The effect of corticosteroid on the acantholysis induced by pemphigus vulgaris IgG

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The abbreviations used are: PV, pemphigus vulgaris; IgG, immunoglobulin G; Dsg, desmoglein; Pkg, plakoglobin; p38 MAPK, p38 mitogen-activated protein kinase; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; PKC, protein kinase C: MKP-1, MAP kinase phosphatase 1; MAPKAP 2, MAP kinase-activated protein kinase 2; RT, room temperature; Dexa, dexamthasone; PBS, phosphate-buffered saline; NH, normal human; HSP, heat shock protein; WB, western blot.

ABSTRACT

The effect of corticosteroid on the acantholysis induced by pemphigus vulgaris IgG

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(Directed by Professor Soo-Chan Kim)

Pemphigus vulgaris (PV) is a potentially fatal blistering disease characterized by autoantibodies against the desmosomal adhesion protein desmoglein (Dsg) 3. It has been proven that autoantibodies against Dsg 3 cause loss of cell-cell adhesion (acantholysis) which is pathognomic histologic finding in PV. Although the pathomechanism of acantholysis still remains unclear, one explanation is that an antibody binding to Dsg extracellular domain simply blocks adhesion by steric hindrance. However, PV pathogenesis involves more than steric hindrance. Recently, increasing evidences showed that variety of intracellular signaling pathways are also involved in the loss of cell-cell adhesion.

Several signaling pathways have been introduced. First, p38 mitogen-activated protein kinase (p38 MAPK) activation, which is necessary for blister formation in response to PV IgG, leads to endocytosis of multiple desmosomal molecules. Second, activation of c-myc and epidermal growth factor receptor (EGFR) can also cause blister formation in PV. Third, PV IgG results endocytosis of Dsg3 by using adhesion-signaling network binding to Dsgs. Fourth, hyperproliferation can lead to acantholysis by unknown mechanisms. Blocking PV IgG induced intracellular signaling and Dsg3

endocytosis or Dsg3 exogenous expression may prevent acantholysis in response to PV IgG.

Corticosteroid is still mainstay of treatment in PV, however, the precise therapeutic role of corticosteroid in PV IgG induced acantholysis has not yet been elucidated. In this study, the effects of corticosteroid on the process of PV IgG induced acnatholysis were investigated.

Dexamethasone attenuated PV IgG induced loss of keratinocyte adhesion in dissociation-based assay. Dexamethasone attenuated PV IgG induced Dsg endocytosis and depletion in immunofluorescence examination of cultured keratinocytes. Immunoblot analysis revealed that the protein level of Dsg3 decreased rapidly until 12 hr incubation at 37℃ with PV IgG and that the decrease of Dsg3 was delayed by dexamethasone. Phosphorylated p38 was increased in dual peaks after 1 hr and 12-24 hrs of incubation with PV IgG, whereas phosphorylated p38 was decreased in the early phase after incubation with PV IgG and dexamethasone. Phosphorylated ERK was increased in peak after 30 mins of incubation with PV IgG. However, elevated peak of phosphorylated ERK was not observed with PV IgG and dexamethasone.

Therefore, this study suggests that corticosteroid plays an important role on the blocking of PV IgG induced acantholysis through attenuating depletion of Dsg3 and phosphorylation of p38 MAPK and ERK signaling.

Key words: pemphigus vulgaris, acantholysis, corticosteroid, desmoglein 3, p38 MAPK, ERK

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I. INTRODUCTION

Pemphigus vulgaris (PV) is an autoimmune blistering disease characterized by autoantibodies against the desmosomal adhesion protein desmoglein (Dsg) 3. Although the pathogenesis of PV still remains unclear, suprabasal acantholysis caused by loss of cell-cell adhesion is pathognomic histologic findings in PV. One explanation is that antibody binding to Dsg extracellular domain simply blocks adhesion by steric hindrance.[1] *In vitro*, PV IgG can directly inhibit Dsg3 homophilic interaction. However, PV pathogenesis involves more than steric hindrance. Recently, increasing evidences showed that variety of intracellular signaling pathways are also involved in the loss of adhesion.

Several signaling pathways have been introduced. First, p38 mitogen-activated protein kinase (p38 MAPK) activation, which is necessary for blister formation in response to PV IgG, leads to endocytosis of multiple desmosomal molecules.[2,3] Inhibition of p38 MAPK prevents blister formation in the passive transfer mouse model.[4] Second, activation of c-myc, epidermal growth factor receptor (EGFR), and Src can also cause blister formation in

PV.[5-7] Third, desmosome assembly is a calcium-dependent process, while matured desmosome does not require calcium to maintain intercellular adhesion.[8,9] Activated protein kinase C (PKC) converts desmosomes from calcium-independent to calcium-dependent so that inhibition PKC signaling promotes desmosomal hyperadhesion.[10,11] Fourth, PV IgG results endocytosis of Dsg3 by using adhesion-signaling network binding to desmogleins.[12] Blocking Dsg3 endocytosis or exogenous Dsg3 expression prevents acantholysis in response to PV IgG.[13,14] Fifth, hyperproliferation can lead to acantholysis by unknown mechanisms.[5,7]

Corticosteroid has been mainstay of treatment in PV. Corticosteroid generates therapeutic effects by suppressing antigen presenting cells, T lymphocytes and B lymphocytes in pemphigus. In addition to the immunological effects, there are some evidences that corticosteroids have direct inhibitory effects on the acantholysis induced by PV IgG. First, autoantibodies in the skin decrease 3-4 weeks after corticosteroid administration, but the clinical symptom improves within 24-48 hrs after steroid pulse therapy. Second, topical steroid cream also improves skin lesions of PV. Third, corticosteroid directly blocked PV IgG induced acantholysis in the neonatal mouse model and also increased Dsg3 transcription in cultured keratinocyte experiment.[15] However, the precise role of corticosteroid on the PV IgG induced acantholysis has not yet been elucidated. In this study, we investigated the effects of corticosteroid on the pathomechanisms of PV IgG induced acantholysis.

II. METHODS

1. Purification of IgG

For the purification of IgG, pooled sera from well established PV patients were used. Sera were tested for reactivity against Dsg1 and Dsg3 by enzyme-linked immunosorbent assay. IgG fractions were purified using Montage purification kit and spin columns with prosep-G media (Millipore, Billerica, MA, USA). The IgG fractions were diluted in culture medium to a final IgG concentration of 1mg/ml.

2. Dispase-based dissociation assay

Primary human keratinocytes were seeded onto 65mm dishes in KGM medium (Lonza, Walkersville, MD, USA). Twenty four hrs after reaching confluence, the medium was changed to 0.6 mM calcium containing KGM for 18 hrs. The cell were treated with normal human (NH) IgG, PV IgG, and PV IgG plus dexamethasone (20 mg/L or 0.05 mM), and then incubated at 37℃ for 6 hrs. After washing, the cells were incubated with 5mg/ml dispase II (Sigma-aldrich, St. Louis, MO, USA) for 1 hr to detach the monolayer from plates. Cell sheets were washed with PBS twice and subject to mechanical stress by pipetting. The sheet fragments were fixed by 4% paraformaldehyde and stained using crystal violet.

