The Members of the Plakin Family of Proteins Recognized by Paraneoplastic Pemphigus Antibodies Include Periplakin

Mỹ G. Mahoney, Sirpa Aho,* Jouni Uitto,* and John R. Stanley

Department of Dermatology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, U.S.A.; *Department of Dermatology and Cutaneous Biology, Jefferson Medical College, and Section of Molecular Dermatology, Jefferson Institute of Molecular Medicine, Thomas Jefferson University, Philadelphia, Pennsylvania, U.S.A.

Sera of patients with paraneoplastic pemphigus (PNP) characteristically immunoprecipitate five proteins, observations confirmed with the sera examined in this study. The proteins characterized thus far as autoantigens in PNP all belong to the plakin family of proteins and include desmoplakin, the 230 kDa bullous pemphigoid antigen, and envoplakin. The pattern of bands precipitated from metabolically labeled human keratinocyte extracts by each PNP serum was different, suggesting varying titers of antibodies against unique epitopes in various plakin family members. To further characterize this PNP antibody response, we produced fusion proteins of the homologous tail region of five plakin family members, including the recently cloned periplakin. Immunoblotting of equal amounts of each plakin tail-glutathione S-transferase fusion protein with PNP sera revealed a strong reaction with the envoplakin tail domain. Each sera also recognized periplakin, and certain sera recognized desmoplakin and plectin, and, weakly, bullous pemphigoid antigen 1. PNP sera were affinity purified with periplakin and envoplakin tail fusion proteins. Immunoprecipitation and immunoblotting with these affinity purified antibodies revealed shared as well as unique epitopes in the tail domains of these plakins. This study indicates that a homologous region in the carboxy-terminus of plakins, including the newly characterized periplakin, serves as an antigenic site in PNP. ***Key words: bullous pemphigoid antigen/desmoplakin/envoplakin/plectin. J Invest Dermatol 111:308–313, 1998***

Paraneoplastic pemphigus (PNP) is a newly characterized autoimmune blistering skin disease associated with thymoma and lymphoproliferative disorders, such as non-Hodgkin's lymphoma, Castleman's disease, and chronic lymphocytic leukemia (Anhalt *et al*, 1990; Anhalt, 1997). In PNP, patients with these tumors develop a severe erosive oral gingivostomatitis and a blistering skin rash resembling erythema multiforme. Histology of the skin and mucous membrane lesions demonstrates two basic patterns. One is suprabasilar acantholysis, as seen in pemphigus vulgaris. The other is an interface dermatitis with basal cell vacuolar changes and individual keratinocyte dyskeratosis, similar to the histology of erythema multiforme (Horn and Anhalt, 1992). Direct immunofluorescence may show IgG and/or the third component of complement deposited on the keratinocyte cell surface or on the epidermal basement membrane zone (Anhalt *et al*, 1990; Fullerton *et al*, 1992; Lam *et al*, 1992). Indirect immunofluorescence shows that PNP patients have serum antibodies that bind to the surface of cells not only in stratified squamous epithelia, similar to pemphigus vulgaris antibodies, but also in transitional, columnar, and simple epithelia as well as to other nonepithelial desmosomal containing tissues (Anhalt *et al*, 1990; Helou *et al*, 1995). Immunoelectron microscopy has shown that the PNP antigens are components of hemidesmosomes and desmosomes and may spread beyond the desmosomes along the keratinocyte cell surface (Joly *et al*, 1994).

PNP antibodies precipitate a characteristic complex of polypeptides of approximate molecular weights 250, 230, 210, 190, and 170 kDa from extracts of cultured human keratinocytes (Anhalt *et al*, 1990; Oursler *et al*, 1992). Three of these polypeptides have been identified as desmoplakin I, 250 kDa, bullous pemphigoid antigen 1 (BPAG1), 230 kDa, and envoplakin, 210 kDa (Hashimoto *et al*, 1995; Kim *et al*, 1997). Some PNP sera also recognize desmoplakin II that migrates on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) slightly slower than envoplakin (Oursler *et al*, 1992; Hashimoto *et al*, 1995). Interestingly, all of these molecules that have been identified are members of the plakin gene family (Green *et al*, 1992; Ruhrberg *et al*, 1996).

Plakins are molecules that associate with cytokeratins, and are thought to link the keratin intermediate filaments to the cell surface, specifically to desmosomes and hemidesmosomes (Ruhrberg and Watt, 1997). In the desmosomes the two known plakins are desmoplakin (Green and Jones, 1996) and envoplakin (Ruhrberg *et al*, 1996), whereas BPAG1 (Green and Jones, 1996) and plectin (Foisner and Wiche, 1991; Uitto *et al*, 1996) are associated with hemidesmosomes; however, there is evidence that plectin is not confined to hemidesmosomes and that it is also associated with desmosomes (Eger *et al*, 1997). The common structure shared by plakins is an amino-terminal globular domain, a central coiled-coil rod domain, and a carboxy-terminal tail containing characteristic repeating amino acid domains (Green *et al*, 1992; Ruhrberg *et al*, 1996). These domains have been labeled as types A, B, or C depending on the amount of homology (Green *et al*, 1992). The number and types of the carboxy-terminal repeats are different in each plakin family member.

