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RECEIVED 04 January 2024

ACCEPTED 07 May 2024

PUBLISHED 22 May 2024

## CITATION

Li N, Oh J-H, Suh JH, Jin S-P, Lee DH, Lee Y  
and Chung JH (2024) Impact of  
fucosyltransferase 1-mediated epidermal  
blood group antigen H on anti-inflammatory  
response in atopic dermatitis.  
*Front. Immunol.* 15:1365430.  
doi: 10.3389/fimmu.2024.1365430

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# Impact of fucosyltransferase 1-mediated epidermal blood group antigen H on anti-inflammatory response in atopic dermatitis

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The presence of the blood group H2 antigen on the membrane of red blood cells determines blood type O in individuals and this H2 antigen serves as a precursor to the A and B antigens expressed in blood types A and B, respectively. However, the specific involvement of ABH antigens in skin diseases is unknown. Therefore, we aim to investigate the expression of ABH antigens in skin tissue of patients with atopic dermatitis (AD) and MC903-induced AD-like mice. We demonstrated that the expression of ABH antigen is primarily located in the granular and horny layers of the skin in healthy control individuals. However, in patients with AD, the expression of the ABH antigen was absent or diminished in these layers, while the H2 antigen expression increased in the spinous layers of the affected skin lesions. Then, we investigated the biological function of blood group H antigen mediated by fucosyltransferase 1 (Fut1) in the skin, utilizing an AD mouse model induced by MC903 in wild-type (WT) and *Fut1*-knockout mice. After the application of MC903, *Fut1*-deficient mice, with no H2 antigen expression on their skin, exhibited more severe clinical signs, increased ear swelling, and elevated serum IgE levels compared with those of WT mice. Additionally, the MC903-induced thickening of both the epidermis and dermis was more pronounced in *Fut1*-deficient mice than that in WT mice. Furthermore, *Fut1*-deficient mice showed a significantly higher production of interleukin-4 (IL-4) and IL-6 in skin lesions compared with that of their WT counterparts. The expression of chemokines, particularly *Ccl2* and *Ccl8*, was notably higher in *Fut1*-deficient mice compared with those of WT mice. The infiltration of CD4<sup>+</sup> T cells, eosinophils, and mast cells into the lesional skin was significantly elevated in *Fut1*-deficient mice compared with that in WT mice. These findings demonstrate the protective role of H2 antigen expression against AD-like inflammation and highlight its potential therapeutic impact on AD through the regulation of blood group antigens.

## KEYWORDS

ABH antigens, fucosyltransferase 1, atopic dermatitis, cytokines, chemokines

## Introduction

Atopic dermatitis (AD) is a multifaceted and chronic inflammatory skin disease, representing one of the most prevalent skin disorders (1). Current estimates indicate that 10–20% of children and 3% of adults in developed countries are affected by this condition (1–4). AD represents a highly heterogeneous clinical and molecular phenotypes (4–6). While the pathogenesis of AD is not fully understood, the disorder is attributed to a combination of genetic and environmental factors, microbial imbalance, immune dysregulation, and abnormalities in the skin barrier (6–9). The amplification of skin inflammation in AD is primarily associated with a T helper-2 (Th2) immune response, characterized by high expression levels of interleukin (IL)-4 and IL-5 (10, 11). The lesional skin of patients with AD exhibited T-cell infiltration, primarily characterized by CD4 expression (1, 12). IL-4 from Th2 cells promotes class switching to immunoglobulin E (IgE) in B cells, leading to the production of antigen-specific IgE (1, 13, 14). Eosinophils and mast cells infiltrate the skin in cases of AD, and their presence was correlated with the severity of the disease (15, 16). Consequently, these immune cells are regarded as potential effector cells due to their production of inflammatory molecules associated with AD (17–19). Moreover, epidermal keratinocytes highly express C-C motif chemokine ligand (CCL) 2 and CCL8 (20, 21) in the skin of patients with AD. CCL2 signaling recruits immune cell infiltration and elicits itch behavior in a mouse model of allergic contact dermatitis (22, 23). CCL8 exhibits chemotactic properties, attracting activated and highly differentiated Th2 cells to the skin during chronic allergic inflammation (24).

The weakening of the skin barrier promotes inflammation and T cell infiltration, subsequently compromising barrier function, intensifying itching, and facilitating microbial dysbiosis, such as colonization with *Staphylococcus aureus* (1, 25, 26). Under inflammatory conditions, keratinocytes produce the epidermal alarmins, such as thymic stromal lymphopoietin (TSLP), leading to tissue damage and driving the recruitment of type 2 inflammatory cells (1, 27–29).

Fucosyltransferase-1 (FUT1) is pivotal in the synthesizing the blood group H2 antigen, a critical determinant for blood type O. This H2 antigen is indispensable for the subsequent formation of A and B antigens, thereby determining blood types A and B, respectively (30, 31). While it is established that ABH antigens determine individual blood types through their expression on the erythrocyte membrane (32), our prior research, along with other studies, have reported that these antigens are predominantly distributed in various tissues. Notably, they are concentrated in regions in direct contact with the external environment, such as the skin, intestines, oral cavity, and liver (33–36). Additional studies have revealed that the expression of FUT1 is restricted to a limited extent within epithelial cells (37–39). The association between the expression of ABH antigens and inflammatory responses, including those induced by UV irradiation, breast and ovarian cancer, respiratory diseases, and pathogen infections, has been explored (33, 39–43). Despite this, the specific involvement of ABH antigens

in skin diseases has remained largely elusive. This study sheds light on the anti-inflammatory role of ABH antigens, especially the H2 antigen, within the context of MC903-induced AD-like inflammation in mouse model.

## Materials and methods

### Antibodies and reagents

Antibodies against A (Z2B-1), B (Z5H-2), H2 (BRIC231), FUT1 (G-13), Filaggrin (N-20), and  $\beta$ -Actin (C4) were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). FUT1 antibody (17956-1) was purchased from Proteintech (Chicago, IL, USA). Antibodies against CD4 (4SM95) and F2RL1 were purchased from Invitrogen (Carlsbad, CA, USA). Eosinophil antibody (BMK-13) was purchased from Novus Biologicals (Littleton, CO, USA). Antibodies against Loricrin (Poly19051) and IL-4 (11B11) were purchased from BioLegend (San Diego, CA, USA). Keratin 10 antibody (EP1607IHCY) was purchased from Abcam (Waltham, Massachusetts, USA). Mcpt6 antibody (MAB3736) was purchased from R&D systems (Minneapolis, MN, USA). MC903 was purchased from Tocris (Bristol, UK). Toluidine blue solution was obtained from Sigma-Aldrich (St. Louis, MO, USA).

