NOTE

SPECIFICITY OF ANTISERA TO SUBUNIT A OF BLOOD CLOTTING FACTOR XIII IN IMMUNODIFFUSION

Serological techniques have been introduced into the assay of human plasma for various blood clotting factors in supplementing the hitherto established functional assay methods. Sometimes, however, discrepancies are observed between the antigenic reactivity and the functional activity of a clotting factor.

Among the serological tests for factor XIII (FSF), single radial immunodiffusion, rocket immunoelectrophoresis and the antibody neutralization test are said to be the methods of choice for quantitative determination of the factor (Schwick, Trobisch and Heimburger, 1973). The last-mentioned method, elaborated by Bohn and Haupt (1968), is based on consumption of factor XIII in the test material by a dilution series of specific antibody directed to it.

Nevertheless, it was soon found that the antigenicity of factor XIII was still detectable in human sera by the immunodiffusion technique in spite of complete absence of the fibrin-stabilizing activity therein. Similarly, plasma of some patients completely devoid of the factor XIII activity was found to contain 25–50% of the normal antigenicity when determined by the immunodiffusion technique (Becker, Gierhake and Schwick, 1969).

It was then discovered by Bohn, Haupt and Kranz (1972) that factor XIII in human plasma is consisted of two subunits A bearing the specific activity and one subunit S having no activity. Thus, it was thought to be only free subunit S that could be detected by the immunodiffusion technique in the serum after clotting had taken place and also in factor XIII-"deficient" plasma. Since then, the antibody neutralization test has been performed with the specific antiserum to only subunit A, excluding any reaction between subunit S and its corresponding antibody.

Attempts were made to see if anti-subunit A serum could satisfactorily be specific when used in various immunodiffusion techniques.

The anti-subunit A serum used, supplied by the Behringwerke, was included in a test-kit for the antibody neutralization test.

As test samples, human plasma, human serum and Liquid Human Plasma (Minimum Requirements, 1971) were used. In some experiments, a partially purified factor XIII concentrate (the Behringwerke) was also used. Some human sera used were so-called “control” sera supplied by the Behringwerke for quantitative determination of serum proteins. Human Plasma Protein Fraction (Minimum Requirements, 1971) was also included in test samples for the Ouchterlony technique.

When undiluted samples were tested by the Ouchterlony technique, only
Human Plasma Protein Fraction gave negative results, whereas, to the author’s surprise, all the remaining samples including human serum formed precipitation bands (Fig. 1). Occasional exceptions were the “control” serum supplied by the Behringwerke, which gave, in some instances, negative results against anti-subunit A serum by the Ouchterlony technique. This serum is said to have been somehow treated for removing unstable proteins and the procedure might be responsible for less frequent occurrence of positive precipitation against anti-subunit A serum.

It was also found that in immunoelectrophoresis, human serum formed a precipitation band against anti-subunit A serum in a region roughly corresponding to that of subunit A, when the test samples were 4 to 5 times concentrated prior to application.

On the other hand, no positive results were obtained with human serum in rocket immunoelectrophoresis against anti-subunit A serum. It seemed that even by rocket immunoelectrophoresis, precise quantitative determination of factor XIII in human plasma may not be so easy as in the case of the factor XIII concentrate. Nor the reaction of human sera against anti-subunit A serum in single radial immunodiffusion could be interpreted as definitely positive.

One of the purposes of the present work was to see if factor XIII could be detected in Liquid Human Plasma, which is as a whole almost devoid of the clotting activity (Miura, in preparation). Even if only such techniques as giving positive results with human plasma and negative with human serum are regarded to be specific, Liquid Human Plasma gave definitely positive results in both
rocket immunoelectrophoresis and single radial immunodiffusion. Therefore, the subunit A antigenicity must be present in Liquid Human Plasma and, consequently, the abnormal clottability can not be attributed to its complete absence of factor XIII. The antibody neutralization technique can not be applied to Liquid Human Plasma because of its inability of forming clot by itself.

In any way, the author is confronted with a strange finding that human serum forms positive precipitation band against anti-subunit A serum in the Ouchterlony technique and immunoelectrophoresis. A finding similar to the author's was also reported by a group of the First Department of Medicine, Teikyo University, mainly by immunoelectrophoresis of human serum as well as of plasma of factor XIII-deficient patients against anti-subunit A serum (Abe, personal communication).

When twofold dilution series of human plasma and that of human serum were tested parallelly against anti-subunit A serum, positive results were obtained up to a 1:8 dilution of both the test materials.

Moreover, the precipitation band was clearer with human serum than with human plasma at the same dilution.

Positive results in the Ouchterlony technique were obtained even in the agar plate containing EDTA in approximately 0.2 w/v%. However, when the plate was prepared with agarose alone containing sodium chloride in 0.9% instead of the conventional mixture of agar and agarose, positive results were no longer obtained with human serum against anti-subunit A serum.

It was further found that the positive result was restricted to the combination of human serum and anti-subunit A serum. No precipitation band was formed between anti-subunit A serum and normal sera of such nonhuman origins as the rabbit, bovine, mouse and guinea pig, nor between normal rabbit serum and normal human serum.

When FITC-labeled goat anti-rabbit IgG serum was applied over the agar plate after the precipitation band had been formed between human serum and anti-subunit A serum, fluorescence was observed on the precipitation band just as on the precipitation band formed with factor XIII. Thus, involvement of rabbit IgG in anti-subunit A serum in forming precipitation band with human serum was demonstrated.

Moreover, the precipitation band formed with human serum against anti-subunit A serum was fused with that against the same antiserum formed by the factor XIII concentrate treated with human thrombin in the presence or absence of fibrinogen (Fig. 2).

The human serum factor causing precipitation with anti-subunit A serum was resistant to heating at 56°C for 10 min. On the other hand, anti-subunit A serum after added with three volumes of human serum no longer formed a precipitation band with human serum in the Ouchterlony technique, while its precipitating reactivity to human factor XIII was still retained.

These findings demonstrate the presence in human serum of a substance capable of forming a precipitation band with anti-subunit A serum. Of course, the antiserum supplied for use in the antibody neutralization technique can not
Fig. 2. Double immunodiffusion tests (the Ouchterlony technique)
A. Antiserum against subunit A of factor XIII
1. Human serum
2. Factor XIII concentrate treated with thrombin and fibrinogen
3. Factor XIII concentrate treated with thrombin
4. Factor XIII concentrate (×16)
5. Human thrombin (40 U/ml, Green Cross Co.)
6. Human fibrinogen (1%, Green Cross Co.)

necessarily be expected to have been guaranteed of its specificity in any other techniques. Furthermore, the use of the agarose plate containing sodium chloride in 0.9% may be recommended if positive results with human serum are to be excluded.

However, there is still no evidence for an assumption that the positive result with human serum must be a "non-specific" one. From the findings thus far obtained, an alternative hypothesis may also be feasible that the precipitation between anti-subunit A serum and human serum could be a specific antigen-antibody reaction, although the factor in human serum can be different antigenically from inactive subunit A. As sometimes weakening or alteration in the antigenicity was noticed in association with activation of enzymes, as in the case of reactivity of plasmin against anti-plasminogen serum (Kazama and Abe, in press), the reactive substance in human serum might be the activated subunit A of the factor XIII itself. It seems, however, inexplicable why no positive precipitation occurred in relatively more sensitive techniques such as rocket immunoelectrophoresis. After all, it has been found that a factor occurring in human serum gives positive precipitation even with anti-subunit A serum recommended by Bohn et al. (1972).

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REFERENCES


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