Pemphigus Vulgaris IgG and Methylprednisolone Exhibit Reciprocal Effects on Keratinocytes*

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Pemphigus vulgaris (PV) is a life-threatening autoimmune disease of skin adhesion associated with IgG autoantibodies against keratinocytes (KC). Treatment of PV with systemic corticosteroids is life-saving, but the mechanism of the therapeutic action has not been fully understood. We have developed an animal model that demonstrates that methylprednisolone (MP) can block PV IgG-induced acantholysis, decreasing the extent of keratinocyte detachment in the epidermis of 3–5-day-old nude mice from 77.5 ± 0.6 to 24.1 ± 1.5% (p < 0.05). We hypothesized that in addition to immunosuppression, MP may exhibit direct anti-acantholytic effects in epidermis, and we compared the effects of PV IgG and MP on KC. The use of DNA microarray showed that PV IgG down-regulated and MP up-regulated expression of the genes encoding keratinocyte adhesion molecules, antigen-processing proteins, regulators of cell cycle and apoptosis, differentiation markers, Na+/K+-ATPase, protein kinases and phosphatases, and serine proteases and their inhibitors. Overall, PV IgG decreased transcription of 198 genes and increased transcription of 31 genes. MP decreased transcription of 14 genes and increased transcription of 818 genes. Specific effects of PV IgG and MP on keratinocyte adhesion molecules were further investigated by Western blot and immunofluorescence assays. By immunoblotting, MP increased the protein levels of E-cadherin and desmogleins 1 and 3 by 300, 180, and 40%, respectively. Specific staining of KC for E-cadherin and desmogleins 1 and 3 increased by 235, 228, and 148%, respectively. In addition, PV IgG increased the level of phosphorylation of E-cadherin by 42%, β-catenin by 37%, γ-catenin by 136%, and desmoglein 3 by 300%, whereas pretreatment with 0.25 mM MP abolished phosphorylation of these adhesion molecules. These results suggested that therapeutic effects of MP in PV include both the up-regulated synthesis and post-translational modification of the keratinocyte adhesion molecules.

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Pemphigus vulgaris (PV) is a life-threatening autoimmune disease of skin adhesion associated with IgG autoantibodies against human keratinocytes (KC), the epithelial cells comprising the superficial layer of the skin termed epidermis. Experimental results obtained in vitro, in skin organ culture or epidermal cell monolayer, and in vivo, in neonatal Balb/c mice, demonstrated that binding of pemphigus antibodies to the cell surface of KC causes these cells to separate from one another and round up (acantholysis) (1–3). Acantholysis leads to extensive intraepidermalclef ting and gross blisters and erosions of the skin and oral mucosa in patients with PV (4). Prior to the development of synthetic corticosteroid hormones (CH) in the late 1940s, PV was almost invariably a fatal illness (5). The prognosis of the disease has improved dramatically because of the introduction of glucocorticosteroid drugs in the treatment of pemphigus patients. Systemic administration of CH allows for establishing control of acantholysis in the skin and oral mucosa of the patient during the acute stage of the disease (6–8). The mortality rate now is at 5–15% (9, 10), and death is almost invariably due to the complications of glucocorticosteroid therapy (11–13). Thus, although modern treatment of pemphigus with CH is life-saving, the patients suffer from severe side effects and complications. Alternative therapies that foster keratinocyte adhesion and/or specifically antagonize the effects of pemphigus autoantibodies are desperately needed. A modified synthetic corticosteroid agent with a specific anti-acantholytic therapeutic action or a novel non-steroidal drug that mimics anti-acantholytic effects of CH should replace CH in future treatment of pemphigus. This goal cannot be achieved without a better understanding of the mechanism of therapeutic action of CH in pemphigus.

The enigma of glucocorticosteroid efficacy in pemphigus stems from the following facts: 1) although it is possible to maintain pemphigus patients in remission using immunosuppressive drugs without CH (14–16), initial treatment of PV relies on the high dose of systemic CH, up to 500–1000 mg of prednisone daily (“pulse therapy”) (14, 17); and 2) the doses required to stop blistering and the maintenance doses of CH in many patients are usually much higher compared with those ordinarily used to control other autoimmune diseases (18). There are several lines of evidence that high doses of systemic CH control acantholysis via actions that are not limited to the
immunosuppressive properties of these drugs but harbor direct anti-acantholytic effects on KC.

First, although the major decline in antibody titers occurs 3–4 weeks after glucocorticosteroid administration (19–21), clinical lesions in PV patients usually improve much more rapidly, within 24–48 h after initiation of a high dose, “pulse” therapy with methylprednisolone (MP) or dexamethasone, when the titer of pemphigus autoantibodies remains unchanged (22–26).

Second, local administration of glucocorticosteroid agents, such as a very potent topical corticosteroid 0.05% clobetasol propionate cream, reportedly can alone initially control cutaneous lesions in mild cases of PV (27).

It has been proposed that assembly and disassembly of desmosomal cadherins to phosphorylate desmoglein (Dsg) (36), desmocollin (37), classical cadherins are accumulated in the adherens junctions due to steric hindrance (42), binding of pemphigus autoantibodies to KC is not restricted to the desmosomal areas (43, 44). Acantholysis in both skin and organ cultures treated with pemphigus IgGs starts from intracellular desmosomal areas (43, 44). The enzyme-linked immunosorbent assay values ranged from 46 to 170 for anti-Dsg 1 antibody and 83–229 for anti-Dsg 3 antibody. The cut-off value was 20.

Animal Model for Quantitative Evaluation of the Potential Anti-acantholytic Effect of MP—Three-day-old littermates of the progeny of pairs of homozygous athymic nude mice breeders (The Jackson Laboratory, Bar Harbor, ME) were divided into two groups. The positive control pups were unmarked and injected intraperitoneally with 7 mg/g of body weight of PV IgG alone. Experimental pups were marked on their foreheads with permanent ink and injected with 15 μg/g of body weight of MP (Solumedrol; Pharmacia & Upjohn) 2 h prior to injecting PV IgG. A group of mice received a second dose of PV IgG together with MP on the 2nd day and were observed for development of gross skin lesions. Approximately 24 h after the first injection, the pups were euthanized using CO2 to quantify the extent of acantholysis microscopically. Euthanized animals were snap-frozen in liquid nitrogen cross-sectioned at the umbilicus level, and embedded into the OCT compound (Miles Scientific, Naperville, IL). Each block was coded with a number, stained by hematoxylin and eosin, and evaluated by light microscopy by an experimenter that was blind to the experimental conditions used. Five random microscopic fields in each skin section...
were captured at magnification ×10, using a Macintosh computer attached to an Axiovert 315 inverted microscope (Zeiss). The images were printed, and the extent of acantholysis was computed directly on the prints by measuring the length of the areas in the epidermis in which suprabasal cell detachment spread along more than 4 adjacent basal cells. In each image, the extent of acantholysis was expressed as the percent of the total length of the epidermis in the print. The extent of acantholysis was then computed for each animal, and the data are presented as mean ± S.D. At the end of experiments, the codes were broken so that the results could be related to each experimental condition used.

