New Approaches to the Treatment of Pemphigus

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In pemphigus vulgaris, treatment with systemic glucocorticosteroids is life saving; it may, however, cause severe side effects, including death. A patient with pemphigus vulgaris and myasthenia gravis was treated for approximately five years with the cholinomimetic Mestinon (pyridostigmine bromide), Imuran (azathioprine), and a topical corticosteroid gel before the need to introduce systemic glucocorticosteroids. Because activation of keratinocyte acetylcholine receptors also has been shown to abolish pemphigus IgG–induced acantholysis in cultured keratinocyte monolayers, a clinical trial of Mestinon was initiated in patients with active pemphigus vulgaris, pemphigus foliaceus, and paraneoplastic autoimmune multiorgan syndrome (also known as paraneoplastic pemphigus). First results indicate that nonsteroidal treatment of pemphigus is possible. Mestinon may be used to slow down progression of the disease and to treat mild cases with chronic lesions on limited areas. Stimulation of the keratinocyte– acetylcholine axis may lead to a therapeutic effect through any of the following mechanisms: (1) stimulating keratinocyte cell-to-cell attachment; (2) accelerating reepithelialization; and (3) competing with the disease-causing pemphigus antibodies, preventing them from attachment to keratinocytes. Glucocorticosteroids and various types of steroid-sparing drugs used to treat pemphigus exhibit cholinergic side effects, including effects on expression and function of keratinocyte adhesion molecules, that are very similar to those produced by the cholinomimetic drugs. Further elucidation of the mechanisms underlying therapeutic efficacy of antiacantholytics may shed light on the immunopharmacological mechanisms of pemphigus antibody–induced acantholysis. ***Key words: acantholysis/acetylcholine receptor/keratinocyte/Mestinon/myasthenia gravis/pemphigus vulgaris. J Investig Dermatol Symp Proc 9:84–91, 2004***

The major objective of pemphigus research is development of a safer treatment regimen. Pemphigus patients need drugs that can replace the glucocorticosteroid hormones (GS) that many of them must take for life. GS therapy is life saving, but patients suffer from severe side effects and complications. Alternative therapies that foster keratinocyte adhesion and/or specifically antagonize the effects of pemphigus antibodies are desperately needed. In the past, I developed treatment modalities (Grando, 1988; Grando *et al*, 1989d; Grando *et al*, 1990; Grando, 1992) that, in addition to GS, included: (1) *ex-vivo* filtration of patients' blood through carbon adsorbent, followed by plasmapheresis and administration of cytostatic drugs, to eliminate disease-causing pemphigus autoantibodies and suppress the synthesis of new ones; (2) antiproteases, antikinins, and antileukotrienes (quercetin, aprotinin, and ε-aminocaproic acid) to inactivate mediators of inflammation present in pemphigus blister fluid (Grando *et al*, 1987; Grando *et al*, 1989a; Grando *et al*, 1989c); and (3) doxycycline to suppress the cell-mediated autoimmunity component of pemphigus' immunopathogenesis (Grando *et al*, 1989b). Unfortunately, none of these approaches allowed complete replacement of GS in pemphigus patients. Recent research results, however, suggest that novel antiacantholytic therapies may be developed by mimicking the antiacantholytic effects of GS with nonsteroidal drugs acting at the acetylcholine receptors (AChR) expressed by keratinocytes (Grando *et al*, 2001). Keratinocyte adhesion is controlled, in part, by acetylcholine (ACh), a cytokine-like chemical (i.e., a cytotransmitter) that is locally produced by keratinocytes (reviewed in Grando, 1997). ACh and its congeners (i.e., cholinomimetic drugs) can reverse pemphigus antibody–induced acantholysis both *in vitro* (Grando and Dahl, 1993) and *in vivo*.[1] Taking into consideration that GS can prevent but not reverse pemphigus IgG-induced acantholysis (Swanson and Dahl, 1983), these observations suggest that cholinomimetic drugs might be a novel and more efficient treatment for pemphigus. A case of pemphigus vulgaris (PV) that improved by cigarette smoking (Mehta and Martin, 2000), studies showing negative correlation between smoking and pemphigus (Brenner *et al*, 2001), and successful use of nicotinamide as a steroid-sparing agent in pemphigus (Chaffins *et al*, 1993) hint that this expectation is realistic for the following reasons: (1) cigarette smoke contains cholinomimetic nicotine; and (2) nicotinamide exhibits cholinomimetic effects (Romanenko, 1987) due to both stimulation of ACh release (Koeppen *et al*, 1997) and inhibition of acetylcholinesterase (AChE) (Stoytcheva and Zlatev, 1996). The rationale behind the use of cholinergic drugs also stems from data demonstrating involvement of keratinocyte AChR in pemphigus pathophysiology (reviewed in Grando, 2000). On these premises, I hypothesized

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Abbreviations: ACh, acetylcholine; AChE, acetylcholinesterase; AChR, acetylcholine receptor; ChAT, choline acetyltransferase; GS, glucocorticosteroid hormone; mAChR, muscarinic acetylcholine receptor; MG, myasthenia gravis; nAChR, nicotinic acetylcholine receptor; PAMS, paraneoplastic autoimmune multiorgan syndrome; PV, pemphigus vulgaris.

