

Early-Life Psychological Stress Exacerbates Adult Mouse Asthma via the Hypothalamus–Pituitary–Adrenal Axis

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Rationale: Despite accumulating evidence that psychological stress has a short-lasting detrimental effect on asthma, little is known about the way stress in childhood predisposes to adult asthma.

Objectives: Using a communication box, we investigated the long-lasting effect of early psychological and physical stress on adult asthma in mice.

Methods: Male BALB/c mice were exposed to either psychological stress or physical stress three times (every other day) during their fourth week of life. The mice were sensitized to ovalbumin at 8 and 10 weeks, and an ovalbumin airway challenge was conducted at the age of 11 weeks.

Results: Twenty-four hours after ovalbumin challenge, both psychological and physical stress–exposed mice exhibited a significant acceleration in the number of total mononuclear cells and eosinophils and airway hyperresponsiveness compared with control mice. No differences in serum anti-OVA-specific immunoglobulin E levels were found between stress-exposed and control animals after antigen sensitization. In the psychological stress group, but not in the physical stress group, an elevation of the serum corticosterone levels during ovalbumin challenge was significantly attenuated in comparison with the control group. Moreover, pretreatment with RU-486, a glucocorticoid receptor antagonist, before ovalbumin challenge completely inhibited a psychological stress–induced exacerbation of asthma. However, pretreatment with GR-82334, a neurokinin-1 receptor antagonist, failed to affect physical stress–induced augmentation of airway inflammation.

Conclusion: Early psychological and physical stresses aggravated adult asthma via hyporesponsiveness of the hypothalamic–pituitary–adrenal axis during antigen challenge and via a pathway(s) distinct from the hypothalamic–pituitary–adrenal axis or neurokinin-1 receptors.

Keywords: allergy; glucocorticoids; mind–body interaction; psycho-neuroimmunology; substance P

From a historical perspective, clinicians have commonly accepted asthma as a “psychosomatic disease.” Before we better understood the underlying inflammatory basis of asthma, it was among the disorders believed to be merely psychogenic in origin and was commonly referred to as asthma nervosa in early medical texts (1). Moreover, leading physicians in the 1930s and 1940s saw improved or exacerbated symptoms of childhood asthma

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Despite evidence that psychological stress has short-term detrimental effects on asthma, little is known concerning the interactions between stress early in life and adult asthma.

What This Study Adds to the Field

Early psychological and physical stresses aggravate adult asthma through inducing hyporesponsiveness of the hypothalamic–pituitary–adrenal axis as well as by additional pathways.

depending on the quality of mother–child interaction (2). Many recent prospective studies have clearly demonstrated a short-lasting detrimental effect of several psychosocial stresses, such as caregiver stress, certain personality types, poor family relationships, and negative life events, on the symptoms of childhood and adult asthma (3–8). However, little is known about whether or not the quality of life in childhood has a long-lasting effect on asthma in adults, although it has been shown to influence the risk for multiple forms of chronic illness, such as type II diabetes, coronary heart disease, gastroenterological disorders, and obstetric outcomes (9).

To investigate possible physiological mechanisms underlying an injurious effect of stress on asthma, animal models of asthma have been commonly used (10–16). However, such asthma models were mainly restricted to an experimental design in which adult animals were exposed to stress during antigen sensitization or during antigen challenge after sensitization, with only one pilot study showing that prenatal psychological stress exacerbated adult asthma (16). In addition, early-life events have been demonstrated to alter the responsiveness of the neuroendocrine–immune axis throughout the life of animals (17, 18). The hypothalamic–pituitary–adrenal (HPA) axis is a neuroendocrine system that is subject to programming by early-life events. For example, neonatally handled animals exhibit dampened HPA responses to stress as adults, compared with nonhandled animals (19). In contrast, adult animals exposed to repeated periods of prolonged maternal deprivation as neonates display increased HPA response to stress (20). Hence, together with the importance of the HPA axis in regulating inflammation and Th1/Th2-mediated immune responses, it seems reasonable to attempt to answer the provocative question of the way psychological stress before antigen sensitization of the young affects the symptoms of adult asthma.

The murine model of asthma reflects two major pathophysiological findings of allergic bronchial asthma: airway inflammation and airway hyperresponsiveness (AHR). In this study, using

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the communication box method, young mice were exposed two distinct types of stress before antigen sensitization. Allergic asthma was induced in adult mice and assessed by the above two asthma parameters for the purpose of scrutinizing the way psychological or physical stress in early life predisposes to adult asthma.

