Systemic Response to Ultraviolet Radiation Involves Induction of Leukocytic IL-1β and Inflammation in Zebrafish

Sanjita Banerjee and Maria Leptin

*J Immunol* 2014; 193:1408-1415; Prepublished online 25 June 2014; doi: 10.4049/jimmunol.1400232

http://www.jimmunol.org/content/193/3/1408
Systemic Response to Ultraviolet Radiation Involves Induction of Leukocytic IL-1β and Inflammation in Zebrafish

Sanjita Banerjee and Maria Leptin

Ultraviolet radiation is a pervasive stimulus with wide-ranging effects on all living forms. The effects of UV vary from physiological to pathological, depending on levels of exposure, but the immune response at the organismal level is not well understood. We used the zebrafish embryo and larva to study immune responses to UV stress in vivo. UV exposure causes inflammation characterized by systemic induction of proinflammatory cytokines. Leukocytes are an important component of this systemic response and upregulate IL-1β expression proportional to the dose of UV exposure. Increased levels of this proinflammatory cytokine counteract the lethal effect of high doses of UV. The Journal of Immunology, 2014, 193: 1408–1415.

Ultraviolet radiation in sunlight has wide-ranging effects in our lives. UV radiation has been subdivided into three wavebands: UVC (200 nm–280 nm), which is filtered out by atmospheric ozone; UVB (280 nm–320 nm); and UVA (320 nm–400 nm). In humans, UVB is mainly absorbed by the epidermis whereas UVA penetrates deeper into the skin. Broad-band UVB has been known to cause sunburn, and narrow-band UVB is used in therapeutic applications (1).

UV is a critical environmental component that causes sunburn, photaging, and cancer (2). UV can also lead to alteration of the immune environment, thereby increasing our susceptibility to cancer (3). However, the beneficial effects of both UVA and UVB radiation are numerous, including synthesis of vitamin D and production of antimicrobial peptides (2, 4). UV has been used as a therapy in several diseases such as psoriasis, graft-versus-host disease, and atopic dermatitis (5–7). It affords protection against multiple sclerosis, and its immunosuppressive effect augments tolerance to allografts (8, 9).

UV exposure can affect mitosis, modulate expression of adhesion molecules, promote apoptosis, and induce cytokine production (10–13). Exposure to UV results in alterations in immune surveillance at the local and systemic levels. Cytokines expressed in the epidermis are thought to play an important role in this process (13). Keratinocytes are the cells that receive the highest doses of radiation, and their response to UV has been documented by various in vitro and ex vivo studies (14). It involves upregulation of several cytokines, such as TNF-α or IL-6, IL-1, and IL-10 (14–17).

Apart from keratinocytes, a few other cell types, such as the Langerhans cells and PBMCs, have been studied in vitro (14, 18), but the effect of UV at the organismal level has not been addressed.

Changes in the levels of these immune modulators can bring about systemic effects that have still not been elucidated.

To understand how an organism responds to UV radiation, we established a zebrafish model of UV stress. In this work we show that UV exposure causes inflammation and concomitant mortality in zebrafish embryos. The myeloid cells are major responders to UV and form the main source of IL-1β, which appears to have a reparative effect at nonlethal doses of UV.

Materials and Methods

Zebrafish care and maintenance
Zebrafish were maintained at 26.5°C with a 14-h light/10-h dark cycle. Embryos were maintained in E3 medium at 28°C. For imaging experiments, phenylthiourea (0.003%) was added to embryos 1 d postfertilization to avoid melanization.

Transgenic fish lines
To create the stable transgenic line that reports NF-κB activation as a function of GFP, Tg(NF-κB binding site:Kal4.6UAS:GFP), hereafter referred to as Tg(nfkB-EGFP), the following construct was generated. A minimal conalbumin promoter was cloned downstream to three copies of a NF-κB consensus sequence (19). To amplify the transcriptional activation, the Kal4.6UAS cassette, an “amplification” cassette based on an optimized GAL4-UAS system (20), was inserted between this promoter and the reporter enhanced GFP (eGFP).

A construct including only the NF-κB–conalbumin promoter did not show any eGFP expression (results not shown). An eGFP driven by a minimal El1 promoter was cloned downstream to the Kal4.6UAS cassette (diagrammatized in Supplemental Fig. 1A). The construct was assembled in the Gateway pDEST vector (Life Technologies) containing two tol2 sites. The vector construct, along with transposase RNA (200 ng/μl), was injected into one-cell-stage embryos (F0), which were grown to adulthood and screened for transgenic offspring. A stable F1 line was generated by mating the founder with wild-type WIK. Experiments were carried out using F1 and F2 lines.

