The therapeutic effect of PLAG in the imiquimod-induced psoriasis mice model

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Abstract

Psoriasis is regarded as a typical inflammatory disease triggered by damage-associated molecular patterns (DAMPs) showing phenotype like as proliferation of keratinocytes and infiltration of excessive neutrophils into dermis and epidermis. Imiquimod (IMQ), analogue of nucleotides as DAMPs is commonly used to develop psoriasis-like skin inflammation in the mice.

As the main pathogenesis of psoriasis, IMQ stimulates epithelial cells and tissue resident macrophages and results in secretion of chemo-attractants which initiate neutrophil recruitment into lesion. IMQ induced epidermal expression of IL-17, IL-22 and CXCL8. Daily application of IMQ on mouse back skin induced inflamed scaly skin lesions resembling plaque type psoriasis. These lesions showed increased epidermal proliferation, abnormal differentiation, and epidermal accumulation of neutrophils in microabcesses. Synthetic diacylglycerol derivatives, 1-palmitoyl-2-linoleoyl-3-acetyl-rac-glycerol (PLAG) was studied for evaluation of its therapeutic efficacy on IMQ-induced psoriasis-like skin inflammation. PLAG has a prominent biological effect on amelioration in the IMQ-induced inflammatory disease and on attenuation of IL-17, IL-22, and MIP-2 expression in the dermal tissue of mice.

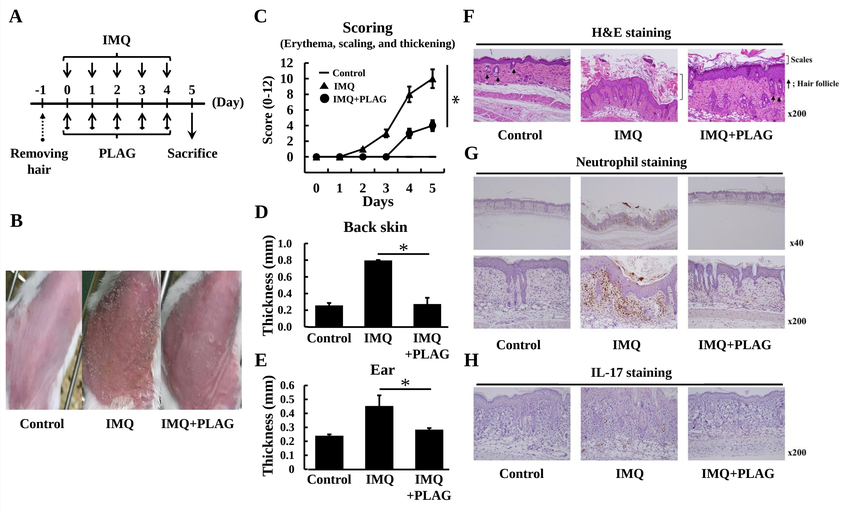
Through an in vitro assay system, the effective eliminating of IMQ as a DAMPs molecule, terminating of successive chemokine and cytokine releasing, blocking of excessive neutrophil migrate activity by PLAG treatment was verified. Promoted resolution of DAMPs successively produced during tissue damage is very crucial function of PLAG for maintenance of homeostasis in the IMQ induced psoriatic skin disorder. Our studies suggest that orally administration of PLAG could be utilized as an effective therapeutic tool for skin inflammatory disease.