METHODS

Mice

Male BALB/c mice (4 wk old) (Charles River Japan, Shizuoka, Japan) were maintained at a constant temperature (23–25°C) on a 12-hour light/dark cycle with food and water available *ad libitum*. Experiments were always done in the morning (between 9:00 A.M.–12:00 P.M.) to minimize variation due to circadian rhythmicity. This experiment was reviewed by the Ethics Committee on Animal Experiments of the Graduate School of Medical Sciences, Kyushu University, and was performed under the control of the Guidelines for Animal Experiments of the Graduate School of Medical Sciences, Kyushu University, and the Law (No. 105) and Notification (No. 6) of the Japanese Government.

Communication Box Stress and Foot-shock Stress

Exposure to either communication box paradigm-induced stress (CS group) or foot-shock stress (FS group) was done according to our previous method (21). Control animals without any induced stress (CON group) were left in a normal compartment, with the same floor space as the others, for the duration of the stress exposure (*see* online supplement, E1).

Stress Protocol, Immunization, and Airway Challenge with Antigen

As shown in Figure 1, the mice were exposed to either FS or CS for 1 hour, three times every other day during the fourth week of life, and thereafter they were reared normally. At 8 and 10 weeks, the mice were sensitized to ovalbumin (OVA). At the age of 11 weeks, the mice were given an airway challenge with OVA for 30 minutes by ultrasonic nebulization for 3 consecutive days. The experiment examining the effect of RU-486 or GR-82334 was conducted according to previous reports (22, 23) (*see* online supplement, E2).

Measurement of Serum Corticosterone Levels

As previously described (24), blood samples were collected from the retroorbital plexus at the indicated time points, and the serum levels of corticosterone were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (online supplement, E3).

Bronchoalveolar Lavage

As previously described (25), animals were killed either 24 or 48 hours after the last airway allergen challenge, and bronchoalveolar lavage (BAL) performed by lavage with 1.0 ml ice-cold phosphate-buffered saline (online supplement, E4).

Airway Responsiveness

At 24 hours after the final OVA challenge, bronchial reactivity to aerosolized methacholine was measured using the Buxco whole-body

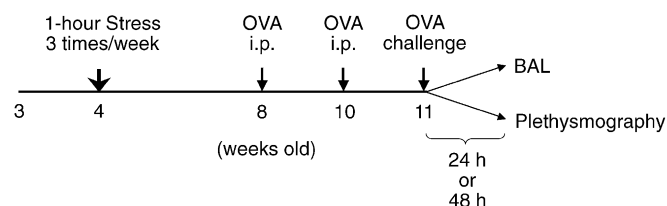


Figure 1. Diagrammatic representation of the protocols for stress exposure, immunization, and airway challenge with antigen (*see* METHODS for details). BAL = bronchoalveolar lavage; OVA = ovalbumin.

plethysmograph (Buxco Electronics, Troy, NY), as previously described (26) (online supplement, E5).

Measurement of Anti-OVA-specific IgE

Anti-OVA-specific IgE was measured by ELISA, according to a previous method (27) with some modifications (online supplement, E6).

Passive Sensitization with Anti-OVA-specific IgE and Airway Challenge with OVA

Using a previous method (28) with some modifications, the mice were passively sensitized by intravenous injection of 2.0 μ g anti-OVA-specific IgE (online supplement, E7).

Statistical Analysis

All data are expressed as means $\pm$ SE. The data were analyzed by one-factor analysis of variance (ANOVA) followed by the Scheffé test. For data on the dose response of the airway responsiveness, we used a repeated-measure ANOVA followed by the Scheffé test. A value of $p < 0.05$ was considered to be significantly different from a corresponding value.

RESULTS

Both psychological and physical stress in early life exacerbate airway inflammation and airway hyperresponsiveness.

As shown in Table 1, the levels of serum corticosterone, a well-known, critical stress hormone, were significantly augmented in the FS and CS groups immediately after a single stress session, in comparison with the CON group, which indicates that each stress strongly stimulated a physiological reaction in these young mice.

