Nevertheless the TNF238.2 polymorphism on the B57.1 haplotype could characterize psoriasis patients with a particular disease course or be related to an impaired clearance of skin infections with candida or staphylococci, and thus predispose individuals to the development of psoriasis.

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IgA Antibodies of Linear IgA Bullous Dermatosis Recognize the 15th Collagenous Domain of BP180

To the Editor:
Linear IgA bullous dermatosis (LAD) is an autoimmune blistering disease characterized by IgA antibodies against epidermal autoantigens. The antigen for the major type of LAD (lamina lucida type) disease characterized by IgA antibodies against epidermal autoantigens was reported to be a 97 kDa protein (Zone et al, 1990; Dmochowski et al, 1993; Ishiko et al, 1998) or 120 kDa protein (Marinkovich et al, 1996). The 97 kDa/120 kDa protein was found to be a degradation product of the 180 kDa bullous pemphigoid antigen (BP180) (Marinkovich et al, 1997; Pas et al, 1997; Hirako et al, 1998; Zone et al, 1998). To further characterize the antigenic sites in LAD, we performed immunoblot studies using various bacterial recombinant proteins of BP180.

Fourteen sera were collected from clinically typical LAD patients and showed IgA antibodies reactive with epidermal side of 1M NaCl-split skin sections with a titer 1:10–1:40 in immunofluorescence. Ten BP sera and 10 normal sera were used as controls. A monoclonal antibody D-20, which reacts with the 120 kDa LAD antigen (Hirako et al, 1998) and an antiglutathione-S-transferase (GST) rabbit antiserum (Sigma, St Louis, MO) were also included as controls. Normal human epidermal extracts (Dmochowski et al, 1993) and concentrated supernatant of cultured DJM-1 cells (Hirako et al, 1998) were used as antigen sources for immunoblotting. The first four fusion proteins were reported previously (Matsumura et al, 1996; Nie and Hashimoto, 1999). BP1050, BP963, and BP915 encode N-terminus, central part, and C-terminus of BP180 extracellular domain, respectively. By the similar method described previously (Matsumura et al, 1996; Nie et al, 1999), we prepared two new recombinant proteins; BP1050 encoding the entire 15th collagenous domain, present just downstream of the NC16A domain; and BP144 encoding 38 amino acids, which is further downstream of BP1050. NC16A, BP15, and BP144 cover the entire BP1050, although BP1050 lacks the N-terminus of the NC16A domain. The constructs for BP1050 and BP144 were confirmed by DNA sequencing and their correct sizes

(about 46 kDa for BP1050 and about 61 kDa for BP144) as revealed by anti-GST antiserum in immunoblotting. The positions of all the recombinant proteins are depicted in Fig 1.

All the results of immunoblotting were summarized in Table I. In immunoblotting of epidermal extracts, none of the 14 LAD sera showed specific IgA reactivity either with BP180 or with protein bands in 97 kDa/120 kDa areas, whereas BP180 was detected by IgG antibodies of 60% BP sera. In contrast, in immunoblotting of concentrated culture supernatant of DJM-1 cells, IgA antibodies of 12 LAD sera clearly reacted with a 120 kDa protein (Fig 2, panel 1). This protein was also reacted by IgG antibodies of six BP sera and the monoclonal antibody D-20.

Because BP1050, BP963, and BP915 roughly cover the whole extracellular domain of BP180, we first examined IgA reactivity of the 14 LAD sera with these fusion proteins. Seven LAD sera reacted clearly with BP1050 (Fig 2, panel 2), and four sera reacted relatively weakly with BP915 (data not shown), whereas no LAD sera reacted with BP963. BP1050 is a large fragment containing 350 amino acids. To further characterize the epitope(s) within BP1050, we examined IgA reactivity of BP1050 with three other fusion proteins, i.e., NC16A, BP15, and BP144, which in total cover BP1050. Interestingly, five of the 11 LAD sera examined reacted with BP15 (Fig 2, panel 3), whereas no sera reacted with either NC16A (Fig 2, panel 4) or BP144. The monoclonal antibody D-20 also recognized BP1050 and BP15, but not other fusion proteins. As expected, IgG antibodies of the control BP sera reacted with

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both BP1050 and NC16A, which contain major epitopes for BP; however, neither IgG nor IgA antibodies of any BP or normal control sera reacted with BPC15.

