Pemphigus and bullous pemphigoid are autoimmune bullous diseases of the skin. Pemphigus, an intraepidermal blistering disease, is characterized by autoantibodies reactive with antigens located in the intercellular spaces or on the surfaces of epidermal cells. These antibodies, which have recently been shown to activate complement, appear to be the cause of the basic pathologic process of pemphigus, acantholysis. The complement system and the plasminogen-plasmin system may be important mediators in the detachment of epidermal cells. Bullous pemphigoid, a subepidermal blistering disease, is characterized by autoantibodies reactive with an antigen located in the lamina lucida region of the basement membrane zone. These autoantibodies, which will avidly fix complement, appear to mediate subepidermal separation by attraction of a variety of inflammatory cells. Anaphylatoxins, released by activation of C4 and C3, or specific IgE antibodies, may activate mast cells with release of ECF-A attracting eosinophils. With activation of C5, C5a is released which could attract polymorphonuclear leukocytes. Antigen-specific lymphocytes, which can also contribute histamine releasing substances, may also be involved. The exact mechanism by which the epidermis separates from the dermis in bullous pemphigoid, however, remains unresolved.

Pemphigus and bullous pemphigoid are members of the chronic nonhereditary blistering skin diseases of man. Based upon similar histopathologic and immunopathologic findings, pemphigus may be considered a disease group which includes pemphigus vulgaris, pemphigus vegetans, pemphigus foliaceus, pemphigus erythematosus, Brazilian pemphigus foliaceus or “fogo selvagem” and drug-induced pemphigus usually associated with d-penicillamine administration. By histopathology, the pemphigus group of diseases may be separated into deep and superficial forms. Pemphigus vulgaris, and the rare variant pemphigus vegetans exhibit suprabasilar intraepidermal bulla formation, while pemphigus foliaceus and its variants exhibit superficial intraepidermal bulla formation. All forms of pemphigus share the histologic feature of acantholysis or loss of cohesion of epidermal cells [1,2]. This acantholytic mechanism in pemphigus has received considerable investigative attention in recent years.

Bullous pemphigoid, on the other hand, is a bullous disorder which affects mainly middle-aged and older persons [2]. Like pemphigus, bullous pemphigoid is a member of a group of diseases which share similar histopathologic and immunopathologic findings. Other members of this disease group include cicatricial pemphigoid, localized scarring pemphigoid (Brunsting-Perry type), and herpes gestationis. Lesions of bullous pemphigoid may appear as grouped bullae of vesicles arising in greatest numbers in flexural areas (groin, axillae, antecubital fossae, etc.) usually healing without scarring. Remissions and exacerbations usually occur during a 6–10 year course of the disease, and spontaneous complete resolution is customary. Histopathologically, bullous pemphigoid is a subepidermal blistering skin disease.

Pemphigus is an autoimmune disease of the skin. By direct immunofluorescence (IF) staining, numerous studies have confirmed the presence of serum autoantibodies with reactivity for antigens localized to the intercellular substance (ICS) of epidermis or the surfaces of individual epidermal cells [1–4]. These autoantibodies are of the IgG type, reside in all subclasses of IgG [5], and are present in all types of pemphigus, an additional feature which unifies members of this disease group. Levels of pemphigus antibodies often fluctuate with activity of disease, being high during periods of disease activity and low or entirely absent during periods of remission [6,7].

In recent years, considerable investigative effort has been focused upon the antigens reactive with pemphigus antibodies. To review all such attempts published thus far would be beyond the scope of this review. Suffice it to say, some controversy still exists concerning the size of the antigen or antigens. Diaz and co-workers [8] isolated a glycoprotein from human saliva (presumably produced by oral mucosa cells) with a molecular weight of approximately 50,000 daltons, while Stanley et al [9] have identified a 210,000 dalton protein which when reduced has 130,000 and 80,000 molecular weight chains. Stanley and co-workers [10] have also recently shown some but not all pemphigus foliaceus sera are reactive with antigens which differ from those associated with pemphigus vulgaris. Whatever their composition, these antigens are clearly produced by and expressed on the surfaces of epidemical cells [4,11] and appear to be important in cell-cell adhesion [12].

Several lines of evidence suggest that antibodies found in the sera of patients with pemphigus are the cause of the disease process. By direct IF methods, early acantholytic areas of pemphigus skin and oral lesions have heavy ICS deposits of IgG [13,14]. Thus, pemphigus antibodies, in addition to their presence in serum, are capable of leaving the circulation and reacting with epidermal cell surface antigens in vivo. In addition to finding IgG deposits in these skin lesions, a variety of complement components, including C1q, C4, C3 (Fig 1A), B and properdin have also been identified in these ICS deposits [15–17].

