

Regular Article

Dimethylglycine, a Methionine Metabolite, Participates in the Suppressive Effect of Methionine on 1-Fluoro-2,4-dinitrobenzene-Induced Dermatitis

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Allergic contact dermatitis (ACD) is a common skin disorder caused by contact with allergens. The optimal treatment for ACD is to avoid contact with allergens. However, in some cases, avoiding exposure is not possible when the allergens are unknown. Therefore, establishing treatment methods other than allergen avoidance is important. We previously reported that the continuous administration of methionine, an essential amino acid, in a mouse model of atopic dermatitis alleviated its symptoms. In the present study, we investigated the effect of methionine on a mouse model of ACD caused by 1-fluoro-2,4-dinitrobenzene (DNFB). Differences in the effect of methionine were observed in DNFB-induced ACD model mice based on the mouse strain used. This difference was attributed to the suppression of hepatic dimethylglycine (DMG) production, which is associated with the suppression of hepatic betaine-homocysteine methyltransferase (*Bhmt*) expression by ACD. Although we did not reveal the mechanism underlying DMG suppression, our study suggests the presence of interactions between the liver and skin in dermatitis, such as the regulation of hepatic metabolic enzyme expression in dermatitis and the alleviation of dermatitis symptoms by the hepatic metabolism status of DMG.

Key words allergic contact dermatitis, dimethylglycine, methionine, betaine-homocysteine methyltransferase

INTRODUCTION

Allergic contact dermatitis (ACD) is a common skin disorder caused by contact with allergens and a growing environmental and occupational health issue.^{1–3} These contact allergens are diverse and include metals, cosmetics, fragrances, dust mites, and even fruits such as mangoes.^{4–6} ACD treatment is based on patient education to avoid contact with allergens, which is a simple and effective method.^{1,7,8} However, in some cases, exposure avoidance is not possible; therefore, these patients are often prescribed ACD suppressors,^{7,9} which can be categorized into two types: topical (ointment) and oral drugs. Ointments are more commonly used than oral medications to treat ACD. Steroid ointments are used to control inflammation, and oral antihistamines are used to control itching.^{1,10} These treatments have long been used and are generally effective; however, their clinical application is limited owing to their numerous side effects. For instance, certain steroids cause side effects, such as skin withering, thinning, and redness,¹⁰ and antihistamines cause sleepiness and have anticholinergic effects.¹¹ Therefore, a novel treatment for ACD is needed to avoid the side effects associated with the standard treatments.

Several factors, including physiological and environmental stimuli (mites and dust), play a role in the onset and progression of atopic dermatitis.^{12,13} We previously reported that the

continuous administration of methionine, an essential amino acid, alleviated the symptoms of atopic dermatitis in an animal model.¹⁴ The immune responses to allergens are also thought to play a role in the establishment of atopic dermatitis.^{15,16} Therefore, we hypothesized that the continuous supplementation of methionine would also suppress ACD symptoms. This study aimed to investigate the effects of methionine in a 1-fluoro-2,4-dinitrobenzene (DNFB)-induced ACD mouse model to evaluate its therapeutic application in dermatitis.

MATERIALS AND METHODS

Materials Methionine and dimethylglycine (DMG) were purchased from Nacalai Tesque Inc. (Kyoto, Japan), and 1-fluoro-2,4-dinitrobenzene (DNFB) was purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). All other reagents were of the highest commercially available grade.

Animals and Treatments All experiments were approved by the Institutional Animal Care and Experimental Committee of the Daiichi University of Pharmacy (Approval Nos: 17007 and 2021004). Male ddY, ICR, BALB/c, DBA/2, and C57BL/6J mice (6-week-old each) were purchased from Japan SLC Co., Ltd. (Shizuoka, Japan). All mice were acclimated for 1 week before experimentation. Fifteen mice of each strain were equally divided into three groups, namely non-treated control (NTC), control, and methionine. A mouse

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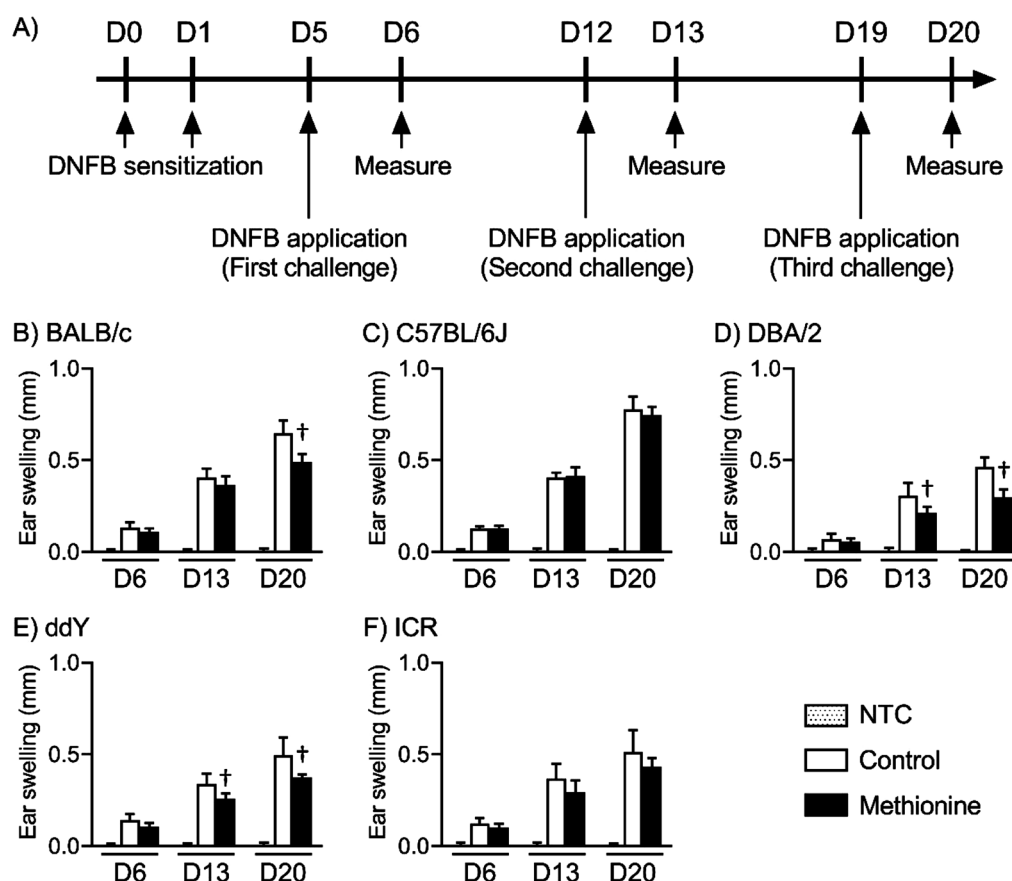


