# The Complement Fixing Ability of Anti-basement Membrane Zone IgG Subclass Antibodies of Herpes Gestationis and Bullous Pemphigoid

MASAYUKI SUZUKI, CHIEKO WATANABE and HIDEO YAOITA

*Department of Dermatology, Jichi Medical School, Minamikawachimachi, Kawachigun, Tochigi, Japan*

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The antibody activities and complement fixing ability of the IgG subclass antibodies of herpes gestationis (HG) and bullous pemphigoid (BP) antibodies were investigated. IgG was prepared by DEAE Affi-Gel Blue column. IgG3 and IgG 1.2.4 fractions were isolated through Protein-A Sepharose CL-4B column. In HG, neither IgG nor C3 deposits were detected at the basement membrane zone (BMZ) in the skins incubated with IgG3 fractions, although C3 deposits were detected without IgG deposits in the skins incubated with IgG 1.2.4 fractions. In BP, IgG and C3 deposits were detected in the skins incubated with IgG 1.2.4 fractions, while neither IgG nor C3 deposits were detected in the skins incubated with IgG3 fractions. These findings suggest that IgG3 might not be able to fix complement at BMZ in BP and HG, although there are some reports that complement activating ability of IgG3 is the strongest among IgG subclasses. (Accepted June 16, 1988.)

M. Suzuki, Department of Dermatology, Jichi Medical School, Minamikawachimachi, Kawachigun, Tochigi, 329-04, Japan.

Herpes gestationis (HG) is a rare blistering disease which occurs during or shortly after pregnancy (1). Bullous pemphigoid (BP) is an acquired chronic blistering disease of skin (2). Histologically, subepidermal basement membrane zone (BMZ) separation and variable infiltrates of eosinophils, mononuclear cells and neutrophils are seen in both diseases. In the immunohistopathology, the finding of the skin of the patient with BP shows deposition of immunoglobulin (most commonly IgG) and complements at BMZ (3, 4). Immunoelectronmicroscopical (IEM) finding of the skin of the patient with BP is the localized immunoglobulin deposition in the lamina lucida area (5). C3 is also demonstrated in this location (6), as well as "lumpy-bumpy" immunoglobulin deposition (7).

On the other hand, in HG, Provost & Tomasi have demonstrated linear deposits of C3 in the absence of immunoglobulin at the BMZ, and the serum factor (HG factor) which deposited C3 along the BMZ (8). Jablonska et al. confirmed these findings and also found in vivo-bound IgG at the BMZ (9). IEM findings of HG show immunoglobulin and C3 depositions to the lamina lucida area (10, 11). Yaoita & Katz suggested that HG factor was identical with IgG, because HG factor had many characters of IgG (12, 13).

As for IgG subclass, using ion-exchange-chromatography, Sams & Schur demonstrated that the IgG subclass from the sera of the patients with BP were exclusively IgG4 (14). Recently, the distribution of IgG subclasses deposition was demonstrated at the BMZ of the skins and in the serum of the patient with BP using monoclonal antibodies specific for IgG subclass. It showed that IgG4 was the most common immunoglobulin (15, 16). On the other hand, there is a hypothesis that IgG3 might be a main IgG subclass which activates complements in BP (14) and HG lesions. In order to make the problem in this discrepancy clear, we studied the complement fixing ability of IgG subclass in BP and HG, which is reported in this paper.

## MATERIALS AND METHODS

1. *Patient sera.* The sera were taken from untreated patients with clinically, histopathologically typical BP and HG (Table I). Normal human sera were obtained from healthy volunteers, who were one female (32 years) and four males (year range 24–28). These sera were divided into aliquots of 0.5 ml and stored at −70°C until used. Their anti-BMZ antibody titers were determined by indirect immunofluorescent staining methods, using normal human skin as substrate.

2. *Skins used as substrate.* Human skins used as substrate were obtained from healthy volunteers. Skin specimens were embedded in OCT Embedding Media (Labo teck). They were stored at −70°C for no longer than 7 days, cut into 4 μm sections in a cryocut and processed, using indirect or complement immunofluorescence staining method.