In analysis of the total number of BAL mononuclear cells (MNCs) after saline or OVA airway challenge in adult mice, the FS and CS groups both exhibited a significant acceleration of the increase in the total MNC number at 24 hours after antigen challenge in comparison with the CON group, although the significant differences between the groups no longer remained at 48 hours (Figure 2). Evaluation of the BAL subpopulation MNC number 24 hours after OVA challenge also revealed a significant elevation of eosinophils in the FS and CS groups in comparison with the CON group, but the other subpopulations did not (Figure 3). Furthermore, AHR in FS and CS mice after OVA airway challenge was significantly higher than that of CON mice (Figure 4). None of three analyses of the total BAL MNC number, BAL subpopulation MNC number, or AHR exhibited any changes by challenge with saline. These findings clearly indicate that

TABLE 1. SERUM CORTICOSTERONE LEVELS IMMEDIATELY AFTER A SINGLE STRESS SESSION

Group	Corticosterone (ng/ml)
Basal (n = 5)	27.6 $\pm$ 6.0
CON (n = 8)	117.5 $\pm$ 46.5
FS (n = 8)	640.7 $\pm$ 80.7*
CS (n = 8)	299.5 $\pm$ 58.6†

Definition of abbreviations: CON = control; CS = communication box paradigm-induced stress; FS = foot-shock stress.

Data represent mean $\pm$ S.E. Basal indicates a group without any exposure, and CON, FS, and CS indicate groups exposed to the control conditions (left in a stress-unburdened compartment during one stress session), physical stress by electric FS, and psychological stress by CS.

* $p < 0.001$ considered to be significantly different from the corresponding value of the CON group.

† $p < 0.05$ considered to be significantly different from the corresponding value of the CON group.

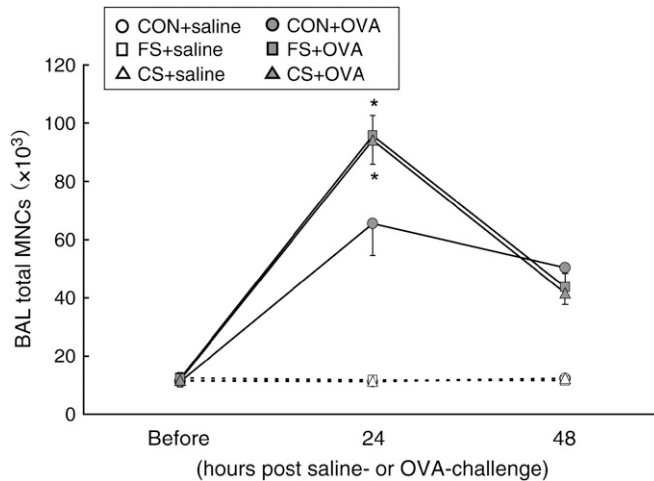


Figure 2. Alterations in the total cell number of BAL mononuclear cells (MNCs) after antigen challenge in actively sensitized mice. CON, FS, and CS indicate the groups exposed to the control conditions, physical stress by electric foot-shock, and psychological stress by communication box, respectively. The total cell number of BAL mononuclear cells was assessed before OVA or saline airway challenge and at 24 and 48 h after OVA (CON + OVA, FS + OVA, CS + OVA) or saline (CON + saline, FS + saline, CS + saline) airway challenge. All values are expressed as the mean $\pm$ SE (CON + saline: $n = 6$; FS + saline: $n = 6$; CS + saline: $n = 6$; CON + OVA: $n = 9$; FS + OVA: $n = 10$; CS + OVA: $n = 10$). * $p < 0.05$ was considered to be significantly different from the corresponding value of the CON + OVA group.

psychological and physical stress in young mice both aggravated the symptoms of adult asthma.

Stress Is More Involved in Antigen Challenge than Antigen Sensitization

The pathophysiological mechanisms behind allergic reactions consist of two components: antigen sensitization and antigen

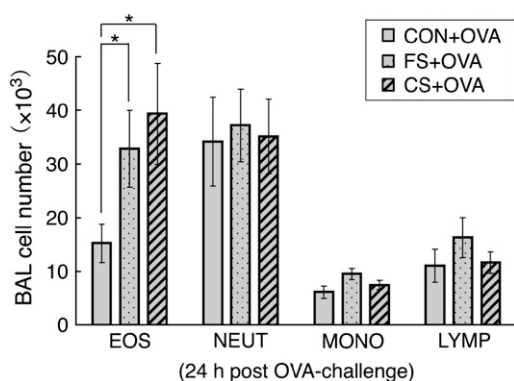


Figure 3. Subpopulation cell number of BAL MNCs after antigen challenge in actively sensitized mice. CON, FS, and CS indicate the groups exposed to the control conditions, physical stress by electric foot-shock, and psychological stress by communication box, respectively. The subpopulation cell number (EOS [eosinophils]; NEUT [neutrophils]; MONO [monocytes]; LYMP [lymphocytes]) of BAL MNCs was assessed at 24 h after OVA airway challenge. All values are expressed as the mean $\pm$ SE (CON: $n = 9$; FS: $n = 10$; CS: $n = 10$). * $p < 0.05$ was considered to be significantly different from the corresponding value of the CON group.