Introduction

- Psoriasis is a chronic inflammatory disease in skin. Its major histopathological phenotype is increased proliferation and uncontrolled differentiation of keratinocytes and infiltration of various leukocytes. **Stenderup K *et al. J Invest Dermatol.* (2011) 131(10):2033-9**
- Expression of cytokines and chemokines [C-X-C chemokine ligand (CXCL)1 and CXCL2] increased imiquimod (IMQ)-induced psoriasis mouse model , leading to the reduction of neutrophilic abscess in the skin. **Zin-Chan Lin *et al. FASEB J.* (2018) 19:fj201800354.**
- Neutrophil-keratinocyte interaction in the early pathogenesis of psoriasis, showing the importance of neutrophil in the early phase of psoriasis, because they are involved in T-cell recruitment and keratinocyte proliferation/differentiation. **Mahil, S.K, *et al. Semin. Immunopathol.* (2016) 38, 11-27**
- DAMP molecules, Extracellular ATP and IL-23, form a local inflammatory circuit leading to the development of a neutrophil-dependent psoriasiform dermatitis. **Diaz-Perez JA *et al. J Invest Dermatol*. (2018) (18)32040-2.**
- PLAG (1-palmitoyl-2-linoleoyl-3-acetyl-rac-glycerol) is an acetylated form of diacylglycerol and a mono-acetyl-diglyceride that was first isolated from the antlers of sika deer. PLAG can be chemically synthesized using glycerol, palmitic acid, and linoleic acid, and the synthetic form has been confirmed to be identical with the naturally isolated form. **Yang HO *et al.*, (2004) Biol Pharm Bull 27(7):1121-1125.**
- PLAG was shown to exert a therapeutic effect with pegfilgrastim to treat chemotherapy-induced neutropenia by modulating neutrophil transmigration. **Yoo N., *et al* (2016) Cancer Letters 377(1), 25-31**
- PLAG alleviated pathogenesis of psoriasis via effective elimination of extracellular IMQ as DAMP

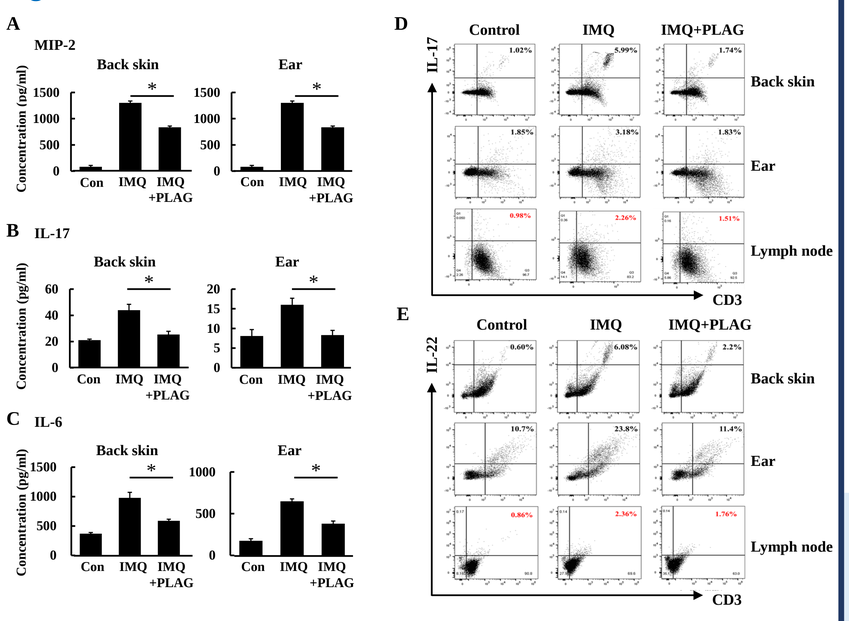
Result

1. PLAG attenuated psoriasis in the imiquimod-induced mouse model



Mice were divided into three groups: 1) control group, 2) IMQ cream-treated group, and 3) PLAG/ IMQ cream-treated group. Mice were treated with IMQ cream on the shaved back and one ear every day for 5 days. PLAG were administered 250 mg/kg/day orally. On Day 5, mice were sacrificed and the isolated tissues were analyzed (**Figure 1A**). On Day 5, the back of mice were photographed (**Figure 1B**). IMQ cream treated back skin effectively induced psoriatic lesion and PLAG significantly attenuated severity of psoriasis which was verified with scoring (**Figure 1C**), measurement of back skin and ear (**Figure 1D, 1E**), IHC staining (**Figure 1F**), infiltration of neutrophil (**Figure 1G**) and IL-17 staining (**Figure 1H**).

