Effect of Both Ultraviolet B Irradiation and Histamine Receptor Function on Allergic Responses to an Inhaled Antigen


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Effect of Both Ultraviolet B Irradiation and Histamine Receptor Function on Allergic Responses to an Inhaled Antigen


Exposure of skin to UV radiation (290–320 nm) modulates the immune system, with most studies showing a suppression of Th1-driven immune responses. This study investigated the effects of UVB on Th2-associated immune responses using a murine model of allergic respiratory inflammation. C57BL/6, histamine receptor-1 knockout (H1RKO), and histamine receptor-2 knockout (H2RKO) mice were exposed to a single 4 kJ/m² dose of UVB (twice a minimal edemal dose) on shaved dorsal skin 3 days before intranasal sensitization with papain, a cysteine protease homologue of the dust mite allergen Der p 1. H1RKO mice demonstrated enhanced papain-specific inflammatory responses in the lung-draining lymph nodes (LDLNs), whereas the responses of H2RKO mice closely mimicked those of C57BL/6 mice. UVB irradiation 3 days before sensitization reduced in vitro papain-specific proliferation of LDLN cells of C57BL/6 and H1RKO mice but not H2RKO mice 24 h after challenge. The regulatory effect of UVB was transferred by adoptive transfer of unfractionated LDLN cells from UVB-irradiated, papain-sensitized C57BL/6 and H1RKO donor mice in naive recipients of the corresponding strain that were subsequently sensitized and challenged with papain. Additionally, UVB exposure suppressed papain-induced IL-5 and IL-10 production in vitro by LDLN cells from H1RKO mice but not from C57BL/6 mice or H2RKO mice. The results of this study demonstrate systemic immunomodulation of responses to intranasally delivered Ag by UVB irradiation and implicate a role for the H2 receptor in UVB-induced suppression of Ag-specific responses in the draining lymph nodes. The Journal of Immunology, 2007, 178: 2794–2802.

Exposure of skin to UV radiation causes erythema and initiates skin cancers and can have long-lasting effects on the immune system (1). These include not only localized immunomodulatory effects at the site of irradiation but also systemic immunity against nonskin-associated infectious agents, tumor Ags, and autoantigens (2). DNA damage (3) and UV-induced photodegradation of urocanic acid (4), a chromophore found in the stratum corneum, can play important roles in the initiation of UV-induced immunomodulation. Furthermore, because exposure to UVB initiates the production of 1α,25-dihydroxyvitamin D3 in the skin, this molecule has been implicated in UVB-induced effects on immune responses (5) and has been shown to inhibit Ag presentation by dendritic cells, and proliferation of and cytokine production by T cells (6).

UV exposure can alter expression both locally and systemically of inflammatory mediators such as IL-4 (7), IL-10 (8), IL-12 (9), TNF-α (10), and IFN-γ (11). Cytokines are important immune mediators and are indicative of the type of immune response elicited (12). Additionally, alterations to APCs (13) and the induction of regulatory T cells (14) have been implicated in pathways of control by UV of immune responses.

Studies of the mechanisms of UV-induced immunomodulation have examined the responses to Ags applied to either the irradiated site (local immunosuppression) or a distant nonirradiated site (systemic immunosuppression). These investigations have focused on contact hypersensitivity to haptons painted onto the skin (15) and delayed-type hypersensitivity to alloantigens, yeast Ags, or tumor Ags injected s.c. (16). UVB-induced suppression of delayed-type hypersensitivity responses to Borrelia burgdorferi (17), Leishmania major (18), Trichinella spiralis (19), and herpes simplex virus (20) has also been reported. These responses are characteristically driven by CD4+ Th1 cells. For local immunosuppression, Ag can be applied immediately after irradiation, whereas for systemic responses 3 days after UV exposure are required (21).

It is not clear from these studies whether UVB immunomodulation reflects a switch from a Th1-driven immune response to a Th2-associated response, or just a down-regulation of the Th1 response. Few studies have examined the effects of UV radiation on Th2-driven immune responses. In studies from one laboratory, BALB/c mice were UVB-irradiated (2.2 kJ/m² for four consecutive days) and then sensitized i.p. to OVA seven times over 13 days before daily challenges for 8 days with aerosolized OVA. UVB decreased OVA-specific serum IgE and IgG1 levels and nonspecific airway hyperresponsiveness. In these same UVB-irradiated mice, mitogen-driven IL-10 production by spleen cells in vitro was increased (8, 22). In another study using a fungal model of allergic lung disease, BALB/c mice were UVB-irradiated (8.2 kJ/m²) before involuntary aspiration of *Metarhizium*
Our study investigated the effects of UVB irradiation on immune responses in a murine model of allergic airway inflammation using as the sensitizing agent papain, a cysteine protease homologous to the house dust mite allergen Der p1. Intranasal delivery of this Ag induced high titters of Ag-specific serum IgE Ab and Th2 cytokine production and eosinophil influx in the BALF of sensitized mice. Unlike other models that incorporate i.p. sensitization and aerosol challenge (8), this protocol depended on intranasal delivery of Ag for sensitization, boost, and challenge. Furthermore, in contrast to other murine models of asthma (24–26), the Ag was delivered without adjuvants such as aluminum hydroxide, which skewed the immune response toward a Th2 (allergic) response.

