Putative therapeutic mechanisms of simvastatin in the treatment of alopecia areata

To the Editor: Alopecia areata (AA) is a common T-cell–mediated autoimmune disorder, and the exact pathomechanism remains elusive. Statins are hydroxy-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors widely used around the world as lipid-lowering agents and to treat cardiovascular disease. Recently, there are human prospective

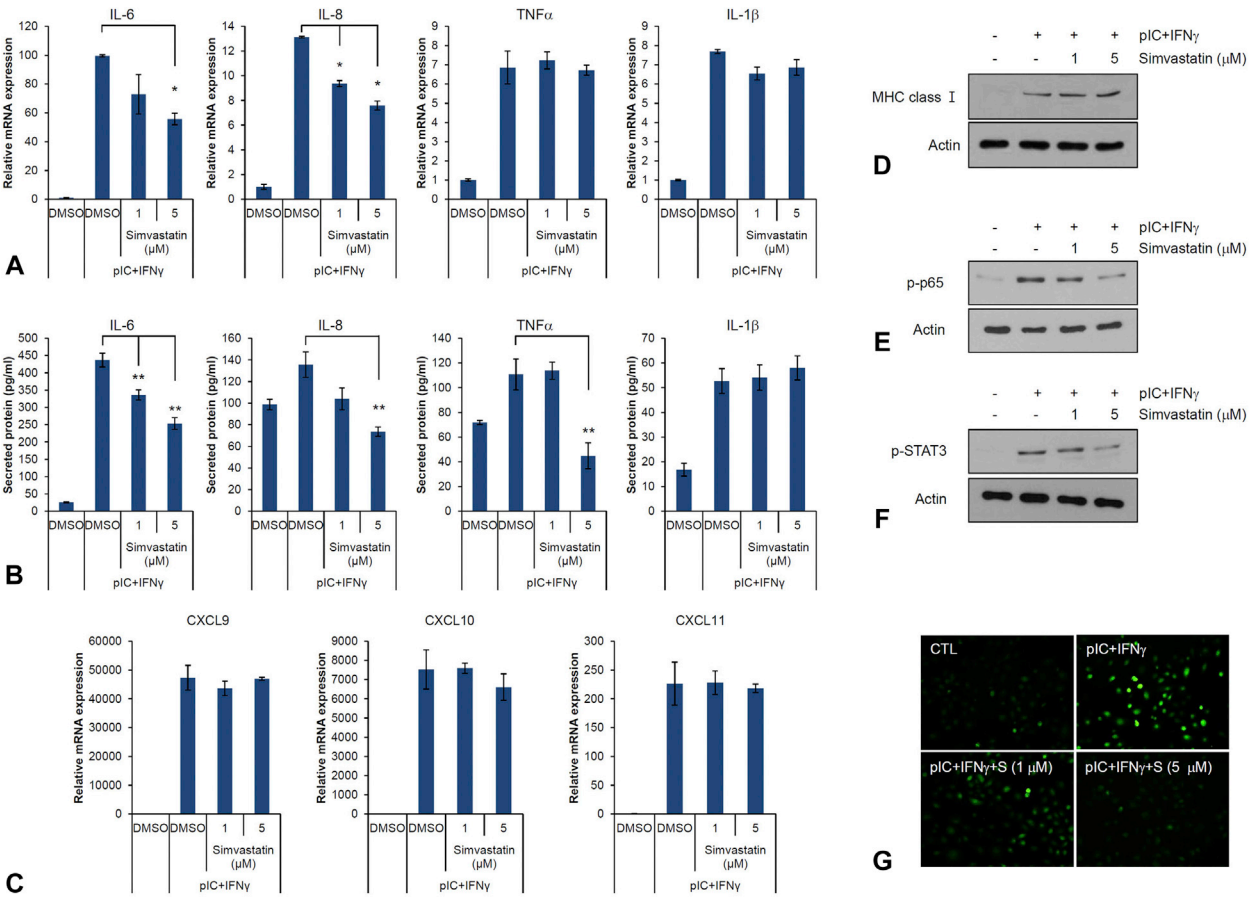

Fig 1. Anti-inflammatory effect of simvastatin on poly(I:C)- and IFN-γ–induced inflammation. **A**, SV-ORS cells were pretreated with simvastatin at the indicated concentrations for 1 hour; subsequently, poly(I:C) (10 μg/mL) and IFN-γ (5 ng/mL) were added for 2 hours. Relative mRNA expression was assessed by qRT-PCR (*$P < .05$). **B**, SV-ORS cells were pretreated with simvastatin at the indicated concentrations for 1 hour; subsequently, poly(I:C) (10 μg/mL) and IFN-γ (5 ng/mL) were added for 24 hours. Secretion of cytokines was measured by enzyme-linked immunosorbent assay (**$P < .01$). **C**, SV-ORS cells were pretreated with simvastatin at the indicated concentrations for 1 hour; subsequently, poly(I:C) (10 μg/mL) and IFN-γ (5 ng/mL) were added for 2 hours. Relative mRNA expression was assessed by qRT-PCR. **D**, SV-ORS cells were pretreated with simvastatin at