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**Psychological stress with long-standing allergic dermatitis causes  
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Abbreviations:

AD: atopic dermatitis

ACD: allergic contact dermatitis

BV: betamethasone-17-valerate

GH: group housing

IH: individual housing

TPA: 12-O-tetradecanoylphorbol-13-acetate

TNCB: 2,4,6-trinitrochlorobenzene

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**Abstract**

Psychological factors have long been assumed to be involved in the pathogenesis of allergic skin diseases. The effects of psychological stress on allergic contact dermatitis (ACD) have been experimentally well investigated; however, the effects of ACD on stress responses are largely unknown. Here, we report that preceding chronic ACD dramatically affects the behavioral and physiological stress responses to social isolation (a psychological stressor). In male BALB/c mice, social isolation combined with long-standing (>2 months) ACD by repeated hapten application caused characteristic symptoms, including chronic dermatitis from persistent self-scratching, behavioral changes related to fear/anxiety, and elevated serum IgE levels. The symptoms were maintained by social isolation alone without further hapten application after the onset, and improved by resocialization. Treatment with topical corticosteroids exacerbated the chronic scratch dermatitis, whereas it was effective for chronic ACD. These results show that the symptoms represent a *de novo* development of a specific disease state and not a mere exacerbation of a preexisting allergic inflammation. With this experimental protocol, similar results were obtained in several other strains of mice. This murine model provides a tool for investigating the pathogenesis and treatment of allergic skin disease with psychodermatological aspects.

## Introduction

In the last 2 decades, there has been great progress in the understanding of the etiopathogenesis of allergic skin diseases such as allergic contact dermatitis (ACD) and atopic dermatitis (AD), especially concerning the genetic factors and molecular mechanisms underlying the skin barrier and immune abnormalities (Bieber, 2008; Boguniewicz and Leung, 2011). A better insight into the pathogenesis and treatment has been made possible by the different murine models (Dubrac *et al.*, 2010; Jin *et al.*, 2009; Shiohara *et al.*, 2004). Although each of these models has some features that are characteristic of human inflammatory skin disorders, there is no suitable animal model in which to examine the effect of the complex interaction between immunological and psychological factors on the development of psychodermatological conditions. There is increasing evidence to support the bidirectional relation between psychological factors and inflammatory skin disorders; for example, psychological stress can trigger or exacerbate AD symptoms, and AD is associated with significant psychiatric morbidity (Chida *et al.*, 2008; Hashizume and Takigawa, 2006; Ring *et al.*, 2006; Yaghmaie *et al.*, 2013). In the search for more clinically relevant models of human skin disorders, it is clear that the scope must be broadened from classic “inflammatory” models, to encompass the wide range of psycho-neuro-endocrine-immunological factors.

AD affects up to 20% of children worldwide, and the prevalence is increasing especially in developing countries (Asher *et al.*, 2006). Such an increase in the prevalence cannot be explained only by genetic factors. The most recent US data (Shaw *et al.*, 2011) also showed a wide range of prevalence of AD (9–18%), and its association with metropolitan

living, higher education, and black ethnicity, suggesting that social or environmental factors can affect the expression of AD. Similarly, many previous studies have reported an increase in AD prevalence in urban areas compared with rural areas (Schram *et al.*, 2010). Moreover, mental disorders such as mood and anxiety disorders are also more prevalent among city dwellers (Peen *et al.*, 2010), and the incidence of schizophrenia is strongly increased in persons born and raised in cities (Krabbendam and van Os, 2005). In this regard, a recent neuroimaging study revealed that city living and an urban upbringing affect neural social stress processing (Lederbogen *et al.*, 2011). These findings imply that daily life stress, in addition to allergic sensitization, is crucial for the development of human allergic disease and related psychodermatological disorders. To investigate this possibility, it is necessary to establish an animal model for psychodermatological disorders in which the bidirectional interaction between psychological stress and allergic inflammation is directly involved in the pathogenesis.

