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Restraint stress applied prior to chemical sensitization modulates the development of allergic contact dermatitis differently than restraint prior to challenge

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Abstract

BALB/c mice were sensitized and challenged on the skin with the contact sensitizer, dinitroflourobenzene. Acute restraint applied before sensitization diminished, whereas restraint administered before challenge enhanced, chemical-induced ear swelling and leukocytic infiltration in the dermis. Administration of RU486, a glucocorticoid receptor antagonist, partially reversed restraint modulation of the ear swelling response in both restraint paradigms. Restraint did not modulate significantly the concentration of TNF-α and IL-1β in ear tissue homogenates. These data show that acute restraint modulates cutaneous sensitization differently than challenge, but the changes are not reflected in TNF-α or IL-lβ production.

Keywords: Stress; Corticosterone; RU486; Skin; Sensitization; Cytokine

1. Introduction

The cutaneous immune system and the hypothalamic–pituitary–adrenal axis (HPAA) are activated by external stimuli, and both events may occur simultaneously in the workplace. The cutaneous immune system is stimulated by antigenic materials that penetrate the stratum corneum, whereas the HPAA integrates physical and emotional stimuli and coordinates a systemic neurohormone and catecholamine response. There are multiple points at which the HPAA and the immune response are integrated and act in concert to restore physiologic equilibrium (Misery, 1997; Tomaszewska and Przekop, 1997). Corticosterone, an important neurohormone in the HPAA response, is capable of acting directly on most cells involved in immune and inflammatory responses, affecting parameters such as leukocyte trafficking, cytokine and cell adhesion molecule expression, B/T cell ratios and $CD4^+/CD8^+$ ratios (Belsito et al., 1982; Chrousos and Gold, 1992; Steer et al., 1998; Maes et al., 1998; Wiegers and Reul, 1998). GC have been shown to modulate antigen-stimulated cytokine production, to inhibit IL-1, IL-6 and TNF-α production in monocytes and macrophages and GM-CSF, IL-8 and IFN-γ in fibroblasts in vivo, but to enhance production of IL-1β in vitro. Furthermore, the modulatory effect of cytokines on serum corticosterone production documents the importance of the bidirectional flow between the immune system and HPAA. For example, TNF-α, IL-1 and IL-6 can activate the hypothalamus alone or in concert with each other to increase serum levels of adrenocorticotropin (ACTH) and corticotropin releasing hormone (CRH). These hormones increase serum glucocorticoids which act through a classic negative feedback loop to decrease TNF-α and IL-1 production (van Deventer et al., 1990; Zhou et al., 1993; Van Dam et al., 1998).

Multiple studies have documented the anti-inflammatory and immunosuppressive effects of chronic stress and corticosteroid therapy, however, recent reports have suggested that acute stress enhances the cutaneous immune response, or allergic contact dermatitis (ACD). In these

Abbreviations: ACD, allergic contact dermatitis; HPAA, hypothalamic–pituitary–adrenal axis; DNFB, 2,4 di-nitrofluorobenzene; AOO, acetone olive oil; GC, glucocorticoids; LC, Langerhans cells; TNF-α, tumor necrosis factor alpha; IL-1β, interleukin 1 beta; FITC, fluoroscein isothiocyanate

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studies, a two hour acute restraint applied prior to chemical challenge increased pinnae edema and cellularity associated with chemical challenge in rats sensitized and challenged epicutaneously with DNFB (Dhabhar and McEwen, 1996). In contrast, restraint applied to naive mice prior to sensitization with FITC decreased ear thickness in BALB/c mice in a restraint-time dependent manner (Kawaguchi et al., 1997).

ACD is considered a delayed type hypersensitivity response that requires two exposures to chemical, a sensitizing exposure and a challenge exposure. Although many of the events associated with sensitization and with challenge are similar, there are differences in the content, magnitude, and timing of the cellular and cytokine response, particularly in the cellular composition of the dermis. During sensitization, Langerhans cells (LC) acquire and process antigen, and migrate from the epidermis to the draining lymph nodes, where they present antigen to T lymphocytes. Cutaneous IL1-β and TNF-α stimulate phenotypic changes in the LC that are necessary for LC migration to the lymph nodes and for maturation into antigen presenting cells (Enk and Katz, 1992; Kimber and Cumberbatch, 1992). During the challenge phase, antigen-specific lymphocytes, predominantly $CD4^+$ and $CD8^+$ T cells, mononuclear cells and macrophages infiltrate the skin by 48 h after chemical challenge.