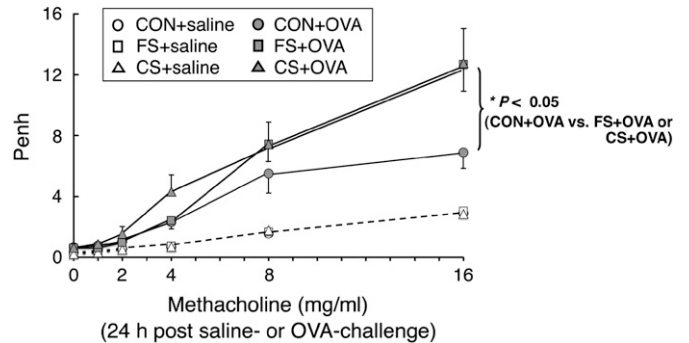


Figure 4. Effect of stress on airway hyperresponsiveness after antigen challenge in actively sensitized mice. CON, FS, and CS indicate the groups exposed to the control conditions, physical stress by electric foot-shock, and psychological stress by communication box, respectively. Airway responsiveness to methacholine was evaluated by changes in enhanced pause (Penh) at 24 h after airway challenge with OVA (CON + OVA, FS + OVA, CS + OVA) or saline (CON + saline, FS + saline, CS + saline). All values are expressed as the mean $\pm$ SE (CON + saline: $n = 6$; FS + saline: $n = 6$; CS + saline: $n = 6$; CON + OVA: $n = 8$; FS + OVA: $n = 8$; CS + OVA: $n = 8$). * $p < 0.05$ was considered to be significantly different from the corresponding value of the CON + OVA group.

challenge. Therefore, we first investigated the effect of stress on antigen sensitization by estimating the serum anti-OVA-specific IgE levels in each group after OVA immunization.

As shown in Table 2, no significant differences in the serum anti-OVA-specific IgE levels were found between the FS or CS group and the CON group, which suggests that antigen sensitization is not involved in the stress-induced exacerbation of adult asthma. This was confirmed by a further passive sensitization experiment in which both psychological and physical stresses significantly accelerated an increase in the number of total MNCs in BAL 24 hours after OVA airway challenge after pretreatment of all groups with the same amount of anti-OVA-specific IgE (Figure 5).

The Involvement of the HPA Axis in the Psychological Stress-induced Exacerbation of Adult Asthma

To investigate the role of HPA axis responsiveness to antigen challenge in stress-induced exacerbation of asthma, we assessed the serum corticosterone levels during OVA airway challenge.

As shown in Figure 6, the CS group exhibited a significant attenuation of increase in the serum corticosterone levels during OVA challenge in comparison with the CON group, but the FS group did not (area under curve, $h \cdot ng/ml$: FS, 363.5 ± 39.7 , $n = 7$; CS, 321.0 ± 22.3 , $n = 6$; CON, 440.8 ± 48.8 , $n = 6$; CS vs. CON, $p < 0.05$). Furthermore, the mice were pretreated with

TABLE 2. SERUM ANTI-OVALBUMIN-SPECIFIC IgE LEVELS AT THE AGE OF 11 WEEKS

Group	Anti-OVA-specific IgE (ng/ml)
CON ($n = 9$)	98.5 $\pm$ 16.9
FS ($n = 10$)	104.9 $\pm$ 13.7
CS ($n = 10$)	97.8 $\pm$ 13.7

Definition of abbreviations: CON = control; CS = communication box paradigm-induced stress; FS = foot-shock stress; OVA = ovalbumin.

Data represent mean $\pm$ S.E.

