

BP180 ELISA using bacterial recombinant NC16a protein as a diagnostic and monitoring tool for bullous pemphigoid

Masakazu Kobayashi ^{a,*}, Masayuki Amagai ^a, Keiko Kuroda-Kinoshita ^b,
Takashi Hashimoto ^c, Yuji Shirakata ^d, Koji Hashimoto ^d, Takeji Nishikawa ^a

^a Department of Dermatology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan

^b Medical and Biological Laboratories Co. Ltd., Nagoya, Japan

^c Department of Dermatology, Kurume University School of Medicine, Fukuoka, Japan

^d Department of Dermatology, Ehime University School of Medicine, Ehime, Japan

Received 27 June 2002; received in revised form 20 August 2002; accepted 22 August 2002

Abstract

Bullous pemphigoid (BP) is an acquired autoimmune subepidermal blistering disease against hemidesmosomal cytoplasmic BP230 and transmembrane BP180 proteins. Epitope mapping studies have shown that the membrane-proximal noncollagenous (NC) 16a domain of BP180 harbors clusters of antigenic sites recognized by the vast majority of BP sera. In this study, we developed an enzyme-linked immunosorbent assay (ELISA) using bacterial recombinant NC16a protein and evaluated its clinical benefit for diagnosis and monitoring disease activity. Fifty four (84.4%) of 64 sera from BP patients were positive, while only one (1.1%) of 91 sera from collagen disease patients and five (1.5%) of 336 sera from normal control barely exceeded the cut-off value. None of 69 pemphigus vulgaris sera and none of 42 pemphigus foliaceus sera exceeded the cut-off value. Thus, the sensitivity and specificity of NC16a ELISA were 84.4 and 98.9%, respectively. The correlation between ELISA scores and disease activity along the time course was examined using seven BP patients. NC16a ELISA scores tended to fluctuate in parallel with the disease activity along the time course and reflected the disease activity much better than indirect immunofluorescence. These findings indicate that NC16a ELISA will be a valuable tool not only for the diagnosis of patients with BP but also for the monitoring of the disease activity.

© 2002 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Autoimmunity; Autoantibody; Collagen; Hemidesmosome

1. Introduction

Bullous pemphigoid (BP) is an autoimmune subepidermal blistering disease characterized by

* Corresponding author. Tel.: +81-3-5363-3823; fax: +81-3-3351-6880

E-mail address: masakazu@saichu.jp (M. Kobayashi).

the linear deposition of IgG and C3 at cutaneous basement membrane zone (BMZ). IgG autoantibodies in BP are directed against two hemidesmosomal proteins designated BP230 and BP180 [1]. BP230 is localized to the intracellular hemidesmosomal plaque, and thus, autoantibodies to this antigen are thought to be not directly involved in the initiation of the blister formation because IgG cannot penetrate cell membranes. Antibodies against BP230 are accessible to the antigen only after preceding inflammation [2]. In contrast, BP180 is a hemidesmosomal transmembrane protein with the extracellular C-terminal end. BP180 consists of 15 interrupted collagen domains and 16 noncollagenous (NC) extracellular domains [3]. A NC stretch of the BP180 extracellular domain, NC16a, has recently been shown to harbor a major antigenic site [4–7]. In a passive transfer mouse model, rabbit antibodies raised against mouse NC16a domains are probed to be pathogenic in inducing BP-like blisters in neonatal mice [8]. These observations indicate that IgG autoantibodies against BP180 play a critical role in the pathogenesis of human BP.

Although it is clear that BP is an autoantibody-mediated disease, it is generally considered that the titers of circulating IgG autoantibodies determined by indirect immunofluorescence are not a sensitive indicator for the clinical activity in BP [9,10]. The poor correlation of indirect immunofluorescence titers and the disease activity is probably because indirect immunofluorescence represents combined reaction of antibodies against BP230 and BP180. Using recombinant NC16a proteins as the antigenic substrate, enzyme-linked immunosorbent assay (ELISA) was developed as a highly sensitive and specific assay for the detection of IgG autoantibodies against BP180 [11]. Furthermore, it was demonstrated that the titers of antibodies against NC16a domain of BP180 tended to fluctuate in parallel with the disease activity [12,13]. In this study we independently developed an ELISA using a NC16a bacterial recombinant protein as a coated antigen and demonstrated that ELISA scores against NC16a domain are a helpful indicator of the disease activity.

