Topical Mevalonic Acid Stimulates *De Novo* Cholesterol Synthesis and Epidermal Permeability Barrier Homeostasis in Aged Mice[1]

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Extracellular lipids of the stratum corneum, which are composed of cholesterol, fatty acid, and ceramides, are essential for the epidermal permeability barrier function. With damage to the barrier, a decreased capacity for epidermal lipid biosynthesis in aged epidermis results in an impaired repair response. Mevalonic acid is an intermediate after the rate-limiting step in cholesterol biosynthesis, which is catalyzed by 3-hydroxy-3-methylglutaryl coenzyme A reductase. In the present study, we investigated the effect of topical mevalonic acid on the murine epidermal permeability barrier function, comparing it with that of cholesterol. Topical treatment with acetone caused linear increases in transepidermal water loss, in proportion to the number of treatments more rapidly in aged mice than in young mice. Administration of mevalonic acid on aged murine epidermis enhanced its resistance against damage and the recovery rate of barrier function from acute barrier disruption. In contrast, although cholesterol also had the same effect, it required a much higher amount than mevalonic acid. In young mice, neither mevalonic acid nor cholesterol had any effect on resistance against acetone damage nor the recovery rate from acetone damage. In the skin of mice topically administered with mevalonic acid, stimulation of cholesterol synthesis and 3-hydroxy-3-methylglutaryl coenzyme A reductase activity were both observed, whereas none was seen with stimulation by equimolar cholesterol. These data indicate that a topical application of mevalonic acid enhances barrier recovery in aged mice, which is accompanied by not only acceleration of cholesterol synthesis from mevalonic acid but also stimulation of the whole cholesterol biosynthesis. ***Key words: acetone treatment/aging/cholesterol synthesis/damaged skin/epidermal permeability barrier/HMG-CoA reductase/homeostasis/mevalonic acid/ transepidermal water loss. J Invest Dermatol 114:247–252, 2000***

One of the major functions of epidermis is as a permeability barrier against excess body water loss and cutaneous permeability. The stratum corneum is the permeability barrier site, and is comprised of stratum corneum cells and intercellular lipids with a lamellar structure (Elias and Friend, 1975; Elias, 1983). Intercellular lipids are mainly composed of cholesterol, ceramides, and free fatty acids which are biosynthesized in the epidermis (review, Feingold, 1991; Jackson *et al*, 1993). Disruptions of the barrier by treatment with organic solvents, detergent, or tape stripping results in an increase of transepidermal water loss (TEWL). This barrier abrogation is followed by increased lipid synthesis in the epidermis which is necessary for the synthesis of new lamellar bodies and the secretion of lamellar body lipids, thus leading to the recovery of normal barrier functions (review, Feingold, 1991a).

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Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; TEWL, transepidermal water loss.

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Focally decrease numbers of the stratum corneum lamellar bilayers, with an accompanying decrease in secretion of lamellar body content and total intercellular lipids quantity of intercellular lipids have been reported as changes that occur in the epidermal permeability barrier of intrinsically aged skin, whereas the thickness of the stratum corneum remains normal and distribution of ceramides, cholesterol, and free fatty acids unchanged (Ghadially *et al*, 1995). After either acetone treatment or tape stripping, the barrier recovers more slowly in aged skin than in young skin (Ghadially *et al*, 1995, 1996). In addition, with damage to the barrier, a decreased capacity of aged epidermis for lipid synthesis, in particular cholesterol synthesis, results in an impaired repair response (Ghadially *et al*, 1996). Several attempts have been performed to improve such impaired repair response. Ghadially *et al* (1996) reported that a topical application of cholesterol after barrier abrogation accelerated barrier recovery in aged epidermis. Furthermore, a topical application of cholesterol-dominant stratum corneum lipid mixture (cholesterol/ceramide/palmitate/linoleate; 3:1:1:1) after barrier disruption by tape stripping was found to accelerate barrier recovery in chronologically aged murine and human skin (Zettersten *et al*, 1997). It is considered that topical physiologic lipid mixtures influence barrier recovery after transport to subjacent, nucleated layers, followed by internalization, then an apparent transport to the distal Golgi apparatus, incorporation into

nascent lamellar bodies, and secretion of the lamellar body into the stratum corneum (Mao-Qiang *et al*, 1995; Madison *et al*, 1996). Thus, *de novo* biosynthesis of lipids is an important mechanism for the acceleration of barrier recovery by topical administration with a physiologic lipid.

