

ORIGINAL ARTICLE

UVA irradiation of the eye modulates the contact hypersensitivity of the skin and intestines by affecting mast cells in mice

Yurika Yamate^{1,2}, Keiichi Hiramoto^{1,2}, Emiko Kasahara¹ & Eisuke F Sato²

¹Department of Physiology, Osaka City University Graduate School of Medicine, Osaka, Japan.

²Department of Pharmaceutical Science, Suzuka University of Medical Science, Mie, Japan.

Key words:

4-ethoxymethylene-2-phenyl-2-oxazolin-5-one; contact hypersensitivity; fluorescein isothiocyanate; ultraviolet A

Correspondence:

Dr Keiichi Hiramoto, Ph.D.,
Department of Pharmaceutical
Science, Suzuka University of
Medical Science, 3500-3
Minamitamagakicho, Suzuka, Mie
513-8670, Japan.
Tel: +81 59 340 0575
Fax: +81 59 368 1271
e-mail: hiramoto@suzuka-u.ac.jp

Accepted for publication:

7 December 2014

Conflict of interests:

The authors have no conflicts of interest to declare.

ABSTRACT

Background

Ultraviolet A (UVA) irradiation before allergic sensitization induces immunosuppression, but the precise mechanism remained unclear. In this study, we examined the influence of UVA irradiation of the eye on contact hypersensitivity (CHS) and the role of mast cells in CHS.

Methods

We used two types of haptens, fluorescein isothiocyanate (FITC: a Th2 type hapten) and 4-ethoxymethylene-2-phenyl-2-oxazolin-5-one (oxazolone: a Th1 type hapten). A 300 kJ/m² dose of UVA irradiation was delivered to the eyes. After UVA irradiation, we sensitized abdominal shaved skin and challenged the ear epidermis and colons of these mice with each hapten.

Results

After UVA irradiation, the CHS of the skin and colon were not inhibited in the FITC-sensitized mice. However, in the oxazolone-sensitized mice, only the CHS of the skin was inhibited by UVA irradiation. The inflammation of the colon became more severe after UVA irradiation. In mast cell-deficient (W/W^v) mice sensitized to FITC, the CHS was weaker than that in WT mice. Moreover, the reduction of immunosuppression in ear swelling was seen for one of the two models they used.

Conclusions

These results suggest that the mast cells induced by UVA irradiation of the eye have different roles in the epidermis and colon and have different responses to different haptens.

Photodermatol Photoimmunol Photomed 2015; ●●: ●●–●●

The ultraviolet (UV) radiation emitted by the sun represents one of the most important environmental factors affecting humans and animals. The neuroendocrine functions of the skin are regulated by external stressors, includ-

ing UV radiation, and can regulate the whole body or specific organ homeostasis by signals generated in the skin and coordinated by the brain and/or other organs (1–4). Each wavelength of UV has specific characteristics. Short

wavelength UVB (280–320 nm) has been well studied with regard to its effects on pigmentation, skin carcinogenesis and photoimmunology (5–7). On the other hand, long wavelength UVA (320–400 nm) has been reported to induced skin carcinoma (8, 9) and aging (10, 11) when the skin is exposed to high doses. However, the details of the mechanism of UVA are not known. Ullrich *et al.* demonstrated that each type of irradiation led to immunological suppression in the skin and that mast cells had important roles in this response (12). Mast cells are immunological cells that secrete chemical mediators, such as histamine, leukotriene and prostaglandin, and contribute to the inflammatory and allergic reactions to constrict smooth muscles, enhance the vascular permeability, induce mucus secretion and increase leukocyte migration. Contact hypersensitivity (CHS) is a representative type of delayed hypersensitivity (type IV allergy) that is induced by the epicutaneous application of contact allergens. CHS occurs after epidermal Langerhans cells (LCs), which recognized the applied contact allergen, move to the regional lymph nodes and encounter naïve T cells, transforming them into antigen-specific sensitized T cells. These sensitized T cells then migrate to the skin if the same allergen is encountered again and recognized it to release many cytokines, inducing inflammatory responses (13). These reactions cause different types of inflammation in response to different types of haptens. For example, it was reported that fluorescein isothiocyanate (FITC) elicits a Th2-dependent allergic reaction (14), whereas 4-ethoxymethylene-2-phenyl-2-oxazolin-5-one (oxazolone) elicits a Th1-dependent allergic reaction (15). UV skin irradiation stimulates the secretion of hormones from the pro-opiomelanocortin (POMC) system of a keratinocytes, and the expression of many receptors increases simultaneously. In addition, the UV stimulus controls the expression of POMC through the hypothalamic/pituitary system. The response to POMC/the receptor of light is induced not only by a skin irradiation, but also eye irradiation. The eye has been revealed to have not only receptors for visible light, but also receptors that respond to UV (16), and the UV receptor of the eyes may also participate in the influence of skin exposure to UV. We previously showed that UVA irradiation of the eye modulates mast cells in the intestine through the sympathetic nervous system (17). How the mast cells are affected by UVA irradiation of the eye and how this influences the CHS have been poorly understood. In this study, we examined the effects of UVA irradiation of the eye using FITC and oxazolone sensitized mice. In addition, to examine the importance of mast cells in this process, we performed the same experiments with male mast cell-deficient mice (W/W^v).

MATERIALS AND METHODS

Mice

Specific pathogen-free male C57BL/6j, WBB6F1-W/W^v, WBB6F1-+/+ mice were purchased from SLC (Hamamatsu, Aichi, Japan). The mice were 8 weeks old at the beginning of each experiment and were housed in filter-protected cages. The ambient light was controlled automatically, and sterile water was provided *ad libitum*. The animals were subjected to experiments according to the animal care regulations of Osaka City University Medical School.

Chemicals

The 4-ethoxymethylene-2-phenyloxazolin-5-one (oxazolone) was obtained from Wako (Osaka, Japan). FITC was obtained from Sigma-Aldrich Chemical (St Louis, MO, USA). Oxazolone was dissolved in ethanol : olive oil (3:1 v/v) solution, and FITC was dissolved in acetone : dibutyl phthalate (1:1 v/v) solution. The solutions were prepared immediately prior to dosing.

Ultraviolet irradiation

Under urethane anesthesia (1 mg/kg, i.p.), the eyes in mice were exposed to UVA irradiation after covering the remaining body surface with aluminum foil (Fig. S1). Using an L20SBLB-A lamp (wavelength 320–400 nm, peak emission 352 nm, Toshiba Co., Tokyo, Japan), the mice were irradiated by UVA at 300 kJ/m²; the dose was confirmed by an UV radiometer (Topcon CO., Tokyo, Japan). The localized irradiation of the eye without exposure of the surrounding tissues was ensured by the topical application of 0.1 ml of 10% 2-ethylhexyl-*p*-methoxy cinnamate, a potent UV absorbent, to the periorbital skin 10 min before irradiation. The application of this absorbent completely prevented the UVA-induced injury of the periorbital skin, as judged by the failure to detect any cells with the typical characteristics of sunburn. In addition, no injury of the eyes was seen with at the dose of UVA energy, which was used for this experiment. The time for the single irradiation was 3 h, from 9:00 to 12:00. Three days after UVA irradiation of the eye, the mice were sensitized to either oxazolone or FITC. After 5 days of sensitization, the mice were sacrificed, and blood, ear skin sheets and colon specimens were obtained. The mice were divided into six groups that were either not irradiated (un-sensitized, oxazolone-sensitized or FITC-sensitized) or irradiated (un-sensitized, oxazolone-sensitized or FITC-sensitized). Each group included six animals.