Injections
The pu.1 morpholino (21) and il-1b morpholino were obtained from Gene Tools and resuspended in sterile water. Morpholinos were injected at a concentration of 0.5 mM with 0.2% Phenol Red (Sigma-Aldrich) and 0.1 M KCl (Sigma-Aldrich) into one-cell-stage embryos. The il-1b ATG mor- pholino (5’-TAACCAGCTCTGAAATGATGCA-3’) was designed by targeting the start codon. To test the efficacy of the morpholino, pCS2- IL1b-ATGmo-eGFP was generated by inserting a 68- bp fragment of il-1b encompassing the region of ATG morpholino into the pCS2-eGFP plasmid, in-frame with eGFP (Supplemental Fig. 1B). The plasmid was linearized using NotI, and capped mRNA was transcribed using an SP6 RNA polymerase in vitro transcription kit (mMESSAGE mMACHINE SP6; Life Technologies). A concentration of 200 ng/μl was used for all injections. The IL-1β coding region was amplified from zebrafish cDNA and cloned into pCS2*plasmid. The resultant pCS2-IL1b plasmid was

Institute of Genetics, University of Cologne, 50674 Cologne, Germany; and European Molecular Biology Laboratory, 69117 Heidelberg, Germany

Received for publication January 27, 2014. Accepted for publication May 30, 2014.

This work was supported by Deutsche Forschungsgemeinschaft (SFB 670) and the European Molecular Biology Organization.

Address correspondence and reprint requests to Prof. Maria Leptin, Institute of Genetics, University of Cologne. Zülpicherstr 47a, 50674 Cologne, Germany. E-mail address: maria.leptin@embo.org

The online version of this article contains supplemental material.

Abbreviations used in this article: eGFP, enhanced GFP; FISH, fluorescent in situ hybridization; hpf, h postfertilization; NA, numerical aperture.
linearized, and in vitro transcribed; capped mRNA (200 ng/ml) was injected into one-cell-stage embryo.

**UV and drug treatments**

Embryos 24 h old (n = 50–100) were sorted into a petri dish with a thin layer of E3 medium and exposed to different doses of UV. The embryos, placed at a distance of 15 cm from a bank of four broad-band UV lamps (Phillips) with an average combined energy output of 1.4 mW/cm² at 320 nm (measured by X-Cite Power Meter, XR2100 Luminan Dynamics), were exposed for different periods, ranging from 2 s (corresponding to 3 ml/cm²) to 90 s (corresponding to 120 ml/cm²) to achieve the different doses of UV exposure. Following UV irradiation, E3 medium was added and the embryos were maintained at 28°C. For survival studies, triplicate petri dishes were irradiated for each dose (n = 3) and monitored over time. For drug inhibition studies embryos were dechorionated using pronase and placed in E3 medium containing the caspase-1 inhibitor Z-YV AD-FMK (Bachem; dissolved in DMSO at 200 mM, final concentration 300 μM), or the pan-caspase inhibitor Z-VAD(Ome)-FMK (Bachem; dissolved in DMSO at 200 mM, final concentration 300 μM) or an equivalent volume of DMSO without inhibitor.

**Confocal and time-lapse imaging**

For live imaging, embryos were anesthetized with 0.01% tricaine, embedded in 1.2% low-melting-point agarose, and imaged using the PerkinElmer Ultraview ERS Spinning Disk (x40/numerical aperture (NA) 1.2 objective). A total of 25–30 z-stacks were captured spanning 50–60 μm. Image processing and analysis were done using Velocity and Fiji software. Quantification of GFP intensities was accomplished using Fiji software. For each embryo, the same region of interest was selected and total intensity within the region of interest measured. Signal intensities for control and experimental groups were determined following background subtraction. The intensities were normalized to control values and plotted as fold increase (n = 9–12 for each group for each time point).

