1. Introduction

Photocontact dermatitis is a specialized form of contact dermatitis evoked by various chemicals, such as halogenated salicylanilides, musk ambrette, benzophenone-3 (oxybenzone), and non-steroidal anti-inflammatory drugs [1]. Patients develop dermatitis, when their skin is exposed to these agents and subsequent ultraviolet (UV) light. This disorder is pathohysiologically divided into two types, phototoxic and photoallergic ones. While the phototoxic dermatitis is mediated by oxygen intermediates, the photoallergic type, also known as contact photosensitivity, is a well-organized cutaneous sensitivity that is immunologically induced and elicited with photoallergic agent and UVA. Recently, the incidence of the photoallergic type is higher than the phototoxic one, because the major causative agents are non-steroidal anti-inflammatory drug with photoallergic properties [2].

It has been clarified that the majority of photoallergic agents are photohaptens, which bind covalently to protein via the formation of free radicals resulting from UV irradiation [2]. Because of this photobinding ability, cells are easily photomodified with photohapten under exposure to UVA, which is the action spectrum of photocontact dermatitis. The main sequential events in photocontact dermatitis have been investigated with 3,3′,4′,5-tetrachlorosalicylanilide (TCSA) in mice [3,4] and are virtually the same as those of ordinary contact dermatitis except for the requirement of UV irradiation in sensitization and challenge. Photoconjugation of epidermal cells with TCSA is the initial step. Langerhans cells (LC), which are professional antigen-presenting cells in the epidermis, play an important role. Photohapten-bearing LC migrates to draining lymph nodes in the sensitization phase [5]. T cells sensitized by photohapten-bearing LC induce the photosensitivity [3] and suppressor or regulatory T cells involved in this sensitivity have been identified [6].

Ketoprofen (KP), widely used as a topical non-steroidal anti-inflammatory drug, is clinically well known to induce the allergic type of photocontact dermatitis [7–11]. In addition to the high incidence of occurrence of photocontact dermatitis, KP is an interesting drug in its cross-photoreactivity with thiaprofenic acid, suprofen, phenofibrate, and benzophenone-3 [7–11]. These substances have a photohaptenic moiety [2] as well as a phototoxic ability [12,13].

A model of KP photocontact dermatitis has been reported using guinea pigs [11]. However, little is known regarding the immunological characteristics of photocontact dermatitis to KP. In this study, we established a murine model of KP photosensitivity and investigated the immunological mechanism, focusing on the involvement of Th1 and Th2 cells.

2. Materials and methods

2.1. Animals

AKR/N (H-2^k), CBA/J (H-2^k), C3H/He (H-2^k), BALB/c (H-2^d), DBA/2 (H-2^d), A/J (H-2^a), C57BL/6 (B6; H-2^b) were obtained from Kyudo Co. Ltd. (Kumamoto, Japan). BALB.K/Ola (H-2^k) mice were kindly provided by National Institute of Genetics (Mishima, Japan). Female mice, 8-week old, were used in this study.

2.2. Chemicals

KP was obtained from Hisamitsu Pharmaceutical Co. Inc. (Tokyo, Japan) and 3,3′,4′,5-tetrachlorosalicylanilide (TCSA) was purchased from Kanto Chemical Co. Inc. (Tokyo, Japan).

2.3. Light source

Black light (FL20SBL-B) emitting UVA ranging from 320 to 400 nm with a peak emission at 365 nm was purchased from Toshiba Electric Co. (Tokyo, Japan). With a UV radiometer (Topcon Technohouse Corp., Tokyo, Japan), the energy output of three 20 W tubes of black light at a distance of 20 cm was 2.4 mW/cm^2 at 365 nm and 0.17 mW/cm^2 at 305 nm.

2.4. Photosensitization and photochallenge to KP

The basic method for photosesitization and photochallenge was described previously [3,4]. Mice were painted with 50 μl of 1, 2 or 4% KP in acetone to the clipped abdomen. Within 30 min, the painted site was irradiated with three tubes of black light at a distance of 20 cm for 2.5 h (20 J/cm^2 at 365 nm) unless otherwise mentioned by placing cages containing mice over the lights. We used a pane of window glass 3 mm thick to insure that no radiation below 320 nm reached the skin. The painting plus irradiation was performed on two consecutive days, i.e., days 0 and 1. Before challenge, the basal line thickness of both ears on all mice was measured with a dial thickness gauge. On day 5, all mice were challenged on both sides of each earlobe with 25 μl of 2% KP in ethanol unless otherwise described. Within 30 min, the mice received irradiation under black light at a distance of 20 cm at 20 J/cm^2 at

365 nm. Ear thickness was measured 24 h after irradiation and was expressed as the mean increment in thickness above basal line control value.

2.5. Preparation of single cell suspension of lymph node cells (LNC) and epidermal cells

Axillary and inguinal lymph nodes were collected on day 3 or 5 from mice photosensitized with KP on days 0 and 1. Single cell suspensions were prepared by teasing lymph nodes. For preparation of epidermal cells, excised murine earlobes were incubated in 0.2% trypsin. Epidermal cells were dispersed and washed three times in PBS, as described previously [14].

2.6. Adoptive transfer of sensitivity with immune LNC

Immune LNC were prepared on day 5 from KP-photosensitized AKR/N mice. To obtain purified $CD4^+$ or $CD8^+$ T cells, LNC were incubated with anti-CD4 or anti-CD8 monoclonal antibody (mAb)-conjugated magnetic beads (Dynal Inc., Oslo, Norway) and the bound cells were detached from the beads with Detachabeads (Dynal Inc.) according to the manufacture's directions. Unfractionated LNC (2×10^7 cells/mouse) or varying ratios of $CD4^+$ or $CD8^+$ T cells in 0.4 ml of phosphate buffered saline (PBS; pH 7.4) were injected i.v. into naïve recipients. The control mice were injected with PBS alone. Within 1 h after cell transfer, the recipient and control mice were challenged on the ears with 2% KP plus 20 J/cm^2 UVA, and ear swelling response was measured after 24 h. In a comparison, BALB/c mice were sensitized with 1% TCSA plus 12 J/cm^2 UVA on days 0 and 1, as described previously [3,4], and LNC were transferred to naïve syngeneic mice. Epicutaneous sensitization and challenge with TCSA plus UVA was reported [3].

2.7. Reverse transcription-polymerase chain reaction (RT-PCR) assay

Total RNA was extracted from LNC or epidermal cells using the SVTotal RNA Isolation system (Promega Co., Madison, WI, USA). To prepare first strand cDNA, 1 μg of RNA was incubated in 100 μl of buffer containing 10 mM dithiothreitol, 2.5 mM $MgCl_2$, dNTP mix, 200 U of reverse transcriptase II (Invitorogen, Carlsbad, CA, USA) and 0.1 mM oligo $(dT)_{12-18}$ (Invitorogen). Each cDNA were amplified in a 50 μl PCR solution containing 0.8 mM $MgCl_2$, dNTP mix and DNA polymerase (Roche Applied Science, Penzberg, Germany) with synthesized primers. Samples were heated to 95 °C for 2 min, 55 °C for 2 min and 72 °C for 3 min, and cycled 40 times through 95 °C for 1 min, 55 °C for 2 min and 72 °C for 3 min. The final incubation was 72 °C for 7 min. The mixture was subjected to 1% agarose gel for electrophoresis with the indicated markers and primers for the internal standard β-actin. Each sample was applied more than two lanes in the same gel. The agarose gel was stained with ethidium bromide and photographed with UV transillumination.

The sense/antisense primer sequences were as follows. Interferon-γ (IFN-γ): 5′-TGA ACG CTA CAC ACT GCA TCT TGG-3′ and 5′-CGA CTC CTT TTC CGC TTC CTG AG-3′; IL-4: 5′-ATG GGT CTC AAC CCC CAG CTA GT-3′ and 5′-GCT CTT TAG GCT TTC CAG GAA GTC-3′; CXCR3: 5′-GCC GAT GTT CTG CTG GTG TTA A-3′ and 5′-TTT TCG ACC ACA GTT GCG GGC-3′ CCR4: 5′-TCG GAT TTG CTG TTC GTC CTG T-3′ and 5′-TAA GGC AGC AGT GAA TGA AGC C-3′; IP-10; 5′-CGC ACC TCC ACA TAG CTT ACA G-3′ and 5′-CCT ATC CTG CCC ACG TGT TGA G-3′; Mig: 5′-TGA TAA GGA ATG CAC GAT GCT C-3′ and 5′-TTC CTT GAA CGA CGA CGA CTT T-3′; TARC: 5′-CAG GAA GTT GGT GAG CTG GTA TAA-3′ and 5′-TTG TGT TCG CCT GTA GTG CAT A-3′; MDC: 5′-TCT GAT GCA GGT CCC TAT GGT-3′ and 5′-TTA TGG AGT AGC TTC TTC ACC CAG-3′; β-actin: 5′-TGG AAT CCT GTG GCA TCC ATG AAA C-3′ and 5′-TAA AAC GCA GCT CAG TAA CAG TCC G-3′.

2.8. Statistical analysis

Student's *t*-test was employed to examine the significance between the means, and $p < 0.05$ was considered statistically significant.

3. Results

3.1. Induction of photocontact dermatitis by KP plus UVA

As shown in Fig. 1, AKR/N mice were sensitized by topical painting of 4% KP and subsequent irradiation with 20 J/cm^2 UVA, or by KP alone. They were challenged on the earlobes with 2% KP and/or 20 J/cm^2 UVA. A significant ear swelling response was observed in mice challenged with both KP and UVA, whereas elicitation with KP or UVA alone did not induce the response. In mice sensitized with 4% KP alone, KP plus UVA evoked a detectable swelling response. Since elicitation with KP alone did not yield any response, this was considered to be a phototoxic response, and was significantly lower than the photoallergic response to KP. Thus, treatment with KP plus UVA was capable of inducing the allergic type of photocontact dermatitis in mice.

