Paraneoplastic pemphigus (PNP) is a life-threatening autoimmune mucocutaneous blistering disease associated with malignancy, particularly lymphoproliferative neoplasms. Clinically, it is characterized by severe and intractable mucositis and polymorphous cutaneous eruptions, ranging from blisters to lichenoid lesions. The histologic features are also diverse according to the morphology of the clinical lesions, ranging from suprabasal acantholysis to interface changes with necrotic keratinocytes. PNP is characterized by the production of autoantibodies against the plakin family proteins as well as the desmoglein 1 and 3, which are target antigens of ordinary pemphigus. Thus, indirect immunofluorescence on substrates other than skin is useful in the diagnosis of PNP. The presence of anti-desmoglein antibodies have been demonstrated by enzyme-linked immunosorbent assay. The gold standard for the diagnosis of PNP however, is the detection of the characteristic circulating autoantibodies against 210-kDa envoplakin and 190-kDa periplakin by immunoblotting or immunoprecipitation. It is now accepted that both humoral and cellular immunities are involved in the pathogenesis of PNP. Anti-desmoglein 3 antibodies are known to play a pathogenic role in the initiation of acantholytic blister formation. Autoreactive CD8+ cytotoxic T cells induced by antitumor immune response are also involved in the development of mucocutaneous manifestations and bronchiolitis obliterans. The prognosis of PNP depends on the nature of the underlying neoplasm, with high mortality rate due to sepsis or multi-organ failure, particularly bronchiolitis obliterans. Although the combined use of immunosuppressive agents and rituximab has been administered in treating PNP, to date, there are no consistently effective treatments for PNP.

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ABSTRACT
Paraneoplastic pemphigus (PNP) is a life-threatening autoimmune mucocutaneous blistering disease associated with malignancy, particularly lymphoproliferative neoplasms. Clinically, it is characterized by severe and intractable mucositis and polymorphous cutaneous eruptions, ranging from blisters to lichenoid lesions. The histologic features are also diverse according to the morphology of the clinical lesions, ranging from suprabasal acantholysis to interface changes with necrotic keratinocytes. PNP is characterized by the production of autoantibodies against the plakin family proteins as well as the desmoglein 1 and 3, which are target antigens of ordinary pemphigus. Thus, indirect immunofluorescence on substrates other than skin is useful in the diagnosis of PNP. The presence of anti-desmoglein antibodies have been demonstrated by enzyme-linked immunosorbent assay. The gold standard for the diagnosis of PNP however, is the detection of the characteristic circulating autoantibodies against 210-kDa envoplakin and 190-kDa periplakin by immunoblotting or immunoprecipitation. It is now accepted that both humoral and cellular immunities are involved in the pathogenesis of PNP. Anti-desmoglein 3 antibodies are known to play a pathogenic role in the initiation of acantholytic blister formation. Autoreactive CD8+ cytotoxic T cells induced by antitumor immune response are also involved in the development of mucocutaneous manifestations and bronchiolitis obliterans. The prognosis of PNP depends on the nature of the underlying neoplasm, with high mortality rate due to sepsis or multi-organ failure, particularly bronchiolitis obliterans. Although the combined use of immunosuppressive agents and rituximab has been administered in treating PNP, to date, there are no consistently effective treatments for PNP.

Introduction
Paraneoplastic pemphigus (PNP) was first described in 1990 by Anhalt et al1 as a mucocutaneous autoimmune syndrome associated with neoplasms, most commonly of lymphoproliferative origin. The original criteria for diagnosis of PNP included: (1) painful mucosal erosions and a polymorphous skin eruption; (2) histopathologic features of intraepidermal acantholysis, dyskeratosis, and vacuolar interface dermatitis; (3) direct immunofluorescence findings of immunoglobulin(Ig)G and complement deposition at the keratinocyte cell surfaces and/or along the basement membrane zone; (4) serum autoantibodies detected by indirect immunofluorescence that bind to cell surfaces of stratified squamous epithelia as well as simple, columnar, and transitional epithelium; and (5) serum immunoprecipitation with a complex of four proteins of 250, 230, 210 and 190kDa.

The concept of PNP has evolved and many clinical variations have been described since then. Recently, the more inclusive term of paraneoplastic autoimmune multi-organ syndrome, was introduced to encompass those patients who show the heterogeneous clinical features and autoantibody
profiles of PNP. The term suggests that internal organs other than the skin and mucous membranes are also targeted by this autoimmune reaction. Indeed, the deposits of autoantibodies in different organs, such as lung, muscle, bladder and kidney have been demonstrated in autopsy specimens, and respiratory involvement with clinical features of bronchiolitis obliterans are also observed in many cases of PNP.

