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Nojirimycin-1-sulfonic acid regulates macrophage polarization to suppress psoriatic inflammation in epidermal cells

Min Ju Jung
CHA University
Hye Jin Lim
Graduate School of CHA University
Jin Hyeop Kim
CHA University
Yeon-Jun Lee
CHA University
Ha Hui Park
CHA University
Ji Hyung Chung
jhchung@cha.ac.kr

CHA University

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Abstract

Psoriasis is a chronic autoimmune skin disease characterized by excessive epidermal proliferation and immune-cell infiltration. Current therapies, including steroids and cytokine inhibitors, can relieve symptoms but are limited by transient efficacy and immunosuppressive side effects. Here, we investigated the anti-inflammatory effects of nojirimycin-1-sulfonic acid (NSA), a derivative of 1-deoxynojirimycin, with a focus on macrophage polarization and macrophage–keratinocyte crosstalk. In LPS-stimulated macrophages, NSA significantly reduced the expression of proinflammatory mediators, including IL-6, TNF-α, COX-2, iNOS, and NF-κB. NSA also promoted polarization toward an anti-inflammatory M2 phenotype, as indicated by increased CD163 and CD206 expression. Conditioned media from LPS-stimulated macrophages induced psoriasis-associated responses in keratinocytes, including increased S100A7, S100A8, S100A9, and HBD-2 expression. In contrast, conditioned media from NSA-treated macrophages markedly attenuated these responses and suppressed keratinocyte hyperactivation without affecting cell viability. These findings indicate that NSA alleviates psoriatic inflammation indirectly by reprogramming macrophage polarization and modulating macrophage–keratinocyte communication. Collectively, this study highlights a macrophage-centered immunomodulatory strategy and suggests NSA as a potential therapeutic candidate for psoriasis.

1. Introduction

Psoriasis is a chronic autoimmune skin disorder defined by the excessive proliferation of epidermal cells and the infiltration of immune cells in the dermis [1]. It affects approximately 1%–3% of the global population, with prevalence rates demonstrating regional variability [2]. Although psoriatic symptoms include itchy skin and rash, psoriasis also involves various internal organs, with approximately one in four patients with psoriasis also being affected by psoriatic arthritis [3, 4]. The pathophysiology of this disease is driven by dysregulated interactions between immune cells and keratinocytes [5]. Activated immune cells release proinflammatory cytokines such as TNF-α, IL-6, IL-17, and IL-23, which stimulate the proliferation of keratinocytes and the production of antimicrobial peptides. In turn, keratinocytes produce cytokines and chemokines that amplify immune activation, generating a self-sustaining inflammatory circuit [6]. Studies have demonstrated hyperactivation of macrophages in the skin tissue of patients with psoriasis, suggesting a vital role of macrophages in skin inflammation [7, 8]. This reciprocal communication is considered an essential mechanism underlying the persistence of psoriatic lesions.

Macrophages respond to dynamic and variable microenvironmental signals with remarkable flexibility and can polarize into distinct functional phenotypes. M1 macrophages constitute a proinflammatory phenotype, whereas M2 macrophages represent an anti-inflammatory phenotype [9]. When the body is stimulated by various factors such as bacterial lipopolysaccharide (LPS), LPS-driven M1 macrophage activation releases proinflammatory cytokines, such as TNF-α, IL-1β, iNOS, and COX-2 [10, 11]; secretes psoriasis-inducing factors, such as IL-23, IL-17A, and IL-22 [12]; and induces transcriptional regulators, such as NF-κB and STAT3 [13]. The differentiation of M2-type macrophages is driven by IL-4 or IL-13, resulting in the increased production of anti-inflammatory mediators such as IL-10 and profibrotic

factors such as TGF-β [14, 15]; these M2-type macrophages are characterized by markers such as CD163 and CD206 [16]. Studies have shown that blocking the JAK/STAT3 signaling cascade facilitates the polarization of macrophages toward the M2 phenotype, which suppresses proinflammatory responses along with increasing the secretion of anti-inflammatory and profibrotic cytokines, thereby contributing to the attenuation of tissue inflammation and damage [17]. Antimicrobial peptides (AMPs) such as S100A7, S100A8, S100A9, and HBD-2 not only amplify local inflammatory responses that contribute to the development of psoriatic plaques but also regulate the function of keratinocytes. In particular, S100A7 and the S100A8/S100A9 complex promote the differentiation of keratinocytes, whereas S100A8/S100A9 additionally suppresses their proliferation, indicating that S100A proteins act as dual regulators of epidermal differentiation and growth as well as important markers of psoriasis pathogenesis [18].

Natural compounds and their derivatives have been used since a long time as valuable sources of novel therapeutics [19]. In this context, nojirimycin-1-sulfonic acid (NSA) is a structural derivative of the natural compound 1-deoxynojirimycin (DNJ) extracted from mulberry leaves [20]. Recent studies on DNJ have demonstrated varied biological properties, including anticancer [21], anti-inflammatory, and antioxidant effects [22, 23]. In contrast, reports on NSA are scarce and largely confined to its modulation of acid α-glucosidase activity in Pompe disease [24]. Consequently, the biological roles of NSA are largely unknown, and its anti-inflammatory potential and underlying biological mechanisms remain unexplored, particularly in psoriasis. The major treatment methods for psoriasis include local therapies, phototherapy, complementary medicine practices, and biologic agents [25]. Nevertheless, biologic agents are associated with risks such as steroid resistance and infections caused by immunosuppression [26]. Hence, there exists a need for the development of safer and more effective psoriasis treatments.

Therefore, we conducted this study to investigate the psoriasis-suppressing potential of the DNJ derivative NSA, focusing on the interaction between hyperactivated macrophages and epidermal cells. In particular, we explored whether NSA modulates the phenotypic shift of macrophages from a proinflammatory M1 state toward an anti-inflammatory M2 phenotype. For this purpose, we first investigated its effects on LPS-stimulated RAW 264.7 macrophages by measuring the production of nitric oxide (NO), secretion of IL-6 and TNF-α, and expression of M1/M2 markers. We further determined the effect of NSA-conditioned macrophage medium on normal human epidermal keratinocytes stimulated with IL-17A and TNF-α, focusing on psoriasis-associated markers and the activation of NF-κB and STAT3 signaling pathways. Accordingly, rather than directly targeting keratinocytes within an already established inflammatory environment, we determined whether keratinocyte inflammatory responses could be indirectly suppressed by modulating the inflammatory microenvironment through changes in macrophage polarity, a major source of inflammatory signals.

2. Materials & Methods

2.1. Chemical structure

NSA, a sulfonated derivative of the iminosugar DNJ, was used in this study. The chemical structures of DNJ and NSA are depicted in Fig. 1A. DNJ and NSA were purchased from Sigma-Aldrich (St. Louis, MO, USA). All compounds were dissolved in sterile distilled water and prepared for *in vitro* experiments.

2.2. Cell culture

RAW 264.7 cells (mouse macrophage line, ATCC, Manassas, VA, USA) were maintained in high-glucose Dulbecco's modified Eagle medium (Welgene, Gyeongsan, Korea) supplemented with 10% fetal bovine serum (FBS; Corning, New York, NY, USA) and 1% penicillin–streptomycin (P/S; Thermo Fisher Scientific, Waltham, MA, USA) at 37°C in a humidified incubator with 5% CO2. Normal human epidermal keratinocytes (NHEKs; Thermo Fisher Scientific) were cultured in EpiLifeTM serum-free medium (Thermo Fisher Scientific) containing human keratinocyte growth supplement (HKGS; Thermo Fisher Scientific) and 1% P/S under identical conditions.

