

Topical glucocorticoids application induced an augmentation in the expression of IL-1 α while inhibiting the expression of IL-10 in the epidermis in murine contact hypersensitivity

K. IGAWA, H. YOKOZEKI, Y. MIYAZAKI, K. MINATOHARA, T. SATOH, I. KATAYAMA* and K. NISHIOKA

Department of Dermatology, Tokyo Medical and Dental University, School of Medicine, Yushima, Bunkyo-ku, Tokyo and

*Department of Dermatology, Nagasaki University, School of Medicine, Nagasaki, Japan

Summary

Background The repeated application of glucocorticoids (GC) on the skin augmented the inflammatory response of both allergic and irritant contact dermatitis in our studies.

Objective In order to further clarify the mechanism of such an augmentation of contact hypersensitivity (CHS), we investigated the modulatory effects of cytokines in the epidermis after the administration of GC at challenged sites in CHS.

Methods Diflucortolone valerate was applied to BALB/c mice on alternate days for a total of nine times. On day 12, they were contact sensitized with dinitrofluorobenzene (DNFB). Next, on day 17, one day after the last application of GC, they were challenged with DNFB on the ear. The whole challenged ear lobes were removed after a hapten challenge and then were analysed by the RT-PCR method or underwent an immunohistochemical analysis. To clarify the modulatory effects of cytokines *in vivo*, DNFB sensitized mice pre-treated with GC were injected with rIL-10, IL-1 receptor antagonist (ra) and anti-IL-1 α monoclonal antibody (mAb) and thereafter were challenged with DNFB.

Results A RT-PCR analysis has demonstrated IL-10 mRNA to be detected in the challenged skin of non-GC-pretreated mice but not in that of GC-pre-treated mice after challenge. On the other hand, the expression of IL-1 α mRNA in the challenged skin of mice pretreated with GC was more strongly detected than that in mice without GC-pretreatment. Furthermore, an immuno-histochemical analysis in the challenge showed the expression of IL-10 in the skin showed the expression of IL-10 in the challenged epidermis of the non-GC-pretreated mice but not in the GC-pretreated mice and IL-1 α was also strongly expressed in the epidermis of the GC-pretreated mice. A subcutaneous injection of anti-IL-1 α mAb or IL-1 ra inhibited the augmented CHS reaction in the GC-pretreated mice. A subcutaneous injection of rIL-10 also inhibited the augmentation of the CHS reaction in the GC-pretreated mice; however, no such inhibition was observed in the non-GC-pretreated mice.

Conclusion These results indicated that both an up-regulation of IL-1 α production and the inhibition of the IL-10 production in the epidermis at the challenged skin sites in the GC-pretreated mice appear to play a critical role in the GC-induced augmentation of murine CHS.

Keywords: glucocorticoid, keratinocytes, IL-1 α , IL-10, contact hypersensitivity

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Introduction

In the past decade, new types of skin manifestations such as persistent erythema on the face and glucocorticoid (GC)-resistant skin inflammatory response, have been recognized in patients with atopic dermatitis (AD), especially in Japan [1–3] and the USA [4]. Such manifestations are frequently observed in adult patients with AD after the long-term use of topical GC, and have been called adult type-AD. It is speculated that these skin manifestations are induced by either a withdrawal effect of topical GC or a direct and/or indirect effect of topical GC [1–3]. To clarify whether topical GC modulates cutaneous inflammatory reactions, we previously found topical GC to augment both the contact hypersensitivity (CHS) reaction and irritant reactions in mice [5]; however, the mechanism of this enhanced cutaneous inflammatory response induced by GC still remains unclear.

The mechanism of immunosuppression in GC has been steadily elucidated to a greater extent in recent reports [6]. For example, the anti-inflammatory properties of GC have been reported to be mediated by an inhibition in the recruitment of neutrophils and monocytes/macrophages into inflammatory sites [7] and an inhibition in both cytokine production [8,9] and action [10]. However, several reports have demonstrated a paradoxical effect of GC in cytokine receptor expression. GC can up-regulate the expression of IL-1 receptor (R) [11], IL-6R [12] and IFN- γ R [13]. Pretreatment with hydrocortisone also significantly augmented LPS-stimulated IL-6 and TNF- α expression [14]. In addition, pretreatment with GC was also shown to be associated with an enhanced T cell effector function upon subsequent antigenic stimulation [15]. We recently reported the augmentation of CHS to only be observed after the epicutaneous application of GC, but not after the intrapenetratorial injection of GC [5]. These results indicated that the topical but not the systemic application of GC might augment cutaneous inflammation through a possible modulation of the local cytokine production from epidermal cells. Very recently, we also demonstrated that GC enhanced the production of IL-1 α but not that of either TNF- α or IL-10 secreted by a chemically stimulated keratinocyte cell line *in vitro* [16]. We therefore investigated the modulatory effect of IL-1 α and IL-10 derived from keratinocytes on the augmented CHS reactions induced by GC pretreatment in the present study.

Materials and methods

Animals

Female BALB/c mice, from 7 to 8 weeks of age, were purchased from the Sankyo Co., Tokyo, Japan. They were fed *ad libitum* at our animal centre until use.

