VON WILLEBRAND FACTOR

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Human urine was analysed using a sensitive enzyme linked immunosorbent assay (ELISA) for von Willebrand factor (VWF) antigen. Urine of healthy persons contained VWF immunoactivity. In the urine of a patient with acute renal failure, the VWF antigen was only detectable after intravenous infusion of VWF-Factor VIII concentrate. The VWF antigen in normal urine was analysed by gel permeation high performance liquid chromatography (HPLC) and gel electrophoresis combined with immunoblotting.

These analyses revealed three immunoreactive components of Mr 350 kDa, 60 kDa, and 20 kDa, respectively, the 60 kDa being the major component. Monoclonal antibodies of known specificity to VWF molecule were used in ELISA and immunoblotting to analyze urinary VWF. The three components reacted with an antibody to the central part of VWF, which is called fragment I, and one which reacts with the antibody that blocks the binding of VWF to collagen.

VWF derivatives of molecular size similar to the largest urinary antigens were also observed in normal plasma. However, there is no obvious relationship between these plasma forms and the products in urine since reduction of plasma and urine yields different products.

These results indicate that VWF antigens excreted in normal urine are most likely fragments of VWF produced by limited degradation in vivo. This degradation preserves the central part of VWF molecule, one which reacts with the antibody that blocks the binding of VWF to collagen.

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PRODUCTION OF ANTIBODIES TO HUMAN VON WILLEBRAND FACTOR IN LAYING HENS. ISOLATION OF IMMUNOGLOBULINS AND APPLICATIONS TO THE DIAGNOSIS OF WILLEBRAND'S DISORDER FROM IMPLANTATION OF EGG YOLK VON WILLEBRAND FACTOR. A. Stierl, M.-L. Wiesel, A. Schwarts, J.-M. Freyssinet and J.-P. Cazenave. INSERM U391, Service d'Hématologie et de Thrombose, Centre Regional de Transfusion Sanguine, Strasbourg, France.

Von Willebrand disease (VWD) is an inherited disorder of primary hemostasis caused by deficiency or structural abnormalities of von Willebrand factor (VWF). VWF circulates in plasma and is also present in platelets. Plasma VWF, the carrier protein for factor VIII is a large multimeric glycoprotein composed of identical subunits linked by disulfide bridges. Plasma and platelet VWF display distinct multimeric electrophoretic patterns. The different VWF subtypes can be classified either by the determination of VWF antigen (VWF:Ag) and/or by multimer analysis. Antibodies to human VWF were raised in laying hens by intramuscular injections of purified human VWF. Immunoglobulins were isolated from egg yolks by selective polyethylene glycol and ammonium sulfate precipitations. These antibodies appeared to be monospecific, as they did not react with the plasma proteins of a patient with severe VWD. The pullets received weekly 50 μg VWF for 4 weeks and then had monthly injections. The antibodies occurred as early as the third injection, the yield being 300 to 500 mg of immunoglobulins per week (6-7 eggs). The titre could be constant over periods greater than 1 year. These immunoglobulins to VWF were tested in VWF:Ag electrowinssommes and for the multimer analysis of plasma and platelet VWF by electrophoresis and immunoblotting techniques. In no case could a difference be detected between assays performed with rabbit monoclonal antisera or with polyclonal antisera to VWF. Ten to 12 multimers could be revealed for normal plasma and up to 12 to 14 bands for normal platelet VWF (1.7 % aggregase). In the case of VWD, the electrophoretic patterns were identical with those of the antibodies. Thus, antibodies to VWF raised in laying hens are a suitable tool to detect and to characterize VWD. Although they do not interact with protease, polyclonal antibodies are certainly useful to produce, as do not contain IgM or IgA. Immunoglobulin fractions can contain up to 10 % of specific antibodies. Since they are available in larger quantities and are easy to isolate, large homogeneous batches of antibodies can be obtained. This method may easily be applied to develop antibodies to a variety of antigens.

