

# The protective effect of sunscreens against ultraviolet B-induced immunosuppression. A study on Langerhans cell depletion

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## ABSTRACT

Ultraviolet B (UVB) radiation can act as immunosuppressant by inducing an epidermal Langerhans cells (LC) depletion which could be inhibited by topical sunscreen. Several kinds of sunscreens with various SPF (Sunscreen Protection Factor) are now available. The minimal SPF which able to inhibit the immunosuppressive effect of UVB among people with skin photo-type IV has not been established yet. The aim of this study is to determine the minimal SPF capable to inhibit UVB-induced immunosuppression among people with skin photo-type IV. A simple experimental (post test only experimental) study was conducted among 5 people's circumsized foreskins with Fitzpatrick's skin photo-type IV. Each of them was divided into equal 5 pieces of 0.5 cm<sup>2</sup>. Each of three pieces of skin was treated by sunscreen SPF 15, SPF 30, and SPF 50, a single piece of skin was treated with placebo, and all of them then were treated by a single 100 mJ/cm<sup>2</sup> of UVB 30 minutes later. A rest single piece of skin was used as control. After 24 hours of incubation in incubator of 37<sup>o</sup> C and 5% CO<sub>2</sub>, all of them then was fixed by buffer formalin, blocked by paraffin, cut in 2 mm of thickness, and then stained with anti CD 1<sup>a</sup> antibody with AEC as chromogen and Mayer's hematoxylin as counterstaining. The number of LC was counted by *Image J Analysis* programmed and the mean of LC were analyzed by Kruskal-Wallis test dan Mann-Whitney test. There were very significantly different of the mean number of LC between UVB placebo group and control group ( $p < 0.01$ ). Compared to the control group, mean number of LC among SPF 30 and SPF 50 treated groups were not significantly different ( $p > 0.05$ ). Sunscreen with SPF 15 had LC number lower than control group significantly ( $p < 0.05$ ). The lowest SPF for preventing UVB induced LC depletion among people with skin photo-type IV was 30.

**Key words** : UVB - immunosuppression – sunscreens – SPF - CD1<sup>a</sup>expression

## INTRODUCTION

The suppressive effect of ultraviolet B (UVB) radiation on the human immune system has been revealed over the last four decades. Kripke and Morison<sup>1</sup> stated that the suspicion against the UVB immunosuppression effect began since Hanisko and Suskind (1963) reported the fact of less severity contact allergic reaction among guinea pigs exposed to UVB compared with control. Toews *et al*<sup>2</sup>. reported an inhibition of contact hypersensitivity reaction among skin C57BL mice exposed to UVB. In addition similar reaction was also occurred among human skin exposed to UVB.<sup>3</sup> The supporting data can be observed in the reactivation and replication of herpes simplex virus (HSV) due to UVB

exposure.<sup>4</sup> The main point of UVB radiation is on the induction of LC migration from epidermis.<sup>5,6</sup> and can reduced the ability of LC to recognize antigens and present them to T cell.<sup>7</sup>

It is known that sunscreen is not only capable to protect human skin against sunburn but also it can prevent the formation of actinic keratoses<sup>8,9</sup> and possibly squamous cell carcinoma.<sup>9</sup> Sunscreen protective effect against the Langerhans cell (LC) depletion has been reported by several researchers. Research by Horchberg and Enk<sup>10</sup>, Israel *et al*<sup>11</sup>. in Austria showed that sunscreen with the low-moderate SPF categories (SPF 2-16) can inhibit depletion of LC partially on healthy adult skin phototype II and III.

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Differs with those researches, Indonesian people are dominated by Fitzpatrick's skin photo-type IV. The minimum SPF required to inhibit the immunosuppressive effect of UVB on skin photo-type IV has not been recommended yet. The aim of this study was to determine the minimum SPF that can provide immunoprotection for Indonesian people who are dominated by photo-type IV skin based on sunscreen's ability in inhibiting UVB-induced LC depletion.

## MATERIALS AND METHODS

A simple experimental study was performed among 5 foreskins collected from circumcision. Each foreskin was cut into 5 pieces of 0.5 cm<sup>2</sup> and they were divided randomly to be (UVB + placebo) group, (UVB + sunscreens SPF 15, 30, 50) group, and control (Non UVB) group. Sunscreens (4 mg/cm<sup>2</sup>) were applied on the epidermal side of skin. After 30 minutes, UVB radiation was performed at 100-mJs/cm<sup>2</sup>. The skin explant was cultured in RPMI complete medium with air interface of epidermal sides in 0.5 uM filter surrounded by metal rings (FIGURE 1).



