

dosages have been used, from 2 mg/kg every 24 hours to 13 mg/kg every 8 hours. Though some evidence suggests every 8 hours would be optimal, the most common dose range appears to be 10 to 20 mg/kg every 12 hours. Since in humans and dogs it is synergistic with cyclosporine in prevention of acute graft-versus-host disease (GVHD) and has a steroid-sparing effect, its use as a combination agent should be evaluated further.<sup>71-73</sup> The every-12-hours and lower doses are more often used when the drug is used in combination. Principal side effects include bone marrow suppression, nausea, vomiting, diarrhea, and increased incidence of infections. Other side effects were minimal, but the most common included pyoderma, *Malassezia* infection, diarrhea, and leukocytosis.<sup>65</sup> Gastrointestinal side effects are more common when the drug is given as a sodium enteric-coated tablet.<sup>74</sup>

### TETRACYCLINE AND NIACINAMIDE

The drugs tetracycline and niacinamide, alone or together, are either beneficial or have steroid-sparing effects for over 20 inflammatory skin diseases.<sup>75</sup> There are also reports of beneficial effects in human immune-mediated diseases, particularly bullous pemphigoid and pemphigus.<sup>75-78</sup> As a result, the combination was tried in dogs.<sup>34</sup> The exact mechanism of action of either drug or the combination for treating inflammatory or immune-mediated disease is unknown.<sup>51,75</sup> Tetracyclines possess various antiinflammatory and immunomodulatory properties, including suppression of *in vitro* lymphocyte blastogenic transformation and antibody production, inhibition of matrix metalloproteinases, downregulation of cytokines, suppression of *in vivo* leukocyte chemotactic responses, inhibition of the activation of complement component 3, inhibition of lipases and collagenases, and inhibition of prostaglandin synthesis.<sup>75,79,80</sup> Niacinamide or nicotinamide have been shown to block antigen IgE-induced histamine release *in vivo* and *in vitro*, prevent degranulation of mast cells, act as photoprotectants from inducing immunologic damage, act as a cytoprotectant that blocks inflammatory cell activation and apoptosis, inhibit phosphodiesterases, and decrease protease releases.<sup>34,75,81,82</sup> There is little documentation of what these two drugs do in combination and whether they have additive or synergistic effects.

The combination of tetracycline and niacinamide has been recommended for the treatment of discoid lupus erythematosus, pemphigus erythematosus, and pemphigus foliaceus in dogs, with some being retained in remission long term with just this therapy.<sup>31,34,34a,65,83</sup> Reported results are variable, but 25% to 65% of cases have an excellent response. It has been used for a variety of diseases, many with unknown pathogenesis that may involve immunologic mechanisms. The diseases described include sterile histiocytosis, idiopathic onychodystrophy, vesicular cutaneous lupus erythematosus, lupoid onychitis (onychodystrophy), German shepherd dog metatarsal fistulae, sterile panniculitis, sterile granulomatous/pyogranulomatous dermatitis, and vasculitis.

The initial dosage for dogs weighing more than 10 kg is 500 mg of tetracycline and 500 mg of niacinamide given every 8 hours. If response is favorable, the dosage may be decreased to every 12 hours and then to every 24 hours. Anecdotal reports exist that this combination may have shown benefit when used concurrently with vitamin E or glucocorticoids as well as prednisone and azathioprine. It was also reported that tetracycline alone may be beneficial in treating discoid lupus erythematosus. Doxycycline has been recommended in place of tetracycline, although no studies or reports of its relative efficacy are available. Because of its longer half-life, a lower dose and longer interval (5 mg/kg every 12 hours) is acceptable.<sup>51</sup> Side effects are uncommon, although vomiting, anorexia, lethargy, and diarrhea have been reported. Two cases were reported of

anorexia with increased liver enzyme activity that resolved with discontinuation of therapy.<sup>389</sup> In some cases, tetracycline administration was continued, suggesting that the niacinamide was responsible for the adverse reactions. Another author (KLC) has observed an increased frequency of seizures in epileptic dogs following treatment with niacinamide and no longer prescribes niacinamide to dogs with a history of seizures.

## AUTOIMMUNE DISEASES

### THE PEMPHIGUS COMPLEX

The pemphigus complex is a group of uncommon autoimmune skin diseases that affect dogs, cats, and horses, with individual cases also reported in goats.<sup>19</sup> They are classically characterized by *acantholysis*, which is the breakdown of the epidermal intercellular desmosomal connections, with separated keratinocytes becoming rounded up as darkly staining acantholytic cells. The two major subsets of pemphigus in animals, as in man, are *pemphigus foliaceus* (PF) and *pemphigus vulgaris* (PV), with the latter occurring relatively much less frequently in animals than in man. Uncommon or rare variants are pemphigus vegetans (PVeg), paraneoplastic pemphigus (PNP), and pemphigus erythematosus (PE). In man, drug eruptions can be a cause of pemphigus, and it is believed this may be responsible for some cases in dogs and cats. However, as discussed later, a cause-and-effect relationship is very difficult to establish. The term *panepidermal pustular pemphigus* has been proposed to encompass cases in which acantholysis with a neutrophilic and/or eosinophilic infiltrate occurs throughout the epidermis, as seen in the descriptions of some cases of PVeg and PE.<sup>84</sup> However, this term has not found general usage, in that many of the described cases could be considered variants of PF.<sup>19</sup>

### AUTOIMMUNITY IN PEMPHIGUS

In the great majority of cases of human pemphigus, intercellular deposition of antibody within the epidermis is demonstrable, and circulating autoantibody is detectable employing indirect immunofluorescence on cultured keratinocyte or sections of normal skin.<sup>85</sup> The antibody binds to the interdesmosomal cell membrane and also within the desmosomes themselves.<sup>86,87</sup> Indeed, clinicians are reluctant to make a definitive diagnosis in the absence of such evidence, and a specific diagnosis is achieved in some 90% of cases of human pemphigus by determination of the autoantibody profile.<sup>87,88</sup> The desmosomal antigens reportedly targeted are desmoglein 1 (Dsg1) in PF, and Dsg3 in PV with predominantly mucosal involvement, and additional activity against Dsg1 where there is significant cutaneous involvement.<sup>87</sup> In general, localization of the lesions parallels the ultrastructural distribution of the respective antigens within the epidermis.<sup>89</sup> However, other desmosomal antigens may be targeted in man, including desmocollin 3 in PV,<sup>90</sup> and the plakin family antigens envoplakin and periplakin in PNP.<sup>91</sup>

Although the clinical and pathologic spectrum of the pemphigus diseases in animals is quite well defined, the classical immunologic abnormalities are not always evident. Thus in an early study, immunoglobulin deposits were seen in only 56% of 45 cases in which a final diagnosis of pemphigus was made based upon a combination of clinical signs, direct immunofluorescence, histopathology, and subsequent clinical course.<sup>92</sup> In two later studies involving 37 and 18 cases of PF, intercellular deposits were seen in 66% and 88%, respectively.<sup>93,94</sup> In one third of the cases in the latter study, deposits were primarily restricted to the outer layers of the epidermis. Subclass-specific staining in another study revealed that the deposits in some cases were restricted to the IgG2 and/or IgG4 subclasses.<sup>95</sup>

Deposits of other immunoglobulin classes or complement components were rarely seen.<sup>95</sup> Unfortunately, intraepidermal immunoglobulin deposits are not specific for pemphigus and may be found in up to 20% of cases with miscellaneous dermatoses.<sup>92</sup>

Indirect immunofluorescence has previously been regarded as unreliable in veterinary medicine and is infrequently positive, but sensitivity has been shown to vary with the substrate employed. In one study employing sera from 14 cases of PF, 9/14 were positive using bovine esophagus, 1/8 employing bovine tongue, 0/11 with bovine nose, and 4/8 were positive on monkey esophagus.<sup>96</sup> In another study, 4/4 sera from cases of PF and two from dogs with PV showed intercellular deposits using freshly cultured canine keratinocytes.<sup>97</sup>