During the last 20 years, our laboratory has been involved in the analysis of a murine model for chronic ACD (Kitagaki *et al.*, 1995; Kitagaki *et al.*, 1997; Shiohara *et al.*, 2004). In this model, a human AD-like inflammation characterized by chronic eczema, increased serum IgE levels, and Th2-dominated immune responses is induced by repeated hapten challenge. The onset and maintenance of the inflammatory state depend on the hapten application, and the inflammation rapidly resolves after the cessation of hapten application. During the resolution process, however, we noted that some of these mice when housed alone maintained skin lesions along with severe scratching behavior despite no further hapten application. Because social isolation, such as by housing the animals

individually, represents a psychological stress for rodents (Hall, 1998), we interpreted this finding as indicating that psychological stress in conjunction with chronic allergic inflammation leads to a specific disease state. On the basis of this idea, we established a murine model in which allergic dermatitis and psychological stress are directly involved as triggering and perpetuating factors of psychodermatological conditions.

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## Results

### **Social isolation during the progression of chronic ACD causes idiopathic dermatitis at unchallenged skin sites**

To initially investigate the effect of social isolation on chronic ACD, male BALB/c mice were assigned to either individual housing (IH) or group housing (GH) conditions, and repeatedly challenged on the right ear with 2,4,6-trinitrochlorobenzene (TNCB) at 2-day intervals. In IH mice, but not in GH mice, an “idiopathic dermatitis” that was accompanied by severe scratching developed at unchallenged skin sites, such as the left ear, head, and face, but rarely on the trunk, as shown by increases in left ear thickness (Figure 1a) and clinical skin score (Figure 1b). Untreated normal mice in either IH or GH conditions did not show any signs of skin symptoms (data not shown). The skin lesions of idiopathic dermatitis were qualitatively distinct from those of chronic ACD; these included excoriation, ulcer, scab, scar, small hemorrhage, and/or alopecia (Figure 1c). There was a delay of about 8 weeks between the initiation of hapten challenge/IH and the onset of idiopathic dermatitis, suggesting that a sufficient duration of ACD, social isolation, or both is required for the onset.

### **Idiopathic dermatitis is triggered by social isolation immediately after long-standing ACD and maintained by social isolation alone**

We next examined the timing of exposure to social isolation necessary for the subsequent development of idiopathic dermatitis. IH, when started immediately after the cessation of repeated hapten challenge, triggered idiopathic dermatitis, which developed without a delay of weeks after IH and persisted for 28 weeks (Figure 2a–c). In contrast, IH, when

started 4 weeks after the cessation of the hapten challenge, did not induce idiopathic dermatitis (data not shown). Resocialization (i.e., transfer from IH to GH conditions), when initiated 4 months after the onset, significantly improved the idiopathic dermatitis (Figure 2d).

To investigate the duration of chronic ACD required for the subsequent development of idiopathic dermatitis, mice were repeatedly challenged with TNCB for various durations (30–180 days) under GH conditions, and then they were transferred to IH conditions immediately after the cessation of the hapten challenge. Repeated challenge for at least 60 days was required to induce idiopathic dermatitis. The incidence and clinical skin score increased as the duration of hapten challenge increased up to 120 days (Figure 2e).

Taken together, these results demonstrate that idiopathic dermatitis is 1) triggered by social isolation combined with chronic ACD which persists for at least 2 months; 2) maintained by social isolation alone without further hapten application; and 3) improved by resocialization. Idiopathic dermatitis is considered to represent the *de novo* development of a specific disease state and not a mere exacerbation of preexisting ACD.

In further experiments, the disease state was induced by >2 months of chronic ACD and >2 months of IH immediately after the cessation of the ACD challenge (AI mice), unless stated otherwise. The control mice included age-matched normal mice in GH (NG mice) or IH conditions (NI mice), and mice in GH conditions that had been subjected to chronic ACD for the same period (AG mice).

**Idiopathic dermatitis is caused by self-scratching**

An intense and long-lasting behavior of scratching with the hind limbs was frequently observed in AI mice, and there was a significant correlation between the number of scratching events and the clinical skin score (Figure 3a), implying that idiopathic dermatitis in AI mice was induced by self-scratching. To prove this, a plastic collar was placed on each mouse to prevent scratching of the ears, head, and face (Figure 3b). As a result, the idiopathic dermatitis improved with time and disappeared completely after 8 weeks (Figure 3c,d). However, the scratching behind the collar continued throughout the experiment, and after removal of the collar, the skin lesions rapidly relapsed (Figure 3d), suggesting that the desire to scratch persisted even after the complete resolution of skin symptoms. These results demonstrate that the idiopathic dermatitis is caused by self-scratching, which is consistent with the diagnosis of scratch dermatitis based on macroscopic and histological features of the skin lesions (Supplemental Figure 1).

