Bivalent Effect of UV Light on Human Skin Mast Cells—Low-Level Mediator Release at Baseline but Potent Suppression Upon Mast Cell Triggering

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Ultraviolet (UV) irradiation is an established treatment for inflammatory skin diseases, although the precise mode of action is still unclear. Activating and suppressive effects on mast cell (MC) mediator release have been described. The aim of this study was to investigate systematically the effects of UVB, UVA-1, and psoralen plus UVA-1 at therapeutic doses on skin-derived human MC. Baseline and stimulated release of histamine, tryptase, and of interleukin (IL)-6, IL-8 and tumor necrosis factor-alpha (TNF-α) were examined. In resting MC, UV light induced a slight, yet significant histamine release corresponding to enhanced surface levels of lysosome-associated membrane proteins (LAMP). In contrast, UV pre-treatment caused a marked suppression of the anti-IgE-induced histamine release, accompanied by a diminished, anti-IgE-mediated increase in LAMP expression. The secretion of IL-6, IL-8, and TNF-α was inhibited in resting and activated MC, suggesting a different mode of action. Regarding the importance of MC in a variety of allergic and inflammatory processes, our data show a high susceptibility of this cell type towards UV light, which seems to partially depend on the state of cellular activation. Immunosuppressive effects predominate in activated MC, thus corresponding with the beneficial effects in inflammatory diseases, whereas in resting MC, both stimulatory and inhibitory effects are observed.

Key words: cytokines/mast cells/mediators/LAMP/UV light
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Ultraviolet (UV) light is known to cause proinflammatory effects, as is well illustrated by overdosing in the context of sunburn. Its anti-inflammatory and immunomodulatory functions, however, have also been recognized since many decades. Selected wavelengths and controlled dosage of UV irradiation are applied widely in the treatment of diverse skin diseases such as psoriasis and atopic dermatitis. The possible mechanisms associated with the beneficial effects of this therapy have been extensively studied in keratinocytes, lymphocytes, Langerhans cells, and infiltrating leukocytes (reviewed in Krutmann and Morita, 1999). Little is known, however, about UV-induced changes in skin mast cells (MC), even though UV treatment is well appreciated as beneficial in pruritic diseases and in cutaneous mastocytosis (reviewed in Hartmann *et al*, 2001).

A limited number of studies have been published on the *in vitro* effect of UV light on rodent MC, which differ substantially from their human counterparts, such that results can be hardly transferred to the human system (Metcalfe *et al*, 1997). Although the reported data are not always in accord (Fjellner and Hagermark, 1982; Danno *et al*, 1988; Glover *et al*, 1989; Yen *et al*, 1990; Amano *et al*, 1998; Mio *et al*, 1999; Graevskaya *et al*, 2000), they clearly revealed that MC histamine release is subject to modulation by UV light. In fact, MC-derived histamine has been suspected to play a dominant role in UV-induced immunosuppression (Hart *et al*, 2001). Furthermore, there are clinical observations to support a significant role for human MC in the beneficial effects imparted by UV light in the therapy of skin diseases (Kolde *et al*, 1984; Yen *et al*, 1991). Moreover, we recently showed that human skin MC are resistant to UV-induced apoptosis, whereas immature leukemic MC are highly susceptible to UV-triggered cell death (Guhl *et al*, 2003). Other clinical findings that support the concept of human MC as targets of UV light include the lower density of MC granules after psoralen plus ultraviolet light A (PUVA) treatment in mastocytosis patients, the markedly reduced tissue levels of histamine, and a diminished ability of the skin to generate leukotrienes (Kolde *et al*, 1984; Czarnetzki *et al*, 1985). Mueller and Meffert (1994) reported on increased histamine serum levels after UVA-1, but not after UVB treatment, whereas in contrast, UVB irradiation was found to be an effective histamine liberator by Walsh (Walsh, 1995). In summary, these data imply that UV light may affect human MC directly by altering the cell's potential of mediator release. Obviously, however, the precise outcome seems to differ, depending on the specific conditions such as irradiation wavelengths and the presence of stimulating agents in the cell's environment.

