Increased serum levels of a proliferation-inducing ligand in patients with bullous pemphigoid

著者	Watanabe R., Fujimoto Manabu, Yazawa N., Nakashima H., Asashima N., Kuwano Y., Tada Y., Maruyama N., Okochi H., Tamaki K.
journal or publication title	Journal of Dermatological Science
volume	46
number	1
page range	53-60
year	2007-04-01
URL	http://hdl.handle.net/2297/3662

Increased serum levels of a proliferation-inducing ligand in patients with bullous pemphigoid

Increased serum APRIL levels in bullous pemphigoid

R. WATANABE, M. FUJIMOTO[†], N. YAZAWA, H. NAKASHIMA, N. ASASHIMA, Y. KUWANO, Y. TADA, N. MARUYAMA[‡], H. OKOCHI[§], and K. TAMAKI

Department of Dermatology, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo, Tokyo, 113-8655, Japan

[†]Department of Dermatology, Kanazawa University Graduate School of Medical Science, 13-1 Takaramachi, Kanazawa, Ishikawa 920-8641, Japan.

[‡]Division of Biostatistics, School of Pharmaceutical Science, Kitasato University, 5-9-1 Shirokane, Minato, Tokyo, 108-8641, Japan

[§]Department of Tissue Regeneration, International Medical Center of Japan, 1-21-1 Toyama, Shinjuku, Tokyo, 162-8655, Japan

Correspondence to:

M Fujimoto

E-mail: fujimoto-m@umin.ac.jp Department of Dermatology, Kanazawa University Graduate School of Medical Science, 13-1 Takaramachi, Kanazawa, Ishikawa 920-8641, Japan.

Phone: +81-76-265-2341

Fax: +81-76-234-4270

Keywords.

APRIL, pemphigus vulgaris, bullous pemphigoid, autoimmunity, anti-BP180 antibody

Abstract

Background: B cells have been demonstrated to have critical roles in developing autoimmune bullous diseases. Recently identified tumor necrosis factor-like molecules, B cell-activating factor of the TNF family (BAFF) and a proliferation-inducing ligand (APRIL) are essential molecules for B cell development, survival, and proliferation. Although the functions of APRIL have not been fully evaluated, recent studies suggest that circulating levels of APRIL are increased in various autoimmune diseases, including systemic lupus erythematosus and rheumatoid arthritis.

Objectives: To determine serum APRIL levels in patients with pemphigus vulgaris (PV) and bullous pemphigoid (BP), and compare those with clinical findings and laboratory findings.

Patients/Methods: Sera from 15 PV patients, 43 BP patients, and 15 normal controls were subjected to ELISA assays to measure serum APRIL, BAFF, Dsg3, and BP180

levels.

Results and Conclusions: Circulating APRIL levels were significantly elevated in BP patients but not in PV patients, and correlated with serum BAFF levels. Our study revealed that serum APRIL levels tended to be increased in the quite early stage of disease. In conclusion, circulating APRIL levels may be a useful marker for early activation of autoimmune diathesis, and furthermore, an effective therapeutic target molecule in patients with BP.

Introduction

Autoimmune bullous diseases are characterized by autoantibodies against specific adhesion molecules of the skin and/or mucous membrane. For instance, patients with pemphigus vulgaris (PV) carry autoantibodies specific to a desmosomal protein desmoglein 3 (Dsg3), while bullous pemphigoid (BP) is distinguished by autoantibodies directed against a hemidesmosomal protein called BP180. Although these autoantibodies are widely known to play a primary role in the disease manifestation[1-5], both in animal models and human, it remains unknown how these disease-specific autoreactive B cells and autoantibodies are induced.

Recent evaluation on the role of B cells in autoimmune diseases has indicated that B cells have more critical functions in regulating immune responses than just the precursors of antibody-secreting cells. Furthermore, B cell depletion therapy with anti-CD20 monoclonal antibody turned out to be effective in the management of

autoimmune diseases, including autoimmune blistering diseases[6-19]. Several studies have also demonstrated that cytokines, such as tumor necrosis factor (TNF)-α, play important roles in these blistering diseases, supported by the validity of the treatment with monoclonal anti-TNF-α antibodies in severe PV[20, 21].

Recently identified two TNF family molecules, B cell-activating factor of the TNF family (BAFF) and a proliferation-inducing ligand (APRIL), have received increasing attention as key regulators of normal B cell functions and autoimmune B cell induction[22]. BAFF is an essential factor for B cell survival, and its three receptors, transmembrane activator and calcium modulator ligand interactor (TACI), B cell maturation antigen (BCMA), and BAFF receptor (BAFF-R), are variably expressed on B cells during their differentiation[23]. APRIL is homologous to BAFF, but binds only to TACI and BCMA. APRIL shares many functions in common with BAFF, while they also have still distinct functions [24-26]. Notably, serum levels of BAFF and

APRIL are increased in autoimmune diseases, including SLE and rheumatoid arthritis[27, 28], and blockade of BAFF and APRIL prevents autoimmunity in animal models of disease[29-32] and is being developed for human use[33].

