Solar-Simulated Ultraviolet Radiation Induces Abnormal Maturation and Defective Chemotaxis of Dendritic Cells

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Exposure to ultraviolet (UV) light induces immunosuppression. Different evidences indicate that this phenomenon is mainly a consequence of the effect of UV light on skin dendritic cells (DC). To investigate the cellular and molecular basis of this type of immunosuppression, we assessed *in vitro* the effect of solar-simulated UV radiation on the phenotypic and functional characteristics of human monocyte-derived DC and Langerhans-like DC. UV radiation induced a decreased expression of molecules involved in antigen capture as DC-SIGN and the mannose receptor. This effect was accompanied by a diminished endocytic capacity, an enhanced expression of molecules involved in antigen presentation such as major histocompatibility complex-II and CD86, and a significant increase in their capability to stimulate T cells. Furthermore, irradiated DC failed to acquire a full mature phenotype upon treatment with lipopolysaccharide. On the other hand, solar-simulated radiation induced the secretion of tumor necrosis factor-αand interleukin (IL)-10 by DC, but no IL-12. Interestingly, solar-simulated UV radiation also caused an altered migratory phenotype, with an increased expression of CXCR4, and a lack of induction of CCR7, thus correlating with a high chemotactic response to stromal cell-derived factor 1 (SDF-1) (CXCL12), but not to secondary lymphoid tissue chemokine (SLC) (CCL21). These data indicate that solar-simulated UV radiation induces a defective maturation and an anomalous migratory phenotype of DC.

Key words: chemotaxis/costimulation/cytokines/dendritic cells/human
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Exposure of the human skin to ultraviolet (UV) wavelengths (290–400 nm) of sunlight results in deleterious effects on the skin such as sunburn, immune suppression, photo-aging, and skin cancer. UVB (290–320 nm) radiation is known to produce disturbances of some cell-mediated immune responses both in a local and systemic fashion (Morison, 1989). Locally, UV radiation inhibits the development of contact hypersensitivity reactions to haptens including tumor antigens, which are considered a major contributing factor for skin cancer development (reviewed by Ullrich, 2002). UV photons penetrate the skin and reach the epidermis and the papillary dermis, leading, at least, to DNA damage of epidermal and dermal cells, including keratinocytes, Langerhans cells (LC), and dermal dendritic cells (DC). Both LC and DC are potent professionally antigen-presenting cells (APC) and play a pivotal role in the initiation and regulation of T cell-mediated immune responses (Banchereau and Steinman, 1998). Compelling data demonstrate that human skin after exposure to UVB radiation results in reduced LC number with altered morphology (Aberer *et al*, 1981). In addition, UVB irradiation inhibits the ability of LC to stimulate antigen-specific CD4+ T-helper type 1 (Th1) (Simon *et al*, 1991), whereas they retain the capacity to activate T-helper type 2 (Th2) cells (Simon *et al*, 1990).

Studies on the effects of UV light have mostly been performed with UVB since this type of radiation is the most effective in inducing skin cancer. UVA has been also involved, however, in the induction of immune suppression (Clement-Lacroix *et al*, 1996; Iwai *et al*, 1999; Dumay *et al*, 2001; Moyal and Fourtanier, 2002).

Skin DC are immature DC characterized by a high antigen internalization capacity and a low expression of molecules involved in antigen presentation. Following antigen capture, DC migrate toward the draining lymph nodes (DLN). During their migration, DC undergo a maturation process, becoming highly immunogenic, upregulating the expression of major histocompatibility complex (MHC)-class II and costimulatory molecules, and diminishing their capacity to internalize antigen (Banchereau and Steinman, 1998). The chemokine secondary lymphoid tissue chemokine (SLC) (CCL21) plays an important role in DC migration from skin to T cell zones of DLN (Gunn *et al*, 1999). Once DC reach the DLN, they interact and activate T lymphocytes, thus initiating the immune response.

Abbreviations: APC, antigen-presenting cell; DC, dendritic cell; DLN, draining lymph node; GM-CSF, granulocyte macrophage-colony simulating factor; IL, interleukin; LC, Langerhans cell; LPS, lipopolysaccharide; MHC, major histocompatibility complex; SDF, stromal cell-derived factor 1; SLC, secondary lymphoid tissue chemokine; TGF-β, transforming growth factor; Th1, T-helper type 1; TNF-α, tumor necrosis factor-α; UV, ultraviolet

To further understand the effects of UV radiation on the skin immune response, we, *in vitro* analyzed the changes in receptors involved in antigen uptake, antigen presentation, and chemotactic responses induced by a solar-simulated UV radiation on monocyte-derived DC.

Results

Effect of UV radiation on DC maturation Human monocytes were induced to differentiate for 6 d to immature DC and Langerhans-like DC with granulocyte macrophage-colony simulating factor (GM-CSF)/interleukin (IL)-4 or with GM-CSF/IL-4 and transforming growth factor (TGF)-β, respectively (Sallusto *et al*, 1995; Geissmann *et al*, 1998). Both the DC and the Langerhans-like DC were $CD14^-$, $CD1a^+$, with a low expression of maturation markers (CD83 and CD86) (Fig 1). In contrast to DC, Langerhans-like DC expressed E-cadherin and the LC-specific antigen, Langerin. Treatment with lipopolysaccharide (LPS) for 20 h induced the maturation of these cells, enhancing the expression of $CD83^+$ and $CD86^+$ (Fig 1).