The first convincing evidence that the antibodies found in sera of pemphigus patients are responsible for acantholysis was presented several years ago by Michel, Schiltz, and co-workers.
[18,19]. With explants of normal human skin in the presence of pemphigus IgG in organ culture, histologic evidence of acantholysis occurred within 48–72 h. Later, Farb, Singer, and co-workers [20,21] demonstrated that epidermal cell detachment occurs when pemphigus IgG was added to monolayers of epithelial cells in culture. They further showed that pemphigus IgG would stimulate the release of proteinases in their culture systems, studies confirmed by Woo et al [22]. Hashimoto and co-workers [23] recently identified the proteinase released by cultured epidermal cells when treated with pemphigus IgG as plasminogen activator (PA). They have proposed that activation of plasminogen to plasmin by PA may be responsible for acantholysis. To substantiate this hypothesis further, Moriya, Jensen, and Lazarus [24] have recently shown that the addition of plasmin to the epidermal culture systems will result in epidermal cell detachment.

As the above investigations were all performed in the absence of complement [18–21,29], the involvement of the complement system in the process of acantholysis has remained controversial. Despite the fact that complement deposits are present in pemphigus lesions by direct IF [15–17], early attempts to demonstrate that pemphigus antibodies would fix complement failed [25]. Later, Nishikawa et al [26] and Hashimoto et al [27] were able to demonstrate by in vitro complement IF staining that some pemphigus antibodies would fix complement to normal human skin. We have recently confirmed their studies and extended them by showing that pemphigus antibodies will fix complement to organ culture skin explants and to epidermal monolayers (Fig 1B) in tissue culture [11]. As these antibodies would fix the early components C1q and C4, and in addition C3 (Table I), pemphigus antibodies appear to fix complement via the classical pathway.

The next question we wished to address was whether complement might enhance pemphigus IgG induced detachment of epidermal cells in culture. Thus, we chose to utilize a system as described by Woo and co-workers [22], but choosing a pemphigus IgG concentration (1 mg/ml) reported by them to be insufficient to cause detachment of monolayers of cultured epidermal cells. Thus, in our system, minimal cell detachment occurred when 1 mg/ml pemphigus was added to the 48-h cultures alone. When complement was added to such cultures (Table II), significant detachment of cultured epidermal cells became apparent [28]. By using C1q depleted serum of heat inactivated serum as the complement source (Table II), epidermal cell detachment was completely inhibited. Depletion of plasminogen from both the pemphigus IgG fractions and the complement source on the other hand failed to inhibit cell detachment*. Thus, complement activation by pemphigus antibodies provides an additional mechanism for loss of epidermal cell cohesion in addition to the plasminogen-plasmin system.

Passive transfer studies also suggest that pemphigus antibodies are pathogenic. Buschard et al [29] developed a model in athymic mice using explanted oral mucosa. One week following explantation, ICS antibodies were injected intraperitoneally. Binding of ICS antibodies could be detected and histologic evidence of acantholysis could be seen in the sites of the oral mucosal explants. More recently, daily i.p. injections of pemphigus serum into 24 h old mice by Anhalt et al [30] resulted

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**TABLE I. In vitro complement staining reactions with mouse epidermal monolayers and organ cultured normal human skin**

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<thead>
<tr>
<th>Complement treatment</th>
<th>Staining reaction</th>
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<td></td>
<td>C1q</td>
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<tr>
<td>Pemphigus IgG (3 mg/ml)</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>+++</td>
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<tr>
<td>Purified C1q</td>
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<td>C 56°C, 30 min</td>
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<td>C2 deficient serum</td>
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**TABLE II. In vitro complement staining reactions with mouse epidermal monolayers and organ cultured normal human skin**

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<td>Pemphigus IgG (3 mg/ml)</td>
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<td>Purified C1q</td>
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<td>C2 deficient serum</td>
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* Kawana S, Geoghegan WD, Jordon RE: Complement fixation by pemphigus antibody. II. Complement enhanced detachment of epidermal cells. Submitted for publication.
in blistering skin lesions at about 1 week. These lesions were histologically identical to true pemphigus lesions and with binding of ICS antibodies to the mouse skin. These studies have recently been confirmed and extended by Peterson and Wuepper [31] who, in addition to passively transferring the disease in neonatal mice, used a rabbit antiserum to purified pemphigus antigen to induce the experimental lesions. Thus, the disease pemphigus has been successfully passively transferred to experimental animals.