The effect of UVB on papain sensitization in histamine receptor-1 (H1R) knockout (H1RKO) and histamine receptor-2 (H2R) knockout (H2RKO) mice was also studied. Histamine is involved in acute inflammatory processes and plays a role in the edema, vasodilation, contraction of smooth muscle, up-regulation of adhesion molecules and hypersecretion of mucus associated with the allergic asthmatic reaction (27). Additionally, histamine has also been described as a chemotactic factor, the effect of which may be inhibited by blockade of the H1R, leaving T cells unable to home to the sites of Ag exposure (27). UVB irradiation of skin results in degummation of mast cells and the release of histamine (28).

Previous studies have demonstrated that the application of H1R and H2R antagonists before and after UVB irradiation partially reversed the immunosuppressive effects of UVB and cis-urocanic acid (29, 30). To further examine the role of the H1R and H2R in UVB-induced immunosuppression, the papain-specific responses of these mice were investigated. This study identified histamine acting on the H1R as a modulator of papain-induced responses in both the lymph nodes draining the lung and at the lung mucosal surface. In contrast, histamine acting on the H2R was involved in the immunomodulatory effects of UVB irradiation. Furthermore, the induction by UVB of a regulatory cell, identified in adoptive transfer experiments between mice of the same strain, was able to suppress papain-induced proliferative responses in the lung-draining lymph node (LDLN) of recipient C57BL/6 and H1RKO, but not H2RKO, mice.

Materials and Methods

Animals

Eight-week-old female C57BL/6 mice and 22-wk-old male Sprague-Dawley rats were obtained from Animal Resources Centre (Murdoch, Western Australia). H1RKO and H2RKO mice (C57BL6 background) were derived as published previously (31) and bred at the Telethon Institute for Child Health Research. Female H1RKO and H2RKO mice were used at 8 wk of age. All experiments were approved by the Animal Experimentation Ethics Committee of the Telethon Institute for Child Health Research.

UV irradiation

The UV source was a row of six FS40 UVB lamps (Westinghouse) 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 (UV Products). As previously described (15, 29), a 2 × 4 cm dorsal patch of skin was cleanshaven. 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 mice were exposed to a single UVB dose of 4 kJ/m² on their shaved backs (approximately two minimal edemal doses) 3 days before initial sensitization. Control mice were shaved but were not exposed to UVB radiation.

Sensitization and challenge of mice to papain

Papain (Sigma-Aldrich) dissolved in water was delivered intranasally (1 µg in 40 µl) each day for 5 days. After 2 wk, the immune responses of mice were boosted by the same regimen. Two weeks postboost, the mice were challenged by daily intranasal delivery of papain (100 µg in 40 µl) for three consecutive days.

Preparation and culture of single-cell suspensions from LDLNs

The posterior mediastinal, tracheobrachial, and parathympic lymph nodes (LDLNs) were removed from mice and stored in glucose-potassium-sodium buffer (137 mM NaCl, 5.5 mM KCl, 23 mM NaHPO₄, 5.5 mM NaH₂PO₄, 2H₂O, and 11 mM glucose) supplemented with 5% FCS (GKN-5) and kept on ice. Nodes were pooled within experimental groups, physically disaggregated, and the cells were filtered through FCS-washed 100-µm nylon mesh with GKN-5. Cells were washed three times (1,300 rpm, 4°C, 7 min) in RPMI 1640 (Invitrogen Life Technologies) supplemented with 10% FCS, 2 mM t-glutamine, 50 µM 2-­ME, and 5 µg/ml gentamicin (RPMI 10). Single-cell suspensions in RPMI 10 were aliquoted in round-bottom, 96-well plates (10⁴ cells/200 µl/well; 6 replicates per treatment) and incubated with or without papain (10 µg/ml). Methyl-[³H]thymidine (Amersham Biosciences; 0.25 µCi; 10 µl/well) was added at 72 h, and cells were harvested at 96 h with [³H]thymidine incorporation measured as a measure of cellular proliferation. Culture supernatants were obtained at 96 h.

Phenotypic analysis of cells from LDLNs

LDLN cells were incubated with rat anti-mouse CD16/CD32 mAb (F4, block) in GKN supplemented with 0.2% BSA for 10 min at 4°C. Conjugated Abs (FITC-labeled CD8, FITC-labeled IA/IE, PE-labeled CD4, PE-labeled CD11c, allophycocyanin-labeled CD25, biotinylated CD3e, biotinylated CD19, FITC-labeled rat IgG2a, PE-labeled rat IgG2b, PE-labeled hamster IgG1, allophycocyanin-labeled rat IgG2b, biotinylated hamster IgG1, and biotinylated rat IgG2a; BD Biosciences) were added at optimized concentrations and incubated for 30 min on ice. Where a biotin Ab was used, cells were washed with GKN 0.2% BSA and then incubated with streptavidin-CyChrome conjugate (1/200 in GKN 0.2% BSA; BD Biosciences) for 30 min on ice. Cells were washed and resuspended in 300 µl of fixative buffer (1% formaldehyde in PBS). Flow cytometric analysis was performed using a FACSCalibur flow cytometer (BD Biosciences), and data were analyzed using FlowJo software (version 4.6.1; Tree Star).