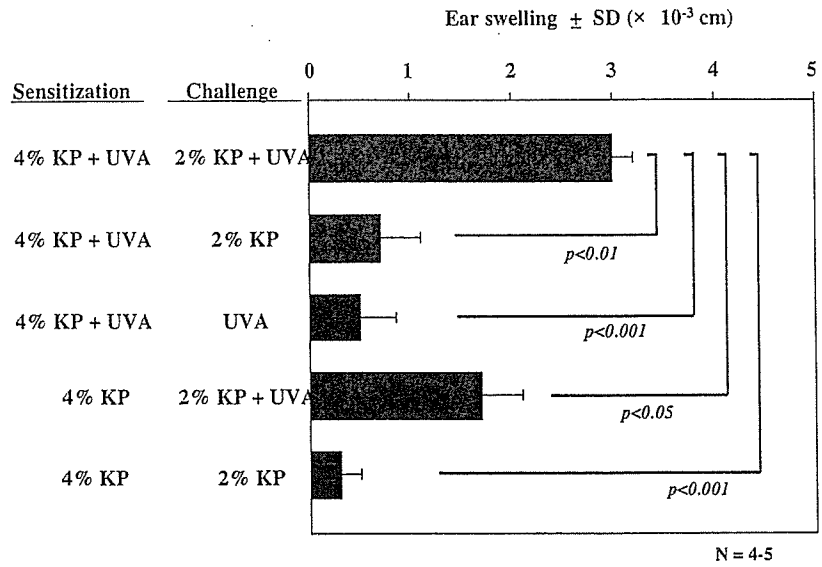

Fig. 1 Requirement of both KP and UVA for induction and elicitation of allergic photocontact dermatitis. AKR/N mice were sensitized with KP (4%) plus UVA (20 J/cm^2) or KP alone on days 0 and 1. On day 5, they were challenged on both sides of each earlobe with KP (2%) and/or subsequent UVA (20 J/cm^2). Ear swelling was measured 24 h after irradiation. Each column represents the mean ± S.D.

3.2. KP concentration and UVA dose effective for induction and elicitation of photocontact dermatitis

AKR/N and C3H/He mice were sensitized with 1, 2, or 4% KP in combination with 20 J/cm^2 UVA, and challenged with 2% KP plus 20 J/cm^2 UVA (Fig. 2A). KP at both concentrations of 2 and 4% produced significant ear swelling responses, with the latter being slightly more effective than the former. When 4% KP-photosensitized mice were challenged with 1, 2, or 4% KP in combination with 20 J/cm^2 UVA, 2 and 4% KP induced comparable responses in AKR/N mice, while all three concentrations of KP produced significant responses in C3H/He (Fig. 2B). When AKR/N mice were sensitized with 4% KP alone and challenged with 2% or 4% KP plus UVA, photochallenge with 4% KP produced two-fold higher swelling than 2% KP (data not shown), indicating that the phototoxic response of 2% KP was low.

AKR/N mice were sensitized with 4% KP and UVA at 10, 20, 30 or 40 mJ/cm^2, and challenged with 2% KP plus 20 mJ/cm^2 UVA (Fig. 2C). UVA at 20, 30, and 40 mJ/cm^2 yielded significant and comparable responses. Therefore, we used 4% KP for photosensitization and 2% KP for photochallenge in combination with 20 mJ/cm^2 UVA in the following experiments.

3.3. Different reactivity in photocontact dermatitis among various mouse strains

Eight strains of mice were sensitized and challenged with KP plus UVA. AKR/N, CBA/J, C3H/He, BALB.K/Ola, and A/J exhibited higher responses than did BALB/c, DBA/2 and B6 mice (Fig. 3). Considering that BALB/c and BALB.K/Ola mice are H-2-congenic strains and thus differ only at the H-2 complex, it seems that H-2^k mice are high responders in this sensitivity.

3.4. Adoptive transfer of photocontact dermatitis

Immune LNC were taken from AKR/N mice photosensitized with KP 5 days before and injected i.v. into naïve recipients, which were challenged with KP plus UVA. As positive control, a group of mice were epicutaneously sensitized and challenged in parallel. Fig. 4A shows that mice receiving 2 × 10^7 LNC exhibited a significant degree of photocontact dermatitis but to a lesser degree than the epicutaneously sensitized mice. Along with this study, immune LNC from BALB/c mice photosensitized with TCSA were transferred to naïve syngeneic recipients. They had a stronger but similarly reduced level of response as compared to the epicutaneously sensitized mice. When donor mice were treated with UVA alone, transfer of their LNC did not induce the sensitivity in recipients (Fig. 4B).

3.5. Essential and augmentative roles of CD4$^+$ and CD8$^+$ cells, respectively, in photocontact dermatitis

CD4$^+$ or CD8$^+$ T cells (5 × 10^6 cells/mouse) purified from KP-immune LNC of AKR/N mice were trans-

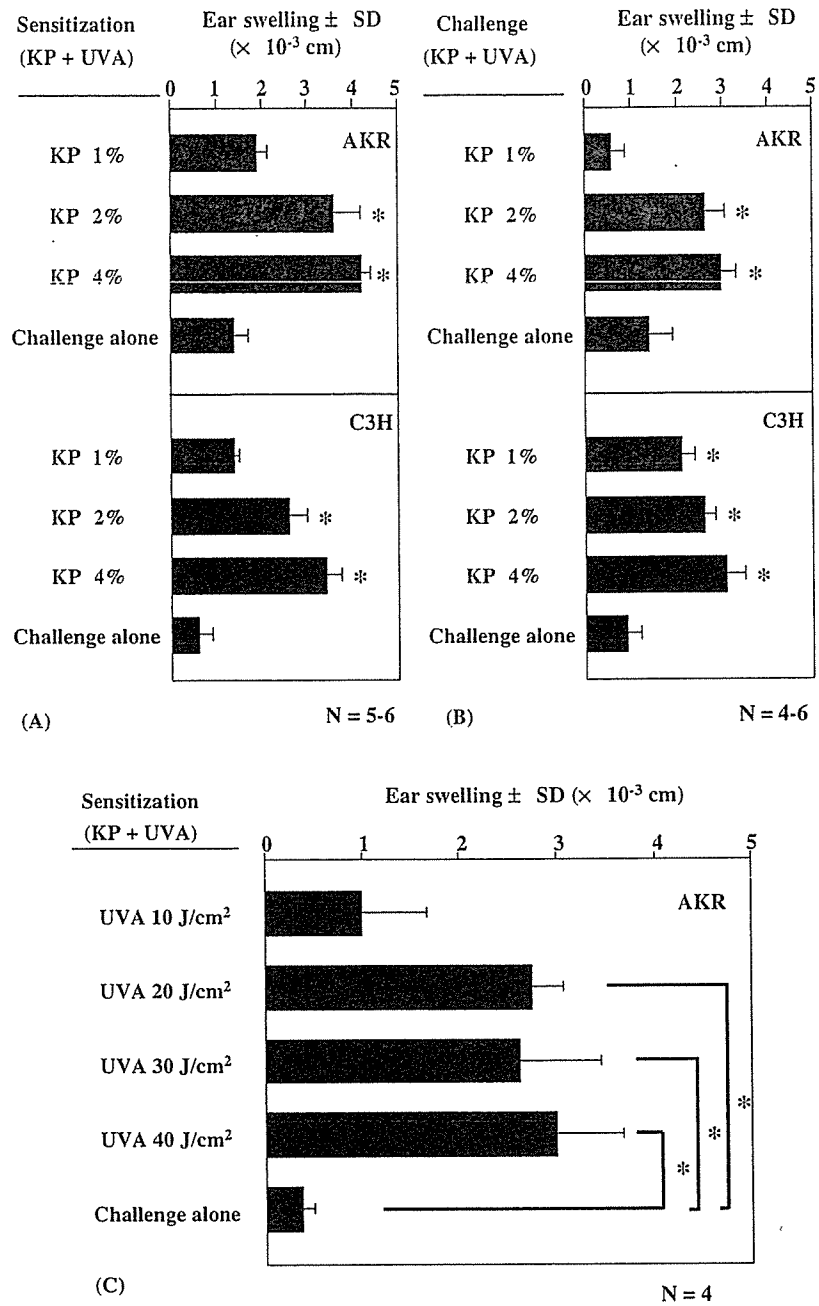

Fig. 2 Effects of KP concentration and UVA dose on sensitization and challenge of photocontact dermatitis. AKR/N and C3H/He mice were sensitized with varying doses of KP (1–4%) (A) or 4% KP (B) plus UVA (20 J/cm^2) on days 0 and 1. On day 5, they were challenged on both sides of each earlobe with 2% KP (A) or varying doses of KP (1–4%) (B) plus UVA (20 J/cm^2). In (C), AKR/N mice were sensitized with 4% KP plus varying doses (10–40 J/cm^2) of UVA and challenged with 2% KP plus 20 J/cm^2 UVA. Ear swelling was measured 24 h after irradiation. Each column represents the mean ± S.D. $^*p < 0.05$.

ferred to naïve syngeneic recipients. Upon challenge with KP plus UVA, mice injected with CD4$^+$ cells, but not CD8$^+$ or CD4$^-$CD8$^-$ cells, exhibited a significant swelling response compared to the non-injected control mice (Fig. 5). When mice were injected with increasing numbers of CD8$^+$ cells additionally with CD4$^+$ cells, 5×10^6 cells, but not 1 or 2.5×10^6 cells, enhanced the CD4$^+$ cell-induced response. This combination of CD4$^+$ and CD8$^+$ cells produced a comparable response to the epicutaneously sensitized mice. The results suggested that CD4$^+$ T cells mediate the sensitivity and CD8$^+$ T cells participate in the full responses.

