Ultraviolet Light and Malaria
Final Report to IDRC

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1. Restatement of Rationale

Abundant evidence in animals and more limited data in humans suggest that ultraviolet (UV) radiation can act as an immunomodulator. Due to stratospheric ozone depletion, incident UV radiation is increasing throughout the world. Even if all of the international agreements negociated to date are respected, it can be anticipated that UV radiation will continue to increase until 2020 at least. Incident UV light is several orders of magnitude greater in tropical regions than in temperate areas. Several animal models have been developed to explore the possible influence of UV exposure on the expression and outcome of infectious diseases. A chance clinical observation by Dr Salem Rashed in Benin raised the possibility that the incidence or severity of malaria might be increased by UV exposure. We undertook a series of experiments to explore this hypothesis in a mouse model of malaria.

2. Specific Objectives

To determine if exposure to UV-light influences the expression of malaria in mice

To establish the optimal dose and timing of UV exposure

To study the clinical impact/immunopathogenesis of UV-influenced malaria

To evaluate the impact of photoprotective agents in UV-influenced malaria

To examine the impact of UV exposure on vaccination with malaria antigens

3.1 Development of Model

3.1 Background

3.1..1 UV-induced immunosuppression

Susceptibility to UV-induced immunosuppression is a genetic characteristic. The 'suppressible' phenotype is present in approximately 40-50% of humans and is independent of skin pigmentation. The effect of UV exposure is cummulative and some time is required for the development of the full effect (24-48 hours). Attention has focused primarily on UVB but UVC radiation is highly bioactive and UVA has also been reported to have immunologic effects. UV-induced immunologic changes can be short-lived (days to weeks) but tolerance induction has been demonstrated in animals and in man. Mouse strains which are susceptible (B57) and resisistant (AJ) to the immunomodulatory effects of UV radiation have been identified.

3.1.2 Background information regarding the mouse malaria model

Dr Mary Stevenson works with a well-characterized mouse model of malaria. Similar to the situation with UV exposure, suscepibility or resitance to malaria is genetically determined and mouse strains with these phenotypes have been identified. For example, B57 mice are resistant to infection with Plasmodium chabaudii while AJ mice are susceptible. Infection of adult B57 mice typically

results in a monophasic illness lasting approximately 15-17 days. Parasitemia generally peaks at 45-50% between 11 and 13 days after intraperitoneal injection of 10^5 viable parasites. AJ mice infected in a similar fashion fail to control the parasitemia and die between 11 and 15 days after infection.

3.2 Techniques

In preliminary experiments we developed a number of techniques which were used in all subsequent work. Mice were initially anesthetized with CO_2 for shaving of the dorsal skin. We subsequently purchased a miniature animal clipper which allowed us to remove the hair from a 2 square centimeter area on the animals' backs without anesthesia. The mice tolerated the clipping well. A plexiglass box was built to allow up to 16 animals to be exposed to UV radiation at one time through a spacious wire mesh grill on the top of the cage. Initial experiments were performed using the clinical phototherapy unit in the Department of Dermatology (FS40 lamps without filters). Subsequently, two irradiation boxes were constructed to free the experimental schedule from the clinical schedule and to allow more convenient handling of the animals. One box allowed us to irradiate animals with predominantly UVA (plus small amounts of long wavelength UVB - see below) and the second box contained the same FS 40 lamps as the clinical unit.

3.3 Preliminary Studies

Our initial experiments using modest doses of UVB demonstrated that single or repeated UV exposures prior to infection with 10^5 organisms resulted in altered kinetics of the parasitemia (more rapid appearance of parasites, peak parasitemia and clearance) and a second wave of parasitemia after initial clearance (see Figures I and 2). In light of these observations, we felt that our initial efforts should be directed towards developing a simpler model with more definitive measureable end-points (eg: death, increased parasitemia).

3.4 Virulence Factors

Several factors were thought to influence the virulence of P chaubaudii in the B57 model. These included age at infection (more virulent in younger animals), sex (more virulent in males), parasite inoculum and passage history of the infecting parasite (passage in susceptible AJ mice increases virulence). A series of experiments was performed to evaluate these variables in a model of malaria after UV exposure. We used male mice in all of the experiments descibed below.

3.5 Control Animals

In all experiments, control animals were handled in an identical fashion to experimental animals except for the UV irradiation (eg: clipping, handling and injections, placement in exposure box, mock irradiation).

