Immunoglobulin G subclass distribution of bullous pemphigoid autoantibodies and complement fixation studies

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ABSTRACT

Objective: The purpose of this study was to examine the immunoglobulin G (IgG) subclass distribution of tissue bound and circulating antibasement membrane zone antibodies in bullous pemphigoid. In addition, the complement fixing capability of circulating IgG subclasses was also investigated.

Methods: Seventeen skin biopsies and 25 serum samples obtained from 25 cases of bullous pemphigoid were analyzed by direct and indirect immunofluorescence using mice anti-human IgG subclasses monoclonal antibodies. Indirect complement immunofluorescence was used to measure the avidity with which antibodies activate the complement. The study was carried out between 1992 and 1996 at the King Fahd Hospital of the University, Al Khobar, Kingdom of Saudi Arabia and the Western Infirmary Immunopathology Laboratory, Glasgow, Scotland.

Results: Immunoglobulin G_1 deposits were detected in 10 of 17, IgG_2 in one, IgG_3 in 3 and IgG_4 in 15 biopsies. Immunoglobulin G_1 circulating antibodies were observed in 14, IgG_2 in 0, IgG_3 in 4 and IgG_4 in 24 serum samples. Complement fixing antibodies were detected in 15 sera, 4 of which contained only IgG_4 subclass.

Conclusion: The predominance of IgG_4 subclass observed in this study is similar to previously reported results. Complement fixation capability studies revealed non-complement fixing sera and the results were not compatible with the complement binding characteristics of IgG subclasses. This suggests that other mechanisms, which do not involve the complement, may participate in blister formation in bullous pemphigoid.

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Bullous pemphigoid (BP) is an autoimmune blistering disease of the elderly. The disorder is associated with linear deposition of immunoglobulin G (IgG) and complement along the epidermal basement membrane. Circulating IgG autoantibodies are present in the majority of patients and bind to a normal component of the basement membrane zone, the bullous pemphigoid antigen.[1] Studies on animal models have demonstrated a definite pathogenic role for IgG autoantibodies in BP.[2,3] The process of blister formation in BP has also been shown to require the presence of complement.[3,4] Early studies in the subtyping of BP IgG autoantibodies have demonstrated that the antibodies are heterogeneous; composed of various IgG subclasses.[5] The different IgG subclasses are characterized by variable ability to fix complement. Immunoglobulin G_1 and IgG_3 are potent complement activators. An IgG_2 binds less well to complement and IgG_4 is a non-complement fixing antibody.[6] It has been shown that BP antibodies from

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some patients lack the capacity to activate complement and that the non-complement fixing sera were composed of IgG_4, whereas, sera containing complement fixing antibodies were found in the subclasses G_1, G_3 and G_4.[5] In the present study, the distribution of tissue bound and circulating IgG subclasses in patients with active BP was investigated by direct and indirect immunofluorescence (IF) using monoclonal antibodies specific for the 4 human IgG subclasses. In addition, indirect complement IF was used to determine the complement fixing abilities of the BP antibodies.

Methods. ***Patients.*** The study was carried out between 1992 and 1996 at the King Fahd Hospital of the University, Al Khobar, Kingdom of Saudi Arabia and the Western Infirmary Immunopathology Laboratory, Glasgow, Scotland. Twenty-five patients with BP were studied. The diagnosis was confirmed by direct IF of perilesional skin demonstrating linear deposits of IgG or 3rd component of complement (C_3), or both at the basement membrane zone. Sera were collected from 25 patients and biopsy specimens were obtained from 17 patients. None of the female patients included were pregnant. All samples were snap frozen in liquid nitrogen and stored at -70^{0}C until use.

Immunofluorescence procedures. For direct IF staining 5um cryostat sections were cut and washed in phosphate buffer saline (PBS) for 30 minutes. The tissue sections were then incubated for 30 minutes at 37^{0}C with mouse monoclonal antibodies against the human IgG subclasses G_1, G_2, G_3 and G_4. The antibodies were used at a working dilution of 1:50 in PBS. After washing with PBS, the section were incubated for 30 minutes at 37^{0}C with fluorescein-conjugated rabbit antimouse IgG antibody and subsequently washed. In addition, fluorescinated polyclonal antisera were used to detect IgG and C_3. For indirect IF 5um cryostat sections of monkey esophagus were used as tissue substrate. The sections were washed in PBS for 30 minutes, incubated with patients sera for 30 minutes at 37^{0}C then after 30 minutes it was washed in PBS and incubated with monoclonal mouse antibodies as described for direct IF. In addition, fluorescinated polyclonal antisera were used to detect circulating IgG. Indirect complement IF was used to measures the avidity with which antibodies fix complement. Normal human skin was used as substrate. Sections were first incubated with patient's sera then with fresh human serum as a source of complement, and finally with fluorescein-conjugated rabbit antihuman C_3 antibody. All incubations were for 30 minutes at 37^{0}C and sections were rinsed for 30 minutes with PBS.

Results. ***Skin subclass distribution.*** A summary of the subclass distribution of anti-basement

Table 1 - Immunoglobulin G subclasses of tissue bound antibodies in 17 patients with bullous pemphigoid.

Fluorescence	IgG	IgG_1	IgG_2	IgG_3	IgG_4	C_3
Negative	0	7	16	14	2	1
Positive	17	10	1	3	15	16
Positive (%)	(100)	(58.8)	(5.9)	(17.6)	(88.2)	(94.1)

IgG - Immunoglobulin G, C_3 -3rd component of complement

Table 2 - Immunoglobulin G subclasses of circulating antibodies in 25 patients with bullous pemphigoid.