Chemicals

The following chemicals were commercially obtained; dinitrofluorobenzene (DNFB), trinitrochlorobenzene (TNCB) and croton oil (Nakarai Chemicals, Kyoto, Japan), oxazolone (Aldrich Chem., Milwaukee, WI, USA), mouse-rIL-10 (Pepr Tech. Inc. from the Cosmo Chem. Co. Ltd, Tokyo, Japan), mouse-rIL-1 α (Genzyme Co. Ltd, Cambridge, MA, USA), antimouse-IL-10 monoclonal antibody (mAb) (Upstate Biotech. Inc., New York, USA) and antimouse IL-1 α mAb (Genzyme), FITC-labelled antihamster IgG (EY Lab. Inc., USA), and IL-1 ra (China Newtech Development & Trade Corp., Beijing, China). In addition, the 0.1% Diflucortron valerate solution was kindly donated from by Schering, Tokyo, Japan.

Treatment with glucocorticoid (diflucortron valerate)

BALB/c mice were painted nine times with 50 μ l of 0.1% diflucortron valerate solution on their shaved dorsal skin every other day from day 0 to day 16.

Sensitization and challenge with haptens

The mice were sensitized with 50 μ l of either 0.5% DNFB, 7% TNCB or 3% oxazolone in a 4 : 1 acetone-olive oil mixture on the dorsal skin pretreated with the GC application on day 12. On day 17, the mice were challenged on the right ear of each mouse with 20 μ l of either 0.15% DNFB, 0.8% TNCB or 0.3% oxazolone in a 4 : 1 acetone-olive oil mixture on day 17. The increment of ear thickness was measured using a dial thickness gauge (Peacoc 2, Ozaki Engineering Co., Tokyo, Japan) at 24, 48, 72 h after challenge and expressed in units of 10^{-2} mm. The change in ear thickness was calculated as ear thickness at the indicated time post-challenge minus the ear thickness prior to challenge. At least three independent experiments were performed using five mice in each group. Typical results were shown for each experiment.

Subcutaneous injection of cytokines and mAbs

The mice pretreated with topical diflucortron valerate and contact sensitization were subcutaneously injected in the right ear with either 30 μ l of rIL-10 in PBS (2, 20 or 200 ng/ear), 30 μ l of anti-IL-1 α mAb in PBS (15 or 150 ng/ear) or 30 μ l of IL-1ra in PBS (0.05, 0.5 or 5 ng/ear) 1 h prior to the challenge. Next, 30 μ l of phosphate-buffered saline (PBS, pH 7.4) or isotype IgG1 diluted in PBS were injected into the left ear. Both the right and left ears were challenged with 20 μ l of 0.15% DNFB.

Reverse transcription-coupled PCR (RT-PCR)

At various times after the challenge with the hapten, the ear lobes were removed, washed with cold water containing 0.2% DEPC immediately, embedded in OCT-compound, frozen in liquid nitrogen and stored at -80°C until use. In some experiments, the epidermis was separated from the dermis by incubation in 0.5 M ammonium thiocyanate at 37°C for 20 min. Total cellular RNA was extracted from $80 \times 10 \mu\text{m}$ cryostat sections of the ear lobe by the guanidinium isothiocyanate method using RNAzol^R (Biotex CS 101). Twenty-two microlitres of RT mixture was prepared by mixing 4 μl of $10 \times$ buffer (100 mM Tris-HCl, pH 8.3, 500 mM KCl, 15 mM MgCl_2), 4 μl of hexanucleotide mixture (62.5 A₂₆₀ U/mL, Boehringer Mannheim, Mannheim, Germany), 4 μl of 100 mM dithiothreitol, 2 μl of dNTP (2.5 mM each), 4 μl of ribonuclease inhibitor (20 U/ μL , TaKaRa, Ohtsu, Japan), 12 U of reverse

transcriptase (RAV-2; TaKaRa), and water. The RT mixture was made for all samples and aliquoted at 22 μl /tube with 18 μl of total RNA (800 ng). The tubes were vortexed, spun briefly and left for 10 min at room temperature and then at 42°C for 60 min. RT was inactivated at 95°C for 5 min. Samples were stored at -70°C . A polymerase chain reaction (PCR) was performed by the previously reported method.

The PCR mixture contained 5 μl reverse-transcribed RNA (100 ng total RNA), 5 μl of $10 \times$ buffer, 4 μl of dNTP (2.5 mM each), 2.5 μl of 20 μM up-primer and 2.5 μl of 20 μM down-primer, 0.4 μl of 5 U/ μl Taq polymerase (TaKaRa), and 30.6 μl of water. Oligonucleotide primers specific for β -actin, IL-1 α and IL-10 were used. The sequences of cytokine specific primer pairs, 5' and 3', were as follows:

β -actin:

5'-GGAGAAGATCTGGCACCACACC

5'-CCTGCTTGCTGATCCACATCTGCTGG

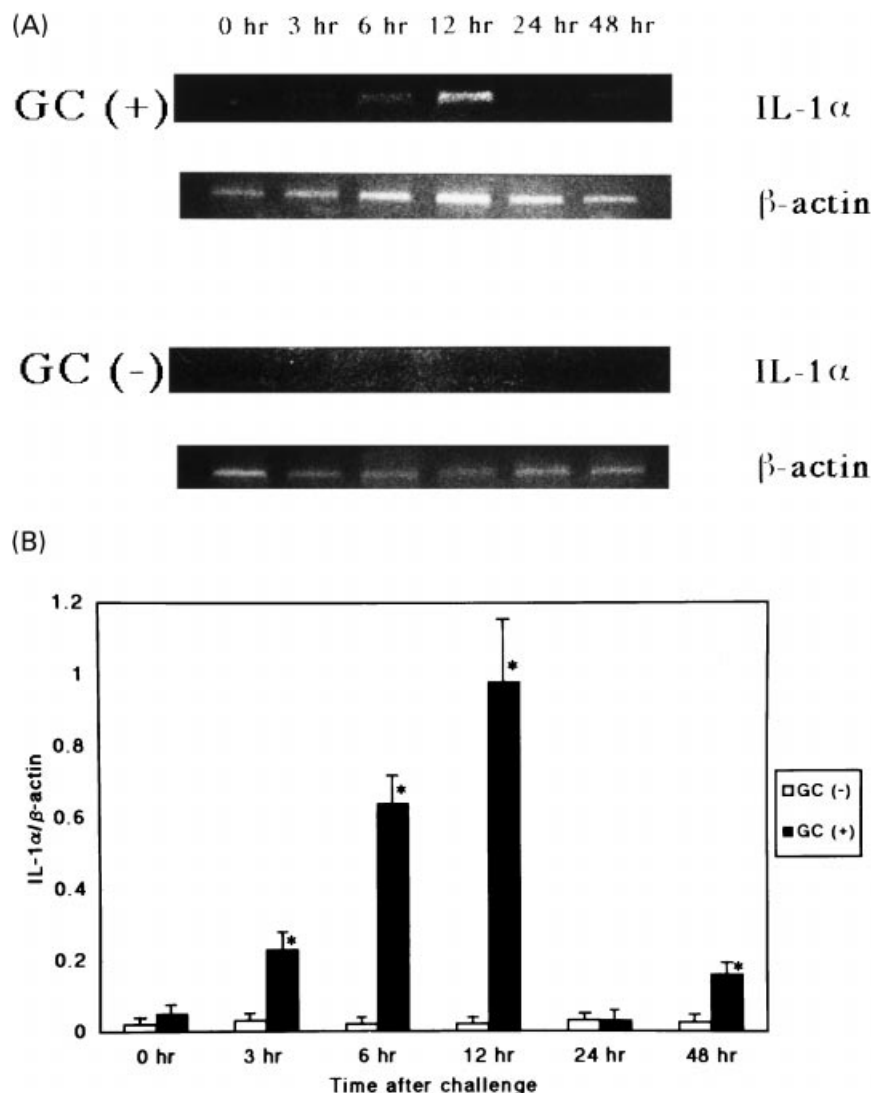


Fig. 1. Upregulation of the expression of IL-1 α mRNA in the GC-pretreated skin of mice. As previously described, challenged ear skin specimens were taken at 0, 3, 6, 12, 24 and 48 h after the DNFB challenge. To determine the expression of IL-1 α , total RNA was extracted from the skin which was then reverse transcribed to cDNA, and PCR was conducted using primers for IL-1 α and β -actin. The PCR products were resolved on agarose gel and photographed under UV light (A). The results presented in this figure are representative of three independent experiments. The relative amount of PCR products was determined by scanning of the PhosphorImager using a laser densitometer. For relative quantification, the densitometric value of IL-1 α was normalized to that of β -actin (B). The presented data represent the mean values $\pm$ SD of three independent experiments. * P , 0.05, indicates a significant difference compared to the non-GC-pretreated mice.

IL-1 α :

5'-AAGATGTCCAACCTTCACCTTCAAGGAGAGCCG

5'-AGGTCGGTCTCACTACCTGTGATGAGTTTGG

IL-10:

5'-ATGCAGGACTTTAAGGGTTACTTGGGTT

5'-ATTTTCGGAGAGAGGTACAAACGAGGTTT

PCR amplification was performed employing a 35-cycle programme (β -actin: 25 cycle) consisting of denaturation, annealing and extension; 1 min of denaturation at 94° C, 1 min of annealing at 60° C, and 2 min of extension at 72° C for β -actin; 1 min of denaturation at 94° C, 1 min of annealing at 62° C, and 2 min of extension at 72° C for IL-1 α ; 1 min of denaturation at 94° C, 1 min of annealing at 55° C, and 2 min of extension at 72° C for IL-10. The PCR

products were electrophoresed in 1.2% agarose gel. The gel was stained with ethidium bromide (1 μ g/mL) and visualized under a UV transilluminator. Photographs were taken and negatives were traced on a densitometric optical scanner (EPA-3000, Chemiway Co., Tokyo, Japan). The signal strength of each cytokine was normalized using β -actin signals.

