Differential Effects of Ultraviolet Irradiation in Neonatal *versus* Adult Mice Are Not Explained by Defective Macrophage or Neutrophil Infiltration

Mathieu P. Rodero[1,4], Herlina Y. Handoko[2,4], Rehan M. Villani[3], Graeme J. Walker[2,5] and Kiarash Khosrotehrani[1,3,5]

Epidemiological studies suggest that ultraviolet B exposure (UVR) during childhood is the most important environmental risk factor for melanoma. In accordance, neonatal, but not adult, UVR exacerbates melanoma incidence in mouse models. The inability of neonates, as opposed to adults, to mount a proper neutrophil inflammatory response in the skin upon UVR exposure has been one of the driving hypotheses explaining this observation for the past decade. However, this aspect remains controversial. Here, we evaluated the UVR-induced inflammatory response in neonatal versus adult mice. In neonates, a significant neutrophil infiltration could be identified and quantified using three different antibodies by flow cytometry or immunohistochemistry. On day 1 after UVR, neutrophils were increased by 84-fold and on day 4 macrophages increased by 37-fold compared with nonexposed age-matched skin. When compared with adults, neonatal skin harbored a higher proportion of neutrophils in the myeloid compartment without significant differences in absolute counts. This response was reproduced with different kinetics in C57Bl/6 and FVB mice with a more rapid attenuation of neutrophil counts in the latter. Overall, our results suggest that the greatly increased sensitivity to melanomagenesis in neonates does not result from their incompetence in terms of myeloid inflammatory response to UVR.

Journal of Investigative Dermatology (2014) **134,** 1991–1997; doi:10.1038/jid.2014.78; published online 6 March 2014

INTRODUCTION

Epidemiological studies as well as genetically modified murine models indicate that UVB exposure (UVR) during the first stage of life greatly increases melanoma risk (Noonan *et al.*, 2001; Whiteman *et al.*, 2001; Ferguson *et al.*, 2010). A special feature of UVR exposure during the early stages of life is the promotion of immune tolerance that supports tumorigenesis (Wolnicka-Glubisz *et al.*, 2007; McGee *et al.*, 2011). This hypothesis is supported by the fact that neonates tend to develop more Th2 or tolerogenic responses after encountering antigen compared with adult mice (Adkins *et al.*, 2004). In accordance, neonatal UVR has been shown to affect delayed-type hypersensitivity immune responses, suggesting persistent immune tolerance that could favor skin cancer development (McGee *et al.*, 2011). In addition to these observations of reduced adaptive immune responses, myeloid and innate responses essential in UVR-induced inflammation have also been examined, and a deficient myeloid-related inflammatory response to UVR has been found in neonatal mice (Wolnicka-Glubisz *et al.*, 2007). More recently, the importance of macrophages in the melanocyte response to UVR has been reported in neonatal skin, suggesting an important myeloid link with melanoma induction (Zaidi *et al.*, 2011; Handoko *et al.*, 2013).

In most acute inflammatory reactions, neutrophils are the first myeloid population to be recruited. Once in the tissue, they promote the recruitment of the macrophages (Soehnlein *et al.*, 2009; Williams *et al.*, 2011) that arise from the differentiation of circulating monocytes upon tissue infiltration. Murine monocytes have been subdivided in two subpopulations on the basis of their expression of Ly6c: the Ly6c^{hi} inflammatory monocytes that infiltrate tissues under inflammatory conditions through a CCR2-dependent mechanism and the Ly6c^{lo}-resident monocytes that infiltrate under normal conditions to help maintain tissue homeostasis (Geissmann *et al.*, 2003). As the ability of myeloid cells, and in particular neutrophils, to infiltrate neonatal UVB-irradiated skin is a matter of debate (Wolnicka-Glubisz *et al.*, 2007; Zaidi *et al.*, 2011), the purpose of our study was to characterize quantitatively and qualitatively the recruitment

[1]*University of Queensland Centre for Clinical Research, Brisbane, Queensland, Australia;* [2]*QIMR Berghofer Medical Research Institute, Brisbane, Queensland, Australia and* [3]*The University of Queensland, UQ Diamantina Institute, Translational Research Institute, Brisbane, Queensland, Australia*

[4]*These authors contributed equally to this work.*

[5]*These authors contributed equally as senior authors.*

Correspondence: Kiarash Khosrotehrani, University of Queensland Centre for Clinical Research, Royal Brisbane Hospital, Building 71/918, Herston, Brisbane, Queensland 4029, Australia. E-mail: k.khosrotehrani@uq.edu.au

Abbreviations: UVR, ultraviolet B exposure; H&E, hematoxylin and eosin

Received 1 November 2013; revised 21 December 2013; accepted 7 January 2014; accepted article preview online 7 February 2014; published online 6 March 2014

of myeloid cells to the skin of neonates after a single burning and melanomagenic dose of UVB radiation, and to compare it with the response in adults.