To address this issue in a systematic manner, we investigated MC purified from human skin and compared the

Abbreviations: IL, interleukin; LAMP, lysosome-associated membrane protein; MC, mast cells; PUVA, psoralen plus ultraviolet light A; TNF, tumor necrosis factor; UV, ultraviolet

effects of different irradiation protocols on spontaneous and induced release of typical MC mediators.

Results

Effects of UV light on stimulated mediator release First, we tested the effects of UV irradiation on the release of histamine induced by cross-linking of high-affinity IgE receptor (FcεRI). Skin MC were exposed to various doses of UVB (0,5, 25, 50, 75, 100 mJ per cm^2), UVA-1 (0, 5, 10, 15, 20, 25 J per cm^2), or PUVA-1 (0, 1, 2, 3, 4, 5 J per cm^2) and then left in culture for 24 h prior to anti-IgE challenge. All types of UV treatment led to striking inhibition of stimulated histamine liberation in a clearly dose-dependent manner (Fig 1, black bars) Interestingly, UVA-1 turned out most potent with over 90% inhibition at 25 J per cm^2, whereas maximum suppression of UVB and PUVA-1 treatment was in the range of 60%–70%. In all settings, the suppression of histamine release induced by calcium ionophore A23187 (Fig 1, hatched bars) closely approximated that of anti-IgE (Fig 1, black bars), indicating a largely receptor-independent effect.

UV light triggers histamine release from resting MC To address the issue of whether MC would release higher levels of histamine following irradiation, cells were treated with UV light as above, left in culture for 24 h, and then subjected to histamine release assays for 30 min in the absence of additional stimuli. In stark contrast to the reduction of stimulated histamine release, all types of UV light caused a concentration-dependent release of histamine from non-stimulated MC (data shown in Fig S7 of full-text version; Fig S8). The next issue was to determine histamine levels that had accumulated in the cell culture supernatant 24 h post-irradiation. Indeed increased extracellular histamine levels were detectable in all UV settings compared with control (Fig 2). In summary, it thus appears likely that histamine secretion from irradiated MC constantly supplies low to intermediate levels of histamine to surrounding cells and that this process still takes place long after irradiation has ceased.

UV light inhibits baseline and stimulated cytokine secretion from MC Both spontaneous and anti-IgE-triggered levels of interleukin (IL)-6 and IL-8 were substantially suppressed by the three types of UV treatment, with UVA-1 being most potent (see Fig S11 full-text version online). As observed by us before, IL-8 release is only slightly enhanced by FcεRI cross-linking, whereas IL-6 is apparently not affected at all or even slightly downregulated (Babina *et al*, 2004). Anti-IgE, however, displays a very clear inducing