Cholesterol synthesis is required for the permeability barrier. In the biosynthetic pathway of cholesterol, the conversion of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) to mevalonic acid is an early and rate-limiting step in the formation of endogenous cholesterol (Lea and McTavish, 1997). Lovastatin, an inhibitor of HMG-CoA reductase, interfered with barrier recovery after acute perturbation (Feingold *et al*, 1990, 1991b). Barrier recovery, however, was restored to normal when lovastatin-treated young mice were simultaneously treated with mevalonate (Feingold *et al*, 1990). Mevalonate is also an intermediate compound of other synthetic pathways. It was previously shown that parts of applied mevalonate are incorporated in the cholesterol synthetic pathway, and applied cholesterol and mevalonate are similarly incorporated into keratinocytes, then recovery of the barrier occurs (Feingold *et al*, 1990). Therefore, it was expected that an administration of mevalonic acid would be able to accelerate *de novo* cholesterol synthesis more than cholesterol treatment in aged epidermis, resulting in an improvement of the epidermal permeability barrier homeostasis.

The purpose of this study was to investigate the effect of topical mevalonic acid on the murine epidermal permeability barrier function, comparing it with topical cholesterol. We found that: (i) the effect of topical cholesterol in aged mice was confirmed, and (ii) a topical application of mevalonic acid enhances barrier recovery in aged mice, which is accompanied by not only an acceleration of cholesterol biosynthesis from mevalonic acid, but also stimulation of whole cholesterol biosynthesis via increasing the HMG-CoA reductase activity. We conclude that acceleration of *de novo* cholesterol synthesis might be significant for the homeostatic function of the murine epidermal permeability barrier, especially with aged mice.

MATERIALS AND METHODS

Animals and materials Hairless mice (Skh:hr-1), 5 or 6 wk old, were purchased from Japan SLC (Shizuoka, Japan). The animals were kept under controlled conditions (ambient temperature, 22 ± 1°C; relative humidity, 55 ± 5%; light condition, 12 h light/12 h dark cycle). The mice were fed commercial pellets (CE-2, Clea Japan, Tokyo, Japan) and well water *ad libitum*. Young mice were 10 wk of age, and aged mice were 90 wk old at the time of study (life span approximately 105 wk). As with a previously described hairless mouse strain (Ghadially *et al*, 1995), these mice develop chronologic aging without significant tumor development, and tumor-bearing mice were excluded. Cholesterol, sodium acetate, acetone, glucose-6-phosphate, NADP, glucose-6-phosphate dehydrogenase, 2-mercaptoethanol, sucrose, and dithiothreitol were purchased from Wako Pure Chemical Industries (Osaka, Japan). [1-^{14}C]acetic acid was from ICN Biomedicals (Costa Mesa, CA). [3-^{14}C]HMG-CoA (40–60 mCi per mmol) and RS-[5-^{3}H]mevalonolacton (5.7 Ci/mmol) were purchased from New England Nuclear. Ethylenediamine tetraacetic acid (EDTA) was from Kanto Chemical (Tokyo, Japan) and R(–)mevalolactone (R(–)-3-hydroxy-3-methyl-5-pentanolide:mevalonic acid) from Asahi Denka Kogyo (Tokyo, Japan).

Samples for application The application samples, cholesterol or mevalolactone, were solubilized in a vehicle ([0.5% nonion surfactants (W/V, N-NP-15, Nikko Chemicals, Tokyo, Japan]) solution at a final concentration of 7.68 mM. The control was vehicle alone.

Acute barrier perturbation Acetone was gently applied to the dorsal surface of hairless mice with acetone soaked cotton balls. Transepidermal water loss (TEWL) was measured by using a HIDROGRAPH (AMU-100, K and S, Aichi, Japan), as described previously (Haratake *et al*, 1997), before treatment, and immediately after each five treatments until 100 times in young mice or 70 times in aged mice. Successive cellophane tape strippings were utilized (Sumitomo 3M, Tokyo, Japan) six to 13 times for disruption of the barrier in aged mice. Data are expressed as milligrams per centimeter squared per min (mean ± SEM).

Barrier integrity and barrier recovery The samples (vehicle, cholesterol, or mevalonic acid, 50 μl of 7.68 mM sample) were applied on the dorsal surface (2.5 cm^2 area) of mice for 5 d (once a day) before the acetone treatments or tape stripping. For assessment of barrier integrity, TEWL was measured immediately after each five acetone treatments. Barrier integrity (strength against artificial damage) was reflected by the number of acetone treatments or tape strippings required to attain TEWL levels of $\geq$ 0.15 mg per cm^2 per min. For assessment of barrier recovery from artificial damage, mice received acetone treatment or tape stripping to attain TEWL levels of 0.15 mg per cm^2 per min after the sample applications for 5 d, then had the sample applied once again. TEWL was measured before, 2, 4, 24, and 48 h after barrier disruption by artificial damage. Data are expressed as the percentage of barrier recovery (0 h = 0% recovery, normal = 100%, mean ± SEM).

