ulin might contribute to the inhibition of neurite elongation. Furthermore, epidermal sema3A mRNA expression was significantly increased by olopatadine (fig. 5c). Based on this result, it might be speculated that oral administration of olopatadine might affect keratinocyte homeostasis. This phenomenon was also found previously regarding the olopatadine-induced improvement of skin barrier function [34]. Keratinocytes have been considered as an immunocompetent cell type for the production of cytokines or other biological substances [39]. When keratinocytes were cultured with antihistamines, decreased expression of proinflammatory cytokines, MHC class II and CD54 were detected [39]. Thus, systemically administered olopatadine might affect epidermal keratinocytes and result in a decreased dermatitis score. Whether this effect only occurred in the affected area remains to be clarified.

The balance of Th1/Th2 cytokines in affected skin was unaltered by olopatadine treatment. Additionally, we failed to observe any Th1/Th2 cytokine imbalance in NC/Nga mice which developed dermatitis (table 1), despite evidence for increased Th2 cytokines [40]. This may be due to the fact that the Dfb ointment used in SPF mice [32] differed from the treatment applied under conventional conditions [40]. Thus, stimulants other than mite antigen contributed to the development of a Th1/Th2 cytokine imbalance in dermatitis induced under conventional conditions in NC/Nga mice. Furthermore, this mouse model is known to develop a greater increase in IgE, and our DDW- and Dfb-treated NC/Nga mice develop a hyper-IgE response. Interestingly, olopatadine reduced the Dfb-specific IgE titer although the expression level of Th2 cytokines was unaffected (table 1, fig. 6b). Recent studies revealed that exposure to allergens through barrier-disrupted skin might promote the production of allergen-specific IgE [41]. Olopatadine may decrease the antigen-specific IgE titer by improving the skin barrier function. However, further study will be required to confirm this possibility.

The present study did not evaluate the presence of a cytokine imbalance in both acute- and late-phase reactions, or the influence of olopatadine during the sensitization or challenge periods. However, it has been reported that olopatadine might downmodulate the antigen-presenting ability of epidermal Langerhans cells [42]. Thus, the inhibitory effects of olopatadine may have modulated the induction or the elicitation phase in the present study.

In this study, we used the commercially available 0.1% tacrolimus ointment as a positive control of our treatment model. As concerns the administration of tacrolimus, we adopted the methodology of Yamamoto et al. [20], i.e. twice per week, since this protocol successfully improved the dermatitis score and reduced the inflammation of Dfb-evoked dermatitis in NC/Nga mice. Topical treatment with tacrolimus favorably affected NGF, IL-1β, GM-CSF, amphiregulin, and E-selectin expression in the lesional skin. Unexpectedly, tacrolimus did not affect the scratching behavior or the number of intraepidermal neurites, nor did it improve the dermatitis score. We observed that NC/Nga mice scratched their backs and showed a disturbed behavior immediately after the tacrolimus treatment. Based on this observation, it seemed that tacrolimus ointment itself might act as a stimulus, which may partly explain the discrepancy in the effect of tacrolimus. Moreover, the scratch behavior was evaluated 24 h after the final application of tacrolimus; this long interval of time may have contributed to the resulting low scores. Concerning the effect of tacrolimus on neurite elongation, it was assumed that these data resulted from a different application method [31]. As we know, the mechanisms of formation of abnormal innervation in skin are complicated. The two main mechanisms of intraepidermal neurite outgrowth are increased peripheral nerve elongation factors (e.g. NGF, amphiregulin) and decreased axonal chemorepellant factors (e.g. sema3A). These axon guidance factors regulate and maintain normal skin innervation. In this study, olopatadine significantly decreased the tissue concentration of NGF and amphiregulin, and increased sema3A. However, tacrolimus also decreased NGF and amphiregulin, but failed to increase sema3A. In generating a hypothesis from these data, it was assumed that not only NGF but also other factors, such as sema3A, might contribute to skin innervation.

Surprisingly, total serum IgE was significantly increased in the tacrolimus group. Inagaki et al. [31] reported that tacrolimus failed to decrease the elevation of serum IgE levels in a mouse model of dermatitis evoked by 2,4-dinitrofluorobenzene. Yamamoto et al. [32] also evaluated the effect of topical tacrolimus treatment that we used. However, we could not reproduce the results of their experiment, especially with regard to the improvement in the dermatitis score, and could not replicate the result for the effect of tacrolimus on serum IgE levels [32]. However, the administration of tacrolimus ointment in daily clinical practice differs from that of these animal studies; therefore, the effect of tacrolimus on dermatitis in NC/Nga mice versus its effect in humans is still controversial.

In the present study we provided insight into novel therapeutic effects of olopatadine, especially with regard to decreasing the number of intraepidermal neurites via increased sema3A expression and decreased NGF and amphiregulin expression in the epidermis. Further examination will yield valuable information for lowering the itch threshold in chronic AD.

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CASE REPORT

Good's syndrome (hypogammaglobulinemia with thymoma) presenting intractable opportunistic infections and hyperkeratotic lichen planus

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ABSTRACT

Good's syndrome (GS) is a rare acquired combined T- and B-cell immunodeficiency accompanying thymoma. This report concerns a case of a 57-year-old man with GS manifesting intractable opportunistic infections and hyperkeratotic lichen planus. He had a past history of extended thymectomy for removal of thymoma. He consulted us about scaly and exudative intractable erythematous plaque on his right forearm. The histology was compatible with phlegmon coexisting with lichen planus. Laboratory examination results indicated hypogammaglobulinemia accompanied by complete absence of B cells, which is consistent with GS. Combined treatment with immunoglobulin replacement and administration of antibiotics and antifungal drugs was effective for the phlegmon and overlying fungal infection. The patient also presented with hyperkeratotic lichen planus on both knees and the right elbow, suggesting that intractable opportunistic infection and lichen planus may be associated with GS.

Key words: agammaglobulinemia, Good's syndrome, lichen planus, opportunistic infections, thymoma.

INTRODUCTION

Immunodeficiency syndrome associated with thymoma was first reported in 1954 by Robert Good and is now known as Good's syndrome (GS).[1] This syndrome is a rare acquired disease of combined T- and B-cell immunodeficiency accompanying thymoma and with an incidence of 6–11% in thymoma cases.[2] It is generally accepted that cases with thymoma occasionally develop other autoimmune diseases, such as myasthenia gravis, pure red cell aplasia, pernicious anemia, idiopathic thrombocytopenia or diabetes mellitus.[3] It is also well known that various autoimmune skin diseases including pemphigus, lupus erythematosus or lichen planus are associated with thymoma.[4,5] This report concerns a case of Good's syndrome complicated with intractable opportunistic infections and hyperkeratotic lichen planus.

CASE REPORT

In August 2006, a 57-year-old man consulted us because he had been suffering for several months from scaly and exudative erythematous plaque on his right forearm. He had a past history of pneumomycosis in May 2003 and of extended thymectomy for removal of thymoma in July 2005. In December 2005, redness and swelling appeared suddenly on his right arm which was initially diagnosed as chilblain at a previously visited hospital. Although application of heparinoid ointment was not effective, the patient refused more accurate and detailed examinations such as skin biopsy. Moreover, since June 2006, multiple annular erythemas had been present on his back and chest, which had been diagnosed as urticarial vasculitis at the same previously visited hospital. Microscopic examination of a specimen from the scale on his forearm showed superficial tinea

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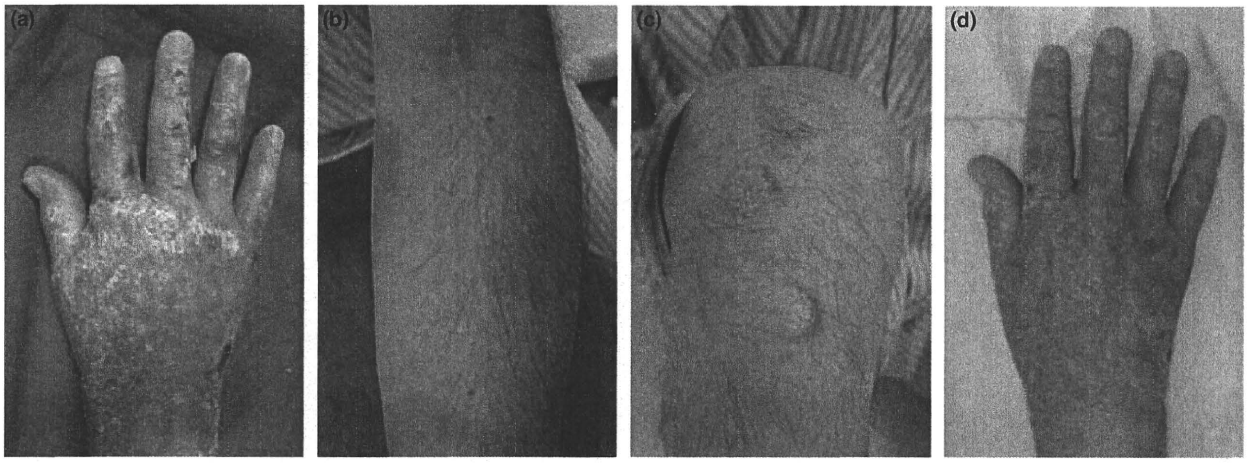

Figure 1. Before treatment. (a) Swelling on the right hand and forearm. (b) Multiple annular erythemas on the left arm. (c) Hyperkeratotic nodule on the right knee. (d) After treatment. Reduced swelling on the right hand and forearm.

infection. Because application of antifungal drug ointment had little effect on the swollen erythematous plaque on his right arm, the patient was admitted to our hospital in October 2006. On admission, he had scaly, exudative, swollen, tender and erythematous plaque on the right hand and forearm (Fig. 1a), and hyperkeratotic nodule-like lesions on both knees and the right ankle, as well as multiple generalized annular erythemas (Fig. 1b,c). The onset of the hyperkeratotic nodule-like lesions was not clear, but it appears to have been more than several months before phlegmon occurred.