Mechanistically, then acantholysis in pemphigus appears to result from the interaction of pemphigus antibodies with epidermal cell surface antigens thought to be important in cell-cell adhesion. Both activated complement and plasmin appear to mediate the cell detachment associated with this disease process (Fig 2). The role cells play, such as eosinophils, which are commonly found in early infiltrates, remains to be determined.

**BULLOUS PEMPHIGOID**

Like pemphigus, bullous pemphigoid is also an autoimmune bullous skin disease. This subepidermal blistering disease, however, is characterized by circulating IgG autoantibodies which react with antigens located in the lamina lucida region of the basement membrane zone (BMZ) [1,32,33]. The antigen(s) reactive with these antibodies, which have been partially characterized [34,35], appear to be produced by basal keratinocytes [4] and deposited along the BMZ [36].

By histopathology, a variety of inflammatory cells have been identified in bullous pemphigoid infiltrates. Noninflammatory appearing lesions show a sparse perivascular infiltrate of eosinophils and mononuclear cells. By contrast, inflammatory lesions show a dense perivascular and subepidermal infiltrate consisting predominantly of eosinophils with mononuclear cells and polymorphonuclear leukocytes (PMNL) present in smaller numbers. Low eosinophil and high eosinophil lesions [37,38] have been noted by several investigators; close light microscopic and electron microscopic studies of very early lesions reveal prominent mast cells in the reticular and papillary dermis showing focal irregular loss of granules as well as degranulation, karyorhexis, and karyolysis of eosinophils [39,40]. Tissue injury which precedes frank dermal-epidermal separation is also present in early lesions [41]. Basal keratinocytes show loss of hemidesmosomes, and widening of intercellular spaces. Vascular injury is apparent with endothelial hypertrophy, focal luminal obliteration, and focal endothelial cell death [39].

The earliest clues to the pathogenic mechanisms which might be responsible for the tissue injury outlined above came from IF and immunoelectron microscopic (IEM) studies. In vivo bound gamma globulin was noted at the BMZ in lesions of bullous pemphigoid [32]. Later, classes of immunoglobulins were defined and evidence of complement activation [42] was noted. Most commonly, IgG and/or C3 are found at the BMZ (Fig 3). IgA and IgM are found in about 25% of the cases [38,43]. In addition, IgE has been noted at the BMZ [44] and on mast cell surfaces in involved skin of bullous pemphigoid patients [45]. Evidence for classical complement pathway activation [46,47] as well as alternate pathway activity has been established [48–50]. β1H globulin, a cofactor for C3bINA, has also been detected using IF studies in involved skin [51–53]. More recently, Dahl and associates [54] have demonstrated deposition of the membrane attack complex (C5–9) in bullous pemphigoid lesions (Table 11).

IEM of lesional skin has localized the immunoglobulin deposition to the sub-basalar lamina lucida area [33]. Others have further noted C3 in this location [55], as well as “lumpy-bumpy” immunoglobulin deposition sub-basally and intercellularly suggesting immune complex deposition [56]. Immune complexes have also been noted in some BP patients with active disease, but negative indirect IF [57]. This suggests that bullous pemphigoid antigen may be produced in excess and may even circulate, thus providing a nidus for immune complex formation. Immune complexes have also been detected in bullous pemphigoid blister fluids [58].

The IF studies of lesional tissue provide evidence that the humoral aspect of the immune system plays a role in lesion formation, with a cellular component as well. This latter evidence, however, is more difficult to interpret.

The mast cell could be viewed as a bridge linking the cellular and humoral components in lesion development. It is observed to be present and to have a degranulated appearance in early lesional tissue [59]. Under the influence of an appropriate stimulus such as IgE, C3a or C4a [60], the mast cell will release histamine, slow reacting substance of anaphylaxis (SRS-A), eosinophilic chemotactic factor of anaphylaxis (ECF-A), high molecular weight neutrophil chemotactic factor (HMW-NCP) platelet-activating factors, and enzymes. C4a and C3a are derived following the activation of C4 and C3 [61]. As many

![Fig 2. Proposed mechanism for pemphigus acantholysis.](image-url)
b Bullous pemphigoid antibodies have been shown to activate complement in vivo and in vitro, one could postulate that complement activation, rather than causing direct tissue injury, triggers mast cell degranulation with histamine release resulting in "leakiness" of blood vessels and chemotactraction of eosinophils, (ECF-A) and PMNL (HMW-NCF). C4a, C3a and C5a produced by complement activation could further amplify the chemotactic effect [62,63].