3. Immunofluorescence

HaCaT cells were grown in chamber slides to confluence in DMEM with 10% fetal calf serum and incubated with NH IgG, PV IgG, and PV IgG with

dexamethasone for 0, 1, 3, 6, 12, or 24 hrs at 37℃. After incubation with NH IgG and PV IgG, culture medium was removed, and cells fixed for 5 mins at 4℃ with ice-cold acetone for immunofluorescence. These cells were treated with 0.1% Triton X-100 in PBS for 5 mins at room temperature (RT). After being rinsed with PBS at RT, cells were incubated for 1 hr with blocking solution, and then incubated for overnight at 4℃ mouse anti-desmoglein 3 antibody (Invitrogen, Camarillo, CA, USA) diluted 1: 100 in TBS-T. After several rinses with PBS (5 mins X 4 times), cells were incubated for 60mins at RT with Alexa Flour 555 goat anti-mouse IgG (diluted 1: 500 in PBS, Invitrogen), and Alexa Flour 488 goat anti-human IgG (diluted 1: 500 in PBS, Invitrogen). Cells were counterstained with DAPI (Invitrogen). Images were acquired at X400 magnification using a Carl Zeiss microscope and AxioVision software.

4. Western blotting

HaCaT cells were grown to confluence as described above and incubated with NH IgG, PV IgG, and PV IgG with dexamethasone for 0, 1, 3, 6, 12, or 24 hrs at 37℃. After incubation with NH IgG and PV IgG, total protein was isolated using RIPA buffer (Cell Signaling Technology, Danvers, MA, USA) added 1mM PMSF. After protein isolation, equal amount of proteins from each experimental group were loaded onto Nupage Novex Bis-Tris Gels (Invitrogen) and electrophoresis was performed on the X-cell SureLock Mini-Cell (Invitrogen). After electrophoresis, proteins were transferred onto PVDF membranes, and membranes were incubated with primary antibodies: mouse anti-desmoglein 3 antibody (Invitrogen), mouse anti-plakoglobin antibody

(Invitrogen), rabbit anti-phospho p38 MAP kinase (Thr180/Tyr182) antibody (Cell Signaling Technology), rabbit anti-p38 MAP kinase (Thr180/Tyr182) antibody (Cell Signaling Technology), mouse anti-phospho p44/42 MAPK (Erk1/2) antibody (MAP kinase (Thr180/Tyr182) antibody) (Cell Signaling Technology), rabbit anti-p44/42 MAPK (Erk1/2) antibody (MAP kinase (Thr180/Tyr182) antibody) (Cell Signaling Technology) diluted in TBS containing 0.05% Tween 20 (TBS-T). Blots were washed with 0.05% TBS-T and then incubated with appropriate HRP-conjugated secondary antibodies diluted in 0.05% TBS-T. Blots were developed using ECL PLUS reagent (Pierce, Rockford, IL, USA).

5. Statistical analysis

All analyses were performed using IBM SPSS statistics 22.0 (IBM corporation, Amonk, NY, USA). Comparison among the three groups was done by analysis of variance (ANOVA). Comparison between two groups was done by unpaired Student's *t*-test. Statistical results are presented as mean ± standard deviation (SD). *P* values less than 0.05 were considered to represent significant differences.

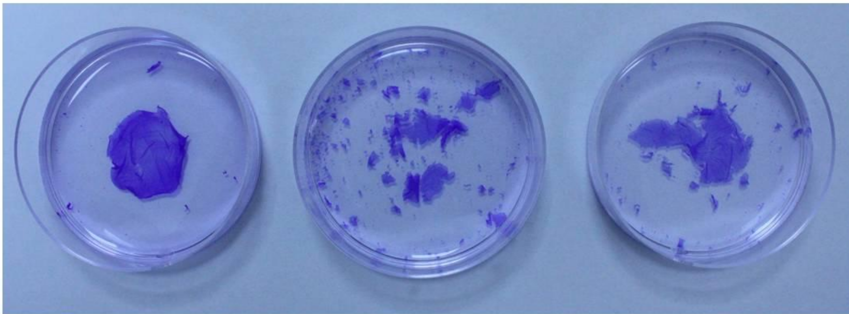
IV. RESULTS

1. Dexamethasone attenuated PV IgG induced loss of keratinocyte adhesion *in vitro*

Interference with keratinocyte adhesion is considered as the pathogenic hallmark of autoantibodies in pemphigus.[16,17] Thus, a well-established *in vitro* system for evaluating the capability of dexamethasone to attenuate the loss of keratinocyte adhesion was applied. PV IgG was applied to a dispase-based keratinocyte dissociation assay using cultured human keratinocytes. Confluently grown normal human keratinocytes were incubated with PV IgG fractions. Next, mechanical stress was applied to the keratinocyte monolayer to obtain cellular fragments illustrating the loss of keratinocyte adhesion in these keratinocyte cultures.

Figure 1A illustrates that PV IgG induced an earlier weakening of keratinocyte adhesion, leading to a higher number of keratinocyte fragments in 6 hrs of incubation at 37℃, whereas PV IgG plus dexamethasone attenuated weakening of keratinocyte adhesion, leading to a lower number of keratinocyte fragments in 6 hrs. The differences were statistically significant with ANOVA (p = 0.001). The differences with post hoc multiple comparisons using least significant difference (LSD) were significant (p = less than 0.0005, 0.005 and 0.012 respectively). The graph represents an average of three separate experiments quantified by a blinded observer in Figure 1B. Pooled IgG from healthy individuals did not affect keratinocyte adhesion.

A



NH IgG PV IgG PV IgG+Dexa

B

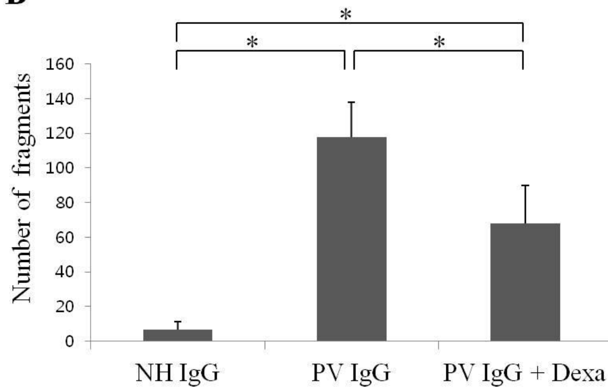


Figure 1. Dissociation assay to evaluate the effect of dexamethasone on the pathogenesis of PV IgG

A) Monolayers of normal human epidermal keratinocytes were treated with NH IgG, PV IgG, and PV IgG + Dexa. After 6 hrs of incubation, monolayers were separated from culture dishes by treatment with dispase. Subsequently, they were subjected to mechanical stress by pipetting. PV IgG induces a dramatic weakening of keratinocyte adhesion, leading to a high number of keratinocyte fragments in 6 hrs of incubation at 37℃, whereas PV IgG with dexamethasone (20 mg/L, 0.05 mM) attenuated weakening of keratinocyte adhesion, leading to a lower number of keratinocyte fragments in 6 hrs.

B) The graph represents an average of three separate experiments quantified by a blinded observer. Comparison among three groups was done by using analysis of variance with post hoc least significant difference analysis. Data are expressed as means ± SD. Statistical significance was set as $p<0.05$(*).

2. Dexamethasone attenuated PV IgG induced desmoglein 3 endocytosis and depletion in immunofluorescence examination

HaCaT cells were exposed to none, NH IgG, PV IgG, and PV IgG plus dexamethasone at 4℃ for 1 hr and subsequently shifted to 37℃ for 1, 3, 6, and 24 hrs. The quantity and localization of desmoglein 3 (Dsg3) and human IgG were monitored by a fluorescence microscopy. In cells incubated at 4℃ for 1 hr, both antibodies to Dsg3 and PV IgG labeled cell borders (Fig 2A).

After 1 hr incubation at 37℃ with PV IgG and PV IgG plus dexamethasone, coloclaized clumping of Dsg3 and IgG was observed (Fig 2B).

