Pemphigus Vulgaris Acantholysis Ameliorated by Cholinergic Agonists

Vu Thuong Nguyen, PhD; Juan Arredondo, PhD; Alexander I. Chernyavsky, PhD; Mark R. Pittelkow, MD; Yasuo Kitajima, MD, PhD; Sergei A. Grando, MD, PhD, DSc

Background: Pemphigus vulgaris (PV) is an autoimmune, IgG autoantibody-mediated disease of skin and mucosa leading to progressive blistering and nonhealing erosions. Patients develop autoantibodies to adhesion molecules mediating intercellular adhesion and to keratinocyte cholinergic receptors regulating cell adhesion.

Observations: To determine whether a cholinergic agonist can abolish PV IgG-induced acantholysis, litter mates of neonatal athymic nude mice were injected with PV IgG together with carbachol (0.04 µg/g body weight). None of these mice developed skin lesions. Through in vitro experiments, we measured the expression of adhesion molecules in monolayers of normal human keratinocytes incubated overnight in the presence of 0.25mM carbachol using semiquantitative Western blot and immunofluorescence. Carbachol caused an elevation of the relative amount of E-cadherin in keratinocytes (P < .05) without changing that of plakoglobin (P > .05). The phosphorylation level of E-cadherin and plakoglobin was increased by PV IgG, whereas this effect of PV IgG was attenuated in the presence of 0.5mM carbachol. Pyridostigmine bromide, an acetylcholinesterase inhibitor, produced effects similar to those of carbachol, which helps explain its clinical efficacy in a patient with active PV that was resistant to treatment with systemic glucocorticosteroids. Treatment with pyridostigmine bromide (360 mg/d) in a patient with PV allowed to keep his disease under control at a lower dose of prednisone than that used before starting pyridostigmine bromide treatment.

Conclusion: Elucidation of the cholinergic control of keratinocyte adhesion merits further consideration because of a potential for the development of novel antiacantholytic therapies using cholinergic drugs.

Arch Dermatol. 2004;140:327-334

Pemphigus Vulgaris (PV) is an autoimmune-mediated disease of skin and mucosa leading to progressive blistering and nonhealing erosions. Therapy for patients with PV relies on the long-term use of systemic glucocorticosteroids in relatively large doses, which, although lifesaving, may cause severe adverse effects, including death. Active disease state is characterized by the presence of serum IgG autoantibodies binding to the keratinocyte cell membrane proteins. Although the antiacantholytic effect of glucocorticosteroids is attributed to immunosuppression, high doses of glucocorticosteroids can directly block PV IgG–induced acantholysis in vitro and rapidly (within 48 hours) stop blistering in patients with pemphigus without altering the titer of autoantibodies or blocking antibody binding to keratinocytes (“pulse therapy”). Patients develop autoantibodies to keratinocyte cholinergic receptors regulating cell adhesion. Activation of these receptors mimics antiacantholytic effects of glucocorticosteroids in vitro. We report that stimulation of the keratinocyte cholinergic receptors controls PV IgG–induced acantholysis in neonatal mice and ameliorates the natural course of disease in a patient with PV. Cholinergic agonists stimulated expression of E-cadherin and abolished phosphorylation of E-cadherin and plakoglobin in keratinocytes caused by PV IgG. Thus, novel antiacantholytic therapies may be developed based on the antiacantholytic effects of cholinomimetic drugs.

METHODS

PASSIVE TRANSFER OF PV

Neonatal athymic nude mice were used to test direct antiacantholytic effects of cholinergic agonists. At the third day of life, athymic nude mice weigh approximately 1.5 g and can develop gross skin blisters on passive transfer of PV antibodies. This study had been approved by the University of California Davis Review Committee on the Use of Animals in Research, Sacramento, Calif. We injected 52 mice intraperitoneally through a 30-gauge needle with PV (ex-
nification cross sections of each mouse body were placed on the glass slide may vary between different anatomical regions. Three 6-µm thick region stemmed from the fact that acantholysis in neonatal mice animals were cross-sectioned at the umbilicus level and freshly fro-
disease (ie, approximately 20 hours after the injection). The ani-
chrest from the stimulation of ACh release and inhibition of
acetylcholinesterase (AChE). To determine whether a
pharmacologic stimulation of keratinocyte acetylcholine (ACh) axis may be a novel antiacan-
lymphocyte or plakoglobin (dilution, 1:1000) or with an-
tripled from Sigma Chemical Co, St Louis, Mo.
assess the extent of acan-
photography by measuring the length of the in-
traepidermal split (at least 4 basal cells long) and expressed the results as percentage of the total length of epidermis in the field, taken as 100%.