More recently, indirect immunofluorescence was examined using sera from 27 dogs with PF, of whom 15/17 exhibited positive direct immunofluorescence. Substrates included a cell line from a canine oral acanthomatous epulis (MCA-B1), a canine mammary tumor cell line (CF33-MT), and cultured keratinocytes.<sup>98</sup> The latter two gave negative or inconsistent results. Only 4/27 were positive using the MCA-B1 cell line, but this appeared very specific in that 0/29 sera from other dermatoses reacted. Canine lip gave positive results in 5/27 (with 1/29 false positive), and bovine esophagus was positive in 8/27, but with 4/29 false positives. A later study employed neonatal mouse skin.<sup>99</sup> Results were uniformly positive, so reciprocal dilutions were employed. When whole anti-IgG was used, this was nondiscriminatory, with almost equal proportions of sera from PF cases (84%) reacting with high titers, as did sera from dogs with other dermatoses (80%). Subclass-specific antisera gave better results, with 35/44 sera (80%) yielding strongly positive results using anti-IgG4, compared to only 2/20 sera from dogs with other dermatoses. Furthermore, in some animals, the titer of IgG4 antibody paralleled disease activity — a finding not noted when using whole anti-IgG or other subclass-specific antisera. This implies that IgG4 may have a significant pathogenic role, a suggestion corroborated by the finding some 13 years earlier of a predominance of deposits of IgG4 on direct immunofluorescence.<sup>95</sup>

In summary, in canine PF, indirect immunofluorescence using substrates other than neonatal mouse skin is generally insensitive and gives inconsistent results. A note of caution is necessary when comparing results from different studies, in that the sensitivity and specificity of fluorescein-conjugated antibodies will inevitably vary between preparations. Further studies employing neonatal mouse skin and subclass-specific antisera are warranted, as is the use of such antisera on other substrates.

In canine PV, results appear to be more reliable employing canine gingival mucosa, with 10/11 sera being positive in titers of up to 1/1000 in one study, but only 5 positive when cultured keratinocytes were employed.<sup>100</sup>

### ROLE OF AUTOANTIBODIES IN DISEASE PATHOGENESIS

Although putative target autoantigens have been identified, there is still considerable controversy regarding the mechanisms by which they exert their effects, and whether all of the relevant autoantigens have been identified. The fact that acantholysis is induced by sera from human patients with pemphigus vulgaris when injected intradermally into neonatal mice is a clear indication of the pathogenicity of the antibodies.<sup>101</sup> The autoreactivity appears to be directed against Dsg1 and/or Dsg3, since the pathogenic effect is removed following preabsorption with recombinant antigen.<sup>102</sup> Additionally, such sera have cytopathogenic effects on keratinocyte cultures,<sup>103</sup> and it is known that cross-linking of antigen is not required; use of monovalent Fab and divalent F(ab)<sub>2</sub> fragments have a similar pathogenic

effect.<sup>104</sup> Furthermore, the lesions can be produced in the absence of complement.<sup>104,105</sup>

Three possible immunopathogenic pathways have been proposed. The first is that the antibodies may act simply by steric hindrance.<sup>104</sup> The second proposes that antibody binding triggers a number of intracellular signaling events leading ultimately to aberrant phosphorylation of Dsg3 and depleted desmosome formation.<sup>106,107</sup> Protein kinases are purportedly involved in this process, which can be modulated by protein kinase inhibitors.<sup>108</sup> It was believed that release of urokinase plasminogen activator (uPA) and subsequent plasmin activation with resultant proteolytic activity played a pivotal role in these events.<sup>109</sup> However, recent work casts doubt on this because IgG from patients with pemphigus can still induce lesions in neonatal mice lacking uPA.<sup>110</sup> Furthermore, addition of a monoclonal anti-Dsg3 to a cultured squamous cell carcinoma cell line did not result in increased uPA activity, although this antibody was pathogenic to cultured keratinocytes. Yet paradoxically, addition of serum from a patient with PV led to a dramatic increase in uPA.<sup>111</sup> Conclusions are that uPA activation does not play a pivotal role in acantholysis.

The relevance of anti-Dsg3 antibodies has been further questioned by experiments in which injections of human PV serum have been shown to produce acantholysis in genetically modified mice lacking Dsg3.<sup>112</sup> Thus, the third mechanism believed to be relevant stems from the knowledge that intercellular cohesion is dependent upon cholinergic mechanisms, and the acetylcholine receptor plays an important role in controlling phosphorylation of adhesion molecules. The possible relevance of this mechanism receives support from the observation that serum from human patients with both PV and PF precipitate cholinergic receptors.<sup>113</sup> Furthermore, atropine and other muscarinic acetylcholine antagonists increase Dsg phosphorylation, again leading to abnormal desmosome formation.<sup>114</sup> Similarly carbachol, an acetylcholinesterase inhibitor, inhibits both the *in vitro* acantholytic effects of PV serum and also the effects of PV serum on neonatal mice.<sup>115</sup> Final proof of the relevance of this pathway are reports of therapeutic efficacy of pilocarpine, an acetylcholine receptor agonist, and successful use of nicotineamide, another cholinomimetic, as a steroid-sparing agent in human PV.<sup>116,117</sup>

Upregulation of the proto-oncogene *c-Myc* has been shown in both human and canine PV and may provide another target for pharmacologic intervention.<sup>118</sup>

### AUTOANTIBODY SPECIFICITY IN CANINE PEMPHIGUS

Over the past 2 decades, techniques employed in these investigations have moved from the relatively crude, and thus only indicative, to the highly sophisticated and thus very specific. Early studies used Western blot analysis in an attempt to demonstrate antibody reactivity on epidermal protein extracts using molecular-weight markers as indicators of probable protein identity. Thus in one study, sera from two cases of canine PF reacted with a protein bearing the same molecular weight as a serum from a case of human PF, which suggested reactivity with Dsg1<sup>119</sup>; in a later study, 8/16 sera from dogs with PF recognized the same 160-kD protein,<sup>97</sup> which was further evidence suggesting that Dsg1 was the target autoantigen in PF. Antiplakin antibodies have also been implicated on this basis in a case of PV<sup>120</sup> and in paraneoplastic pemphigus.<sup>121</sup>

More robust evidence in favor of Dsg3 as a target autoantigen in PV was derived from studies employing canine cell homogenates in which Dsg3 was removed by immunospecific precipitation with a polyclonal antibody against human Dsg3 cross-reactive with the canine homolog,<sup>100</sup> and also by the development of enzyme-linked immunosorbent assays (ELISAs) using recombinant human Dsg 1 and Dsg 3.<sup>120</sup> Both



of these studies implicated Dsg3 as the target autoantigen in canine PV.

Definitive data were enabled by production of recombinant canine Dsg1<sup>122</sup> and Dsg3.<sup>123</sup> When the former was expressed on the surface of a human keratinocyte cell line, only 5/68 sera that showed positive indirect immunofluorescence employing neonatal mouse skin or canine footpad bound to extracellular Dsg1.<sup>124</sup> The conclusion was that Dsg1 is at best a minor autoantigen in canine PF. A further study employed three sera from cases of PF, only one of which recognized recombinant canine Dsg1. Yet upon immunoelectron microscopy, all three co-localized with a human PF antibody to the extra- and intracellular region of normal canine skin.<sup>125</sup> When the canine cell line MCA-B1 was employed, localization was not seen where complete desmosomes were formed, but rather at the area between desmosomes where cytoplasmic projections are formed between adjacent cells.<sup>125</sup> The authors hypothesized that autoantigens recognized by these sera might be desmoplakin or other antigens expressed during the early stages of desmosome formation.

