# Autoimmune diseases

## INTRAEPIDERMAL DISEASES

### PEMPHIGUS VULGARIS

#### Lexicon-format morphological terminology

- **Primary lesions:** bulla
- **Secondary (and predominant) features:** crusting; erosion; post-inflammatory hyperpigmentation
- **Individual lesions:** round or oval
- **Multiple lesion arrangements:** scattered
- **Distributions:** generalized skin and oral cavity
- **Locations:** generalized skin and oral cavity; other mucous membranes
- **Signs:** Nikolskiy; Asboe-Hansen
- **Textures and patterns:** none
- **Consistency:** flaccid
- **Color of lesions:** skin; erythematous
- **Color of body:** normal

#### Epidemiology

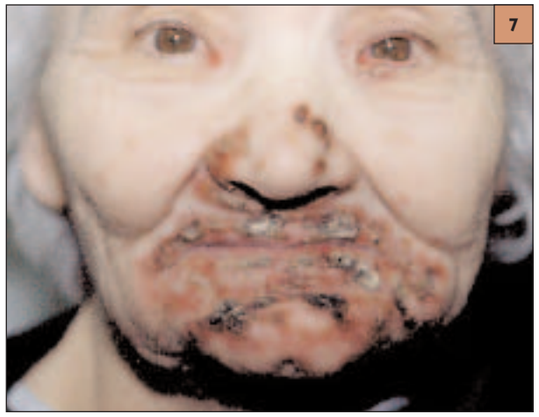
Pemphigus vulgaris affects men and women in approximately equal numbers. The average age of onset is around 55 years, but some patients are children and others are elderly; however, in some regions, such as Kuwait and Pakistan, the age of onset is reported to be mid-30s [4; 22]. Pemphigus vulgaris affects patients of all major ethnic groups but appears to be more common among Jews. The incidence and prevalence vary widely according to the studied population. For example, the annual incidence varies from 0.76 per million in Finland, to 1.3 per million in France, to 1.6 per million in southern Saudi Arabia [46], to 16 per million in Jerusalem.

Numerous studies have been performed on the immunogenetic aspect of this disease. Unfortunately, a common HLA allele among all ethnic groups of patients affected by pemphigus vulgaris cannot be identified; instead, several HLA-allele associations have been observed, but they are different depending greatly on the patients' specific ethnic groups. For example, as early as 1979, HLA-DRW4 was observed to be present in 91% of Jewish patients with pemphigus vulgaris (compared with 25% in normal Jewish control), with a relative risk of 31.5 [37]. In 1990, complotype SC21 was observed to be increased exclusively in Ashkenazi Jewish patients with a relative risk of 17 and complotype SB45 was observed solely in non-Jewish Caucasian patients with a relative risk of 57 [1]. Subsequently, in Ashkenazi Jewish patients with the disease, the class-II susceptibility gene, on [HLA-B38, SC21, DR4, DQw8], HLA-B35, SC31, DR4, DQw8, or their segments, was suggested to be disease associated [2], whereas in non-Jewish American patients two haplotypes have been suggested to confer susceptibility to the disease: (1) HLA-B38 (35), SC21 (SC31), and DR4, DQw8; and (2) HLA-Bw55, SB45, DRw14, and DQw5 [3]. In Japanese patients, a significant association of either HLA-DQB1*0503 or HLA-DRB1* 1405 was observed [36]. In Indian patients, a significant increase of haplotype of HLA-DRB1*1404, DRB3* 0202, DQA1*0101, and DQB1*0503 was observed [17]. In Pakistani patients, significantly increased frequencies of DRB1*1404, DQA1*0101, and DQB1* 0503 were determined [17]. In Korean patients, a significant increase of DRB1*01 allele was observed [26]. More recently, not only class-II, but also class-I molecule in the HLA-A-region genes, were determined to be associated with Jewish patients [44].