The results of laboratory examination showed a white blood cell count of $5.2 \times 10^3/\mu L$, hemoglobin 14.0 g/dL, platelet count $3.27 \times 10^5/\mu L$, C-reactive protein 39 mg/L, total protein 5.8 g/dL, albumin 3.6 g/dL, immunoglobulin (Ig)G 188 mg/dL, IgA 8 mg/dL, IgM not detected, IgD of less than 1.0 mg/dL, CD4/CD8 37.9/37.2, CD3/CD19 71.0/0.0, β-D-glucan 5.3 pg/mL, HIV-antibody negative and anti-acetylcholine receptor antibody (AchR) negative. A combination of *Pseudomonas aeruginosa* and methicillin-sensitive *Staphylococcus aureus* (MSSA) infections was identified in a bacteriological culture from skin biopsy specimens from the erythematous plaque on the dorsum of the right hand. X-rays of the right forearm showed no bone involvement. The result of the skin biopsy disclosed dense infiltration of lymphocytes and neutrophils throughout the dermis. In addition, liquefaction degeneration, irregular acanthosis and Civatte bodies were also

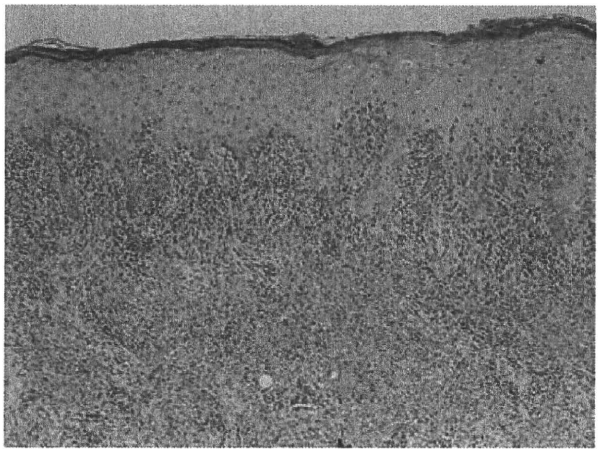

Figure 2. Histology of a biopsy specimen from the erythematous plaque on the dorsum of the right hand. Hematoxylin–eosin staining indicates dense infiltration by lymphocytes and neutrophils in all the layers of dermis with liquefaction degeneration, which suggests phlegmon with lichenoid reaction (original magnification ×40).

detected. No fungal components were detected, not even by periodic acid Schiff (PAS) stain (Fig. 2). We therefore diagnosed the lesion of the right forearm as phlegmon with lichen planus induced by *P. aeruginosa* and MSSA infections. Immunodeficiency due to GS explains why the phlegmon had been intractable for several months. Intravenous Ig (2.5/day for 9 days) in combination with antibiotics (ceftazidime, 2 g/day for 13 days) and an antifungal drug (itraconazole, 200 mg/day for 13 days) caused the phlegmon to subside rapidly, but showed little clinical

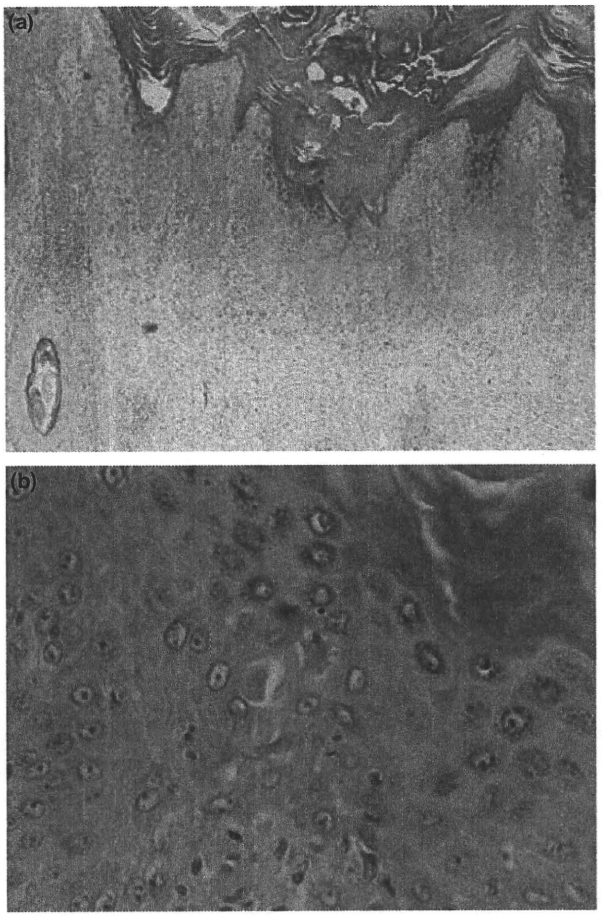

Figure 3. Histology of a biopsy specimen from the hyperkeratotic nodule on the left knee. Hematoxylin–eosin staining indicates liquefaction degeneration, band-like dermal lymphocytic infiltration and irregular acanthosis (a) as well as Civatte bodies (b), which suggests lichen planus (original magnifications: [a] ×40; [b] ×100).

effect on the lichen planus (Fig. 1d). A hyperkeratotic nodule-like lesion on the left knee was histopathologically diagnosed as lichen planus (Fig. 3). Monthly Ig replacement therapy prevented occurrence of compromising infection, but failed to clear the hyperkeratotic lichen planus.

DISCUSSION

The immunological characteristics of GS are hypogammaglobulinemia, few or no B cells, a reduced $CD4^+/CD8^+$ T-cell ratio, $CD4^+$ T-cell lymphopenia and impaired T-cell mitogenic responses. Although the pathogenesis of GS remains obscure, two hypotheses have been put forward. One is that cytokines, possibly secreted by bone marrow stroma cells, may influence both growth and differentiation of T- and B-cell precursors in GS. One candidate cytokine is limitin, an interferon-like cytokine.[6] The other hypothesis is that T cells isolated from thymoma or autoantibodies may inhibit Ig production by B cells and pre-B-cell growth.[2,7]

Extended thymectomy, radiation therapy or chemotherapy are options for treatment of thymoma in combination with Ig replacement therapy to maintain adequate IgG values in order to prevent opportunistic infections.[2] Unfortunately, there are no case reports of thymectomy resolving the immunodeficiency state associated with GS.[2,8,9] Moreover, there is one case report of hypogammaglobulinemia occurring several years after thymoma resection.[10] Our patient had suffered from pneumomycosis approximately 2 years before the extended thymectomy, while the laboratory examination before the thymectomy had already revealed the presence of hypoglobulinemia. These episodes suggest that the onset of his GS might have occurred at least 2 years before the operation and that the thymectomy may not have resolved the immunodeficiency as reported previously.

The most common compromising infection in GS is sinopulmonary infection with encapsulated bacteria, followed by cytomegalovirus, herpes simplex virus, human herpesvirus 8, varicella zoster virus, *Pneumocystis carinii* pneumonia and *Candida albicans* infections.[2] Ig replacement for GS has been reported to be effective for prevention of compromising infections including bacterial sinopulmonary infections. Clinical assessment and follow up for compromising infections in GS patients is vital, including appropriate microbiological investigations and prophylactic antibiotics.[2,8,9] In our case, the fact that intractable phlegmon was caused by bacterial infections provided us with the necessary clues for a diagnosis of GS.

As mentioned above, various skin diseases occur in association with thymoma, including graft-versus-host disease-like erythroderma, lichen planus, pemphigus and alopecia areata.[4,5,11–14] There are several case reports of lichen planus associated with thymoma but its pathogenic significance has not yet been clarified.[4,5] Autoimmune mechanisms might be involved in the pathogenesis of lichen planus, because attacks of T lymphocytes on keratinocytes

and liquefaction degeneration of the basal layer are characteristic findings.[15] One possible mechanism connecting lichen planus and thymoma may be the synthesis of specific autoreactive T lymphocyte clones, which cross-react with specific keratinocyte antigens and secrete tumor necrosis factor-α (TNF-α) and matrix metalloproteinase-9.[16] This accounts for the fact that lichen planus can be accompanied by dysregulation of the immune system in thymoma as mentioned elsewhere.[4] The histology of our patient's hyperkeratotic nodules indicated liquefaction degeneration both on the knees and the right ankle, while the erythematous plaque on his right forearm persisted after the treatment for phlegmon. This suggests that the lichen planus lesion overlapped with the phlegmon on his right forearm. On the other hand, multiple generalized annular erythemas, which had lasted for several years, disappeared soon after the administration of Ig. These erythemas may have been toxic eruptions, possibly accompanied by a compromising viral infection.

In conclusion, when a patient is found to have an intractable infection accompanied by thymoma, an immunological examination should be performed for a differential diagnosis of compromising infections associated with GS. In cases of thymoma, care must also be taken to prevent the development of various skin diseases including lichen planus.

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Effects of a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor and low-density lipoprotein on proliferation and migration of keratinocytes

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Conflicts of interest
None declared.

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Summary

Background Keratinocytes can obtain cholesterol either by *de novo* synthesis or by extraction, primarily from low-density lipoprotein (LDL). LDL is internalized following binding to the LDL receptor (LDLR). Because LDLR is expressed at a higher level in the cells of the basal layer of the epidermis, it might be assumed that LDLR upregulation is associated with keratinocyte proliferation. However, the effect of LDLR stimulation on keratinocyte function remains unclear.