**Neoplasms associated with paraneoplastic pemphigus**

Almost all cases of PNP are associated with tumors, mostly with hematologic malignancies. One review studied 163 cases of PNP and reported that hematologic-related neoplasms or disorders were associated with 84% of the cases, with non-Hodgkin lymphoma (NHL) (38.6%) as the most frequent, followed by chronic lymphocytic leukemia (CLL) (18.4%) and Castleman’s disease (18.4%). Other reported hematologic conditions associated with PNP included thymoma, Waldenström macroglobulinemia, Hodgkin lymphoma and anaplastic large cell lymphoma. The non-hematologic neoplasms associated with PNP were comprised of epithelial-origin carcinomas or mesenchymal-origin sarcomas. Of the 39 cases of PNP associated with non-hematologic neoplasm reported in the English literature between 1990 and 2008, a total of 19 cases were related to carcinoma, and 20 cases were related to sarcoma.

The carcinomas associated with PNP include adenocarcinoma of the colon, pancreas, breast, prostate, liver, and squamous cell carcinoma of the tongue, uterus and kidney. In one case, bronchogenic carcinoma was described. An association with cutaneous malignancies such as basal cell carcinoma, malignant melanoma, and two kinds of cutaneous T cell lymphoma have also been reported. Of the 20 cases of sarcoma, seven were follicular dendritic cell sarcomas and four were inflammatory myofibroblastic tumors. The vesicles, flaccid blisters and erosions that mimic pemphigus vulgaris usually affect the upper trunk, head and neck, and proximal extremities; they might also coalesce to confluent eruptions resembling toxic epidermal necrolysis. Tense blisters resembling bullous pemphigoid are also described in PNP with a predilection for distal extremities. The vesicles, flaccid blisters and erosions that mimic pemphigus vulgaris usually affect the upper trunk, head and neck, and proximal extremities; they might also coalesce to confluent eruptions resembling toxic epidermal necrolysis. Tense blisters resembling bullous pemphigoid are also described in PNP with a predilection for distal extremities.

**Clinical features**

The clinical manifestations of PNP are highly variable, however, painful and intractable mucositis is a constant feature of PNP. The stomatitis usually presents as extensive erosions and ulcerations affecting all surfaces of the oropharynx with preferential involvement of the lateral borders of the tongue. The ulcers typically extend onto the vermilion of the lips, resulting in the characteristic hemorrhagic crusts (Figure 1). The mucosal involvement of PNP is more extensive, necrotic and extremely resistant to therapy than that of pemphigus vulgaris, and in some cases, lichenoid lesions are also found. The mucositis of PNP can extend to the pharynx, larynx and esophagus, causing soreness and dysphagia. The involvement of conjunctival mucosa is frequent with occasional progression to visual impairment. The anogenital and gastrointestinal mucosa can also be affected. Mucositis appears to be the initial presentation in nearly half of PNP patients. Moreover, there have been several reports of PNP patients who presented with localized mucosal involvement as the sole manifestation.

Cutaneous lesions are polymorphic, ranging from blisters to lichenoid lesions, and can vary in the same patient according to the stage of the disease (Figures 1 and 2). The vesicles, flaccid blisters and erosions that mimic pemphigus vulgaris usually affect the upper trunk, head and neck, and proximal extremities; they might also coalesce to confluent eruptions resembling toxic epidermal necrolysis. Tense blisters resembling bullous pemphigoid are also described in PNP with a predilection for distal extremities. The other characteristic cutaneous lesions in PNP are lichenoid eruptions consisting of infiltrated and erythematous macules, papules and plaques resembling lichen planus and graft-versus-host disease. In addition, targetoid lesions resembling erythema multiforme are also observed. In the chronic form of the disease, the lichenoid lesions may develop at the sites of previous blisters and become the predominant features. However, there have been increasing reports of PNP cases in which the lichenoid eruptions are the only cutaneous manifestations. Cummins et al reported four PNP patients with clinical features of lichenoid eruptions in the absence of detectable antibodies and described these cases as lichenoid variants of PNP. Lichenoid lesions are more commonly observed in childhood and adolescent PNP patients. Skin lesions may be psoriasiform, vegetative, or pustular eruptions, and sometimes involve the palm and sole. Alopecia has also been found in PNP patients with corresponding immunofluorescence findings in the intercellular spaces of the follicular epithelium.
Paraneoplastic pemphigus

Figure 1 Characteristic clinical features of paraneoplastic pemphigus (PNP). Severe involvement of oral and conjunctival mucosa is typical for PNP. (A,B) Severe erosions, ulcerations, and hemorrhagic crusts on the vermilion borders of lips. (C,D) Polymorphous cutaneous eruptions, with erythematous macules, papules, flaccid bullae, and erosions on the trunk and extremities.