**Whole-mount immunostaining and in situ hybridization**

Whole-mount immunostaining for F-actin was performed as described (22). Briefly, embryos 48 h postfertilization (hpf) were fixed in 4% paraformaldehyde plus 0.4% Triton X-100 overnight at 4°C, blocked in 5% normal goat serum in PBS plus 0.1% Triton X-100, and incubated overnight with F-actin Ab (1:500 in blocking solution). Anti-rabbit Alexa Fluor 568 was used as the secondary Ab, and the samples were visualized using a PerkinElmer Ultraview ERS Spinning Disk microscope (x40/NA 1.2 objective). The signal in the two populations rose further to 2.8-fold (medium) and 4.7-fold (high) after 4 h. For in situ hybridization, the il-1b coding region was PCR amplified with primers for the flanking T3 and T7 promoters. The PCR product was used as a template for digoxigenin-labeled sense and antisense RNA probes using a kit from Roche Diagnostics. Whole-mount in situ hybridization was performed as previously described (23). Images were visualized using an Olympus SZX16 stereomicroscope. Fluorescent in situ hybridization (FISH) and Ab staining were based on the work of Orygkkozko et al. (24). The bound probe was detected with anti-digoxigenin–POD (1:1000; Roche Diagnostics) and TSA–Alexa Fluor 488 (PerkinElmer), followed by F-actin Ab staining. Images were captured using a PerkinElmer Ultraview ERS Spinning Disk microscope (x40/NA 1.2 objective).

**Quantitative PCR analysis**

Total RNA was extracted from pools of 15–20 embryos from each treatment group at different time points using TRIzol (Life Technologies) following the manufacturer’s protocol. Reverse transcription was done using MultiScribe Reverse Transcriptase (Life Technologies). Gene-specific primers were designed using Universal ProbeLibrary (Roche), and real-time PCR was performed with SYBR Green (Applied Biosystems) on the ABI 7500 Real-Time PCR System. The primer sequences are furnished in Supplemental Table I.

**Statistical analysis**

Data were plotted and analyzed using GraphPad PRISM. One-way ANOVA (Figs. 2, 6B) and two-way ANOVA with Bonferroni posttests were used for statistical analysis; p < 0.05 at a confidence interval of 95% was considered significant.

**Results**

**UV exposure causes mortality**

Embryos 24 h old were exposed to increasing doses of UV, and their survival was monitored over 5 d. Based on the mortality profile, the doses were grouped as low-, medium-, and high-stress stimuli (Fig. 1). Embryos exposed to low doses (3 ml/cm² and 6 ml/cm²) resembled the untreated controls. The embryos irradiated with medium doses (12 ml/cm² and 24 ml/cm²) showed 20–30% mortality by 72 h post–UV treatment. The high UV doses (48 ml/cm² and 120 ml/cm²) killed all the embryos. The data indicate that UV exposure causes mortality in zebrafish embryos and that mortality depends on the dose of UV.

**UV induces an inflammatory response via the NF-κB pathway**

To understand whether an inflammatory process underlies this dose-dependent death, we examined the NF-κB pathway, given its established role in inducing transcription of proinflammatory genes (25). To achieve this, we established a transgenic NF-κB reporter line carrying a construct that has three repeats of the NF-κB consensus binding sequence followed by a minimal conalbumin promoter cloned upstream of an eGFP cassette (Supplemental Fig. 1A). To test whether the reporter responds to known NF-κB activators, 24-h-old transgenic zebrafish embryos were treated with LPS. LPS is a well-established activator of the NF-κB pathway (26, 27) and causes inflammation and death in zebrafish (28). Upon LPS treatment, an increase in GFP expression was seen by 4 h, with a steady rise in signal at 10 h (Fig. 2A) until the embryos died by 24 h. Compared with the untreated transgenic controls, the LPS-treated fish showed a 3.2-fold increase in GFP signal (p < 0.001) by 4 h, which became 4-fold by 10 h post-LPS (p < 0.0001). This reporter is therefore a useful tool to study inflammatory signaling via NF-κB. We used these transgenic NF-κB fish to test whether exposure to UV led to activation of the NF-κB pathway. Zebrafish embryos 24 h old were exposed to the doses that caused clear responses, that is, medium (24 ml/cm²) and high (48 ml/cm²) UV. Because the highest dose (120 ml/cm²) had killed more than half of the fish early in the time course, this dose was excluded from further studies. Expression of the NF-κB reporter was monitored over a period of 24 h (Fig. 2B). In the embryos given medium UV, GFP-positive cells were seen by 10 h post-UV with a further increase in GFP intensity at 24 h post-UV (Fig. 2B, second column). In contrast, embryos exposed to high UV had GFP-positive cells already by 4 h post-UV, and a greater number 10 h post-UV, which increased further by 24 h (Fig. 2B, third column). We quantified the increase in GFP signal over time and plotted it as fold increase over the control embryos receiving no UV. At 4 h post-UV, no significant increase was noted in GFP intensity in embryos exposed to medium dose, but those irradiated with high UV had ~2.8-fold increase in GFP intensity. The signal in the two populations rose further to ~2.8-fold (me-
UV exposure results in inflammation and IL-1β induction.

