Expression of the Alloantigen Zw* (or P14) on Human Vascular Smooth Muscle Cells and Foreskin Fibroblasts: A Study on Normal Individuals and a Patient With Glanzmann's Thrombasthenia

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The cytoadhesin family consists of platelet glycoprotein (GP) IIb-IIIa and the endothelial vitronectin receptor. The β subunit (GP IIIa) of these complexes expresses the alloantigen Zw* (or Pt4). This alloantigen is not expressed by members of other integrin subfamilies. By using immunoprecipitation and immunoblot techniques, we found that the β subunit of a heterodimer, expressed by cultured human arterial smooth muscle cells and cultured foreskin fibroblasts, carries the Zw* antigenic determinant. Furthermore, the mobilities of the α and β subunits of these two heterodimers are indistinguishable from those of the α and β subunits of the endothelial vitronectin receptor. Therefore, we propose that the smooth muscle cell and fibroblast heterodimer are members of the cytoadhesin family. In Glanzmann's thrombasthenia, platelet GP IIb-IIIa is absent or severely reduced. Previously, we showed that endothelial cells from a thrombasthenic patient normally synthesize and express a GP IIb-IIIa-related molecule (the vitronectin receptor). Here we show that arterial smooth muscle cells, obtained from the same patient, express a surface molecule indistinguishable from the endothelial vitronectin receptor. We also demonstrate that both the endothelial and the smooth muscle cell GP IIb-IIIa-related molecule in this Glanzmann patient express Zw*. Our data indicate that (a) GP IIb-IIIa-related molecules on cell types other than platelets and endothelial cells can express Zw* in vitro, and (b) patients with Glanzmann’s disease can express the Zw* antigen. This study substantiates our view that the defect in Glanzmann’s disease is restricted to the megakaryocytes/platelets.

A LARGE NUMBER of different cell types express structurally related surface molecules involved in cell-adhesive events.1-3 These molecules (integrins) are a family of noncovalently linked heterodimers, each consisting of an α and a β subunit. Human umbilical vein endothelial cells, venous smooth muscle cells, and a number of cell lines, including fibroblast cell lines, express an integrin similar to the platelet glycoprotein (GP) IIb-IIIa complex.4-7 The endothelial molecule, which appeared to be identical to the vitronectin receptor (VnR),8 and platelet GP IIb-IIIa were proposed to make up an integrin subfamily termed cytoadhesins.9 The β subunits of this subfamily, endothelial and platelet GP IIIa, are (nearly) identical, whereas the α subunits, GP IIb and the VnR α subunit, are homologous but distinct.9 Two other integrin subfamilies have been defined, both with identical β subunits and different α subunits.10,11 These subfamilies are the Leu-Cam family,10 expressed on leukocytes, and the more widely distributed VLA family.11

The GP IIb-IIIa-related molecules expressed by smooth muscle cells and fibroblasts have not been classified as members of the cytoadhesin family or one of the other two integrin subfamilies. Because the Zw* (or Pt4) determinant is associated only with the β subunit of cytoadhesins (endothelial and platelet GP IIIa), and not with other integrins,12,13 association of Zw* with the β subunit of certain integrins would underscore the close relationship of these molecules with the members of the cytoadhesin subfamily. Therefore, we studied the smooth muscle cell and fibroblast integrin for their ability to express the Zw* antigen.

We also studied the smooth muscle cells obtained from a patient with Glanzmann’s thrombasthenia. Previously, we showed that the endothelial cytoadhesin of this patient is normally expressed, although the platelet cytoadhesin (GP IIb-IIIa) is absent.15 This observation does not exclude that cells other than platelets may be defective in Glanzmann’s thrombasthenia.16 Therefore, we examined whether Glanzmann smooth muscle cells either lack, or normally express a GP IIb-IIIa-like molecule. We also studied the Glanzmann endothelial cells and smooth muscle cells for their ability to express the Zw* antigen.