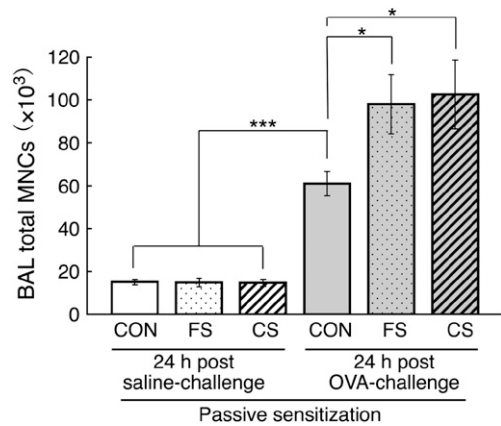


Figure 5. Effect of stress on the total cell number of BAL MNCs after antigen challenge in passively sensitized mice. CON, FS, and CS indicate the groups exposed to the control conditions, physical stress by electric foot-shock, and psychological stress by communication box, respectively. The total cell number of BAL MNCs was assessed at 24 h after OVA (CON + OVA, FS + OVA, CS + OVA) or saline (CON + saline, FS + saline, CS + saline) airway challenge after passive sensitization with anti-OVA-specific IgE. All values are expressed as the mean $\pm$ SE (CON + saline: $n = 6$; FS + saline: $n = 6$; CS + saline: $n = 6$; CON + OVA: $n = 9$; FS + OVA: $n = 8$; CS + OVA: $n = 9$). * $p < 0.05$ and *** $p < 0.001$ were considered to be significantly different from the corresponding value of the CON + OVA group.

RU-486, a glucocorticoid (GC) receptor antagonist, before OVA airway challenge for the purpose of blocking the involvement of corticosterone during antigen challenge. Pretreatment with RU-486 completely inhibited psychological stress-induced increases in the number of eosinophils and total MNCs in BAL, although it did not affect physical stress-induced elevation in BAL (Figure 7). Likewise, psychological stress-induced enhancement of AHR was also completely suppressed by pretreatment with RU-486, but the physical stress group had a tendency toward accelerated AHR (Figure 8). Hence, these findings indicate that hyporesponsiveness of the HPA axis during antigen

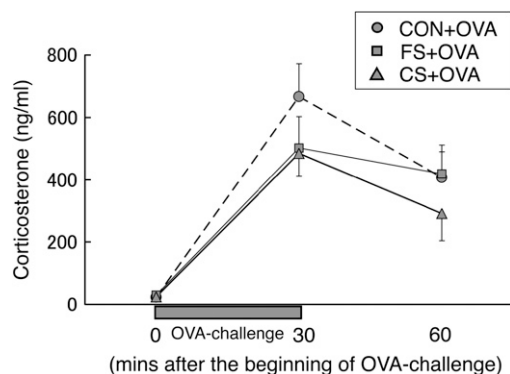


Figure 6. Alterations in serum corticosterone levels after antigen challenge in actively sensitized mice. CON, FS, and CS indicate the groups exposed to the control conditions, physical stress by electric foot-shock, and psychological stress by communication box, respectively. The serum corticosterone levels were assessed at 0, 30, and 60 min after OVA airway challenge. All values are expressed as the mean $\pm$ SE (CON + OVA: $n = 6$; FS + OVA: $n = 7$; CS + OVA: $n = 6$).

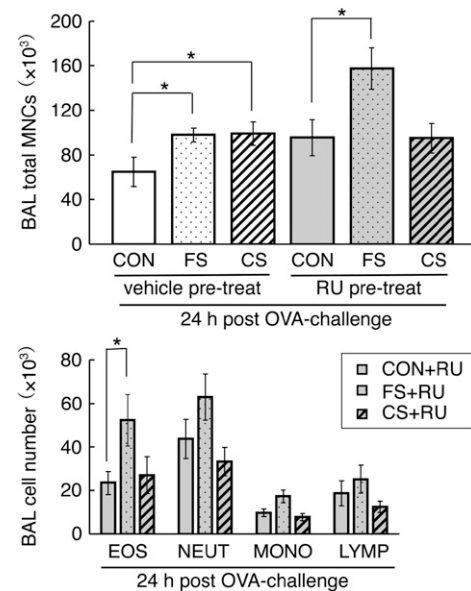


Figure 7. (Upper panel) Total cell number and (lower panel) subpopulation cell number of BAL MNCs after antigen challenge in RU-486-pretreated mice. CON, FS, and CS indicate the groups exposed to the control conditions, physical stress by electric foot-shock, and psychological stress by communication box, respectively. The total cell number and the subpopulation cell number (EOS [eosinophils]; NEUT [neutrophils]; MONO [monocytes]; LYMP [lymphocytes]) of BAL MNCs were assessed at 24 h after OVA airway challenge after pretreatment with RU-486, a glucocorticoid receptor antagonist (CON + RU, FS + RU, CS + RU), or a vehicle (CON + vehicle, FS + vehicle, CS + vehicle). All values are expressed as the mean $\pm$ SE (CON + vehicle: $n = 8$; FS + vehicle: $n = 10$; CS + vehicle: $n = 10$; CON + RU: $n = 10$; FS + RU: $n = 9$; CS + RU: $n = 8$). * $p < 0.05$ was considered to be significantly different from the corresponding value of the CON + vehicle or CON + RU group.

challenge plays a critical role in the psychological stress-induced exacerbation of adult asthma.