Contact sensitization

The skin on the back of each mouse was shaved with electric clippers and was used as the sensitization area. The mice were sensitized by applying 100 μ l of 1.0% oxazolone or FITC solution. The mice were challenged at 5 days after sensitization by applying the oxazolone (5 μ l) or FITC solution (5 μ l) to both the dorsal and ventral surfaces of the right and left ears. The ear thickness was measured with a micrometer (Mitsutoyo, Kanagawa, Japan), and the CHS was examined by determining the difference between the ear thickness measured before the challenge and that measured 24 h after the challenge.

In the colon, either oxazolone or FITC solution was administered through the rectum via a 3.5 F catheter equipped with a 1-ml syringe. The catheter was inserted so that the tip was 3 cm proximal to the anal verge, and oxazolone or FITC was injected in a total volume of 100 μ l. To ensure the distribution of oxazolone or FITC within the entire colon, mice were held in a vertical position for 30 s after the injection. The colon was removed 24 h after challenge, and then the morphological and immunological features were observed.

Preparation and staining of epidermal sheets

At the end of treatment, mice were killed, and skin samples (0.5 $\times$ 0.5 cm) were obtained from the ear. The skin samples were incubated for 5 h at 37°C in 20 mmol/L ethylene diamine tetra acetate phosphate-buffered saline (PBS). The epidermis was then separated from the dermis to obtain epidermal sheets. The sheets were fixed in acetone for 10 min. After washing the epidermal sheets with PBS, they were incubated with a rat anti-I-A/I-E monoclonal antibody (as LCs, 1:100 BD Biosciences, Franklin Lakes, NJ, USA) at 4°C for overnight. The sheets were then washed in PBS and incubated at room temperature for 2 h with rat biotin-conjugated immunoglobulins (1:200 Dako, Glostrup, Denmark). After washing these samples with PBS, these were incubated at room temperature for 2 h with streptavidin-RPE (1:50 Dako). These samples were put on a slide and enclosed with a cover glass. The slides were observed under a microscope (OLYMPUS, Tokyo, Japan). Approximately 20 fields of view were chosen for each of the epidermal sheets, and a stack of about 30 optical sections was scanned with a z-increment of 0–4 μ m. From each optical stack, extended focus projections (summation of all optical sections in the stack, with each section in focus) were performed, and then the numbers and areas of LCs were calculated using a software program provided with the BioRad MRC500 instrument.

The number of LC/mm² and areas were then determined by counting 20 fields at random at $\times$ 400 magnifications.

The histological analysis on the colon

The colon specimens were fixed in phosphate-buffered paraformaldehyde (4%), embedded in frozen Tissue-Tek optimal cutting temperature compound (Sakura. Co. Ltd., Tokyo, Japan) and cut into 5- μ m thick sections. The thin sections were stained with hematoxylin-eosin and analyzed histologically to evaluate the degree of colon inflammation (CHS response) caused by either the oxazolone or FITC. After washing with PBS, other thin sections were incubated with a rat anti-I-A/I-E monoclonal antibody (as dendritic cells, 1:100; BD Biosciences, San Jose, CA, USA) or goat anti-mouse IgA polyclonal antibody (1:100; ZYMED, San Francisco, CA, USA) overnight at 4°C. The specimens for I-A/I-E were then washed in PBS and incubated at room temperature for 2 h with biotin-conjugated rat immunoglobulin (1:200 Dako, Glostrup, Denmark). After washing with PBS, these samples were incubated at room temperature for 2 h with streptavidin-RPE (1:50 Dako, Glostrup, Denmark). The specimens for IgA staining were washed with PBS and incubated at room temperature for 2 h with FITC-conjugated anti-goat immunoglobulin (1:30; Dako, Glostrup, Denmark). The expression of dendritic cells or IgA on the colon was evaluated immunohistochemically under the fluorescence microscopy.

Immunohistochemical staining for histidine decarboxylase (HDC)

To identify mast cells, intestinal specimens were stained immunohistochemically using a primary antibody against HDC. To eliminate blood, the mice were perfused with PBS for 30 sec *via* the left ventricle and were subsequently perfused for 2 min with a fixative containing 4% paraformaldehyde, 0.5% glutaraldehyde and 0.2% picric acid in PBS (pH 7.4) at 4°C. The colon segment was dissected and stretched on a wet paper. The segment was then immersed for 24 h in a post-fixative containing 4% paraformaldehyde and 0.2 % picric acid in PBS (pH 7.4) at 4°C. The specimens were washed, changed to frozen Tissue-Tek and cut into 5 μ m sections using a cryostat.

For immunohistochemistry, the sections were treated with a mixture of 0.01% trypsin and 0.7 mM CaCl₂ in 50 mM Tris-HCl buffer (pH 7.6) at 20°C for 10 min. The sections were subsequently washed and incubated with 0.1 M PBS (0.1% H₂O₂) at room temperature for 10 min to inactivate endogenous peroxidase activity. After being

washed for 10 min, the sections were incubated with blocking buffer (Dako Cytomation, Glostrup, Denmark) for 10 min, then were incubated with a primary antibody against HDC (Progen, Heiderberg, Germany) at 4°C overnight.

The washed sections were incubated with a HRP-conjugated second antibody at room temperature for 30 min. After being washed with 0.1 M PBS for 10 min, these sections were incubated with 3,3'-diamino benzidine substrate (Wako) at room temperature for 5–10 min. The immune reaction was stopped by washing with distilled water for 5–10 min. The stained sections were dehydrated by ethanol, penetrated by xylene, put on cover slips with Entellan New (Merck, Darmstadt, Germany) and observed by light microscopy.

Quantification of cytokines by an enzyme-linked immunosorbent assay (ELISA)

Blood samples were taken from the heart at 24 h after challenge, and then the plasma was fractionated. The levels of tumor necrosis factor- α (TNF- α), interleukin-10 (IL-10) and interleukin-12 (IL-12) were determined using

a commercial ELISA kits (eBioscience, San Diego, CA, USA) according to the manufacturer's instructions. The optical density was measured with a microplate reader (Molecular Devices, Sunnyvale, CA, USA).

Statistical analysis

The data were expressed as the means $\pm$ standard deviation (SD) derived from six animals. Data were compared using an analysis of variance and the Stat-View 512 software program (Abacus Concepts, Berkeley, CA). A value of $P < 0.05$ was considered to be statistically significant.