Fluorescence	IgG	IgG_1	IgG_2	IgG_3	IgG_4
Negative	0	11	25	21	1
Positive	25	14	0	4	24
Positive (%)	(100)	(56)	(0)	(16)	(96)

IgG - Immunoglobulin G

Table 3 - Relationship between complement fixing basement membrane zone antibodies and IgG subclasses with complement activating ability in 25 patients with bullous pemphigoid.

Total tested	IgG_1	IgG_2	IgG_3	IgG_4	Complement fixing antibodies
8	+ve	-ve	-ve	+ve	+ve
3	+ve	-ve	+ve	+ve	+ve
4	-ve	-ve	-ve	+ve	+ve
2	+ve	-ve	-ve	+ve	-ve
1	+ve	-ve	+ve	-ve	-ve
7	-ve	-ve	-ve	+ve	-ve

IgG - Immunoglobulin G, +ve = positive immunofluorescence, -ve = negative immunofluorescence

membrane zone antibodies in perilesional skin biopsies is shown in **Table 1**. Deposits of IgG were present in all 17 cases studied. In 2 cases was IgG_4 autoantibody not detected. However, both cases were positive for IgG_1, and in one of the 2 cases IgG_3 was also demonstrated. In 5 (29%) of 17 biopsies IgG_4 was the only IgG subclass detected, whereas IgG_1 was the only subclass observed in one (6%) of the 17 biopsy specimens. C_3 deposition was noted in all cases but one. It was positive only for IgG_4.

Serum subclass distribution. The results of the distribution of circulating IgG subclasses in serum samples are summarized in **Table 2.** Indirect IF examination showed IgG pemphigoid antibodies in all 25 serum samples tested. Immunoglobulin G_4 was the only subclass detected in 11 (44%) of the total samples examined. Furthermore, in the majority of cases IgG_4 produced a more intense fluorescence than other IgG subclasses.

Complement immunofluorescence. Complement fixing antibodies were detected in 15 (60%) out of the 25 BP sera. To determine the relationship between complement fixing ability and the distribution of IgG subclasses, cases were grouped based on their ability to fix complement and the presence of one of the complement fixing subclasses, IgG_1, IgG_2 or IgG_3 (**Table 3**). In the 15 sera with complement fixing antibodies, 8 showed IgG_1 as well as IgG_4 and 3 samples were positive for IgG_3 in addition to IgG_1 and IgG_4. The remaining 4 samples contained only IgG_4. In the 10 sera without complement binding antibodies IgG_4 was the only subclass observed in 7 sera; however, 2 samples were also positive for IgG_1 in addition to IgG_4. One sample was positive for IgG_1 and IgG_3.

Discussion. Several IF studies have indicated that the subclass of circulating BP antibodies is of the IgG_4 subclass.[7-10] A similar finding has also been noted in immunoblotting studies.[11,12] In addition, IgG_4 was also found to be the predominant subclass in other autoimmune bullous diseases such as Pemphigus and cicatricial pemphigoid.[13-15] The predominance of IgG_4 antibody in the sera and skin of BP patients are a surprising finding because it does not correlate with its distribution in normal serum, which is 3% of total IgG.[6] The cause of the prominent IgG_4 production in BP has not been established, but it is possible that it may be the result of specific genetic factors.[16] It has also been speculated that continued antigenic stimulation affects the normal distribution of IgG subclasses and leads to IgG_4 restricted response.[17]

In contrast to the results obtained in this study, Yamada et al[18] detected more frequent binding of complement by BP antibodies in vitro. Furthermore, analysis of the relationship between IgG subclasses distribution and their capability of complement fixation showed that in addition to IgG_4 at least one of the IgG subclasses which are capable of activating complement was also observed in all complement fixing sera. On the other hand, the non-complement fixing sera were only positive for IgG_4. Thus, the results were in accordance with the complement activating characteristics of IgG subclasses. In another report IgG_1, a potent complement activator, appeared to be the only subclass capable of complement fixation in BP.[19] Conversely, the distribution of IgG subclasses in BP sera examined in this study did not correlate with their complement activating capacity. Although the cause for this discrepancy is not clear, it is possible that complement activation that requires at least 2 closely spaced IgG molecules to bind antigen,[20] did not occur due to few antigenic sites were available. The demonstration that sera containing only IgG_4 do activate complement has also been noted by Kelly et al.[21] The prominent IgG_4 response was also an unexpected finding in this study since almost all patients demonstrated C_3 deposits in the basement membrane zone and 5 of the 16 cases with complement deposits had an IgG_4 restricted response. Similar observation was reported previously.[21] Although IgG_4 does not activate complement by the classical pathway, it is possible C_3 deposits may have occurred via the alternative pathway[22] or that small amount of IgG_1 to IgG_3 subclasses autoantibodies activated the classical pathway.[23] Although, complement appears to be important for the development of lesions in BP, the existence of lesions that lack complement deposits, the presence of non-complement fixing sera and the predominance of IgG_4 in BP tissue and sera have led to the suggestion that the inflammatory response in BP may occur through a different mechanism which involves mast cells.[7] Immunoglobulin G_4 is an antibody that has been shown to have homocytotropic properties for mast cells.[24] Therefore, the interaction of IgG_4 antibodies with mast cells in the skin may represent an alternative or additional mechanism of tissue injury leading to mast cell degranulation, inflammation and blister formation in BP.

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