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Inhibition of Langerhans cell function by UVB radiation

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The functional disruption of Langerhans cells (LC) by UVB radiation is involved in antigen-specific immunosuppression of contact hypersensitivity. We tested whether UVB radiation inhibits the endocytotic activity of LC, which leads to impaired subsequent migration and maturation. Human monocyte-derived LC that took up lucifer yellow (LY) or FITC-dextran (Fd) exclusively migrated in response to 6Ckine and matured. Exposing LC to 10-40 mJ/cm^2 of UVB radiation reduced their endocytotic activity in fluid phase pinocytosis (measured by uptake of LY) and in receptor-mediated endocytosis (measured by uptake of Fd). Membrane ruffling and CD32 expression were also suppressed by UVB radiation. UVB-irradiated, endocytosing LC had less movement towards 6Ckine, expressed less CD54 and CD86, and had less effective stimulatory activity in allo-MLR than nonirradiated, endocytosing LC. Endocytosis up-regulated TNF-α production by LC, but prior UVB radiation inhibited this enhancement. The finding that impaired endocytosis of LC by UVB radiation inhibits subsequent migration and maturation was also confirmed in murine epidermal cells obtained from unirradiated and 20mJ/cm^2 of UVB-irradiated skin.

Key words : endocytosis, Langerhans cells, UV-induced immunosuppression, migration, maturation

INTRODUCTION

Dendritic cells (DC) are the most potent antigen-presenting cells (APC) involved in the initiation of primary immune responses [1]. DC present in peripheral tissues, as immature cells and efficiently take up an antigen (Ag). Langerhans cells (LC) represent such immature DC in the epidermis. Uptake of Ag by immature DC is mediated through two distinct mechanisms. One is macropinocytosis. During this process, macropinosomes are formed at the site of membrane ruffling and require an actin-based cytoskeleton. The other mechanism is endocytosis mediated through receptors such as mannose receptor (MR) and Fcγ receptor II (FcγRII). In contrast to efficient Ag uptake by immature DC, this property is lost when DC mature and migrate into secondary lymphoid organs.

Applying an Ag onto skin previously exposed to UVB radiation results in the development of hapten-specific immunological tolerance, which is correlated with the induction of suppressor T cells [2,3]. Since the number of LC is reduced and their morphology is altered in UVB-irradiated skin, tolerance was considered to be associated with impaired APC in the skin. The hypothesis was supported by the fact that the Ag presenting function of LC is suppressed in an *in vitro* experimental system. However, the effects of UVB radiation on the endocytotic activity of LC as well as the migration activity and maturation properties of irradiated LC after endocytosis are not well defined. Here we examined whether UVB radiation inhibits the migration and maturation of LC by impairing endocytotic activity.

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MATERIALS AND METHOD

Preparation of Langerhans Cells from Human Monocytes We isolated LC as described by Geissmann et al. [4]. Human monocytes were isolated from peripheral blood mononuclear cells (PBMC) by depleting non-monocytes with an indirect magnetic labeling system Monocyte Isolation Kit (Miltenyi Biotec). LC were differentiated from monocytes by culturing with 10% FCS, rhGM-CSF (250ng/mL), rhIL-4 (100ng/mL) and TGF-β (10ng/ml) for 6 days.

UVB radiation UVB radiation was delivered from a bank of seven fluorescent tubes (FL20SE) with an emission spectrum of 275-375nm peaking at 305nm, emitting mainly within the UVB range. The irradiance was 0.35mW/cm^2 at a distance of 35 cm measured using a radiometer (UVR-305/365D (II)).

Quantitation of Fluid phase Endocytosis and Receptor-mediated Endocytosis Fluid phase endocytosis and receptor-mediated endocytosis were measured as the cellular uptake of lucifer yellow (LY) CH dipotassium salt and fluorescein isothiocyanate-dextran (Fd) quantified by flow cytometry, respectively. Approximately 2×10^5 cells per sample were incubated in medium containing LY (1

mg/ml) or Fd (1 mg/ml) for 120 minutes. After incubation, cells were fixed in cold 1% formalin. The quantitative uptake of LY and Fd by the cells was determined using the FACScan.