Fig. 1. Methionine Suppresses Ear Swelling on ACD Development in a Strain-Dependent Manner

(A) The schedule of ACD establishment using DNFB application. The mice were sensitized by application of DNFB to the back on D0 and D1. Then ACD was induced by applying DNFB to the dorsal surface of the ear lobe on D5, D12, and D19. The degree of ACD was assessed by ear swelling 24h after application (*i.e.*, D6, D19, and D20). Methionine was administered in drinking water for the entire period, *i.e.*, from D0 to D20. (B–F) Reducing effect of methionine on ACD progression in each mouse strain. Dotted bars, open bars, and closed bars indicate NTC, control, and methionine groups, respectively. Panel B, C, D, E, and F represent BALB/c, C57BL/6J, DBA/2, ddY, and ICR mice, respectively. Each bar represents the mean $\pm$ standard deviation ($N=5$). $^{\dagger}p<0.05$ compared with control group. ACD, allergic contact dermatitis; DNFB, 1-fluoro-2,4-dinitrobenzene; NTC, non-treated control.

model of chemically-induced ACD was established by topical application of DNFB, as previously described, with some modifications^{17,18)} (Fig. 1A). DNFB (11.2mg/kg body weight) dissolved in acetone-olive oil mixture (acetone:olive oil, 4:1) was continuously applied to the shaved-back skin of mice for 2d (D0 and D1). On D5, 6.7mg/kg of DNFB was applied to the back of the right ear as the first challenge. The vehicle was simultaneously applied to the back of the left ear. Earlobe thickness was measured 24h after the first challenge using a digimatic micrometer (Mitutoyo, Kanagawa, Japan) at a measuring force of 0.5N. ACD symptoms were evaluated based on the difference in thickness between the left and right earlobes. On D12 and D19 (weeks 1 and 2 after D5, respectively), the right and left ears were treated with DNFB and vehicle as the second and third challenges, respectively, as described above for the first challenge. Earlobe thickness was measured 24h later as described above. For the NTC group, DNFB was replaced by the solvent. The livers and sera of the mice were collected after the final measurement for additional analyses.

In few experiments, methionine was administered in drinking water from D0–D20 at a concentration of 30mg/L, as described previously.¹⁴⁾ In a separate experiment, DMG (0.3 or 3 mg/L) was administered in drinking water from D0–D20. Methionine and DMG were diluted every morning to the desired concentration from concentrated stock solutions (3000mg/L)

stored at -20°C .

Real-Time RT-PCR RNA extraction was performed using TRIzol reagent according to the manufacturer's instructions (Invitrogen, Thermo Fisher Scientific, Waltham, MA, U.S.A.). cDNA synthesis was performed using ReverTra Ace[®] qPCR RT Master Mix with a gDNA Remover kit (TOYOBO Inc., Osaka, Japan) according to the manufacturer's instructions on a GeneAmp[®] PCR system 9700 (Applied Biosystems, Thermo Fisher Scientific). RT-PCR analysis was performed using FastStart Essential DNA Green Master (Roche, Basel, Switzerland), according to the manufacturer's instructions on a LightCycler[®] 96 system (Roche), with primers for target genes. Primers for methionine adenosyltransferase 2A (*Mat2a*), betaine-homocysteine methyltransferase (*Bhmt*), 5-methyltetrahydrofolate-homocysteine methyltransferase (also known as methionine synthase; *Mtr*), and β -actin were as follows: *Mat2a*, Forward 5'-GATCAAGGCTGTTGTACCTGC-3', reverse 5'-CCAACGCCATAAGTATCCAC-3'; *Bhmt*, forward 5'-AGGGGCTATGTAAAGGCTGG-3', reverse 5'-AACTCCCGATGAAGCTGACG-3'; *Mtr*, forward 5'-GCAGCCTTGTTTGCATCC-3', reverse 5'-GTCCGAATGAGACACGCTGG-3'; β -actin, forward 5'-TTTCCAGCCTTCCTTCTTGGG-3', reverse 5'-AGGTCTTTACGGATGTCAACG-3'. The expression levels of each gene were calculated by LightCycler[®] 96 software, using stan-

dard curves for each gene generated by using serial dilutions of standard cDNAs.

Enzyme-Linked Immunosorbent Assay (ELISA) Serum cytokine levels were determined using commercial ELISA kits for immunoglobulin E (IgE; FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan), interferon gamma (INF γ ; FUJIFILM Wako Pure Chemical Corporation), interleukin-4 (IL-4; R&D Systems, Inc., Minneapolis, MN, U.S.A.), and tumor necrosis factor alpha (TNF α ; R&D Systems, Inc.). The absorbance of each sample was measured using a Multiskan GO microplate spectrophotometer (Thermo Scientific) with skanlt software 5.0 (Thermo Scientific).