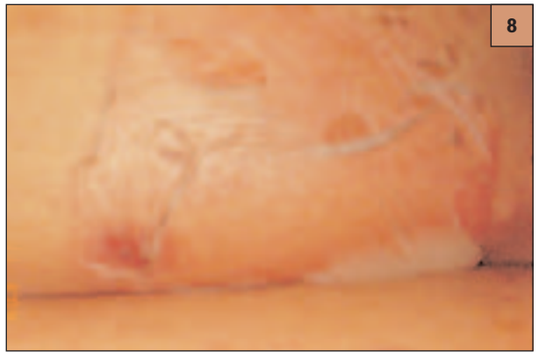
#### Clinical features

Pemphigus vulgaris manifests with mucocutaneous flaccid bullae in a generalized manner. Oral lesions precede skin lesions in most patients (7). In many patients, dentists are the first to make the diagnosis. The oral lesions manifest with rare intact blisters, but most do so with scattered or widespread erosions, primarily on buccal mucosae. The lesions which involve the pharynx and larynx manifest with hoarseness. Oral and pharyngeal lesions are very painful and hinder patients substantially from normal dietary and fluid intake. Other mucosae, such as esophagus, conjunctiva, penis, vagina, and anus, can also be affected. Many patients have oral lesions for many months or years before they develop skin lesions. When skin lesions surface, they are flaccid bullae arising on normal or, less commonly, on erythematous skin (8), scattered in different parts of body. These bullae break easily, leaving behind large areas of erosions; thus, intact bullae are rare (9). Therefore, differential diagnosis of pemphigus vulgaris should be included in a patient who presents with multiple superficial erosions without any observable blisters. By exerting lateral pressure on normal skin adjacent to an active blister, shearing of the epidermis can be-elicited—the 'Nikolskiy sign' In addition, pressing the top of an existing bulla will lead to spread of the bullae to adjacent normal-appearing skin—the 'Asboe–Hansen sign' or 'bulla spread sign.' These lesions heal with post-inflammatory hyperpigmentation and without scarring. Nail involvement manifests most commonly with chronic paronychia and onychomadesis, but also with vegetative and verrucose lesions, onycholysis, onychorrhexis, erosive lesions, and nail destruction has been documented (10).



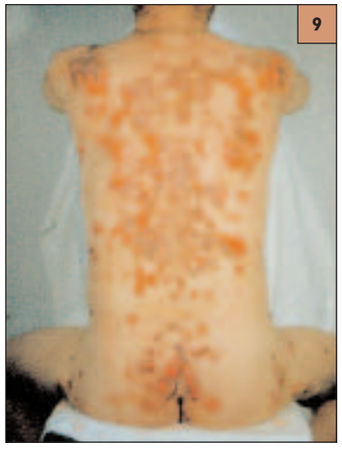


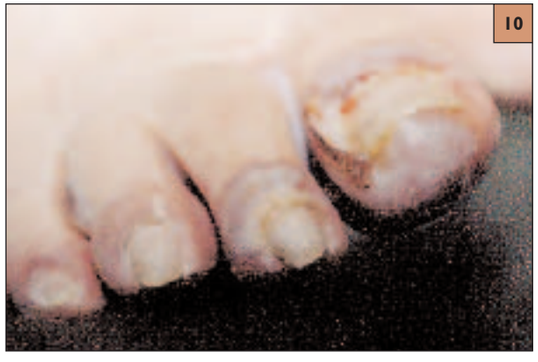

**7** Oral lesions in a patient with pemphigus vulgaris.




**8** Flaccid bullae pictured here are characteristic of pemphigus vulgaris.

**9** Erosive lesions in pemphigus vulgaris.




**10** Nail involvement in pemphigus vulgaris.

## Differential diagnoses

The differential diagnosis can be any of the following: paraneoplastic pemphigus; pemphigus herpetiformis; mucous membrane pemphigoid (oral stage of pemphigus vulgaris); or erythema multiforme.