RESULTS

Effects of UVA irradiation of the eye on the CHS of the ear epidermis

Twenty-four hours after FITC or oxazolone (OXA) challenge in the C57BL/6j mice, the ear swelling was measured. The CHS of the ear epidermis induced by FITC or OXA was suppressed by prior UVA irradiation of the eye (Fig. 1a). The expression of LCs in the ear epidermis was

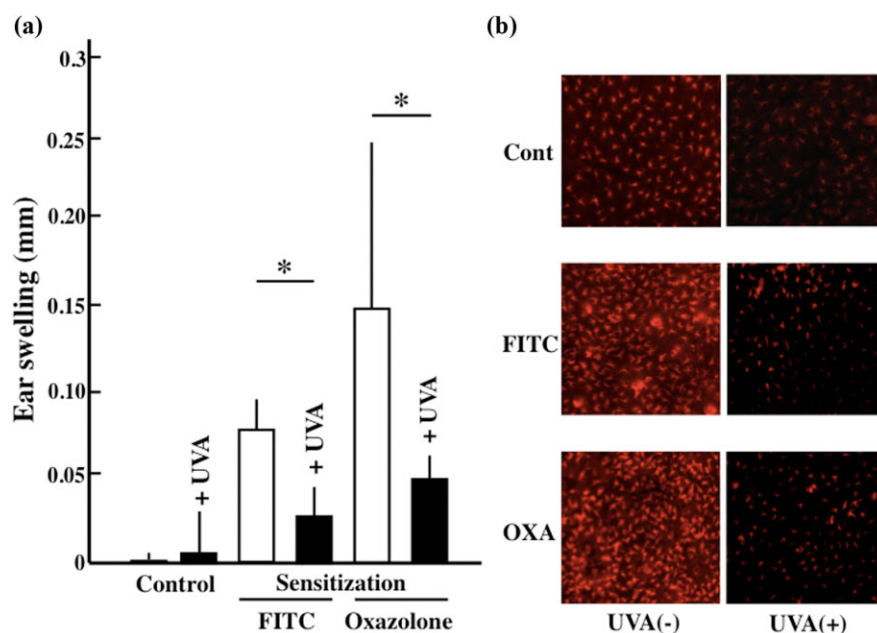


Fig. 1. The effects of UVA irradiation of the eye on the CHS response and the changes in the Langerhans cells of the ear epidermis in C57BL/6j mice sensitized by FITC or OXA. Three days after UVA irradiation ($100 \text{ kJ/m}^2 \times 3 \text{ h}$) of the eye and 24 h after FITC or OXA challenge, the ear swelling was examined (a). Epidermal sheets obtained from the ear were treated with an anti-I-A/I-E antibody and stained by a FITC-conjugated secondary antibody (b). The contact hypersensitivity was expressed as the amount of ear swelling. The data are presented as the means $\pm$ SD from six animals. The open and closed columns are the control and UVA-irradiated mice, respectively. $*P < 0.05$ in comparison with the nonirradiated controls (a). An increase in Langerhans cells was observed immunohistochemically under fluorescence microscopy. Magnification: $\times 200$ (b). CHS, contact hypersensitivity; FITC, fluorescein isothiocyanate; OXA, oxazolone; SD, standard deviation; UVA, ultraviolet A.

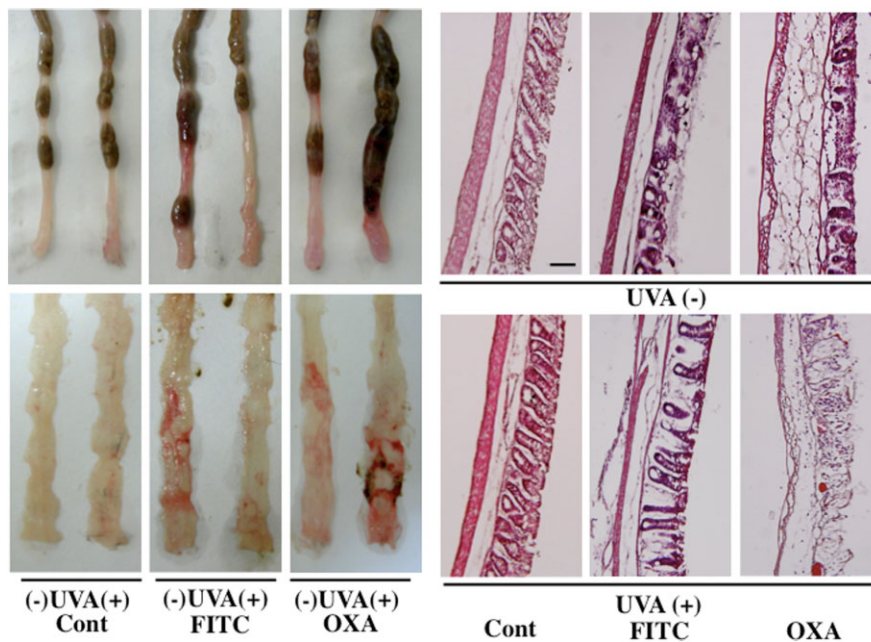


Fig. 2. The effects of UVA irradiation of the eye on the CHS response and inflammation of the colon in C57BL/6j mice sensitized to FITC or OXA. Three days after UVA irradiation of the eye and 24 h after FITC or OXA challenge, colon samples were collected, and the morphological findings are shown in the left photographs. The colon specimens were frozen, cut into thin sections and then were stained with H-E. The microscopic findings of the colon specimens are shown in the right photographs. The data show one typical result from six male C57BL/6j mice. Magnification, $\times 200$. Scale bar = 100 μm . CHS, contact hypersensitivity; H-E, hematoxylin and eosin; FITC, fluorescein isothiocyanate; OXA, oxazolone; UVA, ultraviolet A.

increased by FITC or OXA. However, this increase in the number of LCs was inhibited by UVA irradiation of the eye (the percent inhibition; FITC: 66.7%, OXA: 66.7%) (Fig. 1b).

Effects of UVA irradiation of the eye on the CHS of the colon

The morphological observations of the colon are shown in Fig. 2. Remarkable edema and congestion were observed due to the CHS induced by FITC or OXA. UVA irradiation of the eye suppressed the CHS of the colon only in FITC-sensitized mice, but not in OXA-sensitized mice. In contrast, UVA irradiation of the eye induced a severe CHS response in the colons of OXA-sensitized mice. Microscopic observation of the colon samples stained with hematoxylin and eosin revealed the infiltration of many lymphocytes and mucosal destruction. These results showed that UVA irradiation of the eye decreased the CHS response of the colon only in FITC-sensitized mice, and actually led to increased inflammation in OXA-sensitized mice.

Effects of UVA irradiation of the eye on the immunological changes in the colon

The expression of Immunoglobulin A (IgA) and histidine decarboxylase (HDC)-positive mast cells in the colon was immunohistochemically observed in the FITC- and OXA-sensitized mice. The colonic expression of IgA markedly

increased after either FITC or OXA challenge in nonirradiated mice. UVA irradiation of the eye suppressed the expression of IgA only in FITC-sensitized mice, not in OXA-sensitized mice (Fig. 3a). The colonic expression of HDC-positive mast cells was remarkably increased after FITC or OXA challenge in nonirradiated mice. The degree of increase in mast cells was more intense in the FITC-sensitized mice compared with the OXA-sensitized mice. UVA irradiation of the eye inhibited the increase in colonic mast cells to a level similar to that observed in control mice in both FITC- and OXA-sensitized mice (Fig. 3b).