2. Materials and methods

2.1. Human sera

All sera tested in this study were collected from three different dermatological institutions: Kurume University, Ehime University and Keio University. The diagnosis of BP was made on the basis of clinical features, histology and immunofluorescence findings. All BP sera tested showed positive reactivity against BMZ by indirect immunofluorescence. Sera were obtained from 84 patients with BP. Among them 64 sera were taken at active stage and 20 sera were taken in remission. Sera were also obtained from 69 patients with pemphigus vulgaris, 42 patients with pemphigus foliaceus, and 91 patients with collagen disease patients (dermatomyositis, nine; mixed connective-tissue disease, 11; polymyositis, ten; systemic sclerosis, 12; rheumatoid arthritis, 13; Sjogren's syndrome, 20; systemic lupus erythematosus, 13; overlap syndrome, three) and 336 sera from normal individuals.

2.2. Production of recombinant NC16a protein

The NC16a domain of BP 180, which is known to contain the major epitopes for BP180, was produced by bacterial expression as previously reported [5,6]. In brief, the NC16a domain of BP180 was produced by fusion protein in *Escherichia coli*. The recombinant protein encoding the NC16a domain of BP180 was produced as a fusion protein with glutathione *S*-transferase, and purified on a glutathione sepharose 4B column (Amersham Pharmacia Biotech, Uppsala, Sweden).

2.3. ELISA with NC16a

ELISA plates were coated with approximately 10 µg/ml of rNC16a at 4 °C over night and subsequently blocked with 1% BSA, 0.1% Na₂S₂O₃ and 5% sucrose in phosphate-buffered saline (PBS) at 4 °C over night. The plates were incubated with 1:100 diluted sera for 1 h at room temperature, washed and then incubated with 1:10 000 diluted horseradish peroxidase-conjugated goat polyclonal anti-human IgG antibody (MBL, Nagoya,

Japan) for 1 h at room temperature. After washing the color development was achieved by substrate solution TMBS-1000 (Moss Inc., Maryland, USA). The substrate solution was incubated for 30 min at room temperature and color development was stopped by adding 0.5 M H₂SO₄. The absorbance was measured at 450 nm by microtiter plate readers (Bio-Rad Laboratories, Hercules, CA; Tosoh Co. Tokyo, Japan).

The index value was defined by the following formula: index = (optical density [OD] of tested serum – OD of negative control) / (OD of positive control – OD of negative control) × 100. A positive control and negative control was included in every plate. Positive control for NC16a ELISA was a diluted standard BP serum. The negative control was a diluted standard serum obtained from a normal control. The negative control revealed the non-specific, background noise of the system, which was subtracted from all values on the plate.

2.4. Indirect immunofluorescence

To obtain indirect immunofluorescence titers, BP sera were serially diluted at 1:10, 1:40, 1:160, 1:640, 1:2560 and 1:10 240 with PBS, and incubated on cryosectioned normal human skin for 1 h, which was followed by incubation with 100-fold diluted FITC-conjugated anti-human IgG Abs (DAKO, Copenhagen, Denmark) as a second antibody.

2.5. Immunoblotting analysis with epidermal extracts and recombinant NC16a protein

Epidermal extracts were obtained from ethylene-diamine tetraacetic acid (EDTA)-separated normal human epidermis as described previously [14]. Purified rNC16a was also used as a substrate for immunoblot analyses. Briefly, epidermal extracts or recombinant NC16a dissolved in Leammli's sample buffer were applied to SDS polyacrylamide gel electrophoresis, and transferred to PVDF membranes (Millipore, Bedford, MA). The membranes were incubated with 20-fold diluted sera, and the reactivity was visualized with horseradish peroxidase-conjugated anti-human

IgG antibodies (Zymed Laboratories, San Francisco, CA).

2.6. Statistical analysis

A receiver-operating-characteristic (ROC) analysis was performed to determine a cut-off value for NC16a ELISA. The sensitivity and specificity were plotted when different scores were used for cut-off value and the one which gave the highest score for the sum of sensitivity (percentage) and specificity (percentage) was determined to be the cut-off value to be used. Data obtained with BP sera at active stage and normal control sera were used for this analysis.

2.7. Assessment of disease activity

Disease activity was arbitrarily assessed on a scale of 0–4. The score 4 was set at the highest activity for each patient during the observation period and relative activity was assessed arbitrarily.

3. Results

3.1. Analysis of sera obtained from patients and controls with NC16a enzyme-linked immunosorbent assay

To determine a cut-off value we performed a ROC analysis with 64 BP sera taken at active stage and 336 normal control sera with NC16a ELISA (Fig. 1). The one, which gave the highest sum of sensitivity (percentage) and specificity (percentage), was defined as the cut-off value to be used. For NC16a ELISA, this was determined to be 9.1.