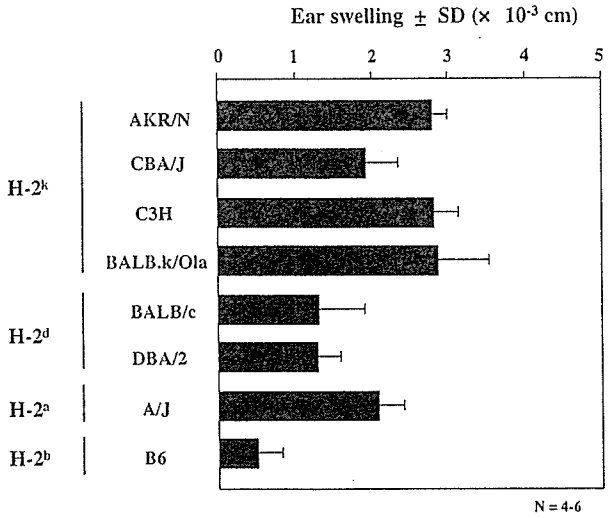

Fig. 3 Photocontact dermatitis to KP in various mouse strains with different H-2 haplotypes. Mice were sensitized with 4% KP plus 20 J/cm^2 UVA on days 0 and 1. On day 5, they were challenged with 2% KP and 20 J/cm^2 UVA. Data are expressed as: Δear swelling, representing (sensitization and challenge) − (challenge alone). Each column represents the mean ± S.D.

3.6. Elevated mRNA expression of cytokines and chemokine receptors of both Th1 and Th2 cells in immune LNC

AKR/N mice were sensitized with KP plus UVA on days 0 and 1, and single cell suspensions of immune LNC were prepared on day 1 (immediately after sensitization), 3 and 5. The expression of Th1 and Th2 cytokines, as represented by IFN-γ and IL-4, respectively, was examined by RT-PCR. As shown in Fig. 6A, KP photosensitization increased the expression of both cytokines compared to vehicle alone. Notably, IL-4 was markedly augmented by KP plus UVA on day 5.

Th1 and Th2 cells express chemokine receptor CXC chemokine receptor 3 (CXCR3) and CC chemokine receptor 4 (CCR4), respectively. The expression of these chemokine receptors was also tested in immune LNC. As most discernibly seen in day 5 LNC, CCR4 expression was remarkably enhanced by sensitization with KP plus UVA, while CXCR3 was increased to a lesser degree (Fig. 6B).

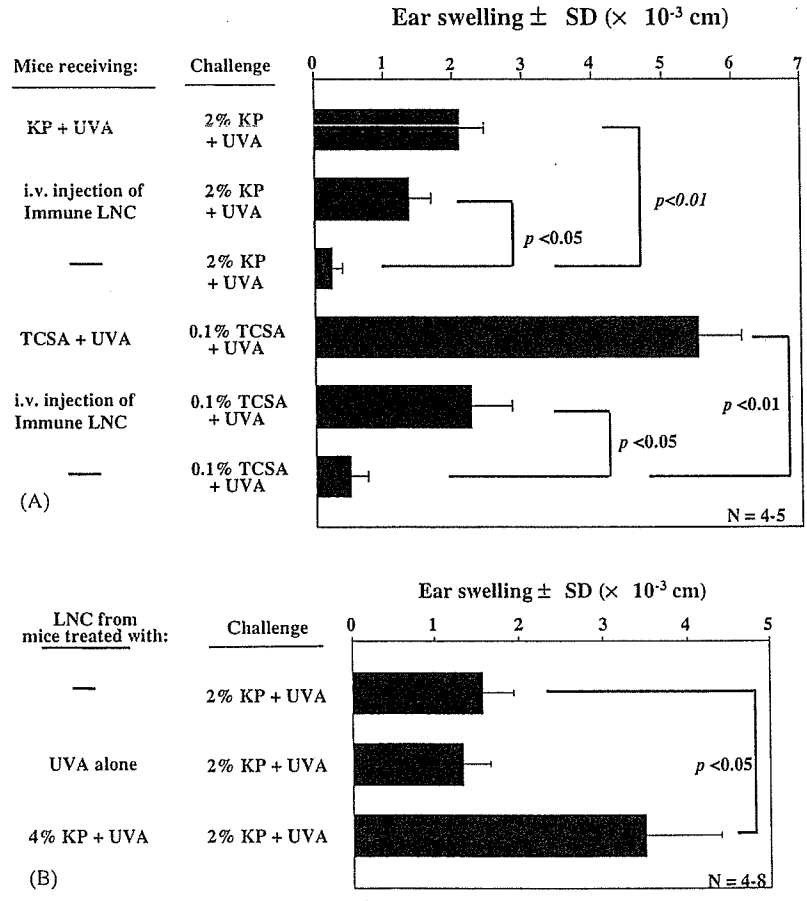

Fig. 4 (A) and (B) Transfer of LNC from KP- or TCSA-photosensitized mice. AKR/N (for KP) and BALB/c (for TCSA) mice were injected i.v. with immune LNC (2 × 10^7 cells/mouse) from KP- or TCSA-photosensitized mice. The control mice were not injected. Within 1 h after cell transfer, the recipient and control mice were challenged with 2% KP plus 20 J/cm^2 UVA or 0.1% TCSA plus 20 J/cm^2 UVA, and ear swelling response was measured after 24 h. Each column represents the mean ± S.D.

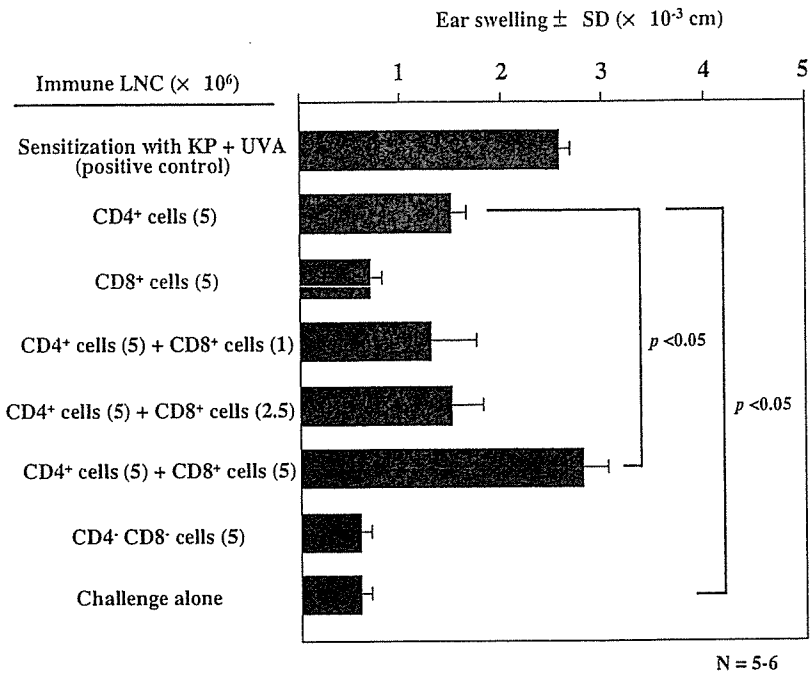

Fig. 5 Transfer of $CD4^+$ and/or $CD8^+$ cells from KP-photosensitized mice. Mice were injected with intravenous injection of purified $CD4^+$ and/or $CD8^+$ T cells from KP-photosensitized AKR/N mice. The control mice were not injected. Within 1 h after cell transfer, the recipient and control mice were challenged with 2% KP plus UVA irradiation. Each column represents the mean ± S.D.

These results suggested that Th2 cells as well as Th1 cells are stimulated in photocontact dermatitis to KP, with the former being more enhanced by this phototreatment.

3.7. Elevated mRNA expression of chemokines of both Th1 and Th2 cells in challenged epidermis

Murine epidermal keratinocytes produce Th1 chemokines, interferon-inducible protein-10 (IP-10/CXCL10) and monokine induced by interferon-γ (MIΓ/CXCL9), and Th2 chemokines, thymus and activation-regulated chemokine (TARC/CCL17) and macrophage derived chemokine (MDC/CCL22). These Th2 chemokines bind to CCR4 on Th2 cells, while the Th1 chemokines have affinity to CXCR3 on Th1 cells [15]. To address the role of these chemokines in infiltration of Th1 and Th2 cells at the challenged site, AKR/N mice were sensitized with KP and UVA, and 5 days later, challenged on the earlobes with KP or vehicle in combination with UVA. Epidermal cell suspensions were prepared from the ears 24 and 48 h after challenge and subjected to RT-PCR. At 24 h after challenge, the expression of Mig and TARC was increased by treatment with KP plus UVA, as compared to no treatment or vehicle alone (Fig. 7). The expression at 48 h was virtually the same as that at 24 h, but less discernible. IP-10 and MDC were not substantially changed. Thus, both certain Th1 and Th2 chemokines, but not all, were expressed increasingly in the challenged epidermis.

4. Discussion

The present study was aimed to establish a murine model of photocontact dermatitis to KP. The photosensitivity was successfully induced and elicited by skin application of KP and subsequent irradiation with UVA. The optimal concentration of KP was 4% for sensitization and 2% for elicitation, and the dose of UVA was 20 J/cm^2. In a comparison with a representative allergic photocontactant TCSA [3,4], these concentration and dose are high, and the degree of ear swelling response is low. Patients with photocontact dermatitis to KP exhibit a strong erythematous reaction, and even bulla formation occurs in some patients [8–11,16]. Our present system, therefore, is not a complete mimicry to the clinical photosensitivity. Nevertheless, the photoallergic potential of KP can be evaluated by this murine model.