## Pathogenesis

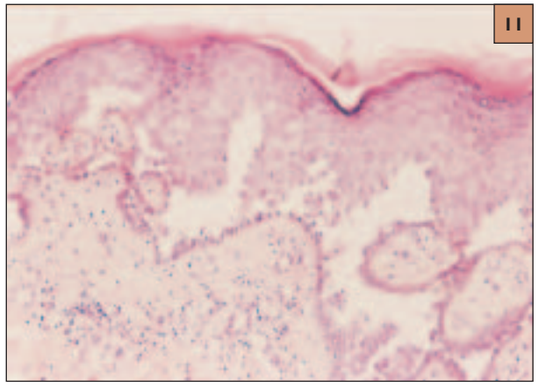
Pemphigus vulgaris is induced by autoantibodies of the IgG class that target the cell–cell connection of the most common cell types of the epidermis/keratinocyte, and this pathomechanism has been documented in a study of reproducible blister formation where patients' IgG was passively transferred to newborn mice [6]. The pathogenicity of patients' IgG autoantibodies is further supported by their ability to cross the placenta and cause neonatal pemphigus vulgaris in naturally occurring clinical cases [14]. The patients' IgG autoantibodies predominantly recognize desmoglein 3 [5], but also recognize desmoglein 1 [31], and most recently desmoglein 4 [24]. In patients with only mucous membrane lesions, their serum autoantibodies target exclusively desmoglein 3. When both mucous membrane and skin lesions surface in the patients, their serum autoantibodies recognize not only desmoglein 3 but also desmoglein 1 [25]. Furthermore, these anti-desmoglein-1 autoantibodies from patients with pemphigus vulgaris are indeed pathogenic and are able to induce blisters in animals which receive passively transferred autoantibodies [19]. Ultrastructurally, it is now determined from an animal model that anti-desmoglein-3 antibodies can directly access the protein present in desmosomes and cause the subsequent desmosome separation, leading to blister formation [43]. A unique and distinct distribution of desmoglein proteins between the skin and the mucous membrane provides an explanation for the above phenomena [32]. In the skin, desmoglein 1 spans the entire epidermis and desmoglein 3 localizes in the lower epidermis, whereas desmoglein 1 localizes in the upper epithelium and desmoglein 3 spans the entire epithelium in the mucous membrane; thus, in patients whose sera contain only desmoglein-3 antibodies, their disease will manifest exclusively with mucosal lesions, since the desmoglein 1 that spans the entire epidermis is capable of stabilizing the skin. But when both desmoglein 3 and desmoglein 1 antibodies are present, skin lesions occur in addition to mucosal lesions. Moreover, experiments have the roles of complement and antibody valence in the blister formation [7; 33]. Since bivalent F(ab')2 fragments of IgG derived from patients with pemphigus vulgaris were capable of inducing blister without the involvement of complement, it was suggested that complement is not essential for blister formation [7]. Subsequently, monovalent Fab fragments, when injected into animals by subcutaneous route, were shown to be able to induce blister formation, suggesting that these autoantibodies might trigger acantholysis by binding to an 'adhesive site' [33]. In addition, the role of plasminogen activator has been ruled out, since the patients' autoantibodies are capable of inducing blister formation in urokinase and tissue-type plasminogen-activator double-knockout mice [32].

While the mechanisms of blister formation after the autoantibodies are formed have been delineated to some degree of certainty, how the autoantibodies develop in the first place has not yet been explored in a systematic fashion. Autoreactive T cells to desmogleins 1 and 3 have been identified in patients affected by pemphigus vulgaris [27; 28]. More recently, regular T cells, a subset of T cells that functions to suppress autoimmunity, have been observed in a reduced number in patients affected by this disease [45]. A *de novo* autoantigen-induced active animal model of pemphigus vulgaris, which is not yet available, may help answer this question in full.