2.3. NO production and cell viability

NO production was detected using the Griess assay. RAW 264.7 cells were seeded in 96-well plates (5×10^4 cells/well), pretreated with NSA at concentrations of 1, 10, 50, 100, 200, 300, and 400 μM (Sigma-Aldrich, St. Louis, MO, USA) for 1 h, and then stimulated with 1 μg/mL LPS (Sigma-Aldrich) for 24 h. Supernatants were mixed 1:1 with Griess reagent (Sigma-Aldrich) and incubated for 15 min, after which the absorbance was measured at 540 nm using a microplate reader (BioTek, Winooski, VT, USA). Cell viability was evaluated in parallel using a WST-1 assay kit (Ez-cytox; Dogenbio, Seoul, Korea). RAW 264.7 cells were seeded in 48-well plates (5×10^4 cells/well), treated under similar conditions, and incubated with Ez-cytox reagent for 2 h, after which the absorbance was measured at 450 nm.

2.4. Cytokine measurements

The concentrations of IL-6 and TNF-α in RAW 264.7 cell culture supernatants were measured using mouse IL-6 ELISA kits (BioLegend, San Diego, CA, USA) and mouse TNF-α ELISA kits (BioLegend). RAW 264.7 cells and NHEKs were pretreated with NSA and stimulated with LPS as described in Section 2.2, and the cell supernatants were collected and centrifuged before analysis.

2.5. Western blot analysis

Both RAW 264.7 cells and NHEKs were pretreated with NSA. RAW 264.7 cells were then stimulated with LPS, whereas NHEKs were stimulated with IL-17A/TNF-α or cultured with conditioned medium derived from NSA-treated RAW 264.7 cells (5%). After treatment, the cells were lysed with RIPA buffer (Biosesang, Gyeonggi, Korea) containing protease inhibitors, and protein concentrations were determined using the BCA protein assay kit (Thermo Scientific Pierce, Rockford, IL, USA). Equal amounts of protein (20–50 μg) were separated by SDS-PAGE and transferred to PVDF membranes (0.2 μm pore size). The membranes were then blocked with 5% skim milk in TBS-T and incubated overnight at 4°C with the following primary antibodies: GAPDH, α-tubulin, COX-2, NF-κB, IκB-α, p-IκB-α, S100A8, S100A9, and STAT3 (Santa Cruz Biotechnology, Dallas, TX, USA); iNOS (Thermo Fisher Scientific); S100A7 (Abcam,

Cambridge, UK); and p-STAT3 and p-NF-κB (Cell Signaling Technology, Danvers, MA, USA). After primary antibody incubation, the membranes were washed three times with 0.1% TBS-T followed by one wash with TBS, each for 7 min, and then incubated with HRP-conjugated secondary antibodies, anti-mouse IgG, or anti-rabbit IgG (Santa Cruz Biotechnology). Protein bands were visualized using the Clarity™ Western ECL substrate (Bio-Rad, Hercules, CA, USA).

2.6. Immunocytochemistry

RAW 264.7 cells were seeded onto sterile glass coverslips, pretreated with NSA, and stimulated with LPS as described earlier. The cells were then fixed with 4% paraformaldehyde, permeabilized with 0.25% Triton X-100 for iNOS staining, and blocked with 5% BSA. Next, they were incubated overnight at 4°C with iNOS (Thermo Fisher Scientific), CD163, or CD206 antibodies (Santa Cruz Biotechnology), followed by Alexa Fluor™488–conjugated goat anti-mouse or Alexa Fluor™594–conjugated goat anti-rabbit secondary antibodies (Sigma-Aldrich). Nuclei were counterstained with DAPI (Thermo Fisher Scientific), and fluorescence images were acquired using a microscope.

2.7. Flow cytometry analysis

RAW 264.7 cells were pretreated with NSA and stimulated with LPS as described earlier. After washing and blocking with 0.5% BSA, the cells were incubated with primary antibodies to CD163 and CD206 at a dilution of 1:250, followed by Alexa Fluor™ 488–conjugated goat anti-mouse secondary antibody. Fluorescence signals were analyzed using a CytoFLEX flow cytometer (Beckman Coulter, Brea, CA, USA), and surface protein expression was quantified.

2.8. IL-17A/TNF-α stimulation of NHEKs to establish a psoriasis-like inflammatory model

To establish an in vitro psoriasis-like inflammatory model, NHEKs were seeded in 48-well plates (1.2×10^4 cells/well) and pretreated with NSA as described earlier. At ~ 60% confluency, the cells were stimulated with IL-17A (100 ng/mL; R&D Systems, Minneapolis, MN, USA) and TNF-α (10 ng/mL; PeproTech, Rocky Hill, NJ, USA) for 24, 48, 72, 96, or 120 h [27]. Then, the expressions of S100A7, S100A8, and S100A9 were determined by western blotting. The secretion of HBD-2 in the culture supernatants was quantified using a human HBD-2 ELISA kit (PeproTech).

2.9. Preparation of conditioned media

RAW 264.7 cells were seeded in 60-mm dishes (1×10^6 cells/dish) and cultured for 24 h. The cells were then pretreated with different concentrations of NSA for 1 h and stimulated with 1 μg/mL LPS for 24 h. The supernatant collected from culture media was referred to as activated conditioned medium (activated-RCM, supernatant obtained from LPS-stimulated RAW 264.7 cells, with or without NSA). Activated-RCMs were prepared from samples stimulated with only LPS, and LPS-stimulated samples were pretreated with various concentrations of NSA (1, 10, 50, 100, 200. 300, or 400 μM). Conversely, the culture medium of RAW 264.7 cells without LPS or NSA treatment was referred to as naive conditioned medium (naive-RCM, supernatant derived from LPS-untreated RAW 264.7 cells). In experiments

performed to observe the effect on keratinocytes, the conditioned media were applied at 5% (v/v) to NHEKs at ~ 60% confluency, followed by incubation for 72 h.

2.10. Statistical analysis

All experiments were conducted in triplicate, and results are expressed as mean ± SD. Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test. All statistical analyses were conducted using GraphPad Prism 9 (GraphPad Software, San Diego, CA, USA), and densitometric quantification was performed using ImageJ (NIH, Bethesda, MD, USA). Statistical significance was defined as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$.

3. Results

3.1. Effect of NSA on NO production and cell viability

The LPS stimulation of RAW 264.7 cells resulted in a substantial increase in NO production, with levels approximately 10-fold higher than those in the control group (Fig. 1B). Pretreatment with NSA at concentrations of 1, 10, 50, 100, 200, 300, and 400 μM reduced the production of NO by 1.14%, 1.23%, 5.75%, 8.44%, 20.44%, 31.7%, and 38.82%, respectively. Significant inhibition of NO production was detected at NSA concentrations of ≥ 100 μM. Cell viability determined using the WST-1 assay exhibited no significant differences between the NSA-treated and control groups at all tested concentrations (Fig. 1C).

3.2. Effects of NSA on the production of the proinflammatory cytokines IL-6 and TNF-α

The effect of NSA treatment on the secretion of inflammatory cytokines was explored in RAW 264.7 cells. For this purpose, the cells were pretreated with the same concentration of NSA as described earlier for 1 h and then stimulated with LPS. The expression of IL-6 and TNF-α was analyzed using ELISA experiments, which confirmed a significant increase in expression in the LPS-treated group compared with that in the NT group ($p < 0.001$). However, after treatment with NSA, the expression of IL-6 and TNF-α was significantly suppressed in a concentration-dependent manner (Fig. 2A, B). In particular, at 400 μM NSA, the levels of IL-6 and TNF-α reduced by 28% and 25%, respectively, compared with those in the LPS-alone-treated group ($p < 0.001$).