After 3 hrs incubation at 37℃ with PV IgG and PV IgG plus dexamethasone, colocalized clumping of Dsg3 and IgG advanced. Linear distribution of colocalized Dsg3 and PV IgG was relatively well preserved with PV IgG plus dexamethasone. Intensity of Dsg3 staining was markedly decreased with PV IgG and PV IgG plus dexamethasone (Fig 2C).

After 6 hrs incubation at 37℃ with PV IgG and PV IgG plus dexamethasone, colocalized clumping of Dsg3 and IgG was also observed. Linear distribution of Dsg3 and PV IgG was relatively well preserved with PV IgG plus dexamethasone. Decreased Dsg3 staining was more pronounced with PV IgG (Fig 2D).

After 12 hrs incubation with PV IgG and PV IgG plus dexamethasone at 37℃, colocalized clumping of Dsg3 and IgG was also observed. Decreased Dsg3 staining was more obvious with PV IgG. Cell-cell separation was more accelerated with PV IgG (Fig 2E).

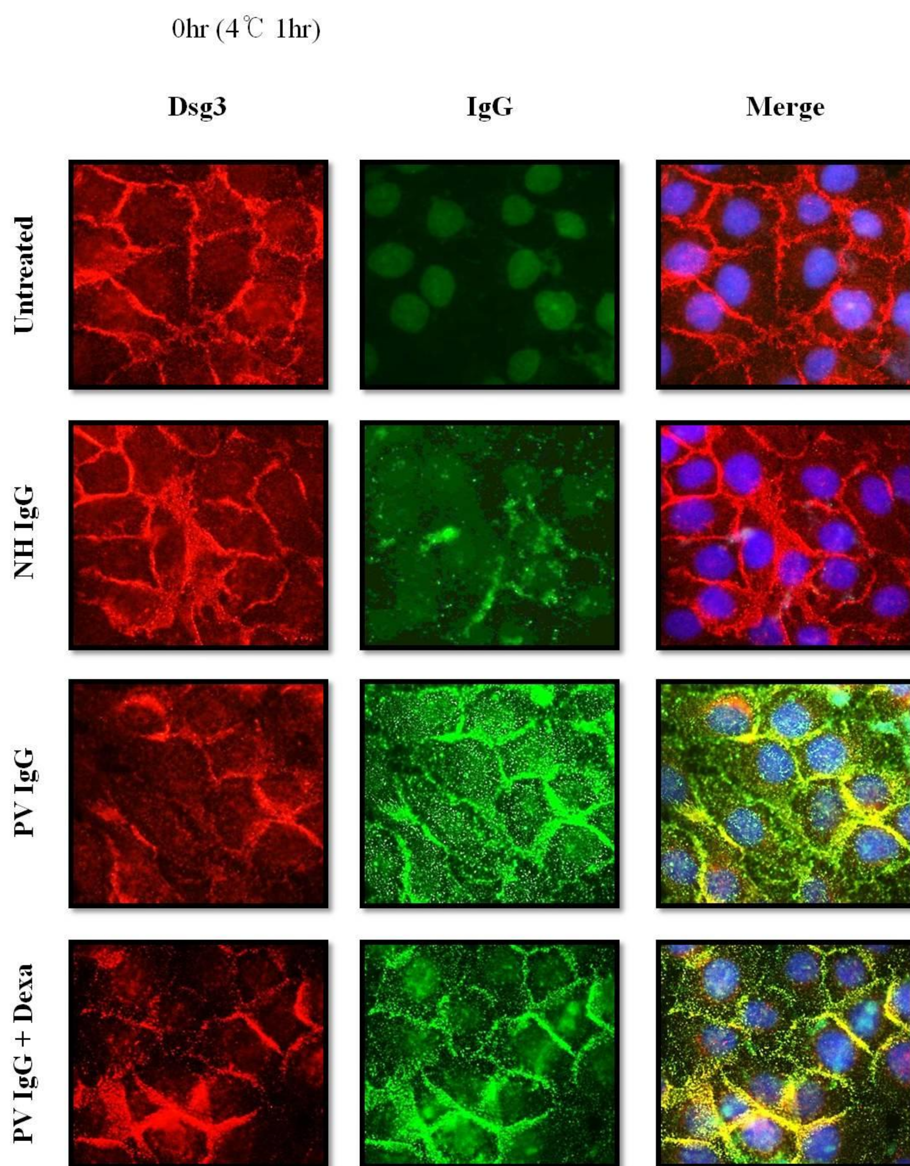
After 24 hrs incubation with PV IgG and PV IgG plus dexamethasone at 37℃, both Dsg3 and IgG were markedly decreased. Decreased Dsg3 staining

was more obvious with PV IgG. Cell-cell separation was more accelerated with PV IgG (Fig 2F).

The intensities of stains for Dsg3 by immunofluorescence examination were analyzed using software for semi-quantitative image analysis (Fig 2G). Keratinocytes treated with PV IgG plus dexamethasone relatively well preserved linear distribution of Dsg3 after 3-6 hrs incubation and Dsg3 depletion was attenuated in cells treated with PV IgG plus dexamethasone after 6–24 hrs incubation. These results suggest attenuated internalization and depletion of Dsg3 in cells treated with PV IgG plus dexamethasone.

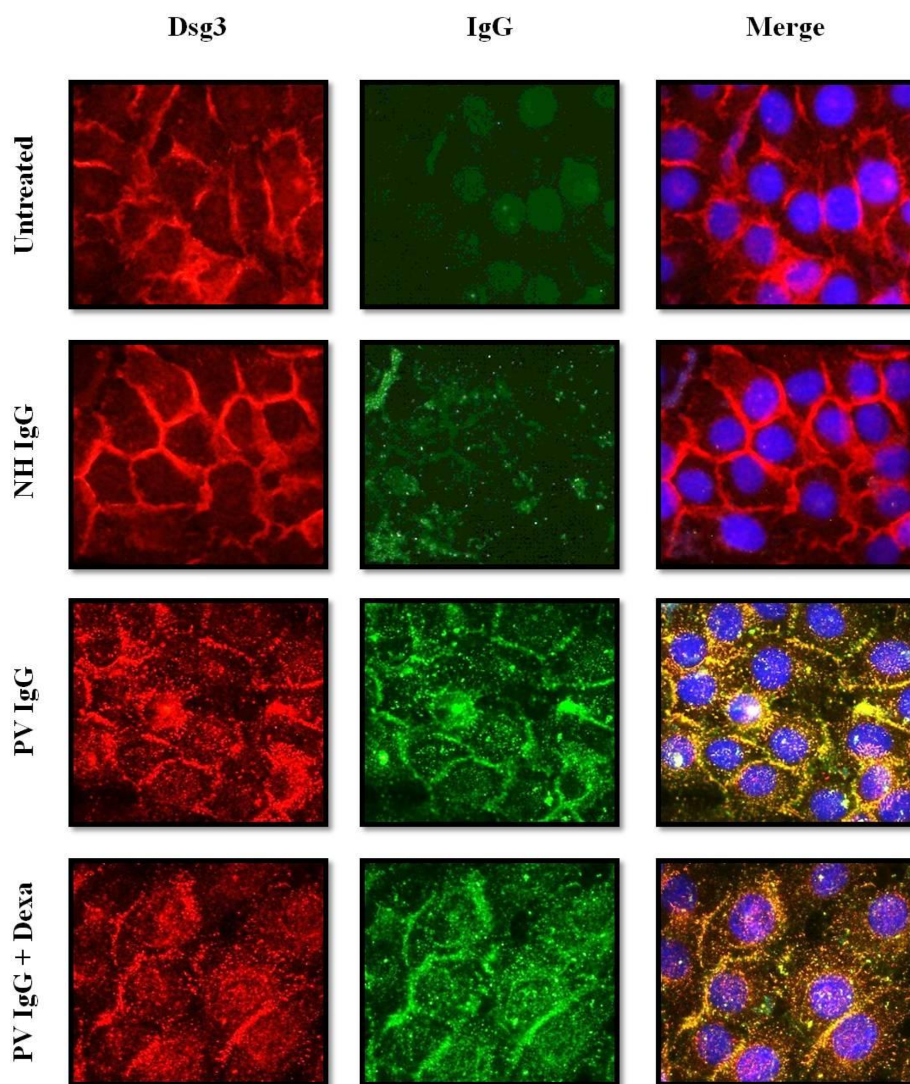
Figure 2. Immunofluorescence examination to evaluate the effect of dexamethasone on the temporal changes in the intensities of Dsg3 and IgG stains induced by PV IgG.