NF-κB-responsive cytokines are upregulated subsequent to UV exposure

Some of the major targets of NF-κB signaling are the genes encoding cytokines. Having shown that NF-κB signaling occurs after UV exposure, we wanted to know which cytokines responded to UV. We therefore tested the transcriptional profiles of various NF-κB-responsive cytokines in 24 hpf zebrafish embryos that were exposed to medium (12 mJ/cm²) and high (48 mJ/cm²) UV (Fig. 3). After UV irradiation, embryos were collected at different time points, RNA was prepared, and levels of mRNAs encoding cytokines were tested by quantitative real-time PCR. We found elevated levels of mRNA for the proinflammatory cytokines il-1b, il-8, and tnf-a. The il-1b showed the highest transcriptional induction, detectable 10 h post-UV at all doses, with differences in profiles between medium and high UV doses. At 24 h post-UV, a 10- to 40-fold increase was seen in the medium doses. The mRNA levels subsided thereafter, although not to the basal level. In the high dose, UV stress resulted in a 75- to 100-fold increase and no abatement until death (Fig. 3A). The il-8 transcripts were elevated by 24 h post-UV in a dose-dependent manner (Fig. 3B). The embryos treated with high dose showed no decrease in il-8 levels at 36 h post-UV. The tnf-a mRNA was not detectable in untreated zebrafish embryos. Upon UV stress, a spike in tnf-a transcript was seen by 20 h (Fig. 3C). In contrast to il-1b and il-8, tnf-a levels decay thereafter also in embryos treated with high UV doses. The prominent anti-inflammatory cytokine il-10 was also elevated upon UV exposure in a dose-dependent manner (Fig. 3D).
In summary, the data show induction of various cytokines involved in immune activation, such as *il-1b*, *il-8*, and *tnf-a*. In contrast, *il-12b*, a cytokine involved in regulating the Th 1 cell response (29), was not induced (Fig. 3E). Elevation of *il-10*, an anti-inflammatory cytokine, was also seen, albeit at much lower levels.

**Leukocytes are the major source of UV-induced il-1b**

The transcriptional profiles of inflammatory cytokines indicate a gradual and progressive rise in inflammation after UV exposure, with elevated mRNA levels detectable by 10 h and continuing to rise until 24 h. The most likely first responders to UV are the epithelial cells of the skin. However, the continual rise in inflammation might reflect a spread of inflammation regulated by immune cells. The immune cells of a 24 hpf zebrafish comprise mainly cells of the myeloid lineage, namely, the neutrophils and the macrophages. We ablated myeloid cells with the help of a *pu.1*-specific morpholino to understand the role of leukocytes in UV-induced inflammation. Pu.1 is a transcription factor with a key role in myeloid differentiation, and its knockdown leads to a loss of the myeloid cell lineage (21). The efficacy of the *pu.1* morpholino was tested by its ability to reduce the number of leukocytes. Immunostaining control and *pu.1* morpholino–injected (“morphant”) embryos for *l-plastin*, a marker for leukocytes (30), showed a decrease in the number of *l-plastin*–positive cells in the morphant embryos (Supplemental Fig. 2). Control and *pu.1* morphants, 24 h old, were irradiated with innocuous (low), damaging (medium), and lethal (high) UV, and were survival monitored to test whether leukocytes contributed toward the survival of embryos. At high UV doses, the mortality profiles of control and *pu.1* morphants were comparable (Fig. 4C), indicating that myeloid cells are unable to counteract the damaging effects of high doses of UV stress.

The data shown so far indicate that a UV response includes involvement of leukocytes and induction of several inflammatory cytokines. To test whether leukocytes contribute toward the inflammatory milieu, we monitored expression of *il-1b*, the cytokine that showed maximal induction by UV. Recent studies have reported the induction of *il-1b* in leukocytes following injury (24). UV irradiation led to a sharp rise in *il-1b* levels, as seen by quantitative real-time PCR and *il-1b* whole-mount in situ hybridization (Supplemental Fig. 3). To find out whether leukocytes are the major source of *il-1b* in embryos exposed to medium UV, we performed *il-1b* FISH in conjunction with immunostaining for *l-plastin*. In embryos exposed to medium UV, leukocytes stained positive for *il-1b* mRNA 24 h postirradiation (Fig. 4D). We also quantified levels of *il-1b* in control and *pu.1* morphants at different time points after low and medium UV exposure (Fig. 4E). Because no difference was observed in the mortality profiles of control and *pu.1* morphants receiving high UV, they were not included for further analyses. As shown earlier, the control embryos exposed to UV showed an elevation of *il-1b* transcript levels compared with nonirradiated embryos. The *pu.1* morphants showed a significant attenuation in the degree of *il-1b* elevation, which was most
pronounced at 24 h after UV exposure. Assuming that the il-1b levels in controls reflect the combined contribution from leukocytic and nonleukocytic populations and the il-1b levels in pu.1 morphants reflect contribution from the nonleukocytic population only, the relative proportion of il-1b from leukocytic and nonleukocytic cells 24 h post-UV was plotted for each UV dose (Fig. 4F). In the populations exposed to low UV, the control embryos showed ∼6-fold il-1b induction compared with ∼4-fold for the morphants, 24 h post-UV. The effect after medium-level UV irradiation was greater 24 h post-UV. The morphants showed ∼5-fold il-1b induction as opposed to the ∼53-fold in the corresponding controls. The data indicate leukocytes as responsible for il-1b upregulation after UV irradiation. Although it is possible that they act in an indirect manner by triggering a third cell type to upregulate il-1b transcription, the most likely interpretation is that they themselves are the source of il-1b. Furthermore, the level of il-1b induction in leukocytes is dependent on the degree of UV radiation.