### Patients and specimens

Skin tissue samples included 39 patients diagnosed with AD (27 males and 12 females, age range = 19 to 46 years) and 66 healthy controls (10 males and 56 females, age range = 20 to 81 years). Informed consent was obtained from all participants before their involvement, and the study received ethical approval from the Institutional Review Board (IRB No. C-1312-084-543) of Seoul National University Hospital. The study adhered to the principles outlined in the Declaration of Helsinki. Patients with AD who had concurrent acute illnesses were excluded from the study.

### Mice

Fut1-deficient mice (B6.129-Fut1tm1Sdo/J) and wild-type (WT) C57BL/6 mice were obtained from Jackson Laboratory (Bar Harbor, ME, USA). They were housed in a pathogen-free barrier facility. Male mice aged six weeks were used in this study, which was approved by the Animal Care and Use Committee (IACUC No. 20-0112-S1A0) of Seoul National University Hospital. The study was performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

### Immunohistochemical staining

Briefly, 4  $\mu$ m sections from the formalin-fixed paraffin-embedded skin specimens of lesional and nonlesional skin from

patients with AD and healthy skin were dewaxed in xylene, rehydrated in graded alcohol, and washed with distilled water. For frozen tissue specimens, the sections were fixed in acetone for 5 min at -20°C. Endogenous peroxidase activity was quenched by a 6-min incubation with a 3% hydrogen peroxide solution. Following a 30-min block with the blocking solution from the SPlink HRP Broad Bulk Kit (Golden Bridge International, Mukilteo, WA, USA), the specimens were incubated with monoclonal primary antibodies against A, B, or H2 antigen in a humidified chamber for 18 h at 4°C. Subsequently, they were incubated with biotinylated secondary antibodies for 15 min and then with streptavidin-horseradish peroxidase conjugate for 15 min at 25°C (Golden Bridge International). Immunostaining signals were developed using 3-amino-9-ethylcarbazole (Golden Bridge International) for 3 to 5 min, depending on the types of antibodies, and the slides were mounted with Paramount medium (DAKO, Carpinteria, CA, USA). Images were captured using a Leica DFC280 camera (Leica, Heerbrugg, Switzerland) coupled with an Olympus BX51 microscope (Olympus, Tokyo, Japan). Semiquantitative evaluation of the images was independently performed by three dermatologists on a scale of 0–4. Standard images, ranging from grade 0 (no staining) to grade 4 (strongest staining), were selected from all staining images. The grade values of each group were statistically analyzed using the Mann–Whitney U Test for comparison between healthy skin and lesional or nonlesional skin from patients with AD. The Wilcoxon signed-rank test was applied for paired samples of lesional and nonlesional skin from the same patients with AD.

## MC903-induced AD-like skin inflammation in mice

In the MC903-induced AD-like model, 1.0 nmol of MC903 in 25  $\mu$ L ethanol was topically applied on both ears for 12 d (44). The ear thickness was measured daily using a digital caliper (Mitutoyo Corp., Tokyo, Japan). At the end of experiment on day 13, the ear skin was snap-frozen in liquid nitrogen for RNA and protein isolation, and the ear was embedded in Optimal Cutting Temperature compounds (Invitrogen) to prepare frozen sections.

## Histology, immunofluorescence analysis, and toluidine blue staining

Skin was fixed in 4% paraformaldehyde at 4°C overnight, paraffin-embedded, sectioned into 4  $\mu$ m, and stained with hematoxylin and eosin. Images were acquired using a Nikon ECLIPSE Ci-L microscope (Nikon Instruments Inc., USA). Epidermal and dermal thicknesses were measured using ImageJ 1.53e software (NIH). For immunofluorescence, skin sections were blocked in pre-blocking solution (GBI Labs, Bothell, WA, USA) and stained with the indicated primary antibodies at 4°C overnight. After washing, the sections were incubated with an Alexa Fluor 488-conjugated secondary antibody at 25°C for 1 h and stained with 4'-6-diamidino-2-phenylindole dihydrochloride (R37606; Thermo Fisher Scientific, Waltham, MA, USA) at 25°C for 5 min. Images

were acquired using a confocal microscope (Leica STED CW; Leica Microsystems, Mannheim, Germany). For the detection of mast cells, ear sections were stained with 0.5% toluidine blue at 25°C for 15 min and then washed three times in PBS. Images were acquired using a Nikon ECLIPSE Ci-L microscope. The number of immune cells was counted every five fields (40 $\times$  objective), and the average was calculated.

## ELISA

The level of *in vivo* cytokine and IgE production in mice was measured using ELISA according to the manufacturer's protocol (BioLegend).

## Quantitative PCR

Total RNA was isolated from skin and cells using RNAiso Plus (Takara Bio Inc., Shiga, Japan) according to the manufacturer's instructions. Subsequently, 1.0  $\mu$ g of the total RNA was converted to cDNA using a First-Strand cDNA Synthesis Kit (Thermo Fisher Scientific), according to the manufacturer's instructions. Quantitative RT-PCR was performed using AccuPower<sup>®</sup> 2X GreenStar<sup>™</sup> qPCR Master Mix (Bioneer, Daejeon, Korea), and an ABI 7500 Real-Time PCR instrument (ABI, Indianapolis, IN, USA) was used to measure relative mRNA expression levels. Each sample was analyzed in duplicate, and the expression levels were normalized to those of the housekeeping gene, *36B4*.

## Flow cytometry

Single-cell suspensions from the ear-draining lymph nodes (LNs) were pre-treated with an anti-CD16/32 antibody (BD Biosciences, San Jose, CA, USA) prior to staining with the specified antibodies. Intracellular staining was conducted using the BD Cytotfix/Cytoperm<sup>™</sup> Fixation/Permeabilization Kit (BD Biosciences) in accordance with the manufacturer's protocol. Samples were assessed with the BD FACSCanto<sup>™</sup> II (BD Biosciences), and data were analyzed using FlowJo software (BD Biosciences).