The results indicate that fibroblasts and smooth muscle cells of normal individuals and smooth muscle cells of a thrombasthenic patient all express a heterodimer that is very similar if not identical to the endothelial vitronectin receptor. Analogously to this vitronectin receptor and GP IIb-IIIa, the β subunits of these heterodimers express the Zw* antigen, at least in vitro.

MATERIALS AND METHODS

Cell culture. Human umbilical vein endothelial cells were isolated, cultured, and characterized as described.13-15 Smooth muscle cells were isolated from human umbilical cord arteries. The isolation procedure and culture conditions of these cells have been described in detail elsewhere.10,13 Fibroblasts were isolated by outgrowth of explants from foreskins of normal healthy individuals, according to the method of Ross.14 Culture conditions were as described for endothelial cells and smooth muscle cells. Both smooth muscle cells and fibroblasts stained negatively with anti-von Willebrand factor antibodies as judged by indirect immunofluorescence. The smooth muscle cells could be distinguished from fibroblasts by the following

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criteria. First, they each had their own typical morphological aspects. Second, the relative amounts of the different types of prostaglandins synthesized by smooth muscle cells were essentially different from those synthesized by fibroblasts (Table 1).

All cell types were labeled in suspension with \(^{14}\text{C}\)-labeled prostaglandins and cell lysates were analyzed by high-performance liquid chromatography (HPLC) as previously described.

**Immunoprecipitation and immunoblot.** Immunoprecipitation experiments were performed as described before. We used affinity-purified antibodies (eluates) from anti-Zw\(^{a}\) antisera. These eluates were prepared as described. Proteins were examined by electrophoresis on 9% sodium dodecyl sulfate polyacrylamide gels (SDS-PAGE) under nonreducing conditions. Immunoblot experiments were carried out as reported, with some minor modifications: instead of eluates of antisera we used antisera diluted 1:100 in phosphate buffered saline (PBS) containing 1% bovine serum albumin (BSA; Organon, Oss, The Netherlands) and 0.1% Tween-20 (Sigma Chemical Co, St Louis). Proteins of the various cell types that had been subjected to 9% SDS-PAGE, were transferred to nitrocellulose paper. The nitrocellulose was incubated for one hour at room temperature with the antisera, thereafter washed three times with PBS, 0.1% BSA, 0.1% Tween-20, dried, and autoradiographed.

**Patient.** Patient YO is a neonate who was previously reported to fulfill the diagnostic criteria for type I Glanzmann's thrombasthenia. The endothelial cells were isolated from the umbilical cord vein. The cultured cells were shown to normally synthesize and express a GP IIb-IIIa-related molecule (the vitronectin receptor). The smooth muscle cells were isolated from one of the umbilical cord arteries, as described for normal smooth muscle cells.

**Monoclonal antibodies and alloantiserum.** Monoclonal antibody CLB-HEC-75 recognizes the vitronectin receptor from endothelial cells. It also recognizes an epitope that is present only on the intact platelet GP IIb-IIIa complex, not on either subunit of the dissociated complex. CLB-HEC-75 recognizes a protein from platelets and endothelial cells indistinguishable from platelet membrane glycoprotein IIa. The anti-Zw\(^{a}\) sera and control AB serum were obtained and characterized as described.

**RESULTS**

**Expression of the Zw\(^{a}\)-antigenic determinant on the GP IIIa-like subunit of a heterodimer in umbilical artery smooth muscle cells and foreskin fibroblasts.** Monoclonal antibody CLB-C17 precipitated two proteins from both smooth muscle cells and fibroblasts with the same mobilities as the subunits of the vitronectin receptor, precipitated from endothelial cells by CLB-C17 (Fig 1). The apparent molecular weight of the vitronectin receptor \(\alpha\) subunit is 140,000 and that of the \(\beta\) subunit (GP IIIa) 90,000, both under nonreducing conditions (see reference 5). Anti-Zw\(^{a}\) antibodies from two different individuals precipitated two proteins with the same mobilities as those precipitated by CLB-C17 from either smooth muscle cells or fibroblasts.

