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 1A, 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 1B). As expected, the original, unfractionated PNP serum 3023 precipitated characteristic bands of 250, 230, 210, 190, and 170 kDa (Fig 1B, 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 1B, 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
In summary, our data confirm that plakin is an autoantigen in PNP, and collectively, these findings contribute to better understanding of the pathogenesis of PNP.

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