We hypothesized that acute activation of the HPAA would modulate the cellular events associated with immunologic sensitization differently than the events associated with antigenic challenge, and that the timing of a stressor is a critical factor in the development of an enhanced or suppressed immune response. To test this hypothesis, we applied the psychological stressor, acute restraint, to stress-sensitive BALB/c mice (Anisman et al., 1998) for 2 h immediately before chemical sensitization or before challenge. The effect of this HPAA manipulation on the development of ACD was evaluated using the well-established measures of chemical-induced increases in ear thickness, leukocytic infiltration of the dermis, and cutaneous production of the proinflammatory cytokines, TNF-α and IL-1β. We evaluated these measurements 24, 48 and 72 h after chemical challenge for both restraint paradigms, timepoints at which the cutaneous immune response has been well characterized. We investigated the role of corticosterone with the subcutaneous injection of the glucocorticoid receptor antagonist, RU486, prior to sensitization and prior to challenge.

2. Materials and methods

2.1. Mice

Young adult male BALB/c mice (20–25 g) were housed five per cage. The animal room was maintained on a 12 h light/dark cycle. Lights went on at 6:00 h and off at 18:00 h. Animals were given food and tap water ad libitum according to ALAC approved guidelines. The protocols for these studies were approved by the NIOSH Animal Care and Use Committee. No anesthesia was used throughout the course of experiments.

2.2. Induction of ACD

Prior to the experiment, animals were weighed, randomly assigned to cages, numbered and shaved on the flanks. On days 1 and 2 of the experiment, 100 μl of 0.5% DNFB (in acetone:olive oil (AOO) 4:1) was applied slowly to the flank with a micropipette. On day 5, baseline ear thickness was measured for the right and left pinnae. On day 6, the right pinnae was challenged with 50 μl of 0.25% DNFB, and the left pinnae were treated with AOO. For application of restraint prior to sensitization, mice were restrained for 2 h prior to chemical application on day 1. For application of restraint prior to challenge, mice were restrained for 2 h on day 6. To evaluate the irritant property of 0.25% DNFB onto the ear, a control group of mice were treated on the flank on days 1 and 2 with AOO and challenged onto the ear on day 6 with 0.25% DNFB. The thickness of the right and left ear pinnae were measured 24, 48 and 72 h after challenge using a digital micrometer.

2.3. Manipulation of the HPA axis

2.3.1. Restraint

Each mouse was placed in an adequately ventilated 50 ml conical plastic tube (Corning Inc., Corning, NY) for 2 h. Mice were not physically squeezed and felt no pain. They could rotate from a supine to prone position, but not turn head to tail. Restraint was applied at 10:00 h for all experimental manipulations. Non-restrained mice were left in their home cages.

2.3.2. RU486 treatment

The protocol for administration of the glucocorticoid receptor antagonist, RU486, was based on methodology established by Matamoros and Levine (Matamoros and Levine, 1996). Briefly, RU486 was dissolved in polyethylene glycol 400 (PEG), and each mouse received 6 mg/kg in 0.1 ml volume subcutaneously on the back. RU486 or the vehicle, PEG, was administered 1 h prior to restraint on day 1 or 1 h prior to restraint on day 6.

2.4. Corticosterone analysis

Mice were euthanized with CO_2 immediately following the restraint period. Whole blood was obtained by cardiac puncture and collected in serum separator EDTA-coated tubes (Becton-Dickenson and Co., NJ, USA). The concentration of corticosterone was analyzed in duplicate by RIA using radiolabelled I^{125} corticosterone (COAT-A-COUNT RIA, Diagnostic Products Corporation, Los Angeles, CA, USA).

2.5. Quantification of dermal cellularity

To assess histologic changes, mice were restrained, sensitized and challenged, as described above and, at 48 h post-challenge, ear tissue was removed and fixed in 10% buffered formalin phosphate (Fisher, NJ), processed overnight and embedded in paraffin wax. Tissue sections (5 μm) were cut and stained with standard Harris hematoxylin-eosin (Surgipath, Illinois) staining protocols. Photomicrographs were taken using an Olympus AX70 Photomicroscope, connected to a Sony 3CCD Color Video Camera, DXC9000.

A semi-quantitative analysis of leukocyte density in mouse ear skin was obtained for each animal by scanning images of hematoxylin-eosin stained skin sections with an Optimas image analysis system (Media Cybernetics, Silver Spring, MD). Positive cells in five randomly selected regions of the dermis (excluding areas of muscle and cartilage) on each slide were enumerated on a digitized image obtained directly from a Nikon light microscope at 40× magnification. All five animals per treatment group were evaluated. Leukocyte density was standardized per 1 mm^2 area, and data are presented as the mean±S.D.

2.6. Cytokine analysis

At 24, 48 and 72 h after challenge, the animals were sacrificed, and the ears were removed. The dorsal half of each ear was weighed, chopped and placed in 1 ml of RPMI (Gibco BRL, Rockville, MD). The samples were freeze–thawed, homogenized for 10 s, freeze–thawed again and sonicated at 50 Hz for 15 s. Homogenates were collected following centrifugation for 15 min at 3100 rev./min. The concentrations of TNF-α and IL-1β were analyzed in duplicate using commercially available solid-phase, two-site ELISAs (R&D systems). The lowest concentration of the standard curve was defined as the minimal level of detection for the assay. ELISA data are reported as the mean of duplicate wells normalized to the weight of the recovered tissue for each sample. Data from all mice within each experimental group were averaged and are presented as the mean±SEM.