Histological features

The histological features of PNP vary according to the lesion sampled because of the varied morphologies of the clinical lesions. Samples from noninflammatory bullae or erosions show suprabasal acantholysis with sparse inflammatory infiltrates. Whereas, in lichenoid lesions, the biopsy specimen may show vacuolar interface change with necrotic keratinocytes and satellite lymphocytic infiltration resembling erythema multiforme or lichenoid infiltrates, as well as interface dermatitis with dyskeratotic keratinocytes resembling lichen planus. Lesions with a mixed clinical pattern might show mixed histological features of concomitant acantholysis and lichenoid interface dermatitis. The underlying immunologic mechanism may shed light to the spectrum of clinical and histological features for a given case of PNP.2,47 If the dominant mechanism of mucocutaneous lesions of PNP is antibody-mediated cytotoxicity, bullae and erosions resembling classic pemphigus with suprabasal and/or intraepidermal blisters might be prominent. In contrast, if the dominant mechanism is cell-mediated cytotoxicity, lichenoid skin lesions with lymphocytic interface dermatitis and dyskeratotic keratinocytes might be prominent (Figure 3).

Immunopathology

PNP is characterized by the production of autoantibodies against various target antigens, mainly plakin family proteins and desmogleins (Table 1).56 The most characteristic and consistently recognized plakin antigens are envoplakin57 and periplakin58 (210 and 190 kDa, respectively), followed by desmoplakins I and II (250 and 210 kDa, respectively). Bullous pemphigoid antigen 1 (BPAG1, 230 kDa), plectin (>500 kDa), plakoglobin, and the unidentified 170 kDa antigen are the less frequently recognized antigens from PNP patient sera. The presence of anti-desmoglein (Dsg) 3 and anti-Dsg 1 autoantibodies in PNP sera also have been demonstrated by enzyme-linked immunosorbent assay (ELISA).59

Envoplakin, periplakin, desmoplakin, BPAG1 and plectin all belong to the plakin family proteins.60 Envoplakin and periplakin have been shown to bind to each other and co-localize with desmoplakin at desmosomal plaques and on keratin filaments in keratinocytes, suggesting that they serve as a scaffold for cornified cell envelope assembly.61 Plakin proteins are present in simple and transitional epithelium as well as in stratified epithelium; in contrast, desmogleins are only expressed in stratified epithelial tissues.60,61 Therefore, indirect immunofluorescence (IIF) of plakin proteins yields a positive reaction not only on normal human skin, but also on monkey esophagus, rat bladder, myocardium, and rat lung.62 This might explain why PNP is the only form of pemphigus in which internal organs can be affected by autoimmune injury. Diagnosis of PNP depends on the demonstration of serum autoantibodies against plakin proteins which can be detected with IIF, immunoblotting, and immunoprecipitation.
Diagnostic methods

Direct immunofluorescence (DIF)

Direct immunofluorescence (DIF) is a useful technique in the diagnosis of PNP; it works by detecting IgG autoantibodies bound to the keratinocyte cell surfaces of perilesional skin and mucosa (Figure 4). Three main patterns of DIF have been noted in PNP patients: (1) deposition of IgG and/or its complement in the epidermal intercellular spaces (ICS); (2) granular/linear deposition of IgG and/or its complement along the basement membrane zone; and (3) a combination of the above two patterns. A previous histologic review showed that the most frequently observed pattern of DIF was IgG and/or C3 deposition on the ICS alone. The combined ICS and basement membrane zone deposition of IgG and/or C3 was observed in less than 50% of PNP patients. In addition, false-negative DIF is more commonly noticed in PNP than in other forms of pemphigus.

Indirect immunofluorescence (IIF)

Since PNP is characterized by the presence of autoantibodies against plakin family proteins that are present in simple, transitional, columnar as well as stratified squamous epithelia, IIF on substrates other than skin is useful in the diagnosis of PNP. An IIF study of PNP sera showed cell surface binding of IgG on both normal human skin and other patterns.
In addition, IIF on rat bladder is known to be highly specific (83%) in the diagnosis of PNP because rat bladder is rich in desmoplakins and lacks desmogleins. As a result, IIF using rat bladder is now considered to be a useful screening test for PNP. However, it should be noted that about 25% of PNP serum samples show negative reactivity with IIF using rat bladder. Therefore, more sensitive and specific tests such as immunoblotting and immunoprecipitation are often needed to confirm the diagnosis of PNP.