Cholesterol biosynthesis in organ cultured epidermis Full-thickness skin samples were obtained from aged mice at 3 h after topical application of the samples (vehicle, cholesterol, or mevalonic acid, 50 μl of sample applied to a 2.5 cm^2 area). Subcutaneous fat was scraped off full-thickness murine skin. Cholesterol biosynthesis in the epidermis was assessed by measuring the incorporation of [1-^{14}C]acetic acid into tissue in a skin organ culture. Briefly, the organ culture (2 cm^2 skin pieces) were incubated in phosphate-buffered saline (pH 7.4) including 10 mmol EDTA, acetic acid sodium salt, and [1-^{14}C]acetic acid (0.74 MBq) for 2 h at 37°C, and the epidermis was separated by gently scraping with a scalpel blade. Incorporation activity of [^{14}C] into cholesterol was measured according to the method of Feingold *et al* (1986). Cholesterol syntheses are expressed as dpm per hour per gram of epidermis (mean ± SEM).

HMG-CoA reductase activity assay The samples (vehicle, cholesterol, or mevalonic acid, 50 μl) were applied on the dorsal surface (2.5 cm^2 area) of mice once a day for 5 d. For measurement of HMG-CoA reductase activity, skin samples were incubated in phosphate-buffered saline (pH 7.4) including 10 mmol EDTA for 45 min at 37°C, after the epidermis was separated and a microsomal was prepared by the method of Proksch *et al* (1990). HMG-CoA reductase activity was determined by the method of Feingold *et al* (1983). Protein was determined with a Bio-Rad Laboratories protein assay dye reagent (Gotham *et al*, 1988). HMG-CoA reductase activities are expressed as pmol per minute per milligram microsomal protein (mean ± SEM).

Cholesterol and free fatty acid content assay The samples (vehicle, cholesterol, or mevalonic acid, 50 μl) were applied on the dorsal surface (2.5 cm^2 area) of aged mice once a day for 5 d. Full-thickness skin samples were obtained from aged and young untreated mice, and the subcutaneous fat was scraped off. Skin samples were then heat split at 60°C for 30 s to remove the intact epidermis from the dermis. Thereafter, the epidermal lipids were extracted by the method of Bligh and Dyer (1959), dried and stored at –80°C until analyzed. Lipids were fractionated and quantified by high-performance thin layer chromatography (HPTLC) followed by charring and scanning densitometry. Lipids were solubilized in chloroform/methanol (2:1 vol) and spotted on HPTLC plates (Merck, Darmstadt, Germany). The plates were developed once in benzene/hexane (1:1 vol) for 80 mm, and then developed once in hexane/diethyl ether/acetic acid (70:30:1 vol) for 40 mm. After development, the plates were dipped in a charring solution (distilled water containing 10% w/v copper sulphate, and 8% w/v phosphoric acid), drained briefly, and then dried for 15 min at room temperature. Next the plates were transferred to a 180°C oven for 15 min. After cooling to room temperature, the plates were scanned immediately and quantitated using a densitometer (CS9300, Shimadzu, Kyoto, Japan). The content of total cholesterol and free fatty acid in murine epidermis are expressed as micrograms per square centimeter (mean ± SEM).

Statistical analysis Statistical analysis was performed using a Kruskal–Wallis test and then a Dunnett multiple comparison test of the nonparametric type or Dunnett multiple comparison test of parametric type, where appropriate.

RESULTS

Integrity against acute barrier disruption by acetone treatment The barrier was more readily disrupted with acetone in aged than in young mice, and the TEWL rates as well increased more steeply with successive acetone applications (**Fig 1**). Although the barrier function appeared to be unchanged under basal conditions, disruption of the barrier in aged epidermis was developed with a

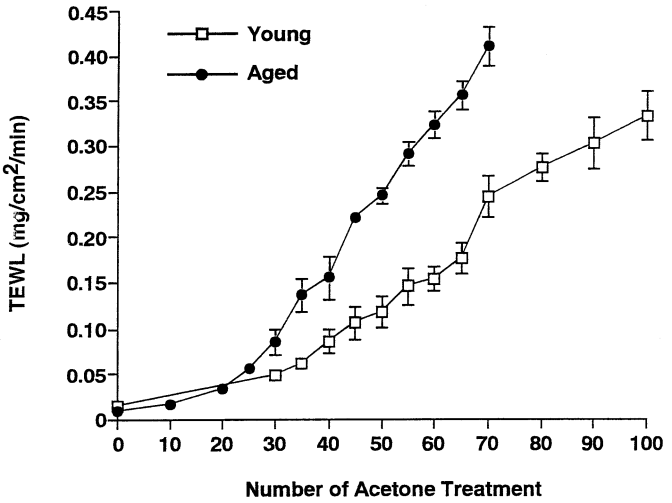

Figure 1. Number-dependent barrier disruption by acetone treatment on murine epidermis. Young (10 wk old, n=5) and aged mice (90 wk old, n=8) were treated with an acetone-soaked cotton ball. TEWL was measured before treatment and immediately after each of five treatments. Data shown are mean ± SEM.

lag-phase of over 20 times, this more rapid than in young epidermis which had a lag-phase of over 30 times. Barrier integrity against acetone damage can also be expressed as the number of acetone treatments required to attain TEWL levels of ≥ 0.15 mg per cm^2 per min. As shown in **Table I**, the barrier integrity with acetone treatment in aged mice was significantly weaker than in young mice ($p < 0.05$).

