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Restraint-Induced Modulation of Allergic and Irritant Contact Dermatitis in Male and Female B6.129 Mice

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Recent studies in rats have indicated that acute restraint enhances cutaneous hypersensitivity. We hypothesized that acute restraint would also modulate the development of allergic and irritant dermatitis in mice and that these restraint-induced changes would be reflected in the cutaneous cytokine profile and be gender-specific. For these studies, male and female B6.129 mice were sensitized and challenged with the contact sensitizer dinitrofluorobenzene or challenged with the irritant croton oil. Two-hour restraint was applied prior to chemical challenge. Restraint combined with chemical increased ear swelling in both genders in ACD, a change that was blocked by administration of RU-486 prior to restraint. Neither restraint nor RU-486 administration modulated development of ICD; however, IL-1β was decreased by restraint in females only. TNF-α and IFN-γ production were modified in ACD; TNF-α in both genders and IFN-γ in female mice only. Our data demonstrate that acute restraint increases serum corticosterone in B6.129 male and female mice to comparable levels. Restraint modulated the murine ear swelling in ACD, but not ICD, in both genders, and the change in the ear swelling response and cytokine production were, at least in part, corticosterone-dependent.

INTRODUCTION

Perturbation of homeostasis by physical or emotional manipulations triggers physiological responses that are initiated or modulated, in part, by the hypothalamic-pituitary-adrenal axis (HPA axis). Activation of the HPA axis results in increased serum corticosterone and catecholamines, which, in turn, may modulate immune and inflammatory responses. These hormones, which include corticotropin releasing factor and adrenocorticotropin hormone, upregulate serum glucocorticoids, which historically have been reported to suppress inflammatory responses, including the production of TNF-α and IL-1β (Imura et al., 1991; Chrousos, 1995). However, these proinflammatory cytokines, through the systemic circulation, can activate the HPA axis, stimulating the release of neurohormones, thus completing a negative feedback loop.

Multiple studies have demonstrated that the magnitude of the response of the HPA axis to stressors, and the inflammatory/immune system response to increased serum corticosterone, are gender-specific (Lesniewska et al., 1990; Viau & Meaney, 1991; Da Silva, 1995; Broug-Holub & Kraal, 1996; Kawaguchi et al., 1997; Denda et al., 1998). In particular, female and male rats have similar basal corticosterone levels, but females produce a greater increase in serum corticosterone than male rats in response to a stressor (DaSilva et al., 1992). Other studies have demonstrated that females of many species, including mice, rats, and humans have greater humoral and cell-mediated immune responses to antigen and higher resistance to infection (Grossman, 1990). Linking activation of the HPA axis more directly to cytokine regulation, Polan and colleagues showed that gonadal steroids modulate directly the

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synthesis of IL-1β and that female mice exhibit a greater HPA activation and glucocorticoid production than males following IL-1 stimulation (Polan et al., 1988; Frederic et al., 1993).

Recently, Dhabhar and colleagues hypothesized that acute stress, or short-term activation of the HPA axis, enhances the immune response, thus returning the animal to homeostasis more efficiently, whereas chronic stress suppresses the immune response and makes the animal more susceptible to disease (Boumpas et al., 1991; Chrousos, 1995; Dhabhar & McEwen, 1999). Using chemical induction of allergic contact dermatitis (ACD) in rats as a model system, these researchers demonstrated that acute restraint prior to antigenic challenge enhanced two parameters of ACD, edema and lymphocytosis. Kawaguchi, using a similar experimental paradigm with mice, measured no acute restraint-induced change in pinnae thickness 24 h postchallenge (Belsito et al. 1982; Dhabhar & McEwen, 1996; Dhabhar et al. 1996; Kawaguchi et al., 1997).

To address the discrepancy in these findings and to determine if acute manipulation of the HPA axis modulated development of the inflammatory and immune responses in a gender-specific manner, we evaluated the effect of acute restraint on the development of allergic contact dermatitis and irritant contact dermatitis (ICD) in male and female mice. Allergic contact dermatitis involves an antigen-specific, T-cell-mediated immune response that is characterized by edema, erythema, and influx of inflammatory cells (Kimber, 1994). ACD requires two exposures to chemical, a sensitizing exposure and a challenge exposure 5–7 days later. In contrast, irritant contact dermatitis (ICD) is characterized as a nonimmunological inflammatory response that appears rapidly after a single application of chemical on the skin. We evaluated the effect of 2-h restraint on the murine ear swelling response, a well-established measure of contact dermatitis, as well as changes in the production of cytokines. In addition to glucocorticoid modulation of TNF-α and IL-1β, previous research has demonstrated glucocorticoid modulation of the Th1 cytokine, IFN-γ, and the Th2 cytokine, IL-4 (Stanulis et al., 1997). To verify the involvement of corticosterone in restraint-induced modulation of contact dermatitis, selected mice were injected with the glucocorticoid receptor antagonist, RU-486, prior to restraint.