Immunoblotting, immunoprecipitation and ELISA

Immunoblotting and immunoprecipitation have been considered as the gold standard for the diagnosis of PNP. Immunoprecipitation can detect antibodies against multiple epidermal antigens including plakin family proteins and an unidentified 170-kDa protein. Immunoblot analysis of normal human epidermal extracts has shown that PNP sera constantly react with the 210-kDa envoplakin and 190-kDa periplakin (Figure 6). This characteristic reactivity with two plakin proteins is highly sensitive and specific for PNP. The detection of plectin by either immunoblotting...
According to a previous study with 129 pemphigus patients (81 with pemphigus vulgaris and 48 with pemphigus foliaceus), the sensitivity and specificity of Dsg 3 ELISA were 97.5% and 97.8%, respectively, while the sensitivity and specificity of Dsg 1 ELISA were 97.9% and 98.9%, respectively. Anti-Dsg 3 and anti-Dsg 1 autoantibodies have been shown to be pathogenic in PNP. The majority of immunoprecipitation is difficult because of its large size (518 kDa). For that reason, the sequential immunoprecipitation and immunoblot analysis has been used as a more sensitive technique for the detection of anti-plectin autoantibodies.

ELISA for recombinant Dsg 1 and Dsg 3 is a highly sensitive and specific diagnostic tool for the diagnosis of pemphigus. According to a previous study with 129 pemphigus patients (81 with pemphigus vulgaris and 48 with pemphigus foliaceus), the sensitivity and specificity of Dsg 3 ELISA were 97.5% and 97.8%, respectively, while the sensitivity and specificity of Dsg 1 ELISA were 97.9% and 98.9%, respectively. Anti-Dsg 3 and anti-Dsg 1 autoantibodies have been shown to be pathogenic in PNP. The majority

Figure 4 Direct immunofluorescence in paraneoplastic pemphigus. (A) Deposition of immunoglobulin G on the keratinocyte cell surfaces and (B) complement (C3) along the basement membrane zone.

Figure 5 Indirect immunofluorescence in paraneoplastic pemphigus. Circulating immunoglobulin G antibodies bind to multiple epithelia including (A) esophagus, (B) rat bladder, (C) lung, and (D) rat myocardium.
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in their report on PNP patients with prominent lichenoid mucositis or dermatitis. Their revised diagnostic criteria included the presence of antiplakin and/or anti-desmoglein autoantibodies, lack of correlation of mucocutaneous disease with anti-Dsg 3 and Dsg 1, respiratory injury evolving to bronchiolitis obliterans, and lichenoid mucocutaneous lesions.

Currently, the four features that have been consistently found in the majority of PNP patients are generally accepted as the minimal criteria of PNP with a high degree of confidence. These include (1) clinical features of severe and protracted mucosal involvement and polymorphic cutaneous eruptions, (2) histologic features of acantholysis or lichenoid or interface dermatitis, (3) demonstration of antiplakin autoantibodies, and (4) the presence of an underlying neoplasm, especially lymphoproliferative tumors.

DIF is now thought to be non-essential for the diagnosis of PNP because of the frequent false negatives. IIF on rat bladder epithelia and monkey esophagus is accepted as an adequate screening test for PNP. However, IIF is not highly reliable, and immunochemical techniques are considered more precise. Therefore, a negative IIF does not exclude the diagnosis of PNP, and further tests with immunochemical techniques such as immunoblotting and immunoprecipitation should be performed to confirm or veto a diagnosis.

Pathogenesis

The mechanisms by which the underlying neoplasms induce autoimmunity have been proposed in many previous studies (Figure 7).8,20,50,77,78 Foreign tumor antigens cross-react with epidermal antigens

One explanation is that PNP results from an antitumor immune response cross-reacting with the normal epithelial proteins and thereby inducing autoimmunity by molecular mimicry.77,78 This theory is based on the hypothesis that the tumors constitutively or anomalously express epithelial proteins.63 In line with this theory, thymomas and Castleman’s disease have been noted to express desmoplakin.63 To date, however, there is no evidence that the majority of PNP-associated tumors such as NHL and CLL produce desmosomal proteins including desmoplakins.

Epitope spreading

Epitope spreading refers to the development of immune responses to epitopes, which are distinct from and non-cross-reactive with the disease-causing epitopes; this may lead to propagation of autoimmunity.79,80 This process has