FIGURE 1. Skin organ culture

Tissue fixation, paraffin blocking, and cutting were performed 24 hours later. Histological images were captured digitally by using 8 mega pixels camera connected to ocular lens of 400 magnification light microscope (Olympus) in quadruplets for each histological slide.

Measurement of CD1<sup>a</sup> number was based on the cell counter of the program ImageJ analysis. Data which were unnormal distributed were analyzed using Kruskal-Wallis with post hoc test by Mann Whitney.

The study has been approved by the Health Research Ethics Committee of the Faculty of Medicine, Gadjah Mada University, Yogyakarta.

## RESULTS

The mean number of LCs of placebo (UVB) group compared to control (Non UVB) group ( $0.92 \pm 0.88$  vs  $4.12 \pm 2.64$ ;  $p < 0.001$ ) was very significantly different as shown on FIGURE 2. The mean number of LCs in placebo (UVB) group ( $0.92 \pm 0.88$ ) compared to sunscreen (SPF 15, SPF 30, and SPF 50) treated groups ( $2.24 \pm 1.25$ ,  $2.48 \pm 1.86$ , and  $2.80 \pm 2.00$  respectively) was very significantly different as shown on FIGURE 3. FIGURE 4 showed, compared to the control group, mean number of LCs among SPF 30 ( $24.8 \pm 1.86$ ) and SPF 50 treated groups ( $2.80 \pm 2.00$ ) were not significantly different ( $p > 0.05$ ). Sunscreen with SPF 15 group had significantly lower number of LC than control group ( $2.24 \pm 1.25$  vs  $4.12 \pm 2.84$ , with  $p < 0.05$ ).

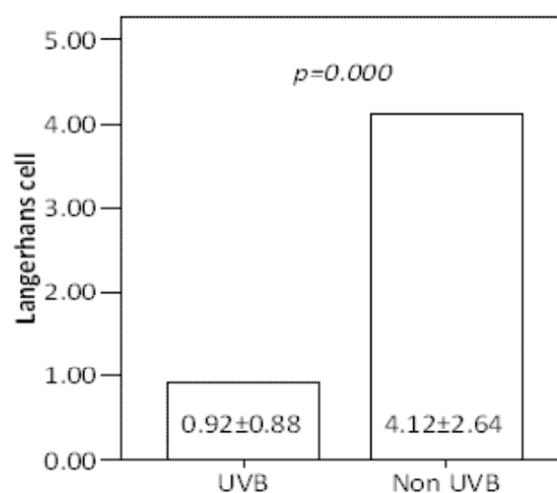


FIGURE 2. The figure showed depletion of Langerhans cell induced by UVB. The mean number of Langerhans cells of placebo (UVB) group was very significantly different compared to control (Non UVB) group ( $p < 0.001$ )

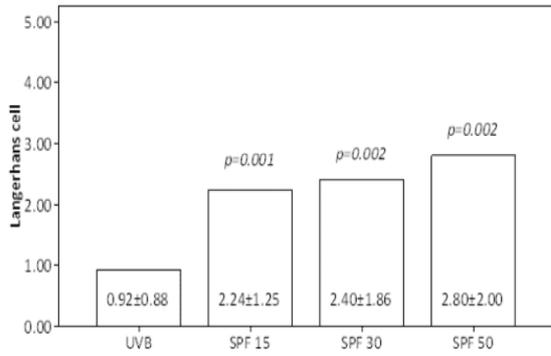


FIGURE 3. The figure shows the protection against depletion of Langerhans cell induced by UVB offered by sunscreens. The mean number of Langerhans cell of placebo (UVB) group was significantly different compared to sunscreen treated group ( $p < 0,05$ )

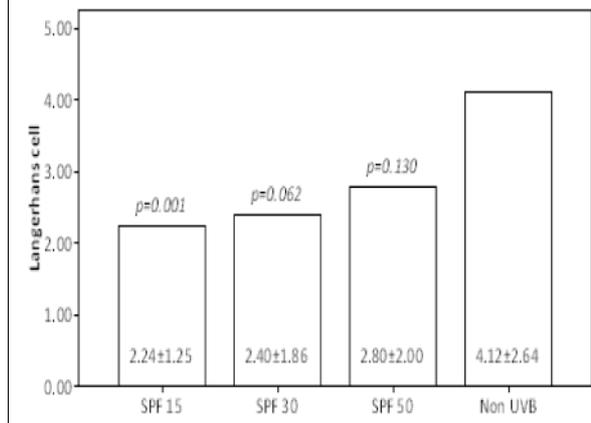


FIGURE 4. The figure showed that sunscreen with SPF 15 had Langerhans cell number lower than control group significantly ( $p < 0,05$ ). Compared to the control group, the mean number of Langerhans cell among SPF 30 and SPF 50 treated groups were not significantly differ ( $p > 0,05$ ).