With this cut-off value, 54 (84.4%) of 64 sera from BP patients in active stage showed positive scores above the cut-off value, while one (1.1%) of 91 sera from collagen disease patients and five (1.5%) of 336 normal control sera slightly exceeded the cut-off value, and none (0%) of 69 pemphigus vulgaris sera and none (0%) of 42 pemphigus foliaceus sera were positive (Fig. 2). Thus, the sensitivity and specificity of NC16a ELISA were 84.4% (54/64) and 98.9% (532/538), respectively.

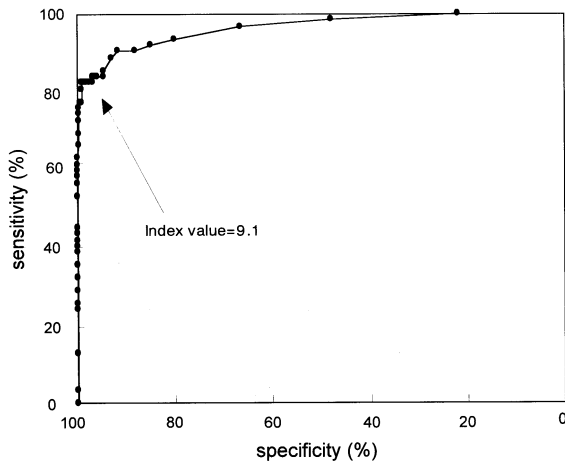


Fig. 1. Receiver-operating-characteristic curve to determine a cut-off value for BP180 ELISA.

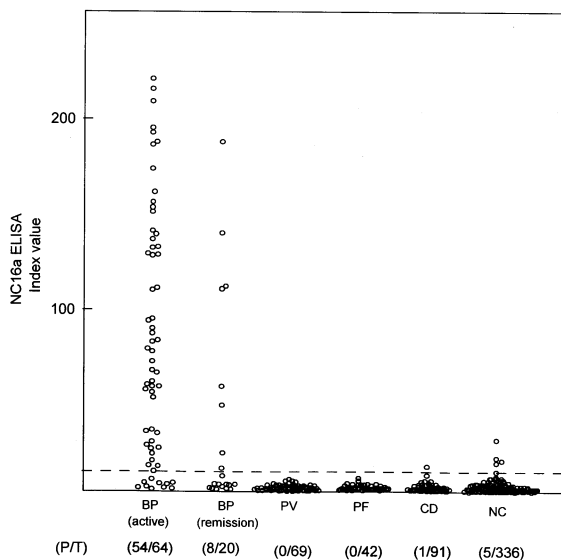


Fig. 2. Anti-NC16a IgG autoantibody titers by ELISA using recombinant NC16a. BP, bullous pemphigoid; PV, pemphigus vulgaris; PF, pemphigus foliaceus; CD, collagen diseases; NC, normal controls; P/T, positive vs. total number examined. Dashed line indicates the cut-off value at 9.1.

When BP patients in remission were examined, eight (40%) of 20 sera exceeded the cut-off value.

To investigate the antigen specificity of the BP sera which showed negative reactivity by NC16a ELISA, we performed immunoblot analysis with human epidermal keratinocytes and recombinant

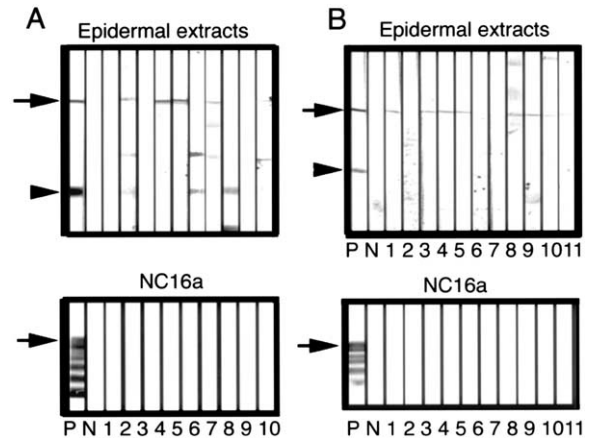


Fig. 3. Immunoblot analysis with ELISA-negative BP sera. Negative sera obtained from patients with active BP (A) and in remission (B) were subjected to immunoblot analysis with epidermal extracts (upper panel) and recombinant NC16a (lower panel) as a substrate. In the upper panel arrow and arrow head indicate 230 and 180 kDa bands, respectively. In the low panel arrow indicates the recombinant NC16a protein and ladders with lower molecular weights are degraded products. P, a positive BP serum; N, a normal control serum.

