1916

IMMUNE TOLERANCE INDUCTION IN HEMOPHILIA A WITH INHIBITOR. C.K. Kasper (1),N.L.Sanders (1) and N.P.Ewing (2). Departments of Medicine (1) and Pediatrics (2), University of Southern California; and Orthopaedic Hospital, Los Angeles, California, U.S.A.

Factor VIII (FVIII), 50 U/Kg/day, was given without immunosuppressive drugs to 11 patients with hemophilia A and inhibitor. Interval since inhibitor diagnosis was 4-6 years (yr) in 3 cases and 12-27 yr in others. Six had received no FVIII since inhibitor diagnosis. Intervals since last FVIII use were 2 months (mo) in 3 cases and 3-15 yr in others. Historic peak inhibitor levels were 3,8,9,10,25,27,64,375,809,1100 and 2780 Bethesda units (BU). Levels at start of protocol (baseline) were under one BU in 7 cases and 2,42,265 and 398 in others. Evidence of HIV infection exists in 10 patients: 8 of 9 tested have HIV antibody, 7 of 11 tested have low T4 cell counts and 4 currently have AIDS-related complex. Results after 4-31 mo (median 10) are as follows. Peak inhibitor levels on protocol exceeded baseline in only 6 cases and exceeded historic peaks in only 2 cases. All peaks occurred during the first mo and levels fell markedly by the second. All patients but one had levels below baseline by the third mo. Among 7 patients with one BU or less baseline, no inhibitor was detected after one mo in 4 cases and after 3 mo in 2 cases; another child still has trace inhibitor after 8 mo. A child with baseline 2 BU had no inhibitor after 4 mo. Three older patients have not responded completely. In 2 adults who began the protocol 2 mo after intense FVIII use for hemorrhages, baselines of 275 and 398 BU fell rapidly in the first 4 mo but then plateaued around 20-30 BU despite 9 and 26 mo further therapy. In a teenager who began the protocol 4 yr after intense FVIII use, the baseline of 42 BU has not fallen in 4 mo of therapy. Thus, rapid induction of immune tolerance was achieved in 7 of 8 patients with low baselines; the program was less useful in patients with high baselines. Of 4 patients resistant to full induction of immune tolerance, all have evidence of HIV infection; 3 were tested and have HIV antibody, 2 have low T4 counts and 2 have ARC. Thus, the immune suppression of HIV infection may not potentiate induction of FVIII tolerance. All patients achieving FVIII tolerance remain of FVIII prophylaxis. In some, low-level inhibitors emerged on attempts at withdrawal. Induction of immune tolerance with FVIII is advised for patients with currently-low inhibitor levels because of the high success rate.

1917

REMOVAL OF HUMAN ANTIBODIES TO FACTOR VIII:C FROM HEMOPHILIAC PLASMA USING NEW SYNTHETIC SORBENTS. H. Messaïkeh (1), N. Belattar (1), D. Gulino (1), J. Jozefonvicz (1), Y. Sultan (2). Laboratoire de Recherches sur les Macromolecules, Université Paris-Nord, Villetaneuse, France (1) and Laboratoire d'Hémostase Hôpital Cochin, Paris, France (2).

Human antibodies that neutralize factor VIII procoagulant activity (Anti VIII:C) detected in polytransfused patients with hemophilia A cause serious difficulties for the affected patients as they inactivate any injected FVIII preparations.

We hypothesized that FVIII:C might possess active sequences composed of amino acids able to bind Anti VIII:C antibodies. Consequently, completely synthetic resins with suitable chemical substituents mimicking these sequences might interact with Anti VIII:C antibodies. Based upon this hypothesis, crosslinked polystyrene was substituted by various amino acids or their derivatives in order to obtain completely synthetic adsorbents able to remove Anti VIII:C antibodies from hemophiliac plasmas. To establish the relationship between chemical composition of the resins and their affinity towards Anti VIII:C antibodies, the "in vitro" removal of these inhibitors from hemophiliac's immunoglobulins G was tested by measuring simultaneous adsorptions of either IgG or Anti VIII:C antibodies. Specific and accurate methods well adapted for studying the adsorption of these two kinds of proteins were used for evaluating either the IgG concentrations (rocket immunoelectrophoresis) or the Anti VIII:C concentrations (immunoradiometric method). To determine the most suitable chemical groups able to develop a specific adsorption, a first screening on amino acid substituents was undertaken and allowed the selection of glutamic acid and hydroxyproline. In fact, among the twenty resins tested, the most interesting one is obtained by linking glutamic dimethyl ester derivative onto the polystyrene matrix. This resin possesses a pseudo-specificity towards Anti VIII:C antibodies as it is possible to remove 12 % of Anti VIII:C antibodies and only 3 % of IgG on 5 mg of this resin. Furthermore, the hydrophobic sites on the surface seems to be involved in the Anti VIII:C adsorption as demonstrated by comparing selectivity obtained with mono and diester derivatives either in aspartic or glutamic series.