MATERIALS AND METHODS

Young adult (5–6 weeks, 20–25 g) male and female B6.129 mice were housed four per cage. The animal room was maintained on a 12-h light/dark cycle. Lights went on at 6 A.M. and off at 6 P.M. All animals were given food and tap water *ad libitum* according to ALAC guidelines, and all animal protocols were approved by the NIOSH Animal Care and Use Committee.

Induction of ACD

Animals were weighed, numbered, and shaved on the flanks. On days 1 and 2 of the experiment 100 μl of 0.5% 2,4-dinitrofluorobenzene (DNFB) (Sigma, St. Louis) in acetone:olive oil (4:1; AOO) was applied slowly to the flank with a micropipette. On day 5, baseline pinna thickness was measured for the right and left pinnae. On day 6, designated animals were restrained for 2 h. All mice were then challenged with 50 μl of 0.25% DNFB on the right ear and with AOO only on the left ear. Because of the extended time course for the development of the antigen-specific immune response, the thicknesses of the right and left pinnae were measured prior

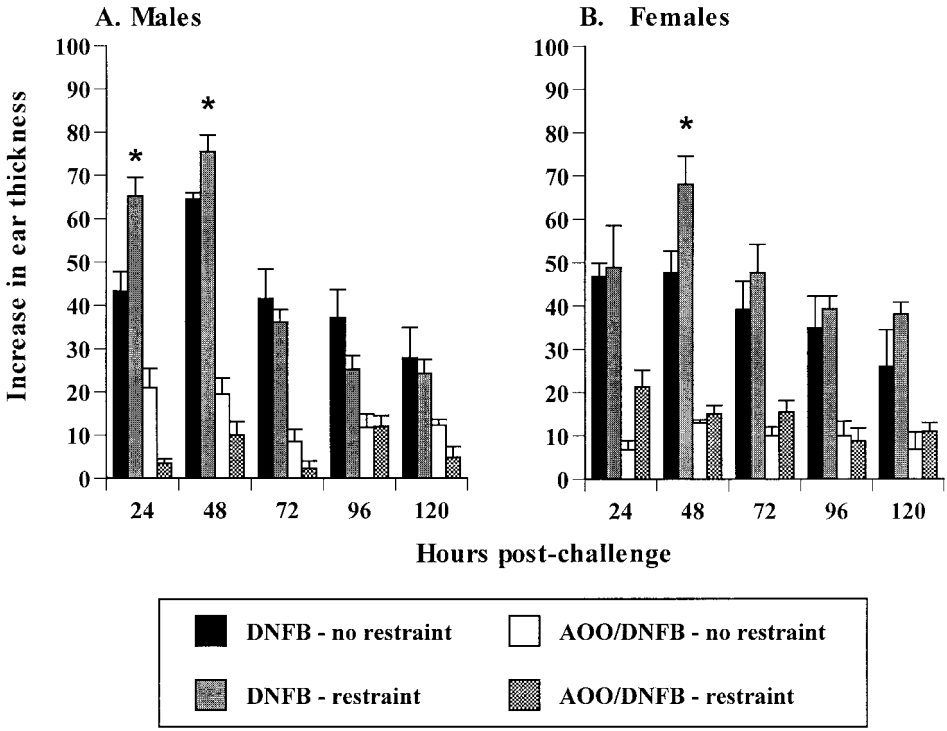

FIG. 1. Restraint modulates DNFB-induced ear swelling in male (A) and female (B) mice. Nonrestrained and restrained mice were sensitized and challenged with DNFB or sensitized with AOO and challenged with DNFB as described under Materials and Methods. Changes in pinna thickness were calculated as the percentage change from baseline measurements. Data are presented as the mean ± *SEM* ($n = 4$ mice per test group) and are representative of three independent experiments. Asterisk (*) indicates significant change ($p < .05$) in ear thickness between nonrestrained, DNFB-exposed mice and restrained, DNFB-exposed mice.

to the first dose of chemical on day 1 (baseline measurement) and 24, 48 and 72 h after chemical challenge on day 6 using an engineer's micrometer. To evaluate the inflammatory response to a single dose of DNFB on the ear, mice was treated on the flank with 100 μl of AOO on days 1 and 2 and restrained or left in their home cages and challenged onto the ear with 50 μl of 0.25% DNFB on day 6. The percentage of change in pinna thickness was calculated as (the measurement of ear thickness at 24, 48, or 72 h postchallenge minus the baseline measurement divided by the baseline measurement) × 100.