3. *Preparation of IgG 3 fractions and IgG 1.2.4 fractions from sera.* The sera of patients and normal human volunteers were dialyzed and applied to DEAE Affi-Gel Blue column Bio-Rad Lab. The column was developed with a linear gradient of phosphate buffer (0.02 M $K_2HPO_4$ to 0.04 M $K_2HPO_4$ pH 8.0) to elute IgG. The purified IgG was chromatographed in a Protein A-Sepharose CL-4B (Pharmacia Fine Chemicals AB) column measuring 1.6/3 cm, with a bed volume of 5.2 ml. Unbound protein was eluted with 0.02 M phosphate-buffered saline (pH 7.4). Then bound protein was eluted with 0.1 M acetic acid. All chromatographic procedures were performed at 4°C. Fractions containing bound and unbound protein were pooled separately and concentrated by ultrafiltration through an Amicon Diaflow membrane at the final concentration of 10 mg/ml phosphate-buffered saline (as UV method). These fractions were then dialyzed against phosphate-buffered saline. The concentrated pools were characterized for their IgG subclass content by double immunodiffusion (17) in 1% agar in 0.85% sodium chloride solution at neutral pH using porcine antibody against each of the subclasses of human IgG 1–4 (obtained from Nordic Pharmaceuticals and Diagnostics Co., The Netherlands) (Fig. 1). The contamination of proteolytic enzyme and plasminogen in the IgG fraction was checked by using Bio-Rad's Substrate Gel Tablets which contained a bovine casein in 1% agar gel (18). The proportion of IgG in each IgG subclass fractions was checked by polyacrylamide gel electrophoresis (19).

4. *Immunofluorescence (IF).* The IgG 3 and IgG 1.2.4 fractions which were purified from the sera of patients and healthy volunteers were incubated with the human skins described above from detection of the antibody activity against BMZ and stained using the standard indirect IF technique (20). The complement IF technique was according to the method described by Jordon et al. (21). FITC labeled rabbit antisera against human IgG and C3 (Behring Institute, W. Germany, specific antibody concentration 1.0 mg/ml, F/P ratio: 2.1, 2.6) were used at 1 : 10 dilution.

## RESULTS

The specificity of both IgG 3 fraction and IgG 1.2.4 fraction was checked by the Ouchterlony method (Fig. 1). Kronvall & Williams showed that protein A derived from the cell wall of *Staphylococcus aureus* bound IgG 1, 2 and 4 but not IgG 3 paraproteins (22). We ensured that IgG 3 was purified in this system, using the Ouchterlony method, since protein A has been shown to bind IgG 3 proteins bearing allotypic markers characteristic of Mongoloid populations, whilst it does not bind IgG 3 bearing allotypic markers characteristic of Caucasian populations (23). The results of indirect and complement IF are shown in Table II. IgG 1.2.4 from HG-3 bound along BMZ, but IgG 3 from the same patient did not bind. IgG 1.2.4 from

Table I. *Characteristics of patient's sera which were used to purify IgG subclass fractions*

HG = herpes gestationis, BP = bullous pemphigoid, M = male, F = female

| | Age/sex | Anti-BMZ antibody titer | Complement fixing titer |
|---|---|---|---|
| HG-1 | 26 F | – | 1 : 8 |
| HG-2 | 30 F | – | 1 : 8 |
| HG-3 | 32 F | 1 : 320 | 1 : 40 |
| BP-1 | 65 F | 1 : 640 | 1 : 160 |
| BP-2 | 71 M | 1 : 10 | 1 : 10 |
| BP-3 | 77 M | 1 : 320 | 1 : 160 |