RESULTS

A single UVB irradiation exposure induces macrophage and neutrophil recruitment to C57Bl/6 neonate skin

We first used hematoxylin and eosin (H&E)–stained sections of neonatal skin to observe the inflammatory process upon UVR. We observed a noticeable thickening of the epidermis and a dense inflammatory infiltrate mostly in the deep dermis when compared with age-matched nonexposed neonatal skin (Figure 1a). It is thought that neutrophils are recruited to UVB-exposed skin in adult mice but not in neonates (Strickland *et al.*, 1997; Teunissen *et al.*, 2002; Wolnicka-Glubisz *et al.*, 2007). However, using immunohistochemical staining with two different monoclonal antibodies recognizing Ly6g, a neutrophil-specific marker, we found that, although they were almost undetectable in unexposed skin, neutrophils could be observed in large numbers in the dermis and hypodermis 4 days after UV irradiation (D4) (Figure 1b; Supplementary Figure S1 online). In many inflammatory conditions, the first wave of infiltration of the inflamed tissues by neutrophils is proposed to promote the recruitment of a second wave composed of macrophages. As we have previously published (Handoko *et al.*, 2013), we found a greatly increased number of macrophages in the dermis at D4 after UVR compared with unexposed skin (Figure 1c).

Given the contrast between our findings and previous reports in neonates, we extracted skin-infiltrating leukocytes to perform flow cytometry analysis. Myeloid cells were defined as CD11b+ cells, and neutrophils and macrophage subpopulations were further discriminated on the basis of Ly6g and Ly6c expression, respectively, as previously described for skin (Handoko *et al.*, 2013; Rodero *et al.*, 2013). A skin infiltrate composed of CD11b+ myeloid lineage cells could be clearly identified by flow cytometry at Day 4, whereas this population was barely detectable in age-matched unexposed skin (Figure 1d and data not shown). Flow analysis confirmed that both neutrophils and macrophages composed this infiltrate (Figure 1e). The presence of neutrophils was then further confirmed by using an additional neutrophil-specific antibody clone 7/4 staining (Figure 1f). All assays reliably displayed a specific staining as controlled by isotype control antibodies.

Overall, these findings strongly demonstrated the infiltration of neonatal skin with neutrophils and macrophages upon UVB irradiation.