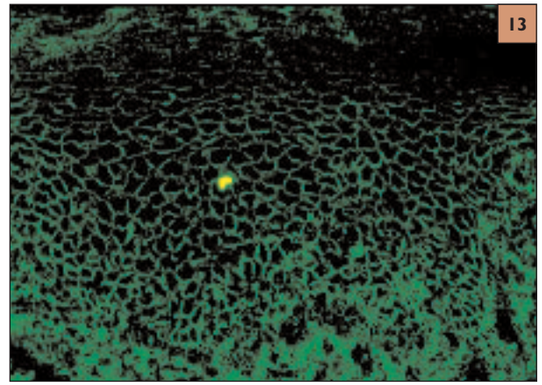
## Laboratory findings

Lesional skin biopsy obtained for routine histopathology reveals an intraepidermal blister located in the suprabasal level, with prominent acantholysis and a characteristic feature of 'tombstoning', *i.e.*, retaining of basal keratinocytes on the basement membrane at the dermal side (**11**). Acantholysis of the adnexal structures is also commonly observed (**12**). Eosinophils, associated with spongiosis, can be observed within the epidermis. Dermal infiltration of lymphocytes, eosinophils, and neutrophils are commonly detected. Perilesional skin biopsy obtained for direct immunofluorescence microscopy detects IgG (**13**) and C3 deposition around the keratinocyte cell surfaces like a chicken-wire pattern. Indirect immunofluorescence microscopy detects IgG-class autoantibodies from patients' serum, recognizing the epithelial cell surfaces in monkey esophagus (**14**) or human skin substrate. Similarly, ELISA detects IgG-class autoantibodies from patients' serum, recognizing cell–cell adhesion molecule desmoglein 3, and sometimes also desmoglein 1.

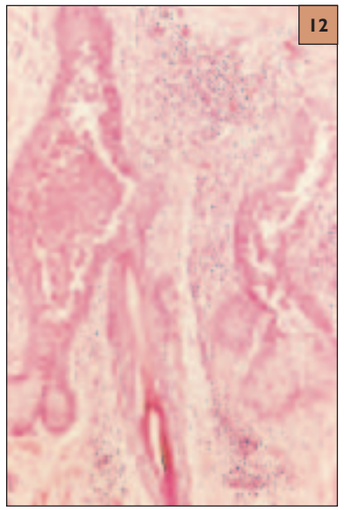




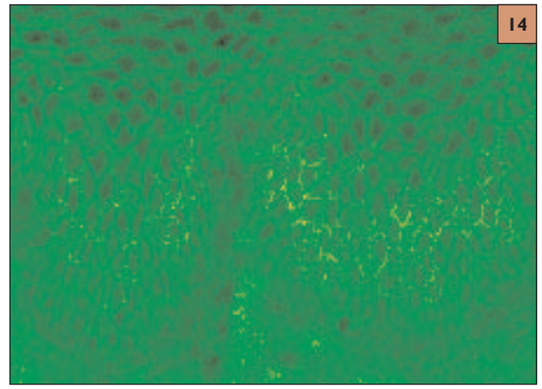

**11** Histopathological findings in pemphigus vulgaris. Suprabasal intraepidermal blister with acantholysis and tombstoning.




**13** Direct immunofluorescence microscopy examination of perilesional skin in a patient with pemphigus vulgaris reveals IgG deposits at the epidermal cell surfaces.




**12** Histopathological findings in pemphigus vulgaris. Acantholysis is also commonly observed in the adnexal structures. Hematoxylin and eosin staining.




**14** Indirect immunofluorescence microscopy using serum from a patient with pemphigus vulgaris on monkey esophagus substrate reveals circulating IgG autoantibodies binding to the epithelial cell surfaces.

## Therapeutic strategy

Before corticosteroid treatment became available, most patients affected by pemphigus vulgaris died. Mortality is now very low (approximately 5–10%), due to the availability of many effective immunosuppressive medications. For patients with the oral stage of the disease, topically applied high-potency corticosteroid, such as clobetasol gel, should be started. In addition, azathioprine (100 mg/day) can be considered, since it has a very slow onset of action (4–8 weeks). When oral lesions become severe, low-dose systemic corticosteroid, such as prednisone 20 mg/day, could be added. When both oral and skin lesions appear in the patient, both a systemic corticosteroid, such as prednisone (~1 mg/kg day$^{-1}$) and azathioprine (100 mg/day) should be included in the regimen. Another immunosuppressive option is mycophenolate mofetil, to which most pemphigus vulgaris patients would respond [39]. Because of the