Adoptive transfer of LDLN cells into naive recipients

The LDLNs were removed from papain-sensitized and challenged C57BL/6, H1RKO, and H2RKO donor mice (with or without UVB irradiation 3 days before sensitization) 24 h after the final challenge and stored in GKN-5 on ice. Nodes were pooled within experimental groups, physically disaggregated, and the cells were filtered through FCS-washed 100-µm nylon mesh with GKN-5. Cells were washed in saline (1300 rpm,
4°C, 7 min), resuspended in saline, and injected into the tail vein of naive recipient mice of the corresponding strain at 5 × 10^6 cells/mouse in a 200-μl volume. Twenty-four hours after adoptive transfer, recipient mice were then sensitized, boosted, and challenged with papain in accordance with the protocol described earlier. Twenty-four hours after the final challenge, LDLN were removed from recipient mice and single-cell suspensions cultured in vitro, with or without papain (10 μg/ml).

**Bronchoalveolar lung lavage and removal of lung tissue for histology**

Twenty-four hours after the final challenge with papain, all mice were euthanized and bronchoalveolar lavages 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 (3 × 400-μl aliquots) of 0.2% BSA in PBS was flushed into the lungs and recovered using 1-mm diameter polyethylene tubing (Boots) and 1-ml syringes. 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 (H&E and alcian blue stain for mucus-secreting cells).

**Inflammatory cell counts**

Lavage samples were centrifuged (1700 rpm, 4°C, 10 min) and the supernatant stored at 20°C until cytokine analysis. The pellet was resuspended and a total cell count performed. Cells (5 × 10^4 in 100 μl) were then centrifuged onto slides and stained using DIFF-Quik Stain Set 64851 (Lab-Aids) per the manufacturer’s instructions.

**Detection of cytokines in BALF and culture supernatants**

Nunc Maxisorp (96-well; Nunc) plates were coated with 100 μl of anti-IL-4, IL-5, IL-10, and IFN-γ capture Abs (BD Pharmingen) in carbonate buffer (100 mM Na_2CO_3, 100 mM NaHCO_3; pH 9.6) at a concentration of 2 μg/ml. After overnight incubation at 4°C, the wells were incubated with 200 μl of blocking buffer (1.0% BSA in Tris-HCl; pH 7.4) for 1 h at room temperature (RT) with shaking. Recombinant murine (rm)IL-4, rmIL-5, rmIL-10, and rmIFN-γ (BD Pharmingen) were used as standards and incubated, with test samples, for 2 h at RT with shaking. Anti-IL-4, anti-IL-5, anti-IL-10, and anti-IFN-γ-biotinylated Abs (BD Pharmingen) were added to the corresponding wells (2 μg/ml) and incubated for 1 h at RT with shaking. Streptavidin-europium (1/1000; Wallac) was added and incubated for 30 min at RT with shaking. Enhancer (75 μl; Wallac) was added and the plates incubated in the dark for 15 min at RT. Time-resolved fluorescence was measured at 615 nm on a Wallac Victor 2 counter (Wallac). The sensitivities of the assays were as follows: IL-4 (4 pg/ml), IL-5 (2 pg/ml), IL-10 (8 pg/ml), and IFN-γ (8 pg/ml).

**FIGURE 2.** Significant papain-induced IL-5 production in vitro by LDLN cells from papain-sensitized and -challenged mice. Papain-induced cytokines in culture supernatants of LDLN cells from nonirradiated, papain-sensitized and -challenged C57BL/6 mice cultured with (+) and without (−) papain (10 μg/ml) for 96 h. Bars show mean ± SEM (n = 3 replicate culture supernatants). * Significantly different from cultures without papain added in vitro.

**FIGURE 3.** Papain sensitization and challenge induced significant levels of IL-4 and IL-5, and increased cellular infiltration, into the BALF. A. Levels of IL-4, IL-5, IL-10, and IFN-γ, and (B) cell numbers in BALF taken from naive (−) and papain-sensitized and -challenged (+) C57BL/6 mice at 24 h after challenge. Bars show mean ± SEM (n = 5 mice). * Significantly different from naive; nd, not detectable.