A



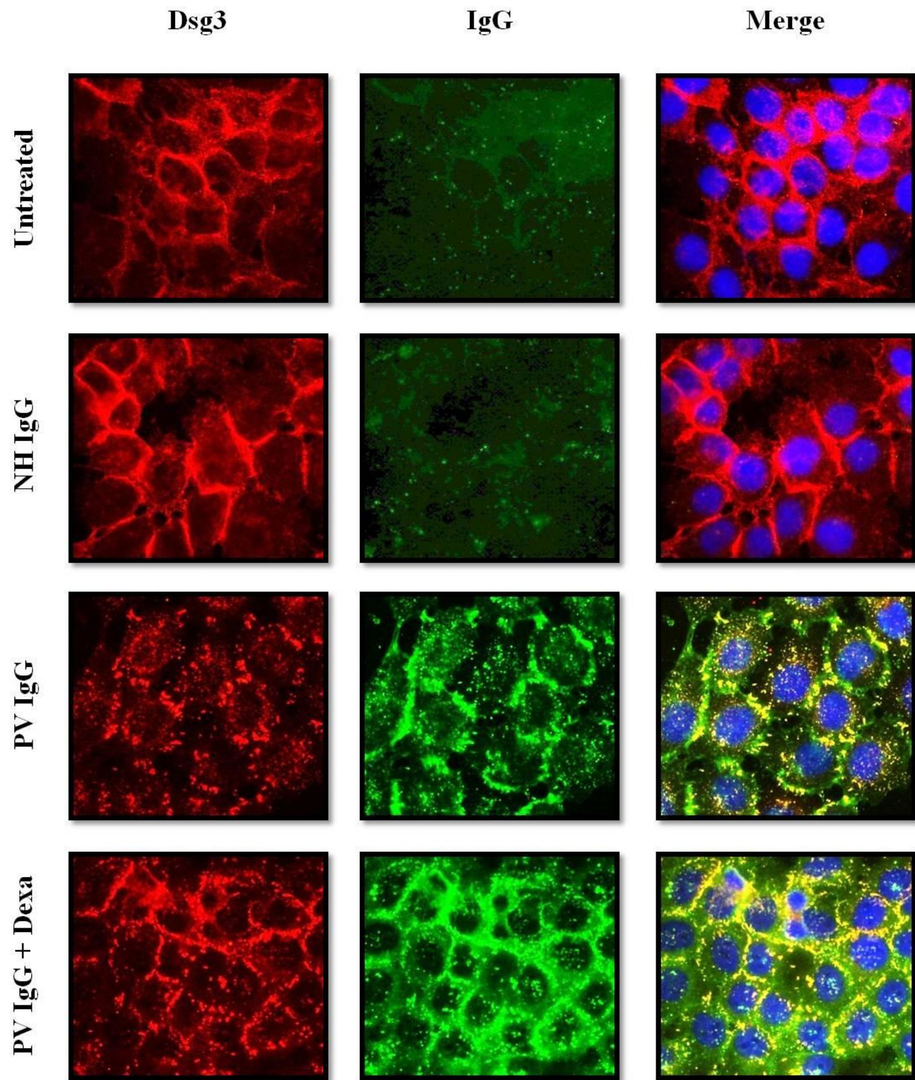
B

1hr (4℃ 1hr + 37℃ 1hr)



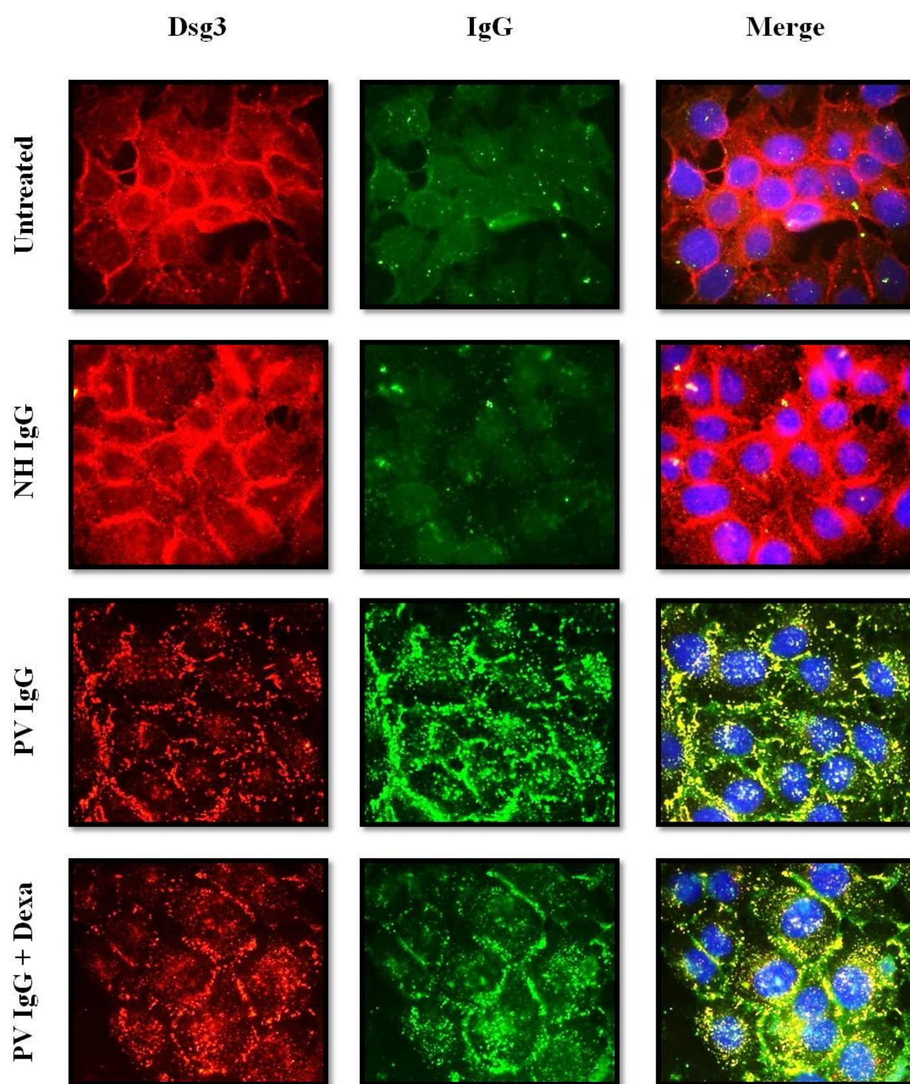
C

3hr (4℃ 1hr + 37℃ 3hr)