2.7. Statistical analysis

Analyses of ear swelling data were conducted using the SAS software program. Descriptive statistics, such as means and standard deviations, were calculated and compared using PROC MIXED, and adjusted for the covariate, initial ear thickness. When analyzing repeated measures data, the use of the REPEATED option is used to model the within subjects covariance structure. The decision process of picking the best covariance structure is done by using two model-fit criteria, Akaike's Information Criterion (AIC) and Schwarz' Bayesian Criterion (SBC). The covariance structure that produces the largest value for these criterion are considered the best model-fit. The LSMEANS option was used in PROC MIXED for calculating means of treatment groups. The LSMEANS option adjusts for unequal sample sizes among treatment groups with all covariates at their mean value. The DIFF option was also used to calculate the differences between two adjusted means.

For analysis of cytokine production and leukocyte infiltration, statistically significant differences ($P<0.05$) between restrained and non-restrained groups were determined by the Student's *t*-test.

3. Results

3.1. Timing of restraint alters ear swelling response in mice treated with DNFB

We first verified restraint-induced increase in serum corticosterone. Two hour restraint significantly increased serum corticosterone in male BALB/c mice 10-fold from 90±27 to 931±56 ng/ml ($P<0.05$, data not shown).

To determine if sensitization and challenge were modulated differently by manipulation of the HPAA, we sensitized and challenged mice using the chemical treatment paradigm described in Materials and methods, and applied restraint prior to sensitization on day 1 or prior to challenge on day 6. We evaluated restraint-induced changes in the DNFB-induced ear swelling response and cellular infiltrates in the dermis. Previous studies in our laboratory and others have demonstrated clearly that mice sensitized and challenged with AOO displayed no significant increase in pinnae thickness (Dhabhar et al., 1997). Mice sensitized and challenged with DNFB and restrained prior to sensitization displayed a significantly decreased mean ear thickness with respect to non-restrained chemically-treated mice whereas, DNFB-treated mice restrained before challenge showed an enhanced ear swelling response (Fig. 1). DNFB-treated mice restrained prior to sensitization demonstrated decreased ear swelling that measured approximately 10–20% increase over baseline measurements, compared to 30–40% observed in non-restrained, DNFB-treated mice. BALB/c mice restrained prior to challenge on day 6 demonstrated significantly increased ear swelling 48 h post-challenge, an increase of 55% compared to a 38% increase measured in non-restrained mice.

3.2. Timing of restraint alters cellular infiltration in mice treated with DNFB

To determine if restraint-induced changes in pinnae thickness correlated with a redistribution of leukocytes in

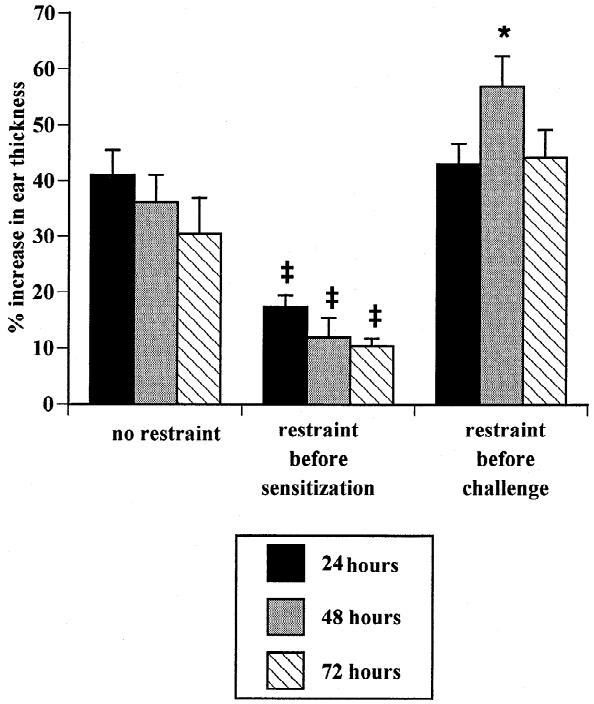

Fig. 1. Restraint on day 1 diminished, whereas restraint on day 6 enhanced, the DNFB-induced ear swelling response in BALB/c mice. Mice (n=5 per group) were sensitized on the flank with 0.5% DNFB and challenged on the ear with 0.25% DNFB. Two hour restraint was applied prior to sensitization on day 1 or prior to challenge on day 6. Data are expressed as mean percent increase in ear swelling±SEM and are representative of three independent experiments. Statistically significant differences between non-restrained, DNFB-exposed and restrained, DNFB-exposed mice are indicated as (*) increased ear swelling; (‡) suppressed ear swelling. Restraint before sensitization: at 24 h, $P<0.004$; at 48 h, $P<0.0021$; at 72 h, $P<0.0008$. Restraint before challenge: at 48 h, $P<0.0326$.

