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Suppression of the asthmatic phenotype by ultraviolet B-induced, antigen-specific regulatory cells

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Clinical and Experimental Allergy

Summary

Background Over recent decades, there has been a significant global increase in the prevalence of asthma, an inflammatory disease of the respiratory system. While ultraviolet radiation (UV) has been used successfully in the treatment of inflammatory conditions such as psoriasis, studies of UV-induced regulation of allergic respiratory responses have been rare, and have not analysed *in vivo* measurements of airway hyperresponsiveness (AHR) or the antigen specificity of the UV-induced effects.

Objective To investigate the regulatory properties of erythematous ultraviolet B (UVB) irradiation of the skin and the induction of allergen-induced airway immunity in a murine asthma model, and to examine the mechanisms involved.

Methods BALB/c mice were exposed to a single erythematous dose of UV 3 days before intraperitoneal sensitization (day 0) and boost (day 14) with the antigen, ovalbumin (OVA). Airway-associated, asthma-like responses to aerosolized OVA at day 21 were analysed including (a) AHR measured *in vivo*, (b) OVA-specific proliferative responses and cytokine production by cells from the lung-draining lymph nodes (LDLN), and (c) inflammatory cells and cytokines in the bronchoalveolar lavage fluid. To determine UVB-induced mechanisms of regulation, LDLN cells from UVB irradiated, OVA-sensitized mice were adoptively transferred into naïve BALB/c mice that were subsequently sensitized and challenged with OVA, or a non-specific antigen.

Results UVB irradiation of skin significantly suppressed AHR to methacholine and OVA-specific responses in the LDLN and in the lung compartment. Reduced OVA-specific responses by LDLN cells from both UVB irradiated mice and mice that received 5×10^6 LDLN cells from UVB irradiated, but not from non-irradiated, OVA-sensitized mice suggested that UVB-induced regulatory cells are responsible for many of the asthma-reducing effects of dorsal UVB exposure.

Conclusion UVB irradiation of skin suppresses AHR and cellular responses of the airways to respiratory allergens. Further, this study implicates UVB or its downstream mediators as a potential approach to reducing the severity of asthma.

Keywords asthma, immune regulation, regulatory cells, respiratory, ultraviolet

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Introduction

Asthma is a chronic disease of the airways characterized by wheeze, shortness of breath, chest tightness and cough. It is associated with the infiltration of inflammatory cells, particularly eosinophils, into the airways, hyperresponsiveness (AHR) of the bronchial smooth muscle and airway remodelling. Increased levels of IL-4, IL-5, IL-9 and IL-13 in the bronchoalveolar lavage fluid (BALF) and increased antigen-specific IgE in serum are also associated with asthma [1].

Ultraviolet B (UVB) irradiation of skin alters immune responses to antigens administered both locally and systemically. Previous reports have described the effectiveness of ultraviolet radiation (UV) in inhibiting the development of T-helper type 1 (Th1)-driven allergic reactions such as contact hypersensitivity to haptens painted onto the skin [2] and delayed-type hypersensitivity to microbial, tumour or allo-antigens [3]. To date, few studies have extensively studied the systemic effects of UVB exposure of skin on the asthmatic allergic response, particularly at the cellular

level, or indeed the effects of UVB on Th2-driven responses in general.

While the prevalence of allergic diseases such as asthma has increased in recent decades, epidemiological studies suggest that our average level of sun exposure has decreased due to raised awareness of the risks associated with sun exposure and lifestyles that involve more time spent indoors [4]. Although a definitive link between these observations cannot be made, the evidence for UVB-induced immunomodulation, coupled with epidemiological data of reduced sun exposure and increased prevalence of asthma, certainly warrants further investigation into the effects of UVB exposure on the allergic-asthmatic response.

In this study, we investigated the effects of erythema UVB irradiation on a murine model of asthma using ovalbumin (OVA) as the sensitizing allergen. Mice received two intraperitoneal (i.p.) doses of OVA adsorbed in aluminium hydroxide and were challenged 21 days after the initial sensitization with a single OVA-in-saline aerosol. A number of physiological and immunological parameters were analysed including *in vivo* changes in lung function, partitioned into components for the airways and lung parenchyma, using a custom-designed ventilator and a low-frequency forced oscillation technique [5]. In addition, OVA-specific *in vitro* responses by the cells from lung-draining lymph nodes (LDLN), cytokines and inflammatory cells in the BALF, histology of the lung tissue and OVA-specific IgE levels in the serum were also measured. Our integrated approach focused on the effects of UVB exposure on the development and severity of asthma not only at the level of airway and tissue mechanics but also at the antigen-specific and lung-specific cellular level.

Methods

Animals

BALB/c female mice, 8 weeks old, and Sprague-Dawley male rats, at 22 weeks old, were obtained from The Animal Resources Centre (Murdoch, WA, Australia). All experiments were approved by the Animal Experimentation Ethics Committee of the Telethon Institute for Child Health Research.

Ultraviolet irradiation

The UV source was a row of six FS40 UVB lamps (Westinghouse Corporation, Pittsburgh, PA, USA) emitting a broad band of UV, 250–360 nm, with 65% of the output in the UVB range (290–320 nm). The radiation output for each experiment was measured using a UVX radiometer with a UVX-31 sensor (Ultraviolet Products Inc., San Gabriel, CA, USA). As described previously [2], a 2 × 4 cm dorsal patch of skin was clean shaven. Mice were irradiated in 4 × 6 cm compartments of a clear perspex box

with wire mesh roofing and a polyvinylchloride plastic sheet (to remove wavelengths <290 nm) placed between the mice and UV lamps. The sunlamps were positioned 20 cm above the cages. The dose used was a single UVB exposure of 8 kJ/m² (approximately three to four minimal edema doses) 3 days before sensitization with OVA or β -lactoglobulin (BLG). Control mice were shaved but were not exposed to UV radiation.

