American Journal of Pathology, Vol. 134, No. 3, March 1989

Role of Plasminogen Activator in Pemphigus Vulgaris

J. Erby Wilkinson, C. A. Smith, Maja M. Suter, W. Falchek, and R. M. Lewis

From the Department of Pathobiology, University of Tennessee, Knoxville, Tennessee, and the Department of Pathology, Cornell University, Ithaca, New York

The role of plasminogen activator (PA) in the pathogenesis of acantholysis in canine pemphigus vulgaris (PV) was evaluated using differentiated cultures of canine oral keratinocytes. Both the secreted and cell-associated PA activity in cultured canine keratinocytes were completely inhibited by specific anti-urokinase antibodies. Anti-tissue type PA antibodies did not inhibit either secreted or cell-associated PA activity. Immunoblots and fibrin zymography revealed a single 57,000 molecular weight urokinase-type PA in the conditioned media of the canine oral keratinocytes. Incubation of the differentiated cultures with PV Ig resulted in a significant increase in the levels of PA activity and both canine and human PV Ig were effective at inducing acantholysis typical of that seen in the clinical disease. The addition of urokinase inhibitor to the cultures treated with PV Ig prevented the development of acantholysis. These data strongly support the conclusion that PA is involved in acantholysis which is the cardinal feature of PV. (Am J Pathol 1989, 134:561–569)

Pemphigus vulgaris (PV) is a severe, immune-mediated, bullous dermatosis that affects the skin and other stratified squamous epithelia.[1,2] The clinical, histologic, and immunopathologic characteristics of PV have been well documented in humans and domestic animals and are similar between species.[1–5] The characteristic lesion in all variants of PV is the formation of intraepithelial bullae as a result of a unique process of cell detachment termed acantholysis.[6,7] This auto-antibody mediated loss of cell cohesion has been linked to the synthesis, activation, and/or release of certain cellular enzymes, particularly serine proteases.[8–13]

A prominent role has been suggested for plasminogen activator (PA) in the induction of acantholysis.[14–16] PAs are ubiquitous serine proteases that are thought to mediate a variety of physiologic and pathologic processes that require extracellular proteolysis.[17–20] Both PA and PA inhibitors are present in the stratified squamous epithelium.[21–28] The activity of both cell-associated and secreted PA is reported to be increased after *in vitro* binding of PV antibodies to keratinocytes and this increase in PA activity is associated with acantholysis.[9,14,15] Although such studies have suggested a prominent role for PA in the induction of acantholysis, considerable doubt persists based on studies using various protease inhibitors,[12] steroids,[15,29–31] and an *in vivo* model of PV in neonatal mice.[32] Some evidence suggests that the increase in PA activity associated with PV antibody induced acantholysis may be secondary to the loss of cell cohesion.[33]

Using recently developed methods for producing cultures of differentiated canine oral keratinocytes, the role of PA in canine PV was investigated. Urokinase type PA (uPA) was determined to be the only type PA produced by cultured canine keratinocytes under normal culture conditions and inhibition of PA markedly reduced the number and severity of lesions in this model.

Materials and Methods

Keratinocyte Cultures

Canine oral keratinocyte cultures were established and subcultured as described previously.[34] Canine oral keratinocytes grown on plastic have previously been shown to express the PV antigen.[35] Levels of secreted and cell associated PA were determined in cultures grown in 48-well plates or on glass microdot slides. Conditioned medium for PA isolation was obtained from cultures grown

Supported by NIH Grant 1-F32-AM07562 to J.E.W. and Training Grant CA09399.

Accepted for publication November 2, 1988.

Address reprint requests to J. Erby Wilkinson, Department of Pathobiology, University of Tennessee, P.O. Box 1071, Knoxville, TN 37901.

in 25 sq cm tissue culture flasks. Differentiated cultures of canine oral keratinocytes were produced by growing keratinocytes at passage 3–6 on either cellulose acetate membranes (Millicell-HA, Millipore Corp.) or nylon membranes (Puropor-200R, Gelman Scientific, Bedford, MA) at the air-liquid interface as described previously.[36] Canine keratinocytes grown in this manner were evaluated for expression of PV antigen and reactivity with monoclonal antibody 1.11D9, which binds to the cell surface of canine keratinocytes in a pemphigus like pattern,[35] using immunohistochemical methods as previously described.[35,36]

Pemphigus Antibodies

Human PV sera were provided by Dr. Ernst Beutner. The human and canine PV sera used in these studies had titers of 1:160 by indirect immunofluorescence tests using canine esophagus as a substrate. Both the human and canine PV sera were obtained from patients with the clinical, morphologic, and immunopathologic characteristics of PV.[1]

Antibodies to PAs

New Zealand white rabbits were injected subcutaneously at 10 to 15 sites with 100 μg human urinary uPA or Bowes melanoma tPA emulsified in complete Freunds adjuvant. After 6 weeks and at 6-week intervals thereafter, booster injections of 50 μg of the appropriate PA were given in incomplete Freund's adjuvant in the same manner. Ten days after the booster injections, the rabbits were bled. Antibody activity and specificity were analyzed by double immunodiffusion for each antiserum at each collection. The anti-uPA antibody recognized only uPA and the anti-tPA antibody recognized only tPA.