Figure 1

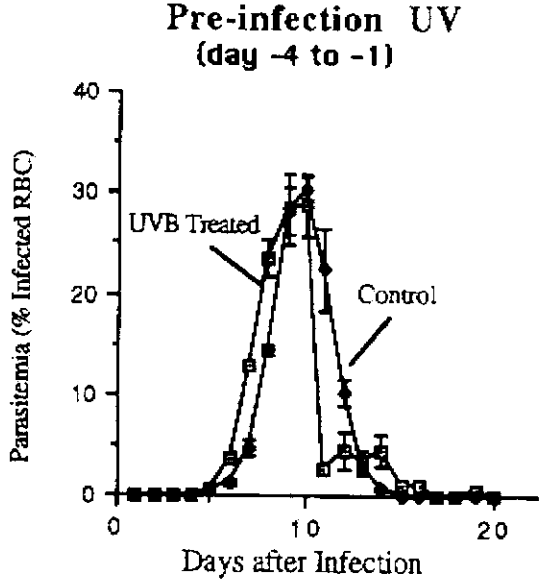
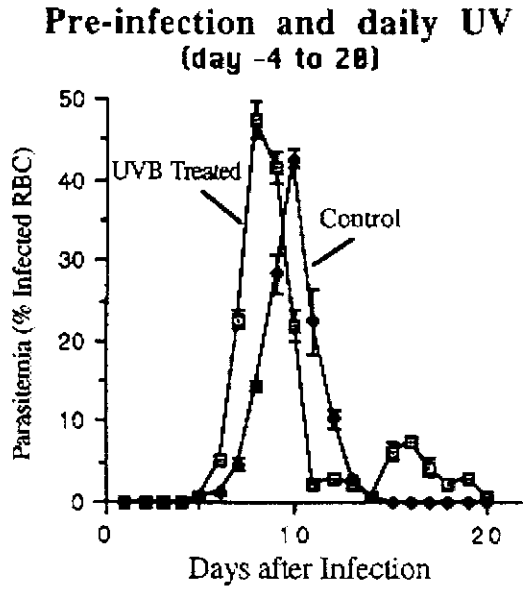


Figure 2



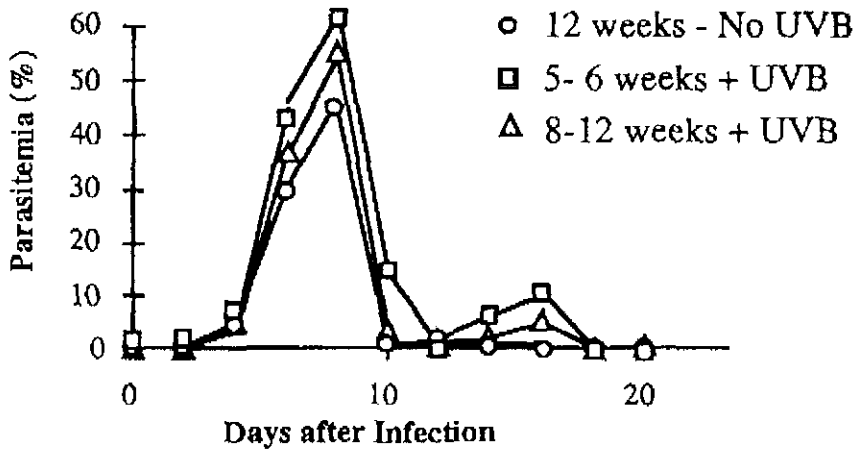
3.6 Experiments

3.6.1 Age of mice

We investigated the impact of UVB exposure in mice at various ages in an attempt to increase either the lethality of the infection or the peak parasitemia.

Group	N	Age	UV Dose	Inoculum
1	5	5 weeks	1.5 kJ/m^2 in 4 doses	10^5
2	5	6 weeks	1.5 kJ/m^2 in 4 doses	10^5
3	5	8 weeks	1.5 kJ/m^2 in 4 doses	10^5
4	4	12 weeks	1.5 kJ/m^2 in 4 doses	10^5
5	5	6 weeks	control	10^5
6	4	12 weeks	control	10^5

Impact of Age on Parasitemia



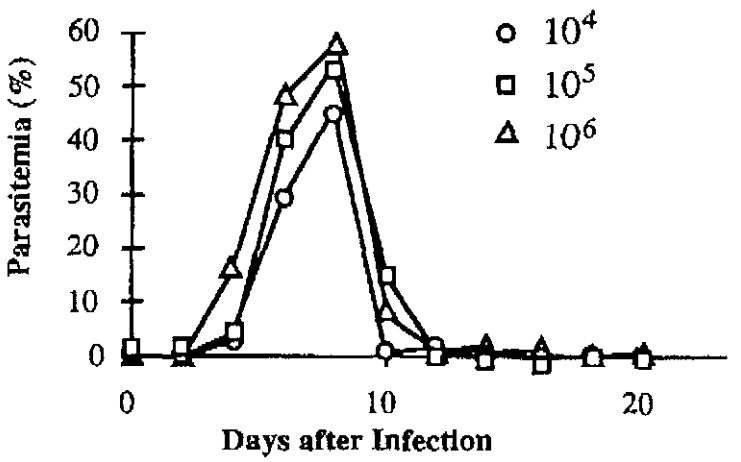
Comments - There was a small but definite effect of age on both parasitemia (5-6 weeks vs 8-12 weeks; $p<.04$) and mortality (Fisher Exact test; $p = .08$). We chose to use male mice at 6 weeks of age in all subsequent experiments.