Accordingly, it is considered important to examine the correlation between bullous diseases and BAFF/APRIL system to infer the function of these TNF family molecules in the process of skin blistering diseases, and to suggest the efficacy of BAFF and/or APRIL-targeting therapy in these skin disorders. Recently, we have demonstrated that serum BAFF levels are increased in BP patients[34]. In this study, we determined serum APRIL levels of the patients with PV and BP, and compared them with BAFF as well as other clinical findings. Serum APRIL levels were elevated in BP patients and closely correlated with serum BAFF levels, suggesting a role of BAFF/APRIL system in the disease development.

Materials and Methods

Patients and clinical assessment

This analysis includes 15 patients with PV (8 males and 7 females) and 43 patients with BP (20 males and 23 females) who visited Tokyo University Hospital from January 1985 to August 2006, and 15 normal controls (NC, 7 males and 8 females). Their ages (mean±SD) were as follows: PV patients were 57.1±13.1 (range from 26 to 74), BP, 70.0±16.4 (range from 17 to 93), and NC, 52.5±22.7 (range from 24 to 88) years old. There was no evident difference of sex among the three groups, and BP patients turned out to be significantly older than the other two groups ($p<0.05$) by Kruskal Wallis one-way analysis of variance (ANOVA) and Dunn's test. Blood samples were obtained from patients with clinically active PV or BP, i.e., patients who had been diagnosfed as PV or BP respectively, but not yet received systemic corticosteroid and/or other immunosuppressive treatments.

Diagnosis of PV was based on the following criteria[35]: characteristic clinical findings of mucous membrane ulcerations and /or multiple fragile vesiculobullous lesions of the skin; histological evidence of intraepithelial acantholysis; evidence of in-vivo bound IgG autoantibodies on the cell surfaces of affected epithelium by direct immunofluorescence; evidence of circulating antiepithelial autoantibodies by indirect immunofluorescence; and autoantibodies specific to Dsg3 by immunochemical techniques. Diagnosis of BP was based on the following criteria[35]: characteristic clinical findings of pruritic plaques and tense blisters of the trunk and extremities; histological evidence of subepidermal blisterings with many infiltrating polymorphonuclear cells, especially eosinophils, along the basal membrane and within the blister cavity; in-vivo linear IgG and/or C3 deposits at the dermoepidermal junction by direct immunofluorescence, evidence of circulating autoantibodies to the basement membrane zone by indirect immunofluorescence, and autoantibodies specific to BP180

by immunochemical techniques. Patients and healthy volunteers gave consent to participate in this study, which has been approved by the institutional review board.

Disease duration was defined as the time period from the onset of symptoms till diagnosis.

ELISA assays for detection of human soluble APRIL, BAFF, anti-Dsg3 antibodies, and anti-BP180 antibodies

Serum APRIL levels were measured using human APRIL EIA kit (IBL, Takasaki, Japan) according to the manufacturer's protocol. Briefly, standards and serum samples were added to microplate wells pre-coated with mouse monoclonal antibodies specific for human APRIL. Next, biotin-conjugated polyclonal anti-APRIL antibodies, and then HRP-conjugated streptavidin, were added to the wells after rinsing any unbound substances at each step. Following color development with TMB substrate solution and

1 M phosphoric acid, absorbance of each well was read using a microplate reader set to 450 nm. Similarly, human BAFF/BlyS/TNFSF13B immunoassay kit (R&D Systems, Minneapolis, MN, USA) was used for detection of serum BAFF levels, anti-Dsg3 ELISA kit (Mesacup Desmoglein Test “Dsg3”, MBL, Nagoya, Japan) for the serum titers of anti-Dsg3 antibodies, and BP180 ELISA kit (MBL) for anti-BP180 antibodies, according to the manufacturer’s protocol.

Indirect immunofluorescence analysis

Normal human skin was taken from healthy volunteers, who had also given consent to participate in this study, and frozen in OTC compound (Sakura Fineteck, Torrance, CA). Cryostat-cut tissue sections were fixed, blocked, and then incubated with patients’ sera diluted 1:20, 40, 80, 160, and 320 with PBS (20min, 37˚C). After washing, the sections were incubated sequentially with FITC-conjugated anti-human IgG antibody (BD

PharMingen, San Diego, CA) at predetermined optimal concentration (20min, 37˚C). The autoantibody titer at which IgG deposition to the intracellular space or BMZ was demonstrated, was depicted as indirect immunofluorescence dilution index.