3.3. Effect of NSA on the expression of the M1 markers iNOS and COX-2

We examined the expression of iNOS and COX-2, inflammatory markers associated with M1 macrophage activation, and found that both proteins showed markedly increased expression after LPS stimulation compared with that in the NT group (Fig. 2C, D). However, NSA treatment resulted in a progressive reduction in the expression of these markers. Remarkably, iNOS expression was restored to levels

comparable to those in the NT group at NSA concentrations of > 200 μM. The expression of COX-2 also significantly reduced, exhibiting an ~ 41% decrease compared with that in the LPS-treated group at 400 μM NSA ($p < 0.001$). On the basis of these findings, we selected NSA concentrations of 50 and 300 μM for subsequent immunocytochemistry analysis. Consistent with the western blot findings, immunofluorescence staining revealed that NSA treatment markedly reduced the fluorescence intensity of iNOS compared with that in the LPS-alone-treated group, with a greater reduction found at 300 μM (Fig. 2E). NSA treatment also significantly reduced the expression of iNOS (Fig. 2C) and COX-2 (Fig. 2D) in LPS-stimulated RAW 264.7 cells. At 400 μM, NSA reduced the expression of COX-2 by ~ 41% compared with that in the LPS-treated group ($p < 0.001$). On the basis of these data, we selected NSA concentrations of 50 and 300 μM for further immunocytochemical analyses. Under these conditions, NSA treatment significantly reduced the fluorescence intensity of iNOS (Fig. 2E) compared with that in the LPS-alone-treated group.

3.4. Effect of NSA on the expression of the M2 markers CD163 and CD206

To determine the effect of NSA treatment on the M2 phenotype, RAW 264.7 cells were pretreated with 50 or 300 μM NSA followed by stimulation with LPS. Immunocytochemistry revealed a concentration-dependent increase in the expression of CD163 (Fig. 3A) and CD206 (Fig. 3E) compared with that in the LPS-treated group. Flow cytometry analysis (Figs. 3B, 3F) demonstrated that NSA treatment increased both the proportion of CD163- and CD206-positive cells (Figs. 3C, 3G) and the mean fluorescence intensity (MFI) (Figs. 3D, 3H) in a concentration-dependent manner compared with that in the LPS-alone-treated group.

3.5. Therapeutic effects of NSA-treated activated-RCM on NHEKs in psoriasis

We established an *in vitro* psoriasis-like inflammatory model by stimulating NHEKs with IL-17A and TNF-α. Western blot analysis revealed a time-dependent increase in the expression of S100A7, S100A8, and S100A9, which became evident from 48 h and persisted up to 120 h. In parallel, the expression of HBD-2, determined by ELISA, also increased in a time-dependent manner after treatment with IL-17A and TNF-α. Based on these findings, we selected a stimulation period of 72 h for subsequent experiments (Supplementary Fig. 1A, B). To determine the effects of macrophage-derived factors on psoriatic inflammation, RAW 264.7 cells were pretreated with NSA and then stimulated with LPS for 24 h. The resulting conditioned media were collected and applied to NHEKs at a concentration of 5% (v/v) for 72 h to examine the macrophage-mediated effects on keratinocyte inflammatory responses (Fig. 4A). Based on the expression of HBD-2, it was confirmed that IL-17A/TNF-α treatment increased it by ~ 3.4 times compared with that in the NT group (No Treatment), and a tendency of more than three-fold increase was confirmed between naive-RCM, which is a RAW 264.7 cell medium treatment group that was not treated with anything, and activated-RCM induced by LPS. These data indicate that LPS-activated macrophage-derived factors can significantly elevate the inflammatory response of keratinocytes.

Conversely, RCM pretreated with NSA tended to significantly reduce this inflammatory response. The expression of HBD-2 gradually decreased with increases in NSA concentration; in particular, NSA concentrations of 300 and 400 μM restored the expression of HBD-2 to a level similar to that observed in naive-RCM (Fig. 4B). Remarkably, the cell viability analysis confirmed that none of the tested concentrations of NSA induced cytotoxicity (Fig. 4C), thereby suggesting that the observed effects were attributable to immunomodulation rather than nonspecific toxicity. The western blot analysis revealed that treatment of NHEKs with conditioned media derived from LPS-stimulated macrophages markedly increased the expression of S100A7, S100A8, and S100A9 compared with that in naive-RCM. In contrast, treatment with conditioned media derived from NSA-pretreated, LPS-stimulated macrophages significantly attenuated the expression of these psoriasis-associated proteins (Fig. 4D).

3.6. Effect of NSA-treated activated-RCM on NF-κB/IκB-α and STAT3 signaling in keratinocytes

We next confirmed the protein expression of NF-κB and IκB-α in NHEK cells. The activated-RCM derived from LPS-treated RAW 264.7 macrophages significantly increased the phosphorylation of NF-κB and IκB-α in NHEK cells compared with naive-RCM. In contrast, the groups treated with NSA and LPS-treated, RAW 264.7 macrophage–derived cultures exhibited a dose-dependent decrease in the phosphorylation levels of NF-κB and IκB-α, with the most marked inhibitory effect detected at 300 μM NSA (Fig. 5A–C). Similarly, STAT3 phosphorylation also increased in activated-RCM but was significantly suppressed in the NSA-treated group, with the greatest reduction detected at 300 μM NSA (Fig. 5D, E).

4. Discussion

This study explored the anti-inflammatory effects of NSA, a derivative of the natural iminosugar DNJ. Results demonstrated that NSA treatment suppressed M1 polarization, improved the M2 phenotype, and reduced keratinocyte hyperactivation. These effects were accompanied by the inhibition of NF-κB and STAT3 signaling, two vital regulators of psoriatic inflammation. Psoriasis is characterized by keratinocyte hyperproliferation, epidermal thickening, and immune dysregulation, often accompanied by systemic comorbidities such as psoriatic arthritis, metabolic syndrome, and cardiovascular disease. These characteristics underscore psoriasis as not only a skin disease but also a systemic inflammatory condition [28, 29]. Although advances in biological therapies have significantly improved disease management, limitations related to cost, route of administration, and safety necessitate the investigation of novel small-molecule therapies. Among the immune players involved in psoriasis, macrophages have emerged as pivotal regulators of both the initiation and persistence of inflammation [30].

Classically activated M1 macrophages secrete proinflammatory mediators, including NO, IL-6, and TNF-α, thereby amplifying immune responses [31]. In contrast, alternatively activated M2 macrophages produce anti-inflammatory cytokines and contribute to tissue repair and homeostasis [32]. The predominance of M1 activity and the relative deficiency of M2 function contribute to sustained inflammation in psoriatic lesions [33]. Consequently, therapeutic strategies capable of reprogramming

macrophage polarization hold strong promises in restoring immune balance. One of the earliest hallmarks of macrophage activation is the production of NO via inducible nitric oxide synthase (iNOS) [34]. NO is a reactive mediator that amplifies inflammatory cascades and has been associated with the severity of psoriasis [35]. Our experiments demonstrated that NSA treatment reduced the NO levels in a concentration-dependent manner without compromising the viability of macrophages, suggesting that NSA does not exert general cytotoxicity but selectively interferes with inflammatory signaling. Accordingly, we conducted a preliminary screening for DNJ and 12 structurally related derivatives and found that although most compounds exhibited some degree of inhibition of NO production, NSA demonstrated the strongest suppression, reducing NO release by ~ 25% at 25 μM (Supplementary Fig. 2). We also observed the effects of various concentrations of DNJ and NSA on NO production in LPS-stimulated RAW 264.7 cells and found that DNJ exhibited no significant change in NO production depending on the concentration, whereas NSA treatment tended to decrease NO production in a concentration-dependent manner (Supplementary Fig. 3). These preliminary results guided the selection of NSA as the main candidate for subsequent mechanistic studies, thereby providing the rationale for the present investigation. Hence, the ability of NSA to inhibit NO production suggests that it attenuates acute macrophage-driven inflammatory responses. Proinflammatory cytokines, such as IL-6 and TNF-α, are well-established drivers of psoriatic pathology. Both these cytokines are abundantly produced by activated macrophages and contribute to the proliferation of keratinocytes [36–38]. In the present study, the ELISA results demonstrated that NSA reduced the secretion of IL-6 and TNF-α from LPS-stimulated macrophages in a concentration-dependent manner, thus supporting its ability to modulate key cytokines that drive psoriatic inflammation. In addition to cytokine inhibition, NSA significantly modulated macrophage polarization by downregulating classical M1-associated proteins. The results of western blotting showed that NSA markedly suppressed the expression of iNOS, consistent with the observed reduction in the production of NO. NSA also reduced the expression of COX-2, a remarkable finding considering its frequent upregulation in inflammatory conditions and its role in the synthesis of inflammatory mediators [39]. These findings were confirmed by immunocytochemistry, which showed diminished intracellular iNOS staining.