3.6.2 Dose of parasite

We investigated the impact of parasite inoculum in 6 week old mice in an attempt to increase either the lethality of the infection or the peak parasitemia.

Group	N	Age	Inoculum
1	4	6 weeks	10^4
2	8	6 weeks	10^5
3	8	6 weeks	10^6
4	6	6 weeks	10^7

Impact of Inoculum on Parasitemia



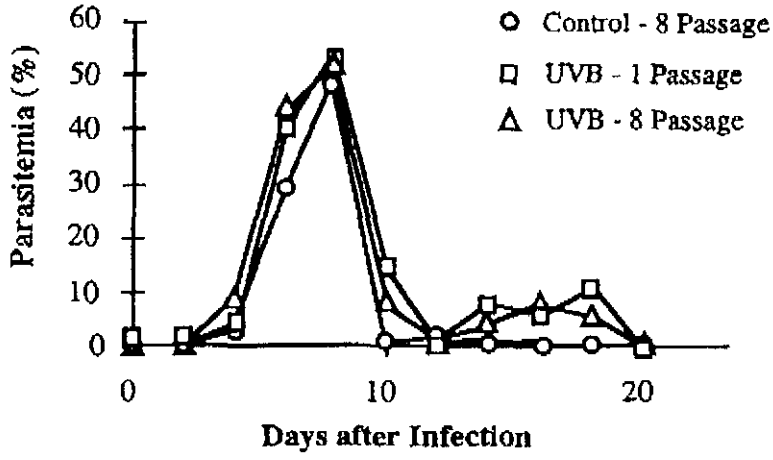
Comments - There was a subtle effect of inoculum on the kinetics and height of the parasitemia as well as a trend towards increased mortality. None of the animals receiveing the two lower doses died while 1/8 (12.5%) of the 10^6 group and 3/6 (50%) of the mice recieving 10^7 organisms died. Since we wanted a model which would allow us to detect small changes in parasitemia and survival, we chose to use an inoculum of $5x10^6$ organisms in all subsequent experiments.

3.6.3 Virulence of parasite

We investigated the impact of increasing parasite passage in AJ (susceptible) mice in an attempt to increase the lethality of the infection in 6 week old B57 mice. All animals in this experiment received 1.5 kJ/m^2 in 4 divided doses (as above).

Group	N	Age	Inoculum	Passage History
1	6	6 weeks	10^6	1 passage in AJ
2	6	6 weeks	10^6	4 passages in AJ
3	6	6 weeks	10^6	8 passages in AJ
4 (control)	5	6 weeks	10^6	1 passage in AJ
5 (control)	4	6 weeks	10^6	4 passages in AJ
6 (control)	4	6 weeks	10^6	8 passages in AJ

Impact of Passage on Parasitemia



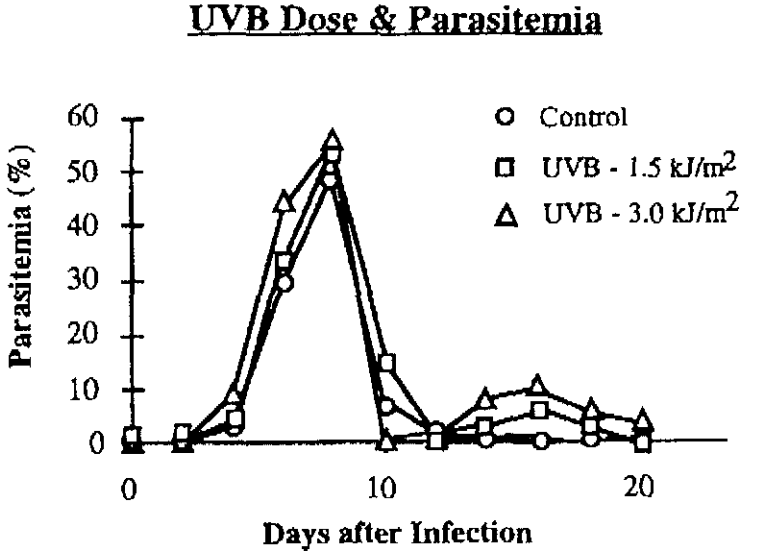
Comments - Increasing number of passages in AJ mice had relatively little impact on parasitemia or mortality in the model. This is likely due to the fact that we had already made several manipulations to increase the virulence of the organism. We therefore used organisms which had been passaged 1-4 times in AJ mice in subsequent experiments.