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Reprint requests to: Dr. John R. Stanley, Department of Dermatology, 415 Curie Blvd, 211 Clinical Research Building, University of Pennsylvania, Philadelphia, PA 19104.

Abbreviations: BPAG1, bullous pemphigoid antigen 1; COL17, mouse collagen XVII α1 chain; GST, glutathione-S-transferase; PNP, paraneoplastic pemphigus.

Originally identified along with envoplakin as a precursor to the cornified envelope (Simon and Green, 1984), periplakin, a new member of the plakin family, was recently characterized by cDNA cloning and immunologic studies (Ruhrberg *et al*, 1997; Aho *et al*, 1998). Periplakin has also been localized to the desmosomes (Ruhrberg *et al*, 1997). The deduced amino acid structure revealed a protein with a calculated mass of 204 kDa and with a domain structure resembling other plakin proteins. Periplakin has the shortest tail domain among the plakins because it is lacking the A, B, and C domains; however, its tail is homologous to the region preceding the C domain in other plakin proteins. Because it is a plakin, like other PNP antigens, and because it has a molecular weight of ≈190 kDa, the size of one of the polypeptides immunoprecipitated with PNP sera, we hypothesized that periplakin is one of the PNP antigens. In this study we characterized the PNP antibody response against periplakin and compared it with the response against other plakins.

MATERIALS AND METHODS

Sera PNP sera were kindly provided by Drs. Richard Sontheimer (PNP899), Lois Matsuoka (PNP906), and Grant Anhalt (PNP1081, PNP1341, PNP3021, and PNP3023).

Immunoprecipitation Metabolic labeling of human keratinocytes and immunoprecipitation were performed as previously described (Stanley *et al*, 1984). Primary human keratinocytes from neonatal foreskin were propagated, without feeders and without fetal calf serum, in complete MCDB 153 medium (Sigma, St. Louis, MO) containing 30 μM calcium and bovine pituitary extract, insulin, epidermal growth factor, hydrocortisone, and nonessential amino acids as described in detail by Ando and Jensen (1996). Cells were grown to confluency, washed three times with methionine- and cysteine-deficient Dulbecco's minimal essential medium (Gibco BRL, Grand Island, NY), and incubated overnight with 1 mCi ^{35}S- methionine and cysteine (Amersham, Arlington Heights, IL) in 3 ml methionine- and cysteine-deficient Dulbecco's minimal essential medium containing 10% fetal bovine serum (Gibco BRL), penicillin/streptomycin (Gibco BRL), and 1 mM $CaCl_2$. Cells were washed three times with phosphate buffered saline (PBS; Gibco BRL) and extracted in lysis buffer containing 10 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.5% NP-40, 10 μg aprotinin per ml, 10 μg leupeptin per ml, and 1 mM phenylmethylsulfonyl fluoride. The cell lysate was vortexed for 30 s then centrifuged at 16,000 rpm for 10 min. Bovine serum albumin (BSA; Sigma) was added to the supernatant at a final concentration of 0.1%.

The labeled cell extract was precleared with normal human serum for 2 h at 4°C and antibodies were precipitated with protein A-bearing staphylococci (Pansorbin; Calbiochem, La Jolla, CA). Human PNP anti-sera (1–5 μl) or affinity purified antibodies (0.2–100 μg) were added to 10^7 cpm of the precleared extract, then incubated overnight at 4°C. Antibodies were precipitated with Pansorbin that was then washed three times each with DEThi/BSA (10 mM Tris, pH 7.4, 0.5% Nonidet P-40, 0.5% sodium deoxycholate, 0.5 M NaCl, 0.2% NaN_3 and 0.1% BSA), DEThi (no BSA), and TBS (10 mM Tris, pH 7.4 and 150 mM NaCl). The final pellet was re-suspended in Laemmli buffer (Bio-Rad Laboratories, Hercules, CA) and heated at 100°C for 5 min. The labeled proteins in the supernatant were separated by 6% SDS-PAGE and then visualized by autoradiography.