NC16a as a substrate (Fig. 3). We tested the 21 BP sera, which were negative by ELISA. Among them ten sera were taken at active stage and 11 were taken in remission. None of the 21 sera tested reacted with the recombinant NC16a by immunoblotting, which was consistent with the results of ELISA. Three of ten active BP sera reacted with the 180 kDa band while five of them reacted with the 230 kDa band in the epidermal extracts. None of the 11 remission BP sera reacted with the 180 kDa band while eight of them reacted with the 230 kDa band.

3.2. Enzyme-linked immunosorbent assay scores to monitor the disease activity

We examined the correlation between ELISA scores and disease activity over time and compared that with indirect immunofluorescence titers. We tested seven BP patients whose serial sera and clinical records were available.

The first BP patient was a 58-year-old Japanese woman (Fig. 4A). She had been well controlled with low dose of prednisolone with minocycline and nicotinamide. After 3 years of remission she

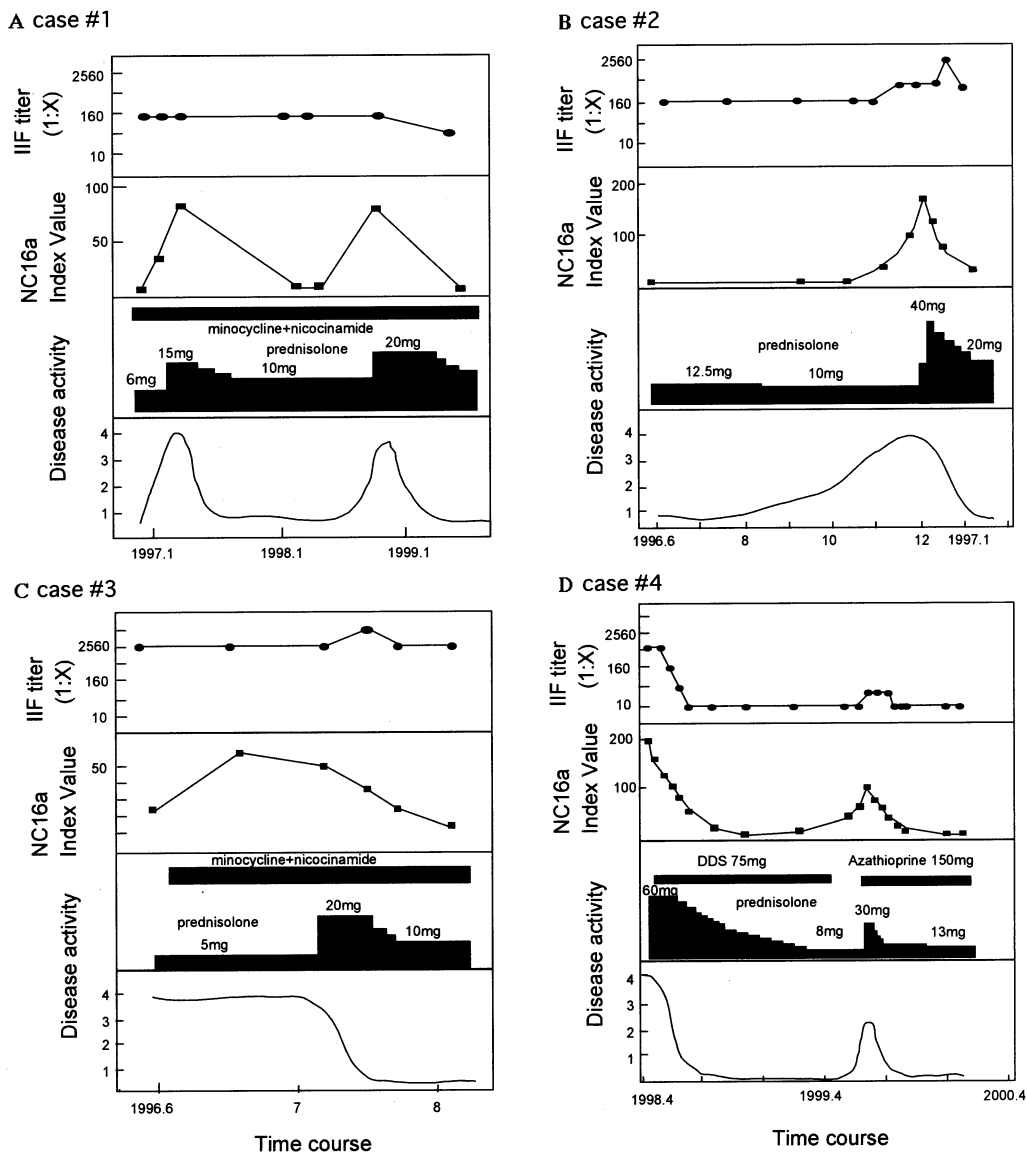


Fig. 4. NC16a ELISA scores tend to fluctuate in parallel with the disease activity over a time course. DDS, diaphenylsulfone; PE, plasmapheresis; pulse, methylprednisolone pulse therapy.

experienced a flare. When prednisolone was increased to 15 from 6 mg/day, she responded well. However, she showed second flare with extensive skin lesions while she was maintained with 10 mg/day prednisolone. When prednisolone was increased to 20 mg/day her skin lesions rapidly improved. NC16a ELISA demonstrated rapid increases at the time of flares and decrease at the

time of remission. In contrast, indirect immunofluorescence titers did not show any fluctuation during the time course and stayed in high reactivity.

The second BP patient was a 86-year-old Japanese man (Fig. 4B). The cutaneous lesions were well controlled with a low dose of prednisolone. When prednisolone was tapered to 10 mg/

