Increased Susceptibility of Mice to Malarial Infection Following UV-B Irradiation

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Using a mouse model, we examined whether UV-B was a risk factor for malarial infection. Two mouse strains, susceptible (BALB/c) and resistant (C57BL/10) to murine malaria (Plasmodium chabaudi), were UV-preirradiated and infected with a sub-lethal dose of malaria parasite (104 and 105, respectively). Parasite growth was assayed with tail-blood smears counting parasitized red blood cells. Mice resistant to malaria were bled by heart puncture and the plasma cytokines were determined.

Our results showed that UV-B irradiation worsened the malarial infection and 100% of the malaria-resistant mice strains died due to a usual infection at sub-lethal dose following UV-B irradiation. In the resistant mice strain infected with the parasite, the plasma IFN-γ production was inhibited by UV-B irradiation and the maximum titer was about one-fifth of the non-irradiated mice. Furthermore, activation of macrophages from UV-irradiated mice also decreased compared with that of non-irradiated mice. IFN-γ administration prevented the death of UV-B irradiated resistant mice and the cure ratio was 60%.

In conclusion, UV-B increased the susceptibility of both strains of mice and impaired IFN-γ production in the malaria-resistant mice strain. J Epidemiol, 1999 ; 9 : S93-S96.

UV-B, murine malaria, susceptibility, IFN-γ

INTRODUCTION

There have been many reports that UV exposure affects the immune systems, and thus immunological modulation by UV irradiation may influence the course of some infectious diseases. UV-B exposure is known to affect infectious diseases involving many kinds of microorganisms including bacteria, viruses, protozoa and fungi such as yeast 1. Regarding the effects of UV exposure on human health, the United Nations Environmental Programme, UNEP, consider the effects of UV on infectious diseases as important as skin cancer. In particular, in the UNEP report, they warn that malaria is an important disease in terms of increasing risk of infection due to ozone depletion and subsequent increased UV-B radiation on earth. However, there is as yet no epidemiological or experimental evidence indicating that UV-B exposure enhances malarial infection 2.

In this study, we investigated whether UV-B is a risk factor for malarial infection using a mouse model 3.

MATERIALS AND METHODS

Female BALB/c and C57BL/10 mice were used at the age of 7-10 weeks. One day before UV irradiation, mouse dorsal hair was removed with clippers and remaining hair was cleaned off with a depilatory cream. The next day, mice were irradiated at a dose of 200 mJ/cm2 using a UV-B lamp. After several hours, mice were challenged intraperitoneally with mouse malaria (Plasmodium chabaudi) at an infectivity of 10⁶ PRBC (parasitized red blood cells) which represents the infectivity of the parasite (abbreviated simply as parasite titer or parasites).
After infection with malaria, selected mice were bled from
the heart or suborbital sinus on the desired days and IFN-γ,
interleukin-10 and parasite titers were assayed in these sam-
ples. Furthermore, experiments with exogenous administration
of IFN-γ and FACScan analysis were performed. The
remaining mice were observed for survival or death.

RESULTS AND DISCUSSION

Effects of UV-B irradiation on the susceptibility of mice

BALB/c mice are susceptible to mouse malaria at a dose of
10⁴ PRBC, while C57BL/10 mice are resistant.

Figure 1 shows typical survival patterns of these two mouse
strains infected with malaria. When BALB/c mice were infect-
ed with a sublethal dose of 10⁴ parasites, as shown in Fig.1A, 4
of 5 mice survived. However, when infection followed UV-B
preirradiation, all mice died within 10 days.

On the other hand, C57BL/10 mice are resistant to malaria.
Therefore, all mice survived even when infected with 10⁵ para-
sites (Fig. 1B). Interestingly, when these mice were UV-B
preirradiated, all mice died within 11 days postinfection. These
results indicated that UV-B exposure enhanced the susceptibil-
ity of both mouse strains.

Parasite growth

Parasite growth in mouse red blood cells was determined as
the percentage of parasite-positive red blood cells to total red
cells per microscopic field.

Until the middle stage at 4 to 6 days postinfection, no differ-
ences were seen between the two groups of UV-irradiated and
unirradiated mice of both strains. Parasite growth peaked at 7
days postinfection in both groups. At later stages (7 to10 days
postinfection) UV-B-irradiated mice tended to have slightly
higher parasite titers than the unirradiated mice of both strains
(data not shown).

Correlation between IFN-γ level and subsequent malaria
growth

In this experiment, individual mice were bled from the sub-
orbital sinus at 5 days after infection for determination of IFN-
γ level and parasite titers were determined 7 days postinfec-
tion. The IFN-γ mean concentration of the unirradiated mice
was 134 pg/ml, while that of irradiated mice was 55 pg/ml
(data not shown). The difference was statistically significant.
The same mice were assayed for parasite growth. The mean
parasite titer of 7 unirradiated mice was 36.5%. On the other
hand, the mean titer of the 3 irradiated mice reached 63.1%. This
result again confirmed that UV-B irradiation suppressed
IFN-γ production and that low IFN-γ level was correlated
with higher parasite growth in irradiated mice.