The Role of Neurokinin-1 Receptor in Physical Stress-induced Exacerbation of Asthma

Because previous animal studies (12, 13) have suggested that substance P mediates stress-induced aggregation of asthma via neurokinin-1 (NK-1) receptor, GR-82334, an NK-1 receptor antagonist, was used to further evaluate the possibility that this receptor might link physical stress to the exacerbation of asthma. However, pretreatment with GR-82334 before OVA challenge failed to affect a physical stress-induced acceleration of the number of total MNCs in BAL (Figure 9).

DISCUSSION

Stress can be defined as the psychophysiological reaction of the body to a variety of psychological or physical stimuli that threaten homeostasis. In animal experiments, a number of types of stress have been created and used in several disease models. In general, each stress is characterized according to a distributed ratio of physical and psychological burdens: for example, a physical burden is far more dominant in forced swimming, immobilization, and electrical FS stress (22, 29–31), whereas a psychological burden is created by social isolation stress, water avoidance stress, and social disruption stress (24, 32, 33). The communication box method, designed by Ogawa and Kuwahara (34), has

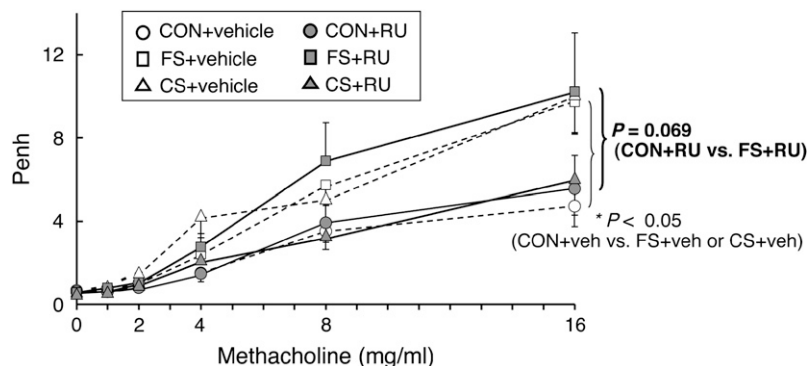


Figure 8. Effect of stress on airway hyperresponsiveness after antigen challenge in RU-486-pretreated mice. CON, FS, and CS indicate the groups exposed to the control conditions, physical stress by electric foot-shock, and psychological stress by communication box, respectively. Airway responsiveness to methacholine was evaluated by changes in Penh at 24 h after airway challenge with OVA after pretreatment with RU-486, a glucocorticoid antagonist (CON + RU, FS + RU, CS + RU), or a vehicle (CON + vehicle, FS + vehicle, CS + vehicle). All values are expressed as the mean $\pm$ SE (CON + vehicle: $n = 7$; FS + vehicle: $n = 7$; CS + vehicle: $n = 7$; CON + RU: $n = 9$; FS + RU: $n = 10$; CS + RU: $n = 10$). The p value was calculated for comparison of corresponding values in the CON + vehicle and CON + RU groups. $*p < 0.05$ was considered to be significantly different from the corresponding value of the CON + vehicle group.

been used worldwide to investigate behavioral and physiological changes caused by purely psychological stress (21, 35, 36). The important feature of this method is that some animals that are exposed to the physical stress of an electrical FS can induce psychological stress in other animals by means of intraspecies emotional communication. Therefore, we used a communication box to evaluate the effects of two types of stress, physical (FS) and psychological (CS), on asthma in mice.

In this study, mice were exposed to either FS or CS for 1 hour three times (every other day) during the fourth week of life. This stress protocol was determined by the innate nature of young mice, which are usually weaned at three weeks of age, and by a preliminary experiment in which stress induced two times during the fourth week of life was insufficient for a significant stress-induced exacerbation of adult asthma (total MNCs in BAL at 24 h after antigen challenge, $\times 10^3$: FS, 71.8 ± 6.0 , $n = 10$; CS, 81.4 ± 11.8 , $n = 10$; CON, 64.1 ± 8.8 , $n = 10$;

ANOVA, $p = 0.411$). Furthermore, it should be noted that stress 1 week later (three times during the fifth week of life) also failed to significantly exacerbate adult asthma (total MNCs in BAL at 24 h after antigen challenge, $\times 10^3$: FS, 87.0 ± 14.3 , $n = 10$; CS, 79.7 ± 5.1 , $n = 10$; CON, 66.2 ± 4.1 , $n = 10$; ANOVA, $p = 0.275$). Taken together, the finding that stress at the age of 4 weeks exacerbated adult asthma and the same stress at the age of 5 weeks did not show that a narrow age window seems to be quite important for determining whether or not early stress has a harmful effect on adult asthma.