Immunoprecipitation experiments were also carried out using biotin-labeled keratinocyte proteins with the Cellular Labeling and Immunoprecipitation Kit (Boehringer, Mannheim, Germany). Confluent cultured human keratinocytes were incubated with 1 mM $CaCl_2$ for 2 d. The cells were then washed extensively with ice-cold PBS and lysed in biotinylation lysis buffer (50 mM sodium borate, pH 8.0, 150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, 10 μg aprotinin per ml, 10 μg leupeptin per ml, and 1 mM phenylmethylsulfonyl fluoride). Cells were then scraped into microfuge tubes, vortexed for 30 s, incubated on ice for 15 min, and then centrifuged at 16,000 rpm for 10 min. The supernatant was collected and total cellular proteins were biotinylated on ice for 15 min with 0.25 mg D-biotinoyl-ε-aminocaproic acid-N-hydroxy succanimide ester per ml. The reaction was stopped with 50 mM NH_4Cl. The remaining immunoprecipitation steps were as described above.

The precipitated biotinylated proteins were separated by 6% SDS-PAGE and transferred to nitrocellulose membranes (Trans-Blot, Bio-Rad Laboratories, Hercules, CA). Membranes were incubated for 1 h each with blocking solution (PBS, 0.05% Tween-20, 1% BSA, and 1% normal goat serum) and then with streptavidin-horseradish peroxidase conjugate in blocking solution. After three quick rinses followed by five rinses over the course of 30 min with washing solution (PBS + 0.05% Tween-20), the blots were developed for chemiluminescence by ECL (Amersham).

Molecular cloning, production, and purification of plakin fusion proteins Homologous regions within the tail domains of periplakin, envoplakin, plectin, desmoplakin, and BPAG1 were cloned by polymerase chain reaction from a human keratinocyte matchmaker cDNA library (Clontech Laboratories, Palo Alto, CA). Primers were: periplakin, forward 5′AGAATTCAAGCGGGAGCAGCGGGAG3′ and reverse 5′AGTCGACCTTCTGCCCAGATACCAAGA3′ (Genbank accession #AF13717); envoplakin, forward 5′AGAATTCGAGACCCAGACGCGAGAG3′ and reverse 5′ AGTCGACGGTCTCCCCAGCTACAAGC3′ (Genbank accession #U53786); plectin, forward 5′AGAATTCAAGACGTCCTCCAAGTCCTC3′ and reverse 5′AGTCGACGGCGTTGCCCGAGAGCAT3′ (Genbank accession #U53204); desmoplakin, forward 5′AGAATTCCAGACATCACAAAAGAATACCC3′ and reverse 5′AGTCGACCTGCACCAAGGAGATCATG3′ (Genbank accession #J05211); BPAG1, forward 5′AGAATTCAACATTTCCAATCTCAATGTCAA3′ and reverse 5′AGTCGACTAACCGGCTCAGCAAAGAATC3′ (Genbank accession #M63618). The polymerase chain reaction products were digested with EcoRI and SalI and ligated into pGEX-4T-1 (Pharmacia, Piscataway, NJ). Glutathione-S-transferase (GST)-fusion proteins consisting of either the 111 or the 112 amino acid conserved region of the tail domains of envoplakin, periplakin, desmoplakin, plectin, and BPAG1 were expressed in *Escherichia coli* XL1-Blue cells (Stratagene, La Jolla, CA). The fusion proteins were purified over a glutathione Sepharose 4B column according to the manufacturer's protocol (Pharmacia). Envoplakin-GST and periplakin-GST were used to affinity purify the PNP serum #3023. As a negative control, a GST-fusion protein with mouse type XVII collagen α1 chain domain 16 A (COL17-GST) was used (a kind gift from Dr. Kehua Li).

Affinity purification and adsorption of PNP antibodies with plakin fusion proteins PNP3023 serum was affinity purified on envoplakin-GST, periplakin-GST, and, as a control, COL17-GST columns. The fusion proteins (1.5 mg) were N-cross linked to CM Affi-Gel-10 column (1 ml) (Bio-Rad Laboratories) overnight at 4°C in 100 mM MOPS (pH 7.5) (periplakin-GST) or 100 mM MOPS (pH 7.5) + 80 mM $CaCl_2$ (envoplakin-GST and COL17-GST). The gel matrix was washed with 10 column volumes each of 10 mM Tris (pH 7.5), 10 mM Tris (pH 7.5) + 0.5 M NaCl, and 10 mM Tris (pH 2.5) + 100 mM glycine + 0.5 M NaCl and re-equilibrated with 20 ml of 10 mM Tris (pH 7.5). PNP3023 serum (5 ml) was diluted with 15 ml of 10 mM Tris (pH 7.5) and run through the gel matrix three times. The column was subsequently washed with 20 column volumes each of 10 mM Tris (pH 7.5) and 10 mM Tris (pH 7.5) + 0.5 M NaCl. Bound antibodies were eluted in 18 ml of 10 mM Tris (pH 2.5) + 100 mM glycine + 0.5 M NaCl into a tube containing 2 ml of 1 M Tris (pH 7.5). Eluted antibodies were dialyzed overnight against PBS (2 liters) at 4°C, concentrated by Centricon-10 (Amicon, Lexington, MA), then BSA was added to a final concentration of 0.1%.

