Classification, Clinical Manifestations, and Immunopathological Mechanisms of the Epithelial Variant of Paraneoplastic Autoimmune Multiorgan Syndrome

A Reappraisal of Paraneoplastic Pemphigus

Vu Thuong Nguyen; Assane Ndoye, MD; Karl D. Bassler, MD; Leonard D. Shultz, PhD; Molly C. Shields, MD; Beth S. Ruben, MD; Robert J. Webber, PhD; Mark R. Pittelkow, MD; Peter J. Lynch, MD; Sergei A. Grando, MD, PhD, DSc

Background: Recent studies suggest that paraneoplastic pemphigus (PNP) is a heterogeneous autoimmune syndrome involving several internal organs and that the pathophysiological mechanisms mediating cutaneous, mucosal, and internal lesions are not limited to autoantibodies targeting adhesion molecules.

Objective: To classify the diverse mucocutaneous and respiratory presentations of PNP and characterize the effectors of humoral and cellular autoimmunity mediating epithelial tissue damage.

Methods: We examined 3 patients manifesting the lichen planus pemphigoideslike subtype of PNP. A combination of standard immunohistochemical techniques, enzyme-linked immunosorbent assay with desmoglein (DSG) baculoproteins, and an immunoprecipitation assay were used to characterize effectors of humoral and cellular autoimmunity in patients with PNP and in neonatal wild-type and DSG3-knockout mice with PNP phenotype induced by passive transfer of patients’ IgGs.

Results: In addition to the known “PNP antigenic complex,” epithelial targets recognized by PNP antibodies included 240-, 150-, 130-, 95-, 80-, 70-, 66-, and 40/42-kd proteins but excluded DSG1 and DSG3. In addition to skin and the epithelium lining upper digestive and respiratory tract mucosa, deposits of autoantibodies were found in kidney, urinary bladder, and smooth as well as striated muscle. Autoreactive cellular cytotoxicity was mediated by CD8+ cytotoxic T lymphocytes, CD56+ natural killer cells, and CD68+ monocytes/macrophages. Inducible nitric oxide synthase was visualized both in activated effectors of cellular cytotoxicity and their targets. Keratin 14+–positive basal epithelial cells sloughed from the large airways and obstructed small airways.

Conclusions: The paraneoplastic disease of epithelial adhesion known as PNP in fact represents only 1 manifestation of a heterogeneous autoimmune syndrome in which patients, in addition to small airway occlusion and deposition of autoantibodies in different organs, may display a spectrum of at least 5 different clinical and immunopathological mucocutaneous variants (ie, pemphiguslike, pemphigoidlike, erythema multiforme–like, graft-vs-host disease–like, and lichen planus–like). We suggest that the more encompassing term “paraneoplastic autoimmune multiorgan syndrome,” or PAMS, be applied. The pathophysiological mechanisms of PAMS involve both humoral and cellular autoimmunity responses. Epithelial cell membrane antigens other than DSG1 or DSG3 are targeted by effectors of PAMS autoimmunity. Apoptosis of damaged basal cells mediates epithelial clefting, and respiratory failure results possibly from obstruction of small airways with sloughed epithelial cells.

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Paraneoplastic pemphigus (PNP) was first described 10 years ago as atypical pemphigus occurring in patients with associated neoplasm.1 The lesional tissues in patients with this disease demonstrate 3 pathognomonic microscopic features of classic pemphigus: epidermal cell separation (acantholysis); intraepithelial blister formation; and deposition of immunoreactants in intercellular spaces of epithelial tissue. However, in contrast to the lesions of classic pemphigus, a marked degree of inflammation and other findings are present both clinically and microscopically.2

As additional patients have been described, heterogeneity of the clinical features has become apparent. Some patients have mucocutaneous features that are largely noninflammatory and mimick pemphigus, whereas others have marked inflammatory lesions similar to those seen in erythema multiforme, pemphigoid, li-
METHODS

IMMUNOHISTOCHEMICAL ASSAYS

Both DIF and IIF were performed following standard protocols. For the DIF assay, the tissue specimen was incubated for 1 hour at room temperature with a fluorescently labeled antibody to human IgG (Pierce, Rockford, Ill; dilution 1:200 in phosphate-buffered saline), CD4, CD8, CD56, CD68 (all from Zymed, San Francisco, Calif; all neat), keratins 8 (K8; Sigma Chemical Co, St Louis, Mo; 1:200) and 14 (K14; Babco, Richmond, Calif; 1:300), or murine CD8 or CD32/16 (both from Pharmingen, San Diego, Calif; both diluted 1:100). For the IIF experiments, a tissue substrate was first treated with primary antibody and then exposed to the fluorescein isothiocyanate–labeled secondary antibody. As a source of primary antibody in IIF assays, we used pemphigus or control serum, or polyclonal antibody to human inducible nitric oxide synthase (iNOS) raised in rabbits immunized with the peptide representing the human iNOS amino acid sequence 1137 to 1153 conjugated to keyhole limpet hemocyanin, following the procedure detailed elsewhere. Double staining experiments were performed following the protocol previously described.

IP ASSAY

The serum IgG fractions were isolated using 40% ammonium sulfate followed by dialysis in phosphate-buffered saline, lyophilized, and reconstituted in phosphate-buffered saline, as detailed elsewhere. Cultured keratinocyte monolayers were metabolically labeled for 16 hours at 37°C with 3.7 × 10^–7 Bq/mL [35S] methionine (3.7 × 10^3 Bq/mmol, Amersham Life Science Inc, Arlington Heights, Ill) in 1.8-mmol/L Ca++ labeling medium, and the [35S] methionine-labeled proteins were separated by centrifugation and used as a source of naturally folded keratinocyte proteins in a standard IP assay. The immune complexes were precipitated with a protein A–sepharose suspension, washed, and resolved on 7.5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). The gels were fixed and enhanced, and the radioactivity was analyzed using the storage phosphor autoradiography feature of the Storm system (Molecular Dynamics, Mountain View, Calif), as described previously.