Statistical analyses

Statistical analyses of serum APRIL levels among the three groups were performed using Kruskal Wallis one-way analysis of variance (ANOVA) and then Dunn's test was applied because the data of BP patients' serum APRIL levels were not strictly normally distributed. Correlations between serum APRIL and serum autoantibody titers, blood eosinophil number, serum LDH level, serum IgE level, serum IgA level, serum BAFF levels, disease duration, indirect immunofluorescence dilution index, and extent of skin lesion, that is, the extent of skin blistering, erosive, or erythematous lesion (which was decided in a blinded way), were analyzed with Spearman's correlation coefficient by

rank test. A p-value less than 0.05 was considered statistically significant.

Results

Serum APRIL levels were elevated in the patients with PV and BP

Serum APRIL levels were measured by ELISA in PV patients, BP patients, and NC. Median (first, third quantile) of serum APRIL levels was, 7.84 (0, 15.35) ng/ml in NC group, 19.36 (0, 27.79) ng/ml in PV patients, and 20.31 (6.19, 64.36) ng/ml in BP patients (Figure 1). Statistical analysis revealed significant differences between the groups (ANOVA; $p < 0.05$, $df = 2$). Serum APRIL levels in BP patients were significantly higher than those in NC (Dunn's test; $p < 0.05$). Although PV patients also tended to have higher levels of APRIL than NC, there was no significant difference. When values higher than third quantile of NC group (15.35 ng/ml) were considered to be elevated, serum APRIL levels were elevated in 66.7% (10/15) of PV and 58.1% (25/43) of BP patients. In BP patients, there was no correlation between serum APRIL levels and sex, age, or complications found, including internal malignancy (data not

shown). Accordingly, it can be concluded that BP patients had significantly elevated serum levels of APRIL.

Serum APRIL levels were elevated in the early stage of BP disease

We next analyzed the relationship between serum APRIL levels and clinical findings in BP patients. However, there was no significant association with blood eosinophil number, serum LDH level, serum IgE level, serum IgA level, extent of skin lesion, or reactivity to treatment (Figure 2A, B, and data not shown).

We then investigated the direct role of APRIL on pathogenic antibody production in BP. However, in respect of anti-BP180 antibody levels determined by ELISA, there was no evident correlation with APRIL levels (Figure 2C), nor was indirect immunofluorescence dilution index (data not shown). Also in PV patients, no correlation was found between serum APRIL levels and anti-Dsg3 antibody levels (data

not shown).

By contrast, there found a significant negative correlation between serum APRIL levels and disease duration in BP patients, that is, the patients in the shorter period from BP onset turned out to carry significantly higher levels of serum APRIL than those with longer disease duration (Figure 2D, r=-0. 55, $p < 0.001$). Meanwhile, there was no statistical correlation proven between serum APRIL levels and disease durations in PV patients (data not shown). Attending to BP, serum APRIL levels were longitudinally assessed in three BP patients treated with oral corticosteroid (Figure 2E). In patients 1 and 2, serum APRIL levels were highest in the early point and decreased immediately, to normal level in patient 1, while anti-BP180 antibody levels were still higher after APRIL declined. It is interesting to note that serum APRIL level was already elevated even before skin lesion emerged in patient 3. This patient experienced recurrence on day 53 through the disease course. Unfortunately, we could not follow up the serum APRIL

or anti-BP180 antibody levels of disease-free period due to the absence of preserved sera. However, at least, all the three patients found in Figure 2E showed the highest levels of serum APRIL at the earliest point of investigation. In addition, the elevation of anti-BP180 antibody levels delayed those of APRIL levels in all the three patients. Especially in patient 1 and 2, anti-BP180 antibody levels were still higher even after skin lesion almost disappeared. While, APRIL levels decreased along with improved disease activity in all the patients. These data may suggest that serum APRIL levels tend to reflect the disease activity more subtly than anti-BP180 antibody titers in patients with BP.

APRIL levels correlated with BAFF levels in sera of BP patients

Association of serum APRIL levels with the same TNF family BAFF levels was also examined. As shown in Figure 3, serum APRIL levels in BP patients showed a positive

correlation with serum BAFF levels (r=0.46, $p<0.01$). Thus, most patients with high levels of serum APRIL also had elevated serum BAFF levels, suggesting that elevation of BAFF and APRIL may result from common mechanisms. In respect of PV patients, however, we did not detect significant correlation between serum APRIL and BAFF levels (data not shown).