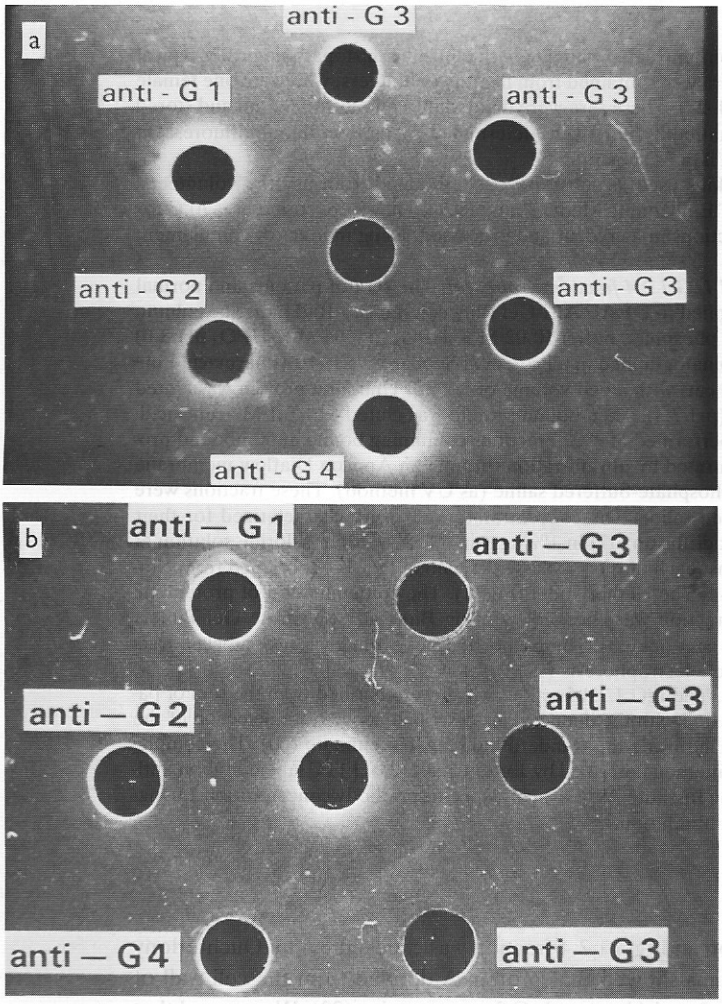


*Fig. 1.* Immunodiffusion (Ouchterlony) of $IgG_{1.2.4}$ fraction (*a*) and $IgG_3$ fraction (*b*) from the sera of the patient with HG. Each IgG subclass fraction was placed in the center hole. The IgG subclass specific porcine antibody were placed in the peripheral holes.

all patients with BP bound along BMZ, but IgG 3 from all patients with BP and HG did not bind. IgG 1.2.4 from all patients with BP and HG bound C3 along BMZ, while IgG 3 from all patients with BP and HG did not bind (Fig. 2).

Both IgG 1.2.4 and IgG 3 from all healthy volunteers did not bind IgG and C3 along BMZ.

## DISCUSSION

Four subclasses of human IgG were firstly recognized by Grey & Kunkel on the basis of antigenic differences among myeloma proteins (24). In comparing subclass behavior, it is important to distinguish C1q fixing, C1 fixing, C1 activation and whole complement activation (as measured in hemolytic assays). All the subclass in monomeric form bind C1q with measurable affinity with the order of binding constants IgG 3>IgG 1>IgG 2> IgG 4. IgG 1 and IgG 3 activate C1 and the whole complement efficiently. IgG 2 is less efficient in complement activation. IgG 4 does not appear to fix C1 and does not activate complement (25).

Therefore we made a hypothesis that HG factor was IgG 3, because IgG 3 was the lowest volume IgG and had the strongest complement fixation ability. But in this result, the com-