**FIGURE 4.** UVB irradiation did not regulate papain-specific Ab levels in the serum of C57BL/6 mice. Levels of papain-specific (A) IgE, (B) IgG1, and (C) IgG2a in C57BL/6 mice at 2 wk post-sensitization (Bleed 1) and 2 wk postboost (Bleed 2). The effect of UVB irradiation before papain sensitization and challenge is shown. Bars show mean ± SEM for (n = 15 mice). nd, Not detectable.
**Table 1.** UVB irradiation did not alter the phenotype of LDLN cells in papain-sensitized and -challenged mice

<table>
<thead>
<tr>
<th></th>
<th>C57BL/6 Control</th>
<th>H1RKO Control</th>
<th>H2RKO Control</th>
<th>H2RKO UVB</th>
<th>H1RKO UVB</th>
<th>C57BL/6 UVB</th>
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<tr>
<td>Number of LDLN cells/mouse (%) (×10⁶)</td>
<td>8 ± 4</td>
<td>6 ± 2</td>
<td>7 ± 2</td>
<td>6 ± 2</td>
<td>5 ± 2</td>
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<td>CD3⁺/CD4⁺</td>
<td>21 ± 4</td>
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<td>25 ± 3</td>
<td>27 ± 8</td>
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<td>25 ± 3</td>
</tr>
<tr>
<td>CD3⁺/CD8⁺</td>
<td>19 ± 3</td>
<td>19 ± 2</td>
<td>17 ± 2</td>
<td>20 ± 1</td>
<td>18 ± 2</td>
<td>17 ± 1</td>
</tr>
<tr>
<td>CD4⁺/CD25⁺</td>
<td>2 ± 0.2</td>
<td>2 ± 0.5</td>
<td>2 ± 0.2</td>
<td>3 ± 0.0</td>
<td>2 ± 0.4</td>
<td>2 ± 0.1</td>
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<tr>
<td>CD25⁺ of CD3⁺CD4⁺</td>
<td>9 ± 2.2</td>
<td>10 ± 3</td>
<td>9 ± 3.3</td>
<td>8 ± 0.3</td>
<td>9 ± 2</td>
<td>8 ± 2</td>
</tr>
<tr>
<td>CD11c⁺/IA/IE⁺</td>
<td>7 ± 0.9</td>
<td>7 ± 0.7</td>
<td>6 ± 0.6</td>
<td>8 ± 1</td>
<td>7 ± 2</td>
<td>6 ± 2</td>
</tr>
<tr>
<td>CD19⁺/IA/IE⁺</td>
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<td>42 ± 2</td>
<td>43 ± 3</td>
<td>36 ± 6</td>
<td>40 ± 3</td>
<td>41 ± 5</td>
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*Phenotypic profile of LDLN cells removed 24 h after challenge of papain-sensitized and -challenged C57BL/6, H1RKO, and H2RKO mice, with and without UVB irradiation before sensitization. Values show means ± SEM (n = 3 experiments).*

**Papain-specific IgE detection via passive cutaneous anaphylaxis assay**

Two weeks after sensitization and boost, mice were bled (Bleeds 1 and 2, respectively). Serum samples were serially diluted in PBS. Sprague-Dawley rats were pacified by Rhodia Halothane (Merial Australia) and then sedated using 5.7% chloral hydrate (Sigma-Aldrich) 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 s.c. in marked rows. Twenty-four hours later, papain (4 mg/ml in water) was diluted 1/1 with 1% Evan’s blue dye solution administered i.v. After 20 min, areas of extravasation of the dye due to IgE-mediated mast cell degranulation were examined. The IgE titer of each sample was determined as the highest dilution of serum at which extravasation had occurred (expressed as −log₂ values).

**Detection of papain-specific IgG1 and IgG2a**

Nunc Maxisorp plates (96-well; Nunc) were coated with papain (0.1 μg) in carbonate buffer. After overnight incubation at 4°C, 200 μl of blocking buffer (0.5% BSA in Tris-HCl; pH 7.4) was added and incubated for 2 h at RT with shaking. A pool of serum samples known to be high in anti-papain IgG1 and IgG2a was used as a standard with undiluted serum assigned the value of 1.0. Dilutions of standards and test serum samples were incubated for 2 h at RT with shaking. Goat anti-mouse IgG1 and goat anti-mouse IgG2a-biotinylated Abs (Southern Biotechnologies Associates) were added (0.5 μg/ml) and incubated for 1 h at RT with shaking. Streptavidin-peroxidase (1/1000) was added and incubated for 30 min at RT with shaking. Enhancer (75 μl) was added, and the plates were incubated in the dark for 15 min at RT. Time-resolved fluorescence was measured on a Wallac Victor 2 counter (Wallac).

**Statistical analyses**

Levels of significance were calculated using one-way ANOVAs. The statistical program used was GraphPad Prism 4.00, where a p value of < 0.05 was considered to show a significant difference between groups. Each experimental group consisted of five mice, with each experiment performed at least three times. Data were shown as one of at least three representative experiments.