**DNA Microarray Assay**—The cultures of normal human KC were started from neonatal foreskins. The cells were isolated from the epidermis using collagenase and dispase (1.75–2 units/ml) in fibroblast-free keratinocyte growth medium (KGM) containing 0.09 mM Ca²⁺ in a humid 5% CO₂ incubator, and then incubated for additional 48 h in medium (control) or medium containing 0.25 mM MP (experiment), and incubated for 8 h at 37 °C in a humid 5% CO₂ incubator. The doses of PV IgG and MP used in this study were chosen based on results published previously (60) showing that similar concentrations of PV IgG induce reversible acantholysis in keratinocyte monolayers, and that the 0.25 mM concentration of MP inhibits pempigus acantholysis in skin cultures (28), respectively. The experiments were designed based on the results of pilot studies that revealed appreciable acantholysis in PV IgG-treated keratinocyte monolayers after 8 h of exposure and the absence of acantholysis in cultures pretreated with MP (data not shown). These cell cultures were then used to extract mRNA used in the DNA microarray assay, as well as total cell proteins used in WB assay described below. These experiments, as well as in vitro experiments, tested the ability of MP to block or attenuate rather than to reverse the effects of PV IgG, as CH do not reverse PV IgG-induced acantholysis. The DNA microarray assay was performed using the Affymetrix hU95Av2 arrays (Affymetrix, Inc., Santa Clara, CA) in accordance to the Affymetrix protocol. In order to identify genes with differential expression, the intergenic and U74A expression values were corrected by pooling RNA from different cell donors per each treatment condition (61). Keratinocyte mRNA was isolated using the OligoT Direct mRNA kit (Qiagen, Santa Clarita, CA), and double-strand cDNA was synthesized using the SuperScript Choice system (Invitrogen) primed with the T7-(dT)24 primer. Biotin-labeled cRNA was prepared using the SuperScript Choice system (Invitrogen), and then hybridized to Affymetrix hU95Av2 arrays using the Fluidics Station 400, and scanned using the ImageScaner feature of the Storm™ scanner (Amersham Biosciences) overnight. The membranes were scanned using the ImageQuant feature of the Storm™ scanner (Amersham Biosciences). The relative amount of protein in each band was determined following normalization based on the content of the housekeeping protein β-actin that was determined in each lane by the same technique using a monoclonal antibody from Sigma. Because CH do not change the cellular levels of β-actin, it is commonly used as a housekeeping protein in quantitative Western blot assays (65). In pilot studies, we did not see appreciable differences in cellular levels of β-actin due to MP exposures; therefore, we used it for normalization purposes only. The results were expressed as densitometry values of the protein bands representing adhesion molecules after normalization for protein concentration in each lane based on post-exposure staining. The specificity of staining was controlled in negative control experiments, in which the primary antibody was omitted.

**Quantitative Phosphorylation Assay**—Quantitative phosphorylation assay was designed based on the established protocols described elsewhere (51, 52, 66, 67). Briefly, the DJM-1 cells were grown to ~75% confluence in 75-cm² flasks in KGM containing 0.09 mM Ca²⁺ without hydrocortisone supplement (Clonetics, Walkerville, MD) at 37 °C in a humid 5% CO₂ incubator, and then incubated for additional 48 h in minimal essential medium containing 10% dialyzed fetal bovine serum, 1.8 mM Ca²⁺, and 10 ng/ml epidermal growth factor (all from Invitrogen). At this point, the culture medium was changed to phosphate-free minimal essential medium (without fetuin), and after 18 h of incubation, the cells were labeled with 100 μCi/ml [³²P]phosphate (Amersham Biosciences) overnight. The next morning, cells were exposed for 1 h at 37 °C to either 1 mg/ml pooled normal human IgG (Sigma) or 1 mg/ml PV IgG used in all other experiments described in this paper. Some cells were pretreated for 2 h with 0.25 mM MP before addition of PV IgG. The cultures were washed three times with cold Tris-NaCl buffer (20 mM Tris-HCl, 150 mM NaCl, pH 7.4), solubilized in SDS-PAGE sample buffer (29.2 mM Tris-HCl, 1% NaCl, 1% Triton X-100, 1% Nonidet P-40, 1 μg/ml aprotinin, 10 mM phenylmethylsulfonyl fluoride, 1 mM leupeptin and 5 mM dithiobis-nitrobenzoate (all from Sigma)) containing 1% SDS, and centrifuged at 18,000 × g at 4 °C for 20 min. The keratinocyte adhesion molecules
were immunoprecipitated (57) from the supernatants using primary monoclonal antibodies to Dsg 3, E-Cad, β-Cat, or γ-Cat and protein A-agarose (Sigma). The immune complexes were dissolved in a sample buffer (50 mM Tris-HCl, 10% glycerol, 2% SDS, and 1% β-mercaptoethanol (pH 6.8)), resolved by 7.5% SDS-PAGE, electroblotted, and analyzed with the PhosphorImager feature of the Storm™ system, as described by us previously (57). Both the amount of radioactivity in each band and the intensity of its staining by a specific antibody were analyzed using the ImageQuant analytic software (Amersham Biosciences). The results were expressed as ratios of the radioactivity value of each adhesion molecule to its protein quantity in each sample, compared with the ratios obtained in control cultures, and taken as 1.00.

Statistics—The results of quantitative experiments were expressed as mean ± S.D. Significance was determined using the Student’s t test.

RESULTS

Development of an In Vivo Model for Testing Clinical Efficacy of MP—Passive transfer of PV IgG to neonatal Balb/c mice caused gross and microscopic changes consistent with experimental pemphigus in vivo, as reported elsewhere (3, 57, 58). We first determined the dose of PV IgG appropriate for testing the clinical efficacy of MP. Whereas 7 of 8 mice injected with 7 mg of PV IgG/g/day survived for 2 days, 7 of 8 mice that received 10 mg of PV IgG/g/day died, suggesting that this relatively high total dose of PV IgG killed them. Therefore, the anti-acantholytic efficacy of MP was tested in mice injected with 7 mg of PV IgG/g/day. Simultaneous administration of 15 μg/g body weight of MP with 7 mg of PV IgG/g/day did not abolish appearance of gross skin blisters 24–30 h after injection (n = 8). Pretreatment of neonates with MP 2 h prior to PV IgG injection (n = 4) lessened the extent of skin blistering, compared with their non-treated littermates (n = 2). Skin blisters in mice pretreated with MP developed several hours later than in non-treated littermates. We speculated that skin of 1-day-old Balb/c mice might be either highly susceptible to acantholytic action of pemphigus antibodies or not yet sensitive enough to anti-acantholytic action of MP, or both.

