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Immunosuppressive Effect of Restraint Stress on the Initiation of Allergic Rhinitis in Mice

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Key Words
Allergic rhinitis · IgE · IgG1 · IgG2a · Immunosuppression · Mouse model · Nasal sensitization · Phospholipase A2 · Restraint stress

Abstract

Background: Exposure to acute stressors modulates both innate and acquired immune function. However, little is known about whether stress has the potential to modulate the pathogenesis of allergic rhinitis. ***Objectives:*** To determine the effects of acute restraint stress on the initiation of allergic rhinitis in a murine model. ***Methods:*** CBA/J mice were repeatedly intranasally sensitized with phospholipase A2 (PLA2) from honeybee venom without adjuvant. Restraint stress was applied using uniform cylinders once a week for a continuous 8-hour period, on five occasions in total. Production of PLA2-specific antibodies and degree of nasal and blood eosinophilia were compared between stressed and control mice. ***Results:*** Repeated intranasal sensitization with PLA2 induced PLA2-specific IgE and marked eosinophilia in both the nose and blood in CBA/J mice. Exposure to restraint stress significantly inhibited production of PLA2-specific IgE, IgG1 and IgG2a. Conversely, the stress exerted no significant effect on eosinophilia. ***Conclusions:*** Exposure to acute restraint stress inhibits antigen-specific antibody production, but not local or systemic eosinophilia. The results of this study suggest that acute stress has the potential to modulate the initiation of allergic rhinitis.

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Introduction

Exposure to physical, neurological, or emotional stress can affect both innate and acquired immunity [1–4]. In general, stressor exposure can increase many measures of innate immunity, such as natural killer cell activity and nitric oxide synthesis [5, 6]. Exposure to acute stress also modulates features of acquired immune functions such as antigen-specific T-cell responses [7, 8]. Such stresses reportedly suppress Th1 responses, since Th1 cells selectively express β2-adrenergic receptor [9]. Controversial results have been reported regarding the effect of stressor exposure on the production of antigen (i.e. keyhole limpet hemocyanin)-specific IgG or IgM [10, 11]. Although exposure to stress affects the pathophysiology of allergic disease, little is known regarding the effect of stress on the initiation of allergic rhinitis [12, 13]. This may be due to a lack of adequate animal models reflecting the pathogenesis of allergic rhinitis.

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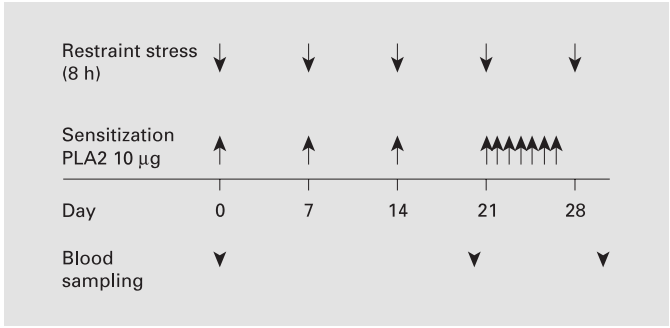


Fig. 1. Treatment schedule. Mice were intranasally sensitized to 10 μg of PLA2 in 20 μl of saline. Sensitization was repeated in the same manner. Following sensitization, restraint stress applied to mice using a single transparent cylindrical chamber was repeated once every week, on a total of five occasions. Blood samples were taken from each tail vein 0, 20, and 30 days after primary sensitization.

We have previously generated a murine model of allergic rhinitis using PLA2, a major allergen in venom from the honeybee, as a sensitizing allergen [14, 15]. In this model, CBA/J mice display production of PLA2-specific IgE and nasal eosinophilia on repeated intranasal sensitization with PLA2 without adjuvants. The present study sought to determine for the first time the role of restraint stress on the pathogenesis of allergic rhinitis in vivo using this murine model.

