Plectin Serves as an Autoantigen in Paraneoplastic Pemphigus

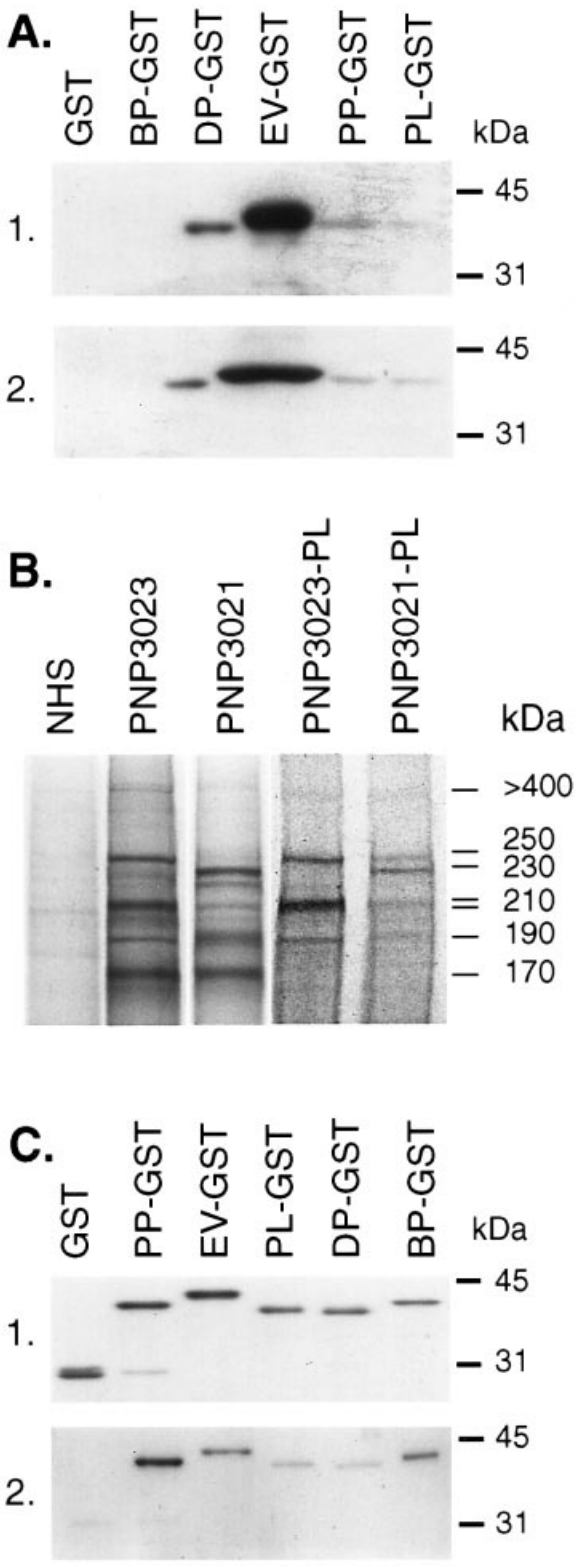
To the Editor:

Paraneoplastic pemphigus (PNP) is an autoimmune disease associated with malignant lymphoproliferative disorders, manifesting with extensive blistering of the skin and erosions of the oral mucosal membranes (Anhalt *et al*, 1990; Anhalt, 1997). We have previously shown by immunoblot analysis that PNP autoantibodies recognize various plakin proteins, including plectin, a high-molecular weight, ubiquitously expressed adhesion molecule (Mahoney *et al*, 1998). Quite recently, plectin has been shown to be recognized by PNP sera using a combination of immunoprecipitation and western blotting techniques (Proby *et al*, 1999). In this study, we have utilized affinity purified autoantibodies to demonstrate that, in addition to other plakins, plectin definitively serves as an autoantigen in PNP. Furthermore, our data suggest that the conserved carboxy-terminal region of these plakin proteins functions as a common antigenic epitope recognized by autoantibodies in PNP.

In the first set of experiments, we used glutathione-S-transferase (GST) fusion proteins corresponding to the linker region of five members of the plakin family of proteins, including BPAG1, desmoplakin, envoplakin, periplakin, and plectin (PL). Antibodies from two previously characterized PNP sera, PNP3023 and PNP3021, were affinity purified using PL-GST fusion protein. The affinity purified antibodies were then concentrated and tested in western analysis. The antibodies from both PNP sera purified with PL-GST recognized, in addition to plectin, epitopes in periplakin, envoplakin, and desmoplakin fusion proteins. Interestingly, the reaction with envoplakin was the strongest, possibly reflecting the presence of multiple antigenic sites and/or higher affinity for a single epitope within this molecule (**Fig 1*A*,** *panels 1* and *2*). Exogenously added PL-GST (1 μg per ml) was able to compete off the binding to all proteins (data not shown). Thus, the PNP antibodies specific to certain protein epitopes also recognize other members of the plakin family, suggesting the presence of shared epitopes.