**AI mice show low body weight, and behavioral, endocrine, and immune abnormalities**

AI mice had a significantly lower body weight compared to that of age-matched control mice (Figure 4a,b), and there was a significant correlation between body weight and clinical skin score (Figure 4c). Resocialization restored the body weight of AI mice significantly (Figure 4d).

AI mice also showed the following behavioral changes compared with NI mice: a frequent appearance of fear behaviors during human handling, such as freezing, vocalization, urination, and defecation (Figure 4a); an increase in defecation during novel environment exploration (Figure 4e), which indicates increased anxiety-like behavior (Okuyama *et al.*, 1999); a lack of nest-building behavior (Figure 4f); and a change in the circadian pattern of locomotor activity (hypoactivity in the dark phase and hyperactivity in the light phase) (Figure 4g). Furthermore, basal plasma corticosterone levels were significantly higher in AI mice than in control mice (Figure 4h). There was no significant correlation between the corticosterone levels and clinical skin score in AI mice, which may be due to the technical limitation of blood sampling in the presence of artificial disturbances.

Serum total IgE levels were markedly increased by repeated hapten challenge (Shiohara *et al.*, 2004) and decreased after the cessation of the challenge. However, the levels were maintained significantly higher in AI mice than in AG mice (Figure 4i). There was a significant correlation between serum total IgE levels and the clinical skin score in AI mice (Figure 4j). In addition, the total IgE and IL-4 productions by splenocytes were much higher in AI mice than in control mice (Supplemental Figure 2). Importantly, the serum total IgE levels in AI mice were significantly reduced by resocialization (Figure 4k), but not by prevention of scratching (Figure 4l), showing that the immunological change is dependent on psychological stress but not skin inflammation.

**Topical corticosteroid treatment improves chronic ACD but exacerbates chronic scratch dermatitis**

To evaluate the effect of topical corticosteroid on chronic ACD and the subsequent development of psychodermatological conditions, 0.1% betamethasone-17-valerate (BV) was topically applied to the challenged right ear once daily. The treatment markedly improved the chronic ACD and simultaneously prevented the onset of idiopathic dermatitis (Figure 5a).

Next, to evaluate the therapeutic effect on established chronic skin lesions in AI mice, which is considered to be “pure” scratch dermatitis, 0.1% BV was topically applied to the right and left ears once daily for 6 weeks. Unexpectedly, the treatment resulted in an exacerbation of the skin lesions, as evidenced by a significant increase in the clinical skin score (Figure 5b) and distinctive macroscopic features: development of necrotic skin lesions and reduction of the size of ear pinna (Figure 5e). We also compared the dose-response effects of BV on chronic scratch dermatitis with those on chronic ACD. The lower doses of BV (0.001% or 0.01%) showed no significant effects on the chronic scratch dermatitis and 0.1% BV exacerbated it in the same fashion (Figure 5c), whereas 0.001% to 0.1% BV significantly suppressed chronic ACD (Figure 5d).

**Idiopathic dermatitis can be induced with another hapten or in some other mouse strains**

Finally, to test the generality of our experimental protocol, we evaluated the incidence of idiopathic dermatitis with different chemicals or mouse strains (Supplemental Table). The

use of oxazolone, a different hapten, instead of TNCB induced idiopathic dermatitis with a similar incidence, whereas 12-O-tetradecanoylphorbol-13-acetate (TPA), a primary irritant, did not cause the development of idiopathic dermatitis although it induced chronic dermatitis on the challenged right ear comparable to that induced by TNCB. Idiopathic dermatitis could also be induced in some other mouse strains, including mast cell-deficient mice (Supplemental Figure 3); however, there were clear differences in the incidence among mouse strains. On the other hand, female BALB/c mice showed a lower incidence of idiopathic dermatitis than male mice. These results indicate that this pathological event is a general phenomenon neither limited to the BALB/c strain nor to a specific hapten, although there are some strain and sex differences in the susceptibility.

## Discussion

The nervous and immune systems interact in a bidirectional fashion; various inflammatory diseases, including skin disorders, are influenced by psychological stress, and vice versa produce mental problems (Ader *et al.*, 1995; Chida *et al.*, 2008). The effects of stress on ACD have been experimentally well investigated in rodents, the results of which demonstrate that ACD is differentially influenced by different types and durations of stressors; for example, acute restraint stress enhances whereas chronic restraint stress suppresses ACD (Dhabhar and McEwen, 1997), and acute isolation stress suppresses whereas chronic isolation stress enhances ACD (Nakano, 2004). In contrast, the effects of ACD on stress responses have been poorly investigated in animal models. In this study, we demonstrated that preceding chronic ACD dramatically affects the behavioral and physiological stress responses to a psychological stressor (social isolation).