Effects of UVA irradiation of the eye on the plasma levels of IL-10, TNF- α , and IL-12 in sensitized mice

The plasma levels of IL-10, TNF- α , and IL-12 were measured by ELISAs (Fig. 4). The level of IL-10 was markedly increased in both the FITC- and OXA-sensitized mice compared with control mice in the nonirradiated animals. In addition, UVA irradiation of the eye led to a greater increase in the levels of IL-10 in both FITC- and OXA-sensitized mice. The level of TNF- α was significantly increased in both FITC- and OXA-sensitized mice compared with the control mice in the nonirradiated animals. UVA irradiation of the eye increased the level of TNF- α in un-sensitized mice, but not in either of the sensitized mice. Conversely, UVA irradiation of the eye slightly decreased the level of TNF- α in both the FITC- and OXA-sensitized mice. The level of IL-12 was not changed in the FITC- or

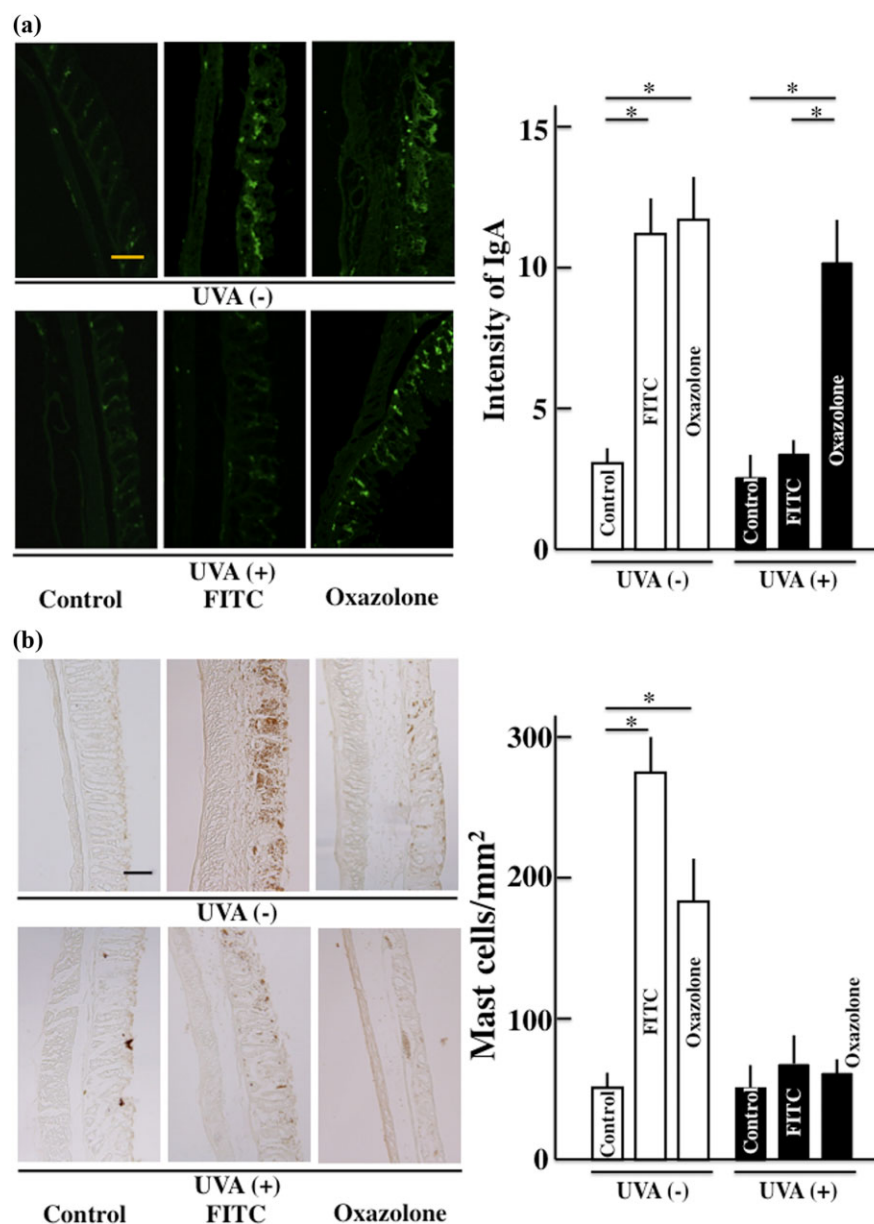


Fig. 3. The effects of UVA irradiation of the eye on the expression of IgA and anti-histidine decarboxylase (HDC)-positive mast cells of the colons in C57BL/6j mice sensitized to FITC or OXA. Three days after irradiation and 24 h after FITC or OXA challenge, colon specimens were obtained and treated with an anti-IgA antibody, and then were stained with a FITC-conjugated second antibody (a). To identify mast cells, the colon specimens were immunohistochemically stained using an HDC-antibody (b). The data for A and B show one typical experiment from six C57BL/6j mice. Scale bar = 100 μ m. FITC, fluorescein isothiocyanate; HDC, histidine decarboxylase; UVA, ultraviolet A.

OXA-sensitized mice compared with the control mice in the nonirradiated animals. UVA irradiation of the eye did not affect the level of IL-12 in the FITC-sensitized mice but remarkably increased it in the OXA-sensitized mice.

Effects of UVA irradiation of the eye on the CHS and LCs of the ear epidermis in WT (WBB6F1) and mast cell-deficient (W/W^v) mice

The ear swelling was measured 24 h after FITC or OXA challenge in WT and mast cell-deficient mice. The CHS of the ear epidermis was induced by FITC or OXA in both types of mice. In the WT mice, UVA irradiation

of the eye suppressed the CHS of the ear epidermis, regardless of the type of hapten. However, in mast cell-deficient mice, UVA irradiation of the eye suppressed the CHS of the ear epidermis only in FITC-sensitized mice but not in OXA-sensitized mice. The expression of LCs in the ear epidermis was increased, and the cells were activated by FITC or OXA in both the WT and mast cell-deficient mice. In WT mice, these effects were inhibited by UVA irradiation in both FITC- and OXA-sensitized mice. On the other hand, in mast cell-deficient mice, these effects were inhibited by UVA irradiation only in the FITC-sensitized mice not in OXA-sensitized mice (Fig. 5).

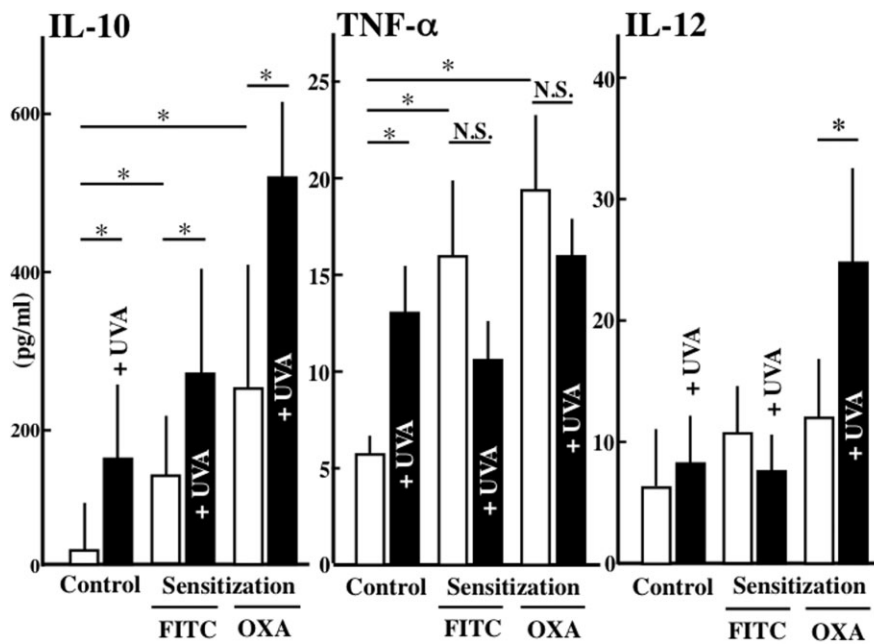


Fig. 4. The effects of UVA irradiation of the eye on the changes in the plasma levels of IL-10, TNF- α , and IL-12 in mice sensitized mice to FITC or OXA. Three days after irradiation and 24 h after challenge with FITC or OXA, the plasma levels of IL-10, TNF- α , and IL-12 was determined by ELISA. The open and closed columns indicate the data for the control and UVA-irradiated C57BL/6j mice, respectively. The data are presented as the means $\pm$ SD from six animals. * $P < 0.05$. ELISA, enzyme-linked immunosorbent assay; FITC, fluorescein isothiocyanate; IL-10, interleukin-10; TOXA, oxazolone; TNF- α , tumor necrosis factor- α ; UVA, ultraviolet A.