**IL-1β induction subsequent to UV exposure is beneficial**

We have shown that loss of pu.1-positive cells resulted in an increased sensitivity to UV and decreased il-1b induction. These outcomes could be causally related, or parallel and independent. If the main function of the pu.1-positive cells in protecting against UV is the production of IL-1β, then loss of IL-1β should also lead to increased mortality, and conversely, providing additional IL-1β might fully or partially protect embryos depleted of leukocytes from damage caused by radiation.

We tested the effect of reducing il-1b by morpholino injection. The morpholino was effective in knocking down the levels of IL-1β (Supplemental Fig. 1C). Control and il-1b morphant embryos were exposed to medium UV and their mortality monitored (Fig. 5A). The UV-treated il-1b morphants showed significantly higher death rates than did the corresponding control siblings by 48 h post-UV (Fig. 5A). Thus, reducing IL-1β levels has an effect similar to that of removing pu.1 cells. The mortality profiles of pu.1 morphants and il-1b morphants exposed to medium UV was similar, with ∼60% mortality 52 h post-UV (Figs. 4B, 5A). To test whether additional IL-1β could afford protection against UV irradiation, we increased the level of IL-1β expression by injecting mRNA into one-cell-stage embryos (il-1bhi). Overexpression of IL-1β alone had no effect on the survival of unexposed control embryos. The il-1bhi experimental group exhibited greater resilience to medium UV exposure (Fig. 5B). The UV-exposed control embryos showed ∼22% mortality 24 h post-UV, compared with 6% mortality in the UV-exposed il-1bhi group. The difference in their mortality profiles remained significant throughout the experiment, with 66% of il-1bhi survivors compared with only 54% control embryos at the end of the experiment. The experiments show that mortality subsequent to UV irradiation inversely correlates with IL-1β levels, with higher IL-1β contributing to greater survival and its absence resulting in higher susceptibility.

To find out whether IL-1β production was partly or fully responsible for the protective effect of pu.1-positive cells, we overexpressed il-1b in the pu.1 morphants (il-1bhi + pu.1 morphants). As
before, nonirradiated embryos from all groups were fully viable, UV-irradiated pu.1 morphants were more susceptible, and il-1b+ embryos were less so than the control counterparts (Fig. 5C). The overexpression of il-1b was sufficient to restore the survival rate of UV-irradiated leukocyte-deprived embryos (il-1b+ + pu.1 morphants) to similar levels observed with il-1b overexpression (il-1b) alone. This finding indicates that in the presence of artificially raised IL-1β levels, the absence of pu.1-positive cells no longer compromised the ability of the animal to withstand UV damage. At the end of the experiment, 52 h post-UV, 67% il-1b and 65% il-1b+ + pu.1 morphants survived, compared with 58% control and 43% pu.1 morphants. Thus, raising the level of IL-1β completely obviates the requirement for pu.1 cells in this assay, indicating that the main role of pu.1-positive cells in protecting against medium levels of UV damage is the production of IL-1β.

If the protective effect of pu.1-positive cells in the response to UV exposure is mediated mainly or exclusively by IL-1β and, conversely, IL-1β is produced mainly by these cells, then the detrimental effect of removing pu.1-positive cells should not be enhanced by also removing IL-1β or vice versa. To test this, il-1b levels were knocked down in pu.1 morphants (il-1b + pu.1 morphants) and mortality to medium UV exposure monitored. As shown earlier, UV-treated pu.1 morphants and il-1b morphants were more susceptible than the controls (Fig. 5D). Knocking down il-1b levels in pu.1 morphants did not significantly worsen the survival of the embryos post-UV exposure. By 52 h post-UV, 62% controls survived, compared with 52% pu.1 morphants, 44% il-1b morphants, and 42% il-1b + pu.1 morphants.