devastating side effects to bone, potentially induced by systemic corticosteroids, the search for better treatment for pemphigus vulgaris is an ongoing process. Plasmapheresis, a method of removing serum immunoglobulins, has also been used with success in some patients, especially in combination with immunosuppressives to prevent antibody rebounding [11; 47]. Intravenous immunoglobulin (IVIG) is one of the successful alternatives to immunosuppressives for the treatment of pemphigus vulgaris and can be considered when the combined prednisone and azathioprine regimen cannot control the disease [12; 42]. Some investigators have observed that IVIG rapidly and selectively lowers the IgG autoantibodies in pemphigus, and reported an average of 70% reduction in 1 week after a single cycle of treatment, without lowering other antibodies [12]. Antibody rebound, one of the difficulties encountered in IVIG or plasmapharesis treatment, can be prevented by concurrent administration of IVIG and immunosuppressives such as cyclophosphamide or azathioprine [8; 9; 48]. Most recently, a humanized mouse monoclonal antibody against B-cell antigen CD20, rituximab, has been used successfully in several cases [41; 21]. A well-controlled clinical trial in the future will help establish the true therapeutic benefits of rituximab by examining its clinical effectiveness and side effects in a large number of patients. Monitoring patients' autoantibodies against their target antigens by ELISA may aid physicians in making the decision to change medication dosage [13]. In patients with several oral lesions, topically applied medium- to high-potency corticosteroid ointments (0.025% clobetasol proprionate or 0.025% fluocinonide) have proven effectiveness without significant suppression of the hypothalamus–pituitary–adrenal axis, as measured by plasma cortisol level [29; 30]. (For those clinicians who prefer the algorithmic approach to treatment, a recent article by Mutasim is available for consideration [35].)

Recently, monoclonal autoantibodies isolated by phage display from patients with pemphigus vulgaris have been characterized both genetically and functionally [38]. Since this technique is capable of separating antibodies that are pathogenic from those that are non-pathogenic, and is able to determine the restricted patterns of heavy and light-chain gene usage in these pathogenic antibodies, it may lead to more target-specific therapeutic options in the future [38].

For patients with autoimmune blistering diseases (pemphigus or pemphigoid groups) who require systemic corticosteroid medication, monitoring, treatment and prevention of side-effects, particularly those on bone, are essential part of patient management, as corticosteroid-induced osteoporosis is the leading cause of secondary osteoporosis [16]. Calcium (1 g/day) and vitamin D (400 IU/day) supplements should be taken by all patients on systemic corticosteroids, and should be sufficient for those patients receiving less than three months of corticosteroids [16; 23]. For those patients who are prescribed corticosteroids for more than 3 months, bisphosphonate is the treatment of choice, with alendronate (10 mg/day) [10; 16] and risedronate (5 mg/day) [15; 20; 40] being the two medications currently approved by FDA of the U.S. government for this specific purpose [16]. As a second-line agent, calcitonin should be used for patients who cannot tolerate bisphosphonate or experience pain due to vertebral fracture [16]. A recent study showed that salmon calcitonin nasal spray at 200 IU/day can reduce the risk of vertebral osteoporotic fractures by 33% [34].

## PEMPHIGUS VEGETANS

### Lexicon-format morphological terminology

- **Primary lesions:** vesicle and bulla
- **Secondary (and predominant) features:** exudates; pustule; fissure; vegetating plaque
- **Individual lesions:** round or oval
- **Multiple lesion arrangements:** grouped
- **Distribution:** flexural skin
- **Locations:** axillae; groin; scalp
- **Signs:** Nikolskiy
- **Textures and patterns:** cauliflower-like
- **Consistency:** flaccid
- **Color of lesions:** skin (primary lesion); dark red (secondary lesion)
- **Color of body:** normal

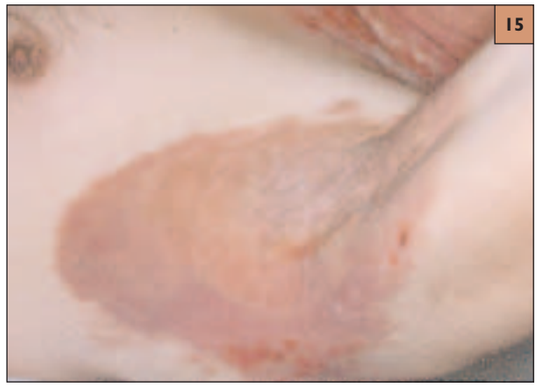
### Epidemiology

The incidence and prevalence for this rare disease entity have not yet been determined. In a survey performed in eastern Sicily (Italy), 2% of all pemphigus cases were determined to be pemphigus vegetans [54].

