The effect of UV-B irradiation on secondary epidermal infection of mice with herpes simplex virus type 1

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Previous studies have indicated that suberythemal ultraviolet B (UV-B) irradiation of C3H mice before primary infection with herpes simplex virus (HSV) type 1 does not result in increased morbidity or mortality, but a suppressed delayed type hypersensitivity (DH) to the virus can be demonstrated. Any effect of UV radiation on pathogenesis during secondary epidermal HSV infection has not been previously examined. Mice were immunized by subcutaneous injection of inactivated HSV and, 5 days later, one group was UV-B-irradiated. The next day all mice were challenged epidermally with HSV. Most of the mice (92%) in the irradiated group developed severe lesions, whilst 59% of the non-irradiated group had mild lesions and 30% no lesions. Infectious virus was not isolated from the adrenal glands after challenge in either group. In addition, the DH to the virus was not affected by the UV exposure. The numbers of lymphocytes and dendritic cells in the lymph nodes draining the site of epidermal infection were increased in the UV group compared with the non-irradiated group. Following challenge, the percentage of CD4+ and CD8+ lymphocytes in lymph nodes was unaltered but the MHC class II expression on dendritic cells in these lymph nodes was reduced by UV exposure. The lymphoproliferative response in vitro of lymph node cells revealed a suppressed response to HSV and to the mitogen concanavalin A in the irradiated group. Thus, UV irradiation prior to epidermal secondary infection with HSV led to more severe infections due, perhaps, to a modulation in local antigen presentation.

Introduction

Exposure to ultraviolet light (UV), particularly in the UV-B range, is recognized as the environmental cause of skin cancers in humans and other species. In addition to the genotoxic effect of UV, irradiation is known to suppress specific immune responses by producing defects in antigen presenting cell function and by inducing the formation of suppressor T cells (recently reviewed by Goettisch et al., 1993). These outcomes are likely to be mediated by various factors in the skin, such as urocanic acid, prostaglandins, tumour necrosis factor-α and interleukin-10. As well as its role in malignancy, UV exposure may affect the immune response to microbial infections (Norval et al., 1994). This could result in increased incidence of disease and severity of clinical symptoms, or in an altered outcome in the balance between the micro-organism and the host in the case of persistent infections. Several murine models have been examined including infection with Mycobacterium bovis (Jeevan & Kripke, 1990), Candida albicans (Denkins & Kripke, 1993), Leishmania major (Giannini, 1986), Schistosoma mansoni (Jeevan et al., 1992), Trichinella spiralis (Goettisch et al., 1994) and murine leukaemia virus (Brozek et al., 1992). Thus, for example, we have previously examined the effect of suberythemal UV-B in a murine model of primary infection with herpes simplex virus (HSV) type 1. If the mice were irradiated before infection, suppression of delayed hypersensitivity (DH) occurred on subsequent challenge with inactivated virus (Howie et al., 1986 a). T cells were generated in the spleens of the irradiated animals which were antigen specific and capable of transferring the suppression of DH to mice already infected with HSV (Howie et al., 1987). In addition, epidermal cells from irradiated animals had a decreased capacity to present HSV as measured in an in vitro antibody induction system (Howie et al., 1986 b).

In general, these models have consisted of UV exposure before primary infection with the organism and few have considered the effect of UV at some stage following primary infection. One documented instance where UV affects a microbial infection in the natural host is orolabial HSV in human subjects. During the
primary infection the virus enters sensory neurons innervating the portal of entry and remains there in a latent form. It can be reactivated at intervals and is found again in the periphery where it may replicate in epithelial cells to form a recrudescent lesion. One of the most frequently recognized stimuli for recrudescence (30% of our study group in Edinburgh; Vestey et al., 1994) is sudden exposure to erythemal doses of sunlight.

Most of the effector arms of the immune system are involved in the control of HSV infections, with cell-mediated mechanisms being considered more important than humoral in recovery and clearance of virus from the epidermis (Nash & Cambourpoulos, 1993; Sprecher & Becker, 1986). T cells mediating cytotoxicity and DH are required locally, and crucial roles for macrophages and natural killer cells are indicated, as well as for cytokines such as interferon-γ and interleukin-2.