The magnitude of response depended on the strain of mice, and at least the major histocompatibility complex (MHC) seems to influence the response. H-2^k mice are high responders compared to H-$2^{d,b}$ mice. This is strikingly in contrast to photocontact dermatitis to TCSA, in which H-$2^{d,b}$ mice are high responders, while H-2^k is the low

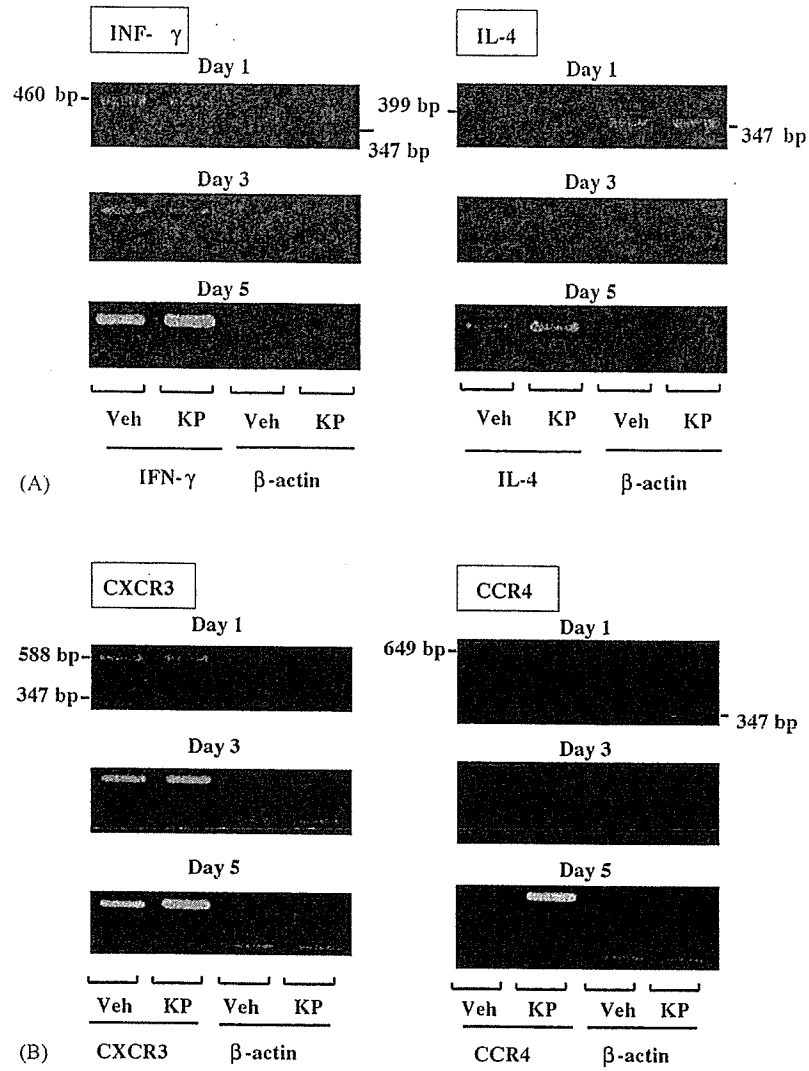

Fig. 6 RT-PCR analysis of mRNA expression of cytokines and chemokine receptors in immune LNC. LNC were taken from mice 1 h (day 1), 48 h (day 3) and 96 h (day 5) after sensitization with KP plus UVA. Each assay was performed four times using two independent samples. Representative data were shown. Veh: vehicle alone.

responder haplotype [4]. Thus, the susceptibility to photocontact dermatitis in individuals appears to be different depending on each photocontactant. The majority of exogenous photoallergic substances have a photohaptenic property [2,17]. Photohaptens are capable of binding to MHC class II molecules/self peptides on LC upon exposure to UVA [18]. In this context, the T cell response is likely controlled by MHC molecules.

In the adoptive transfer study, injection of $CD4^+$ T cells was crucial to evoke the sensitivity, but transfer of both $CD4^+$ and $CD8^+$ cells resulted in a higher response. In accordance with the present study, cutaneous photoallergy to exogenous agents is mediated by $CD4^+$ T cells [4,17,18]. The roles of $CD4^+$ and $CD8^+$ T cells in ordinary contact hypersensitivity remains disputed. Several independent studies have shown mediation of the sensitivity by $CD8^+$ T cells [19–21]. On the other hand, the contribution of $CD4^+$ cells has been variously reported, as $CD4^+$ cells are unnecessary [21], helpful [22,23], or suppressive [24,25]. Circumstantial evidence may indicate that $CD4^+$ cells participate more profoundly in photocontact hypersensitivity than ordinary contact hypersensitivity. For example, in vitro stimulation of immune LNC with photohapten results in the preferential propagation of $CD4^+$ cells, and the sensitivity can be transferred to naïve mice with $CD4^+$ T cell line [17]. In such a case, $CD8^+$ T cells may be required for the full development of the sensitivity.

In the draining LNC, mRNAs for not only IFN-γ and CXCR3 but also IL-4 and CCR4 were increasingly expressed. Rather, the expression levels of these Th2-relevant molecules were higher than those of Th1. Such a Th2 dominant state was also found in photosensitivity to TCSA [26]. In the skin, keratinocyte-derived chemokines initiate migration of T cells. mRNAs for TARC (a ligand for CCR4) and Mig

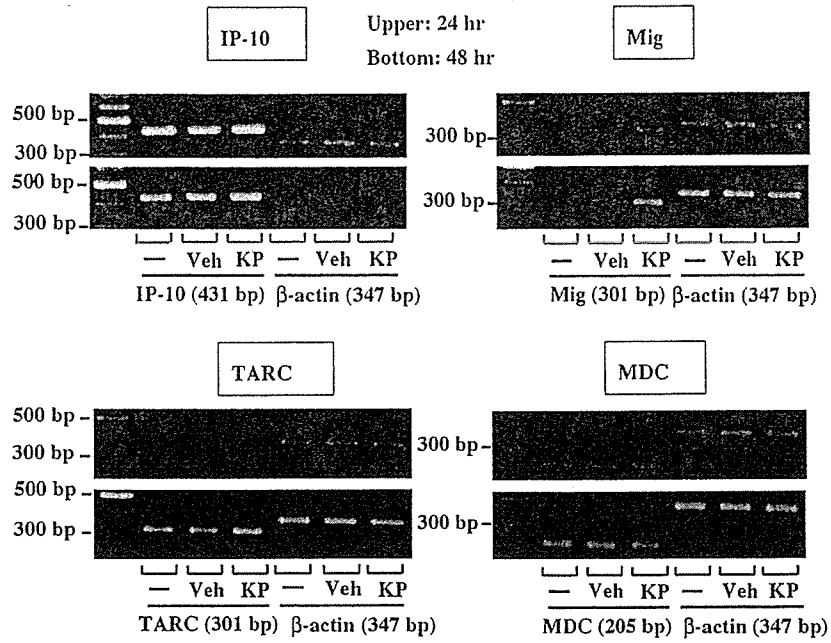

Fig. 7 RT-PCR analysis of chemokine mRNA expression in epidermal cells. Epidermal cell suspensions were prepared from earlobes of KP/UVA-sensitized mice 24 or 48 h after challenge with KP plus UVA. Each assay was performed four times using two independent samples. Veh: vehicle alone.

(a ligand for CXCR3) were also enhanced in the epidermal cells from earlobes of mice sensitized and challenged with KP plus UVA. This chemokine expression is different from that of ordinary contact sensitivity to picryl chloride, which shows apparent Th1 chemokine mRNA expression but no TARC expression in the challenged ears [27]. Of particular importance is whether these Th2 cells serve as effectors or suppressors in the sensitivity. We measured the percentage of $CD4^+CD25^+$ cells, indicative of regulatory T cells [24,25], in immune LNC from KP- or TCSA-photosensitized AKR/N or BALB/c mice, and found no increment of cells bearing this phenotype (data not shown). Together with the ability of $CD4^+$ T cells to transfer the sensitivity, these findings implies that $CD4^+$ cells play a helper or effector role in photocontact dermatitis to KP.

Photocontact dermatitis to KP is known to prolong at the applied skin site even several months after cessation of application [9–11]. This enigmatic phenomenon cannot be clarified from the present study. In addition to its photohaptenic moiety, KP might exert its pharmacological effect on LC [28], possibly leading to the prolongation. Elucidation of this phenomenon may characterize this photosensitivity more specifically.

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Formation of 8-hydroxy-2′-deoxyguanosine in the DNA of cultured human keratinocytes by clinically used doses of narrowband and broadband ultraviolet B and psoralen plus ultraviolet A

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Psoralen plus ultraviolet A (PUVA) and narrowband ultraviolet B (UVB) are widely used in skin disease phototherapy. Recently, the efficacy of UVB therapy has been greatly improved by narrowband UVB, compared to conventional broadband UVB. The objectives of the current study were to evaluate the influence of UVB-induced and PUVA-induced oxidative stress on cultured keratinocytes. We analyzed 8-hydroxy-2′-deoxyguanosine (8-OH-dG) in human keratinocytes (HaCaT cell line) using a high-performance liquid chromatography system equipped with an electrochemical detector. Non-irradiated human keratinocytes contained a baseline of 1.48 ± 0.22 (mean ± SD) 8-OH-dG per 10^6 deoxyguanosine (dG) residues in cellular DNA, which increased linearly with higher doses of UVB. When their abilities to induce 8-OH-dG were compared to each other, based on the minimal erythemal and therapeutically used doses, by irradiating them with broadband UVB at 100 mJ/cm², the amount of 8-OH-dG increased to 3.42 ± 0.46 residues per 10^6 dG, while a narrowband UVB treatment at 1000 mJ/cm², with biological effects comparable to those elicited by 100 mJ/cm² broadband UVB, increased it to 2.06 ± 0.31 residues per 10^6 dG. PUVA treatment, with 100 ng/mL 8-methoxypsoralen and 5000 mJ/cm² UVA, increased the 8-OH-dG level to 4.52 ± 0.42 residues per 10^6 dG. When HaCaT cells treated with 2000 mJ/cm² narrowband UVB were cultured and the amount of 8-OH-dG was monitored in the living cells, 65.6% of the residues were repaired 24 h after treatment. Our study provides a warning that widely used narrowband UVB and PUVA induce cellular oxidative DNA damage at the therapeutically used doses, although to a lesser degree than broadband UVB with the same clinically effective dose. (*Cancer Sci* 2006; 97: 99–105)

Eight-hydroxy-2′-deoxyguanosine (8-OH-dG), also known as 7,8-dihydro-8-oxo-deoxyguanosine (8-oxo-dG),[1] has been proposed as a key biomarker of oxidative DNA damage relevant to carcinogenesis[1,2] and pathogenesis of autoimmune disorders.[3,4] This DNA damage is induced by the reactions of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), superoxide anions (O_2^-), singlet oxygen and hydroxyl radicals (·OH).