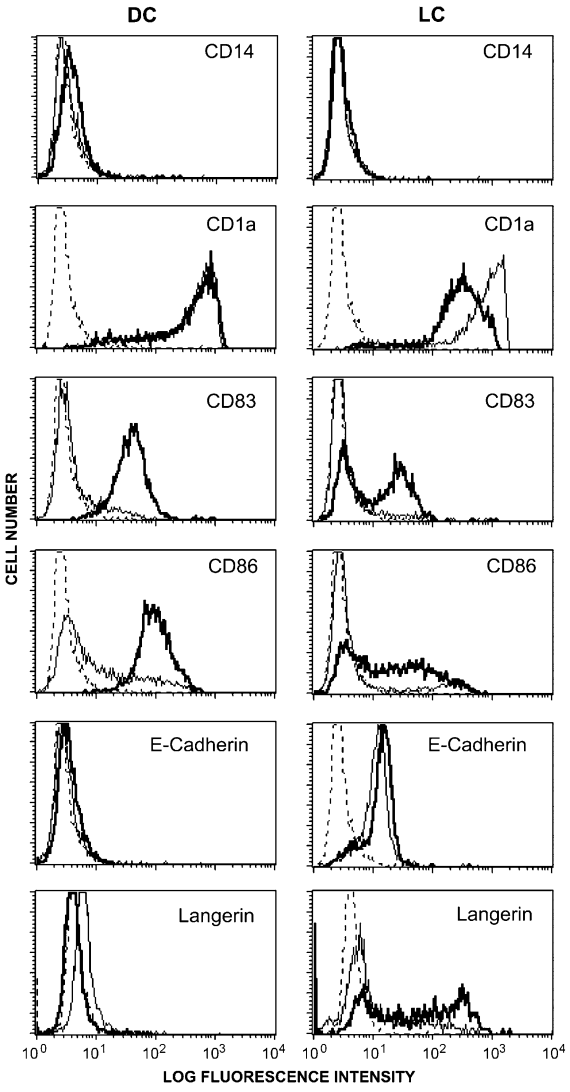

Figure 1
Phenotype of human monocyte-derived dendritic cells (DC) and Langerhans cells (LC). Human monocytes were induced to differentiate to immature DC, and Langerhans-like DC for 6 d with granulocyte macrophage colony simulating factor/interleukin-4 (GM-CSF/IL-4) or with GM-CSF/IL-4 and transformation growth factor-β, respectively. The phenotype of immature (*thin line*) and mature (lipopolysaccharide treatment for 20 h, *solid line*) DC and LC was analyzed by flow cytometry. Dotted line corresponds to the negative control.

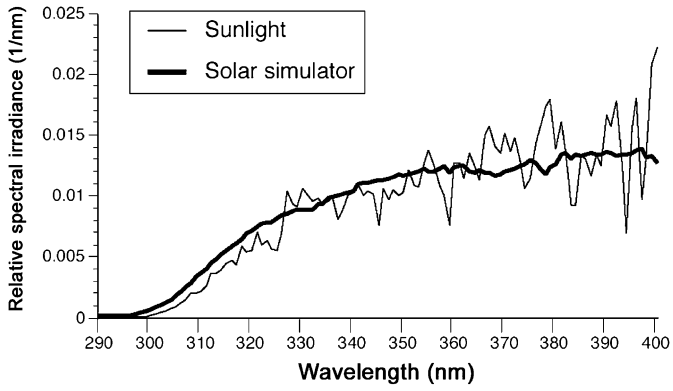

Figure 2
Solar-simulated ultraviolet (UV) radiation spectrum. UV spectrum emission of the solar simulator used in this study. The sunlight spectrum determined by the DIN-67501 standard is also shown.

To determine the effect of UV light on the maturation of DC, immature DC were exposed to UV radiation with a solar simulator (Fig 2, see Material and Methods), and the expression of several surface markers was analyzed 20 h later. Non-irradiated immature DC expressed high levels of C-lectin receptors, as DC-SIGN and mannose receptor, moderate levels of MHC-II, and low levels of CD83, and the costimulatory molecule CD86 (Fig 3*A* and Figure S1 and Table S1). When these cells were treated with the potent maturation agent LPS (10 ng per mL, 20 h), a decrease in the expression of DC-SIGN and mannose receptor, and an upregulation of MHC-II, CD86, and CD83 was observed. Interestingly, solar-simulated radiation (4.91 J per cm^2 UVA + 0.4 J per cm^2 UVB, corresponding to 2 standard erythemal doses) induced the downregulation of DC-SIGN and the mannose receptor to an extent similar to LPS and the upregulation of CD86 and CD83 ($p < 0.05$). The effect of UV radiation on CD86 and CD83 expression was however lower compared with LPS. On the other hand, we observed an increment of MHC-II expression induced by UV, although in this case, the difference was not statistically significant. Similar results were observed with Langerhans-like DC, obtained upon monocyte differentiation with GM-CSF/IL-4 and TGF-β (Fig 3*B*). Thus, we conclude that solar-simulated UV radiation induced a partial maturation of DC.

UV radiation induced the upregulation of MHC-II and CD86, and the downregulation of DC-SIGN in a dose-dependent manner (Fig 4*A*). Time-course experiments revealed that the upregulation of MHC-II and CD86 occurred 18 h after solar-simulated radiation (4.91 J per cm^2 UVA + 0.4 J per cm^2 UVB (Fig 4*B*).

To further confirm the phenotype of irradiated DC, we assessed their antigen uptake capacity 20 h after irradiation. As expected, non-irradiated immature DC took up more dextran—fluorescein isothiocyanate (FITC) than mature DC (Fig 3*C*). Solar-simulated UV radiation significantly diminished the endocytic activity of DC ($p < 0.05$), confirming the partial mature state of irradiated DC.

UV radiation modifies DC response to LPS To study the effect of UV light on the induction of maturation of DC by LPS, immature DC were irradiated (4.91 J per cm^2 UVA + 0.4 J per cm^2 UVB), and then incubated in the presence of LPS (10 ng per mL). In contrast with non-irradiated cells, the expression of CD83, CD86, and MHC-II on irradiated DC was not apparently enhanced in response to LPS (Fig 5*A*). Time-course experiments, however, revealed a late upregulation of CD86 by LPS in irradiated cells. Similar results were obtained with Langerhans-like DC (data not shown). We conclude that irradiated-DC maintain their partial maturation phenotype, and are transiently unresponsive to maturation stimuli.

Influence of solar-simulated radiation on the stimulatory capacity of DC The effect of UV radiation on the antigen-presenting function of DC was also evaluated by analyzing the ability of irradiated DC to stimulate allogenic $CD4^+$ T cells. As shown in Fig 6, UV radiation significantly increased the stimulatory capability of immature DC ($p < 0.05$), an effect that was not observed when DC were incubated with LPS for 30 h ($p > 0.05$).

Effect of UV radiation on cytokine secretion by DC To determine the pattern of cytokine production by DC upon solar-simulated UV radiation, levels of IL-10, p70 IL-12, and tumor necrosis factor-α (TNF-α) in culture supernatants were measured by ELISA. Non-irradiated immature DC did not produce detectable levels of IL-10, p70 IL-12, or TNF-α. DC secreted TNF-α and IL-10, but not p70 IL-12 upon solar-simulated UV radiation (Fig 7*A, C,* and *D*). Dose–response assays confirmed the induction of TNF-α secretion by UV radiation (Fig 7*B*). Interestingly, whereas non-irradiated DC produced predominantly IL-12 and TNF-α after LPS treatment, irradiated DC produced more IL-10 than IL-12 after incubation with LPS (Fig 7*C–D*). A similar pattern of cytokine production was observed with Langerhans-like DC (data not shown).