Methods

Animals

Young adult (10 weeks old) female CBA/J mice (Charles River Japan, Yokohama, Japan) weighing about 18–20 g were used in this study. Mice were maintained in an animal house according to the guidelines of the Animal Study Committee at the Kagawa Prefectural College of Health Sciences. All animals were housed in groups of 3, each in an opaque polycarbonate mouse cage (30 × 20 × 30 cm) with access to food and water ad libitum, and were maintained on a 12-hour light-dark cycle for 2–3 weeks before experiments began. Temperature in the animal house was maintained at 25°C.

Reagents

ELISA plates were purchased from Corning, (Corning, N.Y., USA). Purified rat anti-mouse IgE was purchased from Biosource (Camarillo, Calif., USA), extraAvidin-peroxidase conjugate, PLA2, carbonate buffer and fetal calf serum from Sigma (St. Louis, Mo., USA), tetramethylbenzidine substrate from Kirkegaard & Perry Laboratories (Gaithersburg, Md., USA), phospholic acid from Wako Pure Chemical Industries (Osaka, Japan), goat anti-mouse IgG1/IgG2a monoclonal antibody peroxidase conjugate from Boehringer-Mannheim (Indianapolis, Ind., USA) and biotin (long-arm) N-hydroxy succinimide ester from Vector Laboratories (Burlingame, Calif., USA). Contamination of endotoxin was negligible as determined using an Endospec assay kit (Seikagaku Kogyo, Tokyo, Japan) in accordance with the manufacturer's instructions.

Sensitization of Mice

Mice (n = 6–8/group) were sensitized by nasal administration of 20 μl of saline containing 10 μg of PLA2 using a microsyringe (Hamilton, Reno, Nev., USA). PLA2 was carefully administered as 7–8 drops of aqueous solution into each nostril in turn. Sensitization was repeated in the same manner after 1 and 2 weeks. On day 21 and on the following 7 consecutive days, the same amount of PLA2 was administered in the same manner. Blood samples were taken from the tail vein on days 0, 20, and 30 after primary sensitization (fig. 1).

Induction of Restraint Stress

Following sensitization, restraint stress was applied to mice (n = 8) using a single transparent polymethylmethacrylate cylindrical chamber (20 mm diameter, 100 mm long) commonly used for drawing blood from mice. This chamber was placed in a horizontal position in the mouse cage, and the mice were maintained therein for a continuous 8-hour period without food or water. This manipulation was performed once a week, on a total of five occasions (fig. 1). Control mice were maintained in their cages without food and water at the same times. Three separate experiments were performed to confirm reproducibility. One mouse escaped from the cylinder during the restraint period, and was excluded from analysis.

PLA2-Specific IgE, IgG1, and IgG2a in Serum

Serum levels of PLA2-specific IgE were determined using sandwich ELISA, and PLA2-specific IgG1 and IgG2a were detected using indirect ELISA as described elsewhere [14, 15]. Results are expressed as endpoint titers, where the endpoint equals the final serum dilution yielding an absorbance twice the background. Absorbance was measured at 450 nm using an automatic microplate reader (BioRad, Hercules, Calif., USA).

To determine whether restraint stress affected antigen-specific immune responses, differences in values of PLA2-specific IgE, IgG1 and IgG2a serum levels were compared between stressed and control animals.

Histological Examination

Twelve hours after drawing the final blood sample, mice were killed by cervical dislocation. The nasal cavity with paranasal sinuses was removed, fixed in 10% formalin and decalcified. Coronal nasal sections were stained using hematoxylin/eosin and Luna staining techniques. Eosinophils in the nasal mucosa were counted under light microscopy.

Blood Eosinophils in Mice

Eosinophils were counted in a blood smear stained using May-Grünwald Giemsa.

Statistical Analysis

Data are expressed as means ± SEM for each subject group. Statistical analysis was performed using Student's unpaired t test to compare titers of PLA2-specific IgE, IgG1 and IgG2a for restrained and control groups. Values of $p < 0.05$ were considered statistically significant.