We next tested 3-day-old Balb/c mice that weighed 2.5–3 g. In these older mice, the skin lesions were not observed 24 h after injection of PV IgG and MP, which allowed a second injection. Administration of MP prevented skin blistering in all mice (n = 8). However, gross blisters and Nikolskiy sign, (i.e. peeling off the normal-looking skin by lateral traction with a Q-tip (68)) did not develop in 5 of 9 control mice, suggesting that rapidly developing hair follicles reinforce epidermal integrity in older Balb/c mice. Therefore, we next tested hairless (nude) mice (below).

MP Inhibits PV IgG-induced Acantholysis in 3-Day-old Nude Mice—The control 3-day-old athymic nude mice received 7 mg/g body weight per day of PV IgG alone, and their littermates comprising the experimental group received the same dose of PV IgG together with 15 μg/g/day body weight of MP. Because no skin lesions could be observed on the following day in either group of mice, a second dose of PV IgG and MP was administered. The next day, 6 of 9 control mice developed skin blisters (Fig. 1A). The Nikolskiy sign could be induced in the skin of the remaining 3 mice in this group. In marked contrast, none of 8 mice of the experimental group developed any visible skin lesions (Fig. 1B), indicating that nude mice provided an appropriate in vivo model for testing anti-acantholytic activity of MP.

In a time course study with neonatal athymic nude mice, we determined that microscopic features of acantholysis can be observed in the epidermis of both control and experimental pups as early as the next day after the first injection of PV IgG. The skin of control mice showed extensive intraepidermal acantholysis (Fig. 1C), whereas the skin of experimental mice showed only limited areas of intraepidermal splitting (Fig. 1D). Therefore, the occasional presence of Nikolskiy sign in the skin of experimental mice (4 of 8) could be explained by exaggeration of the microscopic acantholytic changes by mechanical trauma. To standardize assessment of the extent of acantholysis, the intraepidermal splitting was measured in the skin of control and experimental mice in which we did not attempt to induce the Nikolskiy sign. Administration of MP resulted in a significant (p < 0.05) decrease of the extent of acantholysis from 77.5 ± 0.6% (n = 4), seen in control mice, to 24.1 ± 1.5% (n = 5), found in experimental mice.

One of the hypothetical mechanisms that could explain direct anti-acantholytic effect of MP was its competition with pemphigus antibodies for binding to KC. To test this hypothesis, we measured the intensity of fluorescent staining of the epidermis of control and experimental mice using FITC-labeled goat anti-human IgG antibody (Fig. 1, E and F). Both in control and experimental mice, direct IF assay revealed intercellular, pemphigus-like staining produced by deposition of PV IgG. Surprisingly, semi-quantitative analysis showed more than a 2-fold increase of the intensity of specific staining in the epidermis of mice treated with PV IgG and MP versus PV IgG alone, 186.0 ± 6.1 versus 80.3 ± 13.7, respectively (p < 0.05). Thus, steric hindrance at the keratinocyte cell membrane an-
tigen(s) could not account for direct anti-acantholytic activity of MP. An unexpected increase of the intensity of fluorescent staining of the epidermis in experimental mice might be explained through a hypothesis that MP up-regulated expression of adhesion molecule(s) targeted by PV IgG on the cell surface of murine KC.

Reciprocal Modulation of Keratinocyte Gene Expression by PV IgG and MP—The cRNA generated from mRNA isolated from the keratinocyte monolayers grown from 3 cell donors and chips that contained probes for more than 10,000 human gene transcripts were used to measure effects of treatments of KC with 1 mg/ml PV IgG versus 1 mg/ml normal IgG. In pilot studies, we determined that an 8-h incubation provided a sufficient amount of time to observe early acantholytic changes in PV IgG-treated keratinocyte monolayers. According to different reports, acantholysis can develop as early as several hours and as late as several days after exposure of KC to PV IgG (2, 50, 60, 69, 70), which may reflect the “strength” of PV antibody used.

Overall, PV IgG decreased transcription of 198 genes and increased transcription of 31 genes. MP decreased transcription of 14 genes and increased transcription of 818 genes. Among 93 genes down-regulated by PV IgG, 34 genes were up-regulated by MP. Only genes that have the Detection value above the arbitrary value of 50 and the FC value of 2-fold or more are listed in Table I. The reciprocal changes were observed in transcription of the genes coding for one-and-the-same proteins with known biological functions as follows: (i) keratinocyte adhesion molecules (e.g. Dsg 3 and periplakin); (ii) antigen processing/presentation (e.g. tapasin); (iii) regulators of cell cycle progression and apoptosis (e.g. p53<sup>onc</sup>); (iv) components of cell organization and structure (e.g. the differentiation markers cytokeratin 10, transglutaminase, and involucrin); (v) cell signaling proteins (e.g. Na<sup>+</sup>-K<sup>+</sup>-ATPase, a kinase, a phosphatase, and RhoE); (vi) enzymes involved in general cellular metabolism (e.g. acid ceramidase); (vii) serine proteases and their inhibitors; and (viii) tumor-associated antigens. In addition, both PV IgG and MP produced reciprocal effects, down-regulation and up-regulation, respectively, of different genes within the same functional group (Table I). These included classical cadherins and other types of adhesion molecules, such as desmoplakin, cytokeratins, integrins, transduction factors, cyclin D2, galectins, annexins, cytokines, proton ATPase, protein kinases and phosphatases, cellular enzymes, oncogenes, proteolytic enzymes, and receptors to endocrine and local hormones, and cytokines. The only gene that was up-regulated due to PV IgG treatment with the cut-off level of >2-fold was one encoding the collagenase.

MP modified cell response to PV IgG. Compared with the effect of PV IgG alone, the cells exposed to PV IgG in the presence of MP showed up-regulation of 6 genes and down-regulation of 7 genes, using the FC of >2 (Table II). Additionally, the MP-dependent modification of the cell response to PV IgG in the FC range from 1.5 to 2 included up-regulation of the gene transcription of putative G protein-coupled receptor (FC = 1.87), cytokeratin 13 (FC = 1.87), interleukin-1 receptor antagonist (FC = 1.62), and human small G protein (FC = 1.52) and down-regulation of human skin collagenase (FC = 1.52).

These screening experiments demonstrated that the biological effects of PV IgG and MP on KC are multifactorial, which might explain plethoric effects of both mediators in the skin (reviewed in Refs. 24, 58, 71, and 72). An increase in the expression of the cell adhesion genes suggested that MP stimulates expression and/or function of adhesion molecules in the epidermis and thus directly antagonizes the acantholytic activity of PV IgG. This hypothesis was further tested in a series of in vitro experiments measuring the expression of the keratinocyte adhesion molecules that are believed to play an important role in the pathophysiology of PV.