Diagnosis criteria

Recently, there has been a surge in the number of reports of PNP patients who did not satisfy all of the diagnostic criteria originally proposed by Anhalt et al. As a result, the diagnostic criteria for PNP have been revised. Camisa and Helm75 have proposed major and minor criteria. The major criteria include polymorphic mucocutaneous eruption, concurrent internal neoplasia and characteristic serum immunoprecipitation findings. The minor criteria include histologic evidence of acantholysis, DIF showing intercellular and basement membrane staining, and positive cytoplasmic staining of rat bladder by IIF. Patients must satisfy three major, or two major and two minor criteria to be diagnosed with PNP. Later on, Mimouni et al54 developed their own criteria of patients with PNP have been reported to have circulating anti-Dsg3 IgG as determined by ELISA, although patients without anti-Dsg autoantibodies have also been reported.71 A recent study72 using an epitope mapping technique, demonstrated that the linker subdomains of envoplakin and periplakin contain major antigenic epitopes targeted by autoantibodies in PNP patients. ELISAs using recombinant proteins containing linker subdomains of envoplakin and periplakin expressed in a human cell line as the antigens were reported to be highly sensitive and specific for the diagnosis of PNP.73 In addition, a specific bead-based assay using the recombinant envoplakin-linker subdomain-conjugated beads was also shown to be a useful diagnostic technique with high sensitivity (69.7%) and specificity (94.6%) in 33 PNP patients.74

![Figure 6](https://example.com/figure6.png)

**Figure 6** Immunoblotting analysis against human epidermal extracts. Control pemphigus vulgaris (PV) serum reacts with the 160-kD anti-Dsg 1 and 130-kD Dsg 3 (lane 1); control bullous pemphigoid (BP) serum reacts with BP 230 and BP 180 (lane 4); paraneoplastic pemphigus (PNP) serum reacts with the 210-kD envoplakin and the 190-kD periplakin (lanes 2 and 3).
been proposed as a possible mechanism for autoimmune skin diseases. Dysregulated cytokine production by tumor cells may induce the development of autoimmunity. IL-6 is known to promote differentiation of B cells and autoantibody production. Autoantibodies against Dsg 3 can induce acantholysis, thus initiating the blister formation. Autoantibodies against plakin family proteins are constantly present in the sera of PNP patients. However, its role in pathogenicity remains unknown.

Figure 7 Pathogenesis of paraneoplastic pemphigus (PNP). Both humoral and cellular immunity are involved in the pathogenesis of PNP. Autoimmune cellular cytotoxic reactions may be generated by an antitumor immune response. These autoreactive CD8+ cytotoxic T cells can induce pulmonary injury resulting in bronchiolitis obliterans. Lichenoid interface dermatitis can also be induced by these cytotoxic T cells, thereby generating humoral immune response through the mechanism of epitope spreading. The tumor itself can produce autoantibodies against antigens in epidermis or produce the large amount of IL-6, resulting in the activation of B cells and autoantibody production. Autoantibodies against Dsg 3 can induce acantholysis, thus initiating the blister formation. Autoantibodies against plakin family proteins are constantly present in the sera of PNP patients. However, its role in pathogenicity remains unknown.
and maturation of B cells as well as the production of immunoglobulins and acute phase reactants; in addition, it also stimulates the activity of cytotoxic T cells and NK cells. Markedly elevated serum IL-6 levels have been demonstrated in a majority of PNP patients. Furthermore, it has been observed that in a subset of tumors associated with PNP, such as NHL, CLL and Castleman’s disease, the tumor cells secrete large amounts of IL-6 in vitro. These tumors are characterized by dysregulated IL-6 production and are known to be associated with other autoimmune phenomenon. For example, CLL has been reported to be associated with autoimmune thrombocytopenia, pure red cell aplasia and Coomb’s positive hemolytic anemia. Castleman’s disease is known to be associated with myasthenia gravis; in those with Castleman’s disease-associated autoimmune phenomenon, the administration of anti-IL-6 antibodies as a treatment has been effective. This evidence suggests that dysregulated cytokine production, including up-regulation of IL-6 by tumor cells is responsible for inducing the autoantibodies and stimulating cytotoxic T cells in the pathogenesis of PNP.

Lymphoid tumor itself produces autoantibodies against epithelial antigens

Recently, increasing evidence has shown support for the hypothesis that lymphoid tumor itself can produce autoantibodies against epithelial antigens. Wang et al demonstrated that cultured tumor cells from patients with PNP associated with Castleman’s disease produced autoantibodies that recognized normal human epidermal proteins of 210-kDa and 190-kDa. These findings suggest that secreted autoantibodies from Castleman’s disease, which react against epidermal proteins might have an important role in the pathogenesis of PNP. Later, the same group also found autoantibodies against plakin family (periplakin, envoplakin, and desmoplakin) and Dsg 3 in cultured medium of thymoma and follicular dendritic cell sarcoma from PNP patients.