Concurrently, NSA treatment increased the expression of M2 markers, including CD163 and CD206, as confirmed by immunocytochemistry and flow cytometry. We observed that after NSA treatment, there was a significant increase in the proportion of CD163- and CD206-positive cells, as well as their mean fluorescence intensity. CD163 and CD206 are surface receptors associated with alternative activation, tissue remodeling, and anti-inflammatory cytokine secretion, and their induction emphasizes a shift toward immune resolution [40, 41]. This dual activity, characterized by the suppression of M1 markers and the promotion of the M2 phenotype, indicates that NSA does not merely inhibit inflammation but actively reprograms macrophages toward an anti-inflammatory state. Similar immunomodulatory effects have been reported for natural compounds, such as quercetin and luteolin, which suppress NF-κB activity and promote M2 polarization, thereby reducing the release of inflammatory cytokines [42, 43]. Our results indicate that NSA shares this dual modulatory profile, as it simultaneously inhibited M1-associated pathways and increased the expression of M2 markers. Importantly, NSA demonstrated a

concentration-dependent regulation of both phenotypes, suggesting a stronger and more consistent effect than that exerted by previously described compounds. Such reprogramming is therapeutically relevant, because M2-dominant environments support tissue repair and contribute to the resolution of psoriatic inflammation [44]. Furthermore, the structural uniqueness of NSA as a DNJ derivative, combined with its remarkable ability to suppress NO production, emphasizes its potential as a particularly promising candidate among small molecules possessing dual immunomodulatory capacity. Hence, by promoting M2 dominance, NSA may help restore immune balance within the cutaneous microenvironment and mitigate psoriatic pathology.

To extend our findings from macrophages to epidermal cells, we established an *in vitro* psoriasis-like model using NHEKs stimulated with IL-17A and TNF-α. These cytokines are key mediators in psoriatic inflammation and drive the overexpression of antimicrobial peptides and S100 family proteins in keratinocytes, thereby contributing to the development of psoriatic plaques [45–47]. Consistent with previous studies, our model demonstrated a time-dependent increase in the expression of S100A7, S100A8, and S100A9, accompanied by an increased secretion of HBD-2. The induction of these markers validates the reliability of the model and emphasizes the importance of keratinocyte-derived signals in amplifying psoriatic inflammation. Moreover, selecting a 72-h stimulation window for subsequent experiments allowed us to capture the peak of marker expression and also minimize the potential secondary effects associated with prolonged cytokine exposure. This model provided a robust platform to explore whether NSA-mediated macrophage modulation could alter keratinocyte responses, thereby enabling us to investigate immune–epidermal interactions in a controlled setting. In addition, to determine whether NSA-mediated macrophage modulation could impact epidermal inflammation, we investigated the effects of conditioned media on keratinocyte responses. Activated conditioned medium (activated-RCM) derived from LPS-stimulated macrophages induced robust psoriatic changes in keratinocytes, characterized by the markedly increased secretion of HBD-2 and elevated expression of S100A7, S100A8, and S100A9 [48, 49]. These data recapitulate the paracrine interactions observed in the psoriatic skin, where macrophage-derived cytokines drive keratinocyte activation and antimicrobial peptide production [50].

In contrast, in this study, the culture medium derived from macrophages treated with LPS and NSA (activated-RCM) significantly reduced the abovementioned effects. NSA treatment reduced the secretion of HBD-2 in a concentration-dependent manner, restoring its levels comparable to those observed with naive-RCM, and suppressed the protein expression levels of S100. Importantly, these changes occurred without compromising the viability of keratinocytes, suggesting that conditioned media derived from NSA-treated RAW 264.7 macrophages exerted protective effects without inducing cytotoxicity. The dual effects of NSA, namely, reprogramming macrophages and protecting keratinocytes, emphasize its therapeutic potential as a regulator of intercellular communication in psoriatic pathology. Hyperactivation of keratinocytes is a critical driver of epidermal thickening and chronic inflammation in psoriasis [51]. Among intracellular signaling pathways, NF-κB and STAT3 represent two central regulators of these processes [38]. NF-κB is activated after the degradation of IκB-α, enabling the nuclear translocation and transcription of proinflammatory mediators [52]. In parallel, STAT3 is activated by

cytokines such as IL-6 and IL-22, thereby promoting the proliferation and survival of keratinocytes and the production of cytokines [53, 54]. Constitutive activation of both NF-κB and STAT3 has been consistently demonstrated in psoriatic lesions and is considered a hallmark of disease progression [55, 56]. In our study, conditioned medium derived from activated macrophages strongly induced the phosphorylation of NF-κB and STAT3 in NHEKs, reflecting the paracrine environment of the psoriatic skin, where macrophage-derived cytokines amplify keratinocyte activation [57]. In contrast, NSA-treated activated-RCM markedly suppressed these responses. Conditioned media derived from NSA-treated RAW 264.7 macrophages prevented IκB-α degradation, thereby reducing NF-κB activation, and simultaneously suppressed STAT3 phosphorylation in a dose-dependent manner. These inhibitory effects were accompanied by the downregulation of psoriasis-associated proteins, including S100A7, S100A8, S100A9, and HBD-2. Importantly, the expression of NF-κB and STAT3 was suppressed under conditions where keratinocyte viability was preserved, as confirmed by the cytotoxicity assays depicted in Fig. 4. This supports the interpretation that the observed effects are mediated by conditioned media derived from NSA-treated RAW 264.7 macrophages rather than by direct or nonspecific cytotoxic effects on keratinocytes. Altogether, these findings demonstrate that NSA exerts anti-inflammatory effects indirectly by reprogramming macrophage polarization—suppressing M1-associated mediators and promoting M2-associated markers—which in turn results in the attenuation of NF-κB and STAT3 signaling in keratinocytes. This integrated mechanism is illustrated in Fig. 6, which schematically depicts how NSA modulates macrophage–keratinocyte interactions to attenuate psoriasis-associated inflammation.

Our study emphasizes that macrophage modulation represents an essential therapeutic avenue for psoriasis. Excessive inflammation in macrophages can propagate inflammatory cascades that ultimately drive the activation of keratinocytes and the formation of psoriatic lesions [38, 58]. By reprogramming macrophages and restoring a favorable M1/M2 balance, NSA effectively diminishes this pathogenic loop. Similar immunomodulatory strategies have been reported for natural products such as luteolin, quercetin, and Paris saponin VII, which attenuate psoriatic inflammation by modulating NF-κB and STAT3 signaling [59–61]. Hence, NSA may be added to this group of promising compounds, although it is distinguished by its strong dual action on both macrophages and keratinocytes. Regarding the clinical significance of the results of this study, although current biologics that target IL-17 or TNF-α have demonstrated significant efficacy in psoriasis, they require long-term administration and carry risks of systemic immunosuppression [62, 63]. Hence, as a small-molecule derivative, NSA may provide an alternative or complementary therapeutic strategy with potentially fewer side effects. Its ability to restore immune balance at both macrophage and keratinocyte levels suggests that it could be developed as either a standalone therapy or an adjunct to existing biologics. Nonetheless, all experiments in our study were conducted *in vitro*, and therefore, the *in vivo* efficacy and safety of NSA remain to be established. Animal models of psoriasis will be required to confirm whether the immunomodulatory effects of NSA translate into meaningful clinical benefits. Moreover, although NSA increased the expression of M2 markers, the specific cytokines and mediators secreted by these M2 macrophages remain unidentified. Detailed proteomic and transcriptomic analyses of NSA-treated macrophages could elucidate the

soluble factors that contribute to the observed suppression of keratinocyte inflammation. Altogether, these results support a macrophage-centered immunomodulatory mechanism in which NSA restores immune balance by reprogramming macrophage polarization and indirectly attenuating keratinocyte-driven inflammatory responses.