## Western blot analysis

Tissue lysates were prepared in 1 $\times$ RIPA buffer (50 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, and 1% NP-40), supplemented with a complete mini protease inhibitor cocktail (Roche Applied Science, Indianapolis, IN, USA), and a phosphatase inhibitor cocktail (Sigma-Aldrich). Subsequently, total protein extracts were loaded and resolved on a 12% SDS-polyacrylamide gel, then transferred onto a polyvinyl difluoride membrane. After blocking with 5% skim milk in PBS/Tween 20 (0.1%) overnight at 4°C with specified primary antibodies, the membrane underwent PBS/Tween washes. Afterwards, the membrane was incubated with HRP-

conjugated secondary antibodies for 1 h at 25°C and visualized using WestGlow™ PICO PLUS or FEMTO (Biomax Co., Ltd., Seoul, Korea).

presented as mean ± standard error. A significance threshold of  $p < 0.05$  was set for all analyses.

### Statistical analysis

Statistical analyses were performed using Prism software (version 9.0; GraphPad Software, La Jolla, CA, USA). Dunnett’s multiple range test or Student’s t-tests were used to compare differences between two groups, whereas comparisons between multiple groups utilized the two-way ANOVA. All graphs are

## Results

### Aberrant expression of ABH antigen was observed in the skin of patients with AD

We have determined that ABH antigen expression was primarily localized in the granular and horny layers for each blood type (A, B, or O) of healthy skin (Figure 1A,

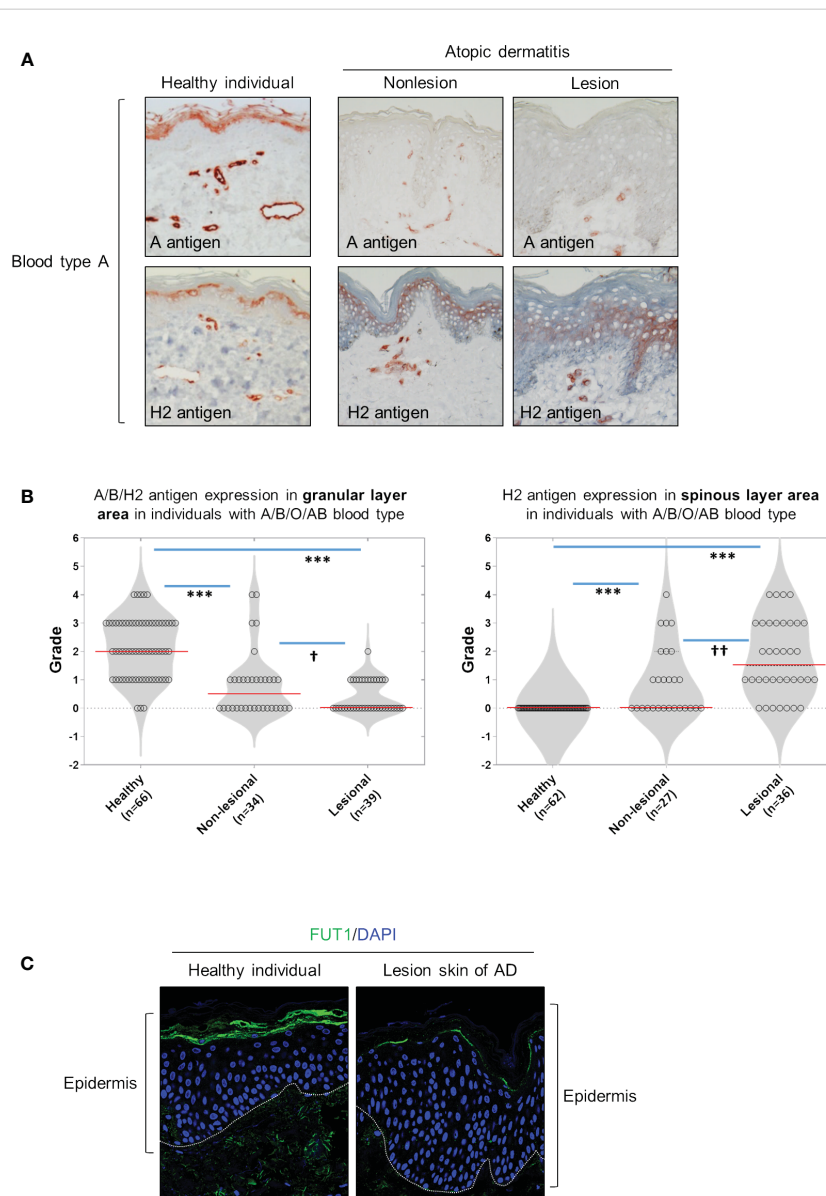


FIGURE 1

The granular layers of healthy skin exhibit ABH antigen and FUT1 expression which is diminished in nonlesional and lesional AD skin. (A) Immunohistochemical staining to detect A and H2 antigens in healthy skin, as well as on nonlesion and lesional AD skin from individuals with blood type A. (B) Evaluating the expression of A, B, and H2 antigens in the granular (the left graph) and spinous layers (the right graph) of healthy skin, and comparing these expressions in nonlesion and lesional AD skin among individuals with blood types A, B, O, or AB. (C) Immunofluorescent labeling of FUT1 (green) and DAPI (blue) in healthy skin and lesional AD skin. The data are representative of the mean ± SEM from 39 patients and 66 healthy controls. †  $p < 0.05$ , ††  $p < 0.01$ , \*\*\*  $p < 0.001$ . AD, atopic dermatitis.



