Neonatal exposure to solar simulated ultraviolet radiation leads to deviation of immune development

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Abstract. Exposure to ultraviolet radiation (UVR) during childhood reduces the likelihood of developing auto-immune disease, but increases the risk of developing melanoma. As immune suppression is likely to contribute to both of these alterations, we propose that exposure to UVR during the neonatal period modifies development of the immune system. To test this hypothesis, neonatal mice were exposed to a single erythemal dose of solar simulated UVR at 3 days of age. At eight weeks post-exposure the contact hypersensitivity response to oxazalone was significantly reduced in the neonatally irradiated mice. Analysis of the lymph nodes of such mice revealed an increase in the percentage of T regulatory cells. Furthermore, during the challenge phase of the contact hypersensitivity response, the number of T regulatory cells within the medullary cord region of lymph nodes was significantly increased, suggesting that these cells are responsible for the suppressed response in neonatally irradiated mice. When an equivalent number of T regulatory cells was isolated from irradiated or control mice, eight weeks after neonatal irradiation, the suppression was not transferred to naïve mice to a greater extent by the cells from the irradiated mice. As such, we propose that exposure to UVR during the neonatal period leads to a deviation of immune development, leading to suppressed immunity which is not due to the induction of T regulatory cells.

Introduction

Childhood exposure to burning doses of sunlight increases the risk of developing melanoma, the most lethal form of skin cancer. In contrast, low dose childhood exposure may protect against the development of autoimmune diseases including multiple sclerosis and rheumatoid arthritis. These findings imply that childhood exposure to sunlight may modulate the immune response, most likely through the induction of immunosuppression. In support of this, exposure to various other agents, including pathogens, chemicals and allergens in early life have a major impact in directing development of the immune response. It is likely that sunlight is another of these agents. As such, we analysed changes in the skin immune system following neonatal exposure to solar simulated ultraviolet radiation (ssUVR) with the aim of identifying alterations that may play a role in the development of immunosuppression.

Methods

To test this hypothesis, neonatal BALB/c mice were exposed to a single dose of solar simulated UVR at three days of age from Cleo Natural Lamps. Doses used ranged from two to four erythemal doses. Changes in the skin immune system, including the contact hypersensitivity response and the proportions of immune cells in skin draining lymph nodes, were assessed in mice when they reached adulthood, eight weeks following neonatal irradiation.

Contact hypersensitivity and tolerance

Neonatal mice were irradiated and 8 weeks later, the contact hypersensitivity response to oxazalone was assessed. Increase in ear thickness following antigen challenge was measured using a spring-loaded micrometer. Neonatally-irradiated mice had a significantly reduced contact hypersensitivity response compared to their unirradiated counterparts (Figure 1). Additional experiments showed that when antigen sensitisation occurred during the neonatal period at 3 days post-irradiation, followed by challenge in adult life, there was a greater reduction in the contact hypersensitivity response, reflecting increased tolerance induction (Figure 2).

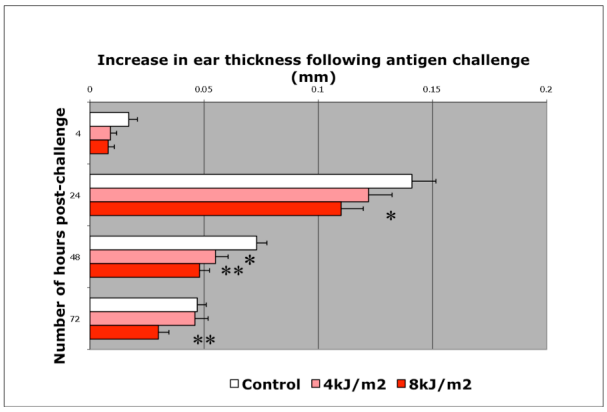

Figure 1. Contact hypersensitivity at eight weeks post-neonatal irradiation. The response is significantly suppressed in mice exposed to ultraviolet radiation than their control counterparts. (* $p<0.05$)

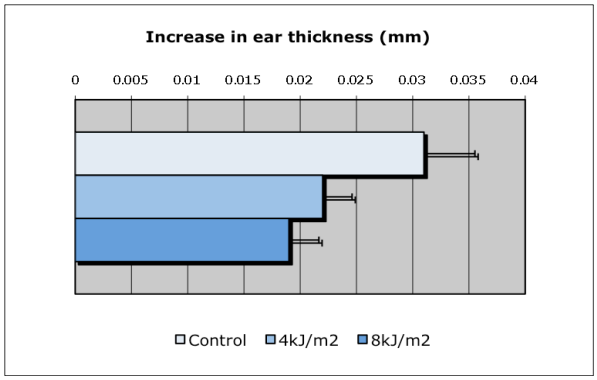

Figure 2. Neonatal tolerance induction. The level of tolerance is increased (and therefore ear swelling decreased) in mice that were exposed to ultraviolet radiation as neonates.