[1]Nguyen VT, Grando SA: Novel animal model for testing antiacantholytic treatments of pemphigus. *J Invest Dermatol* 117:543, abstr. #919, 2001

that cholinomimetics should abort or alleviate pemphigus. This therapeutic approach should be successful even if keratinocyte AChR are not targeted by disease-causing pemphigus antibodies in a particular patient, because ACh and its muscarinic and nicotinic congeners can accelerate the rate of keratinocyte migration, thus fostering re-epithelialization of pemphigus erosions (Grando, 1997). As a test of this hypothesis, an open clinical trial of a nontoxic AChE inhibitor, Mestinon (pyridostigmine bromide), was initiated approximately two years ago in the dermatology clinic of the University of California at Davis. In the clinical trial, Mestinon was chosen as a systemic cholinomimetic drug because an index patient with both PV and myasthenia gravis (MG) had successfully treated her pemphigus for almost five years, without using systemic GS, by manipulating her daily dose of Mestinon from 180 to 360 mg, taking Imuran (azathioprine) and applying a glucocorticoid gel to fresh lesions.

RESULTS

The results of the clinical trial reported herein were obtained in eight patients with active pemphigus without MG, including all three major clinical forms of autoimmune pemphigus. The diagnosis of pemphigus was based on the results of comprehensive clinical and histological examinations, together with immunological studies, following standard protocols (Beutner *et al*, 1985). This study had been approved by the University of California, Davis, Human Subjects Review Committee.

Treatment of PV without systemic GS in the index patient The index patient was a 39-year-old Ashkenazi Jewish female with both PV and MG. At the age of 18, she developed arthralgias and was given the diagnosis of lupus erythematosus on the basis of positive results of ANA, nDNA and VDRL antibody tests and LE cell preparations. She was treated with NSAIDs until the symptoms resolved by the age of 20 and the above tests became normal. After that, she had two pregnancies that resulted in miscarriages during the first trimester due to large subchorionic hemorrhages associated with borderline titers of anticardiolipin antibodies. A third pregnancy was complicated by another large subchorionic hemorrhage in the first trimester, but the pregnancy continued and resulted in a normal vaginal delivery by induction. At the age of 30, she developed symptoms of nasal speech at five months into the next pregnancy. Three weeks prior to delivery of the second child, she developed ptosis of the left eyelid. Three days after delivery, her speech became much more slurred and she developed difficulty in deep breathing. The finding of an anti-AChR antibody at the titer of 25 and the results of a Tensilon test confirmed the clinical diagnosis of MG. A chest CT scan revealed a hyperplastic thymus, but was negative for thymoma. She was started on 60 mg Mestinon three times a day. Initial improvement was followed by an increase of muscular weakness, difficulty swallowing, and worsening of slurred speech. At the age of 31, she had a thymectomy, which was preceded by five sessions of plasmapheresis. The postoperative course was relatively uncomplicated. Since then, she has been controlling her myasthenia symptoms with daily doses of Mestinon ranging between 120 and 210 mg.

At the age of 33, this patient developed the initial symptoms of PV. She first noticed occasional bleeding and inflammation of her gums that did not disturb her much. Approximately one year later, multiple white and ulcerated areas on gingival crest and crevice, which bled readily, were found during an annual dental checkup. Superficial mucosa could be wiped away, indicating the presence of a positive direct Nikolskiy sign (Grando *et al*, 2003). A crusted erosion of approximately 1×1 cm that bled easily upon mechanical stimulation was also found behind the right ear. She was referred to an oral surgeon, who made an incisional biopsy of the right posterior mandibular alveolar mucosa. The histopathological study revealed suprabasilar acantholysis consistent with PV. This clinical and histological diagnosis was confirmed by the results of immunological studies performed at the Beutner Laboratories (Buffalo, New York) during exacerbation of her skin disease. Intercellular antibodies were found in the perilesional skin by direct immunofluorescence, and low-titer intercellular antibodies were demonstrated by indirect immunofluorescence.