Supplementary Figure S1). However, the expression of ABH antigens was absent or diminished in both the granular and horny layers of nonlesioned and lesioned skin from patients with AD (Figure 1A; Supplementary Figure S1). Moreover, H2 antigen expression abnormally manifested in the spinous layers, exhibiting a randomly dispersed pattern in many cases of patients with AD (Figure 1A; Supplementary Figure S1). The grading of immunostaining intensity revealed a significant decrease in the staining of A, B, and H2 antigens in granular layers in both lesional and nonlesional skin of patients with AD compared with those in healthy skin (Figure 1B). Additionally, the staining of H2 antigen in the spinous layers significantly increased in both lesional and nonlesional skin of patients with AD compared with that in healthy skin (Figure 1B). Notably, these changes were more pronounced in lesional skin than in nonlesional skin (Figure 1B). Furthermore, FUT1 expression was identified in the granular layers of healthy skin, while in the lesional skin of AD, it was either absent or reduced in these layers. Conversely, FUT1 expression exhibited no increase in the spinous layers of the lesional skin of AD (Figure 1C). The data showed a correlation between the patterns of FUT1 expression and H2 antigen expression in the granular layers, while no such correlation was observed in the spinous layers. These findings indicate the potential relationship between ABH antigen expression and inflammation in AD.

## FUT1 deficiency enhances AD-like inflammation in a mouse model

Subsequently, we investigated the biological implications of ABH antigen expression in context of AD-like inflammation. To do this, we conducted a comparative analysis of MC903-induced AD-like inflammation in both WT and *Fut1*-deficient mice. H2 antigen and FUT1 expression were exhibited in the granular layers of control skin of WT mice, whereas they were notably absent in vehicle-treated *Fut1*-deficient mice (Figures 2A, B). The expression of the H2 antigen decreased in the granular layer, but increased in the spinous layers of WT mice treated with MC903 on day 12, whereas no such expression was observed in *Fut1*-knockout (KO) mice (Figures 2A, B). Additionally, the expression of *Fut1* mRNA and protein in the skin of WT mice treated with MC903 decreased notably compared to the vehicle-treated skin of WT mice (Supplementary Figures S2A, B). Conversely, no expression of *Fut1* mRNA and protein was detected in the skin of *Fut1*-KO mice treated with either vehicle or MC903 (Supplementary Figures S2A, B).

Upon daily application of 1 nmol MC903 or EtOH (vehicle) on the ears for 12 d, the ear skin of *Fut1*-deficient mice exhibited greater redness and attained higher clinical scores compared with that of WT mice (Figures 2C, D). From day 6 of MC903 application onward, the caliper measurements indicated a significantly higher ear thickness in *Fut1*-deficient mice compared with that of WT mice (Figure 2E). *Fut1*-deficient mice exhibited elevated serum IgE levels compared with that in WT mice following MC903 induction (Figure 2F). The expression levels of keratinocyte differentiation markers, such as Loricrin, Filaggrin, and Keratin 10, showed no significant

differences between the epidermal layers of vehicle-treated WT and *Fut1*-KO mice, as well as between MC903-treated WT and *Fut1*-KO mice (Figures 2G–I). This indicates that the diminished expression of the H2 antigen was not caused by abnormal epidermal differentiation. Our findings suggest that FUT1 plays a crucial role in providing protection against AD-like inflammation.

## FUT1 deficiency exacerbates both epidermal and dermal thickening and inflammatory cell infiltration following MC903 application

Histological analysis revealed increased inflammatory cell infiltration as well as greater epidermal and dermal thickening in *Fut1*-deficient mice compared with that in WT mice following MC903 application (Figures 3A–C). Nevertheless, in the control skin, there were no discernible differences in epidermal and dermal thickness between WT and *Fut1*-deficient mice (Figures 3A–C). To identify the specific immune cell types infiltrating the skin, we conducted staining on ear skin using CD4, Eosinophil marker, and toluidine blue for the detection of CD4<sup>+</sup> T cells, eosinophils, and mast cells, respectively. The results revealed elevated numbers of infiltrating CD4<sup>+</sup> T cells in the epidermis and dermis, along with increased counts of eosinophils and mast cells in the dermis of *Fut1*-deficient mice compared with those in WT mice following MC903 application (Figures 3D–I). However, no apparent differences in the numbers of CD4<sup>+</sup> T cells, eosinophils, and mast cells were observed in the control skin between WT and *Fut1*-deficient mice (Figures 3D–I). Nevertheless, the expression levels of mast cell activation markers, F2RL1 and Mcpt6, exhibited variability depending on mast cell morphology in the MC903-treated skin of *Fut1*-KO mice (Supplementary Figures S3A, B). Our findings disclosed that *Fut1* deficiency leads to heightened infiltration of inflammatory immune cells, concurrently resulting in increased dermal and epidermal thickening in AD-like inflammation.

## FUT1 deficiency amplifies the expression of AD-related cytokines and chemokines following MC903 application

We have discerned differences in the expression of AD-related cytokines and chemokines between WT and *Fut1*-deficient mice. ELISA results revealed elevated expression of AD-related cytokines, such as IL-4 and IL-6, in the skin of *Fut1*-deficient mice compared with that in WT mice following MC903 application (Figures 4A, C). Nevertheless, no noticeable differences were observed in the production of IL-5 and TSLP (Figures 4B, D). Quantitative PCR analysis showed a significant increase in the expression of *Ccl2* and *Ccl8* mRNA in the skin of *Fut1*-deficient mice compared with those in WT mice (Figures 4E, F). Our results conclusively demonstrated that the enhanced expression of chemoattractive factors, specifically CCL2 and CCL8, contributes to immune cell infiltration, subsequently leading to increased expression of IL-4 and IL-6 from immune cells in the skin of *Fut1*-deficient mice.

