Characterization of an acquired factor VIII inhibitor and plasmapheresis therapy in a patient with bullous pemphigoid

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Dear Sir,

Acquired factor VIII inhibitor is rarely found to be associated with bullous pemphigoid. In the literature, only eight patients with acquired FVIII inhibitor in association with bullous pemphigoid have been described (1–4). Information on identifying inhibitor types or recognized epitope is essentially non-existent. We present a 49-year-old woman with bullous pemphigoid who manifested episodes of unexplained bleeding. The FVIII inhibitor was detected and its characterization was investigated.

Case history

A 49-year-old woman presented with multiple episodes of bleeding manifested by soft tissue hemorrhages in both forearms, intramuscular hematoma (up to 12 cm in size) following an intramuscular injection, intermittent ecchymoses on the extremities and upper gastrointestinal bleeding. These bleeding episodes developed several months after a bout of skin blisters on the extremities and erosions of oral mucosa, which led to the diagnosis of bullous pemphigoid. The bullous pemphigoid was treated with prednisone and cyclophosphamide, and her skin and mucosal symptoms resolved. There was no family or prior bleeding history. She had previously undergone extracorporeal shock wave lithotripsy (ESWL) therapy for a kidney stone without incident.

Physical examination revealed a large hematoma on the left buttock with ruptured skin and tenderness. Both forearms were swollen with ecchymoses. Hemoglobin was 80 g/dL, leukocytes 4.2 x 10^9/L, and platelets 503 x 10^9/L. Liver or kidney function tests were normal. Rheumatoid factor, anti-nuclear antibody, anti-cardiac phospholipids antibody and anti-double strands DNA were negative. Prothrombin time was 12.0 s (normal range: 11~14 s) and activated partial thromboplastin time (APTT) was 80.5 s (normal range: 25~35 s). The abnormal APTT could not be corrected by normal plasma. Platelet aggregation studies and clotting factors I, II, V, VII, IX, X, XI were all normal. Thromboplastin generation test (TGT) showed factor VIII: C deficiency, and the VIII:C activity (one-stage method) was 1.4%. FVIII: C inhibitor titer in patient’s plasma was 147.8 Bethesda Unit (BU/ml) as measured by the Bethesda assay (5).

The patient was treated with fresh frozen plasma (FFP) transfusion (200 ml per time, for 6 times) and prednisone in a dose of 30 mg daily for two weeks. However, her symptoms did not improve. Plasmapheresis was performed (plasma exchange volume, 2000 ml) and oral cyclophosphamide (100 mg daily) was given. After one week, her hemotoma slowly resolved and skin ecchymoses disappeared. The VIII:C activity went up to 13.2% and FVIII inhibitor titer dropped to 28 BU/ml. She was discharged, and no further hemostatic problems occurred during one year’s follow up.

To confirm the inhibitory effect on FVIII:C of the patient’s plasma, IgG was purified from her serum on a protein A-Sepharose 4B column according to the manufacturer’s instruction (Pierce, USA). Purified IgG concentration was determined by absorbance at 280 nm (E_{280}^1%=13.5). Equal amounts of patient’s IgG or normal human IgG were incubated with normal pooled plasma at different dosages (0 µg, 6.25 µg, 12.5 µg, 25 µg, 50 µg, 75 µg) and APTT assay was completed. The result exhibited that patient’s IgG was able to prolong normal human plasma APTT significantly in a dose-dependent manner (Fig. 1B), while normal IgG had no effect on APTT.

In order to determine the IgG subclass of FVIII inhibitor in bullous pemphigoid, we used immunoblotting and immuno-nephelometric methods to examine the immunoglobulin subclass with monoclonal antibodies specific for human IgG_1, IgG_2, IgG_3 and IgG_4. The immunoblotting assay, combined with optical density (OD) scanning, confirmed that IgG_4 and IgG_1 were the patient’s FVIII inhibitors of which IgG_4 was predominant. Immuno-nephelometric quantitative assay for IgG subclass concentration also was carried out on a Beckman Coulter immunochemical analysis system using detecting kit containing anti-human IgG_1 or IgG_2 serum and anti-human IgG_3 or IgG_4 latex (the Binding Site, USA). The tested samples included the plasma and purified IgG from both the patient and normal human plasma. Results documented that the concentration of IgG_4 in patient’s plasma (681 mg/l) or purified IgG (148 mg/l) was significantly higher than that in normal plasma (363 mg/l) or purified IgG (73.4 mg/l). The patient’s IgG_4 subtype (both plasma and IgG) was also somewhat higher than that of normal controls. These results were consistent with Western blotting results.
These findings represent the first documentation of the IgG subclass of F VIII inhibitor in bullous pemphigoid.

Applying solid-phase binding method followed by Western blotting, we identified the epitopes of F VIII inhibitors (solid-phase binding combined with Western blotting assays). The results indicated that the F VIII epitope was located at a 44 KD polypeptide and could be recognized by against IgG4 or IgG1 antibody. 1, 1', 1'': Thrombin cleaved F VIII+ normal plasma incubation + anti-IgG subclass Ab -> negative. 2, 2', 2'': Normal IgG+ normal plasma incubation + anti-IgG subclass Ab -> positive control. 3, 3', 3'': Thrombin cleaved F VIII + patient plasma incubation + anti-IgG subclass Ab -> IgG4 and IgG1 showed positive; IgG2 was negative. 4, 4', 4'': Normal IgG+ patient plasma incubation + anti-IgG subclass Ab -> positive control.

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plasma, no signal was detected (Fig. 1C). The results support strongly the fact that the specific epitope of FVIII recognized by the inhibitor is within the A2 domain. This is the first evidence of a FVIII-binding epitope of the inhibitor found in bullous pemphigoid. The epitopes recognized by inhibitory antibodies for FVIII are generally located in specific regions of FVIII. The most frequent sites of inhibitory binding occur within the A2 and C2 domain (7, 8). The A2 domain directly interacts with factor IXa through residues 484–509 (9). Previously published data showed that most of the epitopes recognized by the inhibitors were localized within the FVIII 44-KD fragment, corresponding to the A2 domain as well as the light chain (6). More detailed epitope mapping was obtained by using recombinant FVIII fragment expressed in E. coli. It appeared that the light-chain epitopes are multiple with a predominance in the C2 domain (10). Lollar et al. elegantly mapped the A2 domain epitopes localized within the N-terminal part of the domain, between residues 379–538 (11). Huang et al. investigated the epitopes of FVIII inhibitors in 22 Chinese patients, and results showed that the FVIII inhibitors in Chinese patients, from very different genetic backgrounds, shared epitopes, most of which are localized in A2-a2 and A3-C1-C2 of FVIII. Combined with our case’s FVIII epitope distribution, it suggests a common mechanism for the development of FVIII inhibitors (12).

In summary, we identified an acquired FVIII inhibitor developed in a patient with bullous pemphigoid. Furthermore, our investigation also revealed the presence of the IgG subclass (IgG1, IgG4 predominantly) inhibitor against the 44KD FVIII: C A2 domain. Although acquired FVIII deficiency is often associated with a poor prognosis, our case responded well to prednisone, plasmapheresis and cyclophosphamide. Even though factor FVIII inhibitor complicating bullous pemphigoid is a rare occurrence, this finding, and particularly the clinical assessment and the application of various techniques, which led to this finding, are clinically relevant and of significant importance.

References