3.6.4 Dose and pattern of ultraviolet exposure

Several experiments were performed to explore the role of UV dose in the model. In these experiments, we had to balance increasing the dose (for presumed increased biologic effect) and the risk of burning the animals' skin. We wished to avoid UV burns not only to prevent pain and suffering but also to prevent possible confounding by non-specific inflammation.

3.6.4.1 Increasing doses of UVB exposure

Group	N	Age	UV Dose (Total)	Pattern of UV
1	4	6 weeks	1.5 kJ/m^2	4 doses pre-infection
2	4	6 weeks	3.0 kJ/m^2	4 doses pre-infection
3	5	6 weeks	6.0 kJ/m^2	4 doses pre-infection
4	4	6 weeks	12 kJ/m^2	4 doses pre-infection
5	4	6 weeks	control	4 doses pre-infection
6	4	6 weeks	control	4 doses pre-infection
7	4	6 weeks	control	4 doses pre-infection
8	4	6 weeks	control	4 doses pre-infection

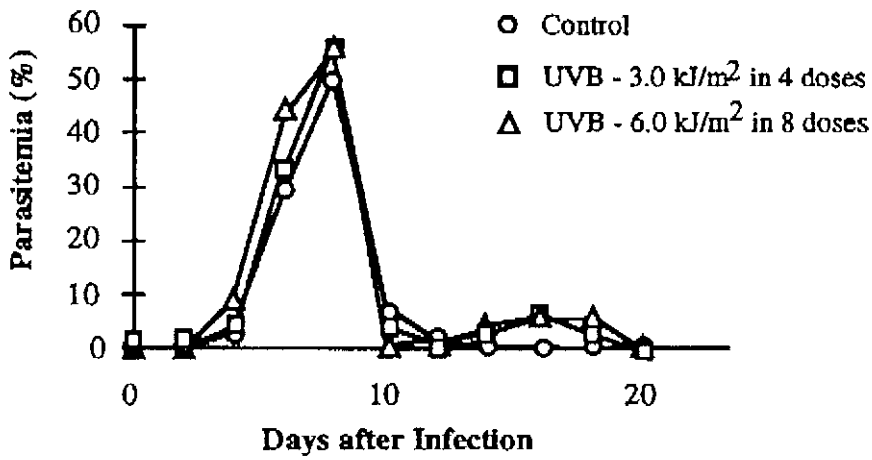


Comment - 3.0 kJ/m^2 (total) in 4 daily doses was found to be the maximum dose tolerated by the mice without significant burning. Higher doses resulted in erythema (6.0 kJ/m^2) and frank blistering (12 kJ/m^2). Animals with UV burns were killed by CO_2. Although burning and blistering did not occur at a dose of 3.0 kJ/m^2, pigmentation was pronounced in many of these animals in responses to UV exposure.

3.6.4.2 Increasing dose of UVB by extending period of exposure

Group	N	UV Dose (Total)	Pattern of UV
1	6	3.0 kJ/m^2	4 doses pre-infection
2	5	3.0 kJ/m^2	8 doses (4 at 1 week before and 4 immediately prior to infection
3	6	6.0 kJ/m^2	8 doses (4 at 1 week before and 4 immediately prior to infection
4 (control)	4	control	4 doses pre-infection
5 (control)	5	control	8 doses (4 at 1 week before and 4 immediately prior to infection
6 (control)	4	control	8 doses (4 at 1 week before and 4 immediately prior to infection

