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Complement-Fixing Abilities and IgG Subclasses of Autoantibodies in Epidermolysis Bullosa Acquisita

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Epidermolysis bullosa acquisita (EBA) is an autoimmune-mediated subepidermal bullous disease in which the target of the autoantibodies is type VII collagen, a major component of anchoring fibrils. The purpose of this study was to evaluate the complement-fixing abilities and IgG subclass distribution of autoantibodies in EBA, and to also attempt to investigate the relation between inflammation, complement fixation and IgG subclass distribution in EBA patients. Only 2 sera of 18 patients (11%) showed weak complement-fixing abilities. IgG1 and IgG4 were the most frequently and intensely stained IgG subclasses in EBA sera. We could not find any relationship between the clinico-pathologic types, complement-fixing abilities and IgG subclasses in EBA. These results suggested that complement activation may not be a key factor of bulla formation in EBA.

Key Words: EBA, complement-fixing ability, IgG subclasses

Epidermolysis bullosa acquisita (EBA) is a rare autoimmune subepidermal bullous disease characterized by autoantibodies targeting the NC1 domain of type VII collagen of the skin's basement membrane zone (BMZ) (Gammon *et al.* 1982; Woodley *et al.* 1984; Sakai *et al.* 1986; Woodley *et al.* 1988; Tanaka *et al.* 1994).

Clinically, EBA can be divided into three types. Classic type is a noninflammatory mechanobullous eruption that presents with marked skin fragility, blisters and erosions at sites of trauma. This type mimics porphyria cutanea tarda and hereditary dystrophic epidermolysis bullosa. The second type is an inflammatory blistering disease mimicking bullous pemphigoid (BP). The third type is a mucosal type that affects oral, ocular, genital and even esophageal and laryngeal mucous membranes, forming scars and erosions mimicking cicatricial pemphigoid. Most patients belong to the first two types (Woodley, 1988; Briggaman *et al.* 1990; Woodley, 1990; Lapiere *et al.* 1996). During the course of the disease, transitions may be seen from the inflammatory type to the noninflammatory mechanobullous type of the disease (Briggaman *et al.* 1990). Pathologically, inflammatory lesions show many inflammatory cell infiltrations of neutrophils and eosinophils around and below the subepidermal bulla. Noninflammatory lesions show subepidermal blisters with a dermis nearly devoid of inflammatory cells (Woodley, 1988; Briggaman *et al.* 1990; Lapiere *et al.* 1996; Cohen *et al.* 1997).

Several hypotheses on the pathogenesis of blister formation in EBA have been proposed. In the inflammatory type, IgG at the BMZ leads to complement-mediated inflammation. The inflammatory cellular infiltrate induces proteolytic digestion of the essential component of the BMZ and upper papillary dermis. This results in fracturing of the dermoepidermal junction at the lamina lucida

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because the lamina lucida is the most sensitive to proteolytic enzymes (Gammon *et al.* 1984; Fine *et al.* 1989). In the noninflammatory type, the autoantibodies may directly disrupt the interaction of type VII collagen with one or more basement membrane components or prevent the assembly of anchoring fibrils. This process results in splitting of the dermoepidermal junction below the lamina densa (Woodley *et al.* 1990).

Antibodies found in EBA patients are mostly IgG antibodies (Woodley *et al.* 1990). Of the four subclasses of IgG, IgG1 and IgG3 have strong complement-fixing abilities, IgG2 has moderate but IgG4 has little (Roitt, 1997).

We hypothesized that IgG of the inflammatory type has a strong complement-fixing ability and leads to complement-mediated inflammation, whereas IgG of the non-inflammatory type has a weak complement-fixing ability. To test this hypothesis, we evaluated the complement-fixing abilities and IgG subclass distribution of autoantibodies in two clinico-pathologic types of EBA.