B lymphocytes in these tumors carried a similarly rearranged variable region of the immunoglobulin gene and a high incidence of somatic mutations in the complementarity-determining regions and framework regions, suggesting that B cell clones from these hematological tumors can produce autoantibodies. Although it is not known whether these B cells originate in the tumor itself or migrate from other immunologic origins, this evidence supports the theory that B cells in the associated tumors produce autoantibodies against desmosomal and hemidesmosomal proteins, resulting in the development of PNP. While this theory could explain the pathogenesis of PNP associated with lymphoid tumors such as Castleman’s disease, thymoma, and follicular dendritic cell sarcoma, there is no evidence of the production of autoantibodies from non-hematological solid tumors associated with PNP.

Humoral immunity in the pathogenesis of paraneoplastic pemphigus

Humoral immunity

Using ELISA, Amagai et al found anti-Dsg 3 antibodies in all 25 PNP patients, and anti-Dsg 1 antibodies in 16 of these patients. The authors demonstrated that anti-Dsg 3-specific antibodies from PNP sera can cause blisters by injection in neonatal mice. Reversely, removal of anti-Dsg 3 antibodies was sufficient to eliminate the ability of PNP sera to induce blisters. Recently, the same authors identified anti-Dsg 3 IgG in canine PNP serum and showed that these autoantibodies could cause dissociation of keratinocytes in canine PNP. Based on these observations, they suggested that Dsg 3 and Dsg 1 are the cell surface target antigens in PNP and that anti-Dsg 3 antibodies play a pathogenic role in the initiation of blister formation. They proposed that anti-Dsg 3 autoantibodies initiate the primary acantholytic process, damage the cell membranes, and subsequently induce the production of autoantibodies to the intracellular proteins of plakin family, thereby resulting in the disease condition. The constant mucosal involvement and typical histological features of suprabasal acantholysis in PV and PNP might be explained by the presence of anti-Dsg 3 autoantibodies.

However, Nguyen et al demonstrated that autoantibodies against nondenomsomal targets, possibly including keratinocyte cholinergic receptor, of patients with pemphigus are also pathogenic and that IgG from PNP patients can induce extensive skin blistering with suprabasilar acantholysis in Dsg 3-knockout mice. Based on these findings, they proposed that anti-Dsg 3 antibody may not be necessary for the development of mucocutaneous lesions in patients with PNP. In addition, several patients with PNP were reported to have no detectable circulating anti-Dsg 3 autoantibodies using ELISA. A recent study using recombinant Dsg 3 with its extracellular domain expressed in mammalian cells, as the ELISA antigen also reported that only 11 of 16 PNP sera showed positive reactivity. In spite of the absence of anti-Dsg 3 IgG, these patients showed clinical manifestations of PNP including mucosal involvement and acantholytic lesions, except in one case, where the patient had no mucosal involvement or reactivity with Dsg 3 and Dsg 1 using ELISA. These cases support the possibility that acantholysis in PNP may be induced by antibodies against epithelial antigens other than Dsg 3 or cell-mediated immunity.

Although the pathogenicity of anti-Dsg autoantibodies in PNP has been demonstrated previously, the pathogenic role of anti-plakin autoantibodies in PNP remains unknown. Since most of the sera from PNP patients recognize several members of plakin family, including desmoplakin I and II, BPAG1, envoplakin, periplakin and plectin by immunoprecipitation and immunoblotting, autoantibodies against plakin proteins are considered to be the most constant diagnostic
markers of PNP. In particular, antibodies against envelopakin and periplakin are the most consistently recognized in the serum of PNP patients by immunoblotting.

Among the anti-plakin antibodies, autoantibodies directed against desmoplakins I and II have been reported to have pathogenic effects in a subset of patients with erythema multiforme, as demonstrated by passive transfer studies into newborn mice. Li et al. reported that application of purified plakin and periplakin from PNP sera to cultured human epidermal keratinocytes induced the internalization of these antibodies into the keratinocytes, and the retraction of keratin filaments from cell-cell borders in cultured human keratinocytes. They concluded that in addition to anti-Dsg 3 antibodies, autoantibodies against envelopakin and periplakin might also play a pathogenic role in PNP. However, a previous report described four patients with PNP who presented with predominantly lichenoid mucocutaneous lesions of their PNP patient and demonstrated a selective epidermal accumulation of activated CD8+ T cells, with an increased local productions of interferon-γ and tumor necrosis factor-α, and strong expressions of HLA-DR and ICAM-1 on keratinocytes. In addition, Nguyen et al. demonstrated that the mononuclear cell infiltrates at the dermoepidermal junction in PNP lesions consisted of both MHC-restricted CD8+ cytotoxic T lymphocytes and non-MHC-restricted CD56+, CD68+ Natural killer (NK) cells. Based on these findings, they proposed that both direct cytotoxic reactions and antibody-dependent cellular cytotoxicity may mediate target-cell damages in PNP. Moreover, IL-6, which is known to be increased in PNP sera, has been observed to enhance the activity of both cytotoxic T cells and NK cells in vitro. In addition, T cells have also been reported to be necessary for the development of intraluminal fibrosis associated with bronchiolitis obliterans, organizing pneumonia using a reovirus 1/L-induced murine model. These findings further provide evidence for the important role of cellular immunity and suggest that both humoral and cellular immunity are involved in the pathogenesis of PNP.