ENZYME-LINKED IMMUNOSORBENT ASSAY

The immunoreactivities of test serum samples with the extracellular portions of the DSG1 and DSG3 baculoproteins that reportedly display the pathogenic epitopes of PV and pemphigus foliaceus (PF) antigens, respectively, were determined using DSG1- and DSG3-coated ELISA plates purchased from MBL (Nagoya, Japan) together with the positive and the negative controls and following the protocols provided by the manufacturer. The results were expressed as ELISA scores calculated from optical density values using the equation suggested by the manufacturer. As controls, we also used serum samples from normal subjects and from patients with confirmed PV, PF, and bullous pemphigoid (BP).

PASSIVE TRANSFER EXPERIMENTS WITH NEONATAL MICE

The PAMS phenotype was induced in neonatal mice by passive transfer of serum IgG fractions. Both DSG3-containing normal BALB/c mice and DSG3-knockout, ie, Dsg3null (129; C57BL/6) mice were used. The serum IgG fractions were injected intraperitoneally at a dose of 20 mg/g of body weight per day into 10- to 12-hour-old pups. In each litter, the Dsg3null mice that are homozygous for a targeted mutation of the Dsg3 gene were identified by genotyping at the end of each passive transfer experiment. Polymerase chain reaction (PCR) was used to amplify the sequences from the genomic DNA isolated from pieces of mouse tails using PCR primers and reaction conditions described previously. The neonates in each progeny always received the same amount of PAMS or normal human IgG isolated from serum purchased from Sigma Chemical Company.

chren planus, or even cutaneous graft-vs-host disease. Within this clinical spectrum, painful erosions are typically present in the mouth and also are often found on the mucous membranes of the pharynx, larynx, esophagus, eyes, nose, and genitalia. Almost all of the described patients have had associated malignancy, usually of the lymphoproliferative type. Death, often due to respiratory failure, has occurred shortly after the onset of the disease in most patients.

A spectrum of pathological changes also occurs histologically. In some patients, epithelial cell acantholysis with pemphigus vulgaris (PV)–like blister formation is the most prominent feature, whereas in others, little or no acantholysis occurs, and the dominant feature is an intense inflammatory infiltrate at the dermoepidermal junction (DEJ). This infiltrate consists primarily of mononuclear cells and is accompanied by varying degrees of satellite cell necrosis, keratinocyte dyskeratosis (or apoptosis), and vacuolar damage to the epithelial basal cell layer. These inflammatory changes are very similar to those characterizing erythema multiforme, lichen planus, lupus erythematosus, and acute graft-vs-host disease.

A clinical diagnosis of PNP can be confirmed by the presence of a distinctive profile of antibodies directed toward the plakin family of adhesion proteins. Direct immunofluorescence (DIF) of involved skin and mucosa reveals deposits of IgG and complement localized in an intercellular (ie, pemphiguslike) and/or linear (ie, pemphigoidlike) pattern. By indirect immunofluorescence (IIF), PNP antibodies stain the simple, columnar, and transitional epithelial tissue substrates in addition to the stratified squamous epithelium. By immunoprecipitation (IP), PNP antibodies react with intracellular proteins of the plakin gene family: plectin (>400 kd), desmoplakin I (250 kd), bullous pemphigoid antigen 1 (230 kd), envoplakin, desmoplakin II (a 210-kd double band), and periplakin (190 kd) as well as with an unidentified keratinocyte polypeptide with an apparent molecular weight (MW) of 170 kd. Paraneoplastic pemphigus antibodies have also been reported to react with recombinant desmoglein (DSG) 3, and it was postulated that anti-DSG3
antibodies cause acantholysis. Recently, Mahoney et al.19 concluded that anti-DSG3 antibody alone is inefficient at causing skin blistering. Further, the conjecture that pulmonary epithelial injury is caused by autoantibodies against plakin proteins20 was challenged with new evidence that a distinctive intrinsic lung disease can cause respiratory failure in patients with PNP.21

A pathophysiological role for mononuclear cells infiltrating PNP lesions is suggested strongly by the fact that PNP resembles closely the abnormality caused by autoreactive cell-mediated reactions, such as those found in graft-vs-host disease,14,22 lichen planus,13 and erythema multiforme.23 Moreover, there is a body of evidence that cell-mediated autoimmunity also develops in patients with classic forms of pemphigus and pemphigoid.24-30

In this study, we characterized the antigenic profile of PNP antibodies using a combination of an IP assay and enzyme-linked immunosorbent assay (ELISA) using standard DSG1 and DSG3 baculoproteins. We found that in addition to the known “PNP antigenic complex” patients’ antibodies react specifically with several other self-antigens, but not with DSG1 or DSG3. In addition to skin and upper digestive and respiratory tract epithelia, deposits of autoantibodies were found in the kidney, urinary bladder, and muscle. We analyzed the cellular infiltrate of PNP lesions and obtained evidence that cellular cytotoxic reactions contribute to damage of the tegumental epithelial lining of the mucocutaneous, upper digestive and respiratory tract tissues. Sloughed bronchial cells obstructed the small airways, which may provide a mechanism for respiratory failure. These new and critical aspects of the pathophysiological mechanism of PNP were confirmed in passive transfer experiments with neonatal mice.

Although the historical value of the term paraneoplastic pemphigus is recognized, we believe that the phenotypic heterogeneity of the patients with this disease coupled with a pathophysiological course that is substantially different from classic pemphigus favors adoption of a more encompassing term. We therefore designate the term paraneoplastic autoimmune multiorgan syndrome (PAMS) to describe this distinctive paraneoplastic syndrome.

REPORT OF CASES

CASE 1

A 63-year-old man developed pharyngitis that failed to respond to a course of antibiotics. Subsequently, he noticed the occurrence of painful erosions of the mouth, bilateral conjunctival effusions, and painful blisters and erosions of the glans penis associated with a diffuse morbilliform eruption. During examination, circinate, polycyclic erythematous plaques with overlying peripherally distributed small vesicles and desquamating, colllarette-type scale were present over the upper trunk. The Nikolsky sign was negative. The lips were covered with hemorrhagic crusts. Intractable stomatitis accompanied painful erosions and ulcerations of the oropharynx and vermilion borders of the lips. Medical history revealed that 2 years earlier he had been successfully treated with 4 cycles of fludarabine for non-Hodgkin lymphoma. A computed tomographic scan revealed no evidence of active lymphoma.