Immunoblotting GST, COL17-GST, BPAG1-GST, plectin-GST, desmoplakin-GST, periplakin-GST, and envoplakin-GST were resolved by 14% SDS-PAGE (Novex, San Diego, CA) and transferred to nitrocellulose membrane (Trans-Blot, Bio-Rad Laboratories). Membranes were incubated with blocking solution (PBS, 0.05% Tween-20, 4% dry milk, 1% BSA, and 1% normal goat serum) for 1 h at room temperature. Membranes were probed at 4°C overnight with primary anti-sera, PNP899 (1:1000), PNP906 (1:1000), PNP3021 (1:1000), or antibodies from PNP sera affinity purified on COL17-GST (0.2 μg per ml), envoplakin-GST (0.3 μg per ml), and periplakin-GST (0.8 μg per ml). After three quick rinses followed by five rinses over the course of 30 min with washing solution (PBS + 0.05% Tween-20), membranes were probed with horseradish peroxidase-conjugated goat anti-human IgG (1:2000, Bio-Rad Laboratories) for 1 h at room temperature and developed for chemiluminescence by ECL (Amersham).

RESULTS

PNP sera bind to a homologous region within the carboxy-terminus of members of the plakin gene family, including periplakin PNP sera immunoprecipitated multiple polypeptides from extracts of human keratinocytes (**Fig 1**). Several of these polypeptides have been identified as PNP antigens, including desmoplakin I (250 kDa), BPAG1 (230 kDa), and envoplakin (210 kDa), all members of the plakin family of proteins (Anhalt, 1997; Ruhrberg and Watt, 1997). The unidentified 190 kDa band could be the newly cloned periplakin, also a member of the plakin family; however, it has been suggested that envoplakin and periplakin form heterodimers (Ruhrberg *et al*, 1997), therefore periplakin may be simply coprecipitated with envoplakin and may not be an antigen *per se*. As shown in **Fig 1**, however, the 210 and the 190 kDa polypeptides are not always precipitated in the same stoichiometric ratio by various PNP sera

(**Fig 1**, lanes PNP906, PNP1081, and PNP3021). Therefore, we hypothesized that each polypeptide contains an epitope recognized by PNP antibodies, and that these epitopes might be in the homologous regions of these various plakin molecules.

Periplakin protein contains only a short carboxy-terminal globular domain and does not contain the A, B, or C repeats found within the carboxy-terminal domains of other plakin family members (**Fig 2*A***). The computer alignment of the amino acid sequences of these tail regions showed highest homology within the region immediately before the C repeat of each plakin family protein (**Fig 2*B***). The homology domain of each protein, also referred to as the linker region by Ruhrberg *et al* (1997), was cloned as a GST-fusion protein and expressed in bacteria (**Fig 2*A***). The fusion proteins were affinity purified on glutathione-Sepharose 4B columns, then separated by SDS-PAGE. Coomassie Blue staining of these gels showed purified GST-fusion proteins of the expected molecular sizes (see **Fig 6*A***).

The GST-fusion proteins were then used to analyze, by immunoblotting, five PNP sera, and data for four of these sera are shown in Fig 3 and for one of these sera (PNP3023) in **Fig 6(*B*)**. All five PNP sera detected the periplakin-GST and envoplakin-GST fusion proteins. PNP906 bound only to these two fusion proteins. Three PNP sera (PNP899, PNP1081, and PNP3023) also bound the desmoplakin-GST fusion protein and, surprisingly, two sera (PNP899 and PNP1081) bound strongly and one sera (PNP3021) bound weakly to the plectin-GST fusion protein. Binding to the plectin fusion protein was unexpected because plectin has not been identified in immunoprecipitates of keratinocyte extracts with PNP sera. Finally, two sera (PNP899 and PNP3021) bound weakly to the BPAG1-GST fusion protein. None of the sera bound to GST alone. Normal human serum did not bind to any of these fusion proteins (data not shown).

