

STUDIES ON THE RELATIONSHIP BETWEEN FACTOR VIII RELATED ANTIGEN (VIII_RAg) AND FACTOR VIII CLOTTING ANTIGEN (VIII_CAg) BY IMMUNOELECTROPHORESIS AND AUTORADIOGRAPHY USING ¹²⁵I ANTI VIII_CAg. B.L. Davies, R.A. Furlong and I.R. Peake, Department of Haematology, Welsh National School of Medicine, University Hospital of Wales, Cardiff, U.K.

In order to determine the relationship between factor VIII related antigen (VIII_RAg) and factor VIII clotting antigen (VIII_CAg), a technique has been developed which involves preincubation of plasma and sera samples with ¹²⁵I anti VIII_CAg antibodies, prior to immunoelectrophoresis against anti VIII_RAg. Autoradiography of the VIII_RAg immunoprecipitin line indicates the presence or absence of anti-VIII_CAg associated with the factor VIII complex. In normal citrated plasma, the presence of ¹²⁵I radioactivity associated with the precipitin arc suggests that VIII_RAg and VIII_CAg are associated. The lack of radioactivity associated with VIII_RAg in serum suggests complete dissociation of VIII_RAg and VIII_CAg. This pattern was also seen in recalcified normal plasma and in purified factor VIII concentrates which had previously been incubated with purified thrombin, the radioactivity in the latter case being concentration dependent, with complete dissociation of VIII_RAg and VIII_CAg at levels >0.1 u/ml thrombin. This result suggests that the dissociation of VIII_RAg and VIII_CAg in serum may be due to the action of thrombin in the clotting process. In VIII_CAg positive (CRM+ve) plasma samples, radioactivity was associated with the top of the VIII_RAg rocket only, which is similar to that seen for heparinised normal plasma. Reduction of radioactivity occurred with increasing ionic strength when high molar NaCl was incubated with normal citrated plasma. This technique is useful in studying the relationship between VIII_RAg and VIII_CAg, and in the identification of CRM+ve haemophilia.

0381

A GENETIC VARIANT OF FACTOR IX WITH AN ABNORMAL HIGH MOLECULAR WEIGHT. R.M. Bertina and I.K. van der Linden. Haemostasis and Thrombosis Research Unit, Leiden University Hospital, Leiden, The Netherlands.

In 1979 we reported a genetic variant of factor IX that behaved very similarly to acarboxy-factor IX (Br J Haematol 1979;42:623-35). With respect to normal FIX it showed an²⁺ increased electrophoretic mobility in the presence of Ca²⁺, a low specific coagulant activity, strongly reduced affinity for adsorption to Al(OH)₃ and faint precipitation lines against anti-factor IX serum. These properties strongly suggested abnormal Ca²⁺ binding properties of this FIX-variant.

The FIX-variant was isolated from the patients plasma using affinity chromatography on anti-human FIX-Sepharose, heparin-Sepharose chromatography and DEAE-Sephadex chromatography. Purification of the FIX antigen was followed by counter immunoelectrophoresis or electroimmunoassay; 50-70% of the FIX-variant was recovered in the final preparation.

Analysis by SDS-PAGE revealed that the FIX-variant has an apparent MW of ca 85,000, which is much higher than that of either FIX or acarboxy-FIX (MW 63,000). After reduction two bands appear: one of 63,000 and one of 35,000. Apparently an additional polypeptide chain is associated with the FIX molecule in this variant. Incubation of the isolated FIX-variant with RVV-X, XIa or Xa does not result in cleavage of either α or β bond.

The abnormal MW of the FIX-variant was also demonstrated by gel-chromatography of the patients plasma or plasma of an obligatory carrier from the patients pedigree. Using a radioimmunoassay, which is specific for the FIX-zymogen, it was found that also in plasma the FIX-variant is not 'activated' by XIa.

It is hypothesized that the association of a FIX molecule with a 35,000 polypeptide interferes both with Ca²⁺ binding and with proteolytic degradation by its physiological activators.