Immunohistochemical analysis

An ear lobe of each mouse was removed and frozen with OCT-compound in liquid nitrogen. 8 μ m sections were incubated with either antimouse-IL-10 mAb or antimouse-IL-1 α mAb overnight at 4° C. This incubation was followed

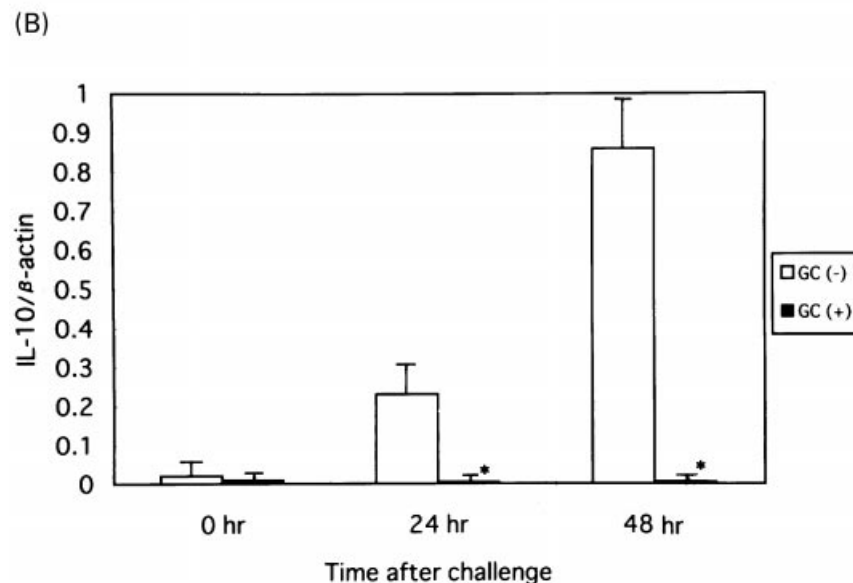
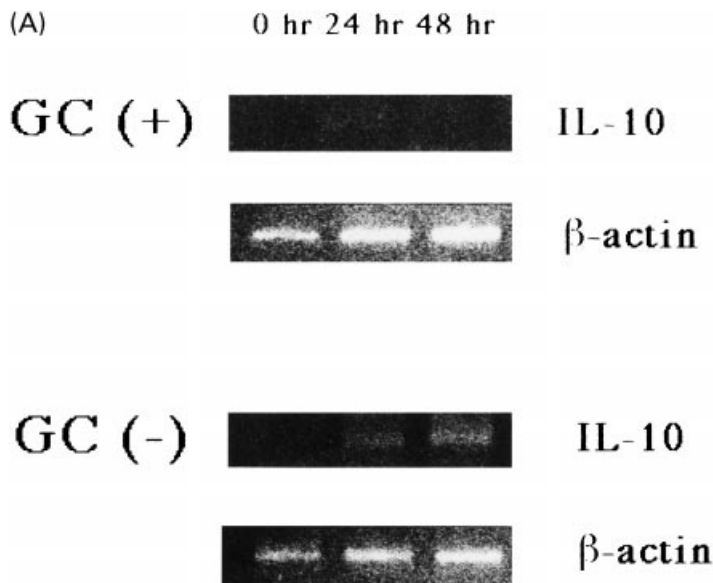


Fig. 2. Down-regulation of the expression of IL-10 mRNA in the GC-pretreated skins of mice. As previously described, challenged ear skin specimens were taken at 0, 24 and 48 h after the DNFB challenge. To determine the expression of IL-10, the challenged pinnae were taken at 0, 24 and 48 h after the DNFB challenge. Total RNA was extracted from the skin which was then reverse transcribed to cDNA, and PCR was conducted using primers for IL-10 and β -actin. PCR products were resolved on agarose gel and photographed under UV light (A). The results presented in this figure are representative of three independent experiments. The relative amount of PCR products was determined by scanning Phoroloid negatives using a laser densitometer. For the relative quantification, the densitometric value of IL-10 was normalized to that of β -actin (B). The presented data represent the mean values $\pm$ SD of three independent experiments. **P*, 0.05, indicates a significant difference compared to the non-GC-pretreated mice.

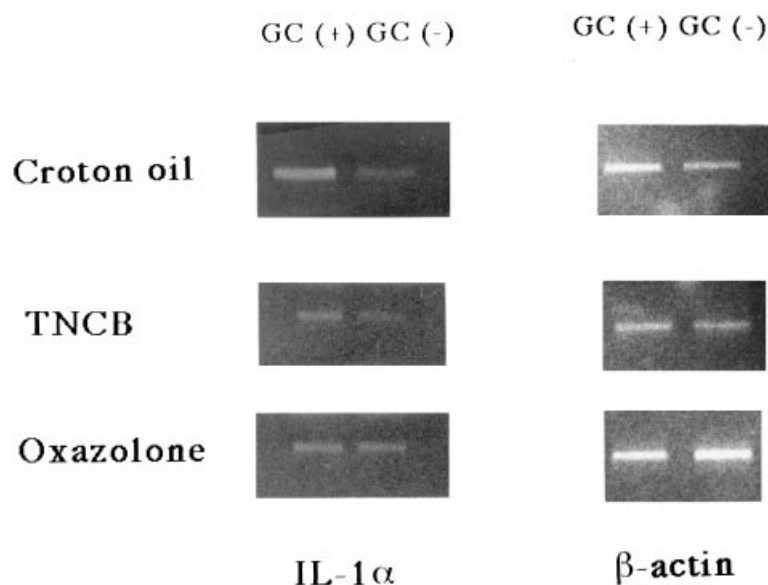


Fig. 3. Expression IL-1α mRNA in the various haptens or irritant substance-challenged ear. As described in Fig. 1, challenged skin samples were taken at 6 h after either haptens or irritant substance challenge, and then the IL-1α mRNA expression was analysed by RT-PCR. The PCR products were resolved on agarose gel and photographed under UV light. The results presented in this figure are representative of three independent experiments.