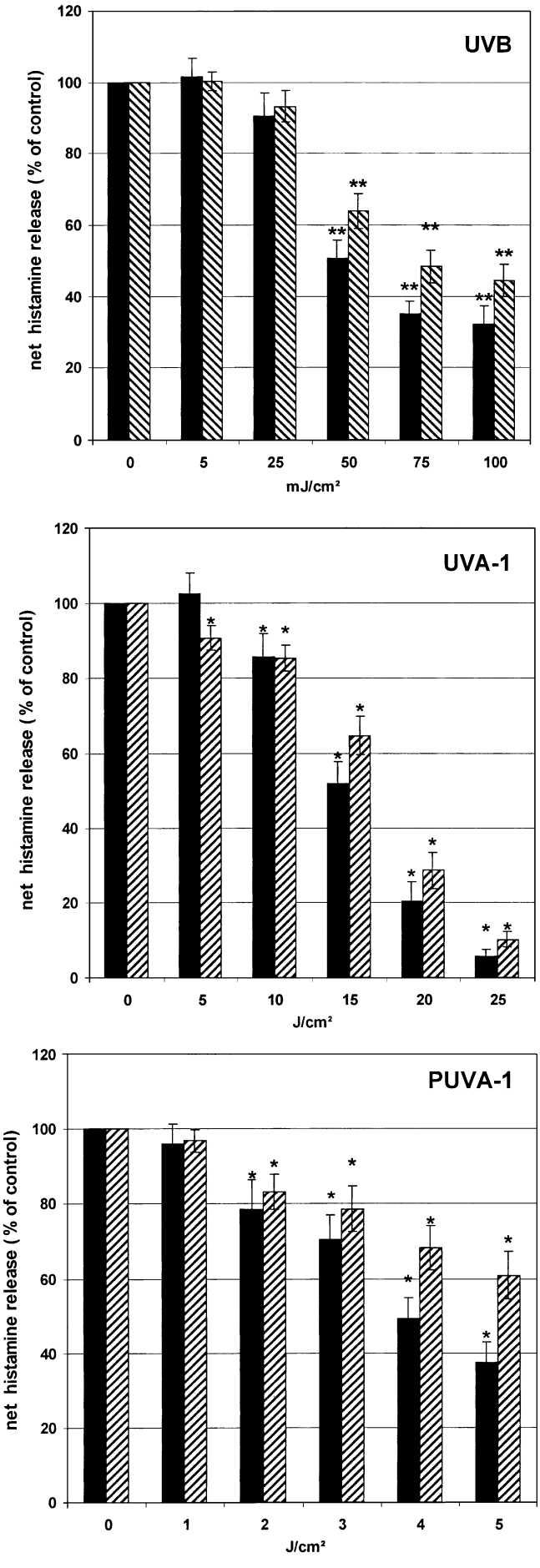

Figure 1
Ultraviolet (UV) treatment inhibits stimulated histamine release from skin mast cells (MC). Skin MC were irradiated with UV light at the doses indicated, kept in medium for 24 h, and then stimulated by either anti-IgE (black bars) or the calcium ionophore A 23187 (hatched bars). Results (net histamine release = (stimulated histamine release − spontaneous histamine release)/total cellular histamine) are expressed in relation to the value obtained for non-irradiated control cells (sham or 8 methoxypsoralen were set at 100%) and are the mean ± SEM of 7–10 independent tests. $^{*}p<0.05$; $^{**}p<0.01$; compared with non-irradiated control cells.

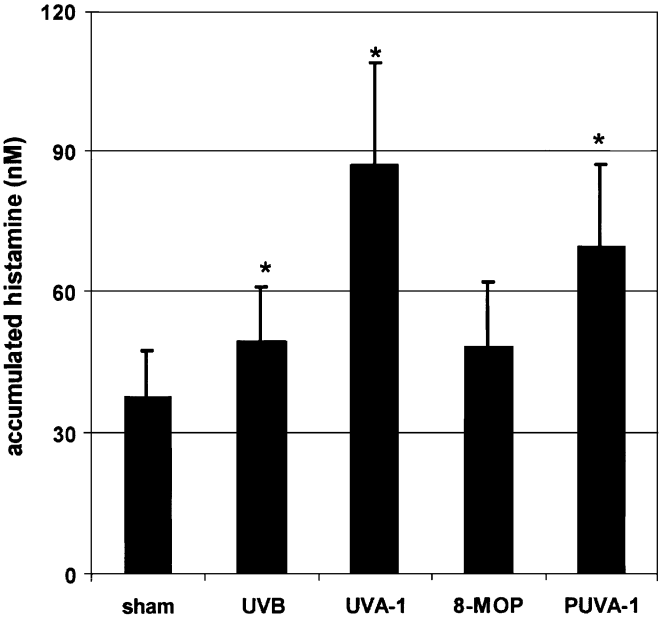

Figure 2
Effects of ultraviolet (UV) light on accumulated histamine levels. Cells were irradiated with 75 mJ per cm^2 (UVB), 15 J per cm^2 (UVA-1), and 5 J per cm^2 (PUVA-1), and left in culture for 24 h. Sham- and MOP-treated cells represent non-irradiated controls. Accumulated histamine content in supernatants was assessed by the enzyme immuno assay (EIA) technique. Results are the mean ± SEM of nine independent assays. $^*p < 0.05$, compared with sham- and 8-methoxypsoralen-treated cells, respectively.

effect on tumor necrosis factor-alpha (TNF-α) secretion. This up-modulating activity of anti-IgE on TNF-α was completely abrogated by UV irradiation, whereas the very low baseline level remained unaffected. In contrast to what was observed for the preformed mediator histamine, the release of these mainly *de novo* synthesized MC mediators were inhibited irrespective of whether cells were stimulated or not.