IgG Preparation

Immunoglobulins from all sera including normal human, dog, and rabbit were prepared by sequential affinity chromatography on lysine-sepharose[28] followed by Protein A sepharose.[37] Monoclonal antibody 1.11D9 was similarly purified from ascites fluid. Eluted Ig fractions were adjusted to neutral pH, dialyzed against MEM with 0.01M HEPES, pH 7.4, and sterilized by filtration. Anti-uPA antibodies were determined to inhibit uPA in a dose-dependent fashion by incubating serial dilutions of Ig with a standard solution of uPA (1 Pu/ml) and determining the subsequent PA activity. Anti-uPA antibodies had no inhibitory activity against tPA. Anti-tPA antibodies were determined to be specific for tPA in a similar fashion. Antibodies to PAs and normal rabbit Ig were adjusted to 400 μg/ml antibody protein and frozen at −70 C until use. At this concentration, the anti-uPA and anti-tPA antibodies completely inhibited 1 PU/ml uPA and tPA respectively. All PV antibodies, normal human and dog Ig, and monoclonal antibody 1.11D9 were concentrated to 10 mg/ml and frozen at −70 C.

PA Assay

A two-step assay was used to measure both secreted and cell associated PA.[38,39] This assay is based on the conversion of canine plasminogen to plasmin that is then measured using the fluorogenic substrate [Cbz-Ile-Pro-Arg-NH]$_2$-Rhodamine [BZIPAR].[38] BZIPAR was the gift of Dr. Walter Mangel (Brookhaven National Laboratory). BZIPAR is nonfluorescent until the plasmin cleaves a single amide bond which converts the rhodamine from the lactone state into the highly fluorescent quinone state. The rate of cleavage of BZIPAR by canine plasmin has been shown to be linear with time under the conditions used in these experiments.[38] The fluorescence also has been shown to be directly proportional to the initial plasmin concentration.[38] Canine plasminogen was purified by lysine-sepharose chromatography and fraction I (90 kd) and fraction II (95 kd) canine plasminogen were pooled.[38] Following the isolation, the plasminogen was exhaustively dialyzed against MEM with Earles salts, 0.01 M HEPES, 0.1 M lysine, pH 7.2. This pooled fraction has been shown to contain 60–80 percent activatable plasminogen.[38] For the PA assay, 0.05 ml aliquot of plasminogen [1 mg/ml] previously incubated with either conditioned medium or cells for 1 hour at 20 C was removed and added to 1.20 ml of a 5 μM BZIPAR in 0.01 M HEPES, pH 7.2, containing 15% (vol/vol) ethanol and 5% (vol/vol) dimethylformamide at 20 C. After 15 minutes, the fluorescence was measured in a Perkin-Elmer LS-5 fluorescence spectrophotometer. The excitation wavelength was 492 nm and the emission wavelength was 526 nm. The fluorescence spectrophotometer was standardized for each assay using a polymethacrylate block embedded with Rhodamine B.[39] The assay was determined to be linear over the range of 0.01–0.0001 Plough units (Pu) PA per μl. Controls run with every assay included an appropriately diluted plasminogen negative control and a standard solution of human urokinase (0.01 Pu/10 μl) positive control.

Cell associated PA was determined by rinsing cells in a 48-well plate three times with MEM before incubation with 100 μl of plasminogen in MEM with 0.01 M HEPES, 0.1 M lysine, pH 7.2, for 1 hour. A 0.05 ml aliquot was then removed and the plasmin formed determined with the BZIPAR assay. In preliminary experiments, the amount

of secreted PA during a 1-hour incubation was determined to account for less than 5% of the total PA activity.

To determine the effects of PV Ig on cell associated PA levels, confluent cultures of keratinocytes on microdot slides (0.5 sq cm/well) were washed three times with MEM and incubated for 24 hours in 25 μl of MEM with 10 mg/ml PV or normal human Ig before determination of the level of cell associated PA.

To determine the levels of secreted PA, confluent cultures in 1 sq cm wells were rinsed vigorously in serum-free medium prior to incubation with 300 μl of MEM for 24 hours. Ten ul of serum-free conditioned medium was then removed and incubated for 1 hour at 20 C with 90 μl of plasminogen and the amount of plasmin formed determined using the BZIPAR assay.

Characterization of the PA in Conditioned Media

Confluent cultures in 25 sq cm flasks were rinsed three times with MEM before incubation for 24 hours in serum free MEM. The conditioned medium was removed, centrifuged to remove cellular debris, and frozen at −70 C before analysis. The pooled conditioned medium was thawed and concentrated 100 times by microfiltration in a stirred cell using a 10,000 MW cutoff filter (Amicon). The concentrated conditioned medium was then dialyzed against 0.1 M TRIS, pH 8.1 containing 0.5% Triton X-100 overnite at 4 C. Forty ul samples were then subjected to discontinuous SDS-PAGE using a 4.5% stacking gel and a 10% resolving gel.[40] For Western blotting the proteins were transferred to nitrocellulose.[41] Protein transfer was monitored by following the prestained molecular weight standards. The electrophoretic blot was then placed in gelatin (0.25%) in NET buffer overnite at 4 C before incubation with either rabbit anti-uPA or anti-tPA (100 μg/ml). Exposure to a 1:1000 dilution of HRP conjugated anti-rabbit Ig for 2 hours followed by substrate (4 chloro-1-napthol + H_2O_2) developed the blot.