Objectives To investigate the effects and mechanism of action of pitavastatin and effects of LDL on proliferation and migration of keratinocytes.

Methods Pitavastatin, an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase, was used to induce upregulation of LDLR. LDLR expression was evaluated by immunofluorescence staining, fluorescence-activated cell sorting, immunohistochemical staining and real-time polymerase chain reaction (PCR). HaCaT cells and normal human keratinocytes (NHKs) were used for evaluation of migration. 5-Bromo-2′-deoxyuridine incorporation was used to evaluate keratinocyte proliferation and differentiation. C57BL6 mice were used for *in vivo* evaluation of the effect of topical pitavastatin or lovastatin.

Results Pitavastatin was most effective in LDLR induction at a concentration of 1 $\mu mol\ L^{-1}$ in NHKs. Real-time PCR showed that pitavastatin significantly increased LDLR and liver X receptor (LXR) β mRNA expression in these cells. Similar results were obtained *in vivo*. However, pitavastatin had no effect on the migration of NHKs. After the addition of LDL and/or mevalonate concomitantly with pitavastatin to NHK cultures, or topical application of pitavastatin on mouse skin, keratinocyte proliferation was significantly increased.

Conclusions Pitavastatin significantly upregulates LDLR in both NHKs and C57BL6 mouse skin, resulting in increased keratinocyte proliferation. LXRβ may be involved in the pitavastatin-induced keratinocyte proliferation.

About 20% of the low-density lipoprotein (LDL) content of the blood reaches the basal cell layer of the epidermis, and it can be assumed that amounts reaching suprabasal layers are even lower.[1] LDL binds to its receptor (LDLR); following internalization these molecules are catabolized in lysosomes, providing cholesterol for membrane and steroid hormone synthesis. Therefore, the uptake of LDL regulates both the circulating level of LDL and the synthesis of cholesterol.[2]

The LDLR is similarly distributed in normal human epidermis and in reconstructed epidermis.[3,4] In basal cells the LDLR is evenly distributed between the cell surface and within the cell. Cells of the suprabasal layer contain a similar number of receptors; however, they are almost entirely intracellular.[3,4] In the upper layers of the epidermis very few LDLR molecules are detected and in differentiating keratinocytes there is a diminution in LDL-binding capacity.[3,4] Conversely, intrinsic cholesterol synthesis via the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase-mediated pathway increases in differentiated keratinocytes, which express low levels of LDLR. Synthesis is decreased in LDLR-upregulated proliferating keratinocytes.[5] From these observations, it might be proposed that keratinocytes use cholesterol derived from both LDL and *de novo* synthesis depending on physiological conditions, and that proliferating keratinocytes rely to a much greater extent

on LDL-derived cholesterol than on *de novo* synthesized cholesterol. Whether upregulation of the LDLR is preceded, or followed by, increased keratinocyte proliferation remains to be determined.

More recently, liver X receptor (LXR) α and LXRβ were reported to regulate intracellular cholesterol homeostasis through activation of cholesterol sulfotransferase and, hence, may play a role in keratinocyte proliferation and differentiation.[6] Pitavastatin has been shown to reduce serum LDL-cholesterol by inhibiting the *de novo* synthesis of cholesterol and increasing the transport of serum LDL into the cell by upregulation of cell-surface LDLR expression.[7,8] Based on these data, it might be assumed that treatment of keratinocytes with pitavastatin would result in keratinocyte proliferation.

In this study, we have investigated the effect of pitavastatin on LDLR expression. We have also explored the effect of pitavastatin and/or LDL on keratinocyte migration and proliferation, and whether this is modulated by interaction with other receptors, which may be important for maintenance of homeostasis in the skin. Mevalonate (Mev), as an antidote for the HMG-CoA reductase inhibitors, was also used to elucidate further the mechanism of action of pitavastatin.

Materials and methods

Animals

C57BL6 mice were purchased from CLEA (Tokyo, Japan). Animal care was in accordance with the institutional guidelines of Osaka University. All mice were used at 8–12 weeks of age in the animal centre at Osaka University. The mice were separated into three groups of three mice each and were treated with lovastatin, pitavastatin, or vehicle (propylene glycol/alcohol 7 : 3) alone. The skin of the back of the mice was shaved daily and the mice were treated according to the protocol described by Feingold *et al.*[9] The mice were killed after 0, 1, 3 and 7 days of treatment. Skin biopsies were taken and fixed with 10% formalin and the remainder of the treated back skin was used for mRNA extraction.

Immunohistochemical staining

In addition to the paraffin-embedded biopsies of mice tissue obtained as described above, 51 formalin-fixed, paraffin-embedded human skin biopsies [psoriasis, 17; atopic dermatitis (AD), 16; healthy control, 18] were obtained from the Department of Dermatology of Osaka University Hospital, following approval by the ethics committee of Osaka University.

Paraffin sections of 3 μm were cut and dried, followed by deparaffinization and rehydration. Sections were heated for antigen retrieval, blocked with 3% hydrogen peroxide, washed twice with phosphate-buffered saline (PBS) containing 0·05% Tween 20 (PBS/Tween 20), and incubated in blocking solution (Protein Block, Serum-Free; Dako, Carpinteria, CA, U.S.A.) for 15 min. Sections of human tissue were incubated for 1 h at room temperature with mouse monoclonal anti-LDLR antibody (United States Biological, Marblehead, MA, U.S.A.). Mouse sections were incubated with rabbit polyclonal anti-involucrin antibody (BAbCO, Berkeley, CA, U.S.A.) for mouse sections. All sections were then washed twice and processed using the ChemMate Envision kit (Dako) according to the manufacturer's protocol. The diaminobenzidine reaction product was visualized and sections were counterstained with haematoxylin.

Alternatively, mouse sections were incubated overnight with rabbit polyclonal anti-LDLR antibody (Abcam, Cambridge, U.K.), washed twice, and processed using the LSAB+System-AP kit (Dako) according to the manufacturer's protocol. Reaction products of the new Fuchsin substrate system were visualized and sections were counterstained with haematoxylin.

Cell culture

HaCaT cells were cultured in Dulbecco's modified Eagle's medium (Gibco-BRL, Gaithersburg, MD, U.S.A.) containing 10% fetal bovine serum (BioWhittaker Inc., Walkersville, MD, U.S.A.), L-glutamate, penicillin G and streptomycin at 37 °C in an atmosphere of 5% CO_2/95% air. Normal human keratinocytes (NHKs; DS Pharma Biomedical Co., Osaka, Japan) were cultured in a serum-free medium formulated for human keratinocytes (KJB-200; Kohjinbio, Saitama, Japan) at 37 °C in an atmosphere of 5% CO_2/95% air.

Slide culture for immunofluorescence staining

For immunofluorescence (IF) staining NHKs were seeded in Lab-Tek® chamber slides (Nalgene Labware, Rochester, NY, U.S.A.). After 48 h of incubation, cells were treated with 0·1, 1 and 10 μmol L^{-1} pitavastatin (gift of Kowa Pharmaceutical Company, Tokyo, Japan) in dimethylsulphoxide (DMSO; Nacalai Tesque Inc., Kyoto, Japan). Control cultures were treated with vehicle alone or received no treatment.

Fluorescence-activated cell sorting

HaCaT cells and NHKs were seeded in six-well dishes. After 1 day in culture, cells were incubated with 0·1, 1 or 10 μmol L^{-1} of pitavastatin or DMSO alone for 24 h. Cultures were rinsed once with PBS, incubated in trypsin for 10 min at 37° C, harvested, and washed twice with PBS. Cells were then incubated with 0·1–10 μg mL^{-1} monoclonal anti-LDLR antibody or isotype control monoclonal antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA, U.S.A.) in PBS supplemented with 1% bovine serum albumin (1% BSA-PBS) for 20 min. Cells were washed twice, fixed in 2% formaldehyde in PBS, washed twice again, and incubated in 50 μL fluorescein isothiocyanate (FITC)-labelled polyclonal goat antimouse antibody (Dako; 1:10) for 30 min, followed by two washes in PBS. The cells were then rinsed with 1% BSA-PBS and analysed by FACScan using the Cell Quest software program (Becton Dickinson, Franklin Lakes, NJ, U.S.A.).

Immunofluorescence staining

Cells cultured in chamber slides were fixed with 4% paraformaldehyde (Nacalai Tesque Inc.) for 10 min. Mouse paraffin sections were deparaffinized and hydrated. Slides and sections were then processed for IF staining according to the protocol of Simon *et al.*[10] Briefly, sections were incubated with ammonium chloride (50 mmol L^{-1}; Nacalai Tesque Inc.) for 10 min followed by washing with PBS. They were then heated in a Taitec (Saitama, Japan) steamer for 20 min in citric acid buffer followed by washing with PBS/Tween 20. After blocking with 5% normal goat serum (NGS) (Vector Laboratories Inc., Burlingame, CA, U.S.A.) in PBS/Tween 20, slides were incubated with the monoclonal anti-LDLR antibody for 2 h and mouse sections were incubated overnight with rabbit polyclonal anti-Ki-67 antibody (Novocastra, Wetzlar, Germany). All sections and slides were then washed with 1% NGS in PBS/Tween 20 for 5 min and rinsed in water. Slide cultures were incubated with an FITC-labelled polyclonal goat antimouse secondary antibody and sections were incubated with an FITC-labelled polyclonal swine antirabbit secondary antibody (Dako) together with Hoechst 33342 (Invitrogen Molecular Probes, Eugene, OR, U.S.A.) for 40 min. Slides were washed again in 1% NGS in PBS/Tween 20 for 5 min, washed with water, and mounted. The cells were photographed using a BZ-8000 microscope (Keyence, Osaka, Japan).