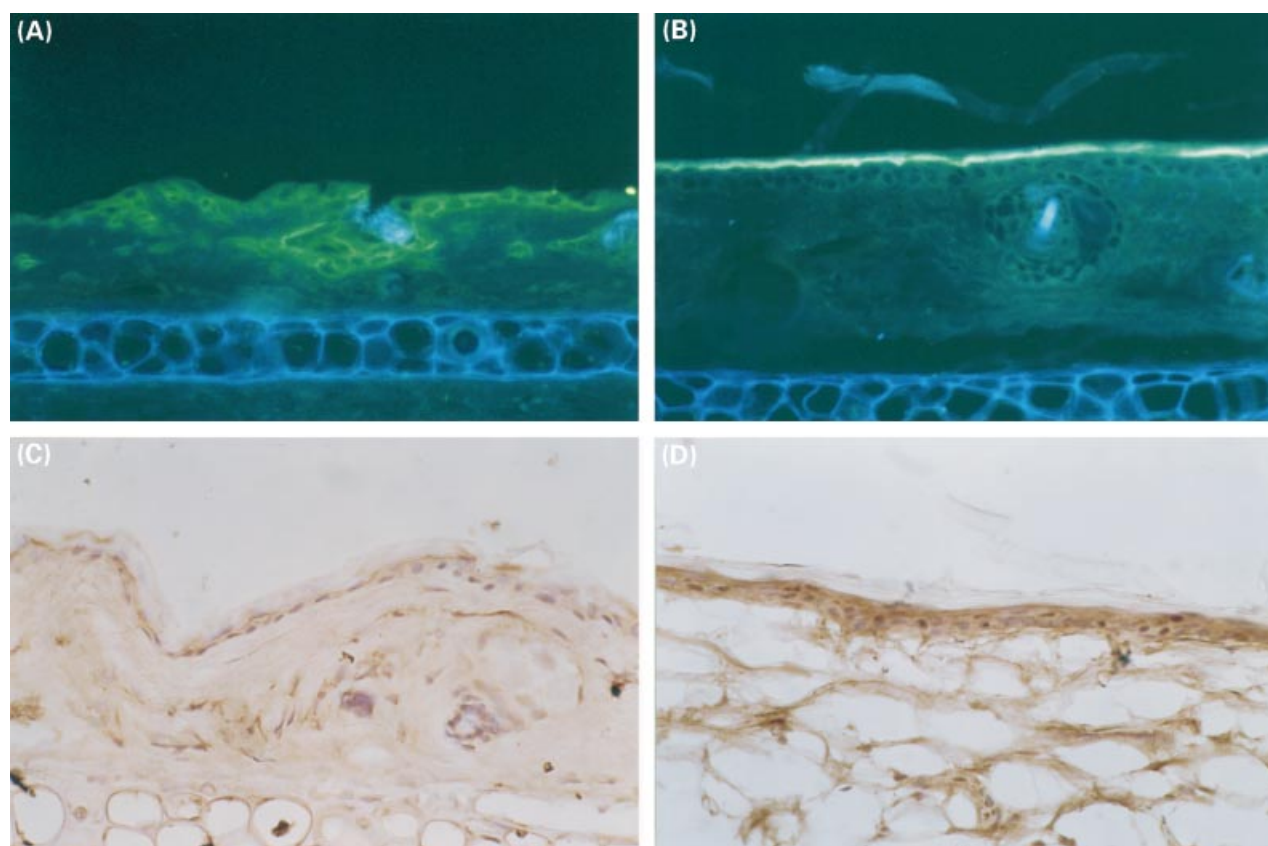
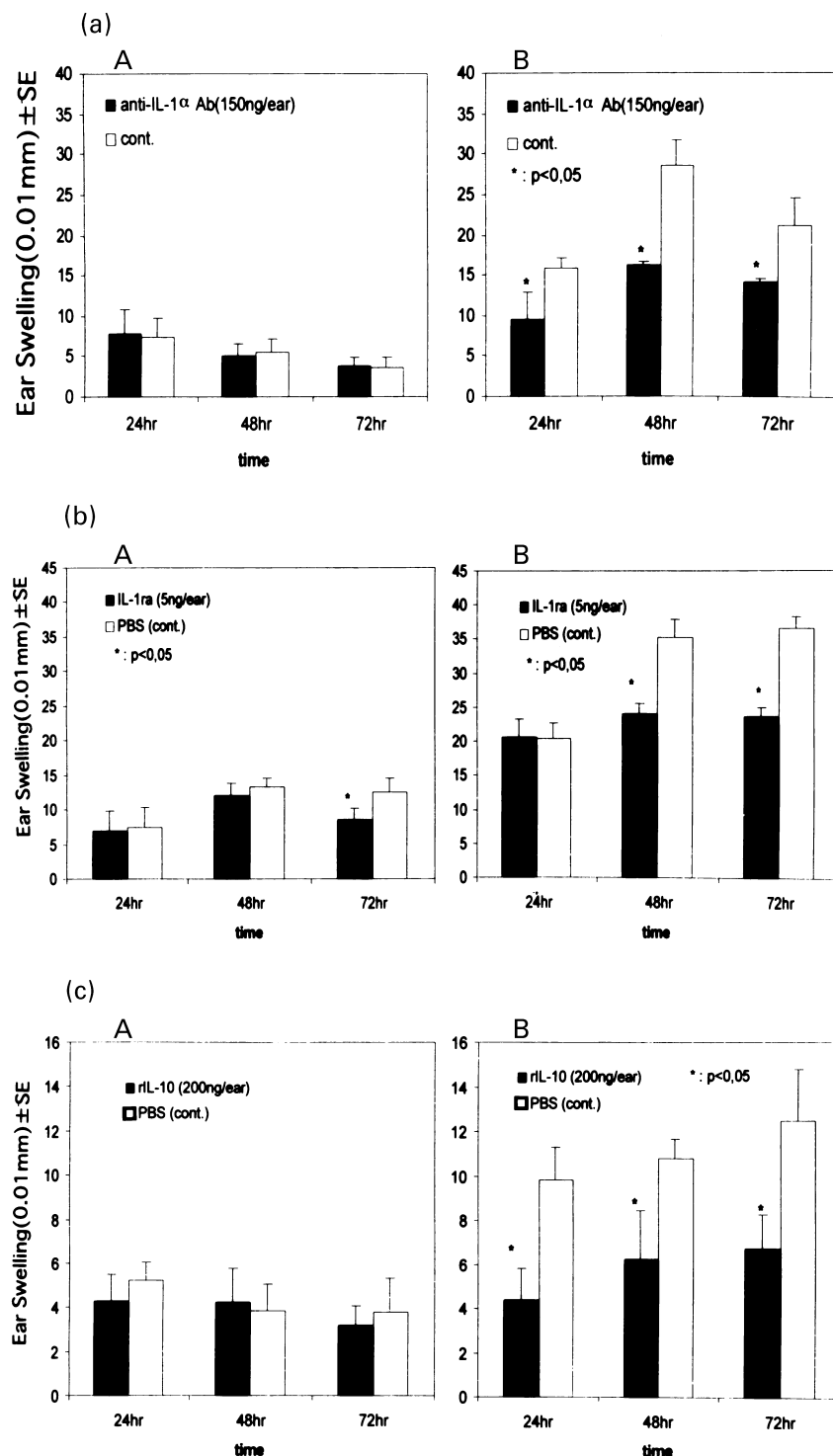