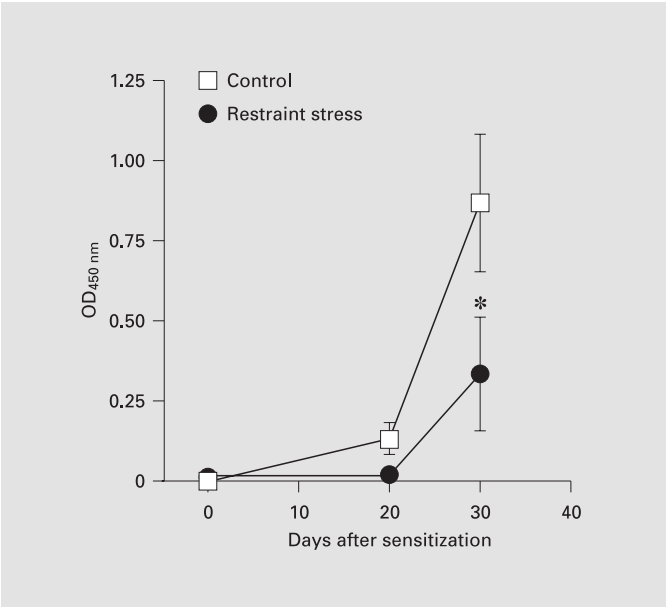


Fig. 2. Effect of restraint stress on PLA2-specific IgE production. Production of PLA2-specific IgE in mouse serum was significantly increased 30 days after the first sensitization. The level of PLA2-specific IgE was significantly decreased in stressed mice compared with control mice on day 30 (* $p < 0.05$). Data are representative of three separate experiments.

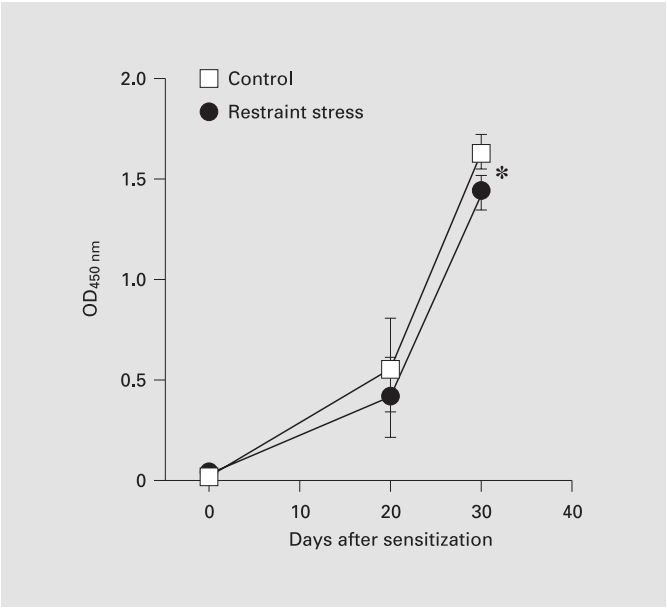


Fig. 3. Effect of restraint stress on PLA2-specific IgG1 production. Production of PLA2-specific IgG1 in mouse serum was significantly increased 30 days after the first sensitization. The level of PLA2-specific IgG1 was significantly decreased in stressed mice (* $p < 0.025$). Data are representative of three separate experiments.

Results

Effect of Restraint Stress on Antibody Production

Production of PLA2-specific IgE, IgG1 and IgG2a in mouse serum after PLA2 nasal sensitization was observed 20 days after the first nasal sensitization, and significant increases in serum IgE, IgG1 and IgG2a levels were detected 30 days after the first sensitization (fig. 2–4). As shown in figure 2, levels of PLA2-specific IgE were significantly lower in stressed mice than in control mice on day 30 ($p < 0.05$). Similarly, levels of PLA2-specific IgG1 and IgG2a were significantly decreased in stressed mice compared to control mice ($p < 0.025$ and $p < 0.05$, respectively) (fig. 3, 4).

Effect of Restraint Stress on Eosinophilia in the Nose and Blood

After sensitization with PLA2, the number of eosinophils that infiltrated the mucous membranes of the mouse nasal septum was significantly increased, in line with our previous reports [15]. Stressed mice tended to display a lower degree of nasal eosinophilia compared with control mice, but this difference was not significant ($p > 0.05$).