Biopsy specimens from the glans penis, mouth, and skin lesions were similar and showed lichenoid infiltrate at the DEJ with basal layer vacuolar changes and occasional dyskeratotic epithelial cells. Minimal basal layer clefting was present. Findings of DIF studies of skin and mucosa revealed a linear deposition of IgG both at the DEJ and in a pemphiguslike intercellular pattern in the epidermis. Results of IIF studies demonstrated circulating IgG, which created a pemphigoidlike pattern at the DEJ and a pemphiguslike pattern in the epithelial intercellular spaces (Table 1). A diagnosis of PAMS (also known as PNP) was confirmed by the presence of a characteristic IP pattern (Figure 1).

Prednisone, in a dose of 80 mg/d, led to some improvement of his mucocutaneous lesions, but within a short time he developed a chronic cough and shortness of breath. Continued oral and topical steroid therapy led to eventual clearing of the mucocutaneous lesions. However, when the daily prednisone dose was tapered to 30 mg/d, his lesions returned and his respiratory symptoms worsened. Papular erythematous lesions progressed to blisters and erosive lesions affecting the trunk, extremities, palms, and soles. Confluent erythema developed in the V area of the upper chest and back, and diffuse erosions appeared on the lips and oral and nasal mucosa. Chest radiogram results showed a right lower lobe infiltrate that failed to clear with intravenous antibiotics. Results of a ventilation perfusion scan for pulmonary embolism were negative. Bronchoscopy revealed occlusion of small bronchi by desquamated epithelial cells.

Despite an increase in prednisone dose to 100 mg/d, the patient died of respiratory failure a few days later. The results of a postmortem lung examination revealed diffuse upper and lower airway mucosal erythema. Histologically, the mucosa and submucosa of the airways showed nonspecific chronic inflammatory changes, including infiltration of lymphocytes, plasma cells, and macrophages. Bronchial epithelium was partially or entirely absent with bronchial scarring, fibrous obliteration, and dilatation of bronchial lumens.

CASE 2

A 48-year-old man was admitted with thymoma (malignant to the lungs and liver), oral erosions, and widespread lichenoid eruption with exfoliation involving the face, arms, and upper trunk that was accentuated in radiation ports. The palms and soles were reddened and desquamating. Findings of a skin biopsy demonstrated a subacute vacuolar interface pattern with associated dyskeratotic (apoptotic) keratinocytes. Other medical problems included acquired pure red blood cell aplasia with chronic anemia requiring repeated transfusions.

While hospitalized, the patient developed respiratory problems and died shortly thereafter. Autopsy re-
vealed disseminated, invasive *Pseudallescheria boydii* infection involving multiple organs. The cut surface of the left lung showed multifocal areas of induration with multiple, light brown, spherical lesions with central necrosis. The bronchi appeared of normal caliber with hyperemic mucosa. Microscopically, focal pneumonia with alveolar damage and hyaline membrane formation was found. Immunological studies performed with the patient’s serum post mortem showed the presence of characteristic antibodies against the classic PNP antigenic complex (Table 1; Figure 1).

### CASE 3

A 62-year-old man presented with a 1-year history of marked weight loss, recent respiratory distress, including dyspnea, painful oral erosions, hoarseness, and dysphagia. No skin lesions were present. Findings of previous oral biopsies were said to show focal pemphiguslike acantholysis on one occasion and a pemphigoidlike subepidermal bulla on another occasion. Our oral biopsy results showed a lichenoid lymphocytic infiltrate at the DEJ and slight basal layer clefting. Direct immunofluorescence, IIF, and IF studies confirmed the clinical diagnosis of PAMS (Table 1; Figure 1).

A search for an associated malignancy revealed a suprarenal mass, which was excised and histologically identified as a Castleman tumor (ie, angiofollicular lymph node hyperplasia). Bronchial washings revealed clusters of bronchial epithelial cells. The patient’s mucocutaneous lesions responded to prednisone at a dose of 80 mg/d. His prednisone dose was tapered over an 18-month period and continued for approximately 2 months. This clinical remission was associated with transient disappearance of those circulating autoantibodies that stained monkey esophagus in a fishnetlike, intercellular pattern, whereas the titer of anti–basal membrane zone antibodies remains unchanged (Table 1). Later, the patient developed a flare of mucocutaneous lesions without evidence of metasases of Castleman tumor. He is currently on the maintenance dose of prednisone and is doing well. He occasionally experiences respiratory symptoms, which he controls using a corticosteroid inhaler.

### RESULTS

**EFFECTORS OF HUMORAL AUTOIMMUNITY**

The IP assay was used to characterize the antigenic profile of PAMS autoantibodies (Figure 1). The serum samples...
from all 3 of our patients with PAMS uniquely immunoprecipitated keratinocyte proteins with apparent MWs of 40/42, 66, 70, 80, 95, 150, 190, 210 (double band), 230, and 250 kd. Additionally, the serum samples from patients 1 and 2 reacted with 130- and 170-kd proteins, and the serum sample from patient 2 uniquely recognized a 240-kd protein. The serum sample from patient 3 lacked an autoantibody against the 170-kd protein, a finding noted occasionally in other PAMS cases.41

The presence of anti-DSG1 and anti-DSG3 antibodies in test and control serum samples was investigated using ELISA with DSG1 and DSG3 baculoproteins (Table 1). Repeated ELISA testing with different batches of DSG1 and DSG3 showed that the serum samples from all 3 patients with PAMS as well as from a patient with BP were always negative for DSG1 and DSG3 antibodies, as were the samples from normal subjects. High titers of DSG1 and DSG3 were detected in the positive control samples, PV, and PF serum samples, indicating that the lack of reactivity of PASM serum with DSG baculoproteins was due to the absence of anti-DSG antibodies rather than to technical limitations. In addition, since the IgG fraction of the normal subjects whose ELISA scores were equivalent to those of PAMS patients 1 and 2, did not precipitate either DSG1, 160 kD,42 or DSG3, 130 kD,43 the 130-kD keratinocyte protein immunoprecipitated by the IgG fractions from PAMS patients 1 and 2 is not DSG3 but instead an unknown antigen bearing the same MW as DSG3.