Solar-simulated radiation induces apoptosis on DC We have found that after radiation (24 h), the number of viable

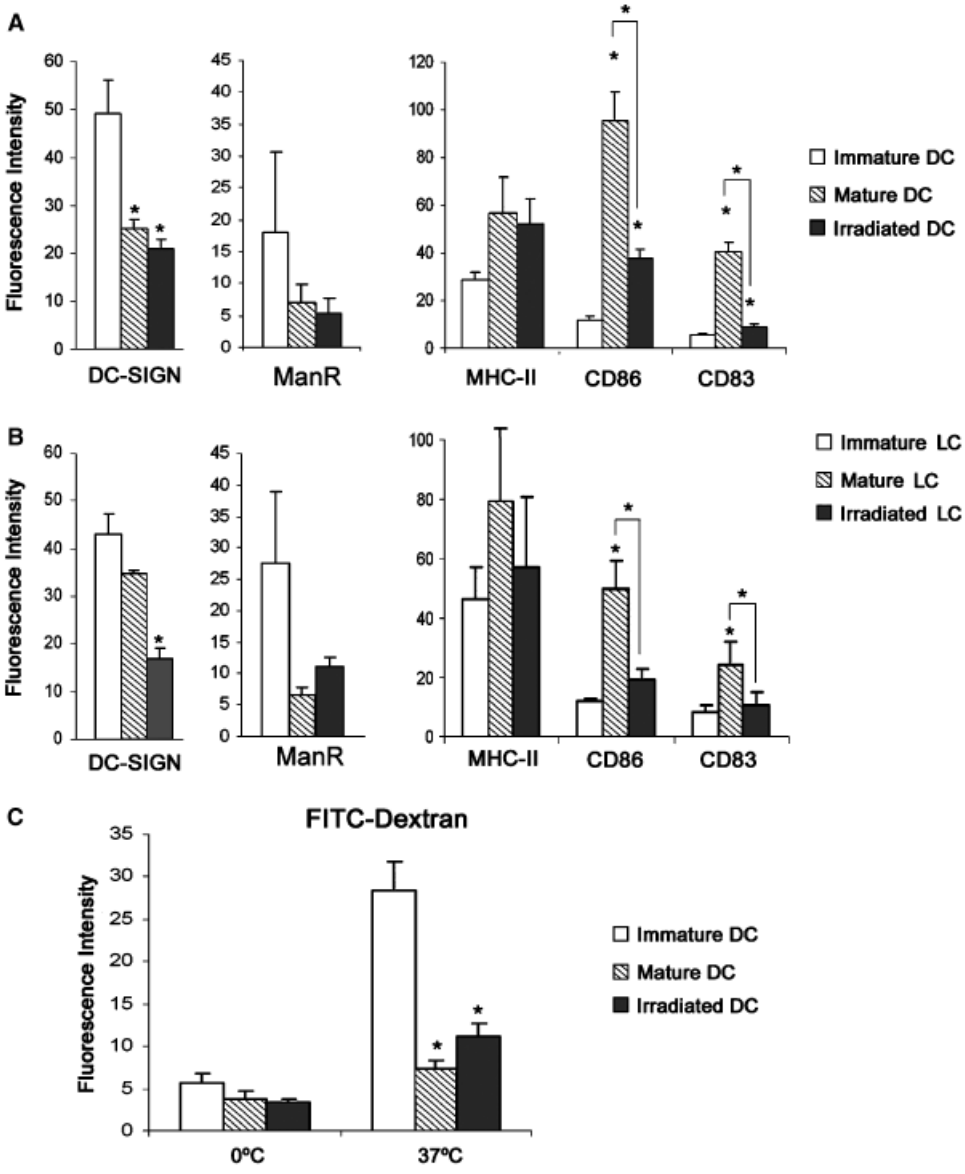

Figure 3
Induction of a partial mature dendritic cell (DC) phenotype by solar-simulated ultraviolet (UV) radiation. (*A*) Human immature monocyte-derived DC (*open bars*), induced to maturate with lipopolysaccharide 10 ng per mL) for 20 h (*striped bars*), or 20 h after exposure to solar-simulated UV radiation (5.0 J per cm^2 UVA + 0.4 J per cm^2 UVB) (*solid bars*) were analyzed for the surface expression of DC-SIGN, mannose receptor, major histocompatibility complex-II, CD86, and CD83 by flow cytometry. Data represent the arithmetic mean ± SEM of fluorescence intensity of 10 different experiments *($p < 0.05$ compared with immature DC or indicated). (*B*) Langerhans-like DC were treated and analyzed as in *A*. Data represent the arithmetic mean ± SEM of fluorescence intensity of three different experiments. ManR: mannose receptor *C*. Endocytic activity of immature (*open bars*), mature (*striped bars*), or irradiated (*solid bars*) DC was analyzed. Cells were incubated with fluorescein isothiocyanate–dextran for 30 min at 0°C (negative control) or at 37°C and fluorescence capture was analyzed by flow cytometry. Data represent the arithmetic mean ± SEM of fluorescence intensity of six different experiments.* ($p < 0.05$ compared with immature DC).