Sensitization and challenge of mice to ovalbumin and β -lactoglobulin

OVA (Sigma Chemical Company, St Louis, MO, USA) in an aluminium hydroxide suspension (Serva, Heidelberg, Germany) was delivered i.p. on day 1 (20 μ g OVA in 4 mg aluminium hydroxide per mouse; 200 μ L volume) and again on day 14. Mice were then challenged on day 21 with a 1% OVA-in-saline aerosol delivered using an ultrasonic nebulizer (UltraNeb[®], DeVilbiss, Somerset, PA, USA) for 30 min. For sensitization of mice to BLG, a single dose of BLG (Sigma) in an aluminium hydroxide suspension (Serva) was delivered i.p. (100 μ g BLG in 2 mg aluminium hydroxide per mouse; 200 μ L volume).

Preparation and culture of single-cell suspensions from lung-draining lymph nodes

Twenty-four hours after OVA challenge or 7 days after BLG sensitization, the posterior mediastinal, tracheobronchial and parathymic lymph nodes (LDLNs) were removed from mice, pooled within experimental groups, physically disaggregated and cultured at 10⁵ cells/200 μ L/well (six replicates per treatment) in Roswell Park Memorial Institute medium (Gibco, Auckland, New Zealand) with 10% fetal calf serum (FCS), 2 mM L-glutamine, 50 μ M 2-ME and 5 μ g/mL gentamicin. Single-cell suspensions were incubated at 37 °C with 5% CO₂, with or without OVA or BLG (100 μ g/mL). Methyl-³H-thymidine (Amersham Pharmacia Biotech, Piscataway, NJ, USA; 0.25 mCi; 10 μ L/well) was added at 72 h and cells harvested at 96 h with ³H-thymidine incorporation used as a measure of cellular proliferation. Culture supernatants were also obtained at 96 h.

Phenotyping lung-draining lymph node cells

LDLN cells were incubated with rat anti-mouse CD16/CD32 monoclonal Ab (BD Biosciences, San Diego, CA, USA) for 10 min on ice. Conjugated antibodies (FITC-labelled CD8, FITC-labelled I-A/I-E (MHC class II), PE-labelled CD4, PE-labelled CD11c, APC-labelled CD25, biotinylated CD3e, biotinylated CD19, FITC-labelled rat IgG_{2a}, PE-labelled rat IgG_{2a}, PE-labelled hamster IgG₁, APC-labelled rat IgG_{2b}, biotinylated hamster IgG₁ and biotinylated rat IgG_{2a}; BD Biosciences) were added at optimized concentrations and incubated for 30 min on

ice. Where a biotin antibody was used, cells were washed and then incubated with a streptavidin-CyChrome conjugate (BD Biosciences) for 30 min on ice. Cells were washed and resuspended in 300 µL fixative buffer [1% formaldehyde in phosphate-buffered saline (PBS)]. For intracellular FoxP3 staining, an anti-mouse/rat FoxP3-FITC staining kit was obtained and used according to the manufacturer's instructions (eBioscience, San Diego, CA, USA). Flow cytometric analysis was performed using a FACSCalibur flow cytometer (BD Biosciences), and data were analysed using FlowJo software (version 4.6.1; TreeStar, Ashland, OR, USA).

Bronchoalveolar lung lavage and removal of lung tissue for histology

Twenty-four hours after the aerosol challenge with OVA, bronchoalveolar lavages (BALs) were performed via an incision in the trachea. After perfusion of the lung tissue with PBS, the left lung was clamped at the bronchus and 1.2 mL 0.2% BSA in PBS was flushed into the right lung and recovered. Lavage samples were centrifuged (300 g, 4 °C, 10 min) and the supernatant was stored at -20 °C until cytokine analysis. The pellet was resuspended and cells (5×10^4 in 100 µL) were then centrifuged onto slides and stained using the DIFF-Quik Stain Set 64851 (Lab Aids, Narrabeen, NSW, Australia) as per the manufacturer's instructions. After the lavage, the left lung was removed at the bronchus and fixed in 10% phosphate-buffered formalin. Tissue sections were stained according to routine procedures [haematoxylin and eosin (H&E) and alcian blue].

Detection of cytokines in bronchoalveolar lavage fluid and culture supernatants

IL-4, IL-5, IL-10 and IFN- γ were determined using rat anti-mouse IL-4, IL-5, IL-10 and IFN- γ ELISA capture and detection monoclonal antibodies (BD Biosciences) in a dissociation-enhanced time-resolved fluorescence immunoassay with Europium as the label. Recombinant murine IL-4, IL-5, IL-10 and IFN- γ (BD Biosciences) were used as standards. The sensitivities of the assays were as follows: IL-4 (4 pg/mL), IL-5 (2 pg/mL), IL-10 and IFN- γ (8 pg/mL).

