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Histamine involvement in UVB- and *cis*-urocanic acid-induced systemic suppression of contact hypersensitivity responses

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SUMMARY

Studies in experimental models have implicated histamine and prostanoids in ultra-violet B (UVB)- and *cis*-urocanic acid (UCA)-induced systemic immunosuppression. This study examined the hypothesis that UVB irradiation and *cis*-UCA suppressed contact hypersensitivity responses to hapten by induction of histamine, which in turn evoked a prostanoid-dependent component of immunosuppression. BALB/c mice were administered with a *cis*-UCA monoclonal antibody, a combination of histamine types 1 and 2 receptor antagonists, or indomethacin. Mice were sensitized to 2,4,6-trinitrochlorobenzene (TNCB) on their ventral surface 5 days after UVB irradiation, or *cis*-UCA or histamine administration. Ears were challenged with TNCB 5 days later. *Cis*-UCA antibody inhibited the suppressive effects of UVB by approximately 60% and confirmed that suppression of contact hypersensitivity responses by UVB was due, at least in part, to mechanisms involving *cis*-UCA. Histamine suppressed contact hypersensitivity responses and the effects of *cis*-UCA and histamine were not cumulative, suggesting that *cis*-UCA and histamine signal largely through the same pathway. The immunosuppressive effects of histamine were not affected by the *cis*-UCA antibody, consistent with the model that histamine acts downstream of *cis*-UCA. Administration of histamine receptor antagonists and indomethacin each approximately halved the UVB- and *cis*-UCA-induced systemic suppression of contact hypersensitivity responses. The effects of the reagents that inhibited the action of histamine and prevented prostanoid production were not cumulative, and suggested involvement in the same pathway. These results support the involvement of *cis*-UCA, histamine and prostanoids, in a sequence, in UVB-induced systemic suppression of contact hypersensitivity responses.

INTRODUCTION

Ultra-violet B (UVB) irradiation (wavelength 280–320 nm) is immunosuppressive and allows the growth of highly antigenic UV-induced tumours.[1,2] The immunosuppression can be both local and systemic, and results in reduced expression of contact hypersensitivity (CHS) and delayed-type hypersensitivity (DTH) responses to a variety of antigens in mice and humans. Examination of the suppression of CHS responses to haptens in experimental animals has allowed some dissection of the mechanisms of the UVB-induced effects.[2–4]

As less than 10% of UVB irradiation reaches the dermis,[5] it seems likely that a UVB photoreceptor exists in the epidermis which, in turn, initiates immunosuppressive signals. There is some evidence that DNA may be a UVB photoreceptor,[6,7] but *trans*-urocanic acid (deaminated histidine), a molecular species located superficially in the stratum corneum of the skin and which isomerizes to its *cis* form on UVB irradiation, has also been implicated in the mechanisms whereby UV irradiation generates systemic immunosuppression.[8–12] Skin painting or parenteral inoculation with *cis*-UCA can reduce systemic CHS responses and is associated with an alteration in antigen-presenting cell ability *in vivo*. However, *in vitro* studies have shown that the defect is not due to the direct effect of *cis*-UCA on antigen-presenting cells of the spleen.[8] It is generally hypothesized that UVB (and *cis*-UCA) regulates the production of immunomodulatory mediators by epidermal and dermal cells.[13–15]

Whole-animal experiments have previously determined that UVB- and *cis*-UCA-induced immunosuppression is, in part, indomethacin-reversible[3,16] and suggested prostanoids as important mediators of UVB- and *cis*-UCA-induced systemic suppression of CHS responses. In a study with skin explants and keratinocytes in culture, histamine was implicated in

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Abbreviations: CHS, contact hypersensitivity; DTH, delayed-type hypersensitivity; H1, histamine type 1; H2, histamine type 2; TNCB, 2,4,6-trinitrochlorobenzene; UCA, urocanic acid; UVB, ultra-violet of wavelength 280–320 nm.

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signficant UVB-induced production of prostaglandin E_2 (PGE_2), 6-keto $PGF_1\alpha$ and $PGF_2\alpha$.[17] We found that *cis*-UCA was not stimulatory for PGE_2 production by keratinocytes in culture, but synergized with histamine for increased PGE_2 production.[16] Furthermore, *cis*-UCA, but not *trans*-UCA, could increase the sensitivity of keratinocytes to very low concentrations of histamine.[16] These studies implicated histamine as another important mediator in UVB- and *cis*-UCA-induced systemic immunosuppression.