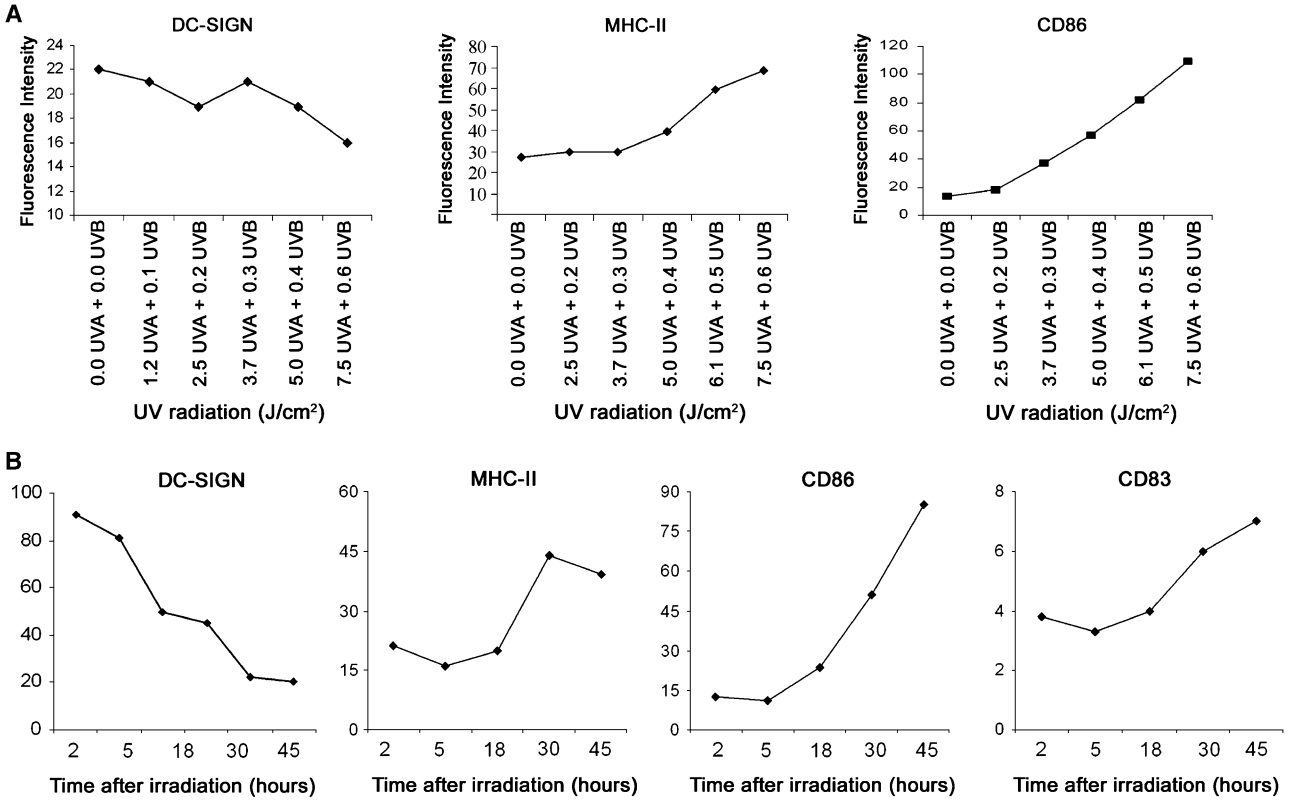

Figure 4
Dose–response and time course of costimulatory/antigen receptor expression in dendritic cells (DC) after solar simulated ultraviolet (UV) radiation. (*A*) Immature human monocyte-derived DC were exposed to different doses of solar-simulated UV radiation. DC-SIGN, major histocompatibility complex (MHC)-II, and CD86 levels were analyzed 20 h after irradiation. (*B*) Time course of costimulatory/antigen receptors expression in DC after 5.0 J per cm^2 UVA + 0.4 J per cm^2 UVB radiation. Data represent the arithmetic mean of fluorescence intensity of one representative experiment of three.

cells was lower compared with control cells (24% ± 9% and 55% ± 15% of viable cells, respectively). To assess the effect of UV light on cellular viability of DC, immature DC were exposed to different doses of solar-simulated UV radiation. Cells were then cultured for 24 h, and the percentage of apoptotic cells was analyzed by two different flow cytometry methods: analysis of DNA content and binding to Annexin V. The percentage of hypodiploid cells was obtained by flow cytometry DNA content analysis. Solar-simulated UV radiation induced both the appearance of hypodiploid cells and the binding of Annexin V in DC in a concentration-dependent manner (Fig 8), indicating that UV light was able to trigger apoptosis of these cells. Irradiation of DC at 5.0 J per cm^2 UVA + 0.4 J per cm^2 UVB induced 25%–30% of cell apoptosis. We assumed that the decrease of cell number is a result of cellular death as well as to experimental procedures (such as centrifugations) and cell adherence to the plate.

Effect of UV light on DC migration To determine the molecular changes driving the migration of DC upon UV radiation, the expression pattern of chemokine receptors of irradiated DC was analyzed. Immature DC expressed high levels of CCR1 (regulated on activation, normal T cells expressed and secreted (RANTES) receptor), and low expression of CXCR4 (stromal cell-derived factor 1 (SDF-1) receptor) and CCR7 (SLC and Epstein-Barr virus-induced molecule 1 ligand chemokine (ELC) receptor) (Fig 9*A*). In contrast, mature DC expressed high levels of CXCR4 and CCR7 and a low expression of CCR1 (Fig 9*A* and Table S1). Remarkably, we found that solar-simulated UV radiation induced the downregulation of CCR1 and upregulation of CXCR4 ($p < 0.05$), whereas no significant effect was observed on CCR7, a receptor that is characteristic of mature DC (Fig 9*A*).

In order to confirm whether the pattern of expression of chemokine receptors by DC was associated with a migratory behavior, the chemotactic response to different chemokines was analyzed using Transwell assays. As shown in Fig 9*B*, immature DC showed a low migratory capability. Upon incubation with LPS for 20 h, however, more than 80% of cells migrated in response to SLC, and 35% to SDF-1. On the other hand, cells exposed to solar-simulated UV radiation increased their chemotactic capability to SDF, but did not significantly increase their chemotaxis toward SLC. These data indicate that upon UV radiation, the changes observed in the expression pattern of chemokine receptors are associated with significant changes in the migratory behavior of DC.

To assess whether irradiated DC in response to LPS are able to acquire CCR7 expression, immature DC were irradiated (4.91 J per cm^2 UVA + 0.4 J per cm^2 UVB), and then incubated in the presence of LPS (10 ng per mL). In contrast with non-irradiated cells, UV-treated DC incubated for 20 h