Examination of blister fluid helps to substantiate this theory of pathogenesis. Complement activation leading to a decreased CH₅₀ of blister fluid as compared to serum CH₅₀ in the same patient has been observed [64]. Immune complexes which form and have been detected in these blister fluids [58] might contribute to these depressed blister fluid CH₅₀ levels. This reduced CH₅₀ is associated with increased chemotactic activity of blister fluid [65]. A number of immune modulating factors have also been observed in bullous pemphigoid blister fluid, including ECF-A [66,67], eosinophil colony stimulating material or ESM [68], lymphocyte chemotactic material [69], and prostaglandin E₂ [70]. Enzymatic activity in blister fluid has also been noted [71]. These findings can be interpreted as evidence for humoral and cellular activity in the bullous pemphigoid lesion.

The difficulty lies in determining a causal relationship between cellular and humoral aspects and in proving which if any actually produces the histologic change of dermal epidermal separation. Dubertret et al [72] using histologic studies propose that direct keratinocytic killing and enzyme release by eosinophils leads to blister formation. The eosinophil, abundant in most inflammatory lesions of bullous pemphigoid is capable of antibody dependent cellular cytotoxicity (ADCC) killing of a variety of cell types in culture [73]. An associated eosinophilia is often noted in cases of bullous pemphigoid [74].

Attempts to develop an in vitro model have focused on the role of complement and PMNL in lesion formation. Anhalt et al have produced microblebbing and a PMNL rich inflammatory infiltrate by injecting bullous pemphigoid antibody into rabbit cornea [75]. Gammon et al, using an in vivo model of the dermal-epidermal junction, have demonstrated directed migration of PMNL to the BMZ in the presence of bullous pemphigoid antibody and complement [76]. Although activation of PMNL has been demonstrated [77], no true separation of epidermis from dermis has been demonstrated in vitro using bullous pemphigoid antibody and complement [78] or PMNL [76,79,80]. It appears then that some other cell(s) or factor(s) may be necessary for full lesion development. Indeed, the role of the PMNL may be to elaborate ECF-A after ingestion of immune complexes [58,67]. However, other possible roles for the PMNL could be elaboration of lytic enzymes or direct keratinocyte killing or injury via ADCC.

The mononuclear cell is also present in inflammatory infiltrates of bullous pemphigoid. The role of the lymphocyte and/or monocyte macrophage in bullous pemphigoid is poorly understood. As noted above, a lymphocyte chemotactic factor has been isolated from blister fluid [69]. The source of this factor is unknown, although peripheral blood mononuclear (Ficoll Hypaque separated cells) will elaborate a factor chemotactic for lymphocytes when stimulated with mitogen in vitro [81].

It has also been noted that PPD-specific lymphocytes will accumulate within and beneath artificial blisters overlying dermal injections of PPD in vivo [82]. Thus, lymphocytes may accumulate in response to specific antigen and/or chemotactants elaborated by stimulated monocytes or other mononuclear cells. The role of the lymphocyte is also unclear since these cells are capable of a number of varied activities. One possible role for the lymphocyte is as a source of histamine releasing factors [83]. This would permit long-term stimulation of mast
cells to elaborate chemoattractants and histamine thus perpetuating the immune response locally. Another possible role would be direct cytotoxic effect on basal keratinocytes. These functions remain to be experimentally tested in vitro using a model that is capable of full lesional expression, that is, dermal-epidermal separation, such as organ culture or epidermonolayers on dermal-epidermal junction-like glycoprotein matrix.

Based on the evidence presented, we propose the following mechanism of tissue injury in bullous pemphigoid: Bullous pemphigoid antigen in some way rendered immunogenic to stimulate an antiself clone of B cell/plasma cells. Various classes of antibullous pemphigoid antigen antibodies are elaborated with IgG predominating. The specific IgG binds bullous pemphigoid antigen in its normal lamina lucida location and begins fixing and activating complement (Fig 4). Among other things, C3a and C4a are elaborated. Mast cells degranulate elaborating ECF-A, HMW-NCF, perhaps ESM, histamine, and enzymes. Eosinophils and PMNLs are recruited and possibly bind via C3b receptors to the dermal-epidermal junction. By direct cytotoxic action or because of enzymes elaborated, a rupture of the dermal-epidermal attachment is produced and a blister formed. Lymphocytes in the area elaborate HRF (histamine releasing factor) to up regulate mast cell degranulation and the lesions progressed to show bullae, often in groups extending over weeks and months. This proposed model would account for most of the findings to date in bullous pemphigoid and the task now is to isolate and sequence the discrete events leading to clinical expression of the disease.

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### IMMUNOPATHOLOGIC MECHANISMS IN PEMPHIGUS AND BULLOUS PEMPHIGOID