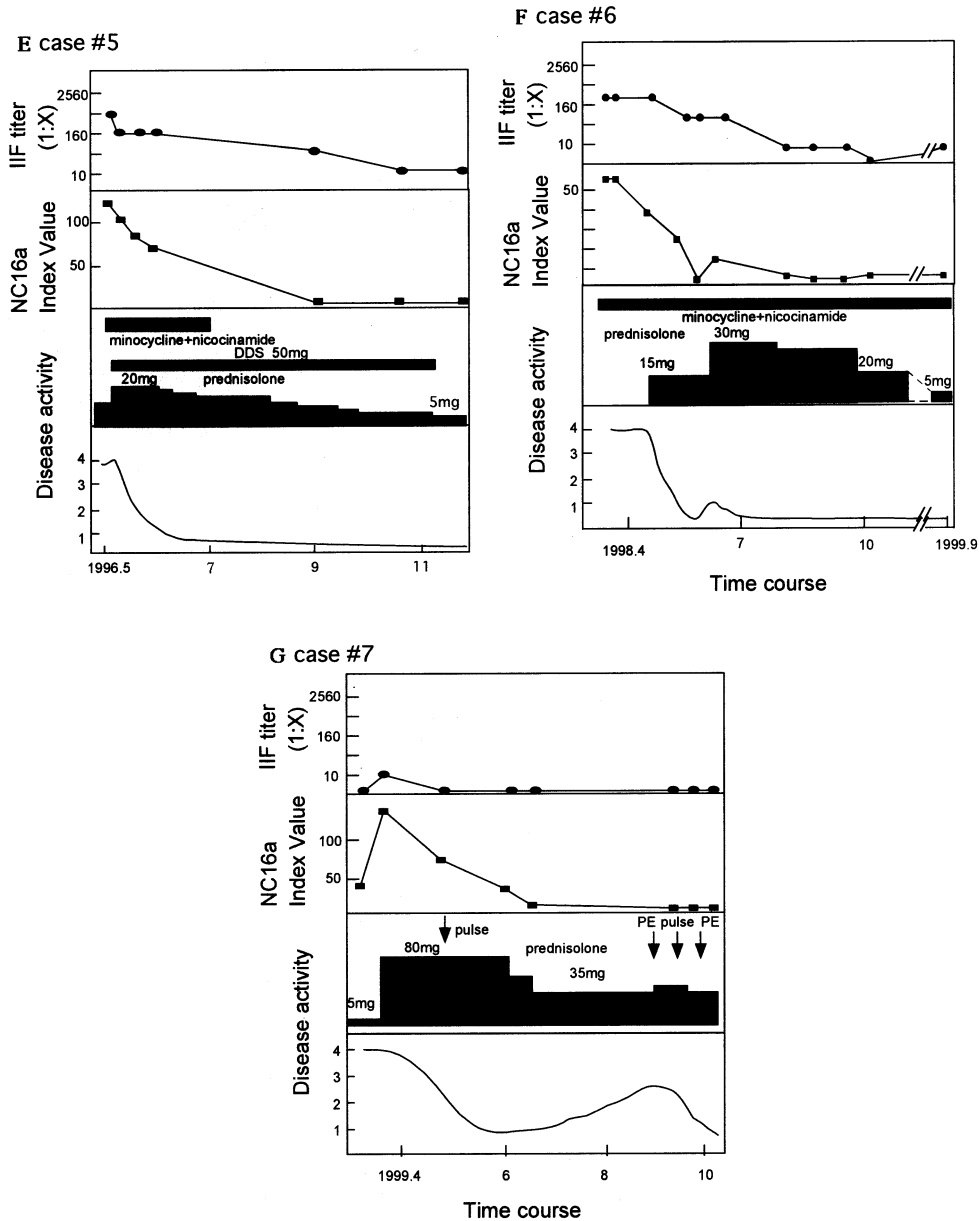


Fig. 4 (Continued)

day, the skin condition got gradually exacerbated. He responded well with 40 mg/day prednisolone. NC16a ELISA scores increased as the disease activity went up and they showed a sharp decline after the treatment. Although indirect immunofluorescence titers showed slight elevation of the

titer around the time of the flare, the fluctuation was not as clear as that of ELISA.

The third BP patient was a 71-year-old Japanese woman (Fig. 4C). This patient had a 4 years history of the disease. When she came to our hospital, she had blisters and erosions on her arms

and trunk with 5 mg/day of prednisolone. Although minocycline and nicotinamide were added, new skin lesions continued to appear. When prednisolone was increased to 20 mg/day, she responded well and the cutaneous lesions disappeared completely within 1 month. NC16a ELISA scores showed gradual increase as new lesions kept appearing, and declined after prednisolone was increased. In this case, indirect immunofluorescence titers did not show any significant fluctuation throughout the time course and stayed in high reactivity.

The fourth BP patient was a 38-year-old Japanese man (Fig. 4D). He presented with small erosions, blisters and diffuse erythema on his face and new lesions continued to appear on his trunk and extremities. Treatment was initiated with 60 mg/day prednisolone and 75 mg/day of diaphenylsulfone, which resulted in clinical improvement with epithelization of erosive lesions. When prednisolone was tapered to 8 mg/day and diaphenylsulfone was discontinued, the disease relapsed with small papules, erosions and blisters on his face and extremities. Prednisolone was increased to 30 mg/day in combination with 150 mg/day of azathioprine, which resulted in significant improvement of the skin lesions. Then prednisolone was gradually tapered to 13 mg/day. NC16a ELISA scores fluctuated very well in parallel with the disease activity along the time course. ELISA scores showed a sharp decline in response to the treatment and picked up the flare. In this case indirect immunofluorescence titers reflected the disease activity relatively well.