**Results**

**Sensitization of C57BL/6 mice to papain**

Single-cell suspensions from LDLNs prepared 24 h after the final papain challenge proliferated significantly in vitro in response to papain, providing evidence of sensitization (Fig. 1), and secreted increased levels of IL-5 into the culture supernatants (Fig. 2). In the BALF harvested 24 h after the final papain challenge, significantly higher levels of IL-4 and IL-5 were detected (Fig. 3A) and there was increased infiltration of lymphocytes, neutrophils, and eosinophils into the BALF with sensitization to papain (Fig. 3B). H&E staining of lung tissue from papain-sensitized mice showed cuffs of inflammatory cells around the bronchioles with frequent perivascular inflammation and increased numbers of inflammatory cells in the alveolar walls compared with naïve mice. There were a number of mucus-secreting cells (most likely goblet cells) lining the bronchiole with mucus plugs occasionally occluding the airways (data not shown). Papain-specific IgE and IgG1 were observed 2 wk postsensitization (Bleed 1) and 2 wk post-boost (Bleed 2). Papain-specific IgG2a was only detectable at 2 wk postboost (Fig. 4).

**Effect of UVB irradiation on papain sensitization of C57BL/6 mice**

Exposure to 4 kJ/m² UVB (equivalent to two minimal edematous doses) before sensitization significantly reduced papain-specific proliferation in vitro by LDLN cells 24 h after the last papain challenge (Fig. 1). In contrast, papain-induced cytokine production at 96 h by single-cell suspensions isolated from LDLNs was not affected by UVB irradiation (data not shown). There was no significant difference in the phenotypic profile of the LDLN cells from control and UVB-irradiated mice (Table 1). By comparison with nonirradiated, papain-sensitized mice, the total number of cells, the cellular profile, and levels of IL-4, IL-5, IL-10, and IFN-γ in the BALF were not affected by UVB exposure (data not shown). There were no observable effects of UVB on lung tissue histology in papain-sensitized and challenged mice (data not shown). UVB had no significant effect on papain-specific IgE, IgG1, or IgG2a titers in papain-sensitized animals. There was a tendency for increased papain-specific IgG1 and decreased IgG2a by Bleed 2; however, these changes were not statistically significant (Fig. 4).

**FIGURE 5.** Increased papain-specific proliferation by LDLN cells from papain-sensitized and -challenged H1RKO mice. Papain-induced proliferation by single-cell suspensions from LDLNs of papain-sensitized and -challenged C57BL/6, H1RKO, and H2RKO mice cultured with (+) and without (−) papain (10 μg/ml) for 96 h. Bars show means ± SEM (n = 6 replicate cultures). *p < 0.05.
Sensitization of H1RKO and H2RKO mice to papain

Studies using H1RKO mice have suggested that Th1-driven responses are enhanced by the action of histamine binding to the histamine-1 receptor (31), and that histamine may act as a chemotactic factor to facilitate T cell migration to the site of Ag exposure (27). Because histamine is released from mast cells in the skin upon UV irradiation (28), the responses of H1RKO mice were therefore studied to determine 1) the effect of papain sensitization on potentially Th2-susceptible mice and 2) the role of the H1R in UVB-induced immunosuppression. Sensitization of H2RKO mice was also investigated to examine the specificity of the changes for the deletion of the H1R and to determine whether this receptor is involved in 1) responses to papain and 2) immunosuppression by UVB.

The papain-specific proliferation by LDLN cells taken from H1RKO mice 24 h after challenge was significantly higher than that observed in LDLN cells from similarly treated C57BL/6 and H2RKO mice (Fig. 5). LDLN cells from papain-sensitized and -challenged H1RKO mice secreted significantly higher baseline (unstimulated) levels of IL-4, IL-5, and IFN-γ compared with those from C57BL/6 mice (Fig. 6) and higher levels of IL-4, IL-5, IL-10, and IFN-γ when stimulated with papain in vitro compared with single-cell suspensions of papain-stimulated LDLN cells from C57BL/6 mice. LDLN cells from H1RKO mice secreted significantly higher levels of IL-4, IL-5, and IL-10 in response to papain in vitro compared with unstimulated H1RKO LDLN cells. In contrast, there was reduced IFN-γ production by LDLN cells cultured with papain in vitro from H1RKO mice (Fig. 6). The responses of H2RKO mice were not significantly different from those observed in C57BL/6 mice (Fig. 5; cytokine production in vitro not shown). There was no significant difference in the phenotypic profile of LDLN cells between the different mouse strains (Table I).

Significantly higher levels of all cytokines (IL-4, IL-5, IL-10, and IFN-γ) were measured in the BALF of naive H1RKO mice compared with naive C57BL/6 mice. H1RKO mice sensitized and challenged with papain produced higher levels of IL-4 in the BALF than papain-sensitized and -challenged C57BL/6 mice, and lower levels of IL-10 and IFN-γ than naive H1RKO mice. The levels of IL-5, IL-10, and IFN-γ were similar in H1RKO and C57BL/6 mice that had been sensitized and challenged with papain (Fig. 7, A–D).

The infiltration of lymphocytes and eosinophils into the BALF of papain-sensitized and -challenged H1RKO mice was similar to the C57BL/6 mice, but there was a significant increase in neutrophils (Fig. 7 E). Papain-specific IgE (Fig. 7 F), IgG1, and IgG2a (data not shown) in H1RKO mice were similar to the responses of C57BL/6 mice. Responses in the BALF and serum of H2RKO mice were not significantly different from those in C57BL/6 mice (data not shown).