Induction of ICD

Animals were weighed and numbered. Baseline pinna thickness was measured for the right and left ears. Animals were left in their home cages or restrained for 2 h, as described below. Following restraint, the right pinnae were challenged with 50 μl of 5% croton oil, and the left pinnae were treated with AOO. Because of the rapid onset of irritant dermatitis, the thicknesses of the right and left ear pinnae was measured every 6 h for 24 h using an engineer's micrometer.

Manipulation of the HPA Axis

Restraint manipulation. Nonrestrained mice were left in their home cages for the 2-h restraint period, and restrained mice were placed in a separate room. Restraint was applied by placing the animal in a well-ventilated 50-ml plastic centrifuge tube (Corning Inc., Corning, NY) for 2 h at 10:00 A.M. The mice could rotate from a supine to prone position, but could not turn head to tail. They were not physically squeezed and felt no pain.

RU-486 treatment. RU-486, a glucocorticoid receptor antagonist, peaks in the serum 1 h after injection and has an elimination half-life of 86 h (Foldesi et al., 1996). Based on a pilot study in the laboratory, RU-486 was dissolved in polyethylene glycol 400 (PEG), and each mouse received one subcutaneous injection of RU-486 (6 mg/kg in 0.1 ml volume in PEG) or PEG only 1 h prior to restraint. The effect of RU-486 administration on restraint modulation of ACD was evaluated 3 h following administration, well within the peak response of the drug.

Collection of Blood for Hormone Assays

Mice were sacrificed immediately following the 2-h restraint period, and blood was obtained by cardiac puncture and collected into plasma separator tubes containing lithium heparin (Becton-Dickinson & Co., NJ). Serum was separated by centrifugation. Fifty-milliliter samples were assayed in duplicate for corticosterone content, reported as nanograms per milliliter, using an anti-rat corticosterone-coated tube and ^{125}I-corticosterone tracer protocol (Coat-A-Count RIA kit; DPC Inc., Los Angeles CA). The RIA was performed according to the manufacturers protocol, and samples were analyzed by gamma scintillation. Duplicates were averaged, and data are presented as the mean $\pm$ *SEM* for each group of mice.

Cytokine Analyses

To evaluate corticosterone modulation of proinflammatory and Th1- and Th2-specific cytokines DNFB-treated ears were harvested at 8 and 24 h following challenge, and, to match the time course for ICD ear measurements, croton oil-treated ears were harvested at 6 and 24 h after chemical application. The dorsal half of each ear was weighed, chopped, and placed in 1 ml of RPMI medium supplemented with 10% fetal calf serum and 1% penicillin/streptomycin solution. The samples were then freeze-thawed, homogenized for 10 s, freeze-thawed again, sonicated at 50 Hz for 15 s, and centrifuged. Homogenates were collected and frozen at -80°C until analysis. The concentrations of TNF-α, IL-1β, and the Th1-specific cytokine IFN-γ and the Th2-specific cytokine IL-4 were analyzed in duplicate by a quantitative sandwich ELISA (R & D Systems) according to the manufacturer's protocol. The limit of detection was set to the lowest concentration of the standard curve: 23 pg/ml for TNF-α, 8 pg/ml for IL-1β and IL-4, and 9 pg/ml for IFN-γ. Duplicates were averaged, and data from three replicate experiments were pooled and are presented as the mean $\pm$ *SEM* for each group of mice.

Statistical Analysis

For the cytokine data, statistically significant differences between restrained and nonrestrained groups were determined by the *t* test. Significant differences in the ear

swelling response were determined by repeated-measures analysis of variance and the Tukey–Kramer multiple-comparison analysis.

RESULTS

To verify that 2-h restraint increased serum corticosterone, we measured the concentration of corticosterone in restrained and nonrestrained mice. Following restraint, plasma corticosterone levels were increased in males from 96 ± 35.7 ng/ml to 436 ± 65.7 ng/ml and in females from 110.5 ± 39 to 539 ± 96 ng/ml. These increases were statistically significant in both males and females and neither nonrestrained nor restraint-modified concentrations were significantly different between genders ($p < .05$). These data demonstrate that acute restraint activation of the HPA axis is similar in male and female B6.129 mice.

Restraint Modulates Ear Swelling in ACD, But Not ICD

To test gender-related differences in restraint modulation of ear swelling in ACD, male and female mice were sensitized, restrained, and challenged as previously described under Materials and Methods. DNFB is a well-established cutaneous sensitizer that, like all sensitizing chemicals, also induces a modest inflammatory, or irritant, response to a single application (Grabbe et al., 1997). To evaluate first the effect of restraint on the inflammatory response to a single dose of DNFB, mice were sensitized with AOO on the flank and challenged with DNFB once on the ear. These mice displayed an approximately 10–20% increase in ear thickness in both genders that was modified by restraint. At 24 h only, restraint decreased the mean ear swelling response in males, whereas females displayed an increased ear thickness. Pinna thickness was significantly enhanced in male B6.129 mice sensitized with DNFB and restrained prior to chemical challenge. At 24 and 48 h postchallenge, ear thickness was increased an additional 23 and 14% compared to nonrestrained mice (Fig. 1A). Restrained female mice sensitized and challenged with DNFB displayed an additional 20% increase in pinna thickness, with respect to nonrestrained females, at 48 h only (Fig. 1B). These data suggest a dichotomy in the gender response to restraint modulation of the irritant/inflammatory response to a sensitizer but an enhanced response to chemical challenge in B6.129 males and females.