Laser Confocal Imaging of Actin and Membrane Ruffles We visualized membrane ruffles containing F-actin using a modification of the protocol described by Cheresh et al. [5]. After fixation in 4% paraformaldehyde, cells were permeabilized with 0.2% Triton X-100 and stained with TRITC-labeled phalloidin. Cells were observed using a personal confocal microscope system FLUOVIEW. Cells with prominent actin-rich membrane ruffles represent the average percentage of LC with ruffles of five fields.

Chemotaxis assay The migration of LC was assayed in multiwell chemotactic chambers fitted with a 8-μm pore size polycarbonate membrane. This membrane separated cells from 300μl of 50ng/ml 6Ckine or control medium placed in the lower compartments. 100μl of cell suspension was seeded into the upper compartments. After a 2hr incubation, the membranes were removed, fixed, and stained with Giemsa. Migrated cells were counted under a microscope (×400). Values are expressed as a percentage of the activity in non-endocytosing LC.

ELISA for cytokine determination Protein levels of TNF-α and IL-10 in culture supernatants of LC were measured using a commercially available enzyme immunoassay kit (Genzyme Corp) according to the manufacturer's instructions.

Allogenic MLR Allogenic T cells were distributed at a density of 5×10^4 cells/well into flat-bottom 96-well microplates and incubated for 5 days in the presence of graded numbers of irradiated LC stimulators (3000 rad, ^{137}Cs source). T cell proliferation was assessed using a cell proliferation ELISA, BrdU (Roche) according to the manufacturer's instructions.

Preparation of murine epidermal cell suspension Ear skin of female C57BL/6 mice (8-10wk) was exposed to 20mJ/cm^2 of UVB radiation. Twenty-four hours later ear skin was split into two pieces and incubated in 20mM ethylenediamine tetraacetic acid solution (pH7.4) for preparation of epidermal sheets. The sheets were then treated with 0.5% trypsin solution for 15min and LC were partially enriched by density gradient centrifugation.

Calculations and statistical analysis Statistical significance was determined using Student's *t*-test. Results are presented as means ± SD. $P < 0.05$ was considered statistically significant.

RESULTS

Effect of UVB radiation on endocytotic activity of LC We exposed LC to 10, 20 or 40mJ/cm^2 UVB radiation and analyzed their endocytotic activities 24 hours later. UVB radiation inhibited both fluid phase pinocytosis and receptor-mediated endocytosis [Fig.1]. The extent of inhibition between fluid phase pinocytosis and receptor-mediated endocytosis by UVB radiation did not differ. The inhibition of endocytotic activity peaked at 24 hours after irradiation. The kinetics of Fd uptake by UVB-treated LC were the same as those of LY uptake.

Effect of UVB radiation on CD32 and MR expression CD32 (FcγRII) and MR are Ag uptake molecules associated with receptor-mediated endocytosis. FACS analysis showed that monocyte-derived LC expressed large amounts of CD32. On the other hand, as reported by Mommaas et al. [6], the expression of MR was negligible. A low dose of UVB radiation significantly down-regulated CD32 expression.

Effect of UVB radiation on membrane ruffling Activation of fluid phase pinocytosis is characterized by the assembly of actin into membrane ruffles. Membrane ruffling was observed in approximately 90% of unexposed LC and decreased in about 50% after UVB radiation. This indicates that the inactivation of membrane ruffling by UVB radiation leads to the reduction of fluid phase pinocytosis in UVB-LC.

Effect of UVB radiation on migration of LC toward chemoattractants The CCR7 ligand, 6Ckine, helps LC migrate towards lymph nodes. When LC were treated with LY or Fd, the migratory activity increased by 137.2 ± 4.3% or 197.8 ± 17.9%, respectively. However, when LC were exposed to UVB radiation before LY or Fd, the enhancement of migratory capacity to 6Ckine by endocytosis was significantly suppressed.