Infectious virus was titrated as plaque forming units (p.f.u.) on monolayers of Vero cells overlaid with complete medium containing essential medium (Gibco) supplemented with 7% newborn calf serum, throughout (Howie et al., 1994). Infectious virus was titrated as plaque forming units (p.f.u.) on monolayers of Vero cells overlaid with complete medium containing essential medium (Gibco) supplemented with 7% newborn calf serum, throughout (Howie et al., 1994). Infectious virus was titrated as plaque forming units (p.f.u.) on monolayers of Vero cells overlaid with complete medium containing essential medium (Gibco) supplemented with 7% newborn calf serum, throughout (Howie et al., 1994). Infectious virus was titrated as plaque forming units (p.f.u.) on monolayers of Vero cells overlaid with complete medium containing essential medium (Gibco) supplemented with 7% newborn calf serum, throughout (Howie et al., 1994).

In humans, UV exposure would be most likely to occur after the primary HSV infection when the virus is already in the latent state. Therefore, in the study reported here, mice were immunized with HSV before UV irradiation and challenge with HSV. The virus was inoculated epicutaneously to try to mimic a natural infection as far as possible. The appearance of the clinical lesions, virus isolation, antibody and T cell responses, and cell types in the lymph nodes draining the site of challenge were monitored.

**Methods**

**Mice.** Female C3H HeN mice, age 6–9 weeks, were used in all experiments and were maintained as previously described (El-Ghorr et al., 1994).

**Virus.** A plaque purified strain of HSV-1, isolated from a clinical case and passed four times in Vero cells at an m.o.i. of 0.2, was used throughout (Howie et al., 1986). It was cultured in Eagle's minimal essential medium (Gibco) supplemented with 7% newborn calf serum, 100 i.u./ml penicillin, 200 mg/ml streptomycin and 2 mM-L-glutamine (Sigma). Infectious virus was titrated as plaque forming units (p.f.u.) on monolayers of Vero cells overlaid with complete medium containing 0.25% SeaPlaque agarose (Sigma). Inactivated HSV was prepared by UV irradiating a virus stock for 1 h (2880 J/m² UV-B).

**Immunization and secondary HSV-1 infections.** Twenty mice per group were vaccinated subcutaneously with inactivated HSV-1 (3 x 10⁴ erstwhile p.f.u. per mouse). Six days later, the mice were shaved on their dorsal side and anaesthetized with halothane. The backs of the mice were tape-stripped eight times using Sellotape and a 10 μl volume containing 5 x 10⁴ p.f.u. of HSV-1 was rubbed on the back of each mouse. Sellotape stripping in itself may induce some changes in the skin but this was controlled for in the non-UV irradiated group which was treated in the same manner. At regular intervals up to 12 days post-infection, groups of four mice were killed at each time-point. Serum was collected for antibody titration, the adrenal glands for plaque assay, and lymph nodes for phenotyping and lymphoproliferation studies. For each experiment, the mice were infected on different days as appropriate and were all killed subsequently on the same day in order to minimize variation within a single experiment. In separate studies, mice were observed for up to 20 days post-infection for herpetic lesions on their backs and the DH response to HSV-1 was measured as described below.

**UV irradiation of mice.** The mice were irradiated on their shaved dorsal surfaces as previously described (El-Ghorr et al., 1994). A 30 min exposure (1440 J/m²; 0.9 minimal erythemal dose) was administered 5 days after vaccination and 1 day prior to the secondary HSV infection.

**ELISA to detect HSV antibody levels.** The plates were coated with 0.5 μg HSV antigen (antigen prepared as described by Vestey et al., 1990) per well and incubated overnight at 4 °C. PBS containing 0.05% Tween 20 was subsequently used as a diluent and washing solution between every step. The pooled sera from four mice per group, at 1/100 dilution, were added to the plate which was incubated for 3 h at room temperature (rt). Anti-mouse Ig alkaline phosphatase conjugate (Sigma) was placed in the wells at 1/500 dilution and incubated for 2 h at rt. The substrate was then added and allowed to develop at rt for 30 min.