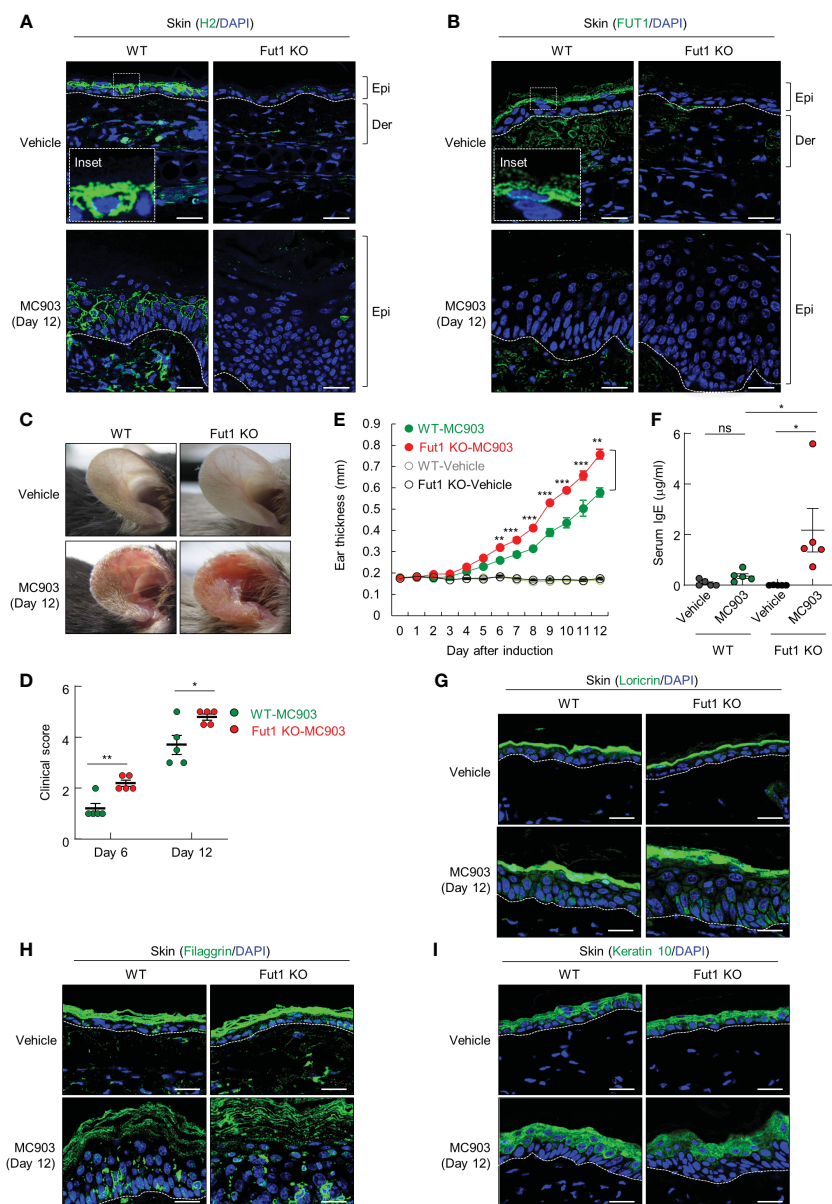


FIGURE 2

The deficiency of FUT1 exacerbates AD-like inflammation induced by MC903 in a murine model. **(A)** Immunofluorescent labeling of H2 antigen (green) and DAPI (blue) in the ears of both WT and *Fut1*-KO mice treated with either vehicle or MC903 on day 12. Scale bars, 20 µm.

**(B)** Immunofluorescent labeling of FUT1 (green) and DAPI (blue) in ear sections from both WT and *Fut1*-KO mice. Scale bars, 20 µm. **(C)** Ear skin inflammation induced by MC903 in both WT and *Fut1*-KO mice on day 12.

**(D)** Clinical scores of MC903-induced ear skin inflammation in both WT and *Fut1*-KO mice on days 6 and 12. **(E)** Measurement of ear swelling in both WT and *Fut1*-KO mice treated with MC903 using a digital caliper.

**(F)** ELISA analysis of serum IgE levels in both WT and *Fut1*-KO mice treated with either vehicle or MC903 on day 12. The data are representative of the mean ± SEM of three independent experiments, with five mice per group. ns., not significant; \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  and \*\*\*\*  $p < 0.0001$ . AD, atopic dermatitis; WT, wild-type.

## FUT1 deficiency enhances the population of CD4<sup>+</sup> T cells producing IL-4 in the ear-draining LNs following MC903 application

Finally, we performed FACS analysis to evaluate the population of IL-4 producing CD4<sup>+</sup> T cells in the ear-draining LNs of both WT and *Fut1*-KO mice after a 12-d treatment with MC903. The results

revealed an elevated ratio and greater numbers of IL-4 producing CD4<sup>+</sup> T cells in the ear-draining LNs of *Fut1*-KO mice compared with those in WT mice (Figures 5A, B). This observation correlated with elevated levels of IL-4 production and a greater number of CD4<sup>+</sup> T cells in the skin of *Fut1*-KO mice compared with that in WT mice. This suggests an increased proliferation of IL-4 producing CD4<sup>+</sup> T cells in the ear-draining LNs.