Effects of UVA irradiation of the eye on the CHS of the colon in WT and mast cell-deficient mice

The morphological observations of the colon are shown Fig. 6. In the WT mice, edema and congestion were observed due to CHS induced by FITC or OXA. These changes of the colon were especially severe in the OXA-sensitized mice. UVA irradiation of the eye suppressed the CHS of the colon only in FITC-sensitized mice, but not in OXA-sensitized mice. Microscopic observation of colon samples stained with hematoxylin and eosin revealed the infiltration of large numbers of lymphocytes and mucosal destruction in both WT and mast cell-deficient mice induced by either FITC- or OXA-sensitization. In WT mice, UVA irradiation inhibited these responses of the colon only in FITC-sensitized mice, but not in OXA-sensitized mice. Conversely, UVA irradiation of the eye led to more severe inflammation in OXA-sensitized mice. In the W/W^v mice, UVA irradiation of the eye did not inhibit the inflammation in the FITC-sensitized mice, but it did inhibit the inflammation in OXA-sensitized mice (Fig. 6).

Effects of UVA irradiation of the eye on the plasma levels of IL-10, TNF- α , and IL-12 in WT and mast cell-deficient mice

The plasma levels of IL-10, TNF- α , and IL-12 were measured by ELISA in WT and mast cell-deficient mice (Fig. 7). In the WT mice, these changes in cytokines were similar to those in C57BL/6j mice (Fig. 4). In the mast cell-deficient

mice, the level of IL-10 was increased only by OXA sensitization, but not by FITC sensitization, in the nonirradiated animals. UVA irradiation of the eye did not affect the FITC-sensitized mice but suppressed the IL-10 level in the OXA-sensitized mice. In the mast cell-deficient mice, the level of TNF- α was not changed by either FITC- or OXA-sensitization in the nonirradiated animals. In addition, UVA irradiation did not affect the levels of TNF- α in either the FITC- or OXA-sensitized mice. In the mast cell-deficient mice, the level of IL-12 was significantly increased in both FITC- and OXA- sensitized mice compared with control mice in the nonirradiated animals. However, UVA irradiation of the eye had no major effects in either the FITC- or OXA-sensitized mice (Fig. 7).

DISCUSSION

In this study, we demonstrated that UVA irradiation of the eye induced immunosuppression of the ear epidermis in the FITC- or OXA-sensitized mice. On the other hand, in the colon specimens, UVA irradiation of the eye induced immunosuppression in FITC-sensitized mice but not in OXA-sensitized mice. These results suggested that UVA irradiation of the eye differently affected the CHS between the FITC (Th2 type)-sensitized mice and OXA (Th1 type)-sensitized mice.

In our previous report, we revealed that UVA irradiation of the eye modulated the intestinal mucosal functions and properties of mast cells in mice (17). Mast cells are important immune cells that participate in the inflammatory,

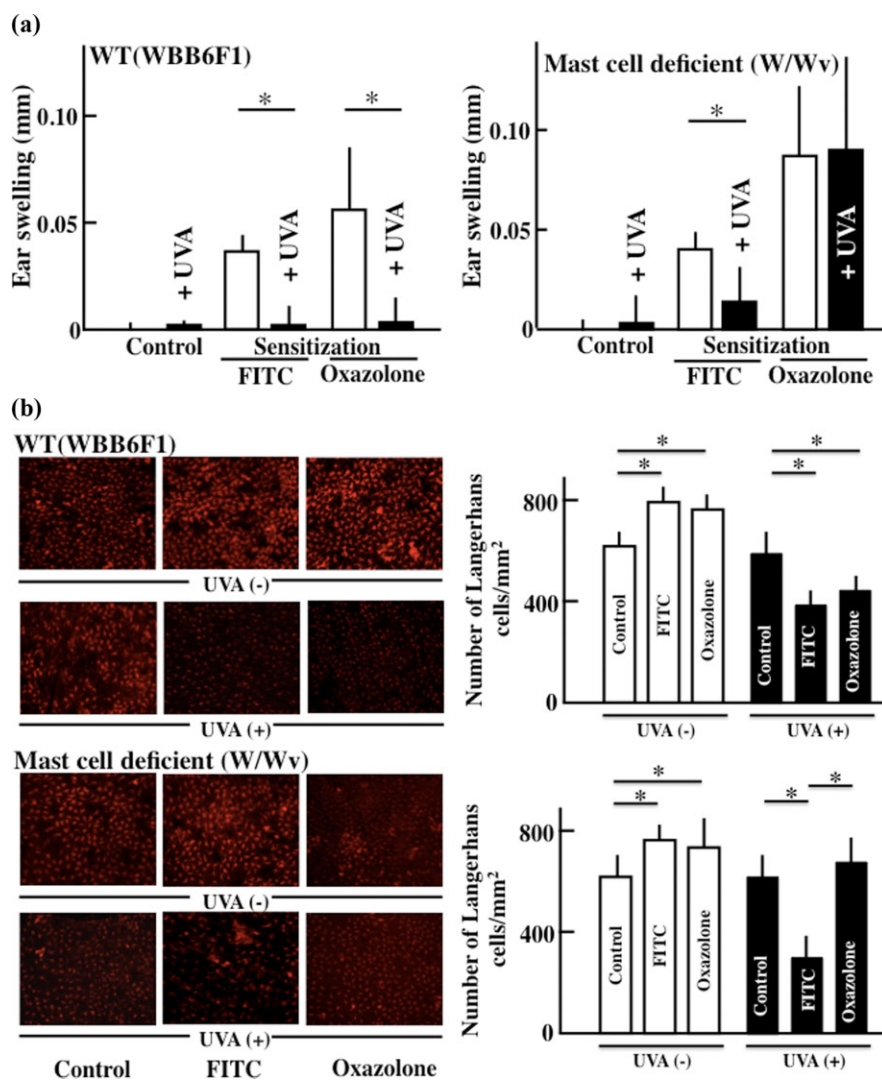


Fig. 5. The effects of UVA irradiation of the eye on the CHS response and the changes in the LCs of the ear epidermis in mast cell-deficient (W/Wv) mice sensitized to FITC or OXA. Under the identical conditions (Fig. 4), the ear swelling was measured 24 h after FITC or OXA challenge in WBB6F1/J (WT) or mast cell-deficient (W/Wv) mice. The contact hypersensitivity was expressed as the amount of ear swelling. The open and closed columns indicate nonirradiated and UVA-irradiated mice, respectively. The data are presented as the means $\pm$ standard deviation (SD) from six animals. * $P < 0.05$. CHS, contact hypersensitivity; FITC, fluorescein isothiocyanate; OXA, oxazolone; UVA, ultraviolet A.

allergic, and mucosal defense systems. In the present study, we investigated the changes in the CHS after UVA irradiation of the eye, to examine the relationship between mast cells and UVA irradiation in the pathology of delayed-type allergic reactions.