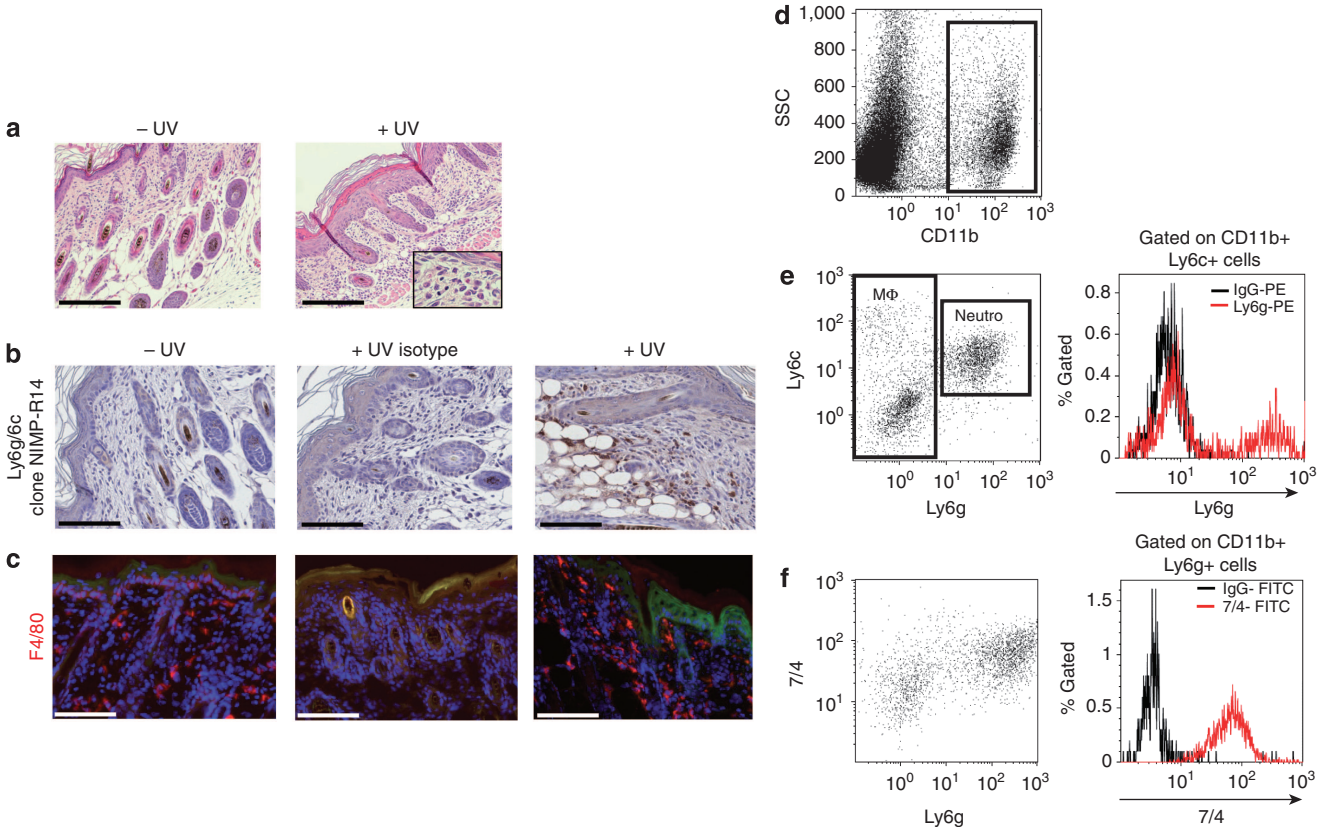

Figure 1. Single UVB irradiation to C57Bl/6 neonate skin induces macrophage and neutrophil recruitment. (**a**) Hematoxylin and eosin staining reveals that single UVB exposure induces an increase in the number of nuclei in the dermis compared with age-matched nonexposed neonates at 4 days (D4) after exposure. This could be explained at least to some extent by the recruitment of (**b**) neutrophils (Ly6g, brown) and (**c**) macrophages (F4/80, red). (**d**) By flow cytometry, we identified a massive infiltrate of CD11b+ myeloid cells in neonatal skin at D4 after irradiation. (**e**) On the basis of Ly6c and Ly6g expression levels, we confirmed here that this infiltrate is composed of macrophages and neutrophils (**f**) as further validated by 7/4 expression. Bar = 100 μm.

Dynamic recruitment of myeloid cells to neonatal skin induced by single UVB exposure

We next aimed to characterize the kinetics of recruitment of these myeloid cells. H&E staining of neonatal skin revealed that the recruitment of inflammatory cells started as early as 1 day after UV exposure (data not shown). To assess the respective participation of neutrophils and macrophages to the myeloid infiltration upon UVR over time, we studied the skin infiltrate by flow cytometry from D0 to D7 (Figure 2a). As previously reported, we observed an increase in the number of skin-infiltrating macrophages up to D4 (D0: 284, D4: 10,472, $P<0.001$; 37-fold increase) followed by a return to steady state value by D7 (Handoko *et al.*, 2013). The neutrophil infiltration showed a bimodal trend, with a first wave starting before 12 hours and peaking at D1 (D0: 234, D1: 19,824, $P<0.001$; 85-fold increase), followed by a second one beginning on D2 and peaking at D4 after irradiation (D0: 234, D4: 10,196, $P=0.004$; 43-fold increase) (Figure 2b). Overall, our results indicate that neutrophils are the main myeloid cell population in UVR-damaged neonatal mouse skin between 12 and 48 hours (Figure 2c) after exposure, and that they remain present until the resolution of the inflammation at D7.

During inflammatory processes, neutrophils are recruited from the circulation, whereas macrophages may be tissue-resident cells with proliferative properties. To assess the relative contribution of recruitment versus local proliferation to the increase in macrophage numbers observed between D1 and D4, we co-stained neonatal skin at D3 with F4/80 and KI67. Although the proliferative activity of the cells from developing hair follicles was clearly identified, less than one macrophage per section was found to be positive for KI67 (Supplementary Figure S2 online). This suggested that macrophage proliferation is a rare event during the few days following skin irradiation by UVB and does not significantly contribute to macrophage infiltration of the skin.

A single UVB dose induces myeloid cell recruitment in both adult and neonatal mice

As it is hypothesized that the importance of neonatal UVR in melanoma induction is due to lower inflammatory response in the neonate, and in particular the lack of neutrophil infiltration, we aimed to compare neonatal with adult responses. A single full dose of 5.9 kJ m^{-2} caused severe sunburn and epidermal damage in adult mice that were not acclimatized with repetitive UVR exposures. It was therefore necessary to explore lower doses. With half the usual dose (2.95 kJ m^{-2}), apoptosis could be observed in both adult and neonatal skin (Supplementary Figure S3a online). In addition, the study by De Fabo *et al.* (2004) showed that neonatal melanoma induction is dose sensitive and that reduced doses can still induce melanoma. The 2.95 kJ m^{-2} exposure did not result in a significant reduction in the number of macrophages recruited into neonatal skin compared with full doses at D1 or D4 after UVR (Figure 3a). However, the proportion of ''inflammatory'' Ly6c^{hi} macrophages was significantly reduced at D1 in half dose–exposed mice (43 ± 4% versus 21 ± 7%, $P=0.0095$; Figure 3 b). The number of neutrophils was also strongly affected at D1 (19,824 ± 11,410 versus 2,774 ± 1,163, $P=0.011$) and to a lesser extent at D4 (8,943 ± 8,101 versus 1,486 ± 1,538, $P=0.024$; Figure 3c). These data clearly show that increased doses of UVR result in a more inflammatory infiltrate.