Very recently, evidence implicating desmocollin 1 as a major autoantigen in canine PF has emerged. Sera from 63% of 48 cases of PF, all of which exhibited positive indirect immunofluorescence utilizing canine footpad, recognized the recombinant canine antigen.<sup>126</sup>

The first study employing cloned canine Dsg3 for assessment of autoreactivity of sera from dogs with PV confirmed recognition of this antigen, but autoreactivity against intercellular epidermal antigens was not completely removed by immunoadsorption with Dsg3, implying that one or more additional autoantigens were recognized<sup>123</sup> (possibly Dsg1). Another study examined the autoantibody profile of sera from 5 dogs with PV and one with paraneoplastic pemphigus.<sup>127</sup> Two cases that exhibited both mucosal and skin involvement had antibodies against both Dsg1 and Dsg3, whereas one dog had only mucosal involvement and the serum detected only Dsg3. Noteworthy was a case whose serum had the highest titer of reactivity against intercellular antigens of the keratinocyte cell line MCA-B1, and yet no reactivity with either Dsg1 or Dsg3. More recently, an ELISA has been developed for the detection of anti-Dsg3 antibodies employing baculovirus-expressed Dsg3.<sup>4</sup> Antibody levels above the cutoff point of 1/25 were found in 78.5% of 14 sera from dogs with PV, but also in 37.8% of sera from 37 cases of PF and around 50% of sera from dogs with autoimmune subepidermal bullous dermatoses. The PF sera failed to show intercellular staining on direct immunofluorescence using MCA-B1 cells, which implies a lack of specificity of this assay.

In summary, the autoantibody specificity of sera from dogs with PF and PV remains unclear. Although it was tempting to hypothesize that the varying clinicopathologic signs of PV and PF in the dog could be explained by the regional variations in Dsg1 and Dsg3 expression<sup>128</sup> proposed for man,<sup>3</sup> this almost certainly represents an oversimplification. Further elucidation of the role of desmocollin 1 is awaited with interest; the spectrum of autoreactivity is likely heterogeneous, and multiple autoantigens may be involved.

### **PATHOGENICITY OF SERA FROM DOGS WITH PEMPHIGUS**

Both in vitro and in vivo studies have been used to demonstrate pathogenicity, but employing only a limited number of sera. A disperse dissociation assay was developed using normal human keratinocytes. Serum from one dog with PV that recognized Dsg3 caused dissociation of the cell culture, and the anti-Dsg3 specificity of this reaction was confirmed by abrogation of the effect following prior absorption of the serum with human

recombinant Dsg3.<sup>127</sup> Similar results were obtained with serum from a case of paraneoplastic pemphigus. Intradermal injections into neonatal mice have also been employed.<sup>99</sup> Four mice were injected with IgG from pooled PF sera, and a further four employing individual sera. Blistering with subgranular acantholysis resulted, with absence of neutrophils. This finding was surprising in light of electron microscopic studies of the spontaneous disease that showed close association of neutrophils with the acantholytic process.<sup>129</sup>

### **ROLE OF DRUGS IN CANINE AND FELINE PEMPHIGUS**

In humans, the role of drug eruptions in triggering pemphigus is well established. They can either initiate acantholysis or cause disease flares in patients predisposed to the disease (drug-induced vs. drug-triggered).<sup>130</sup> In drug-induced disease, the condition ordinarily goes into remission following drug withdrawal, whereas in drug-triggered disease, it is hypothesized that the drug initiates a disease already latent in the patient, and the condition usually persists following drug withdrawal. The existence of drug-induced canine pemphigus has been questioned.<sup>19</sup> Ideally, a cause-and-effect relationship between a drug and a disease should be confirmed by (1) showing clinical improvement upon withdrawal and (2) exacerbation upon challenge. However, drug challenges in patients suspected to have adverse drug reactions are dangerous because the next drug reaction may well be far more serious. Nonetheless, reports in the literature suggest more than an anecdotal association. PF-like drug eruptions have been reported in 4 dogs in one report,<sup>131</sup> with one or more in other reports.<sup>31,132-134</sup> A PV-like drug eruption was also the focus of a case report where polymyxin-B was implicated.<sup>135</sup> PF-like drug eruptions have also been suspected in five cats.<sup>53,136,137</sup> In the first of these, the diagnosis was confirmed by reintroduction of the implicated drug (cimetidine), and in one of the last series of three cases, either itraconazole or lime sulfur was implicated, and the lesions resolved spontaneously upon discontinuation of the therapy.<sup>53</sup>

Recently, a convincing report documenting 22 cases of a PF drug eruption following application of an ectoparasiticide containing amitraz and metaflumizone (Promeris Duo [Fort Dodge]) was published.<sup>138</sup> Large-breed dogs appeared to be overrepresented, although the data were not analyzed statistically. In a third of cases, pustular lesions were limited to the area surrounding the point of application, but in two thirds of cases, lesions appeared at distant sites. In 5 cases, the eruptions developed after the first application, but in other cases, up to 10 applications were required. Immunosuppressive therapy was necessary in the majority but not all cases, and in some instances, long-term maintenance was necessary to prevent relapse, particularly in cases with distant disease. This led the authors to suggest that the condition was drug-triggered rather than drug-induced. The cases with distant rather than localized disease also had a higher incidence of systemic signs, including fever, lethargy, anorexia, and lameness. Immunologic studies revealed intercellular IgG deposition in two thirds of cases, evidence of circulating antikeratinocyte antibody in 6 cases and reduced or absent Dsg1 immunostaining in most cases,<sup>138</sup> a feature often noted in spontaneous PF.<sup>139</sup>

Such studies provide convincing evidence of the existence of drug-associated pemphigus and emphasize that a careful drug history is essential in the workup of all cases of pemphigus.

### **CANINE PEMPHIGUS FOLIACEUS**

Since the first report of canine PF in 1977,<sup>140</sup> subsequent publications have documented a large number of cases,<sup>1,31,32,34a,93,141,142</sup> and it appears that this is the most common of the autoimmune

skin diseases in dogs though one author (KCL) sees more discoid lupus erythematosus. Although the incidence in the general population is unknown, PF accounts for 2% of cases seen at a major referral center.<sup>65</sup> Reports fail to document any sex incidence except that from the University of Pennsylvania, which showed a significant male bias.<sup>32</sup> The disease typically develops in middle-aged to older dogs, but it can commence at any age. Occasional cases commence at 1 year or less, with one case developing lesions at 3 months.<sup>142</sup> Data on breed incidence is not always compared to a relevant base population, but Akitas and chow chows appear to be overrepresented,<sup>32,93</sup> with cocker spaniels, dachshunds, and Labrador retrievers prominently featured in most studies. The English bulldog was the most commonly affected breed in the latest case series from Louisiana.<sup>142</sup> There is a report of the disease appearing in two Shetland sheepdog female littermates at 6 months of age, again suggesting a genetic trait.<sup>143</sup>

In most cases, the disease appears to be idiopathic in nature, and predisposing causes are not apparent. The possible association with drug administration has already been discussed. It is widely held that a subset of cases develop subsequent to chronic skin disease—usually of an allergic nature. In a study in California, a history of flea allergy dermatitis often preceded the diagnosis of pemphigus, but the former was a very common disease when the study was undertaken.<sup>93</sup> In the study from Pennsylvania, 11.6% had a diagnosis of concurrent canine atopic dermatitis.<sup>32</sup> In a more recent study from Louisiana, 15/40 dogs (37.5%) had a previous history consistent with allergic dermatitis, and it was noteworthy that these cases had a significantly greater incidence of eosinophilic infiltrates on histopathology samples.<sup>142</sup> Definitive proof for this widely held contention is lacking, however, and will be difficult to obtain. A further complicating factor is that these allergic animals will likely have been treated with a plethora of drugs, so the possibility of an adverse drug reaction cannot be excluded. The possible role of UV light in exacerbating the condition has been the subject of a number of epidemiologic and laboratory studies, with conflicting results.<sup>19</sup>