**Effects of UVB irradiation on papain sensitization in H1RKO and H2RKO mice**

UVB exposure (4 kJ/m²) before sensitization of H1RKO mice significantly reduced proliferation of LDLN cells in vitro (Fig. 8A).

**FIGURE 6.** LDLN cells from H1RKO mice produced significantly higher levels of IL-4, IL-5, IL-10, and IFN-γ in response to papain in vitro compared with LDLN cells from C57BL/6 mice. Papain-induced cytokine production at 96 h by LDLN cells from papain-sensitized and -challenged C57BL/6 and H1RKO mice. □, C57BL/6 mice; ■, H1RKO mice. Papain addition in vitro (+) or not (−) is shown (10 μg/ml). Bars show mean ± SEM (n = 3 replicate culture supernatants). *p < 0.05.

**FIGURE 7.** H1RKO mice had higher basal cytokine levels in the BALF compared with C57BL/6 mice, and higher IL-4 upon papain sensitization and challenge. Levels of (A) IL-4, (B) IL-5, (C) IL-10, (D) IFN-γ, and (E) cell numbers in BALF at 24 h after challenge. F, Papain-specific IgE in serum from naive (−) and papain-sensitized and -challenged (+) C57BL/6 and H1RKO mice, with (+) and without (−) UVB irradiation before sensitization. □, Represent C57BL/6 mice; ■, H1RKO mice. Bars show mean ± SEM (n = 5 mice). *p < 0.05.
However, UVB had no significant effect on these responses in H2RKO mice (Fig. 8B). In contrast to the C57BL/6 mice examined previously, UVB irradiation significantly decreased papain-induced IL-5 and IL-10 production by LDLN cells from H1RKO mice, with little effect on IL-4 and IFN-γ production (Fig. 9). UVB irradiation had no significant effect on cytokine production from LDLN cells from H2RKO mice (data not shown). There was no significant difference in the phenotypic profile of the LDLN cells from control and UVB-irradiated mice in either the H1RKO or the H2RKO mice (Table I). In both H1RKO and H2RKO mice, the total number of cells, the cellular profile, and levels of IL-4, IL-5, IL-10, and IFN-γ in the BALF were not affected by UVB exposure (data not shown). There was no significant difference in papain-specific IgE (Fig. 7F), IgG1, or IgG2a (data not shown) between UVB-irradiated and nonirradiated papain-sensitized H1RKO mice at either Bleed 1 or Bleed 2, similar to that observed in C57BL/6 mice. Levels of papain-specific IgE, IgG1, or IgG2a were also unaffected by UVB exposure in H2RKO mice (data not shown).

Adoptive transfer of LDLN cells from UVB-irradiated, papain-sensitized, and papain-challenged mice

UVB-induced, Ag-specific regulatory T cells have been previously reported (32). To determine whether the suppressive effects of UVB on papain-induced proliferation of LDLN cells were due to UVB-induced regulatory cells, 5 × 10^6 unfractionated LDLN cells from UVB- or nonirradiated, papain-sensitized and -challenged C57BL/6, H1RKO and H2RKO mice were adoptively transferred into naive mice of the same strain. These recipient mice were subsequently sensitized and challenged with papain, and 24 h after the final challenge, proliferative responses to papain by single-cell suspensions from the LDLN were assessed. Papain-induced proliferation of LDLN cells from recipients of unfractionated LDLN cells from UVB-irradiated, papain-sensitized and -challenged mice had significantly reduced proliferation compared with recipients of LDLN cells from nonirradiated, papain-sensitized and -challenged mice of the C57BL/6 and H1RKO mouse strains, similar to that observed in the respective donor mice (Fig. 10). The proliferation in response to papain by LDLN cells from H2RKO mice that received LDLN cells from UVB-irradiated H2RKO mice was not suppressed, also in accordance with responses observed in the H2RKO donor mice (Fig. 10).

Discussion

Sensitization and challenge intranasally with papain induced allergic airway inflammation in C57BL/6 mice. Papain-specific IgE, IgG1, and IgG2a Abs were detected in serum, and the BALF had increased cell numbers and levels of IL-4 and IL-5. These allergen-specific immune responses are similar to those observed in human inflammatory respiratory diseases such as asthma (33). Additionally, single-cell suspensions of LDLNs from mice sensitized and challenged with papain proliferated and produced IL-5 in response to papain in vitro. In contrast to other animal models of allergic respiratory disease that used the i.p. route for sensitization (8, 34), this model was completely intranasal for sensitization, boost, and challenge, and as such better mimics the route through which human respiratory sensitization to allergens occurs. Furthermore, this model was adjuvant-free and thus the immune responses observed were due entirely to Ag exposure and were not skewed by the use of adjuvants such as aluminum hydroxide.
Papain-specific proliferation in vitro was reduced for LDLN cells from UVB-irradiated, papain-sensitized, and papain-challenged C57BL/6 mice. The effect of UVB was greater in the H1RKO mouse, in which papain sensitization induced significantly higher levels of papain-specific proliferation and cytokine production (IL-4, IL-5, IL-10, and IFN-γ) in LDLN cultures and higher levels of IL-4 in the BALF compared with C57BL/6 mice. This increased reactivity of the H1RKO mice to papain reflects the absence of histamine enhancement of Th1 responses following its interaction with the H1R. This supports an earlier study in mice in which IFN-γ production was suppressed when the H1R was deleted and the secretion of Th2 cytokines including IL-4 and IL-13 was enhanced (31). A similar bias in cytokine production was also observed in splenocytes of H1RKO mice in an OVA asthma model, suggesting that the deletion of the H1R resulted in a systemic Th2 phenotype upon Ag exposure (27).