Liquid Chromatography Coupled with Mass Spectrometry (LC-MS/MS) The levels of essential amino acids, metabolites of methionine, homocysteine, cysteine, cystine, taurine, and DMG in the serum and liver were determined using LC-MS/MS (LCMS-8040; Shimadzu, Kyoto, Japan). Serum collected on D20 was added to a four-fold volume of methanol. The liver was homogenized by adding cold saline (nine times its weight), followed by the addition of a two-fold volume of methanol. The mixture was then vigorously stirred and centrifuged (MRX-150; TOMY, Tokyo, Japan) at 9000 $\times g$ for 10 min at 4°C. The upper (aqueous) layer was collected, the solvent was removed using a centrifugal evaporator (Concentrator 5301; Eppendorf, Hamburg, Germany), and the residues were dissolved in 0.1% formic acid. The resulting sample was filtered using a syringe and analyzed using an LC-MS/MS instrument. The operating conditions for LC were as follows: column, Intrada Amino Acid (3- μ m particle size, 2 $\times$ 100 mm; Imtakt, Kyoto, Japan); column temperature, 40°C; sample temperature, 10°C; mobile phase, water with 100 mM ammonium formate (solvent A) and acetonitrile with 0.1% formic acid (solvent B); elution program (% of B in A [min]): 86% (0–3), 86 to 0% (3–10), 0 to 86% (10–11), and 86% (11–15); and flow rate, 0.3 mL/min. Mass spectrometry was performed using electrospray ionization in multiple reaction monitoring (MRM) in positive ion mode. The data were quantified using the LabSolution software (Shimadzu). Cysteine, an irreversible metabolite of methionine, is converted nonenzymatically and spontaneously to the dimer, cystine. Therefore, hepatic and serum levels of cysteine and cystine were measured and presented as cysteine equivalent level (calculated by adding the cysteine level and twice of cystine level; shown as Cys/Cys-Cys). The MRM transition and retention times were set as listed in Table 1.

Statistical Analysis Statistical differences were determined by two-way and one-way ANOVA with Tukey–Kramer *post hoc* test for comparing ACD progression among the three groups (Fig. 1) and other comparisons, respectively. Statistical significance was set at $p < 0.05$ for all analyses. These statistical analyses were performed with GraphPad Prism 9 (GraphPad Software Inc., Boston, MA, U.S.A.).

RESULTS

Effects of Methionine on the Development of ACD To determine the effects of methionine on the development of ACD, we administered methionine solution at a concentration of 30 mg/L to five strains of mice, namely BALB/c, C57BL/6J, DBA/2, ddY, and ICR, during the induction of ACD. We used the same effective concentration that suppressed atopic derma-

Table 1. MRM Transition and Retention Time of Amino Acids and Metabolites of Methionine

	MRM transition	Retention time (min)
Isoleucine (Ile)	132.18 > 86.10	5.382
Leucine (Leu)	132.18 > 86.10	4.975
Threonine (Thr)	120.13 > 74.10	6.525
Valine (Val)	118.16 > 72.10	5.861
Phenylalanine (Phe)	166.20 > 120.05	4.578
Tryptophan (Trp)	205.24 > 188.00	4.841
Histidine (His)	156.16 > 110.10	10.048
Lysine (Lys)	147.20 > 84.10	10.722
Methionine (Met)	150.22 > 56.10	5.629
Dimethylglycine (DMG)	104.13 > 58.10	5.274
Homocysteine (Hcy)	136.19 > 89.95	6.073
Cysteine (Cys)	122.17 > 58.85	6.289
Cystine	241.31 > 74.00	7.786
Taurine (Tau)	124.1422 > 80.0500	6.126

MRM, multiple reaction monitoring.

titis in our previous study.¹⁴⁾ In the present study, we evaluated the degree of ACD progression by measuring earlobe swelling with a digimatic micrometer. We observed that the degree of swelling worsened with each additional challenge in the control group for all strains (Figs. 1B–F). In contrast, the continuous administration of methionine suppressed the swelling in BALB/c, DBA/2, and ddY mice (defined as methionine-sensitive strains) but not C57BL/6J and ICR mice (defined as methionine-insensitive strains) (Figs. 1B–F). Although these results indicate that methionine may be a potential therapeutic target for ACD, they also suggest that methionine could not be a panacea for ACD because of strain differences in its suppressive effects.

We then focused on the mechanism underlying the differences between each strain (Fig. 1). DNFB-induced ACD induces immune dysfunction by affecting the balance of helper T cells (Th), *i.e.*, Th1/Th2.³⁾ Therefore, the strain differences in the suppressive effects of methionine could be due to differences in the degree of the ACD-induced immune dysfunction. Furthermore, if methionine suppresses ACD by regulating immune disturbances, strain differences in its effects would also correlate with strain differences in the immune responses it induces in mice. Therefore, we evaluated methionine-induced changes in immune responses in the different groups. The serum level of IgE was increased by DNFB only in methionine-sensitive strains but not in methionine-insensitive strains (Fig. 2A). However, methionine reduced the IgE level in ddY but not in BALB/c and DBA/2 mice, although these strains were methionine-sensitive (Fig. 2A). These results suggest that IgE-mediated immune responses could be involved in the mechanism of strain differences in methionine-induced suppression of ACD. Moreover, it is possible that methionine-mediated suppression of ACD is not attributable to the correction of disturbed IgE levels. In addition, no correlation was observed between strain differences in methionine-induced ACD suppression and changes in serum levels of the other cytokines, IL-4, INF- γ , and TNF- α (Figs. 2B–D). These results suggest that the observed strain differences in the effect of methionine are not mediated by ACD/methionine-induced dysfunction/modulation of the immune system.