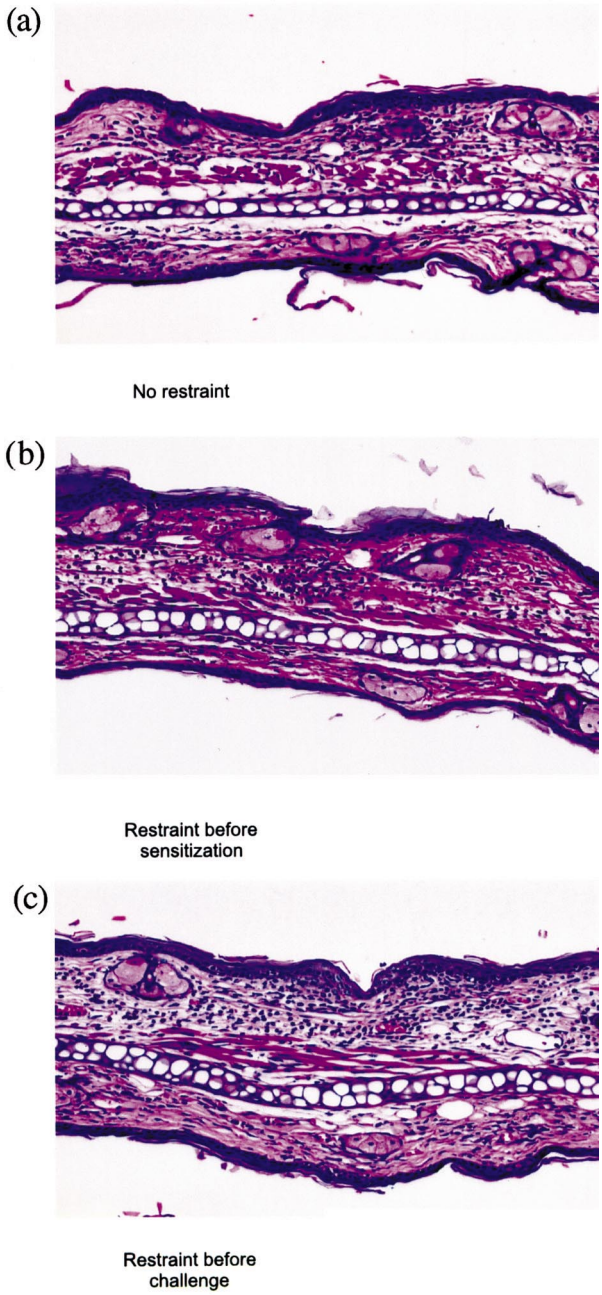

Fig. 2. Restraint-induced changes in cellularity paralleled DNFB-induced changes in pinnae thickness. Mice (n=5 per group) were sensitized, challenged and restrained as described in Materials and methods. At 48 h post-challenge, ear tissue was harvested, fixed and stained with hematoxylin and eosin. Representative tissue sections from sensitized and challenged mice are shown: (a) nonrestrained, (b) restrained prior to sensitization and (c) restrained prior to challenge. Semiquantitative analysis of leukocytic infiltration for all mice in each experimental group (d) is presented as the mean±S.D. Statistically significant differences between non-restrained and restrained mice are indicated as (*) increased cellularity; (‡) decreased cellularity. Restraint before sensitization, $P<$ 0.0025; restraint before challenge, $P<0.0049$).

the dermis, we evaluated hematoxylin and eosin stained cross-sections of ear skin taken from DNFB-treated mice that were not restrained (Fig. 2a), or were restrained either prior to sensitization or to challenge (Fig. 2b and c, respectively) at 48 h post-challenge, the time point at which restraint-induced differences were the largest. Semi-quantitative analysis of leukocytic infiltration demonstrated that mice restrained prior to sensitization had significantly fewer leukocytes in the dermis compared to non-restrained mice, with 2722±268 cells/mm^2 in restrained mice compared to 4529±484 cells/mm^2 in non-restrained mice (Fig. 2d). Mice restrained prior to challenge had significantly more cells than non-restrained mice, approximately 6660±540 cells/mm^2.

3.3. The glucocorticoid receptor antagonist, RU486 partially blocks restraint-induced changes in ear swelling

To determine if the restraint-induced increase in serum corticosterone contributes to restraint-induced modulation of the ear swelling response, DNFB-treated mice were injected subcutaneously with the Type II glucocorticoid receptor antagonist, RU486 1 h prior to restraint on day 1 or on day 6, as described in the Materials and methods.