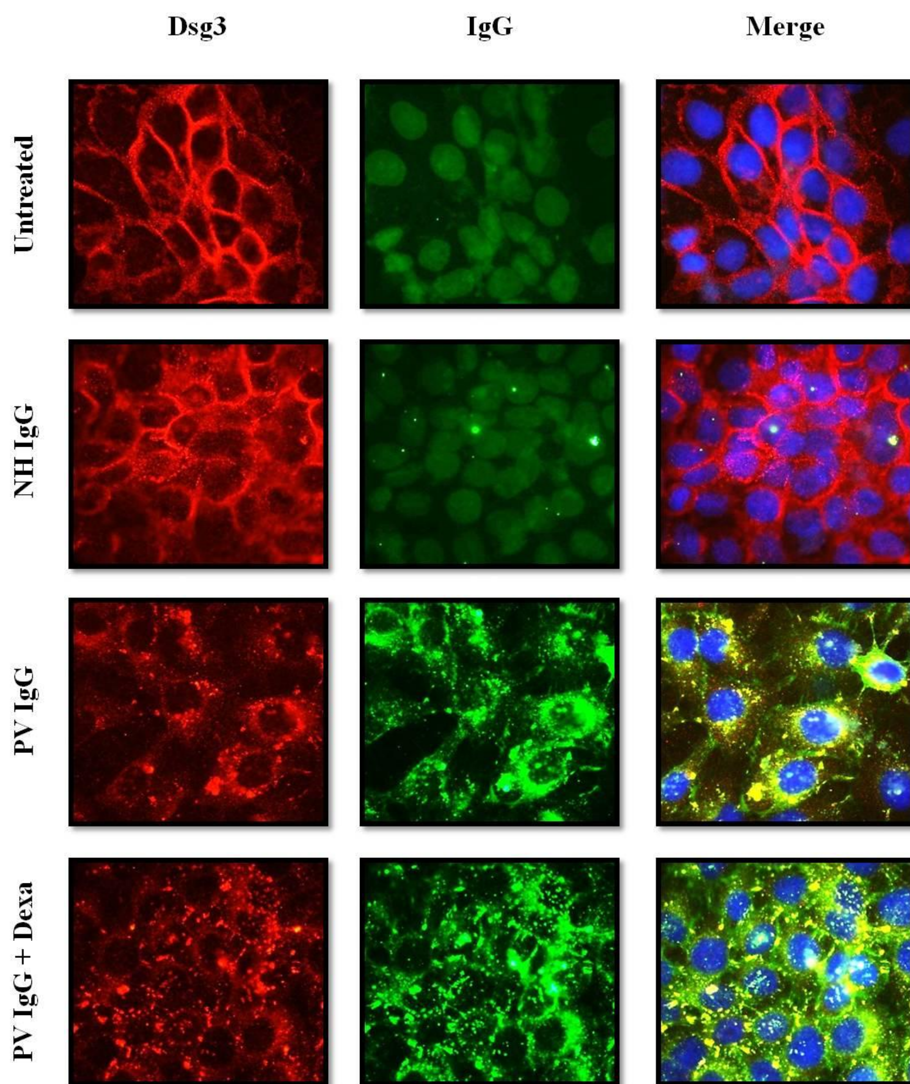
D

6hr (4℃ 1hr + 37℃ 6hr)



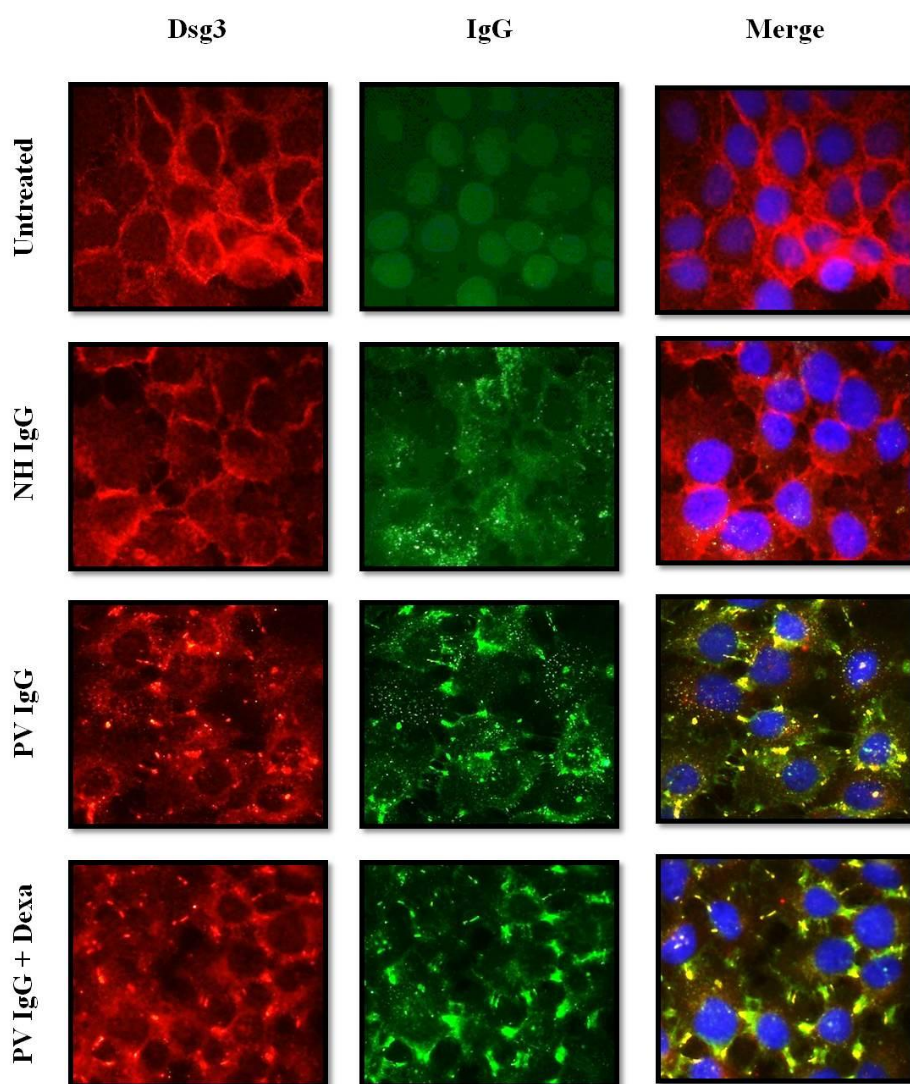
E

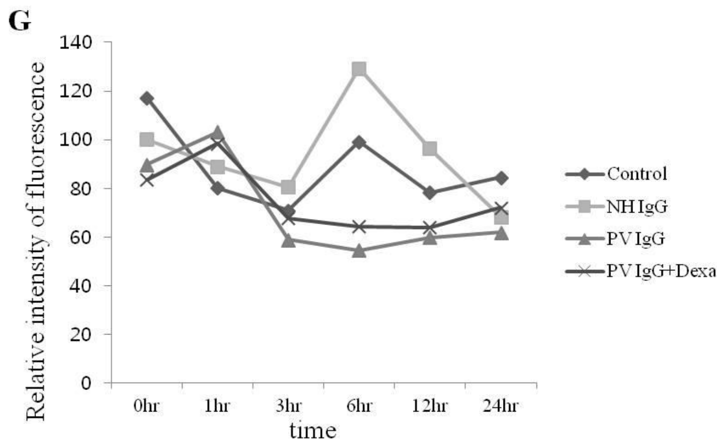
12hr (4℃ 1hr + 37℃ 12hr)



F

24hr (4℃ 1hr + 37℃ 24hr)





A) In cells incubated at 4℃ for 1 hr, both antibodies to Dsg3 and PV IgG labeled cell borders.