Barrier recovery rate from acute disruption The barrier recovery rate from acute disruption by acetone treatment was compared between the young and aged epidermis. In young epidermis, TEWL recovered to 50% of the control (non-treated) at 2 h, then 90% at 48 h after disruption, whereas TEWL in aged epidermis recovered to only 15% at 2 h, 30% at 4 h, 50% at 6 h, and 70% at 48 h, a significantly slower recovery rate than in young epidermis (**Fig 2**).

Effect of topical mevalonic acid treatment on barrier integrity and barrier recovery rate in aged epidermis A topical application of mevalonic acid to aged mice significantly improved the integrity of the permeability barrier against acetone damage to a level similar to normal in young mice (63 ± 3 times, $p < 0.05$, **Table I**). Barrier integrity against tape stripping in aged mice was also significantly increased by topical applications of mevalonic acid (**Table I**). In contrast, a topical application of cholesterol to aged epidermis also had the same effect, but required a 10-fold higher amount than mevalonic acid (**Table II**).

Figure 3 shows the effect of mevalonic acid on barrier recovery from acetone damage. The recovery rates of the permeability barrier following acetone treatment in young and aged mice are shown in **Fig 3**. The barrier recovery by topical applications of the vehicle was quite similar to the results of nontreated mice. Therefore, only the vehicle results are shown as a control. The recovery rate of the permeability barrier in aged mice was significantly accelerated by a topical application of mevalonic acid before and after the acetone treatment ($p < 0.01$). An application of cholesterol had the same tendency as mevalonic acid, but was not significant when compared with the control, except at 48 h (**Fig 3*a***). The recovery rate after tape stripping in aged mice was the same as the acetone treatment (data not shown). In contrast, the barrier integrity and recovery rate in young mice was not affected by either a topical application of cholesterol or mevalonic acid (**Fig 3*b***).

Cholesterol biosynthesis in aged mice pretreated with topical mevalonic acid As it was observed that topical

Table I. Barrier integrity against with acetone treatment or tape stripping[a]

	Acetone treatment		Tape stripping
	Young[b]	Aged[c]	Aged[c]
Group	Times (n)	Times (n)	Times (n)
Vehicle	57 ± 4 (5)	35 ± 3[d] (5)	8.6 ± 0.8 (7)
Cholesterol	58 ± 3 (5)	50 ± 4 (5)	9.4 ± 0.9 (7)
Mevalonic acid	63 ± 3 (5)	63 ± 3[e] (5)	11.6 ± 0.6[e] (7)

[a]Mice received acetone treatment or tape stripping after sample application for 5 d (50 µl of 7.68 mM sample applied to 2.5 cm^2 area per d). Barrier integrity is reflected by the number of either acetone treatments or tape strippings required to attain TEWL levels of ≥ 0.15 mg per cm^2 per min.
[b]Young mice were 10 wk old.
[c]Aged mice were ≥ 90 wk old.
[d]$p < 0.05$ *versus* vehicle in young mice.
[e]$p < 0.05$ *versus* vehicle in aged mice. Data shown are mean ± SEM (n=5 or 7).

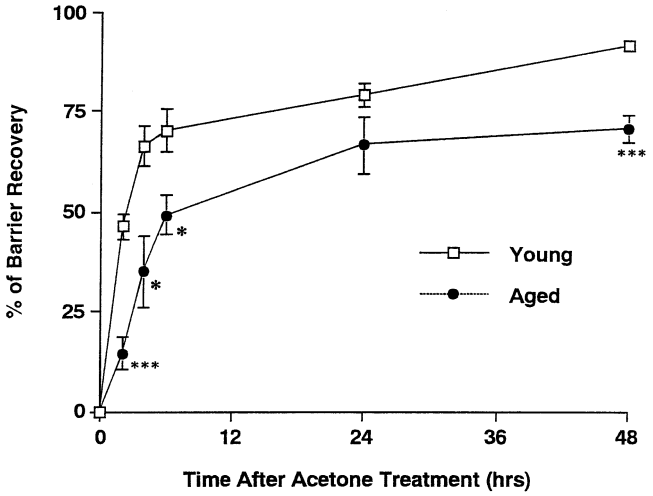

Figure 2. Murine barrier recovery from acetone damage. Murine barrier recovery after acetone treatment was delayed in aged as compared with young epidermis. Mice received an acetone treatment to attain TEWL levels of 0.15 mg per cm^2 per min. TEWL was measured at 0, 2, 4, 6, 24, and 48 h after acetone treatment. Values are expressed as % barrier recovery. Data shown are mean ± SEM (n=5). *$p < 0.05$, ***$p < 0.001$.

mevalonic acid improved the integrity and recovery function on the permeability barrier in aged mice, we examined the effect of mevalonic acid on cholesterol biosynthesis and HMG-CoA reductase activity. Without application of the samples, the rates of cholesterol synthesis were significantly lower in aged than in young mice ($p < 0.05$, **Fig 4**). Cholesterol synthesis at 3 h after topical application of mevalonic acid in aged mice was about 1.6-fold greater than in nontreated aged mice ($p < 0.1$), whereas a topical application of cholesterol revealed no effect.