Effect of UVB irradiation on TNF-α and IL-10 production of LC TNF-α and IL-10 are implicated in LC migration. TNF-α production by LC was highly enhanced by fluid phase pinocytosis or receptor-mediated endocytosis. Therefore, we tested whether UVB radiation affects the cytokine production by LC, thus inhibiting migration activity. Although UVB radiation did not affect TNFα production by non-endocytosing LC, it reduced the production by LY-treated LC and Fd-treated LC by 60% and 77%, respectively. On the other hand, IL-10 production by LC was not affected by either type of endocytosis or by UVB radiation.

Effect of UVB radiation on LC maturation After uptake of Ags, LC mature while migrating into the draining lymph nodes (DLN). Therefore, we examined whether UVB radiation affects the maturation process of LC, by phenotypic and functional means. CD54 moderately and CD86 were weakly expressed in untreated LC, whereas high levels of both surface Ags were expressed in LY-

treated LC and Fd-treated LC endocytosis, indicating that fluid phase pinocytosis and receptor-mediated endocytosis induced LC maturation. The expression of CD86 was more dramatically enhanced than that of CD54. UVB radiation before exposure to LY or Fd inhibited the up-regulation of CD54 and CD86 expression by fluid phase pinocytosis and by receptor-mediated processes.

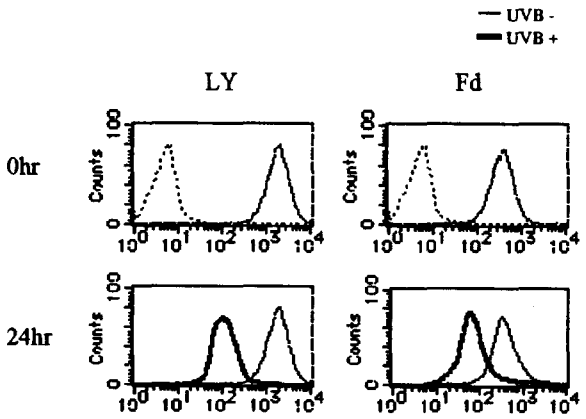

Fig. 1 Effects of prior UVB radiation on endocytotic activity of human monocyte-derived Langerhans cell-like cells.

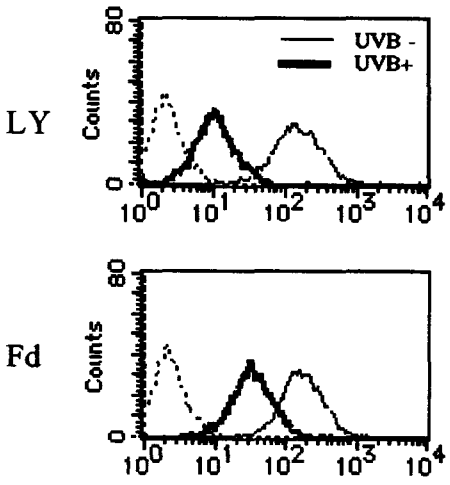

Fig. 2 Endocytosis by CD11$^+$ epidermal cells from normal or UVB-treated murine skin.

We tested whether both types of endocytosis up-regulate the allostimulatory capacity of LC. LY-treated LC and Fd-treated LC stimulated more proliferation of allogenic T cells than unendocytosing LC, indicating that both types of endocytosis induce maturation of LC. However, when LC were exposed to UVB radiation before LY or Fd, the allostimulatory capacity was significantly lower than that of non-irradiated LC. These results suggest that the impairment of fluid phase pinocytosis and receptor-mediated endocytosis caused by UVB radiation inhibits LC maturation.

Endocytotic activity of murine CD11c$^+$ epidermal cells

Fig.1 shows that CD11$^+$ epidermal cells of untreated mice had high endocytotic activity in both types of endocytosis. In contrast, CD11$^+$ epidermal cells from 20mJ/cm^2 of UVB irradiated skin exhibited the suppressed activity. Migration ability in response to 6Ckine was enhanced by endocytosis, but suppressed in endocytosing epidermal cells from UVB-irradiated skin. CD86 in normal epidermal cells was also enhanced by uptake of LY or Fd, but their enhanced activity was not observed in cells from UVB-irradiated skin.