During the first four years, the course of PV in this patient was rather mild. One to two new lesions on oral mucosa and/or skin would develop monthly and heal quickly, either spontaneously or upon the use of topical GS. These symptoms developed in the background of a maintenance dose of Mestinon. At the age of 37, the patient developed a moderately severe exacerbation characterized by the appearance of approximately 50 small, 1–2-cm-diameter, bullous lesions on skin and oral mucosa soon after these preceding events: (1) five sessions of intravenous gammaglobulin injections over a period of one week in an attempt to improve myasthenia symptoms; (2) slight tapering of Mestinon because improvement of myasthenia had been achieved; and (3) severe emotional stress. Systemic GS were not initiated because of the threat of a myasthenic crisis. Instead, the patient was treated with plasmapheresis. For several years after that, in addition to the maintenance dose of Mestinon, she took Imuran at a daily dose of 150 mg and used 0.05% clobetasol propionate gel (Temovate) on new pemphigus lesions that occasionally appeared on her skin and oral mucosa. Her pemphigus antibody titer remained at 1/320, as determined by indirect immunofluorescence using monkey esophagus as a substrate. She infrequently developed side effects from the Mestinon, such as skin flushes, sweating, and diarrhea. Otherwise, she had a good quality of life. After approximately five years of treatment, the lesions on her skin and oral mucosa began to develop more frequently, which required initiation of prednisone therapy to control her PV. Currently, she takes 20 mg prednisone per day.

First results of clinical trial of mestinon in pemphigus patients The intriguing aspect of the management of the index patient was that conventional GS therapy was not instituted for the first five years of her disease. Although it is possible to maintain pemphigus patients in remission using immunosuppressive drugs without GS (Lever and Schaumburg-Lever, 1977; Lever and Schaumburg-Lever, 1984; Stemm and Thivolet, 1995), initial treatment of PV relies on systemic GS in a high dose (Holubar and Fellner, 1986; Muller and Stanley, 1990; Carson *et al*, 1996). To the best of my knowledge, neither Imuran alone nor Imuran in combination with plasmapheresis and/or Temovate gel has ever been reported to allow complete avoidance of systemic GS at the initial stage of pemphigus treatment. Therefore, I considered Mestinon as a therapeutic agent that ameliorated the natural course of disease in this patient, and I initiated an open clinical trial. An overall goal was to evaluate the efficacy of Mestinon in terminating the spread of pemphigus erosion and in fostering re-epithelialization of already existing lesions. Both new patients with pemphigus who had not received GS and established patients with disease exacerbation on the background of immunosuppressive therapy were enrolled. Patients with generalized, life-threatening forms of disease whose well being might be jeopardized by any delay in initiating systemic GS therapy were excluded from the study, as were children less then than 16 years of age, pregnant women, and nursing mothers.

During approximately two years of the clinical trial, eight patients with active pemphigus used Mestinon for at least four weeks (**Table 1**). They took Mestinon tablets at a total daily dose of 360 mg. Three PV patients (patients 1, 6, and 8) and a patient with paraneoplastic autoimmune multiorgan syndrome [(PAMS; also known as paraneoplastic pemphigus (Nguyen *et al*, 2001)] showed a very good response (the patients are hereafter referred to as the responders). The other three PV patients and one pemphigus foliaceus patient showed no significant improvement. Among the responders, two PV patients (patients 1 and 6)

Table 1. Summary of the Results of Open Trial of Mestinon in Pemphigus Patients[a]

Patient	Age/Sex	Diagnosis	IF tests	Nikolskiy sign	Prior systemic therapy	Systemic treatment during the trial	Outcome of the trial	Systemic treatment after the trial
1	45/M	PV	positive	positive	GS , IS	none	permanent remission	taper Mestinon over 3 months
2	64/F	PV	positive	positive	GS, IS	GS	no improvement	GS , IS
3	38/M	PV	positive	positive	GS, IS	GS (lower dose)	no improvement	GS, IS
4	33/M	PF	ND	positive	GS	GS (lower dose)	no improvement	GS, IS
5	65/M	PAMS	positive	negative	GS, IS	none	temporary remission	GS (lower dose); Mestinon
6	53/F	PV	positive	positive	none	none	improvement	Mestinon
7	51/F	PV	positive	positive	GS, IS	GS, IS	no improvement	GS , IS
8	82/M	PV	positive	ND	GS, IS	GS (lower dose)	improvement	GS (lower dose); Mestinon

[a]Abbreviations: GS, glucocorticosteroid hormones; IF, direct and/or indirect immunofluorescence; IS, immunosuppressors; ND, not done; PAMS, paraneoplastic autoimmune multiorgan syndrome; PF, pemphigus foliaceus; PV, pemphigus vulgaris.