The hypothesis to be tested was that the immunomodulatory effects of UVB were due, at least in part, to the activity of *cis*-UCA and that histamine was involved in the biological effect of *cis*-UCA. Finally, the effects of UVB, *cis*-UCA and histamine were manifest by increased prostanoid production by, for example, cells of the skin. The effects of inhibitors of the activity of *cis*-UCA and histamine, and the production of prostanoids, were examined in a murine model of UVB-induced systemic immunosuppression in which the sensitization phase involved hapten application to a non-irradiated site 5 days after irradiation. The immunosuppressive effects of UVB were compared directly with those of *cis*-UCA, and similarly the effects of *cis*-UCA were compared with those of histamine. Using the same *cis*-UCA antibody, both positive[18] and negative[19,20] involvement of *cis*-UCA in UVB-induced suppression of CHS responses has been reported recently. Previous investigations of the effect of histamine receptor antagonists on UVB- and *cis*-UCA-induced immunosuppression[21] had been performed with the hypothesis that *cis*-UCA may act through receptors for histamine. The studies presented herein support the involvement of *cis*-UCA in UVB-induced systemic suppression of CHS responses, and the involvement of histamine in the prostanoid-associated component of UVB- and *cis*-UCA-induced immunosuppression.

MATERIALS AND METHODS

Mice

Pathogen-free female BALB/c mice, aged 8–12 weeks, were obtained from the Animal Resource Centre of the South Australian Department of Agriculture. All experiments (five animals/group) were performed according to the ethical guidelines of the National Health and Medical Research Council, the Commonwealth Scientific and Industrial Research Organization and the Australian Agricultural Council.

UV irradiation

The UV source was a bank of FS40 sunlamps (Westinghouse Corp., Pittsburgh, PA) emitting a broad band of UV, 250–360 nm, with 65% of the output in the UVB range (280–320 nm). A PVC plastic film was used to screen out wavelengths <290 nm. The dose rate was monitored using a UVX radiometer with a UVX-31 sensor (Ultraviolet Products Inc., San Gabriel, CA).

For irradiation of the mice, a uniform dorsal area (8 cm^2) was clean-shaven, the ears protected with black adhesive insulation tape and the mice housed in individual compartments of perspex cages. The sunlamps were held 20 cm above the cages. Mice were UVB-irradiated 5 days prior to induction of CHS.

In the mice irradiated and administered the histamine receptor antagonists or indomethacin, immediately before irradiation (18 kJ/m^2/mouse), 50 μl of a commercial alcohol-based SPF 15 + sunscreen (containing 4-tertiary-butyl-4-methoxy dibenzoyl methane, 2-ethyl-hexyl-paramethoxy cinnamate and 2-hydroxy-4-methoxy-benzophenone) was evenly applied to the shaved dorsal area of each mouse. The sunscreen components had maximum absorption at 358, 311 and 325 nm, respectively.[22] The sunscreen inhibited the acute skin damage induced by UV irradiation but minimally reduced the suppression of CHS responses in UVB-irradiated mice. In nine previous experiments in which this sunscreen was applied to the backs of mice and UVB-irradiated (18 kJ/m^2), the CHS response was reduced by $42 \pm 9\%$ (mean $\pm$ SD). In five previous experiments in the absence of sunscreen,[16] the CHS response was reduced by $50 \pm 6\%$.

Urocanic acid and histamine

The *trans* isomer of UCA was purchased from Sigma Chemical Co. (St Louis, MO) and UV-irradiated. *Cis*-UCA was purified from irradiated *trans*-UCA by ion-exchange chromatography.[23] For experimentation, both isomers were dissolved in mouse–osmolality–phosphate–buffered saline (PBS) (330 mOsm/kg H_2O) at 1 mg/ml. For the animal experiments, 0·2–200 μg was injected subcutaneously (s.c.) into the backs of mice (control mice received an equal volume of PBS), 5 days prior to induction of CHS. Histamine (0·2–200 μg/mouse) (Sigma) was administered in an identical manner to that described for *cis*-UCA.