IMMUNOHISTOCHEMISTRY
Direct and indirect immunofluorescence assays were per-
formed as detailed elsewhere. For the direct immunofluores-
cecence assay, a tissue specimen was incubated for 1 hour at room temperature with a fluorescently labeled goat antihuman IgG an-
tibody (Pierce, Rockford, Ill; dilution 1:200 in PBS). For the im-
direct immunofluorescence experiments, a tissue substrate was first treated with a primary antibody to E-cadherin or plakoglo-
bin (both from BD Transduction Laboratories, Lexington, Ky; dilution, 1:200) and then exposed to a secondary fluorescently labeled antibody (Pierce). Semiquantiative indirect immu-
nofluorescence assay was performed as detailed previously,10,11 us-
ing a computer-assisted image analysis with a software package purchased from Scanalytics (Fairfax, Va). For each tissue spec-
imen, a minimum of 3 different segments in at least 3 different microscopic fields were analyzed and the results compared.

WESTERN BLOT
Immunoblotting was performed as detailed by us previ-
ously. Briefly, monolayers of normal human keratinocytes were either untreated (control) or incubated overnight with 0.25mM carbachol or 0.25mM pyridostigmine bromide at 37°C. They were then washed and lysed, and the lysates were resolved by a 7.5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis, followed by electroblotting. The membranes were blocked and stained with a primary antibody to either E-cadherin (dilution, 1:2000) or plakoglobin (dilution, 1:1000) or with an-
tibody to β-actin (Sigma Chemical Co; dilution, 1:2000) to stan-
dardize the measurements. Binding of the primary antibody was visualized with horseradish peroxidase–conjugated secondary antibodies and developed with the chemiluminescence method of the ECL+Plus system (Amersham Pharmacia Bio-
tech, Inc, Piscataway, NJ). The results were expressed as ac-
tual densitometry values of each protein band representing an adhesion molecule.

PHOSPHORYLATION ASSAY
Quantitative phosphorylation assay was designed based on the established protocols, as detailed elsewhere.13 Briefly, cul-
tured DJM-1 cells were grown to approximately 75% conflu-
ence in 75-cm² flasks (Corning Costar, Cambridge, Mass) in serum-free keratinocyte growth medium (Clonetics Corp, San Diego, Calif) containing 0.09mM Ca²⁺ at 37°C in a humid 5% carbon dioxide incubator. The cells were then treated for 14 hours with phosphate-free minimal essential medium containing 1.8mM Ca²⁺ and 5% dialyzed fetal calf serum (Gibco BRL) and then treated with the same medium containing 200 µCi/mL of ³²P orthophosphate (Amersham Pharmacia Biotech, Inc). The cells were labeled for 8 hours and then exposed for 1 hour to 1 mg/mL of pooled normal human IgG (Sigma Chemical Co) or PV IgG pooled from 5 PV sera samples in the absence or pres-
ence of 0.5mM carbachol or pyridostigmine bromide. After in-
cubation, the cells were lysed and the lysates were cleared by centrifugation at 14000g for 10 minutes. The adhesion mol-
ecules under consideration were immunoprecipitated, re-
solved by 7.5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis, electroblotted, and analyzed with the Phos-
phorImager feature of the Storm system (Molecular Dynam-
ics, Mountain View, Calif). To determine protein concentra-
tion of each precipitated adhesion molecule, the samples were analyzed by Western blotting, as described above. The results were expressed as ratios of the radioactivity value of each ad-
hesion molecule to its protein quantity in each sample, com-
pared with the ratios obtained in control cultures (taken as 1.00).