Human skin is constantly exposed to environmental stresses, and is vulnerable to the effects of ROS generated by exposure to ultraviolet (UV) radiation.[5] Yamamoto *et al.*[6] reported that the formation of 8-OH-dG in DNA might be one of the mechanisms of daylight-induced mutagenesis. In fact, irradiation with a fluorescent sun lamp or with UVB does induce 8-OH-dG in the epidermis of hairless mice.[7,8]

Parrish and Jaenicke[9] found that 313 nm UVB radiation is the most effective wavelength for the treatment of psoriasis. This finding provided the impetus for developing the Philips TL-01 fluorescent bulb, a narrowband UVB light source that produces a spectral emission between 310 and 315 nm. Narrowband UVB phototherapy has thus significantly improved the therapeutic efficacy of conventional broadband UVB (290–320 nm) phototherapy for skin diseases such as psoriasis, atopic dermatitis, vitiligo and others.[10–13]

Narrowband UVB is widely used in the treatment of skin disease, and the current trend toward the increased use of narrowband UVB phototherapy is justified.[14] Its carcinogenic potential is judged to be substantially less than that of psoralen plus UVA (PUVA) photochemotherapy.[15] Although the results of studies in mice indicate that narrowband UVB could induce more skin cancers than broadband UVB therapy,[16] the participants in a workshop on the use of narrowband UVB in phototherapy concluded that the long-term human cancer risk should be no greater than that with broadband phototherapy.[17]

When the DNA damage in keratinocytes induced by narrowband or broadband UVB was measured by single cell gel electrophoresis (comet assay), narrowband UVB produced less DNA damage than broadband UVB at equal doses.[18] The formation of 8-OH-dG has also been reported in fibroblasts after UVA irradiation[19–21] and in normal human epidermal keratinocytes after broadband UVB exposure.[22] Using immunofluorescence staining methods, Budiyanto *et al.*[23] observed that in both mouse skin and organ cultured human skin cells, 250 and 500 mJ/cm² narrowband UVB yielded levels of cyclobutane pyrimidine (CPD/Py–Py) dimers similar to those induced by 25 and 50 mJ/cm² broadband UVB, respectively, which have biological effects comparable to 250 and 500 mJ/cm² narrowband UVB, respectively. However, the yields of 8-OH-dG after irradiation with 1000 and 3000 mJ/cm²

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narrowband UVB were 1.5–3 times higher than those obtained using 100 and 300 mJ/cm^2 broadband UVB, respectively.

The ratio of Py–Py dimers to 8-OH-dG formation in cellular DNA after UVB irradiation is 80–100:1,[19,24] while the ratio of psoralen-adducts to 8-OH-dG formation by PUVA treatment is 25:1.[25] Although UVB and PUVA treatments induce Py–Py dimers and psoralen-DNA adducts, respectively, as major cellular DNA modifications, our study focused on the analysis of 8-OH-dG as a marker of cellular oxidative stress for the following reasons: (1) not only initiation but also chronic inflammation-induced promotion and progression may be involved in UVB-induced skin carcinogenesis;[26,27] (2) antioxidants inhibit both UVB-induced 8-OH-dG formation and carcinogenesis in mouse skin;[28,29] and (3) in *Ogg1* (8-OH-Gua glycosylase) knockout mice, UVB irradiation induced both 8-OH-dG formation and an increase in skin tumors, suggesting that 8-OH-dG is involved in UVB-induced skin carcinogenesis.[30]

The purpose of the present study was to assess the oxidative stress induced by clinically used UV wavelengths, doses and apparatus. There has been no accurate analysis reported using a high-performance liquid chromatography (HPLC) system equipped with an electrochemical detector (ECD) of 8-OH-dG in human keratinocytes irradiated with narrowband UVB. PUVA is another modality whose potential to form 8-OH-dG should be investigated, because PUVA is the most widely used phototherapy for skin diseases. However, the PUVA-induced formation of 8-OH-dG has been reported only for human epidermoid carcinoma cells.[31]

In the present study, we quantified the 8-OH-dG formed in keratinocytes (HaCaT) after irradiation with clinically used doses of broadband and narrowband UVB, and PUVA. Our results provide information about the oxidative DNA damage-inducing potencies of these three phototherapies and the repair of 8-OH-dG.

Materials and Methods

Cells and culture conditions

The HaCaT cell line[32] was cultured in Dulbecco's Modified Eagle's Medium (DMEM; Nissui Pharmaceutical, Tokyo, Japan), supplemented with 10% fetal bovine serum, L-glutamine (2 mM), 100 units/mL penicillin, 100 μg/mL streptomycin sulfate and sodium pyruvate (1 mM), and was maintained at 37°C in a humidified atmosphere containing 5% CO_2 in air. Unless otherwise mentioned, all culture supplies were purchased from Gibco-Invitrogen (Carlsbad, CA, USA).

Ultraviolet irradiation of cells

The cells were seeded into 100-mm tissue culture dishes and allowed to attach for a period of 16–24 h at 37°C. Before UV irradiation, the culture medium was removed and 5 mL of phosphate-buffered saline (PBS, pH 7.4) were placed over the monolayer, so that the depth of the solution was always 0.1 cm, to prevent cell drying and reflection of UV. A total of approximately 5×10^6 keratinocytes in a 100-mm dish were exposed to UV irradiation at room temperature. Broadband UVB irradiation was applied at a wavelength range of 280–370 nm, peaking at 305 nm, using a bank of five FL.20SE.30 medical sun lamps (Toshiba, Tokyo, Japan) emitting mainly UVB, but also small amounts of UVA and UVC. The irradiation was 1.0 mW/cm^2 at a distance of 33 cm, as measured with a radiometer (UVR-3036/S; Toshiba). Narrowband UVB irradiation was carried out with a bank of four TL-20 W/01 lamps (Philips, Eindhoven, Holland) at a wavelength range of 310–315 nm (emission maximum at 313 nm, almost monochromatic) housed in a luminaire (type UV801 KL-1; Waldmann, Villingen-Schwennigen, Germany). For PUVA treatment, keratinocytes were exposed to UVA produced by six 40-watt CLEO lamps (Philips) at a wavelength range between 315 and 400 nm with a peak emission at 355–365 nm, housed in a Waldmann luminaire. The distance from the light source was maintained at 25 cm. The dosimetry was monitored with a UV meter (type 585200000; Waldmann) equilibrated for the UV sources according to the manufacturer's instructions. Control cells were incubated in PBS without irradiation. At several time points after irradiation, the adherent cells were harvested, washed with ice-cold PBS and processed immediately for DNA isolation.

Cell viability

A portion of each cell suspension obtained from the control and irradiation experiments was used to determine cell viability. Cell viability was determined using the trypan blue dye exclusion test (0.4%) (Gibco-BRL, Grand Island, NY, USA). Due to the toxicity of UV light, we collected the adherent cells immediately after irradiation unless otherwise mentioned. As damaged cells gradually became detached during the culture period, depending on the UV irradiance, only adherent cells with viability above 90% were subjected to the analysis in the time course experiments.

PUVA treatment

Stock solutions were prepared by dissolving crystalline 8-methoxypsoralen (8-MOP) (Sigma, St Louis, MO, USA) in absolute ethanol (100 μg/mL). Before UVA irradiation, 10 μL of the 8-MOP stock solution were added to 10 mL of PBS for the keratinocyte culture. A final 8-MOP concentration of 100 ng/mL was chosen, as the mean plasma concentration in humans receiving PUVA therapy is approximately 100 ng/mL.[33] After an incubation at 37°C for 30 min in the dark, the cells were irradiated with UVA.

Determination of 8-OH-dG in cellular DNA

Cellular DNA was isolated using a DNA extractor WB kit containing NaI (Wako, Osaka, Japan).[34,35] Desferal (deferoxamine mesylate; Sigma) was added to the lysis solution (1 mM) to prevent DNA oxidation.[36] The isolated DNA was digested with 8 units of nuclease P1 (Yamasa, Choshi, Japan) in a 100 μL solution containing 1 mM ethylenediaminetetracetic acid (EDTA) and 10 mM sodium acetate (pH 4.5), and was then treated with alkaline phosphatase (2 units) in a 250 mM Tris-HCl (pH 8.0) buffer. This solution was filtered with an Ultrafree-Probind filter (Millipore, Bedford, MA, USA) and a 70 μL aliquot of the sample was injected onto an HPLC column (Shiseido Fine Chemicals, Tokyo, Japan 5 μM, 4.6 × 250 mm, 27°C, flow rate 1.0 mL/min) equipped with an ECD (Coulochem II, ESA, Chelmsford, MA, USA; electrode 1, 150 mV; electrode 2, 300 mV; guard cell, 350 mV). The mobile phase consisted of 10 mM phosphate buffer (pH 6.7)

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containing 8% methanol. As the standard samples, 20-μL aliquots of the deoxyguanosine (dG) (0.5 mg/mL) and 8-OH-dG (5 ng/mL) solutions were injected. The concentration of test samples was determined by comparison to the standards. The 8-OH-dG level in the DNA was expressed as the number of 8-OH-dG per 10^6 dG.

Efficiency of DNA synthesis in HaCaT cells after UVB irradiation

HaCaT cells were cultured in 96-well plates (Corning Glass Works, Corning, NY, USA) until semiconfluent. After the culture medium was replaced by PBS, the cells were irradiated with UVB. The irradiated cells were further cultured in medium for 24 h, and ^{3}H-thymidine (1 μCi/well; Amersham International, Amersham, UK) was added for the last 12 h. Adherent cells were detached with EDTA/trypsin and collected on glass fibers using a cell harvester, and radio-uptake was measured in a scintillation counter.

Statistical analysis

All analyses were carried out using the StatView-J® 5.0 program (SAS Institute, Cary, NC, USA). All of the data are expressed as the mean ± SD from four to five independent measurements. Statistical significance was determined by the Student's *t*-test, using $P < 0.05$ as the level of significance.

Results

Quantification of 8-OH-dG in HaCaT cells irradiated with broadband or narrowband UVB

HaCaT cells were exposed to broadband or narrowband UVB at various doses, and the 8-OH-dG formed in the cells was measured. Figure 1 shows a representative 8-OH-dG analysis. The hatched peak in Fig. 1a is derived from authentic 8-OH-dG. Untreated cells had a small but discernible amount of 8-OH-dG (Fig. 1b). Irradiation of cells with 1000 mJ/cm^2 narrowband UVB increased the amount (Fig. 1c).