Social isolation is known to produce wide-ranging behavioral and neuroendocrine effects in rodents depending on the developmental stage of the animals: social isolation during the preweaning or early post-weaning period profoundly affects brain development and adult behavior, whereas that during adulthood produces relatively mild and reversible changes (Fone and Porkess, 2008; Hall, 1998; Lukkes *et al.*, 2009). Indeed, it has been reported that serum corticosterone levels are elevated by short-term social isolation (2 days), but are returned to the normal range by long-term social isolation (1 month) in adult mice (Nakano, 2004). Likewise, in our experiments, social isolation (>2 months) had no significant effect on plasma corticosterone levels and body weights in normal

mice. In mice subjected to long-standing ACD, however, social isolation induced significant elevation of corticosterone levels and weight loss, in addition to behavioral changes such as increased self-scratching and anxiety-related behaviors. These results show that chronic ACD leads to substantial changes in stress responses to social isolation.

Chronic ACD by repeated hapten application causes a shift from a Th1-dominated to a Th2-dominated immune response (Kitagaki *et al.*, 1997). In the chronic ACD model, serum levels of IgE, which predominantly reflect activation of Th2 immunity, gradually increase up to 4 months during repeated hapten application (data not shown)— a time period similar to that when the incidence of idiopathic dermatitis reaches a peak. In contrast, chronic irritant contact dermatitis by repeated TPA application does not influence stress responses to social isolation. These findings suggest that overactivation of Th2 immune responses by long-standing allergic inflammation leads to changes in stress responses to social isolation. In addition, the Th2 dominance, characterized by elevated levels of serum IgE and IL-4 production from splenocytes, is maintained by social isolation alone without further hapten application after the onset of psychodermatological conditions. Because stress hormones such as glucocorticoid and catecholamines can influence the Th1/Th2 balance (Calcagni and Elenkov, 2006), a vicious circle between the Th2 dominance and psychological stress may be involved in the pathogenesis of psychodermatological conditions in this model.

Pruritus is one of the most troublesome symptoms of skin disorders such as AD that substantially impair the patients' quality of life (Koblenzer, 1999). Sleep loss that

accompanies nighttime scratching can lead to daytime fatigue, mood disorder, and poor school or work performance (Bender *et al.*, 2008; Slattery *et al.*, 2011). Our model reproduces these pruritus-related symptoms, such as persistent scratching behavior, and disturbed circadian pattern and nest building. The causes of chronic pruritus can be broadly categorized into 4 major groups: dermatologic, systemic, neuropathic, and psychogenic (Yosipovitch and Bernhard, 2013). The “pruritus” in this model seems to have both psychogenic and dermatologic causes, because 1) social isolation is critical for the onset and maintenance, and 2) the scratch dermatitis develops on untreated normal skin sites (although it localizes around the hapten-challenged right ear) such as the left ear, head, and face, but rarely on the trunk. In this regard, recent studies have suggested the existence of a “central sensitization to itch” in chronic pruritus (Ikoma *et al.*, 2006; Paus *et al.*, 2006; Schmelz, 2010). This mechanism may be involved in the pathogenesis of pruritus in this model, in which we assume that long-standing repeated pruritic stimuli by ACD facilitate central itch processing involving pathways around the originally activated one, and the subsequent psychological disturbances further lowers the threshold, resulting in chronic pruritus evoked by innocuous stimuli, such as tactile stimuli perceived by low-threshold mechanoreceptors (A- $\beta$  fibers). In fact, a light tactile stimulus easily evokes scratching or head-shaking behavior in AI mice (data not shown).