We previously reported that the severity of dermatitis le-

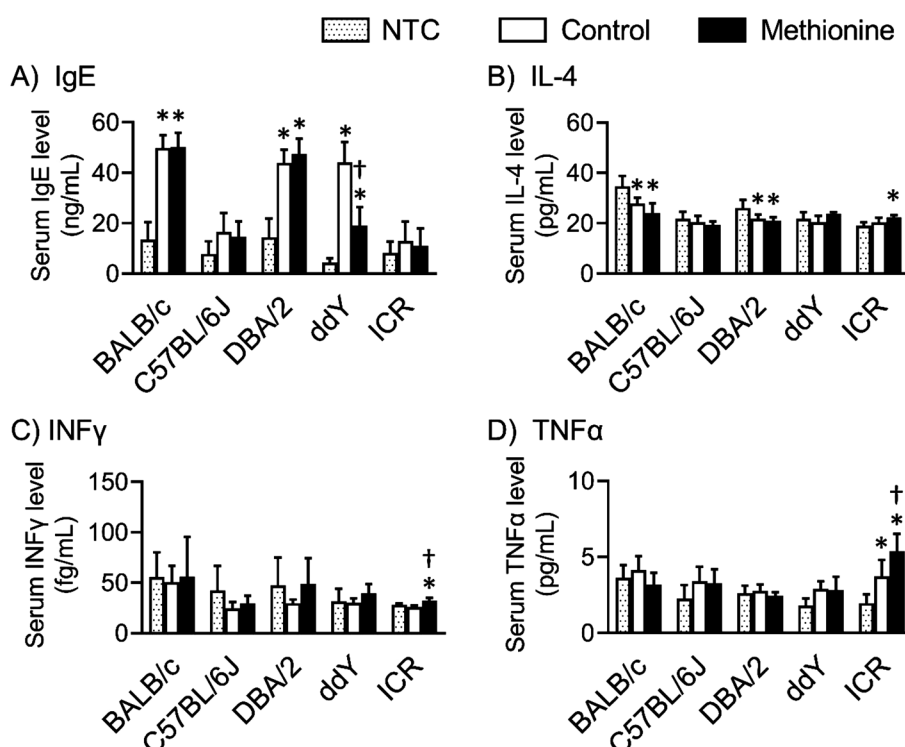


Fig. 2. ACD Induces Serum IgE Production in a Strain-Dependent Manner

(A) Serum IgE levels were increased by ACD compared with NTC group in BALB/c, DBA/2 and ddY, which were defined as methionine-sensitive strains. (B) Serum IL-4 levels were decreased by ACD in BALB/c and DBA/2 but not in other strains. (C) Serum INF γ level was increased by methionine only in ICR mice. (D) Serum TNF α level was increased by ACD and methionine only in ICR mice. Dotted bars, open bars, and closed bars indicate NTC, control, and methionine groups, respectively. Each bar represents the mean $\pm$ standard deviation ($N=5$). * $^{\dagger}p<0.05$ compared with NTC or control group, respectively. ACD, allergic contact dermatitis; IgE, immunoglobulin E; IL-4, interleukin-4; INF γ , interferon gamma; NTC, non-treated control; TNF α , tumor necrosis factor alpha.

sions is inversely correlated with serum methionine levels in NC/Nga mice, a mouse model of atopic dermatitis,¹⁴⁾ indicating that dermatitis and serum methionine levels influence each other. Therefore, strain differences are possible in dermatitis-induced reduction in serum methionine levels, and that these strain differences may contribute to the difference in ACD-suppressing effects of methionine. However, serum methionine levels were not reduced in either methionine-sensitive or methionine-insensitive strains (Fig. 3A, NTC vs. control groups). Surprisingly, the continuous administration of methionine did not increase serum methionine levels in either strain (Fig. 3A, control vs. methionine groups). Moreover, other essential amino acids did not correlate with the strain differences in the suppressive effects of methionine (Figs. 3B–I). These results indicate that serum methionine levels do not reflect the degree of methionine-induced ACD suppression.

Effects of Methionine Metabolites on the Development of ACD We hypothesized that the observed strain differences might be due to differences in methionine metabolism in the liver.^{19,20)} Methionine is usually metabolized to homocysteine via *S*-adenosylmethionine by enzymes such as methionine adenosyltransferase (Mat). Homocysteine is metabolized to methionine by 5-methyltetrahydrofolate-homocysteine methyltransferase (also known as methionine synthase; Mtr) and betaine-homocysteine methyltransferase (Bhmt). This metabolic cycle is called “methionine cycle”²¹⁾ (Fig. 4A). Additionally, homocysteine is metabolized to cysteine and eventually to taurine in an irreversible manner by cystathionine- β -synthase and cystathionine- γ -lyase²²⁾ (Fig. 4A). In the methionine cycle, decreased *Bhmt* expression was observed in methionine-in-

sensitive mice but not in methionine-insensitive mice (Fig. 4B); however, administering methionine did not alleviate *Bhmt* downregulation (Fig. 4B). In contrast, ACD induced the expression of *Mat2a*, a subtype of *Mat*, in DBA/2 and ICR, but not in the other strains (Fig. 4C). Moreover, methionine did not affect *Mat2a* expression in both methionine-sensitive and -insensitive mouse strains (Fig. 4C). Additionally, no change in *Mtr* expression was observed in either methionine-sensitive or methionine-insensitive mice (Fig. 4D). These results suggest that the decreased expression of *Bhmt* is associated with species differences in methionine-induced suppression of ACD. Therefore, we hypothesize that the strain differences in ACD suppression by methionine may be due to differences in DMG, a metabolite produced by *Bhmt* from homocysteine and betaine in the methionine cycle.²¹⁾ We measured hepatic and serum DMG levels and investigated the effect of ACD on DMG levels in both methionine-sensitive and methionine-insensitive strains. ACD reduced DMG levels in the liver of C57BL/6J and ICR mice (Fig. 5A, NTC vs. control groups), which are methionine-insensitive mice, and the decrease in hepatic DMG levels was presumably due to ACD-induced suppression of hepatic *Bhmt* expression (Fig. 4B). Moreover, methionine did not affect hepatic DMG levels in any strain. ACD did not affect serum DMG levels in both methionine-sensitive and methionine-insensitive strains (Fig. 5E, NTC vs. control groups) whereas methionine increased serum DMG levels only in ICR mice, a methionine-insensitive strain (Fig. 5E, control vs. methionine groups). These results suggest that the strain differences in methionine-induced ACD suppression are based on differences in hepatic DMG levels but not serum