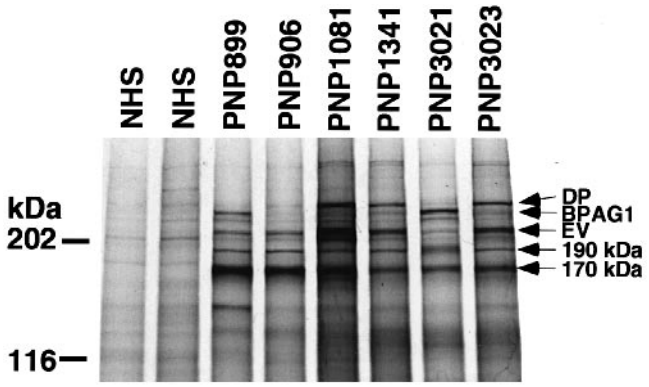

Figure 1. PNP sera precipitate plakin proteins in different stoichiometric ratios. Immunoprecipitation with two normal human sera (NHS) and six different PNP sera (PNP899, PNP906, PNP1081, PNP1341, PNP3021, and PNP3023) of metabolically labeled human keratinocyte cell lysates. Five specifically precipitated proteins of Mr 250 (DP, desmoplakin), 230 (BPAG1, bullous pemphoid antigen 1), 210 (EV, envoplakin), 190, and 170 kDa are marked. Molecular weight markers on the left are 202 and 116 kDa.

These data indicated that PNP sera recognize homologous regions in the carboxy-terminal tails of plakin proteins, including that of the newly cloned periplakin, and confirm a recent report in which IgG from a PNP serum was shown to bind the carboxy-terminal region (containing the homologous domain used in our fusion proteins) of envoplakin (Kim *et al*, 1997).

PNP antibodies recognize shared as well as unique epitopes on periplakin and envoplakin We affinity purified PNP3023 serum on either envoplakin-GST or periplakin-GST and used the affinity purified antibodies to immunoprecipitate extracts of human keratinocytes that were labeled either by ^{35}S-methionine/cysteine or by biotinylation. **Figure 4** shows that both affinity purified antibodies precipitated the 190 and 210 kDa molecules but with different stoichiometry. Comparison with the intensity of the periplakin band shows that anti-envoplakin-GST antibodies precipitated relatively more envoplakin compared with anti-periplakin-GST antibodies. These data suggest that either envoplakin and periplakin tail domains contain both shared and unique epitopes, or that there are only unique epitopes but coprecipitation of a complex of envoplakin and periplakin brings down both proteins as suggested by Ruhrberg *et al* (1997).

To further investigate these possibilities we performed immunoblotting of envoplakin-GST and periplakin-GST with PNP sera, unadsorbed or adsorbed with envoplakin-GST or periplakin-GST (**Fig 5**). PNP899 and PNP906 sera both bound to envoplakin-GST and periplakin-GST; however, envoplakin-GST adsorbed out from PNP899 antibodies binding to both envoplakin-GST (as expected) and periplakin-GST, demonstrating that the antibodies against envoplakin cross-reacted with periplakin (i.e., shared epitopes). On the other hand, adsorption of PNP906 serum with envoplakin-GST did not adsorb out staining against periplakin-GST and adsorption with periplakin-GST did not adsorb out staining against envoplakin-GST, demonstrating that this serum has antibodies against unique epitopes on both envoplakin and periplakin.