Chronic pruritus leads to persistent scratching and consequent variable skin damage (scratch dermatitis) (Stander *et al.*, 2007). Clinically, scratch dermatitis generally coexists with primary skin lesions such as allergic dermatitis, making it difficult to investigate them separately. It is therefore important to experimentally dissect scratch dermatitis to

directly address whether conventional treatment such as topical corticosteroids is indeed beneficial to it as well as to allergic dermatitis. In this study, we show that topical treatment with corticosteroids has no beneficial or detrimental effects on “pure” chronic scratch dermatitis in AI mice, whereas it suppresses chronic ACD. Consistent with this, it is known that human skin lesions that may be induced by prolonged scratching, such as prurigo nodularis, lichen simplex chronicus, or lichen amyloidosis, are highly resistant to therapy (Lotti *et al.*, 2008; Yosipovitch and Bernhard, 2013), although topical corticosteroids are extremely effective for severe flares of AD. Finally, the intractable psychodermatological symptoms, including scratch dermatitis, in this model can be restored by removing the social isolation stressor (resocialization); likewise, psychological interventions have been reported to be beneficial for person with pruritic skin disorders such as AD, although further studies are needed to explore and evaluate the appropriate strategies (Chida *et al.*, 2007; Ersser *et al.*, 2007; Lavda *et al.*, 2012).

The results of this study indicate that there are at least 2 distinct disease states in our mouse model: an “inflammatory disease state” driven by allergic dermatitis and a “psychiatric disease state” driven mainly by psychological stress. The use of controllable environmental factors (hapten application and social isolation) with predisposing genetic factors (male BALB/c mice) makes it possible to develop and dissect both disease states in this model. In the psychiatric disease state (i.e., psychodermatological conditions), elevated IgE levels are maintained by social isolation alone without further hapten application and improved by resocialization but not by prevention of scratching, suggesting a differential regulation of IgE that is more dependent on psychological

factors than the initial skin barrier perturbation and subsequent cutaneous inflammation. This infers that such psychiatric disease states underlie the development of intractable symptoms in human allergic skin diseases such as ACD and AD. The psychosocial stress associated with city living and modern lifestyles may have a strong impact on the development of allergic disorders and offer a potential target for interventions designed to reduce the prevalence in westernized countries. Our model provides a tool for investigating the pathogenesis and treatment of allergic skin disease with psychodermatological aspects.

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## **Materials and Methods**

### **Mice and housing conditions**

Male BALB/c mice were purchased from Charles River Japan (Yokohama, Japan) or CLEA Japan (Tokyo, Japan) at 5 weeks of age. All mice were housed in groups of 6 to 11 per cage (22 × 38 × 20 cm) and acclimated for at least 1 week before use. In each experiment, some mice were transferred from GH to IH conditions in smaller cages (18 × 28 × 13 or 10 × 18 × 11 cm). Animals were maintained under specific pathogen-free conditions on a 12-h light/dark cycle (lights on at 6:30 am and off at 6:30 pm) and received food and water ad libitum. The protocols of this study were approved by the Institutional Ethics Committee on Animal Experiments, and all procedures were in accordance with the Guidelines for Animal Care and Use of Otsuka Pharmaceutical Co., Ltd.

### **Induction of chronic ACD**

Mice were sensitized by a single epicutaneous application of 20 µL of 1% TNCB (Tokyo Chemical Industry, Tokyo, Japan) in acetone to the right ear 7 days before the first challenge, and then repeatedly challenged with 20 µL of 1% TNCB on the right ear at 2-day intervals. Because repeated hapten application induces severe dermatitis, the treated area was restricted to the right ear pinna (approximately 3% of all skin surface areas). The left ear and other skin sites were left untreated.

### **Ear thickness**

Ear thickness was measured with a dial thickness gauge (Ozaki MFG, Tokyo, Japan) under light anesthesia with diethyl ether. During the period of hapten challenge, the measurement was performed just before each challenge.

### **Clinical skin score**

To assess the severity of idiopathic dermatitis (scratch dermatitis), we used the following clinical skin score based on the area of skin lesions with scratch-induced damage such as ulcers, excoriations, erosion, small hemorrhages, crusts, or scabs: score for the left ear or the right ear (0 = no lesion, 1 = lesion area <12.5%, 2 = lesion area from 12.5% to 25%, 3 = lesion area from 25% to 50%, 4 = lesion area  $\geq$ 50%) and score for the head, left face, or right face (0 = no lesion, 1 = lesion area <50%, 2 = lesion area  $\geq$ 50%). The incidence of idiopathic dermatitis was calculated from the number of mice with a total clinical skin score  $\geq$ 2.

