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The Role of Epidermal Langerhans Cells in NB-UVB-Induced Immunosuppression

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ABSTRUCT

Narrowband ultraviolet B (NB-UVB) induces different immunological features from broadband ultraviolet B and is effective for the treatment of various cutaneous diseases. UV exposure alters the morphology and function of epidermal Langerhans cells (LCs), which can elicit cutaneous immunosuppressive responses. Recent studies have proposed that LCs serve as immunoregulatory cells in UV-induced immune suppression. This study investigated the cellular mechanisms of NB-UVB-induced immune suppression, including its effects on LC migration. NB-UVB irradiation induced the migration of epidermal LCs from the skin to the draining lymph nodes in a time- and dose-dependent manner. Experiments in Lang-DTR knock-in mice confirmed that epidermal LCs rather than Langerin$^+$ dermal dendritic cells are essential for NB-UVB-induced immune suppression. These findings indicate that LCs play a critical immunoregulatory role in NB-UVB-induced immune suppression and NB-UVB phototherapy.

INTRODUCTION

Ultraviolet (UV) radiation in sunlight can adversely affect human health. However, UV therapy is commonly used to treat skin disorders. Phototherapy with UVB can be classified as either conventional broadband UVB (BB-UVB) therapy, which has a wavelength of 280–320 nm, or narrowband UVB (NB-UVB) therapy, with a peak wavelength of 311 nm [1]. Pioneering studies demonstrated that wavelengths around 311 nm provoked the least amount of erythema, while NB-UVB was most effective for clearing psoriasis [2, 3]. Clinical studies found that psoriasis responded better to NB-UVB than BB-UVB therapy [4-7], and NB-UVB phototherapy has subsequently been used to treat a variety of skin diseases, including atopic dermatitis, vitiligo, and mycosis fungoides.

UV therapy is thought to act through the induction of immune suppression, as demonstrated by the inhibition of contact hypersensitivity (CHS). Mechanisms include local immune suppression where the hapten is applied directly to the UV-irradiated skin, and systemic immune suppression [8, 9]. UV-induced immune suppression involves the disruption of Langerhans cell (LC) function, DNA damage, induction of soluble epidermal factors such as IL-10, and the induction of regulatory T cells [9, 10]. This inducible immune suppression is hapten-specific and can be transferred to naïve mice [8, 11]. A recent report suggested that LCs were dispensable for BB-UVB-induced local immune suppression, while

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in contrast, other reports have suggested that they are essential [12-15]. Moreover, BB-UVB exposure can trigger the migration of mature, but not immature IL-10-producing LCs from the skin to the draining lymph nodes (DLNs) [12, 15]. The mechanism of BB-UVB-induced immune suppression has been well studied, whereas the mechanism of NB-UVB action remains unclear. Although one report demonstrated NB-UVB irradiation induced suppression of CHS and tolerance in C3H/HeN mice, the cellular mechanism requires further investigation [16].

Because UV radiation in sunlight is the primary cause of skin cancer, for which UV-induced immune suppression is a major risk factor [17, 18], clinicians need to be aware of the potential carcinogenicity of NB-UVB therapy. No definitive relationship between NB-UVB phototherapy and skin cancer has been observed in retrospective studies in any ethnic group [19-21]. However, a recent study in mice found that the ratio of malignant skin tumors induced by chronic exposure of NB-UVB was significantly higher than that induced by BB-UVB, and epidermal cyclobutane pyrimidine-dimer formation following a minimum erythema dose (MED) of NB-UVB was significantly higher than that following a MED of BB-UVB [1]. Thus, the increased carcinogenic risk of NB-UVB phototherapy should be weighed against its greater therapeutic benefits. A better understanding of the cellular mechanisms involved in NB-UVB therapy is therefore important when considering the balance between carcinogenicity and efficacy.

This study investigated the cellular mechanisms of NB-UVB-induced immune suppression, including its effects on LC migration and suppression of the CHS response in *Lang-DTR* knock-in mice, in which LCs can be depleted by administration of diphtheria toxin (DT). Our data demonstrate that epidermal LCs are stimulated to migrate from the skin to the draining lymph nodes by NB-UVB irradiation, and are essential for NB-UVB-induced immune suppression.

