Alloantigenic Composition of the Endothelial Vitronectin Receptor


Endothelial cells synthesize a heterodimeric adhesion molecule, the vitronectin receptor (VnR), which is similar to the platelet glycoprotein (GP)IIb/IIIa complex. The subunits of the endothelial VnR (VnRa and GPIIa) have been studied for their ability to express alloantigens associated with platelet GPIIb and IIIa. We previously showed that endothelial GPIIa can express the platelet alloantigen Zw or Pt, which is associated with GPIIIa. We studied the relationship between the expression of Zw on platelets and endothelial cells in neonates (n = 13). Using immunoprecipitation and immunofluorescence techniques, we showed that the Zw antigen is either expressed or absent from both platelets and endothelial cells of the same individual.

This finding indicates that in both cell types the same gene is expressed. We also showed that Zw-negative endothelial cells express Zw (P), in analogy to Zw-negative platelets. Moreover, our results strongly suggest expression on endothelial cells of Yuk, a recently described platelet alloantigen, also located on GPIIIa. However, we could not demonstrate expression on the endothelial VnRa subunit of Bak, an alloantigen located on platelet GPIIb. These findings are in agreement with the concept that the endothelial GPIIa subunit is more closely related to its platelet counterpart than to the endothelial VnRa subunit. 1988 by Grune & Stratton, Inc.

IT HAS BECOME increasingly apparent that the endothelial and platelet plasma membranes have many structural and antigenic similarities.7,8 Endothelial cells synthesize a plasma membrane protein complex, biochemically and immunologically similar to the platelet glycoprotein (GP)IIb/IIIa complex.8 This endothelial complex was recently shown to be identical to the vitronectin receptor (VnR). The VnR and GPIIb/IIIa belong to a family of structurally and functionally related proteins,9-12 of which the α subunits (including VnRa and GPIIb) are apparently different, although they have significant homology, whereas the β subunits (including GPIIIa) have stronger homology.6,14 Platelet GPIIb and IIIa are genetically polymorphic GPs, because both of them can express one or more alloantigens. The Bak (or Lek) and Bak alloantigens are associated with GPIIb,15-17 whereas the Zw (P), Zw (P), and Pen alloantigens are associated with GPIIIa.18

The structural homology between endothelial and platelet GPIIa has been illustrated by the fact that endothelial GPIIa carries, just like its platelet counterpart, the Zw antigen.20 We now have extended our studies on the expression of platelet alloantigens by the endothelial VnR. To determine whether the same gene is expressed in platelets and endothelial cells, Zw expression on platelets, obtained from umbilical cord blood, was compared with that in cultured endothelial cells, isolated from the same umbilical cord, in normal healthy neonates (n = 13). Both Zw-positive and Zw-negative individuals were studied.

The Zw system on platelets is biallelic, implicating that Zw-negative platelets express the Zw or P alloantigen.21 Therefore, endothelial cells isolated from the umbilical cord of a neonate with Zw-negative platelets were tested for their ability to express Zw, in analogy to the platelets.

Furthermore, endothelial cells as well as platelets were tested for their ability to express the recently discovered platelet alloantigen Yuk,22 located on GPIIIa.23 We also studied whether the Bak or Lek alloantigen can be expressed by endothelial VnRa.

The results of our study provide evidence that the alloantigens associated with platelet GPIIIa are coexpressed by endothelial GPIIIa in vitro, whereas endothelial VnRa fails to express the alloantigen associated with platelet GPIIb.

MATERIALS AND METHODS

Endothelial cell isolation and culture,1 indirect immunofluorescence on endothelial cells in suspension,29 and immunoprecipitation from 125I-labeled endothelial cells24,25 were performed as previously reported. For immunoprecipitation with human antisera, immunopurity-purified antibodies (eluates)26 were used. Sodium dodecyl sulfate (SDS) 9% polyacrylamide gels were run under nonreducing conditions.