These observations indicate that both smooth muscle cells and fibroblasts express a surface molecule similar to the endothelial vitronectin receptor. They also indicate that this molecule carries the Zw\(^{a}\) antigen, analogously to the endothelial vitronectin receptor. On endothelial cells, the Zw\(^{a}\) determinant is associated with GP IIa,\(^{12}\) the vitronectin receptor \(\beta\) chain. To confirm that Zw\(^{a}\) on smooth muscle cells and fibroblasts was also associated with the GP IIa-like molecule, and not with the \(\alpha\) subunit of the heterodimer, an immunoblot experiment was performed (Fig 2). The two different anti-Zw\(^{a}\) antisera both recognized a protein with the same mobility as endothelial GP IIIa. Hence, a GP IIa-like molecule in smooth muscle cells and fibroblasts expresses the Zw\(^{a}\) antigen.

Monoclonal antibody CLB-HEC-75, which recognizes a GP IIa-like protein from platelets and endothelial cells but not from other cell types,\(^{26}\) was included as a control antibody in this study. This antibody did not precipitate a GP IIa-like molecule from smooth muscle cells and fibroblasts.

**Expression on Glanzmann smooth muscle cells of a molecule indistinguishable from the vitronectin receptor.** We have isolated the smooth muscle cells from one of the umbilical cord arteries of a previously described patient (YO) with Glanzmann's thrombasthenia.\(^{15}\) CLB-C17 precipitated two proteins from these cells with the same mobilities as the subunits of the vitronectin receptor, precipitated by CLB-HEC-75 from either of these two cell types. Hence, ClotBank endothelial cells failed to express a GP IIa-like molecule recognized by CLB-HEC-75 (Fig 3).

Zw\(^{a}\) expression on Glanzmann endothelial cells and smooth muscle cells. The patient's platelets were Zw\(^{a}\) negative because the protein complex on which Zw\(^{a}\) is expressed, GP IIb-IIIa, was absent.\(^{15}\) However, anti-Zw\(^{a}\) antibodies precipitated two proteins from both the smooth muscle cells and the endothelial cells of the patient. These two proteins had the same mobilities as the proteins precipitated by CLB-C17 from either of these two cell types. Hence, Glanzmann endothelial cells and smooth muscle cells express a heterodimer that carries the Zw\(^{a}\) antigen. This heterodimer is indistinguishable from the vitronectin receptor, expressed by endothelial cells obtained from normal individuals. Since the expression of Zw\(^{a}\) is genetically controlled, at least one of

| Table 1. Relative Amounts of Prostaglandins Produced by Umbilical Artery Smooth Muscle Cells and Foreskin Fibroblasts |
|------------------|------------------|------------------|------------------|
|                   | 6-keto-PGF\(_{10}\) | PGF\(_{2}\) | PGE\(_{2}\) | PGD\(_{2}\) |
| Umbilical artery smooth muscle cells (n = 4) | 1 ± 1 | 4 ± 3 | 94 ± 4 | 1 ± 1 |
| Foreskin fibroblast (n = 5) | 51 ± 10 | 49 ± 10 | 1 ± 1 |

The given numbers are percentages of the total amount of [1-\(^{14}\text{C}\)]-labeled prostaglandins detected after incubation with [1-\(^{14}\text{C}\)]-arachidonic acid, as analyzed by HPLC. The smooth muscle cells produce mainly PGE\(_{2}\), whereas the fibroblasts produce 6-keto-PGF\(_{10}\) and PGE\(_{2}\) in about equal amounts. - , not detectable.
We have provided evidence that cultured human foreskin fibroblasts and umbilical artery smooth muscle cells express a surface molecule that is very similar if not identical to the endothelial vitronectin receptor. The β subunit of this molecule carries the Zw<sup>a</sup> or Pl<sup>41</sup> antigen. So far, Zw<sup>a</sup> was found to be associated only with the β subunit of the two members of the cytoadhesin family, the endothelial vitronectin receptor and the platelet GP IIb-IIIa complex<sup>12-14</sup>. The two subunits of the fibroblast and smooth muscle cell molecule have the same mobilities on SDS-PAGE (under nonreducing conditions) as the α and β subunit of the vitronectin receptor.