Administration of RU486 prior to sensitization mini-

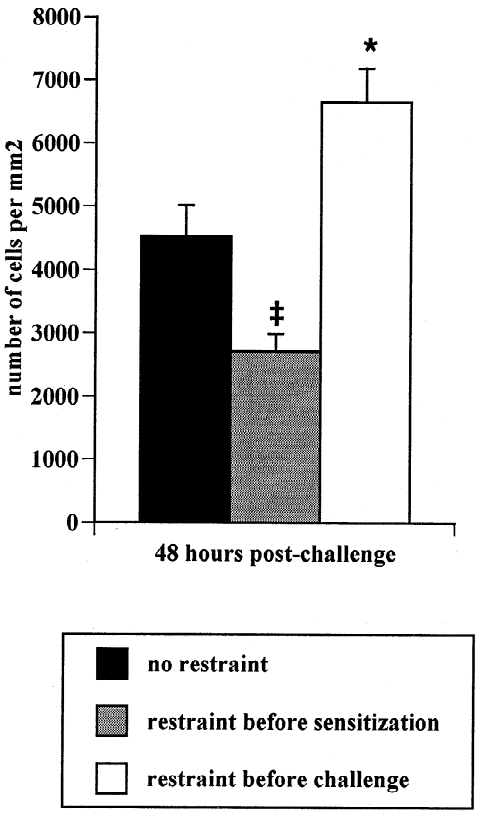

Fig. 2. (*continued*)

mized the restraint-induced decrease in the DNFB ear swelling response at 24 and 48 h (Fig. 3a). Restraint prior to sensitization reduced the mean ear swelling 5- to 19-fold over the 72-h time course, whereas mice who received the RU486 injection 1 h prior to restraint and DNFB application on day 1 displayed an ear swelling response comparable to non-restrained mice at 24 and 48 h. Injection of RU486 to DNFB-treated mice 1 h before restraint prior to challenge decreased the restraint-induced increase in mean ear swelling approximately 18% at 24 and 10% at 48 and 72 h (Fig. 3b).

Under our laboratory conditions, the procedure for subcutaneous injection of vehicle causes a 2- to 3-fold elevation in murine serum corticosterone, compared to serum levels in mice left in their home cages. DNFB-treated mice injected with the vehicle, PEG, 1 h prior to restraint on day 1 exhibited a diminished mean ear swelling response that was intermediate to non-restrained and restrained mice at 24 and 48 h. Mice receiving an injection of PEG 1 h prior to restraint and chemical challenge on day 6 demonstrated a further enhancement of the restraint-induced increase in ear swelling. These data suggest that the vehicle and injection procedure are themselves a stressor, augmenting the ear swelling response in both restraint paradigms. However, these changes in the ear swelling response were not significantly different from those measured in restrained mice.

3.4. Restraint does not alter significantly cytokine protein production in mouse ear skin

TNF-α and IL-1β are proinflammatory cytokines that increase vascular permeability and cellular infiltration at an inflammatory site and are downregulated by GC. Therefore we asked if restraint modulated their production in the dermis in a pattern consistent with restraint-induced changes in ear thickness. For this study, mice were sensitized and challenged with DNFB and restrained prior to sensitization or prior to challenge. Ears were harvested 24, 48 and 72 h post-challenge, the dorsum was homogenized, and cytokine concentrations were measured by ELISA.

In mice sensitized on the flank with AOO and challenged on the ear with DNFB, the concentration of TNF-α did not exceed the cut-off established for this assay at any time point, and the concentration of IL-1β remained below 4 ng/g tissue. DNFB-treated mice restrained prior to sensitization displayed a time-dependent increase in the mean concentration of TNF-α that matched the concentration in non-restrained mice at 24 and 48 h. At 72 h, the mean concentration of TNF-α was elevated in mice restrained before sensitization, 3.6 ± 0.692 ng/g of tissue compared to 2.4 ± 0.264 ng/g of tissue in non-restrained mice. The concentration of TNF-α in mice restrained before challenge corresponded to levels measured in non-restrained mice at all timepoints. The pattern and magnitude of IL-1β production were similar for non-restrained and restrained mice at all time points (Fig. 4).

4. Discussion

These studies demonstrate that acute restraint applied to naive mice before sensitization diminished, whereas acute restraint applied before chemical challenge augmented, the cutaneous immune/inflammatory response in BALB/c mice. Although direct comparison of restraint techniques from multiple laboratories is difficult, these data are consistent with murine studies by Kawaguchi and colleagues who reported a restraint duration-dependent decrease in the FITC-induced ear swelling in naive BALB/c mice (Kawaguchi et al., 1997). Our observation that restraint applied prior to chemical challenge on day 6 enhanced the DNFB-induced ear swelling response and leukocyte infiltration in sensitized mice is consistent with observations previously made in rats and mice (Blecha et al., 1982; Dhabhar and McEwen, 1996; Dhabhar et al., 2000). In contrast, in separate studies. Blecha and Dhabhar observed enhanced ear swelling in mice which were restrained prior to sensitization. These authors applied restraint on both days 1 and day 2 prior to each sensitizing dose whereas in the studies presented here, restraint was applied only on day 1 prior to chemical. We have also observed that restraint prior to chemical application on two