MATERIALS AND METHODS

Patients

Eighteen patients with EBA (8 male, 10 female), with an average age of 42 (range, 27~60) were included in this study. The diagnosis of EBA was confirmed by the results of indirect immunofluorescence (IIF) on salt-split skin and immunoblotting. Control sera were obtained from 5 patients with BP.

Indirect immunofluorescence studies

Six-μm-thick cryostat sections were prepared from normal human skin and incubated with serially-diluted serum from each EBA and BP patient for 1 hour at room temperature in a humidified chamber. The serum was diluted four-fold from 1 : 10 to 1 : 640 in phosphate buffered saline (PBS, pH 7.4). After incubation, each specimen was briefly rinsed in PBS, further incubated for 30 min in the presence of FITC-conjugated anti-human IgG (1 : 40 dilution; DAKO, Copenhagen, Denmark), further rinsed in PBS, and then mounted with 10% glycerol in PBS. The slides were examined by immunofluorescence microscope (Olympus, Tokyo, Japan).

Complement-fixing indirect immunofluorescence studies

Complement of the serum from each EBA and BP patient was inactivated by being placed in a 56°C warm bath for 30 min. Cryostat sections of normal human skin were incubated with complement-inactivated serum of patients diluted in Hanks' balanced salt solution (Ca^{2+} 0.14g/ ℓ, Mg^{2+} 0.2 g/ ℓ, HBSS No. 24020, Gibco, Grand island, NY, USA)) at room temperature for 1 hour. The slides were further incubated for 40 min in the fresh human serum (1 : 10 dilution, in Hanks' balanced salt solution containing calcium and magnesium) as a fresh complement supplement. After rinsing in PBS, the tissues were incubated for 1 hour in the presence of FITC-conjugated anti-human IgG (1 : 40 dilution; DAKO) and anti-C3 (1 : 40 dilution; DAKO), further rinsed in PBS, and then mounted with 10% glycerol in PBS.

Detection of IgG subclasses by indirect immunofluorescence studies

Cryostat sections of normal human skin were incubated with serially-diluted sera from each EBA and BP patient at room temperature for 1 hour. After incubation, each specimen was briefly rinsed in PBS, further incubated for 40 min in the presence of FITC-conjugated anti-human IgG1 (1 : 40 dilution; Sigma, St Louis, MO, USA; clone number 8 c/6-39), IgG2 (1 : 40 dilution; Sigma; clone number HP-6014), IgG3 (1 : 40 dilution; Sigma; clone number HP-6050) and IgG4 (1 : 20 dilution; Sigma; clone number HP-6025), further rinsed in PBS, and then mounted with 10% glycerol in PBS.

RESULTS

Routine histologic examination

Routine biopsy slides were obtained from 10 of the 18 EBA patients. Many inflammatory infiltrates were noted in 7 patients (7/10). The most common

Table 1. The pathologic type, DIF studies, complement-fixing IIF studies and IgG subclasses of EBA patients