Measurement of airway hyperresponsiveness

A modified low-frequency forced oscillation technique was used to measure change in respiratory system input impedance (Zrs) [5]. Briefly, 24 h after the aerosol challenge, mice were anaesthetized with xylazine (2 mg/mL; Troy Laboratories, Smithfield, NSW, Australia) and ketamine (40 mg/mL; Troy Laboratories) delivered i.p. at a dose of 0.01 mL/g. Mice were tracheostomized and ventilated (*flexiVent*, Scireq, Montreal, QC, Canada) at 450 breaths/min with a tidal volume of 8 mL/kg and a positive end expiratory

pressure of 2 cm H₂O. Baseline values were obtained by measuring Zrs five times at 1-min intervals. A 90-s saline aerosol was delivered with an ultrasonic nebulizer (DeVilbiss UltraNeb[®]) and Zrs was measured five times at 1-min intervals. This was repeated with $\frac{1}{2} \log_{10}$ incremental doses of methacholine (0.1–30 mg/mL) and the peak response for each parameter was recorded for analysis. The constant-phase model [6] was used to partition Zrs into components representing the conducting airways (airway resistance) and the lung parenchyma (tissue damping and tissue elastance).

Ovalbumin-specific immunoglobulin E detection via a passive cutaneous anaphylaxis assay

At 2 weeks after sensitization (Bleed 1) and at 1 week after boost (Bleed 2), mice were bled. Serum samples were serially diluted in PBS. Sprague-Dawley rats were sedated using 5.7% chloral hydrate (Sigma) in PBS delivered i.p. at 0.5 mL/100 g body weight. The back of the rat was shaved and each of the serum samples (50 µL) was delivered subcutaneously in marked rows. Twenty-four hours later, OVA (4 mg/mL in PBS) was diluted 1 : 1 with 1% Evan's blue dye (Sigma) in PBS. The rats were sedated and 1.8 mL of the OVA in Evan's blue dye solution was administered intravenous (i.v.) After 20 min, areas of extravasation of the dye due to IgE-mediated mast-cell degranulation at the sites of subcutaneous delivery of the serum samples were examined. The IgE titre of each sample was determined as the highest dilution of serum at which extravasation had occurred. IgE titres are expressed as $-\log_2$.

Adoptive transfer of lung-draining lymph node cells into naïve recipients

The LDLNs were removed from OVA-sensitized and challenged donor mice (with or without UVB irradiation before sensitization) 24 h after challenge, pooled within experimental groups, physically disaggregated and filtered into single-cell suspensions. Cells were washed, resuspended in saline and delivered to naïve recipient mice i.v. at 5×10^6 cells/mouse in a 200 µL volume. Recipient mice were then sensitized to OVA or BLG 24 h after adoptive transfer. Mice sensitized to OVA also received an OVA boost and OVA challenge.

Statistical analyses

Levels of significance were calculated using one-way ANOVA. The statistical programme used was GraphPad Prism 4.00, where a *P*-value of <0.05 was considered to indicate a significant difference between groups. Each experimental group consisted of five mice, with each experiment performed at least three times. Airway hyperresponsiveness (AHR) measurements were performed

