0583

08:45 h

EVIDENCE FOR A SECOND MOLECULAR DEFECT IN HEMOPHILIA A. J.E. Brown and C. Hougie. Department of Pathology, University of California, San Diego, School of Medicine, USA

Immunoradiometric assays (IRMAs) for factor VIII coagulant antigen (VIII:CAg) utilize naturally occurring antibodies arising in hemophiliacs or previously normal individuals. Factor VIII:C loses its activity in the presence of these antibodies, and when they are used in an IRMA, little or no binding material, referred to as VIII:CAg, is detected in severe hemophilia A or von Willebrand's disease plasmas. It is therefore generally assumed that such antibodies are specifically directed against a site on the VIII:C molecule. We have observed that factor VIII:C and VIII:CAg fractionate in different regions when gel-filtered factor VIII/vWf concentrates are chromatographed on QAE Sephadex; the vWf appears first, followed by VIII:CAg and then VIII:C. Sucrose density gradient ultracentrifugation on plasma from either a resting or exercised donor demonstrated two separate regions of activity with VIII:C sedimenting significantly faster than VIII:CAg. These separation experiments occurred using plasma prepared from blood collected into DFP and Trasylol and were obtained using two different antibodies in the IRMA. The VIII:CAg in a gel-filtered factor VIII/vWf concentrate was more heat labile (56°C) than VIII:C. These findings suggest that some of the antibodies used in the IRMA may not be directed against a site on the VIII:C molecule, but against sites on another protein (factor VIII coagulant related antigen?).
A sequitur of this hypothesis is that there is a second protein deficiency in hemophilia A.

0584

09:00 h

ANTIBODIES TO F.VIII/vWF SUBUNIT AND CONFORMATIONAL ANTIGENIC DETERMINANTS. DEMONSTRATION OF DISTINCT ABNORMALITIES IN VARIANTS OF vWD. J.P. Girma, G. Pietu, D. Meyer, J.M. Lavergne & M.J. Larrieu. Hôpital de Bicêtre, Paris, France.

Antibodies (Ab) specific for either the subunit (MW 2.4 x 10^5) or conformational antigenic determinants of F.VIII/vWF were prepared in order to investigate further the abnormal antigenic reactivity in variants of von Willebrand's disease (vWD). Goat anti-human F.VIII/vWF Fab were immunoadsorbed with F.VIII/vWF subunits bound to activated thiol-Sepharose and the "anti-subunit" Ab was eluted at pH 2.5. Antibodies which did not bind to the beads were designated as "anti-conformation" Ab. The "anti-subunit" Ab specifically reacted by IRMA with the F.VIII/vWF subunits and with all subunit-containing multimers (MW 4.8 x 10^5 to 20 x 10^6). The "anti-conformation" Ab failed to react with F.VIII/vWF subunits but reacted with native (MW 1 to 20 x 10^6) or partially reduced (MW 4.8 x 10^5) F.VIII/vWF. It was thus specific for antigenic sites resulting from the association of subunits in dimers or multimers of F.VIII/vWF. In 8 patients with a variant of vWD (Type II A), an abnormal antigenic reactivity in plasma, characterized by a dose-response curve non parallel to that of control, was consistently observed by IRMA using the "anti-subunit" Ab. Six showed a normal dose-response curve using the "anti-conformation" Ab while two unique patients also demonstrated an abnormal (non-parallel) response. This study 1) allows the distinction between subunit and conformational antigenic sites on F.VIII/vWF, 2) demonstrates that the decreased antigenicity in type II A vWD is associated in all cases with an abnormality of F.VIII/vWF subunits and in some patients with an additional defect of polymerization and 3) further emphasizes the heterogeneity of variants of vWD.

0585

09:15 h

IMMUNORADIOMETRIC ASSAY OF VIII:CAG, A POTENTIAL TOOL TO DETECT HUMAN ANTI-VIII:C ANTIBODIES. J.A. Hellings, J. Over, F.R. van Leeuwen and J.A. van Mourik. Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands.

A modification of a two-site, solid-phase immunoradiometric assay (IRMA) for Factor VIII coagulant antigen (VIII:CAg) has been evaluated for its potential to detect antibodies against Factor VIII coagulant activity (VIII:C) in patient plasma samples. For this purpose the assay system comprised four steps: 1) coating of test tubes with human anti-VIII:C, 2) incubation with normal Factor VIII-VWF complex, 3) incubation with test sample, and 4) binding of radiolabeled human anti-VIII:C as marker.

Of eight hemophilic plasma samples containing antibodies against VIII:C (as detected in a clotting assay) five were able to prevent binding of radiolabel partially and three prevented this completely. One of these three (which actually was the antibody used in the IRMA), was effective even at very high dilutions. Two hemophilic plasma samples without detectable antibodies in the clotting assay, a severe Von Willebrand's disease plasma and normal plasma samples showed no significant interference with binding of radiolabeled human anti-VIII:C. Also, a plasma sample containing a high titer of spontaneous human antibody to VIII:C as well as a heterologous antiserum against Factor VIII-VWF complex did not interfere with binding of radioactivity.

It is concluded that the test system described is a sensitive tool to detect antibodies of the same specificity as those used in the IRMA. It may also detect antibodies of differing specificity. The lack of crossreactivity with some antibodies points to interindividual differences in specificity of antibodies against VIII:C.

0586

09:45 h

SPECIFIC PRECIPITATING ANTIBODIES TO VIII:CAg IN TWO PATIENTS WITH HAEMOPHILIA. J.M. Lavergne, D. Meyer, J.M. Girma & M.J. Larrieu. Hôpital de Bicêtre, Paris, France.

IgG and Fab fragments were prepared from the plasma of two haemophilia A patients whose anti-VIII:C titres were 2,000 and 400 Bethesda U./ml. 125-I-IgG and Fab specific for VIII:CAg were purified by a solid phase procedure and the reactivity of these two antibodies with VIII:CAg was studied by immunoprecipitation in agarose. With double diffusion and autoradiographic methods, both the IgG and Fab antibodies showed a precipitin line against normal plasma, serum, haemophilia A+ plasma, cryoprecipitate, purified F.VIII/vWF or VIII:C free of vWF. No precipitin line was observed in haemophilia A- or severe von Willebrand's disease. With electroimmunoassay (radio-Laurell) using both IgG and Fab antibodies, results of VIII:CAg were in agreement in all samples with those obtained by immunoradiometric assay using the same antibodies. The specificity of the immunoprecipitation observed in agarose with IgG or Fab fragments was assessed by modifying the pH (7.5 to 9.5) of the buffer, the ionic composition (0.15, 0.5 and 1M sodium chloride, 0.15 and 0.5M potassium iodide, 0.15 and 0.5M sodium thiocyanate) of the washing fluid, or by carbamylation of the anti-VIII:CAg IgG. In all cases, specific precipitation was observed. In addition, 125-I-IgG or Fab isolated by the same technique from normal plasma gave no precipitin line with VIII:CAg. This study demonstrates that, contrary to previous evidence, 1) human anti-VIII:CAg antibodies are both precipitating as well as neutralizing when studied by highly sensitive techniques; and 2) monovalent Fab can also precipitate VIII:CAg. The results of this study raise some questions about the lattice theory of immunoprecipitation.