Discussion

In the current study, we demonstrated that serum APRIL levels were elevated in patients with BP. This is the first report demonstrating elevated serum APRIL levels in organ-specific autoimmune diseases. Although there was no statistical relationship proven between serum APRIL levels and anti-BP180 antibody titers (Figure 2C), longitudinal analysis in three BP patients suggested that serum APRIL levels tended to be high in the early stage of disease and reduced in response to treatment with oral corticosteroid, ahead of the changes in anti-BP180 antibody titiers (Figure 2E). In especial, serum APRIL level of patient 3 turned out to have been elevated even before the manifestation of skin lesion. Furthermore, while serum APRIL levels decreased rapidly, anti-BP180 antibody titers remained higher even after serum APRIL levels declined. In patient 2, we unfortunately could not follow up the change of anti-BP180 antibody titers or serum APRIL levels from day 14 to day 529, due to the absence of

sera. Accordingly, we were not able to decide whether anti-BP180 antibody titer kept high or once reduced. There was no recurrence experienced through disease course, thus, the high titer of anti-BP180 antibody did not affect directly to disease continuance or recurrence. Rather, as regarding patient 2, serum APRIL levels may reflect properly the disease activity. Therefore, although these results do not prove that elevations in serum APRIL alone cause clinical symptoms, APRIL is assumed, at least, to participate in production of anti-BP180 antibodies, conductive to BP manifestation.

These features in longitudinal change of serum APRIL levels are quite similar with that of the same TNF family member BAFF levels. In respect of BAFF, the same distinction was reported recently in SLE[27]. Elevated BAFF levels preceded the formal fulfillment of criteria for SLE, suggesting the efficacy of BAFF as a marker for early activation of autoimmune diathesis. Adding our data, APRIL also has prospects of carrying the similar validity to evaluate autoimmune activity in the early stage. This is

supported by the result that serum APRIL levels were significantly higher in the patients with shorter duration of BP disease (Figure 2D).

While serum APRIL and BAFF levels correlated with each other in the majority of patients with BP (Figure 3), some patients carried high APRIL level and low BAFF level, and others expressed inverse data. As for these patients with ‘mismatched’ value, no evident character was found in either pathogenic anti-BP180 antibody level or disease activity markers. Despite similar structure, shared receptor-specificity, and overlapping features as the same TNF superfamilies, BAFF and APRIL have distinct functions. APRIL appears to play a role in T-cell-independent type II antigen responses and T-cell survival, while BAFF induces B-cell proliferation and differentiation[25, 26]. In a murine SLE model NZB/W F1, blockade of BAFF alone, and both BAFF and APRIL, exhibited similar effects, including B cell and B cell subset depletion, and prevention of the progressive T cell activation and dendritic cell accumulation.

Meanwhile, inhibition of both BAFF and APRIL, but not BAFF alone, reduced the serum levels of IgM antibodies, decreased the frequency of plasma cells in the spleen, and inhibited the IgM response to a T cell-dependent antigen[24]. We did not find any characteristics reflecting these functional differences of BAFF and APRIL in BP patients. Presumably, main role of both BAFF and APRIL in manifestation of BP is quite similar, that is, involvement in early activation of self-antigen-driven B and T cells along with loss of tolerance. Actually, APRIL and BAFF are reported to form active heterotrimeric molecules when coexpressed, in addition to homotrimeric molecules that is common to TNF superfamily, and the circulating heterotrimers are recognized in the sera of patients with systemic immune-based rheumatic disease[36]. Thus, both APRIL and BAFF may play a role, in consort with each other, in autoimmune disorders through the active BAFF/APRIL heterotrimers. Although only BAFF, not APRIL, contributes to B cell class-switch leading to production of pathogenic IgG autoantibodies, other

additional factors might determine progression and elongation of autoimmune diseases. Leastwise, still quite small number of patients attended to this study, so that accumulation of patients might be able to explain any functional segregation in the autoimmune skin blistering disorders.

As for serum immunoglobulin levels, in addition, serum IgA levels are reported to be significantly decreased in APRIL knock out mice[37], suggesting that APRIL promotes IgA class switching. In fact, TACI, the common receptor of BAFF and APRIL, and BAFF-R are important for transducing signals that result in isotype switching in B cells to IgG1, IgA, and IgE[38]. In our study, there was no correlation found between serum APRIL level and serum IgA or IgE level (Figure 2A and data not shown). So as to further elucidate the role of APRIL in the manifestation of BP, it would be interesting to examine the detailed correlation between serum APRIL levels and especially serum IgA levels, during disease course.

While serum APRIL levels were significantly elevated in BP patients, and were correlated with disease durations, we did not find the similar statistical relationship in patients with PV. While it may indicate that the factors involved in the induction of PV and BP are different, this may be because of the disease duration in PV patients. While serum APRIL levels were proved to be higher in patients with shorter disease duration in BP patients, PV patients that we were able to examine carried longer disease duration than BP patients (mean; 3.5 months in PV). Therefore, the assessment of early time points in PV patients will be necessary to determine more exact relation between serum APRIL and pemphigus. In regard to other factors explaining the difference between BP and PV, for instance, elevated eosinophil and neutrophil number both in circulating blood and skin blistering lesion is more frequently seen in BP patients, rather than PV patients. The activation of these cells may be through T-cell mediated inflammatory reactions. These augmented T cell functions may have respect to some APRIL

functions.