In vitro migration assay

HaCaT cells and NHKs were used to assess the migratory activity of keratinocytes after exposure to 1 μmol L^{-1} pitavastatin in the presence or absence of LDL using the procedure described by Kira *et al.*[11] Briefly, HaCaT cells and NHKs were each plated in 18-well plates. After 24 h of incubation, HaCaT cell culture medium was replaced by serum-free medium. After HaCaT cell starvation for 24 h, and after 48 h incubation for NHKs, cells were treated with 10 μg mL^{-1} mitomycin C (Nacalai Tesque Inc.) for 1 h to prevent cell proliferation. A cell-free area was created in each well by scraping the monolayer with a yellow pipette tip. Three wells in each plate were each treated with 1 μmol L^{-1} pitavastatin, 50 μg mL^{-1} LDL (Biomedical Technologies Inc., Stoughton, MA, U.S.A.), 1 μmol L^{-1} pitavastatin together with 50 μg mL^{-1} LDL, 30 ng mL^{-1} epidermal growth factor (EGF; Upstate Biotechnology, Lake Placid, NY, U.S.A.), DMSO, or no treatment. After 48 h cultures were photographed using a phase contrast microscope (DIA-PHOT 300; Nikon, Tokyo, Japan) and keratinocytes that had migrated into the cell-free areas in each well were counted.

In vitro proliferation assay

NHKs were used to assess the proliferation of keratinocytes after exposure to 1 μmol L^{-1} pitavastatin in the presence or absence of LDL and/or Mev (500 μmol L^{-1}; Sigma, St Louis, MO, U.S.A.) using the ELISA 5-bromo-2′-deoxyuridine (BrdU) assay kit (Roche, Basel, Switzerland). Briefly, NHKs were plated in 27-well plates. Twenty-four hours after plating, cells were supplemented with the same substances as in the *in vitro* migration assay and at the same concentrations (three wells each). In addition, nine wells were supplemented with Mev, Mev and pitavastatin, or Mev with LDL and pitavastatin (three wells each). Cells were then incubated for 24 h, followed by addition of BrdU and another 24-h incubation. Cells were fixed, DNA was denatured, and anti-BrdU-POD was added. This immune complex was detected following a substrate reaction, which was quantified by measuring the absorbance at wavelength 450:655 nm using a Model 680 microplate reader (BioRad, Hercules, CA, U.S.A.).

Normal human keratinocyte toxicity assay

The effect of different pitavastatin concentrations on cell viability was measured by the 3-dimethylthiazol-5carboxymethoxyphenyl-2-sulfophenyl-2H-tetrazolium (MTS) method using the Cell Titer 96® Aqueous One Solution Cell Proliferation Assay (Promega, Madison, MI, U.S.A.). MTS assays were performed as directed by the manufacturer. NHKs were plated in 30 wells in a 96-well microtitre plate (100 μL each). Twenty-four hours after plating, cells were supplemented with the same substances as in the fluorescence-activated cell sorting (FACS) analysis, and at the same concentrations (six wells each) in addition to six untreated wells. Cells were then incubated for 24 h. Twenty microlitres of MTS reagent was added to the culture medium in each well. Cells were incubated for an additional 3 h at 37 °C. The amount of coloured formazan product in metabolically active cells was then measured at wavelength 490–655 nm using a Model 680 microplate reader (BioRad).

Extraction of mRNA and quantitative real-time polymerase chain reaction

NHKs were plated in nine wells. After 24 h, cells were treated with 1 μmol L^{-1} pitavastatin, DMSO alone, or left untreated (three wells each) followed by incubation for another 24 h.

Mouse skin samples were incubated overnight in 4% Dispase (Invitrogen, Carlsbad, CA, U.S.A.). The epidermis was then separated from the dermis using the protocol described by Kira *et al.*[11] and total epidermal RNA was extracted using the SV Total RNA Isolation System Kit (Promega) according to the manufacturer's protocol. Total RNA was extracted from cultured NHKs using the same protocol. One microgram of extracted RNA was used to prepare cDNA using the SuperScript VILO cDNA Synthesis Kit (Invitrogen) according to the manufacturer's instructions.

Real-time polymerase chain reaction (PCR) primers for the human EGF receptor, LXRα, LXRβ and mouse LDLR were from JBioS (Saitama, Japan). Primers for the human LDLR were from Sigma and primers for human and mouse glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were designed

Table 1 Primer sequences for real-time polymerase chain reaction

	Forward	Reverse
mLDLR	5′-TGGCCATCTATGAGGACAAA-3′	5′-GTGTGACCTTGTGGAACAGG-3′
hLDLR	5′-GTGTCACAGCGGCGAATG-3′	5′-CGCACTCTTTGATGGGTTCA-3′
hLXRα	5′-CAACCCTGGGAGTGAGAGTATCAC-3′	5′-CATTCATGGCCCTGGAAGAACT-3′
hLXRβ	5′-AAGGACTTCACCTACAGCAAGGA-3′	5′-CCGCGAGAACTCGAAGATG-3′
hGAPDH	5′-GGAGTCAACGGATTTGGTCGTA-3′	5′-GCAACAATATCCACTTTACCAGAGT-3′
mGAPDH	5′-CATGGCCTTCCGTGTTCCTA-3′	5′-TGTCATCATACTTGGCAGGTTTCT-3′
hEGFR	5′-GCGTCTCTTGCCGGAATGT-3′	5′-GGCTCACCCTCCAGAAGGTT-3′

LDLR, low-density lipoprotein receptor; LXR, liver X receptor; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; EGFR, epidermal growth factor receptor; m, mouse; h, human.

using Primer3 software. All primer sequences are shown in Table 1.

Real-time PCR was performed in an ABI 7900 HT Fast Real-Time PCR System using SYBR Green dye (Applied Biosystems, Foster City, CA, U.S.A.) according to the manufacturer's protocol. Data were analysed using the SDS 2.1 software program.

Statistical analysis

Results are presented as the mean ± SEM. Data were analysed using the two-tailed unpaired t-test and SPSS 16 software for Windows (SPSS, Chicago, IL, U.S.A.) with the level of significance set at $P < 0{\cdot}05$.

Results

Pitavastatin induced upregulation of the low-density lipoprotein receptor in keratinocytes

Morikawa *et al.*[8] had observed that pitavastatin was the most efficient of the statins tested in the induction of LDLR upregulation in a cultured human hepatoma cell line. In this study, we confirmed the ability of pitavastatin to upregulate LDLR expression in NHK slide cultures using concentrations within the range used by Morikawa *et al.* LDLR expression level was evaluated using IF staining. Time-course analysis showed that maximum LDLR expression was achieved 2 h after pitavastatin application and that the expression level remained stable for up to 24 h (Fig. 1a). Therefore, IF staining of cells treated with different concentrations of pitavastatin was performed after 24 h of incubation. Cells treated with 0·1 μmol L^{-1} pitavastatin showed an increase in LDLR expression compared with DMSO-treated and untreated cells, with staining being strongest in the cytoplasm. Cytoplasmic and membranous staining was more intense in cells treated with 1 μmol L^{-1} pitavastatin while cells treated with 10 μmol L^{-1} pitavastatin showed LDLR expression levels similar to untreated cells (Fig. 1b).

FACS analysis of HaCaT cells (Fig. 1c), with the fluorescence intensity set as more than 10^2, revealed that cells treated with all concentrations of pitavastatin had a similar level of fluorescence intensity, which was higher than that of DMSO-treated cells. FACS analysis of NHKs (Fig. 1d), with the fluorescence intensity set as more than 10^1, revealed that cells treated with 1 μmol L^{-1} pitavastatin had more intense LDLR fluorescence than DMSO-treated cells and cells treated with other concentrations of pitavastatin.

In an attempt to elucidate the relatively decreased LDLR upregulation in the cells treated with 10 μmol L^{-1} pitavastatin, an NHK toxicity assay was performed and revealed that there is a significant toxic effect of 10 μmol L^{-1} pitavastatin, as compared with other pitavastatin concentrations and non-treated cells (Fig. 1e).

Real-time PCR analysis of mRNA extracted from NHKs (Fig. 2a) showed a significant increase in the level of LDLR mRNA in NHKs treated with 1 μmol L^{-1} pitavastatin compared with DMSO-treated and untreated cells.

LDLR expression levels in C57BL6 mice treated topically with pitavastatin were compared with those in mice treated with lovastatin or vehicle alone after 0, 1, 3 and 7 days. Immunohistochemical staining (IHC) revealed an increase in LDLR expression that became particularly strong after 7 days treatment in both lovastatin- and pitavastatin-treated mice (Fig. 2b). mRNA obtained from these mice showed a tendency towards upregulation of LDLR expression after 1 and 3 days of treatment with lovastatin and pitavastatin, respectively, followed by downregulation in subsequent days. None of these changes was statistically significant (Fig. 2c).

Effect of pitavastatin on keratinocyte migration

In HaCaT cells there was no significant difference in the migratory activity of cells treated with pitavastatin or LDL, either alone or in combination (Fig. 3a); however, treatment with EGF significantly increased migratory activity compared with all other treatments. Results obtained for NHKs (Fig. 3b) were comparable with those for HaCaT cells; however, there was a nonsignificant increase in the migration of pitavastatin-treated cells compared with untreated and DMSO-treated cells.