MP-induced Changes in the Expression of Adhesion Molecules in Keratinocyte Monolayers—To correlate changes in the transcription of genes encoding keratinocyte adhesion molecules with the amounts and distribution of these molecules in MP-treated KC, we used semi-quantitative WB and indirect IF assays. In these studies, in addition to Dsg 3, which showed up-regulation at the transcriptional level, we also investigated the effects of MP on other important adhesion molecules, Dsg 1, β-Cat, and γ-Cat. For WB assay, proteins were extracted from the same cultures that were used in the DNA microarray assay. For IF assay, the monolayers were grown in chamber slides and exposed to 0.25 mM MP overnight, to allow sufficient time for translocation of the adhesion molecules to the cell membrane compartment.

By WB, the protein level of E-Cad was increased approximately 300% in the cultures treated with MP, compared with non-treated control monolayers (Fig. 2A). Similarly, the relative amounts of the desmosomal cadherins Dsg 1 and Dsg 3 were increased by 180 and 40%, respectively. Statistical analysis of the densitometry values revealed that all these MP-induced changes significantly (p < 0.05) differed from controls. In contrast, the protein amounts of neither β-Cat nor γ-Cat significantly differ from the control values (p > 0.05) (Fig. 2A).

By indirect IF, we observed that due to treatment with MP, specific staining of KC for E-Cad, Dsg 1, and Dsg 3 increased by 235, 228, and 148%, respectively (p < 0.05), compared with control KC (Fig. 2B). Additionally, β-Cat staining increased by 313% (p < 0.05). These results were consistent with overexpression of both classical and desmosomal cadherins to the keratinocyte cell membrane in response to MP. The representative images of KC expressing adhesion molecules are shown in Fig. 2C.

An apparent discrepancy between degrees of increase of each particular adhesion molecule in KC treated with MP detected by two different techniques, WB and IF, could be explained, in part, through an hypothesis that in addition to its effects on the gene expression, the anti-acantholytic action of MP also involves post-translational modification of these molecules, such as via changes in their phosphorylation status. Phosphorylation has been recently recognized as a major factor in the regulation of intercellular adhesion (73), particularly in disassembly of keratinocyte intercellular junctions caused by PV IgG (51, 52).

Reciprocal Modulation of Phosphorylation of Keratinocyte Adhesion Molecules by PV IgG and MP—To elucidate further the molecular mechanism of action of PV IgG and MP on KC, we investigated their effects on the phosphorylation status of the keratinocyte adhesion molecules under consideration. In these series of experiments, we used the DJM-1 cutaneous squamous cell carcinoma cell line. This cell line features high levels of phosphorylation of adhesion molecules and, therefore, is customarily used in phosphorylation assays (52, 72, 74–76). As expected, treatment with PV IgG increased the level of phosphorylation of E-Cad by 42%, β-Cat by 37%, γ-Cat by 136%, and Dsg 3 by 300% (Fig. 3). In the presence of 0.25 mM MP, phosphorylation of these adhesion molecules was either abolished or completely blocked. Notably, in the presence of MP the phosphorylation level of E-Cad and β-Cat and γ-Cat decreased below the base line, i.e. by 84, 79, and 34%, respectively (Fig. 3). Taken together, these results indicated that both PV IgG and MP can alter adhesion of KC through a combination of their genomic and non-genomic effects that affected phosphorylation of adhesion molecules in these cells. Dsg 1 was excluded from phos-
Gene expression was determined in cultures of KC from three different donors treated with 1 mg/ml of PV IgGs versus 1 mg/ml normal IgG (PVIgG versus NIgG), or 0.25 mM MP versus no drugs control (MP versus C). The DNA microarray data were analyzed using the MicroArray Suite software version 5.0, as described under “Experimental Procedures.” The Fold change (FC) values were calculated from the Signal log ratio, i.e. FC = $\frac{\text{Signal log ratio}}{H_{11005}}$. The results are expressed as both the FC value and the direction of the Change, i.e. an increase (I), a decrease (D), or no change (NC) in gene transcription. The genes are grouped in accordance to the known general function of the relevant proteins. LDL, low density lypoprotein; IL, interleukin; TNF, tumor necrosis factor; INF, interferon; MAP, mitogen-activated protein.