Secondly, immunoprecipitations of radio-labeled proteins in keratinocyte extracts with two PNP sera were performed (**Fig 1*B***). As expected, the original, unfractionated PNP serum 3023 precipitated characteristic bands of 250, 230, 210, 190, and 170 kDa (**Fig 1*B*,** *lane 2*). In addition, at least two closely migrating bands, >250 kDa, were noted. The PNP serum 3021 immunoprecipitated clearly detectable bands of 230, 190, and 170 kDa, whereas bands of 250 and 210 kDa were barely detectable (**Fig 1*B*,** *lane 3*). Immunoprecipitation analysis using antibodies from PNP sera 3023 and 3021, which were affinity purified with PL-GST fusion protein, revealed precipitation of multiple bands similar to those seen in the original precipitations with PNP serum. Interestingly, immunoprecipitates with affinity purified antibodies did not include the 170 kDa band suggesting that this protein does not share epitopes with the plakin proteins, but instead, the PNP sera may contain antibodies specifically recognizing the 170 kDa protein. The nature of the 170 kDa protein is currently unknown, but it is clearly larger than desmoglein 3, which also has been shown to be recognized by PNP sera (Amagai *et al*, 1998). Furthermore, immunoprecipitation with antibodies purified with PL-GST fusion protein also recognized, although weakly, one of the high molecular weight bands, >400 kDa, further suggesting the presence of plectin.

The monoclonal antibody AE11 has been previously reported to recognize a 195 kDa protein by immunoblotting in human epidermal keratinocytes (Ma and Sun, 1986), which may be periplakin based on its approximate size. To test this possibility, we used the GST



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Reprint requests to: Dr. Sirpa Aho, Department of Dermatology and Cutaneous Biology, Jefferson Medical College, 233 S 10th Street, Suite 450 BLSB, Philadelphia, PA 19107, U.S.A. E-mail: Sirpa.Aho@mail.tju.edu

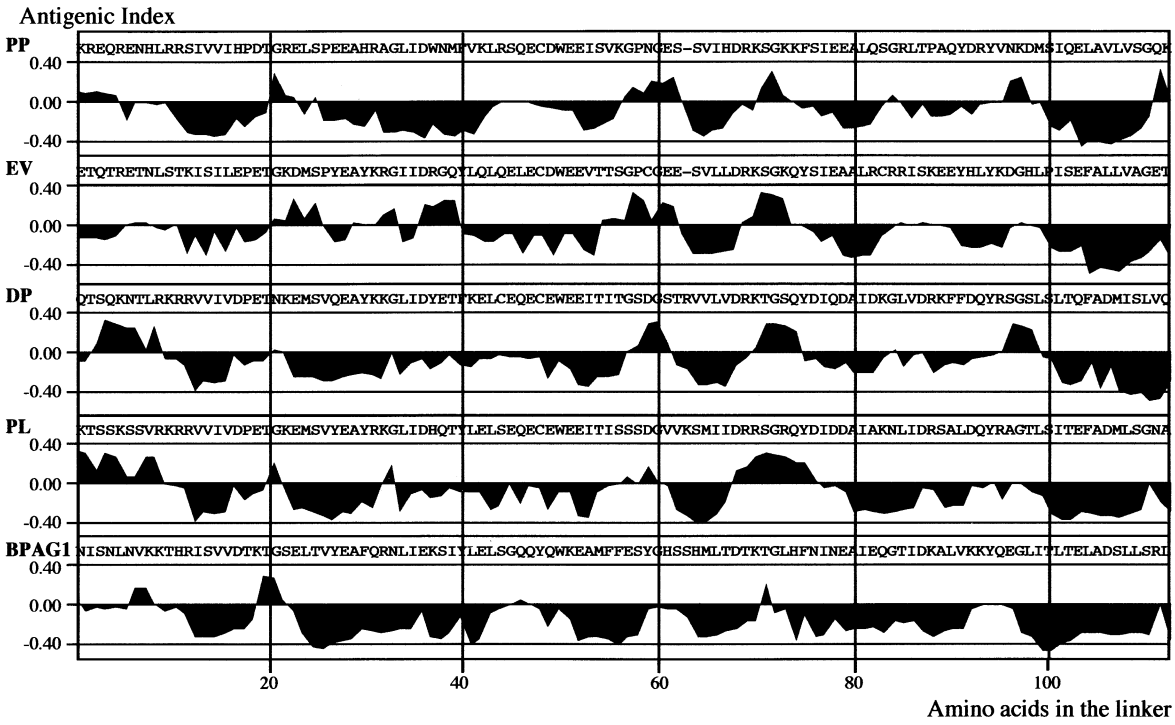

Figure 2. The antigenic profile for plakin protein linker domains. Each profile was generated using MacVector 6.0 program (Oxford Molecular, Campbell, CA). The corresponding amino acid sequences are shown above each antigenic profile. PP, periplakin; EV, envoplakin; DP, desmoplakin; PL, plectin; BPAG1, bullous pemphigoid antigen 1.