In summary, serum APRIL levels were increased in BP patients. APRIL tended to altitude in the early phase of disease, preceding the elevation of pathogenic anti-BP180 antibodies especially in patient 1 and 3 in Figure 2E. This change of APRIL along disease course is similar with that of BAFF, indicating the overlapping roles in disease activation. These results suggest that APRIL may predict not only first onset but also recurrence of BP, and that blockade of BAFF/APRIL system may be potential therapeutic targets in this autoimmune bullous disorder.

References

1. Amagai M, Klaus-Kovtun V, Stanley JR: Autoantibodies against a novel epithelial cadherin in pemphigus vulgaris, a disease of cell adhesion. Cell 67:869-877, 1991.

2. Tanaka M, Hashimoto T, Dykes PJ, Nishikawa T: Clinical manifestations in 100 Japanese bullous pemphigoid cases in relation to autoantigen profiles. Clin Exp Dermatol 21:23-27, 1996.

3. Amagai M, Karpati S, Prussick R, Klaus-Kovtun V, Stanley JR: Autoantibodies against the amino-terminal cadherin-like binding domain of pemphigus vulgaris antigen are pathogenic. J Clin Invest 90:919-926, 1992.

4. Stanley JR, Tanaka T, Mueller S, Klaus-Kovtun V, Roop D: Isolation of complementary DNA for bullous pemphigoid antigen by use of patients' autoantibodies. J Clin Invest 82:1864-1870, 1988.

5. Rico MJ, Korman NJ, Stanley JR, Tanaka T, Hall RP: IgG antibodies from patients with bullous pemphigoid bind to localized epitopes on synthetic peptides encoded by bullous pemphigoid antigen cDNA. J Immunol 145:3728-3733, 1990.

6. Arin MJ, Engert A, Krieg T, Hunzelmann N: Anti-CD20 monoclonal antibody (rituximab) in the treatment of pemphigus. Br J Dermatol 153:620-625, 2005.

7. Belgi AS, Azeez M, Hoyle C, Williams RE: Response of pemphigus vulgaris to anti-CD20 antibody therapy (rituximab) may be delayed. Clin Exp Dermatol 31:143, 2006.

8. Cecchi R, Gasperini U: Severe pemphigus vulgaris treated with rituximab (Mabthera). J Dermatol 32:862-864, 2005.

9. Cooper HL, Healy E, Theaker JM, Friedmann PS: Treatment of resistant pemphigus vulgaris with an anti-CD20 monoclonal antibody (Rituximab). Clin

Exp Dermatol 28:366-368, 2003.

10. Dupuy A, Viguier M, Bedane C, et al.: Treatment of refractory pemphigus vulgaris with rituximab (anti-CD20 monoclonal antibody). Arch Dermatol 140:91-96, 2004.

11. Espana A, Fernandez-Galar M, Lloret P, Sanchez-Ibarrola A, Panizo C: Long-term complete remission of severe pemphigus vulgaris with monoclonal anti-CD20 antibody therapy and immunophenotype correlations. J Am Acad Dermatol 50:974-976, 2004.

12. Herrmann G, Hunzelmann N, Engert A: Treatment of pemphigus vulgaris with anti-CD20 monoclonal antibody (rituximab). Br J Dermatol 148:602-603, 2003.

13. Kong HH, Prose NS, Ware RE, Hall RP, 3rd: Successful treatment of refractory childhood pemphgus vulgaris with anti-CD20 monoclonal antibody (rituximab). Pediatr Dermatol 22:461-464, 2005.

14. Morrison LH: Therapy of refractory pemphigus vulgaris with monoclonal anti-CD20 antibody (rituximab). J Am Acad Dermatol 51:817-819, 2004.

15. Salopek TG, Logsetty S, Tredget EE: Anti-CD20 chimeric monoclonal antibody (rituximab) for the treatment of recalcitrant, life-threatening pemphigus vulgaris with implications in the pathogenesis of the disorder. J Am Acad Dermatol 47:785-788, 2002.

16. Schmidt E, Herzog S, Brocker EB, Zillikens D, Goebeler M: Long-standing remission of recalcitrant juvenile pemphigus vulgaris after adjuvant therapy with rituximab. Br J Dermatol 153:449-451, 2005.

17. Virgolini L, Marzocchi V: Anti-CD20 monoclonal antibody (rituximab) in the treatment of autoimmune diseases. Successful result in refractory Pemphigus vulgaris: report of a case. Haematologica 88:ELT24, 2003.