5. Conclusion

NSA, a derivative of the natural iminosugar DNJ, is a promising small-molecule candidate for psoriasis therapy. NSA reprogrammed macrophage polarization by suppressing the expression of M1-associated mediators and increasing that of M2-associated markers in RAW 264.7 macrophages. Importantly, conditioned media derived from NSA-treated macrophages attenuated keratinocyte hyperactivation by suppressing NF-κB and STAT3 signaling, without compromising cell viability. By modulating macrophage-driven inflammatory signals, NSA effectively disrupted the macrophage–keratinocyte crosstalk that sustains psoriatic inflammation. This study provides a mechanistic basis for considering NSA as a novel immunomodulatory strategy to restore immune balance in psoriasis. Future *in vivo* and clinical studies are necessary to validate the therapeutic efficacy, safety, and translational potential of NSA.

Abbreviations

***ANOVA*:** Analysis of variance

***CD163*:** Hemoglobin scavenger receptor, a marker of M2 macrophages

***CD206*:** Mannose receptor, a surface marker of M2 macrophages

***COX-2*:** Cyclooxygenase-2

***DNJ*:** 1-Deoxynojirimycin

***ECL*:** Enhanced chemiluminescence

***ELISA*:** Enzyme-linked immunosorbent assay

***iNOS*:** Inducible nitric oxide synthase

***IL*:** Interleukin

***LPS*:** Lipopolysaccharide

***M1 macrophage*:** Classically activated, pro-inflammatory macrophage

***M2 macrophage*:** Alternatively activated, anti-inflammatory macrophage

***MFI*:** Mean fluorescence intensity

***NF-κB*:** Nuclear factor kappa-light-chain-enhancer of activated B cells

***NHEKs*:** Normal human epidermal keratinocytes

***NO*:** Nitric oxide

***NSA*:** Nojirimycin-1-sulfonic acid

***PBS*:** Phosphate-buffered saline

***PVDF*:** Polyvinylidene difluoride

***RCM*:** RAW 264.7 cell–derived conditioned medium

***SD*:** Standard deviation

***SDS-PAGE*:** Sodium dodecyl sulfate–polyacrylamide gel electrophoresis

***STAT3*:** Signal transducer and activator of transcription 3

***TBS-T*:** Tris-buffered saline with Tween-20

***TNF-α*:** Tumor necrosis factor-alpha

Declarations

Data statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

The authors declare that no generative AI or AI-assisted technologies were used in the writing of this manuscript.

Author contributions: CRediT

Min Ju Jung: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. Hye Jin Lim: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. Jin Hyeop Kim: Validation, Supervision, Methodology, Investigation. Yeon-Jun Lee: Software, Formal analysis. Methodology, Investigation. Ha Hui Park: Software, Formal analysis, Data curation. Ji Hyung Chung: Writing – review & editing, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

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Figures

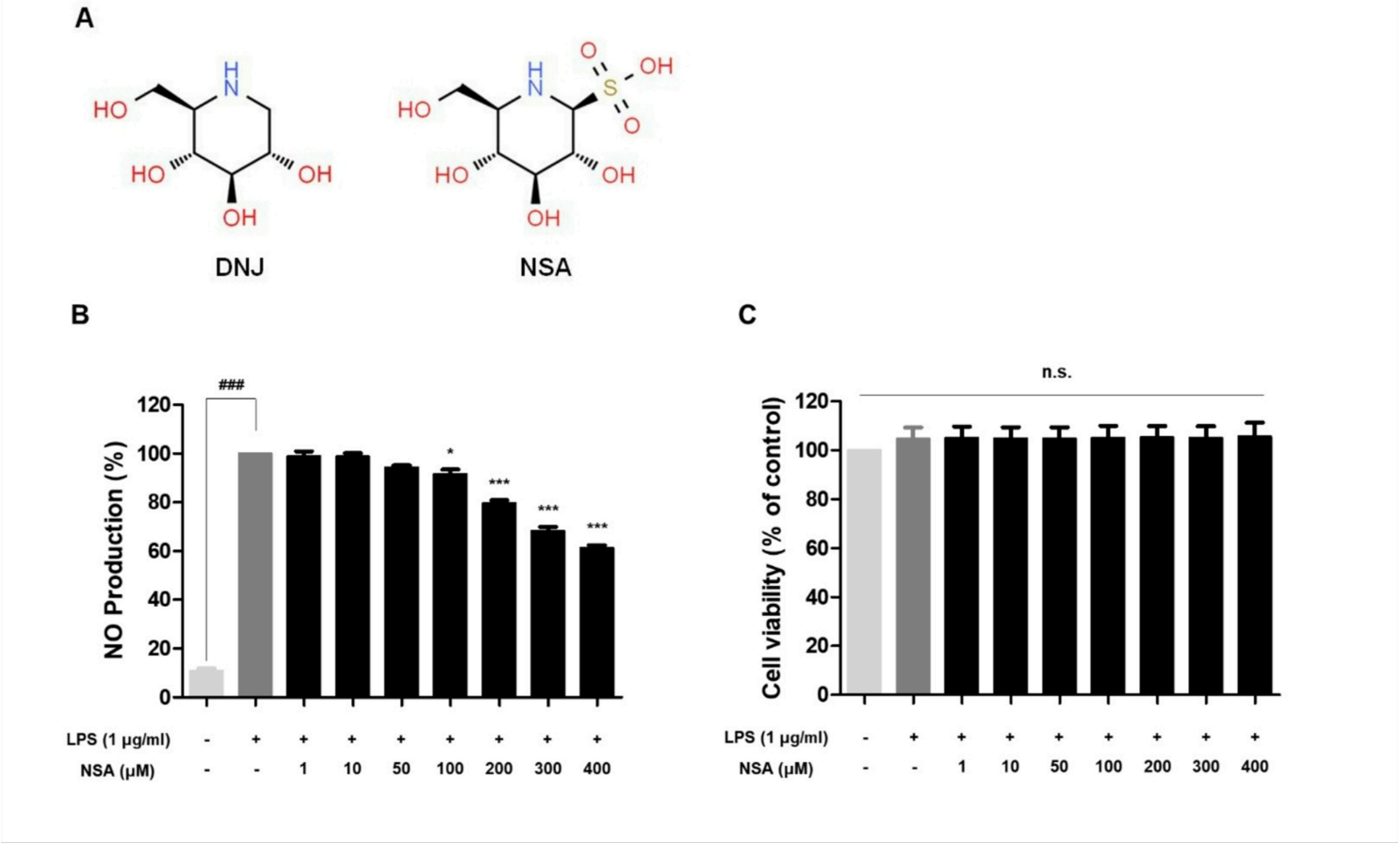


Figure 1

Effect of NSA on NO production and cell viability in LPS-stimulated RAW 264.7 cells.