As shown in Fig. 2, the level of 8-OH-dG in untreated cells was 1.48 ± 0.22 per 10^6 dG. Irradiation of cells with broadband UVB (50–500 mJ/cm^2) induced 8-OH-dG formation in a dose-dependent manner (Fig. 2a), and 0.0113 residues per mJ/cm^2 were estimated to be increased by broadband UVB on the per-dose basis.

At the low doses of 250 and 500 mJ/cm^2 of narrowband UVB, the amount of 8-OH-dG was not increased compared to that of the non-irradiated control (Fig. 2b). A significant augmentation of 8-OH-dG was found at 1000 mJ/cm^2 of narrowband UVB. The amount of 8-OH-dG produced by narrowband UVB at 2000 mJ/cm^2 (3.51 ± 0.83) was comparable to that generated by 3.42 ± 0.46 of broadband UVB at 100 mJ/cm^2. Therefore, broadband UVB seemed to induce approximately 20-fold higher oxidative DNA stress than narrowband UVB when compared at the same exposure dose.

The minimal erythema doses (MED) of broadband and narrowband UVB were 70–150 and 500–1200 mJ/cm^2 in Japanese normal subjects and patients with psoriasis or cutaneous T-cell lymphoma. Thus, approximately 10-fold higher doses of narrowband UVB than broadband UVB are used clinically. Given this 10-fold difference in the biological activities of the two UVB sources, the level of 8-OH-dG in

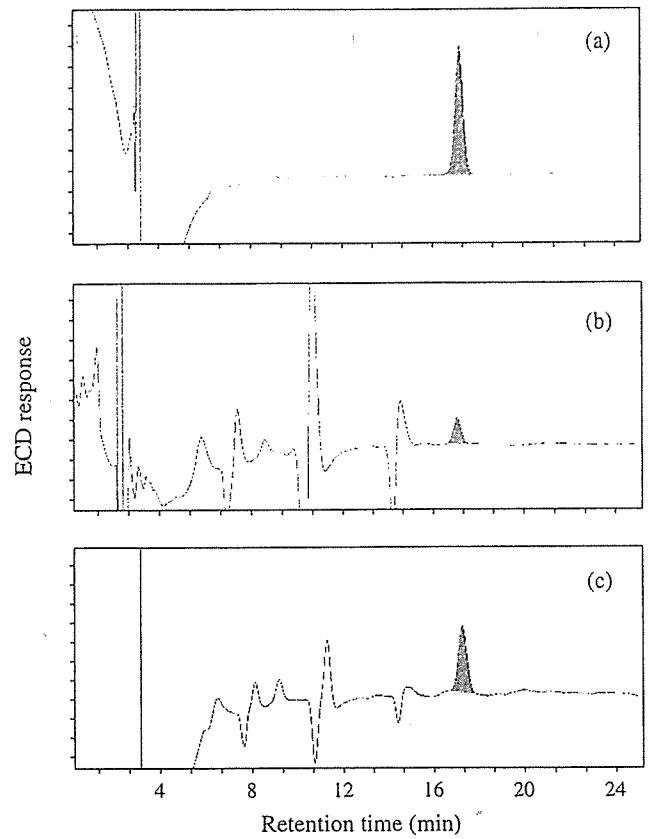

Fig. 1. Representative high-performance liquid chromatography (HPLC)-electrochemical detector (ECD) analyses of 8-OH-dG. DNA isolated from HaCaT cells was treated with the nuclease P1 protein, and a 70-μL aliquot of each sample was subjected to HPLC-ECD analysis. (a) Authentic 8-OH-dG (100 pg), (b) DNA from unirradiated cells and (c) DNA from 1000 mJ/cm^2 narrowband ultraviolet B-irradiated cells. The amount of DNA in the injected samples (b,c) was adjusted.

1000 mJ/cm^2 narrowband UVB-treated cells (2.97 ± 0.44), for example, was less than that in 100 mJ/cm^2 broadband UVB-treated cells (3.42 ± 0.46). However, it should be considered that narrowband UVB yields considerable amounts of 8-OH-dG in clinical settings.

Quantification of 8-OH-dG in HaCaT cells treated with 8-MOP plus UVA

HaCaT cells were treated with 100 ng/mL of 8-MOP and various doses of UVA, or UVA alone. As shown in Fig. 3, UVA alone produced low levels of 8-OH-dG in a dose-dependent manner. Endogenous photosensitizers, such as porphyrins and flavins, which have UV absorption in the UVA range (320–400 nm), may be involved in this process. In contrast, the incubation of cells with 8-MOP before UVA irradiation (2000–10000 mJ/cm^2) significantly enhanced 8-OH-dG formation. Because PUVA therapy usually starts with 100% of the minimal phototoxic dose (ranging from 500–5000 mJ/cm^2 UVA),[37] the amount of 8-OH-dG produced by narrowband UVB exposure is considered to be lower than that generated by PUVA therapy.

In the control experiments without UVA and with 8-MOP, the 8-OH-dG levels were higher than in those without UVA

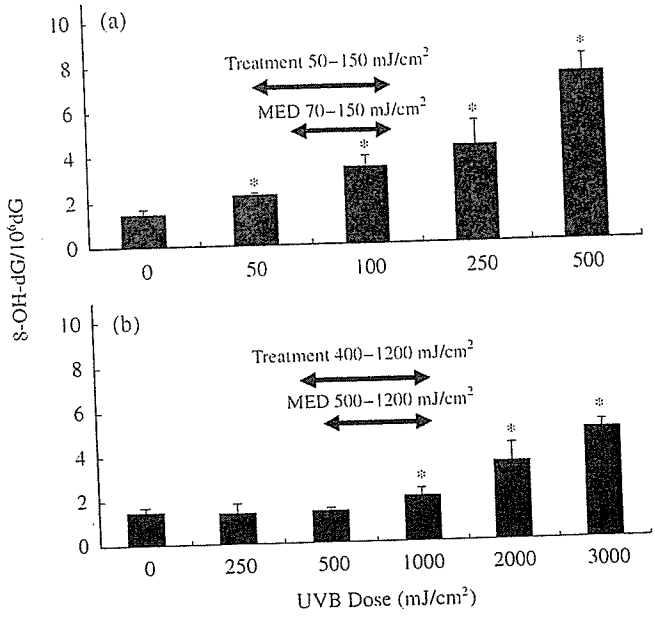

Fig. 2. Formation of 8-OH-dG in the cellular DNA of HaCaT cells irradiated with (a) broadband and (b) narrowband ultraviolet (UV) B. Data are expressed as the mean ± SD of determinations on four to five independently irradiated dishes of keratinocytes. $*P < 0.05$, compared with the unirradiated samples (0 mJ/cm², the background level). MED, minimal erythema doses of Japanese individuals whose skin types were III to IV, defined according to the Fitzpatrick classification.[54] Treatment: broadband UVB and narrowband UVB doses for psoriasis vulgaris in our institution.

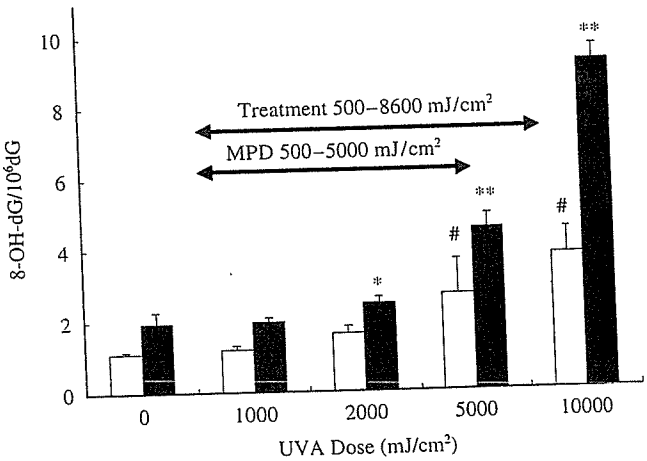

Fig. 3. Formation of 8-OH-dG in HaCaT cells treated with ultraviolet (UV) A or psoralen plus UVA (PUVA). HaCaT cells were incubated with 100 ng/mL 8-MOP and irradiated with various doses of UVA. The data represent the mean ± SD of four to five experiments. $*P < 0.05$, $**P < 0.001$, compared without UVA and with 8-MOP. $\#P < 0.01$, compared without UVA and 8-MOP. MPD, minimal phototoxic doses. Skin was exposed to UVA 2 h after the ingestion of 8-MOP tablets. The MPD is defined as the dose that induced minimally perceptible erythema 72 h after irradiation.[35] Treatment: oral PUVA therapy doses for psoriasis vulgaris.[35]

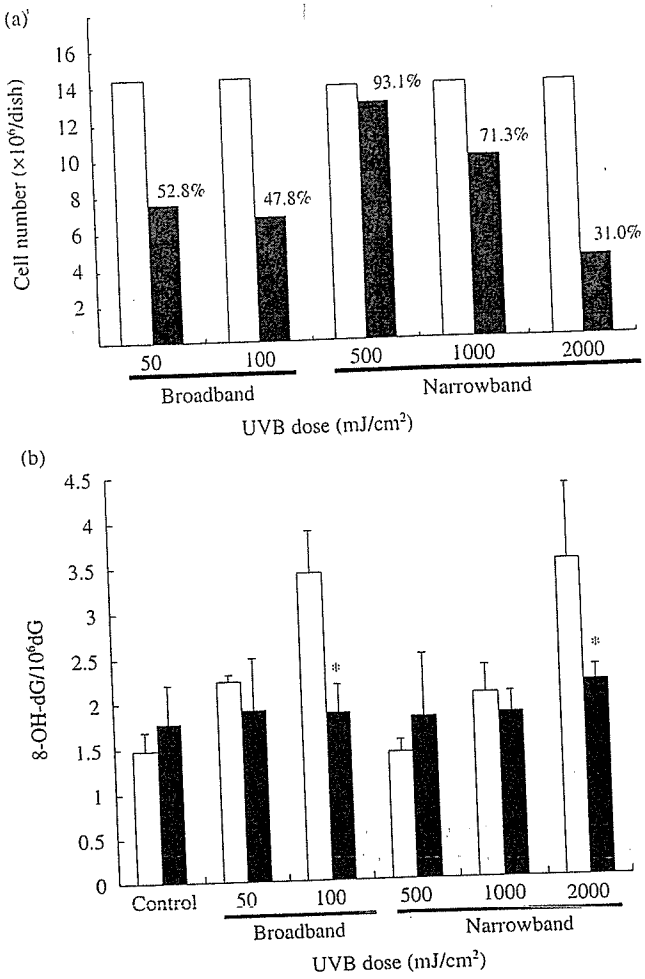

Fig. 4. (a) Viability levels of cells 24 h after ultraviolet (UV) B irradiation. □, Non-irradiated cells; ■, irradiated cells. (b) 8-OH-dG levels immediately (□) and 24 h after (■) UVB irradiation. HaCaT cells were irradiated with the indicated doses of broadband or narrowband UVB. $*P < 0.05$, compared with the value immediately after UVB irradiation. The results represent the mean ± SD of four to five experiments.

and 8-MOP (Fig. 3). This may be due to artifactual formation of 8-OH-dG during DNA isolation under light.