Protection of UV-B-irradiated mice by exogenous administra-
tion of IFN-γ

In this experiment, UV-B-irradiated mice were divided into
two groups. One group was injected with 400 ng of recombi-
nant IFN-γ every day for 5 days from 3 to 7 days postinfection,
while controls were injected with diluent medium alone
according to a similar protocol.

Four of 5 irradiated control mice died within 12 days postin-
fecction. One mouse survived the infection resulting in a sur-

Figure 1. Effects of UV-B irradiation on parasite susceptibility of two mouse strains. BALB/c (A) and C57BL/10 (B) mice were infected
with 10⁴ and 10⁵ PRBC. Symbols: ○, Unirradiated. ●, UV-B-irradiated.
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Figure 2. Effects of UV-B irradiation on IFN-γ levels in C57BL/10 mice. Plasma IFN-γ levels of 3 mice per day were assayed with a commercial ELISA kit (R & D systems Inc., Minneapolis, USA), and the mean values of the 3 mice were plotted. Symbols: ○, Unirradiated; ●, UV-B-irradiated.

...vival ratio of 20%. On the other hand, only one of 5 mice injected with IFN-γ died corresponding to an 80% survival rate. IFN-γ administration prevented the death of UV-B-irradiated mice and the cure ratio was 60%. This result confirmed that induction of susceptibility of resistant mice by UV irradiation involved low IFN-γ production (data not shown).

Effects of UV-B irradiation on macrophage activation

A main target of IFN-γ is macrophages. Therefore, we examined whether the reduced IFN-γ levels after UV-B irradiation affected macrophage activation.

In this study, splenic macrophages at 7 days postinfection were analyzed by FACScan. Spleen cells were double-stained for macrophage-specific (Mac-1) antigen and activation-marker (I-Ab) antigen with fluorescent isothiocyanate (FITC) and phycoerythrine (PE)-labeled specific antibodies, respectively.

The peak of macrophage activation in unirradiated mouse was shifted to the left in irradiated mouse, demonstrating low activation. Furthermore, low activation was also shown by a gated cell number representing the numbers of more activated macrophages. Unirradiated mouse-cell number was 2,563 cells (relative activation, 57%), while that of irradiated mouse was 1,605 cells (relative activation, 34%).

Based on this result, it was concluded that UV-B irradiation resulted in decreased activation of macrophages in comparison with unirradiated mice.

Is interleukin-10 (IL-10) released following UV-B irradiation?

The decreased IFN-γ production after UV-B irradiation suggested the release of an inhibitory cytokine following UV irradiation. IL-10 which is an inhibitory cytokine was assayed. Plasma IL-10 levels of UV-irradiated mice were lower than those of unirradiated mice until 6 days postinfection but they increased markedly on day 7 or 8 (data not shown). The appearance of IL-10 occurred later than that of IFN-γ and did not precede the appearance of IFN-γ. IL-10 levels increased in response to mouse malaria infection and increased further even after IFN-γ titer had decreased.

This experiment indicated that IL-10 levels were related to malarial infection but were not involved in suppression of IFN-γ production in UV-B-irradiated mice. These results suggested that UV-B irradiation of mice induces another inhibitory mediator other than IL-10. It is interesting to speculate as to whether production of an unknown mediator is induced by UV-B exposure.

In summary, the following points should take into consideration:

1. After UV-B preirradiation, BALB/c mice showed increased susceptibility to mouse malaria and died at sublethal doses of the parasite by day 10. C57BL/10 mice, which are naturally resistant to the parasite, showed decreased resistance after UV-B irradiation and died by day 11.
2. Parasitemia (growth of the parasite) in the late stage of infection was slightly higher in UV-B-irradiated than unirradiated mice.
3. Plasma IFN-γ levels in C57BL/10 mice were decreased by about 80% after UV-B irradiation.
4. The increased susceptibility and ultimate death of C57BL/10 mice after UV-B irradiation was prevented by exogenous administration of recombinant IFN-γ.

Thus the decreased IFN-γ production after UV-B irradiation was shown to be involved in the loss of resistance.

5. Suppression of IFN-γ production by UV-B irradiation resulted in a lower rate of activation of macrophages as compared to unirradiated controls.

6. IL-10 which is an inhibitory cytokine was not involved in the suppression of IFN-γ production after UV-B irradiation.

IFN-γ is a cytokine secreted by T helper 1 (Th 1) lymphocytes. Therefore, it is supposed that in C57BL/10 mice infected with mouse malaria, Th 1 dependent immune
response would be induced. The observation that IFN-γ levels in malaria-infected C57BL/10 mice were decreased by UV-B irradiation suggested that UV-B irradiation down-regulates Th1 cell-dependent immune responses.

This mouse model may be useful for assessment of the risk of increased UV-B irradiation due to ozone depletion in human infectious diseases.

REFERENCES