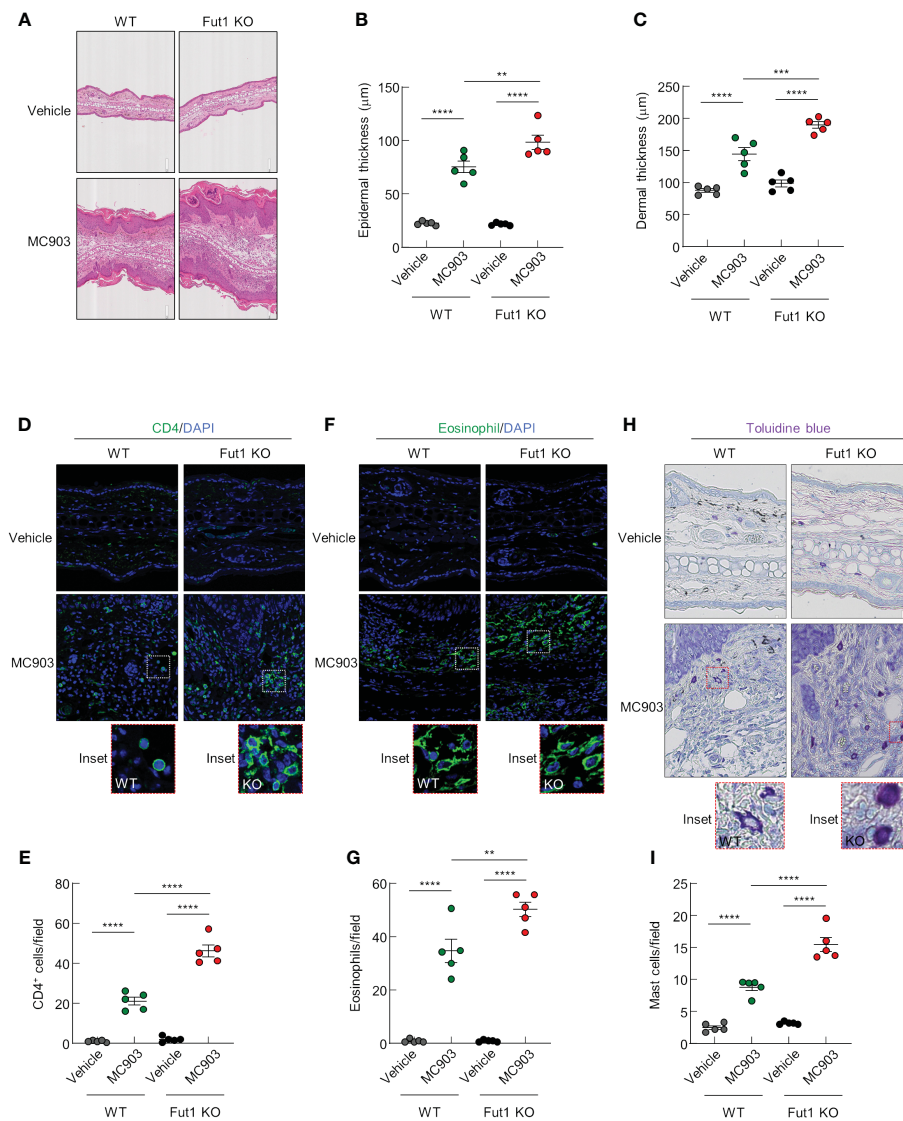


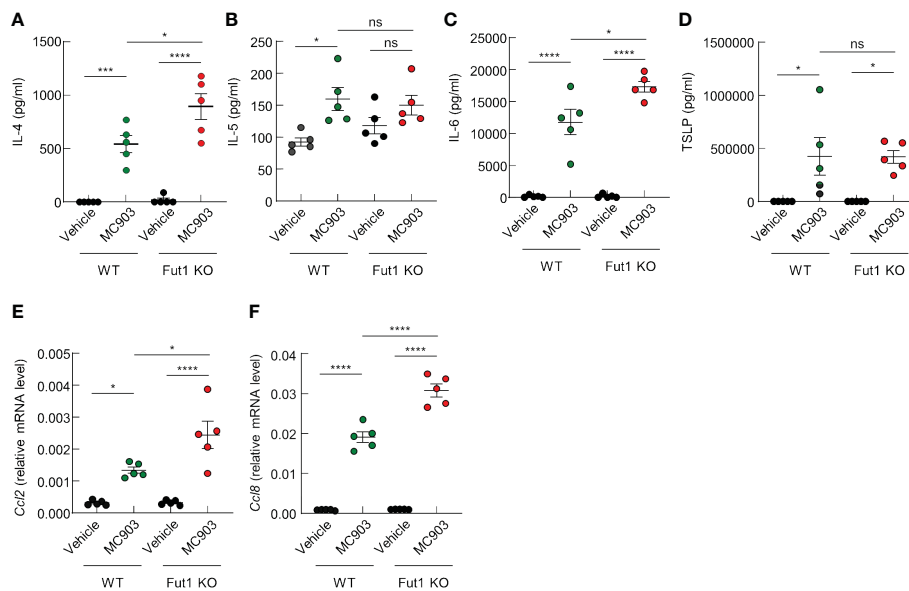
FIGURE 3

FUT1 deficiency amplifies MC903-induced ear thickening and immune cell infiltration. (A) H&E staining of the ear in both WT and *Fut1*-KO mice treated with either vehicle or MC903 on day 12. (B) Epidermal thicknesses measurements derived from H&E-stained ear sections. (C) Dermal thicknesses measurements obtained from H&E-stained ear sections. (D) Immunofluorescent labeling of CD4 (green) and DAPI (blue) in ear sections on day 12. (E) Quantitation of CD4<sup>+</sup> T cells in immunofluorescent-labeled ear sections. (F) Immunofluorescent labeling of Eosinophil (green) and DAPI (blue) in ear sections on day 12. (G) Quantitation of eosinophils in immunofluorescent-labeled ear sections. (H) Toluidine blue staining of mast cells in ear sections. (I) Quantitation of mast cells in toluidine blue-stained ear sections. The data are representative of the mean  $\pm$  SEM of three independent experiments, with five mice per group. \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  and \*\*\*\*  $p < 0.0001$ . AD, atopic dermatitis; H&E, hematoxylin and eosin; WT, wild-type.

## Discussion

In this study, we elucidated the protective role of H2 antigen expression, induced by FUT1, in the epidermal layers against AD-like inflammation triggered by MC903 treatment in a murine model. Additionally, we demonstrated the diminished expression of ABH antigens in the granular and horny layers of lesional skin in AD compared with that in healthy skin. However, there is an observed increase in H2 antigen expression in spinous layers of lesional skin in AD. This result aligns with our earlier findings, indicating a significant decrease in the expression of ABH antigens in the granular layers of photoaged skin and acutely UV-irradiated

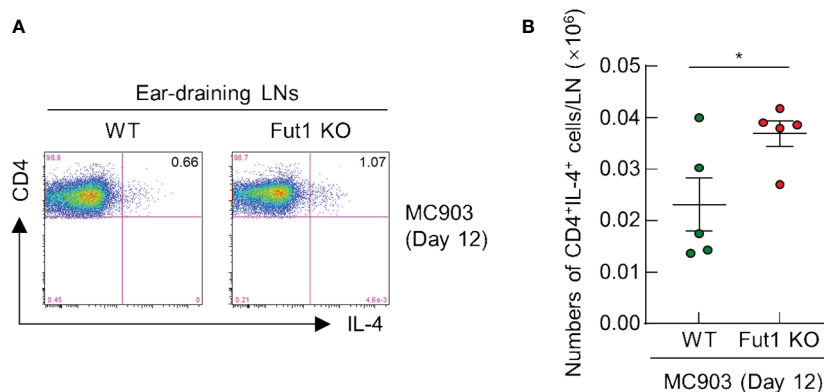
skin (33). Notably, there is an elevation in H2 antigen expression in the spinous layers of these skin conditions (33). Our findings suggest a robust link between skin inflammation and the diminished expression of ABH antigens in the epidermal granular layers, accompanied by an elevation of H2 antigens in the spinous layer. This heightened expression of the H2 antigen, a precursor to A and B antigens, in the spinous layers of inflamed skin may represent compensatory mechanisms in response to the reduced expression of ABH antigens in the granular and horny layers. Alternatively, it could be attributed to a decrease in ABO glycosyltransferase expression or activation. Consequently, this may result in the accumulation of H2 antigen without its