18. Wenzel J, Bauer R, Bieber T, Tuting T: Successful rituximab treatment of severe

pemphigus vulgaris resistant to multiple immunosuppressants. Acta Derm Venereol 85:185-186, 2005.

19. Yeh SW, Sami N, Ahmed RA: Treatment of pemphigus vulgaris: current and emerging options. Am J Clin Dermatol 6:327-342, 2005.

20. Jacobi A, Shuler G, Hertl M: Rapid control of therapy-refractory pemphigus vulgaris by treatment with the tumour necrosis factor-alpha inhibitor infliximab. Br J Dermatol 153:448-449, 2005.

21. Pardo J, Mercader P, Mahiques L, Sanchez-Carazo JL, Oliver V, Fortea JM: Infliximab in the management of severe pemphigus vulgaris. Br J Dermatol 153:222-223, 2005.

22. Dillon SR, Gross JA, Ansell SM, Novak AJ: An APRIL to remember: novel TNF ligands as therapeutic targets. Nat Rev Drug Discov 5:235-246, 2006.

23. Mackay F, Schneider P, Rennert P, Browning J: BAFF AND APRIL: a tutorial

on B cell survival. Annu Rev Immunol 21:231-264, 2003.

24. Ramanujam M, Wang X, Huang W, et al.: Similarities and differences between selective and nonselective BAFF blockade in murine SLE. J Clin Invest, 2006.

25. Moore PA, Belvedere O, Orr A, et al.: BLyS: member of the tumor necrosis factor family and B lymphocyte stimulator. Science 285:260-263, 1999.

26. Mackay F, Ambrose C: The TNF family members BAFF and APRIL: the growing complexity. Cytokine Growth Factor Rev 14:311-324, 2003.

27. Zhang J, Roschke V, Baker KP, Wang Z, Alarcon GS, Fessler BJ, Bastian H, Kimberly RP, Zhou T: Cutting edge: a role for B lymphocyte stimulator in systemic lupus erythematosus. J Immunol 166:6-10, 2001.

28. Cheema GS, Roschke V, Hilbert DM, Stohl W: Elevated serum B lymphocyte stimulator levels in patients with systemic immune-based rheumatic diseases. Arthritis Rheum 44:1313-1319, 2001.

29. Gross JA, Johnston J, Mudri S, et al.: TACI and BCMA are receptors for a TNF homologue implicated in B-cell autoimmune disease. Nature 404:995-999, 2000.

30. Kayagaki N, Yan M, Seshasayee D, et al.: BAFF/BLyS receptor 3 binds the B cell survival factor BAFF ligand through a discrete surface loop and promotes processing of NF-kappaB2. Immunity 17:515-524, 2002.

31. Ramanujam M, Wang X, Huang W, Schiffer L, Grimaldi C, Akkerman A, Diamond B, Madaio MP, Davidson A: Mechanism of action of transmembrane activator and calcium modulator ligand interactor-Ig in murine systemic lupus erythematosus. J Immunol 173:3524-3534, 2004.

32. Wang H, Marsters SA, Baker T, et al.: TACI-ligand interactions are required for T cell activation and collagen-induced arthritis in mice. Nat Immunol 2:632-637, 2001.

33. Ramanujam M, Davidson A: The current status of targeting BAFF/BLyS for

autoimmune diseases. Arthritis Res Ther 6:197-202, 2004.

34. Asashima N, Fujimoto M, Watanabe R, Nakashima H, Yazawa N, Okochi H, Tamaki K: Serum levels of BAFF are increased in bullous pemphigoid but not in pemphigus vulgaris. British Journal of Dermatology 155 330-336, 2006.

35. Nousari HC, Anhalt GJ: Pemphigus and bullous pemphigoid. Lancet 354:667-672, 1999.

36. Roschke V, Sosnovtseva S, Ward CD, et al.: BLyS and APRIL form biologically active heterotrimers that are expressed in patients with systemic immune-based rheumatic diseases. J Immunol 169:4314-4321, 2002.

37. Castigli E, Scott S, Dedeoglu F, Bryce P, Jabara H, Bhan AK, Mizoguchi E, Geha RS: Impaired IgA class switching in APRIL-deficient mice. Proc Natl Acad Sci U S A 101:3903-3908, 2004.

38. Castigli E, Wilson SA, Scott S, Dedeoglu F, Xu S, Lam KP, Bram RJ, Jabara H,

Geha RS: TACI and BAFF-R mediate isotype switching in B cells. J Exp Med 201:35-39, 2005.

Figure legends

Figure 1. Serum levels of APRIL measured by ELISA in 15 patients with PV, 43 with BP, and 15 normal controls (NC). The measured values from individual patients were plotted by dots. *$p < 0.05$ by Kruskal Wallis test followed by Dunn's test.