B) After 1 hr incubation at 37℃ with PV IgG and PV IgG plus dexamethasone, coloclaized clumping of Dsg3 and IgG was observed.

C) After 3 hrs incubation at 37℃ with PV IgG and PV IgG plus dexamethasone, colocalized clumping of Dsg3 and IgG advanced. Linear distribution of colocalized Dsg3 and PV IgG was relatively well preserved with PV IgG plus dexamethasone. Intensity of Dsg3 staining was markedly decreased with PV IgG and PV IgG plus dexamethasone.

D) After 6 hrs incubation at 37℃ with PV IgG and PV IgG plus dexamethasone, colocalized clumping of Dsg3 and IgG was also observed. Linear distribution of Dsg3 and PV IgG was relatively well preserved with PV IgG plus dexamethasone. Decreased Dsg3 staining was more pronounced with PV IgG.

E) After 12 hrs incubation with PV IgG and PV IgG plus dexamethasone at 37℃, colocalized clumping of Dsg3 and IgG was also observed. Decreased Dsg3 staining was more obvious with PV IgG. Cell-cell separation was more accelerated with PV IgG.

F) After 24 hrs incubation with PV IgG and PV IgG plus dexamethasone at 37℃, both Dsg3 and IgG were markedly decreased. Decreased Dsg3 staining was more obvious with PV IgG. Cell-cell separation was more accelerated with PV IgG.

G) Analysis of the expression of Dsg3 by immunofluorescence examination. The images were analyzed using software for semi-quantitative image analysis.

3. Dexamethasone attenuated the PV IgG induced depletion of Dsg3

To compare the temporal changes in the protein levels of Dsg3 and plakoglobin (Pkg) in HaCaT cells with PV IgG and those with PV IgG plus dexamethasone, semi-quantitative western blotting was performed. The protein level of Dsg3 decreased rapidly until 12 hrs incubation at 37℃ with PV IgG as measured by immunoblotting. The decrease of Dsg3 was delayed with PV IgG plus dexamethasone. There was no significant difference between temporal changes in the protein levels of Pkg with PV IgG and those with PV IgG plus dexamethasone. The protein levels of Dsg3 and Pkg were not affected by incubation with NH IgG.

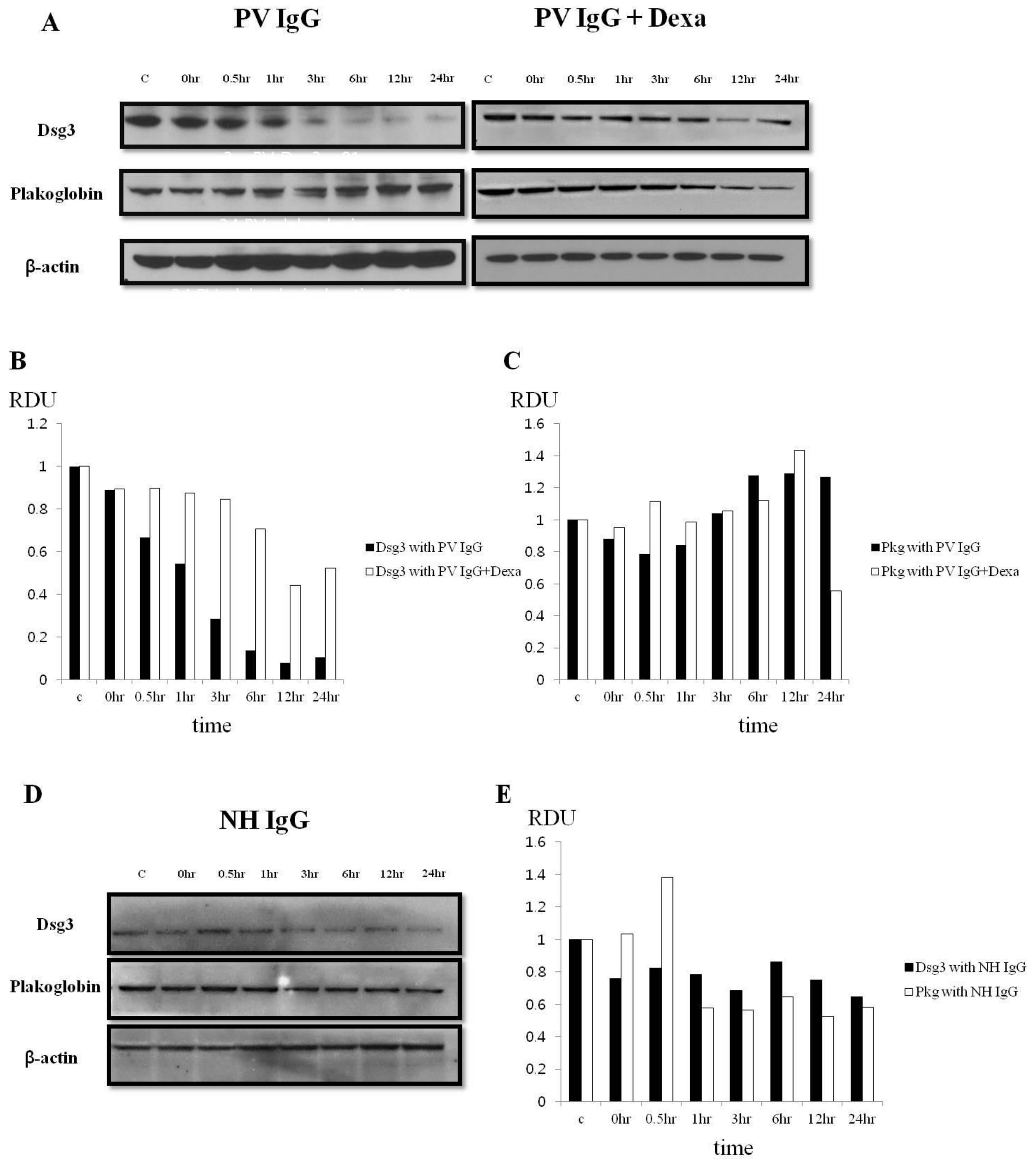


Figure 3. Effects of dexamethasone on the PV IgG induced depletion of Dsg3.

A) The protein level of Dsg3 decreased rapidly until 12 hr incubation at 37℃ with PV IgG as measured by immunoblotting with steep slope (B). The

decrease of Dsg3 was delayed with PV IgG with dexamethasone, showing gentle slope in the graph (B).

B) Densitometry of the Dsg3 reactivity in western blot shown in (A).

C) Densitometry of the plakoglobin reactivity in western blot shown in (A).

Western blot of Dsg3 with NH IgG and densitometry of its reactivity shown in (D) and (E).

β-actin was used to show equal protein load. To standardize results, levels of Dsg3 and plakoglobin are expressed as a ratio against β-actin protein quantity, as compared with the ratios obtained in controls (0 hr of incubation at 37℃, taken as 1). RDU, relative densitometric units.

4. Dexamethasone attenuated the PV IgG induced cell signaling.

To evaluate the temporal changes in phosphorylation levels of p38 MAPK and ERK in HaCaT cells with PV IgG and those with PV IgG plus dexamethasone, semi-quantitative western blotting was performed. Phosphorylated p38 was increased in dual peaks at 1 hr and 12-24 hrs after incubation with PV IgG, whereas phosphorylation of p38 was decreased in the early phase after incubation with PV IgG plus dexamethasone. Phosphorylation of ERK was increased in peak at 30 min with PV IgG. However, elevated peak of phosphorylated ERK is not observed with PV IgG plus dexamethasone.