STATISTICS
The results of quantitative experiments were expressed as mean ± SD. Significance was determined using the t test. The results were deemed significant if the P value was less than .05.

REPORT OF A CASE
A recently reported case of PV that had improved by ciga-
rette smoking14 and a study showing successful use of nico
tinamide as a steroid-sparing agent in pemphigus,15 sug-
gested that pharmacological regulation of keratino-
cyte acetylcholine (ACh) axis may be a novel antiacan-
tholytic therapy for pemphigus because (1) cigarette smoke contains the cholinomimetic agent nicotine and (2) nicotinamide exhibits cholinomimetic effects16 owing to the stimulation of ACh release17 and inhibition of acetylcholinesterase (AChE).18 To determine whether a pharmacologic stimulation of keratinocyte cholinergic recep-
tors can be used as a steroid-sparing regimen in the treat-
ment of pemphigus, we administered pyridostig-
mime bromide (Mestinon; ICS Pharmaceuticals, Costa Mesa, Calif; 60-mg tablets) to a patient with active PV at the dose of 360 mg/d. The use of Mestinon in a patient with PV had been approved by the University of Cali-
fornia Davis Human Subjects Review Committee. This patient, an 82-year-old white man, had been treated for almost 8 years with a mid-dose of prednisone, ranging from 15 to 30 mg/d, and occasional intraleisonal corti-
coosteroid injections. He had a recalcitrant erosion on his nose, which would never completely heal and would be-
come active (ie, turn red and/or get painful, enlarge in size, and produce exudate). No other lesions were seen. The lesion began to improve starting from the third week of treatment with pyridostigmine bromide. After 2 months of treatment with pyridostigmine bromide, the patient’s
condition dramatically improved (Figure 1). The dose of pyridostigmine bromide was then decreased to 300 mg/d. The patient was treated with pyridostigmine bromide for an additional 3 months. Occasional redness and/or itching, burning, or tingling sensations of the skin lesion could be alleviated by increasing the pyridostigmine bromide dose from 300 to 360 mg/d without changing the dose of prednisone. While receiving pyridostigmine bromide treatment, the daily dose of prednisone was tapered to 10 mg. Further decrease of prednisone dose was associated with a flare of his skin lesion. No other therapy, except for prednisone tapering, was used. The abdominal skin was then examined by light microscopy and direct immunofluorescence, revealing intraepidermal epidermal staining consistent with binding of PV IgGs to murine keratinocytes, respectively. None of the 4 negative control mice that were injected with pooled normal human IgG at the daily dose of 10 mg/g of body weight for 2 days developed any gross or microscopic signs of pemphigus or showed any deposition of human IgGs in their skin during 40 hours of observation. To standardize assessment of the extent of acantholysis, we computed the areas of intraepidermal splitting in the images of skin harvested from the euthanized mice at the umbilical level 20 hours after the injection. The results showed that in mice treated with PV IgG alone, the acantholysis extended to 73.4%±11% of the epidermis (Table). To determine whether a cholinergic agonist can abolish PV IgG–induced acantholysis, litter mates of the athymic nude mice were injected with the same dose of PV IgG together with carbachol (0.04 µg/g body weight). In addition to being a mixed, nicotinic, and muscarinic agonist, carbachol is also a reversible AChE inhibitor. We chose carbachol because in the past this cholinomimetic agent has been shown to antagonize PV IgG–induced acantholysis in keratinocyte monolayers. In contrast to mice in the positive control group, none of the mice injected with PV IgG together with carbachol developed any visible skin lesions 40 hours after the first injection (Figure 2B). At this point, our most vigorous efforts to induce Nikolskiy sign failed in the skin of 4 of 7 mice in this sub-