Figure 2: A-C: The correlation of serum APRIL levels with blood eosinophil number (A), extent of skin lesion (B), anti-BP180 antibody titer (C), and disease duration (D) in patients with BP. In C, anti-BP180 antibody levels were determined by ELISA. E: Longitudinal analysis of the serum APRIL and anti-BP180 antibody levels in three patients with BP. The arrows indicated in each figure show the period when oral corticosteroid treatment was started. The curved lines colored gray mean relative extent of skin lesion.

Figure 3. The correlation of serum APRIL levels with serum BAFF levels in BP patients.

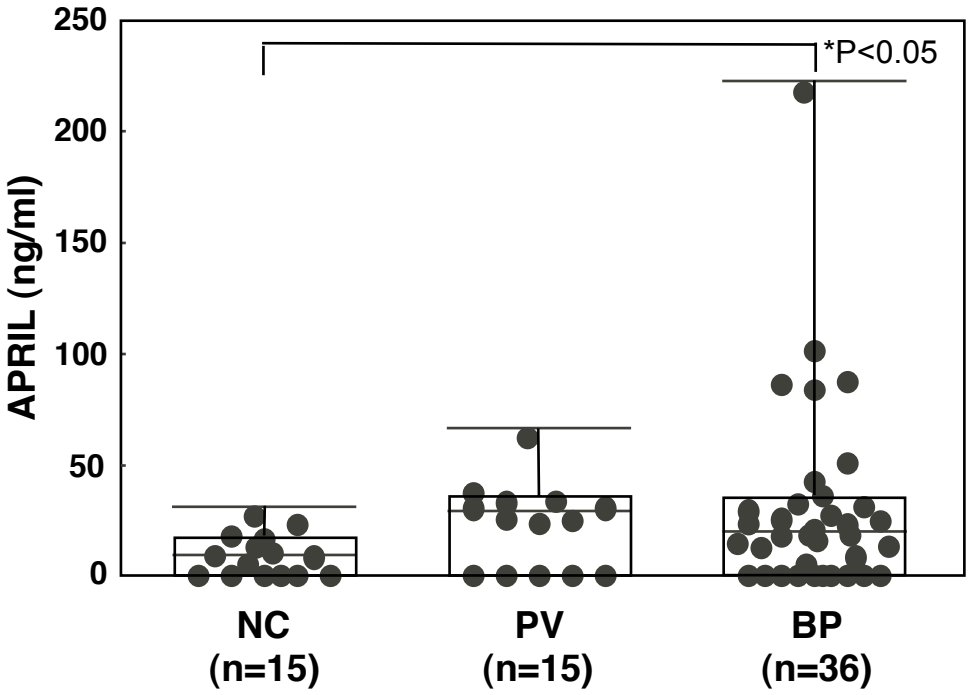
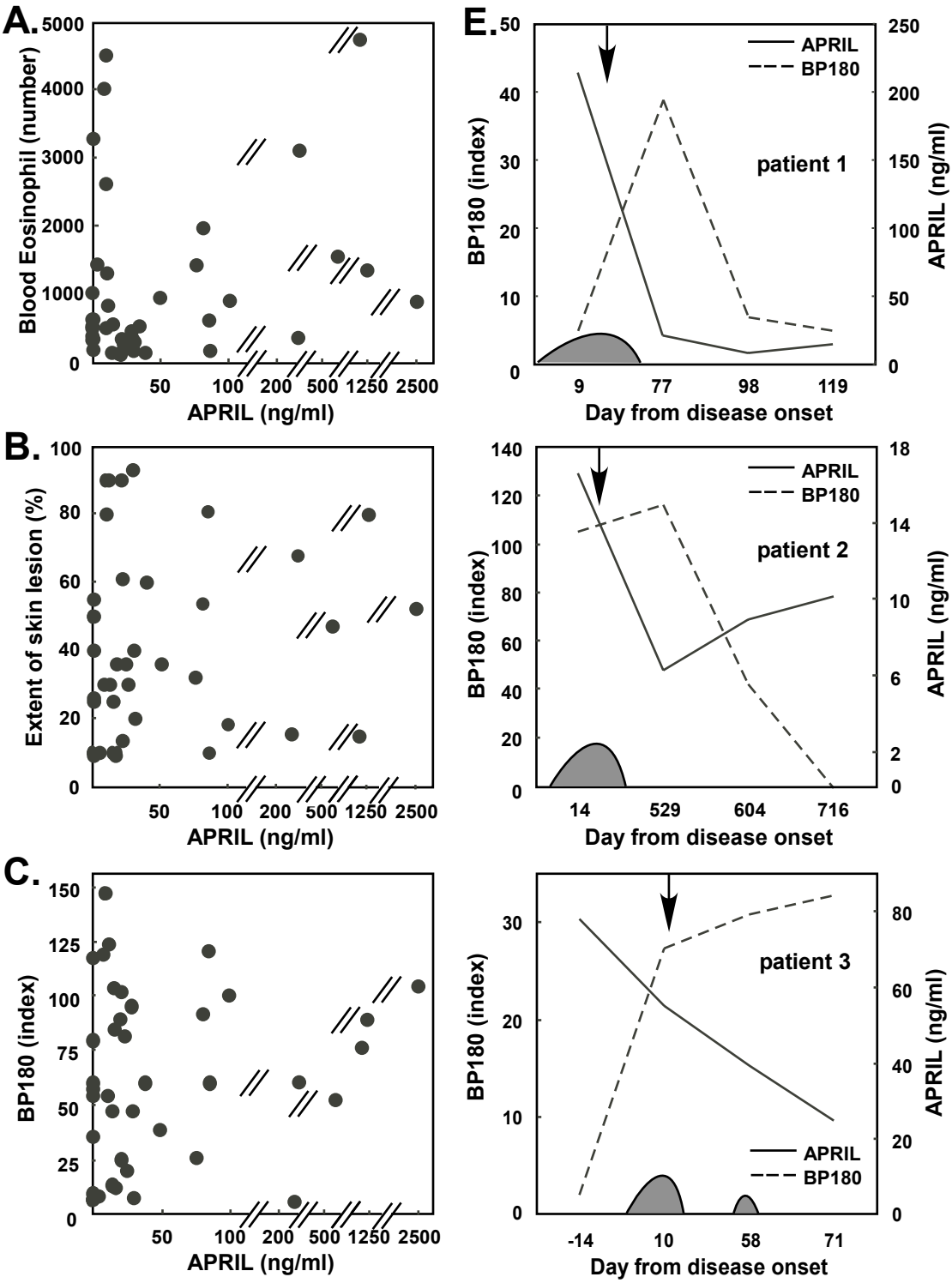