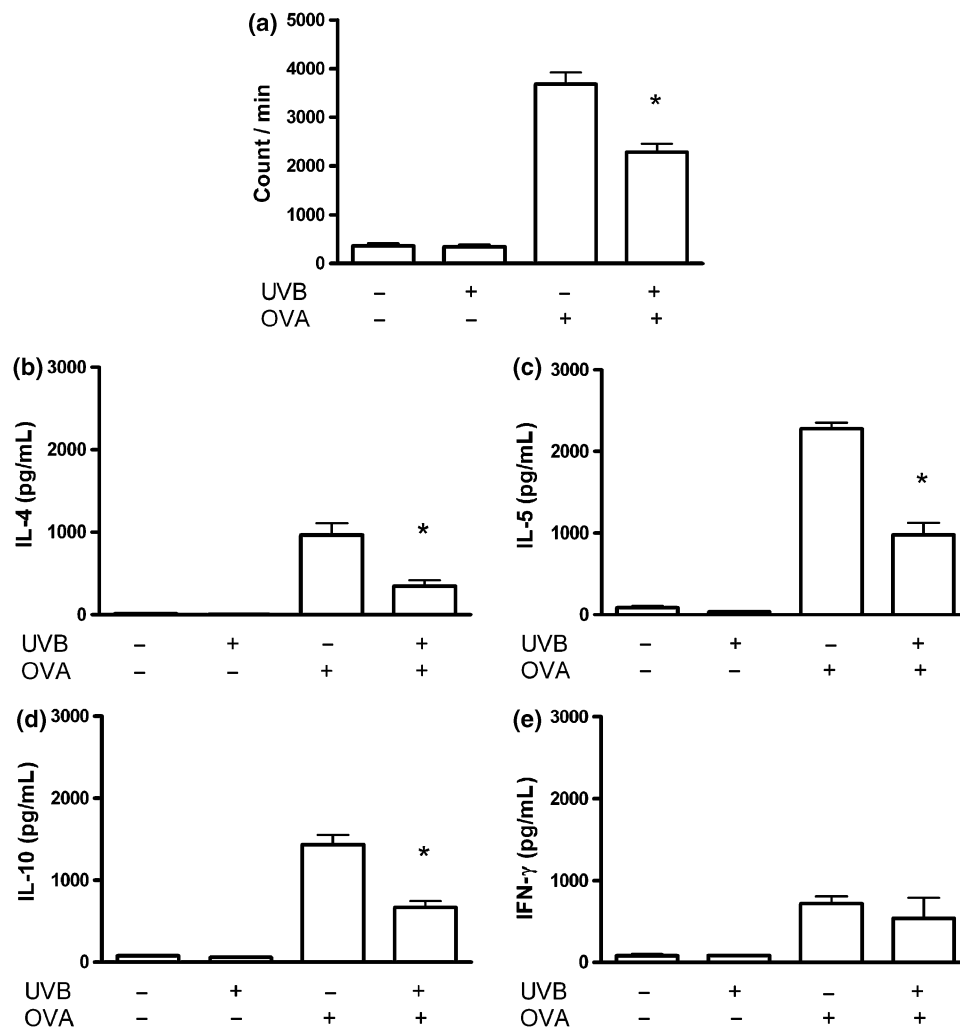


Fig. 1. (a) Proliferation and (b) IL-4, (c) IL-5, (d) IL-10 and (e) IFN- γ production *in vitro* by lung-draining lymph nodes (LDLN) cells from ovalbumin (OVA)-sensitized and -challenged mice, with or without ultraviolet B exposure before sensitization. LDLN cells were cultured *in vitro* with or without OVA (100 μ g/mL). Bars show mean $\pm$ standard error of the mean, $n = 6$ replicate cultures. * $P < 0.05$.

twice, with each experimental group consisting of eight mice. Data shown are from a representative experiment.

Results

Reduced Ovalbumin-induced proliferation and production of interleukin-4, -5 and -10 by lung-draining lymph node cells from mice irradiated with ultraviolet B

There was significantly reduced proliferation by the LDLN cells removed 24 h after challenge from OVA-sensitized and -challenged mice irradiated with UVB when cultured with OVA protein *in vitro* (Fig. 1a). The production of Th2 cytokines (IL-4, IL-5 and IL-10), but not IFN- γ , in response to OVA *in vitro* was also reduced for LDLN cells from UVB irradiated, OVA-sensitized and -challenged mice (Fig. 1b-e). UVB irradiation of mice did not significantly alter the yield or the proportion of CD3⁺CD4⁺, CD3⁺CD8⁺,

CD4⁺CD25⁺, CD4⁺CD25⁺FoxP3⁺, CD19⁺I-A/I-E⁺ or CD11c⁺I-A/I-E⁺ cells from the LDLNs (data not shown).

Ultraviolet B irradiation decreased interleukin-5 levels in the bronchoalveolar lavage fluid

Levels of IL-5 and IFN- γ in the BALF were significantly elevated in mice sensitized and challenged with OVA compared with the levels detected in the BALF of naïve mice (Fig. 2a). If mice were UVB irradiated, IL-5 levels in the BALF of OVA-sensitized and -challenged mice were significantly reduced to a level not significantly different from that of naïve mice. Levels of IL-4, IL-10 and IFN- γ in the BALF of OVA-sensitized and -challenged mice were not significantly altered by UVB irradiation (Fig. 2a). OVA sensitization and challenge did not significantly increase the number of cells per mouse in the BALF compared with naïve mice (approximately 2×10^6 cells/mouse; data not

shown). However, the ratio of macrophages:lymphocytes:neutrophils:eosinophils in the lavage fluid of naïve mice shifted from 95:4:0.5:0.5 to 81:6:9:4 upon OVA sensitization and challenge, representing a 20- and 10-fold increase in the prevalence of neutrophils and eosinophils, respectively (Fig. 2b). Upon UVB irradiation of mice, the absolute numbers of cells (data not shown) and relative percentages of cells (Fig. 2b) in the BALF of OVA-sensitized and -challenged mice were not significantly altered. Histologically, no differences in cellular infiltration or mucus production in the lung tissue were observed between UVB irradiated and non-irradiated mice by H&E and alcian blue staining, respectively (data not shown).

Ultraviolet B irradiation decreased airway hyperresponsiveness

OVA sensitization and challenge significantly increased changes in airway resistance (Fig. 3), tissue damping and tissue elastance (data not shown) compared with naïve mice in response to methacholine. UVB irradiation significantly decreased the airway resistance component of AHR in OVA-sensitized and -challenged mice such that there was no significant difference between naïve and UVB irradiated, OVA-sensitized and -challenged mice (Fig. 3). UVB irradiation did not alter AHR in non-sensitized mice, indicating that UV exposure alone does not suppress AHR (data not shown). While UVB irradiation did not reduce tissue damping and tissue elastance in response to methacholine in OVA-sensitized and -challenged mice, these components were not significantly different from naïve mice (data not shown).

Ultraviolet B irradiation did not alter ovalbumin-specific immunoglobulin E in serum

Detectable levels of OVA-specific IgE were measured in the serum of mice 2 weeks after OVA i.p. sensitization (Bleed 1) and 1 week after OVA i.p. boost (Bleed 2). UVB irradiation of mice did not significantly alter OVA-specific serum IgE titres at Bleed 1 (non-irradiated = 4.6 ± 0.6 ; UVB-irradiated = 4.6 ± 0.7) or Bleed 2 (non-irradiated = 4.0 ± 0.3 ; UVB irradiated = 3.4 ± 0.2), although there was a trend for reduced IgE titres in UVB irradiated mice at Bleed 2.