RESULTS

EXPERIMENTAL AMELIORATION OF PV IgG–INDUCED ACANTHOLYSIS

To induce experimental PV, for 2 consecutive days we injected intraperitoneally 3-day-old athymic nude mice with IgG fraction (7 mg/g of body weight per day) pooled from the sera of 5 patients with acute PV containing anti-desmoglein 1 and anti-desmoglein 3 antibodies. Gross skin blisters developed approximately 40 hours after the first injection of PV IgG (Figure 2A). At the onset of skin blistering, the Nikolskiy sign representing a loss of intraepidermal cohesion could be elicited in mice by applying lateral traction with a pencil eraser to the skin. When blisters became generalized, the mice were euthanized. The abdominal skin was then examined by light microscopy and direct immunofluorescence, revealing intraepidermal clefting due to extensive acantholysis and intercellular epidermal staining consistent with binding of PV IgGs to murine keratinocytes, respectively. None of the 4 negative control mice that were injected with pooled normal human IgG at the daily dose of 10 mg/g of body weight for 2 days developed any gross or microscopic signs of pemphigus or showed any deposition of human IgGs in their skin during 40 hours of observation. To standardize assessment of the extent of acantholysis, we computed the areas of intraepidermal splitting in the images of skin harvested from the euthanized mice at the umbilical level 20 hours after the injection. The results showed that in mice treated with PV IgG alone, the acantholysis extended to 73.4%±11% of the epidermis (Table). To determine whether a cholinergic agonist can abolish PV IgG–induced acantholysis, litter mates of the athymic nude mice were injected with the same dose of PV IgG together with carbachol (0.04 µg/g body weight). In addition to being a mixed, nicotinic, and muscarinic agonist, carbachol is also a reversible AChE inhibitor. We chose carbachol because in the past this cholinomimetic agent has been shown to antagonize PV IgG–induced acantholysis in keratinocyte monolayers. In contrast to mice in the positive control group, none of the mice injected with PV IgG together with carbachol developed any visible skin lesions 40 hours after the first injection (Figure 2B). At this point, our most vigorous efforts to induce Nikolskiy sign failed in the skin of 4 of 7 mice in this sub-

Figure 1. Clinical results in a patient with pemphigus vulgaris treated with pyridostigmine bromide (Mestinon; ICS Pharmaceuticals, Costa Mesa, Calif). A, Before pyridostigmine bromide treatment; B, 2 months later. Prior to starting treatment with pyridostigmine bromide, this patient took prednisone at the dose of 20 mg/d. Pyridostigmine bromide was administered at the daily dose of 360 g. While the patient was receiving pyridostigmine bromide treatment, the dose of prednisone was decreased to 10 mg/d.
group. Microscopic examination of the skin from euthanized mice, however, was performed 20 hours after the injection because at this time visible skin lesions were also absent in the positive control mice. Compared with an extensive intraepidermal splitting seen in the positive control litter mates (Figure 2C), skin samples from carbachol-treated mice showed only limited areas of epidermal acantholysis (Figure 2D). Morphometric analysis revealed that administration of carbachol resulted in a significant (P<.001) decrease of the extent of epidermal splitting from approximately 73% to 37% (Table).

Since epidermal keratinocytes synthesize and release ACh, which serves as an endogenous agonist of both the nicotinic and muscarinic classes of cholinergic receptors expressed in keratinocytes, we sought to determine whether increasing the level of free ACh in the epidermis can ameliorate the signs of experimental pemphigus. Toward this goal, we injected a group of mice with PV IgG

### Results of Microscopic Analysis of Skin in Control and Experimental Mice

<table>
<thead>
<tr>
<th>Treatment (Control/Experimental)</th>
<th>No. of Mice</th>
<th>Morphometric Assay of the Extent of Acantholysis, %</th>
<th>Semiquantitative Assay of Epidermal IgG Binding (Relative Values)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PV IgG/PV IgG + cholinomimetic carbachol</td>
<td>4/7</td>
<td>73.4 ± 11/36.8 ± 2.4</td>
<td>67.0 ± 9.3/106.87 ± 7.9</td>
</tr>
<tr>
<td>PV IgG/PV IgG + pyridostigmine bromide</td>
<td>4/7</td>
<td>78.2 ± 1.9/31.3 ± 7.2</td>
<td>116.8 ± 7.6/188.9 ± 7.3</td>
</tr>
</tbody>
</table>