The fifth BP patient was a 80-year-old Japanese woman (Fig. 4E). When she came to us after a year history of the disease, she presented with tense blisters and erosions on her arms and trunk despite of 10 mg/day of prednisolone. When minocycline and nicotinamide were initiated and prednisolone was increased to 20 mg/day in combination with 50 mg/day of diaphenylsulfone, the patient showed rapid clinical improvement. Prednisolone was gradually tapered to 5 mg/day in the following 6 months without any flares. NC16a ELISA scores initially showed a high reactivity and demonstrated a gradual decrease as the disease subsided. Indirect immunofluorescence titers showed a de-

cline but its decline speed was much slower than that of ELISA.

The sixth BP patient was a 62-year-old Japanese woman (Fig. 4F). She had several scattered tense blisters on her trunk as well as erosions in her oral mucosa. Treatment was initiated with minocycline and nicotinamide without any clinical improvement. She showed a quick response with 15 mg/day prednisolone. However, her skin lesions showed exacerbation and prednisolone was increased to 30 mg/day which was effective. Then prednisolone was tapered in following 2 years to 5 mg/day without any flares. NC16a ELISA scores reflected the clinical activity well and even picked up the small exacerbation. Indirect immunofluorescence gradually and slowly decreased but did not necessarily reflect the disease activity and failed to pick up the exacerbation.

The seventh BP patient was a 49-year-old Japanese woman (Fig. 4G). She presented herself with extensive blisters and erosions on her trunk and extremities and erosions in her oral mucosa with maintenance dose of 6 mg/day of prednisolone. Prednisolone was increased to 80 mg/day, which resulted in clinical improvement. When prednisolone was tapered to 35 mg/day, she showed a flare with wide-spread erythema. Plasmapheresis and methylprednisolone pulse therapy were administered with clinical improvement. ELISA scores reflected the disease activity in the first half of the time course although they failed to fluctuate in parallel with the activity in the second half. Indirect immunofluorescence showed positive reaction at 1:10 only once at the peak of the disease activity and showed negative reactivity throughout the time course.