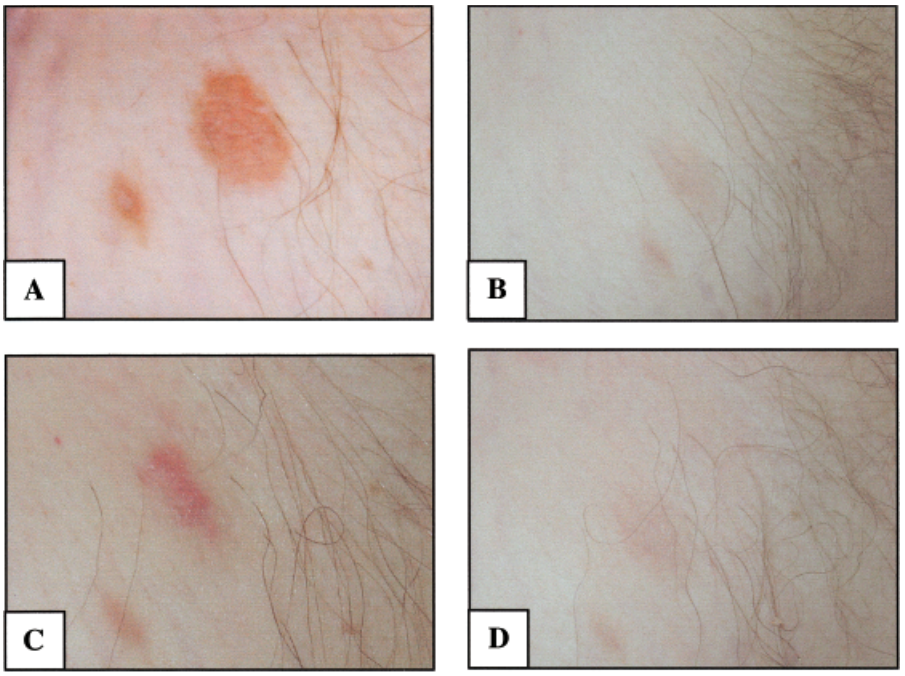

Figure 1. Treatment of Pemphigus Vulgaris with Mestinon. (A) Before treatment. (B) Three months on Mestinon. (C) Three weeks after discontinuing Mestinon. (D) One month after restarting Mestinon.

and the PAMS patient were able to control their disease using Mestinon alone. One responder demonstrated a direct strong interrelationship between the use of Mestinon and the ability to control his PV (patient 1; **Fig 1**). After achieving stable control, he discontinued Mestinon. Approximately two weeks later, he reported redness and itching/burning sensations at the sites of the fully healed pre-existing lesions, which were followed by microvesiculation and advert lesional weeping. At this point, Mestinon was restarted. Within several days, the progression of the lesions aborted and the erosions began to dry. It took about three weeks for the lesions to completely heal, after which the patient slowly tapered his Mestinon daily dose to zero. He has remained free from lesions for almost 18 months without any need of medication for pemphigus. This patient provides a "proof of concept" case of the efficacy of Mestinon in pemphigus because he showed (1) rapid improvement of his disease at the time the drug was taken, although this might be attributed to a coincidental spontaneous improvement of the disease; (2) rapid exacerbation of PV after the drug had been abruptly discontinued; and (3) rapid reversal of the flare-up of his PV after the drug had been restarted.

DISCUSSION

Possible mechanisms of the therapeutic efficacy of Mestinon in pemphigus The first results of the clinical trial of Mestinon are encouraging, indicating that this cholinomimetic can be used to slow down progression of disease in patients with acute pemphigus and to treat mild cases with limited areas of nonhealing erosions. No controls needed to be allocated to standard therapy because the success of the new treatment could be judged against the well-known standard prognosis. The course of pemphigus is well characterized, and it is well known that practically all pemphigus patients relapse if systemic GS are withdrawn during the disease's active phase (Herbst and Bystryn, 2000). In a classic example, Dr. Morton established the efficacy of anesthesia by demonstrating that one anesthetized patient felt none of the excruciating pain that had invariably accompanied surgery. Therefore, the "proof of concept" result with Mestinon in the PV patient shown in **Fig 1** indicates that an approach to the treatment of pemphigus that employs cholinomimetics is practical. The obtained results do raise a question, however: How can Mestinon suppress acantholysis in pemphigus? The answer may provide a new lead in solving the pemphigus enigma.

It is well known that Mestinon increases tissue levels of endogenous ACh because of the reversible inhibition of AChE that hydrolyzes ACh (Taylor, 1985). Keratinocytes actively metabolize ACh, employing the synthesizing enzyme choline acetyltransferase (ChAT) and the degrading enzyme AChE, and they use ACh as an autocrine and paracrine hormone or cytotransmitter (reviewed in Grando, 1997). Additionally, Mestinon can act directly on keratinocyte AChR because it has

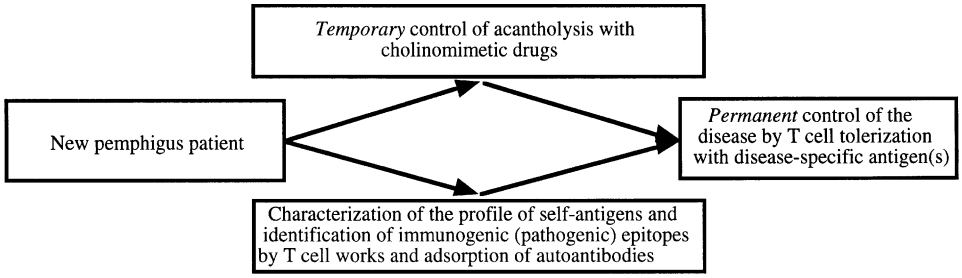

Figure 2. Hypothetical plan of treatment of pemphigus in the post-corticosteroid era.