### **Scratching behavior**

The observations of scratching behavior were made 0.5 to 2 h after lights-off in a 12-h light/dark cycle. Mice in their familiar home cages were video-recorded using an infrared camera (VC-D900; Sanyo, Osaka, Japan) without human disturbance. Only scratching of the right or left ear with the hind limbs was counted for 30–60 min. Mice generally showed a series of scratches for a second; therefore, a series of scratching movements between lifting the hind limb and putting it down was counted as 1 event (Kuraishi *et al.*, 1995).

**Emotional defecation**

Mice were transferred individually from their home cages to a novel environment ( $18 \times 28 \times 13$  cm cage with a clean aluminum foil sheet on the floor), and allowed to freely explore the environment for 10 min. The number of fecal boli excreted during the period was counted.

**Nest-building behavior**

Under IH conditions, normal mice consistently formed an identifiable nest in a new cage by using shredded strips of paper chips for bedding. To assess this nest-building behavior, 1 week after each mouse was moved to a new clean cage with fresh paper chip bedding, the cage was checked for the presence of a newly built nest.

**Locomotor activity**

The locomotor activity of the mice was measured using a Supermex system (Muromachi Kikai, Tokyo, Japan). The infrared sensors were placed above their home cages, and locomotor activity was monitored continuously over 24 h without human disturbance.

**Basal plasma corticosterone levels**

Between 8:30 am and 11:00 am, blood was collected from the tail vein within 2 min after mice were removed from their home cages. The plasma was stored at  $-80^{\circ}\text{C}$  until analyzed. The samples were extracted with diethyl ether, evaporated, reconstituted in assay buffer, and determined by ELISA using goat anti-rabbit IgG ( $1.5 \mu\text{g/mL}$ ; Vector Laboratories, Burlingame, CA), horseradish peroxidase-labeled corticosterone ( $0.1$

µg/mL; Cosmo Bio, Tokyo, Japan), and rabbit anti-corticosterone IgG (1:600,000, Cosmo Bio), according to the manufacturer's instructions.

### **Serum total IgE levels**

Blood was collected from the tail vein, and the serum was stored at – 80°C until analyzed. Serum levels of total IgE were measured using an ELISA kit (OptEIA; BD Pharmingen, San Diego, CA) according to the manufacturer's instructions.

### **Topical corticosteroid treatment**

BV (Sigma-Aldrich Japan, Tokyo, Japan) dissolved in acetone/methanol (1:1, v/v) was epicutaneously applied to the ears of mice (20 µL/ear, once daily).

### **Statistics**

Data are presented as mean ± SEM. All statistical analyses were performed using SAS software (SAS Institute Japan, Tokyo, Japan).  $P < 0.05$  was considered statistically significant.

**Conflict of Interest**

HK, HH, and TK are employees of Otsuka Pharmaceutical Co., Ltd. TS states no conflict of interest.

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## Figure Legends

**Figure 1. Effect of social isolation during the progression of chronic allergic contact dermatitis (ACD).** Mice were assigned to either individual housing (IH) or group housing (GH) conditions ( $n = 16$  each), and repeatedly challenged on the right ear with 2,4,6-trinitrochlorobenzene at 2-day intervals for 28 weeks. (a) Changes in left and right ear thickness. (b) Changes in total clinical skin score for the left ear, head, right face, and left face. (a, b)  $**P < 0.01$ , repeated-measures ANOVA. (c) Photographs of IH and GH mice at 20 weeks.

**Figure 2. Effect of social isolation immediately after chronic allergic contact dermatitis (ACD).** (a–c) Mice subjected to 12 weeks of ACD were subsequently exposed to individual housing (IH) ( $n = 16$ ) or continuous group housing (GH) conditions ( $n = 15$ ). (d) Mice with established idiopathic dermatitis induced by 12 weeks of ACD and subsequent 17 weeks of IH were assigned to either resocialization ( $n = 6$ ) or continuous IH conditions ( $n = 5$ ). (e) Mice subjected to ACD for various durations (30–180 days;  $n = 11–17$ ) were exposed to 7 weeks of IH. (a, b, d)  $**P < 0.01$ , repeated-measures ANOVA. (e)  $*P < 0.05$ ,  $**P < 0.01$  versus unchallenged mice (0 day), Fisher's exact test with Bonferroni correction or one-way ANOVA followed by Dunnett's test.