Pattern of UVB Exposure & Parasitemia

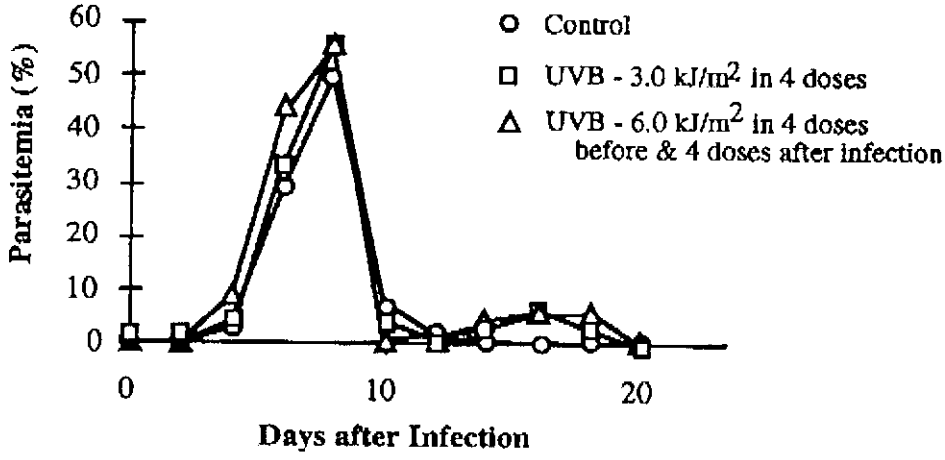


Comment - Increasing the total UV exposure prior to infection by splitting the dose over a period of a week had little additional impact on parasitemia and no effect on mortality.

3.6.4.3 Increasing dose of UVB by irradiating before and after infection

Group	N	UV Dose (Total)	Pattern of UV
1	6	3.0 kJ/m^2	4 doses pre-infection
2	6	3.0 kJ/m^2	8 doses (4 before infection and 4 immediately following infection
3	5	6.0 kJ/m^2	8 doses (4 before infection and 4 immediately following infection
4 (control)	5	control	4 doses pre-infection
5 (control)	5	control	8 doses (4 before infection and 4 immediately following infection
6 (control)	5	control	8 doses (4 before infection and 4 immediately following infection

Pattern of UVB Exposure & Parasitemia - II



Comments - Increasing the total UV exposure by splitting the dose and extending exposure into the period of infection had no impact on parasitemia or mortality.

3.6.5 Final model

The maximum impact of UV exposure in this murine malaria model was therefore observed after exposing 6 week-old, male B57 mice to a total dose of 3.0 kJ/m^2 over the 4 days immediately prior to intraperitoneal infection with $5x10^6$ organisms.

This series of experiments was sobering and somewhat discouraging. While we were able to confirm our preliminary data and make modest improvements in the model, the impact of UV exposure in the P chabaudii model of malaria was obviously limited.

Normally, B57 mice are completely resistant to re-challenge with virulent parasites after they have recovered from P chabaudii infection. In several experiments, animals were allowed to recover for 2-3 weeks before rechallenge. There was no difference between the UVB-exposed and the control animals. All were solidly immune to re-challenge.

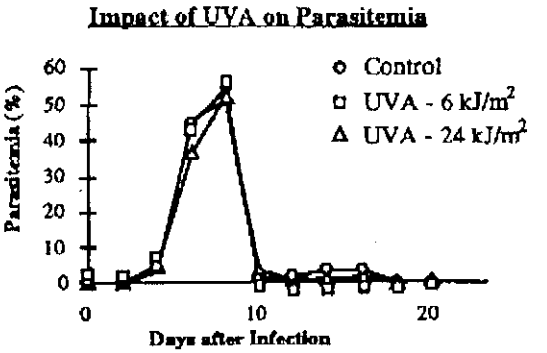
4. Possible role of UVA and UVC

We felt that it was important to define precisely the effective UV wavelength in our model. Immunological effects attributed to UVA have been reported in some studies and the UV energy which reaches the earth's surface is largely UVA. We therfore performed several experiments to examine the possible impact of UVA.

Clinical phototherapy lights (FS 40) are known to emit small amounts of UVC radiation. Although the energy emitted in the UVC range is miniscule, these wavelengths are particularly biologically active. We therefore obtained a filter-film from Schering Plough which blocks all UVC (absolute 285 nm cut-off) to determine if the effects we observed in our model were due to 'contaminating' UVC radiation emitted by the FS 40 bulbs.

4.1 UVA

Group	N	Age	UV Dose (Total)	Pattern of UV
1	6	6 weeks	1.5 kJ/m^2	4 doses pre-infection
2	6	6 weeks	3.0 kJ/m^2	4 doses pre-infection
3	5	6 weeks	6.0 kJ/m^2	4 doses pre-infection
4	6	6 weeks	12 kJ/m^2	4 doses pre-infection
5	4	6 weeks	24 kJ/m^2	4 doses pre-infection
6	4	6 weeks	control (24 kJ/m^2 duration of exposure)	4 doses pre-infection