| Accession no. | Common gene name | Known function | PVIgG vs. NIgG | MP vs. C |
|---------------|------------------|----------------|----------------|--------|
| Reciprocal effects of PV IgG and MP | | | 2.5 D | 4.3 I |
| M76482 | Desmoglein 3 | Adhesion | | |
| AF001691 | Periplakin | Same | 2.6 D | 3.0 I |
| AF029750 | Tapasin (NGS-17) | Antigen processing | 2.1 D | 3.2 I |
| AI002619 | β2-Microglobulin | Same | 2.6 D | 2.8 I |
| U93106 | p53-activated fragment-1 (WAF1) | Apoptosis/Cell cycle | 2.5 D | 2.6 I |
| AF001294 | IPl protein (TSSC5) | Same | 2.6 D | 3.7 I |
| X14487 | Cytokeratin 10 | Cell organization and structure | 16.0 D | 2.8 I |
| X07696 | Cytokeratin 15 | Same | 4.9 D | 3.2 I |
| M22299 | T-plastin | Same | 2.1 D | 4.0 I |
| M21302 | Small protein-rich protein (sprII) | Same | 2.3 D | 2.8 I |
| 98447 | Transglutaminase | Same | 2.5 D | 3.7 I |
| M13903 | involucrin | Same | 2.6 D | 3.0 I |
| X81637 | clathrin | Same | 2.0 D | 2.5 I |
| M38690 | CD9 antigen | Cell regulation and signaling | 2.6 D | 3.7 I |
| M30448 | Casein kinase II β subunit same | Same | 2.1 D | 2.6 I |
| NM004417 | CL100, Tyr/Thre-protein phosphatase | Same | 2.6 D | 2.8 I |
| U16799 | Na⁺,K⁺-ATPase | Same | 3.2 D | 3.0 I |
| NCSA2240 | RhoE | Same | 3.5 D | 2.3 I |
| M92943 | Zinc finger transcriptional regulator | Same | 2.6 D | 2.4 I |
| D38305 | Tob (Transducer of Erb B-2) | Same | 2.8 D | 3.7 I |
| L00352 | LDL receptor | Metabolism | 2.5 D | 3.5 I |
| M20681 | Glucose transporter-like protein-III | Same | 2.5 D | 3.2 I |
| U92315 | Hydroxysteroid sulfotransferase | Same | 2.6 D | 3.7 I |
| D50840 | Ceramide glucosyltransferase | Same | 2.5 D | 4.6 I |
| U70063 | Acid ceramidase | Same | 2.5 D | 12.1 I |
| M94856 | Fatty acid-binding protein | Same | 2.5 D | 3.5 I |
| L42403 | Ataxia-telangiectasia group | Same | 2.5 D | 3.0 I |
| L33404 | Stratum corneum chymotryptic enzyme | Proteolysis | 6.0 D | 3.5 I |
| AB012917 | Serine protease (TTLSP) | Same | 3.7 D | 4.6 I |
| NM002773 | Prostasin (serine protease) | Same | 2.6 D | 4.0 I |
| AF027866 | Megsin (serine protease inhibitor) | Same | 2.1 D | 3.5 I |
| U08839 | Urokinase-type plasminogen activator receptor | Same | 2.8 D | 3.2 I |
| NM0008114 | Cathepsin C (cyteine proteinases) | Same | 2.3 D | 4.9 I |
| J04152 | GAT35-1 protein | Tumor-associated antigen | 2.3 D | 3.7 I |
| U43916 | Tumor-associated membrane protein | Same | 3.2 D | 3.2 I |
| Unilateral effects of PV IgG | | | 2.5 D | NC |
| AL031058 | Desmoplakin | Adhesion | 2.0 D | NC |
| X56841 | HLA-E | Antigen processing | 2.1 D | NC |
| U39819 | Apoptosis-associated protein (GADD34) | Apoptosis/cell cycle | 2.6 D | NC |
| X86809 | Phosphoprotein PEA-15 | Same | 2.6 D | NC |
| X86452 | Cyclin D2 | Same | 2.3 D | NC |
| AB000712 | Clostridium perfringens enterotoxin (CPE)-receptor | Cytolysis | 2.6 D | NC |
| U47834 | α-Actinin | Cell organization and structure | 2.1 D | NC |
| X14640 | Cytokeratin 13 | Same | 12.1 D | NC |
| M26329 | Cytokeratin 18 | Same | 2.6 D | NC |
| X86570 | Cytokeratin 1 | Same | 2.5 D | NC |
| AF061812 | Cytokeratin 16 | Same | 2.1 D | NC |
| AF141349 | Tubulin-β | Same | 3.0 D | NC |
| X51521 | Ezrin | Same | 2.6 D | NC |
| J05008 | Endothelin 1 | Cell regulation and signaling | 2.6 D | NC |
| Z23090 | 28 kDa heat shock protein | Same | 2.3 D | NC |
| AF099670 | Estrogen-responsive B box protein | Same | 2.6 D | NC |
| XM084028 | JunD | Same | 2.5 D | NC |
| M68831 | Transcription factor ETR101 | Same | 2.0 D | NC |
| AB006780 | Galectin 3 | Same | 2.1 D | NC |
| AAO10777 | Galectin 7 | Same | 5.7 D | NC |
| AI535946 | Galectin 1 | Same | 2.5 D | NC |
| D18431 | Hepatoma-derived growth factor | Same | 2.5 D | NC |
| AJ031001 | TMTXN1 protein | Same | 2.6 D | NC |
| AF095448 | RAIG1 | Same | 2.5 D | NC |
| M19481 | Follistatin | Same | 3.5 D | NC |
| X04409 | Coupling protein G, α-Subunit (α-S1) | Same | 2.1 D | NC |
| M60047 | Heparin-binding protein | Same | 2.8 D | NC |
phorylation studies because its expression in DJM-1 under conditions used in phosphorylation assays is uncertain.

Surprisingly, treatment with normal IgG reproducibly decreased the level of phosphorylation of adhesion molecules compared with untreated cells (Fig. 3).

**DISCUSSION**

One of the major objectives of pemphigus research is to develop a safer treatment for patients suffering from this serious disease. Patients need drugs that can replace CH that many of them must take for life. A non-steroidal treatment of pemphigus may be achieved by pharmacologically interceding at the intracellular biochemical events mediating acantholytic effects of pemphigus antibodies. Although previous models of PV have suggested that the effects of PV IgG are "passive," disrupting Dsg-mediated adhesion (reviewed in Ref. 77), recent advances in the study of the pathophysiology of pemphigus indicate strongly that PV is an acquired signal transduction disease activated by PV IgG (reviewed in Ref. 78). Results of our study clearly indicate that the effects of pemphigus anti-

| Accession no. | Common gene name | Known function | PV IgG vs. NC IgG | MP vs. C | FC |
|---------------|------------------|----------------|-------------------|---------|----|
| L78833 | Rho7 | Same | 2.3 D | NC |
| M59465 | TNF-α-inducible protein A20 | Same | 2.8 D | NC |
| X52015 | IL-1 receptor agonist | Same | 3.2 D | NC |
| M15330 | IL-1β | Same | 2.1 D | NC |
| M53367 | NF-IL6-β | Same | 3.5 I | NC |
| X52560 | NF-IL6 | Same | 3.2 D | NC |
| M13755 | INF-inducible 7-kDa protein | Same | 3.5 D | NC |
| X57352 | INF-inducible protein 1-8U | Same | 2.5 D | NC |
| J04164 | INF-inducible protein 9-27 | Same | 2.3 D | NC |
| AB000095 | Growth factor activator inhibitor | Same | 2.8 D | NC |
| NM001878 | Retinoic acid-binding protein 2 (CRAPB2) | Same | 3.0 D | NC |
| D14874 | Adrenomedulin | Same | 3.2 D | NC |
| M80254 | Cyclophilin isoform (hCyP3) | Same | 2.5 D | NC |
| M84739 | Calreticulin | Same | 2.5 D | NC |
| X71490 | Proton ATPase | Same | 2.5 D | NC |
| U51478 | Na⁺, K⁺-ATPase-β3 | Same | 3.0 D | NC |
| NM000858 | Guanylate kinase 1 | Same | 2.0 D | NC |
| X75346 | MAP-kinase activated protein kinase | Cell regulation and signaling | 2.1 D | NC |
| NM000858 | Guanylate kinase (GUK1) | Same | 2.0 D | NC |
| AF024636 | STE20-like kinase | Same | 2.0 D | NC |
| AF038844 | MKP-1 like protein tyrosine phosphatase | Same | 3.2 D | NC |
| U14187 | Receptor tyrosine kinase ligand LERK-3 | Same | 2.3 D | NC |
| AF002688 | Fatty-acid desaturase LDL | Metabolism | 2.5 D | NC |
| AF034544 | δ7-Sterol reductase | Same | 2.0 D | NC |
| U35719 | Aldehyde dehydrogenase 8 | Same | 2.3 D | NC |
| M54915 | h-pim-1 Protein | Oncogene | 2.3 D | NC |
| NCM13929 | c-Myc-P64 | Same | 2.1 D | NC |
| U28501 | Protease M | Proteolysis | 6.0 D | NC |
| U29429 | SNC 19 (matrix serine protease) | Same | 2.5 D | NC |
| AB008390 | Neurpsolin type 1 (serine protease) | Same | 2.5 D | NC |
| Y13834 | Farnesylated protein-converting enzymes 1 | Same | 2.0 D | NC |
| M13508 | Collagenase | Same | 2.1 I | NC |