Grabbe and colleagues have demonstrated that the inflammatory/irritant response to a single dose of a sensitizer produces a qualitatively different response that the one evoked by an irritant chemical (Grabbe et al., 1997). We next asked if restraint modified the development of ICD. To induce irritant contact dermatitis, croton oil was applied to the ear once immediately following restraint, and, because of the rapid onset of ICD, pinna thickness was measured every 6 h for 24 h. No statistically significant differences in pinna thickness were measured between restrained and nonrestrained mice for either gender over the 24-h time course (Figs. 2A and 2B). In combination, our ear swelling data suggest that restraint modulates molecular events in single application, sensitizer-induced inflammation that are not activated in irritant-induced inflammation and that restraint enhances the cutaneous response to chemical challenge in B6.129 males and females.

RU-486 Decreases Ear Swelling in ACD, But Not ICD

To test the hypothesis that restraint-induced modulation of ear swelling is corticosterone-dependent in the mouse, male and female mice were sensitized with DNFB,

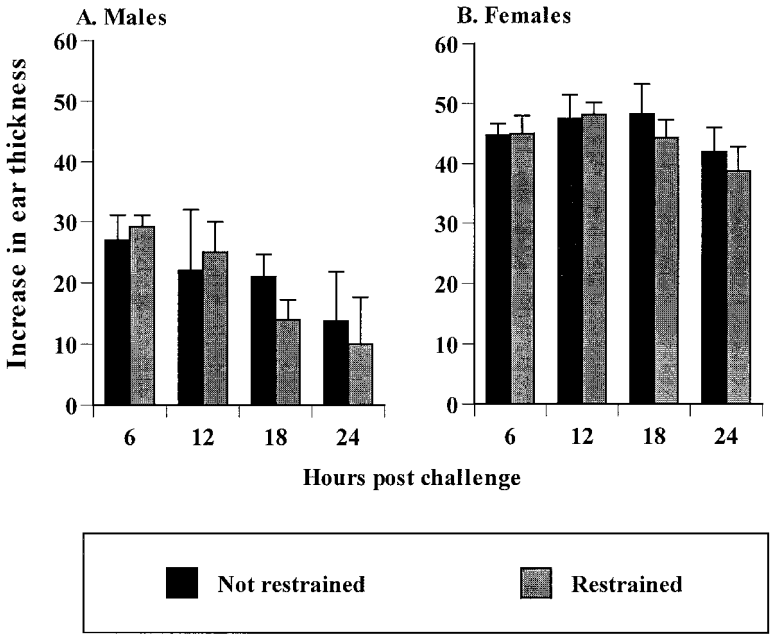

FIG. 2. Restraint does not alter croton oil-induced ear swelling in male (A) and female (B) mice. Croton oil was applied to the ears of restrained and nonrestrained mice, and ear measurements were obtained 6, 12, 18, and 24 h after chemical application. Changes in pinna thickness were calculated as the percentage change from baseline measurements. Data are presented as the mean ± *SEM* ($n = 4$ mice per test group) and are representative of three independent experiments.

restrained, and challenged as previously described under Materials and Methods. Selected groups of mice received subcutaneous injections of the glucocorticoid receptor antagonist RU-486 1 h prior to restraint. Ear thickness was measured 24 h postchallenge. Restrained, DNFB-sensitized, and challenged male mice displayed a mean increase in ear thickness of 46% (Fig. 3A).

Administration of RU-486 blocked significantly restraint-modulated, DNFB-induced ear swelling, reducing the mean increase to 7%. Restrained, DNFB-treated female mice displayed a 50% increase in pinna thickness, an increase that decreased to 2.5% in RU-486-treated mice (Fig. 3B).

In contrast, administration of RU486 did not change pinnae thickness in restrained, croton oil-treated male or female mice at 6 or 24 h (Figs. 4A and 4B). Application of PEG only did not alter significantly croton oil- or DNFB-induced ear swelling in restrained mice of either gender.