HMG-CoA reductase activity in aged mice pretreated with topical mevalonic acid The effect of mevalonic acid application on HMG-CoA reductase activity was also assessed in aged mice. A topical application of mevalonic acid for 5 d resulted in a significant increase of HMG-CoA reductase activity ($p < 0.01$, **Table III**). On the other hand, the activity of HMG-CoA reductase was unchanged by the topical cholesterol treatment.

Total cholesterol and free fatty acid content in aged epidermis pretreated with topical mevalonic acid Finally, we examined the effect of mevalonic acid on total cholesterol and free fatty acid content in aged epidermis. The contents of total

Table II. Effective dose of cholesterol on barrier integrity against with acetone treatment in aged murine epidermis[a]

Group		Times (n)
Vehicle		37 ± 3 (6)
Cholesterol	0.768 mm	43 ± 5 (5)
	7.68 mm	55 ± 3 (5)
	76.8 mm	59 ± 3 (7)[b]
Mevalonic acid	(7.68 mm)	60 ± 2 (5)[b]

[a]Aged mice were ⩾ 90 wk old. Mice received acetone treatment after sample application for 5 d (50 μl of sample applied to 2.5 cm^2 area per d). Barrier integrity is reflected by the number of acetone treatments required to attain TEWL levels of ⩾ 0.15 mg per cm^2 per min.

[b]$p < 0.05$ *versus* vehicle. Data shown are mean ± SEM (n=5–7).

cholesterol and free fatty acid were decreased in aged epidermis in a significantly greater way than in young epidermis ($p < 0.01$, $p < 0.001$, respectively, **Fig 5**). Moreover, a topical application of mevalonic acid for 5 d resulted in a significant increase of total cholesterol and free fatty acid contents ($p < 0.05$, $p < 0.01$, respectively, **Fig 5**). In contrast, the total cholesterol and free fatty acid contents were not significantly increased by topical cholesterol treatment.

DISCUSSION

Barrier disruption induces metabolic changes in the underlying epidermis that results in the rapid return of lipids to the stratum corneum interstices leading to barrier recovery. These changes include stimulation of the epidermal cholesterol, fatty acid, and ceramides *de novo* synthesis (Menon *et al*, 1985; Grubauer *et al*, 1989; Holleran *et al*, 1991). After either acetone treatment or tape stripping, the permeability barrier recovered more slowly in aged than in young skin (Ghadially *et al*, 1995, 1996). In aged epidermis, cholesterol synthesis of one of the three key lipid classes is decreased under basal conditions, and sterologenesis fails to attain the levels reached in young epidermis following comparable acute perturbation. In contrast, fatty acid and sphingolipid synthesis in aged epidermis increases sufficiently to approach the levels attained in stimulated young epidermis (Ghadially *et al*, 1996). HMG-CoA reductase, a rate-limiting enzyme in the cholesterol biosynthetic pathway, is also decreased in aged epidermis. Thus, with damage to the barrier, the decreased capacity of aged epidermis for lipid synthesis, in particular cholesterol synthesis, results in an impaired repair response (Ghadially *et al*, 1996). Furthermore, a topical application of cholesterol-dominant stratum corneum lipid mixture after barrier disruption by tape stripping accelerates barrier recovery in chronologically aged murine and human skin (Zettersten *et al*, 1997). These aspects led us to apply mevalonic acid, an intermediate compound after the rate-limiting step in cholesterol synthesis, for aged epidermis to accelerate the barrier repair process.