Injection of the cis-UCA antibody

The production of the monoclonal antibody to *cis*-UCA has been described elsewhere[24] and was used as previously optimized.[19] Mice were injected intraperitoneally (i.p.) with 300 μl of 1/500 dilution of the *cis*-UCA antibody ascitic fluid (equivalent to 0·1 μg IgGl) 4 h prior to UVB irradiation or *cis*-UCA administration. As controls, an equal number of mice was injected with 300 μl mouse–osmolality PBS or with PBS containing 0·1 μg X63, a non-specific isotype-matched control antibody.[25]

Histamine receptor antagonists

The histamine receptor antagonists were administered to mice on each of four consecutive days, starting one day before UVB-irradiation or administration of histamine or *cis*-UCA. The antagonists were administered in two equal doses/day approximately 10 h apart. The histamine type 1 (H1) receptor antagonist, cyproheptadine[26] (Merck, Sharp & Dohme, South Granville, Australia), 330 μg/mouse/day, was administered i.p. in 5% ethanol in PBS. The histamine type 2 (H2) receptor antagonist was cimetidine (Sigma), 100 μg/mouse/day.[21] In preliminary studies, neither cyproheptadine or cimetidine, at the doses defined above, alone consistently reversed UVB-induced systemic suppression of CHS responses. Thus, the combination of an H1 with an H2 receptor antagonist, which was shown in preliminary studies to inhibit UVB-induced immunosuppression consistently (data not shown but illustrated in Fig. 4b), was administered.

Indomethacin

Pellets containing 0·05 mg indomethacin in a biodegradable carrier (Innovative Research of America, Toledo, OH) were implanted into mice s.c., by trochar, at the base of the neck[3,27]

4 days prior to UVB exposure. The pellets released indomethacin at a constant rate (2·4 μg/day) over a 21-day period. Previous studies have shown that a daily dose of 1·25–2·5 μg indomethacin blocks prostaglandin synthesis.[3] Placebo pellets contained the biodegradable carrier alone. There was no evidence of gastrointestinal bleeding in mice receiving the indomethacin pellets.

Assay of CHS

Mice (five animals/group) were sensitized on the shaved ventral skin with 100 μl freshly prepared 5% (w/v) 2,4,6-trinitrochlorobenzene (TNCB, Tokyo Kasei Kogyo Co. Ltd., Tokyo, Japan) in acetone. Five days later, and after coding the identities of the mouse groups, a CHS response was elicited by applying 10 μl freshly prepared 1% TNCB in acetone to each of the ventral and dorsal surfaces of both ears. Twenty-four hours after challenge, the ear thickness was measured with a micrometer (Mitutoyo Corp., Tokyo, Japan) and the extent of ear swelling for each mouse was calculated by subtracting the ear thickness before challenge. From this value was subtracted the mean swelling measured in mice that were challenged, but not sensitized, with TNCB (0·03 mm).

Expression of results and statistical analysis

For each *in vivo* study, the mean values ± SD for changes in the ear thickness upon challenge with TNCB for all five mice in a group were calculated. For some treatments, the mean results from individual studies was used to calculate the mean value ± SD for *n* experiments. Within an experiment, a multiple comparison procedure employing a one-way analysis of variance and Fisher's test was used to determine the statistical significance of differences between experimental and control groups. For comparison of mean values from multiple experiments, a paired Student's *t*-test was used. Probabilities less than 0·05 were considered significant.

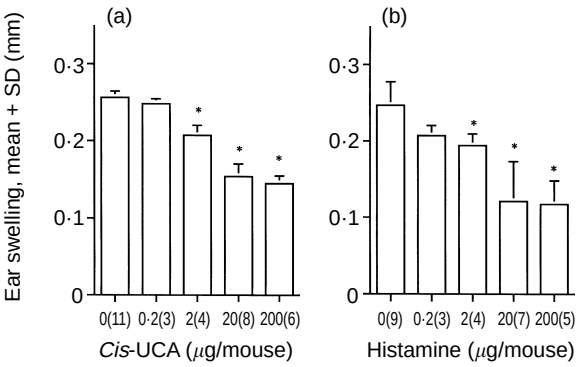

Figure 1. The effect of *cis*-UCA and histamine on CHS responses in BALB/c mice. Mice were administered s.c. different doses of (a) *cis*-UCA or (b) histamine in a volume of 200 μl mouse-osmolality PBS. Five days later a CHS response to TNCB was assessed. Mean ear swelling + SD is shown for (*n*) experiments. An asterisk represents a significant difference in ear swelling due to *cis*-UCA or histamine administration.