Alloantigen typing of platelets obtained from umbilical cord blood. Healthy neonates were screened for platelet Zw expression. Blood from umbilical cords was collected in 0.1 mL 17% K-EDTA. Platelet-rich plasma (PRP) was prepared by centrifugation of whole blood at 1,000 g for 15 minutes. PRP was centrifuged at 2,500 g for eight minutes, and the platelet pellet was resuspended in 10 mmol/L EDTA/150 mmol/L NaCl/10 mmol/L Tris-HCl, pH 7.4. This washing procedure was repeated twice. Alloantigen typing of the washed platelets was performed by indirect immunofluorescence, according to the method of von dem Borne et al.29

Antiseras and monoclonal antibody CLB-C17. Anti-Zw antisera were obtained and characterized as previously described.20 Anti-Zw and anti-Yuk sera were kindly provided by Dr Taaming (Glostrup Hospital, Glostrup, Denmark) and Dr Shibata (Toranomon Hospital and Okinaka Memorial Institute for Medical Research, Tokyo), respectively. CLB-C17 is a monoclonal antibody directed against an epitope expressed only on the intact GPIIb/IIIa complex (P.W. Modderman et al, personal communication, October, 1987).

From the Central Laboratory of The Netherlands Red Cross Blood Transfusion Service, Amsterdam.

Supported by Grant No. 900-526-069 from the Foundation for Medical Research MEDIGON, which is subsidized by The Netherlands Organization for the Advancement of Pure Research (ZWO).

Address reprint requests to J. A. van Mourik, PhD, c/o Publication Secretariat. Central Laboratory of The Netherlands Red Cross Blood Transfusion Service. P.O. Box 9406, 1006 AK Amsterdam, The Netherlands.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1988 by Grune & Stratton, Inc.
0006-4971/88/7201-0041$3.00/0

RESULTS

Expression of Zw\textsuperscript{a} on endothelial cells and platelets. Expression of the Zw\textsuperscript{a} antigen on platelets, as determined by indirect immunofluorescence,\textsuperscript{25} was always (n = 10) accompanied by expression of this antigen on endothelial cells. We used either indirect immunofluorescence or immunoprecipitation or both to determine Zw\textsuperscript{a} expression on endothelial cells.

Anti-Zw\textsuperscript{a} antibodies bound to protein A-Sepharose precipitated two proteins with the same mobility as endothelial VnR\textsubscript{a} and GPIIIa from endothelial cells obtained from platelet Zw\textsuperscript{a}-positive neonates (Fig 1). Although Zw\textsuperscript{a} antigen is located on GPIIIa,\textsuperscript{29} VnR\textsubscript{a} is coprecipitated probably because the VnR\textsubscript{a}/IIIa complex is not dissociated under the conditions used.\textsuperscript{3,4}

Only 2.4% of the Dutch population has a Zw\textsuperscript{a}-negative phenotype. However, we succeeded in obtaining platelets and endothelial cells from three neonates with absent platelet Zw\textsuperscript{a} expression.

When the cultured endothelial cells obtained from these donors were used for immunoprecipitation studies, two different anti-Zw\textsuperscript{a} antisera were unable to precipitate proteins with the mobility of VnR\textsubscript{a}/IIIa, whereas CLB-C17,\textsuperscript{4} a monoclonal antiplatelet GPIIb/IIIa antibody, did precipitate such proteins (Fig 1). When the endothelial cells were tested by indirect immunofluorescence and analyzed by a fluorescence-activated cell sorter, no binding of anti-Zw\textsuperscript{a} antibodies was found (Fig 2D) as compared with Zw\textsuperscript{a}-positive control endothelial cells (Fig 2B).

We had at our disposal a small amount of anti-Zw\textsuperscript{b} antisera, which enabled us to test the endothelial cells of one Zw\textsuperscript{a}-negative donor. The anti-Zw\textsuperscript{a} antibodies did precipitate VnR\textsubscript{a}/IIIa (Fig 3, panel B).

These findings indicate that in Zw\textsuperscript{a}-negative individuals the endothelial VnR lacks Zw\textsuperscript{a} but does carry, in analogy to Zw\textsuperscript{a}-negative platelets, the Zw\textsuperscript{b} antigen.

Expression of Yuk\textsuperscript{b} on endothelial cells. Anti-Yuk\textsuperscript{b} antibodies precipitated VnR\textsubscript{a}/IIIa from either Zw\textsuperscript{a}-positive or Zw\textsuperscript{a}-negative endothelial cells and GPIIb/IIIa from platelets (Fig 3).