Inhibition of caspase-1 activity affects survival as well as il-1b induction
A core element in the inflammatory signaling cascade is caspase-1. We used the caspase-1 inhibitor YVAD to test the role of caspase-1 in survival after UV treatment (Fig. 6A). YVAD has been reported to inhibit the activity of caspase-A, the zebrafish homolog of caspase-1 (31, 32). Treatment with YVAD or the vehicle DMSO alone had no effect on mortality. Inhibition of caspase-1 resulted in significant increase in mortality with medium UV. UV-irradiated controls showed 65% survival as opposed to 52% survival of embryos when caspase-1 was inhibited. Pan-caspase inhibition with VAD also showed a trend toward reduced survival but did not reach significance (data not shown).

The effect of caspase-1 inhibition on downstream effects of inflammatory signaling was monitored by measuring il-1b transcript levels in these groups (Fig. 6B). In the group without YVAD, the UV-treated embryos showed the expected induction of il-1b, as described above. Inhibition of caspase-1 resulted in a significant dampening of il-1b induction. Exposure to medium UV resulted in 46-fold increase of il-1b in DMSO-controls, compared with only 8-fold in YVAD-treated embryos. These results show that at least part of the response to UV is mediated by a caspase-dependent signaling pathway.

Discussion
This study establishes the zebrafish as an in vivo model for UV stress and elucidates aspects of the immune response elicited by UV irradiation. We show that upon UV exposure, leukocytes become activated and contribute toward inflammation by elevating IL-1β.

FIGURE 5. Overexpression of il-1b protects zebrafish embryos against UV irradiation. Effects of lowering (A) or raising (B) the levels of il-1b in normal fish embryos and in pu.1 morphants (C and D). Embryos were injected with il-1b (A and D) or pu.1 (B) morpholinos (0.5 mM) or il-1b (200 ng/µl) mRNA alone or in combination with pu.1 morpholino (C and D) and subjected to medium (24 mJ/cm²) UV at 24 hpf. Their survival was monitored over the following 60 h. The ordinates have been interrupted for illustration purposes, ∗p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, comparing UV-treated controls with corresponding experimental groups; #p < 0.0001, comparing UV-treated controls with corresponding experimental groups.

Inhibition of caspase-1 activity affects survival as well as il-1b induction
A core element in the inflammatory signaling cascade is caspase-1. We used the caspase-1 inhibitor YVAD to test the role of caspase-1 in survival after UV treatment (Fig. 6A). YVAD has been reported to inhibit the activity of caspase-A, the zebrafish homolog of caspase-1 (31, 32). Treatment with YVAD or the vehicle DMSO alone had no effect on mortality. Inhibition of caspase-1 resulted in significant increase in mortality with medium UV. UV-irradiated controls showed 65% survival as opposed to 52% survival of embryos when caspase-1 was inhibited. Pan-caspase inhibition with VAD also showed a trend toward reduced survival but did not reach significance (data not shown).

The effect of caspase-1 inhibition on downstream effects of inflammatory signaling was monitored by measuring il-1b transcript levels in these groups (Fig. 6B). In the group without YVAD, the UV-treated embryos showed the expected induction of il-1b, as described above. Inhibition of caspase-1 resulted in a significant dampening of il-1b induction. Exposure to medium UV resulted in 46-fold increase of il-1b in DMSO-controls, compared with only 8-fold in YVAD-treated embryos. These results show that at least part of the response to UV is mediated by a caspase-dependent signaling pathway.

Discussion
This study establishes the zebrafish as an in vivo model for UV stress and elucidates aspects of the immune response elicited by UV irradiation. We show that upon UV exposure, leukocytes become activated and contribute toward inflammation by elevating IL-1β.
Innate immune responses to UV exposure have been studied mainly in vitro in keratinocyte cultures (33). The initial response of keratinocytes includes inflammasome formation, resulting in activation of proinflammatory cytokines (34). Cytokines such as IL-1β, TNF-α, and IL-10 are key players in maintaining cutaneous homeostasis, and alteration in the transcriptional levels of these molecules is a characteristic feature of immune response to sunburn (35–37). Exposure to UV radiation resulted in upregulation of these cytokines in zebrafish embryos, indicating induction of a similar inflammatory response. UV exposure also results in immunosuppression by inducing regulatory T cells (38). IL-10 has been implicated as a key regulator in bringing about the systemic immunosuppression (39, 40). This model of UV stress also recapitulates the immunosuppressive effects of UV, as seen by a systemic rise in the levels of IL-10. However, at this age the zebrafish lack regulatory T cells (41); hence it will be interesting to study whether the rise in IL-10 levels affects immunosuppression in this instance.