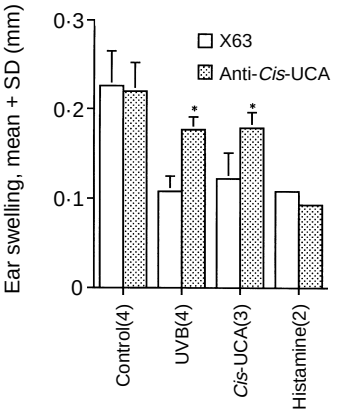

Figure 2. The effect of a *cis*-UCA antibody on UVB- and *cis*-UCA-induced suppression of CHS responses. Mice were injected i.p. with 0·1 μg of the *cis*-UCA antibody, or with the isotype-matched control antibody, X63, 4 h before UVB irradiation (18 kJ/m^2) or *cis*-UCA or histamine administration (20 μg s.c.). The CHS response to TNCB was assessed commencing 5 days after UVB irradiation or *cis*-UCA administration. The mean ear swelling + SD in each group for (*n*) experiments is shown. An asterisk represents a significant difference in ear swelling by similarly treated mice administered X63 or the *cis*-UCA antibody.

RESULTS

The involvement of cis-UCA and histamine in the mechanisms by which UVB irradiation suppresses CHS responses

Figure 1a demonstrates the effect of increasing doses of *cis*-UCA on suppression of CHS responses in BALB/c mice. The dose-dependent immunosuppressive effects of histamine on CHS responses in BALB/c mice are shown in Fig. 1b.

To determine the relationship of studies with *cis*-UCA to those with UVB irradiation, 0·1 μg *cis*-UCA antibody or the control antibody, X63, was administered 4 h before UVB irradiation (18 kJ/m^2) in each of four experiments (Fig. 2). It was first demonstrated that the effect of injecting X63 was not different from injecting an equal volume of PBS (data not shown). UVB was found to suppress the CHS response in mice pre-treated with X63 by a mean of 52%. The suppression was reduced to 20% in mice pre-treated with the antibody to *cis*-UCA, and this change in the CHS response induced by administration of the *cis*-UCA antibody was significant ($P = 0·02$). In one of the experiments, the *cis*-UCA antibody totally blocked the suppressive effects of UVB irradiation. Indeed, when the results of the four experiments were combined, the CHS response of UVB irradiated mice pre-treated with the *cis*-UCA antibody was not significantly different from that of unirradiated animals pre-treated with the same antibody. The specificity of the antibody was confirmed in three experiments in which mice were administered 20 μg *cis*-UCA s.c. in place of UVB irradiation 4 h after *cis*-UCA antibody (Fig. 2). In mice pre-treated with X63, *cis*-UCA administration caused a 46% suppression of the CHS response. Administration of the *cis*-UCA antibody changed this to 19% suppression of the CHS response ($P = 0·05$). In contrast, in two separate experiments, administration of the *cis*-UCA antibody had no effect on the suppressive properties of histamine (Fig. 2).

That *cis*-UCA and histamine may signal systemic immunosuppression by similar pathways is suggested by the lack of

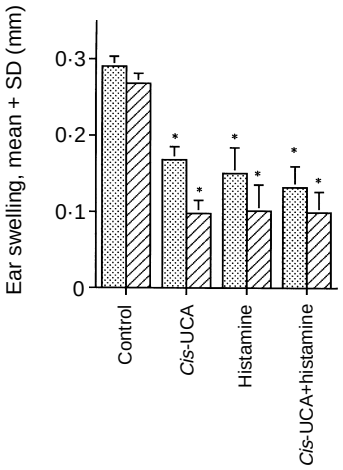

Figure 3. The effect of histamine, without and with *cis*-UCA, on CHS responses in BALB/c mice. Mice were administered *cis*-UCA (200 μg) or histamine (200 μg), alone or together. Five days later, a CHS response to TNCB was assessed. The mean ear swelling + SD for the five mice/group in each of two experiments (different shadings) is shown. An asterisk represents a significant difference in the ear swelling by the *cis*-UCA-, histamine- or *cis*-UCA with histamine-treated mice and control mice.

cumulative effects of maximal doses of histamine (200 μg) with *cis*-UCA (200 μg) in each of two experiments (Fig. 3).