Effect of pitavastatin on keratinocyte proliferation

It was previously reported that LDLR expression is upregulated in proliferating keratinocytes.[4] We used human psoriatic skin as a model of proliferating keratinocytes to investigate LDLR expression compared with expression in AD, a model of

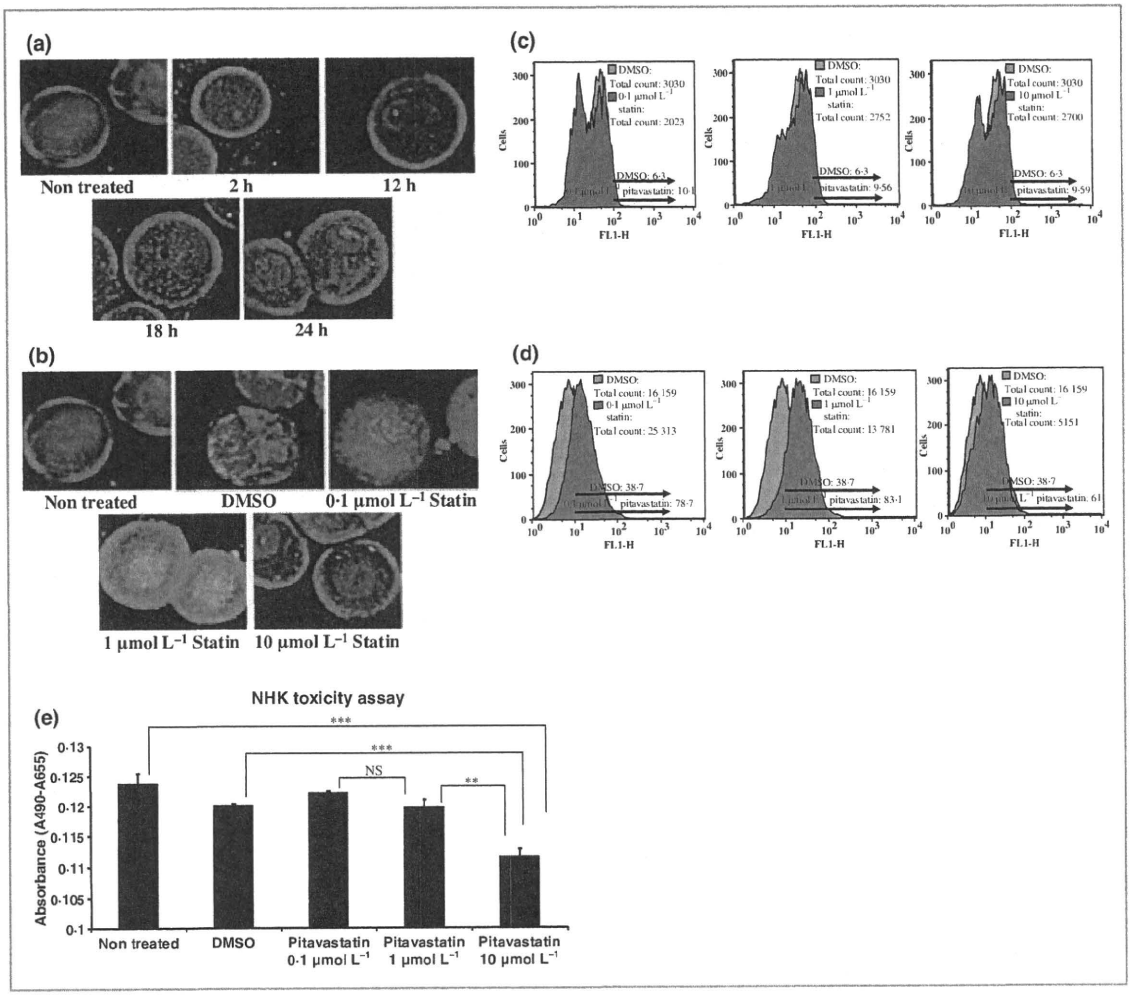

Fig 1. (a) Immunofluorescence (IF) staining for low-density lipoprotein receptor (LDLR) after different time intervals of pitavastatin (1 μmol L^{-1}) supplementation. The maximum LDLR expression was achieved 2 h after pitavastatin application and the expression level remained stable, with increased cytoplasmic level, for up to 24 h. (b) IF staining for LDLR 24 h after application of pitavastatin (0·1, 1, 10 μmol L^{-1}) in comparison with untreated and dimethylsulphoxide (DMSO)-treated cells. Pitavastatin at a concentration of 1 μmol L^{-1} was the most effective in induction of LDLR in both the cytoplasm and membrane. (c) Fluorescence-activated cell sorting (FACS) analysis of HaCaT cells. The proportion of cells which gave intense fluorescence was similar among cells treated with different concentrations of pitavastatin and was higher than in DMSO-treated cells, with the fluorescence intensity set as > 10^2. (d) FACS analysis of normal human keratinocytes (NHKs). The highest proportion of cells giving intense fluorescence corresponded to cells treated with 1 μmol L^{-1} pitavastatin. All pitavastatin-treated cells showed a higher proportion of cells with intense fluorescence compared with DMSO-treated cells. The fluorescence intensity was set as > 10^1. (e) 3-Dimethylthiazol-5-carboxymethoxyphenyl-2-sulfophenyl-2H-tetrazolium toxicity assay for NHK culture treated with different pitavastatin concentrations. The cell viability is significantly decreased in cells treated with 10 μmol L^{-1} pitavastatin as compared with other pitavastatin concentrations and nontreated cells. Data are shown as mean ± SEM. NS, not significant, **P < 0·01, ***P < 0·001.

acanthotic nonproliferating skin, and in healthy control skin. We found that, consistent with previous reports, LDLR is highly expressed throughout the psoriatic epidermis, both in basal and suprabasal layers (Fig. 4a). To determine whether LDLR upregulation is a cause or an effect of increased keratinocyte proliferation we investigated the effect of LDLR induction on keratinocyte proliferation. Because pitavastatin was shown to be an inducer of LDLR, we used pitavastatin, both *in vitro* and *in vivo*, to explore this issue. An *in vitro* BrdU incorporation assay (Fig. 4b) showed that proliferation in keratinocytes treated with pitavastatin was significantly inhibited compared with untreated cells. Surprisingly, addition of LDL and/or Mev to pitavastatin-treated cells significantly increased proliferation, whereas neither LDL alone nor Mev alone induced any significant proliferative activity. Taking into consideration the availability of LDL in the *in vivo* environment, the results of the *in vitro* assay are consistent with those of the *in vivo* study, where topical treatment with pitavastatin, compared with vehicle alone, resulted in a marked increase in epidermal thickness (Fig. 5a). This occurred in association

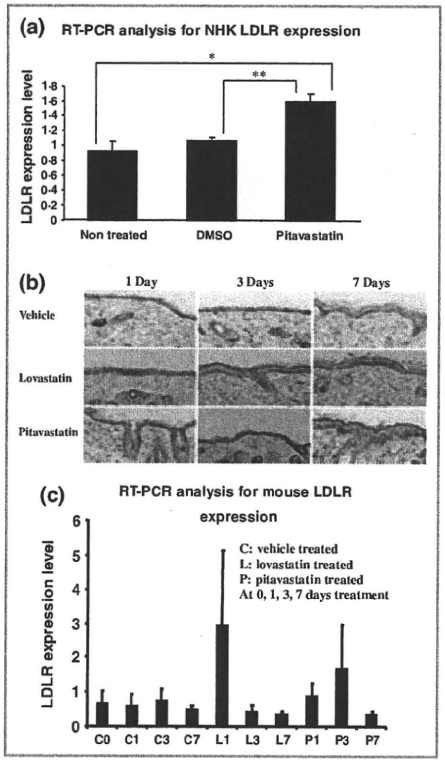

Fig 2. (a) Real-time reverse transcription–polymerase chain reaction (RT-PCR) analysis of low-density lipoprotein receptor (LDLR) expression in mRNA of cultured normal human keratinocytes (NHKs). Pitavastatin at a concentration of 1 μmol L^{-1} significantly induced LDLR expression at the transcriptional level. Data are shown as mean ± SEM. DMSO, dimethylsulphoxide. *P < 0·05, **P < 0·01. (b) Immunohistochemical staining for LDLR expression in mouse epidermis. Lovastatin and pitavastatin induced upregulation of LDLR; staining became strong after 7 days of treatment. (c) Real-time RT-PCR analysis of LDLR expression in mRNA of mouse epidermis. Lovastatin induced LDLR upregulation faster and more markedly than pitavastatin. Following treatment with either lovastatin or pitavastatin the LDLR mRNA level returned to the basal level on day 7. None of these changes was significant compared with untreated and vehicle-treated mice. Data are shown as mean ± SEM.

with an apparent decrease in the expression of involucrin, a marker of keratinocyte differentiation (Fig. 5b), and an obvious increase in the expression level of Ki-67, a marker of proliferating cells (Fig. 5c). The results of topical application of lovastatin were similar to those of pitavastatin, but less marked.

Relationship of pitavastatin to epidermal growth factor receptor and liver X receptor expression

Because previous reports have shown a relationship between keratinocyte proliferation and expression of both the EGF receptor[12] and LXR,[6] and because pitavastatin induces keratinocyte proliferation in the presence of LDL, we investigated the effect of pitavastatin on receptor expression. Real-time PCR analysis of mRNA of pitavastatin-treated NHKs showed a significant increase only in LXRβ expression compared with untreated cells (Fig. 6).