Discussion

UV light can mediate either pro- or anti-inflammatory effects on the skin through its action on different cells including keratinocytes, melanocytes, fibroblasts as well as infiltrating leukocytes. Our work provides evidence that human MC need to be viewed as additional major targets in the skin that are involved in immunomodulation by UV light, as has been suspected previously (Hart *et al*, 2001, 2002). Interestingly, UV light displayed a dual effect on MC by triggering slight but significant histamine release from resting MC, but suppressing this same release up to 90% when cells were appropriately stimulated. The inhibition of histamine release was accompanied by reduced secretion of tryptase (data shown in full-text version), suggesting that UV light had a negative impact on the discharge of MC granules into the outer cell environment.

MC are well known to produce several cytokines in addition to the preformed histamine (Artuc *et al*, 1999; Bradding and Holgate, 1999). One significant finding of this study was therefore the inhibition of proinflammatory cytokines in human skin MC detected after all types of UV irradiation and irrespective of the activation state of the cell. Together with the data on preformed mediators, UV light obviously turns skin MC insensitive or even inert towards different types of stimulation, which is illustrated by the counteracting effect of UV light on the production of TNF-α where UV light fully impedes the stimulatory capacity of anti-IgE.

From a more physiological or clinical viewpoint, our data demonstrate that the bivalent effects of UV light on the skin are impressively reflected at the level of the skin MC, thereby possibly explaining some clinical observations. The low, but long-lasting histamine release from resting MC may contribute to UV-induced erythema, either directly (Clydesdale *et al*, 2001) or through coupled prostaglandin synthesis, a key feature of sunburn-associated inflammation (Pentland *et al*, 1990). Our findings are largely in line with data showing UV-mediated reduction of skin histamine levels in cutaneous mastocytosis (Kolde *et al*, 1984; Czarnetzki *et al*, 1985), a condition in which MC appear to be slightly but constantly activated (Escribano *et al*, 2002). But the fact that inhibitory effects are not limited to histamine provides a plausible explanation for the advantageous anti-inflammatory actions of UV treatment in a number of other dermatological diseases that are not or not primarily histamine dependent, such as atopic dermatitis and psoriasis. Taken together, the present findings identify human skin MC as important targets of UV-induced immunomodulation. They help explain both the adverse effects of UV light, with sunburn reactions being the most prominent example, and the beneficial anti-inflammatory effects during therapy.

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Supplementary Material

The following material is available from http://www.blackwellpublishing.com/products/journals/suppmat/JID/JID23523/JID23523sm.htm

Figure S1
Spectral irradiance as a function of wavelength. Spectral irradiance as a function of wavelength for the UVB device, UV 800 equipped with TL-12 tubes (1) and the UVA-1 fluorescence tube TL-10 (2) determined at the distance of use (50 cm, 5 cm) without solution (a) and after transmission of the solution (b).

Figure S2
UV-treatment inhibits stimulated histamine release from skin mast cells. Skin mast cells were irradiated with UV-light at the doses indicated, kept in medium for 24 h and then stimulated by either anti-IgE (black bars) or the calcium ionophore A 23187 (hatched bars). Histamine levels in the supernatants and lysates were assessed as described under Materials and Methods. Results [net histamine release = (stimulated histamine release – spontaneous histamine release)/total cellular histamine] are expressed in relation to the value obtained for non irradiated control cells (sham or 8-MOP were set at 100%) and are the mean ± SEM of 7–10 independent tests. $^*p < 0.05$; $^{**}p < 0.01$; compared to non irradiated control cells.