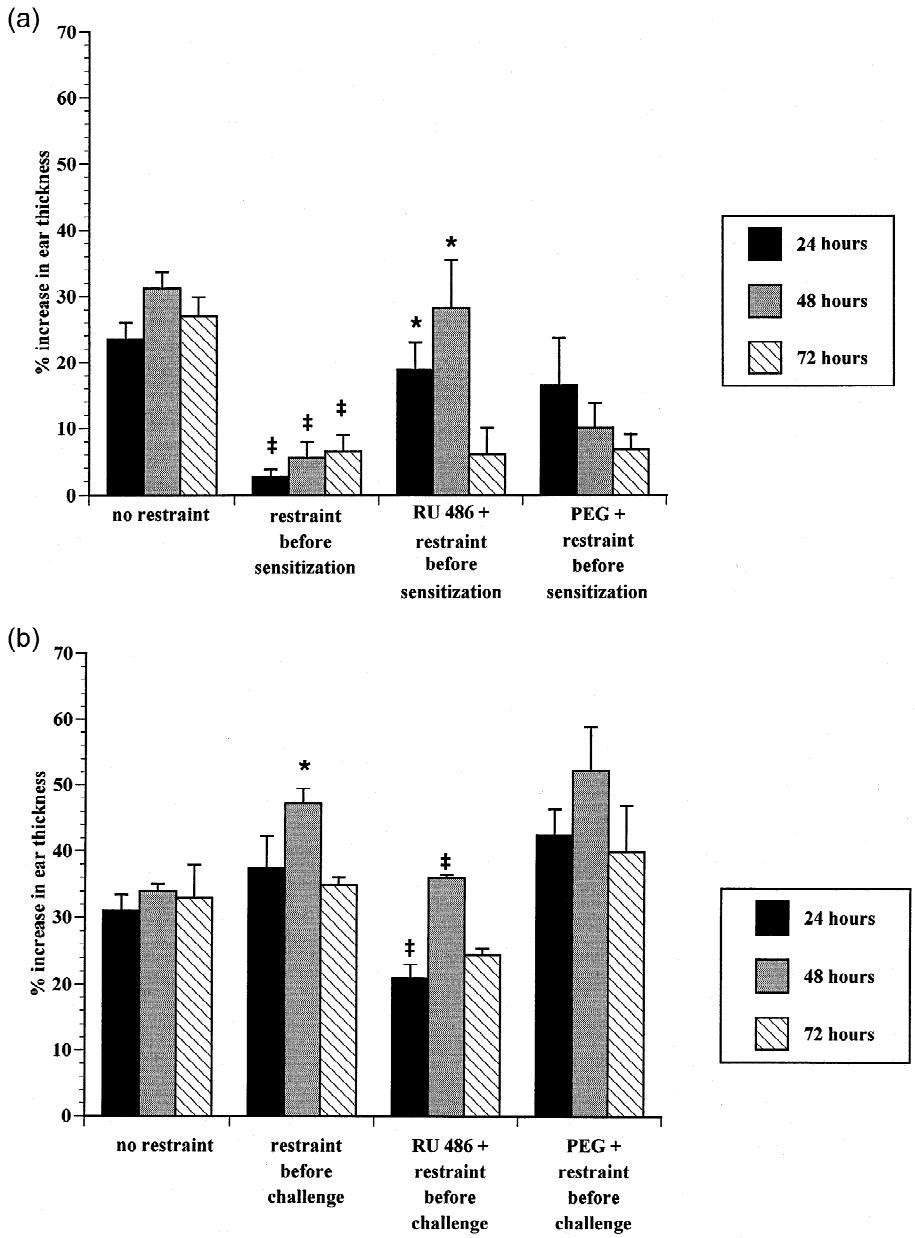

Fig. 3. RU486 applied either prior to sensitization or prior to challenge partially reverses restraint modulation of DNFB-induced ear swelling in mice. Mice were sensitized onto the flank with 0.5% DNFB and challenged onto the ear with 0.25% DNFB. Designated mice ($n=5$ per group) were injected sub-cutaneously with 6 mg/kg of RU486 dissolved in PEG (0.01 ml/g body weight) or PEG alone 1 h prior to restraint before sensitization on day 1 (a) or prior to challenge on day 6 (b). Data are expressed as mean percent increase in ear swelling±SEM and are representative of three independent experiments. Statistically significant differences between non-restrained and restrained mice and restrained DNFB-treated mice and RU486 and DNFB-treated mice are indicated as (*) increased ear swelling or (‡) suppressed ear swelling. Restraint before sensitization, at 24 h, $P<0.0473$. Restraint before challenge, at 24 h, $P<0.0262$; at 48 h, $P<0.0053$.

consecutive days results in the elevation of the mean of ear swelling however, this was not significantly different from non-restrained control mice (data not shown). These data indicate that the consequence of restraint on cutaneous contact hypersensitivity is influenced by the chemical status of the mouse: naive versus sensitized.