These results suggest that major epitope(s) for IgA antibodies of LAD sera locate(s) in the fifteenth collagenous domain of BP180. This finding, however, is different from that in the previous study (Zillikens et al., 1999), which showed that some LAD sera reacted with NC16a domain. This discrepancy may be due to differences in subsets of LAD patients between the two studies; however, our conclusion is supported by the following observations; (i) the epitope(s) for LAD sera disappeared by collagenase treatment (Pas et al., 1997); (ii) the epitope(s) for LAD sera localized in the lamina lucida between NC16a and C-terminal domain of BP180 indicated by immunoelectron microscopy (Ishiko et al., 1998); (iii) the 97 kDa LAD antigen is a truncated form of BP180 started from the amino acid 566, 41 amino acids downstream from the transmembrane domain, which includes the 15th collagenous domain (Zone et al., 1998); and (iv) some BP sera reacting with the 97 kDa LAD antigen reacted with a mid-portion of BP180 extracellular domain, or with epitope(s) present in the area distal from the NC16a domain (Egan et al., 1999).

The most interesting and mysterious issue for LAD is why IgA antibodies of LAD sera recognize the degraded 97 kDa/120 kDa LAD antigen (Marinkovich et al., 1996; Pas et al., 1997) and small fusion proteins, BP1050 and BPC15 (in this study), but not with the intact BP180. One possible explanation is that the proteins may not be totally degenerated and may still possess some conformation even under the presence of SDS, a potent detergent. In this situation, amino acid sequences in either N-terminus or C-terminus of BP180 may produce some difference in conformational structures between the 97 kDa/120 kDa LAD antigen and intact BP180; however, further studies should be necessary to unravel this issue.

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Zalikens D, Herzske K, Georgi M, et al.: Anti-LAD antibodies is a subgroup of patients with BP180. Panell 1 (concentrated culture supernatant of DJM-1 cells): IgA antibodies of five representative LAD sera (lanes 3±7), but not a LAD serum (lane 8), reacted with the 120 kDa LAD antigen, which is indicated by an arrow in the left. The same protein band was also recognized by IgG antibodies of a BP serum (lane 1) and the monoclonal antibody D-20 (lane 2), but not by IgA antibodies of a normal serum (lane 9). In panels 2±4, the lane with the same number showed the reactivity of the same sera or antibodies, and an arrow in the left indicates the position of intact GST-fusion protein. Panel 2 (BP1050 GST-fusion protein): three of the five representative LAD sera (lanes 3±7) showed IgA antibodies reactive with this fusion protein, which was also reacted by the anti-GST antiserum (lane 1) and the monoclonal antibody D-20 (lane 2), but not by IgA antibodies of a normal serum (lane 8). Panel 3 (GST-fusion protein BPC15): the results are the same as those of panel 2. Panel 4 (NC16a GST-fusion protein): this fusion protein was not reacted by IgA antibodies of any LAD sera or normal serum. The anti-GST antiserum, but not the monoclonal antibody D-20, reacted with this fusion protein.
Mechanisms other than Shunting are Likely Contributing to the Pathophysiology of Erythromelalgia

To the Editor:

We read with interest the article by Mörk et al, “Microvascular arteriovenous shunting is a probable pathogenetic mechanism in erythromelalgia,” in the April 2000 issue of the Journal of Investigative Dermatology, in which the authors proposed that the common pathogenetic mechanism for erythromelalgia is increased thermoregulatory arteriovenous shunt flow. We have also noted increased local perfusion during symptoms of heat, redness, and pain (Sandroni et al, 1999) and have observed a concomitant local hypoxemia during symptoms, which is consistent with the hypothesis of shunting.

We have been intrigued by the following additional observations in patients we have studied (Sandroni et al, 1999): (1) the temperature of the symptomatic extremity occasionally exceeds core temperature; shunting alone would not explain this finding; (2) of the patients studied, 63% had evidence of small-fiber neuropathy, most commonly that of a severe postganglionic pseudomotor impairment, implying that neuropathy is prevalent. Whether the observed neuropathy led to erythromelalgia, or vice versa, is unclear.

Therefore, we submit that erythromelalgia is a complex disease, and shunting alone does not explain the findings observed during symptoms. Increased local metabolism may explain the increase in local heat, above that of core temperature. The contribution of small-fiber neuropathy to the syndrome needs to be delineated further.

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Table I. Summary of immunoblotting using various antigen sources

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*Epidermal, epidermal extracts; the results show the reactivity with BP180.
†DJM-1, concentrated culture supernatant of DJM-1 cells. (+) means that the LAD serum reacted with the 120 kDa LAD antigen.
nd, not done due to shortage of the serum.