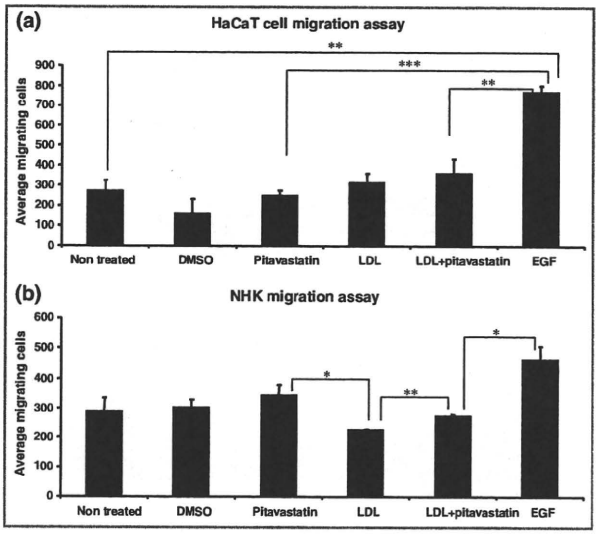

Fig 3. (a) *In vitro* migration assay of HaCaT cells. Treatment with pitavastatin and/or low-density lipoprotein (LDL) resulted in no significant change in the migratory activity of keratinocytes. Epidermal growth factor (EGF) significantly increased migratory activity. (b) *In vitro* migration assay of normal human keratinocytes (NHKs). Treatment with pitavastatin and/or LDL resulted in no significant change in the migratory activity of keratinocytes. EGF-induced migratory activity was less marked than seen in HaCaT cells. Data are shown as mean ± SEM. DMSO, dimethylsulphoxide. *P < 0·05, **P < 0·01, ***P < 0·001.

Discussion

As cholesterol is an essential component of plasma membranes and because LDL is an important source of cholesterol, it is reasonable to propose that LDL might have an impact on keratinocyte functions, such as proliferation and differentiation. Statins are known to be effective antihyperlipidaemic agents. We hypothesized that statins, as lipid-reducing agents, may act through LDLR upregulation. After confirmation of this hypothesis, we explored the use of statins to amplify the effects of LDLR on keratinocyte function. To do this we used IF staining, FACS analysis and real-time PCR to assess LDLR expression in cultured keratinocytes after exposure to different pitavastatin concentrations. We also assessed the effect of topical application of pitavastatin on mouse back skin on LDLR expression using IHC and real-time PCR. Taking into consideration that upregulation of LDLR was previously reported to be associated with hyperproliferation of keratinocytes,[5] the finding that pitavastatin treatment made no significant difference in HaCaT cell LDLR expression might be explained by the high proliferative activity of HaCaT cells. Higher proliferative activity might be associated with a higher baseline level of LDLR expression in HaCaT cells compared with NHKs, precluding significant upregulation by pitavastatin, regardless of the concentration used. Also, the toxic effect of pitavastatin,

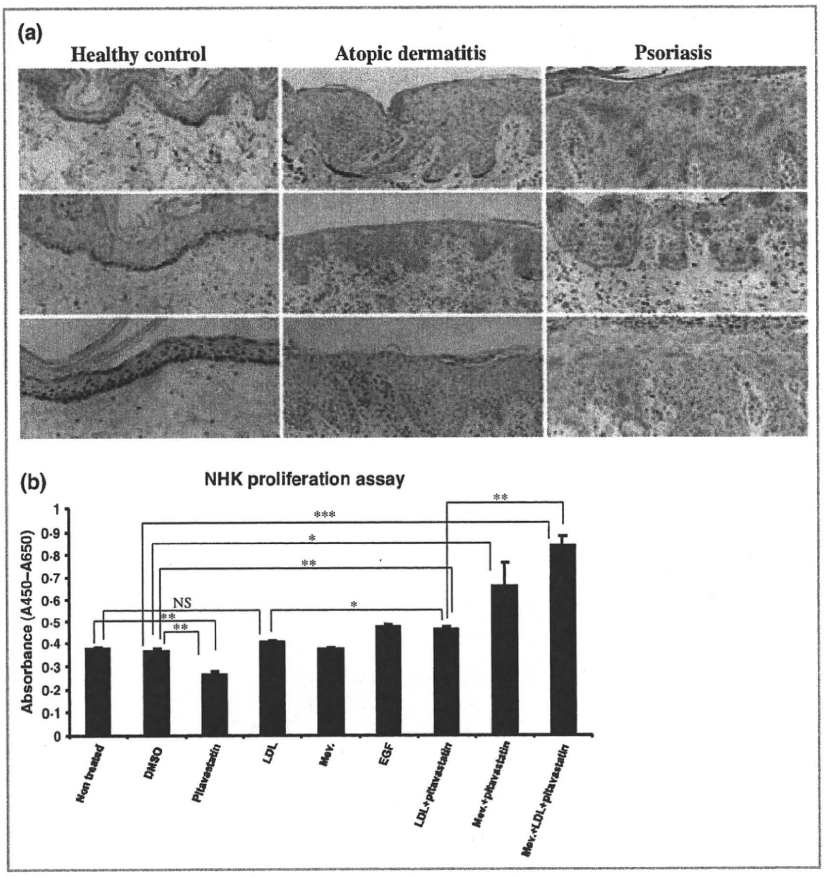

Fig 4. (a) Immunohistochemical staining for low-density lipoprotein (LDL) receptor (LDLR) expression in human skin samples. Hyperproliferation in psoriasis, but not acanthosis in atopic dermatitis, was associated with upregulation of LDLR compared with healthy skin samples. (b) 5-Bromodeoxyuridine incorporation assay in normal human keratinocytes (NHKs). Pitavastatin significantly inhibited keratinocyte proliferation, but after supplementation of the culture medium with LDL and/or mevalonate (Mev), significantly induced keratinocyte proliferation to an extent approaching or exceeding the effect of epidermal growth factor (EGF). Neither LDL alone nor Mev alone induced any significant proliferative activity as compared with untreated cells. Data are shown as mean ± SEM. DMSO, dimethylsulphoxide. NS, not significant, $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$, $^{***}P < 0{\cdot}001$.

estimated by MTS assay, provided a possible explanation for the relative decrease in the LDLR expression in cells treated with 10 μmol L^{-1} as compared with 1 μmol L^{-1} pitavastatin. Moreover, real-time PCR analysis of mRNA from pitavastatin-treated NHKs supported the results of IF staining and FACS analysis and showed a significant increase in LDLR mRNA over DMSO-treated and untreated cells. These findings are in agreement with *in vivo* results where a clear increase in LDLR expression was observed, particularly after 7 days of topical statin application; this effect was more marked in pitavastatin-treated than in lovastatin-treated mice. Although real-time PCR analysis of mRNA revealed no significant change in LDLR expression, the results were informative with regard to the interval between lovastatin or pitavastatin application and the beginning and termination of upregulation of LDLR mRNA. Whereas LDLR expression was more highly upregulated by pitavastatin, LDLR mRNA expression was upregulated sooner after lovastatin application.

Keratinocyte migration is critical for proper wound healing.[13] To our knowledge, no reports on the effect of pitavastatin on keratinocyte migration have been published. EGF, which is well known to be effective in wound healing,[14] significantly induced migration in HaCaT cells and increased migration, with a tendency toward significance, in NHKs, compared with untreated cells. Treatment with neither pitavastatin nor pitavastatin together with LDL resulted in any significant difference in HaCaT cell or NHK migratory activity compared with DMSO-treated and untreated cells. These data suggest that pitavastatin may have neither a positive nor a negative role in wound healing.

Having confirmed the role of pitavastatin as an upregulator of LDLR and the association of keratinocyte hyperproliferation with increased LDLR expression, we were surprised that pitavastatin significantly downregulated keratinocyte proliferation compared with untreated and DMSO-treated cells. This downregulation might be explained by the lack of LDL in the culture medium; notably, the addition of LDL to the pitavastatin-treated cells resulted in a significant increase in keratinocyte proliferation as compared with DMSO-treated as well as LDL-treated cells. This explanation is supported by the results of the *in vivo* study, where keratinocyte hyperproliferation after topical application of pitavastatin was accompanied by acanthotic changes, decreased involucrin expression, and increased Ki-67 expression.

Moreover, Mev, which is known to antagonize the inhibitory effect on HMG-CoA reductase, together with pitavastatin, have induced keratinocyte proliferation. The same effect was induced by LDL, together with pitavastatin. The effects of both Mev and LDL might be through providing the precursor for cholesterol synthesis, which is essential for cell proliferation. In this context, the additional increase in the proliferative activity with concomitant pitavastatin, LDL and Mev supplementation might be explained by the availability of cholesterol from both cellular uptake and *de novo* synthesis.