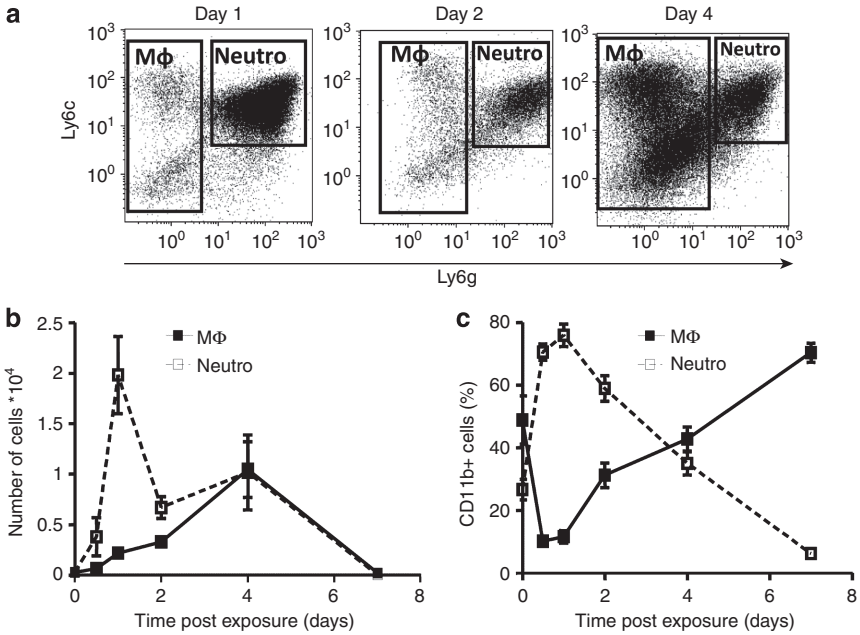

Figure 2. Dynamic recruitment of myeloid cells to neonate skin induced by single UVB exposure. (**a**) Representative dot plot of Ly6c and Ly6g levels of expression on skin-infiltrating CD11b+ myeloid cells at different time points after UV exposure. (**b**) Representation of the total number of skin-infiltrating macrophages and neutrophils per neonatal dorsal skin after UV exposure. (**c**) Representation of the respective proportion of skin-infiltrating macrophages and neutrophils among CD11b+ cells after UV exposure. $N=3$–9 mice depending on time points.