In this study, we describe the novel effect of mevalonic acid on barrier homeostasis in aged epidermis. In acetone or tape stripping damaged aged mice, a pre- and postadministration of mevalonic acid increased the recovery rate of epidermal barrier functions, as compared with the vehicle or cholesterol (**Fig 3*a***). To investigate the mechanism for this improvement, whole cholesterol synthesis and HMG-CoA reductase activity were assessed in aged epidermis that had an application of mevalonic acid. It is of interest that mevalonic acid demonstrated an ability to increase not only whole cholesterol synthesis but also HMG-CoA reductase activity (**Table III** and **Fig 4**). Our results demonstrated that applied mevalonic acid is incorporated in the cholesterol biosynthetic pathway. The proportion of the volume of incorporation into cholesterol biosynthesis as compared with the total applied volumes, however, was not clarified. In contrast, cholesterol had no effect on increasing HMG-CoA reductase activity, which may

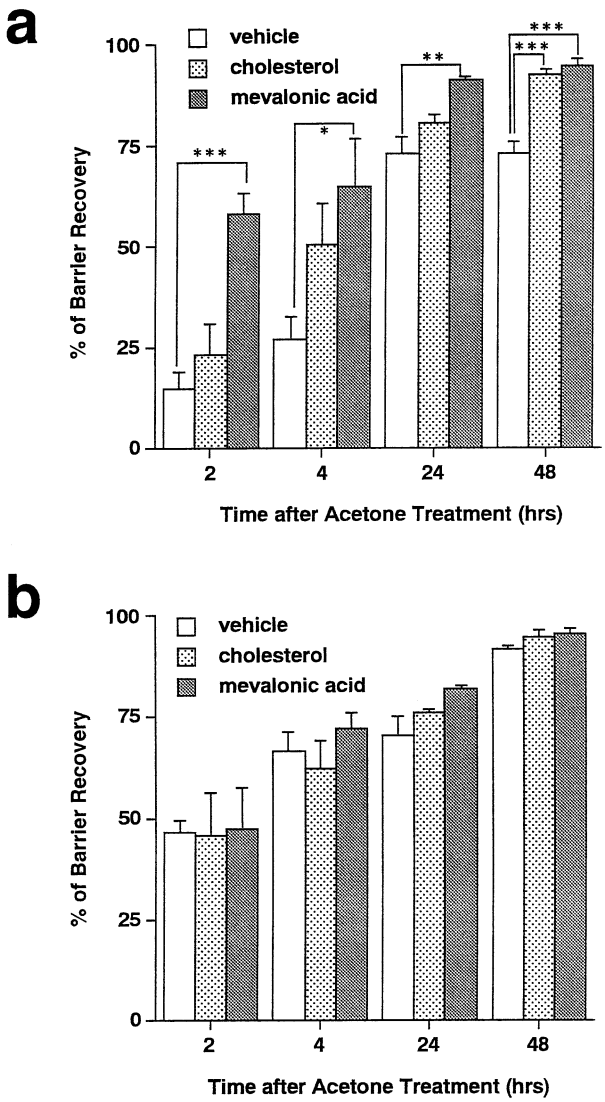

Figure 3. Effect of mevalonic acid on murine barrier recovery after acetone treatment. (a) Aged epidermis, (b) young epidermis. Aged mice were 90 wk old (n=5), young mice were 10 wk old (n=5). Mice received acetone treatment to attain TEWL levels of 0.15 mg per cm^2 per min after sample application for 5 d, then the sample was applied once again (50 μl of 7.68 mM sample or vehicle applied to 2.5 cm^2 area once). TEWL was measured at 0, 2, 4, 24, and 48 h after acetone treatment. Values are expressed as % barrier recovery. Data shown are mean ± SEM (n=5). $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

be why it had less of an effect on increasing whole cholesterol synthesis and repair response than mevalonic acid (**Table III**, **Figs 3** and **4**). We observed that a daily topical application of high-dose cholesterol (50 μl of 76.8 mM applied to a 2.5 cm^2 area) for 5 d significantly increased the barrier integrity in aged mice (**Table II**). Therefore, we conclude that a topical application of cholesterol is effective for improving the barrier function of aged epidermis, as our results agreed with a previous study (Ghadially *et al*, 1996). We believe that this effect, however, was caused by another mechanism and not by the effect of mevalonic acid. Our data strongly suggest that stimulation of *de novo* cholesterol synthesis via an increase of the HMG-CoA reductase activity might have significance for the homeostatic function of the murine epidermal permeability barrier, especially in aged mice.

The barrier requirements regulate epidermal cholesterol synthesis by modulating both the HMG-CoA reductase amount and activation state (Proksch *et al*, 1990). By an *in vitro* examination, Bradfute and Simoni (1994) reported that two analogies of farnesyl