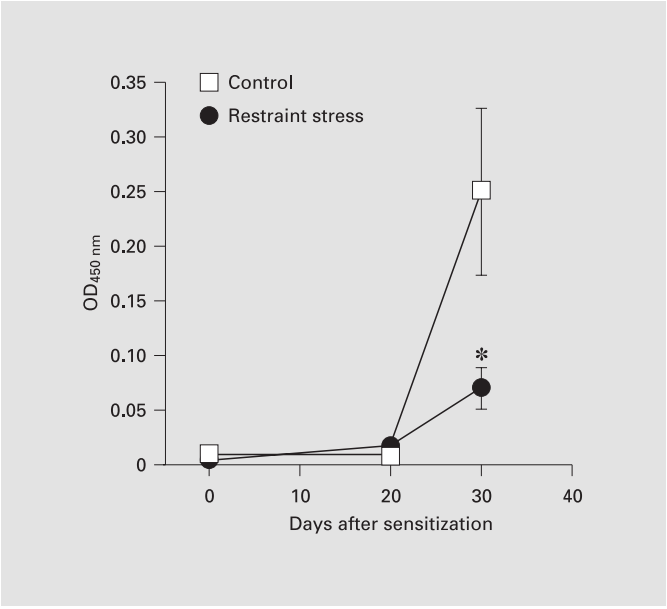


Fig. 4. Effect of restraint stress on PLA2-specific IgG2a production. Production of PLA2-specific IgG2a in mouse serum was significantly increased 30 days after the first sensitization. The level of PLA2-specific IgG2a was also significantly decreased in stressed mice (*$p < 0.05$). Data are representative of three separate experiments.

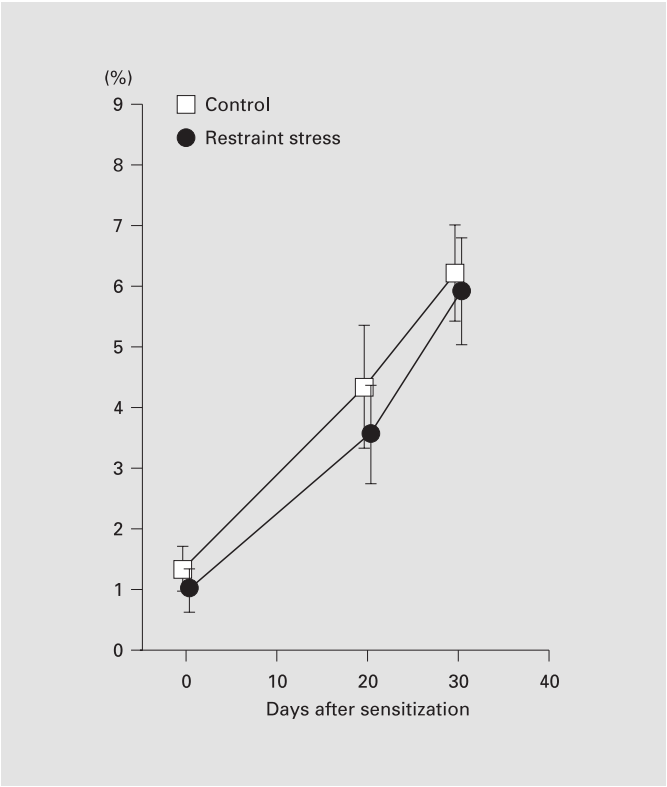


Fig. 5. Effect of restraint stress on blood eosinophilia. After sensitization with PLA2, blood eosinophil counts increased significantly (day 20, 4.3%, $p < 0.005$; day 30, 6.2%, $p < 0.0001$) after the first sensitization (day 0, 1.3%). In contrast, in restraint-stressed mice, eosinophil counts did not significantly change compared with control mice on days 20 and 30. Data are representative of three separate experiments.

