DEMONSTRATION OF βIH GLOBULIN IN PEMPHIGUS

Takeji Nishikawa,1 Makoto Sugiura, Takashi Hashimoto, Seiichi Kurihara and Noboru Tamura

1Department of Dermatology, Keio University School of Medicine, Tokyo 160, and 2Department of Immunology, Institute of Basic Medical Sciences, University of Tsukuba, Ibaraki 305, Japan

Abstract. βIH globulin is a plasma protein which regulates the biologic activities of the major fragment of the 3rd complement component, C3b. The role of βIH globulin in pemphigus was investigated using immunofluorescence in the present study. Lesional skin biopsies from patients with confirmed pemphigus demonstrated in-vivo deposition of βIH 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 βIH and other complement components. All eight pemphigus sera showed fixation of βIH 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 βIH by intercellular antibodies requires the activation of the classical complement pathway. These data suggested that βIH, 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: βIH 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). βIH 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 βIH 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 βIH 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 βIH is present in the lesional skin of pemphigus and to ascertain if IC antibodies will fix βIH 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 βIH, using immunofluorescence (IF) technique. For the staining, commercially available conjugates for human IgG, C1q, C4 and βIH, using immunofluorescence (IF) technique. For the staining, commercially available conjugates for human IgG, C1q, C4 and C3 were used. Anti-βIH antiserum was prepared by immunizing rabbits with βIH in Freund’s complete adjuvant. The βIH globulin was purified as previously described (4) and showed a single stained band upon analysis by sodium dodecyl sulfate polyacrylamide gel electrophoresis (8). For the staining of βIH, the antiserum should occur via the classical complement pathway, followed by activation of the C3b amplification loop (3).
Table I. Demonstration of \( \beta 1H \) globulin and complement components in the lesional skin of pemphigus

<table>
<thead>
<tr>
<th>No.</th>
<th>Patients</th>
<th>Diagnosis</th>
<th>( \beta 1H )</th>
<th>C3</th>
<th>C1q</th>
<th>C4</th>
<th>IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M. H.</td>
<td>P. vulgaris</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>R. A.</td>
<td>P. foliaceus</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>T. T.</td>
<td>P. foliaceus</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>T. S.</td>
<td>P. erythematosus</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

The results of staining for IgG, C3, C1q, C4 and \( \beta 1H \) in the lesional skin of pemphigus patients are shown in Table I. IgG and C3 were deposited in the IC areas of the lesional skin in all four biopsies. All of these skin biopsies showed positive \( \beta 1H \) binding in the same fashion as for IgG and C3. The intensity of the staining was similar as that of C3 (Fig. 1). Clq 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 \( \beta 1H \) in the IC areas of the normal human skin at 1:5 serum dilution. Fixation of other early complement components of the classical pathway, Clq 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 \( \beta 1H \), while the staining of Clq and C4 was not affected at all.

DISCUSSION

The role of \( \beta 1H \) in the complement system has only recently been appreciated. \( \beta 1H \), 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 C3bNA in regulating C3b activity (4). In vitro studies have shown an absolute requirement for \( \beta 1H \) in the conversions of fluid phase C3b to the inactive products C3c and C3d (10). Recently, a role for \( \beta 1H \) in regulating complement activity in vivo has been suggested by studies showing that \( \beta 1H \) is deposited with complement and immunoglobulin 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 \( \beta 1H \) 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 \( \beta 1H \) in vitro and that \( \beta 1H \) binding by BMZ antibodies requires the activation of the classical complement pathway. In the present study, \( \beta 1H \) 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 \( \beta 1H \) 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 \( \beta 1H \) is demonstrated in the exactly same site as that if complement components including Clq, C4 and C3. In addition, we have demonstrated that \( \beta 1H \) can be fixed in the IC areas of the normal skin by C3 fixing 1C antibodies. This fact also suggests the association of \( \beta 1H \) 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 \( \beta 1H \) staining, while the positive binding of Clq and C4 was not affected.
at all, suggesting that \( \beta H \) fixation by IC antibodies requires the activation of the classical complement pathway.

Thus, the presence of the complement regulating protein, \( \beta H \), in the IC areas of the pemphigus skin and the fixation of \( \beta H \) 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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REFERENCES


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T. Nishikawa, M.D.
Department of Dermatology
Keio University School of Medicine
Tokyo 160
Japan