Chemical structures of 1-deoxynojirimycin (DNJ) and nojirimycin-1-sulfonic acid (NSA) (A). Cells were pretreated with NSA for 1 h, followed by stimulation with 1 μg/mL LPS for 24 h. NO production levels were measured using the Griess reagent (B), and cell viability was determined using a WST-1 assay (C). All data are expressed as mean ± SD (n = 3). Statistical significance was evaluated relative to the untreated control (### $p < 0.001$) and the LPS-alone-treated group (* $p < 0.05$; *** $p < 0.001$); n.s., not significant.

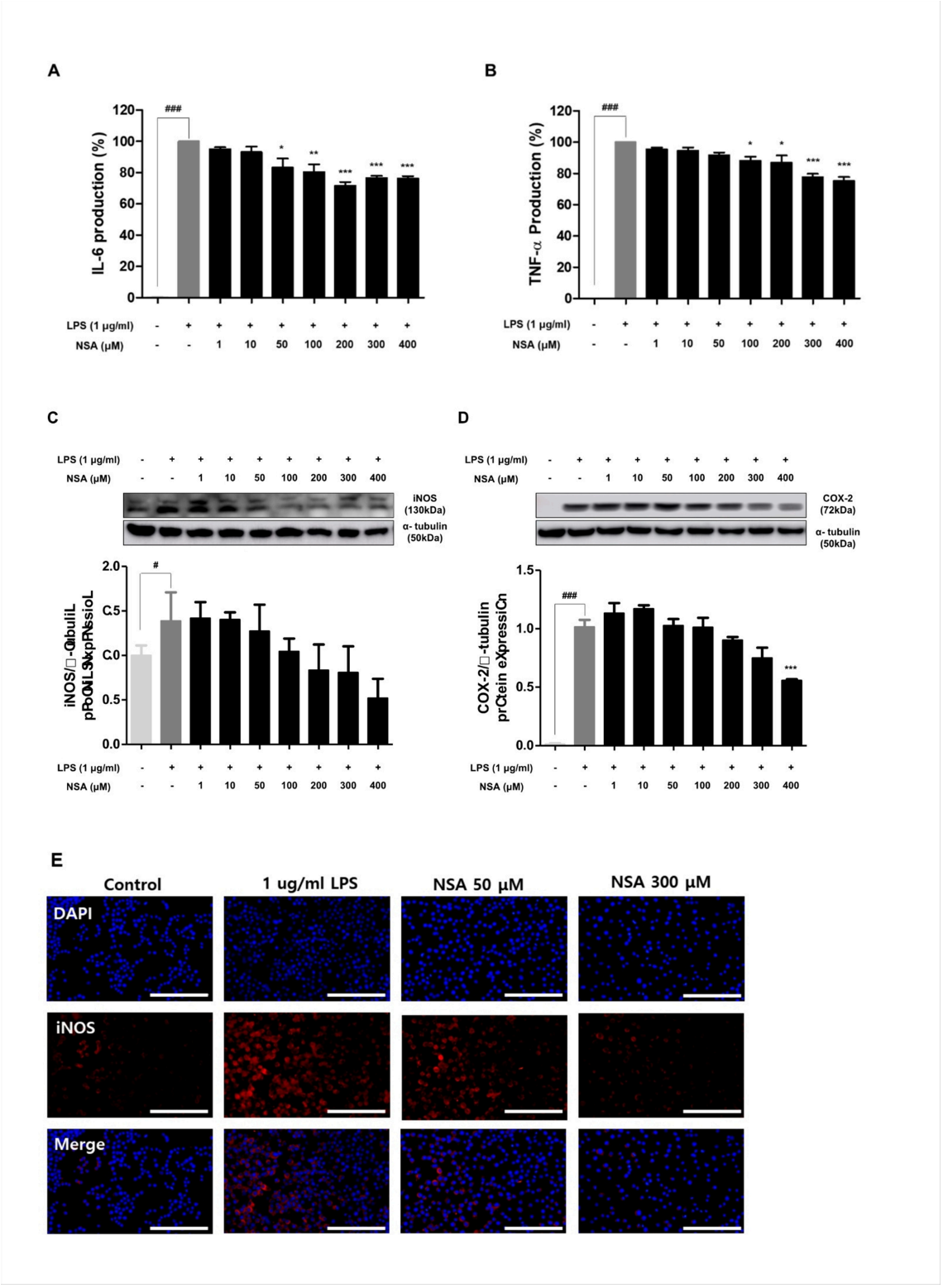


Figure 2

Effects of NSA on the production of proinflammatory cytokines and expression of M1 markers in LPS-stimulated RAW 264.7 cells.

Cells were pretreated with NSA for 1 h, followed by stimulation with 1 μg/mL LPS for 24 h. The levels of IL-6 (A) and TNF-α (B) in culture media were measured by ELISA. The expression levels of iNOS (C) and COX-2 (D) were determined by western blotting. The expression level of intracellular iNOS was also

observed by immunocytochemistry using a fluorescence microscope at a magnification of × 400 (Scale bar: 10 μm) (E). All data are expressed as mean ± SD (n = 3). Statistical significance was determined relative to the untreated control (# $p < 0.05$; ### $p < 0.001$) and the LPS-alone-treated group (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$); n.s., not significant.