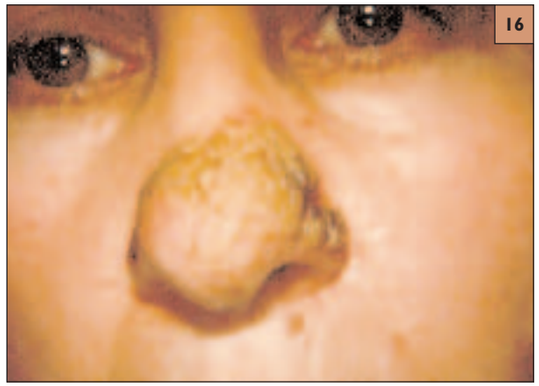


### Clinical features

Pemphigus vegetans is a very rare clinical phenotype. In an epidermal analysis of 84 consecutive cases of pemphigus presented in eastern Sicily, only 2% of all pemphigus cases were diagnosed as pemphigus vegetans [54]. Two distinct clinical phenotypes have been observed: Neumann type and Hallopeau type [49]. The Neumann type differs from the Hallopeau type in that it starts with the clinical phenotype of pemphigus vulgaris but subsequently develops hypertrophic vegetative plaques primarily in flexure skin areas, such as neck, axilla (**15**), and groin, but also on the nose (**16**) and mouth. Exudates, pustules, and fissures are commonly observed within these plaques. The Hallopeau type is recognized as a chronic pustular and vegetative phenotype that is initiated in, and remains primarily in, flexure skin areas and is known to have a more benign clinical course. Recently, a localized Hallopeau-type pemphigus vegetans presenting as acrodermatitis continua suppurativa was reported [58]. In addition, pemphigus vegetans has been reported to occur in children. Pemphigus vegetans needs to be differentiated from the heritable Hailey–Hailey disease and a subepidermal blistering disease, pemphigoid vegetans—which has a very similar clinical phenotype—by histopathological and immunopathological tests. In addition, patients with paraneoplastic pemphigus can present with the clinical phenotype of pemphigus vegetans.



**15** Flexural skin involvement in a patient with pemphigus vegetans.



**16** A pemphigus vegetans lesion on a patient's nose.

### Differential diagnoses

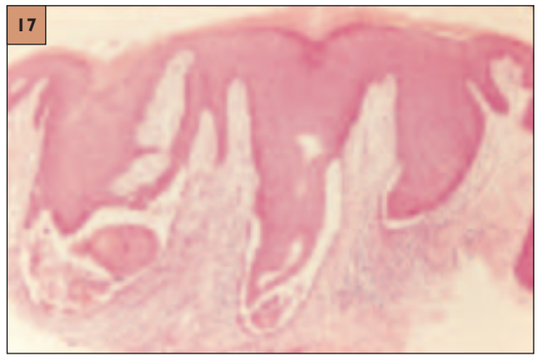
The differential diagnosis can be any of the following: pemphigoid vegetans; paraneoplastic pemphigus; or Hailey–Hailey disease (familial benign pemphigus).