The effect of histamine receptor antagonists on UVB- and *cis*-UCA-induced systemic suppression of CHS responses

To investigate the role of histamine in UVB- and *cis*-UCA-induced suppression of contact hypersensitivity to TNCB, cyproheptadine, 330 μg/mouse/day, with cimetidine 100 μg/mouse/day, were administered to BALB/c mice on each of four consecutive days, namely days -1, 0, $+1$ and $+2$ of UVB irradiation (18 kJ/m^2). This dosage of histamine receptor antagonists on days -1, 0, $+1$ and $+2$ of s.c. administration of histamine was sufficiently potent to inhibit by 77% the effects of 200 μg histamine (Fig. 4a). The histamine receptor antagonists had no significant effect on CHS responses in non-irradiated mice (Fig. 4b). The inhibitory effects on UVB-induced suppression of CHS responses of the combination of an H1 with an H2 receptor antagonist were significant in each of the five experiments (Fig. 4b). However, the response by irradiated, receptor-antagonist-treated mice was significantly less than that measured in non-irradiated receptor-antagonist-treated mice, indicating that the reversal of immunosuppression was only partial. Similarly, in five experiments, administration of identical doses of cyproheptadine and cimetidine as used in the UVB-irradiated mice diminished the significant inhibitory effects of *cis*-UCA on CHS responses (Fig. 4b).

The effect of indomethacin on UVB- and *cis*-UCA-induced systemic suppression of CHS responses

The effect of indomethacin or placebo pellets administered 4 days prior to UVB irradiation or *cis*-UCA administration is shown for six and nine experiments, respectively, in Fig. 5. As previously published,[16] indomethacin caused a consistently

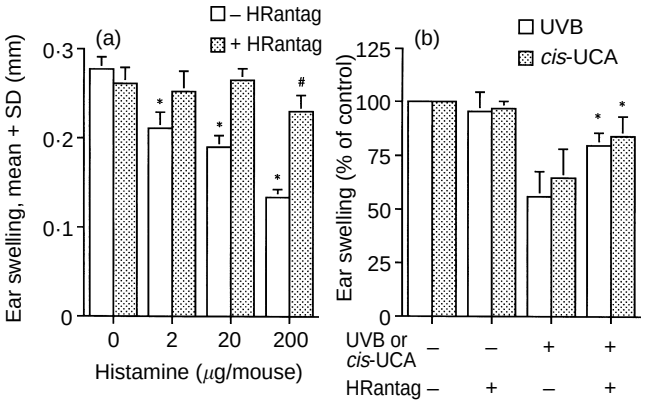

Figure 4. The effect of histamine receptor antagonists (HRantag) on immunosuppression due to (a) histamine and (b) UVB and *cis*-UCA. Cyproheptadine (330 μg/mouse/day) plus cimetidine (100 μg/mouse/day) was injected into mice on each of four consecutive days, starting from 1 day prior to (a) histamine (0–200 μg) or (b) UVB irradiation (18 kJ/m^2) or *cis*-UCA administration (200 μg). The CHS response to TNCB was assessed commencing 5 days after administration of histamine or *cis*-UCA, or UVB-irradiation. In (a) the ear swelling for mice from a single experiment (five animals/group) is shown. An asterisk and a hash represent a significant effect of histamine in the absence and presence, respectively, of histamine receptor antagonists (HRantag). In (b) ear swelling in each experiment was normalized with the swelling of control mice calculated as 100%; the mean swelling + SD for mice from five experiments is shown. In (b) an asterisk represents a significant difference in the ear swelling by the UVB-irradiated or *cis*-UCA-administered mice that were administered the histamine receptor antagonists (HRantag) and both (a) those UVB-irradiated or administered *cis*-UCA, and (b) those non-irradiated but administered the HRantag.

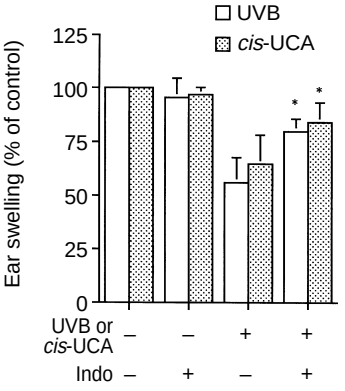

Figure 5. The effect of indomethacin on UVB- and *cis*-UCA-induced immunosuppression. Indomethacin (Indo) or placebo pellets were implanted s.c. into mice 4 days prior to UVB-irradiation (18 kJ/m^2) or *cis*-UCA administration (200 μg). The CHS response to TNCB was assessed commencing 5 days after UVB irradiation or *cis*-UCA administration. Ear swelling in each experiment was normalized, with the swelling of control mice calculated as 100%. The mean swelling + SD for mice from six and nine experiments, respectively, is shown. An asterisk represents a significant difference in the ear swelling by the UVB-irradiated or *cis*-UCA-administered mice that were administered indomethacin and both (a) those UVB-irradiated or administered *cis*-UCA, and (b) those non-irradiated but administered indomethacin.