the indicated concentrations for 1 hour; subsequently, poly(I:C) (10 μg/mL) and IFN-γ (5 ng/mL) were added for 24 hours. The protein level of MHC class I was determined by Western blotting. Actin was used as a control. **E** and **F**, SV-ORS cells were pretreated with simvastatin at the indicated concentrations for 1 hour; subsequently, poly(I:C) (10 μg/mL) and IFN-γ (5 ng/mL) were added for 1 hour. The protein level of phosphorylated p65 (p-p65) and phosphorylated STAT3 (p-STAT3) was determined by Western blotting. Actin was used as a control. **G**, SV-ORS cells were pretreated with simvastatin at the indicated concentrations for 1 hour; subsequently, poly(I:C) (10 μg/mL) and IFN-γ (5 ng/mL) were added for 2 hours. The reactive oxygen species production was detected by 2′,7′-diclorodihydrofluorescein diacetate (H_2-DCFDA) staining. *CTL*, Control; *DMSO*, dimethyl sulfoxide; *IFN*, interferon; *IL*, interleukin; *M*, mol/L; *MHC*, major histocompatibility complex; *mRNA*, messenger RNA; *pIC*, polyinosinic:polycytidylic acid; *poly(I:C)*, polyinosinic:polycytidylic acid; *qRT-PCR*, quantitative reverse-transcription polymerase chain reaction; *S*, simvastatin; *STAT*, signal transducer and activator of transcription; *SV-ORS*, simian virus 40 T antigen-transformed outer root sheath cells; *TNF*, tumor necrosis factor.