MATERIALS AND METHODS

Animals

Female C57BL/6N mice aged 6–8 weeks were purchased from Charles River Laboratories Japan, Inc. *Lang-DTR (diphtheria toxin receptor) enhanced green fluorescent protein* knock-in transgenic mice, in which the Langerin promoter drives expression of the diphtheria toxin (DT) receptor [22, 23], were obtained from Dr. Bernard Malissen (INSERM, Paris, France). Animals were housed under specific pathogen-free conditions. Animal care was provided by expert personnel in compliance with the relevant laws and institutional guidelines of Kobe University Graduate School of Medicine (Kobe, Japan).

UV source

The irradiance from the TL 20W/01RS lamp was 11.3 J/m^2/second at a distance of 40 cm, as measured with an IL1400A radiometer/photometer (International Light Inc., Peabody, MA, USA), respectively. The dorsal hair of mice was removed and mice were exposed to UV using a TL 20W/01RS lamp.

Antibodies and reagents

Monoclonal antibodies specific for CD8 (clone 53-6.7), CD207 (Langerin) (clone RMUL.2), Alexa Fluor 647 rat IgG2a isotype control and Armenian hamster IgG isotype control Alexa Fluor 488 were purchased from eBioscience (San Diego, CA, USA). Alexa Fluor 594 anti-rat IgG (H+L), streptavidin-Alexa Fluor 594 conjugate, biotin Rat IgG2a isotype control, streptavidin-Alexa Fluor 594 conjugate, and biotin rat IgG2a isotype control were purchased from Invitrogen (Carlsbad, CA, USA). Antibodies to CD11c (clone HL3) and streptavidin Per-CP were purchased from BD Pharmingen (San Diego, CA, USA).

2,4-dinitro-1-fluorobenzene (DNFB) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Preparation of epidermal sheets and immunofluorescence analysis

Epidermal sheets were prepared as previously described [15]. Epidermal sheets were fixed in acetone for 3–5 minutes at −20 °C, and stained with rat anti-mouse Langerin antibody overnight at room temperature, and Alexa Fluor 594 anti rat IgG (H+L) was used as the secondary antibody. After washing with PBS, they were mounted using Prolong Gold antifade reagent with DAPI. The number of Langerin$^+$ cells was counted using a fluorescence microscope (Keyence, BZ-8100, Osaka, Japan).

Induction of migration of skin-derived DCs to DLNs by NB-UVB irradiation

The dorsal hair of mice was removed and mice were exposed to 10 kJ/m^2 of NB-UVB. Inguinal DLNs from control or irradiated mice were removed 7 days after UV exposure and stained for CD11c, CD8α and CD207, followed by flow cytometric analysis using FACSCaliber (BD Biosciences, San Diego, CA, USA).

Suppression of CHS by UV radiation in *Lang-DTR enhanced green fluorescent protein* knock-in mice

LCs were depleted in *Lang-DTR enhanced green fluorescent protein* knock-in mice by intraperitoneal injection of 1 μg DT (Calbiochem, San Diego, CA, USA) [22]. At 1 or 7 days after DT treatment, mice were exposed to 10 kJ/m^2 of NB-UVB on the shaved area on the back of the mice. Seven days post-UV exposure, mice were sensitized by painting 50 μl of DNFB solution (0.5% in acetone/olive oil; 4:1) on the shaved back. After a further 6 days, 20 μl of 0.2% DNFB was applied to the ear. Ear swelling was quantified with a micrometer 24 hours after elicitation. The degree of CHS was determined by measuring the change in thickness of the ear due to swelling, and was expressed in centimeters × 10^{-3} (mean ± SD). Each group consisted of at least five mice.

Statistical analysis

Data (except those in Fig. 1 and 6) were analyzed statistically using the Student's *t*-test. The data in Fig. 1 and 6 were analyzed using Dunnett's and Tukey's tests, respectively. In all cases, values of $p<0.05$ were considered statistically significant.

RESULTS

NB-UVB irradiation decreases LC density in the epidermis in a dose- and time-dependent manner

A previous study of immune suppression induced by BB-UVB irradiation in terms of CHS response demonstrated that immune suppression correlated with the degree of epidermal LC depletion 7 days after BB-UVB irradiation [15]. We initially determined that NB-UVB irradiation also reduced epidermal LC density in the ear in C57BL/6 mice in a dose-dependent manner (**Figure. 1A**). Epidermal LC numbers were significantly decreased 7 days after treatment with 5, 10, and 15 kJ/m^2 NB-UVB (**Figure. 1A**). Doses of 10 and 15 kJ/m^2 NB-UVB irradiation clearly reduced epidermal LC density. Next, we assessed the time course of LC depletion in ear epidermis in C57BL/6 mice exposed to 10 kJ/m^2 NB-UVB. LC numbers started to decrease on day 1 and minimal epidermal LC numbers were observed on day 7. NB-UVB irradiation decreased the number of epidermal LCs in a time-dependent manner (**Figure. 1B**). This suggests that NB-UVB can induce the migration of LCs from the epidermis.