Fig. 4. The expression of IL-1α and IL-10 in the epidermis of challenged skin in GC treated or non-treated mice. Twenty-four hours after the DNFB-challenge, the challenged skins of the mice were removed and prepared for an immunohistochemical analysis as described in the Materials and methods. For staining, we used monoclonal mouse anti-IL-1α antibody and FITC-labelled rat anti-IgG antibody. In the GC-pretreated skin (A), the expression of IL-1α increased more than in the non-GC-pretreated skin (B). At 24 h after the DNFB-challenge, the challenged skin of the mice was removed and prepared for an immunohistochemical analysis as described in the Materials and methods. For staining, we used a monoclonal mouse anti-IL-10 antibody. In the GC-pretreated skin (C), the expression of IL-10 decreased more than in the non-GC-pretreated skin (D). The results presented in this figure are representative of three independent experiments.

by an application of biotinylated antirat Ig mAb at room temperature for 30 min in order to analyse the IL-10 expression. After extensive washing with PBS they were visualized with diaminobenzidine. For the analysis of IL-1 α expression, the sections were incubated with FITC-labelled antihamster IgG.

Statistical analysis

The statistical significance of difference in the means of the experimental groups was calculated using Student's *t*-test. The mean differences were considered significant when $P < 0.05$.



Results

The expression of cytokines mRNA in the challenged sites

The mRNA expression of IL-1 α was assessed at 0 h, 3 h, 6 h, 12 h, 24 h and 48 h after the challenge to examine the modulatory effects of GC on the expression of cytokine mRNA in the skin in CHS. The expression of IL-1 α mRNA was enhanced between 6 h and 12 h after the challenge in the GC pretreated mice, but it decreased at 24 h after the challenge and was detected as a very weak band at 48 h in GC-pretreated mice, whereas IL-1 α mRNA was detected only weakly in non-pretreated mice (Fig. 1A,B). The challenged skin from non-pretreated mice showed IL-10 mRNA expression that peaked at 48 h after challenge, whereas IL-10 mRNA expression was not observed in the GC-pretreated mice (Fig. 2A,B). These results indicated that GC pretreatment enhanced the expression of IL-1 α mRNA and the suppression of IL-10 mRNA in challenged skin.

The CHS induced by TNCB or oxazolone and the irritant response induced by croton oil were examined in order to determine whether a GC induced up-regulation of IL-1 α mRNA can be observed in CHS induced by different haptens and irritant responses. As shown in Fig. 3, GC-pretreatment enhanced the expression of IL-1 α mRNA in the skin challenged with TNCB and croton oil, but not in the skin challenged with oxazolone. The main source of IL-1 α mRNA was epidermal cells because only the epidermis, but not the dermis, separated by 0.5 M ammonium thiocyanate, showed an expression of IL-1 α mRNA (data not shown).