### CLINICAL SIGNS

PF is a pustular disease, but the primary lesion may commence as a papule that progresses very rapidly to the pustular phase, leading to widespread areas of erosions and yellow crusting (Fig. 9-4). Large pustules spanning multiple follicular units are characteristic, and postinflammatory alopecia commonly develops and can be extensive. In some cases, the papular phase may persist longer, and the clinical appearance is one of papules admixed with pustules (Fig. 9-5). Onset of clinical signs may be quite rapid, with extensive disease appearing within 1 or 2 weeks, or insidious and developing over 1 or more months. The majority develop extensive disease within 1 month.<sup>53</sup> The degree of pruritus is variable, but was reported as moderate to severe in 17% in one study<sup>31,134</sup> and 36% in another.<sup>141</sup> Particularly when there is an acute onset, the animal may be depressed, anorectic, and febrile, sometimes with an accompanying lymphadenopathy.

The head, face, and ears are sites of predisposition, and are involved in more than 80% of cases, with the lesions exhibiting a striking bilateral symmetry (Fig. 9-6, A and B). More widespread distribution may not be evident upon initial examination, but is readily revealed upon shaving (Fig. 9-6, C). The pinna is often affected, showing well-demarcated lesions rather than diffuse inflammation (Fig. 9-6, D). Nasal depigmentation may develop (Fig. 9-6, E), but this is a late event, in contrast to discoid lupus erythematosus where it is usually the first change seen. The head and facial distribution is an important distinction from a staphylococcal pyoderma (Fig. 9-6, F). The ventral



**FIGURE 9-4** A, Pustular lesions in a case of pemphigus foliaceus (PF). B, Pustular lesions of ventral abdomen in a case of PF. Primary pustules have ruptured, and multiple secondary pustules have erupted from an erythematous base.

abdomen and trunk are sites of predilection for the latter; facial and cranial involvement is uncommon. The footpads are often affected (Fig. 9-6, G), with fissures and crusting developing, and occasionally the disease may be restricted to the footpads.<sup>144</sup> Mucosal lesions are extremely rare,<sup>31</sup> and a mucocutaneous distribution is not a feature. The disease has an unpredictable course; most cases progress relentlessly and often rapidly (Fig. 9-7), whereas in other cases it waxes and wanes. Animals suffering from PF often acquire a secondary staphylococcal infection that may complicate the picture by showing partial antibiotic responsiveness.

### DIAGNOSIS

Where the typical clinical signs are evident, a tentative diagnosis is readily made. Key features are those of a pustular disease that does not behave like a staphylococcal pyoderma, which is the major differential. Bacterial pyodermas do not commence on the face or involve the ears. Neither do they cause crusting of the footpads. Care must be taken to exclude demodicosis and dermatophytosis, particularly cases caused by *Trichophyton*,<sup>145,146</sup>





**FIGURE 9-5** Lesions of pemphigus foliaceus showing the papular phase with crusting and less evident pustule formation.

which can readily mimic PF both clinically and histopathologically. Other differentials include pemphigus erythematosus, systemic and discoid lupus erythematosus, dermatomyositis, and leishmaniosis in geographic locations in which this condition is endemic. The rare condition subcorneal pustular dermatosis is another pustular disease that should be considered, but extensive crusting is not seen in the latter, which also is not corticosteroid responsive.<sup>147</sup> A careful history of systemic or topical drug administration must be taken to rule out the possibility that an adverse drug reaction may have precipitated the disease.

Examination of intact pustules by cytology and biopsy offers the best prospect of a definitive diagnosis. If intact pustules are not evident and the patient is not acutely ill, it is preferable to await development of further pustules rather than biopsy old crusted lesions. Cytologic identification of acantholytic cells, which are rounded and deeply staining isolated keratinocytes, is highly suggestive (Fig. 9-8, A). These cells may occur in clumps and with neutrophils adhering to the cell wall (Fig. 9-8, B and C). Occasional separated keratinocytes may be seen at the base of pustules of staphylococcal origin, but rarely do they take on the classic form. Secondary bacterial infection may lead to the presence of both intracellular and extracellular cocci.<sup>31</sup> The classic histopathology is of a subcorneal pustule with acantholytic cells admixed with neutrophils (Fig. 9-9). Eosinophils are often present along with the neutrophils, and in one report



**FIGURE 9-6** Clinical signs of pemphigus foliaceus (PF). **A**, Early lesions of PF in a golden retriever. **B**, Typical symmetrical lesions involving the face of a chow chow. **C**, Same case as in **A**. Shaving reveals extensive lesions over trunk region. **D**, Focal pinnal lesions.





**FIGURE 9-6, cont'd** **E**, Nasal depigmentation noted in areas not covered by crusting lesions. **F**, Severe pustular lesions of PF involving the head of a boxer. **G**, Footpad lesions in a dog with PF.