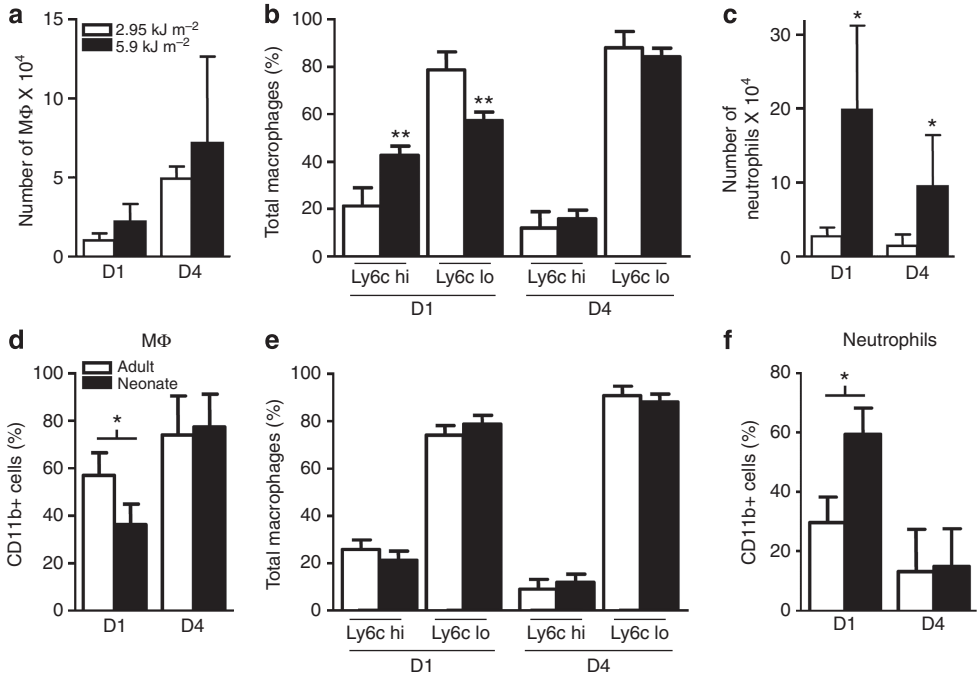

Figure 3. Single UVB irradiation induces distinct myeloid cell recruitment in adults and neonate. (**a**) Number of skin-infiltrating macrophages in neonatal skin for different doses of UV exposure. (**b**) Proportion of ''inflammatory'' (Ly6c^{hi}) and ''resident'' (Ly6c^{lo}) macrophages within skin-infiltrating macrophages (CD11b+ Ly6g-gate) for different doses of UV exposure. (**c**) Representation of the number of skin-infiltrating neutrophils in neonatal skin for different doses of UV exposure. (**d**) Graph showing the proportion of skin-infiltrating macrophages within CD11b+ myeloid cells in neonatal versus adult skin. (**e**) Proportion of ''inflammatory'' and ''resident'' macrophages within skin-infiltrating macrophages in neonates versus adults. (**f**) Proportion of neutrophils within CD11b+ myeloid cells in neonatal versus adult skin. Results shown as mean ± SD, Mann–Whitney test. $N = 4$–7 mice per condition and representative of two independent experiments. *$P<0.05$. **$P<0.01$.

We next compared the myeloid recruitment to the skin in response to UVR in neonates with that in adults. Given that the adult mouse dermis is more fibrous and less cellular than the neonatal dermis, and adult skin has fully formed hair follicles with a shaft, the efficiency of making single-cell suspension is much reduced in adults. Thus, we could not directly compare absolute numbers of infiltrating cells between adults and neonates. Although we observed an increased proportion of macrophages among cells recruited at D1 in adults (57 ± 10%; 36 ± 9; $P = 0.028$; Figure 3d), when looking at the proportion of Ly6c^{lo} and Ly6c^{hi} macrophages, both adults and neonates harbored similar profiles at D1 and D4 (Figure 3e). Importantly, we found an increased proportion of neutrophils in neonatal infiltrate at D1 compared with that in adult mice (30 ± 9% in adults versus 59 ± 9% in neonates; $P = 0.028$), although the proportions did not differ at D4 (Figure 3f). This was corroborated by counting Ly6g+ cells at D1 and D4 by immunohistochemistry (Supplementary Figure S3b online). In sum, the neutrophil response to UVR is more prominent in neonates than in adults.

Modulation of myeloid cell recruitment according to genetic background

Because the lack of inflammatory response in neonatal skin after UVB radiation reported in the literature had been described in FVB mice, we repeated our experiments in FVB neonates. In FVB mice, we observed slightly different kinetics of infiltration. In H&E sections (Figure 4a), as well as by Ly6g-stained skin sections (Figure 4b), a strong neutrophilic infiltrate was observed at D1. These neutrophils mostly disappeared by D4 when macrophages were more prominent (Figure 4a and c). Quantification of immunostaining and comparison with data obtained for C57Bl/6 showed a more rapid neutrophil infiltration on D1 in FVB neonates with a more rapid clearance of these cells (Figure 4d). This was further confirmed by flow cytometry in which, in FVB mice at D4, fewer neutrophils and more macrophages (in proportion) could be observed (Figure 4e and f). These results confirm the neutrophil infiltration upon UVR in FVB mice, albeit with more rapid recruitment and clearance than that in C57Bl/6 mice.

DISCUSSION

UVR is the main environmental risk factor for melanoma. Epidemiological studies have pointed to burning intermittent exposure(s) during early life as one of the most important factors modulating melanoma risk (Whiteman *et al.*, 2001). Similarly, in neonatal mice carrying melanoma-inducing mutations, but not in adults, irradiation has been shown to powerfully accelerate tumorigenesis (Noonan *et al.*, 2001). We report here a detailed characterization of the recruitment of myeloid cell subpopulations after UVB exposure in the skin of neonates as compared to adults in C57Bl/6 mice, and provide evidence that both macrophages and neutrophils are massively increased in both situations. The detection