fusion proteins to test the specificity of the AE11 antibody. This monoclonal antibody recognized not only the periplakin fusion protein but also, with somewhat lower intensity, envoplakin, plectin, desmoplakin, and BPAG1 fusion proteins (**Fig 1*C***). These data further emphasize the presence of shared epitopes in plakin proteins.

Finally, because the primary amino acid sequence of the linker region of all five plakin proteins is known, we examined the potential of these peptide segments to serve as autoantigens. For this reason, each linker domain was first subjected to the computer prediction for antigenic index, based on the hydrophilicity, surface probability, and flexibility of the protein, as predicted from the primary sequence (**Fig 2**). Several segments with high antigenic potential were identified in all proteins, and many of them were shared between two or more. In particular, a region with positive antigenic index was recognized in all sequences surrounding the amino acid 71 (**Fig 2**). Furthermore, the region around amino acid 20 and that spanning amino acids 57–62 showed a positive index in four of five proteins. It was of interest to note that envoplakin had a region, between amino acids 20–40, which showed several peaks of high antigenic potential, whereas the corresponding region in other plakin proteins appeared less antigenic.

Figure 1. PL-GST antibodies affinity purified from PNP sera and a monoclonal antibody AE11 recognize plakin tail-GST fusion proteins. (*A*) Western blot analysis of recombinant GST and GST-fusion proteins composed of the conserved region within the tail domains of periplakin (PP), envoplakin (EV), plectin (PL), and desmoplakin (DP) (1 μg per lane) with PNP3023 PL-GST purified antibodies (*panel 1*) and PNP3021 PL-GST purified antibodies (*panel 2*). Preparation of the fusion proteins has been described elsewhere (Mahoney *et al*, 1998). (*B*) Metabolically labeled keratinocyte cell lysate was subjected to immunoprecipitation by normal human serum (NHS), PNP3023 serum, PNP3021 serum, PL-GST affinity purified antibodies from PNP3023, and PL-GST affinity purified antibodies from PNP3021, as described previously (Mahoney *et al*, 1998). (*C*) Western blot analysis of plakin-GST proteins (20 ng per lane in *panel 1*, and 400 ng per lane in *panel 2*) with anti-GST antibody (*panel 1*) and the monoclonal antibody AE11 (*panel 2*).

In summary, our data confirm that plectin is an autoantigen in PNP, and collectively, these findings contribute to better understanding of the pathogenesis of PNP.

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Sirpa Aho,* Mỹ G. Mahoney,* and Jouni Uitto*†
Departments of *Dermatology and Cutaneous Biology, and †Biochemistry and Molecular Pharmacology, Jefferson Medical College, and Jefferson Institute of Molecular Medicine, Thomas Jefferson University, Philadelphia, Pennsylvania, U.S.A.

REFERENCES

Amagai M, Nishikawa T, Noussari HC, Anhalt GJ, Hashimoto T: Antibodies against desmoglein 3 (pemphigus vulgaris antigen) are present in sera from patients with paraneoplastic pemphigus and cause acantholysis in vivo in neonatal mice. *J Clin Invest* 102:775–782, 1998

Anhalt GJ: Paraneoplastic pemphigus. *Adv Dermatol* 12:77–96, 1997

Anhalt GJ, Kim S, Stanley JR, *et al*: Paraneoplastic pemphigus. An autoimmune mucocutaneous disease associated with neoplasia. *N Engl J Med* 323:1729–1735, 1990

Ma AS-P, Sun T-T: Differentiation-dependent changes in the solubility of a 195-kD protein in human epidermal keratinocytes. *J Cell Biol* 103:41–48, 1986

Mahoney MG, Aho S, Uitto J, Stanley JR: The members of the plakin family of proteins recognized by paraneoplastic pemphigus antibodies include periplakin. *J Invest Dermatol* 111:308–313, 1998

Proby C, Fujii Y, Owaribe K, Nishikawa T, Amagai M: Human autoantibodies against HD1/plectin in paraneoplastic pemphigus. *J Invest Dermatol* 112:153–156, 1999