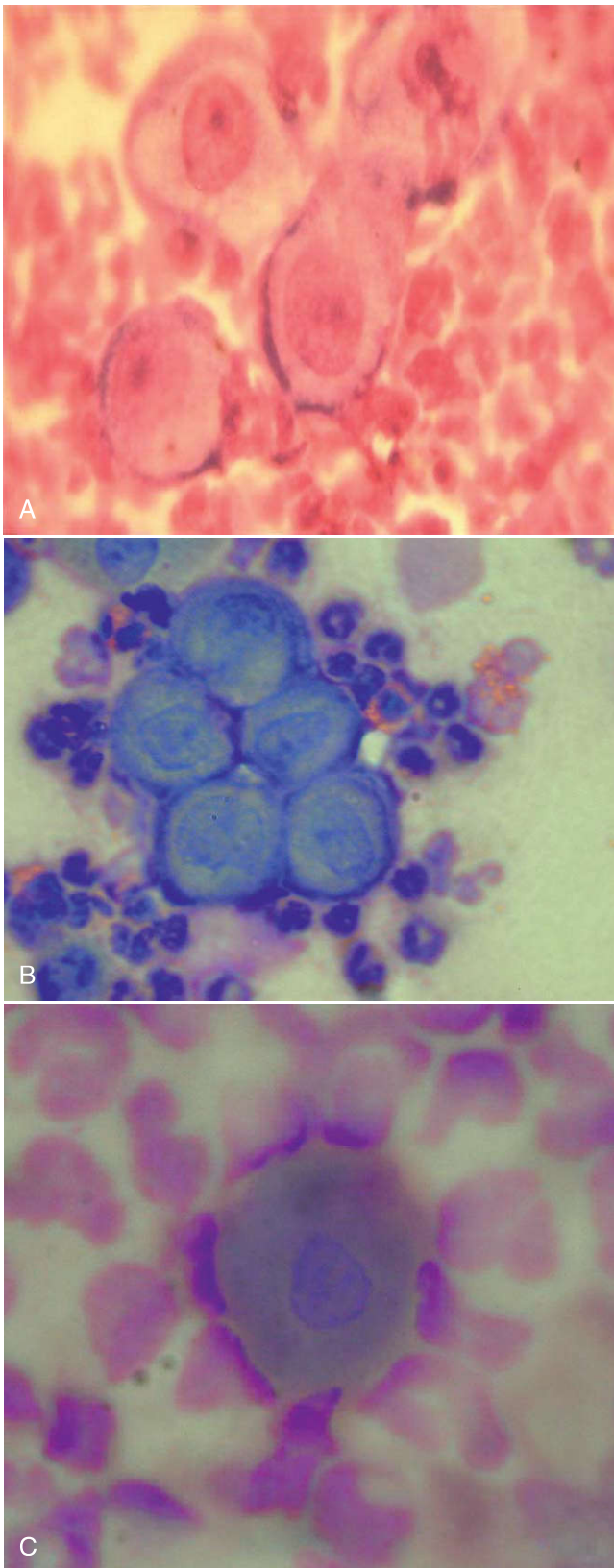


**FIGURE 9-7** Severe lesions of pemphigus foliaceus in a chow chow that progressed very rapidly.

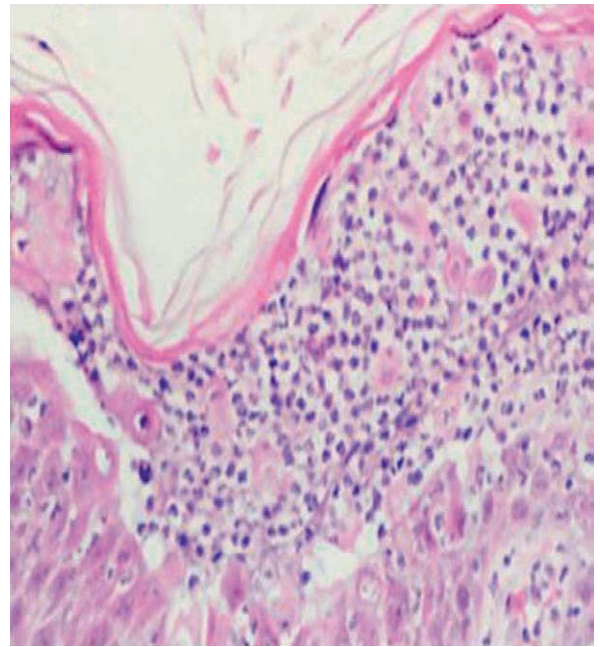
comprised the dominant inflammatory cell in a third of cases.<sup>142</sup> The subcorneal pustule may span a number of hair follicles, and the follicles themselves may be involved. Fungal stains should be employed to rule out the possibility of acantholytic dermatophytosis.<sup>145</sup> If primary pustules are not evident, histologic examination of the undersurface of recently formed crusts can also be diagnostic. Biopsies can also be submitted for direct immunofluorescence (either sent fresh to the laboratory or preserved in Michel medium) or preserved in formalin for immunohistochemistry. Available evidence suggests that both are of equal sensitivity.<sup>7</sup> Evidence of intercellular antibody deposition, particularly if restricted to the outer layers of the epidermis, is strongly supportive but not diagnostic (Fig. 9-10). At this point, the use of indirect immunofluorescence cannot be recommended for routine diagnostic purposes.

Routine hematology and biochemistry should be undertaken primarily to provide a baseline for monitoring of potential side effects of future therapy. A neutrophilia is often seen, which may be severe. Bacterial culture of an intact pustule is indicated. Helpful diagnostic information can then be obtained by treating any resulting isolate, if deemed of potential significance, with an appropriate antibiotic. The response may range from none (in which case the isolate is irrelevant) to 20% to 30% (in which case it is contributing to the disease process) or to a marked improvement, in which case bacterial pyoderma is the likely final diagnosis.

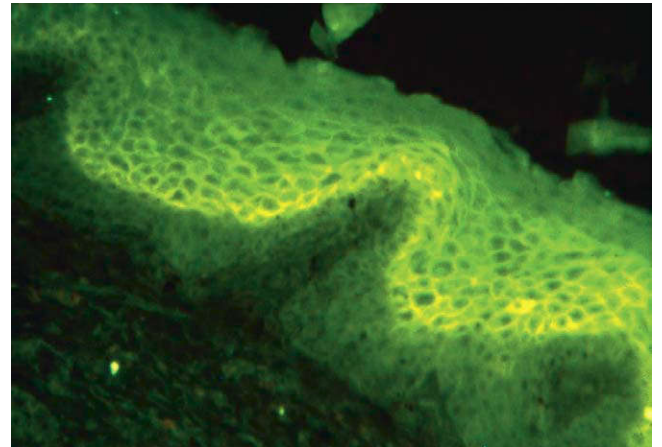




**FIGURE 9-8** **A**, Cytology specimen from a pustule in a case of pemphigus foliaceus (PF). Typical acantholytic cells are seen. **B**, Clump of acantholytic cells from a PF pustule. Also note presence of eosinophils and neutrophils. **C**, Acantholytic cell with neutrophils surrounding and adhered to it, forming a rosette. (**A** Courtesy Dr. Chiara Tieghi.)



**FIGURE 9-9** Histopathology in a case of pemphigus foliaceus. There is a subcorneal pustule with acantholytic cells admixed with neutrophils.



**FIGURE 9-10** Direct immunofluorescence in a case of pemphigus foliaceus. Deposits are seen involving outer layers of epidermis.

### TREATMENT

Corticosteroids are the cornerstone of the treatment approach, and prednisone or prednisolone at doses initially of 2 mg/kg to up to 6 mg/kg administered daily or divided into twice daily are most commonly used.<sup>19,65</sup> Some prefer to use methyl prednisolone, which may have less mineralocorticoid-induced side effects.<sup>65</sup> Most cases will improve with this regimen over 10 to 14 days, and the dosage is gradually reduced over the next 30 to 40 days. The goal of this monotherapy is to achieve an alternate-day regimen of around 1 mg/kg. In two reports, complete control was achieved using corticosteroids alone in 35% and 38% of cases, respectively.<sup>31,65</sup> In severe cases, one or two doses of prednisolone sodium succinate (10 mg/kg) or dexamethasone (1 mg/kg) given with a gastric protectant may be of initial benefit while instituting oral therapy.<sup>65</sup>

In cases that fail to achieve adequate control with corticosteroids alone, azathioprine at 1.5 to 2.5 mg/kg is usually added. Indeed, some clinicians prefer to initiate therapy with this combination. Once control is achieved, both drugs are tapered, with the aim of reaching an alternate-day regimen where the corticosteroid is given on one day and azathioprine on the next. The

desired maintenance dose of the latter is on the order of 0.5 mg/kg. It is unclear whether this combination of drugs has a lower incidence of side effects than are encountered when using glucocorticoids alone, but one report cites two cases of acute pancreatitis in dogs so treated.<sup>39</sup> It has further been suggested that combination therapy does not lead to a quicker response nor a better final outcome.<sup>31</sup> However, this aspect has yet to be addressed by means of a carefully controlled and randomized study, and combination therapy is favored by many dermatologists. An alternative immunosuppressive agent that can be used as a corticosteroid-sparing agent is chlorambucil at a dose of 0.1 to 0.2 mg/kg daily or on alternate days. This is generally preferred to cyclophosphamide because of the propensity of the latter to cause a chemical cystitis. Careful monitoring for myelosuppression is essential when using azathioprine and chlorambucil. Tetracycline together with the cholinomimetic niacinamide (nicotinamide) has been employed with limited success in 1 of 8 cases.<sup>34</sup> In another study of 34 superficial pemphigus cases, which included pemphigus erythematosus and pemphigus foliaceus, 62% responded.<sup>34a</sup> Others have reported that pemphigus erythematosus is more responsive than pemphigus foliaceus and this may explain part of the difference between reports.<sup>65</sup> However because of the long lag phase of induction, typically 4 weeks or longer, it may not be suitable as the sole treatment for dogs that are pruritic or who have extensive lesions. Doses are 500 mg three times daily of each drug for dogs over 10 kg, with half that dose for smaller dogs.<sup>34</sup>

Other recently developed drugs have been assessed for efficacy in limited studies. Cyclosporine has been reported as efficacious in a single case report<sup>148</sup> but was not effective for induction as sole therapy in a series of 5 cases.<sup>149</sup> However, a report of three cases that were refractory to a combination of glucocorticoids and azathioprine responded when cyclosporine and ketoconazole were added (7.5 mg/kg and 2.5-5 mg/kg, respectively), suggesting that cyclosporine, either alone or in combination with ketoconazole which prolongs the drug's activity, warrants further assessment as a corticosteroid-sparing agent.<sup>36</sup> Similarly, reports of the use of mycophenolate mofetil have suggested variable efficacy, with one author claiming a 50% success rate.<sup>64,65</sup> Human intravenous immunoglobulin (IVIG) was employed as the initial treatment of a dog with very severe PF and was clearly efficacious.<sup>150</sup> The dog was later treated with azathioprine and prednisone and responded well to further IVIG following a relapse.