**Delayed type hypersensitivity (DH) assay.** The DH response to HSV was measured as described elsewhere (Norval et al., 1989). Briefly, the ear thicknesses of six mice per group were measured on day 8 post-secondary infection and all the mice were challenged intradermally with 7.5 x 10⁴ erstwhile p.f.u. per ear of inactivated HSV in a 10 μl volume. One day after the challenge, the ear thicknesses were measured again. The mean increase in ear thickness for each individual mouse was first calculated and then the mean increase for each group of mice.

**Phenotyping of lymph node cells.** Four mice in each group were killed and their auricular, axillary and inguinal lymph nodes were removed and pooled. All further procedures were performed at 4 °C. A single cell suspension was made, the red blood cells lysed and the lymph node cells washed twice in RPMI medium containing 10% fetal bovine serum (RPMI-FBS). Rat monoclonal antibodies to mouse phenotypic markers (Serotec) were diluted in RPMI-FBS (CD4, 1/10; CD8, 1/40; Ia, 1/20), added to 10⁵ lymph node cells and incubated for 45 min. The cells were then washed twice and a rabbit-antirat IgG (Fab) FITC conjugate (Serotec) was added. This conjugate was diluted 1/100 in RPMI-FBS containing 10% normal mouse serum and incubated for 45 min. The cells were washed once in RPMI-FBS and resuspended in PBS containing 10% formal saline before being analysed with an EPICS XL flow cytometer. The lymphocyte population (around 80% of lymph node cells) and the dendritic cells (DC) population (approximately 2000 per lymph node; 1% of the cells) were gated on the basis of size and granularity and were examined separately. DC were also purified from the lymph node cell suspension on a 14.5% metrizamide cushion and counted microscopically, as previously described (Moodycliffe et al., 1992).

**Lymphoproliferation studies.** Lymph node cells were isolated at various times post-secondary infection as detailed above and 2 x 10⁵ cells per well were incubated in round-bottom 96-well plates in RPMI medium containing 1% heat-inactivated mouse serum for 4 days at 37 °C in the presence of 1 mg per well concanavalin A (ConA; a non-specific mitogen) or 5 x 10⁴ p.f.u. per well of inactivated HSV.
Results

Effect of UV-B irradiation on the severity of secondary HSV infections

Previously, using the same virus and mouse strains, we showed that irradiation with a suberythemal dose of UV-B prior to primary epidermal HSV infection did not result in more severe lesions or in a higher mortality (Norval et al., 1987). In the present study mice were immunized with inactivated HSV 6 days prior to a secondary epidermal infection. In a series of experiments, it was found that 19/64 (30%) did not develop lesions and 38/64 (59%) developed mild lesions, less than 1 cm² in size, which healed by 8–10 days post-challenge. The remaining 11% (7/64) developed lesions larger than 1 cm². In contrast, almost all the mice (59/64; 92%) that were UV-B irradiated 1 day prior to the secondary infection showed deep lesions, more than 1 cm² in size, which took longer to heal; in 20% of cases they had not resolved by 20 days post-challenge. The remaining mice (8%) developed mild lesions. No virus could be detected in the adrenal glands of any of the groups of mice after secondary HSV infection. Only three deaths were observed out of a total of 209 mice used in all the secondary infection experiments.

Effect of UV-B irradiation on antibody levels and the DH response after secondary HSV infection

Antibody levels to HSV increased gradually after the secondary HSV infection. UV-B irradiation prior to infection did not affect the subsequent level of HSV antibodies as measured by ELISA (Fig. 1). Similarly, irradiation did not affect the DH response as tested 8 days after the secondary HSV infection (Fig. 2). This figure shows the result of one of two DH experiments performed. The same result was obtained on both occasions.

Effect of UV-B irradiation on the number of lymphocytes and DC in lymph nodes after secondary HSV infection

The number of lymph node cells increased gradually during the course of the secondary infection. Following UV-B exposure, however, a much larger increase in the number of lymph node cells was detected (Fig. 3a).

The number of different types of cells present in these lymph nodes was then investigated. Metrizamide gradi-
ent centrifugation showed that DC accumulated in lymph nodes after the secondary infection (Fig. 3b) but then returned to normal numbers as the infection began to resolve. In the UV-B irradiated group, there was an increase in the number of DC 1 day after exposure, at the time of HSV challenge, and the number remained elevated at most time-points after challenge. The same pattern was observed when this experiment was repeated.