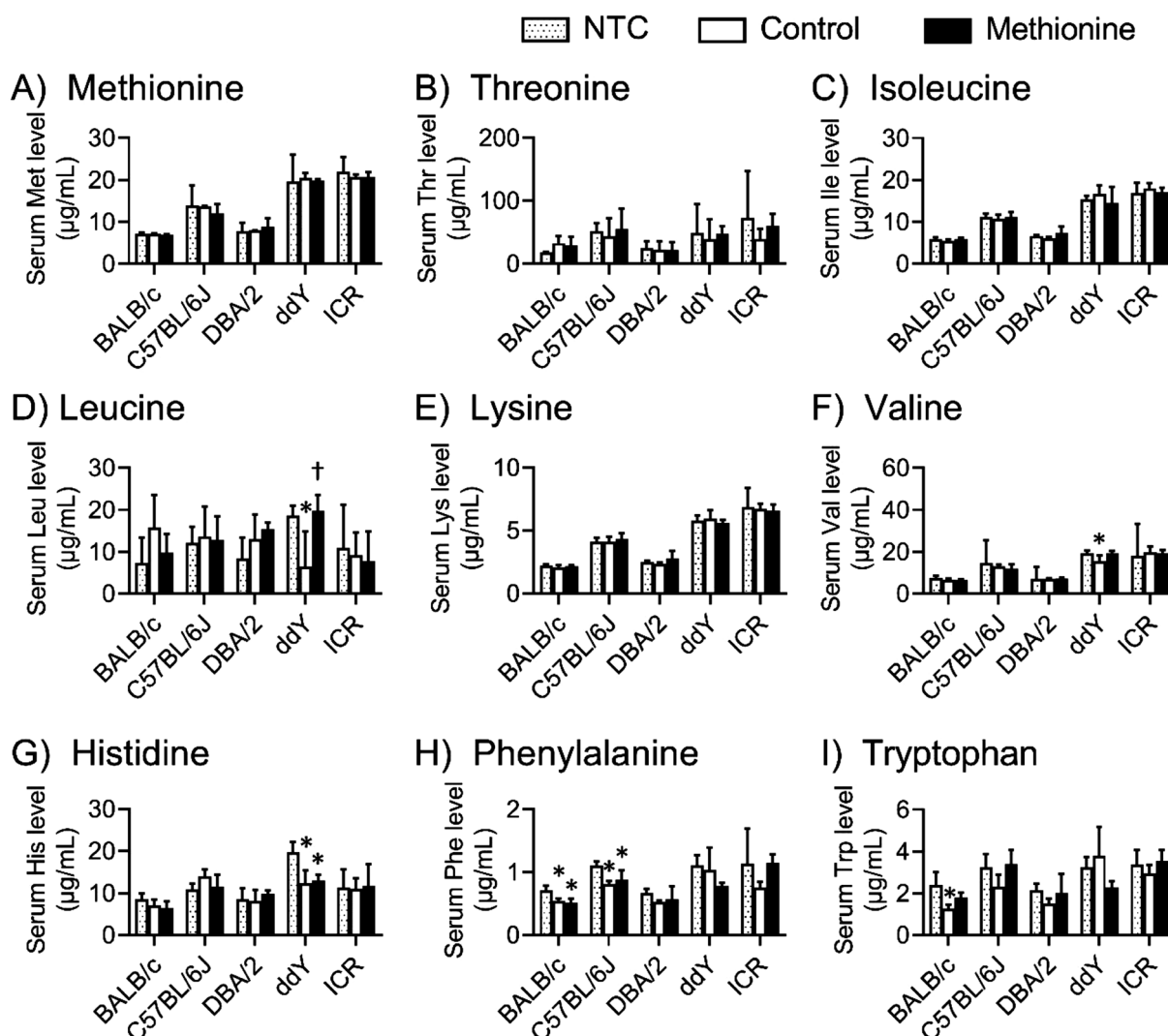


Fig. 3. Free Essential Amino Acid Levels in Serum Are Modulated by ACD or Methionine

Panels A–I represent serum levels of methionine, threonine, isoleucine, leucine, lysine, valine, histidine, phenylalanine, and tryptophan, respectively. Dotted bars, open bars, and closed bars indicate NTC, control, and methionine groups, respectively. Each bar represents the mean $\pm$ standard deviation ($N=5$). * $p < 0.05$ compared with NTC or control group, respectively. ACD, allergic contact dermatitis; NTC, non-treated control.

DMG levels. The decreased expression of *Bhmt* (Fig. 4B) and hepatic DMG level (Fig. 5A) in methionine-insensitive mice suggest that the strain differences in ACD suppression by methionine may be due to disruption of the methionine cycle. However, in both strains, no differences in hepatic or serum levels of homocysteine, an intermediate metabolite of the methionine cycle, were observed with ACD or methionine administration (Figs. 5B, F). Furthermore, no changes in hepatic or serum levels of cysteine or taurine, irreversible metabolites of homocysteine, were observed that correlated with mouse strain differences in methionine-mediated ACD suppression (cysteine, Figs. 5C, D; taurine, Figs. 5G, H). These results suggest that the mouse strain differences in ACD suppression by methionine may be due to the DMG-generating reaction involving *Bhmt*, but not entire the methionine cycle.

To determine whether DMG suppresses ACD, we administered DMG to methionine-insensitive C57BL/6J and ICR mice and methionine-sensitive ddY mice using the same procedure as the administration of methionine. DMG slightly, but significantly, suppressed DNFB-induced ear swelling in a dose-dependent manner in C57BL/6J and ICR mice (Figs. 6A,

B). Moreover, the highest dose of DMG suppressed DNFB-induced ear swelling in ddY mice (Fig. 6C). These results suggest that DMG supplementation suppresses ACD in both methionine-sensitive and -insensitive strains. In addition, DMG induced *Bhmt* expression in the ICR strain but not the C57BL/6J strain (Fig. 7). Therefore, the suppressive effect of DMG on ACD may not be caused by the regulation of *Bhmt* expression. These results suggest that the effects of methionine on ACD are mediated by DMG.

DISCUSSION

The present study showed that methionine suppresses ACD caused by DNFB using ear swelling as a measure of assessment, but mouse strain-based differences in this suppressive effect were observed. These results indicated that methionine cannot be used as a treatment for all ACD. Unfortunately, this study could not clarify the mechanism underlying the strain differences in the suppressive effect of methionine. In the present study, ACD suppression by methionine was observed in BALB/c, DBA/2, and ddY mice, whereas similar effects