Topical corticosteroids are of significant benefit in treating localized lesions. Similarly, reports of 0.1% tacrolimus in topical use are quite impressive.<sup>65</sup>

The use of systemic antibiotics has been somewhat controversial, although in one study a significantly more favorable outcome resulted.<sup>32</sup> Their use would appear to be strongly indicated when cocci are visualized upon cytologic examination or with positive culture results. Additionally, their use may protect against infection in an animal whose immune system is about to be severely compromised by immunosuppressive therapy.

As a final note of caution, the clinician must always ensure that the treatment is not worse than the disease. Although complete remission is usually attainable, it may be preferable to allow persistence of mild disease rather than increase the level of immunosuppression, with a greater risk of side effects, while at the same time further compromising the immune system. Each case is different, each owner is different, and the decision is very much an individual one.

## PROGNOSIS

In one study of 31 cases, 71% were still alive at the time of the report, which comprised a mean follow-up time of 2.7 years.<sup>65</sup>

Of these 31 cases, two were euthanized because of failure of compliance, and 5 due to unrelated causes. Thus a satisfactory response to the disease was achieved in 93% of cases treated. In another report of 88 cases, 46 (52%) underwent complete remission with treatment, and a further 31 (35%) achieved partial remission.<sup>31</sup> In a third series of cases, however, only 17/43 were still alive at the end of the study, with most of the deaths occurring within the first year, and 18 dogs euthanized because of treatment failures, unacceptable side effects, or poor quality of life.<sup>32</sup> Immunosuppressive therapy is usually maintained indefinitely, albeit at the lowest possible dosage. In some cases, it can be reduced to a very low level or even discontinued. In one report following remission that was achieved after 1.5 to 6 months, therapy was gradually withdrawn from 6 cases without any relapse occurring over the ensuing 1.5 to 6 years.<sup>151</sup> There are, however, no data on the proportion of cases where this might be successfully achieved.

In conclusion, a diagnosis of PF is by no means a death sentence, and even the most severely affected animals can usually be successfully treated. Familiarity with the drugs employed, their side effects, and prompt identification and management of these if and when they occur, is the key to success. Nonetheless, even in the most experienced hands, some animals fail to respond, and in others the drug related side effects prove unmanageable.

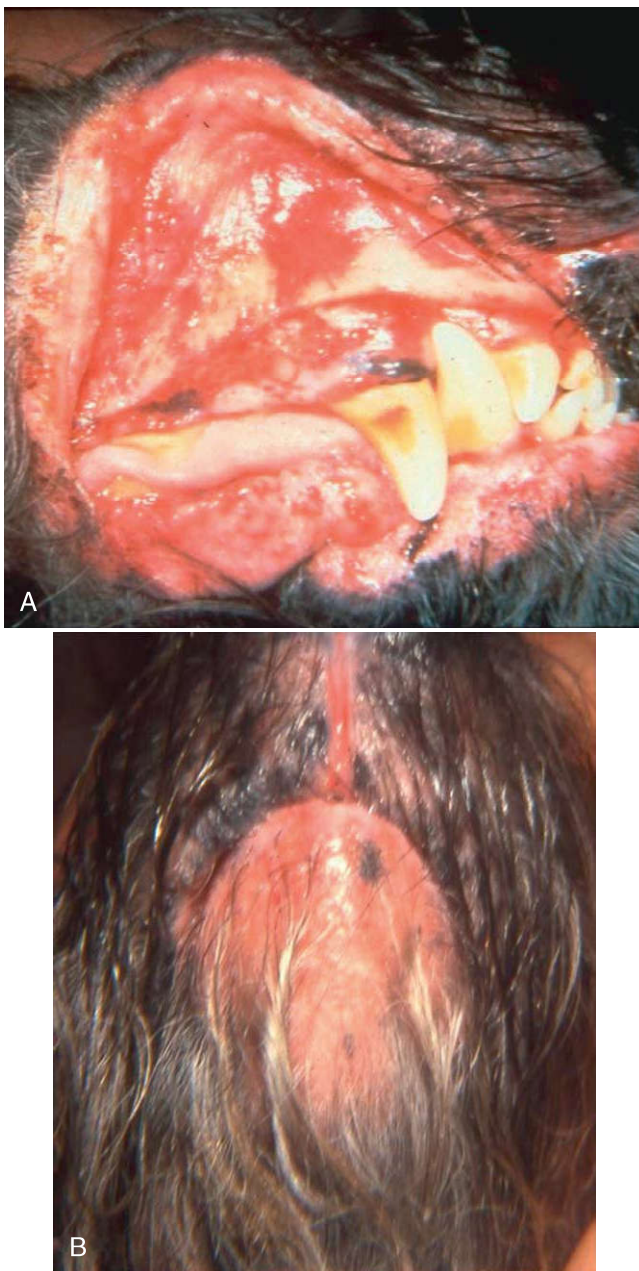
## PEMPHIGUS VULGARIS

PV is more severe than PF but is much rarer, occurring with a frequency of approximately 0.1% of cases in a referral and general practice.<sup>152</sup> The first reports in 1975<sup>153,154</sup> involved three and five dogs, respectively. Since that time, most reports have comprised single or small numbers of cases.<sup>120,155,156</sup> There is no reported breed or sex incidence, and the disease can commence at any age, with the majority developing clinical signs at middle age or older.

Onset may be sudden or gradual, and lesions are initially vesicubullous, rapidly progressing to erosions and ulcerations. Classically, it is a disease of the oral cavity and mucocutaneous junctions (Fig. 9-11), and the majority of animals have oral cavity lesions at the time of diagnosis. In some cases, lesions may start in these regions and then spread to involve other areas of the integument.<sup>120</sup> The Nikolsky sign may be present, where the lack of epidermal cohesion enables the epidermis to be peeled back with a blunt instrument. Footpads may be involved, as may the clawbeds, with resultant onychomadesis; this can sometimes be the only presenting sign.<sup>154</sup> Occasionally, cases lack mucosal involvement,<sup>157</sup> have mucosal involvement only,<sup>158</sup> or present with extensive alopecia following on from erosions.<sup>159</sup> Two cases of severe nasal dermatitis have been reported that satisfied the histopathologic and immunologic criteria for PV.<sup>160</sup> The first of these had lesions in the oral mucosa, scrotum, and feet, whereas in the second the lesions were restricted to the nasal region. Patients are often febrile and anorectic, especially when lesions are widespread and there is oral cavity involvement.

