DIFFERENT FORMS OF FACTOR VIII RELATED ANTIGENS IN ENDOTHELIAL CELL CULTURES, CRYOPRECIPITATE SUPERNATANT AND NORMAL PLASMA. J.E. Peake, D.J. Bloom and S.A.M. Roep. University Hospital of Wales, Cardiff, U.K.

The nature of factor VIII related antigen (FVIIIIRAg) was studied in endothelial cell cultures (EC), cryoprecipitate supernatant (cryo-S) and gel-filtered plasma fractions (GFP). Antigenic reactivity (AR) was assessed using the Laurell electroimmunoassay (LA) and a two-site immunoradiometric assay (IRMA) using a non-coagulation inhibitory rabbit antiserum raised against a Factor VIII concentrate prepared by cryoprecipitation and gel filtration. Electrophoretic mobility (EM) of FVIIIIRAg was assessed by two dimensional crossed immunoelectrophoresis (CIEID). Histoelastin co-factor activity (HCoF) was estimated using fixed platelets.

In normal plasma the results of LA, HCoF assay and IRMA were in agreement. FVIIIIRAg synthesised by EC showed increased EM and, in the IRMA decreased AR when compared to LA often with non-parallel dose response curves (DRC). This effect was not prevented by the presence of protease inhibitors in the culture medium. HCoF activity was reduced compared with LA and was related to the parallelism of the DRC in the IRMA. In cryo-S and the late clotting GFP, increased EM of FVIIIIRAg was seen, and in these preparations also the DRC of the IRMA were not parallel to the normal plasma DRC. AR and HCoF activity were disproportionately reduced when compared to the LA. It is concluded that the IRMA is sensitive to the presence of different forms of FVIIIIRAg, particularly those which exhibit low levels of HCoF activity and increased EM. Some of these forms are present in normal plasma.


Human factor VIII is a glycoprotein and tends to form a variety of large aggregates, the presence of which was demonstrated by a number of techniques including the use of large pore gel chromatography and electrophoresis using a polycrylamide gel system designed for high molecular weight aggregates. Factor VIII aggregates can be fractionated in part according to molecular size. Reduction of these polymers by 2-mercaptoethanol results in presumably identical fragments suggesting that all polymers, though differing in size, are composed of identical subunits.

Factor VIII aggregates are particularly sensitive to proteolytic breakdown by trypsin and plasmin as judged by large pore polycrylamide gel electrophoresis. Short-term incubation of factor VIII with trace quantities (equivalent to plasmin- or trypsin-like activity present in normal plasma) of these enzymes respectively results in substantial fragmentation with concomitant loss of both factor VIII procoagulant and Von Willebrand factor activity. However, factor VIII activity is lost prior to discernible protein fragmentation whereas Von Willebrand factor inactivation is associated with advanced protein degradation. When the ionic strength of the medium is lowered the susceptibility of factor VIII to proteolytic breakdown by trypsin is increased dramatically. Interestingly, degradation of Factor VIII by plasmin is not affected by the ionic strength. These data, then, provide conclusive evidence of microheterogeneity of normal human factor VIII and may account for the observed heterogeneity of factor VIII on crossed immunoelectrophoresis.


When compared with VIII:AMF in normal citrated plasma, VIII:AMF activity showed increased lability at 37°C in the 'late' post-transfusion plasma (VIII:AMF>VIII:VWF) of a patient with von Willebrand’s disease, but not in the 'early' post-transfusion plasma in which VIII:AMF>VIII:VWF. VIII:AMF was also labile in the baseline plasma of 3 patients with von Willebrand’s disease in whom VIII:AMF>VIII:VWF. In two of these patients the lability of Factor VIII antigen (on crossed immunoelectrophoresis) was increased. VIII:AMF was not excessively labile in 4 other patients in whom VIII:AMF>VIII:VWF. In all of the above cases, VIII:AMF was stabilized by the addition of either purified von Willebrand factor or plasma of patients with hemophiliacs, but not by plasma of patients with severe von Willebrand’s disease. Thus, VIII:VWF may serve to stabilize VIII:AMF and this might explain the post-transfusion findings in von Willebrand’s disease.