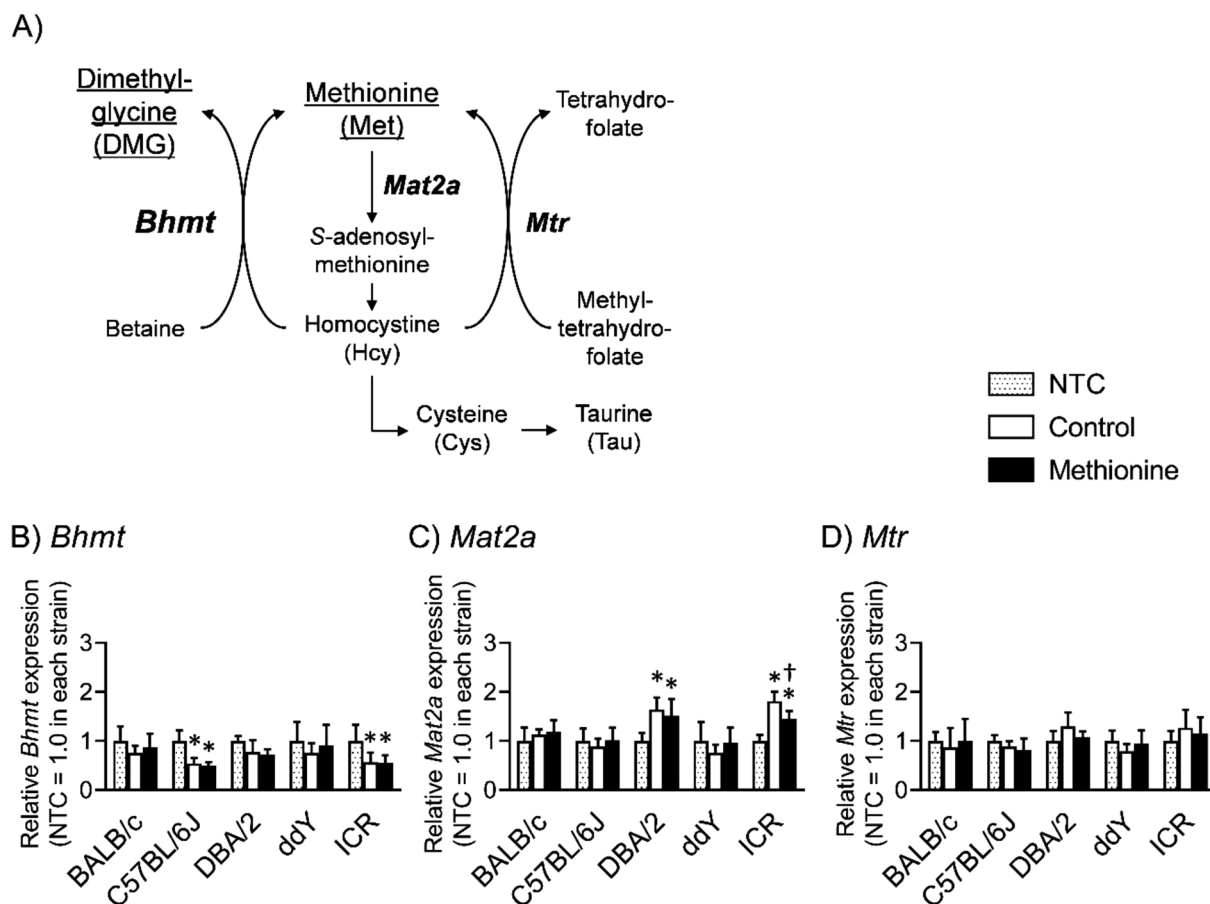


Fig. 4. ACD Suppresses Expression of Hepatic Methionine-Metabolizing Enzymes in a Strain-Dependent Manner

(A) Schematic for methionine metabolism, called as the methionine cycle. (B) Hepatic *Bhmt* expression was decreased by ACD compared with NTC group in C57BL/6J and ICR, which were defined as methionine-insensitive strains. Methionine did not affect hepatic *Bhmt* expression in any strain. (C) Hepatic *Mat2a* expression was increased by ACD only in DBA/2 and ICR mice and was decreased by co-administration of methionine only in ICR mice. (D) Hepatic *Mtr* expression was not affected by ACD or methionine in any strain. Dotted bars, open bars, and closed bars indicate NTC, control, and methionine groups, respectively. Each bar represents the mean $\pm$ standard deviation ($N=5$). * $p < 0.05$ compared with NTC or control group, respectively. ACD, allergic contact dermatitis; *Bhmt*, betaine-homocysteine methyltransferase; *Mat2a*, methionine adenosyltransferase 2A; *Mtr*, 5-methyltetrahydrofolate-homocysteine methyltransferase; NTC, non-treated control.

were not observed in ICR and C57BL/6J mice. Thus, in this study, the former three strains were classified as methionine-sensitive, and the latter two strains were methionine-insensitive. These results indicate that the characteristics of strains, such as mouse hair color, are not associated with strain differences in the suppressive effect of methionine because BALB/c and ddY are methionine-sensitive and ICR is methionine-insensitive, even though the hair color of all three strains was white. Since methionine-insensitive C57BL/6J mice are inbred while ICR mice are outbred, genetic controls, such as outbreeding and inbreeding, do not seem to be related to strain differences in the ACD reduction effect of methionine. Strain differences in the immune response of mice are well-known. For example, immune responses, such as IgE levels, have been previously reported to differ between C57BL/6J (classified as methionine-insensitive mice in this study) and BALB/c (classified as methionine-sensitive mice in this study).^{23,24} Although several reports have described the mechanisms of these mouse strain differences in immune responses,^{24,25} the details of the core mechanisms remain to be elucidated. This study also failed to elucidate the detailed mechanisms of mouse strain differences in immune responses and the suppressive effects of methionine on ACD.

ACD increased serum IgE levels only in methionine-sensi-

tive strains, BALB/c, DBA/2, and ddY, but not in -insensitive strains, C57BL/6J and ICR (Fig. 2A, compared between NTC and control). Therefore, this increase in serum IgE levels is expected to contribute to differences in the inhibitory effects of methionine among mouse strains. Supporting this possibility, selenomethionine, a substitution of methionine with sulfur by selenium, reportedly suppresses chemical-induced skin inflammation by suppressing the IgE-mediated immune system.²⁶ However, even in the mouse strains in which methionine suppressed ACD in this study, there were differences in the effects of methionine on IgE levels, with lower IgE levels observed only in ddY mice treated with methionine, but not in BALB/c or DBA/2 (Fig. 2A, compared between control and methionine). These results indicate that the ACD-induced increase in serum IgE levels contributes to mouse strain differences in ACD suppression by methionine, on the other hand, suppression of serum IgE levels by methionine could not be required for the suppression of ACD by methionine; however, the mechanism remains unclear, and differences in antigen-specific IgE, such as DNP-IgE, production based on mouse strains require further investigation. Furthermore, methionine reportedly does not suppress the degranulation of RBL-2H3 cells, which are basophilic leukemia cells.²⁷ These results suggest that methionine suppresses ACD *via* mechanisms