### Pathogenesis

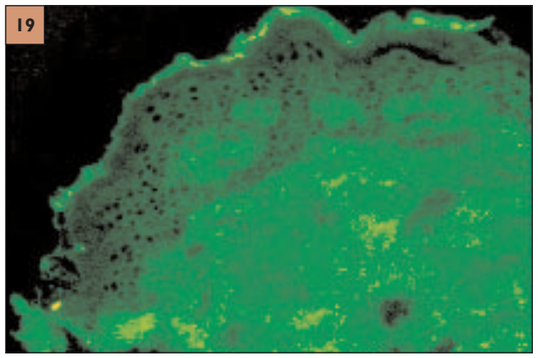
Although it is now established that IgG autoantibodies targeting epidermal cell-surface component desmoglein 3 mediate the acantholysis process in pemphigus, the link between the binding of desmoglein-targeting autoantibodies to the skin and the vegetative skin lesions that characterize pemphigus vegetans remains to be determined. Pemphigus vegetans has been reported to develop in patients who are infected by HIV, but the immunological mechanism for such disease development is not clear [53]. Captopril, an inhibitor for angiotensin-converting enzyme and an anti-hypertensive medication, has also been reported to induce pemphigus vegetans by an unknown pathomechanism [57].

### Laboratory findings

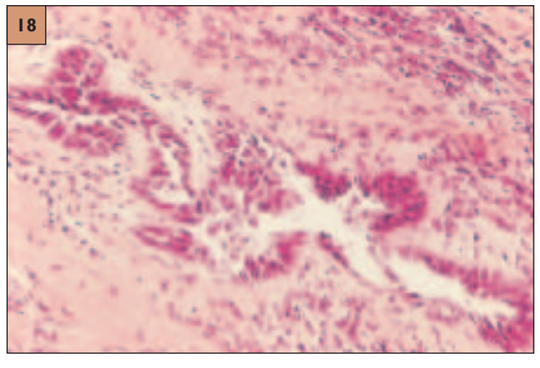
The histopathological and immunological findings in both clinical subtypes of pemphigus vegetans are indistinguishable [49]. Lesional skin biopsy obtained for routine histopathology revealed acantholysis at the suprabasal epidermis, along with pseudoepitheliomatous hyperplasia (**17**) and prominent eosinophil infiltration and intraepidermal eosinophilic abscess. Like that of pemphigus vulgaris, acantholysis is also observed in adnexal structures (**18**). A distinct histopathological feature, localized within the intraepidermal eosinophilic abscess cavity and known as 'Charcot-Leyden crystals,' has been repeatedly observed in lesional skin samples from patients with this disease [57]. Perilesional skin biopsy obtained for direct immunofluorescence microscopy detects IgG (**19**), with or without C3 deposits at the epidermal cell surfaces, identical to that observed in pemphigus vulgaris [49; 55]. Indirect immunofluorescence microscopy reveals that circulating IgG autoantibodies from patients' sera recognizes the epidermal cell-surface component in monkey esophagus substrate (**20**). Immunoprecipitation and Western blot analysis detect IgG autoantibodies, from patients' sera, which recognize desmoglein 3. In some patients their serum autoantibodies not only recognize desmoglein 3 but also desmoglein 1 as well as desmocollins 1, and 2 [50; 51; 56].



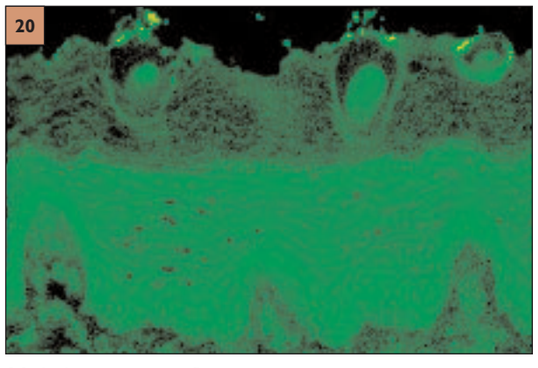

**17** Histopathological findings in pemphigus vegetans. Suprabasal blister with acantholysis, and pseudoepitheliomatous hyperplasia.




**19** Direct immunofluorescence microscopy examination of perilesional skin in a patient with pemphigus vegetans reveals IgG deposits at the epidermal cell surfaces.




**18** Acantholysis observed in adnexal structures. Hematoxylin and eosin stain.




**20** Indirect immunofluorescence microscopy using serum from a patient with pemphigus vegetans on monkey esophagus substrate reveals circulating IgG autoantibodies binding to the epithelial cell surfaces.