Adoptive transfer of lung-draining lymph node cells from ovalbumin-sensitized and -challenged mice irradiated with ultraviolet B before sensitization

UVB-induced, antigen-specific regulatory T cells have been reported previously [7, 8]. It was reasoned that if a regulatory cell was responsible for the UVB-induced suppression of OVA-specific immune responses observed in this study, adoptive transfer of this cell into naïve mice would modulate subsequent OVA sensitization and

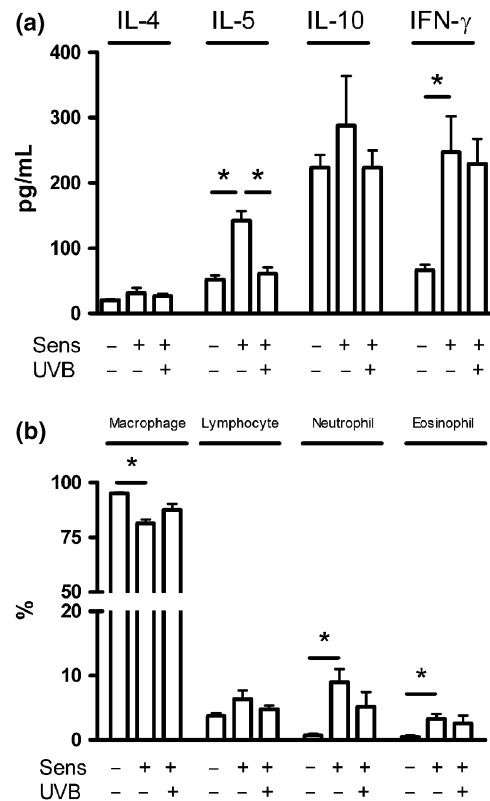


Fig. 2. (a) Levels of (IL)-4, IL-5, IL-10 and IFN- γ , and (b) percentage of macrophages, lymphocytes, neutrophils and eosinophils in the bronchoalveolar lavage fluid of ovalbumin-sensitized and -challenged mice with or without ultraviolet B before sensitization. Data for naïve mice also shown. Bars show mean $\pm$ standard error of the mean, $n = 5$ mice. * $P < 0.05$.

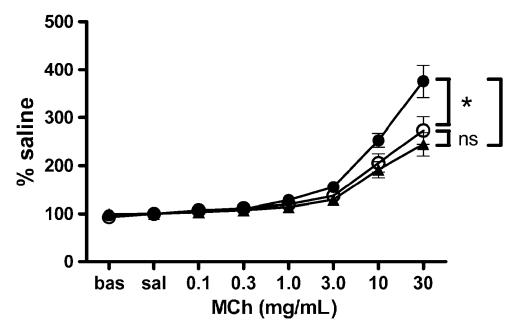


Fig. 3. Airway resistance in response to methacholine in ovalbumin (OVA)-sensitized and -challenged mice with or without ultraviolet B (UVB) before sensitization. Data show dose-response curves to inhaled methacholine (MCh) for non-irradiated, OVA-sensitized and -challenged mice (●), UVB irradiated, OVA-sensitized and -challenged mice (○) and naïve mice (▲). Bas, baseline value obtained by measurement of input impedance (Zrs) five times at 1 min intervals after stabilization of the mouse on the ventilator; sal, saline values obtained by measurement of Zrs five times at 1 min intervals after a 90 s saline aerosol; percent saline, percent increase of saline values for increasing doses of MCh. Data points show mean $\pm$ standard error of the mean, $n = 8$ mice. * $P < 0.05$, NS, not significant.