been shown to interact directly with cholinergic receptors as a weak agonist capable of inducing desensitization, both alone and when combined with ACh (Akaike *et al*, 1984). Human epidermal keratinocytes express both the ACh-gated ion channels—that is, the neuronal-type nicotinic AChR (nAChR), which can comprise α3, α5, α7, α9, α10, β2, and β4 subunits (Grando *et al*, 1995a; Grando *et al*, 1996; Nguyen *et al*, 2000a; Nguyen *et al*, 2000b; Sgard *et al*, 2002)—and G protein–coupled muscarinic AChR (mAChR) of the M_1, M_3, M_4, and M_5 subtypes (Ndoye *et al*, 1998). Oral keratinocytes also express the M_2 mAChR subtype, but lack M_1 (Arredondo *et al*, 2003). Mestinon can interact with the ACh-ionic channel complex, blocking it in open conformation, via at least three distinct, although possibly interacting, mechanisms: (1) a weak agonist action; (2) the formation of desensitized receptor-complex intermediates; and (3) the alteration of the conductance properties of active channels (Albuquerque *et al*, 1984; Pascuzzo *et al*, 1984).

As will be detailed below, stimulation of the keratinocyte ACh axis with Mestinon might lead to a therapeutic effect in pemphigus through any one or a combination of the following mechanisms: (1) stimulating keratinocyte cell-to-cell attachment; (2) promoting faster re-epithelialization; and (3) competing with the disease-causing pemphigus antibodies, preventing them from attachment to keratinocyte AChR.

ACh as a cytotransmitter regulating keratinocyte adhesion
Mestinon might intercede at the intracellular signaling pathway that mediates the acantholytic effects of pemphigus antibodies. The binding of pemphigus IgG to keratinocytes leads to acantholysis through activation of a biochemical cascade that involves activation of phospholipase C, production of inositol 1,4,5-trisphosphate, Ca^{2+} influx and rapid transient increase of intracellular Ca^{2+}, changes in the intracellular cAMP/cGMP ratios, and activation and translocation of protein kinase C from the cytosol to the particulate/cytoskeleton fractions (Grando *et al*, 1988; Esaki *et al*, 1995; Lyubimov *et al*, 1995; Seishima *et al*, 1995; Osada *et al*, 1997). The other messenger systems used by ACh are the same as those used by pemphigus IgG (Grando *et al*, 1988; Esaki *et al*, 1995; Lyubimov *et al*, 1995; Seishima *et al*, 1995; Osada *et al*, 1997). In effect, activation of keratinocyte AChR restores normal morphology of pemphigus IgG–treated acantholytic keratinocytes in cultures (Grando and Dahl, 1993). Therefore, a signal sent by ACh through activating keratinocyte AChR can override the signal evoked by pemphigus antibody binding to keratinocytes.

It has been convincingly demonstrated that nicotinic and muscarinic drugs exhibit dramatic effects on cell-to-cell and cell-to-substrate cohesion of human epidermal and oral keratinocytes (Grando and Dahl, 1993; Grando *et al*, 1993; Grando *et al*, 1995a; Nguyen *et al*, 2000a; Nguyen *et al*, 2000b). Blocking AChR with either muscarinic or nicotinic antagonists—atropine and mecamylamine, respectively—in both cases results in acantholysis in keratinocyte monolayers (Grando and Dahl, 1993; Grando *et al*, 1995a). Notably, systemic use of atropine has exacerbated skin and oral blistering in one of our patients with PV. Results obtained in our pilot studies strongly suggest that cholinergic control of keratinocyte adhesion is exerted through receptor-mediated modifications of both expression and phosphorylation of adhesion molecules.[2] For instance, we demonstrated that the acantholytic effect of atropine is associated with increased phosphorylation of cadherins in DJM-1 cell monolayers. Phosphorylation of classical and desmosomal cadherins is known to be involved in regulation of cell-to-cell adhesion (Parrish *et al*, 1990; Stappenbeck *et al*, 1994; Pasdar *et al*, 1995; Kowalczyk *et al*, 1999), and pemphigus IgG–induced acantholysis involves phosphorylation of desmoglein 3 and its dissociation from plakoglobin (Aoyama *et al*, 1999). Interestingly, ligation of α9 AChR has been reported to induce phosphorylation of cell membrane proteins with molecular weights of 120 and 220 kDa (Szonyi *et al*, 1999). These may represent adhesion molecules, such as the 120-kDa E-cadherin (Mareel *et al*, 1991) and the 220-kDa desmoplakin 2 (Joly *et al*, 1994). Thus, Mestinon might stimulate keratinocyte adhesion by activating classical and desmosomal cadherins, including effects on the phosphorylation status of an adhesion molecule.