CHS is a form of a delayed type hypersensitivity, a classic T-cell-mediated, clinically important phenomenon elicited by small molecular weight molecules (haptens) that bind to host proteins to form a complete allergen (18). In this study, we used two different types of haptens, FITC and OXA, to induce the CHS. It is known that FITC mediates the activation of Th2-type cells and OXA mediates the activation of Th1-type cells (14, 15). By using two types of haptens, we studied the effects of UVA-irradiation of the eye on two different types of contact hypersensitized mice.

Toews revealed that the number of LCs was reduced in the sites of skin irradiated by UVB, and CHS failed to occur in the exposed skin (19). This suggested that UVB irradiation

of the skin causes immunosuppression. There were many subsequent reports that UV irradiation of the skin induces immunosuppression and reduces CHS (20). Our investigation showed that UVA irradiation of the eye inhibited the ear swelling in both FITC- and OXA-sensitized mice and decreased the area of LC infiltration in the ear epidermis. The LCs in the irradiated mice were smaller and more irregular than those in control mice. These findings suggested that local UVA irradiation of the eye affected the distant site (the ear epidermis) systemically.

The skin and intestine are immunologically important organs that protect the body against the outside world. In this study, we showed that UVA irradiation of the eye induced immunosuppression of the colon in the FITC-sensitized mice but not in the OXA-sensitized mice. The inflammation and bleeding of the colons in the FITC-sensitized mice was inhibited by UVA irradiation of the

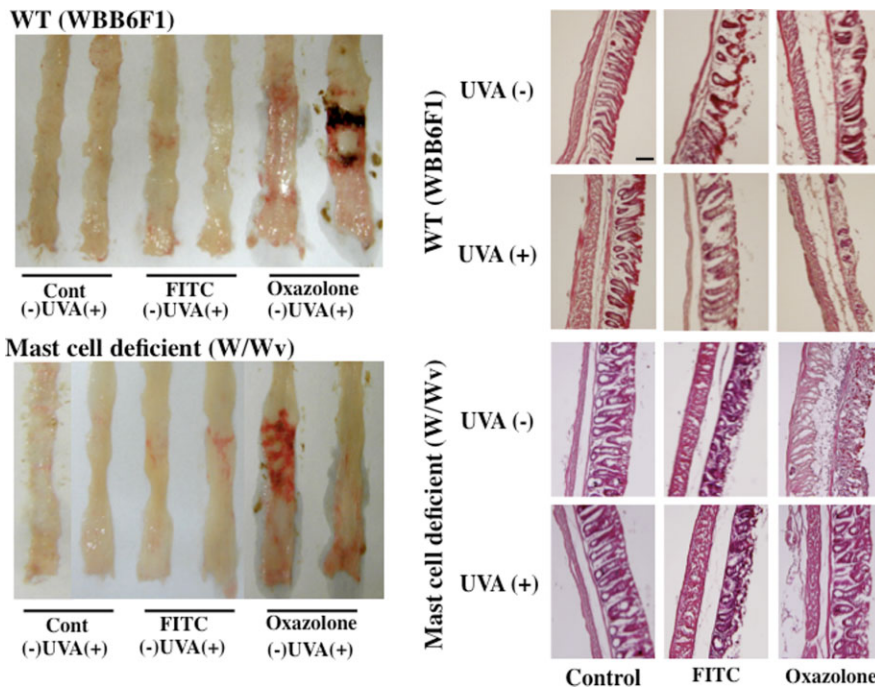


Fig. 6. The effects of UVA irradiation of the eye on the CHS response and inflammation of the colon in mast cell-deficient (W/Wv) mice sensitized to FITC or OXA. Under the identical conditions (Fig. 4), colon specimens were taken from WBB6F1/J (WT) and mast cell-deficient (W/Wv) mice 24 h after FITC or OXA challenge. The morphological findings are shown in photographs. The colon specimens were frozen, cut into thin sections and then stained with H-E. The data show one typical result from a total of six animals. Scale bar = 100 μ m. CHS, contact hypersensitivity; FITC, fluorescein isothiocyanate; H-E, hematoxylin and eosin; OXA, oxazolone; UVA, ultraviolet A.

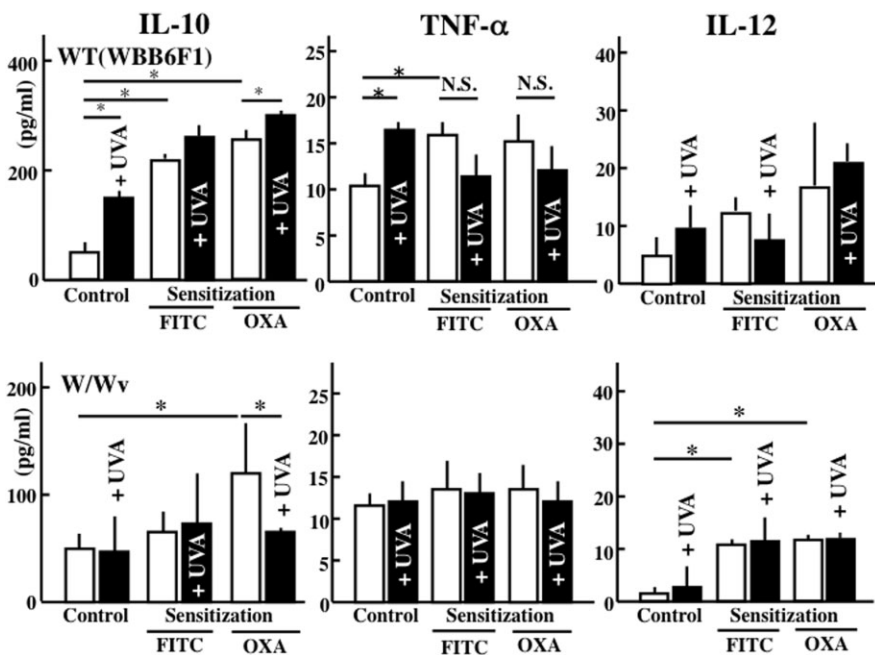


Fig. 7. The effects of UVA irradiation of the eye on the levels of plasma IL-10, TNF- α , and IL-12 in W/Wv mice sensitized to FITC or OXA. Under the identical conditions (Fig. 4), the plasma levels of cytokines such as IL-10, TNF- α , and IL-12 were determined in WBB6F1/J (WT) and mast cell-deficient (W/Wv) mice at 24 h after FITC or OXA challenge by ELISA. The open and closed columns are the control and UVA-irradiated mice, respectively. The data are presented as the means $\pm$ SD from six animals. * P < 0.05. ELISA, enzyme-linked immunosorbent assay; FITC, fluorescein isothiocyanate; IL-10, interleukin-10; OXA, oxazolone; SD, standard deviation; TNF- α , tumor necrosis factor-alpha; UVA, ultraviolet A.

eye, but this did not occur in the OXA-sensitized mice. These results suggested that UVA irradiation of the eye had different effects on the colon between the Th2-type and Th1-type contact hypersensitized mice.