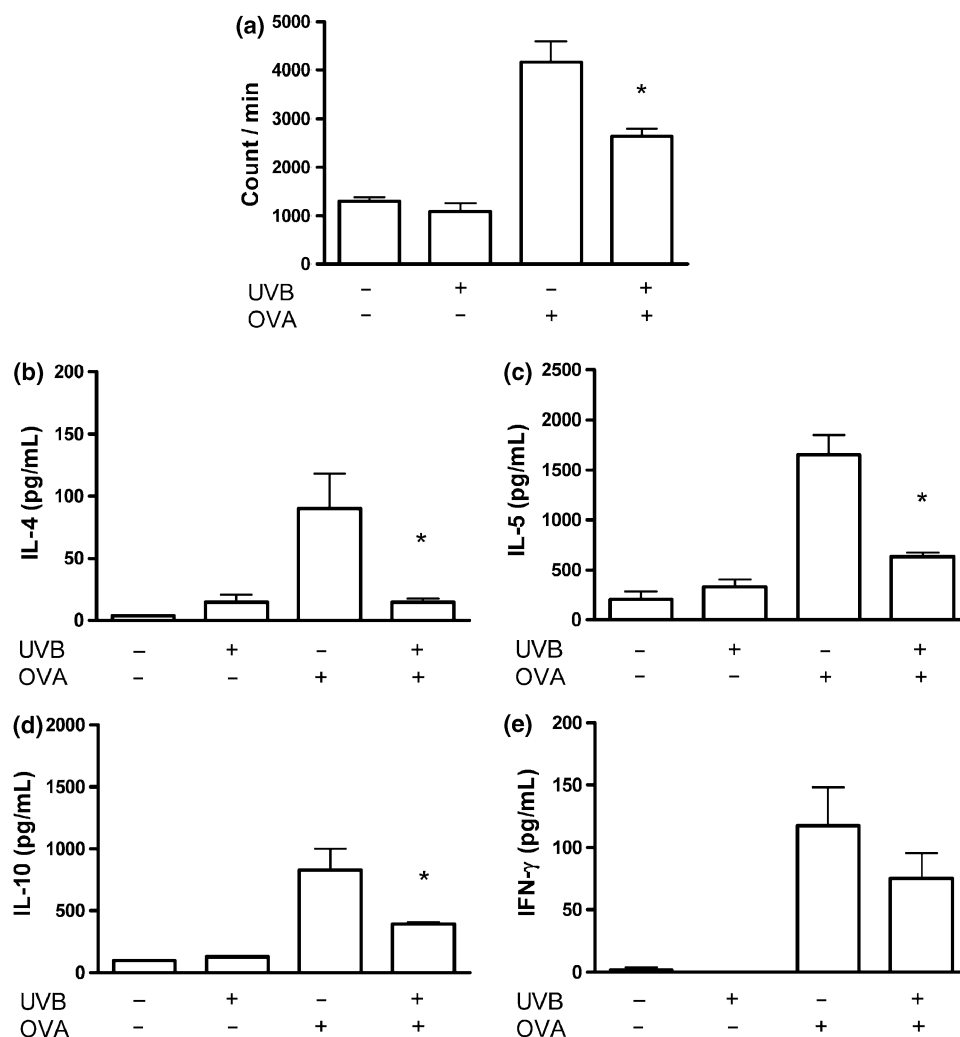


Fig. 4. (a) Proliferation and (b) IL-4, (c) IL-5, (d) IL-10 and (e) IFN- γ production by lung-draining lymph nodes (LDLN) cells from ovalbumin (OVA)-sensitized and -challenged mice that received 5×10^6 LDLN cells intravenously from ultraviolet B irradiated or non-irradiated, OVA-sensitized and -challenged donor mice. LDLN cells were cultured *in vitro* with or without OVA (100 μ g/mL). Bars show mean $\pm$ standard error of the mean, electron microscopy, $n = 6$ replicate cultures. * $P < 0.05$.

challenge in the recipient mice. To test this hypothesis, unfractionated single-cell suspensions were prepared from the LDLNs of UVB irradiated, OVA-sensitized mice 24 h after OVA aerosol challenge and 5×10^6 donor cells were transferred into the tail vein of naïve mice. As a control, the other naïve mice received 5×10^6 cells from the LDLN of non-irradiated, OVA-sensitized and challenged mice. The recipient mice were sensitized to OVA 24 h after cell transfer, boosted with OVA after 14 days and then OVA-challenged by aerosol after 21 days. LDLN cells from recipient mice that had received LDLN cells from UVB irradiated, OVA-sensitized and -challenged donors had significantly reduced proliferation and production of IL-4, IL-5 and IL-10 in response to OVA protein *in vitro* (Figs. 4a-d). IFN- γ production was not altered (Fig. 4e).

In contrast to the responses to OVA sensitization and challenge in UVB irradiated donor mice, mice that received LDLN cells from UVB irradiated, OVA-sensitized mice

and were subsequently sensitized and challenged with OVA did not have reduced IL-5 levels in the BALF. Levels of IL-4, IL-10 and IFN- γ in the BALF were also not reduced (data not shown). Furthermore, airway resistance (Fig. 5), tissue damping and tissue elastance in response to methacholine (data not shown) were not significantly different between recipients of LDLN cells from either UVB- or non-irradiated, OVA-sensitized and -challenged mice.

Antigen specificity of ultraviolet B-induced regulatory cells

To determine whether the transferred regulatory cells were OVA-specific, naïve recipients of unfractionated cells from UVB irradiated and non-irradiated, OVA-sensitized and -challenged mice were subsequently sensitized to a different antigen, β -lactoglobulin (BLG). BLG

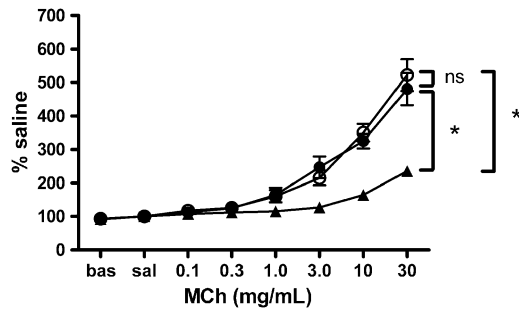


Fig. 5. Airway resistance in response to methacholine in recipients of lung-draining lymph nodes cells from ultraviolet B irradiated or non-irradiated ovalbumin (OVA)-sensitized and -challenged donor mice. Data show dose-response curves to inhaled methacholine (MCh) for non-irradiated, OVA-sensitized and -challenged mice (●), UVB irradiated, OVA-sensitized and -challenged mice (○) and naïve mice (▲). Bas, baseline value obtained by measurement of input impedance (Zrs) five times at 1 min intervals after stabilization of the mouse on the ventilator; sal, saline values obtained by measurement of Zrs five times at 1 min intervals after a 90 s saline aerosol; percent saline, percent increase of saline values for increasing doses of MCh. Data points show mean $\pm$ standard error of the mean, $n = 8$ mice. NS, not significant.

sensitization for allergic reactions in mice has been described previously by other investigators [9].

To confirm that UVB could suppress immune responses to BLG, mice were UVB irradiated and then sensitized to BLG 3 days later. After a further 7 days, LDLN were removed and proliferative responses by single-cell suspensions to BLG *in vitro* were investigated. Responses were significantly suppressed if the mice were UVB irradiated before BLG sensitization (Fig. 6a).