Mean eosinophil counts in stressed and control mice were 21.86 ± 4.46 (10 × 40) and 27.50 ± 5.87 eosinophils/high power field, respectively.

Eosinophil concentration in blood after sensitization with PLA2 increased significantly (day 20, 4.3%, $p < 0.005$; day 30, 6.2%, $p < 0.0001$) from the first sensitization (day 0, 1.3%). No significant inhibition was seen in mice under restraint stress (day 20, 3.6%; day 30, 5.9%) (fig. 5).

Discussion

Many studies have shown that stress can affect IgE production [3, 16–20]. In humans, increased levels of serum IgE are induced by acute stressors such as burns and trauma [16, 17]. More recently, chronic caregiver stress in early childhood has been reported to upregulate IgE production in children [13]. Conversely, several kinds of stressors can suppress IgE production in rodents [3, 18, 19]. However, mild inescapable electric foot-shock stress can augment IgE production in rats [20]. Given these findings, stress should be categorized as either acute stress or chronic continuous stress, and immune reactions should thus be analyzed under each stress condition. The present finding that PLA2-specific IgE production is significantly decreased in stressed CBA/J mice following intranasal sensitization with PLA2 without adjuvants is consistent with earlier reports, and suggests that acute restraint stress plays an inhibitory role in IgE production in a murine model of allergic rhinitis.

In addition, production of both PLA2-specific IgG1 and IgG2a was also inhibited in stressed mice. Interleukin-4 is widely accepted as facilitating development of Th2 responses and inducing IgG1 and IgE production in mice. In contrast, interferon-γ induces a Th1 response that is associated IgG2a production [21]. Although we did not examine cytokine production by nasal mononuclear cells due to the in vitro cytotoxicity of PLA2, our results suggest the possibility that mice under restraint stress display impaired development of both Th1 and Th2 responses to intranasal sensitization with PLA2. Cortisol is known to be able to shift T cells toward a Th2 phenotype [22]. However, Fukui et al. [3] documented that restraint stress significantly suppressed both Th1- and Th2-type immune responses in mice, and this suppression was also closely correlated with the inhibition of interleukin-4 and interferon-γ production. Furthermore, Dhabhar et al. [23] reported that B cells showed greater stress-induced decreases than T cells. This latter report may support our finding, and the current results may suggest that polarized antibody production was not induced under restraint stress in our murine model of allergic rhinitis.

No significant change in nasal eosinophilia was identified in mice under acute restraint stress, as compared with control mice. Several reports have demonstrated that stress enhances eosinophilia in peripheral blood, sputum and bronchoalveolar lavage fluids [24–26]. Although our results were not consistent with these reports, this is the first report to focus on the effect of stress on eosinophil infiltration into the nose. Our results may suggest that eosinophil infiltration into the nose is more resistant to stress than the lower airways. In fact, a recent investigation revealed organ-specific regulation such as the expression of corticotropin-releasing factor receptor 1 following exposure to stress [27]. In addition, several reports have demonstrated that the degree of nasal eosinophilia does

not always parallel IgE production in murine models of allergic rhinitis [15, 28, 29].

A wide range of immunological, endocrinological and neuronal pathways are known to mediate and modulate systemic stress responses [30–32]. Okimura et al. [31], for example, reported that stress-induced production of antibody was stopped by extirpation of the suprarenal glands. Various studies have also revealed that plasma levels of catecholamines, corticosterone and ACTH are increased by the application of restraint stress [33, 34]. Further studies are needed to clarify the direct or indirect involvement of endocrinological and neuronal pathways in the initiation of allergic rhinitis in this model.

In summary, acute restraint stress inhibits antibody production, but not eosinophilia in the murine model of allergic rhinitis. Since the duration, frequency and degree of experienced stress represent important determinants of the impact on health and illness [12], future investigations using various kinds of stresses will help to further our understanding of the roles of stress in the initiation of allergic rhinitis. However, the observations presented herein may provide a new basis for future prophylactic and therapeutic approaches to the management of allergic rhinitis.

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