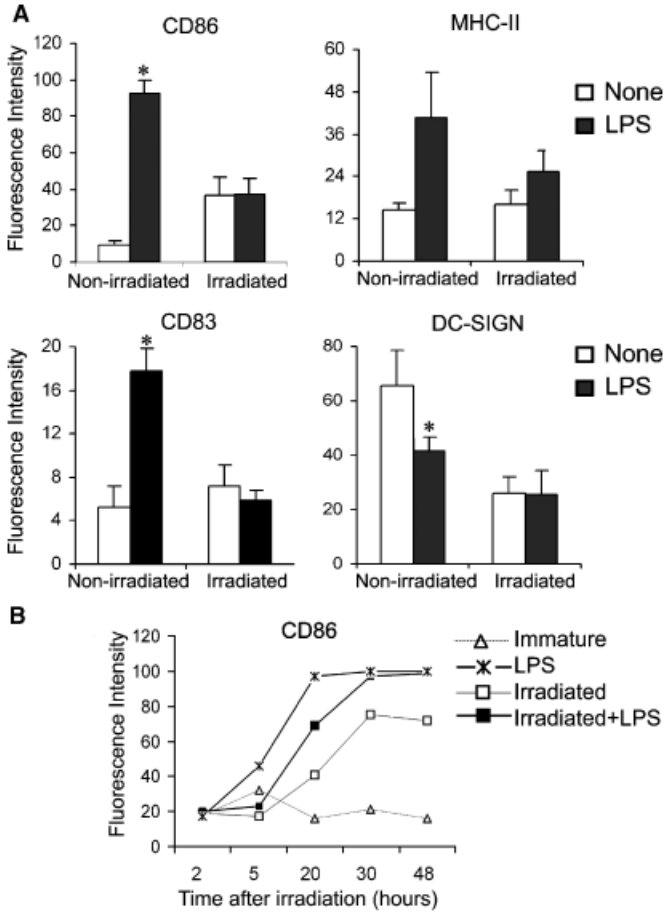

Figure 5
Irradiated dendritic cells (DC) are transiently refractory to a potent maturation stimulus. (*A*) Irradiated and non-irradiated DC were incubated for 20 h in the absence (*open bars*) or presence of lipopolysaccharides (LPS) (10 ng per mL) (*solid bars*). Expression levels of CD83, CD86, major histocompatibility complex (MHC)-II and DC-SIGN were analyzed by flow cytometry analysis. Data represent the arithmetic mean ± SEM of fluorescence intensity of five different experiments *($p < 0.05$ compared with cells cultured in the absence of LPS). (*B*) Kinetics analysis of CD86 expression in DC after 5.0 J per cm^2 ultraviolet (UV)A + 0.4 J per cm^2 UVB radiation in the presence or absence of LPS. Data from a representative experiment out of three performed are shown.

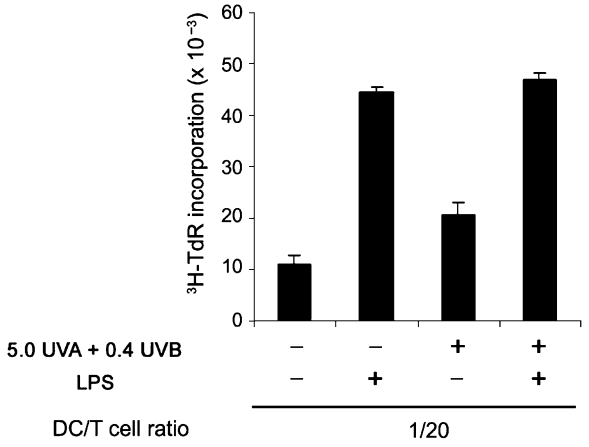

Figure 6
Effect of ultraviolet (UV) radiation on the T cell stimulatory capacity of dendritic cells (DC). DC were irradiated with a solar simulator (5.0 J per cm^2 UVA + 0.4 J per cm^2 UVB) and then incubated in the presence or absence of lipopolysaccharides (LPS). After 30 h, DC were used to stimulate allogenic CD4 + T lymphocytes in 96-well plates, and cell proliferation was measured by 3H-TdR incorporation, as stated in *Materials and Methods*. Data from a representative experiment out of three performed are shown.

with LPS expressed low levels of CCR7 (Fig 9*C*). As occurred with CD86 expression, irradiated DC showed a late upregulation of CCR7 expression in response to LPS (Fig 9*D*). Similar results were observed when Langerhans-like DC were analyzed, although the increment in CCR7 expression after LPS treatment was lower compared with DC (data not shown). We conclude that irradiated DC have altered their migratory behavior, showing a diminished capability to migrate to SLC, the main chemokine involved in DC homing to DLN.

Discussion

It is well-known that UV radiation has an immunosuppressive effect that may be involved in skin cancer development. One of the processes associated with UV-induced immune suppression is the decrease in the number of skin DC, which show a diminished APC function (Meunier, 1999). To gain further insight into the cellular and molecular basis of the immunosuppression induced by solar radiation, we assessed the changes that monocyte derived-DC and Langerhans-like DC undergo upon UV radiation. Monocyte-derived DC with IL-4/GM-CSF have a phenotype comparable to dermal DC (Grassi *et al*, 1998), whereas IL-4/GM-CSF plus TGF-β induces the differentiation of human monocytes into Langerhans-like DC (Geissmann *et al*, 1998). Although studies on the immune effects of UV light have mostly focused on the UVB band, recent reports have shown that both UVA and UVB radiation are immunosuppressive. Therefore, we used as UV source a device that simulates the solar UV radiation that affects human beings.

Partial DC maturation by UV radiation Our data show that upon irradiation immature DC lose their antigen internalization capability, and reduce the expression of receptors involved in antigen capture (DC-SIGN and mannose receptor). Low UVB radiation impairs endocytosis by Langerhans-like DC and inhibits their endocytosis-induced enhancement of migration and maturation (Mizuno *et al*, 2004). We found that irradiated DC and Langerhans-like DC upregulate the expression of molecules involved in antigen presentation (MHC-II, CD86), and show typical maturation markers (CD83). Furthermore, we have found that UV radiation increases the T cell stimulatory capability of immature DC. These data are in accordance with studies that described the upregulation of CD80 and CD86 on human epidermal LC *in vivo* after simulated solar radiation (Laihia and Jansen, 1997). Interestingly, it has been described that p38-mitogen-activated protein kinase mediates the induction of both MHC-II and CD86 and apoptosis on human-monocyte derived DC after UVB induction (Nakagawa *et al*, 2004). Nonetheless, it is important to highlight that we have found that the levels of CD86 and CD83 on irradiated DC and their