DISCUSSION

Monocyte-derived LC-like cells efficiently endocytosed LY and Fd. The migration of LY or Fd-engulfed LC was enhanced in response to 6Ckine. During the process of migration to DLN, LC undergo functional maturation. Our study showed that endocytosing LC exhibited phenotypical and functional maturation properties, including high expression levels of CD54 and CD86, and increased T cell stimulatory capacity. Thus, endocytosis was an induction factor for maturation as well as the enhanced migration of LC. However, a low dose of UVB radiation decreased the endocytotic activity of LC and inhibited the endocytosis-induced enhancement of migration and maturation of LC. These findings were also confirmed in LC-enriched epidermal cells from mouse skin. Since UVB radiation impaired membrane ruffling and down-regulated the expression of CD32, the reduced LC endocytosis caused by UVB radiation was due to damage of the actin cytoskeleton [5] and membrane components.

Inflammatory cytokines such as TNF-α and IL-1β are crucially involved in the induction of DC migration from the skin [7]. The sources of TNF-α in the skin include various types of cells in the epidermis and dermis. However, an early event of the primary immune response in LC *in vivo* is uptake of Ag. Therefore, an encounter between LC and this cytokine produced in a paracrine fashion in the skin should be understood as an event that occurs after Ag-uptake. Once Ag is uptaken, LC subsequently migrate from the skin into DLN. This implies that endocytosis is closely related to a signal for enhanced migration activity. Our findings showed that endocytosing LC acquired a more migration activity without cytokines produced by other skin

components. The level of TNF-α in the culture medium of endocytosing LC significantly increased. This suggests that the enhancement of migration activity in LY-LC and Fd-LC may be induced by the autocrine production of TNF-α.

The number of LC is reduced by UVB irradiation, which is considered to be involved in UVB-induced local immunosuppression of contact hypersensitivity (CHS). One of the reduction mechanisms is the death of LC via necrosis or apoptosis. We reported that a low dose of UVB radiation induces apoptosis in LC as well as in keratinocytes [8]. Another possibility is the loss of cell identification by membrane damage, as shown by ATPase and class II Ag. A third explanation for the reduction mechanism is migration of LC from the irradiated epidermis into the DLN. Modyclifly et al. [9] demonstrated that UVB radiation induces the accumulation of DC in DLN of irradiated skin. Kolgen et al. [10] recently reported that disappearance of LC by UVB radiation is mainly caused by migration but not apoptosis in human skin. This event is considered to be related to cytokines produced in irradiated. Our study showed that prior exposure of UVB radiation before endocytosis significantly inhibited both LC migration to 6Ckine and the production of TNF-α. In contrast, IL-10 was not affected by endocytosis or by UVB radiation. These findings suggested that the decreased migration activity of human LC caused by UVB radiation is related to TNF-α but not to IL-10 produced by LC themselves.

UVB radiation is considered to impair the Ag presenting capacity of LC, mainly by inhibiting costimulatory signals of LC for T lymphocytes. Considering the migration of LC in the UVB-induced local immunosuppression of CHS, some LC may leave the epidermis for DLN after UVB radiation, whereas others stay at the irradiated epidermis. The former contributes to the decreased number of functional APC in UVB-irradiated sites. The results of the current study suggested that residual LC after UVB radiation are down-regulated in their ability to move to DLN after encountering Ag. Simon et al. [11] showed that *in vitro* UVB-irradiated LC can stimulate Th2, but not Th1 clones. Therefore, UVB-irradiated LC with altered or impaired Ag presenting function play a crucial role in the immunosuppression of CHS. However, Ag presentation occurs in DLN and is a subsequent event of Ag uptake and migration of LC from the epidermis. This indicates that residual LC in the irradiated skin are more important in UVB-induced immunosuppression than LC migrating into DLN. Our findings showed that endocytotic activity is impaired and that the trafficking properties and maturation induced by endocytosis are down-regulated in UVB-irradiated LC. Therefore, impairment of the first step in the induction phase of CHS, namely LC endocytosis, is a key event in the immunosuppression of CHS induced by UVB radiation.

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