To discern the epithelial tissues that are targeted by PAMS autoantibodies, we examined by DIF the biopsy and autopsy specimens obtained from our PAMS patients. Deposits of IgG autoantibodies were found within the epidermal lining of the skin, conjunctiva, oral mucosa, esophagus, trachea, bronchi, alveoli, urinary bladder, and renal glomeruli (Figure 2), but not in the glandular or gastrointestinal epithelia. Three staining patterns occurred: (1) intercellular, in a pemphiguslike pattern; (2) linear at the DEJ, in a pemphigoidlike pattern; and (3) homogenous within the cell, in an apoptosislike pattern.

Consistent with the results of DIF assays, specific epithelial binding of PAMS IgGs was observed in the IIF experiments that used as a substrate specimens of human or rodent skin, eyelid, oral mucosa, esophagus, airways, and urinary bladder (Figure 2). The titers and staining patterns of PAMS and control serum samples were determined by IIF using monkey esophagus as a substrate (Table 1). Moderate reactivity toward smooth and striated muscle also occurred in both DIF and IIF assays (data not shown).

EFFECTORS OF CELLULAR AUTOIMMUNITY

Light microscopic examination of PAMS lesions revealed dyskeratotic keratinocytes characterized by condensed and basophilic nuclei and eosinophilic homogenization of the cytoplasm (Figure 3A), which is consistent with keratinocyte apoptosis.44-45 Frequent findings of satellite cell necrosis, vascular interface changes, and lichenoid infiltration implicated cellular cytotoxic reactivity. Therefore, we sought to further characterize effector populations of the cellular immunity by antigen biomarker analysis of autopsies and biopsy samples obtained from the PAMS patients. In lesional skin, CD4+ lymphocytes were found within infiltrates located in the papillary dermis (Figure 3B). In marked contrast, CD8+ cytotoxic T lymphocytes (CTLs) were located within the basal and suprabasal epidermis and often apposing apoptotic keratinocytes (Figure 3C). CD56+ natural killer (NK) cells infiltrated the upper dermis and were also situated at the DEJ (Figure 3D). CD68+ monocyte/macrophages, effectors of natural cytotoxicity, surrounded areas of microvesiculation from both sides of the DEJ (Figure 3E). The most prominent CD68+ infiltrate was present at the dermal side of the blisters and individual cells were seen scattered throughout the epidermis. Double staining experiments that included an antibody to iNOS (which can serve as a marker of both activated cytotoxic mononuclear cells and their target cells46) were performed to determine if the CD68+ cells infiltrating PAMS lesions are activated and can participate in the cytotoxic reactions. As expected, the CD68+ cells abundantly expressed iNOS (Figure 3F). Immunoreactivity of iNOS also occurred in lesional keratinocytes (Figure 3G), suggesting that these iNOS-positive epithelial cells were undergoing apoptosis.47 Both preincubation of the anti-iNOS immune serum samples with the synthetic peptide used for immunization and omission of the primary antibody abolished the fluorescent staining (not shown).

Mononuclear infiltrates containing iNOS-positive CD8+, CD56+, and CD68+ cells were also found in the conjunctival and oral lesions of PAMS patients (not shown). In respiratory tissues, both the epithelial lining of the large (Figure 3H) and the small (Figure 3I) airways demonstrated pronounced infiltrates of CD68+ mononuclear cells. In these locations, CD68+ cells predominated over CD8+ CTL and CD56+ NK cells. Notably, activated CD68+ cytotoxic cells were identified within the sheets of the respiratory epithelium that had sloughed from the bronchial wall (Figure 3H).

MECHANISM OF THE SMALL AIRWAY OCCLUSION

Respiratory problems were present in all 3 of our PAMS patients. Examinations of tracheal and bronchial autopsy specimens of patient 1 revealed detachment of mucosal sheets and individual cells from the lamina propria. The sloughed epithelial cells were found in the lumen of respiratory bronchioles and alveolar sacs. These sloughed cells stained homogeneously with anti-IgG autoantibodies in DIF experiments, producing the apoptosislike pattern (Figure 4A). To determine the origin of sloughed, apoptotic respiratory epithelial cells occluding small airways, the autopsy specimens were stained for K8, a marker of simple epithelial cells that is normally expressed in both upper and lower airways,48,49 and K14, a marker of basal cells in keratinizing stratified epithelium that is not expressed by normal alveolar cells but can be found at low levels in normal human bronchi.50 The sloughed epithelial cells were positive for both K8 and K14. In marked contrast to normal airways, the epithelial cells comprising the mucosal sheets that were sloughed from the walls of the upper airways in PAMS patients abundantly expressed K14 (Figure 4B). Since the epithelium lining small airways was K14 negative, the apoptotic respiratory epithelial cells that were enriched.
with the marker of squamous epithelium K14 had shed from large airways and migrated distally within the airway where they could occlude the lumens of the small airways.

**INDUCTION OF PAMS PHENOTYPE IN WILD-TYPE AND DSG3-KNOCKOUT MICE**

The serum IgG fraction of patient 1 was injected intraperitoneally into nine 10- to 12-hour-old normal BALB/c and 7 Dsg3null neonates, and the IgGs from patient 3 were injected into 6 Dsg3null pups. Approximately 12 to 16 hours after injection, all mice developed generalized cutaneous erythema followed by peeling or prominent blistering of their skin at approximately 24 to 32 hours after injection (Figure 5A). Ruptured blisters revealed superficial erosions with positive Nikolsky sign (Figure 5B). All mice injected with IgG obtained from PAMS patients died by 72 hours after injection with symptoms of generalized cyanosis.

Light microscopic examination of skin lesions representing the “erythematous” stage (16-24 hours after injection) revealed profound mononuclear cell infiltrate in the upper dermis associated with interface microvesiculation (Figure 5C). During the “blistering” stage (> 24 hours after injection), clefting occurred both in the intraepidermal and subepidermal locations. The clefts resulted from disintegration of the basal cell layer caused by separation of basal keratinocytes from both the basal membrane and adjacent cells (Figure 5D). The loss of epithelial cell attachment was also prominent in mouse airways. The respiratory epithelial cells detached from the wall of large airways as intact sheets and individual cells (Figure 5E) that occluded the airway lumens (Figure 5F). The clusters of sloughed epithelial cells that packed respiratory bronchioles and alveolar sacs immunostained positive for K14 (Figure 5G).