### Cellular immunity

With the increasing number of cases of PNP with lichenoid variant, it has been suggested that cell-mediated immune mechanisms play a role in the development of PNP. One previous report described four patients with PNP who presented with predominantly lichenoid mucocutaneous lesions but had no demonstrable circulating autoantibodies. Reich et al. characterized T cells that infiltrated into the lichenoid but had no demonstrable circulating autoantibodies. Reich et al. presented with predominantly lichenoid mucocutaneous lesions of their PNP patient and demonstrated a selective epidermal accumulation of activated CD8+ T cells, with an increased local productions of interferon-γ and tumor necrosis factor-α, and strong expressions of HLA-DR and ICAM-1 on keratinocytes. In addition, Nguyen et al. demonstrated that the mononuclear cell infiltrates at the dermoepidermal junction in PNP lesions consisted of both MHC-restricted CD8+ cytotoxic T lymphocytes and non-MHC-restricted CD56+, CD68+ Natural killer (NK) cells. Based on these findings, they proposed that both direct cytotoxic reactions and antibody-dependent cellular cytotoxicity may mediate target-cell damages in PNP. Moreover, IL-6, which is known to be increased in PNP sera, has been observed to enhance the activity of both cytotoxic T cells and NK cells in vitro. In addition, T cells have also been reported to be necessary for the development of intraluminal fibrosis associated with bronchiolitis obliterans, organizing pneumonia using a reovirus 1/L-induced murine model. These findings further provide evidence for the important role of cellular immunity and suggest that both humoral and cellular immunity are involved in the pathogenesis of PNP.

### Treatment and prognosis

The prognosis of PNP depends largely on the nature of the underlying malignancy. Patients with PNP associated with malignant tumors have poor prognosis and high mortality rate (90%). The course of PNP does not parallel with the course of the underlying malignancy. Mortality frequently results from a complication of immunosuppressive regimens such as sepsis, gastrointestinal bleeding due to high dose of corticosteroids, or bronchiolitis obliterans. In patients with malignant tumor, reducing the tumor burden does not equal to controlling the autoimmunity, and no individual therapeutic regimen has been demonstrated to be consistently effective. Systemic corticosteroids are the first-line treatment. However, only two reports of PNP showed good response with steroids alone. In treating PNP, a combination of prednisone with other immunosuppressive regimens, such as cyclosporine, cyclophosphamide, azathioprine or mycophenolate mofetil as well as intravenous immunoglobulins or plasmapheresis has been used and shown effective in a subset of patients. Several reports have highlighted the effectiveness of high-dose cyclophosphamide in combination with steroids. Intravenous cyclophosphamide without stem cell rescue in a patient with PNP showed a good and safe response. Mucositis is generally resistant to most therapeutic strategies.

Apart from conventional T cell-directed therapies such as cyclosporine and prednisone, daclizumab, a humanized monoclonal antibody to the alpha subunit of the IL-2 receptor of T cells, has been promising as a therapeutic agent of PNP. A small number of PNP patients showed good response to a combination regimen of prednisone, daclizumab, and rituximab, suggesting that downregulating both humoral and cell-mediated autoimmunity is effective in the treatment of PNP. Alemtuzumab, a monoclonal antibody directed against CD52, the high affinity IL-2 receptor, also looks to be a promising treatment, with one case where an ongoing long-term remission of PNP was induced by alemtuzumab in a patient with an underlying B cell chronic lymphocytic leukaemia. In contrast to patients with malignant tumor-associated PNP, cases associated with benign tumors, such as localized Castleman’s disease and benign thymomas show better prognosis with improvement or remission when the tumor is removed. A case series of PNP associated with Castleman’s disease showed that total resection of the tumors resulted in the improvement of cutaneous lesions within 6–11 weeks, recovery from mucositis within 5–10 months, and decreased titer of serum antibody in 6–8 weeks in 7 of the 10 patients. A similar beneficial effect of tumor removal has also been reported in cases of PNP associated with benign thymoma. Therefore, early detection and complete resection of tumors are essential for the treatment of PNP associated with these benign tumors.
To avoid the release of circulating autoantibodies from the tumors during operation, it is important to block the tumor blood supply and avoid squeezing the tumor. The intravenous administration of immunoglobulins before and during the operation was reported to significantly reduce mortality due to bronchiolitis obliterans. After the complete excision of these benign tumors, continued immunosuppressive therapy is required for up to 2 years until the remission of the autoimmune immunity.