1918

TOLERANCE INDUCTION IN HIGH-RESPONDING HEMOPHILIACS WITH F VIII ANTIBODIES BY MEANS OF COMBINED TREATMENT WITH IgG, CYCLOPHOSPHAMIDE AND F VIII. I.M. Nilsson, E. Berntorp and O. Zettervall. Department for Coagulation Disorders, University of Lund, Malmö General Hospital, Malmö, Sweden.

Of 10 patients with hemophilia A and antibodies, 7 have been rendered tolerant by means of combined treatment with high-dose IgG i.v., cyclophosphamide and F VIII. When the initial antibody concentration exceeded 10 Bethesda inhibitor units per ml, the treatment was preceded by antibody adsorption to protein A. Six of the tolerant patients were originally classified as high-responders. After one week of the combined treatment, VIII:C dropped and the inhibitor reappeared in low titer. The F VIII infusions being continued alone, the inhibitor disappeared in the following week and VIII:C increased satisfactorily after infusion, while VIII:Ag (assayed immunoradiometrically) reached very high concentrations. In one patient the treatment had to be repeated once. Except for transient leukopenia, no side effects occurred. Earlier treatments with F VIII in combination with cyclophosphamide gave high anamnestic response. In two of the remaining three non-tolerant patients, anamnestic response decreased dramatically after two courses of the combined treatment. The tolerant state seems to be stable, as the tolerant patients have now been on regular prophylaxis with F VIII concentrate for periods varying from four months to four years. The half-life of infused F VIII is normal, while that of VIII:Ag is prolonged. On the basis of similar findings in hemophilia B patients, we believe the VIII:Ag to have become modified and complexed to a 'new' antibody which lacks VIII:C inhibitory activity. It is known that modified antigen may act as a tolerogen. The tolerant state may thus be sustained by maintaining consistent concentrations of the modified antigen by means of the F VIII treatment. We conclude that the combined treatment described here is a safe and effective method of tolerance induction in hemophilia A patients.

1919

IMMUNOASSAY FOR FACTOR VIII-HEAVY CHAIN. AN INDICATOR FOR IMMUNE COMPLEXES DURING HIGH DOSE FVIII INHIBITOR TREATMENT. O. Nordfang (1), M. Ezban (1). J.B. Knudsen (2). Nordisk Gentofte, Gentofte, Denmark (1) and Rigshospitalet, Copenhagen, Denmark (2).

Specificity studies have shown that most hemophilia A inhibitor antibodies are directed towards the light chain of coagulation factor VIII (FVIII). Thus, conventional immunoassays for FVIII-antigen (FVIII:Ag) presumably have reactivity for FVIII-Light Chain (FVIII-LC). Our sandwich FVIII:Ag assay has been shown to be specific for only FVIII-LC. We have now developed a specific immunoassay for FVIII-Heavy Chain (FVIII-HC). This has made it possible to investigate the FVIII-HC content in hemophilia A plasma, and to study the expression of FVIII-HC in culture medium from transfected cell lines.

By adding purified FVIII-LC and FVIII-HC in coagulation inhibition assay, plasma from one of seven hemophilia A inhibitor patients was found to be reactive with both FVIII-LC and FVIII-HC. IgG from this plasma was used for a FVIII-HC specific inhibition radioimmunoassay. The polyspecific antibodies were coated to microplates with removable wells. The coated wells were incubated with test sample and with purified ^{125}I-FVIII-HC. When normal human plasma pool contains 1 U/ml of FVIII-HC, the sensitivity of the assay was 0.004 U/ml.

For normal plasma and plasma from non inhibitor hemophilia A patients, FVIII-HC measurements correlated with FVIII:C and FVIII-LC measurements. However, after FVIII injection hemophilia A inhibitor patients in high dose FVIII treatment showed a much higher FVIII-HC content (1 - 5 U/ml) than FVIII-LC and FVIII:C (< 0.05 U/ml). These patients have previously been shown to have antibodies towards FVIII-LC. Therefore the antigen measurements indicate that inhibitor patients in high dose FVIII treatment have FVIII/anti-FVIII-LC immune complexes. These circulating immune complexes may be the mediator of an antibody dependent immune tolerance, during the high dose FVIII treatment.