Fibrin Zymography

Concentrated conditioned medium was subjected to polyacrylamide gel electrophoresis according to the method of Laemmli[40] using a 9% acrylamide resolving gel and a 4% acrylamide stacking gel. Fibrin gels (105 mm thick) were prepared as described previously.[42,43] Briefly, a 2% solution of agarose in water was boiled, cooled to 47 C, and mixed with prewarmed PBS containing plasminogen (20 μg/ml) and thrombin (0.6 U/ml). Fibrinogen (10 mg/ml) was added, and the solution rapidly poured onto the surface of a glass plate. Final concentrations were 1% agarose, 6 μg/ml plasminogen, 0.18 U/ml thrombin, and 2 mg/ml fibrin. After SDS-PAGE, the gel was washed in 2.5% Triton X-100, placed directly on the fibrin gel, and incubated in moist chambers at 37 C for 4 hours. Lysis was stopped by removal of the polyacrylamide gel and placing the fibrin gel in normal saline followed by distilled water. The gel was dried, and stained with Buffalo Black.

Induction of Acantholysis

After 10–12 days of growth at the air-liquid interface, the differentiated canine oral keratinocyte cultures were placed in 24-well plates before the addition of antibodies and/or PA inhibitors. One hundred fifty μl of MEM with human PV Ig (10 mg/ml), canine PV Ig (10 mg/ml), normal human or canine Ig (10 mg/ml), or monoclonal antibody 1.11D9 (10 mg/ml) was added to individual wells. After 3 days, the cultures were either fixed in 10% neutral buffered formalin, paraffin embedded, sectioned, and stained with hematoxylin and eosin (H & E), or frozen in isopentane, sectioned on a cryostat, and stained with Mayer's H & E. Sections were examined without prior knowledge of treatment and the extent of acantholysis was determined. Keratinocytes that lacked cell–cell contacts and had a round morphology were considered to be acantholytic. The presence of scattered, individual, suprabasal acantholytic keratinocytes in an otherwise normal culture was considered a mild lesion. Moderate lesions consisted of groups of acantholytic suprabasal cells scattered throughout the section. Severe lesions involved the full thickness of the culture and was widespread in the culture.

Inhibition of PA

At the time of addition of normal or PV Igs, human placental urokinase inhibitor was added to the medium (final concentration, 50 units/ml). Controls included the PA inhibitor without Igs. The cultures were maintained and processed as described above.

Results

Canine Keratinocytes Secrete uPA

PA activity was readily detected in the serum-free conditioned medium from canine keratinocytes. The inhibitory effects of anti-uPA and anti-tPA antibodies on the se-

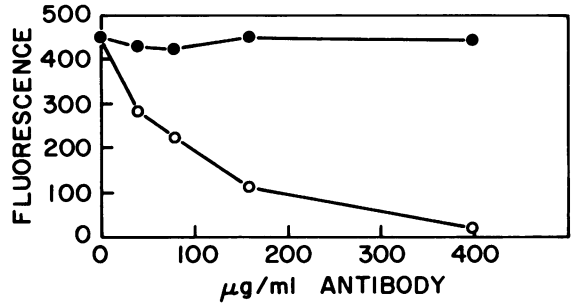


Figure 1. *Inhibition of PA activity in conditioned medium by anti-uPA and anti-tPA antibodies. Open circles, anti-uPA; closed circles, anti-tPA. N = 8.*

creted PA activity is shown in Figure 1. Over 95% of the secreted PA activity was inhibited in a dose-dependent manner by anti-uPA antibody whereas anti-tPA antibody had no inhibitory effects. The secretion of uPA by cultured oral canine keratinocytes is supported by the Western blot analysis that revealed a single PA with an apparent molecular weight of 57,000 daltons, which was detected by anti-uPA antibody. Anti-tPA antibodies did not react with any proteins in the Western blots of serum-free conditioned medium (Figure 2). The presence of a single PA is further supported by the fibrin zymography which also

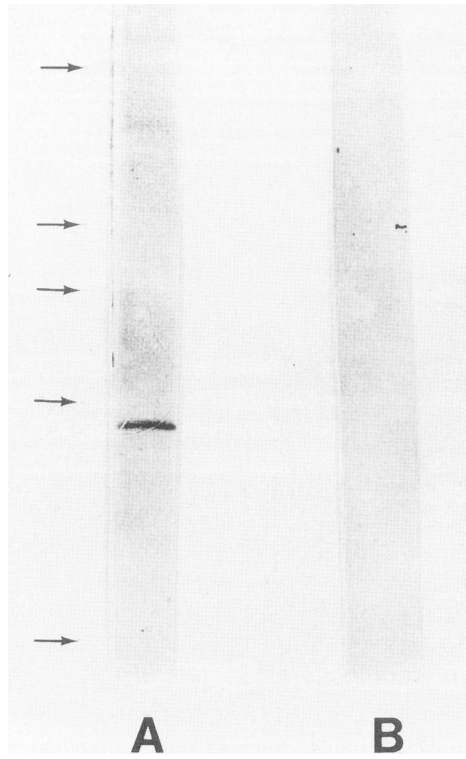


Figure 2. *Immunoblots of conditioned medium.* **A:** *Anti-uPA antibody.* **B:** *Anti-tPA antibody. Arrows, molecular weight standards (200, 116, 97.5, 66, and 44 kd)*