ACh as a cytotransmitter regulating re-epithelialization
Re-epithelialization of pemphigus erosions is a self-sustained process that can be regulated by endogenously secreted mediators such as ACh. ACh can facilitate keratinocyte outgrowth in culture. Both muscarinic and nicotinic agonists produce a stimulatory effect on keratinocyte spreading and migration, whereas inhibiting ACh synthesis and blocking AChR abrogate lateral migration of human keratinocytes (Grando *et al*, 1993; Grando *et al*, 1995a). In neurons, too, ACh regulates the direction of nerve growth cone extension, and blocking ACh signaling inhibits nerve outgrowth (Zheng *et al*, 1994). To characterize cholinergic control of the metamorphosis of keratinocytes during wound healing, we developed an *in vitro* model of skin re-epithelialization that allows accurate evaluation of drug effects on lateral migration of keratinocytes (Grando *et al*, 1993). The cells exposed to carbachol, a muscarinic and nicotinic agonist and reversible AChE inhibitor, moved significantly farther compared to nonexposed keratinocytes.[3] The response to carbachol was dose dependent and was seen starting from the nanomolar concentrations of the drug. Both the nicotinic antagonist mecamylamine and the muscarinic antagonist atropine abrogated the carbachol-induced keratinocyte migration. Recent results obtained in *in vivo* skin-wounding experiments in AChR knockout mice indicate that M_4 mAChR plays a central role in mediating cholinergic control of keratinocyte migration by regulating integrin expression.[4] Therefore, the role of Mestinon as a stimulator of a basic regulatory pathway of keratinocyte migration that might help re-epithelialize pemphigus erosions merits further consideration.

[2]Grando SA, Arredondo J, Chernyavsky A, Kitajima Y, Nguyen VT: Mechanisms of pharmacologic regulation of keratinocyte adhesion by cholinergic drugs. *J Invest Dermatol* 119:225, abstr. #107, 2002

[3]Lee TX, Horton RM, Grando SA: Cholinergic drugs stimulate chemokinesis of human epidermal keratinocytes. *J Invest Dermatol* 106:841, abstr. #215, 1996

[4]Chernyavsky A, Arredondo J, Nguyen VT, Ndoye A, Zia S, Wess J, Grando SA: Molecular mechanisms of stimulatory effect of M_4 muscarinic acetylcholine receptor on keratinocyte migration. *J Invest Dermatol* 119:225, abtsr. #108, 2002

Table 2. Cholinergic "Side-effects" of Drugs Used to Treat Pemphigus

GS	*Via* their *genomic* effects, GS upregulate the cholinergic enzymes ChAT and AChE (Kaufman *et al*, 1988) [although this effect may vary depending on the cell type (Tria *et al*, 1992; Hortnagl *et al*, 1993)] and increase expression of both nAChRs and mAChRs (Ben-Baruch *et al*, 1981; Marquardt *et al*, 1982; Braun *et al*, 1993), which is proposed as a possible explanation for some of their therapeutic effects (Vilquin *et al*, 1992). The *non-genomic* effects of GS are mediated by virtue of their ability to attach in a non-competitive manner to a site of ACh-gated ion channels on the outer cell membrane and alter ACh-induced inward currents (Inoue and Kuriyama, 1989; Bouzat and Barrantes, 1993; Ke and Lukas, 1996; Nurowska and Ruzzier, 1996).
Cyclophosphamide	Behaves like a classic nicotinic cholinergic ligand, because it specifically binds to the ligand-binding sites of both the muscle- and the neuronal-types nAChRs (Minker and Blazso, 1987), but not mAChR (Peroutka, 1987), and also reversibly inhibits AChE activity in a dose-dependent manner (al-Jafari *et al*, 1995).
Cyclosporin	Augments synthesis of ACh (Esquifino *et al*, 1997), and also interferes with protein kinase C-mediated signal transduction from mAChR (Hoecker *et al*, 1994).
Gold	Auranofin and other gold-containing compounds inhibit ACh-mediated effects on non-neuronal cells (Ohlstein and Horohonich, 1989; Fontaine *et al*, 1991).
Nicotinamide	Increases tissue levels of choline [a metabolic precursor of ACh and pharmacologic agonist of AChRs [Sterz, 1986; Ulus, 1988]], leading to increased ACh release (Koeppen *et al*, 1996; Koeppen *et al*, 1997), and also regulates mAChR-coupled K^+ channel (Higashida *et al*, 1995; Higashida *et al*, 1996). Nicotinic acid (*syn:* niacin) acts as a competive inhibitor of AChE (Stoytcheva and Zlatev, 1996), whereas its ester exhibits an ACh-like effect on smooth muscle contraction (Winkelman *et al*, 1969).
Tetracyclines	Tetracycline, chlortetracycline, minocycline and doxycycline cause a concentration-dependent inhibition of ACh release (Anadon and Martinez-Larranaga, 1987).
Tranilast	Inhibits cholinergic neurotransmissions of guinea pig bronchial muscle *in vitro* (Kamikawa, 1989).
Aprotinin	Inhibits activity of AChE (Chasapakis *et al*, 1968)—the enzyme that hydrolyses not only ACh but also various peptides, just like a professional trypsin-like endopeptidase (Small *et al*, 1987).
Quercetin	Inhibits ACh release (Lutterodt, 1989).
Heparin	Enhances agonist binding to an inhibitory-type of mAChRs, due to disruption of the mAChR-G protein interactions (Wang *et al*, 1996), interferes with intracellular signalling from the stimulatory-type mAChR, due to inhibition of inositol 1,4,5-trisphosphate (Olianas and Onali, 1997), and also inhibits AChR aggregation (Hopf and Hoch, 1997) and solubilizes AChE from the cell membrane (Talesa *et al*, 1993).
Suramin	Competitive agonist of nAChRs (Henning *et al*, 1992).
Quinine	Causes a closed-channel block of nAChR (Ssieb *et al*, 1996), inhibits mAChR-induced K^+ currents (Chen *et al*, 1993), and also acts as both non-competitive inhibitor of AChE (Stoytcheva and Zlatev, 1996) and a high affinity competitive inhibitor of choline transport (Porter *et al*, 1992).
Strychnine	Specific pharmacologic ligand of the novel α AChR that was first found in rat (Elgoyhen *et al*, 1994), and then cloned by us from human keratinocytes (Nguyen *et al*, 2000b).
Arsenic compounds	Inhibit mAChRs (Fonseca *et al*, 1991) and both cholinergic enzymes, ChAT and AChE (Kobayashi *et al*, 1987; Sheabar and Yannai, 1989).