Pt	Cellular Infiltration	DIF	IIF	C' fixing IIF	IgG subclasses			
					IgG1	IgG2	IgG3	IgG4
	Neu/Eos	NC	1 : 1280	−	1 : 10	−	−	1 : 20
	Neu/Eos	IgG,C3	1 : 320	−	1 : 80	−	1 : 10	1 : 40
	Neu/Eos	IgG,C3	1 : 320	: 10	1 : 20	1 : 10	1 : 40	1 : 160
	Neu	IgG	1 : 160	−	1 : 10	1 : 10	1 : 10	1 : 10
	Neu	NC	1 : 40	−	1 : 40	1 : 40	1 : 40	1 : 80
	Neu	IgG	1 : 40	−	1 : 10	−	−	1 : 20
7	Neu	IgG	1 : 20	−	1 : 10	−	−	−
8	None	NC	1 : 320	−	1 : 40	−	−	1 : 40
9	None	NC	1 : 160	: 10	1 : 80	1 : 10	1 : 160	1 : 320
10	None	IgG,C3	1 : 40	−	1 : 10	−	−	−
11	NC	IgG	1 : 1280	−	1 : 10	−	1 : 160	1 : 320
12	NC	NC	1 : 1280	−	1 : 40	1 : 10	1 : 80	1 : 40
13	NC	NC	1 : 640	−	1 : 20	1 : 20	1 : 40	1 : 80
	NC	NC	1 : 640	−	1 : 80	1 : 20	1 : 160	1 : 320
15	NC	NC	1 : 160	−	1 : 40	1 : 40	1 : 160	1 : 160
16	NC	IgG,C3	1 : 20	−	1 : 20	−	−	−
17	NC	IgG,C3.	1 : 10	−	1 : 20	−	−	−
18	NC	NC	1 : 10	−	1 : 10	−	−	−
No. of Pt(%)		5(56)	18(100)	2(11)	18(100)	8(44)	10(56)	13(72)
Mean value			1 : 374	: 10	: 30	1 : 9	1 : 40	1 : 89

Pt: patient, Neu: neutrophil, Eos: eosinophil, NC: non-confirmed

Table 2. Complement-fixing abilities and IgG subclasses of BP patients

Pt	C' fixing IIF	IgG subclasses			
		IgG1	IgG2	IgG3	IgG4
1	1 : 160	>1 : 1280	1 : 160	1 : 1280	>1 : 1280
2	1 : 80	1 : 1280	1 : 160	1 : 160	1 : 1280
3	1 : 80	1 : 320	1 : 160	1 : 160	>1 : 1280
4	1 : 40	1 : 80	−	−	1 : 40
5	1 : 40	1 : 160	−	−	1 : 20
No. of Pt(%)	5(100)	5(100)	3(60)	3(60)	5(100)
Mean value	1 : 80	>1 : 624	: 96	1 : 320	>1 : 780

type of inflammatory cell detected was neutrophils (7/10). Eosinophils were also detected in three patients (3/10) and eosinophils predominated in one patient (1/10). Three patients had little inflammatory cell infiltration on the base and lateral sides of bulla (Table 1, Fig. 1A, B).

Direct immunofluorescence findings

Direct immunofluorescence (DIF) result records were available in 9 EBA patients. IgG was present in all 9 along the BMZ. C3 was present along the BMZ in 5 specimens (56%) (Table 1).

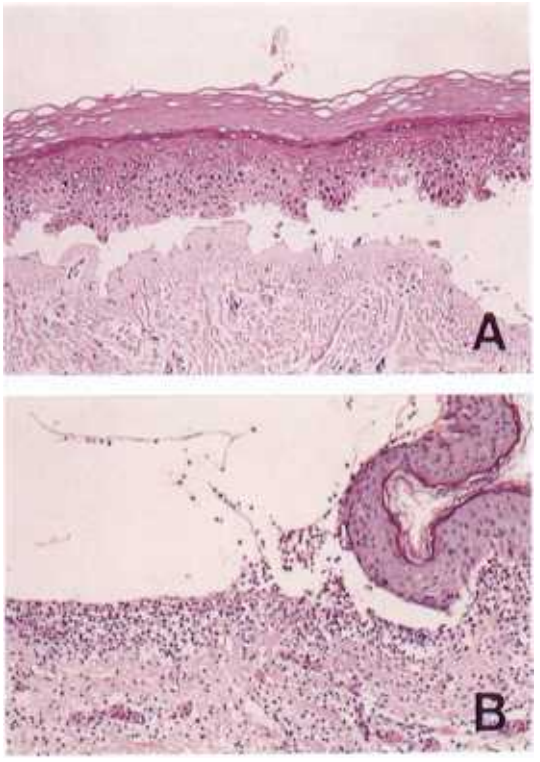

Fig. 1. Histologic findings of EBA (H&E stain, ×100). A: Noninflammatory type showing few inflammatory cell infiltrations (Pt 10), B: Inflammatory type showing many inflammatory cell infiltrations (Pt 2).