Comparison of the expressions of IL-10 and IL-1 α by keratinocytes in GC-pretreated mice

As the enhanced expression of IL-1 α mRNA and the suppressed expression of IL-10 mRNA were noted in the GC-pretreated mice, we conducted an immunohistochemical analysis of the expression of IL-1 α and IL-10 in the challenged skin. As shown in Fig. 4, IL-1 α was more strongly expressed in the epidermis from the mice pretreated with GC at 24 h after challenge than in the epidermis from non-pretreated mice (Fig. 4A,4B). In contrast, GC-pretreatment markedly reduced IL-10 expression in the epidermis (Fig. 4C, 4D). This reduction of the IL-10 expression in the epidermis continued up to 48 h after challenge (data not shown).

Augmented CHS was inhibited by the subcutaneous injection of anti-IL-1 α mAb, rIL-10 or IL-1ra

As we previously reported [4], GC-pretreatment remarkably enhanced CHS in mice. Both a further up-regulation of IL-1 α and down-regulation of IL-10 in the keratinocytes of GC-pretreated mice was observed in CHS. To see whether this augmentation of skin reactions was mediated by the contrasted expression of IL-1 α and IL-10 in keratinocytes, varying doses of neutralized mAb against IL-1 α (1.5, 15, 150 ng/30 μ l PBS) or rIL-1ra (0.05, 0.5, 5 ng/ear) were injected subcutaneously into the ear lobe 1 h prior to challenge. As shown in Fig. 5(a), the injection of anti-IL-1 α mAb (150 ng) inhibited the enhanced response of CHS in GC-pretreated mice at a rate of from 39 to 43%. No such inhibitory effect of IL-1 α mAb was observed in the

Fig. 5. a. Inhibition in the augmented CHS by the subcutaneous injection of anti-IL-1 α mAb. DNFB sensitized mice without GC pretreatment as described in the Materials and methods were injected subcutaneously with 30 μ l of anti-IL-1 α mAb in PBS (150 ng) into the right pinna 1 h prior to challenge. As a control, 30 μ l of PBS were injected into the right pinna (A). The DNFB sensitized mice pretreated with GC pretreatment as described in Materials and methods were injected with either anti-IL-1 α mAb or PBS (B). Solid bars indicate the injection of anti-IL-1 α mAb; blank bars indicate the injection of PBS. The mean increase in ear thickness following DNFB challenge for each group is shown in units of 0.01 mm $\pm$ SE. The data represent the mean $\pm$ SE for groups of four mice and are representative of three independent experiments. **P*, 0.05, indicates a significant difference compared to the control (PBS). **b.** Induction of augmented CHS response by the subcutaneous injection of IL-1 ra. DNFB sensitized mice without GC pretreatment as described in the Materials and methods were injected subcutaneously with 30 μ l of IL-1 ra in PBS (200 ng) into the right pinna 1 h prior to challenge. As a control, 30 μ l of PBS were injected into the right pinna (A). The DNFB sensitized mice pretreated with GC pretreatment as described in Materials and methods were injected with either IL-1 ra or PBS (B). Solid bars indicate the injection of IL-1 ra; blank bars indicate the injection of PBS. The mean increase in ear thickness following DNFB challenge for each group is shown in units of 0.01 mm $\pm$ SE. The data represent the mean $\pm$ SE for groups of four mice and are representative of three independent experiments. **P*, 0.05, indicates a significant difference compared to the control (PBS). **c.** Inhibition in the augmented CHS response by subcutaneous injection of rIL-10. DNFB sensitized mice pretreated by GC as described in the Materials and methods were injected subcutaneously with 30 μ l of rIL-10 in PBS (200 ng) into the right pinna 1 h prior to challenge. As a control, mice were injected subcutaneously with 30 μ l of PBS into the right pinna. Both mice were challenged with 20 μ l of 0.15% DNFB in a 4 : 1 acetone-olive oil mixture in the right pinna (B). The mice sensitized with DNFB without GC treatment as described in the Materials and methods were injected with rIL-10 in PBS or PBS only (A). Solid bars indicate the injection of rIL-10; blank bars indicate the injection of PBS. The mean increase in ear thickness following the DNFB challenge for each group is shown in units of 0.01 mm $\pm$ SE. The data represent the mean $\pm$ SE for groups of four mice and are representative of three independent experiments. **P*, 0.05, indicates a significant difference compared to the control (PBS).

non-pretreated mice. In contrast to the anti-IL-1 α pretreatment, an inhibition of CHS was observed when both the GC-pretreated mice and non-GC-pretreated mice were injected subcutaneously with IL-1ra (Fig. 5b); 35% of the enhanced reaction was suppressed by the injection of rIL-1ra at 72 h after challenge in the GC-pretreated mice and 29% of CHS reaction was suppressed in the non-GC-pretreated mice. Figure 5(c) shows the effect of the subcutaneous injection of rIL-10 on CHS. The suppression of augmented ear swelling was observed in the GC-pretreated mice when 200 ng of rIL-10 was administered into the ear lobe. As a result, from 50 to 58.6% of the reaction was suppressed. In contrast to the GC-pretreated mice, the inhibition of CHS was not significantly observed in the non-GC-pretreated mice by the subcutaneous injection of 200 ng IL-10 (Fig. 5c (A)); however, the injection of 400 ng of IL-10 induced the inhibition of CHS even in the non-GC pretreated mice (data not shown).