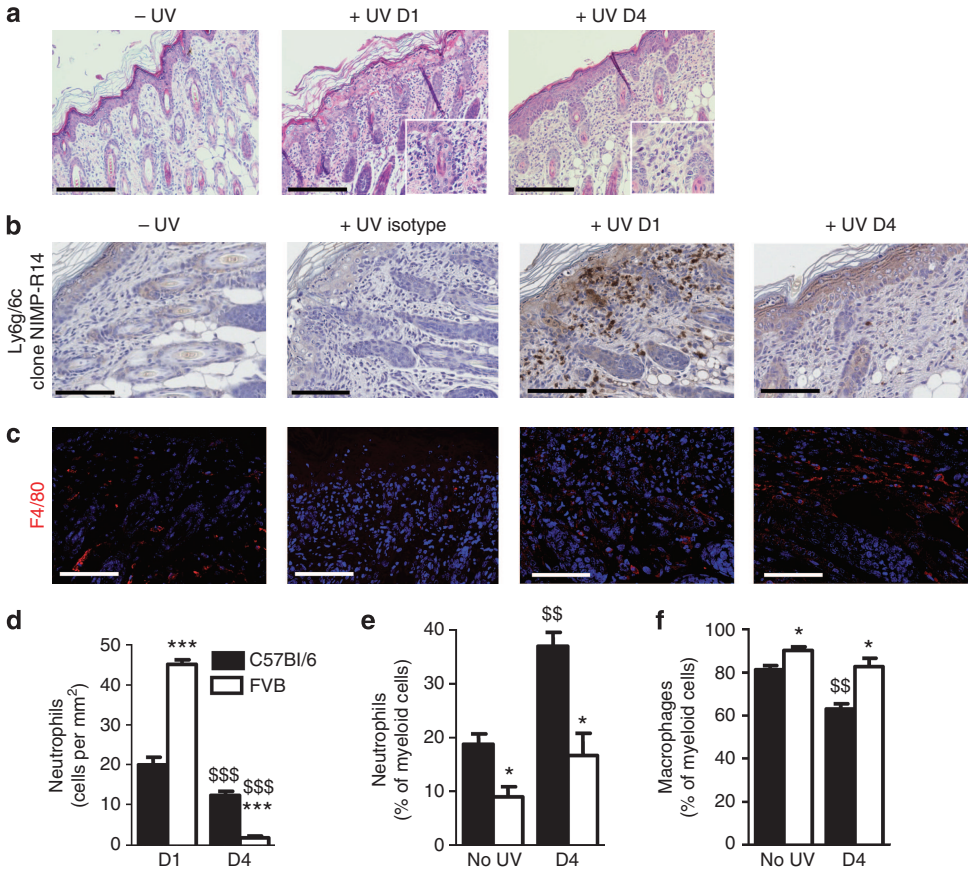

Figure 4. Single UVB irradiation induces distinct myeloid cell recruitment in C57Bl/6 and FVB neonates. Representative (**a**) hematoxylin and eosin, (**b**) Ly6g, and (**c**) F4/80 staining of FVB neonate skin nonexposed to UV (and matched for age for both D1 and D4), or 1 or 4 days after UV exposure with isotype control when appropriate. (**d**) Comparison of the effect of UV exposure on neutrophil number in C57Bl/6 mice with that in FVB mice. (**e**, **f**) Representation of the respective proportion of skin-infiltrated macrophages and neutrophils among CD11b+ cells between C57Bl/6 mice and FVB mice. $^{*}P<0.05$ compared with C57Bl/6, $^{***}P<0.001$ compared with C57Bl/6, $^{\$\$}P<0.01$ compared with no UV. $^{\$\$\$}P<0.001$ compared to D1. Bar = 100 μm.

of neutrophils is noteworthy, as these cells were previously thought to be absent in UVR-treated neonatal skin, despite similarities in terms of macrophage response (Wolnicka-Glubisz *et al.*, 2007; Zaidi *et al.*, 2011). These findings argue against defective recruitment of myeloid cells as a potential mechanism of UVB-induced melanomagenesis.

The differences in neutrophil response between studies are difficult to explain. Indeed, Wolnicka-Glubisz *et al.* (2007) reported a nonlinear neutrophil response with increasing dose in adults, but no neutrophil response in neonates. The differences may be owing to the different antibodies used or use of UV lamps with different spectral characteristics, which make it difficult to compare biologically effective doses. The use of different mouse strains is another common reason for disagreement between studies. Here we used two of the most frequently used strains, C57Bl/6 and FVB. In both strains, we observed infiltration of both neutrophils and macrophages post UVR, albeit with some differences in the kinetics of infiltration. It could be argued that the presence of melanin in pigmented C57Bl/6 but not in albino FVB would result in less DNA damage and inflammation, as in human skin (Tadokoro *et al.*, 2003). However, at D3, when neonates were irradiated, the C57Bl/6 mice were not yet pigmented. Indeed, *HGF* transgenic mice on C57BL/6 and FVB develop neonatal UVR–induced melanoma at similar ages of onset (Noonan *et al.*, 2012). Our observed strain differences could result from different kinetics of release of a variety of factors from the injured epidermis.