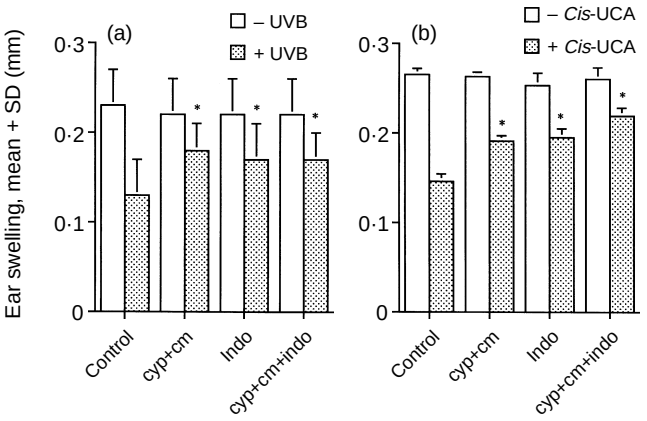

Figure 6. The effect of cyproheptadine with cimetidine, and indomethacin, alone or together, on UVB- and *cis*-UCA-induced immunosuppression. Cyproheptadine (cyp; 330 μg/mouse/day) and cimetidine (cm; 100 μg/mouse/day) were injected into animals on each of four consecutive days, starting from one day prior to (a) UVB irradiation (18 kJ/m^2), and (b) *cis*-UCA administration (200 μg). Indomethacin (indo) or placebo pellets were implanted s.c. into mice 4 days prior to UVB irradiation or *cis*-UCA administration. The CHS response to TNCB was assessed commencing 5 days after UVB irradiation or *cis*-UCA administration. In (a) the mean ear swelling + SD for three experiments is shown. In (b) the mean ear swelling for five mice per group + SD in a single experiment is shown. An asterisk represents a significant difference in the ear swelling by the UVB-irradiated or *cis*-UCA-administered mice that were administered the antagonists and/or indomethacin and both (a) those UVB-irradiated or administered *cis*-UCA, and (b) those non-irradiated but administered the antagonists and/or indomethacin.

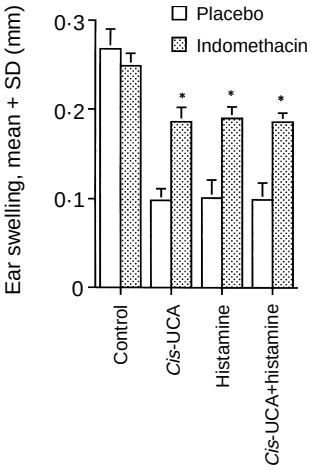

Figure 7. The effect of indomethacin on the immunosuppression induced by *cis*-UCA, histamine, and *cis*-UCA with histamine. Indomethacin or placebo pellets were implanted s.c. into mice (five animals/group) 4 days prior to s.c. injection of 200 μg of *cis*-UCA, 200 μg histamine or *cis*-UCA with histamine, or an equal volume of diluent (control). The CHS response to TNCB was assessed commencing 5 days after injection of *cis*-UCA, histamine or *cis*-UCA with histamine. The mean ear swelling + SD for five mice in each group is shown. An asterisk represents a significant difference in the ear swelling by the indomethacin-treated, *cis*-UCA-, histamine- or *cis*-UCA with histamine-treated mice and both (a) those administered indomethacin alone, and (b) those treated with *cis*-UCA, histamine or *cis*-UCA with histamine.

significant, partial reversal of UVB- and *cis*-UCA-induced suppression of CHS responses.

The effect of histamine receptor antagonists with indomethacin on UVB- and *cis*-UCA-induced suppression of CHS responses

The cumulative inhibitory effects of histamine receptor antagonists (cyproheptadine with cimetidine) and indomethacin on UVB- and *cis*-UCA-induced immunosuppression were investigated in three and two experiments, respectively. For the three experiments summarized in Fig. 6a, UVB irradiation reduced ear swelling by 41% ($P = 0{\cdot}02$). Both cimetidine with cyproheptadine, and indomethacin, significantly increased the CHS responses in UVB-irradiated mice (to 84% of the control response, $P = 0{\cdot}05$, and 80% of the control response, $P = 0{\cdot}02$, respectively). However, the combination of all three drugs did not further reverse the effect of UVB irradiation, the CHS response in this group being 77% of, and significantly different to, the control response ($P = 0{\cdot}04$). In Fig. 6b, the lack of a significant cumulative effect of the histamine receptor antagonists and indomethacin on *cis*-UCA-induced suppression of CHS responses is shown for a representative of two experiments. Thus, there was a significant partial reversal of UVB- and *cis*-UCA-induced immunosuppression by the histamine receptor antagonists. A similar reversal was detected following administration of indomethacin. However, there was no cumulative effect of the histamine receptor antagonists and indomethacin, consistent with a direct relationship between histamine and its action, and prostanoid production.