Our DIF experiments with anti–human IgG antibody demonstrated cell membrane deposits of injected non-DSG3 antibodies within the epidermis (Figure 5H), oral mucosa, esophagus, and respiratory epithelium (Figure 5I) of Dsg3null mice. Monoclonal antibodies to murine CD markers were used to characterize the cellular infiltrate. Examination of cutaneous, esophageal, and
Figure 3. Effectors of cell-mediated cytotoxicity in paraneoplastic autoimmune multiorgan syndrome (PAMS) lesions. A, The interface lichenoid infiltrate in association with satellite-cell necrosis and vacuolar degeneration of basal cells leading to an epidermal split within the basal layer without acantholysis in the penile lesion of PAMS patient 1. B, Abundant infiltrate of CD4 cells in the upper dermis without extension into the epidermis (not shown) in the lesional skin biopsy specimen of the same patient. C, Heavy infiltration of CD8 cytotoxic T lymphocytes involves both the epidermis and the uppermost regions of the lamina propria in the oral lesion biopsy specimen of PAMS patient 3. D, The CD56+ NK (natural killer) cells are positioned along the dermoepidermal junction in the same biopsy specimen. The monocytes/macrophages surrounding an epidermal cleft simultaneously express CD68 (E, F, red) and inducible nitric oxide synthase (iNOS) (F, green). F Also shows iNOS-positive epithelial cells in the basal layer. G, In addition to the basal epithelial layer, the iNOS-positive apoptotic keratinocytes are also found in suprabasilar locations where these cells could be targeted by the effectors of cell-mediated cytotoxicity that invade the upper epidermis, as shown in C. In the autopsy specimen from the respiratory tissue of PAMS patient 1, the CD68+ effectors of natural cytotoxicity invade the epithelial lining that is being sloughed from the bronchial wall (H) and also infiltrate the epithelial linings of smaller airways (I, red). Panel I also shows that CD68+ cells are also iNOS positive (green). (Original magnifications: A-D, G × 200; E × 100; F, × 630, and I, × 1000. Dotted lines outline the epithelial basement membrane. The specificity of antibody binding was demonstrated by abolishing the staining by omitting the primary antibody (not shown).
Figure 4. Visualization of respiratory epithelial cells occluding small airways in patients with paraneoplastic autoimmune multiorgan syndrome (PAMS). A, Clusters of large respiratory epithelial cells occluding a terminal bronchiole of PAMS patient 1 immunostain positive with anti–human IgG. B, The respiratory epithelial cells that compose the epithelial sheet detaching from the wall of a large bronchus immunostain brightly for K14, indicating that the clusters of K14-positive cells occluding the small airways include cells that were sloughed from the walls of the large airways. (Original magnifications, A, ×630; B, ×100.)

Figure 5. Mucocutaneous and respiratory lesions of neonatal Dsg3null mice injected with paraneoplastic autoimmune multiorgan syndrome (PAMS) IgGs. A, Generalized erythema followed by large, flaccid blisters filled with serous fluid developed on the skin of DSG3-negative mice approximately 24 hours after a single intraperitoneal injection of PAMS IgG. B, Light pulling with tweezers of the skin at the edge of the erosions that appeared after spontaneous rupture of the blisters resulted in peripheral enlargement of the detached area toward clinically uninvolved skin. The deg3−/− genotype of this mouse was determined by polymerase chain reaction amplification of the sequences of genomic DNA extracted from the tail.16 A primer set matching within the “Neo” sequence indicative of the targeted disruption of the Deg3 gene amplified a product with the expected size, 280 base pairs, whereas 2 sets of primers matching within the sequences of the wild-type Dsg3 gene amplified no products (not shown). C, Light microscopic examination of early lesions in the skin of a Dsg3null mouse injected with PAMS IgG reveals microvesiculation and lichenoid subepidermal mononuclear infiltrate subjacent to the areas of vacuolization of basal keratinocytes. D, In a fully developed lesion, complete disintegration of the basal cell layer caused by separation of basal keratinocytes from both the basal membrane and the adjacent keratinocytes produces intraepidermal clefting. A loss of epithelial cell adhesion similar to that found in the skin occurs in large airways where the respiratory epithelium cells detach from the lamina propria and neighboring cells (E) and form clusters occluding the airways (F). G, The clusters of respiratory epithelium cells found in the lumen of small airways are positive for the desquamation marker K14. Deposits of PAMS IgG were visualized using fluorescein isothiocyanate–conjugated anti–human IgG antibody. In Dsg3null mice, the non-DSG3 PAMS antibodies are found attached to the surfaces of the epithelial cells comprising the epidermis (H) and bronchial wall lining (I). Omitting the primary antibody abolished the staining in the indirect immunofluorescence assays (not shown). (Original magnifications, C, D, ×400; E, H, I, ×630; F, ×100; and G, ×1000.)
bronchial specimens revealed infiltrates comprised predominantly or exclusively of CD32/16+ NK cells. Only a few CD4+ or CD8+ cells could be seen within the mononuclear cell infiltrates (not shown).

No differences were observed between the wild-type and Dsg3null mice with regard to their response to PAMS IgG injections. Production of gross and microscopic features of the PAMS phenotype, evolution of lesion development, and DIF and IIF findings were identical. Since Dsg3null mice lack the DSG3 target for an anti-DSG3 antibody, and since none of our PAMS patients had anti-DSG3 antibody by ELISA (Table 1), these findings demonstrated that neither the DSG3 antigen nor any anti-DSG3 antibody could play any role in the pathophysiology of mucocutaneous lesions in our PAMS patients.

CLASSIFICATION OF PAMS

“Paraneoplastic pemphigus” has recently emerged as a distinct paraneoplastic disease with an autoimmune pathogenesis.1 It shares some similarity with the classic forms of pemphigus (PV and PF). However, as demonstrated by the clinical and laboratory findings in patients presented in this report and by our investigations in the animal model of PAMS, there are sufficient differences between these 2 conditions to warrant separate terminology. Among the differences are the distinctive mucocutaneous clinical features, histological characteristics, immunofluorescent patterns, systemic involvement, association with neoplasia, responsiveness to therapy, prognosis, and pathophysiological characteristics. Based on these observations, elaborated below, we believe that the term paraneoplastic pemphigus is too restrictive to adequately describe the multiorgan syndrome under consideration. The use of a term containing the word pemphigus may lead to overlooking this possibility when a PAMS is manifested by a nonvesicobullous eruption. Furthermore, the word pemphigus may be misleading because true pemphigus does not usually affect lungs, which is in marked contrast to PAMS, in which lung involvement has proven fatal in many cases. To encompass the aggregate of signs and symptoms associated with the distinct morbidity process affecting the skin, mucosa, and lungs in a heterogeneous group of patients with paraneoplastic disease, we suggest the more inclusive term paraneoplastic autoimmune multiorgan syndrome,” or PAMS. In this study, we characterized patients with the mucocutaneous and respiratory variant of PAMS. In future, this classification system will accommodate subsets of patients with pathological characteristics associated with other systems (eg, endocrine, neural, urinary, etc) as discrete clinical variants of PAMS.