**FIGURE 4** Fut1 deficiency enhances the production of AD-associated cytokines in ear skin induced by MC903. (A–D) ELISA analysis of IL-4 (A), IL-5 (B), IL-6 (C), and TSLP (D) levels in the ear skin of both WT and *Fut1*-KO mice treated with either vehicle or MC903 on day 12. (E, F) Relative mRNA expression of *Ccl2* (E) and *Ccl8* (F) in the ear skin of both WT and *Fut1*-KO mice treated with vehicle or MC903 on day 12. The data are representative of the mean ± SEM of three independent experiments, with five mice per group. ns., not significant; \* p < 0.05, \*\*\* p < 0.001 and \*\*\*\* p < 0.0001. AD, atopic dermatitis; TSLP, thymic stromal lymphopoietin.

conversion into A or B antigens. Previous investigations showed a decrease in *ABO* gene expression in ovarian tumor tissues and human bladder tumors (41, 45). The *ABO* polymorphism has been linked to specific infectious diseases, such as *Helicobacter pylori* infection, *Plasmodium falciparum* malaria, and SARS-CoV-2 (46–49). These findings underscore the broader clinical relevance of the ABH antigens, reaching beyond their role in blood transfusion and transplantation. Moreover, these antigens play a crucial role in various clinically significant aspects, including inflammatory responses, cancer, and other diseases (50–53).

Furthermore, our results suggest that the regulation of H2 antigen expression in the skin epidermal layers is predominantly governed by FUT1, as demonstrated using *Fut1*-KO mice. *Fut1*-deficient mice do not exhibit H2 antigen expression in the granular layers of control skin. FUT1 expression was also observed in the granular layers of healthy human and mouse skin. However, FUT1 expression was notably absent in the spinous layers of AD and MC903-treated mice skin, despite an elevated expression of the H2 antigen in these layers. This observation could be a potential short half-life for FUT1 expression in the spinous layers following H2



**FIGURE 5** Fut1 deficiency heightens the number of IL-4-producing CD4<sup>+</sup> T cells in the ear-draining LN following MC903 treatment. (A) FACS analysis of IL-4-producing CD4<sup>+</sup> T cells in the ear-draining LN after 12 d of MC903 treatment. (B) Quantification of IL-4-producing CD4<sup>+</sup> T cells in the ear-draining LN on day 12. The data are representative of the mean ± SEM of two independent experiments, with five mice per group. \* p < 0.05, LN, lymph node.



antigen induction. Further elucidation is needed to clarify this aspect. In addition, our confirmation that MC903 treatment results in decreased levels of Fut1 mRNA and protein in the skin suggests direct involvement of MC903 in downregulating the H2 antigen expression. This cumulative evidence suggests that the control of H2 expression in the skin is predominantly controlled by FUT1. Importantly, this downregulation does not result from abnormal epidermal differentiation, as evidenced by the expression of epidermal differentiation markers, including loricrin, filaggrin, and keratin 10, within the granular and horny layers of both vehicle and MC903-treated WT and *Fut1*-KO mice. This implies that the absence of H2 antigen expression in the granular and horny layers of MC903-treated skin does not stem from impaired terminal differentiation after MC903 treatment.

Under physiological conditions, the H1 antigen in the intestine serves a protective role by maintaining a balance of commensal bacteria (54, 55). Loss-of-function mutations in FUT2 result in an altered gut microbiome, contributing to the development of inflammatory bowel diseases (55, 56). Nevertheless, amid the inflammatory response, the function of the H1 antigen in the intestine and lung remains controversial, suggesting its potential involvement in either mitigating or exacerbating inflammation (57–62). Our findings indicate that H2 antigens contribute to a protective role against AD-like inflammation triggered by MC903 in the skin. In the absence of H2 antigens in *Fut1*-KO mice, MC903-induced ear swelling, as well as epidermal and dermal thickening, exceeded that observed in WT mice. Corresponding with the heightened dermal thickening, the infiltration of immune cells, including CD4<sup>+</sup> T cells, eosinophils, and mast cells into the dermis, was more pronounced in *Fut1*-KO mice compared with those in WT mice.

The topical application of MC903 on the skin swiftly initiates TSLP production, considered as an initial trigger for Th2 inflammation, in keratinocytes, beginning on day 2 after the initial application (44). Nevertheless, our study revealed no apparent distinction in TSLP production between the skin of WT and *Fut1*-KO mice on day 12, a relatively late time point. This observation could be attributed to the substantial accumulation of TSLP production over the 12-day period with daily application of MC903. IL-4 is primarily produced by Th2 cells and, as non-T cells, by eosinophils (63, 64). The production of IL-4, occurring in the late response following MC903 application, was more pronounced in *Fut1*-KO mice compared with that in WT mice. This correlated with a higher number of infiltrating CD4<sup>+</sup> T cells and eosinophils in the skin of *Fut1*-KO mice compared with those in WT mice. IL-4 stimulates the expansion and activation of mast cells, as well as induces B cells to produce IgE (65–70). This aligns with our findings, which demonstrate higher IgE production and a greater number of mast cells in *Fut1*-KO mice compared with that in WT mice. A study has elucidated that mast cells in MC903-induced AD-like skin are sustained through both the local proliferation of tissue-resident mast cells and the infiltration/differentiation of bone marrow-derived mast cell progenitors. This finding provides a novel perspective on mast cell heterogeneity in allergic conditions (71). Such heterogeneity may contribute to the variability observed in mast cell morphology and the different expression levels of mast

cell activation markers (F2RL1 and Mcpt6) in MC903-treated skin in our study, despite the ongoing ambiguity surrounding the functional distinctions between these cell types.

