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DEMONSTRATION OF β1H GLOBULIN IN PEMPHIGUS

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Abstract. β1H globulin is a plasma protein which regulates the biologic activities of the major fragment of the 3rd complement component, C3b. The role of β1H globulin in pemphigus was investigated using immunofluorescence in the present study. Lesional skin biopsies from patients with confirmed pemphigus demonstrated in-vivo deposition of β1H in addition to C3 in all of four biopsies. Eight serum samples containing C3 fixing intercellular antibodies were then tested for the capacity to fix β1H and other complement components. All eight pemphigus sera showed fixation of β1H to the intercellular areas of normal human skin. C1q and C4 fixation by pemphigus sera was also demonstrated in 7 of 8 sera, respectively. The experiment using C2-deficient serum indicated that the fixation of β1H by intercellular antibodies requires the activation of the classical complement pathway. These data suggested that β1H, a co-factor of C3b inactivator, plays a role in the in-vivo regulation of complement activity and supplies additional evidence for the participation of complement system in the pathogenesis of pemphigus.

Key words: β1H globulin; Pemphigus; Complement system; Complement immunofluorescence

The role of the complement system in pemphigus is not fully understood. In our previous studies, we have demonstrated complement-fixing intercellular (IC) antibodies in some sera of untreated pemphigus patients (1), and several investigations have shown much evidence that the complement system ought to play an important role in pemphigus acantholysis (2). Very recently, we obtained data showing that in-vitro complement activation by IC antibodies should occur via the classical complement pathway, followed by activation of the C3b amplification loop (3).

β1H globulin is a recently characterized plasma protein which regulates the biologic activity of the major fragment of the 3rd complement component, C3b, which is the most biologically active part of the complement system (4). The major function of this protein is to act as a co-factor for the C3b inactivator (C3bINA). Carlo et al. demonstrated the presence of β1H in the dermo-epidermal junction of patients with systemic lupus erythematosus (SLE) (5) and in the basement membrane zone (BMZ) of patients with bullous pemphigoid (6, 7). Since β1H is a co-factor for C3bINA, it binds to C3b and accelerates the cleavage of C3b to C3c and C3d fractions, which explains its presence in the skin.

This study was undertaken to determine whether β1H is present in the lesional skin of pephigus and to ascertain if IC antibodies will fix β1H together with early complement components, C1q and C4 in-vitro.

MATERIALS AND METHODS

Four patients were studied who were clinically and histologically diagnosed as having pemphigus. Biopsy was performed by the standard technique, quick-frozen and cut in a cryostat at 4–6 μm thickness. The sections were used unfixed. Each section of the lesional skin was stained for the presence of IgG, C3, C1q, C4 and β1H, using immunofluorescence (IF) technique. For the staining, commercially available conjugates for human IgG, C1q, C4 and C3 were used. Anti-β1H antiserum was prepared by immunizing rabbits with β1H in Freund's complete adjuvant. The β1H globulin was purified as previously described (*4*) and showed a single stained band upon analysis by sodium dodecyl sulphate polyacrylamide gel electrophoresis (*8*). For the staining of β1H, the antiserum was used at 1:10 dilution and an indirect IF was used, employing fluorescein isothiocyanate (FITC) labelled goat anti-rabbit IgG as the second antibody. The antibody titre and the final dilution of the conjugates were as follows; for human IgG, 1:10 (Dakopatts Co., Denmark, γ-chain specific Lot 089A, DAKO antibody titre 200); for C1q, 1:10 (Behring Institute, W. Germany, Lot 1289D5H, specific antibody content, 2.0 mg/ml); for C4, 1:10 (Behring, Lot 129208A, specific antibody content, 2.5 mg/ml); for C3, 1:10 (Dakopatts Lot 124, DAKO antibody titre 100;

Table 1. *Demonstration of β1H globulin and complement components in the lesional skin of pemphigus*

No.	Patients	Diagnosis	Immunofluorescence				
			β1H	C3	C1q	C4	IgG
1	M. H.	P. vulgaris	+	+	+	+	+
2	R. A.	P. foliaceus	+	+	−	+	+
3	T. T.	P. foliaceus	+	+	−	−	+
4	T. S.	P. erythematosus	+	+	+	−	+

and for rabbit IgG, 1 : 16 (Dakopatts heavy and light chain specific, Lot 037). Eight serum samples from untreated pemphigus patients which contained C3-fixing IC antibodies were tested for the capacity to fix β1H in vitro together with the early components of complement (C1q and C4) using complement IF. Each serum was tested for β1H and complement fixation at 1 : 5 dilution. Fresh normal human serum (NSH) diluted 1 : 5 with Ca^{++} and Mg^{++} enriched phosphate-buffered saline (PBS, pH 7.2) was used as a source of complement and β1H. Heat-inactivated NHS and C2-deficient human serum (9) (a gift from Dr Sheldon R. Pinnell, Duke University Medical Center, USA) were used as controls in complement IF. The procedure and specificity control tests for complement IF are described elsewhere (3).