Indirect immunofluorescence and complement-fixing indirect immunofluorescence findings

The mean value of IgG titers of EBA patients was 1 : 374 (range, 1 : 10 to 1 : 1280). IgG autoantibodies were detected in all sera of EBA patients. However, only 2 of 18 EBA sera (11%) were found to contain complement-fixing abilities (Table 1, Fig. 2A, B). As a positive control, we also performed the same procedures in the sera of 5 BP patients. All sera of BP patients had IgG autoantibodies and complement-fixing abilities (Table 2, Fig. 2C).

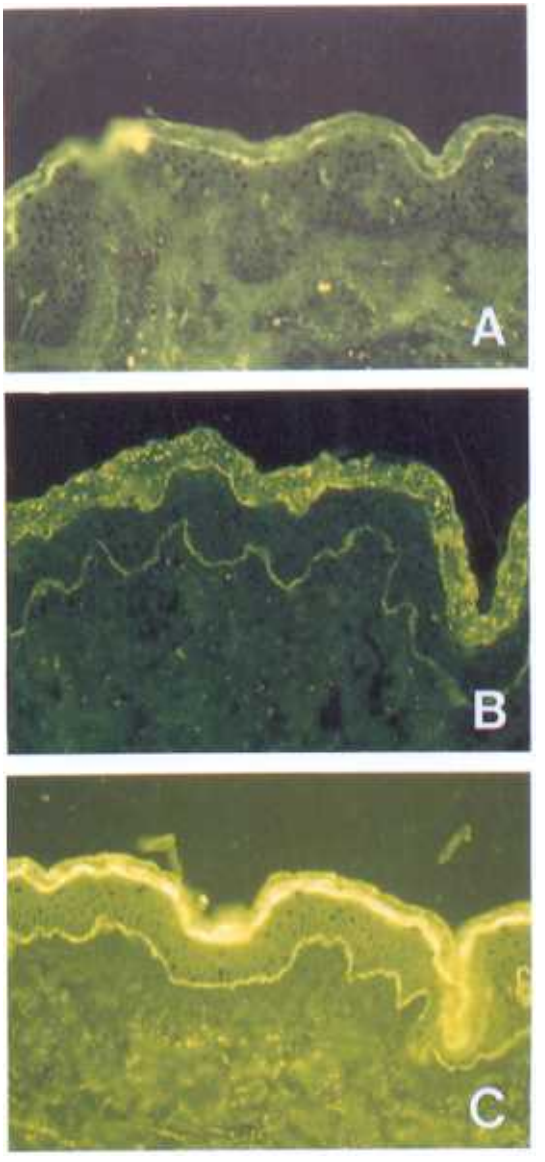

Fig. 2. Complement-fixing IIF studies of EBA and BP patients (×100). A: No complement-fixing abilities found in BMZ of EBA patient (Pt 8), B: Weak complement-fixing abilities detected in BMZ of EBA patient (Pt 9), C: Strong complement-fixing abilities detected in BMZ of BP patient (Pt 2).

IgG subclasses by indirect immunofluorescence studies

All IgG subclasses were found in the sera of 18 EBA patients by IIF studies. IgG1 subclass was the most frequent autoantibody, present in all 18 patients followed by IgG4, IgG3 and IgG2. IgG4 subclass had the highest titer of all the subclasses, followed by IgG3, IgG1 and IgG2. Therefore, IgG1 and IgG4 subclasses were the most frequently detected and intensely stained IgG subclasses of

EBA sera (Table 1).

In 5 BP patients, all IgG subclasses were found in the sera, and also the IgG1 and IgG4 subclasses were the most frequent and intensely stained subclasses (Table 2).