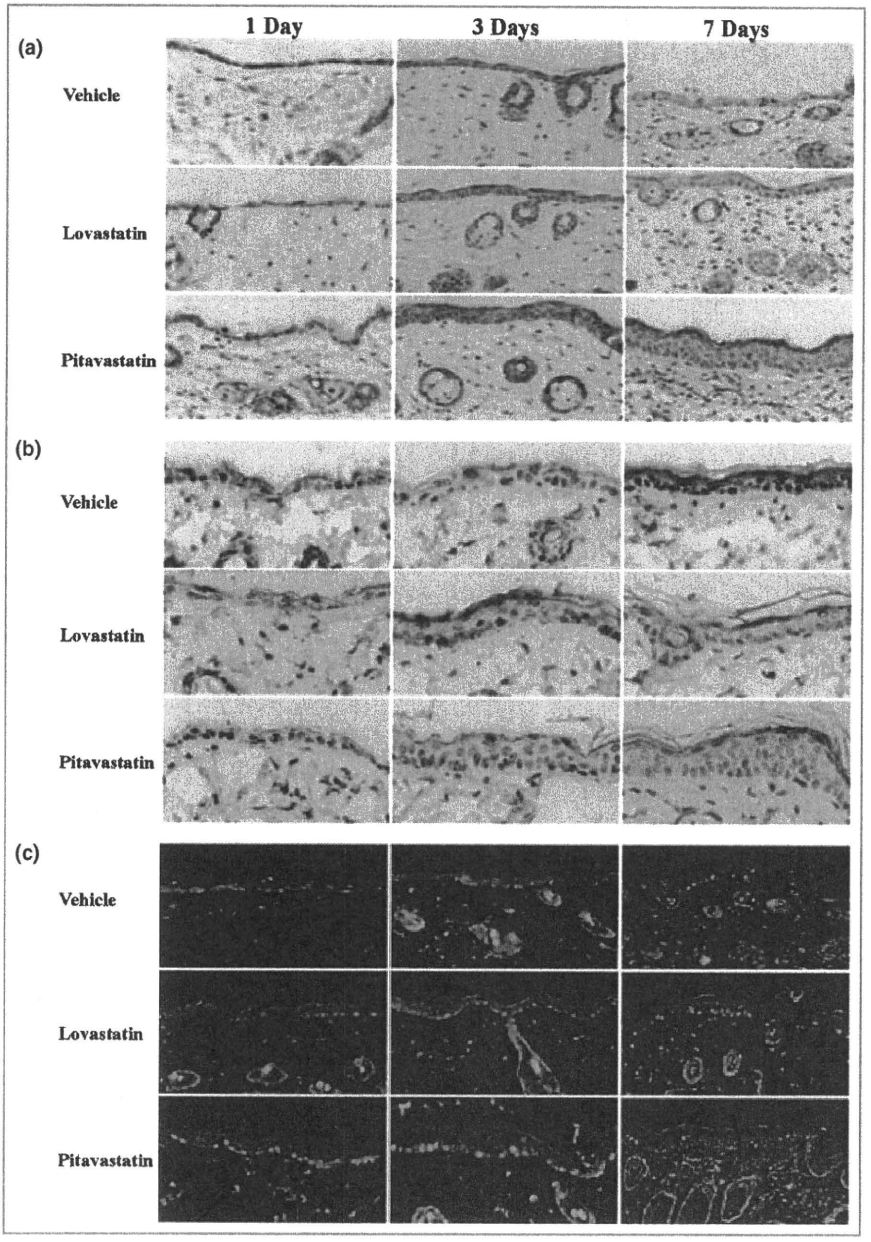

Fig 5. (a) Haematoxylin and eosin staining of mouse skin. Topical application of lovastatin and pitavastatin induced epidermal acanthotic changes observable after 3 days of treatment; the effect was more marked with pitavastatin. (b) Immunohistochemical staining for involucrin expression in mouse epidermis. There was a relative decrease in involucrin expression in lovastatin- and pitavastatin-treated mice compared with mice treated with vehicle alone. The expression level was very low under the stratum corneum after 7 days of pitavastatin treatment. (c) Immunofluorescence staining for Ki-67 expression in mouse epidermis. Pitavastatin induced Ki-67 expression more markedly than did lovastatin; this became more evident after 7 days of treatment.

Our findings also contribute to the debate surrounding the role of statins in cell proliferation and cancer development. Earlier animal studies suggested a possible role for statins in increased cancer risk. However, laboratory evidence suggests that statins inhibit tumour development by inducing cell cycle arrest and apoptosis.[15] In addition, previous experimental evidence suggests that statins may act synergistically with standard chemotherapeutic agents in cancer treatment.[16] In this study, we present evidence that statins are inducers of NHK proliferation, in the presence of cholesterol precursors, whereas in other studies statins were found to be cytotoxic for tumour cells *in vitro*.[17] Consistent with our findings, Schiefelbein *et al.*[18] recently reported an inhibitory effect of simvastatin on proliferation of cultured HaCaT keratinocytes. Taken together, these findings may focus on the role of cholesterol in reversing the effect of statins on cell proliferation.

The onset of keratinocyte proliferation after induction of LDLR, and in the presence of LDL and/or Mev, indicates that

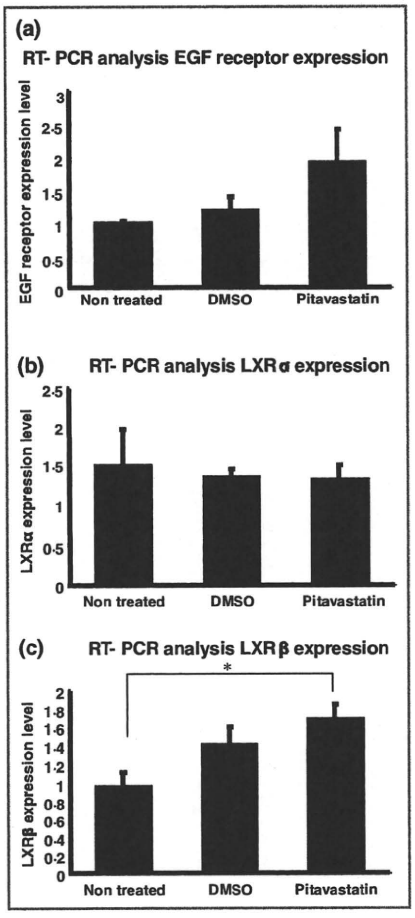

Fig 6. Real-time reverse transcription–polymerase chain reaction (RT-PCR) analysis of normal human keratinocyte mRNA for epidermal growth factor (EGF) receptor (a), liver X receptor (LXR) α (b) and LXRβ (c). Pitavastatin significantly induced only LXRβ expression at the transcriptional level. Data are shown as mean ± SEM. DMSO, dimethylsulphoxide. *$P < 0{\cdot}05$.

LDLR is actively involved in the mechanisms underlying keratinocyte proliferation through different mechanisms, including its role in providing LDL to the cell, and that overexpression of LDLR is not simply an effect of proliferation. In other words, LDLR upregulation might be important for induction of keratinocyte proliferation and not the reverse.

Taken together, our data confirmed the relationship between keratinocyte proliferation and LDLR expression as well as the association between pitavastatin treatment and LDLR upregulation. The data confirmed that upregulated LDLR, in the presence of LDL, induces keratinocyte proliferation but not keratinocyte migration.

Although we show that pitavastatin induced upregulation of LDLR, we cannot exclude the possibility that other mechanisms exist through which pitavastatin might induce keratinocyte proliferation. Our real-time PCR analysis of mRNA from pitavastatin-treated NHKs excluded involvement of the EGF receptor, which is the primary receptor stimulated in EGF-induced keratinocyte proliferation,[12] but also showed that pitavastatin might act through induction of LXRβ, which, when stimulated, induces intracellular cholesterol synthesis.

Our results confirmed the effect of pitavastatin on keratinocyte migration and proliferation. However, the mechanism(s) by which upregulated LDLR induces keratinocyte proliferation in the presence of LDL remain(s) to be elucidated.

What's already known about this topic?

Low-density lipoprotein (LDL) receptor (LDLR) is expressed at a higher level in the cells of the basal layer of the epidermis. Pitavastatin reduces serum LDL-cholesterol by inhibiting the *de novo* synthesis of cholesterol and increasing the transport of serum LDL into the cell by upregulation of cell-surface LDLR expression.

What does this study add?

Pitavastatin significantly upregulates LDLR in both normal human keratinocytes and C57BL6 mouse skin, resulting in increased keratinocyte proliferation. Liver X receptor β may be involved in the pitavastatin-induced keratinocyte proliferation.

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REVIEW ARTICLE

Annular erythema associated with Sjögren's syndrome: review of the literature on the management and clinical analysis of skin lesions

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Abstract Annular erythema has been recognized to be a specific, cutaneous manifestation associated with Sjögren's syndrome. Based on a search of the literature up to 2007, annular erythema with Sjögren's syndrome (AESS) preferentially occurs in Asian but not in Western populations. However, the precise clinical course and standard regimen for the management of AESS have remained obscure, primarily because of its rare occurrence in Western populations and the fact that most Asian cases are isolated reports. In this study, 28 cases of AESS from our department and 92 cases distilled from the literature were enrolled in a retrospective study to evaluate the clinical characteristics and most desirable management of this skin manifestation in Sjögren's syndrome. We found that 75% of all cases with AESS were positive for both anti-SSA and anti-SSB antibodies. Multiple therapeutic options are available to treat AESS, including oral steroids. Several anti-malaria drugs or tacrolimus ointment have also been reported to be effective against AESS. AESS is a distinct clinical entity, and a small dose of prednisolone (approx. 10 mg) is sufficient to control diseases activity, except in some cases with systemic manifestations, and this treatment has a more rapid clinical effect than topical steroids.

Keywords Annular erythema · Management · SCLE · Sjögren's syndrome · Steroids

Abbreviations
AESS Annular erythema with Sjögren's syndrome
LE Lupus erythematosus
SCLE Subacute cutaneous lupus erythematosus
SS Sjögren's syndrome

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Introduction

Sjögren's syndrome (SS) was first reported by the Swedish ophthalmologist Henrik Sjögren in 1933 as a case of keratoconjunctivitis sicca associated with rheumatoid arthritis [1]. Since then, numerous clinical, etiological, and epidemiological analyses have been conducted, and SS is recognized as autoimmune epithelitis or autoimmune sialoadenitis induced by autoreactive T cells or tissue-specific autoantibodies [2]. SS patients develop various types of exocrine manifestations, such as autoimmune thyroiditis, interstitial nephritis, or pneumonitis during the clinical course. B cell lymphoma/pseudolymphoma or several types of skin diseases are known as extra-glandular manifestations of SS.