4. Discussion

Indirect immunofluorescence using normal human skin or monkey esophagus as a substrate has been used as a reliable serological test for the diagnosis of BP since the discovery of the auto-antibodies in BP. However, it is generally considered that the titers measured by indirect immunofluorescence do not necessarily correlated with the disease activity [9,10] probably because it

detects not only anti-BP180 antibodies which was proved to be pathogenic, but also anti-BP230 antibodies which may not be directly involved in the induction of blister formation. Availability of recombinant BP180 and identification of major immunogenic domain on the BP180 molecules allowed us to develop more specific diagnostic tools such as ELISA to measure autoantibodies specific for a particular antigen or a particular domain on the antigen. Giudice et al. developed first ELISA using MCW-1 which is an autoantibody-reactive site in the NC16a domain for detection of IgG autoantibodies in BP, but the sensitivity was only 53% [15]. Subsequently, additional autoantibody-reactive sites were identified adjacent to the MCW-1 in the NC16a domain [7] and the reactivity against the entire NC16a domain was demonstrated in 90% of BP sera by immunoblotting [5,16]. ELISA using the recombinant NC16a domain as a coated antigen was developed and its sensitivity and specificity were 94 and 100%, respectively [11]. Thus, ELISA using NC16a domain was proved to be a highly sensitive and specific diagnostic tool for BP.

Previously, we attempted to express the entire extracellular domain of BP180 by baculovirus expression system because some BP sera contain autoantibodies against epitopes outside of the NC16a domain [16,17]. However, the expression level of the entire extracellular domain of BP180 was too low to develop a large scale of production of ELISA. Furthermore, the sensitivity of ELISA using the entire extracellular domain of BP180 was not necessarily higher than that of ELISA using the NC16a domain [17]. Therefore, we concluded that recombinant NC16a should be practically appropriate to be used as the coated antigen for ELISA.

In this study, we developed an ELISA using bacterial recombinant NC16a and examined the sensitivity and specificity with a large number of BP as well as disease or normal control sera. We used BP sera, which were positive by indirect immunofluorescence. Our ELISA showed positive reactivity in 54 (84.4%) of 64 sera obtained from active BP patients and eight (40%) of 20 sera from BP patients in remission. We then examined the target antigens of those sera, which showed

negative reactivity with the ELISA. None of the 21 BP sera, which were negative by ELISA reacted with the recombinant NC16a; while three of them reacted with the 180 kDa band and 13 of them reacted with the 230 kDa band in the epidermal extracts by immunoblotting. These findings indicate that our ELISA fails to detect autoantibodies in about 15% of active BP patients and a half of BP patients in remission and that many of those false negative sera have autoantibodies against BP230 or the other part of extracellular domain of BP180.

Measurement of autoantibodies against specific antigen directly involved in the pathogenesis of BP enabled us to correlate the titers with the disease activity. Schmidt et al. and Amo et al. reported that autoantibody titer against NC16a domain of BP180 correlated with the disease activity [12,13]. In this study we also found that the ELISA scores against NC16a tended to fluctuate in parallel with the disease activity in seven cases and confirmed their findings. In cases #1, 3, and 7, indirect immunofluorescence was not reliable at all to predict the disease activity although ELISA scores well reflected the disease activity. In cases #2, 4, 5, and 6, indirect immunofluorescence showed some fluctuation, but not as sensitive as ELISA scores. In case #7, the ELISA failed to show a raise in antibody titer in the second flare, indicating that ELISA scores could dissociate with the disease activity. ELISA scores which reflect the disease activity may be helpful to plan tapering schedules of corticosteroids and to predict flares or relapses by detecting increases in antibodies before clinical evidence of disease flares are noticed.

Taking these findings together, ELISA using the recombinant NC16a will provide a valuable tool for the diagnosis of BP and should be used as an adjunct test of indirect immunofluorescence. Furthermore, autoantibody titers obtained by ELISA will be a useful maker for the therapeutic management in most patients with BP.