*Data are mean ± SD value in control/experimental mice. The extent of acantholysis and the intensity of epidermal staining for human IgG were estimated 20 hours after a single injection of pemphigus vulgaris (PV) IgG with or without test drug. P<.001 for all results.*

Figure 2. Carbachol inhibits pemphigus vulgaris (PV) IgG–induced skin blistering of 3-day-old athymic nude mice. A, Positive control: a representative 3-day-old homozygous athymic nude mouse 40 hours after injection of 7 mg/g of body weight per day of PV IgG showing gross skin blistering. B, Experiment: a representative mouse of the same progeny after injection of 7 mg/g body weight per day of PV IgG together with 0.04 µg/g of body weight per day of carbachol. Note lack of gross skin blistering. C, Positive control: extensive acantholysis in murine epidermis 20 hours after injection of PV IgG alone (hematoxylin-eosin, scale bar=100 µm). D, Experiment: limited acantholysis in murine epidermis 20 hours after injection of PV IgG together with 0.04 µg/g body weight per day of carbachol (hematoxylin-eosin, scale bar=100 µm). E and F, Direct immunofluorescence staining of the epidermis in positive control (E) and experimental (F) mice 20 hours after injection of 7 mg/g body weight per day of PV IgG together with 0.04 µg/g body weight per day of carbachol, using fluorescein isothiocyanate–labeled goat antihuman IgG antibody (scale bar=50 µm). Note that the pemphiguslike epidermal staining is present in both cases.
together with the AChE inhibitor pyridostigmine bro-
mide$^{25}$ (0.1 µg/g body weight). The protective effect of pyri-
dostigmine bromide on PV IgG–induced acantholysis was
found to be similar to that of carbachol. The gross skin le-
sions did not appear, Nikolskiy sign was negative, and the
extent of acantholysis in the skin of mice treated with pyri-
dostigmine bromide decreased significantly ($P < .001$)
compared with the positive control litter mates (Table).

Since patients with PV develop antibodies to kerati-
nocyte ACh receptors, along with autoantibodies to des-
mosomal cadherins (reviewed by Grando$^6$), one of the
hypothetical mechanisms that could explain the antiacan-
tholytic effect of carbachol and pyridostigmine bromide
(which, similar to carbachol, can act directly on ACh re-
ceptors in addition to reversibly inhibiting AChE)$^{26}$ was the
direct competition of these drugs with PV IgG for binding
to keratinocytes. To test this hypothesis, we measured the
intensity of fluorescent staining of the epidermis of mice
injected with PV IgG alone (positive control) or together
with carbachol or pyridostigmine bromide (experiment),
using fluorescein isothiocyanate–labeled goat antihuman
IgG antibody (Figure 2E and F). Quantitative analysis of
the intensity of specific staining showed an increased
amount of PV IgG in the epidermis of cholinergic agonist-
treated mice compared with that determined in positive con-
trol mice (Table), indicating that steric hindrance could not
account for the antiantholytic effect of carbachol and pyri-
dostigmine bromide. An unexpected increase of the inten-
sity of fluorescent staining of the epidermis in experimen-
tal mice could be explained through a hypothesis that
cholinergic agonists up-regulated expression of keratino-
cyte adhesion molecules targeted by PV IgG.

UP-REGULATION OF E-CADHERIN EXPRESSION

To investigate molecular mechanism(s) of antiacantho-
lytic action of cholinergic agonists, we measured the ex-
pression of adhesion molecules in monolayers of normal
human keratinocytes incubated overnight (control) (A) or with 0.25 mM carbachol (B) or pyridostigmine bromide (C). After
incubation, keratinocyte monolayers were washed and immunostained. The images were analyzed using software for semiquantitative image analysis.