Additional experiments were performed with PNP3023 serum

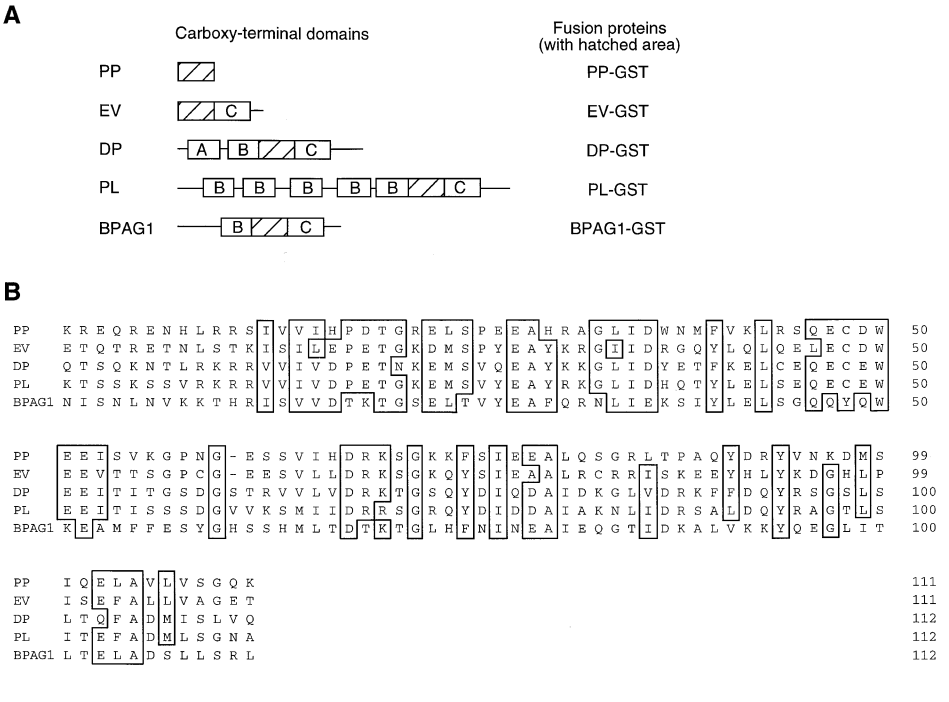

Figure 2. Conserved regions in the carboxy-terminus of plakin proteins. (*A*) Homologous regions within the tail domain of five plakin proteins. The hatched boxes represent the 111/112 amino acid region of highest homology. PP, periplakin; EV, envoplakin; DP, desmoplakin; PL, plectin; BPAG1, bullous pemphigoid antigen 1. (*B*) Alignment of the amino acids encoded by the hatched boxes in (*A*). Conserved amino acids are boxed.

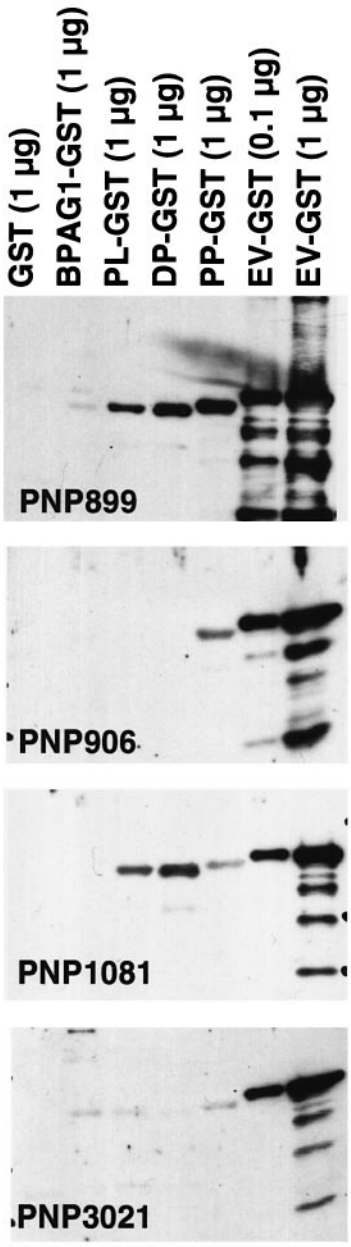

Figure 3. PNP sera recognize plakin tail-GST fusion proteins. Western blot of recombinant GST-fusion proteins of the conserved region within the tail domains of periplakin, envoplakin, plectin, desmoplakin, and BPAG1 (hatched boxes, **Fig 2*A***) with four different paraneoplastic pemphigus sera (PNP899, PNP906, PNP1081, and PNP3021).

affinity purified on envoplakin-GST or periplakin-GST columns or as a control on a COL17-GST column. The unpurified serum bound very strongly to envoplakin-GST on immunoblots, less strongly to periplakin-GST, and very weakly to desmoplakin-GST (**Fig 6*B***). No specific antibody was affinity purified on COL17-GST (**Fig 6*C***). Flow through from the COL17-GST column bound these fusion proteins, as did whole serum (**Fig 6*D***). Affinity purified antibodies against envoplakin-GST bound very strongly to envoplakin-GST and weakly to periplakin-GST and desmoplakin-GST, demonstrating antibodies against shared epitopes (**Fig 6*E***). Flow through from the envoplakin-GST column bound only periplakin-GST (**Fig 6*F***), demonstrating unique epitopes on periplakin not found on envoplakin. Antibodies affinity purified on periplakin-GST bound both periplakin-GST and envoplakin-GST (**Fig 6*G***), showing shared epitopes. Flow through from the periplakin-GST column bound only to envoplakin-GST (**Fig 6*H***), demonstrating epitopes on envoplakin not found on periplakin.

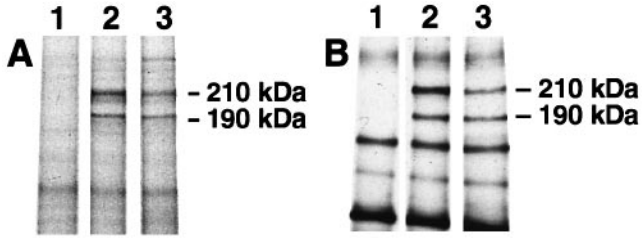

Figure 4. PNP serum affinity purified on envoplakin and periplakin fusion proteins immunoprecipitate the 210 and 190 kDa polypeptides with different stoichiometry. Metabolically labeled (*A*) or biotin labeled (*B*) keratinocyte cell lysates were immunoprecipitated by antibodies affinity purified with COL17-GST (1.6 μg; *lane 1*), envoplakin-GST (0.5 μg; *lane 2*), and periplakin-GST (5 μg; *lane 3*) fusion proteins. Note that the ratio of the 210 kDa envoplakin to the 190 kDa periplakin is greater with antibodies affinity purified on envoplakin-GST compared with those affinity purified on periplakin-GST.