Restraint Modulates DNFB-Induced Cutaneous Cytokine Production

The proinflammatory cytokines TNF-α and IL-1β and the Th1/Th2 cytokines IFN-γ and IL-4 are involved in both the induction and elicitation of the cutaneous immune response (Kimber, 1994; Grabbe et al., 1996; Asada et al., 1997) and are modulated by glucocorticoids. Glucocorticoids decrease TNF-α and IL-1β production and shift the Th1/Th2 ratio in a Th2 direction by augmenting production of IL-4 (Imura et al., 1991; Chrousos, 1995; Stanulis et al., 1997). To link the changes in the ear swell-

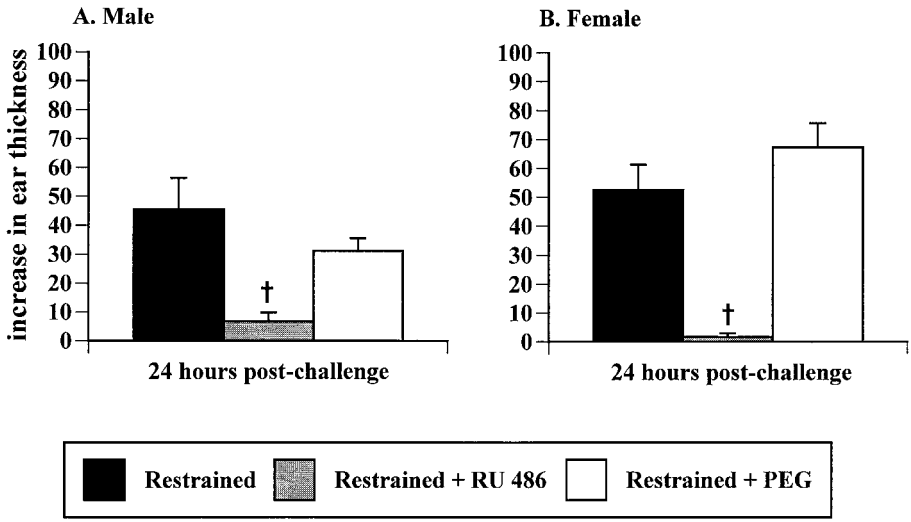

FIG. 3. RU-486 applied prior to DNFB challenge diminished the ear swelling in restrained male (A) and female (B) mice. All mice were sensitized, restrained, and challenged with DNFB as described under the Materials and Methods. Selected mice received a single subcutaneous injection of RU-486 or PEG 1 h prior to restraint. Changes in pinna thickness were calculated as the percentage change from baseline measurements. Data are presented as the mean ± *SEM* ($n = 4$ mice per test group) and are representative of three independent experiments. Statistically significant differences ($p < .05$) are indicated by an asterisk (*).

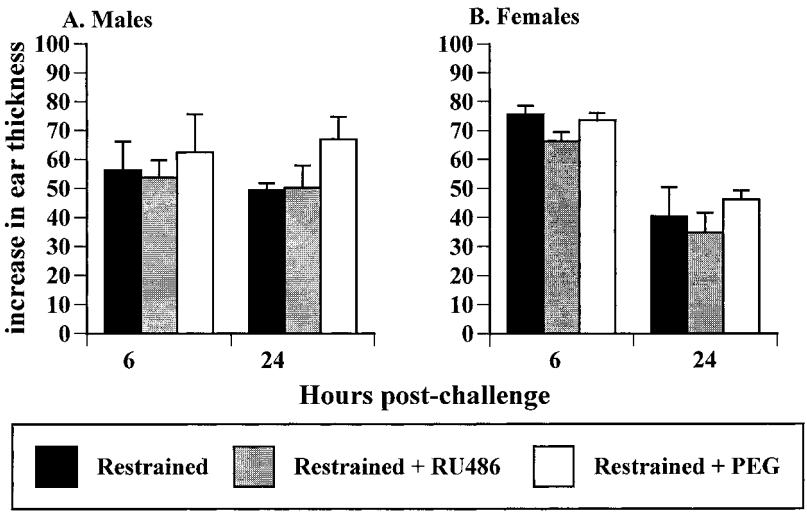

FIG. 4. RU-486 administration has no effect on croton oil-induced ear swelling in restrained male (A) and female (B) mice. All mice were restrained for 2 h and immediately challenged on the ear with 5% croton oil. Changes in pinnae thickness were calculated as the percentage change from baseline measurements. Data are presented as the mean ± *SEM* ($n = 4$ mice per test group) and are representative of three independent experiments.

ing response to restraint modulation of the cytokines underlying that response, we evaluated the production of these cytokines in skin homogenates derived from DNFB-treated male and female mice at 8 and 24 h after challenge. To determine if restraint modulation of cutaneous cytokines was corticosterone-dependent, one group of mice received a single dose of RU-486 subcutaneously 1 h prior to restraint.