Flow cytometry was used to quantify the phenotypes of the lymph node cells. There was no change in the percentage of CD4+ and CD8+ lymphocytes during the course of secondary HSV infection whether the mice had been UV irradiated or not (Fig. 4a, b), indicating that lymphocytes of both phenotypes were accumulating in the lymph nodes of UV irradiated mice. MHC class II expression on a population of gated large cells (mainly

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**Fig. 3.** Effect of UV irradiation on numbers of lymph node cells following secondary HSV infection. (a) Number of cells per lymph node following secondary HSV infection. Data from three experiments are combined and the SEM is shown. (b) Number of dendritic cells per lymph node, isolated on a 14.5% metrizamide cushion, following secondary HSV infection. Twelve lymph nodes from each of four mice per group were pooled for each time-point. ■, HSV infected mice; △, mice UV-B irradiated 1 day prior to infection.

**Fig. 4.** Effect of UV irradiation on the percentage of CD4+ cells (a) and CD8+ cells (b) in the lymphocyte population of lymph node cells following secondary HSV infection. (c) Expression of Ia MHC class II on the gated large cell population (mainly dendritic cells) following secondary HSV infection. Data from two experiments are combined and the range is shown. ■, HSV infected mice; △, mice UV-B irradiated 1 day prior to infection.
UV-B induces more severe HSV-1 infections

ConA stimulation of lymph node cells was significantly reduced 8 days after UV irradiation (Fig. 5a). The HSV-specific stimulation of lymphoproliferation was not detected until day 12 post-infection and, even then, only a small proliferative response was found. At this time-point, however, UV irradiation significantly suppressed the lymphoproliferative response to HSV antigen (Fig. 5b). Similar results were obtained when the ConA and HSV lymphoproliferation assays were repeated in separate experiments.

Discussion

In this study the effect of UV on the pathogenicity of secondary epidermal HSV infection was investigated. We followed the protocol outlined by Denkins & Kripke (1993) for a mouse model of C. albicans infection, in which mice were first immunized with inactive organisms, then irradiated with an erythemal dose of UVB 5 days later and challenged intravenously after 24 h with a lethal dose of C. albicans. It was shown that the survival time was reduced by 50% and that growth of the fungus in various organs was not contained, as happens in the unirradiated animals. Initial experiments showed that this protocol worked well for HSV infected mice. In our model, the immunized mice were irradiated with a suberythemal dose of UV-B and challenged epidermally with HSV in an attempt to mimic some aspects of the natural infection in human subjects. This model may be criticized in that inactivated virus is used to immunize the mice and that the mice were challenged 6 days after the initial immunization. In a natural human infection, virus is encountered first, latency established and then reactivation or secondary challenge may occur subsequently. It has proved difficult to establish a robust murine model of recrudescence (Harbour et al., 1981) and our protocol is a reliable and a useful one in which to study the effects of UV-B irradiation on secondary HSV infections.

We have found that the severity of the lesions, and time to resolution, were increased in the mice exposed to UV before challenge. Infectious virus was not detected in the adrenal glands in any group after the secondary HSV infection. After a primary HSV infection the adrenal glands always contained infectious virus (data not shown). It has been previously shown that the adrenal glands are a preferred site of HSV replication (Hill et al., 1986). These data suggest that 6 days was sufficient time to generate a primary immune response since the secondary infection was generally mild in the immunized mice. In addition, no virus was detectable in the adrenals.
which implies that HSV was probably confined to the local site of inoculation and was not spreading systemically in the immunized animals, as occurs following primary epidermal infection. The increased morbidity is unlikely to be due simply to the inflammation induced by UV exposure as (1) primary HSV epidermal lesions were not more severe and did not result in increased mortality in UV irradiated mice compared with unirradiated mice (Norval et al., 1987), and (2) turpentine, which induces cutaneous inflammation, did not affect the survival rate of mice lethally infected with C. albicans, while UV irradiation before challenge did (Denkins & Kripke, 1993). Therefore, it is possible that UV exposure is mainly influencing the immune response to the virus, such that local replication in the skin is temporarily unchecked.