DISCUSSION

We attempted to divide EBA patients into inflammatory type and noninflammatory type by histologic examinations. We could only obtain routine biopsy slides from 10 EBA patients. Seven of them were of the inflammatory type. Among them, 4 were neutrophil-dominant and 3 showed a mixture of neutrophil and eosinophil infiltration. Three were of the noninflammatory type showing a scanty inflammatory cell infiltrate. Our data showed that the inflammatory type was more common than the noninflammatory type, confirming the report of other authors (Woodley, 1988). However, a strict distinction between inflammatory type and noninflammatory type might be impossible because these 2 types can occur in the same patient and there can be a transition between the 2 types.

In our investigation, DIF studies showed complement depositions in 5 of 9 EBA patients (56%), however, only 2 of 18 EBA patients (11%) showed complement-fixing abilities at 1 : 10 titer in complement-fixing IIF studies. One patient was of the inflammatory type but the other was of the noninflammatory type. Therefore, we could not find any correlation between complement-fixing abilities and clinico-pathologic types. In order to improve the reliability of our complement-fixing IIF test, we performed complement-fixing IIF studies in the sera of 5 BP patients. All of them showed complement-fixing abilities in IIF study. Mooney and Gammon showed similar results to ours (Mooney and Gammon, 1990). They demonstrated that all 10 patients with EBA showed C3 deposition in DIF study, but 8 of 18 sera of patients with EBA showed C3 fixation activities. It is not clear why the sera of EBA patients have lower complement-fixing abilities despite frequent deposition of C3 in the skin of patients with EBA. So far, several studies with variable results have been done to evaluate complement-fixing abilities in patients with EBA. Smaller and Woodley showed that in DIF study, 3 of 8 patients with EBA had C3 deposition in their tissue samples, in contrast, 17 of 18 patients with BP had C3 deposition (Smaller and Woodley, 1992). They suggested that complement-independent chemotaxis may play a role in the pathogenesis of EBA in patients without demonstrable complement deposition in BMZ.

Gammon *et al.* suggested complement binding in BMZ might play an important role in the pathogenesis of EBA (Gammon *et al.* 1984). However, our observations suggest other mechanisms in the pathogenesis of blister formation. The role of complement activation and inflammatory cells in the pathogenesis of blister formation in BP has been well established (Smoller and Woodley, 1992; Liu *et al.* 1996). By contrast, our results suggested that complement activation might not be a key factor of bulla formation in EBA patients because only 2 of 18 patients with EBA showed complement-fixing activities. Some other mechanism of blister formation may be proposed. Autoantibodies bind with EBA antigen (type VII collagen), inducing complement-independent inflammation and disconnection between EBA antigen and an extracellular matrix like fibronectin (Woodley *et al.* 1990). Several chemokines such as eotaxin, IL-8 and lymphotactin (Janeway *et al.* 1997) may be involved in the course of complement-independent inflammation.

IgG1 and IgG4 were more frequent and higher titered IgG subclasses in EBA patients. The same result has been observed in other autoimmune vesicobullous diseases. IgG4 is a predominant subclass in BP, pemphigus vulgaris and non-endemic pemphigus foliaceus (Jones *et al.* 1988; Rock *et al.* 1989) and, IgG1 is the major IgG subclass in herpes gestationis (Kelly *et al.* 1989). IgG4 is known as a protective antibody in many allergic diseases and IgG4 levels increase in situations of chronic antigenic stimulation such as filariasis and hymenoptera venom exposure (Urbanek *et al.* 1986). Although IgG4 has few complement-fixing abilities, the pathogenic role of IgG4 was demonstrated by passive transfer experiments in pemphigus foliaceus (Rock *et al.* 1989). Therefore, increased levels of IgG4 in pemphigus (Jones *et al.* 1988), BP (Bird *et al.* 1986) and EBA (Bernard *et al.* 1991) may be

due either to chronic antigenic stimulation or by some pathogenesis-related mechanism.

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