In the BALF of naive H1RKO mice compared with naive C57BL/6, higher basal levels of IL-4, IL-5, IL-10, and IFN-γ were measured. This supports the findings of Bryce et al. (27) who proposed an elevated constitutive activation of T cells within the airways of H1RKO mice. In papain-sensitized H1RKO mice, levels of IL-4, but not IL-5, IL-10, and IFN-γ, were significantly increased. No effect on cytokine production in sensitized H1RKO mice, other than a decrease in IL-5, had been described and was explained by an unresponsiveness of T cells from H1RKO mice to chemotactic histamine and thus an inability to migrate to sites of respiratory inflammation. As a result, whereas a systemic Th2 phenotype was promoted in the H1RKO mice, this effect was restricted to the spleen because a trafficking defect in the T cells prevented migration to the lungs (27). In our study, the increase in IL-4 at the respiratory mucosal surface may reflect the activation of resident inflammatory cells in response to papain and not necessarily migrating T cells.

It could be argued that once the basal level of inflammatory response to papain was increased in the H1RKO mice, further immunomodulatory effects of UVB were detected. Alternatively, whereas histamine may regulate immune responses to papain, it may also be involved in the mechanisms of UVB-induced immunomodulation. UVB irradiation causes the degranulation of mast cells in the skin, which results in the release of mediators, including histamine (28). Furthermore, mast cell prevalence in the skin also determines susceptibility of mice to UVB-induced systemic suppression of contact hypersensitivity responses (15). Histamine and its derivatives can act via the H2R to inhibit the immune response by the induction of T suppressor cell activity (35) and, in humans, have been shown to inhibit mixed T lymphocyte proliferation (36). More recently, in models of UVB control of contact hypersensitivity responses, H1R and H2R antagonists applied before and immediately after UVB irradiation partially reversed the effects of UVB (29). At the time, it was hypothesized that this modulation was a result of histamine binding to the H2R on dendritic cells and a subsequent increase in cAMP. In turn, elevated cAMP could suppress dendritic cell function via enhanced secretion of IL-10 and reduced MHC class II expression (37, 38). In addition, H2Rs on Langerhans cells are necessary to establish the process by which activated mast cells in murine skin induce Langerhans cell migration (39).

Removal of the H2R in mice had no effect on immune responses to papain. However, papain-induced proliferation and cytokine production in vitro by LDLN cells from H2RKO mice were not suppressed if the mice had been UVB-irradiated before sensitization. The inability of UVB to alter proliferative responses in the H2RKO mice while reducing those of C57BL/6 and H1RKO mice suggests that the H2R is important in the pathway by which UVB is immunomodulatory in the experimental animal and is a subject of further experimentation in our laboratory. Recently, the histamine receptor-4 (H4R) has been implicated in the mediation of allergic airway inflammation via regulation of CD4+ T cell activation (40). When the H4R was genetically removed or pharmaco logically inactivated by the use of H4R antagonists in a mouse OVA asthma model, there were reductions in lung inflammation and Th2 cytokine production from peribronchial T cells. Repeating our papain experimental protocol using H4R knockout mice would allow the definition of the role of the H4R in allergic sensitization or UVB-induced immunomodulation.

Few studies have investigated the effects of UVB irradiation on Th2-driven allergic responses. UVB before sensitization suppressed papain-specific responses by cells from the LDLNs of sensitized and challenged mice, and reduced IL-5 and IL-10 by LDLN cells from UVB-irradiated H1RKO mice. This decrease in IL-10 in response to papain contrasts with a previous report of increased IL-10 levels in the supernatants of mitogen-stimulated spleen cells taken from UVB-exposed animals sensitized with OVA (22). However, the responses measured in our study were Ag-specific and not to a nonspecific mitogen, making our findings more biologically relevant than those of previous investigations.