Although other hormones and neuropeptides are activated in this restraint model and contribute to modulation of contact hypersensitivity (Pastore et al., 1996; Dhabhar and McEwen, 1999; Lundeberg et al., 1999; Seiffert et al., 2000), a role of corticosterone in the ear swelling response was confirmed in this report. RU486, a glucocorticoid receptor antagonist, partially reversed the day 1 and the day 6 restraint-induced changes in ear swelling, demonstrating that corticosterone participates in both restraint-modulated outcomes. Recently, several studies have highlighted the contradictory effects of glucocorticoids. For example, GC have been shown to inhibit cytokine tran-

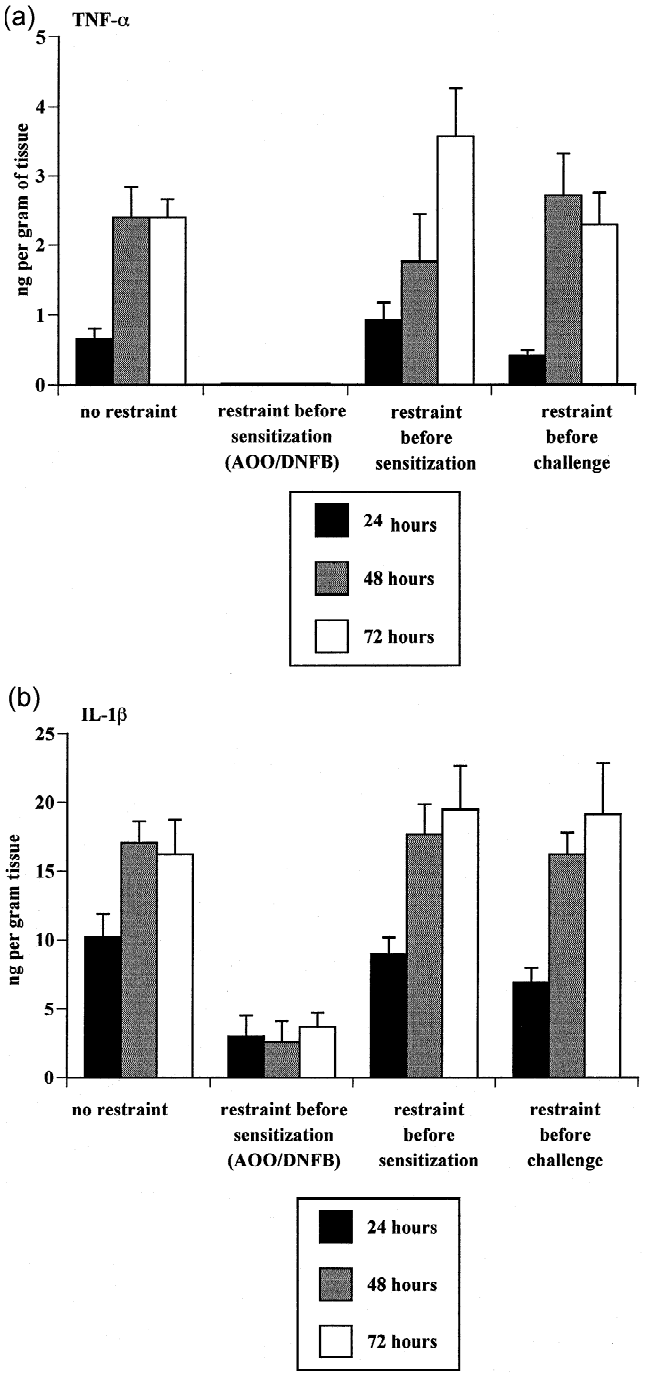

Fig. 4. Restraint does not modify TNF-α and IL-1β production in BALB/c mice. Mice ($n=5$ per group) were sensitized on the flank with 0.5% DNFB or AOO on days 1 and 2 and challenged on the ear with 0.25% DNFB on day 6. Restraint was applied on day 1 or day 6, ear tissue was harvested 24, 48 and 72 h post-challenge, and tissue homogenates prepared. Data from two independent experiments were pooled and are expressed as the mean concentration of cutaneous cytokine±SEM.

scription, translation, mRNA stability and secretion, but to enhance receptor expression for numerous cytokines, including IL-1 and TNF-α (Wiegers and Reul, 1998). Many effects of GC are specific to a cell type or a mechanism of cellular activation. Dexamethasone, a synthetic glucocorticoid, blocks LPS-stimulated production of IL-1 in endothelial cells, but has minimal effect on IL-1 production by LPS-stimulated peripheral blood monocytes (Zuckerman et al., 1989). Again, in contrast to the reported anti-inflammatory properties of GC, studies have documented that GC act synergistically with IL-1 and IL-6 to enhance B cell humoral responses (Emilie et al., 1988), and with IL-6 alone to induce the acute phase protein, a1-acid glycoprotein, in hepatocytes (Nishio et al., 1993). The molecular mechanisms underlying these paradoxical effects of glucocorticoids are not well understood, however, differences in the cell populations and in the degree of cellular activation during sensitization and challenge suggest that different effector pathways will be activated by GC during these two phases of development of ACD.