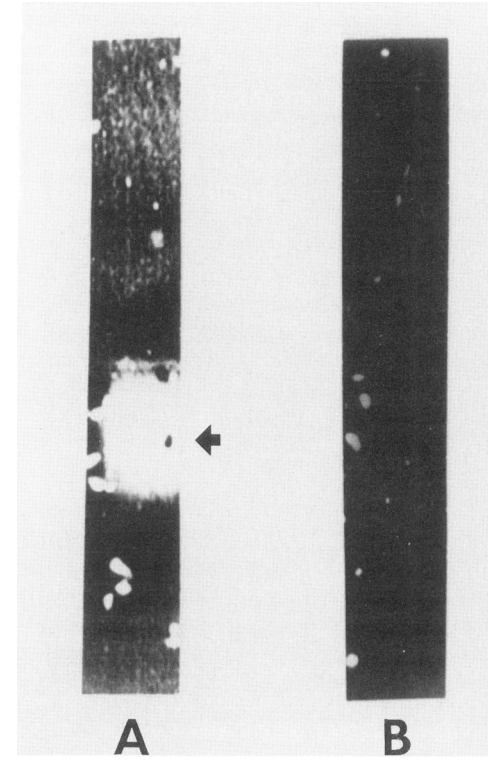


Figure 3. *Fibrin zymography of conditioned medium. Lane A contains plasminogen in the fibrin gel. Lane B does not contain plasminogen. Arrow, 57,000 molecular weight.*

demonstrated a single PA at 57,000 daltons (Figure 3). The fibrinolytic activity was not detected in fibrin gels in which the plasminogen was omitted indicating that the activity was due to a PA.

Cell-Associated PA is Also Urokinase Type

A major component of the canine keratinocyte PA activity was cell associated. Similar to the secreted PA, the cell

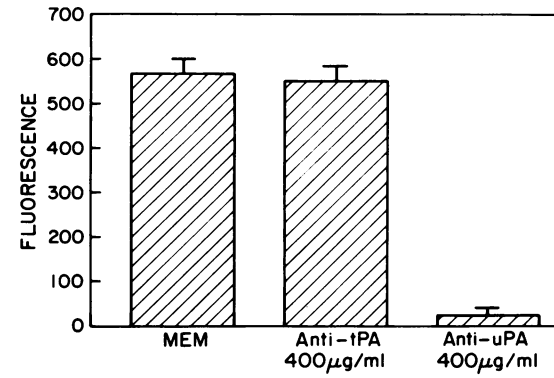


Figure 4. *Inhibition of cell associated PA by anti-uPA and anti-tPA antibodies.*

Table 1. *Cell Associated PA Activity in Cultured Keratinocytes*

Treatment	Fluorescence*	N
Normal human Ig	278 + 21†	6
Pemphigus vulgaris Ig	400 + 105†	6
Human placental urokinase inhibitor	14 + 7†	6

* Mean + SD.
† Means are significantly different ($P < 0.05$).

associated PA was completely inhibited by anti-uPA antibodies whereas anti-tPA antibodies had no inhibitory activity (Figure 4).

PV Antibodies Induce Increased PA Activity in Cultured Canine Keratinocytes

Incubation of cultures of canine oral keratinocytes with human PV Ig for 24 hours resulted in an increase in PA activity of approximately 45% over levels in cultures incubated with normal human Ig (Table 1). Human placental urokinase inhibitor inhibited over 95% of the PA activity in these cultures.

Induction of Acantholysis

Both the PV antibody and monoclonal antibody 1.11D9 stained the cell surface of the canine oral keratinocytes grown at the air–liquid interface on synthetic membranes in a diffuse pattern (Figure 5). Both human and canine PV Ig were effective in inducing lesions in the cultures of differentiated canine oral keratinocytes (Table 2). The extent of the lesions varied from culture to culture and ranged from focal areas of acantholysis (Figure 6) to severe, diffuse acantholysis (Figure 7). Normal human Ig, normal canine Ig, human placental urokinase inhibitor, and monoclonal antibody 1.11D9 never induced any lesions (Figure 8).

Table 2. *Lesion Development in Cultured Keratinocytes*

Treatment	With lesions
Normal human Ig	0/10
Normal canine Ig	0/10
Urokinase inhibitor	0/6
Monoclonal Antibody 1.11D9	0/4
Canine pemphigus Ig	8/8
Human pemphigus Ig	11/12
Canine pemphigus Ig and urokinase inhibitor	1/6
Human pemphigus Ig and urokinase inhibitor	1/10

The canine PV Ig was especially effective in inducing lesions. Lesions were found in all cultures incubated with canine PV Ig and the lesions were always severe. In two of eight cultures treated with canine PV Ig, only an occasional acantholytic keratinocyte remained attached to the membrane following 3 days of culture. Lesions induced by human PV Ig were less severe and were often scattered throughout an otherwise normal culture.

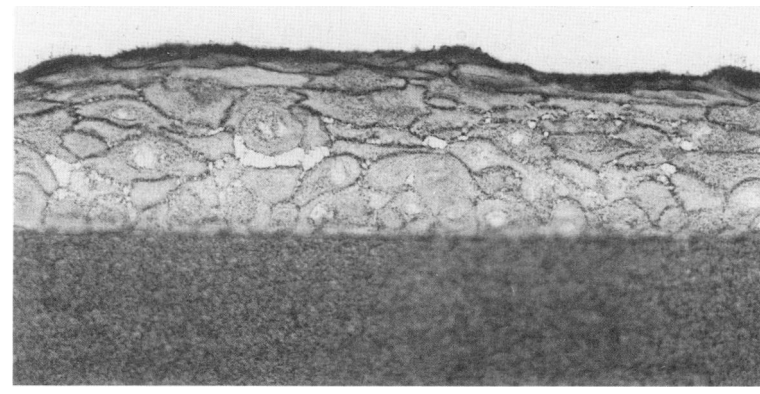
Effects of PA Inhibition on Induction of Acantholysis

The addition of human placental urokinase inhibitor to cultures incubated with either human or canine pemphigus Ig markedly reduced the number of cultures with acantholytic lesions (Table 2). In those cultures that did have lesions, the distribution and severity of the lesions were less severe than in cultures not treated with PA inhibitor.