Acknowledgements

This work was supported by Health Science Research Grants for Research on specific Disease

from Ministry of Health, Labour and Welfare and Grant-in-Aids for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

- [1] Stanley JR. Cell adhesion molecules as targets of autoantibodies in pemphigus and pemphigoid, bullous diseases due to defective epidermal cell adhesion. *Adv Immunol* 1993;53:291–325.
- [2] Hall RP, Murray JC, McCord MM, Rico MJ, Streilein RD. Rabbits immunized with a peptide encoded for by the 230-kDa bullous pemphigoid antigen cDNA develop an enhanced inflammatory response to UVB irradiation: a potential animal model for bullous pemphigoid. *J Invest Dermatol* 1993;101:9–14.
- [3] Giudice GJ, Emery DJ, Diaz LA. Cloning and primary structural analysis of the bullous pemphigoid autoantigen, BP180. *J Invest Dermatol* 1992;99:243–50.
- [4] Giudice GJ, Emery DJ, Zelicson BD, Anhalt GJ, Liu Z, Diaz LA. Bullous pemphigoid and herpes gestationis autoantibodies recognize a common non-collagenous site on the BP180 ectodomain. *J Immunol* 1993;151:5742–50.
- [5] Matsumura K, Hashimoto T, Amagai M, Nishikawa T. The majority of bullous pemphigoid and herpes gestationis serum samples react with NC16a domain of the 180 kDa bullous pemphigoid antigen. *Arch Dermatol Res* 1996;288:507–9.
- [6] Murakami H, Amagai M, Higashiyama M, Hashimoto K, Chorzelski TP, Bhogal BS, Black MM, Zillikens D, Nishikawa T, Hashimoto T. Analysis of antigens recognized by autoantibodies in herpes gestationis: usefulness of immunoblotting using a fusion protein representing an extracellular domain of the 180 kDa bullous pemphigoid antigen. *J Dermatol Sci* 1996;13:112–7.
- [7] Zillikens D, Rose PA, Balding SD, Liu Z, Olague MM, Diaz LA, Giudice GJ. Tight clustering of extracellular BP180 epitopes recognized by bullous pemphigoid autoantibodies. *J Invest Dermatol* 1997;109:573–9.
- [8] Liu Z, Diaz LA, Troy JL, Taylor AF, Emery DJ, Fairley JA, Giudice GJ. A passive transfer model of the organ-specific autoimmune disease, bullous pemphigoid, using antibodies generated against the hemidesmosomal antigen, BP180. *J Clin Invest* 1993;92:2480–8.
- [9] Ahmed AR, Maize JC, Provost TT. Bullous pemphigoid. Clinical and immunologic follow-up after successful therapy. *Arch Dermatol* 1977;113:1043–6.
- [10] Venning VA, Wojnarowska F. Lack predictive factors for the clinical course of bullous pemphigoid. *J Am Acad Dermatol* 1992;26:585–9.
- [11] Zillikens D, Mascaro JM, Rose PA, Liu Z, Ewing SM, Caux F, Hoffmann RG, Diaz LA, Giudice GJ. A highly sensitive enzyme-linked immunosorbent assay for the detection of circulating anti-BP180 autoantibodies in patients with bullous pemphigoid. *J Invest Dermatol* 1997;109:679–83.
- [12] Schmidt E, Obe K, Brocker EB, Zillikens D. Serum levels of autoantibodies in patients with bullous pemphigoid. *Arch Dermatol* 2000;136:253–4.
- [13] Amo Y, Ohkawa T, Tatsuta M, Hamada Y, Fujimura T, Katsuoka K, Hashimoto T. Clinical significance of enzyme-linked immunosorbent assay for the detection of circulating anti-BP180 autoantibodies in patients with bullous pemphigoid. *J Dermatol Sci* 2001;26:14–8.
- [14] Hashimoto T, Ogawa MM, Konohana A, Nishikawa T. Detection of pemphigus vulgaris and pemphigus foliaceus antigens by immunoblot analysis using different antigen sources. *J Invest Dermatol* 1990;94:327–31.
- [15] Giudice GJ, Wilske KC, Anhalt GJ, Fairley JA, Taylor AF, Emery DJ, Hoffman RG, Diaz LA. Development of an ELISA to detect anti-BP180 autoantibodies in bullous pemphigoid and herpes gestationis. *J Invest Dermatol* 1994;102:878–81.
- [16] Nakatani C, Muramatsu T, Shirai T. Immunoreactivity of bullous pemphigoid (BP) autoantibodies against the NC16A and C-terminal domains of the 180 kDa BP antigen (BP180): immunoblot analysis and enzyme-linked immunosorbent assay using BP180 recombinant proteins. *Br J Dermatol* 1998;139:365–70.
- [17] Hata Y, Fujii Y, Tsunoda K, Amagai M. Production of the entire extracellular domain of BP180 (type XVII collagen) by baculovirus expression. *J Dermatol Sci* 2000;23:183–90.