Allergic immune responses are considered to be the sum of allergic sensitization and allergic challenge and caused by a Th1/Th2 imbalance, with an increase in Th2 cytokines. Depending on the experimental model, some research groups have found stress to be associated with a Th1-like proinflammatory cytokine profile, such as tumor necrosis factor- α , interleukin-1, and interferon- γ (37, 38), whereas other studies have shown an opposite effect of stress, with a shift toward Th2-type response (39, 40). In this study, no differences in the levels of serum anti-OVA-specific IgE, one of several parameters of Th2 dominance in the Th1/Th2 balance, before OVA challenge were found between the stress groups and the control group. Furthermore, a passive sensitization experiment with pretreatment of anti-OVA-specific IgE replicated a stress-induced acceleration of the number of total MNCs in BAL of actively sensitized mice. Although these findings appear to preclude the possibility that stress may affect antigen sensitization, the fact that anti-OVA-specific IgE is regarded as one of several markers of antigen sensitization requires further evidence by other evaluations, such as cytokine release and T-cell polarization.

In addition to the whole-body plethysmograph, several methods of measuring airway resistance have been developed to date: for example, electrical field stimulation, tracheal cannula-inserted body plethysmograph, and head-out body plethysmograph. Although the whole-body plethysmograph has such considerable benefits as being a noninvasive, nonanesthetized, and nonrestrained procedure, particularly in stress-related experiments, it should be noted that the whole-body plethysmograph does not directly measure lung resistance. Therefore, in another pilot study, we further evaluated the differences of AHR between a CON group and FS or CS groups using electrical field stimulation (EFS) (12), because it can directly assess airway resistance. ES_{50} , a marker of airway responsiveness, was 2.26 Hz in the CON group ($n = 8$) and was set as 100% ($\pm 18.2\%$). ES_{50} (defined as the frequency that caused 50% of the maximal contraction calculated from logarithmic plots of the contractile response versus the frequency of EFS) was $55.0 \pm 10.4\%$ and

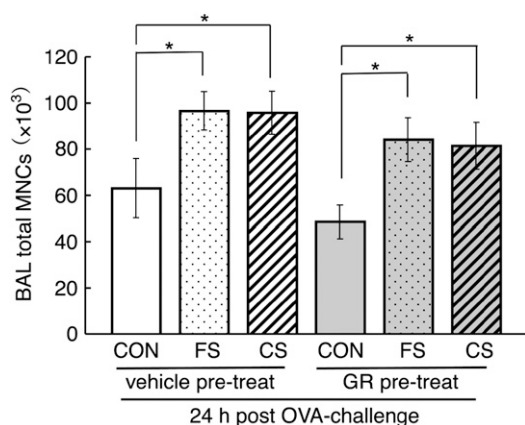


Figure 9. Total cell number of BAL MNCs after antigen challenge in GR-82334-pretreated mice. CON, FS, and CS indicate the groups exposed to the control conditions, physical stress by electric foot-shock, and psychological stress by communication box, respectively. The total cell number of BAL MNCs was assessed at 24 h after OVA airway challenge after pretreatment with GR-82334, a neurokinin-1 receptor antagonist (CON + GR, FS + GR, CS + GR), or a vehicle (CON + vehicle, FS + vehicle, CS + vehicle). All values are expressed as the mean $\pm$ SE (CON + vehicle: $n = 8$; FS + vehicle: $n = 10$; CS + vehicle: $n = 10$; CON + GR: $n = 9$; FS + GR: $n = 10$; CS + GR: $n = 10$). $*p < 0.05$ was considered to be significantly different from the corresponding value in the CON + vehicle or CON + GR group.

58.2 ± 8.6% in the FS (n = 8) and CS (n = 9) groups, respectively (CON vs. FS or CS, $p < 0.05$). In line with the current AHR data by whole-body plethysmograph, this finding confirms that AHR in the FS and CS mice is significantly higher than that in the CON mice.