The suppressed ear swelling response observed with restraint before sensitization is most consistent with the classical understanding of the anti-inflammatory effects of corticosterone. Increased pinnae thickness is characterized by increased vascular permeability and cellular infiltration. Glucocorticoids have been shown to decrease vascular permeability, leukocyte adherence to endothelial cells and cellular infiltration, and the production of TNF-α and IL-1β at an inflammatory site (Schleimer, 1993; Farsky et al., 1995; Ray et al., 1997; Falaschi et al., 1999; Snyder and Unanue, 1982). Other research has confirmed that restraint and dexamethasone induce changes in LC morphology and adhesion molecule expression that could impair LC activation and migration (Belsito et al., 1982; Moser et al., 1995; Kawaguchi et al., 1997). GC also decrease expression of co-stimulatory molecules such as CD80 and CD86 and suppress IL-2 production, molecular events essential to antigen presentation and the development of chemical-specific memory T cells (Munck et al., 1984; de Jong et al., 1999; van den Heuvel et al., 1999).

In contrast, when sensitization has already occurred, acute restraint enhances the cutaneous response to chemical challenge. In addition to our studies, Dhabhar and colleagues showed that, in rat skin, restraint, coupled with chemical challenge, enhanced vascular permeability and cellular infiltration at the inflammatory site (Dhabhar et al., 1996), and, in the peripheral blood, increased circulating neutrophils, decreased leukocytes, and shifted the T lymphocyte $CD4^+/CD8^+$ratio (Dhabhar et al., 1995), systemic changes that could alter indirectly immunocyte subpopulations in chemically-treated skin and enhance the cutaneous response to chemical challenge.

The contradictory effects of glucocorticoids and regulation of cytokine bioactivity in an inflammatory microenvironment are also evident in our TNF-α and IL-1β data. When restraint was applied prior to sensitization or to challenge, the concentrations of TNF-α and IL-1β were comparable to measurements obtained from non-restrained DNFB-treated mice, whereas pinnae thickness was significantly diminished or enhanced at the same timepoints. Many studies have demonstrated that corticosterone de-

creases TNF-α and IL-1β protein production but upregulates IL-1 Type II receptor and TNF-α Type I receptor expression, possible counter-regulatory events that may explain changes in dermal cellularity and edema in the absence of changes in ligand concentration (Akahoshi et al., 1988; Levine et al., 1996; Schwiebert et al., 1996; Corsini et al., 1997; Wiegers and Reul, 1998). Furthermore, these concentrations of TNF-α and IL-1β in mice restrained before sensitization were significantly higher than those measured in the inflammatory response of naive mice challenged with DNFB once on the ear. This finding suggests that many TNF-α and IL-1β-dependent cellular processes associated with sensitization may still occur. Ear swelling is caused by both cellular infiltration and edema. Although TNF-α enhances vascular permeability, it may enhance cellular infiltration but not edema. Also, glucocorticoids reduce permeability which may suggest that glucocorticoids exert a stronger influence in the microenvironment than TNF-α in cutaneous hypersensitivity. In combination with previous reports, our data suggest that regulatory links between the stress response and the immune response, other than the concentration of TNF-α and IL-1β, contribute to modulation of the cutaneous microenvironment at 24–72 h post-challenge. Cytokine receptor expression, production of dermal chemokines, ICAM expression on LC and keratinocytes, endothelial cell-leukocyte adhesion molecules (ELAM) and E-selectin on endothelial cells represent additional important molecular events that are sensitive to GC and critical to development of cutaneous hypersensitivity (Munro et al., 1989; Pober and Cotran, 1990; Hauptmann et al., 1994).

While our studies and others have addressed the role of GC in restraint modulation of cutaneous hypersensitivity, the role of cutaneous neuropeptides also needs to be clarified. Dhabhar and McEwen have reported that epinephrine contributes to the enhanced ear swelling response in adrenalectomized mice, however Granstein and colleagues recently reported that, in CAF1 mice, epinephrine decreased DNFB-induced ear swelling and norepinephrine reduced tumor antigen-induced footpad swelling (Dhabhar and McEwen, 1999; Seiffert et al., 2000). Additionally, the timing of restraint immediately before chemical challenge and 24–72 h before measurement of the ear swelling response and cytokine production may reflect more closely the effects of corticosterone on these cellular and vascular events than measurements associated with restraint prior to sensitization on day 1. However, our data demonstrate that the modulatory effects of acute restraint on contact hypersensitivity can be measured 2–5 days after the restraint is applied and that the timing of the restraint with respect to the development of the cutaneous immune response shifts the outcome. Experiments are underway in our laboratory to evaluate the bidirectional effects of restraint on the cellular and molecular events underlying the cutaneous immune/inflammatory response immediately following restraint on day 1 and day 6.

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