Comparison of skin myeloid infiltration upon UVR in adults with that in neonates did not demonstrate a lack of myeloid cell recruitment. It is clear that macrophages have a role in driving epidermal melanocyte proliferation upon neonatal UVR (Zaidi *et al.*, 2011), and we showed that CCR2-negative Ly6c^{lo} MHCII^{hi} cells were the likely causal macrophage population (Handoko *et al.*, 2013). On comparing adult with neonate myeloid responses, we could not find clear differences in the proportion of Ly6c^{lo} macrophages. Also, despite our finding of similar infiltration, neonatal UVR has been shown to induce a qualitatively different form of immunosuppression than that seen following adult exposure (Wolnicka-Glubisz *et al.*, 2007), including T-regulatory cell responses (McGee *et al.*, 2011). It is therefore tempting to speculate that qualitatively, the cells infiltrating the neonatal or adult skin may differ in their capacity to induce long-term

immunosuppression. An alternative possibility is the receptive state of neonatal but not adult melanocytes to signals generated by macrophages upon UVR.

In summary, we demonstrate by both flow cytometry and immunostaining that a single burning dose of UVB radiation on C57Bl/6 and FVB neonates induced a strong myeloid infiltration of the dermis composed of both macrophages and neutrophils. Our data suggest that the infiltrate is similar in adults and neonates, despite some degree of macrophage/neutrophil imbalance. The current thinking that neonatal UVR can induce melanoma by inducing immunotolerance can, therefore, not be explained by a lack of neutrophil recruitment. However, we cannot exclude qualitative differences in eliciting immune responses and affecting a developing immune system. Further experiments are now required to assess the functional potency of the macrophages and neutrophils that were recruited into neonatal UVR-exposed skin to promote melanocyte proliferation and immunosuppression.

MATERIALS AND METHODS

UVR treatments

Treatments were performed as previously described (Walker *et al.*, 2009). Three-day-old pups (P3) were exposed to UVB from a bank of Philips TL100W 12RS UVB lamps (Total UVB dose 5.9 kJ m^{-2}, or an erythemally weighted dose of 1.8 kJ m^{-2}). UVB dose was measured using a Solar Light (Glenside, PA) PMA2100 radiometer with either a PMA2101 detector to measure biologically weighted UVB or a PMA2106 detector to measure non-weighted UVB. Comparable doses of UVB effectively induced MM in different strains of genetically modified mice (Noonan *et al.*, 2001), probably in a dose-dependent manner (De Fabo *et al.*, 2004). Animals were euthanized at several time points and a portion of the exposed skin was excised. Experiments were undertaken with Queensland Institute of Medical Research animal ethics approval A98004M. We chose postnatal D3 for UVR exposure, as this is the developmental time of maximum epidermal melanocyte density (Walker *et al.*, 2009). Similarly, we chose D4 after exposure to count melanocytes, as this is the developmental time of maximum epidermal melanocyte density (Walker *et al.*, 2009).

Immunofluorescence staining

Ten-micrometer-thick cryosections of skin were prepared. Sections were blocked with 1% BSA and incubated with rat monoclonal anti-F4/80 antibody (ab6640, Abcam, Cambridge, UK), the rat monoclonal anti-Ly6g antibody (Becton Dickinson, Franklin Lakes, NJ), or the rat anti-mouse KI67 antibody (clone 16A8, BioLegend, San Diego, CA). After washing, sections were incubated with AlexaFluor 488–labeled donkey anti-rat (Jackson ImmunoResearch Laboratories, West Grove, PA) or 568-labeled goat anti-rabbit (Jackson ImmunoResearch Laboratories). Slides were mounted with Vector Shield (Vector Laboratories, Burlingame, CA) containing DAPI, and then viewed on a fluorescent microscope. Isotype control or secondary antibody alone were used as negative control.

Immunohistochemistry staining

Paraffin sections were dewaxed, incubated in 2% H_2O_2, and rinsed with tris-buffered saline, before they were blocked with 10% goat serum in 1% BSA. The sections were incubated overnight at 4 °C in antibody ab2557 (Rat Anti-Neutrophil mAb (NIMP-R14), Abcam) in 1:100 dilution. Secondary antibody was Rat-on-Mouse HRP-Polymer (Biocare Medical, Concord, CA). Color development was done with ImmPACT DAB (Vector Laboratories), followed by light counterstain in Mayers' hematoxylin. Isotype controls were used as negative control.