Unilateral effects of MP

| Accession no. | Common gene name | Known function | PV IgG vs. NC IgG | MP vs. C | FC |
|---------------|------------------|----------------|-------------------|---------|----|
| Z35402 | E-cadherin | Adhesion | NC | 3.0 I |
| XM012483 | H-cadherin | Same | NC | 3.0 I |
| X63629 | P-cadherin | Same | NC | 2.3 I |
| X56807 | Desmocollin 2 | Same | NC | 3.7 I |
| Z34974 | Plakophilin | NC | NC | 3.0 I |
| U03100 | α2/E-catenin | Same | NC | 2.6 I |
| AJ011497 | Claudin 7 | Same | NC | 3.0 I |
| AF099730 | Connexin 31 | Same | NC | 2.5 I |
| J03202 | Laminin β-2 chain | Same | NC | 3.5 I |
| L34155 | Laminin-related protein (LamA3) | Same | NC | 2.5 I |
| U24048 | Ladinin (LAD) | Same | NC | 2.5 I |
| X76033 | Integrin α2 | Cell organization and structure | NC | 4.6 I |
| Y00503 | Cytokeratin 19 | Same | NC | 2.8 I |
| X07979 | Integrin β1 | Same | NC | 2.5 I |
| X53002 | Integrin β3 | Same | NC | 2.3 I |
| AF045941 | Sciellin (SCEL) | Same | NC | 4.0 I |
| AF001628 | Ab1BP4-interactor protein | Same | NC | 4.6 I |
| AJ005835 | IL-1 receptor antagonist | Cell regulation and signaling | NC | 16 I |
| X05908 | Lipocortin (phospholipase A2 inhibitor) | Same | NC | 3.0 I |
| M20560 | Lipocortin-III | Same | NC | 2.8 I |
| NM004039 | Annexin A2 (ANXA2) | Same | NC | 3.5 I |
| Y00630 | Arg-serpin (plasminogen activator-inhibitor) | Proteolysis | NC | 2.8 I |
| J03764 | Fibrillin activator inhibitor-1 | Same | NC | 2.5 I |
| D11139 | Tissue inhibitor of metalloproteinases | Proteolysis | NC | 2.6 I |
| M93056 | Elastase inhibitor | Same | NC | 3.0 I |
| AB000832 | Cathepsin V | Same | NC | 4.3 I |
| M21724 | Phosphotyrosyl-protein phosphatase | Same | NC | 4.6 I |
| NM002715 | Protein phosphatase 2A (PPP2CA) | Same | NC | 3.5 I |
| Y13936 | Protein phosphatase 2C-γ | Same | NC | 2.3 I |
bodies on KC are “active,” inducing many changes in keratinocyte functions. Although successful removal of pathogenic autoantibodies by plasmapheresis (79), hemocarboxadorsorption (80), or immunoadsorbsion with a trypthopan-linked polyvinyl alcohol adsorber (81) or staphylococcal protein A (82) alleviates the severity of the disease, the long term control of PV still relies on the maintenance therapy with CH. In pemphigus, CH may play by (i) inhibiting antibody synthesis, (ii) suppressing inflammation, especially eosinophilic spongiosis, and (iii) by stopping acantholysis via the direct pharmacological effect on KC. Pioneer works showing that addition of CH to cultured skin explants can prevent pemphigus antibody-induced acantholysis (28, 29) hinted that a discovery of a non-steroidal treatment of pemphigus could be achieved through a better understanding of the molecular mechanism(s) mediating direct anti-achantolytic action of CH. The results obtained in this study demonstrated for the first time that MP can decrease the extent of PV IgG-induced acantholysis in the skin of neonatal mice, and that this effect is mediated, in part, by a complex action of MP that includes both genomic and non-genomic effects of this drug on KC, keratinocyte adhesion molecules in particular. Although the increased level of caderhins in soluble fractions does not necessarily means the increased level of cell-cell adhesion of KC, the anti-acantholytic effect of MP in nude mice demonstrated that CH-dependent changes of keratinocyte adhesion molecules actually affect the strength of cell adhesion. To be able to detect even subtle changes in expression/function of adhesion molecules, we had to use three different experimental systems, neonatal mice, and cultures of normal human KC and DJM-1 cells, because each provides the most sensitive model for studying regulatory mechanisms of adhesion molecules at the transcriptional, translational, post-translational, and functional levels. The fact that different approaches produced consistent results indicates that certain in vitro findings can be extrapolated to the mechanism of therapeutic action of MP in vivo.

To examine anti-acantholytic activity of MP, we had to develop a reliable experimental technique. Initial testing of the anti-acantholytic activity of CH by Shiltz et al. (83) showed that co-administration of a relatively high dose of PV IgG, 23 mg/ml, and 10 μg hydrocortisone, or the same dose of triamcinolone acetonide, did not block acantholysis in the skin explant cultures. Next, Swanson and Dahl (28) demonstrated that the acantholysis induced by pemphigus plasma diluted 1/3 could be prevented if the skin explants were preincubated for 24 h in a medium containing 0.25 mM MP. Suppression of acantholysis did not occur when MP was added simultaneously with pemphigus plasma. Later studies demonstrated that this phenomenon was dose-related. Jeffes et al. (29) reported that the anti-acantholytic effect of hydrocortisone added simultaneously with pemphigus plasma commenced at a specific dose, 0.5 mM, although at lower doses, i.e. 0.25 mM and below, the drug was ineffective. No graded effect was appreciated. Both the anti-acantholytic dose of MP used by Swanson and Dahl (28) and that of hydrocortisone used by Jeffes et al. (29) approximated the serum concentration of CH used to treat acute PV patients. Since in neither experiment were lymphocytes present in the skin cultures treated with CH, it was clear that the glucocorticosteroid agents were directly affecting KC. However, potential clinical implications of the in vitro findings were diminished by the results of subsequent in vivo experiments showing that dexamethasone did not inhibit PV IgG-induced acantholysis (84). Anhalt et al. (84) administered to 1-day-old Balb/c mice either 14.4 or 20 mg of PV IgG/kg body weight/day together with, or 24 h after, dexamethasone, given at the total dose of either 10 or 20 mg/kg/day. These glucocorticosteroid doses greatly exceeded the maximum therapeutic dose of CH used in PV patients, i.e. >4.5 g of prednisone per day. The extent of disease produced by the PV IgG in the skin of injected mice was not influenced by treatment with dexamethasone. The authors concluded that the major therapeutic effect of CH was due to reduction of antibody synthesis rather than modification of the events that occur within the epidermis after antibody binding. In turn, the adequacy of the conclusion drawn from the in vivo experiment has been challenged by the well established clinical fact that pulse therapy with a high dose of an intravenous administered glucocorticosteroid agent, such as MP, can stop acantholysis in PV patients within 24–48 h, which is too early to induce changes in the serum titer of pemphigus autoantibodies (22, 25, 26). Therefore, we hypothesized that the discrepancy among the results of in vitro findings and in vivo experiments reported previously might be due to limitations of the mouse model used by Anhalt et al. (3, 84), and we sought to develop an adequate model for in vivo testing the anti-acantholytic efficacy of CH in pemphigus.