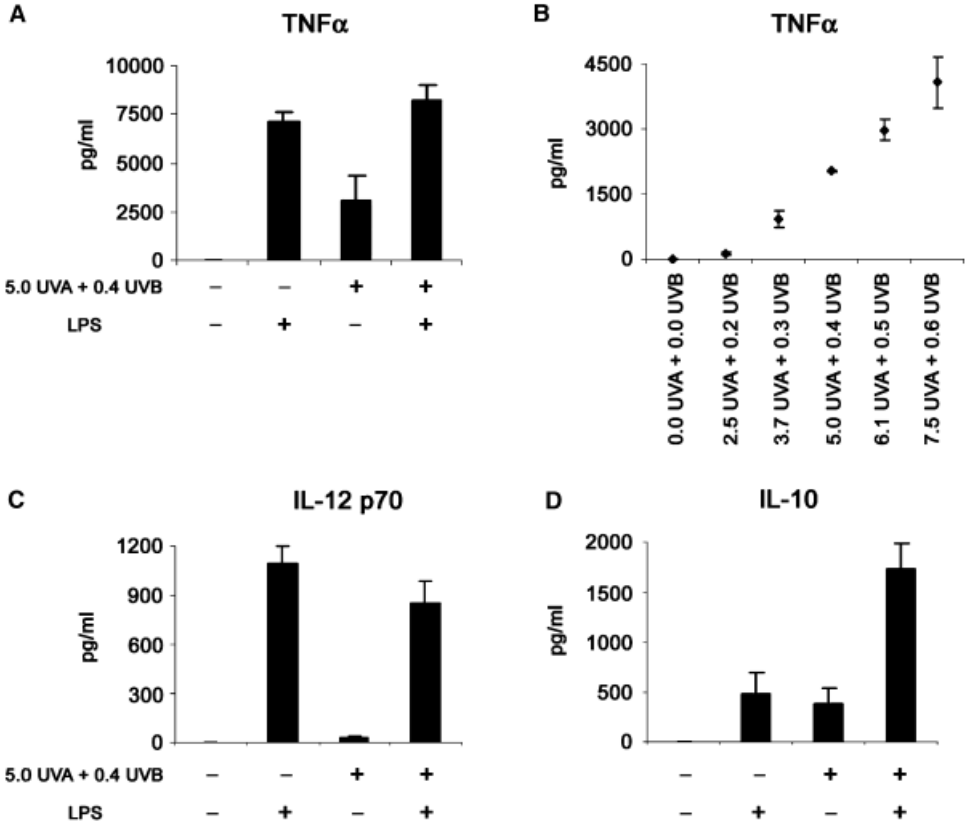

Figure 7
Cytokine secretion by dendritic cell (DC) after solar-simulated ultraviolet (UV) radiation. (*A, C, D*) Human immature DC were irradiated or not with 5.0 J per cm^2 UVA + 0.4 J per cm^2 UVB, and culture for 20 h in the absence or presence of lipopolysaccharide (LPS) 10 ng per mL). Levels of interleukin (IL)-10, p70 IL-12, or tumor necrosis factor (TNF)-α in cultured supernatants were detected by ELISA. (*B*) Immature DC were exposed to different doses of solar-simulated UV radiation. TNF-α in the supernatant was measured by ELISA 20 h after irradiation. The arithmetic mean ± SEM of six different experiments is shown.

T cell stimulatory effect were significantly lower than those induced by LPS. Moreover, the expression levels of CD83, CD86, and MHC-II on irradiated DC did not increase in response to LPS within the first 20 h. In this regard, a previous report showed that the inducible expression of CD80 and CD86 by monocytes during allogenic mixed leukocyte reaction was decreased after UVB radiation (Fujihara *et al*, 1996). It is important to remark that UV radiation delays but does not block the upregulation of CD86. These data suggest that irradiated DC become partially mature, but transiently unresponsive to maturation stimuli. Hence, it is very likely that these effects significantly contribute to the immunosuppression associated with UV light exposure. We also have found, however, that UV irradiation does not impair the T cell stimulatory capacity of DC after potent maturation stimuli such as LPS.

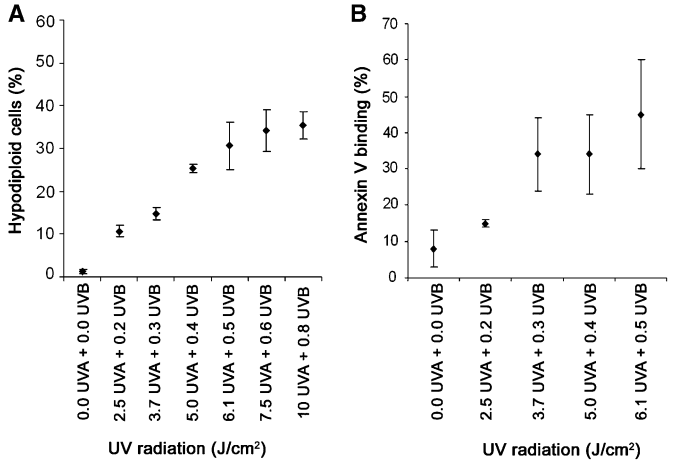

Figure 8
Induction of dendritic cell (DC) apoptosis by solar-simulated irradiation. Human immature DC were irradiated with different doses of solar-simulated ultraviolet (UV) radiation. Apoptotic cells were detected, 24 h later, by two different approaches. (*A*) Cells were analyzed for their DNA content by flow cytometry. Results represent the percentage of hypodiploid (apoptotic) cells from four different experiments. The arithmetic mean ± SEM is shown. (*B*) Cells were incubated with fluorescein isothiocyanate–Annexin V and analyzed by flow cytometry. Results represent the percentage of positive (apoptotic) cells from four different experiments. The arithmetic mean ± SEM is shown.

Cytokines induced on DC by UV radiation Normal LC present antigens equally well to both Th1 and Th2 clones, but UV-irradiated LC lost their ability to present antigens to Th1 cells (Simon *et al*, 1991). The balance between IL-10 and IL-12 plays an important role in Th polarization. Production of IL-12 by APC is an absolute requirement for the activation of Th1 clones (Hsieh *et al*, 1993), whereas IL-10 inhibits Th1 proliferation and induces Th2 responses (Enk *et al*, 1993). We found that monocyte-derived DC and Langerhans-like DC produce moderate levels of IL-10 after irradiation with solar-simulated UV radiation. Moreover, whereas non-irradiated DC produce predominantly IL-12 in response to LPS for 20 h, irradiated DC produces more IL-10 than IL-12 after incubation with LPS. Macrophages and keratinocytes also release IL-10 upon UV exposure (Rivas and Ullrich, 1992; Kang *et al*, 1998). Furthermore, administration of IL-12 prevents UV-induced immunosuppression and overcomes UV-induced tolerance (Schmitt *et al*, 1995). Altogether, our data show a predominant induction of IL-10 secretion by UV radiation, which may explain why irradiated LC fail to present antigen to Th1 cells.