In skin homogenates, constitutive concentrations of IL-1β ranged from 500 to 1600 pg/g tissue, and IFN-γ from 6 to 18 pg/g tissue, below the concentrations measured in DNFB-sensitized and -challenged mice. The constitutive levels of TNF-α were below the limit of detection for the ELISA. The concentration of IL-4 in tissue homogenates was at or below the limit of detection for all conditions in male and female mice, and IL-4 was excluded from further analysis.

Evaluating first the effect of restraint on proinflammatory cytokines in male mice, we measured no restraint-induced change in the concentration of IL-1β, with levels of 2 ng/g tissue in nonrestrained mice and 1.89 ng/g tissue in restrained mice at 8 h and 4.6 ng/g tissue and 3.9 ng/g tissue in nonrestrained and restrained mice, respectively, at 24 h (Fig. 5A). The concentration of IL-1β was not significantly changed by administration of RU-486 at either 8 or 24 h. Cutaneous production of TNF-α was unaffected by restraint at 8 h, but significantly decreased at 24 h from 0.59 ng/g tissue to 0.32 ng/g tissue. Consistent with previous reports that glucocorticoids decrease TNF-α production through a negative feedback loop, administration of the glucocorticoid receptor antagonist RU-486 significantly elevated TNF-α levels from 0.32 ng/g tissue to 1 ng/g tissue at 24 h. We also measured no restraint-induced change in the mean concentration of IFN-γ at 8 and 24 h. Surprisingly, RU-486 decreased IFN-γ production from 1.15 ng/g tissue to 0.51 ng/g tissue at 8 h, but not at 24 h.

Acute restraint had minimal effect on cytokine production in females also. As observed in males, female mice demonstrated no significant change in the concentration of cutaneous IL-1β at 8 and 24 h following challenge, approximately 2.3 ng/g tissue and 4 ng/g tissue respectively (Fig. 5B). Mice treated with RU-486 produced IL-1β at concentrations comparable to restrained mice. In contrast to measurements obtained from male mice, restraint increased the mean production of TNF-α, although these increases did not achieve statistical significance. Females demonstrated approximately 0.8 ng/g tissue in nonrestrained mice and 1.42 ng/g tissue in restrained mice at 8 h and 0.33 ng/g tissue and 1 ng/g tissue at 24 h. Administration of RU-486 1 h prior to restraint blocked this enhanced TNF-α production at both time points. The pattern of TNF-α production exhibited by restrained female mice contrasts with that observed in males, however, both restraint modifications were reversed by administration of RU-486, suggesting partial corticosterone-dependence. The mean concentration of IFN-γ was unaffected by restraint at 8 h, but decreased at 24 h. RU-486 administration provided paradoxical results, increasing IFN-γ at 8 h but not at 24 h. PEG, the vehicle for RU-486 administration, had no significant effect on cytokine production in restrained male and female mice.

Restraint Modulates Croton Oil-Induced Cutaneous Cytokine Production

Researchers have shown that cytokines contribute to a nonspecific inflammatory response and enhance both allergic and irritant contact dermatitis (Piguet et al., 1991; Grabbe et al., 1996). Although we measured no restraint-induced change in pinna thickness in croton oil-treated B6.129 mice, restraint decreased IL-1β production in