It has been shown that Langerhans cells are the major antigen presenting cells for HSV in human epidermis (Vestey et al., 1990) and murine epidermis (Yasumoto et al., 1986), and that the density of Langerhans cells at the site of intradermal inoculation affects the severity of the infection, fewer resulting in greater morbidity (Sprecher & Becker, 1986). UV irradiation is known to have several effects on Langerhans cells. The first of these is to reduce their number in the epidermis (El-Ghorr et al., 1994). The second is suppression of their antigen presenting capacity for HSV (Howie et al., 1986b), which may be due to alterations in the expression of adhesion molecules, such as ICAM-1 (Tang & Udey, 1992), or of costimulatory molecules, such as the B7 family (Young et al., 1993). It is not known whether additional antigen presentation pathways are operational in secondary responses. While the major pathway, where Langerhans cells process antigen in the periphery and present it to specific T cells in the draining lymph node, is thought to be common to all cutaneous immune responses, there may be augmentation by alternative pathways in secondary responses where activated T cells have already been generated.

When humoral and cell mediated responses were assayed following secondary challenge, no differences in HSV-specific antibody levels in the serum of irradiated and unirradiated animals were detected. Perhaps this result was expected as most experimental models and studies in human subjects have shown no effect of UV exposure on general immunoglobulin production. In addition, the DH to HSV was unaffected by the irradiation. This is in contrast to a primary HSV infection where UV exposure prior to infection resulted in a suppressed DH to the virus on subsequent challenge, due to the induction of an antigen-specific subset of splenic T cells (Howie et al., 1986a, b). The effect of UV may vary depending on the timing of exposure with respect to infection, particularly whether it occurs before primary contact with the antigen or after the establishment of immune responses. Two reports indicate that irradiation following sensitization can enhance contact hypersensitivity responses on subsequent challenge, a process possibly mediated by tumour necrosis factor-α (Pollat et al., 1986; Yoshikawa et al., 1992). Further, Denkins et al. (1989) showed that, while a single high dose of UV before or after immunization with C. albicans led to the suppression of DH in all cases, the mechanism was different if the exposure took place after immunisation as no splenic T suppressor cells could be detected.

Changes were noted in the lymph node draining the site of secondary epidermal infection with HSV. It was found that the number of cells per lymph node increased as a result of the HSV challenge and this further increased about 2-fold if the mice were UV irradiated before challenge. However, this was not due to the accumulation of a particular T cell subset as the percentage of CD4+ and CD8+ cells remained unchanged throughout, indicating that both populations were induced to migrate into the lymph nodes. The number of DC per lymph node also increased following secondary infection, and was higher still in the UV group. It has been shown previously that, 24-48 h after UV irradiation, there was an accumulation of DC in the lymph nodes draining the site of exposure, mainly due to the action of tumour necrosis factor-α induced by irradiated keratinocytes (Moodycliffe et al., 1994). It was interesting to note that, of the gated population representing mainly the DC, the percentage expressing MHC class II antigens was reduced in the irradiated group but remained unchanged in the unirradiated group following challenge. Thus, it is possible that migration of antigen presenting cells from the epidermis to the draining lymph nodes takes place following epidermal HSV infection and that this process is enhanced by irradiation. However, in the latter case the DC may be temporarily ineffective at presenting antigen as their expression of MHC class II antigens is reduced. This suggestion is substantiated by observing that the mitogen- and HSV-induced lymphoproliferative response of lymph node cells was suppressed in the UV group. In a study of latently infected human subjects, whole body exposure to UV-B resulted in inhibition of HSV and phytohaemagglutinin-induced proliferation of peripheral blood mononuclear cells, first apparent at 3 days post-irradiation and lasting at least 9 days (Miura et al., 1994). The down-regulation was associated with increased levels of transforming growth factor-β. Recently, Yasumoto et al. (1994) have provided evidence that UV irradiation prior to primary subcutaneous infection of BALB/c mice with HSV-1 resulted in a switch in cytokine synthesis by lymph node and spleen cells from a T helper 1 response (interferon-γ and interleukin-2) to a T helper 2 response (interleukin-
4). It will be interesting to find out in further studies whether UV exposure before secondary HSV infection might have a similar effect and whether the irradiation induces a novel array of cytokines at the site of the epidermal lesion.

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References


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