To reproduce features of pemphigus in vivo, we screened different concentrations of PV IgG and employed pups of two different strains and ages. The extent of acantholysis was assayed by measuring the length of intradermal split, rather than by scoring gross skin lesions using an arbitrary scale, as it was done in the past (84). Among various glucocorticosteroid

| Accession no. | Common gene name | Known function | MP + PV IgG versus PV IgG FC |
|---------------|------------------|----------------|-----------------------------|
| M69225        | Bullous pemphigoid antigen | Adhesion | 2.46 D                       |
| S66213        | Integrin αβ       | Same | 2.30 D                       |
| AY101193      | CD44 antigen      | Same | 2.14 D                       |
| U19999        | BAX-β protein     | Apoptosis | 2.30 I                       |
| NM_001233     | Caveolin-2        | Cell regulation and signaling | 2 D | |
| AF048732      | Cyclo T2b         | Same | 3.03 I                       |
| M80244        | E16 protein       | Same | 2 D                          |
| L13463        | Helix-loop-helix basic phosphoprotein (GOS8) | Same | 2.30 I                       |
| J02931        | Placental tissue factor | Same | 2 D                          |
| M64554        | Factor XIII-β subunit | Coagulation | 9.19 I                       |
| NM_000766     | Cytochrome P450 (CYP2A13) | Metabolism | 2.64 I                       |
| L07077        | Enol-CoA-hydratase 3-hydroxyacyl-CoA dehydrogenase | Same | 6.06 I                       |
| AF054187      | α-NAC protein     | Same | 2 D                          |

Corticosteroids Stimulate Keratinocyte Adhesion

Gene expression was determined in cultures of KC from three different donors pretreated for 2 h with 0.25 mM MP and the exposed for 8 h to 1 mg/ml PV IgGs together versus 1 mg/ml PV IgG alone (MP + PV IgG or PV IgG). The DNA microarray data were analyzed as described in the footnote to Table I. The genes are grouped alphabetically. The abbreviations used are: D, a decrease in gene transcription; I, an increase in gene transcription; FC, fold change.
drugs, MP was selected because it represents an active form of prednisone, the most commonly used glucocorticosteroid drug in the treatment of autoimmune pemphigus (15, 85, 86). Based on the experimental data, we believe that the major problem with using 1–2-day-old neonates was related to a very high fragility of their skin, making it too vulnerable to a relatively high dose of PV IgG required to reproducibly induce pemphigus symptoms. These pups also demonstrated an apparent lack of

**Corticosteroids Stimulate Keratinocyte Adhesion**

**FIG. 2.** MP up-regulates the synthesis and cellular expression of adhesion molecules in the monolayers of normal human KC. A, analysis of the expression of adhesion molecules in normal human KC by WB assay. The cells were treated with 0.25 mM MP or with no drugs (control; C) exactly as described in the legend to Table I, and the total cellular protein was extracted and analyzed by WB, as detailed under “Experimental Procedures.” Identical amounts of proteins from treated and nontreated control cultures were separated by 7% SDS-PAGE and electrophoresed. Each membrane was stained with a primary antibody to an adhesion molecule or for β-actin to standardize the measurements. The intensity of protein bands was determined via standard densitometry, as described under “Experimental Procedures.” The numbers below the bands indicate the mean densitometry value of each protein band ± S.D. obtained in three independent experiments (n = 3). Asterisks denote statistically significant difference from the control value (p < 0.05). B, analysis of the expression of adhesion molecules in normal human KC by indirect IF assay. Monolayers of normal human foreskin KC were grown in 4-chamber culture slides and treated overnight at 37 °C in 5% CO2 with culture medium without any drugs (control; white bar) or containing 0.25 mM MP (experiment; black bar), as detailed under “Experimental Procedures.” After incubation, keratinocyte monolayers were washed and immunostained with specific antibodies. The images were analyzed using software for semi-quantitative image analysis. In each cell culture specimen, at least three different randomly selected segments in at least three different microscopic fields were analyzed, and the results were compared. This analysis revealed considerable changes in the expression levels of the studied adhesion proteins in KC treated with MP. Data are means ± S.D. of the results obtained in keratinocyte monolayers from 3 donors. Asterisks indicate significant (p < 0.05) difference from control. No specific staining could be seen in negative control experiments in which the primary antibody was omitted (not shown). C, representative images of KC-expressing adhesion molecules in control, untreated cultures (C) and cultures treated with MP (MP) as described above. Bar, 100 μm.
sensitivity to anti-acantholytic action of CH. In experiments with older 3–5-day-old Balb/c mice, we found that they might respond to anti-acantholytic treatments with CH, the untreated positive controls did not always produce PV-like lesions either, perhaps due to rapidly developing hair follicles that could reinforce the epidermal integrity. Results of extensive in vivo experiments allowed us to select 3-day-old athymic nude mice weighing ~2 g as an adequate model for testing anti-acantholytic efficacy of test drugs. By using this novel animal model of pemphigus, we demonstrated that at its therapeutic doses, MP can significantly (p < 0.05) diminish the extent of epidermal acantholysis without diminishing PV IgG binding to murine KC.

To elucidate the mechanism(s) mediating the anti-acantholytic effect of MP, we sought to identify potential targets for genomic effects of PV IgG and MP in KC. As a screening procedure, we chose DNA microarray assay because CH exhibit their biological effects on KC predominantly through modulating gene expression at the transcriptional level (87, 88). PV IgG and MP produced both reciprocal effects on the gene transcription of some adhesion molecules, Dsg 3 and periplakin, and unilateral effects on several other types of adhesion molecules, including classical and desmosomal cadherins. Up-regulation of the genes encoding E-, H-, and P-cadherins, desmocollin 2, and plakophilin might be relevant to the anti-acantholytic effect of MP. Unexpectedly, the reciprocal effects of PV IgG and MP in KC encompassed regulatory systems that mediate not only the adhesive function of these cells, but also many other vital components of the cell homeostasis, including regulation of cell organization and structure, cell cycle progression, differentiation, ion exchange, and antigen processing and presentation. The observed changes offered new insights into the mechanism of acantholysis. For example, PV IgG-induced decrease of proton ATPase might lead to alterations in keratinocyte adhesion via mechanisms that cause acantholysis in Hailey-Hailey and Darier diseases, both of which are associated with ATP-powered pump mutations (89, 90). It has been reported recently that in the epidermis of patients with Hailey-Hailey disease abnormal ATP and Ca2+ levels are linked to defects in actin reorganization, which is necessary for normal cell-to-cell adhesion in KC (91, 92).