![Figure 3. Fluorescence images of E-cadherin (scale bar=50 µm). Results of semiquantitative indirect immunofluorescence analysis of the relative amounts of E-cadherin in cultured human foreskin keratinocytes incubated overnight without (control) (A) or with 0.25 mM carbachol (B) or pyridostigmine bromide (C). After incubation, keratinocyte monolayers were washed and immunostained. The images were analyzed using software for semiquantitative image analysis.](http://archderm.jamanetwork.com/pdfaccess.ashx?url=/data/journals/derm/11761/)

![Figure 4. Cholinergic effects on the expression of adhesion molecules by keratinocytes. The results are expressed as the relative amounts of fluorescence intensity expressed by experimental vs nontreated cells (control). Error bars indicate SD.](http://archderm.jamanetwork.com/pdfaccess.ashx?url=/data/journals/derm/11761/)
Figure 5. Cholinergic effects on the synthesis of adhesion molecules in keratinocytes. Triplicate monolayers of normal human foreskin keratinocytes were treated overnight with 0.25 mM carbachol or pyridostigmine bromide at 37°C, after which the total protein was isolated and measured, as detailed in the “Methods” section. Identical amounts of proteins from treated and nontreated control cultures were separated by 7% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and electroblotted. Each membrane was stained with a primary antibody to E-cadherin, plakoglobin, or β-actin (to standardize the measurements). The numbers represent mean ± SD densitometry values of each protein band. Asterisks indicate significant (P < .05) differences from control.

Figure 6. Cholinergic effects on pemphigus vulgaris (PV) IgG–induced phosphorylation of keratinocyte adhesion molecules. The levels of phosphorylation of E-cadherin and plakoglobin in cultured DJM-1 cells exposed for 1 hour to 1 mg/mL of normal human IgG (N IgG) or 1 mg/mL of PV IgG in the absence or presence of 0.5 mM carbachol or pyridostigmine bromide. Each adhesion molecule was immunoprecipitated by the specific monoclonal antibody, resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and electroblotted. The amount of phosphorus 32 incorporated by each adhesion molecule was assayed by autoradiography. Parallel samples were analyzed by Western blot (WB), as described in the “Methods” section. The protein bands were visualized to determine the protein amount of each adhesion molecule in the sample. The results are expressed as ratios of the radioactivity value of each adhesion molecule to its protein quantity in each lane, compared with the ratios obtained in control cultures (taken as 1.00).}

This study demonstrates that activation of the keratinocyte ACh axis can ameliorate pemphigus acantholysis and up-regulate the expression of adhesion molecules and protect them from PV IgG–induced phosphorylation. The knowledge on the pathophysiology of acantholysis converges with that on the physiology of keratinocyte adhesion. We hypothesized that a nonsteroidal treatment of pemphigus can be achieved by pharmacologically interceding at the site of intracellular biochemical events that mediate the acantholytic effects of pemphigus autoantibodies, and studied the immunopharmacology of pemphigus IgG action on keratinocytes. In the past, we reported that PV IgG–induced phosphorylation of keratinocyte adhesion molecules could be abolished in the presence of the corticosteroid methylprednisolone. Increased phosphorylation of desmoglein 3 in pemphigus may lead to the formation of desmoglein 3–depleted desmosomes and altered adhesion. In addition to the phosphorylation of desmoglein, desmocollin, and desmoplakin, assembly and disassembly of desmosomal junctions also involves phosphorylation of the keratin–vimentin-intermediate filaments (reviewed by Eriksson et al). For instance, while phosphorylation of classic cadherins on tyrosine disables the adherence-type junctions, leading to cell-cell detachment, experimentally inhibiting tyrosine-specific phosphatases results in a major changes in cell morphology, as manifested by a rapid rounding up of the cells, followed by reorganization of the cell monolayer. A list of known targets for pemphigus antibodies includes both adhesion molecules (eg, desmogleins 1, 2, and 3, desmocollins, and plakoglobin) and the receptor molecules (FceRIα, α3 and α9 nicotinic ACh receptor subunit, pemphaxin, and other annexins) (reviewed by Grando). Anti-ACh receptor antibodies are found in pa-

