Thromboregulation by Endothelial Cells: Significance for Occlusive Vascular Diseases

To the Editor:

I wish to raise several issues about the “Brief Review” by Dr Aaron J. Marcus and colleagues.

The prior ecto-ADPase or ATP diphosphohydrolase nomenclature is no longer recommended; nucleoside triphosphate diphosphohydrolase or NTPDase is the preferred terminology for this family of enzymes. For example, CD39 can now be classified as NTPDase-1.1

The claim here (and in prior communications) that work by Dr Marcus and colleagues2 was “the first direct demonstration of a physiological function for CD39 . . . blockade of platelet responsive- ness to ADP for recruitment and aggregation than was previously appreciated” is incorrect. Prior to this, we had clearly demonstrated that CD39 cloned from human umbilical vein endothelial cells had all the biochemical and biological properties (inclusive of platelet antiaggregatory capacity) of an ecto-ADPase or ATPDase (old terminology).3 In addition, the pioneering work of Miura and colleagues,4 who demonstrated that CD39 inhibits platelet aggregation to ADP, collagen, and thrombin; additionally, platelets from the mutant mouse null for cd39 are nonresponsive to collagen and thrombin.5

The important cysteine at the N-terminal end of CD39 was omitted in Figure 2 (see also Gayle et al6). This amino acid is crucial for palmitylation of native CD39 and consequent targeting to caveolae.8 This posttranslational modification may be important in modulating the properties of this ectoenzyme and also in blocking endothelial cell reactions to ATP and ADP.9,10

In the area of CD39 mutagenesis and derivation of soluble mutants,6 no reference is made to the work by Guidotti (Wang et al11,12) nor our own.13 Findings that inherently soluble NTPDases (apyrases) could block platelet sequestration in rejected cardiac transplants and prolong xenograft survival have established that these agents have therapeutic potential.14 Because soluble CD39 mutant proteins dramatically lose NTPDase activity, we have also studied gene-based therapies with adenovaliral vectors to upregulate CD39 in the vasculature of cardiac grafts.15

The putative generation of a cd39-null mouse by Marcus and colleagues is described, but surprisingly, there are no data. For unclear reasons, no reference is made to the cd39-null mouse model which we had already generated and characterized in depth.7,16 In keeping with deficient vascular protective mechanisms, fibrin deposition was observed at multiple organ sites in our cd39-null mice. However, we also demonstrated that cd39-null mice displayed prolonged bleeding times secondary to platelet hypofunction.

These data have indicated an important dualistic role for NTPDase-1 in modulating hemostasis and thrombotic reactions that may complicate any planned therapeutic application of this agent.

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Response:
We reply to Dr Simon Robson’s letter as follows. The Editors of Arteriosclerosis, Thrombosis, and Vascular Biology invited us to submit a Brief Review concerning our recent studies on thromboregulation by endothelial cell CD39 and our views on the therapeutic implications of our data. The review, “Thromboregulation by endothelial cells: significance for occlusive vascular diseases,” was not intended as a comprehensive compilation of the literature in the ectonucleotidase field. After peer review, the manuscript was revised and published.1 We were limited in the number of references to be published in the Brief Review and therefore would request that readers interested in the subject matter refer to the publications quoted, as well as the references therein. The main emphasis was on our views of thromboregulation by endothelial cells.

With regard to nomenclature, we stated: “These biochemical activities identify the HUVEC enzyme as an apyrase (ATP diphosphohydrolase), ATPDase, EC3.6.1.5, now classified as E-NTPDase-1.”3 Dr Robson is a coauthor of this quoted publication. For the readers of Arteriosclerosis, Thrombosis, and Vascular Biology interested in thrombosis, we referred to the molecule mainly as CD39/ecto-ADPase.

We claimed to have been the first to demonstrate “a physiological function for CD39 as an ADPase: blockade of platelet responsive-


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ness to the prothrombotic agonist ADP via its metabolism to AMP. While our work and that by Dr Robson and colleagues were submitted in abstract form virtually simultaneously in 1996, final publication of the work of Dr Kaczmarek did precede ours. Our article was the result of a longstanding effort in the study of thromboregulation by human endothelial cells, which began in the 1970s, and our first endothelial cell ecto-ADPase article was published in 1991. In our 1998 and 2000 publications, we quoted Kaczmarek et al. as well as several others by Sévigny and colleagues. Thus, we believe that we have duly recognized the contributions of Dr Robson and associates. In our 1997 article, we did refer to the work by Dr Côté and colleagues as reference 8. In that publication, intimal and medial preparations from bovine aorta were shown to inhibit ADP-induced platelet aggregation. However, the preparations of Côté and colleagues were not pretreated with aspirin and hemoglobin, and therefore, prostacyclin and/or NO could have played a role in the inhibition of platelet aggregation that they observed. Historically, Heyns and colleagues may have been among the first to demonstrate the platelet-inhibitory activity of a vessel wall nucleotidase, and their conclusions are subject to the same limitations concerning possible prostacyclin and NO formation by their tissue extract. In fact, comparable tissue preparations from vessel walls were used to produce prostacyclin.

The statement that “collagen and TRAP depend more on released ADP for recruitment and aggregation than was previously appreciated” was referring to the general thinking in the platelet field, not to the collagen and thrombin experiments of Kaczmarek and colleagues. This statement clearly does not seek priority for us. We do not understand why a cd39-null mouse should be unresponsive to collagen and thrombin. Moreover, as stated by Dr Zimmermann regarding the cd39-null mouse by Enjyoji et al., “some of the results reported in the paper seem paradoxical. Instead of a general increase in the thrombotic state, bleeding times were increased in CD39-deficient mice.”

The aim of Figure 2 was to illustrate the retention of the extracellular domain of CD39 in our construct solCD39, not to illustrate the potential role of posttranslational modifications. We agree with Dr Robson on the potential importance of the cysteine in the N-terminal cytoplasmic domain of CD39. Our review focused mainly on solCD39 as a potential antithrombotic modality. We appreciate the contributions that Drs Guidotti and Robson and their respective colleagues have made in the field of mutagenesis of CD39, especially with regard to the multimerization of CD39 as a transmembrane protein, and the effects that this has on enzyme activity. Our own unpublished studies on truncated C-terminal mutations of solCD39 revealed diminishing activity as the deletion increased. These unpublished observations are complementary to the work of Schulte am Esch and colleagues.

As Dr Robson, we are concerned about the implications of the Enjyoji null mouse for therapeutic applications of CD39. However, our concern is that “the results reported in the paper seem paradoxical.” We find it difficult to reconcile platelet hypofunction with fibrin deposition in multiple organs. We have attempted to comprehend that desensitization of ADP receptors by the presence of circulating ADP is responsible for this phenomenon. In view of the observations that cd39-null and wild-type mice had similar plasma ADP and ATP concentrations and that many mammals have plasma phosphodiesterases that limit ADP and ATP concentrations, we believe that desensitization may not be the entire explanation. We would consider the possibility that the radical deletion in CD39 to accomplish the generation of Enjyoji’s null mouse revealed other, as yet unknown, properties of the cd39 gene that are important in the phenomena that Enjyoji et al. observed.

Our goals focus on the metabolic deletion of adenine nucleotides from the fluid phase of activated platelets in patients with coronary artery disease and stroke. The Brief Review highlights how our research on solCD39 led to development of these objectives.
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