Removal of 8-OH-dG in UVB-irradiated HaCaT cells

We compared the 8-OH-dG levels in HaCaT cells immediately after and 24 h after UVB irradiation. Viability levels of HaCaT cells 24 h after UVB irradiation are shown in Fig. 4a. As UVB exposure induced the detachment of HaCaT cells from the dish, depending on the UVB dose, we quantified the 8-OH-dG in the attached cells, so that only living cells were analyzed. As shown in Fig. 4b, the discernibly elevated 8-OH-dG amounts in the 100 mJ/cm² broadband and 2000 mJ/cm² narrowband UVB-irradiated HaCaT cells were significantly decreased after 24 h of culture. Therefore, the oxidative DNA damage in living keratinocytes seemed to be repaired within 24 h of UVB irradiation.

To further confirm the lack of influence of cell proliferation on the 8-OH-dG reduction, the ^{3}H-thymidine incorporation

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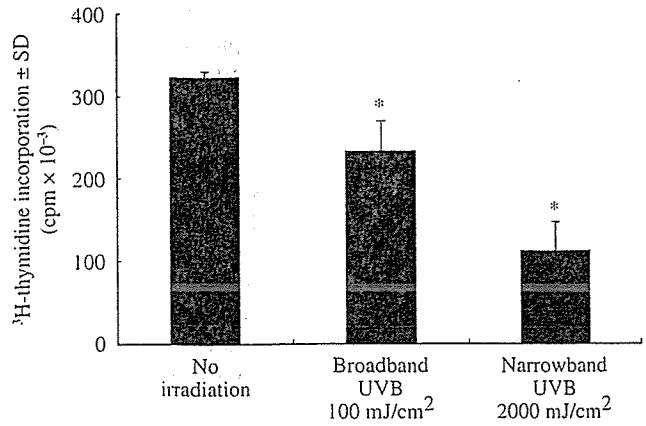

Fig. 5. Reduction of ^{3}H-thymidine incorporation in ultraviolet (UV) B-irradiated HaCaT cells. After irradiation with broadband UVB at 100 mJ/cm^2 and narrowband UVB at 2000 mJ/cm^2, HaCaT cells were cultured for 24 h, and were pulsed with ^{3}H-thymidine for the last 12 h. *$P < 0.05$, compared with no irradiation.

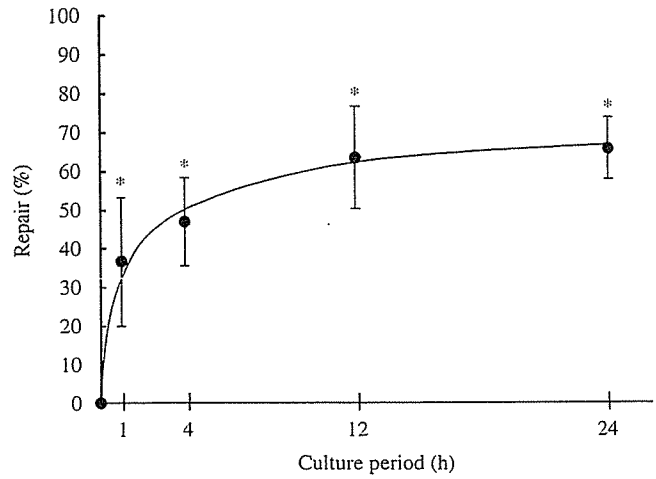

Fig. 6. 8-OH-dG repair rate after irradiation with narrowband ultraviolet (UV) B. HaCaT cells were irradiated with narrowband UVB at 2000 mJ/cm^2, cultured for the indicated period, and subjected to the analysis. Repair (%) = (3.51 [8-OH-dG level immediately after irradiation] – X [8-OH-dG level 1–48 h after irradiation])/(3.51 – 1.48 [8-OH-dG level without irradiation]) × 100 = ([3.51–X]/2.03) × 100. *$P < 0.01$, compared with the value immediately after irradiation.

by UVB-irradiated HaCaT cells was measured. The DNA synthesis levels in the cells treated with broadband UVB at 100 mJ/cm^2 and narrowband UVB at 2000 mJ/cm^2 were decreased after 24 h of culture (Fig. 5). Therefore, the 8-OH-dG formed in HaCaT cells was probably repaired during the cultivation.

The amount of 8-OH-dG was monitored in HaCaT cells at 1–24 h after 2000 mJ/cm^2 narrowband UVB irradiation and the repair rate was calculated. The viability of the cells attached to the dish was similar to that of the control. As shown in Fig. 6, the level of 8-OH-dG was reduced with time, and 65.6% of the 8-OH-dG was repaired at 24 h after UVB exposure.

Discussion

Ultraviolet radiation produces ROS by photodynamic action,[38,39] which causes several kinds of DNA damage, such as 8-OH-dG, and eventually leads to mutations and abnormal cell proliferation.[8,40] Several techniques have been developed to detect 8-OH-dG. The measured background levels of 8-OH-dG differ, depending on both the DNA isolation technique and the 8-OH-dG analysis method.[41] To measure the steady-state level of DNA oxidation, HPLC-ECD is particularly useful because of its selectivity, sensitivity and ease of quantification. During the past two decades, improved DNA isolation techniques and enhanced HPLC-ECD sensitivity have considerably lowered the assayed background levels of 8-OH-dG.[34] Reliable data have been obtained mainly by an improved method that uses an iron chelator, desferal, in the lysis step.[36] In the present study, we also analyzed 8-OH-dG by HPLC-ECD, after DNA was isolated by the improved method.

Previous studies revealed that the number of 8-OH-dG residues in murine keratinocytes treated with UVB increases in an irradiance-dependent manner.[42–44] However, those techniques had a limitation derived from the artifactual oxidation of DNA during its extraction. It was recently reported that relatively low doses of UVB (62.5–500 mJ/cm^2) cause dose-dependent increases in 8-OH-dG, and DNA from unirradiated normal human epidermal keratinocytes contains 1.49 ± 0.11 8-OH-dG residues per 10^6 dG.[22] This is similar to the background level of 8-OH-dG observed in our study using HaCaT cells (1.48 ± 0.22). Furthermore, we report that narrowband UVB at a dose of more than 1000 mJ/cm^2 increases the amount of 8-OH-dG, but to a lesser degree than broadband UVB with the same clinically effective dose. The maximum recommended dose of narrowband UVB for atopic dermatitis and psoriasis is 1500 mJ/cm^2.[45–47] We found that at the highest narrowband UVB dose, such as 1500 mJ/cm^2 used in clinical treatment, 8-OH-dG increased to 2.82 per 10^6 dG.

When the biological effects of broadband and narrowband UVB were assessed by the inhibition of macrophage-derived chemokine production, 10-fold higher doses of narrowband UVB than broadband UVB exerted a comparable inhibitory effect.[48] This is consistent with the observation that the MED and the therapeutic dose of narrowband UVB are approximately 10-fold higher than those of broadband UVB. Even when narrowband UVB at 1000 mJ/cm^2 was compared with broadband UVB at 100 mJ/cm^2, the former induced fewer 8-OH-dG residues than broadband UVB.

Ultraviolet A-induced formation of 8-OH-dG has been observed in human skin fibroblasts,[19–21] and has been detected immunohistochemically in human keratinocytes.[49] Our study demonstrated that UVA induced a dose-dependent increase in 8-OH-dG with a fixed concentration of 8-MOP in keratinocytes. PUVA produced both singlet oxygen and superoxide anions in an *in vitro* system.[50] PUVA has already been reported to induce 8-OH-dG in the human epidermoid carcinoma cell line A431.[31] Upon irradiation of A431 cells with a fixed dose (2500 mJ/cm^2) of UVA, the level of 8-OH-dG increased, depending on the concentration of 8-MOP. However, the background 8-OH-dG level was as high as 27 per 10^6 dG,[31] compared with 1.48 per 10^6 dG in our study.

The amount of 8-OH-dG formed by UVB was reduced in living cells during cultivation. As the cell number and the rate of DNA synthesis were decreased after UVB irradiation, the reduction in 8-OH-dG does not seem to result from cell proliferation and division. Therefore, it is likely that 8-OH-dG is successfully repaired in keratinocytes. The repair rate of 65.6% in 24 h is slightly lower than that of Py–Py dimers[51] and higher than that of 8-MOP-DNA photoproducts.[25] The kinetics of 8-OH-dG repair in the present study seem to be slower than those determined in the previous study by Osterod *et al.*[52] This may be explained by the presence of an overwhelming amount of Py–Py dimers in the irradiated DNA.