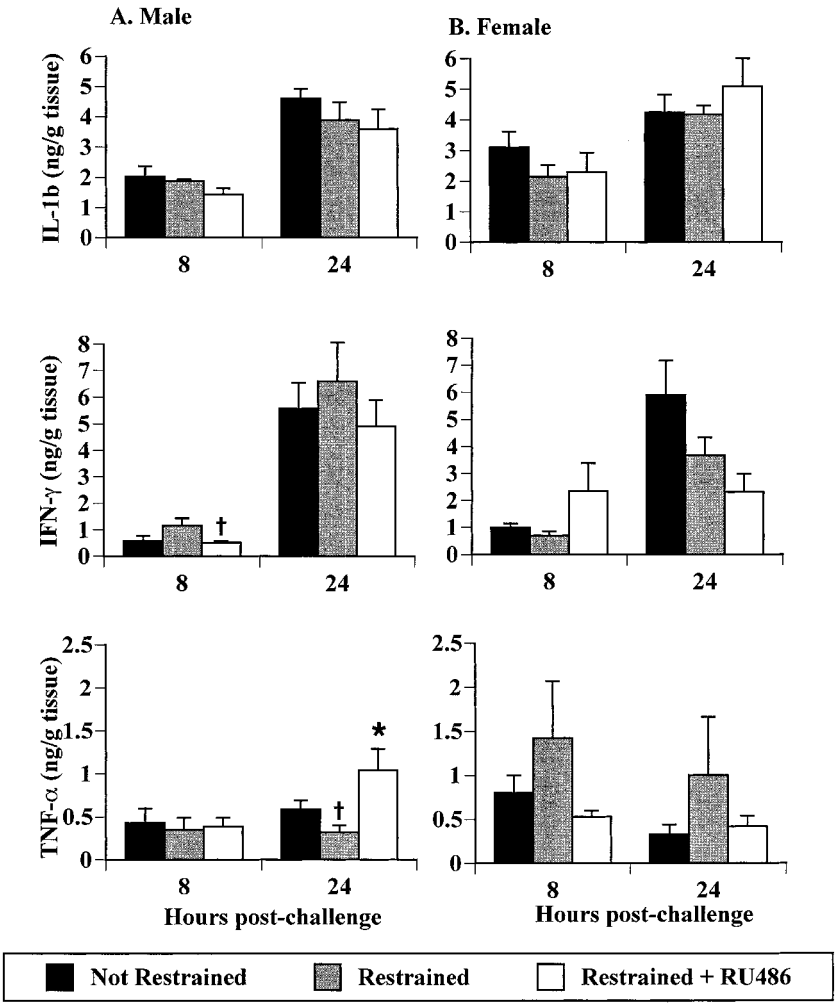

FIG. 5. Restraint modulation of cutaneous cytokine production in ACD in male (A) and female (B) mice. Nonrestrained and restrained mice were sensitized and challenged with DNFB as described under Materials and Methods. Selected mice received a single subcutaneous injection of RU-486 or PEG 1 h prior to restraint. Data are expressed as the means ± *SEM* ($n = 4$ mice per test group) and are representative of three independent experiments. Statistically significant differences ($p < .05$) between restrained mice and mice restrained and injected with RU-486 are indicated by an asterisk (*), which indicates increased with respect to nonrestrained control; a dagger (†) indicates decreased with respect to nonrestrained control.

female mice. At 8 h, restrained females produced levels of IL-1β that were not significantly different than nonrestrained mice, and at 24 hours, restraint halved the concentration. At both time points, the decrement was restored by RU-486 administration. Male mice demonstrated no restraint-induced change in IL-1β; however, administration of RU-486 confirmed the glucocorticoid sensitivity of IL-1β production by augmenting the croton oil-induced levels. We measured no restraint- or RU-486-induced change in croton oil-stimulated production of TNF-α or IFN-γ. Again, ad-

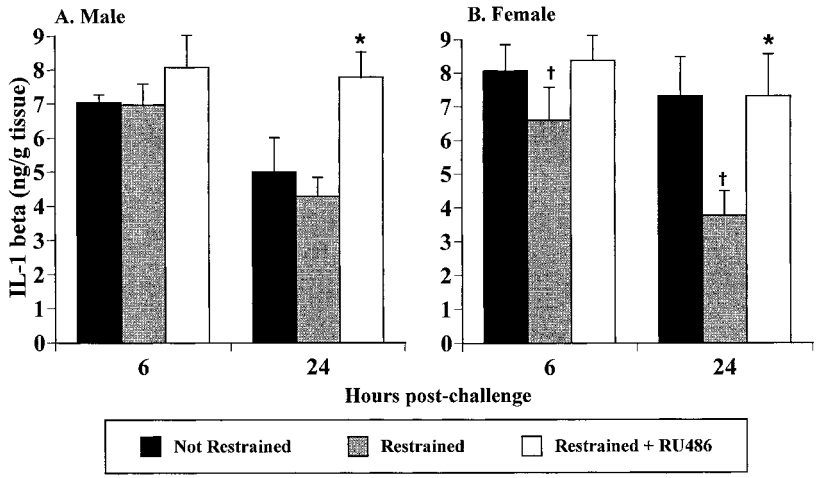

FIG. 6. Croton oil-induced IL-1β production in male (A) and female (B) mice. Croton oil was applied to the ears of restrained and nonrestrained mice, and selected mice received a single subcutaneous injection of RU-486 or PEG 1 h prior to restraint. Data are expressed as the means ± *SEM* ($n = 4$ per test group) and are representative of three independent experiments.

ministration of PEG, the vehicle for RU-486, had no significant effect on croton oil-induced cytokine production at either time point.

DISCUSSION

In this study we examined the effect of acute restraint on the development of allergic and irritant contact dermatitis and potential gender differences in the development of ACD that might reflect underlying gender-related differences in activation of the HPA axis and the immune/inflammatory response.

Two-hour restraint increased significantly serum corticosterone levels in male and female B6.129 mice; however, the increase was similar for both genders. Previous studies demonstrating gender differences in activation of the HPA axis frequently were based on chronic application of a stressor, more severe conditions, or administration of pharmaceuticals (Lesniewska et al., 1990; Da Silva et al., 1993). Our data, in combination with these previous studies, suggest that gender differences in activation of the HPA axis by acute stressors may be minimal, whereas more severe or prolonged stressors have a more pronounced affect. The B6.129 males and females present an opportunity to examine gender differences in the development of a peripheral immune/inflammatory response when baseline and restraint-induced serum corticosterone levels are comparable.