Discussion

The results of these studies strongly implicates PA as a major extracellular protease involved in the induction of acantholysis at the keratinocyte level. Previous studies have clearly implicated extracellular proteolysis as a major factor in acantholysis[8–13] and several studies have sug-

Figure 5. *Lifted culture stained with PV antibody.*



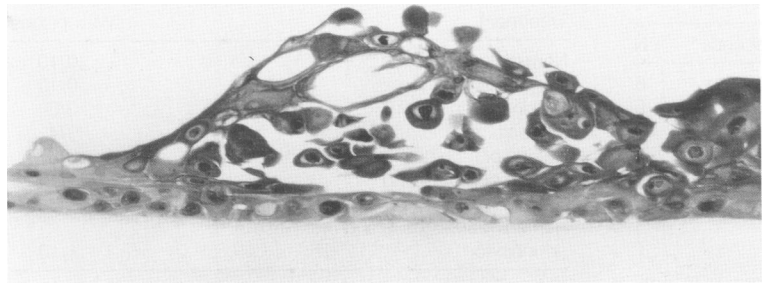


Figure 6. *Typical lesion produced in cultured canine keratinocytes by human PV Ig.*

gested that PA is the primary protease involved.[14–16] Considerable doubt persists regarding the role of PA, however, based on both *in vitro* and *in vivo* studies.[12,15,29–32]

The current studies differ from previous *in vitro* studies of the role of PA in acantholysis in several ways. In these studies differentiated cultures of canine oral keratinocytes were used. The dog is an excellent model for the study of PV. Spontaneous PV is more common in the dog than any other species with the possible exception of man and clinicopathologic characteristics of human and canine PV are similar.[1,3,4] The primary difference in human and canine PV is that dogs with PV rarely have high titers of circulating PV antibody while humans with PV commonly do. The reason for this difference is unknown. As a consequence of this prevalent lack of circulating PV antibodies in canine PV patients, experimental studies such as those reported here are often difficult to perform. Fortunately, PV antibodies are not species specific and canine stratified squamous epithelium is an excellent substrate for human PV antibodies.[44] In addition, we have demonstrated previously that the differentiated cultures of canine oral keratinocytes express the PV antigen in a pattern and titer similar to that of the native canine oral mucosa.[35,36] Because canine and human PV Igs were available for the studies reported here, both were evaluated. Whereas both consistently induced lesions typical of PV, the canine PV antibodies appeared to be more effective. Whether this is a general phenomenon or is specific to the specific antibodies compared in these studies is currently unknown and will require further comparisons using larger numbers of different antibodies.

Another difference in these studies is the use of differentiated cultures of oral keratinocytes. Oral keratinocytes seem especially suitable for *in vitro* studies of PV because the level of expression of the PV antigen in the normal oral mucosa is among the highest in the body[45] and PV often begins in the oral mucosa.[1] More importantly, the use of differentiated cultures of these oral keratinocytes is especially relevant because PV antigen is maximally expressed in stratifying cells,[46] histological lesions of PV are always suprabasilar, certain pemphigus antibodies preferentially bind to upper layers of stratified squamous epithelium[47] and the adhesion characteristics differs between cells at different stages of differentiation in the stratified squamous epithelium.[48]

In past studies, skin explants and keratinocyte cultures have been utilized to study the immunopathogenesis of PV. However, the use of explants is limited due to the short term viability of all keratinocytes except basal cells, the presence of other cell types that complicate PA analysis, and the significant spontaneous release of endogenous PA *in vitro* associated with cellular degeneration.[49] Keratinocytes cultured on plastic have also been used to study the pathogenesis of PV but atypical differentiation[50–53] and spontaneous blistering, which is associated with differentiation in such cultures,[54] limits their usefulness.

Due to these problems, a "lifted" culture system was selected to grow fully-differentiated canine stratified squamous epithelium for use as an *in vitro* model system. Recent advances in cell culture techniques now make it possible to subcultivate differentiated stratified squamous epithelium. By "lifting" such cultures to the air–liquid interface, uniform cultures develop with the morphological and antigenic characteristics of normal stratified squamous epithelium including the expression of PV antigen in a normal pattern.[36,51,55,56] Although this method of culturing stratified squamous epithelium cells has never been used for the study of PV, it is especially suitable for these studies. The absence of feeder cells in the system removes the complications associated with PA and PA inhibitors present in other cell types. Stratified squamous

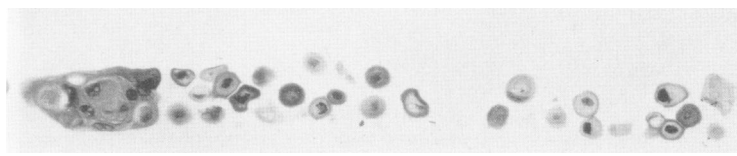


Figure 7. *Severe acantholysis in a culture treated with canine PV Ig.*

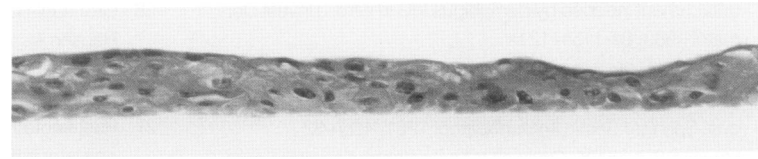


Figure 8. *Typical control culture treated with normal human Ig.*

epithelial cells grown in this manner reflect the morphologic, immunologic, and biochemical characteristics of stratified squamous epithelium, which is lacking in monolayer systems.[36] The viability of the cells distinguishes this system from explants.