This study highlights the systemic suppressive effects of erythemal UVB irradiation of skin before the delivery of Ag to the respiratory tract. Although it has been reported that UV can regulate established immune responses (41), preliminary studies with this model suggest UV is more effective if delivered before sensitization (data not shown). The effects of UVB on responses to papain challenge may be explained by altered APCs that affect the process of sensitization (13), or the induction or accumulation of UVB-induced regulatory T cells that can control subsequent Ag sensitization (42). When LDLN cells from UVB-irradiated, papain-sensitized and -challenged C57BL/6 and H1RKO mice were adoptively transferred into naive mice of the corresponding strain, papain-induced proliferation by the LDLN cells from recipient mice was significantly suppressed compared with the responses of LDLN cells from mice that received cells from nonirradiated, papain-sensitized and -challenged mice of the same strain. Adoptive transfer of LDLN cells from UVB-irradiated, papain-sensitized and -challenged H2RKO mice had no effect on papain-induced proliferation by cells from the H2RKO recipient mice. Thus, for each strain, adoptive transfer of LDLN cells from UVB-irradiated mice resulted in a similar papain-induced proliferative response by LDLN cells from recipient mice compared with their respective donors. The adoptive transfer experiments indicate the induction by UVB of regulatory cells in C57BL/6 and H1RKO mice that can be adoptively transferred into naive mice and actively suppress subsequent papain sensitization in the recipient mice. Furthermore, the induction of these regulatory cells by UVB is dependent on a functionally active H2R.

When Ag is applied to the irradiated site, Ag-specific CD4+CD25+ regulatory T cells induced by UVB exposure have been reported (14, 43); however, in our experiments the proportion of CD4+CD25+ cells in the LDLN was not altered by UVB irradiation. CD4+CTLA4+T cells have also been implicated in UVB-induced immune tolerance (44). Furthermore, UVB-induced regulatory CD3+CD4+DX5+ NK T cells that suppressed tumor rejection and delayed-type hypersensitivity reactions have been described previously (45). Our adoptive transfer experiments indicate the presence of UVB-induced papain-specific regulatory cells that contribute to the reduced immune responses to papain. Consequently, the characterization of this UVB-induced regulatory cell and its Ag specificity is the focus of future work in this laboratory. This will be coupled with studies into whether the H2R is...
required in the donor or recipient animal for the immunomodulatory properties of UVB to be effective.

UVB exposure before sensitization had no effect on Ab production in serum or cellular infiltration, cellular profile, or cytokine production in the BALF. It has been demonstrated recently that UVB-induced regulatory T cells home to the lymph nodes and do not move out to the periphery (14). It is possible in this study that the effects of UVB are localized to the lymph nodes draining the site of Ag presentation and are unable to extend out to the lung tissues. The results obtained in this study contrast with the findings of other investigations that used different sensitization protocols (8, 23). In those studies BALB/c mice were used, compared with mice on a C57BL/6 background used in this study; this may account for some discrepancies in results because strain-dependent susceptibilities to UVB have been described previously (15, 46). The extent of the regulatory effects of UV on reported by other investigators also varied between different experimental models. For example, eosinophilic responses were unchanged by UVB irradiation in an OVA model (22) but significantly reduced in a respiratory model using a fungal extract (23). There is clearly a need to investigate the scope of UV-induced suppression; the decrease in lymph node cell proliferation and cytokine release contributes to this. The response to the fungal extract is more closely allied to this study because it used respiratory and not peritonsilal sensitization. The extract could, however, contain many undefined immunomodulators compared with the protein allergen used in this investigation.

The dose of UVB to which the mice were exposed in this study is equivalent to two minimal edemal doses in mice on a C57BL/6 background and approximately five minimal erythema doses in humans. However, individual phototype and sensitivity to UV radiation should be considered when estimating this value (47). This is analogous to ~30-min exposure to the midday sun in the more populated parts of Australia for people of average skin phototype. It should be noted, however, that whereas sunlight is made up of 1–4% UVB, this study used lamps emitting predominantly UVB wavelengths. As such, extrapolations of this data to natural sunlight should be undertaken with caution. Because many people are exposed to UVB radiation on a daily basis, it is important that the scope of the immunomodulatory effects of UVB and the mechanisms by which it acts are defined. This combination of UVB and allergy research provides a tool not only for the dissection and mechanisms by which it acts but can suppress the induction but can suppress the effector phase of contact hypersensitivity. J. Immunol. 172: 1036–1043.


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The dose of UVB to which the mice were exposed in this study is equivalent to two minimal edemal doses in mice on a C57BL/6 background and approximately five minimal erythema doses in humans. However, individual phototype and sensitivity to UV radiation should be considered when estimating this value (47). This is analogous to ~30-min exposure to the midday sun in the more populated parts of Australia for people of average skin phototype. It should be noted, however, that whereas sunlight is made up of 1–4% UVB, this study used lamps emitting predominantly UVB wavelengths. As such, extrapolations of this data to natural sunlight should be undertaken with caution. Because many people are exposed to UVB radiation on a daily basis, it is important that the scope of the immunomodulatory effects of UVB and the mechanisms by which it acts are defined. This combination of UVB and allergy research provides a tool not only for the dissection and recognition of how UVB modulates immune responses but also allows the elucidation of the mechanisms by which allergic responses can be regulated, thus providing a better understanding of allergy research.

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Disclosures

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