MUCOCUTANEOUS CLINICAL FEATURES OF PAMS

Oral erosive disease occurs in patients with classic pemphigus as well as in patients with PAMS. In PAMS, however, the degree of mucosal involvement is usually more severe in extent and intensity. There are usually erosions extending from the mouth to the aerodigestive tract, and there is often involvement of other mucosal surfaces such as the eyes and genitalia.

In classic pemphigus, blisters and erosions arise from otherwise normal-appearing skin; inflammatory lesions are not usually present.2 In PAMS, inflammatory macules, papules, and plaques are always present and may be more prominent than blisters. These erythematous lesions are polymorphic and vary considerably from one patient to another. They may mimic morphologically the appearance of erythema multiforme, lichen planus pemphigoides, or graft-vs-host disease. Vesicles and bullae are often relatively inconspicuous and when present, arise from the surface of the erythematous lesions rather than from normal, uninflamed skin.

HISTOLOGICAL CHARACTERISTICS OF PAMS

Consistent with the clinical presentation, the microscopic appearance of classic pemphigus is relatively pauci-inflammatory with prominent cell-to-cell desquamation (acantholysis) within the epithelium above the basal layer. In contrast, PAMS exhibits a marked mononuclear, lichenoid inflammatory infiltrate at the DEJ. Vacuolar degeneration of the basal layer is accompanied by suprabasilar clefting. Dyskeratotic (apoptotic) keratinocytes appear predominantly within the more basal regions of the epithelium. Acantholysis is generally present but much less marked than in classic pemphigus. Reflecting these microscopic changes, blisters develop either within the epidermis, in a pemphiguslike pattern, and/or at the DEJ, in a pemphigoidlike or erythema multiforme–like pattern.

IMMUNOFLUORESCENT HALLMARKS OF PAMS

Findings of DIF examination of perilesional tissue in classic pemphigus reveal the deposition of immunoreactants (almost always IgG antibodies with some complement components) in the intercellular spaces of the epithelium. The results of IIF examination of the patients’ serum samples demonstrate the presence of circulating antibodies, which bind to the intercellular spaces of human epidermis and monkey esophageal epithelium. In PAMS, these same immunofluorescent patterns occur but, in addition, DIF studies usually reveal the presence of immunoreactants at the DEJ as well as in an intraepithelial cell location. Findings of IIF studies show circulating antibodies that bind to the intercellular spaces within many additional types of simple, columnar, and transitional epithelia. The titer of intercellular antibodies, but not anti–basal membrane zone antibodies, seems to correlate with the clinical course of PAMS.

SYSTEMIC INVOLVEMENT IN PAMS

Although mucosal involvement of the pharynx and larynx can rarely be found in classic pemphigus, involvement of the bronchial and alveolar airways does not occur. On the other hand, pulmonary involvement, often severe enough to result in death, occurs in nearly all patients with PAMS. All 3 of our patients developed respi-
ASSOCIATION OF PAMS WITH NEOPLASIA

Malignancy, usually thymoma, has been reported in a small number of patients with classic pemphigus. However, this association does not result in any change in the clinical or histological appearance of classic pemphigus lesions. In contrast, patients with PAMS almost always have associated neoplasia at the time when mucocutaneous lesions develop. The neoplasia may be discovered before or after the development of the mucocutaneous disease, but simultaneous recognition occurs most frequently. The neoplasia is typically malignant. Lymphoproliferative disease occurs most often, but a large number of other malignancies have been reported. Two of our 3 patients had concurrent neoplasia (metastatic thymoma and Castleman tumor), and the other had been treated for non-Hodgkin lymphoma 2 years prior to developing PAMS. Establishing the diagnosis of PAMS in 1 of our patients (patient 3) prompted the search for an occult malignancy, which resulted in the diagnosis and subsequent removal of the Castleman tumor.

In addition to the epidermis and other epithelial tissues, the nervous system is also targeted by an aberrant immune response to an underlying malignancy. This results in paraneoplastic with debilitating and frequently life-threatening consequences.

RESPONSIVENESS TO THERAPY OF PATIENTS WITH PAMS

Most patients with classic pemphigus respond well to moderate- or high-dose systemic corticosteroid therapy. Recalcitrant disease can be treated with cytotoxic agents, plasmapheresis, intravenous γ-globulin, cyclosporin, or mycophenolate mofetil. Patients with PAMS respond less consistently. Although 2 of our 3 patients improved or cleared with oral prednisone, many previously reported patients with PAMS have been resistant to all conventional types of therapy. Interestingly, in a few instances, successful treatment of the associated neoplasia was associated with clearing of the mucocutaneous lesions. Excision of the Castleman tumor in our PAMS patient 3 was associated with temporal disappearance of oral and cutaneous lesions.

PROGNOSIS OF PAMS

Prior to the era of corticosteroid therapy, most patients with classic pemphigus died (reviewed by Robinson et al). With current therapies, fewer than 10% of cases are fatal. Mortality frequently results from complications of therapy rather than the disease itself. However, patients with PAMS have a significantly worse outcome. A recent report calculated a fatality rate of 90% in 84 patients cared for at several academic health centers. One of our patients died of respiratory failure shortly after the onset of disease, and another died from fungal sepsis.