Immunopathological similarities of PV and MG Although experimental PV in neonatal mice can be induced with autoantibodies to the adhesion molecules desmoglein 1 and desmoglein 3 (Amagai *et al*, 1992; Arteaga *et al*, 2002), results obtained in my laboratory show that pemphigus symptoms can be induced in neonatal mice lacking desmoglein 3 with passive transfer of the PV IgG that lack desmoglein 1 antibody (Nguyen *et al*, 1998). The pool of disease-causing pemphigus antibodies includes the autoantibodies to keratinocyte AChR, which are found in approximately 85% of patients (Nguyen *et al*, 1998). The antigenic specificities of pemphigus antibodies include the novel human α AChR (Nguyen *et al*, 2000b), with mixed, nicotinic and muscarinic pharmacology (Elgoyhen *et al*, 1994), and pemphaxin, a human annexin that binds ACh (Nguyen *et al*, 2000c). Indeed, pemphigus antibodies have been shown to compete directly with a cholinergic radioligand, [^{3}H]atropine, for binding to keratinocytes (Grando and Dahl, 1993), indicating that binding of anti-AChR IgG to keratinocytes can produce an immunopharmacological effect.

We reported a pemphigus patient with MG who developed an autoantibody binding to keratinocyte α3 nAChR.[5] Myasthenia and pemphigus may therefore share a common immunopathological pathway. MG is caused by autoantibodies against the nAChR expressed at the neuromuscular junction to mediate neuromuscular transmission (reviewed in Conti-Tronconi *et al*, 1994). These antibodies are heterogeneous and can be detected in approximately 85% of patients (Tzartos *et al*, 1982). It is not uncommon to find in some myasthenic patients clinical manifestations of pemphigus (reviewed in Kaplan and Callen, 1983). In such patients, autoantibodies can be directed against both desmosomal and neuromuscular antigens (McKee *et al*, 1978; Beutner *et al*, 2002). Likewise, AChR accumulate at both desmosomal and neuromuscular junctions (Engel *et al*, 1977; Grando *et al*, 1995b). Recently, Dr. Beutner's group proposed that in pemphigus associated with malignancies, autoimmunity may serve primarily as a defense mechanism against such systemic complications, although some forms of it, such as the autoantibodies to AChR, can cause the death of patients with PAMS (Beutner *et al*, 2002). Approximately 70% of MG patients have thymitis, and approximately 10% develop thymoma (reviewed in Marx *et al*, 1992). Like myasthenia, pemphigus may be associated with a tumor of the thymus, and pemphigus, thymoma, and myasthenia may coexist in the same patient (reviewed in Younus and Ahmed, 1990; Sherer *et al*, 1997). This raises the possibility that two seemingly disparate clinical conditions such as PV and MG may have similar immunopathological mechanisms. In addition to mAChR (Maslinski

[5]Grando SA, George PM, Dahl MV, Conti-Tronconi BM: Antibody against keratinocyte nicotinic acetylcholine receptor in patient with coexistent pemphigus foliaceus, myasthenia gravis and thymoma. *J Invest Dermatol* 102:609, abstr. #511, 1994

et al, 1990; Rinner *et al*, 1990), the thymus expresses both the "muscle" α1 and the "neuronal" or "epithelial" α3, α5, α7, and β4 types of nAChR subunits (Wheatley *et al*, 1992; Navaneetham *et al*, 1997; Mihovilovic and Butterworth-Robinette, 2001). Therefore, these two autoimmune diseases might develop as a result of autoimmune responses triggered by autosensitization against the AChR expressed by the thymus. In myasthenia, such sensitization would focus on the AChR expressed by myocytes; in pemphigus, on the AChR expressed by keratinocytes. On the basis of this model, only those patients with thymoma/thymitis would be expected to develop a second or third disease whose antibodies are directed toward the epitope of thymic AChR shared by the AChR expressed in the muscle or skin. Future studies are needed to determine whether the keratinocyte self antigens targeted by autoantibodies in pemphigus patients with MG are the same as those targeted in patients with pemphigus without thymoma/thymitis and/or myasthenia.