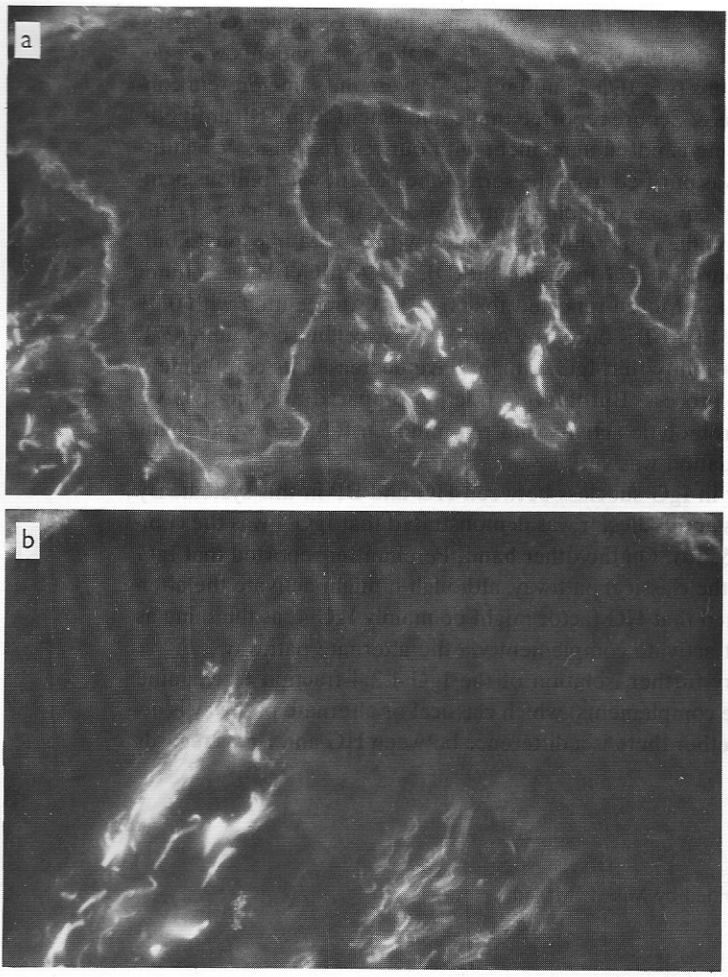


*Fig. 2.* Complement immunofluorescence of $IgG_{1.2.4.}$ and $IgG_3$ fractions of a patient with herpes gestationis, using normal human skins as substrate and fluorescein rabbit antihuman $C_3$ antisera. $C_3$ deposits along BMZ of the skin incubated with $IgG_{1.2.4}$ fraction of HG (*a*). $C_3$ does not deposit along BMZ of the skin incubated with the $IgG_3$ fraction of the same patient (*b*).

plement fixing IgG in HG was contained in IgG 1.2.4 fractions and there was no difference between HG and BP. Carruthers & Ewins investigated the ability of the antibodies of the patients with HG and BP bound to the staphylococcal protein A. In all the HG and BP sera examined, the BMZ complement fixing IgG bound to the protein A was IgG 1, since IgG 2 and

Table II. *Indirect and complement immunofluorescence findings of $IgG_{1.2.4}$ and $IgG_3$ fractions of patients with herpes gestationis and bullous pemphigoid*

HG = herpes gestations, BP = bullous pemphigoid, fr. = fraction

| | IgG | | C3 | |
|---|---|---|---|---|
| | IgG124 fr. | IgG3 fr. | IgG124 fr. | IgG3 fr. |
| HG-1 | – | – | + | – |
| HG-2 | – | – | + | – |
| HG-3 | + | – | + | – |
| BP-1 | + | – | + | – |
| BP-2 | + | – | + | – |
| BP-3 | + | – | + | – |

IgG 4 fixed complement weakly or not at all (26). We confirmed that no complement fixing ability of the purfied IgG 3 was detected but that of IgG 1.2.4 of the same patients were detected, even though both IgG fractions (i.e. IgG 3 and IgG 1.2.4) were the same protein concentration. On the other hand, Sams & Schure reported that complement fixing BP antibody seemed to be IgG 3 (14). This finding is not in agreement with our results. Sams & Schure reported that the first peak which was purified from the sera of the patient with bullous pemphigoid by DEAE-celulose column chromatography contained only IgG 3. However, this peak might contain IgG 1–4, because the peak which was eluted with 0.02 M phosphate buffer contained IgG 1–4 when we purified IgG by using the DEAE Affi-Gel Blue column chromatography. Moreover, Skvaril & Morell reported that IgG 1–3 were eluted with 0.01 M Tris buffer and IgG 4 was eluted 1.0 M Tris buffer by using DEAE-cellulose column (27).

We also have to consider the possibility that IgG 3 might have decreased its complement fixation activity in our purification process. But the IgG fraction prepared by using DEAE Affi-Gel Blue column is free of proteolytic activity and plasminogen (28). So we thought that the possibility of IgG 3 degradation was very low.

We found no differences of quality in IgG subclass between HG and BP in this system. By using monoclonal anti IgG subclass antibodies, it was demonstrated that IgG 4 was the common IgG of the patient with BP (15, 16). On the other hand, Perelmutter reported that IgG 4 did not activate complement via the classical pathway, although it might activate the alternative pathway (29). It was suggested that HG factor might be mainly IgG 4, as the same as BP antibody, and HG factor might activate complement via the alternate pathway.

In future experiments we will try further isolation of the IgG 1.2.4 fraction to examine which of the IgG 1, 2 and 4 activate complements, which classical or alternate pathway is activated by the IgG subclass and whether there is a difference between HG and BP as regards IgG subclass or not.

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