Flow cytometry

Flow cytometry was performed as previously described (Handoko *et al.*, 2013). Briefly, neonate skin (epidermis, dermis, and hypodermis) was removed, cut in small pieces, and incubated for 2 hours in 0.25% trypsin. For adult skin, an approximately 2 cm square of skin was removed, cut into small pieces, and incubated for 2 hours in 0.25% trypsin. Samples were minced through a 70-μM cell strainer to generate single-cell suspensions. All cell suspensions were layered on Histopaque 1083 (Sigma Aldrich, St Louis, MO) for gradient density centrifugation. The mononuclear cell fraction was stained for flow cytometry analysis with the following protocol: dissociated single cells were pre-blocked with anti-CD16/CD32 (Becton Dickinson) to prevent nonspecific Fc binding. Cell preparations were then incubated with Rat anti-mouse CD11b PerCp-Cy5.5, 7/4-FITC, Ly6g-PE, NK1.1-PE, and Ly6c V450 for multiparameter flow acquisition and analysis. A Gallios flow cytometer was used for sample acquisition, whereas unbiased data analyses were performed with Kaluza analysis software (Beckman Coulter, Indianapolis, IN). Isotype controls were used to fix positivity thresholds.

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

We thank Blake Ferguson for assistance with the KI67 staining. KK is supported by an NHMRC career development fellowship. GJW is the recipient of a Senior Research Fellowship from the Cancer Council of Queensland (CCQ). This work is supported by a CCQ project grant.

Author contributions

KK, GJW, HYH, and MPR designed the research study. All the authors analyzed the data and edited the manuscript. HYH, RV, and MPR performed the research, and MPR, KK, and GJW wrote the manuscript.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

REFERENCES

Adkins B, Leclerc C, Marshall-Clarke S (2004) Neonatal adaptive immunity comes of age. *Nat Rev Immunol* 4:553–64

De Fabo EC, Noonan FP, Fears T *et al.* (2004) Ultraviolet B but not ultraviolet A radiation initiates melanoma. *Cancer Res* 64:6372–6

Ferguson B, Konrad Muller H, Handoko HY *et al.* (2010) Differential roles of the pRb and Arf/p53 pathways in murine naevus and melanoma genesis. *Pigment Cell Melanoma Res* 23:771–80

Geissmann F, Jung S, Littman DR (2003) Blood monocytes consist of two principal subsets with distinct migratory properties. *Immunity* 19: 71–82

Handoko HY, Rodero MP, Boyle GM *et al.* (2013) UVB-induced melanocyte proliferation in neonatal mice driven by CCR2-independent recruitment of Ly6c(low)MHCII(hi) macrophages. *J Invest Dermatol* 133: 1803–12

McGee HM, Malley RC, Muller HK *et al.* (2011) Neonatal exposure to UVR alters skin immune system development, and suppresses immunity in adulthood. *Immunol Cell Biol* 89:767–76

Noonan FP, Recio JA, Takayama H *et al.* (2001) Neonatal sunburn and melanoma in mice. *Nature* 413:271–2

Noonan FP, Zaidi MR, Wolnicka-Glubisz A *et al.* (2012) Melanoma induction by ultraviolet A but not ultraviolet B radiation requires melanin pigment. *Nat Commun* 3:884

Rodero MP, Hodgson SS, Hollier B *et al.* (2013) Reduced Il17a expression distinguishes a Ly6c(lo)MHCII(hi) macrophage population promoting wound healing. *J Invest Dermatol* 133:783–92

Soehnlein O, Lindbom L, Weber C (2009) Mechanisms underlying neutrophil-mediated monocyte recruitment. *Blood* 114:4613–23

Strickland I, Rhodes LE, Flanagan BF *et al.* (1997) TNF-alpha and IL-8 are upregulated in the epidermis of normal human skin after UVB exposure: correlation with neutrophil accumulation and E-selectin expression. *J Invest Dermatol* 108:763–8

Tadokoro T, Kobayashi N, Zmudzka BZ *et al.* (2003) UV-induced DNA damage and melanin content in human skin differing in racial/ethnic origin. *FASEB J* 17:1177–9

Teunissen MB, Piskin G, di Nuzzo S *et al.* (2002) Ultraviolet B radiation induces a transient appearance of IL-4+ neutrophils, which support the development of Th2 responses. *J Immunol* 168:3732–9

Walker GJ, Kimlin MG, Hacker E *et al.* (2009) Murine neonatal melanocytes exhibit a heightened proliferative response to ultraviolet radiation and migrate to the epidermal basal layer. *J Invest Dermatol* 129:184–93

Whiteman DC, Whiteman CA, Green AC (2001) Childhood sun exposure as a risk factor for melanoma: a systematic review of epidemiologic studies. *Cancer Causes Control* 12:69–82

Williams MR, Azcutia V, Newton G *et al.* (2011) Emerging mechanisms of neutrophil recruitment across endothelium. *Trends Immunol* 32: 461–9

Wolnicka-Glubisz A, Damsker J, Constant S *et al.* (2007) Deficient inflammatory response to UV radiation in neonatal mice. *J Leukoc Biol* 81: 1352–61

Zaidi MR, Davis S, Noonan FP *et al.* (2011) Interferon-gamma links ultraviolet radiation to melanomagenesis in mice. *Nature* 469:548–53