In the adoptive transfer experiments, mice receiving 5×10^6 cells from OVA-sensitized and -challenged mice were sensitized to BLG 24 h after cell transfer and responses of LDLN cells to BLG *in vitro* examined 7 days later. As a control, other naïve mice receiving 5×10^6 cells from the same OVA-sensitized and -challenged donor mice were sensitized to OVA and responses of LDLN cells to OVA *in vitro* were examined. In contrast to reduced responses to OVA, there was no difference in BLG-induced proliferation *in vitro* by LDLN cells from mice adoptively transferred with cells from UVB irradiated, OVA-sensitized and -challenged mice compared with those from mice that received LDLN cells from non-irradiated, OVA-sensitized and -challenged mice (Fig. 6b). LDLN cells from recipient mice sensitized to OVA had reduced proliferation *in vitro* as observed previously (Fig. 6c). These results suggest that the transferred regulatory cells were OVA-specific and were unable to regulate immune responses to the different antigen, BLG.

Discussion

Using the allergen OVA, a murine model of asthma was established that replicated many of the typical hallmarks

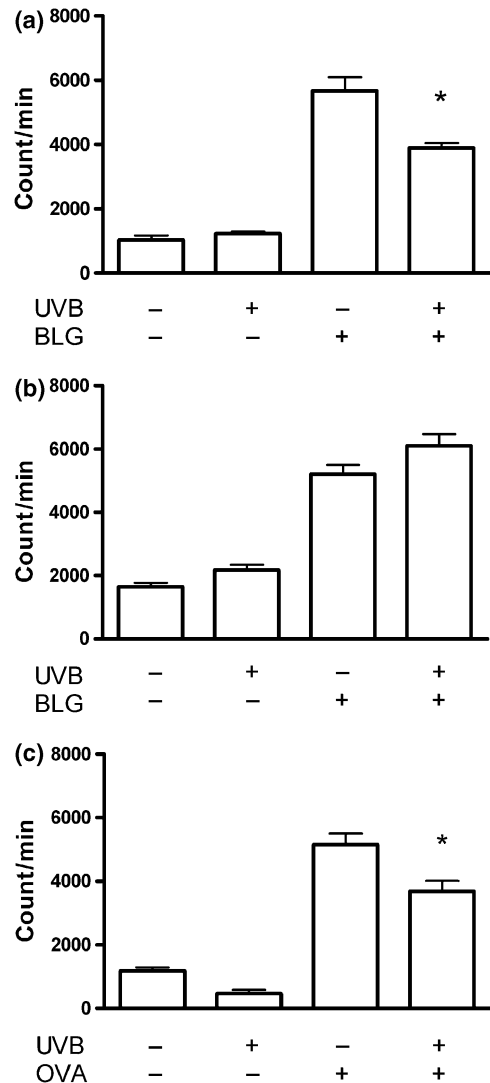


Fig. 6. (a) Proliferation in response to β lactoglobulin (BLG) *in vitro* by lung-draining lymph nodes (LDLN) cells from ultraviolet B (UVB) irradiated, BLG-sensitized mice. (b) and (c) Proliferative responses to (b) BLG and (c) ovalbumin (OVA) by LDLN cells from mice adoptively transferred with 5×10^6 unfractionated LDLN cells from UVB irradiated and non-irradiated, OVA-sensitized and -challenged donor mice, and then subsequently sensitized to (b) BLG or (c) OVA. LDLN cells were cultured *in vitro* with or without (a, b) BLG or (c) OVA (100 μ g/mL). Bars show mean $\pm$ standard error of the mean, $n = 6$ replicate cultures. * $P < 0.05$.

of asthma. When OVA-sensitized mice were challenged with OVA aerosol, there was significant AHR, increased cytokines in the BALF and significant levels of antigen-specific serum IgE. Upon UVB irradiation of mice and subsequent OVA sensitization and challenge, there was a consistent, significant reduction in airway resistance in response to methacholine, as well as significantly reduced levels of IL-5 in the BALF, a key cytokine in the asthmatic response in humans. OVA-specific proliferation and Th2 cytokine production by LDLN cells from UVB irradiated,

OVA-sensitized and -challenged mice were also reduced. These modified immune responses highlight the systemic effects of UVB delivered to the shaved dorsal skin of mice. The erythral dose of UVB to which the mice were exposed is realistic and is a dose that is equivalent to exposure of 20 min to midday sunlight in many areas of Australia for a Caucasian of average skin phototype.

Twenty-four hours after challenge with OVA aerosol, UVB irradiated mice had significantly suppressed airway responses to methacholine that were not significantly different from that observed in naïve mice. This is the first report of UV suppressing airway responses to methacholine using an *in vivo* technique capable of partitioning responses into components representing the airway and lung tissues separately. AHR is mediated by bidirectional interactions between resident dendritic cells in the conducting airways and activated memory T cells [10]. After aerosol challenge, antigen is taken up by airway mucosal dendritic cells, which then interact with memory T cells in the airways and translocate to the draining lymph nodes within 24 h after challenge, leaving activated T cells in the airways responsible for the development of AHR [11]. It is possible that UVB-induced antigen-specific regulatory T cells interact with the dendritic cells and memory T cells in the airways, resulting in modified antigen-presentation and curtailed development of AHR. In addition, activation of dendritic cells in the airways is restricted to the conducting airways, and not the lung parenchyma [11]. This correlates with significantly suppressed airway resistance (which represents responses in the larger airways) in UVB irradiated, OVA-sensitized and -challenged mice, but no significant effect on tissue damping or tissue elastance (which represent responses in the lung periphery).