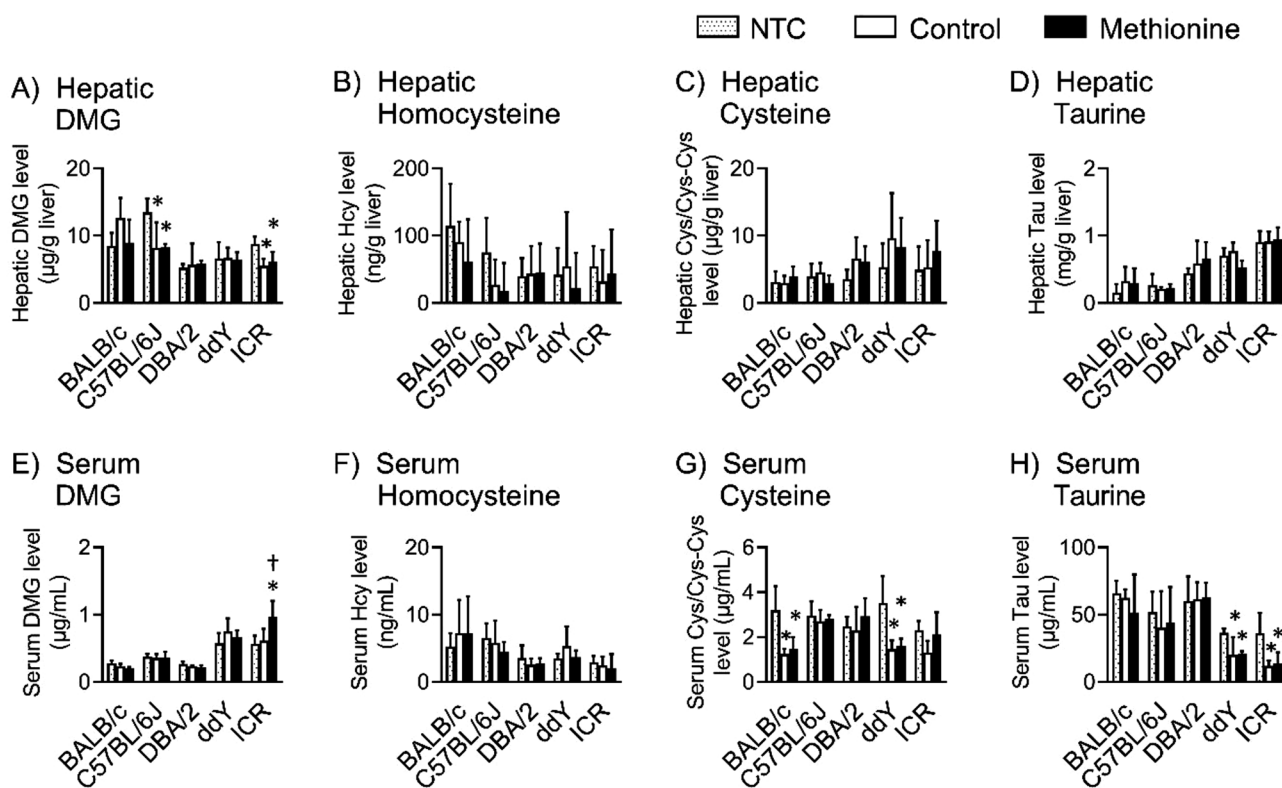


Fig. 5. ACD Decreases Hepatic DMG Level in Methionine-Insensitive Strains

(A–D) Hepatic DMG level was reduced by ACD compared with NTC group, only in C57BL/6J and ICR, both of which were defined as methionine-insensitive strains. (B–D) Hepatic levels of methionine metabolites homocysteine (B), cysteine (C), and taurine (D), were not affected by ACD and methionine. (E) ACD and methionine had no effect on serum DMG levels in all strains except ICR. (F) ACD and methionine had no effect on serum homocysteine levels in all strains. (G) Serum cysteine levels were reduced by ACD compared with NTC group, only in methionine-sensitive BALB/c and ddY strains, but not in the other methionine-sensitive strain, DBA/2. (H) Serum taurine levels were reduced by ACD compared with NTC group, only in ddY and ICR strains. Dotted bars, open bars, and closed bars indicate NTC, control, and methionine groups, respectively. Each bar represents the mean $\pm$ standard deviation ($N = 5$). * $p < 0.05$ compared with NTC or control group, respectively. ACD, allergic contact dermatitis; DMG, dimethylglycine; NTC, non-treated control.

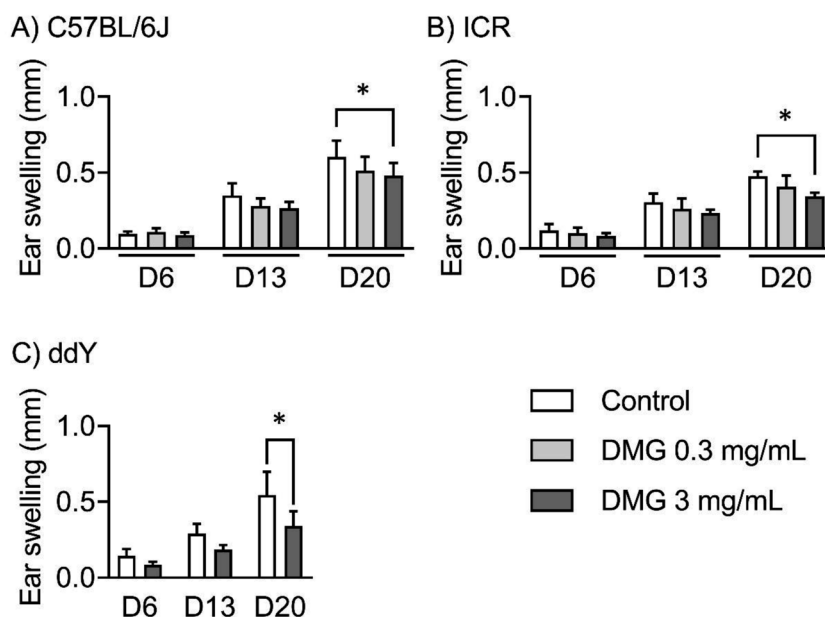


Fig. 6. DMG Suppresses Ear Swelling in a Dose-Dependent Manner in Methionine-Insensitive Strains

DMG suppressed ear swelling caused by DNFB at the highest dose (3 mg/L) in C57BL/6J (A) ICR (B), and ddY (C) mice. Each bar represents the mean $\pm$ standard deviation ($N = 5$). * $p < 0.05$ compared with indicated pairs. DMG, dimethylglycine.

other than the IgE-mediated immune response.