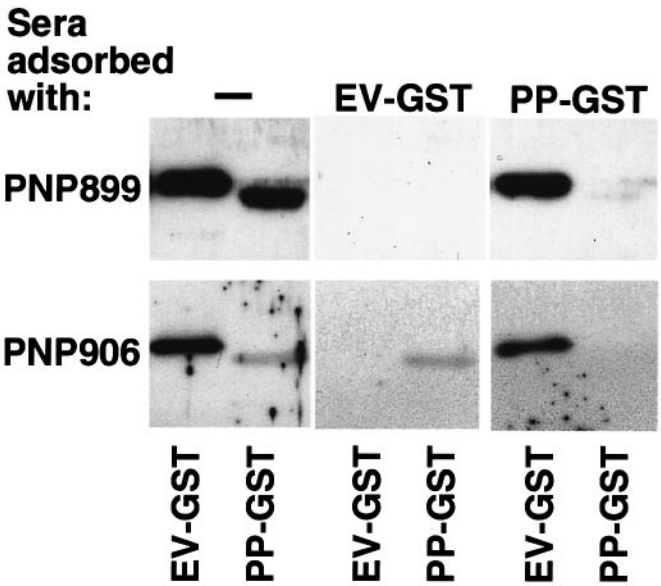

Figure 5. PNP sera contain antibodies that recognize common as well as unique epitopes between envoplakin and periplakin. Western blot of envoplakin-GST and periplakin-GST fusion proteins with two different PNP sera, PNP899 and PNP906. The primary antibodies were incubated in the presence of excess exogenous envoplakin-GST (10 μg per ml) or periplakin-GST (10 μg per ml) fusion proteins.

The flow through from the envoplakin-GST, periplakin-GST, and COL17-GST columns were also used for immunoprecipitation of keratinocyte extracts (data not shown). All these flow through antibodies precipitated envoplakin, periplakin, and desmoplakin with the same stoichiometry, suggesting that the tail domains of envoplakin and periplakin do not contain all the epitopes bound by PNP serum in the full length molecules.

Taken together, these results demonstrate that PNP sera have antibodies against unique as well as shared epitopes on periplakin and envoplakin.

DISCUSSION

In this report we have identified a novel plakin family member as an autoantigen in paraneoplastic pemphigus patients. Thus far the following plakins have been identified as PNP autoantigens: desmoplakin I, desmoplakin II, BPAG1, envoplakin, and, in this report, periplakin. These are not simply coprecipitated by an antibody against one of them, because not all periplakin sera precipitate all these molecules and the stoichiometry of precipitation varies among different PNP sera. In addition, we demonstrate, as have others (Oursler *et al*, 1992; Hashimoto *et al*, 1995; Kim *et al*, 1997), that antibodies from PNP