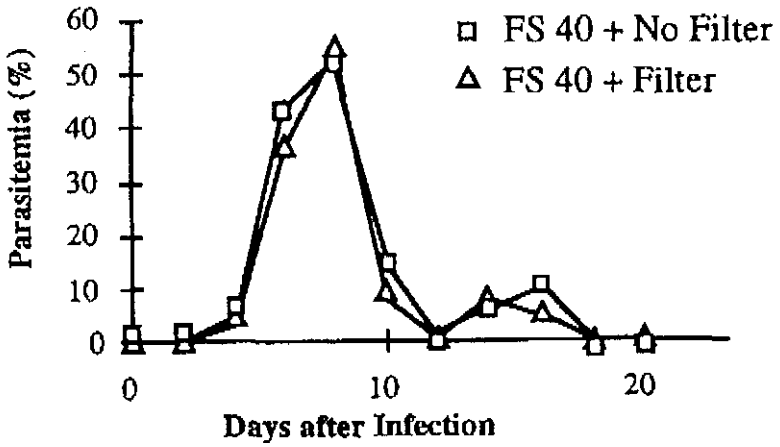


Comment - We found no effect of UVA on parasitemia or mortality in our model.

4.2 UVC

Group	N	UV Dose (Total)	Filter	Pattern of UV
1	8	3.0 kJ/m^2	no	4 doses pre-infection
2	8	3.0 kJ/m^2	yes	4 doses pre-infection
3 (control)	6	control	no	4 doses pre-infection
4 (control)	6	control	yes	4 doses pre-infection

Impact of Filtering UVC on Parasitemia



Comment - Elimination of contaminating UVC emitted by the FS40 lamps had no effect on the UV-induced changes in parasitemia. These effects could therefore be reasonably attributed to energy in the UVB range.

5. Hairless mouse

A hairless mouse has been developed which is widely used in studies of UVB-induced cutaneous neoplasms. These mice are therefore genetically susceptible to the immunomodulatory effects of UVB. Hairless mice are not yet fully characterized and remain 'outbred'. It was therefore unknown if they would be genetically susceptible or resistant to P chabaudii. We performed one experiment with these animals to determine if they could simplify our experimental protocol (ie: no clipping, larger exposed surface area). These animals were also attractive because their dermal architecture closely resembles that of humans.

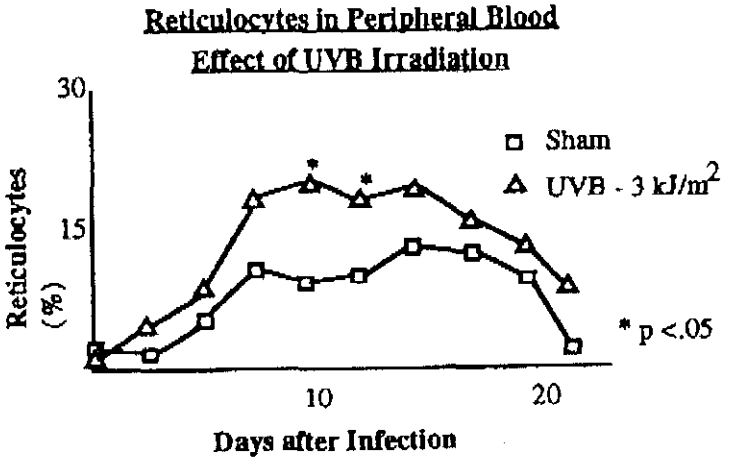
Group	N	Age	UV Dose (Total)	Pattern of UV
1	6	6 weeks	1.5 kJ/m^2	4 doses pre-infection
2	6	6 weeks	3.0 kJ/m^2	4 doses pre-infection
3	6	6 weeks	control (3 kJ/m^2 duration of exposure)	4 doses pre-infection

Comment - The hairless mice were uniformly susceptile to infection with P chabaudii infection. Therefore, they could not be used in the development of our model.

6. Immunopathogenesis of UVB Exposure in malaria

We did not feel that the modest effects we had identified justified an aggressive pursuit of our original aims #3 and #4. The end-points which we had identified were relatively 'soft'. However, we noticed a pronounced reticulocytosis following UVB exposure in some animals and followed-up on this observation in several of the later experiments. Hematopoetic capacity plays a important role in the pathogenesis of P chabaudii infection. The reticulocyte results from the UV dosing experiments 3.6.4.3 (above) are shown:

Group	N	UV Dose (Total)	Pattern of UV
1	6	3.0 kJ/m^2	4 doses pre-infection
2	5	3.0 kJ/m^2	8 doses (4 at 1 week before and 4 immediately prior to infection
3	6	6.0 kJ/m^2	8 doses (4 at 1 week before and 4 immediately prior to infection