2. PLAG reduced IMQ-induced inflammatory cytokine in the psoriatic region



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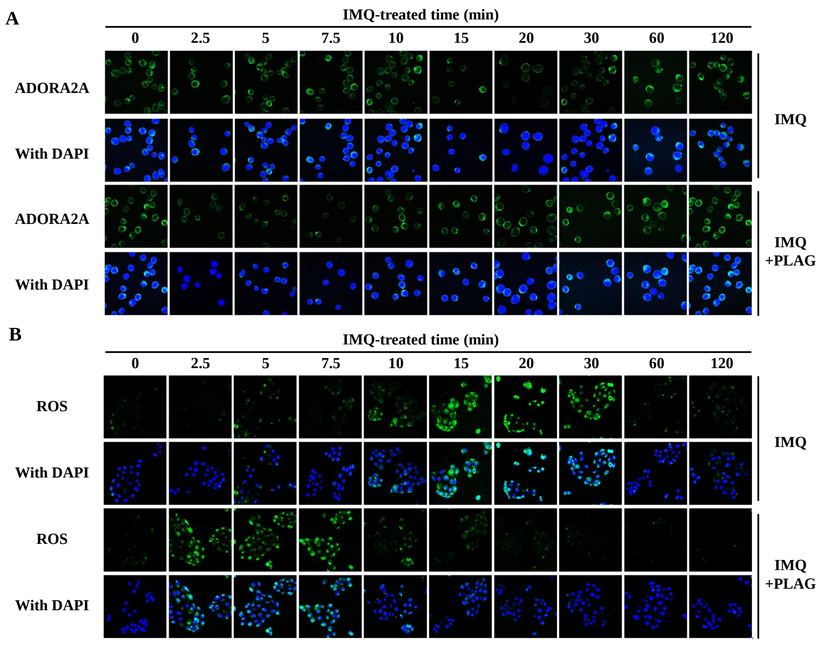
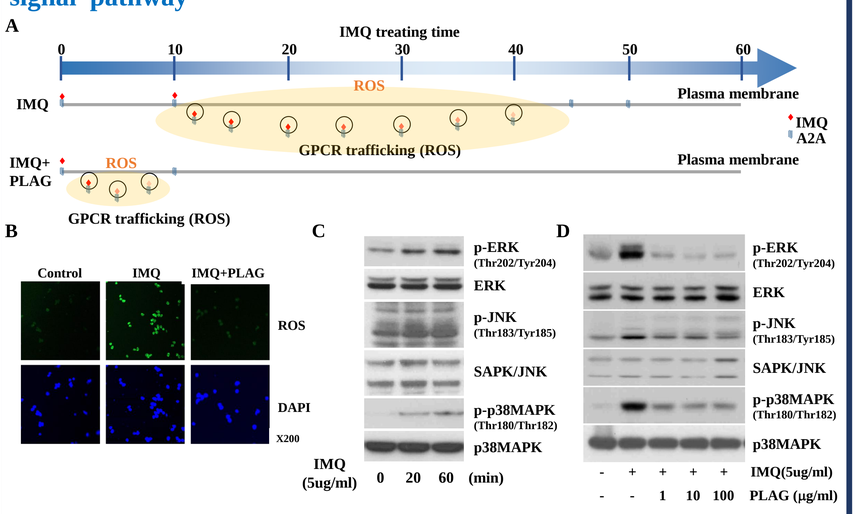
3. Imiquimod-induced intracellular trafficking of A2A and ROS production was accelerated by PLAG