Most previous studies of the effects of UVB have examined models of immunomodulation in which experimental antigens are applied to irradiated sites. From these models, it is proposed that UV damages Langerhans cells in the skin, which then migrate to the draining lymph nodes where they present antigen to T cells suboptimally such that antigen-specific regulatory T cells are induced [12]. The mechanisms involved in systemic immunosuppression, where antigens are applied to non-irradiated sites, are less clear. UVB-induced hapten-specific suppressor cells have been implicated in UVB suppression of contact hypersensitivity responses [13, 14]. In models of UV regulation of delayed-type hypersensitivity responses, UV-induced, IL-4-producing, antigen-specific natural killer-like T cells were described [15]. More recently, regulatory B cells have been described in the nodes of UVB irradiated mice that can alter antigen presentation by CD11c⁺ cells [16]. UVB irradiation of mouse skin, without subsequent antigen sensitization, also causes the induction or accumulation of antigen-non-committed CD4⁺ regulatory T cells in the lymph nodes draining the skin [17].

These cells regulate the proliferation of cells from OVA T cell receptor transgenic mice adoptively transferred into naïve BALB/c congenic mice subsequently sensitized to OVA [17]. In our OVA asthma model, UV-induced CD4⁺ cells may migrate to the site of antigen presentation (LDLN) where they suppress sensitization to OVA and induce the production of antigen-specific regulatory T cells.

When 5×10^6 LDLN cells from UVB irradiated, OVA-sensitized and -challenged donor mice were adoptively transferred into naïve recipients that were subsequently sensitized and challenged with OVA, responses to OVA by cells from the LDLN of the recipient mice were significantly reduced, indicating the transfer of a regulatory cell. When recipient mice were sensitized to BLG, and not OVA, responses to BLG *in vitro* by LDLN cells were not altered, indicating that the transferred regulatory cells were OVA specific. There were no significant UVB-associated differences in the number of CD3⁺ CD4⁺ CD25⁺ cells or FoxP3⁺ cells (regulatory T cells) in the LDLN, leaving the phenotype of the regulatory cells in our study yet to be identified.

While OVA-specific proliferation and cytokine production by LDLN cells from recipients of transferred cells from UVB irradiated donor mice were suppressed, the reduction of AHR and lowering of IL-5 in the BALF observed in the UVB irradiated donor mice were not transferred to the recipients. It is possible that the transferred regulatory cells localize to the LDLN in the recipient mice and are not able to relocate into the periphery to modulate immune responses in the airways and lung [18]. This homing may be due to the expression of molecules on the cell surface such as CD62L (L-selectin), a lymph node-homing receptor [19]. Unlike the well-described pathways of lymphocyte homing to the skin and gut, homing to the lung tissue is yet to be defined. Studies have identified cell surface markers present on lung T cells that are different from those described on skin or gut homing T cells, suggesting a distinct lung-homing pathway; however, the exact mechanisms and markers required for lung homing remain to be clearly defined. Chemokine receptors-3 and -4 have been implicated in mouse models [20], while human studies suggest that P-selectin and binding of lymphocyte function-associated antigen-1 to both intracellular adhesion molecules-1 and -2 are involved in homing to the bronchial mucosa [21, 22]. Definitive identification and up-regulation of these markers on the regulatory cells induced in our model would represent an elegant means of directing these cells to the lung compartment where they may be able to suppress AHR and IL-5 production in the BALF. Alternatively, the failure of the transferred regulatory cells to suppress AHR may be a downstream effect of their inability to suppress IL-5 production in the BALF, as IL-5 has been associated with the development of AHR [23].

In contrast to other investigations [24, 25], OVA-specific serum IgE titres were not significantly suppressed in UVB irradiated mice, although a trend supporting this was evident at Bleed 2. It is possible that in our model, titres of OVA-specific IgE were not sufficient to detect a significant difference between UVB irradiated and non-irradiated mice as the protocol only involved two i.p. sensitizations and a single aerosol challenge compared with other models [25] in which multiple i.p. sensitizations and multiple aerosols over a longer timeframe (approximately 7 weeks) were administered. In a further study [24], the mice were sensitized intranasally to a crude fungal extract and UV irradiation of mice also decreased the number of eosinophils in the BALF. This was not observed in our study, possibly as the single OVA aerosol did not induce a significant increase in the number of inflammatory cells in the BALF compared with naïve mice and thus limited any potential regulatory effects of UVB.

Over recent decades, there has been a significant global increase in the prevalence of asthma [26]. Epidemiological studies indicate that while the incidence of allergic diseases such as asthma has increased, our exposure to UV has decreased. Levels of vitamin D, produced in the skin upon UV exposure, have declined in recent years [4]. Using vitamin D levels as a surrogate measure of UV exposure, these data suggest a considerable decrease in the amount of UV exposure. Further, this is supported by the evidence that community intervention programmes that aim to reduce sun exposure are highly successful in increasing awareness of the risks associated with sun exposure and consequently increasing personal protection from UV radiation [27, 28]. The immunosuppressive effects of UV, coupled with epidemiological data and the findings of our study, suggest that reductions in sun exposure over recent decades may contribute to the concurrent increase in asthma prevalence. In this study, UVB exposure reduced the asthmatic phenotype in mice; thus UVB or its downstream mediators could provide a potential prophylactic approach to reducing the severity of asthma.

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