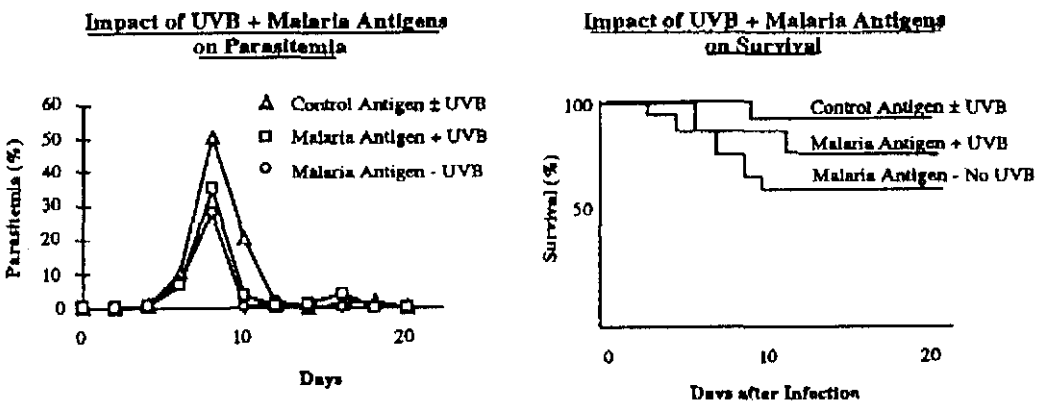


7. UVB and vaccination

Despite the limited impact of UVB radiation on the expression of P chabaudii in our model, we felt that it was important to explore the possibility that physiologic doses of UVB might modulate 'vaccine-type' immune responses. We prepared malaria antigens in two ways; either by isolating whole parasites from RBCs or by using whole parasitized RBC preparations. In the former case, the antigen dose injected was 100mg (with or without complete Freund's adjuvant) and in the latter case, 10^4 B57 RBC at 50-60% parasitemia were injected. The control antigens for these experiments were either lysed RBC (100 mg) or unparasitized RBC. Antigens were injected subcutaneously into UV or mock-irradiated skin once 10-14 days before infection or twice at 2 and 4 weeks before infection. Freund's adjuvant was used only once in each animal. Each UV dose was 3.0 kJ/m^2 of UVC-filtered UVB divided into 4 equal daily doses given immediately prior to the antigen injection(s).

7.1 Isolated parasite antigens

Group	N	Antigen	Adjuvant	Priming	UV
1	3	Purified parasite	No	1 dose	Yes
2	4	Purified parasite	Yes	1 dose	Yes
3	4	Purified parasite	Yes	2 doses	Yes
4 (control)	3	Purified parasite	No	1 dose	No
5 (control)	3	Purified parasite	Yes	1 dose	No
6 (control)	3	Purified parasite	Yes	2 doses	No
7	4	Lysed RBC proteins	No	1 dose	Yes
8	3	Lysed RBC proteins	Yes	1 dose	Yes
9	4	Lysed RBC proteins	Yes	2 doses	Yes
10 (control)	4	Lysed RBC proteins	No	1 dose	No
11 (control)	3	Lysed RBC proteins	Yes	1 dose	No
12 (control)	4	Lysed RBC proteins	Yes	2 doses	No

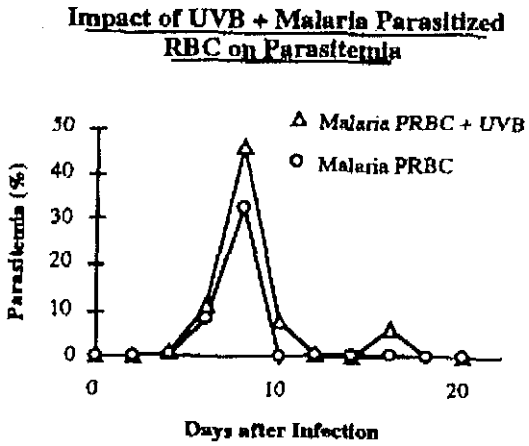


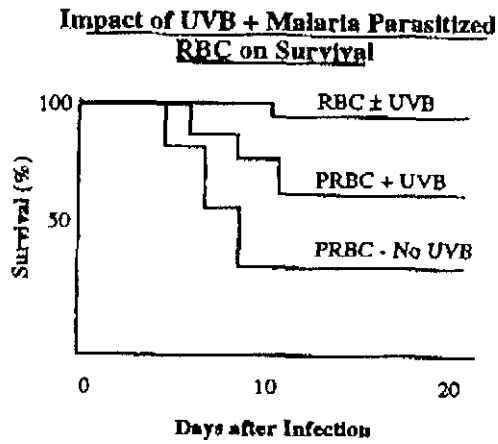
Comments - Injection of purified parasite antigens did not influence the intensity or kinetics of the parasitemia. Mortality however, was increased in the vaccinated animals. Because of the small numbers of animals in each group, this difference only approached significance when all of the vaccinated groups were combined and compared with the unvaccinated animals ($p = .06$). The addition of adjuvant and multiple doses had no effect. UVB exposure at prior to vaccination appeared to protect against increased mortality.