Several lines of study have shown NF-κB activation as a downstream effect of UV radiation. Whereas Fisher et al. (42) showed NF-κB activation even for low UV doses, Vile et al. (43) reported that activation of the NF-κB pathway was contingent on DNA damage. In our studies, NF-κB activation and subsequent induction of NF-κB–responsive inflammatory cytokines following UV irradiation are detectable 6–8 h post-UV (Fig. 3). The transcriptional response is unlikely to be the initial response to UV irradiation.

In vitro studies indicate that generation of reactive oxygen species, inflammasome formation, and IL-1β release are the initial events following UV exposure. In zebrafish, il-1β is upregulated postinfection with Yersinia ruckeri (44), and inflammasome formation and IL-1β release have been shown following Francisella noatunensis infection (31). Our data indicate involvement of caspase-1 in the signaling pathway that eventually leads to the transcriptional upregulation of IL-1β. This means that the caspase-1 homolog in zebrafish, caspase-A, is involved in IL-1β processing or that it has other roles in the pathway, given the lack of conserved caspase-1 cleavage sequence in zebrafish IL-1β.

The continual rise in inflammation over a period of 24 h pointed toward a systemic response, as opposed to being localized in nature. This indicated the possible involvement of additional cells, and we show that leukocytes respond to UV stress and constitute a major source of IL-1β. Although several chemokines such as MCP-1 and IL-8 have been implicated in leukocyte activation and recruitment (45, 46), it will be interesting to discover the cues involved in activating the leukocytes subsequent to UV exposure. In pu.1 morphants, IL-1β is induced to similar levels (~5-fold) by low and medium UV doses. Because most of the inflammation persists despite pu.1 knockdown must arise from nonimmune cells, the contribution of this component to total inflammation appears to be similar in these two doses and might be the result of the local damage caused by UV. The contribution of the immune cells toward IL-1β production is proportional to the degree of UV exposure as seen in the corresponding control populations. Leukocytes contribute minimally toward inflammation in low UV but have a more pronounced effect in the medium dose. The system thus shows nuanced responses to varying degrees of stress. Similarly, different doses of UV produce varying consequences in other model systems. Whereas 15 mJ/cm² of UV induces production of antimicrobial peptides in human keratinocytes, repeated doses of 1800 mJ/cm² administered over time can give rise to tumors in mice (3, 4).

We show the importance of IL-1β levels in the response mounted by the embryos to varying degrees of UV stress. At low and medium UV doses, modulation of IL-1β affects the ability of the fish to withstand the damaging effects of UV. IL-1β is a potent proinflammatory cytokine involved in acute inflammatory responses to several conditions (47–49). However, less well known is the role of IL-1β in mediating tissue repair and reconstitution. Both in vitro and in vivo studies have shown an early IL-1β response upon skin injury, and lack of this cytokine is associated with healing defects (50, 51). In skin repair studies, IL-1β has been shown to act via epidermal growth factor and TGF-α to enhance wound closure (50). Mouse models of colitis have implicated IL-1β in the repair of the epithelial barrier, with IL-1β–deficient mice showing exacerbated disease (52). IL-1β can activate astrocyte and microglia-macrophage–derived insulin-like growth factor-1 to mediate repair in the CNS (53). We show that in the zebrafish model for UV stress, IL-1β has a protective function and might be reparative, as higher levels of IL-1β correspond to better survival rates post–medium UV dose. It is possible that increased IL-1β leads to subsequent induction of various growth factors, which in turn mediate the reparative process after UV damage and result in greater survival.

In conclusion, we have established a zebrafish model for UV exposure and detail the inflammatory response in vivo. The zebrafish response to UV includes a systemic anti-inflammatory as well as a strong proinflammatory response that is dependent on the degree of UV exposure. IL-1β plays a major role in the progress of this response by positively regulating recovery after UV exposure.

