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THE PLATELET ALLOANTIGEN $Zw^a$ ($Pl^{A1}$) IS EXPRESSED BY CULTURED ENDOTHELIAL CELLS.
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$Zw^a$ ($Pl^{A1}$) is a platelet specific alloantigen, located on glycoprotein (GP) IIIa, and is of pathogenic importance in alloimmunologic disorders such as neonatal alloimmune thrombocytopenia and post transfusion purpura. As endothelial cells synthesize a plasma membrane protein complex which is structurally closely related to the platelet membrane GP IIb/IIIa complex, we examined whether these cells also express $Zw^a$. Employing a variety of immunochemical techniques, our studies show that endothelial cells indeed can express this antigen at the plasma membrane surface.
We also compared the expression of $Zw^a$ on platelets, isolated from umbilical cord blood, with the expression of $Zw^a$ on cultured endothelial cells, isolated from the same umbilical cord, of a number of neonates. Both platelets and endothelial cells obtained from the same individual, either expressed $Zw^a$ ($Zw^a$ positive individuals) or lacked expression of $Zw^a$ ($Zw^a$ negative individuals). These findings strongly suggest that endothelial- and platelet $Zw^a$ are encoded by the same genes.
Thus, $Zw^a$ is not exclusively expressed by platelets, also endothelial cells express this alloantigen.
An important consequence could be, that in alloimmunologic disorders in which the $Zw^a$ antigen is implicated, not only the platelets, but also the vessel wall is involved.

Supported in part by the Foundation for Medical Research MEDIGON (grant no. 900-526-069).

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EXPRESSION OF PLATELET ALLOANTIGENS ON HUMAN ENDOTHELIAL CELLS AND HEL CELLS.
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Analogs of platelet membrane glycoproteins IIb and IIIa (GPIIb-IIIa) have been shown to be synthesized and expressed by human endothelial cells (HEC), a human erythroleukemia cell line (HEL) and various other cells. Previous studies from our laboratory demonstrated that the platelet alloantigen $Pl^{A1}$, is expressed on HEC GPIIIa. Other alloantigen systems, namely, Pen and Bak, are known to be localized on platelet GPIIIa and GPIIb, respectively. Utilizing additional antibodies from patients with PTP specific for $Pen^a$, $Bak^a$, and $Bak^b$ allo-antigens, and isoantibodies (iso-ab) from a patient with Glanzmann's Thrombasthenia (GT), we have studied cultured HEC and HEL cells for expression of epitopes recognized by these antibodies. HEC and HEL cells were metabolically labeled with $^{35}$S-methionine and lysed in 0.5% TX-100 in the presence of 5mM EDTA. Soluble antigens were immunoprecipitated with these antibodies coupled to Protein A-Sepharose and subjected to SDS-PAGE and fluorography. Anti-$Pen^a$ and the GT iso-ab reacted with the GPIIb-IIIa complex from both HEC and HEL lysates, but anti-$Bak^a$ and anti-$Bak^b$ failed to immunoprecipitate GPIIb-IIIa analogs from either HEC or HEL. In an immunoblot assay, the GT iso-ab bound to GPIIIa of both HEC and HEL. Anti-$Pen^a$ failed to react with SDS-denatured proteins. HEL GPIIIa migrates slightly faster than HEC GPIIIa and slightly slower than platelet GPIIIa. These results indicate that the epitopes of platelet GPIIIa recognized by alloantibodies and isoantibodies are shared by GPIIIa analogs of HEC and HEL. GPIIb-associated alloantigens are not expressed by HEC and HEL GPIIb analogs, an observation that is consistent with the decreased structural homology between GPIIb analogs derived from different cell types.

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**THE LOCALIZATION OF PLATELET GpIIb-IIIa RELATED PROTEINS IN ENDOTHELIAL CELL ADHESION STRUCTURE.**
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**Different laboratories have reported that human endothelial cells (EC) synthesize and express surface proteins biochemically and immunologically related to platelet GpIIb-IIIa.However the functional role of this glycoprotein complex in EC has not yet been elucidated.In this study we investigated whether these molecules are involved in the process of EC adhesion to different substrata. Cultured human umbilical vein ECs ,seeded on purified fibrinogen(fg), or vitronectin(VN) coated coverslips, adhered ,underwent spreading, organization of thick microfilament bundles and formation of focal contacts as shown by immunofluorescence and interference reflection microscopy.Polyclonal antibodies raised against human platelet GpIIb-IIIa and cross reacting with the EC form,showed by immunofluorescence a discrete and well organized distribution at cell adhesion structures.Indeed they distributed at vinculin rich focal contacts at the membrane insertion of microfilament bundles of stress fiber type.They were also found at cell to cell contacts and in a diffuse pattern at the dorsal surface of EC.GpIIb-IIIa antibodies added to EC suspensions prior to plating inhibited EC adhesion and spreading in a concentration dependent way.This effect was present at different degrees when EC were seeded on fg or VN being clearly more evident on VN and somehow less apparent on fg.In addition when the antibodies were added to confluent EC monolayers for 24 h they disrupted cell to cell contacts and caused cell rounding and detachment Preimmune serum or control antibodies able to bind to EC external membrane but which did not recognized GpIIb-IIIa proteins were unable to inhibit EC attachment and spreading. These results indicate that EC GpIIb-IIIa complex is involved in the adhesion mechanism of these cells to extracellular matrix proteins.**

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PLATELET AND ENDOTHELIAL CELL CYTOADHESINS ARE BIOSYNTHESIZED AND PROCESSED VIA SIMILAR PATHWAYS.
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The cytoadhesin family represents a group of heterodimeric adhesion receptors with common structural, fonctional and immunochemical properties. Platelet and endothelial cell (EC) GPIIbIIIa related proteins exemplify two members of this family. In the present study the biosynthesis and processing of EC GPIIbIIIa were examined and compared with that of platelet GPIIbIIIa to verify whether the diversity of these cytoadhesins was related to post translational events.
Endothelial cells of human umbilical cord vein origin were metabolicaly labeled with 35S-methionine. The newly synthesized proteins were analyzed and immunoprecipitated with polyclonal antibodies against the purified platelet GPIIbIIIa complex and the isolated GPIIb and GPIIIa. Under non-reducing conditions three bands were detected at 135 kD, 125 kD and 90kD with the anti GPIIbIIIa. Samples obtained from pulse chase experiments and analysed under non reducing conditions indicated that the 135 kD band derived from the 125 kD band. Under reducing conditions the 135 kD generated two bands at 118 kD and 25 kD. The 125 KD band also gave a 118 kD band and the 90 kD band shifted to 100 kD. These results indicated that the mature form of 135 kD is composed of two polypeptidic chains which derive from a common single chain precursor similar to that observed in the megakaryocyte. The mature protein and the precursor molecule were not recognised by anti GPIIb antibodies. Also immunoprecipitated by polyclonal anti GPIIIa antibodies was a single chain protein of 100kD. In addition, endoglycosidase treatement showed that both EC GPIIIa and platelet GPIIIa were glycosylated protein of the high mannose type.
These results indicate that although structural differences exists between platelet and endothelial cell GPIIbIIIa, the two membrane glycoproteins have a similar cellular transit.



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