The effect of indomethacin on the systemic suppression of CHS responses caused by histamine or *cis*-UCA, alone or in combination

Both histamine and *cis*-UCA (200 μg/mouse) suppressed CHS responses by an extent similar to that caused by the co-administration of both reagents (Fig. 3). The partial reversal of the immunosuppression by indomethacin was similar in all treatment groups; Fig. 7 shows the effects of indomethacin on the mice of the second experiment of Fig. 3.

DISCUSSION

Many studies have implicated *cis*-UCA in the mechanisms by which UVB suppresses CHS responses in mice (reviewed in refs. 9, 28). However, in this study of systemic suppression of CHS responses by UVB irradiation and *cis*-UCA, it was important to validate this hypothesis and verify that by studying the actions of *cis*-UCA, we were investigating, at least in part, the mechanisms of action of UVB irradiation. A monoclonal antibody to *cis*-UCA[19,24] was used to confirm that UVB-induced suppression of CHS responses was due, at least in part, to mechanisms involving *cis*-UCA. The change in UVB-induced effects on CHS responses from 52% to 20% suppression in mice pretreated with the *cis*-UCA antibody could be considered a minimum, as a single intraperitoneal injection of the antibody was given 4 h prior to UVB irradiation. It is unknown how much of the antibody reached the epidermis, the site of *cis*-UCA formation. We also do not know the half-life of the antibody. It should be noted that the dose of antibody administered also reversed the suppressive

activity of 20 μg *cis*-UCA to a similar extent (Fig. 2). Outcomes of previous studies using this antibody have varied, with one study using C57BL/6 mice implicating *cis*-UCA in UVB–suppression of CHS responses.[18] The other studies[19,20] concluded that this antibody reversed UVB-induced changes to DTH responses but not CHS responses, but this may reflect the species of mice used previously (C3H/HeN) or the availability of the antibody at the epidermis at a crucial time of *cis*-UCA activity. In one study,[20] an amount of *cis*-UCA antibody nine times that used in the present study was administered to each mouse. We have found blocking effects of the *cis*-UCA antibody similar to those shown in the present study (which examined systemic CHS responses in BALB/c mice) on UVB suppression of local CHS responses in BALB/c mice, and on systemic CHS responses in C57BL/6J mice receiving 2 kJ/m^2 UVB (data not shown).

With respect to the amounts of *cis*-UCA used in these studies (0·2–200 μg/mouse; Fig. 1a), it has been estimated previously that the concentration of *cis*-UCA in non-irradiated murine epidermis was 0·2 μg/cm^2, with values of 15 μg/cm^2 after UVB irradiation with 96 mJ/cm^2,[8] or 80 μg/mouse measured after 42 mJ/cm^2 UVB.[29] With respect to histamine, we have estimated that the dermis of 8 cm^2 dorsal skin of BALB/c mice (approximately 270 μm deep, approximately 40 mast cells per mm^2 horizontal section of dermis and 3–5 pg histamine/mast cell)[30] contains approximately 8 μg histamine. This must be considered a crude estimation of available histamine as not all histamine would be released from degranulating mast cells. In addition, histamine may be released by murine keratinocytes.[30] Significant suppression of CHS responses was detected with 2 μg *cis*-UCA/mouse and 2 μg histamine/mouse (Fig. 1).

The relevance and the magnitude of involvement of histamine in UVB- and *cis*-UCA-induced systemic suppression of CHS responses and UVB- and *cis*-UCA-induced prostanoid production, respectively, was the focus of this study. Experiments with the *cis*-UCA antibody confirmed that histamine acts downstream of *cis*-UCA in signalling immunosuppression. Both UVB- and *cis*-UCA-induced suppression of CHS responses was partially reversed by administration of receptor antagonists to histamine (Fig. 4), which may be released by degranulating mast cells[31] or by keratinocytes.[30] Indeed, this study presents the first direct evidence that histamine receptor antagonists inhibit UVB irradiation-induced suppression of systemic CHS responses. In further experiments investigating prostanoid involvement in these responses, the possibility of additive effects of indomethacin, a cyclo-oxygenase inhibitor, with histamine receptor antagonists was investigated (Fig. 6). The lack of any cumulative effects by the combination of reagents suggested that the histamine receptor antagonists inhibited the receipt of stimuli for initiation of synthesis of the same prostanoids, the production of which was blocked by indomethacin. Thus, in the whole animal, the prostanoid-associated component of UVB immunosuppression was linked with the activity of histamine.