7.2 Parasitized red blood call antigens

Since parasite antigens 'seen' by the immune system are predominantly those expressed on the surface of RBC, we used parasitized RBC to immunized animals with or without UVB exposure.

Group	N	Antigen	Adjuvant	Priming	UVB
1	4	Parasitized RBC	Yes	1 dose	Yes
2	5	Parasitized RBC	Yes	2 doses	Yes
3 (control)	5	Parasitized RBC	Yes	1 dose	Yes
4 (control)	5	Parasitized RBC	Yes	2 doses	Yes
5	4	RBC	Yes	1 dose	Yes
6	5	RBC	Yes	2 doses	Yes
7 (control)	5	RBC	Yes	1 dose	Yes
8 (control)	4	RBC	Yes	2 doses	Yes





Comments - The findings with parasitized RBC were similar to those with purified parasite antigen. Vaccination in UVB-exposed skin had little effect on parasitemia but modified survival of the animals. Animals vaccinated with parasitized RBCs (PRBC) had a higher mortality than the control (RBC) animals which approached significance when the mice were grouped ($p = .09$). Although vaccination through skin exposed to UVB also appeared to have a protective effect in this experiment, the difference failed to reach significance ($p = .14$). All animals received adjuvant in this experiment. There was no difference between animals which received a single antigen exposure or two.

8. Discussion

We have demonstrated that murine malaria can be influenced by exposure to doses of UVB which would be considered 'physiologic' for sun-bathing humans. The maximum effect which we could reliably produce with UVB exposure was modest however. Subtle changes in the kinetics of the parasitemia, the height of the parasitemia and (in some experiments) and clearance of parasites were observed. UV exposure increased the number of circulating reticulocytes in many animals. UVB-exposed animals were solidly immune to re-challenge after they had cleared the initial infection. We could not find any dose or pattern of UVB exposure in simple challenge experiments which resulted in a consistent increase in mortality. Mortality was increased only when mice were exposured to malaria antigensprior to challenge. The increases was small however and failed to reach statistical significance despite relatively large numbers of animals studied.

Given these results, it seems unlikely that the P chabaudii model of malaria will be useful for studies exploring the impact of UVB on infectious agents. Several factors may have contributed to the difficulties we experienced in these experiments. First, the *immunopathogenesis* of murine (and human) malaria is complex and this subject is currently under intense investigational scrutiny. There may not be a 'simple' model of malaria in which suppression of one or another aspect of the immune response results in clear-cut effects such as death. Malaria may fall into the category of infections in which too weak an immune response may be as deliterious to the host as a response which is over-vigorous.

This last possibility is supported by our finding that animals which received malaria antigens through UVB irradiated skin had somewhat lower mortality than those infected through normal skin. This observation raises two obvious question:

1) What is the mechanism of the (possible) increased mortality in animals vaccinated with parasite antigens?
2) What is the (possible) protective mechanism of UV irradiation in this setting?

The data we have do not allow us to come to any firm conclusions regarding these two questions. The trends we observed were suggestive but nothing actually reached significance.

IF UVB acts to tolerize the animals to malaria antigens and **IF** mortality in the P chabaudii model is primarily due to an over-vigorous host response ... then our findings make perfect sense.

It is possible that an effect could be better defined with larger numbers of animals, but I am not certain that our data justify further efforts with this model.

9. Conclusions

We have established a model for studying the effects of UVB exposure in P chabaudii malaria. This model is of limited utility however, due to the complexity of the immune response to murine malaria and the subtle nature of the demonstrated effects.

The observation of UVB-induced reticulocytosis is novel and will be followed-up in humans undergoing phototherapy for psoriasis in collaboration with colleagues in Photodermatology at the Montreal General Hospital.

The observation that UVB exposure may modulate the immune response to cutaneously injected antigens is also novel and may be followed-up in a simpler model of immune priming and tolerance (eg: experimental allergic encephalomyelitis (EAE)). Dr Trevor Owens at The Montreal Neurological Institute has an EAE model and is interested in this project. The ability to tolerize animals to an antigen by inoculation in UV-exposed skin has obvious and exciting implications for diseases such as multiple sclerosis, diabetes, etc.

Finally, two of the co-investigators (BW & SR) may generate field data in the coming years concerning the possible impact of UVB in tropical infections. Dr Rashed will look at the effect of reduced UV exposure on malaria expression in Benin and Dr Ward has submitted a proposal to explore the possibility that UV light influences neonatal BCG responses. Dr Ward may also seek funds to explore the role of UVB on the expression of post-kala azar dermal leishmaniasis.