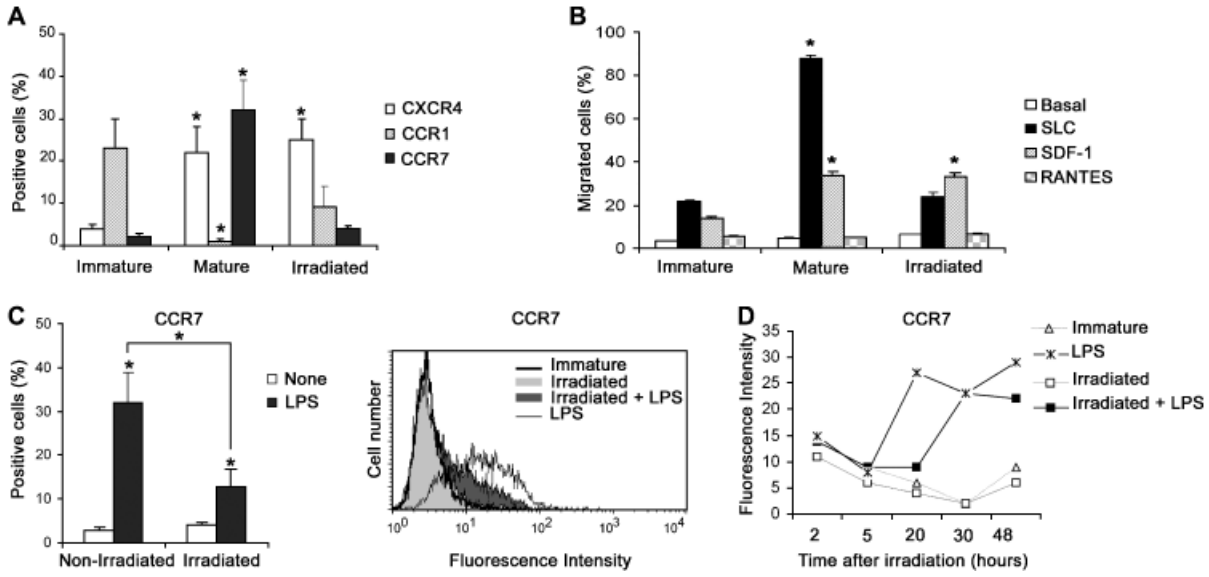

Figure 9
Solar-simulated radiation induces dendritic cell (DC) expression of CXCR4, but not CCR7 and chemotaxis to stromal cell-derived factor 1 (SDF-1), but not SLC. (*A*) Human immature monocyte-derived DC, induced to mature with lipopolysaccharide (LPS) 10 ng per mL) for 20 h, or 20 h after exposition to solar-simulated ultraviolet (UV) radiation (5.0 J per cm^2 UVA + 0.4 J per cm^2 UVB) were analyzed for the surface expression CXCR4 (*open bars*), CCR1 (*gray bars*), and CCR7 (*solid bars*) by flow cytometry. Data represent the arithmetic mean ± SEM of positive cells of seven different experiments. *($p < 0.05$ compared with immature DC). (*B*) Immature, mature, or irradiated DC were allowed to migrate as described in *Materials and Methods*. Data correspond to the arithmetic mean ± SEM of the percentage of migrating cells of four independent experiments. (*C*) Irradiated and non-irradiated DC were incubated for 20 h in the absence (*open bars*) or presence of LPS (10 ng per mL) (*solid bars*). Then, the expression of CCR7 was analyzed by flow cytometry. Data correspond to the arithmetic mean ± SEM of the percent-positive cells of eight different experiments. *($p < 0.05$ compared with immature DC or indicated). A representative histogram of CCR7 expression of DC upon UV radiation is also shown. (*D*) Kinetics analysis of CCR7 expression in DC after 5.0 J per cm^2 UVA + 0.4 J per cm^2 UVB radiation in the presence or absence of LPS. Data represent the arithmetic mean of fluorescence intensity of one representative experiment out of three.

It is well-known that UVB radiation induces programmed cell death of DC (Rattis *et al*, 1998; Okamoto *et al*, 1999; Nicolo *et al*, 2001; Nakagawa *et al*, 2004). Our data show that simulated solar UV radiation induces the appearance of hypodiploid cells and the binding of Annexin V, which correspond to apoptotic DC. Thus, it is very feasible that apoptosis also contributes to the immunosuppression and LC depletion associated with UV exposure. It has been described, however, that UVB-induced LC decrease is mainly attributable to its migration rather than apoptosis (Kolgen *et al*, 2002).

Abnormal chemotactic response of irradiated DC Remarkably, chemokine receptor expression and the chemotactic response of irradiated DC are strongly altered upon UV radiation. Whereas immature DC express CCR1, upon maturation stimuli they express CCR7 and acquire responsiveness to SLC, which is specifically expressed in T cell-rich areas of secondary lymphoid tissue (Dieu *et al*, 1998). As occurs with maturation stimuli, simulated solar radiation induces both CXCR4 expression and chemotactic response of DC to SDF-1. In contrast with mature DC, however, irradiated DC do not express significant levels of CCR7, and consequently show a low response to SLC. Interestingly, even after incubation with LPS, irradiated DC express lower levels of CCR7 receptor than non-irradiated DC. These data suggest that after irradiation, DC have a diminished capability to migrate to SLC, the main chemokine involved in DC homing to DLN (Cyster, 1999; Gunn *et al*, 1999; Saeki *et al*, 1999). Moreover, it has been recently described that CCR7 is an essential mediator for entry of both dermal and epidermal DC into the lymphatic vessels within the dermis whereas this receptor is dispensable for the mobilization of LC from the epidermis to the dermis (Ohl *et al*, 2004). In this regard, we show that the chemotactic migratory activity of DC to SLC was inhibited by solar-simulated UV radiation via prevention of CCR7 expression. Collectively, these observations suggest that UV radiation modulates the trafficking behavior of DC by regulating chemotaxis. Further experimentations are necessary to ascertain whether irradiated skin DC have impaired their homing to DLN or use other chemokine receptors instead of CCR7 to reach DLN. It is feasible that IL-10 secretion induced by UV radiation could mediate the acquisition of the abnormal migratory phenotype observed on irradiated DC, since it has been described that exposure of DC to IL-10 enhances CCR5, but downregulates CCR7 expression, correlating with an *in vivo* impairment of DC homing to DNL (Takayama *et al*, 2001).

Finally, it is important to remark that no appreciable differences were observed between monocyte-derived DC and Langerhans-like DC in the parameters that we analyzed in this report. In both types of cells, UV radiation induces a partial maturation phenotype with predominant production of IL-10 *versus* IL-12 after LPS stimulation, and impairs migration to SLC via prevention of CCR7 expression. Therefore, we conclude that simulated solar UV radiation induces complex changes in the phenotype and functional characteristics of DC, and that this effect may account, at least in part, for the immunosuppression observed after UV exposure.

Material and Methods

Human studies were performed according to the Declaration for Helsinki Principles and were approved by Ethics Committee of Basic Research. Human volunteers gave their informed consent.

Preparation of monocyte-derived DC Human peripheral blood mononuclear cells (PBMC) were isolated from buffy coats from normal donors over a Lymphoprep (Nycomed, Oslo, Norway) gradient according to standard procedures. Monocytes were purified from PBMC by a 30 min adherence step at 37°C in complete medium. Non-adherent cells were washed off and monocytes were immediately subjected to the DC differentiation protocol, as previously described (Sallusto *et al*, 1995). Briefly, monocytes were cultured in RPMI supplemented with 10% fetal calf serum (FCS) containing IL-4 (10 ng per mL, R&D Systems Inc., Minneapolis, Minnesota) and GM-CSF (200 ng per mL, Schering-Plough, Madrid, Spain). Cells were cultured for 6 d, with addition of cytokine every second day, to obtain a population of immature DC. Phenotypic characteristics of these cells were assessed by flow cytometry analysis at day 6 (MHC-II$^+$, CD1a$^+$, DC-SIGN$^+$, CD14$^-$). To obtain Langerhans-like DC, monocytes were cultured for 6 d in the presence of IL-4/GM-CSF and TGF-(10 ng per mL, R&D Systems Inc.) as described (Geissmann *et al*, 1998), and its phenotype was also confirmed by flow cytometry analysis (MHC-II$^+$, CD1a$^+$, DC-SIGN$^+$, CD14$^-$, E-cadherin$^+$). Maturation of DC was induced by treatment with LPS (10 ng per mL, Sigma Chemical Co., St Louis, Missouri) for 20 h. In the case of Langerhans-like DC, TGF-β was removed before incubation with LPS.

