Fixation of Properdin and Factor B by Bullous Pemphigoid Antibody (in vitro study)*

Chang Woo Lee, M.D.

Graduate School of Medicine, Korea University

Abstract

Ten serum samples from the patient with bullous pemphigoid with the basement membrane zone autoantibody titers of 320 or greater were tested, by the method of in vitro complement immunofluorescence, for their ability to fix Factor B and properdin in addition to Clq, C4 and C3.

Five samples yielded positive C3 and properdin staining reactions while four samples demonstrated positive Factor B stainings. All ten samples yielded positive C3, C4 and Clq staining reactions. Heat inactivation or treatment of the complement source with EDTA, Mg2-EGTA abolished both C3, properdin and Factor B staining in all ten cases. This result suggest that pemphigoid antibody will fix properdin and Factor B in addition to Clq, C4 and C3, a phenomenon explained by assembly of the C3b amplification mechanism following activation of the classical pathway of complement system.

Introduction

Activation of complement system appears to play a major role in the pathogenesis of bullous pemphigoid, a blistering skin disease of the elderly which is characterized histologically by subepidermal bulla formation and immunologically by autoantibody reactive with the basement membrane zone (BMZ) of the skin and mucosa as demonstrated by indirect immunofluorescence (IF) staining method (1, 2). Using direct IF staining, C3 deposition to the BMZ occurs in virtually all bullous pemphigoid skin lesions in addition to IgG (3, 4). Components of both the classical (Clq, C4) and alternative (properdin, Factor B) pathways of the complement system are similarly deposited in skin lesions as demonstrated by both direct and modified direct IF method (3, 4).

Total hemolytic complement and individual complement components were also reduced in blister fluid of most serologically positive bullous pemphigoid patients when compared to other blister fluid and serum proteins(5).
Using in vitro complement IF technique it was also reported that circulating pemphigoid antibody will fix C1q and C4 in addition to C3 (6, 7), findings which suggest classical pathway activation of complement and which explain the presence of these components in skin lesions.

In the present study, it was designed to examine if bullous pemphigoid antibody will fix components of the alternative complement pathway (properdin and Factor B) in vitro in addition to C1q, C4 and C3, and to examine the mode of activation of the complement system, which can be compared to the previous studies (6, 7, 8).

Materials and Methods

Ten sera from the patient with active bullous pemphigoid which yielded BMZ antibody titers of 320 or greater were included in this study. Sera were stored at −20°C, while complement source (fresh normal human serum) were stored at −70°C until used.

Antiserum to human C1q, C4 and Factor B were purchased (Behring Diagnostics, New Jersey, U.S.A.). Labeled antiserum to human IgG (Meloy Lab. Inc., Virgina, U.S.A.), labeled rabbit anti-goat and goat anti-rabbit antisera were purchased (Atlantic Antibody Inc., Georgia, U.S.A.). Antiserum to human properdin was made and tested after purifying properdin by the method of Pensky et al. (9) and labeled antiserum to human C3 was also prepared after purifying C3 by the method of Tack and Prahl (10).

Details of the preparation and use of the anti-properdin and anti-Factor B antisera have been summarized previously (7). Before use, all antisera were checked for specificity and activity by both double immunodiffusion (Ouchterlony) and by immunoelectrophoresis. Other characteristics of these labeled antisera-unit, antibody protein assay, F/P ratio and use dilution were confirmed with previously published standards (2).

In vitro C3 staining was performed as previously described (7, 11), using two step procedure. Normal human skin sections were treated with heat inactivated (56°C for 30 min.) patient serum dilutions prepared with fresh normal human serum (NHS) diluted 1:10 in phosphate buffered saline with Mg++ and Ca++ added. This resulted in a complement concentration of approximately 15 hemolytic unit per ml. The second step involving treatment the skin sections with labeled anti-C3. Only sera yielded positive in vitro C3 staining reactions were included in the studies outlined below. In vitro properdin, Factor B, C1q and C4 staining were performed using the three step procedure recently described (7). In step one the normal human skin sections were treated with heat inactivated patient serum dilutions plus human complement (NHS). In step two, the tissues were treated with unlabeled antisera. The final step involved treating the sections with either labeled goat anti-rabbit serum (C4 staining) or labeled rabbit anti-goat serum (C1q, Factor B and properdin staining).

Experimental controls for the various in vitro complement IF staining procedures were indentical to those outlined previously in detail (7) and positive control was high titer pemphigoid serum. Selective control study were designed to determine the mode of complement activation when in vitro C3, properdin and Factor B staining tests were employed. These included addition of Mg++–EGTA and EDTA to the complement sources.

Result

All ten serum samples yielded positive in