Acknowledgments
We thank Gene Core and the Advanced Light Microscopy Facility at the European Molecular Biology Laboratory Heidelberg for crucial support; PerkinElmer for continuous support of the Advanced Light Microscopy Facility; Bernd Klaus for help with the statistical analysis; Yi Feng for the generous gift of L-plastin Ab; Georges Lufailla, Baubak Bajoghli, Martina Rembold, and Paola Kuri for critical comments on the manuscript and discussions throughout this work; and Francesca Peri for critical suggestions throughout the project.
Supplementary Figure S1

(a) Diagram of the NFκB reporter construct Tg(NFκB binding site: Kal4.6UAS:eGFP). 3xNFκB consensus site (red), minimal conalbumin (Con) promoter (blue), Kal4 (grey), 6xUAS (light blue) binding cassette, minimal E1b (black) promoter, eGFP (green).

(b) Construct to test the efficacy of the il-1b ATG morpholino. The 88 bp fragment contained parts of il-1b 5’-UTR (black) and coding sequence (blue). The morpholino sequence is underlined and the start site marked with an arrow. This whole fragment was cloned upstream to eGFP (green).

(c) In vivo test for the efficacy of il-1b morpholino (il-1b mo). Zebrafish were injected with linearized pCS2-il-1b-ATGmo-eGFP mRNA with or without the il-1b morpholino and GFP expression monitored. Zebrafish co-injected with the mRNA and the morpholino did not show any GFP expression (right column).

Supplementary Figure S1. Construct design and validation of the il-1b ATG morpholino.

(a) Diagram of the NFκB reporter construct Tg(NFκB binding site: Kal4.6UAS:eGFP). 3xNFκB consensus site (red), minimal conalbumin (Con) promoter (blue), Kal4 (grey), 6xUAS (light blue) binding cassette, minimal E1b (black) promoter, eGFP (green). (b) Construct to test the efficacy of the il-1b ATG morpholino. The 88 bp fragment contained parts of il-1b 5’-UTR (black) and coding sequence (blue). The morpholino sequence is underlined and the start site marked with an arrow. This whole fragment was cloned upstream to eGFP (green). (c) In vivo test for the efficacy of il-1b morpholino (il-1b mo). Zebrafish were injected with linearized pCS2-il-1b-ATGmo-eGFP mRNA with or without the il-1b morpholino and GFP expression monitored. Zebrafish co-injected with the mRNA and the morpholino did not show any GFP expression (right column).
Supplementary Figure S2. Efficacy of pu.1-positive cell knockdown.
48 hpf control and pu.1-morphant embryos were immunostained with antibodies against L-plastin, a marker for leukocytes. The number of L-plastin positive cells was reduced in pu.1-morphant embryos (right) as compared to controls (left).
Supplementary Figure S3. UV exposure up-regulates *il-1b*.
Whole mount in situ with a probe for *il-1b* was carried out on untreated and UV-treated embryos 24h after UV treatment. While the controls did not show detectable levels of *il-1b* mRNA, UV-treated embryos expressed *il-1b* expression throughout the embryo. High background staining for alkaline-phosphatase in the yolk is seen in both cases, and also in control embryos with antisense probes (data not shown).
**SUPPLEMENTARY TABLE S1.** Sequence of primers used for qPCR.

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Primer sequence</th>
<th>Amplicon (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>b-actin</strong></td>
<td>Forward: 5’-GATCTTCACCTCCCCCTTGTCA-3’&lt;br&gt;Reverse: 5’-GGCAGCGATTTCCTCCTAC-3’</td>
<td>65</td>
</tr>
<tr>
<td><strong>il-1b</strong></td>
<td>Forward: 5’-ATGCTCATGGCGAACGTC-3’&lt;br&gt;Reverse: 5’-TGGTTTTTAGTGTGTAAGACGCGACT-3’</td>
<td>73</td>
</tr>
<tr>
<td><strong>il-8</strong></td>
<td>Forward: 5’-GGCAAAATGACCAGCAAAAT-3’&lt;br&gt;Reverse: 5’-GCCAGACCTCTCAAGCTCAT-3’</td>
<td>92</td>
</tr>
<tr>
<td><strong>tnf-a</strong></td>
<td>Forward: 5’-AGGCAATTTCACCTTCAAGG-3’&lt;br&gt;Reverse: 5’-AGGTCTTGATTCAGTTGTATCC-3’</td>
<td>65</td>
</tr>
<tr>
<td><strong>il-10</strong></td>
<td>Forward: 5’-AAGCGGGATATGTGTAAGATG-3’&lt;br&gt;Reverse: 5’-CCCCCCTTTTCTCCTTCTTT-3’</td>
<td>100</td>
</tr>
<tr>
<td><strong>il-12b</strong></td>
<td>Forward: 5’-CGCTGTAGGAAACGCAA-3’&lt;br&gt;Reverse: 5’-AGGCAGCTGGAAGATTGTG-3</td>
<td>81</td>
</tr>
</tbody>
</table>