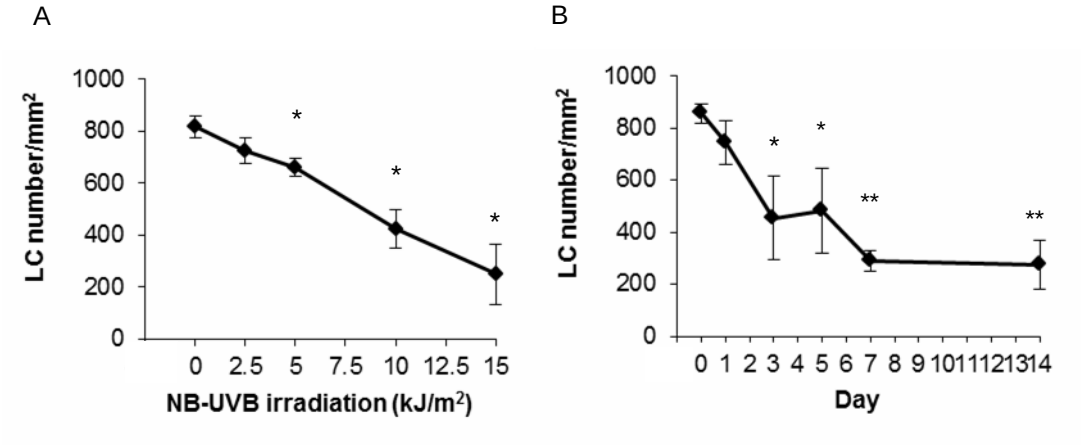

Figure 1.
Dose and time-dependent decrease in epidermal LC density in the epidermis following NB-UVB irradiation. (A) C57BL/6 mice were irradiated on day −7 with 0, 2.5, 5, 10, and 15 kJ/m^2 of NB-UVB. The numbers of epidermal LCs in epidermal sheets from ears were counted on day 0. Density of epidermal LCs in the epidermis was determined.
(B) C57BL/6 mice were irradiated with 10 kJ/m^2 of NB-UVB. Ears were collected on days 0, 1, 3, 5, 7, and 14. Epidermal sheets were stained with anti-Langerin antibody. The density of epidermal LCs was counted in the epidermal sheets. The density of epidermal LCs was decreased by NB-UVB irradiation and reached a minimum on day 7. Each group consisted of five mice. Each experiment was repeated twice. **p<0.01 compared with unirradiated mice (day 0); *p<0.05 compared with unirradiated mice (day 0).

NB-UVB irradiation induces migration of skin-derived Langerin$^+$ dendritic cells (DCs) into DLNs

BB-UVB irradiation can promote LC migration from the skin to DLNs, where they induce immune suppression [12, 15, 16, 24, 25]. We focused on the migration of cutaneous DCs into DLNs after NB-UVB irradiation. DLNs were removed 7 days post-NB-UVB irradiation. Single cell suspensions were analyzed, and the number of Langerin$^+$ CD11c$^+$ CD8α$^-$ cells, representing skin-derived Langerin$^+$ DCs, was counted (**Figure. 2**). The number of DLN cells increased significantly 7 days after NB-UVB irradiation (**Figure. 2A**). In addition, there was a significant increase in skin-derived Langerin$^+$ DCs 7 days after NB-UVB irradiation (**Figure. 2B**).

