDa protein, a Caenorhabditis elegans 61.3-kDa protein, and human and murine CD39 (16). The following observations also strengthen our conclusion. Both ATPDases and CD39 are known to be membrane glycoproteins with the same molecular weight range. Both proteins have a comparable cellular distribution, are postulated to be involved in cell adhesion, and may undergo up-regulation after viral transformation of certain cells (2, 14, 15, 22).

We were able to generate CD39 cDNA from human umbilical endothelial cells RNA by reverse transcription-PCR. This PCR product, which was of expected size, was then subjected to restriction mapping and sequencing, which confirmed that this product represented true CD39 cDNA (data not shown). The CD39 cDNA was then cloned into the pCDNA3 vector and expressed in COS-7 cells. Using cell membranes or whole-cell lysates, we established that CD39 protein expressed by these transiently transfected cells reacted with both monoclonal antibodies to CD39 and to polyclonal antibodies directed at ATPDase. By FACS analysis using mAb to CD39, we clearly demonstrated that ATPDase was expressed at the surface of COS-7 cells (Fig. 2). Both polyclonal antibody generated by us to the porcine ATPDase N-terminal peptide fragment and cross-reactive with the bovine vascular ATPDase and monoclonal antibody to CD39 detected the appropriate and same mobility band on Western blotting (Fig. 3).

Our functional data show for the first time that the ATPDase activity associated with CD39 expressed by COS-7 cells can hydrolyze the substrate ADP. This specific ADPase enzyme activity can be induced over 100-fold by the COS-7 transfection with CD39. Likewise, we were able to show enzymatic activity of CD39 for the substrate ATP to be induced over 30-fold by COS-7 transfection (394 nmol/mg protein/min in representative experiments). We also have demonstrated that intact transfected COS-7 cells were able to hydrolyze radiolabeled ADP. This latter result obtained by TLC clearly indicated that the active site of ATPDase faces the extracellular milieu. Wang and Guidotti (17) have also established that Epstein-Barr virus-transformed B cells express both CD39 and potentially Ca2+, Mg2+ apyrase activity. They were able to show by the DEAE-dextran method that COS-7 cells developed 5.4-fold increased ecto-ATPase activity following transfection with CD39 cDNA prepared from B cells, when compared to vector alone (17).

Of potential significance was our observation that preparations of cell membranes from COS-7 cells transfected with pCDNA3-CD39 could inhibit platelet aggregation in response to ADP, collagen, and thrombin (Fig. 5). ADP release from platelet granules is a vital part of the feedback process that amplifies and propagates platelet activation induced by ADP itself or other more potent agonists (3). Hence, ADP may be an important mediator of vascular thrombosis in inflammatory states. It has been suggested that a major role for the ATPDase may be to inhibit ADP or ATP-induced signal transduction in platelets, leukocytes, and vascular endothelium mediated through the purinergic receptors P2x and P2y (2, 23, 24). The hydrolysis of ATP and ADP by ATPDase would remove these purinergic mediators from the extracellular environment and ultimately favor the generation of adenosine (3, 4) with the associated anti-inflammatory sequelae related to the interaction with P1 receptors (2, 4).

CD39 has been shown to play a role in B-cell adhesion, in part related to cellular integrins (14, 15). It is further possible that the interaction of adenosine nucleotides with CD39 may also influence cell signaling and integrin affinity for their respective ligands. The identification of CD39 as the vascular ATPDase and the documentation of the significant role in modulating platelet reactivity in vitro will further help to test this hypothesis in several experimental models.

Our observation that exposure of COS-7 CD39 transfectants to reactive oxygen intermediates results in loss of platelet antiaggregatory properties coupled to inhibition of biochemical ATPDase activity is in keeping with the oxidant-dependent loss of ATPDase function noted in association with EC activation in vitro and following vascular injury in vivo (8, 9). ATPDase activity has been shown to be lost in vivo with reperfusion injury and this process may be ameliorated by the administration of antioxidants (25). We speculate that this loss, and the resultant decreased capacity to degrade ADP, could play a significant role in the extensive platelet activation and vascular inflammation seen in graft rejection and other forms of vascular injury. Certainly, the intravenous administration of apyrases to experimental animals has been shown to prolong xenograft survival and abrogate the platelet activation and deposition seen in this setting (26).

Because of the variable sensitivity of organs to vascular injury and thrombosis, the tissue-specific distribution for CD39/
ATPase was studied. Northern analysis of RNA extracted from different tissues was, therefore, performed with a full-length CD39 probe (Fig. 7). Among the tissues examined, the strongest signals were observed in certain highly vascularized organs, i.e. placenta, lung, skeletal muscle, and kidney. Heart and liver had lower levels of mRNA transcripts. Two dominant CD39 mRNA transcripts are noted in most tissues akin to the pattern observed when mRNA preparations from cultures of human ECs are studied (data not shown). However, we were also able to detect as many as five different mRNA transcripts reacting with the entire CD39 cDNA probe (Fig. 7) and the 3’ and 5’ cDNA regions. These data suggest that these multiple RNA transcripts probably are alternative splicing variants. The pathophysiologic significance of this observation is presently undetermined. Interestingly, there was no convincing evidence for the presence of CD39 transcripts in RNA isolated from human brain. This last observation suggests that the specialized vascular tissues of the brain may not express CD39 at levels comparable to the other organs tested and does not explain the data published previously showing the presence of ATPase activity in nerve tissues and on the external surface of intact synaptosomes (27–29). Possibly other E-type ATPases or ATPases unrelated to CD39 are expressed in brain and hepatobiliary tissues, as would be suggested by the finding that ATPase activity may be demonstrated in immunoprecipitated neural cell adhesion molecule from rat brain (reviewed in Ref. 2) and in cell-CAM105 from rat liver (30).

Further investigation and determination of the CD39/ATPase ecto-enzymatic active site, putative ATP binding sites (31), and regions sensitive to oxidative reactions by sequential mutagenesis experiments will help elucidate the reason(s) for the potential posttranslational modification or other modulation of ATPase activity with EC activation. This knowledge should permit us to express CD39/ATPase in a active form despite EC activation, as we have done for thrombomodulin (32), and to explore the consequences of this intervention in transplantation models associated with vascular inflammation (33).

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