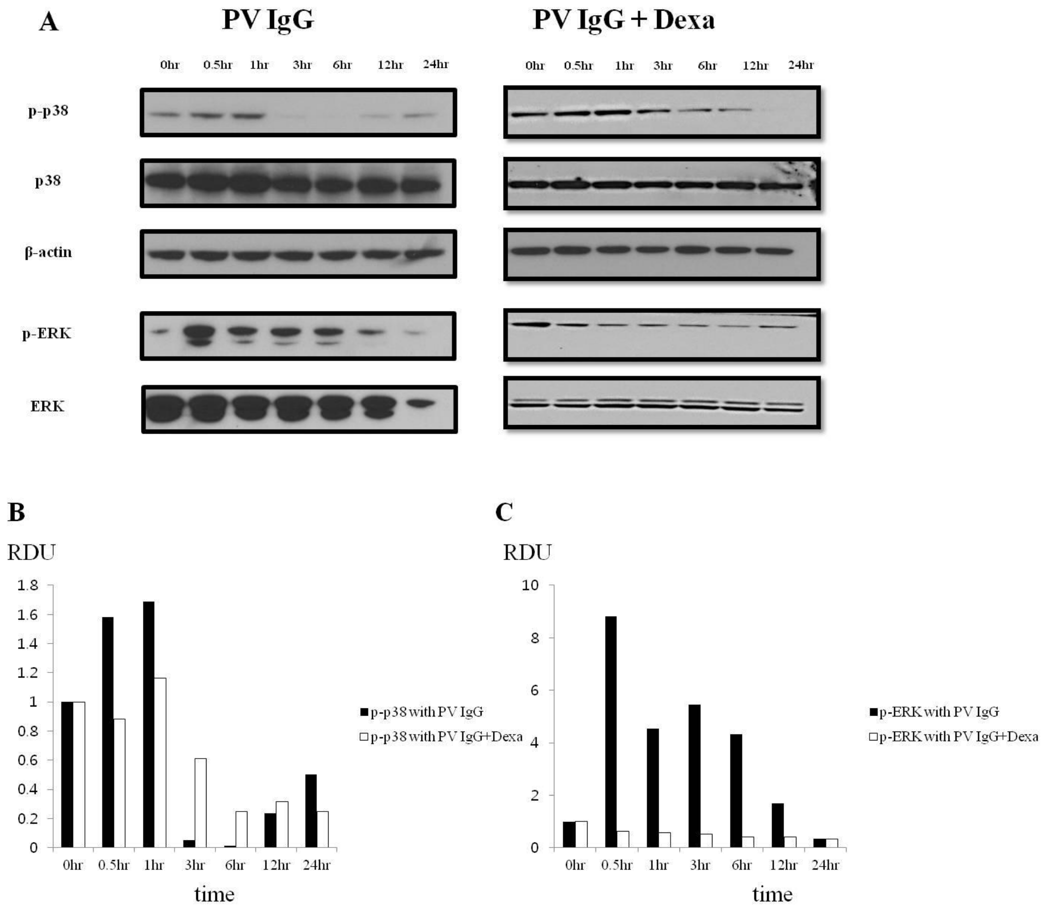


Fig 4. Effects of dexamethasone on the PV IgG induced phosphorylation of signal molecules in keratinocyte.

A) Phosphorylation of p38 was increased in dual peaks at 1hr and 12-24 hrs after incubation with PV IgG, whereas phosphorylation of p38 was decreased in the early phase after incubation with PV IgG plus dexamethasone. Phosphorylation of ERK was increased in peak at 30 mins with PV IgG. However, elevated peak of phosphorylation of ERK is not observed with PV IgG plus dexamethasone.

B) Densitometry of the p-p38 reactivity in western blot shown in (A).

C) Densitometry of the p-ERK reactivity in western blot shown in (A).

β-actin was used to show equal protein load in (B) and (C). To standardize results, levels of p-p38 and p-ERK are expressed as a ratio against β-actin protein quantity, as compared with the ratios obtained in controls (0 hr of incubation at 37℃, taken as 1). RDU, relative densitometric units.

V. DISCUSSION

Pulse therapy refers to discontinuous administration of very high doses of corticosteroids. The choice of the corticosteroid and doses of each pulse are not standardized, but usually are equal to 500–1000 mg methylprednisolone or 100–200 mg dexamethasone.[18] Serum concentration of dexamethasone reached up to mean peak concentration of 4.45 mg/L (0.0113 mM) after administration of 200 mg intravenously.[18] In order to suppress acantholysis in organ culture, high concentration of at least 0.25 mM of methylprednisolone was used to minimize artifacts in skin culture after 72 hrs with longer pre-incubation of skin with lower concentrations of methylprednisolone.[19] In this study concentration of 20 mg/L (0.05 mM) of dexamethasone was used to minimize artifacts in a given incubation time.

Corticosteroids dampen inflammatory responses, and this occurs at least in part by inducing rapid and prolonged expression of MAP kinase phosphatase 1 (MKP-1), which potently inactivates p38 MAPK.[20,21]

Dissociation assay properly evaluates the disease activity among different patients with similar ELISA scores.[16] In this study, PV IgG induced a dramatic weakening of keratinocyte adhesion, leading to a high number of keratinocyte fragments in 6 hrs of incubation at 37℃, whereas PV IgG with dexamethasone (0.05 mM) attenuated weakening of keratinocyte adhesion, leading to a lower number of keratinocyte fragments in 6 hrs.

The results of immunofluorescence examination of cultured keratinocytes demonstrated that polyclonal PV IgG cause clumping and endocytosis of Dsg3 in accordance with the previous result reported by Saito, M., *et al.*[22] Addition of

dexamethasone relatively well preserved linear distribution of Dsg3 after 3-6 hrs incubation. In addition, Dsg3 depletion by PV IgG was attenuated with addition of dexamethasone after 6–24 hrs incubation. These results suggested that the addition of dexamethasone attenuated internalization and depletion of Dsg3 by PV IgG.

p38 MAPK was first discovered to be potentially important in signaling pathway for PV pathogenesis by the detection of increased levels of phosphorylated p38 MAPK and heat shock protein 27 (HSP27) after treatment of primary human keratinocytes with PV IgG, following proteomics effort to determine the set of phosphorylated proteins.[23] The relationship between p38 MAPK activation, Dsg3 endocytosis, and the loss of intercellular adhesion in PV has been difficult to determine.[3] A clear increase in phosphorylation of p38 MAPK was also detected after 30 mins of treatment with AK23 mAb, thus indicating that AK23 mAb binding to Dsg3 augmented the activation of p38 MAPK.[2]

Activation of p38 MAPK resulted in phosphorylation of MAP kinase-activated protein kinase 2 (MAPKAP 2), which in turn phosphorylated HSP27.[24] HSP27 has been shown to regulate both actin[25-27] and intermediate filaments.[28,29] Furthermore, p38 MAPK-mediated phosphorylation of HSP27 had been shown to regulate the cytoskeleton.[30-32] Overwhelming evidence exists demonstrating a prominent role of p38 in short-term regulation of epithelial plasticity that should be distinguished from long-term effects on keratinocyte differentiation and apoptosis.[33] The intermediate filament network tethering desmosomes to the cell membrane has been demonstrated to disintegrate in response to p38 MAPK activation.[33,34] PV IgG-induced phosphorylation of p38

MAPK and HSP27 was followed by remodeling of the actin cytoskeleton and keratin intermediate filament retraction.[4,23] It was also shown that anisomycin-mediated p38 MAPK activation induced both keratin filament retraction and loss of cell adhesion which were abrogated by specific inhibition of p38 MAPK via SB202190.[34]