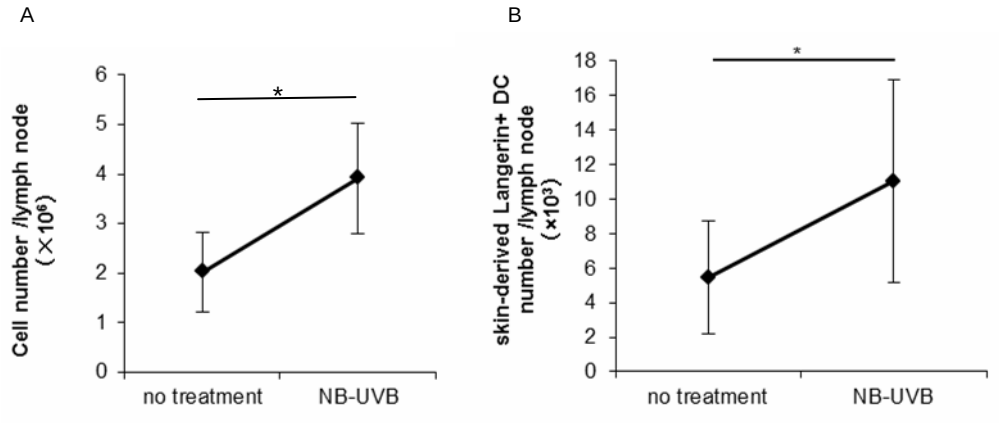

Figure 2.
Acceleration of migration of skin-derived Langerin$^+$ cells into draining lymph nodes by NB-UVB irradiation. C57BL/6 mice were irradiated on the back on day −7 with 10 kJ/m^2 of NB-UVB. Lymph nodes were collected and cell suspensions were stained for CD8α, CD11c, and Langerin, and analyzed by flow cytometry on day 0. The numbers of CD11c$^+$CD8α$^-$Langerin$^+$ cells were counted. (A) Total numbers of cells in the draining lymph nodes were measured 7 days after NB-UVB exposure. (B) The number of skin-derived Langerin$^+$ DCs migrating into DLNs 7 days after NB-UVB irradiation was counted. Each group consisted of five mice. Each experiment was repeated twice. *$p<0.01$ compared with untreated mice.

Epidermal LCs are essential for NB-UVB-induced immune suppression

We used *Lang-DTR* knock-in mice [12, 15, 22, 23, 26], where LCs can be depleted by administration of DT, to confirm the role of LCs in NB-UVB-induced immune suppression. Both epidermal LCs and Langerin$^+$ dDCs are depleted following DT injection in *Lang-DTR* knock-in mice. Langerin$^+$ dDCs start to repopulate the dermis 3 days post-DT treatment, while LCs do not repopulate the epidermis until at least 2 weeks later [22]. This system therefore allows analysis of the individual roles of epidermal LCs and/or Langerin$^+$ dDCs in NB-UVB-induced suppression of the CHS response. DT was injected 7 days (day -7) or 1 day (day -1) before NB-UVB irradiation (day 0), followed by hapten sensitization (day 6) and challenge (day 13). CHS responses were significantly suppressed in mice sensitized through skin exposure to NB-UVB irradiation (#2 and #3, **Figure. 3**). Thus, NB-UVB-induced immune suppression occurred in *Lang-DTR* knock-in mice. The immune suppressive effects of NB-UVB irradiation in *Lang-DTR* knock-in mice following DT injection were also investigated. Interestingly, no suppression of the CHS response by NB-UVB irradiation was observed in mice depleted of both LCs and Langerin$^+$ dDCs by DT treatment 1 day before NB-UVB irradiation, compared with controls without NB-UVB exposure (#5 and #4, **Figure. 3**). These results suggest that either epidermal LCs and/or Langerin$^+$ dDCs are essential for NB-UVB-induced immune suppression. To identify the critical cell type that mediated NB-UVB-induced immune suppression, DT was administered 7 days before NB-UVB irradiation, in the absence of epidermal LCs but in the presence of recovered Langerin$^+$ dDCs. No NB-UVB irradiation-induced suppression of the CHS response occurred in mice possessing Langerin$^+$ dDCs but not LCs (day -7 DT treatment), compared with controls without NB-UVB exposure (#7 and #6, **Figure. 3**). This suggested that epidermal LCs, but not Langerin$^+$ dDCs, were essential for NB-UVB-induced immune suppression.