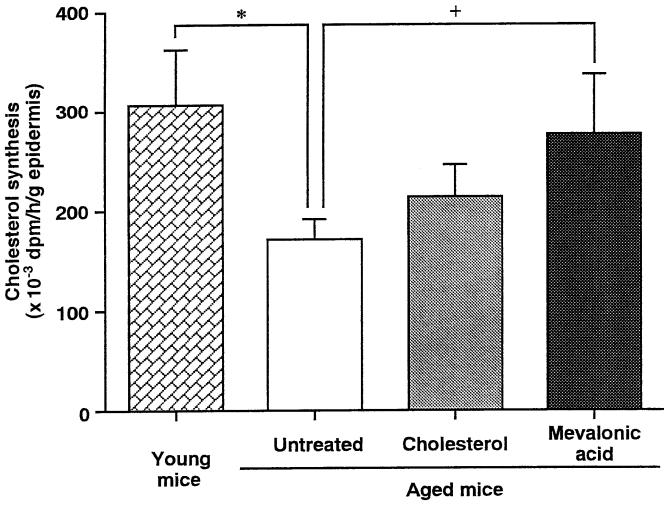

Figure 4. Topical mevalonic acid accelerated cholesterol biosynthesis in aged murine epidermis, but cholesterol was not effective. Full-thickness skin specimens were obtained from aged mice (90 wk old) at 3 h after a topical application of each sample (50 μl of 7.68 mM sample or vehicle applied to 2.5 cm^2 area). Cholesterol biosynthesis in the epidermis was assessed by measuring the incorporation of [^{14}C]acetic acid into tissue in a skin organ culture. Values are activity of [^{14}C] incorporation in cholesterol from acetic acid (dpm per h per g epidermis). Data shown are mean ± SEM (n=5). $^{+}p < 0.10$, $^{*}p < 0.05$.

pyrophosphate, farnesyl acetate and ethyl farnesyl ether, stimulated post-transcriptional downregulation of HMG-CoA reductase. In addition, it has been reported that the addition of mevalonate caused a complete downregulation of HMG-CoA reductase (review, Brown and Goldstein, 1980). Our results showed that topical mevalonic acid increases HMG-CoA reductase activity *in vivo*, probably by upregulation of the basal condition. This discrepancy has not yet been clarified. The mechanism of activation toward HMG-CoA reductase requires further investigation.

The recent discovery of sterol regulatory element binding proteins (SREBP), transcription factors that regulate cholesterol synthesis in response to sterols, has provided important insights into the regulation of lipid synthesis (Brown and Goldstein, 1997). Furthermore, SREBP-2, a kind of SREBP, is an important regulator of cholesterol and fatty acid synthesis after acute barrier disruption (Harris *et al*, 1998). We observed that a daily topical application of mevalonic acid (50 μl of 7.68 mM applied to a 2.5 cm^2 area) for 5 d significantly increased the content of total cholesterol and free fatty acid in aged epidermis, as compared with an application of the vehicle or cholesterol (**Fig 5**). Therefore, we consider that a topical application of mevalonic acid might affect the regulation mechanism of cholesterol and fatty acid synthesis in the epidermis.

On the other hand, an *in vivo* examination following acute disruption of the epidermal permeability barrier disclosed that there is a coordination of mRNA levels for cholesterol synthesis enzymes (HMG-CoA reductase, HMG-CoA synthase, farnesyl pyrophosphate synthetase, squalene synthase), fatty acid synthesis enzymes (acetyl-CoA carboxylase, fatty acid synthase), and serine palmitoyl transferase (SPT) of the ceramide synthesis enzyme (Harris *et al*, 1997). The effects on other key cholesterol and fatty acid synthesis enzyme activities in aged epidermis by topical application of mevalonic acid, as well as the changes of aging, require further investigation.

As reported previously (Ghadially *et al*, 1995), the integrity of the stratum corneum is diminished in chronologically aged epidermis. In addition, aged epidermis displays a diminution in secreted lamellar body-derived contents, and these contents fail to form a continuous series of multilamellar bilayers within the intercellular spaces of the stratum corneum (Ghadially *et al*, 1995). The present study also demonstrated that acetone treatment with cotton balls in

Table III. Topical mevalonic acid increased the activity of HMG-CoA reductase on aged murine epidermis[a]

Group	(n)	HMG-CoA reductase activity (pmol per mg protein per h)
Vehicle	(9)	0.223 ± 0.016
Cholesterol	(9)	0.218 ± 0.019
Mevalonic acid	(9)	0.347 ± 0.037[b]

[a]Aged mice were ≥90 wk old. Skin samples were obtained after sample application for 5 d (50 μl of sample applied to a 2.5 cm^2 area per d). Epidermis was separated from the dermis by EDTA, and HMG-CoA reductase activity was determined as described in *Materials and methods*.

[b]$p < 0.01$ *versus* vehicle. Data shown are mean ± SEM.