The major differentials are the subepidermal blistering diseases, bullous pemphigoid and epidermolysis bullosa acquisita, together with other causes of oral ulceration. A diligent search should be made for any neoplastic condition that could suggest a diagnosis of paraneoplastic pemphigus, and a careful drug history should be obtained to assess the possibility of drug-related pemphigus. Because lesions are deeper within the epidermis than those in PF, cytologic examination is unrewarding, and acantholytic cells are rarely observed. Diagnosis is confirmed by histopathologic analysis, which should reveal a suprabasilar intraepidermal cleft, with the remaining basal cells





**FIGURE 9-11** Lesions of pemphigus vulgaris in a Skye terrier's oral mucosa (A) and oral mucocutaneous junction (B).

exhibiting a tombstone appearance. Direct immunofluorescence or immunohistochemistry is usually positive,<sup>156</sup> and indirect immunofluorescence is overall more likely to be positive than in cases of PF. Presence of autoantibodies against Dsg3 ± Dsg1 may be identified, as may the presence of antiplakin antibodies.<sup>4,120,127</sup>

This is usually an acute and severe disease, and aggressive therapy with a combination of corticosteroids and azathioprine or chlorambucil is indicated. Concomitant antibiotic therapy is mandatory. Despite such therapy, the prognosis is still poor, with only 25% of cases in one report surviving for 1 year.<sup>152</sup>

### PARANEOPLASTIC PEMPHIGUS

Only two cases of confirmed and fully-characterized canine PNP have been reported in the literature, with a third likely case contained in the first report of canine PV.<sup>153</sup> In one full case report, a 7-year-old Bouvier is described with extensive oral ulcerations that later spread to many body areas. Failure of the

animal to respond to immunosuppressive therapy led to euthanasia, and upon autopsy a thymic lymphoma was found.<sup>161</sup> Subsequent immunologic investigations identified autoantibodies to two members of the plakin family, periplakin and envoplakin, which are classically associated with PNP in man.<sup>162</sup> The second case was a 7-year-old golden retriever with similarly widespread lesions.<sup>121</sup> A splenic mass was detected upon ultrasound and was surgically removed. However, the animal died 12 hours later from cardiac complications. A spindle cell sarcoma was diagnosed histologically. Again, the serum was shown to have autoreactivity with autoantigens of the plakin family.<sup>121</sup> Histologically, suprabasilar clefting was seen, characteristic of PV, as well as some suggestion of erythema multiforme characterized by an interface dermatitis with apoptotic keratinocytes. In man, PNP carries a very poor prognosis, with skin lesions generally unresponsive even following tumor removal, with the interesting exception of thymomas, which carry a better prognosis.<sup>163</sup>

### PEMPHIGUS VEGETANS

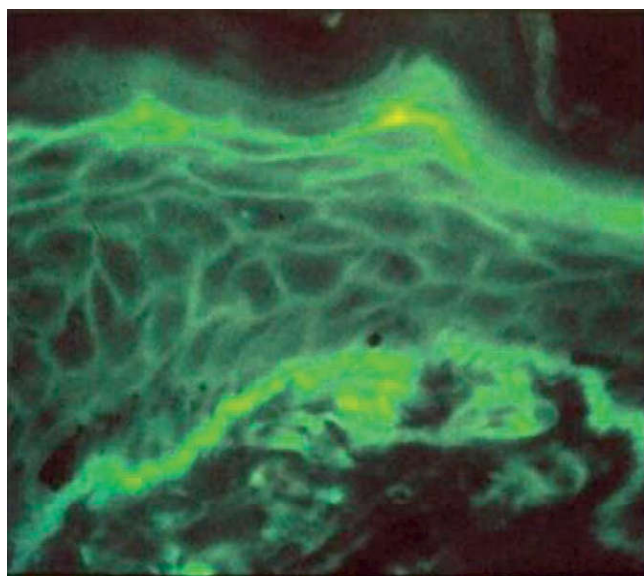
Two types of PVeg are described in man: the Neumann type and the Hallopeau type.<sup>164</sup> In the latter, pustules are followed by proliferative wartlike lesions and plaques, and a relatively benign course follows. In the Neumann type, vesicles and bullae are followed by widespread areas of erosions, and the condition is often refractory to therapy. Oral mucosal lesions may be seen in both. Three canine cases are described in the literature,<sup>165-167</sup> only one of which appears to be truly analogous to the human condition. The patient was a 4-year-old Greater Swiss Mountain dog who developed crusted papules, pustules, and verrucous projections.<sup>167</sup> Histopathology revealed suprabasilar acantholysis that was accompanied by multiple intraepidermal neutrophilic and eosinophilic pustules. Direct and indirect immunofluorescence were both positive, and anti-Dsg1 antibodies were detected. This contrasts with human PVeg, which is usually accompanied by anti-Dsg3. The disease was readily controlled with azathioprine and prednisolone, and the authors commented that the case was most suggestive of the Hallopeau type.

### PEMPHIGUS ERYTHEMATOSUS

Pemphigus erythematosus (PE) is regarded as a crossover between PF and discoid lupus erythematosus, with features of both. The justification for considering this a separate entity rather than a variant of PF has been recently questioned.<sup>19</sup> Case reports of the condition describe dogs showing a gradual onset of symmetrical crusting lesions involving the nose, face, and ears.<sup>168</sup> This may lead to erosions and ulceration of the nasal planum, with loss of pigment. The main differential is discoid lupus, with the latter disease restricted in the early stages to the nasal planum and preceded by loss of pigment. Histopathology reveals intragranular and subcorneal pustules comprising varying combinations of neutrophils and eosinophils, together with a lichenoid interface dermatitis. Direct immunofluorescence characteristically reveals a combination of intercellular epidermal deposits typically associated with PF, and also basement membrane deposits<sup>1</sup> (Fig. 9-12). Indirect immunofluorescence has been reported as negative, but some dogs may have significant although low titers of antinuclear antibody (ANA).<sup>1</sup>

This is not generally an aggressive disease, and control is usually readily achieved with immunosuppression<sup>168,169</sup> or with tetracycline and niacinamide.<sup>34</sup> More recently, topical tacrolimus has been reported to be efficacious.<sup>170</sup> The condition is believed to be exacerbated by light exposure, so restriction from sunlight or use of sunscreens may be helpful (Fig. 9-13).





**FIGURE 9-12** Direct immunofluorescence in pemphigus erythematosus. Immunoglobulin deposits are evident intercellularly within the epidermis and at the dermoepidermal junction.



**FIGURE 9-13** Pemphigus erythematosus in a dog. The lesions were aggravated by sun exposure.

## FELINE PEMPHIGUS

Pemphigus diseases are much less common in the cat than in the dog. Single cases of PV and PE have been reported,<sup>171,172</sup> but PF is by far the most common disease. A number of series of cases have been published,<sup>172,173</sup> with one documenting 57 cases.<sup>53</sup>

No breed or sex predisposition has been documented for feline PF. The disease can commence at any time from younger than 1 year of age to 17 years, with a median of age 5 years.<sup>53</sup> Pustular lesions are not as often noted as in dogs, and focal crusting lesions are the most common presenting sign. As is the case in dogs, the head, face, and ears are most commonly affected ( $\approx 80\%$ ).<sup>53</sup> Lesions exhibit the same striking bilateral symmetry as seen in dogs (Fig. 9-14, A). In many cases, the lesions extend to involve other body regions (Fig. 9-14, B). The legs are involved in some 10% of cases, and a striking feature