The development of ACD combines an inflammatory response to sensitizing chemicals and a specific immune response, whereas ICD, a nonimmunological pathology, involves only an inflammatory response. TNF-α and IL-1β are upregulated in the skin in both ACD and ICD and act directly to promote vascular permeability, leukocyte infiltration into an inflammatory site, and LC migration to the lymph node, and indirectly through upregulation of numerous other cytokines and chemokines and through changes in adhesion molecule expression, all critical steps in the development of inflammation and cutaneous hypersensitivity (Piguet et al., 1991; Cumberbatch &

Kimber, 1992; Enk & Katz, 1992; Kimber, 1994; Enk et al., 1993; McHale et al., 1999). Coincident with the proinflammatory effects of these cytokines in the skin, IL-1β and TNF-α also act systemically to activate the HPA axis (Zhou et al., 1996). Both cytokines are able to stimulate the HPA axis to release corticotropin releasing hormone (CRH), which stimulates the adrenal glands to produce glucocorticoids, which have been shown to suppress immune responses, including further production of IL-1β and TNF-α (Imura et al., 1991; Munck & Naray-Fejes-Toth, 1994; Chrousos, 1995; Schweibert et al., 1996; Wilckens & De Rijk, 1997; Van Dam et al., 1998).

Recently, several laboratories have reported contradictory effects of glucocorticoids, some of which enhance immune and inflammatory responses. For example, glucocorticoids have been shown to inhibit transcription and translation of numerous cytokines, including TNF-α and IL-1β, but to enhance their receptor expression (Weigers & Reul, 1998). The effects of glucocorticoids may also be specific to a cell type or a mechanism of cellular activation. The synthetic glucocorticoid, dexamethasone, has minimal effect on LPS-stimulated peripheral blood monocytes but blocks LPS-stimulated IL-1 production in endothelial cells (Zuckerman et al., 1989).

Our ear swelling data are consistent with these reports of paradoxical effects of glucocorticoids and with the hypothesis of Dhabhar and McEwen, who theorized that acute stress enhances the immune response, thus returning the animal to homeostatic balance more quickly. We have demonstrated that restraint applied prior to chemical challenge enhances the murine ear swelling response to the contact sensitizer DNFB in male mice at 24 and 48 h and in females at 48 h. The magnitude of the restraint-augmented ear swelling in ACD is similar in both genders, as is the ability of RU-486 to block the enhanced response. These data suggest comparable activation of the HPA axis and development of the murine cutaneous immune response in male and female mice subjected to 2-h restraint.

Furthermore, we demonstrated that neither restraint nor RU-486 were able to alter significantly croton oil-induced increases in pinna thickness in male and female mice. It has been suggested that the ability of glucocorticoids to modulate inflammation may be dependent on the mechanism of cellular activation (Weigers & Reul, 1998) and that contact sensitizers and irritants produce qualitatively different inflammatory responses in the skin (Grabbe et al., 1996). Our data are consistent with these reports and suggest that differences in restraint modulation of the ear swelling response in ACD and ICD may be due to qualitative differences in the cutaneous inflammatory response initiated by DNFB and croton oil and to differences in the modulation of the cellular and molecular events associated with the specific immune response but not the inflammatory response (Zhang et al., 2000).

Restraint-induced modulation of cytokines in our study also supports this conclusion, although more research will be necessary to clarify the mechanistic relationship between the observed cytokine patterns and restraint-enhanced development of ACD. We measured no restraint- or RU-486-induced change in IL-1β production in either males or females in ACD, but a glucocorticoid-sensitive reduction in IL-1β in the inflammatory response of ICD at 24 h TNF-α production was diminished in males at 24 h and increased at 8 and 24 h in females in ACD, but below the level of detection for the time points we sampled in ICD for both genders. Consistent with previous reports, both restraint-induced changes in TNF-α in ACD were reversed by administration of RU-486. IFN-γ, a Thl cytokine of the specific immune response, was de-

creased in female mice only in ACD and not detectable in ICD. The effect of RU-486 administration on IFN-γ production was contradictory, increasing IFN-γ at 8 h but decreasing it at 24 h. These cytokines represent only three potential points of intersection between the stress response mediated through the HPA axis and the cutaneous immune/inflammatory responses, two individually intricate, yet interrelated systems. However, the differences we observed in cytokine production and ear swelling between male and female mice in these studies present an opportunity to study immunological differences between genders in a mouse strain that displays minimal gender differences in glucocorticoid response to 2-h restraint.

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