Discussion

Our RT-PCR analysis demonstrated that the mRNA expression of IL-1 α was more strongly detected in the DNFB-challenged site of the GC-pretreated mice than in the mice without pretreatment. In contrast to the IL-1 α expression, the expression of IL-10 mRNA was suppressed at the DNFB-challenged sites of the GC-pretreated mice. Furthermore, immunohistochemical results showed GC-pretreatment to enhance the expression of IL-1 α protein and the down-regulation of IL-10 in the epidermis of DNFB-challenged sites. These data indicated that the GC-pretreatment induced an up-regulation of both the mRNA and protein of IL-1 α and a down-regulation of IL-10 from the epidermis at DNFB-challenged sites. This conclusion is consistent with the findings of our recent report in which the addition of a low dose of GC to the chemically stimulated keratinocytes augmented the IL-1 α production but not the TNF- α or IL-10 production *in vitro* [16]. Furthermore, this up-regulation of IL-1 α mRNA was also detected in both TNFB-induced CHS and irritant response but not in oxazolone-induced CHS. These data are also supported by our recent report in which the early reaction of CHS was augmented by GC in mice sensitized by DNFB and TNFB but not by oxazolone [5]. We also demonstrated that a low dose of GC enhanced DNBS and TNBS-induced NF- κ B activation but not SEB or Oxa-induced NF- κ B activation [16]. These data indicated that the signal transduction system may be different in the GC-induced KC activation in the presence of each chemical or superantigen.

GC inhibits an inflammatory reaction by affecting a variety of different elements. GC has been shown to down-regulate the production of such pro-inflammatory cytokines

as IL-1 [17], TNF- α [17], IFN- γ [18], IL-2 [19] and MIF [20]. In contrast to these observations, GC up-regulates the expression of such cytokine receptors as IL-6R, IL-1R and GM-CSF-R [21,22]. Recently, a topical pretreatment of GC has been demonstrated to enhance both allergic and non-allergic cutaneous reaction [5,15,23], despite the fact that the topical application of GC reduces the number of resident I-A + Langerhans cells in the epidermis for a prolonged period [15,23]. Although the precise mechanism of GC-induced augmentation of cutaneous reactions is still unclear, our data indicate that the up-regulation of IL-1 α and the down-regulation of IL-10 in the epidermis is most likely responsible for the GC-induced augmented inflammatory response. Our previous report also concluded that GC up-regulates the expression of IL-1 α and down-regulates that of IL-10 [16].

To confirm this possibility, anti-IL-1 α mAb or rIL-1ra was injected at the challenged ear in mice with or without GC-pretreatment in DNFB-induced CHS. Interestingly, the injection of anti-IL-1 α mAb inhibited the ear swelling of the GC-pretreated mice but not in the non-GC-pretreated mice at 24 h after challenge; however, the ear swelling in the GC-pretreated mice was three times stronger than that in mice without GC-pretreatment at 48 h after challenge. Furthermore, rIL-1ra also inhibited the enhancement of ear swelling both in the GC-pretreated mice and the non-GC-pretreated mice in DNFB-CHS. These findings indicate that an increased level of IL-1 α therefore appears to play a major role in the enhancement of ear swelling in the GC-pretreated mice in DNFB-induced CHS; however, other factors besides IL-1 α may also contribute to the enhancement of CHS by GC. IL-1ra but not anti-IL-1 α mAb inhibited the response of CHS in non-GC-pretreated mice. These data indicated that IL-1 α but not IL-1 β may play a critical role in the afferent phase of CHS. In line with these findings, conflicting effects of IL-1 in CHS have been reported [24,25].

A low dose administration of GC has been reported to upregulate the number of MIF-positive cells in contact dermatitis [26], thereby significantly enhancing the skin DTH reaction and causing an increase in the number of T cells in the lymph nodes which drain the DTH reaction site [27]. GC enhances the production of cytokines which thus modulate the skin reaction.

In contrast to IL-1 α , the expression of IL-10 was down-regulated at both the mRNA and protein levels to a greater degree in the epidermis of the GC-pretreated mice than that in the non-pretreated mice. The injection of rIL-10 inhibited ear swelling in GC-pretreated mice but such inhibition was only slight in the non-pretreated mice. These data indicate that the inhibition of IL-10 induced by the GC-pretreatment also plays a critical role in the augmented skin reaction of DNFB-induced CHS in the GC-pretreated

mice. IL-10 is well known to inhibit CHS [28]. Dexamethasone has been recently reported to suppress the gene expression and production of IL-10 by LPS-stimulated PBMCs in a dose dependent manner [29]. The suppression of IL-10 induced by GC-pretreatment is therefore suggested to be one of the critical mechanisms by which GC enhances the DNFB CHS in mice. It is not clear why GC-pretreatment upregulates the expression and the production of IL-1 α while downregulating those of IL-10 in the epidermis. One possible mechanism for this is that a low dose of GC enhances the activation of NF- κ B in chemically stimulated keratinocytes [16]. During GC withdrawal, the concentration of GC in serum should decrease. It is likely that a low dose of GC modulates the signal transduction induced by IL-1 α and IL-10 in different types of chemically stimulated keratinocytes. Further studies are thus called for to elucidate the signal transduction in GC-pretreated keratinocytes in the future.

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