Timing of p38 MAPK activation is critical for understanding the hierarchy of signaling events leading to acantholysis.[20] Time course experiments demonstrated that the activities of Src and EGFRK peak at 30–60 mins after exposure to PV IgG suggesting that engagement of Src/EGFRK was a critical step that generates signals from ligated antigens to the intracellular effectors such as p38 MAPK affecting keratinocyte adhesion and viability.[6,20] When neonatal C57BL/6J mice were injected intradermally with PF IgG (0.1 mg/g of body weight) *in vivo*, two peaks of p-p38 MAPK activity were detected by western blot of skin extracts from PF IgG-treated mice.[35] The first peak of p38 MAPK activity was observed at 4 hrs, and the second peak was observed at 8–21 hrs.[35] Normal human keratinocytes were incubated with PV IgG for various times from 0 to 10 hrs; extracts were prepared, separated by SDS PAGE, and probed by immunoblot for phospho-p38 MAPK and total p38 MAPK. Similar to the above *in vivo* experiments, two peaks of phospho-p38 MAPK activity were observed in normal human keratinocytes treated with PV IgG: (i) an early peak observed within 30 mins of the addition of PV IgG to cultures and (ii) a second later peak seen at 6–10 hrs after the addition of PV IgG.[35] The time course studies to elucidate the hierarchy of signaling events demonstrated that the activity of Src peaked at 30 mins, EGFRK at 60 mins, and p38 MAPK at 240 mins in keratinocytes exposed to 1 mg/ml of PV IgG.[6]

Activation of MAP kinase ERK by PV IgG was first observed during investigation of EFGR and its downstream substrates.[36] HaCaT keratinocytes treated with 5 mg/ml PV-IgG showed phosphorylated ERK1/2 after 15 min, with a peak at 1 hr and still elevation after 10 hrs of incubation.[36]

In this study, phosphorylated p38 MAPK was increased in dual peaks at 1hr and 12-24 hrs after incubation with PV IgG, whereas early peak of phosphorylated p38 was knocked out after incubation with PV IgG plus dexamethasone *in vitro*. This study also showed that phosphorylated ERK was increased in peak at 30 min with PV IgG. However, elevated peak of phosphorylated ERK was not observed with PV IgG plus dexamethasone. This study demonstrated that elevated peak of phosphorylated ERK at 30 min and early phase increase in phosphorylated p38 *in vitro* with peak times 1 hr with PV IgG was effectively knocked out with addition of dexamethasone. In conclusion, dexamethasone interfered with the PV IgG induced cell signaling.

VI. CONCLUSION

The results of this study are summarized as follows:

1. Dexamethasone attenuated PV IgG induced loss of keratinocyte adhesion in dispase-based dissociation assay.

2. Dexamethasone attenuated PV IgG induced desmoglein endocytosis and depletion in immunofluorescence examination of cultured keratinocytes.

3. The protein level of Dsg3 decreased rapidly until 12 hr incubation at 37℃ with PV IgG as measured by immunoblotting. However, PV IgG with dexamethasone attenuated the decrease of Dsg3.

4. Phosphorylated p38 was increased in dual peaks at 1hr and 12-24 hrs after incubation with PV IgG, whereas phosphorylated p38 was decreased in the early phase after incubation with PV IgG plus dexamethasone. Phosphorylated ERK was increased in peak at 30 mins with PV IgG. However, elevated peak of phosphorylated ERK is not observed with PV IgG plus dexamethasone.

Therefore, this study suggests that corticosteroid plays an important role on the blocking of PV IgG induced acantholysis through attenuating depletion of Dsg3 and phosphorylation of p38 MAPK and ERK signalings.

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ABSTRACT(IN KOREAN)

보통 천포창 환자의 면역글로불린 G에 의해 유도된 가시세포분리에 스테로이드가 미치는 영향

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장 재 용

보통 천포창은 교소체를 구성하는 desmoglein (Dsg) 3에 대한 자가항체가 특징인 수포성 질환으로 치료를 하지 않으면 치명적일 수 있다. Dsg3에 대한 자가항체는 보통 천포창에서 보이는 특징적 조직학적 소견인 가시세포분리를 유발한다. 가시세포분리의 발생기전은 아직도 명확히 밝혀지지 않았지만, 항체가 Dsg 세포외 도메인에 부착하여 입체장애기전(steric hindrance)으로 세포간 부착을 방해한다는 한가지 사실은 알려져 있다. 하지만, 최근 입체장애기전 뿐만 아니라 다양한 세포내 신호전달체계가 가시세포분리의 발생기전에 관여한다는 사실이 보고되고 있다. 첫째, 보통 천포창 면역글로불린 G에 의한 수포형성에 필요한 p38 MAPK 활성이 다수의 교소체 분자들의 세포내 섭취(endocytosis)를 일으킨다. 둘째, 보통 천포창에서 c-myc과 EGFR의 활성이 수포를 형성한다. 셋째, 보통 천포창 면역글로불린 G는 desmoglein에 부착하는 부착-신호망을 이용한 Dsg3의 세포내 섭취를 유발한다. 넷째, 알려지지 않은 기전으로 과다세포증식이 가시세포분

리를 유발한다. 보통 천포창 면역글로불린 G에 의한 세포내 신호전달과 Dsg3 세포내 섭취를 막거나 외부에서 Dsg3를 보충해 준다면 보통 천포창 면역글로불린 G에 의한 가시세포분리를 예방할 수도 있을 것이다.

스테로이드는 보통 천포창의 치료에서 아직도 중요한 기둥의 역할을 하고 있다. 하지만 스테로이드가 보통 천포창 면역글로불린 G에 의해 유도된 가시세포분리에 정확히 어떤 치료적 역할을 하는지는 아직도 잘 알려지지 않았다. 본 연구에서 저자들은 보통 천포창 면역글로불린 G에 의해 유도된 가시세포분리의 과정에 스테로이드가 미치는 영향에 대하여 조사하였다.

결과적으로 dissociation-based assay상 덱사메타손은 보통 천포창 면역글로불린 G에 의해 유도된 가시세포분리를 억제시켰다. 면역형광검사상 덱사메타손은 보통 천포창 면역글로불린 G에 의해 유도된 Dsg3의 세포내 섭취와 소실을 감소시켰다. 면역블롯팅 분석에서 보통 천포창 면역글로불린 G과 함께 배양했을 때 12시간까지 Dsg3의 단백질양이 급격히 감소하였으나 덱사메타손이 첨가된 경우 Dsg3의 감소가 지연되었다. 보통 천포창 면역글로불린 G과 함께 배양했을 때 배양 후 1시간과 12-24시간 두 번 정점을 보이며 인산화 p38 MAPK가 증가하였으나 덱사메타손이 첨가된 경우 인산화 p38 MAPK가 초기에 감소

하는 양상을 보였다. 인산화 ERK의 경우도 보통 천포창 면역글로불린 G과 함께 배양 30분 후 정점을 보이며 증가했지만 덱사메타손이 첨가된 경우 앞서 언급된 증가양상이 보이지 않았다.

그러므로, 본 연구의 결과는 스테로이드가 p38 MAPK와 ERK 신호전달의 인산화 및 Dsg3의 양적 소실을 방지함으로써 보통 천포창 면역글로불린 G에 의해 유도된 가시세포분리의 방지에 중요한 역할을 한다는 것을 시사한다.

핵심되는 말: 보통 천포창, 가시세포분리, 스테로이드, desmoglein, p38 MAPK, ERK