Another difference in these studies and previous studies is the method of measuring and inhibiting cell associated PA. The BZIPAR method used here permitted the direct measurement of PA activity in the extracellular environment which is the critical parameter in PV. This two-step assay measures PA activity that represents the combined contributions of both PA and PA inhibitors. However, the assay does not indicate absolute amounts of PA and PA inhibitors nor does it indicate the type of PA or PA inhibitor present.

Both PA and PA inhibitors are present in the stratified squamous epithelium.[21-28,33,57] Previous studies indicate that keratinocytes can produce uPA and studies reported during the preparation of this manuscript indicate that under some circumstances keratinocytes produce tPA.[33,57] In particular, normal keratinocytes produce only uPA, but in certain pathological conditions and during wound healing, superficial keratinocytes produce tPA.[33] Jensen et al also have shown that tPA is present in lesional skin of some PV patients.[57] Although the source of the tPA in lesional skin in PV is not known, it may arise from the epidermis.[58] The placental PA inhibitor used in these studies has also recently been shown to be immunologically identical to native epidermal PA inhibitor.[59] Using the BZIPAR assay, it was possible to directly demonstrate that the PA activity in the extracellular environment of PV antibody treated keratinocytes was higher than that of cultures treated with normal Ig and that the PA inhibitor blocked this activity. Because the PA inhibitor also prevented acantholysis, these data strongly support a role of PA in the induction of acantholysis. The exact mechanism by which this occurs is not clear. How PV Ig alters PA and/or PA inhibitor levels is not determined by this assay and clearly requires further investigation. In addition, the mechanism by which the interaction of the PV antibodies with the PV antigen alters PA activity is unknown. Stanley et al have demonstrated that the human PV antigen is a polypeptide with a molecular weight of 210,000 with disulfide-linked chains with molecular weights of 130,000 and 85,000.[60,61] However, the functional characteristics of the PV antigen are unknown. The canine PV antigen has not been characterized but the constant cross reactivity of human PV antibodies and canine stratified squamous epithelia indicates significant similarities.[44]

The lack of dependence of acantholysis on the presence of plasminogen has been previously reported.[15] Recent evidence suggests that cells have receptors for both plasminogen and PA, however,[62,63] and it is possible that plasminogen from the serum may have remained associated with the cells at the time the antibodies and inhibitors were added in serum free medium. uPA is secreted by keratinocytes as an inactive precursor which is activated in the extracellular environment by proteases including plasmin. Thus, a role for plasmin in acantholysis in these studies cannot be ruled out. However, PA could also be directly responsible for the acantholysis because PA is known to directly cleave other biologically important proteins such as cell adhesion molecules.[64]

In conclusion, the role of PA in acantholysis was evaluated in an *in vitro* model of canine PV. In this system of fully differentiated oral keratinocytes, inhibition of PA activity was extremely effective in preventing acantholysis.

References

1. Ahmed AR: Pemphigus: Current concepts. Ann Intern Med 1980, 92:396-405
2. Jordon RE: Pemphigus, Dermatology in General Medicine: Textbook and Atlas. Edited by TB Fitzpatrick, AZ Eisen, K Wolff, IM Freedberg, KF Austen. New York, McGraw-Hill Book Co., 1979, pp 310-317
3. Scott DW, Lewis RM: Pemphigus and pemphigoid in dog and man: Comparative aspects. J Am Acad Dermatol 1981, 5:148-167
4. Scott DW, Wolfe MJ, Smith CA, Lewis RM: The comparative pathology of non-viral bullous skin diseases in domestic animals. Vet Pathol 1980, 17:257-281
5. Hurvitz AI: Animal model of human disease: Pemphigus vulgaris. Am J Pathol 1980, 98:861-864
6. Schiltz JR: Pemphigus acantholysis: A unique immunological injury. J Invest Dermatol 1980, 74:359-362
7. Anhalt GJ, Labib RS, Voorhees JJ, Beals TF, Diaz LA: Induction of pemphigus in neonatal mice by passive transfer of IgG from patients with the disease. N Engl J Med 1982, 306: 1189-1196
8. Anhalt GJ, Patel H, Diaz LA: Mechanisms of immunologic injury: Pemphigus and bullous pemphigoid. Arch Dermatol 1983, 119:711-714
9. Woo TY, Hogan VA, Patel H, Anhalt GJ, Labib RS, Voorhees JJ, Diaz LA: Specificity and inhibition of the epidermal cell

detachment induced by pemphigus IgG in vitro. J Invest Dermatol 1983, 81:115s–121s