PATHOPHYSIOLOGICAL CHARACTERISTICS OF PAMS

Currently, classic pemphigus is considered to be mediated by antibodies directed toward the desmosomal cadherin adhesion molecules, DSG1, and DSG3. In contrast, PAMS is associated with antibodies directed primarily toward desmosomal plakin proteins (Table 2). In paraneoplastic autoimmune neuronal diseases, several novel families of antigen targets have been identified. However, there is mounting evidence that the antibodies detected in both the paraneoplastic mucocutaneous and neuronal syndromes do not necessarily mediate the pathological process directly, but may serve as serological markers of disease (similar to humoral markers for cancer). Instead, cellular immunity seems to mediate the major activity in tumor

Table 2. Self-antigens of Paraneoplastic Autoimmune Multiorgan Syndrome

<table>
<thead>
<tr>
<th>Molecular Weight, kd</th>
<th>Protein Family</th>
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<tbody>
<tr>
<td></td>
<td>Plakin</td>
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<tr>
<td>40/42 †</td>
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<tr>
<td>60 †</td>
<td>...</td>
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<td>70 †</td>
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<td>&gt;400</td>
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*Ellipses indicate not applicable; plus sign, unknown; and BP, bullous pemphigoid.
†Proteins identified in this study. Other target molecules have been reported previously.
‡Although an antibody to a 130-kd protein can be identified, there are conflicting reports as to whether this is DSG3 (see the "Comment" section).
§The 210-kd protein occurs as a doublet.
In this regard, autoantibodies serving as serological markers for cancers have been identified as a useful surrogate to elucidate the cellular effects of immunity, including both CD8+ and CD4+ T cells. Tumor-specific CTLs are present in patients with cancer who experience Purkinje cell loss and paraneoplastic cerebellar degeneration.

Similar observations have recently implicated specific CD8+ T cells in the development of a graft-vs-host–like immunophenotype in a patient with PAMS. However, another report suggested that antibodies against the 130-kd DSG3 are present in the serum of patients with PAMS. The antibodies eluted from the DSG3 chimeric baculoprotein caused acantholysis in neonatal mice, thus raising a possibility that the clinical manifestations of PAMS result from an anti-DSG3 antibody action. This notion was challenged by recent findings that the 130-kd keratinocyte protein targeted by PV IgG may be not DSG3 but some unknown molecule with the same MW. The Dsg3-Ig-His chimeric baculoprotein that was used in the past to show that anti-DSG3 antibodies from patients with PAMS are pathogenic actually absorbs autoantibodies to an array of keratinocyte proteins including a non-DSG3 130-kd polypeptide present in keratinocytes from DSG3-knockout mice. We have therefore attempted to clarify the overall pathophysiological characteristics of PAMS. Based on our previous findings, which questioned the pathogenic importance of DSG antibodies in classic pemphigus (reviewed by Grando), we have specifically addressed what, if any, role DSG antibodies might play in our patients with PAMS.

**Effectors of Humoral Autoimmunity in PAMS**

Our IP studies revealed the presence of the expected antibodies directed against the previously reported members of the plakin protein family. As has also been previously reported, 2 of our patients had antibodies directed toward a 170-kd protein of unknown type. In addition, 2 of our patients had antibodies that recognized a protein of 130 kd, the MW of DSG3. However, using an ELISA with DSG3 recombinant baculoprotein, we could not confirm that this targeted keratinocyte protein was DSG3, which is in keeping with observations reported by de Bruin et al. Moreover, upon passive transfer of our patients’ IgGs, generalized erythema followed by blistering and/or peeling developed in both wild-type mice (that posses DSG3) and DSG3-negative knockout mice. (The DSG3-negative knockout mice lack spontaneous skin lesions at birth, which justifies the use of Dsg3null mice in passive transfer experiments.) The lesions that developed in injected mice resembled closely those in patients with PAMS. Based on these observations, we submit that antibodies to DSG3 did not play a role in the development of the mucocutaneous lesions in our patients with PAMS. Our results indicate that the candidates for the pathophysiologically important targets of humoral autoimmunity include keratinocyte proteins with apparent MWs of 240, 170, 150, 130, 95, 80, 70, 66, and 40/42 kd. The specificities of non-DSG pathogenic antibodies produced by patients with PAMS remain to be discovered. These may include antibodies to cell membrane antigens targeted by antikeratinocyte IgGs produced by patients with PV and/or PF. We have recently identified 2 novel human molecules targeted by PV IgGs. These are α9, a first of its kind acetylcholine receptor with dual, muscarinic, and nicotinic pharmacology; and pemphakin, a novel annexinlike molecule that also binds acetylcholine.

Deposits of autoantibodies alone, without cytotoxic mononuclear cells, occurred in the kidney and urinary bladder, but no clinical or light microscopic evidence suggested that these organs are otherwise involved in the disease process. These findings indicate that a loss of epithelial adhesion and the development of inflammatory lesions in the epithelial tissues of patients with PAMS require participation of both humoral and cellular autoimmunity effectors.

**Effectors of Cellular Autoimmunity in PAMS**

The clinical signs of inflammation and the microscopic presence of a mononuclear inflammatory infiltrate suggest that cell-mediated immune mechanisms play a role in the pathophysiological development of PAMS. We found that the mononuclear cell inflammatory infiltrate present at the DEJ in the lesions of our patients consisted of CD8+ CTL, CD56+ NK cells, and CD68+ monocytes/macrophages. These cells are similar to those found in lichenoid infiltrates characteristic of erythema multiforme, graft-vs-host disease, lichen planus, and lichen planus pemphigoides.

Cell-mediated cytotoxicity in pemphigus involves both autoreactive CD8+ CTLs and patients’ IgGs that recruit allogenic peripheral blood mononuclear cells into antibody-dependent cellular cytotoxicity (ADCC) against epithelial targets. In PAMS, autoimmune cellular cytotoxic reactions may also be generated through an antitumor response similar to the antileukemia immune response, which involves both major histocompatability complex (MHC)–restricted CD4+ and CD8+ lymphocytes, and MHC-nonrestricted NK cells. The infiltrates in PAMS contain effectors of both MHC class I–restricted (CD8+) and nonrestricted (CD56+ and CD68+) cells. Thus, both direct cytotoxic reactions and ADCC likely mediate target cell damage. Activation of both CD8+ CTL and CD56+ NK cells is enhanced by IL 6, and the serum level of this cytokine is significantly increased in PAMS patients.