CHOLINERGIC SIDE EFFECTS OF DRUGS USED TO TREAT PEMPHIGUS

The enigma of pemphigus stems from the fact that the doses of GS required to stop blistering, as well as to sustain remission in many patients, are usually much higher compared to those ordinarily used to control other autoimmune diseases (Myles and Daly, 1974). In pemphigus, GS may work by (1) inhibiting antibody synthesis; (2) suppressing inflammation, especially eosinophilic spongiosis; and (3) stopping acantholysis via direct pharmacologic effect on keratinocyte, given that the addition of GS to skin organ cultures treated with pemphigus antibodies prevents pemphigus IgG–induced acantholysis (Swanson and Dahl, 1983; Jeffes *et al*, 1984). The last of the three mechanisms just listed, reported independently by two different groups, deserves particular attention because the use of very large doses of methylprednisolone ("pulse therapy") suppresses pemphigus in patients within 48 hours (Werth, 1996), and it is believed that the therapeutic effect is mediated by a direct pharmacologic effect of GS on keratinocytes (Hashimoto *et al*, 1984). We recently reported that PV IgG and methylprednisolone exhibit reciprocal effects on the transcription, translation, and phosphorylation of keratinocyte adhesion molecules.[6] Methylprednisolone upregulated transcription of the genes encoding desmoglein 3, desmocollins, plakophilin, E-cadherin, *p*-cadherin, α-catenin, several protein phosphatases, protease inhibitors, and lipocortins, and also suppressed PVIgG-induced phosphorylation of adhesion molecules. Therefore, GS may block PVIgG-induced acantholysis via a complex of intracellular genomic and nongenomic events, some of which are also involved in mediating signaling from keratinocyte AChR. Further elucidation of the mechanisms underlying the therapeutic activity of GS and other drugs that have been or are successfully used to treat pemphigus may shed light on the pharmacologic mechanisms mediating pemphigus IgG–induced acantholysis.

Nonsteroidal treatments of pemphigus reported to date include the following drugs (in chronological order): quinine and strychnine (reviewed in Kartamyshev, 1949), organic arsenic compounds (Oppenheim, 1927), suramin (also known as germanin or nephuride) (Veiel, 1931), vitamin D (Ludy and DeValin, 1932), methotrexate (Lever and Goldberg, 1969), azathioprine (Wolff and Schreiner, 1969), cyclophosophamide (Krain *et al*, 1972), gold (Penneys *et al*, 1973), dapsone (Haim and Friedman-Birnbaum, 1978), heparin (Mashkilleyson, 1985), cyclosporine (Balda and Rosenzweig, 1986), quercetin and doxycyline (Grando, 1988), aprotinin and ε-aminocaproic acid (Grando, 1992), nicotinamide and tetracycline (Chaffins *et al*, 1993), minocycline (Sawai *et al*, 1995), p-aminomethylbenzoic acid (Dobrev *et al*, 1996), mycophenolate mofetil (Enk and Knop, 1997), and tranilast (Miyamoto and Takahashi, 1997). Surprisingly, a single mechanism of action common for GS and many of the above listed nonsteroid drugs is cholinergic activity (**Table 2**). Is this a mere coincidence, or does the pharmacologic modulation of keratinocyte ACh axis provide a common denominator of antiacantholytic action of these drugs?

The post-corticosteroid era in the treatment of pemphigus: it is possible The results of the clinical trial of Mestinon suggest that nonsteroidal treatment of PV patients can be achieved by pharmacologically stimulating keratinocyte cell-to-cell adhesion through the keratinocyte ACh axis. Future studies in this direction will create an opportunity for pemphigus patients to obtain safer treatment of their disabling condition. A successful GS-free treatment regimen should be able to efficiently block the intracellular signaling elicited by pemphigus antibody binding to keratinocytes. Both an immediate and a more distant solution to this problem should be sought. An immediate solution will be to identify a pharmacological substitute for, or an adjunct to, GS that can efficiently control acantholysis. A more distant solution will be to prevent acantholysis by inhibiting synthesis of disease-causing pemphigus antibodies. In addition to their use as a tolerogen for T cell tolerization in the future, the sequences of the pathogenic epitopes of self antigens can be used for *ex-vivo* selective immunoadsorption of disease-causing pemphigus antibodies from patients' blood. Thus, successful development of nonsteroidal treatment of pemphigus will animate patient management, as shown in Fig. 2.

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