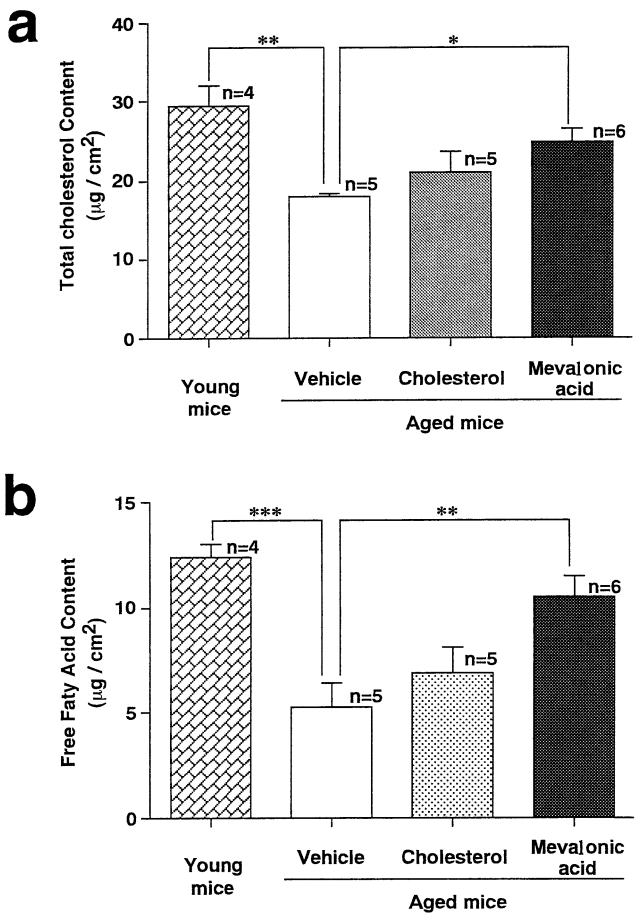

Figure 5. Topical mevalonic acid increased total cholesterol and free fatty acid content in aged murine epidermis, but cholesterol was not effective. Full-thickness skin specimens were obtained from aged mice (90 wk old) after the topical application of each sample (50 μl of 7.68 mM sample or vehicle applied to 2.5 cm^2 area) for 5 d, as well as untreated young mice. Total cholesterol (*a*) and free fatty acid (*b*) contents were normalized to tissue surface area. Data shown are mean ± SEM (n=4–6). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{**}p < 0.001$.

aged mice causes a number-dependent linear increase in TEWL, more rapidly than in young mice (**Fig 1**), resulting in a reduction of barrier integrity in aged mice (**Table I**). This reduction in the integrity of aged stratum corneum may be due to a decrease of quantitative lipid content, causing a change of quality.

In addition, we assessed the thickness of the epidermis, as mevalonate is necessary for the DNA synthesis of cells (melanoma cells, fibroblasts, glial cells, etc.; Fairbanks *et al*, 1984; Dricu *et al*,

1997; Choi and Jung, 1999). A daily topical application of mevalonic acid for 5 d, however, did not influence the thickness of the epidermis (data not shown). We do not consider that the concentrations of mevalonic acid used in this study had any influence on the DNA synthesis of keratinocytes.

Barrier recovery in young epidermis was not affected by cholesterol or mevalonic acid application, in contrast to that in aged epidermis (**Fig 3*b***). Mao-Qiang *et al* (1993) also reported that each of the three major, naturally occurring stratum corneum lipids alone, with the exception of cholesterol, interferes with barrier recovery after a topical application in young epidermis. In their study, however, a topical application of cholesterol after barrier disruption slightly delayed recovery at 2 h. Moreover, barrier recovery 2 h after acetone disruption was at a point different from our data. In order for a topical physiologic lipid to influence barrier recovery, it must be incorporated into the nascent lamellar bodies, before they are secreted into the stratum corneum (Mao-Qiang *et al*, 1995). Our barrier recovery rate data were obtained by a topical application of the samples before and after acetone treatment. Therefore, we think that the difference in results is from a difference of methods. Furthermore, we maintain that the adverse effect of cholesterol does not influence barrier recovery after barrier disruption in young skin, as compared with other lipids. After either acetone treatment or tape stripping, the barrier recovers more rapidly in young than in aged epidermis, and the capacity for lipid synthesis is also higher in young than in aged epidermis. Moreover, electron microscopy of young epidermis shows comparable numbers of lamellar bodies in the granular cell cytosol, and abundant secreted lamellar body contents at the stratum granulosum–stratum corneum interface (Ghadially *et al*, 1995). We observed that a topical application of mevalonic acid (50 μl of 7.68 mM applied to a 2.5 cm^2 area) significantly increased the epidermal cholesterol synthesis in young mice, whereas in contrast, the content of cholesterol was not significantly increased in young epidermis (data not shown). Therefore, young epidermis might have regulatory systems for the related enzymes in amounts large enough to lead to rapid barrier repair that can be induced to the full range by damage, so as not to be affected by an exogenous inducer such as mevalonic acid or cholesterol.

In summary, in this study: (i) the effect of topical cholesterol in aged mice was confirmed, and (ii) a topical application of mevalonic acid was found to enhance barrier recovery in aged mice, which was accompanied not only by acceleration of cholesterol biosynthesis from mevalonic acid, but also by stimulation of whole cholesterol biosynthesis via increasing HMG-CoA reductase activity. We conclude that acceleration of *de novo* cholesterol synthesis might be significant for the homeostatic function of the murine epidermal permeability barrier, especially with aged mice.

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