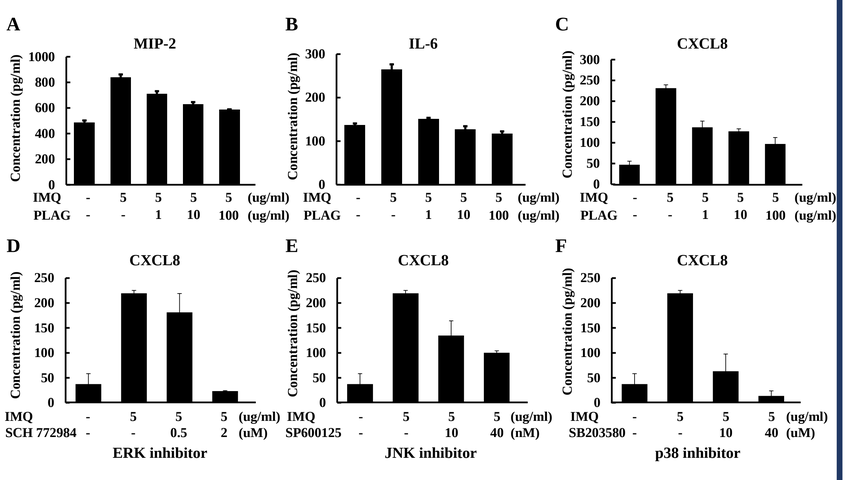
PLAG role for psoriasis protection was investigated *in vitro* system. IMQ was introduced to HaCaT cells, a keratinocyte cell line, and was recognized by its receptor adenosine receptor A2A and followed by endocytosis of the receptor for elimination. *In vitro* culture condition, A2A endocytosis was observed at 15min and A2A was returned to cell surface at 60min after IMQ treatment (**Figure 3A**). Intensive ROS production was also detected only A2A almost disappeared from cell surface (**Figure 3B**). In the PLAG treated cells, the accelerating of endocytosis and ROS production were apparently observed, endocytosis and ROS production was started at 2.5 min and return to membrane of A2A and termination of ROS production were shown up at 10 min. which data indicated that the extracellular of DAMP molecule was promptly eliminated by PLAG treatment via promoted GPCR trafficking.

4. Promotion of ROS production by PLAG modulated the intracellular signal pathway



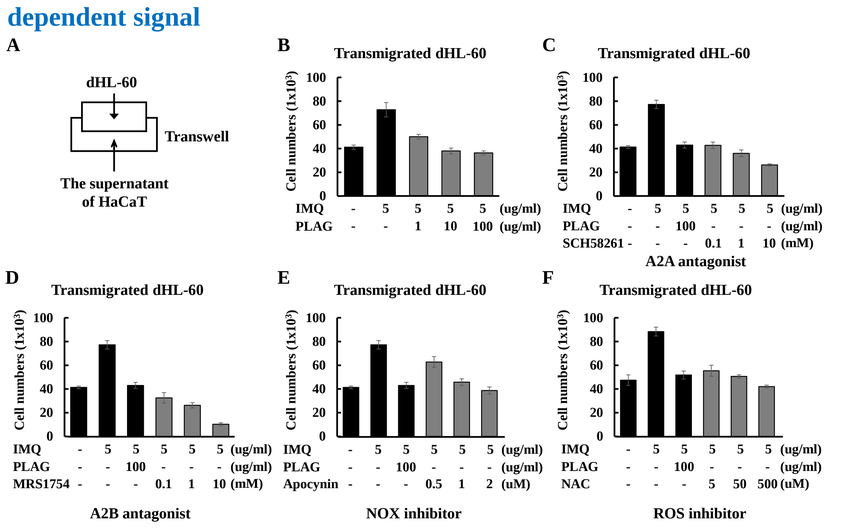
PLAG induced profound changes on the GPCR trafficking in the IMQ treated keratinocytes. PLAG induced the promoted endocytosis and shortened duration of intracellular ROS production (**Figure 4A**). Modulation ROS production means change of ROS dependent signal pathway. Practically, phosphorylation of MAPK including ERK, JNK, p38 was induced in the IMQ treated cells (**Figure 4B**). Decrease of MAPK phosphorylation in the PLAG treated cells was observed (**Figure 4C**). Early termination of ROS by PLAG eventually could effect on negative regulation of MAPK phosphorylation.