A prior study demonstrated elevated IL-6 production by T cells derived from patients with AD (72). Additionally, IL-6 is generated by various cell types, including dendritic cells, mast cells, eosinophils, and keratinocytes (73–76). After the application of MC903 in *Fut1*-KO mice, there was a significantly elevated production of IL-6 in the skin, along with increased infiltration of mast cells and eosinophils compared to WT mice. IL-5 is recognized as the most crucial cytokine in eosinophil maturation within the bone marrow and their subsequent release into the blood (77–79). In humans, IL-5 affects eosinophils and basophils, influencing their growth, activation, and survival (80, 81). However, our findings indicate that IL-5 production did not exhibit a significant difference between the skin of WT and *Fut1*-KO mice. This implies that in mice, alternative chemokines may exert a substantial influence on eosinophil recruitment into the skin, or it could be essential to explore different time points to discern any divergence in IL-5 production. Furthermore, our findings indicate that the expression of chemokines, CCL2 and CCL8, was notably higher in the skin of *Fut1*-KO mice compared with those in WT mice on day 12 after MC903 application. CCL8 has the potential to regulate the recruitment of immune cells in various tissues during inflammatory diseases (82). Mouse CCL8 is constitutively expressed in the skin and may contribute to chronic eosinophilic inflammation by inducing the accumulation of CD4<sup>+</sup> Th2 cells in a mouse model of chronic AD (24). CCL2 secretion in the skin plays a role in recruiting inflammatory monocytes, dendritic cells, and memory T cells (22, 83–85). Mice deficient in CCL2 exhibited diminished recruitment of monocytes and eosinophils in an irritant contact dermatitis model (86). CCL2 induces DC maturation and migration from the skin to draining lymph nodes, facilitating antigen presentation to T cells (87). The data strongly support our findings, indicating that heightened production of CCL2 and CCL8 is likely to contribute to the augmented recruitment of immune cells into the skin of *Fut1*-KO mice. Additionally, our results reveal an increased presence of IL-4-producing CD4<sup>+</sup> T cells in the ear-draining LNs of *Fut1*-KO mice compared with that in WT mice.

We investigated the synthesis of the H2 antigen by Fut1 and its expression patterns within healthy human and murine skin, particularly within the granular cells and stratum corneum. Notably, these patterns differed in skin lesions in patients with AD and in MC903-treated mice. In these pathological contexts, both H2 antigen levels and FUT1 expression within the granular layers and stratum corneum were markedly decreased but were significantly increased in the spinous layers of lesional skin. Based on these findings, complete loss of H2 antigen expression in patients with AD may exacerbate inflammation associated with this condition. Although inflammation typically correlates with reduced H2 antigen expression in the granular cells and stratum corneum of healthy skin, compensatory upregulation of H2 antigen expression throughout the spinous layers in lesional skin suggests a mechanism for counteracting this loss. Further studies are required to fully understand the underlying mechanisms.



The importance of Fut1 or the H2 antigen in biological processes may vary depending on the context. Both Fut1-mediated terminal fucosylation and the resultant H2 antigen may contribute distinct but interconnected roles in cellular interactions and signaling pathways. The presence or absence of FUT1 can influence interactions between cells and molecules in the immune system, as well as cell adhesion and signaling (88–91). The absence of functional FUT1 enzyme activity leads to the Bombay phenotype, in which individuals lack H2 antigen expression; this absence can have important biological consequences (92, 93). Similarly, the presence of the H2 antigen and its modifications may contribute to various physiological processes (94, 95).

In summary, our study reveals that ABH antigens are expressed in the granular and honey layers of healthy human and mice skin. However, in the context of AD and MC903-treated mice skin, there is a significant reduction in the expression of these antigens within these layers. Notably, our findings indicate that the H2 antigen plays a role in a protective capacity against AD-like inflammation, leading to reductions in clinical scores, skin thickening, and the production of AD-related cytokines. While our results underscore the potential therapeutic significance of the H2 antigen in AD by regulating blood group antigens, further investigation is essential to elucidate the specific mechanisms through which the H2 antigen contributes to its protective role in AD inflammation.

## Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

## Ethics statement

The studies involving humans were approved by The Institutional Review Board (IRB No. C-1312-084-543) of Seoul National University Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. The animal study was approved by The Animal Care and Use Committee (IACUC No. 20-0112-S1A0) of Seoul National University Hospital. The study was conducted in accordance with the local legislation and institutional requirements.

## Author contributions

YL: Conceptualization, Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. NL: Data curation, Investigation, Writing – review & editing. J-HO: Writing – review & editing, Funding acquisition, Investigation. JS: Resources, Writing – review & editing. S-PJ: Data curation, Writing – review & editing. DL: Conceptualization, Funding acquisition, Writing – review & editing. JC: Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

## Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This research received support from the Bio & Medical Technology Development Program of the National Research Foundation (NRF) funded by the Ministry of Science and ICT (2019M3A9I3091696), the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2016R1D1A1B03932664), and a grant from the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (HI14C1277).

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fimmu.2024.1365430/full#supplementary-material>

### SUPPLEMENTARY FIGURE 1

The granular layers of healthy skin exhibit ABH antigen expression which is diminished in nonlesional and lesional AD skin. Immunohistochemical staining to detect B and H antigens in healthy skin, as well as on nonlesion and lesioned AD skin from individuals with blood types B or O. AD, atopic dermatitis.

### SUPPLEMENTARY FIGURE 2

Reduced expression levels of Fut1 mRNA and protein in the skin of WT mice treated with MC903. (A) Relative mRNA expression of *Fut1* in the ear skin of both WT and *Fut1*-KO mice treated with vehicle or MC903 on day 12. (B) The protein expression of Fut1 and  $\beta$ -Actin in the ear skin of both WT and *Fut1*-KO mice treated with vehicle or MC903 on day 12. The data represent the mean  $\pm$  SEM of three independent experiments, with five mice per group. ns., not significant; \*\*\*\*  $p < 0.0001$ .

### SUPPLEMENTARY FIGURE 3

Expression of mast cell activation markers in the skin. (A) Immunofluorescent labeling of F2RL1 (green) and DAPI (blue) in ear sections on day 12. (B) Immunofluorescent labeling of Mqpt6 (green) and DAPI (blue) in ear sections on day 12.

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