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Assessment of plasma VWF abnormalities by clinical coagulation laboratories is difficult because the available test systems for VWF antigen quantification and multimer analysis are expensive, laborious, and require days, months or years. Radioactive anti-VWF antibodies and autoradiographic methods are available and simple, rapid, sensitive alternative techniques for VWF quantification and multimer analysis that can be readily installed in clinical laboratories. Plasma VWF antigen quantification is by a 2 hour enzyme immunoassay that accurately detects levels as low as 0.25 % of normal. Plasma VWF to be quantified is bound to polyclonal monospecific anti-human VWF attached to small glass beads, and anti-human VWF conjugated with alkaline phosphatase is added, followed by potassium ferriyaylde. Optical density (at 490-510 nm) of the red color that develops is directly proportional to the plasma concentration of VWF antigen. Plasma VWF multimer analysis is by a one-day electrophoretic immunoblot procedure. Plasma VWF multimer forms are solubilized in SDS-urea-Trit-GDVTA, separated by horizontal % agarose gel electrophoresis, and transferred to a cationic membrane. Other protein binding sites on the membrane are blocked with milk proteins, and the membrane is overlaid with anti-VWF IgG linked to alkaline phosphatase. VWF multimers are then displayed as blue bands by treatment of the histochemical stain, fast blue RR (commonly used for leukocyte alkaline phosphatase scoring) dissolved in methanol 4% HCl phosphate. This procedure, non-radioactive procedure, and the above described antibody will permit the rapid distinction of classical (Type I) von Willebrand's disease (vWD), characterized by low VWF antigen and multimer analysis, and vWD type II, characterized by a relative deficiency of the largest plasma VWF forms. Unusually large VWF multimers, present in remission plasma of patients with chronic active thrombocytopenic purpura (TPP), are also easily detected using this rapid system of multimer analysis.

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We previously reported the presence of an acquired von Willebrand factor (VWF) abnormality characterized by absence of the highest molecular weight (mw) multimers in 12 children with non-cyanotic congenital cardiac lesions. In order to determine the prevalence and define the characteristic of this defect, a prospective series of 17 children were studied at the time of cardiac catheterization. In addition to standard coagulation and VWF assays, VWF multimeric patterns were determined by SDS agarose electrophoresis using two agarose concentrations, low concentration (0.63 %) and high concentration (3.0 %). Nine of the 17 children had absence of high mw VWF multimers on 0.63 % agarose gels as follows:

<table>
<thead>
<tr>
<th>Cardiac lesion</th>
<th>N</th>
<th>N (%)</th>
<th>Abnormal VWF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ventricular Septal Defect</td>
<td>7</td>
<td>6 (86)</td>
<td></td>
</tr>
<tr>
<td>Atrial Septal Defect</td>
<td>7</td>
<td>2 (28)</td>
<td></td>
</tr>
<tr>
<td>Aortic stenosis</td>
<td>1</td>
<td>1 (100)</td>
<td></td>
</tr>
<tr>
<td>Pulmonic stenosis</td>
<td>1</td>
<td>1 (100)</td>
<td></td>
</tr>
<tr>
<td>Coarctation</td>
<td>17</td>
<td>9 (53)</td>
<td></td>
</tr>
</tbody>
</table>

Of 5 children with absence of high mw VWF multimers on 0.63 % agarose gels, 4 had 3 % agarose gel patterns similar to that seen in patients with Type IIA von Willebrand disease (VWD). Two of 3 studied had the presence of VWF-IIa on electroimmuno electrophoresis. The patterns were not different when samples were drawn into a "cocktail" of proteolytic inhibitors. There was no correlation of the absence of high mw VWF multimers with elevated B thromboglobulin, low dose rintocetin induced platelet aggregation or abnormal platelet VWF subunits. Thus, abnormal VWF multimers are not helpful in the diagnosis of congenital cardiac lesions, particularly VSD. The lack of abnormality in platelet VWF multimers, absent low dose rintocetin aggregation, and presence of VWF:Ag suggests that these alterations may be secondary to fragmentation or are of endothelial cell origin.