5. PLAG modulated cytokine production via MAPK dependent signal



RAW 264.7 or HaCaT cells were pretreated with 100 ug/ml of PLAG or DMSO (as solvent control) for 1 hr and then were stimulated with 5 ug/ml of IMQ for 12 hr. MIP-2 (**Figure 5A**), IL-6 (**Figure 5B**) and CXCL8 (**Figure 5C**) in the culture supernatants were analyzed with the cognate antibody using ELISA kit. Modulation of CXCL8 expression in the IMQ treated HaCaT cells by MAPK inhibitors, SCH 772984(ERK inhibitor, **Figure 5D**), SP600125(JNK inhibitor, **Figure 5E**) and SB203580 (p38 inhibitor, **Figure 5F**) was evaluated using ELISA kit. IMQ treated keratinocyte apparently induced chemokine CXCL8 which successively recruits neutrophil into epidermis. Excessive neutrophil infiltration into lesion was prototype of pathogenesis of psoriasis. PLAG attenuated CXCL8 expression in the IMQ treated HaCaT cell via controlling MAPK signaling like as dephosphorylation of ERK, JNK, p38.

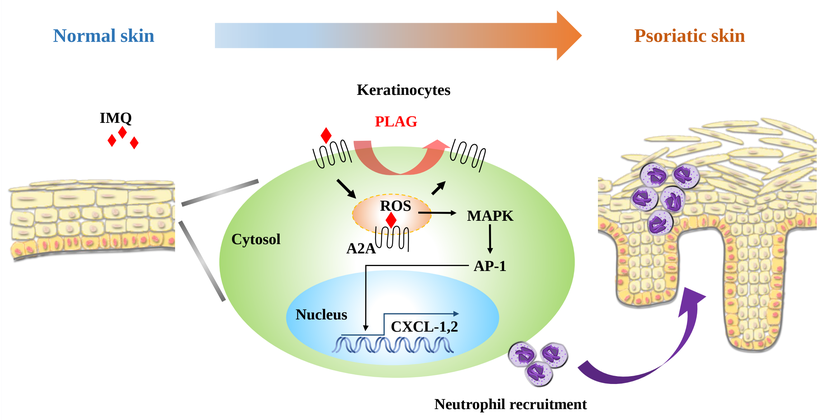
6. PLAG decreased neutrophil transmigration via receptor/ROS dependent signal



To determine that PLAG regulates neutrophil recruitment, in vitro transmigration assay system was used for evaluation of transmigrated cell. $1x10^5$ /ml of differentiated HL-60 was placed upper chamber (**Figure 6A**). For preparation of supernatant of HaCaT cells, cells were pretreated with the 1, 10, 100 ug/ml of PLAG or DMSO (as solvent control) for 1 hr and then were stimulated with 5 ug/ml of IMQ for 12 hr. The supernatants were put down chamber and migrated HL-60 cells were counted (**Figure 6B**). HaCaT cells were pre-incubated with PLAG (100 μg/ml) or SCH58261(A2A antagonist),MRS1754(A2B antagonist), Apocynin(NOX inhibitor), NAC(ROS inhibitor) for 1 hr and then stimulated with IMQ (5ug/ml). After 12 hr, cells were centrifuged and the supernatant was transferred in the bottom chamber of transwell (**Figure 6C-F**). After 24 hr, the migrated dHL-60 were counted using hemocytometer with trypan blue staining. Neutrophil in vitro transmigration by the CXCLs induced in the IMQ treated keratinocyte was observed. PLAG reduced neutrophil transmigration *in vitro* system with dose dependent manner. Inhibition A2A, A2B which is IMQ receptor reduced neutrophil migration. Inhibition of NOX and ROS also effectively reduced neutrophil transmigration.

Summary

Therapeutic effects of PLAG in the pathogenesis of psoriasis with imiquimod



Therapeutic effects of in the IMQ induced psoriatic lesion

- IMQ as a kind of DAMP is recognized by GPCR A2A receptor in keratinocyte
- A2A receptor bound IMQ induces GPCR trafficking
- During intracellular trafficking as form of endosome, intensive ROS production was detected
- Phosphorylation of MAPK was induced with ROS dependent signal
- Increased chemokine CXCLs recruits neutrophils into the epidermis where IMQ introduced.
- PLAG effectively prevent progress of psoriasis induced by IMQ via prompt elimination of DAMP