Figure S3
UV-treatment inhibits anti IgE stimulated tryptase release from skin mast cells. Cells were treated with 75 mJ per cm^2 UVB, 15 J per cm^2 UVA-1 and 5 J per cm^2 PUVA-1 and levels of released tryptase assessed in relation to a standard curve using the recombinant enzyme by proteolytic cleavage of a specific substrate. Results are expressed in percent of total tryptase content and are the mean ± SEM of 7–9 independent tests. $^*p < 0.05$. compared to sham-treated cells and 8-MOP included as a control for PUVA-1 (which were set at 100%).

Figure S4
Time-dependent inhibition by UV-light of stimulated histamine release from skin mast cells. Cells were irradiated with doses of 75 mJ per cm^2 (UVB), 15 J per cm^2 (UVA-1), or 5 J per cm^2 (PUVA-1) and then

left in standard medium for the times indicated on the abscissa prior to challenge with anti-IgE (black bars) or the calcium ionophore A 23187 (hatched bars). Results are the mean ± SEM of 6 independent tests. *$p < 0.05$. compared to non irradiated control cells.

Figure S5

UV-light does not affect mast cell survival. Skin mast cells were irradiated with 75 mJ per cm^2 (UVB), 15 J per cm^2 (UVA-1) and 5 J per cm^2 (PUVA-1) and kept in culture for the times indicated in the figure. Cell numbers were assessed by automatic casy cell counter. The bars show the mean of the cell number of 6 independent experiments +/−SEM. No differences were found between irradiated MC and the two non irradiated control groups (sham and 8-MOP).

Figure S6

UV-light reduces total histamine content of skin mast cells in a time-dependent manner. Cells were irradiated with the UV-doses stated in the figure and then left in standard medium for the times indicated on the abscissa. Total histamine content was assessed in cell lysates relative to a standard curve. 8-MOP and sham treated cells served as negative controls Data are the mean ± SEM of 6 independent tests.

Figure S7

UV-light provokes spontaneous histamine release from skin mast cells. Cells were irradiated with various doses of UV-light. After 24 h in standard medium, spontaneous histamine release was assessed. Data show the mean ± SEM of 7–10 independent tests. *$p < 0{,}05$.

Figure S8

Effects of UV-light on spontaneous histamine release over the time. Cells were irradiated with the indicated doses of UV-light, and kept in culture medium for different times as shown on the abscissa, afterwards spontaneous histamine release was assessed. Data are the mean ± SEM of 6 independent assays.

Figure S9

Effects of UV-light on accumulated histamine levels. Cells were irradiated with 75 mJ per cm^2 (UVB), 15 J per cm^2 (UVA-1) and 5 J per cm^2 (PUVA-1), and left in culture for 24 h. Sham- and MOP-treated cells represent non irradiated controls. Accumulated histamine content in supernatants was assessed by EIA technique. Results are the mean ± SEM of 9 independent assays. *$p < 0.05$, compared to sham- and 8-MOP-treated cells, respectively.

Figure S10

UV-light affects spontaneous and induced surface expression of CD107a. Cells were irradiated with 75 mJ per cm^2 (UVB), 15 J per cm^2 (UVA-1) and 5 J per cm^2 (PUVA-1), and then kept in culture for 24 h. Cell surface expression of CD107a was assessed by flow-cytometry with (black lines) or without (grey lines) stimulation by anti-IgE for 30 min. Representative histograms of 5 independent assays are shown.

Figure S11

UV-light inhibits the release of proinflammatory cytokines from skin mast cells. Cells were irradiated with 75 mJ per cm^2 UVB, 15 J per cm^2 UVA-1 and 5 J per cm^2 PUVA-1, kept in culture for 1 h, and then triggered or not by anti-IgE. Supernatants were removed after 24 h and cytokine content assessed by standard ELISA technique. Control referred to non irradiated and non anti IgE challenged cells, the values for which were set at 100%. Results are the mean ±SEM of 4–10 independent tests. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Tables S1. Irradiance data for UV-sources.

Tables S2. Received UV-doses at mast cell site.

Tables S3. Percentage of dead cells.

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