### Therapeutic strategy

As it is a rare disease, a clinical study of the therapeutic response of patients affected by pemphigus vegetans is not possible; we can rely only on sporadic case reports to generate a strategy. A reasonable initial regimen could consist of a low dose of prednisone (≤40 mg/day) and retinoic acid (acitretin 25–50 mg/day) [52]. This makes good clinical–pathological sense, because prednisone can help reduce the inflammation, whereas retinoic acid can help reduce the epidermal hyperplasia that characterizes the disease. Another approach could be a combination of low-dose prednisone (≤40 mg/day) and cyclosporine A [58]. Alternatively, a combination of low-dose prednisone (≤40 mg/day) and azathioprine (100 mg/day) could be considered. The humanized mouse anti-CD20 antibody, rituximab, has shown great potential in treating patients with pemphigus vulgaris and may also be beneficial for patients affected by pemphigus vegetans.

## PEMPHIGUS FOLIACEUS

### Lexicon-format morphological terminology

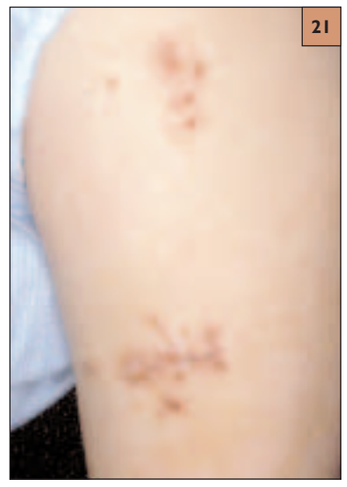
- **Primary lesions:** vesicle and bulla
- **Secondary (and predominant) features:** crusting; erosion; exudates; scale; exfoliation; post-inflammatory hyperpigmentation
- **Individual lesions:** round or oval
- **Multiple lesion arrangements:** scattered
- **Distributions:** seborrheic
- **Locations:** generalized skin, concentrating on scalp, face, upper back, and chest
- **Signs:** Nikolskiy
- **Textures and patterns:** none
- **Consistency:** flaccid
- **Color of lesion:** skin; erythematous
- **Color of body:** normal

### Epidemiology

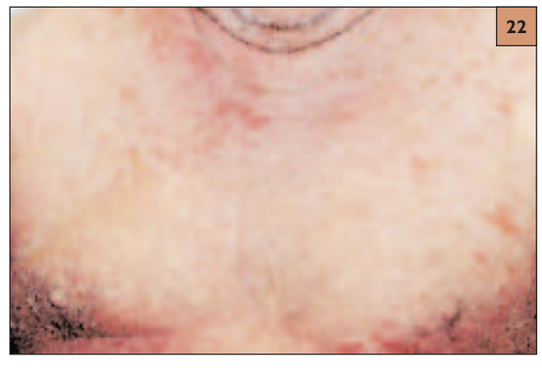
Like pemphigus vulgaris, pemphigus foliaceus (PF) affects men and women equally. The average age of onset for this disease is around 55 years, but some patients are children and others are elderly. Like pemphigus vulgaris, the incidence of PF varies depending on the studied population, but ethnic dominance in Jews has not been correlated. An endemic form of PF, observed in Brazil, is known as fogo selvagem. In addition, a second endemic form of PF has been reported in Tunisia. In both Brazilian and Tunisian forms of endemic PF, antibodies to desmoglein 1 were observed in large numbers of normal individuals living in the endemic areas regardless of their ethnic backgrounds, strongly suggesting an environmental factor [63; 73].

### Clinical features

Flaccid blistering is the initial manifestation (**21**) and can be observed in any patient; however, due to the superficial and fragile nature of the blister, the predominant clinical features in PF are scaly, crusted, erosive lesions with exudates, mostly on an erythematous base, and distributed in seborrheic locations (**22**) [69]. The lesions are




**21** Flaccid blisters mark the initial clinical presentation of pemphigus foliaceus.




**22** Scaly, crusted, erosive lesions with exudates, mostly on an erythematous base and distributed in a seborrheic location, are the most commonly observed clinical manifestations in pemphigus foliaceus.