Solar-simulated UV radiation A 1000 W xenon arc solar simulator (Oriel, Surrey, UK) equipped with an Oriel 81017 filter ("Colipa") was used. Spectral emission is shown in Fig 2. UVB and UVA irradiance measurements were performed daily using an IL-1700 radiometer (International Light, Newburyport, Massachusetts) equipped with the SED240/UVB-1/TD and SED033/UVA/TD photodetectors. The radiometer was calibrated with a Solar Scope (Solatell, Croydon, UK) spectroradiometer.

DC (2×10^6 in 0.75 mL of Hank's balanced salt solution) were irradiated with the indicated doses. After irradiation, DC were cultured for 24 h in RPMI with 10% FCS in the presence of IL-4 and GM-CSF (10 ng per mL and 200 ng per mL, respectively) until analyzed.

Antibodies and reagents The following mouse anti-human monoclonal antibodies (mAb) were used: DCIS1/21 (anti-MHC-II), T3b (anti-CD3), and T6 (anti-CD1a) (Mittelbrunn *et al*, 2002). Anti-DC-SIGN mAb (clone 120507, R&D Systems), anti-CD14 (clone M5E2 from BD Biosciences, San Diego, California), anti-CD83 (clone HB15a from Immunotech, Marselle, France), anti-CD86 (clone BU63 from Caltag Laboratories, Burlingame, California), anti-CXCR4 (BD PharMingen, San Diego, California), anti-CCR1 (clone 53504.111, R&D Systems), anti-CCR7 (clone 2H4 from BD Biosciences), anti-E-Cadherin (clone HECD-1 from Calbiochem, Darmstadt, Germany), anti-Langerin (clone DCGM4 from Immunotech, Marseille, France), and anti-mannose receptor (clone 19.2 from BD Biosciences) mAb were also used. FITC–Dextran (wt 77.000) was from Sigma Chemical Co.

Flow cytometry analysis DC were incubated with human γ-globulin (100 μg per mL) in phosphate-buffered saline (PBS) for 20 min to block Fc receptors, and then stained with primary mAb for 30 min. For Langerin staining, cells were fixed (10 min in 2% formaldehyde–PBS), washed, and permeabilized (20 min 4°C in 0.1% saponin). Cells were then incubated with an FITC-conjugated secondary Ab, stained with propidium iodide (2μg per mL) to exclude non-viable cells, and analyzed in a FACSCalibur flow cytometer (BD Biosciences, Mountain View, California). Data were analyzed using the CellQuest software (BD Biosciences).

Endocytic assays The endocytic activity of DC was measured as described previously (Sallusto *et al*, 1995). Cells were resuspended in PBS and incubated with FITC–dextran (1 mg per mL) for 30 min at 37°C (negative control at 0°C). Incubation was stopped by adding 5 mL ice-cold PBS containing 0.02% sodium azide. Then, cells were washed three times with cold PBS and analyzed by flow cytometry.

Allogenic T lymphocyte proliferation induced by DC CD4$^+$ T lymphocytes were isolated from PBMC by immunomagnetic negative selection using the CD4+T cell Isolation Kit II (Miltenyi Biotec, Bergisch Gladbach, Germany). Then, 2×10^5 T cells were mixed in a 96-well plate with 1×10^4 irradiated (1.5 Gy per min for 10 min) allogenic DC after solar-simulated radiation or LPS addition. After 5 d of incubation, ^{3}[H]-TdR was added (1.0 μ Ci per well) during the last 16 h of cell culture. Thymidine incorporation was determined with a liquid scintillation counter, and T cell proliferation was expressed as cpm incorporated. All assays were performed in triplicate.

Detection of apoptosis Cellular apoptosis was detected by two different methods, as follows:

DNA content assay DC (5×10^5 cells per mL) were washed in PBS and resuspended in 2 mL of hypotonic staining solution (50 μg per mL propidium iodide, 0.1% sodium citrate, 0.1% Triton X-100, and 40 μg per mL RNAse). Cells were vortexed, and incubated at 4°C for 2 h before analysis in a FACSCalibur flow cytometer, using the ModfitLT software from BD Biosciences. Results were expressed as the percentage of hypodiploid apoptotic cells.

Annexin V binding This assay was performed using the BD Apo-Alert kit (BD Biosciences) according to the manufacturer's instructions. Briefly, after UV exposure, DC (5×10^5 cells per mL) were washed and resuspended in binding buffer and stained with FITC-conjugated Annexin V during 15 min at room temperature. Cells were then stained with propidium iodide (2 μg per mL) to identify non-viable cells, and analyzed by FACScalibur flow cytometer. Results were expressed as the percentage of Annexin V-binding cells.

Chemotaxis assay Assays for DC chemotaxis were performed in polycarbonate membranes, 5 μm-diameter pore size Transwell chambers (Costar, Corning, New York). DC (100 μL at 1×10^6 per mL) suspended in complete medium were added to the upper chamber and SDF-1α(100 ng per mL), RANTES (10 ng per mL), or SLC (10 ng per mL) was added to the lower well. Thereafter, cells were allowed to migrate for 2 h at 37°C in 5% CO_2 atmosphere, after which migrated cells were recovered from the lower part of the chemotaxis chamber. The number of migrated cells was assessed by flow cytometry as described (Vicente-Manzanares *et al*, 1999).

Cytokines measurement Irradiated DC (2×10^6 per mL) or non-irradiated DC (1×10^6 per mL) were cultured in the presence or absence of LPS for 20 h. Then, IL-10, IL-12, and TNF-α levels were determined by ELISA (Eli-Pair, Diaclone, Besancon, France) in cell culture supernatants according manufacturer's guidelines. All cytokine determinations were performed in supernatants from plates with similar numbers of DC at the end of cell culture.

Statistical analysis Data were compared by the non-parametrical Mann–Whitney *U*-test with an α value of 0.05.

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Supplementary Material

The following supplementary material is available for this article online.
Figure S1 CD86, CD83 expression and FITC-dextran incorporation on immature, matured, and irradiated DC.
Table S1 Effect of solar-simulated UV radiation on the expression levels of CD86, CD83, DC-SIGN, mannose receptor and CCR7, and on FITC-dextran incorporation.

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