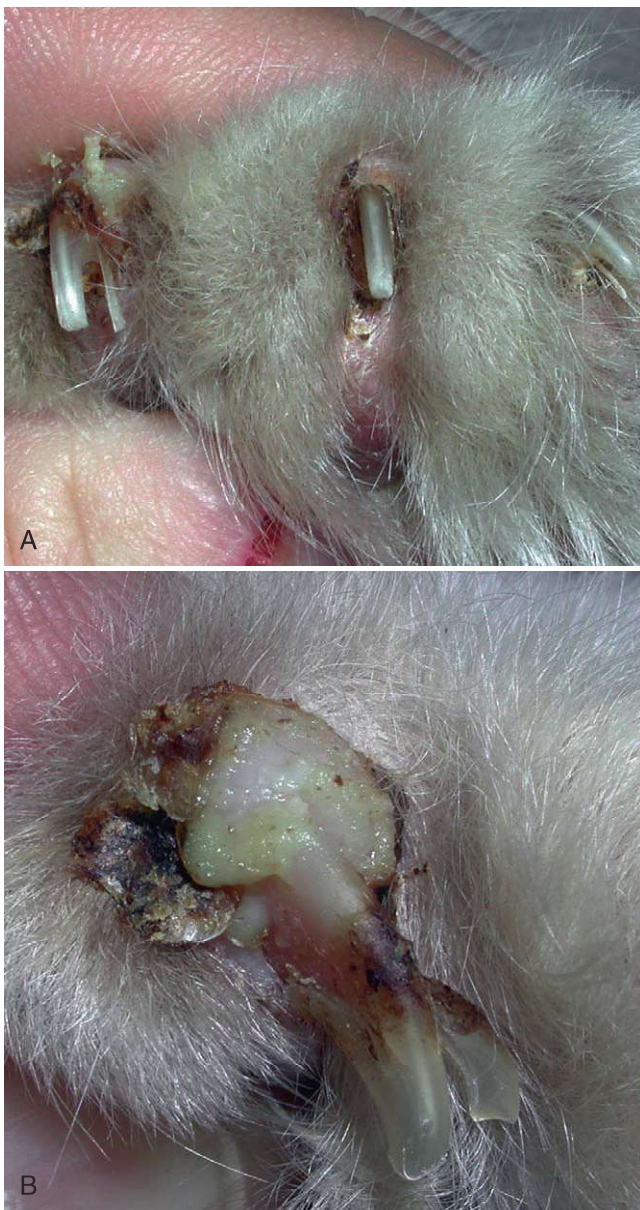
**FIGURE 9-14** A, Typical symmetrical lesions of pemphigus foliaceus (PF) in a 1-year-old Siamese cat. B, Lesions of PF on ventral abdomen of a cat.

of the feline disease is involvement of the claw folds ( $\approx 30\%$  of cases<sup>53</sup>; Fig. 9-15), often presenting as a refractory paronychia. Pruritus is mild to severe in some 80% of cases,<sup>53</sup> and animals are often lethargic, febrile, and anorectic.

Diagnosis is made by histopathology, with subcorneal or intracorneal pustules regarded as the most significant finding. All are neutrophilic, with a minority containing significant proportions of eosinophils. The outer root sheath is involved in a third of cases,<sup>59</sup> and the pustules, where seen, may span from 1 to 15 follicular units. Acantholytic cells are of course a highly significant finding, and 39 cases in the above series had more than 20 acanthocytes per high-power field.<sup>53</sup> An important observation was that prior immunosuppressive therapy rendered the diagnosis more difficult to achieve, in that the characteristic findings were less evident. Immunologic studies have not been routinely performed, so there are no data on their utility as diagnostic aids.

The prognosis for this disease in cats is quite good. Although in an earlier report, azathioprine was used successfully for induction therapy,<sup>173</sup> the myelosuppressive effects of this drug in the cat means that it should be used with great caution and for short periods, if at all.<sup>44</sup> The treatment regimens most often employed are prednisolone (4-5 mg/kg), alone or in





**FIGURE 9-15** **A**, Typical nailfold involvement often seen in cats with pemphigus foliaceus. **B**, Close up of paronychia showing cheesy exudate and crusting in claw fold.

combination with chlorambucil, and triamcinolone (0.6-2 mg/kg). The latter appears to be the most effective glucocorticoid, with a lower incidence of side effects. For maintenance, triamcinolone is used at 0.6 to 1 mg/kg every 2 to 7 days, or prednisone at 2.5 to 5 mg/kg every 2 or 3 days. Follow-up was possible in 44 cases in the above series for periods of 1 to 54 months (median 9 months). Only 4/44 cats died from their disease or treatment-related causes during this period.<sup>53</sup> The most common treatment-related side effect in cats is the development of diabetes mellitus in cats that require high doses of corticosteroids for prolonged periods. Monitoring of serum fructosamine levels is recommended to detect cats in the early stages of developing diabetes mellitus. Cyclosporine at 7.5-10 mg/kg, alone or in combination with prednisolone, has been effective in treating some cats.

### AUTOIMMUNE SUBEPIDERMAL BLISTERING DISEASES

A *blister* is a collection of fluid underneath the epidermis and is a broader term because there can be differences in the fluid, and

there is no restriction in size. Blisters are not common lesions in dogs and often lead to erosions or ulcerations, which are the more common clinical finding. Though there are many causes of blisters in humans, there are fewer in dogs. There are a group of diseases characterized by autoantibodies to components of the basement membrane that result in separations of adjoining structures. These separations may fill with serum or clear transudates, resulting in subepidermal vesicles and/or bulla. Hence these diseases are referred to as *autoimmune subepidermal blistering diseases* (AISBDs). The classic lesion is the *vesicle*, which is a term referring to a lesion that is a clear, fluid-filled, small (<1 cm) blister. The diseases currently described in dogs and cats include acquired junctional epidermolysis bullosa (AJEB), bullous pemphigoid (BP), epidermolysis bullosa acquisita (EBA), linear IgA bullous disease (LAD), mixed AISBD, mucus membrane pemphigoid (MMP), and type 1 bullous systemic lupus erythematosus.<sup>3</sup> These diseases are definitively diagnosed by determining the specific basement membrane antigens being targeted by the autoantibodies. Other subepidermal blistering diseases are due to hereditary deficiencies or functional abnormalities of BMZ structures (see Chap. 12). Some other diseases may create subepidermal vesicles, either grossly or histologically, but also affect other parts of the skin such as the epidermis and hair follicles (erythema multiforme, toxic epidermal necrolysis, dermatomyositis, vesicular cutaneous lupus erythematosus) and will be covered in their respective disease sections of the book.

The AISBDs are very rare diseases we are still in the process of understanding.<sup>3</sup> This is because few cases have been definitively diagnosed with proper identification of the targeted antigens. Many cases described prior to 1995 were usually called *bullous pemphigoid*, and we therefore have only been able to look critically at these diseases in the last 15 years. Unfortunately the tests to determine these autoantibodies are not readily available, and the exact diagnosis of these diseases is not routinely achieved in clinical practice. Therefore we are still learning the complete clinical spectrum of lesions, how often crossover findings occur, and whether a specific disease is associated with a better prognosis, a triggering event, or a preferred therapeutic treatment or protocol. The answer to these questions will only be determined if we make diagnoses based on the best available information.

The initial diagnosis is based on clinical findings and histopathology showing subepidermal vesicles. As we learn more, it appears there are clinical phenotypic differences that may become even more apparent as more cases are definitively identified. Some available tests to help determine which AISBD is present are histopathologic findings, salt-split skin indirect immunofluorescence, and collagen IV immunostaining.<sup>3,10,15,56,174-177</sup> Salt-split canine lip or gingival skin has been used in indirect immunofluorescence testing with the patient's serum. A 1-molar solution of NaCl splits skin through the lamina lucida, allowing recognition of autoantibodies that bind to the top (epidermal side and lamina lucida), bottom (dermal and lamina densa side), or combined both sides of the split, which may indicate multiple antibodies are present. Table 9-3 compares the AISBDs based on current understanding of these tests. As stated, definitive diagnosis requires that the autoantigen targeted by the autoantibodies is determined, and these tests require submission to research facilities, which currently in the United States is Dr. Olivry's laboratory at the College of Veterinary Medicine, North Carolina State University.

### PROGNOSIS AND TREATMENT OF AISBD

The prognosis for this group of diseases is not well known in dogs or cats, because our experience in treating them is limited.