10. Singer KH, Hashimoto K, Lazarus GS: Antibody-induced proteinase activation: Â proposed mechanism for pemphigus. Springer Semin Immunopathol 1981, 4:17–32
11. Morioka S, Naito K, Ogawa H: The pathogenic role of pemphigus antibodies and proteinase in epidermal acantholysis. J Invest Dermatol 1981, 76:337–341
12. Farb RM, Dykes R, Lazarus GS: Anti-epidermal-cell-surface pemphigus antibody detaches viable epidermal cells from culture plates by activation of proteinase. Proc Natl Acad Sci USA 1978, 75:459–463
13. Michel B, Schiltz JR: In vitro studies on the mechanism of acantholysis in pemphigus: Organ culture and cell suspension studies, Immunopathology of the Skin. Edited by EH Beutner, TI Chorzelski, SF Bean. New York, John Wiley and Sons, 1979, pp 231–242
14. Fabbri P, Lotti T, Panconesi E: Pathogenesis of pemphigus: The role of epidermal plasminogen activators in acantholysis. Int J Dermatol 1985, 24:422–425
15. Hashimoto K, Shafran KM, Webber PS, Lazarus GS, Singer KH: Anti-cell surface pemphigus autoantibody stimulates plasminogen activator activity of human epidermal cells: A mechanism for the loss of epidermal cohesion and blister formation. J Exp Med 1983, 157:259–272
16. Morioka S, Jensen PJ, Lazarus GS: The role of plasminogen and plasminogen activator in acantholysis in vitro. J Invest Dermatol 1984, 82:399
17. Saksela O: Plasminogen activation and regulation of pericellular proteolysis. Biochem Biophys Acta 1985, 823:35–65
18. Ossowski L, Quigley JP, Reich E: Plasminogen, a necessary factor for cell migration in vitro. Cold Spring Harbor Conf Cell Prolif 1975, 2:901–913
19. Reich E: Activation of plasminogen: A widespread mechanism for generating extracellular proteolysis, Biological Markers of Neoplasia: Basic and Applied Aspects. Edited by RW Ruddon. New York, Elesevier/North Holland, 1978, pp 491–500
20. Reich E: Activation of plasminogen: A general mechanism for producing localized extracellular protelysis, Molecular Basis of Biological Reproductive Processes. Edited by RD Berlin, M Hermann, IH Lepow, JM Tanzer. New York, Academic Press Inc., 1987, pp 155–169
21. Hashimoto K, Shafran KM, Webber PS, Lazarus GS, Singer KH: Plasminogen activator in cultured human epidermal cells. J Invest Dermatol 1983, 81:424–429
22. Birkedal-Hansen H, Taylor RE: Production of three plasminogen activators and an inhibitor in keratinocyte culture. Biochem Biophys Acta 1983, 756:303–318
23. Morioka S, Lazarus GS, Baird JL, Jensen PJ: Migrating keratinocytes express urokinase-type plasminogen activator. J Invest Dermatol 1987, 88:418–423
24. Isserhoff RR, Fusenig NE, Rifkin DB: Plasminogen activator in differentiating mouse keratinocytes. J Invest Dermatol 1983, 80:217–222
25. Hashimoto K, Nishioka K, Katayama I, Yoshikawa K: Secretion of plasminogen activator inhibitor by cultured human epidermal cells. Br J Dermatol 1986, 115:133–138
26. Grondahl-Hansen J, Nielsen LS, Kristensen P, Grondahl-Hansen V, Andreasen PA, Dan: K: Plasminogen activator in psoriatic scales is of the tissue-type PA as identified by monoclonal antibodies. Br J Dermatol 1985, 113:257–263
27. Hashimoto K, Katayama I, Nishioka K: Tissue plasminogen activator inhibitor in the epidermis. Br J Dermatol 1985, 113: 523–527
28. Morioka S, Jensen PJ, Lazarus GS: Human epidermal plasminogen activator: Characterization, localization, and modulation. Exp Cell Res 1985, 161:364–372
29. Swanson DL, Dahl MV: Inhibition of pemphigus acantholysis by methylprednisolone in vitro (abstr). Clin Res 1980, 280: 583A
30. Hashimoto K, Singer K, Lazarus GS: The effect of corticosteroids, dapsone and gold upon plasminogen activator synthesis and secretion by human epidermal cells cultured with pemphigus antibody. Br J Dermatol 1984, 110:293–297
31. Schiltz JR, Hu C, Michel B: Corticosteroids, aurothioglucose, and soybean trypsin inhibitor do not prevent pemphigus antibody-induced acantholysis in vitro. Br J Dermatol 1979, 101:279–284
32. Anhalt GJ, Patel HP, Labib RS, Diaz LA, Proud D: Dexamethasone inhibits plasminogen activator activity in experimental pemphigus in vivo but does not block acantholysis. J Immunol 1986, 136:113–117
33. Grondahl-Hansen J, Lund LR, Ralfkiaer E, Ottevanger V, Dano K: Urokinase and tissue type plasminogen activators in keratinocytes during wound reepithelization in vivo. J Invest Dermatol 1988, 90:790–795
34. Wilkinson JE, Smith C, Suter M, Lewis RM: Long term cultivation of canine keratinocytes. J Invest Dermatol 1987, 88: 202–206
35. Suter MM, Wilkinson JE, Greenberger L, Smith CA, Lewis RM: Monoclonal antibodies: Cell surface markers for canine keratinocytes. Am J Vet Res 1987, 48:1367–1371
36. Wilkinson JE, Lillie JH, Smith CA, Suter MM, Lewis RM: Morphologic and antigenic differentiation of canine keratinocytes grown on collagen gels and artificial matrices. J Invest Dermatol (Submitted for publication)
37. Ey PL, Prowse SJ, Jenkin CR: Isolation of pure IgG-1, IgG-2a, and IgG-2b immunoglobulins from mouse serum using protein A-sepharose. Immunochemistry 1978, 15:429–436
38. Leytus SP, Peltz GA, Liu HY, Cannon JF, Peltz SW, Livingston DC, Brocklehurst JR, Mangel WF: A quantitative assay for the activation of plasminogen by transformed cells in situ and by urokinase. Biochemistry 1981, 20:4307–4314
39. Peltz SW, Katzenellenbogen BS, Kneifel MA, Mangel WF: Plasminogen activators in tissues of the immature and estrogen-stimulated rat uterus and in the luminal fluid: Characterization and properties. Endocrinology 1983, 112:890–897
40. Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970, 227: 680–685
41. Towbin H, Staehelin T, Sedman D, Gordon J: Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. Proc Natl Acad Sci USA 1979, 76:4350–4354
42. Levin EG, Loskutoff DJ: Cultured bovine endothelial cells produce both urokinase and tissue type plasminogen activators. J Cell Biol 1982, 94:631–636