Clinically, narrowband UVB is as effective as PUVA in patients with psoriasis[37,45] and atopic dermatitis when administered in equi-erythemogenic doses.[46,53] The highest final doses of narrowband UVB and PUVA for these treatments were 2450 and 8600 mJ/cm^2, respectively.[37] In the present study, narrowband UVB at 2000 mJ/cm^2 induced 3.51 8-OH-dG per 10^6 dG, and PUVA at 5000 mJ/cm^2 (8-MOP, 100 ng/mL) induced 4.52 8-OH-dG per 10^6 dG. Thus, narrowband UVB seems to induce less oxidative stress than PUVA at the clinically effective doses. However, this study provides a warning that widely used narrowband UVB and PUVA at the therapeutically used doses induces cellular oxidative DNA damage, which may induce cancer.

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特集：光アレルギーはいま…

1

光線過敏症における光アレルギーの位置

Photoallergy as an important disorder in photosensitivity

要　約

光アレルギーは光がトリガーとなり，免疫学的機序を介して起こる皮膚疾患である．光アレルギーの範疇に入りうる疾患は，①光接触皮膚炎，②薬剤性光線過敏症，③日光蕁麻疹，④慢性光線性皮膚炎(CAD)である．①と②は外因性光感受性物質による疾患であり，③と④は原因物質がはっきりしない疾患である．これら4疾患について解説していただき，光アレルギーという分野を浮き上がらせるのが本企画の目的である．

光アレルギー

アレルギーはCoombsとGellの分類として4タイプに分かれる．これを光アレルギーに踏襲すると，光接触皮膚炎と薬剤性光線過敏症はIV型に属することになる．日光蕁麻疹のかなりの部分はI型になるであろう．慢性光線性皮膚炎(CAD)は何型に属するかは不明であるが，T細胞が起こす疾患であり，IV型かII型になる．

KEY WORDS／光アレルギー／光接触皮膚炎／薬剤性光線過敏症／日光蕁麻疹／慢性光線性皮膚炎

Photoallergy as an important disorder in photosensitivity

1 光線過敏症はいろいろな原因で起こる

光線過敏症は，太陽光線に当たった皮膚が赤くなるなどの異常な反応を起こす疾患の総称である．ひどい場合は水疱形成など熱傷様になることすらあり，決して皮膚疾患として軽いものばかりではない．光に当たりやすい顔，項部，耳，手背，前腕伸側，上胸部などに皮疹が生じ，臨床現場ではまず皮疹の分布状態により光線過敏症を疑うことになる．

光線過敏症の原因は表1のように多種多様である．これらのうちで色素性乾皮症は先天性の光線過敏症の代表的なものである．後天性のものには，ペラグラ，光接触皮膚炎，薬剤性光線過敏症，種痘様水疱症，日光蕁麻疹，多形日光疹，慢性光線性皮膚炎(chronic actinic dermatitis；CAD)がある．ペラグラは先天性のHartnup病と同じようにニコチン酸欠乏による代謝性疾患である．ポルフィリン症には晩発性皮膚ポルフィリン症(PCT)と骨髄性皮膚ポルフィリン症(EPP)がある．ペラグラとPCTはどちらもアルコール多飲によることが多い．EPPは意外と軽症例は見逃されていることがあり，アトピー性皮膚炎(atopic dermatitis；AD)と誤診されている例すらある．種痘様水疱症は，発症以前に慢性のEBウイルス感染が存在することを土台とする疾患と考えられる．多形日光疹はわが国では小丘疹性日光疹という軽い光線過敏性疾患である．

これらのなかで光アレルギーの範疇に入りうる疾患は，外因性光感受性物質による疾患である，①光接触皮膚炎と②薬剤性光線過敏症，そのほか，③日光蕁麻疹，④CADということになる．各疾患についてエキスパートの先生に解説していただくのが今回の特集のねらいである．

2 外因性光感受性物質がはっきりしている光アレルギー

光線過敏症には明瞭な光線過敏性物質が存在する場合と，そうでない場合とがある．さらに明瞭な物質が存在する場合には，光毒性機序によって生ずるものと，光アレルギー性機序によって生ずるものとがある．臨床的には光アレルギー性による頻度のほうが高い．

通常のアレルギーには薬疹，接触皮膚炎を代表とするように抗原物質が明瞭なものと，AD，蕁麻疹などのように必ずしもアレルゲンを決定し得ないものとがある．この事情は光アレルギーについても同様であり，薬剤性光線過敏症，光接触皮膚炎は抗原となる光感受性物質が明らかであり，そのほかは明確でない疾患となる．

光アレルギーのひとつの特殊性として，光がアレルギー症状発現に必須であるため，光が当たる臓器すなわち皮膚だけが病変形成の場となることにある．すなわち，光アレルギーの症状は皮膚炎のみである．別の見方をすれば，光アレルギーはアレルギーのメカニズムを比較的

表1　光線過敏症の原因別分類

1．外因性物質によるもの：光毒性または光アレルギー性機序
　経皮：光接触皮膚炎
　経口：薬剤性光線過敏症(光線過敏型薬疹)

2．内因性物質によるもの：光毒性
　ポルフィリン症(PCT，EPP)，ペラグラ，Hartnup病

3．DNA修復機構の異常
　色素性乾皮症，Cockayne症候群

4．EBウイルス関連
　種痘様水疱症

5．メラニン色素減少による閾値低下
　白皮症，フェニルケトン尿症

6．日光により増悪ないし誘発される疾患
　エリテマトーデス

7．原因不明のもの
　日光蕁麻疹，多形日光疹，慢性光線性皮膚炎(CAD)

ピュアに調べることのできるシステムともいえる．

光接触皮膚炎は抗原が皮膚に塗られて，紫外線が当たって発症する．一方，薬剤性光線過敏症は抗原が薬剤という形で経口投与されて，紫外線が当たって発症する．現在，光接触皮膚炎の原因にはケトプロフェン，スプロフェンなどのNSAIDsや，サンスクリーン剤がある．薬剤性光線過敏症の原因には，ニューキノロンをはじめとして多くの薬剤がある．

通常の抗原とは異なり，光アレルギー性物質が抗原となるには紫外線照射が必要となる．この紫外線の作用による抗原性の獲得については，古くよりいくつかの考えが提唱されてきたが，大きく2つの説に集約される．ひとつはプロハプテンであり，もうひとつは光ハプテンという考えである．プロハプテン説は，光アレルギー性物質はUV照射により化学構造の変化が起き，通常のハプテンのようになり，蛋白との結合能力を獲得する，という単純明快な説である．一方，光ハプテン説は，UV照射がなされるとその化学構造の一部が光分解され，その分解と同時に近傍の蛋白と共有結合し完全抗原ができあがるという考えである．したがって，あらかじめUVAを照射した物質が蛋白と結合すればプロハプテン，一方，その物質と蛋白との共存下でUVAを照射し，両者が共有結合すれば光ハプテンということになる．

多くの光抗原は光ハプテンとしての性格をもっている．したがって，当該物質が光線過敏症の原因になっているかを検証するときは，まず物質を皮膚に塗っておいて，そこに紫外線を当てる方法，すなわち光パッチテストを行う．あらかじめ当該物質に紫外線を当てておいて，それを普通のパッチテストする方法は経験的に避けられてきたが，これはプロハプテンの証明方法であり，プロハプテンの性格をもつ薬剤が少ないことを知らず知らずのうちに実証してきたことになる．

ランゲルハンス細胞はプロフェッショナルな抗原提示細胞であり，通常の接触皮膚炎と同様に，光接触皮膚炎においても抗原提示細胞として働き，薬剤性光線過敏症においても光抗原を提示する細胞として機能する．ランゲルハンス細胞による光抗原の提示において，光ハプテンがランゲルハンス細胞上の主要組織適合抗原複合体(MHC)クラスII分子あるいはクラスII分子によって表出された自己ペプチドに直接光結合するのか，あるいは紫外線照射によってできた光ハプテンと蛋白の複合体がランゲルハンス細胞に一旦取り込まれ，クラスII分子とともに再表出されるのかは不明である．しかし，われわれは直接MHCクラスII分子と自己ペプチドとの複合体に光共有結合するとする実験結果を得ている．こうして光アレルギー性物質はT細胞を感作することになる．

3　原因物質のはっきりしていない光アレルギー

CADは，外因性光抗原を原因としない自己免疫性光線過敏症と呼ぶべき疾患である．このなかには，ある物質に光貼布試験陽性を示す患者がおり，光線過敏症は以前その物質に対する光接触皮膚炎であったものが，光アレルゲンなしに紫外線に感受性をもつようになってしまった状態と解される．同様に，ある薬剤による光線過敏症を示していた患者が，薬剤を中止しても光線過敏症が治癒することなく存続することもある．つまり引き金は光接触皮膚炎であったり，薬剤性光線過敏症であったものが光抗原が除去されても存続することがあることになる．

こうした光抗原なくして光アレルギーが起こるようになる機序はいまだ明瞭ではない．古典的には光感受性物質が皮膚に微量に残っている可能性がいわれていた．しかし，むしろ現在では紫外線が表皮細胞の表面に何らかの物質を誘導し，それを自己反応性T細胞が認識して皮膚炎を起こす可能性が考えられている．あるいは紫外線照射が自己蛋白の修飾を行い，それがアジュバント効果を発揮するのかもしれない．しかし，そもそもの過敏症を引き起こした光抗原反応性T細胞と自己反応性T細胞にはどんな関係があるのかは，まだまだ不明である．

もうひとつ重要な臨床的観察がある．それはCADがHIV陽性患者に多く報告されていることである．CADの病変組織にはCD8陽性T細胞が浸潤し，苔癬型組織反応を形成していることがしばしばある．一般にCD4陽性細胞のなかにはTh2やregulatory T細胞といったCD8陽性細胞傷害性T細胞の機能を抑制する細胞がある．HIV陽性者ではCD4陽性T細胞の数が減少するが，これが結果的にCD8陽性細胞傷害性T細胞を活性化させてしまい，CADを誘導してしまう可能性がある．最近，われわれは成人T細胞性白血病に伴ったCADを経験した[1]．この場合でもCD4陽性T細胞の機能障害を下地としてCD8陽性細胞傷害性T細胞を活性化させてしまい，CADを生じたと思われる．

以上のように，CADの発症には，自己反応性T細胞の抑制の解除が重要な因子となっているのかもしれない．