Large groups of genes coding for the proteins mediating cell signaling and proteolysis were also affected. The group of affected genes included those encoding cytokines and cytokine-inducible proteins, which is in keeping with observations that pemphigus antibodies can modulate the cytokine production in KC (93, 94). Finally, changes of apoptosis-related genes may underlie the appearance of an apoptotic phenotype in KC prior to cell-cell detachment (acantholysis) in the skin of pemphigus patients (95). Evidence that keratinocyte apoptosis occurs in PV is growing (96–98). Because induction of apoptosis is an early event following PV IgG binding to KC, some of the gene changes detected by DNA microarray assay might be associated with apoptosis induction. The studies of a stepwise apoptosis induction in PV IgG-treated KC are currently under way in our laboratory.

In addition to antagonizing genomic effects of PV IgG, MP exhibited non-genomic effects on KC. This was not surprising because of the increasing evidence for a rapid non-genomic action of steroids that is incompatible with the traditional model of late genomic effects via intracellular receptor-mediated transcriptional and translational events (reviewed in Ref. 99). The exact mechanisms of a variety of CH-mediated non-genomic effects remain unknown. Recent results of whole-cell patch clamp technique suggested that CH bind to specific sites on the outer cell membrane, such as the neuronal-type nicotinic acetylcholine receptor-coupled channel (100). In this regard, it is worth noting that acetylcholine receptors in KC are coupled to regulation of cell adhesion, and they can be targeted by pemphigus autoantibodies (reviewed in Refs. 101 and 102). Indeed, both nicotinic and muscarinic classes of acetylcholine receptors have been shown to affect functioning of structural and adhesion proteins through cascade reactions mediated by protein kinases (103–108). The presence of anti-KC autoantibodies may therefore explain why PV IgG but not normal IgG induced phosphorylation of adhesion molecules. Based on these premises, we compared the effects of PV IgG and MP on phosphorylation of Dsg 3 and γ-Cat and some other keratinocyte adhesion molecules that were down-regulated or up-regulated in DNA microarray assay. We knew that binding of PV IgG to KC has been reported to result in marked phosphorylation of Dsg 3, leading to its dissociation from γ-Cat and disappearance from desmosomes (51, 52). As expected, PV IgG induced increased phosphorylation of keratinocyte adhesion molecules that were abolished in the presence of MP. In addition to non-genomic cell membrane receptor-mediated signaling pathways, the observed changes in the phosphorylation status of keratinocyte adhesion molecules under the effects of PV IgG and CH might result from alterations in the gene expression of protein kinases and phosphatases. For instance, PV IgG down-regulated and CH up-regulated expression of the protein phosphatase genes in KC (Table I). Neither we nor other workers (52, 109–111) who studied phosphorylation of β-Cat, γ-Cat, and Dsg 3 found any measurable differences in the migration dis-
tances of the phosphorylated versus non-phosphorylated isoforms of these proteins.

The results of the phosphorylation experiments help reconcile the data obtained by two different semi-quantitative techniques, WB and IF, used to evaluate MP effects on keratinocyte adhesion molecules at the protein levels. Changes in the phosphorylation status of the adhesion molecule might cause changes in its subcellular localization with corresponding alterations in the intensity of fluorescence. This helps explain both the striking increase in the β-Cat immunofluorescence in the face of no differences in WB and increased IF staining of the epidermis in the mice treated with MP. This is also in keeping with observations that phosphorylation of classical and desmosomal cadherins underlies assembly/disassembly of the adherence and desmosomal junctions (30, 33, 37, 112), and that β- and γ-Cat immunoreactivities in epithelial tissues are displaced toward the cytosol and nucleus, respectively, particularly in areas with intense acantholysis (113). Therefore, the results of phosphorylation experiments elucidate the role for protein phosphorylation in the signal transduction pathway mediating both the pro-acantholytic effects of PV IgG and the anti-acantholytic effects of MP. The fact that normal human IgG, just like MP, also decreased the level of phosphorylation of adhesion molecules may provide a novel mechanism that helps explain therapeutic activity of IVIg in patients with pemphigus (114, 115).

Thus, both PV IgG and MP can cause and treat, respectively, pemphigus through a combination of their genomic and non-genomic effects on cell-cell adhesion and other vital functions of KC. Recognition of rapid anti-acantholytic activity of CH has genomic effects on cell-cell adhesion and other vital functions of pemphigus through a combination of their genomic and non-genomic effects on KC with non-steroidal drugs. Recognition of rapid anti-acantholytic activity of CH has genomic effects on cell-cell adhesion and other vital functions of pemphigus through a combination of their genomic and non-genomic effects on KC with non-steroidal drugs. Recognition of rapid anti-acantholytic activity of CH has genomic effects on cell-cell adhesion and other vital functions of pemphigus through a combination of their genomic and non-genomic effects on KC with non-steroidal drugs. 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Recognition of rapid anti-acantholytic activity of CH has genomic effects on cell-cell adhesion and other vital functions of pemphigus through a combination of their genomic and non-genomic effects on KC with non-steroidal drugs. The data obtained by two different semi-quantitative techniques, WB and IF, used to evaluate MP effects on keratinocyte adhesion molecules at the protein levels. Changes in the phosphorylation status of the adhesion molecule might cause changes in its subcellular localization with corresponding alterations in the intensity of fluorescence. This helps explain both the striking increase in the β-Cat immunofluorescence in the face of no differences in WB and increased IF staining of the epidermis in the mice treated with MP. This is also in keeping with observations that phosphorylation of classical and desmosomal cadherins underlies assembly/disassembly of the adherence and desmosomal junctions (30, 33, 37, 112), and that β- and γ-Cat immunoreactivities in epithelial tissues are displaced toward the cytosol and nucleus, respectively, particularly in areas with intense acantholysis (113). Therefore, the results of phosphorylation experiments elucidate the role for protein phosphorylation in the signal transduction pathway mediating both the pro-acantholytic effects of PV IgG and the anti-acantholytic effects of MP. The fact that normal human IgG, just like MP, also decreased the level of phosphorylation of adhesion molecules may provide a novel mechanism that helps explain therapeutic activity of IVIg in patients with pemphigus (114, 115).