43. Loskutoff DJ, Van Mourik JA, Erickson LA, Lawrence D: Detection of an unusually stable fibrinolytic inhibitor produced by bovine endothelial cells. Proc Natl Acad Sci USA 1983, 80:2956–2960
44. Seiler RJ: Interspecies reactivity of human pemphigus antibody with canine pemphigus antigen (PhD Thesis). Ithaca, Cornell University, 1983
45. Sison-Fonacier L, Bystryn JC: Reagional variations in antigenic properties of skin: A possible cause for disease-specific distribution of skin lesions. J Exp Med 1986, 164:2125–2130
46. Stanley JR, Yuspa SH: Modulation of the synthesis of pemphigoid and pemphigus antigens by extracellular calcium concentration which regulate differentiation of epidermal cells. J Invest Dermatol 1983, 80:365
47. Bystryn JC, Rodriguez J: Absence of intercellular antigens in the deep layers of the epidermis in pemphigus foliaceus. J Clin Invest 1978, 61:339–348
48. Watt FM: Selective migration of terminally differentiating cells from the basal layer of cultured human epidermis. J Cell Biol 1984, 98:16–21
49. Wunschmann-Henderson B, Astrup T: Inhibition by hydrocortisone of plasminogen activator production in rat tongue organ cultures. Lab Invest 1974, 30:427–433
50. Holbrook HA, Hennings H: Phenotypic expression of epidermal cells in vitro: A review. J Invest Dermatol 1983, 81:11s–24s
51. Asselineau D, Bernard BA, Bailly C, Darmon M, Prunieras M: Human epidermis reconstructed by culture: Is it "normal"? J Invest Dermatol 1986, 86:181–186
52. Lillie JH, MacCallum DK, Jepsen A: Fine structure of subcultivated stratified squamous epithelium grown on collagen rafts. Exp Cell Res 1980, 125:153–165
53. Regnier M, Schweizer J, Michel S, Bailey C, Prunieras M: Expression of high molecular weight (67K) keratin in human keratinocytes cultured on dead de-epidermalized dermis. Exp Cell Res 1986, 165:63–72
54. Chapman SJ, Eady RA: Blistering in keratinocyte culture: A regular phenomenon associated with differentiation. Eur J Biochem 1985, 39:352–359
55. Asselineau D, Bernhard B, Bailey C, Darmon M: Epidermal morphogenesis and the induction of the 67kD keratin polypeptide by culture of human keratinocytes at the liquid-air interface. Exp Cell Res 1985, 159:536–539
56. Bernstam LI, Vaughan FL, Bernstein IA: Keratinocytes grown at the air-liquid interface. In Vitro Cell Dev Biol 1986, 22:695–705
57. Jensen PJ, Baird J, Morioka S, Lessin S, Lazarus GS: Epidermal plasminogen activator is abnormal in cutaneous lesions. J Invest Dermatol 1988, 90:777–782
58. Baird J, Lazarus GS, Belin D, Bosso N, Gubler P, Vassalli JD, Jensen PJ: mRNA for tissue-type plasminogen activator is detectable in psoriatic lesions (abstr). Clin Res 1988, 36: 631A
59. Hibino T, Izaki S, Ohkuma M, Kon S, Thorsen S, Stedt B: Epidermal plasminogen activator inhibitor (PAI) is immunologically identical to placental-type PAI-2. FEBS Lett 1988, 231:202–206
60. Stanley JR, Yaar M, Hawley-Nelson P, Katz SI: Pemphigus antibodies identify a cell surface glycoprotein synthesized by human and mouse keratinocytes. J Clin Invest 1982, 70: 281–288
61. Eyre RW, Stanley JR: Identification of pemphigus vulgaris antigen extracted from normal human epidermis and comparison with pemphigus foliaceus antigen. J Clin Invest 1988, 81:807–812
62. Plow EF, Freaney DE, Plescia J, Miles LA: The plasminogen system and cell surfaces: Evidence for plasminogen and urokinase receptors on the same cell type. J Cell Biol 1986, 103:2411–2420
63. Stoppelli MP, Corti A, Soffientini A, Cassani G, Blasi F, Assoian RK: Differentiation-enhanced binding of the amino-terminal fragment of human urokinase plasminogen activator to a specific receptor on U937 monocytes. Proc Natl Acad Sci USA 1985, 82:4939–4943
64. Quigley JP, Gold LI, Schwimmer R, Sullivan LM: Limited cleavage of cellular fibronectin by plasminogen activator purified from transformed cells. Proc Natl Acad Sci USA 1987, 84:2776–2780