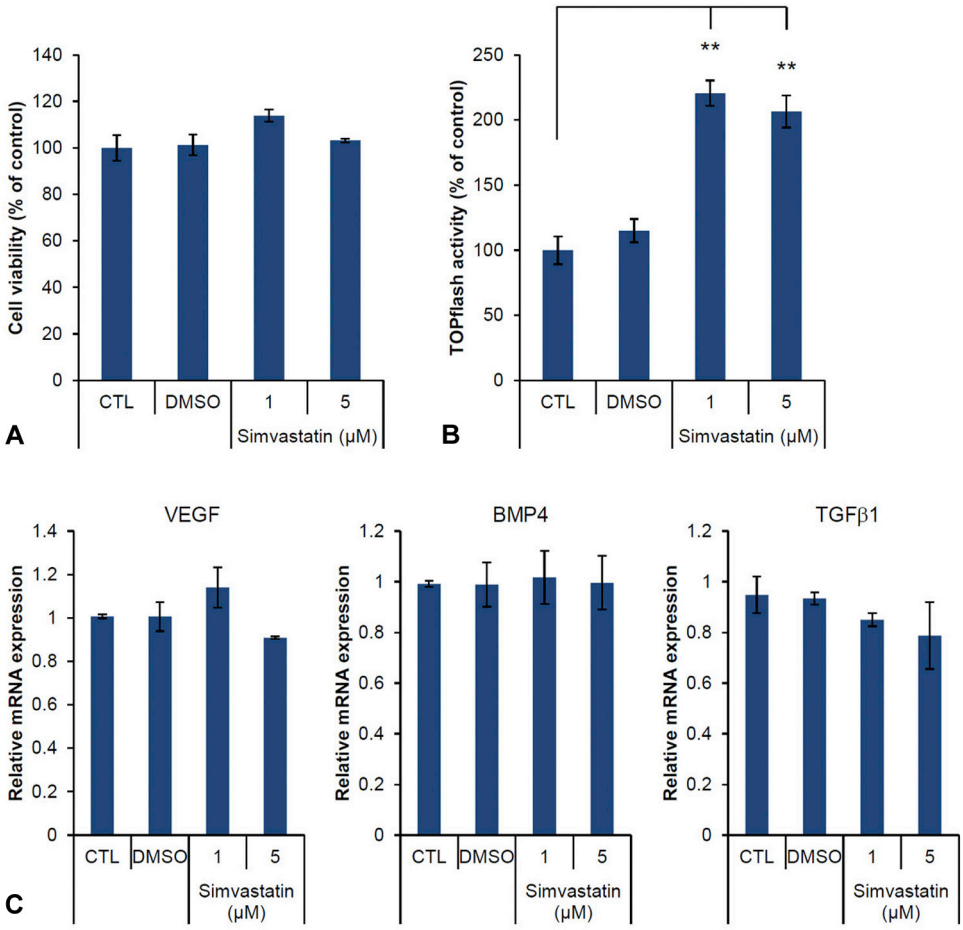

Fig 2. Effect of simvastatin on Wnt/β-catenin signaling pathway. **A**, SV-ORS cells were treated with simvastatin at the indicated concentrations for 24 hours. Cell viability was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. **B**, SV-ORS cells were transduced with an adenovirus-expressing TOPflash luciferase reporter and then treated with simvastatin at the indicated concentrations for 24 hours. Cells were assessed for luciferase activity (**$P < .01$). **C**, SV-ORS cells were treated with simvastatin at the indicated concentrations for 6 hours. Relative messenger RNA expression was determined by quantitative reverse-transcription polymerase chain reaction. *BMP*, Bone morphogenetic protein; *CTL*, control; *DMSO*, dimethyl sulfoxide; *M*, mol/L; *SV-ORS*, simian virus 40 T antigen-transformed outer root sheath cells; *TGF*, transforming growth factor; *VEGF*, vascular endothelial growth factor.

observational, retrospective, and case series of the effectiveness of simvastatin treatment on AA.[1,2] However, the exact mechanism by which statins improve AA is not yet known. Here, we investigate the putative therapeutic effect and mechanism of simvastatin in the treatment of AA through in vitro studies.

To mimic the inflammatory responses in AA, we cotreated polyinosinic:polycytidylic acid [poly(I:C)] and interferon (IFN) γ to primary cultured human outer root sheath (ORS) cells.[3] First, we examined whether simvastatin affects inflammatory reactions in ORS cells. Cotreatment of poly(I:C) and IFN-γ increased the expression of inflammatory cytokines, including interleukin (IL) 6, IL-8, tumor necrosis factor (TNF) α, and IL-1β. Simvastatin pretreatment downregulated poly(I:C)- and IFN-γ–induced IL-6 and IL-8 messenger RNA (mRNA) levels and inhibited secretion of IL-6, IL-8, and TNF-α (Fig 1, *A* and *B*). Next, we checked the effect of simvastatin on the expression of IFN-inducible C-X-C motif chemokines (CXCL) 9-11 and major histocompatibility complex (MHC) I. However, simvastatin did not decrease IFN-γ–induced CXCL9-11 and MHC I expression (Fig 1, *C* and *D*). To investigate putative mechanisms, we examined the effect of simvastatin on the nuclear factor κB signaling pathway, which is a central regulator in inflammatory responses. Simvastatin significantly inhibited poly(I:C)- and IFN-γ–induced phosphorylation of p65 in a dose-

dependent manner. Moreover, simvastatin decreased phosphorylation of signal transducer and activator of transcription (STAT) 3, which is another critical player linked to AA (Fig 1, *E* and *F*). Simvastatin also significantly inhibited poly(I:C)- and IFN-γ–induced reactive oxygen species (ROS) production in a dose-dependent manner (Fig 1, *G*).

Finally, we determined whether simvastatin regulates Wnt/β-catenin signaling and hair growth. Simvastatin increased cell viability and TOPflash activity, indicating transcriptional activation of β-catenin (Fig 2, *A* and *B*). However, simvastatin did not affect other hair growth–related genes (Fig 2, *C*).