Monocytes/macrophages and NK cells mediating ADCC are activated through binding of their Fcγ receptor II (CD32) and Fcγ receptor III (CD16) to the Fc portion of the IgGs attached to the target cell. Therefore, in contrast to what occurs in patients with PAMS, cellular cytotoxicity in neonatal mice injected with PAMS IgGs can be mediated by CD32/16+ MHC-nonrestricted cytotoxic mononuclear cells infiltrating the skin and not by CD8+ CTLs, which were absent from the infiltrates. The CD32/16+ murine NK cells can mediate ADCC against autologous epithelial cells targeted by human IgGs because ADCC is mediated by IgG and nonsensitized effector cells that attack appropriate target cells and induce destruction. Specificity of ADCC is determined by IgGs, and autologous, allogenic, or xenogenic effector cells have been
Figure 6. Effectors of autoimmune-mediated clinical and pathological findings in paraneoplastic autoimmune multiorgan syndrome (PAMS).

Proposed Mechanism of Airway Damage in PAMS

The presence of an inflammatory infiltrate in the respiratory epithelium and the deposition of autoantibodies have been reported in PAMS in association with squamous metaplasia and apoptotic changes of individual cells consistent with epithelial cell death.90,91 Studies from the large and small airways of our patients demonstrated a basal layer inflammatory infiltrate similar to that described above for mucocutaneous lesions. However, at this site, CD68+ monocytes/macrophages were much more numerous than were CD8+ CTL or CD56+ NK cells. The presence of this inflammatory infiltrate correlated with the presence of an inflammatory infiltrate in the respiratory bronchioles and alveoli. Impaired gas exchange due to ventilation-perfusion inequality may explain both hypoxemia of patients with PAMS and signs of cyanosis in neonatal mice with passively transferred PAMS. Furthermore, chronic immune inflammation may lead to the development of reactive changes such as fibrosis. Fibrosis occurring predominantly in the walls and contiguous tissues of membranous and respiratory bronchioles constitutes the histopathological hallmark of constrictive bronchiolitis obliterans found in many patients with PAMS.35-35 We believe that these observations can reasonably explain much of the respiratory failure that occurs in patients with PAMS. Interestingly, exposure to nitric oxide can lead directly to constrictive bronchiolitis (reviewed by Schlesinger et al91).

Possible Roles of iNOS in the Lesions of PAMS

Immune inflammation involves activation of iNOS in both effector and target cells. For example, both significant increase of CD8+ and CD68+ cells in the skin and induction of apoptosis of epidermal cells occurred in an experimental treatment of skin with nitric oxide–releasing cream.92 Subsets of cytotoxic cells kill their targets through release of nitric oxide, which induces programmed cell death.93,94 Nitric oxide sensitizes target cells to Fas-induced apoptosis,95 and represents one major mechanism where target cells are eliminated by activated cytotoxic cells.91 In PAMS lesions, iNOS-positive keratinocytes were seen throughout the epidermis, and thus could have been targeted by CD8+ and CD68+ cytotoxic cells invading the epidermis. Interestingly, most iNOS-positive keratinocytes were localized to the basal layer, which is the specific site of loss of epidermal adhesion in patients with PAMS. Therefore, death and subsequent elimination of apoptotic cells from the basal epithelial layers may provide a general mechanism for a loss of adhesion of the tegumental epithelium in patients with PAMS.

We postulate that damage to basal layer epithelial cells by the NK and ADCC effectors exposes otherwise hidden self-antigens to the immune system, which results in production of autoantibodies to desmosomal/hemidesmosomal proteins, notably those of the intracellularly located plakin family, such as plectin, desmoplakins I and II, bullous pemphigoid antigen 1, envolakin, and perilakin.1,62,63 Approximately 5% and 20% of patients with PAMS may also develop antibodies to DSG1 and DSG3, respectively.90 These and other types of antikeratinocyte antibodies may further aggravate mucocutaneous inflammation, since autoantibodies produced by patients with PV97 or BP98,99 can stimulate production of immunomodulatory cytokines by targeted keratinocytes. Bowen et al100 have recently reported sequential development of autoantibodies to distinct self-antigens in a patient with the lichen planus–like mucocutaneous variant of PAMS. We concur with the notion that the phenomenon of antigenic diversification or epitope spreading may underlie spreading of an autoimmune inflammatory process in patients with PAMS,100,101 and propose that iNOS plays a role in this process.

CONCLUSIONS

1. Patients with PAMS differ from patients with classic pemphigus, and may have lesions that resemble pemphigoid, erythema multiforme, lichen planus, lichen planus pemphigoides, and graft-vs-host disease as well as the pemphiguslike variant that was termed paraneoplastic pemphigus in the index patient with PAMS.1 Multiple reports describing patients with the mucocutaneous variant of PAMS12 indicate that the frequencies of different clinical varieties are similar.
2. The pathophysiological development of the lesions in PAMS occurs through mechanisms that differ appreciably from those supposedly responsible for the lesions of classic pemphigus; PAMS is a multorgan autoimmune syndrome targeting both terminal epithelium and internal organs. We have provided evidence that the mucocutaneous lesions in patients with PAMS occur as the result of both humoral and cell-mediated immune mechanisms. The IgGs putatively responsible for the development of the disease may reflect, or even induce, the cell-mediated immune response that accounts for the localized inflammation, basal layer clefting, and sloughing of clustered epithelial cells. The expansion of the autoimmune inflammatory process to other organs and tissues may be triggered by iNOS-mediated damage of target cells contributing to antigen diversification and epitope spreading. The interrelationships between the predominant immunopathological mechanism of target cell damage and the clinical and histological presentation of the cutaneous variant of PAMS are summarized in Figure 6.

3. Sloughing of bronchial epithelial cells can contribute to occlusion of the small airways, which provides a potential mechanism for the respiratory failure that constitutes a terminal event in many patients with PAMS. We propose that the targeted bronchial epithelial cells undergo dyskeratotic changes manifested by K14 overexpression, detach from the lamina propria and their neighbors, and are aspirated distally where they obstruct the lumen of intermediate and small airways and occlude alveolar sacs.

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Reprints: Sergei A. Grando, MD, PhD, Dsc, Department of Dermatology, University of California Davis Medical Center, 4860 Y St, No. 3400, Sacramento, CA 95817 (e-mail: sagrado@ucdavis.edu).

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