Strain differences, which correlated with differences in ACD suppression by methionine, were also observed in the

suppression of hepatic *Bhmt* expression by ACD. As shown in Fig. 4B, ACD decreased hepatic *Bhmt* expression only in methionine-insensitive strains but not in methionine-sensitive

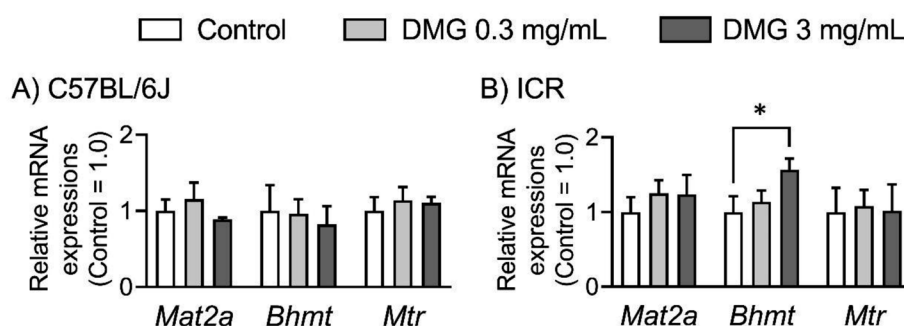


Fig. 7. DMG Does Not Modulate Methionine-Metabolizing Enzymes in Methionine-Insensitive Strains

(A) DMG did not modulate enzyme expression related with methionine-cycle at any dose in C57BL/6J mice. (B) DMG increased hepatic expression of *Bhmt*, but not *Mat2a* and *Mtr* at the highest dose (3 mg/L) in ICR mice. Each bar represents the mean $\pm$ standard deviation ($N=5$). * $p<0.05$ compared with indicated pairs. *Bhmt*, betaine-homocysteine methyltransferase; DMG, dimethylglycine, *Mat2a*, methionine adenosyltransferase 2A; *Mtr*, 5-methyltetrahydrofolate-homocysteine methyltransferase.

strains. In addition, methionine did not influence the expression of *Bhmt*. As described above, methionine is partially metabolized in the methionine cycle and irreversibly metabolized into cysteine and taurine. As shown in Fig. 1, methionine suppressed ACD in ddY mice, whereas neither cysteine nor taurine suppressed ACD (data not shown). These results indicate that ACD suppression by methionine is due to methionine itself, or an intermediate metabolite of the methionine cycle. In this regard, the correlation between strain differences in ACD suppression by methionine and strain differences in decreased expression of *Bhmt* suggests that metabolites of *Bhmt* may influence strain differences in ACD suppression. Indeed, the amount of DMG in the liver was reduced only in ACD-insensitive mice (Fig. 5A). This result is consistent with the mouse strain difference in the ACD-induced decrease in *Bhmt* expression, suggesting that the decrease in DMG levels in the liver is due to suppression of *Bhmt* expression. In contrast, changes in serum DMG levels in the presence of ACD or methionine were not observed, even in methionine-sensitive mice. These results suggest that hepatic DMG levels are involved in the ACD-suppressing effect of methionine; however, this reduction in hepatic DMG levels does not affect serum DMG levels, and DMG does not act directly on the skin.

The results of this study indicate that the amount of DMG, a metabolite of methionine, especially its content in the liver, contributes to the mouse strain difference in the ACD-suppressive effects of methionine. However, this result raises two questions. First, the mechanism by which ACD suppresses hepatic *Bhmt* expression in methionine-insensitive strains is unknown; ACD is a skin disease induced by chemical application to the skin. Therefore, a mechanism is expected to be present by which the liver receives information on the inflammatory response to ACD and that hepatic *Bhmt* expression is regulated by this transmission of information. Strain differences in this mechanism may be the basis for strain differences in the ACD-suppressing effects of methionine in mice. Unfortunately, the present study could not identify this mechanism. Nevertheless, the occurrence of ACD at the site of DNFB application alone suggests that localized skin disease also regulates liver function. In recent years, the interaction between the skin and internal organs, such as the liver and kidneys, has been in focus. For example, it is well known that diseases of internal organs promote skin abnormalities, such as decreased function due to liver or kidney disease increases skin itchiness/dryness.^{28–30} On the other hand, it has also been

reported that skin diseases affect liver function; for example, atopic dermatitis promotes fat accumulation in the liver, and psoriasis patients are at an increased risk of liver disease.^{31–33} However, these skin diseases are systemic in nature, and the effects of local skin diseases, such as ACD, on liver function are unknown and require further investigation. Second, DMG suppressed ACD. As mentioned above, DMG administration suppressed ACD in C57BL/6J and ICR mice (Fig. 6). Moreover, ACD reduced the amount of DMG in the liver but did not change the amount of DMG in the serum (Fig. 5). Furthermore, methionine did not increase serum DMG levels in either methionine-sensitive or -insensitive mice (Fig. 5). These results suggest that DMG suppresses ACD, but this effect is not due to the direct action of DMG on the skin. Further investigation of the indirect mechanism of ACD reduction by DMG is needed to answer these questions.

In recent years, organ–organ interactions have received increasing attention in disease control and progression mechanisms. Although the detailed mechanism could not be elucidated in this study, the fact that dermatitis suppressed *Bhmt* expression in the liver in some mouse strains and, conversely, the metabolic state of the liver affected the suppression of dermatitis strongly suggests, at least, the existence of a skin–liver interaction, especially dermatitis–liver interaction.

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