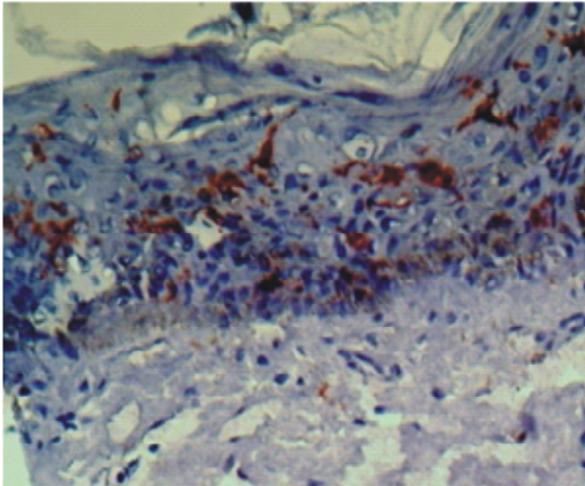


FIGURE 5a. Non UVB (control)

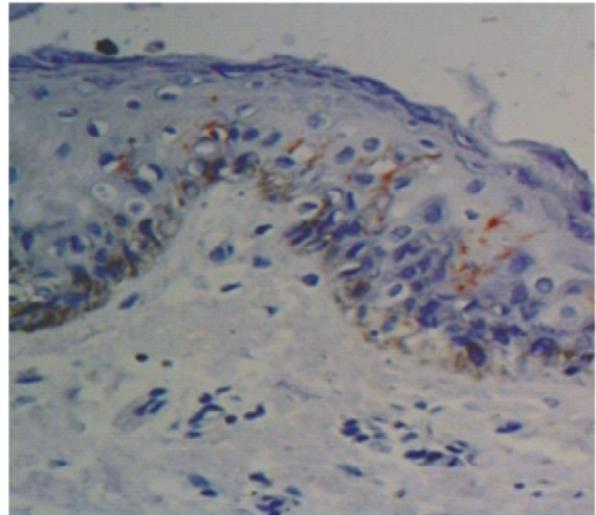


FIGURE 5b. UVB exposure without sunscreen

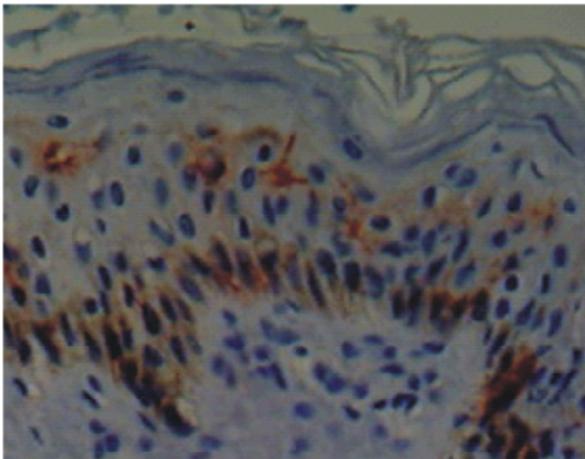


FIGURE 5c. UVB exposure with sunscreen SPF 15

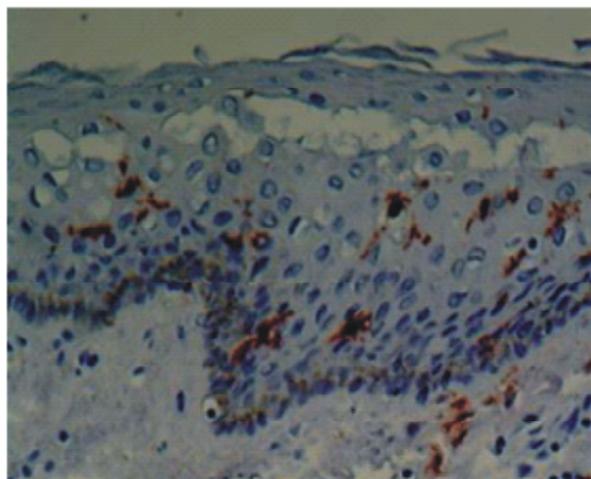


FIGURE 5d. UVB exposure with sunscreen SPF 30

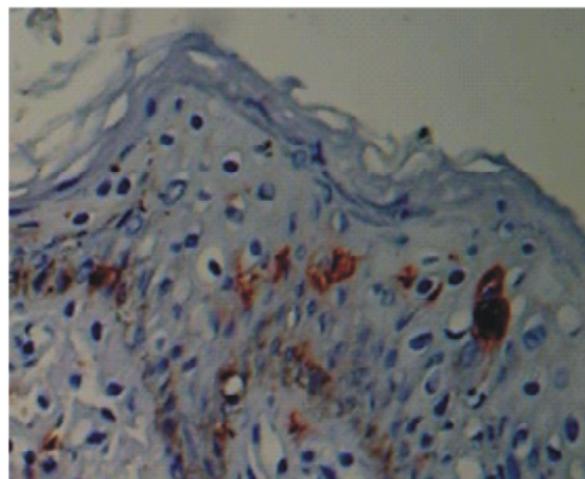


FIGURE 5e. UVB exposure with sunscreen SPF 50

FIGURE 5. Results of epidermal staining of Langerhans cells

## DISCUSSION

Based on FIGURE 2, the mean number of LCs in the placebo (UVB) group was very significantly different ( $p < 0.001$ ) compared to control (Non-UVB) group, indicating that the wavelength and amount of UVB energy used in this study induced the depletion of LC. This finding was in accordance with several studies that reported that UVB causes depletion<sup>3,5</sup> and morphologic changes of LC.<sup>5</sup> The depletion of LC number was due to the LC migration that induced by UVB.<sup>6</sup> Miyagi *et al*<sup>12</sup>. reported that exposure to UVB of 100 mJ/cm<sup>2</sup> dose can induce migration of LC. The migration occurred after 24 hours of exposure to UVB.<sup>13,14</sup> In this study, the skin was exposed to 100 mJ/cm<sup>2</sup> dose of UVB and incubated for 24 hours. Ghaznawie<sup>15</sup> stated that depletion of LC and changes in LC morphology would affect LC function and eventually influence the cutaneous immune response.

FIGURE 3 showed a significant difference in the mean number of LC of placebo (UVB) group compared to sunscreen treated group. It was in accordance with other researches which revealed the protection against depletion of Langerhans cell induced by UVB was offered by sunscreens, both in animal<sup>16,17</sup> and human.<sup>4,18</sup> Elmetts *et al*<sup>19</sup>. stated that both the cinnamate and benzophenone sunscreen combination and an extract of baker's

yeast present in the preparation had photoprotective properties. Pretreatment of skin with one such cosmetic product provided complete protection against UV-induced erythema, sunburn cell formation and Langerhans cell damage in volunteers, skin photo-types II and III.