It is well known that TNF- α plays an important role in many immunological and inflammatory reactions (21, 22). In addition, TNF- α mediates CHS (23). In this study, the

plasma level of TNF- α was increased in the FITC- and OXA-sensitized mice compared with the nonsensitized mice. However, the values in the UVA-irradiated sensitized groups were not significantly different compared with those in the nonirradiated sensitized groups. This may be because the UVA irradiation of the eye might induce the immunosuppression that thus stops the

secretion of TNF- α by immune cells. It was previously reported that IL-10, which is secreted by keratinocytes, mast cells and helper T cells, was increased by CHS (24, 25). We therefore analyzed the plasma level of IL-10 in this study. The level of IL-10 was found to be increased in both FITC-sensitized and OXA-sensitized mice compared with un-sensitized mice. In UVA-irradiated sensitized mice, the level of IL-10 was increased compared with that in the nonirradiated sensitized mice. IL-10 has an important role in the immunosuppression induced by UV irradiation (26), and IL-10 might be one of the major factors that induced the immunosuppression of the ear epidermis mediated by the UVA irradiation of the eye.

Because IL-12 and INF- γ are typical Th1 cytokines, we also analyzed the level of IL-12 in this study (27). The level of IL-12 was increased in the sensitized mice compared with un-sensitized control mice. However, in the UVA-irradiated sensitized mice, this level was not altered compared with that in nonirradiated sensitized mice. These results showed that UVA irradiation of the eye might more strongly affect the Th2 type cytokines than Th1 type cytokines.

To investigate the role of mast cells in the contact hypersensitized mice after UVA irradiation of the eye, we performed the same experiments in mast cell-deficient (W/W^v) mice. Although mast cells were once thought to function primarily as proinflammatory effector cells that could contribute to either allergic reactions or inflammation caused by innate responses to exogenous environmental agents (28), recent evidence indicates that, in certain settings, mast cells can also limit inflammation and tissue injury, including that induced by UV irradiation (29, 30).

In the experiments using W/W^v mice, the ear swelling of FITC-sensitized W/W^v mice was diminished compared with that in FITC-sensitized WT mice. Compared with nonirradiated FITC-sensitized W/W^v mice, the ear swelling of the UVA-irradiated FITC-sensitized W/W^v mice was inhibited. We found that UVA irradiation of the eye in FITC-sensitized W/W^v mice led to suppression of the inflammation in the ear epidermis. However, the ear swelling was not significantly different in the FITC-sensitized W/W^v mice compared with that in FITC-sensitized WT mice. These findings suggested that the mast cells of the ear epidermis have an important role in inducing inflammation associated with the CHS in FITC-sensitized mice.

In contrast, in the OXA-sensitized W/W^v mice, the ear swelling due to CHS was not affected by UVA irradiation of the eye. This suggested that mast cells have an important role in the UVA-derived immunosuppression of the epidermis in OXA-sensitized mice. Moreover, we

revealed that the epidermal mast cells have different roles in FITC (Th2-type)-sensitized mice and OXA (Th1-type)-sensitized mice. Therefore, the mast cells might differently influence the epidermis based on the type of sensitization.

In these experiments using W/W^v mice, the area of LCs in the FITC- or OXA-sensitized ear epidermis was larger than that in un-sensitized controls. In addition, the LCs in both types of sensitized mice were irregular and unbalanced compared with those in the control mice. However, UVA irradiation of the eye had no significant effects on the area of LCs in either of the sensitized-W/W^v mice in this study. Based on this finding, we thought that mast cells might contribute to the UVA-derived changes in the LCs in the ear epidermis. Further studies are thus required to confirm whether or not this is the case.

We demonstrated that the inflammation, bleeding, edema, and white blood cell infiltration of the colon specimens in FITC-sensitized W/W^v mice were decreased compared with those in FITC-sensitized WT mice. The immunosuppressive effect of UVA-irradiation of the eye on the colon was unremarkable in FITC-sensitized W/W^v mice. The different responses of the ear epidermis and colon in FITC-sensitized W/W^v mice suggested that the role of mast cells in the colon might be important to cause inflammation due to CHS after FITC-sensitization. On the other hand, the inflammation, bleeding, edema, and white blood cells infiltration of the colon specimens in OXA-sensitized W/W^v mice and OXA-sensitized WT mice increased to the same extent. In addition, the inflammatory responses of the colon specimens in UVA-irradiated OXA-sensitized W/W^v mice were weaker than those in un-irradiated OXA-sensitized W/W^v mice. This suggested that UVA irradiation of the eye had an immunosuppressive effect in OXA-sensitized mice. We then showed that the mast cells of the colon might be one of the factors that aggravates inflammation in the colon after UVA irradiation in OXA-sensitized mice, and it is possible that another type of cells, other than mast cells, may contribute to immunosuppression after UVA irradiation in OXA-sensitized W/W^v mice.

Regarding the responses of the ear epidermis and colon in OXA-sensitized W/W^v mice, the roles of mast cells appear to be different between the sites in these mice. This study suggested that after OXA-sensitization, the mast cells of the epidermis might contribute to UVA-induced immunosuppression, whereas the mast cells of the colon might contribute to aggravating the inflammation associated with CHS.

Collectively, the present data indicate that the functions of mast cells are different between mice sensitized to FITC

and OXA after UVA irradiation of the eye. Furthermore, there are differences in effects on mast cells at different sites (the epidermis and colon). This might have been caused by the differentiation of two types of mast cells, such as connective tissue mast cells and mucosal mast cells (MMC) (31). Because these two types of mast cells have different characteristics in terms of their release of histamine or protease, there might be different effects induced between the epidermis and intestine (32, 33). On the other hand, in terms of the immunosuppression induced

by UV exposure, steroidogenesis plays an important role (34, 35). We previously reported an increase in an adrenocorticotrophic hormone/corticosterone system by UV eye irradiation (36, 37). Further studies should be performed to analyze the impact on steroidogenesis.

In this study, we demonstrated that UVA irradiation of the eye had different effects on the different types of CHS (induced by different types of haptens), and the role of mast cells was also different between the epidermis and intestinal mucosa.