0380

THE USE OF RADIOLABELLED FVIII:CAG ANTIBODIES TO CHARACTERISE FVIII:C ABNORMALITIES. I.J. Mackie and M.J. Seghatchian. North London Blood Transfusion Centre, Edgware, Middlesex. U.K.

The development of a simple method of characterising various forms of FVIII:C and identifying the functional abnormality in disease states is important for accurate diagnosis, treatment, and in the preparation of clinical concentrates. We report an electrophoretic method using radiolabelled antibodies to monitor the distribution of FVIII:CAG. Normal and patient plasmas were incubated with ¹²⁵I-anti VIII_C (*IgG or *FAB), prior to electrophoresis in 1% agarose gels. The gels were then cut into fractions and the radioactivity was measured. Results were compared with crossed immunoelectrophoresis for FVIII_RAg. In normal plasma, bound *IgG/*FAB migrated in the same area as the slow moving side of the FVIII_RAg peak. In severe classical von Willebrands disease and haemophilia A (no detectable FVIII:CAG) plasmas, the radioactivity pattern was, as expected identical to running antibodies alone. Haemophilia A plasma with 100% FVIII:CAG activity (by fluid phase assay) gave a radioactivity pattern identical to normal plasma. Patients with variant von Willebrands disease (decreased levels of the larger FVIII multimers), also had antibody binding patterns similar to plasma.

These preliminary findings show that the method is suitable for identifying FVIII:C abnormalities. The fact that FVIII:CAG was only seen in association with slow moving FVIII_RAg, could be caused by the method of preparing *IgG/*FAB; this may select antibodies with high affinity for more aggregated forms of FVIII. Better resolution between bound and free antibody was obtained when *FAB was used; increasing the gel strength and plate length may improve separation of smaller FVIII populations. The above method has been successfully applied to the identification of various FVIII forms in clinical concentrates, and has important implications in the management of patients with coagulation abnormalities. Since only small sample volumes are required, the method may be used on finger prick and foetal blood samples.

0382

EVIDENCE OF POSSIBLE LINKAGE BETWEEN VON WILLEBRAND'S DISEASE (VWD) AND 2 POLYMORPHIC GENETIC MARKERS. E.S. Barrow, H.M. Reimer, K.K. Nambodiri, R.C. Elston, and J.B. Graham. Departments of Pathology and Biostatistics, University of North Carolina, Chapel Hill, NC 27514, USA.

VWD shows great variability within and between families. In some kindred the affected persons show decreased amounts of an apparently normal VIII_RAg. In others, abnormal electrophoretic mobility of VIII_RAg has been associated with a defect in the degree of polymerization. Within kindred, expressivity may be so variable that some transmitters have normal laboratory findings. Detection of linkage between VWD and a clearly defined antigenic or biochemical marker might provide the means to make unambiguous diagnoses and to distinguish between the effects of the multiple genes involved in synthesis of VIII_RAg.

We have examined 4 VWD kindred, using 23 genetic markers. Individuals were classified for VWD using clinical and laboratory data, pedigree information and 2 statistical procedures: D I based on 3 measures of F VIII activity, and D II which also included bleeding time and a subjective index of symptoms. Using D I, a LOD score of 0.66 at a recombination frequency (θ) of 0.20 was found with GPT (glucose pyruvic transaminase). Using D II a LOD score of 0.50 at a θ of 0.25 was found. Most of the evidence of a VWD-GPT linkage was provided by a single kindred. Using D II, evidence suggesting a second linkage was observed between VWD and GLO (glyoxylase) with a LOD score of 1.03 at a θ of 0.20, all 4 families contributing.

The LOD scores reported are suggestive of linkage and warrant further study. Since GLO relates to Chromosome 6 while the chromosomal location of GPT is not known and since GPT and GLO are unlinked, VWD may be genetically heterogeneous, 2 or more loci being involved.

Studies to clarify the relationship of VWD to Chromosome 6 are underway using markers known to be linked to GLO.