The present study confirmed that early psychological stress attenuated HPA axis responsiveness during OVA challenge, thereby eventually exacerbating adult asthma. This long-lasting suppressive effect of psychological stress on HPA axis responsiveness is consistent with previous studies showing that several early environmental factors altered a late programming of the HPA axis (17, 18). Especially, neonatally handled animals exhibit dampened HPA response to stress throughout life in comparison with nonhandled animals (19). This inhibiting effect is mediated by an enhancement of GC-negative feedback resulting from increased hippocampal GC receptor expression. In addition, a permanent increase in histone acetylation has recently been reported to lead to such an increase in GC receptor expression in the hippocampus by elevating the capacity of transcriptional factor (nerve growth factor–inducible factor A) to bind to the GC receptor promoter (41). Considering this epigenetic programming, further study is needed to clarify the mechanism(s) behind the current finding that early psychological stress attenuated HPA axis responsiveness in adult mice.

Given the well-known antiinflammatory and immunosuppressive properties of GC, a final effector of the HPA axis, it is reasonable to suppose that a blunted HPA axis responsiveness during OVA challenge ultimately leads to an acceleration of allergic reaction in psychological stress–exposed mice. This supposition is also supported by the present result, as shown in Figure 7, that pretreatment with a GC receptor antagonist *per se* tended to accelerate an increase in total MNCs in BAL. In this regard, it is noteworthy to describe recent clinical studies in which cortisol stress response was blunted among patients with allergic diseases, including asthma, compared with nonallergic control subjects (42–45). This attenuated stress response of endogenous GC has been suggested to participate in an accelerated expression of allergic disease.

To our knowledge, this study is the first evidence that distinct physiological mechanisms that depended on the type of stress, either psychological or physical, were engaged in stress-induced exacerbation of asthma, whereas only one recent experiment reported that the involved mechanisms qualitatively changed as the duration of exposure to restraint stress increased (10). In this study, we found that hyporesponsiveness of the HPA axis during OVA challenge mediated psychological stress–induced exacerbation of adult asthma. However, the precise mechanism(s) underlying the injurious effect of physical stress on asthma remain to be identified, although the possibility of involvement of the HPA axis and NK-1 receptors was successfully excluded. Recently, Akiyama and colleagues (46) have shown that corticotropin-releasing factor (CRF) plays a critical role in a water avoidance stress–induced increase in rat tracheal epithelial response via the activation of tracheal mast cells. Nevertheless, CRF involvement seems unlikely in our physical stress–induced exacerbation of adult asthma, because the failure to find any significant differences in the serum corticosterone levels of the CON and FS groups during OVA challenge (Figure 6) suggests that early physical stress might not have the ability to accelerate the circulating CRF levels, a principal stimulator of the HPA axis, in an adult allergic reaction. Alternatively, as suggested in recent studies (47, 48), an inability to detoxify reactive oxygen species or a decrease in β_2 -adrenergic receptor expression might be the putative mechanism(s).

The answer to another provocative question, why the physiological pathway becomes qualitatively distinct by the type of

stress, remains elusive in this study. Previous studies have suggested that an injurious effect of stress on asthma is mediated through different systems involving the autonomic nervous system, HPA axis, immune system function, oxidative stress pathways, and modified genetic expression (47, 49). Moreover, given that the responsiveness level of each physiological system over a lifetime is considered to be altered by the characteristics of early experiences (50), the sum of their stress-induced alterations may result in the differences of pathway across the types of stress.

In previous research on humans, psychological stress has been shown to have a short-lasting, promoting effect on the manifestation of asthma (3–5, 7, 8). For example, in a study of 90 pediatric patients with asthma, using a within-subject, over time analysis of continuous asthma data collected prospectively for 18 months, severely negative life events (i.e., those that carry a long-term threat to the child's physical or psychological well-being) significantly increased the risk of an acute exacerbation over the subsequent 3 to 6 weeks (4). However, unlike the present animal evidence that psychological stress in childhood exacerbated the symptoms of adult asthma, no human studies have unveiled whether or not psychological stress as a youth has a long-lasting detrimental effect on asthma, even to adulthood, probably due to the inherent difficulty of the many years necessary to conduct a prospective observation from childhood to adulthood.

In conclusion, early psychological and physical stress in the fourth week of life accelerated airway inflammation and AHR in our mouse model of adult asthma. This stress-induced exacerbation of asthma was critically involved in antigen challenge, but not in antigen sensitization. Interestingly, early psychological stress exacerbated adult asthma via HPA axis hyporesponsiveness during antigen challenge, whereas physical stress did so through a pathway(s) distinct from the HPA axis or NK-1 receptors.

Conflict of Interest Statement: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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