Figure 1
Watanabe et.al.


A.
Blood Eosinophil (number)
APRIL (ng/ml)
B.
Extent of skin lesion (%)
APRIL (ng/ml)
C.
BP180 (index)
APRIL (ng/ml)
D.
Disease duration (day)
APRIL (ng/ml)
E.
APRIL
BP180
patient 1
BP180 (index)
APRIL (ng/ml)
Day from disease onset
patient 2
patient 3

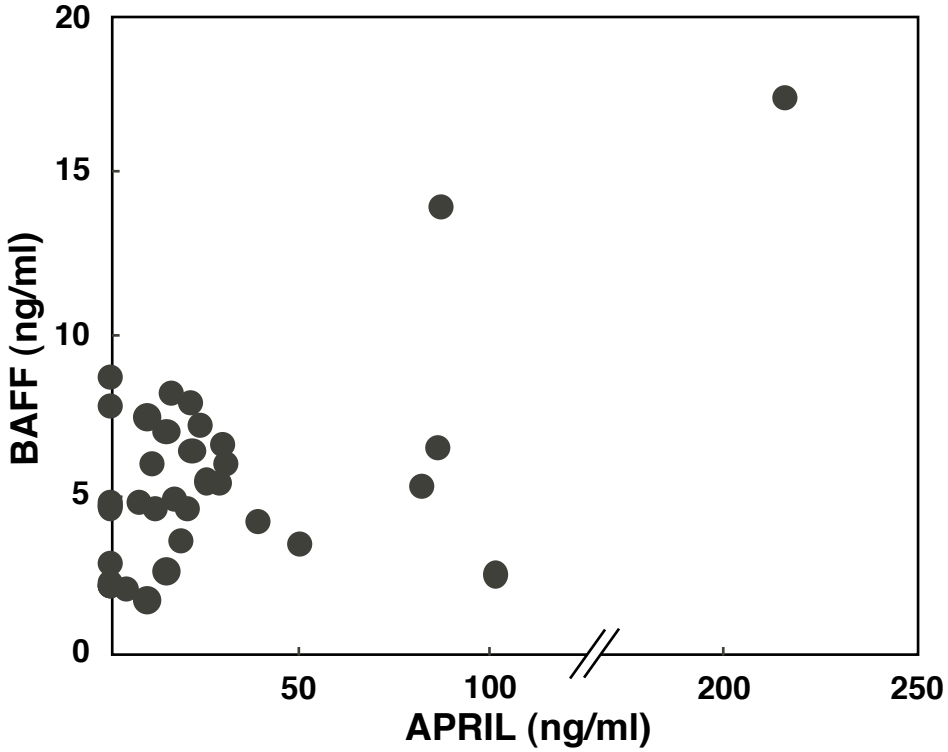


Figure 3
Watanabe et.al.