Immunoprecipitation with anti-Bak\textsuperscript{a} antibodies. Anti-Bak\textsuperscript{a} antibodies, which precipitated GPIIb/IIIa from Bak\textsuperscript{a}-positive platelets (Fig 3), failed to precipitate VnR\textsubscript{a}/IIIa from endothelial cells obtained from a neonate whose platelets were Bak\textsuperscript{a}-positive and Zw\textsuperscript{a}-negative, whereas anti-Zw\textsuperscript{a} antibodies did precipitate VnR\textsubscript{a}/IIIa from these endothelial cells (Fig 3, panel A).

DISCUSSION

The platelet alloantigen Zw\textsuperscript{a} or P\textsuperscript{A1} is of pathogenetic importance in alloimmune disorders, such as neonatal alloimmune thrombocytopenia and posttransfusion purpura.\textsuperscript{27,28} In analogy to its platelet counterpart, endothelial GPIIIa is able to express the Zw\textsuperscript{a} antigen.\textsuperscript{20}

In this study, we provided evidence that endothelial and platelet Zw\textsuperscript{a} are encoded by the same gene. This evidence is based on a comparison of Zw\textsuperscript{a} expression on platelets and cultured umbilical vein endothelial cells obtained from the same individual. Both platelets and endothelial cells always
expressed the Zw\textsuperscript{a} antigen (Zw\textsuperscript{a}-positive individuals, \(n = 10\)) or lacked Zw\textsuperscript{a} expression (Zw\textsuperscript{a}-negative individuals, \(n = 3\)).

In addition, Zw\textsuperscript{a}-negative endothelial cells expressed, analogously to Zw\textsuperscript{a}-negative platelets, the Zw\textsuperscript{b} or PI\textsuperscript{A2} antigen.

To underscore even more the similarity of platelet and endothelial GPIIIa, we showed (Fig 3) that another alloantigen located on platelet GPIIIa, Yuk\textsuperscript{b},\textsuperscript{23} is expressed by cultured endothelial cells. Expression of Yuk\textsuperscript{a} on platelets could not be demonstrated, even though a large panel of platelets was tested, indicating that this antigen is very rarely, if at all, expressed in the Dutch population.

Platelet GPIIb/IIIa has previously been proposed to belong to a cytoadhesion family of membrane proteins\textsuperscript{4,8,12}, endothelial VnR has been proposed to belong to this family of molecules as well.\textsuperscript{6,9,11,12} Molecular cloning of GPIIb and GPIIIa appears to confirm the relationship of these proteins at the DNA level, in that platelet and endothelial GPIIIa are apparently identical,\textsuperscript{13} and that GPIIb and VnR\textalpha, although they are homologous, differ to some extent.\textsuperscript{7,9}

Our results are in agreement with these findings. During the preparation of our manuscript, other researchers\textsuperscript{29} published a preliminary report describing data in accordance with our results. All alloantigens located on platelet GPIIIa which we studied appear to be expressed on endothelial GPIIIa in vitro. However, the Bak\textsuperscript{a} or Lek\textsuperscript{a} alloantigen, which is associated with platelet GPIIb, appears not to be expressed by endothelial VnR\textalpha, possibly because endothelial VnR\textalpha is structurally different from platelet GPIIb.

Studies on cDNA encoding GPIIIa from Zw\textsuperscript{a}-positive and Zw\textsuperscript{a}-positive individuals are expected to clarify the basic molecular properties responsible for alloantigen expression.

ACKNOWLEDGMENT

We are much indebted to Mrs C. van Dalen for excellent technical assistance, and to Dr M. N. Hamers for constructive criticism and careful reading of the manuscript. We thank the Obstetric Department of the Andreas Hospital in Amsterdam for providing us with umbilical cords and umbilical cord blood. The gifts of anti-Zw\textsuperscript{a} antiserum by Dr Taaning and anti-Yuk\textsuperscript{b} antiserum by Dr Shibata are gratefully acknowledged.

REFERENCES

5. Newman PJ, Kawai Y, Montgomery RR, Kunicki TJ: Systhe-
Alloantigenic composition of the endothelial vitronectin receptor

JC Giltay, OC Leeksma, AE von dem Borne and JA van Mourik