The extent of histamine involvement in the mechanisms responsible for *cis*-UCA-induced suppression of CHS responses was not clearly determined. Whether sufficient histamine receptor antagonists and/or indomethacin were present at the site of histamine action and/or prostanoid production to be fully effective may be an issue. The histamine receptor antagonists successfully blocked approximately 80% of the action of 200 μg histamine administered subcutaneously. Optimization of the effects of indomethacin were investigated in a previous study;[16] both one and two pellets of indomethacin (releasing 2·4 and 4·8 μg/day, respectively) blocked UVB- and *cis*-UCA-induced suppression of CHS responses by approximately 50%. The experiments with the histamine receptor antagonists suggested that a significant proportion of the response due to *cis*-UCA was attributable to the actions of histamine. CHS responses were not different in mice treated with relatively high doses of *cis*-UCA and histamine and those administered histamine alone and, thus, did not provide evidence for a histamine-independent component of *cis*-UCA-induced immunosuppression. Indomethacin was able to reverse only partially the immunosuppressive properties of histamine, which suggested that histamine may act through an additional prostanoid-independent pathway.

Cis-UCA is structurally related to histamine[32] and it might be concluded from the experiments with the histamine receptor antagonists that *cis*-UCA bound to histamine receptors. The receptor for *cis*-UCA remains uncharacterized. However, there is functional evidence that the receptor for *cis*-UCA is unlike that for histamine. H1 and H2 receptor antagonists blocked the effects of histamine but were unable to suppress the direct effects of *cis*-UCA on human monocytes.[22] Histamine, but not *cis*-UCA, was able to stimulate PGE_2 production *in vitro* by human keratinocytes.[16] In addition, histamine, but not *cis*-UCA, was able to stimulate PGE_2, IL-6 and IL-8 production by human dermal fibroblasts (P.H. Hart *et al.*, unpublished data). Thus, it is hypothesized that the histamine receptor antagonists inhibited the effects of histamine, rather than blocked *cis*-UCA binding to its receptor.

This study did not identify keratinocytes as the source of prostanoids in UVB-induced immunosuppression; however, keratinocytes are the major structural cells of the epidermis and *trans*-UCA is found in the stratum corneum. As the majority of UVB light does not penetrate beyond the epidermis, keratinocytes reside at sites adjacent to *cis*-UCA formation. Furthermore, keratinocytes express H1 and H2 receptors[17] and both types of receptor play a role in mediating the response by keratinocytes to *cis*-UCA and histamine.[16] Thus, the need for blockade of both receptor types by cyproheptadine and cimetidine, respectively, for optimal inhibition was expected. The source of the bioactive histamine was not confirmed. A report of *cis*-UCA as a mast cell degranulating agent in mice[31] led us to hypothesize that *cis*-UCA directly or indirectly causes mast cell degranulation and contributes to, and perhaps is totally responsible for, UVB-induced mast cell degranulation, particularly as mast cells are located in the dermis and at a site to which minimal UVB irradiation transmits.

The mechanism(s) by which skin-derived prostanoids signal systemic suppression of CHS responses and reduce immune responses to antigens applied to distant body sites is not known. The vasodilatory effects of PGE_2 and PGI_2[33] may allow other immunomodulatory epidermal cytokines access to the vasculature where they can have systemic effects. Prostanoids have been hypothesized to 'force sequestration of sensitized effector cells in peripheral lymph nodes.[3] By inhibiting interleukin-12 (IL-12) production, prostanoids may

play a crucial role in the development of either a T-helper 1 (Th1)-like or a Th2-like immunological response.[34]

A previous study showed the importance of prostanoids in *cis*-UCA-induced systemic immunosuppression in mice. The present study confirms *in vivo* the validity of considering histamine as a critical mediator in the pathway(s) responsible for UVB- and *cis*-UCA-induced systemic suppression of CHS responses. Furthermore, these studies with whole animals suggest that histamine functions in the induction of systemic suppression of CHS responses, at least in part, by induction of prostanoid production.

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