<sup>19</sup> Ho *et al*<sup>20</sup>. showed the ability of *sunscreens* contains octyl dimethyl para-aminobenzoate (Padimate O), 2-ethylhexyl-p-methoxycinnamate (2-EHMC), and benzophenone could protect LC, inhibited UV light from depleting LC from the epidermis of mouse. Walker *et al*<sup>25</sup>. reported that the UVB sunscreen 2-ethylhexyl-4-methoxycinnamate had ability to inhibit LC depletion in hairless albino mouse.

FIGURE 4 showed that sunscreen with SPF 15 had significantly lower number of LC than control group ( $p < 0.05$ ). Compared to the control group, the mean number of LC among SPF 30 and SPF 50 treated groups were not significantly different ( $p > 0.05$ ). Several studies mentioned that sunscreens with SPF  $\geq 15$  could prevent squamous neoplasia.<sup>8,9,22</sup> Edward *et al*<sup>23</sup>. compared the number of LC on UVB exposed skin, non-UVB exposed, and skin treated with SPF 24.5 sunscreen (active ingredient of octyl dimethyl PABA and oxybenzone). The study used anti-Leu 6 monoclonal antibody staining. Based on that study, it was concluded that SPF 24.5 sunscreen had immunoprotective effect.

In this study we used skin organ culture model. Organ culture is a technique whereby small, undisaggregated tissue are cultured at an air-liquid interphase, so that they can retain a three dimensional structure and some or all of the histological and functional feature of the tissue *in vivo*. This technique has provided many advantages including superior control of the physicochemical environment and physiological conditions. Furthermore, they provide more ethical and cheaper alternative to *in vivo* studies.<sup>24</sup> Unfortunately, organ culture can bring with them a number of problems which can severely limit their usefulness such as reproducibility between cultures can be poor, especially if more than one donor was used. To solve the problem, it is necessary to perform further study to investigate the immunoprotection effect of the same sunscreens against UVB induced LC depletion *in vivo* model with a larger sample size.

## CONCLUSION

This study showed that the lowest SPF for preventing UVB induced LC depletion in skin organ culture of Indonesian people with skin photo-type IV is 30 in *in vitro* model.

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