Rituximab is a monoclonal humanized antibody directed against the B cell-specific, cell-surface antigen CD20, which was originally developed for the treatment of B cell lymphomas. Treatment with rituximab has been used for many autoimmune blistering diseases including refractory pemphigus and PNP. The therapeutic mechanism of rituximab in PNP is related to the depletion of both normal and abnormal CD20-expressing B cells, resulting in the reduction of autoantibody-producing neoplastic B cells and the decrease of abnormal immune response. Since 2001, rituximab therapy has been reported in eight cases of PNP within the English literature, with variable results. Two of these cases, which were associated with CD20-positive follicular NHL showed significant improvement of the mucocutaneous lesions and partial remission of NHL after a single course of four weekly infusions of 375 mg/m² of rituximab with or without corticosteroids. One case with gastric B cell lymphoma showed significant improvement in skin lesion, but mild improvement in oral lesion after combined treatment with rituximab, corticosteroids, and mycophenolate mofetil. However, three cases associated with CD20-positive follicular NHL and one case each associated with Waldenström macroglobulinemia and follicular dendritic cell sarcoma showed only partial responses or unsuccessful results.

The adverse effects of rituximab are generally brief and infusion-related; these include headache, fever, chills, rash and hypotension. Serious adverse events such as opportunistic infections are rare. However, infections are often fatal, possibly due to the underlying malignancy and/or immunosuppressive regimens. Because of the small number of cases, the efficacy of rituximab as a treatment of PNP cannot be established. However, rituximab seems to be less effective in treating PNP than pemphigus vulgaris, where rituximab has shown rapid healing of mucocutaneous lesions and long-term remission in approximately 60–100% of the treated patients. The unsuccessful treatment of PNP patients can possibly be attributed to the presence of CD20-negative plasma cells and/or B cells that produce autoantibodies and the involvement of autoreactive T cells in the pathogenesis of PNP. Further case-control studies are required to establish the optimal dosage and cycle of rituximab administration, the possible need for other immunosuppressive therapies, the risk of severe adverse effects and the benefit of repeated rituximab infusions as maintenance therapy.

**Pulmonary involvement**

Respiratory tract involvement with clinical features of bronchiolitis obliterans is frequently found in PNP and may cause irreversible respiratory failure and death. In a previous study of 28 cases of PNP associated with Castleman’s disease, 26 patients were diagnosed to have bronchiolitis obliterans. Another recent study also reported bronchiolitis obliterans in seven of the ten PNP cases associated with Castleman’s disease. It seems that pulmonary involvement is more likely to occur in patients with PNP associated with Castleman’s disease. The presence of desmoplakins in respiratory epithelium coupled with the histologic findings of acantholysis in bronchial epithelium and DIF findings of linear deposition of IgG and complement along the lamina propria suggest that autoantibody-mediated injury may be implicated in the development of bronchiolitis obliterans.

Hoffman et al found intense infiltrates of CD8+ T cells in the bronchiolar wall of PNP patients, and suggested the involvement of CD8+ T lymphocytes in the progression of bronchiolitis. This evidence supports the hypothesis that both humoral and cell-mediated autoimmunities are involved in the development of bronchiolitis obliterans similar to mucocutaneous lesions. Like mucositis, bronchiolitis obliterans is also resistant to various immunosuppressive agents. Lung transplant is the only therapeutic option for the progressive respiratory failure secondary to bronchiolitis. Of note is that 20% or more of patients develop fatal bronchiolitis obliterans-like respiratory involvement, even after the mucocutaneous lesions are improved.

**Conclusion**

PNP is a rare paraneoplastic autoimmune multi-organ syndrome that develops as the result of both humoral and cellular immunities. The prognosis of PNP is poor and often fatal due to sepsis or multi-organ failure, particularly respiratory failure with bronchiolitis obliterans. PNP is characterized by the presence of autoantibodies against all of the plakin family proteins, which makes them the most consistent diagnostic markers. However, the pathogenic roles and the origins of these anti-plakin autoantibodies remains still unknown. Thus, further studies are warranted to investigate the possible role of anti-plakin antibodies in tissue injury, and shed light to their role in the pathogenesis of PNP. In addition, it is also important further investigate the mechanisms of pulmonary injury because bronchiolitis obliterans is a common cause of fatal respiratory failure in PNP patients, even after improvement of the mucocutaneous lesions. Currently, there is no standard treatment for PNP. Further clinical studies on a large patient group with a long-term follow-up are needed in order to find more effective treatment strategies and reduce the high mortality rate.
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