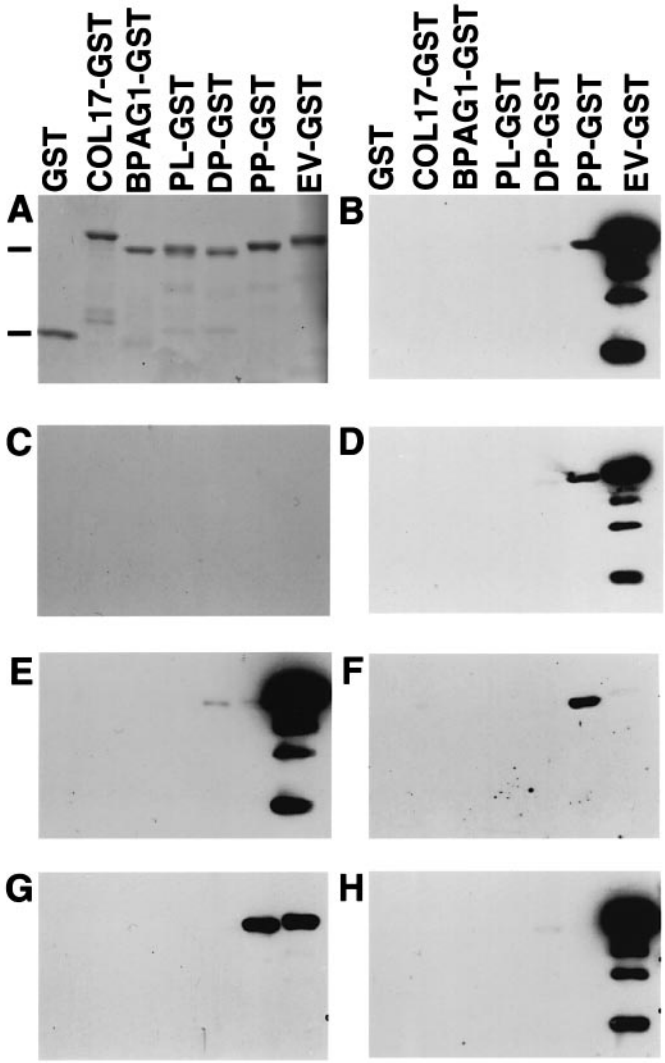

Figure 6. Affinity purification of antibodies against envoplakin-GST and periplakin-GST from PNP serum. PNP3023 was used to affinity purify antibodies against COL17-GST (*C* and *D*), envoplakin-GST (*E* and *F*), and periplakin-GST (*G* and *H*). The flow through (*D*, *F*, and *H*) as well as the bound (*C*, *E*, and *G*) antibodies were collected and subjected to immunoblot analysis. (*A*) Coomassie Blue staining of the SDS-PAGE gel to show equal loading of the GST-fusion proteins (1 μg per lane). Molecular weight markers to the left are 40 and 29 kDa. (*B*) Immunoblot of the proteins shown in (*A*) with PNP3023 serum.

sera bind to the individual plakins. We show here that these antibodies recognize both shared epitopes in homologous regions of the carboxy-terminus of these plakins as well as unique epitopes in each plakin. In addition, PNP antibodies bind to the homologous region of plectin, which has not been implicated to date in PNP. The significance of this finding is not clear as plectin has not been demonstrated in immunoprecipitates of keratinocyte extracts with PNP sera. It may be that immunoprecipitated plectin is difficult to demonstrate on SDS-PAGE due to its high molecular weight (≈600 kDa), or it may be that antibodies bind the denatured plectin-GST on immunoblot but do not bind to the native plectin in immunoprecipitation assays.

Plakins are a family of proteins that are thought to link desmosomal and hemidesmosomal proteins to intermediate filaments in order to maintain structural integrity of the cell. A new member of the plakin family, periplakin, was recently cloned by two independent groups (Ruhrberg *et al*, 1997; Aho *et al*, 1998). Specifically, cDNA cloning allowed identification of a contiguous reading frame encoding a putative polypeptide of ≈204 kDa. Northern and multiple tissue RNA analysis revealed that periplakin is expressed in keratinocytes and in other tissues with prominent epithelial components (Aho *et al*, 1998). By indirect immunofluorescence, periplakin was found expressed in all cell layers of human epidermis, but more strongly in the upper spinous and granular layers (Ruhrberg *et al*, 1997). Periplakin was also found expressed in other stratified squamous epithelia such as oral, cervical, and esophageal mucosa. The expression pattern of periplakin is similar to that of envoplakin in all tissues with the exception of esophagus, where envoplakin was expressed only in the outermost layers, whereas periplakin was expressed in all cell layers. Immuno-electron microscopy suggested that periplakin is a component of desmosomes within epidermal keratinocytes (Ruhrberg *et al*, 1997). Chromosomal assignment by radiation hybrid mapping placed the human periplakin gene to chromosomal region 16p13 (Aho *et al*, 1998). No human genetic disease has been mapped as yet to the periplakin gene locus; however, periplakin clearly serves as an autoantigen in PNP.

Exactly why patients with PNP develop antibodies to periplakin and related molecules and whether any of these antibodies are pathogenic is currently not known. It has been speculated that the types of tumors associated with PNP may produce plakins that result in an immune response (Anhalt, 1997). This is similar to what is thought to happen in patients with some ovarian carcinomas that result in ataxia due to an immune response against Purkinje cells in the cerebellum (Brashear *et al*, 1989; Furneaux *et al*, 1990) and in patients with some oat cell carcinomas who develop retinopathy due to an immune response against tumor antigens also found in retina (Grunwald *et al*, 1987; Thirkill *et al*, 1989); however, tumors of PNP patients have not been carefully examined for plakin production.

Because plakins are located inside cells it is not likely that the anti-plakin PNP antibodies initiate pathology in PNP; however, it has recently been shown that anti-desmoplakin antibodies may perpetuate pathology in erythema multiforme (Foedinger *et al*, 1995). Because histology of PNP lesions may resemble that of erythema multiforme (Horn and Anhalt, 1992), the anti-plakin PNP antibodies might contribute to the pathology.

We would speculate, however, that the pathology is probably initiated by antibodies against either the unidentified 170 kDa PNP antigen or desmoglein 3 (pemphigus vulgaris antigen) (Joly *et al*, 1994). Further studies will be necessary to sort out how the immune response is triggered and how the antibodies contribute to the pathophysiology of disease.

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