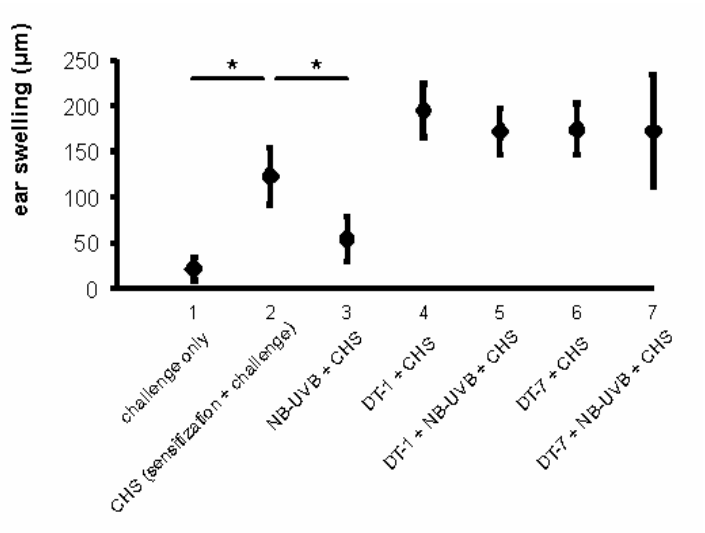

Figure3
Role of epidermal LCs in NB-UVB-induced immune suppression. *Lang-DTR* knock-in mice were injected with DT on days −7 or −1. Mice were irradiated with 10 kJ/cm^2 of NB-UVB on day 0, and the shaved back skin was sensitized with DNFB on day 6, followed by DNFB challenge to the right ear on day 13. The thickness of the right ear was measured after 24 hours. Positive control mice were sensitized and challenged (#2); negative control mice were challenged only (#1); NB-UVB+CHS mice were irradiated with NB-UVB, sensitized and challenged (#3). DT-1+ NB-UVB+CHS mice and DT-7+ NB-UVB+CHS mice were injected with DT at 1 and 7 days prior to NB-UVB irradiation and were then sensitized and challenged, respectively (#5 and #7). As additional controls, DT-1+CHS mice and DT-7+CHS mice were injected with DT on day 8 (1d+7d) and 14 (7d+7d) prior to sensitization and challenged (#4 and #6). Each group consisted of five mice. Each experiment was repeated twice. *$p<0.05$ group 1 *versus* group 2, group 2 *versus* group 3.

DISCUSSION

NB-UVB therapy is more effective and more frequently used than conventional BB-UVB therapy, although the mechanism of NB-UVB action remains unclear [5]. Here, we focused on the cellular mechanisms responsible for the efficacy of NB-UVB therapy by measuring local CHS responses in the ear. A dose of 10 kJ/m^2 of NB-UVB irradiation prior to DNFB sensitization significantly suppressed the CHS response in both C57BL/6 and *Lang-DTR* knock-in mice confirming that the immune suppressive effect of NB-UVB is independent of mouse strain.

LCs are not thought to be involved in the induction of cutaneous immune responses, rather LCs appear to function as immunoregulatory cells in some conditions [23, 24, 27]. We recently proposed a critical role for LCs in immune suppression induced by BB-UVB irradiation [15]. Based on these results, we focused on the role of LCs in NB-UVB-induced immune suppression. Epidermal LCs in C57BL/6 mice migrated in a dose-dependent manner following NB-UVB irradiation. In addition, LC numbers started to decrease on day 1, and minimal epidermal LCs were observed in the epidermis on day 7. A significant increase in the cellularity of DLN cells was also noted 7 days post-NB-UVB irradiation, indicating migration of a variety of bone marrow-derived cells into DLNs. As expected, skin-derived Langerin$^+$ DCs (CD11c$^+$CD8α$^-$Langerin$^+$), including epidermal LCs, were significantly increased 7 days after NB-UVB irradiation. These results were similar to those

demonstrating BB-UVB irradiation-induced epidermal LC migration from the skin into DLNs [15]. Application of DNFB to the irradiated site 7 days post-NB-UVB irradiation resulted in significant suppression of the CHS response. This observation raised the question of whether the depletion of epidermal LCs was responsible for the reduced sensitization, or whether LCs acted as immunoregulatory cells in DLNs.

To clarify this, we took advantage of *Lang-DTR* knock-in mice where Langerin$^+$ cells are depleted by DT administration. Langerin$^+$ dDCs repopulate the skin 3–7 days after treatment while LCs remain absent from the epidermis until 14–28 days after injection. The CHS response was not suppressed by NB-UVB in mice lacking both LCs and Langerin$^+$ dDCs, or in those lacking LCs alone at the timing of NB-UVB irradiation, compared with controls without NB-UVB exposure. The results of these experiments were in accord with previous studies of BB-UVB [12, 14, 15], and demonstrate that epidermal LCs rather than Langerin$^+$ dDCs are essential for activating NB-UVB-induced immune suppression.

In summary, NB-UVB irradiation induces the migration of epidermal LCs from the skin to DLNs and suppresses the CHS respons. The essential role of epidermal LCs in NB-UVB-induced immune suppression supports the recent concept of LCs as important immunoregulatory cells. These findings may contribute to the development of a safe and efficient method of NB-UVB phototherapy.

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