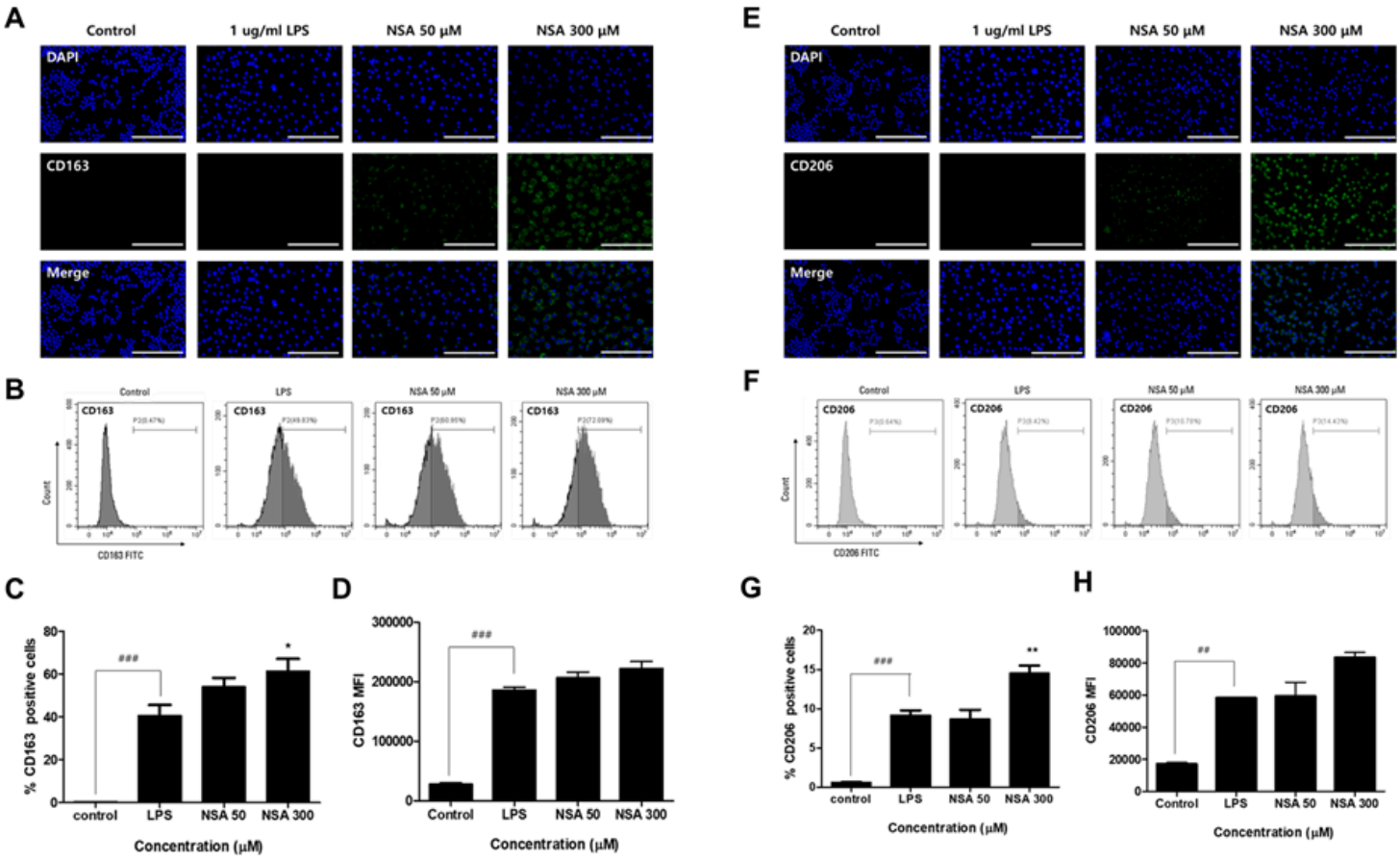


Figure 3

Effects of NSA on M2 macrophage markers in LPS-stimulated RAW 264.7 cells.

Cells were pretreated with NSA for 1 h, followed by stimulation with 1 μg/mL LPS for 24 h. The surface markers CD163 (A) and CD206 (E), representative markers of M2 macrophages, were visualized by immunocytochemistry using a fluorescence microscope at ×400 magnification (scale bar: 10 μm). The expressions of CD163 (B) and CD206 (F) were also detected by flow cytometry. Quantitative analysis was performed using two parameters based on the percentage of positive cells (C and G) and mean fluorescence intensity (MFI) (D and H). All data are expressed as mean ± SD (n = 3). Statistical significance was determined relative to the untreated control (## $p < 0.01$; ### $p < 0.001$) and the LPS-alone-treated group (* $p < 0.05$; ** $p < 0.01$).

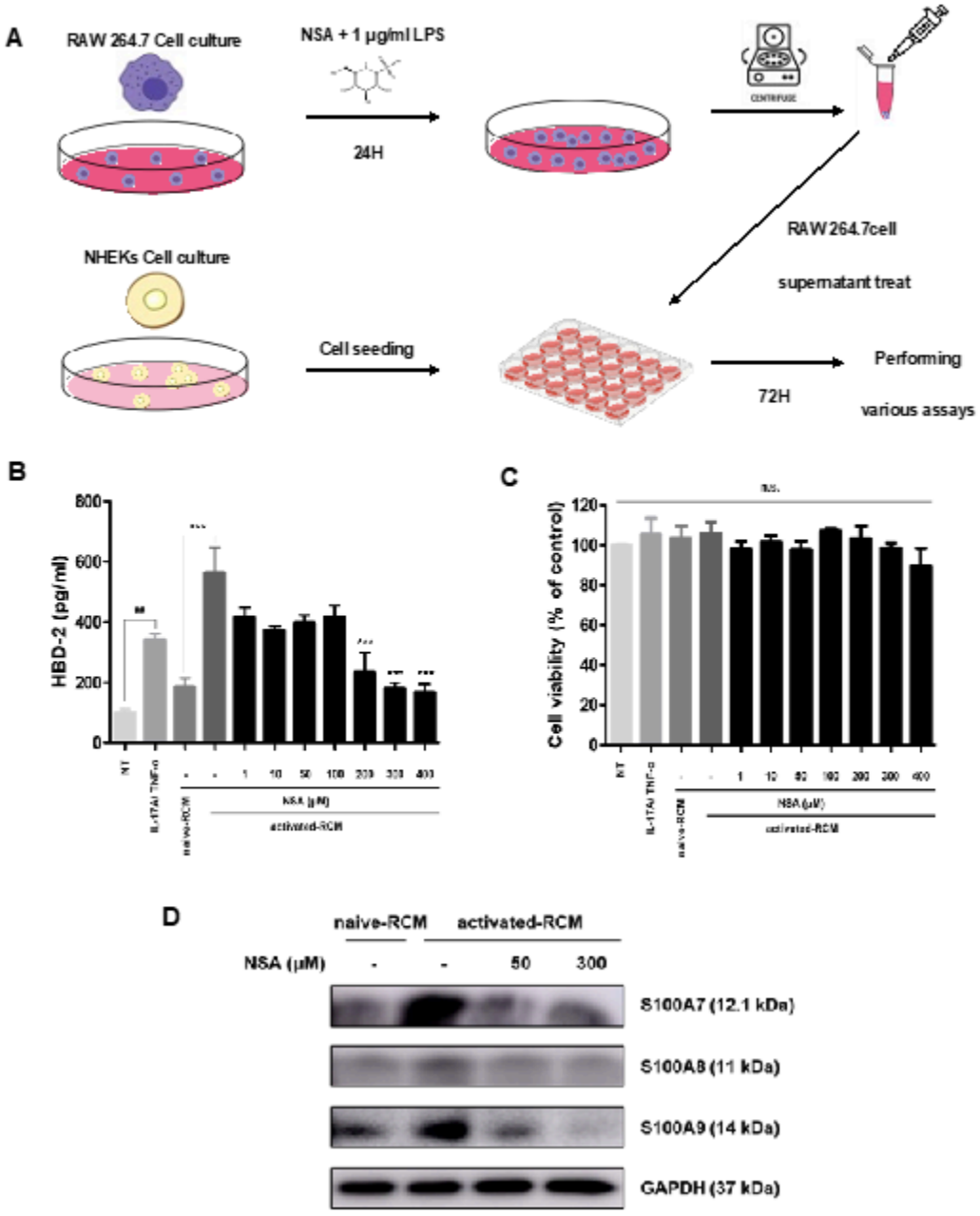


Figure 4

Effects of NSA-treated conditioned media on psoriatic responses in NHEKs

Schematic of the conditioned media assay using naive-RCM (supernatant derived from LPS-untreated RAW 264.7 cells) and activated-RCM (supernatant derived from LPS-stimulated RAW 264.7 cells, with or without NSA) (A). NHEKs were seeded in multi-well plates and treated with each conditioned medium at a final concentration of 5%, followed by culture for 72 h. HBD-2 secretion was measured using an ELISA kit (B). Cell viability was also determined using the WST-1 assay (C). Protein expression of the psoriasis markers S100A7, S100A8, and S100A9 was detected by western blotting (D). All data are expressed as mean ± SD (n = 3). Statistical significance was determined relative to the untreated control (## $p < 0.01$), the naive-RCM group (*** $p < 0.001$), and the activated-RCM group (*** $p < 0.001$); n.s., not significant.