Based on these findings, we propose that the fibroblast and smooth muscle cell heterodimers that we studied here are members of the cytoadhesin family. Since these two molecules are indistinguishable from the endothelial vitronectin receptor in terms of electrophoretic mobility and Zw<sup>a</sup> expression, we cannot conclude whether we are dealing with new members of the cytoadhesin family, or whether the fibroblast and smooth muscle cell molecule are identical to the vitronectin receptor.

Although previously a GP IIb-IIIa-related molecule was found on umbilical vein smooth muscle cells and fibroblast-like cell lines<sup>6,7</sup>, the identification of such a molecule on foreskin fibroblasts was unexpected, because other investigators were unable to detect a GP IIb-IIIa-like molecule on these cells<sup>7</sup>. We have no appropriate explanation for these discrepant findings.

The platelet Zw<sup>a</sup> antigen can be of pathogenic importance in alloimmune thrombocytopenias, such as posttransfusion purpura and neonatal alloimmune thrombocytopenia<sup>28,29</sup>. As endothelial cells and, as shown in this study, smooth muscle cells and fibroblasts express Zw<sup>a</sup>, it is conceivable that not only platelets but also these other cell types are involved in these disorders. In particular endothelial cells, which are directly exposed to the blood, might be involved. Anti-Zw<sup>a</sup> antibodies might cause endothelial damage, similarly to anti-endothelial cell antibodies in autoimmune diseases, such as systemic lupus erythematosus (SLE), which cause endothelial cell lysis in vitro<sup>30</sup>. However, at present we have no direct evidence for this.

It is of importance that the Zw<sup>a</sup> antigen is expressed on cell types (endothelial cells and smooth muscle cells) other than

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**Fig 1.** Immunoprecipitation of <sup>125</sup>I-labeled surface antigens from endothelial cells (EC), umbilical artery smooth muscle cells (SMC), and foreskin fibroblasts (FB). HEC-75 is an anti-GP IIa monoclonal antibody<sup>30</sup>, C17 is an anti-GP IIb-IIIa monoclonal antibody. AB is an eluate<sup>13</sup> of normal human serum that served as a control for the eluates of the anti-Zw<sup>a</sup> antisera (S. and de B.). Proteins were analyzed on 9% SDS-PAGE under nonreducing conditions.

the patient's parents should be Zw<sup>a</sup>-positive. Indeed, the platelets of both parents, obligate heterozygotes for the Glanzmann defect, were Zw<sup>a</sup>-positive.
Fig 2. Immunoblot using two different anti-Zw* antisera (S. and de B.) and normal human control serum (AB). EC, SMC, and FB were solubilized, run on 9% SDS-PAGE, and transferred to nitrocellulose. After incubation with human sera, the nitrocellulose was incubated with 125I-labeled sheep anti-human IgG and submitted to autoradiography.

Fig 3. Immunoprecipitation of 125I-labeled surface antigens from EC and SMC isolated from the umbilical cord of a newborn with Glanzmann's thrombasthenia. The same antibodies were used as in Fig 1. Proteins were analyzed on 9% SDS-PAGE under nonreducing conditions.
platelets in a patient with Glanzmann's thrombasthenia. Previously, we have provided evidence that platelet and endothelial Zw<sup>+</sup> are encoded by the same gene(s). These results indicate that the gene(s) encoding Zw<sup>+</sup> in Glanzmann's disease is (are) not defective, although the platelets are Zw<sup>-</sup>-negative because of a lack of GP IIb. Therefore, these results also directly show that the gene(s) encoding Zw<sup>+</sup> is (are) different from the gene(s) encoding the defect in Glanzmann's disease, at least in this patient. A previous study using a different approach led to the same conclusion.

The identification of cytoadhesins on cell types other than platelets in a patient with Glanzmann's thrombasthenia, strongly suggests that the Glanzmann defect is restricted to the platelets (megakaryocytes). It should be noted that a number of variants of Glanzmann's thrombasthenia were described. Therefore, the genetic defect might be different from one patient to another. So, we cannot exclude that, apart from GP IIb-IIIa, in some Glanzmann patients one or more integrons are defective.

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