Lymph node resident cells

Mice were irradiated as neonates and eight weeks later the skin draining lymph nodes and the spleens were removed and analysed using flow cytometry. A slight increase in T regulatory cells was observed in irradiated compared to control mice (Figure 3), as was a decrease in the level of expression of IgD by B cells in the draining lymph nodes. There were no differences in the proportions of other lymph node cells. In addition, cell populations in the spleen were unaffected, hence a local alteration in immune development is likely.

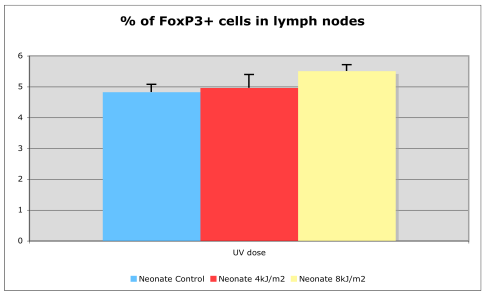

Figure 3. T regulatory cells. When the percentage of T regulatory cells was assessed using flow cytometry, a dose-dependent increase was observed at eight weeks post-neonatal irradiation. y-axis represents percentage of lymph node cells expressing FoxP3.

When assessed *in vitro*, there was a similar capacity for proliferation by lymph node cells obtained from neonatally-irradiated compared to control mice. Despite this, there was an altered cytokine profile, with lymph node cells from irradiated mice producing more IL-10 and IFN-γ than cells from control mice (Figure 4).

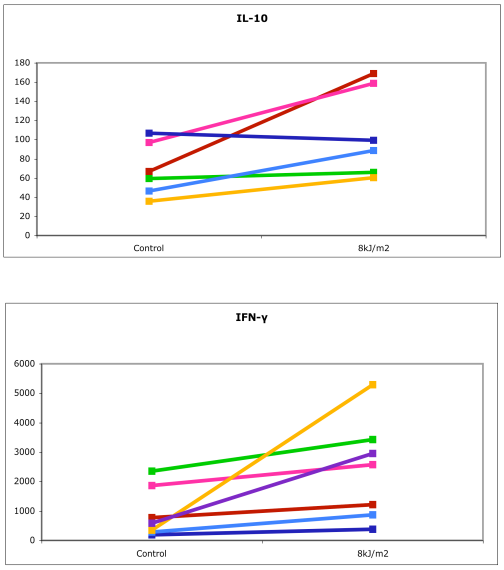

Figure 4. Cytokine production by lymph node cells. Cytokine production was measured on culture supernatants by ELISA. Each line represents a single experiment and the y-axis represents cytokine concentration in pg/mL. The production of both IL-10 and IFN-γ were increased.

Transfer of suppression

As the number of T regulatory cells and production of IL-10 was increased, it could be suggested that T regulatory cells were responsible for the observed immunosuppression. When assessed *in vitro* by isolating T regulatory cells and adding them to a CD3/CD28 stimulated lymphocyte culture, no suppression was observed (Figure 5). When assessed *in vivo* (by isolating T regulatory cells, transferring them into naïve mice, and assessing contact hypersensitivity). suppression was also not observed (results not shown). Furthermore, transfer of whole lymph node cell suspensions could not transfer the observed suppression (data not shown).

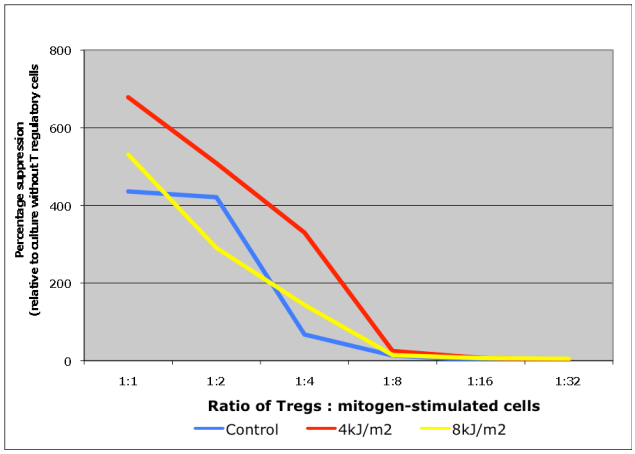

Figure 5. *In vitro* suppression. When T regulatory cells were isolated from mice and co-cultured with mitogen stimulated lymphocytes, there was no difference in suppression from T regulatory cells obtained from irradiated compared to unirradiated mice. (Differences were not statistically different.)

Conclusion

Neonatal exposure to ultraviolet radiation leads to long lasting suppression of the immune response. At the time this suppression is observed, an alteration in the lymph node cell profile can be seen, as well as in the cytokine profile these cells produce. This is not associated with suppression of proliferation. Furthermore, this suppression does not appear to be due only to the induction of a suppressive cell population within the skin draining lymph nodes. We propose that neonatal exposure to ultraviolet radiation alters development of the immune response, thereby altering the contact hypersensitivity response in adult life. This is different to the early suppressive response following adult exposure to ultraviolet radiation which tends to be due to the induction of a single or multiple populations of suppressive cells.