RESULTS

The results of staining for IgG, C3, C1q, C4 and β1H in the lesional skin of pemphigus patients are shown in Table 1. IgG and C3 were deposited in the IC areas of the lesional skin in all four biopsies. All of these skin biopsies showed positive β1H binding in the same fashion as for IgG and C3. The intensity of the staining was similar as that of C3 (Fig. 1). C1q and C4 were also demonstrated in 2 of 4 biopsies, respectively. The staining pattern was the same in all complement components. By in vitro complement IF technique, all 8 pemphigus sera containing C3 fixing IC antibodies showed fixation of β1H in the IC areas of the normal human skin at 1 : 5 serum dilution. Fixation of other early complement components of the classical pathway, C1q and C4, was also demonstrated in 7 of 8 serum samples, respectively. However, the use of C2-deficient serum as the complement source inhibited the fixation of C3 and β1H, while the staining of C1q and C4 was not affected at all.

DISCUSSION

The role of β1H in the complement system has only recently been appreciated. β1H, also known as C3b inactivator accelerator, is an abundant plasma protein and has a molecular weight of 150 000 daltons. It functions with and without C3bINA in regulating C3b activity (4). In vitro studies have shown an absolute requirement for β1H in the conversions of fluid phase C3b to the inactive products C3c and C3d (10). Recently, a role for β1H in regulating complement activity in vivo has been suggested by studies showing that β1H is deposited with complement and immunoglublin and the dermo-epidermal junction of both lesional and non-lesional skin of patients with SLE (5). Further evidence comes from similar findings in patients with bullous pemphigoid in which β1H was demonstrated at the BMZ in every instance where C3 was found (6, 7). In addition, it was also shown that BMZ antibodies of bullous pemphigoid will fix β1H in vitro and that β1H binding by BMZ antibodies requires the activation of the classical complement pathway. In the present study, β1H was demonstrated in the IC areas of the lesional skin in all four biopsies where C3 and other early complement components were shown. The staining pattern of β1H was identical with that of C3 and other complement components. Although the role of the complement system in pemphigus has been very controversial, numerous previous investigations suggest local activation of the complement system (2). Our data provide further evidence favouring a role for complement activation in pemphigus, since β1H is demonstrated in the exactly same site as that if complement components including C1q, C4 and C3. In addition, we have demonstrated that β1H can be fixed in the IC areas of the normal skin by C3 fixing IC antibodies. This fact also suggests the association of β1H in complement activation by IC antibodies in vitro. Substitution of C2-deficient serum as the complement source in the complement IC resulted in the inhibition of both C3 and β1H staining, while the positive binding of C1q and C4 was not affected

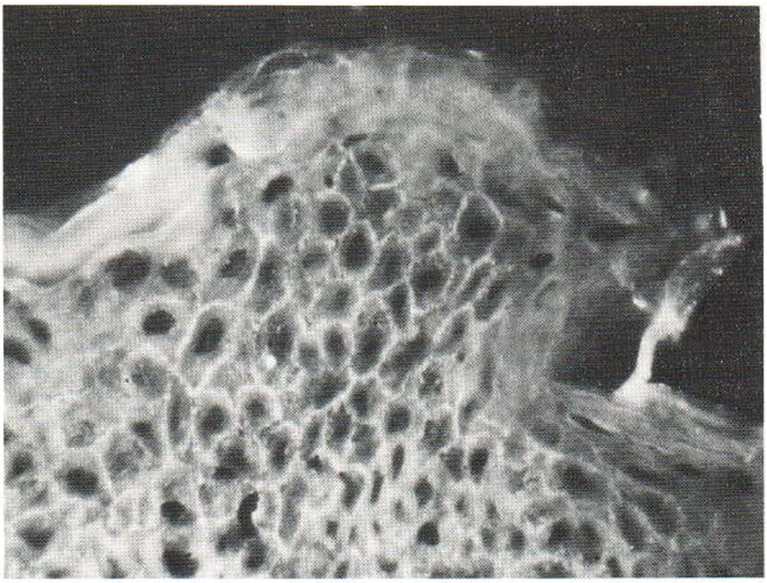

Fig. 1. Deposition of βIH globulin in the intercellular areas of the lesional skin from a pemphigus patient (T. T) (×200).

at all, suggesting that βIH fixation by IC antibodies requires the activation of the classical complement pathway.

Thus, the presence of the complement regulating protein, βIH, in the IC areas of the pemphigus skin and the fixation of βIH by IC antibodies in vitro provides additional evidence of the participation of the complement system in pemphigus, although the definitive role of complement in the pathogenesis of pemphigus remains to be established.

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