©2004 American Medical Association. All rights reserved.
patients with PV or pemphigus foliaceus, ACh signaling in keratinocytes linked to the regulation of expression and function of adhesion molecules and cholinergic drugs affects cell shape, adhesion, and cytoplasmic motility (reviewed by Grando). Because of the lack of a strong correlation between the clinical phenotype of PV and the presence of anti–desmoglein 1 and 3 antibodies and since PV-like lesions can be induced in neonatal mice in the absence of these antibodies, the immunopathogenesis of PV can be explained through the “multiple hit” hypothesis. We propose that acantholysis in PV results from synergistic and cumulative effects of autoantibodies targeting keratinocyte cell membrane antigens of different kinds, including (1) molecules that regulate cell shape and adhesion (eg, ACh receptors) and (2) molecules that mediate cell-to-cell adhesion (eg, desmosomal cadherins). Severity of the disease and exact clinical picture depend on the ratio of different kinds of autoantibodies in each particular patient. Antibodies to ACh receptors can weaken desmosomal junctions by inducing phosphorylation of adhesion molecules, cause desmosome shedding owing to the apoptosis-related cleavage of desmosomal cadherins, and prevent desmosomal reassembly owing to the activation of the proteolytic cascade. In turn, the binding of pemphigus IgG to desmosomal cadherins may prevent formation of new desmosomes because it blocks the extracellular domains of desmogleins mediating homophilic adhesion.

We have previously reported that PV IgG–induced acantholysis can be treated in culture with cholinergic agonists. While glucocorticosteroids or protease inhibitors can only block, but not reverse, acantholysis, cholinomimetics are the only drugs capable of reversing PV IgG–induced acantholysis. The cholinergic effects on cell adhesion observed in cell monolayers have been corroborated by results showing enlargement of the intercellular space between keratinocytes in the epidermis treated with the nicotinic antagonist tubocurarine and since PV IgG in the epidermis of mice treated with cholinergic agonists. Indeed, we have recently found that ACh receptor types expressed in keratinocytes (eg, α9 ACh–gated ion channels) has been reported to induce phosphorylation of the cell membrane–associated proteins, suggests that cholinomimetics ameliorated acantholysis in keratinocytes exposed to PV IgG by inhibiting phosphorylation-mediated alterations in the assembly and disassembly of intercellular attachment units. Additionally and/or alternatively, activation of the keratinocyte ACh axis with cholinomimetic drugs could up-regulate expression of adhesion molecules targeted by pemphigus antibodies, as suggested by increased binding of PV IgG in the epidermis of mice treated with cholinergic agonists. Therefore, we have recently found that ACh receptors expressed by keratinocytes couple a signaling pathway leading to activation of the adhesion molecules that mediate intercellular attachment of these cells. The expression of desmogleins 1 and 3 was increased in keratinocytes treated with carbachol or pyridostigmine bromide. Therefore, we speculate that pemphigus acantholysis in the skin of patients with PV could be treated by pharmacologically stimulating keratinocyte ACh axis. Elucidation of the cholinergic control of keratinocyte adhesion has a potential for the development of treatment regimens using safer drugs to control blistering in a variety of other skin diseases. First results of the clinical trial of pyridostigmine bromide in patients with pemphigus have been recently published.

Accepted for publication July 2, 2003.

This work was supported by a research grant from the Robert Leet & Clara Guthrie Patterson Trust, Hartford, Conn (Dr Grando), and by the International Pemphigus Research Fund, Sacramento, Calif.

This study was presented in part at the Fourth Joint Meeting of the European Society for Dermatological Research, Japanese Society for Investigative Dermatology, and Society for Investigative Dermatology, May 4, 2002, Miyagi, Japan, and was published in the form of an abstract (Grando SA, Arredondo J, Chernyavsky A, Pettitkow MR, Kitajima Y, Nguyen VT. Cholinergic stimulation inhibits pemphigus IgG–induced acantholysis and ameliorates clinical disease in a patient with pemphigus vulgaris. J Invest Dermatol. 2003;121[1]:abstract 42).

Corresponding author and reprints: Sergei A. Grando, MD, PhD, DSc, Department of Dermatology, University of California Davis Medical Center, 4860 Y St, Room 3400, Sacramento, CA 95817 (e-mail: sagrando@ucdavis.edu).