Statins exert pleiotropic anti-inflammatory properties in vitro and in vivo. Previously, statins have been reported to modulate cytokine secretion and T-cell responses and to inhibit the secretion of proinflammatory cytokines.[4] In this experiment, we showed that simvastatin directly attenuates inflammatory reactions in hair follicle cells, especially in ORS cells. However, simvastatin did not produce a significant effect on the expression of CXCL 9-11 and MHC class I. This indicates that simvastatin may not regulate IFN-γ–induced gene expression but that it can inhibit TNF-α–induced nuclear factor κB transcriptional activity in ORS cells, resulting in inflammatory response reduction in AA progression. One of the therapeutic mechanisms of simvastatin in AA is considered to be inhibition of the Janus kinase (JAK)/STAT signaling pathway. We additionally showed the effect of simvastatin on inhibition of the nuclear factor κB pathway, STAT3 pathway, and ROS production, which are closely related to the pathophysiology of AA.

There are in vitro studies showing that statins modulate Wnt/β-catenin signaling in various cells.[5] Our results indicate that simvastatin could induce hair regrowth by activating Wnt/β-catenin signaling and exerting its anti-inflammatory effect simultaneously in patients with AA.

In summary, we suggest that simvastatin improves AA through pleiotropic anti-inflammatory properties; inhibition of NF-κB, the JAK/STAT pathway, and ROS production; and activation of the Wnt/β-catenin signaling pathway.

Jung-Min Shin, PhD,[a] Kyung-Eun Jung, MD,[a] Su-Hyuk Yim, MD,[a] Babar Rao, MD,[b,c] Dong-kyun Hong, MD,[a] Young-Joon Seo, MD,[a] Chang-Deok Kim, PhD,[a] and Young Lee, MD[a,b]

From the Department of Dermatology, School of Medicine, Chungnam National University, Daejeon, Korea[a]; Department of Dermatology, Rutgers Robert Wood Johnson Medical School, Somerset, New Jersey[b]; and Department of Dermatology, Weill Cornell Medical Center, New York, New York.[c]

Drs Shin and Jung contributed equally to this article.

Funding sources: Supported by the Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (NRF-2019R1A2C1004114).

Conflicts of interest: None disclosed.

IRB approval status: This study received ethical approval from the Institutional Review Board at Chungnam National University, Daejeon, Korea (CNUH-IRB-2016-07-009).

Reprints not available from the authors.

Correspondence to: Young Lee, MD, 266 Munhwa-ro, Jung-Gu, Daejeon, Korea

E-mail: resina20@cnu.ac.kr

REFERENCES

1. Cervantes J, Jimenez JJ, DelCanto GM, Tosti A. Treatment of alopecia areata with simvastatin/ezetimibe. *J Investig Dermatol Symp Proc*. 2018;19:S25-S31.
2. Choi JW, Suh DW, Lew BL, Sim WY. Simvastatin/ezetimibe therapy for recalcitrant alopecia areata: an open prospective study of 14 patients. *Ann Dermatol*. 2017;29:755-760.
3. Shin JM, Choi DK, Sohn KC, et al. Induction of alopecia areata in C3H/HeJ mice using polyinosinic-polycytidylic acid (poly[I:C]) and interferon-gamma. *Sci Rep*. 2018;8:12518.
4. Chow SC. Immunomodulation by statins: mechanisms and potential impact on autoimmune diseases. *Arch Immunol Ther Exp*. 2009;57:243-251.
5. Lin CL, Cheng H, Tung CW, et al. Simvastatin reverses high glucose-induced apoptosis of mesangial cells via modulation of Wnt signaling pathway. *Am J Nephrol*. 2008;28:290-297.

https://doi.org/10.1016/j.jaad.2020.03.102

Long-term sequelae from Stevens-Johnson syndrome/toxic epidermal necrolysis in a large retrospective cohort

To the Editor: Stevens-Johnson syndrome/toxic epidermal necrolysis (SJS/TEN) is a rare, severe drug reaction associated with significant mortality.[1] Although the reaction itself is self-limited, associated morbidity may last well beyond the initial hospitalization. However, little is known about the frequency and risk factors associated with long-term SJS/TEN-related sequelae.[2,3]