The known cutaneous manifestations associated with SS include skin dryness, hypergammaglobulinemic purpura, and urticarial vasculitis [2]. In addition to these skin lesions, SS patients occasionally develop annular erythema (AE) with perivascular and periappendageal lymphocytic infiltration, which is characterized by a wide elevated border and central pallor [3–5]. Since only a few cases of AE in SS have been reported in Caucasians [6–8] and because AE in Japanese and Oriental SS patients share many clinical features with subacute cutaneous lupus erythematosus (SCLE), the most photosensitive type of lupus erythematosus (LE) [9], SS manifesting with AESS has been thought to be the Oriental counterpart of SCLE. However, there are substantial differences in clinical and histopathological features between the two types of skin

lesion [3, 4, 9]. Therefore, the differentiation of SS from SCLE/SLE and the management of diversified skin manifestations seen in SS patients remains an important issue.

Methods

In this study, 28 cases from our department [primary SS, 23 cases; secondary SS, 5 (SLE, 2; RA, 2; mixed connective tissue disease,1)] which fulfilled the criteria proposed by the Japanese Ministry of Health and Welfare and presented distinct AESS reported previously were enrolled. In addition, 92 cases which presented AESS were selected from the Japanese and foreign case studies published between 1976 (first Japanese case, reported in the literature [10]) and 2007. Fifty-one English or German reports were accessed by PubMed when 'Annular erythema' and 'Sjögren's syndrome' were entered as key words. One hundred-eighteen Japanese publications are currently listed by access to (Igaku-Chuo-Zasshi) when 'Kanjo Kouhan' and 'She-guren Shoukou-gun' are entered as key words. Eleven foreign studies [6–9, 11–17], except for our articles [3–5], from PubMed and 53 Japanese reports from Igaku-Chuo Zasshi (1983–2007) [10, 18–69] as well as those from our own research (1976–1982) were selected according to the following criteria: (1) fulfilling the diagnostic criteria of SS; (2) clinical and histopathological features, including a coat-sleeve infiltration pattern of T lymphocytes around the blood vessels and skin appendage without the epidermal changes in the cutaneous LE consistent with our first report [3]. Cases of apparent SCLE or Sweet disease with characteristic clinical and histopathological cases were excluded, even though such cases were published as AESS. Cases of both primary and secondary SS were enrolled in this study because other collagen diseases do not present the AESS-type erythema. The clinical characteristics and the most optimal management of these skin manifestations in SS were evaluated, taking into account age, gender, persistent time of AESS before consultation, applied medications, and clinical effects. The diagnostic criteria were Copenhagen, Greece, Japan, and California [70], and the revised form in 2002 [71], as well as the 1999 revised Japanese criteria for SS [72]. Factors differentiating AESS from SCLE are summarized in Table 1 [73–76].

Results

Clinical features of AESS

Three clinical types of AESS have been characterized: isolated donut-ring-like erythema mimicking sweet disease with an elevated border (type I), SCLE-like marginally scaled polycyclic erythema (type II), and papular insect bite-like erythema (type III). All types show common histological characteristics, including a coat-sleeve infiltration pattern of T lymphocytes around the blood vessels and skin appendage without the epidermal changes seen in cutaneous LE (Fig. 1). The demographic/clinical characteristics of the 120 cases were (1) gender ratio (male/female), 20/100; (2) age, 7–78 years (average 36.35 years; Fig. 2); (3) clinical subset: type 1, 101; type 2, 9; type 3, 6; mixed cases of each type, 4; (4) race: Japanese, 113; Korean, 3; Turkish, 2; Chinese, 1; Burmese, 1. Positive anti-SSA/SSB antibodies: (+)/(+), 93; (+)/(−), 17; (−)/(+), 2; (−)(−), 5; unknown, 3. The clinical course of AESS before the patient visited the dermatology clinic: <1 month, 11 cases; 1–2 months, 19 cases; 2–3 months, 18 cases; 3–4 months, 9 cases; >4 months, 3 cases; unknown, 60 cases (Fig. 3).

Management of AESS

Several approaches to manage AESS have been reported (Fig. 4). Oral or topical glucocorticoids are the first line of therapy for AESS. About 12.5% of the patients receive no medication. A recent report described the beneficial effect of tacrolimus [14]. Chloroquine or thalidomide are preferentially used in Korea or Europe [15–17]. The clinical dose of oral glucocorticoid showed two major peaks of prednisolone around 5–15 and 20–30 mg (Fig. 5).

The most effective dose of oral glucocorticoid to control AESS was 5–15 mg oral prednisolone, while AESS recurred at least twice in patients receiving more than 20 mg of prednisolone, which implies there may be several subsets of AESS (Fig. 6). AESS subsided in about 50% of the cases without oral glucocorticoid administration, which may indicate that oral prednisolone only shortened the persistency of AESS but did not downregulate the inflammatory changes underlying AESS. The cases without oral glucocorticoid were treated with topical steroid ointment or other medicine, as described in Fig. 4. The reported time of resolution of AESS following treatment with oral or topical glucocorticoid therapy was compared. Figure 7 shows that AESS responds more rapidly to oral glucocorticoid than to topical glucocorticoid (9 cases vs. 4 cases within 1 month), which is also faster than the natural course of AESS, as shown in Fig. 3. In daily clinical practice, oral glucocorticoid is given to control systemic manifestations of SS, such as parotid gland swelling, joint pain, or high fever that are unresponsive to non-steroidal anti-inflammatory drugs (NSAIDs). However, oral glucocorticoid was administered to the AESS patients without any systemic manifestations in this study (35/52), respectively (Fig. 8).

Table 1 Differentiation of AESS and SCLE

Differentiating factors	AESS	SCLE
Location	Face, upper extremities, sole	Sun exposed area
Clinical features	Annular erythema (infiltrated) type 1	Annular polycyclic erythema
	Polycyclic erythema type 2	Papulosquamous erythema
	Papular erythema type 3	
HLA-DR	DRw52	DR3
Photosensitivity	Occasionally	Frequent
Keratinocyte iNOS	Weak expression in mid-dermis	Strong expression in whole epidermis
Lupus band test (%)	40–50	60
Inflammatory cell infiltration	Deep perivascular, periappendageal infiltration	Superficial perivascular infiltration
Follicular plugging	Rare	Frequent
Epidermal atrophy	Rare	Frequent
Liquefaction degeneration	Rare	Frequent
Topical glucocorticoid	Poor response	Fair response

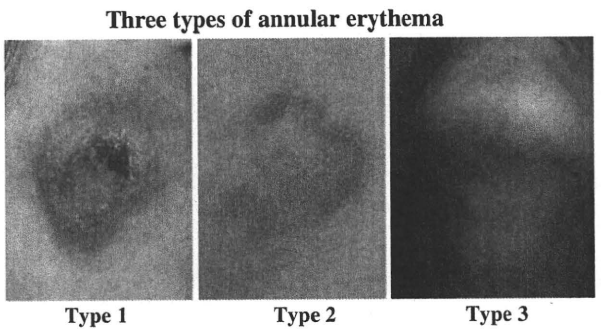

Fig. 1 Clinical characteristics of annular erythema in Sjögren's syndrome (SS). There are three clinical types of annular erythema with Sjögren's syndrome (AESS): isolated donut-ring like erythema with an elevated border (type I), subacute cutaneous lupus erythematosus (SCLE)-like marginally scaled polycyclic erythema (type II), and papular insect bite-like erythema (type III). All types those show common histological characteristics

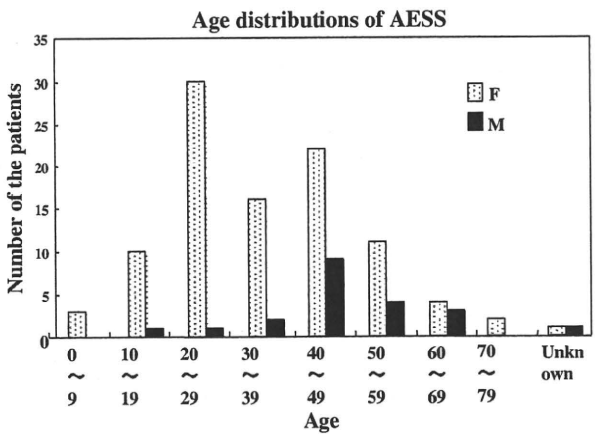

Fig. 2 Clinical characteristics of 120 cases. Gender ratio [male (*M*):female(*F*)] 20:100; age 7–78 years (average 36.35 years)

Discussion

It has been reported that there are more than 2 million SS patients in the USA [77]. Manifestations of SS can be either glandular or extra-glandular. Most of the infiltrating cells are CD4(+) and CD45RO(+) T cells [78]. These cells express the oligoclonal T cell receptor Vbeta2/13, produce interleukin (IL)6, IL16, interferon (IFN)γ, and transforming growth factor (TGF)β, and disrupt epithelial cells through the FAS/FAS-L system [79]. Minor populations of CD8(+) T cells injure the salivary gland through the mediation of perforin/granzyme [80]. Tissue-specific autoantibodies, such as anti-αFodrin antibody or anti-muscarine M3 receptor antibody, are also thought to play some part in the induction of SS [81]. These mechanisms may also trigger AESS.

AESS has been recognized to be a specific, cutaneous manifestation associated with SS. In 1989, four patients with primary SS presented a distinct annular erythema characterized by a wide, elevated border (which can be likened to a doughnut ring) and central pallor [3]. This rather unique erythema, designated AESS, could be clinically and histologically differentiated from both autoimmune annular erythema and SCLE. It preferentially occurs in Asian—but not Western—populations. An immunogenetic analysis by Miyagawa suggested that SS-associated erythema has a close association with HLA-DRw52, a relatively common locus among Orientals [76]. To date, three clinical types have been identified: isolated donut-ring-like erythema mimicking Sweet disease with an elevated border (type I), SCLE-like marginally scaled polycyclic erythema (type II), and papular insect bite-like erythema (type III). All of these show common histological characteristics and meet the diagnostic criteria of SS. The clinical features of annular erythema were analyzed in 22