REFERENCES

- Slominski A, Wortsman J, Luger T, Paus R, Solomon S. Corticotropin releasing hormone and proopiomelanocortin involvement in the cutaneous response to stress. *Physiol Rev* 2000; **80**: 979–1020.
- Slominski A, Wortsman J. Neuroendocrinology of the skin. *Endocr Rev* 2000; **21**: 457–487.
- Slominski AT, Zmijewski MA, Skobowiat C, Zbytek B, Slominski RM, Steketeer JD. Sensing the environment: regulation of local and global homeostasis by the skin's neuroendocrine system. *Adv Anat Embryol Cell Biol* 2012; **212**: 1–115.
- Slominski AT, Zmijewski MA, Zbytek B, Tobin DJ, Theoharides TC, Rivier J. Key role of CRF in the skin stress response system. *Endocr Rev* 2013; **34**: 827–884.
- Kripke ML. Antigenicity of murine skin tumors induced by ultraviolet light. *J Natl Cancer Inst* 1974; **53**: 1333–1336.
- Kripke ML, Fisher MS. Immunologic parameters of ultraviolet carcinogenesis. *J Natl Cancer Inst* 1976; **57**: 211–215.
- Kripke ML, Lofgren JS, Beard J, Jessup JM, Fisher MS. In vivo immune responses of mice during carcinogenesis by ultraviolet irradiation. *J Natl Cancer Inst* 1977; **59**: 227–230.
- Moan J, Dahlback A, Setlow RB. Epidemiological support for an hypothesis for melanoma induction indicating a role for UVA radiation. *Photochem Photobiol* 1999; **70**: 243–247.
- Agar NS, Halliday GM, Barnetson RS, Ananthaswamy HN, Wheeler M, Jones AM. The basal layer in human squamous tumors harbors more UVA than UVB fingerprint mutations: a role for UVA in human skin carcinogenesis. *Proc Natl Acad Sci USA* 2004; **101**: 4954–4959.
- Takeuchi H, Runger TM. Long wave UV light induces the aging-associated progerin. *J Invest Dermatol* 2013; **133**: 1857–1862.
- Hiramoto K, Yamate Y, Kobayashi H, Ishii M. Long-term ultraviolet A irradiation of the eye induces photoaging of the skin in mice. *Arch Dermatol Res* 2012; **304**: 39–45.
- Ullrich SE, Nghiem DX, Khaskina P. Suppression of an established immune response by UVA – a critical role for mast cells. *Photochem Photobiol* 2007; **83**: 1095–1100.
- Grabbe S, Schwarz T. Immunoregulatory mechanisms involved in elicitation of allergic contact hypersensitivity. *Immunol Today* 1998; **19**: 37–44.
- Wang B, Fujisawa H, Zhuang L *et al*. CD4⁺ Th1 and CD8⁺ type1 cytotoxic T cells both play a crucial role in the full development of contact hypersensitivity. *J Immunol* 2000; **165**: 6783–6790.
- Ogawa A, Yoshizaki A, Yanaba K *et al*. The differential role of L-selectin and ICAM-1 in Th1-type and Th2-type contact hypersensitivity. *J Invest Dermatol* 2010; **130**: 1558–1570.
- Slominski A, Pawelek J. Animals under the sun: effects of ultraviolet radiation on mammalian. *Clin Dermatol* 1998; **16**: 503–515.
- Yamate Y, Hiramoto K, Kasahara E *et al*. Ultraviolet-A irradiation to the eye modulates intestinal mucosal functions and properties of mast cells in the mouse. *Photochem Photobiol* 2011; **87**: 191–198.
- Hiramoto K, Imao M, Sato EF, Inoue M, Mori A. Effect of fermented papaya preparation on dermal and intestinal mucosal immunity and allergic inflammations. *J Sci Food Agric* 2008; **88**: 1151–1157.
- Toews GB, Bergstresser PR, Streilein JN. Epidermal Langerhans cell density determines whether contact hypersensitivity or unresponsiveness follows skin painting with DNFB. *J Immunol* 1980; **124**: 445–453.
- Morison WL. Effects of ultraviolet radiation on the immune system of humans. *Photochem Photobiol* 1989; **50**: 515–524.
- Vassalli P. The pathophysiology of tumor necrosis factors. *Annu Rev Immunol* 1992; **10**: 411–452.
- Tracey KJ, Cerami A. Tumor necrosis factor, other cytokines and disease. *Annu Rev Cell Biol* 1993; **9**: 317–343.
- Piguet PF, Grau GE, Hauser C, Vassalli P. Tumor necrosis factor is a critical mediator in hapten induced irritant and contact hypersensitivity reactions. *J Exp Med* 1991; **173**: 673–679.
- Narbutt J, Lesiak A, Sysa-Jedrzejowska A *et al*. Repeated low-dose ultraviolet (UV) B exposures of humans induce limited photoprotection against the immune effects of erythral UVB radiation. *Br J Dermatol* 2007; **156**: 539–547.
- Narbutt J, Lesiak A, Sysa-Jedrzejowska A, Boncela J, Wozniacka A, Norval M. Repeated exposures of humans to low doses of solar simulated radiation lead to limited photoadaptation and photoprotection against UVB-induced erythema and cytokine mRNA up-regulation. *J Dermatol Sci* 2007; **45**: 210–212.
- Hiramoto K, Tanaka H, Yanagihara N, Sato EF, Inoue M. Effect of 17 β -estradiol on immunosuppression induced by ultraviolet B irradiation. *Arch Dermatol Res* 2004; **295**: 307–311.
- Weichenthal M, Schwarz T. Phototherapy: how does UV work? *Photodermatol Photoimmunol Photomed* 2005; **21**: 260–266.
- Galli SJ, Kalensnikoff J, Grimbaldston MA, Piliponsky AM, Williams CM, Tsai M. Mast cells as 'tunable' effector and immunoregulatory cells: recent advances. *Annu Rev Immunol* 2005; **23**: 749–786.
- Grimbaldeston MA, Nakae S, Kalesnikoff J, Tsai M, Galli SJ. Mast cell-derived interleukin 10 limits skin pathology in contact dermatitis and chronic irradiation with ultraviolet B. *Nat Immunol* 2007; **8**: 1095–1104.
- Galli SJ, Tsai M. Mast cells: versatile regulators of inflammation, tissue remodeling,

- host defense and homeostasis. *J Dermatol Sci* 2008; **49**: 7–19.
31. Enerback L. Mast cell in rat gastrointestinal mucosa 1. Effects of fixation. *Acta Pathol Microbiol Scand* 1966; **66**: 289–302.
 32. Bienenstock J, Befus AD, Pearce F, Denburg J, Goodacre R. Mast cell heterogeneity: derivation and function, with emphasis on the intestine. *J Allergy Clin Immunol* 1982; **70**: 407–412.
 33. Woodbury RG, Everitt M, Sanada Y, Katunuma N, Lagunoff D, Neurath H. A major serine protease in rat skeletal muscle: evidence for its mast cell origin. *Proc Natl Acad Sci USA* 1978; **75**: 5311–5313.
 34. Skobowiat C, Dowdy JC, Sayre RM, Tuckey RC, Slominski A. Cutaneous hypothalamic-pituitary-adrenal axis homolog: regulation by ultraviolet radiation. *Am J Physiol Endocrinol Metab* 2011; **301**: E484–E493.
 35. Slominski A, Zbytek B, Nikolakis G et al. Steroidogenesis in the skin: implications for local immune functions. *J Steroid Biochem Mol Biol* 2013; **137**: 107–123.
 36. Hiramoto K, Yanagihara N, Sato EF, Inoue M. Ultraviolet B irradiation of the eye activates a nitric oxide-dependent hypothalamopituitary proopiomelanocortin pathway and modulates functions of α-melanocyte-stimulating hormone-responsive cells. *J Invest Dermatol* 2003; **120**: 123–127.
 37. Hiramoto K. Ultraviolet A irradiation of the eye activates a nitric oxide-dependent hypothalamo-pituitary pro-opiomelanocortin pathway and modulates the functions of Langerhans cells. *J Dermatol* 2009; **36**: 335–345.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1. The experimental set-up for the UVA irradiation.



Fig. S1