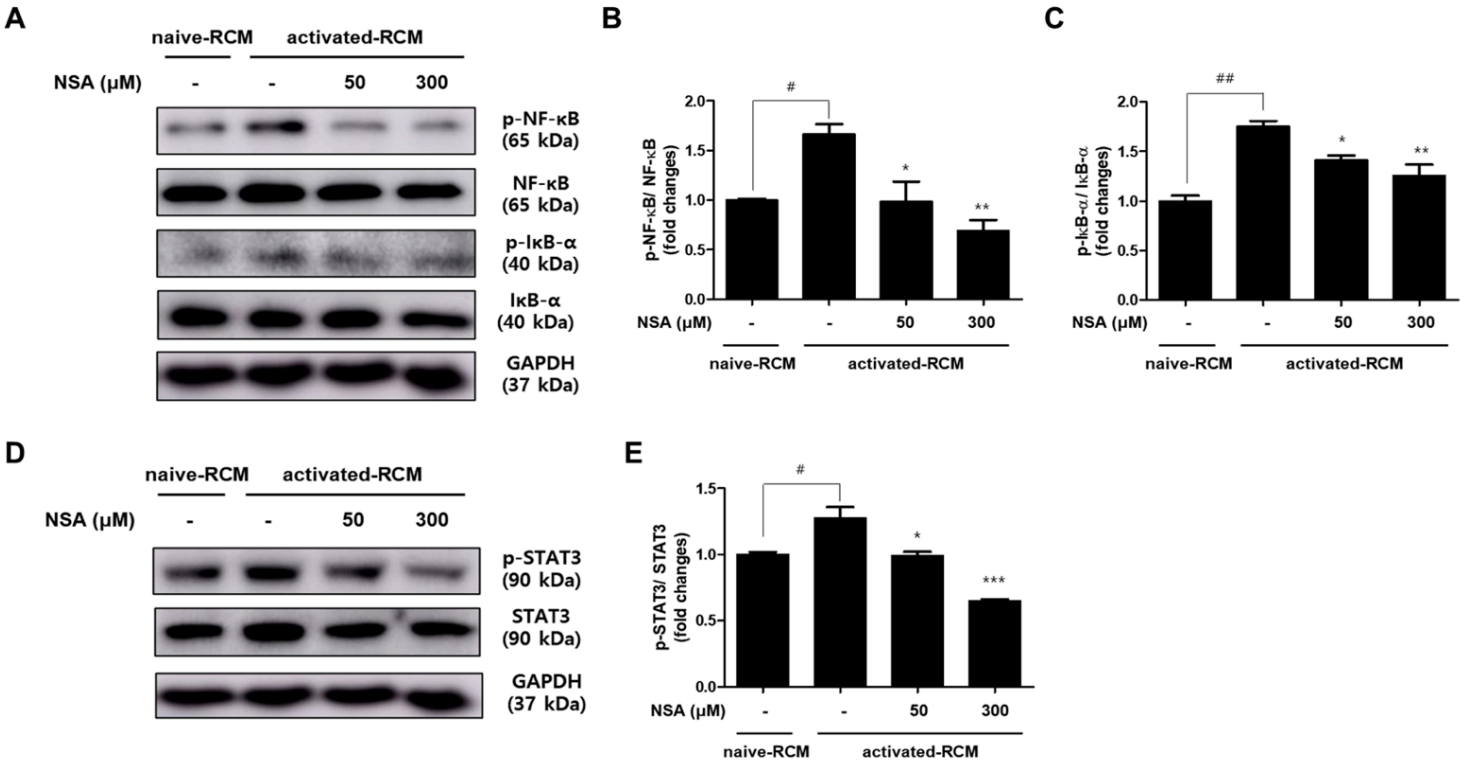


Figure 5

Effects of NSA-treated conditioned media on NF-κB/IκB-α and STAT3 signaling in NHEKs.

NHEKs were seeded in multi-well plates and treated with each conditioned medium at a final concentration of 5%, followed by culture for 72 h. IκB-α and NF-κB phosphorylation was detected by western blot analysis (A-C). STAT3 phosphorylation was also detected by western blot analysis (D, E). Data are expressed as mean ± SD (n = 3). Statistical significance was determined relative to the naive-RCM group (# $p < 0.05$; ## $p < 0.01$) and the activated-RCM group (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$); n.s., not significant.

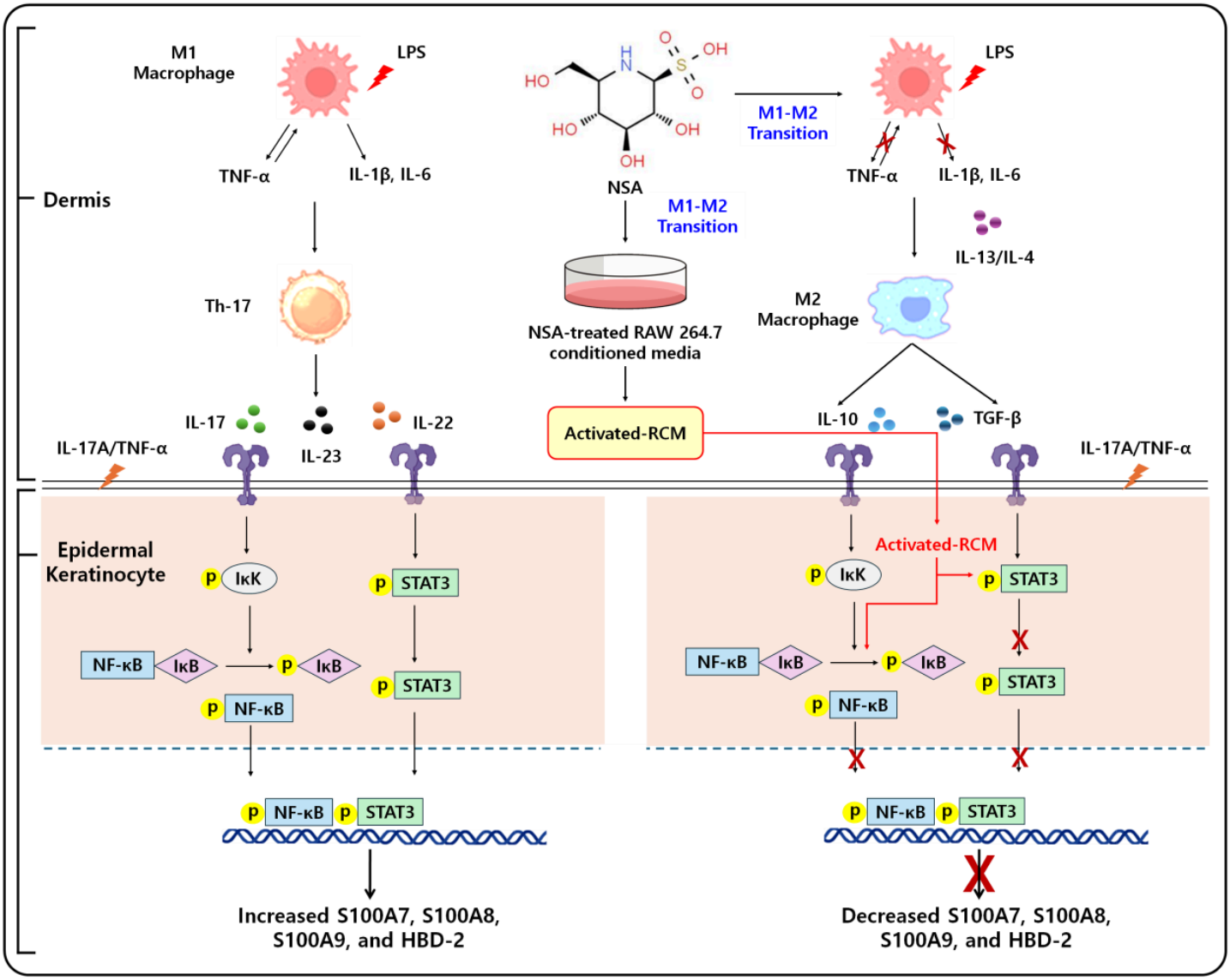


Figure 6

Schematic of the effects of NSA-treated macrophage-derived conditioned media on keratinocyte inflammatory responses in psoriasis.

Activated macrophages release proinflammatory cytokines, which activate NF-κB and STAT3 signaling in NHEKs, resulting in increased expression of psoriasis-associated markers (S100A7, S100A8, S100A9, and HBD-2) and contributing to the formation of psoriatic plaques. In contrast, conditioned media derived from NSA-treated macrophages attenuates NF-κB and STAT3 activation in NHEKs, resulting in reduced expression of these psoriasis-associated markers. Through this indirect regulation of macrophage–keratinocyte crosstalk via macrophage reprogramming, NSA mitigates keratinocyte hyperactivation and suppresses psoriasis-associated inflammation.

Supplementary Files

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