**Figure 3. Idiopathic dermatitis is induced by self-scratching.** (a) Correlation between the number of scratching events and total clinical skin score for the right and left ear in AI mice 6–7 weeks after IH exposure ( $n = 37$ ). Simple linear regression analysis. (b)

Photograph of a mouse wearing a plastic collar to prevent scratching of the ears, head, and face. (c) Changes in total clinical skin score for the right and left ear in AI mice wearing a collar ( $n = 10$ ) or untreated ( $n = 7$ ).  $**P < 0.01$ , repeated-measures ANOVA. (D) Photograph of the ear before (left) and 12 weeks after wearing a collar (middle), and 4 days after removing the collar (right).

**Figure 4. Low body weight, and behavioral, endocrine, and immune abnormalities**

**in AI mice.** Mice subjected to >2 months of IH after >2 months of chronic ACD (AI mice) and age-matched control mice (NG, NI, and AG mice) were employed. (a) Photograph of AI and NI mice. (b) Body weight of AI and control mice ( $n = 9-10$ ). (c) Correlation between body weight and total clinical skin score in AI mice ( $n = 70$ ). (d) Changes in body weight of AI mice after resocialization ( $n = 6$ ) or continuous individual housing (IH) ( $n = 5$ ). (e) Number of fecal boli excreted during novel environment exploration by AI ( $n = 19$ ) and control NI mice ( $n = 18$ ). (f) Incidence of nest-building behavior in AI mice with a total clinical skin score of high (H: >3,  $n = 10$ ), medium (M: 2 or 3,  $n = 12$ ), low (L: 1,  $n = 17$ ), and none (N: 0,  $n = 31$ ), and control NI mice ( $n = 10$ ). (g) Circadian locomotor activity in AI and control NI mice ( $n = 8$  each). (h) Basal plasma corticosterone levels in AI and control mice ( $n = 19-20$ ). (i) Serum total IgE levels in AI and control mice ( $n = 9-10$ ). (j) Correlation between serum total IgE levels and total clinical skin score in AI mice ( $n = 70$ ). (k) Changes in serum total IgE levels in AI mice after resocialization ( $n = 6$ ) or continuous IH ( $n = 5$ ). (l) Changes in serum total IgE levels in AI mice after wearing a collar ( $n = 10$ ) or untreated ( $n = 7$ ).  $*P < 0.05$ ,  $**P < 0.01$ , NS non-significant. (b, h, i) One-way ANOVA followed by Tukey's test. (c, j)

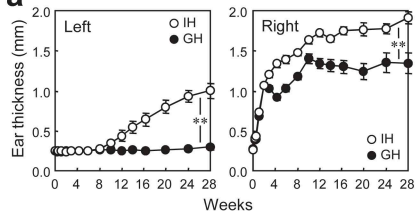
Simple linear regression analysis. (d, k, l) Repeated-measures ANOVA. (e, g) Unpaired Student's t test. (f) Fisher's exact test with Bonferroni correction (vs. NI mice).

**Figure 5. Topical corticosteroid improves chronic allergic contact dermatitis (ACD)**

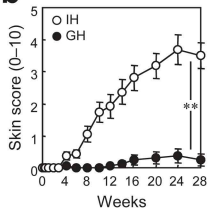
**but exacerbates chronic scratch dermatitis.** (a) Mice subjected to chronic ACD were treated with 0.1% betamethasone-17-valerate (BV) (n = 9) or vehicle (n = 8) on the right ears. (b, e) AI mice were treated with 0.1% BV (n = 10) or vehicle (n = 9) on the right and left ears. (c) AI mice were treated with BV (0.001%, 0.01%, or 0.1%) or vehicle (V) (n = 10–12) on the right and left ears for 6 weeks. (d) Mice subjected to 24 days of ACD were treated with BV (0.001%, 0.01%, or 0.1%) or vehicle (n = 8 each) on the right ears for 2 weeks. \*\*P < 0.01. (a, b) Repeated-measures ANOVA. (c, d) One-way ANOVA followed by Dunnett's test.

# Figure 1

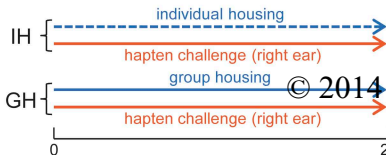
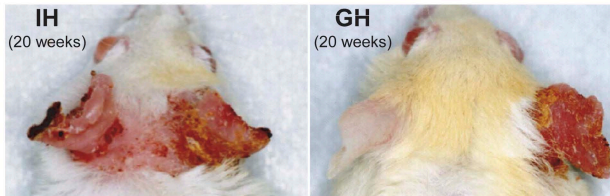
**a**



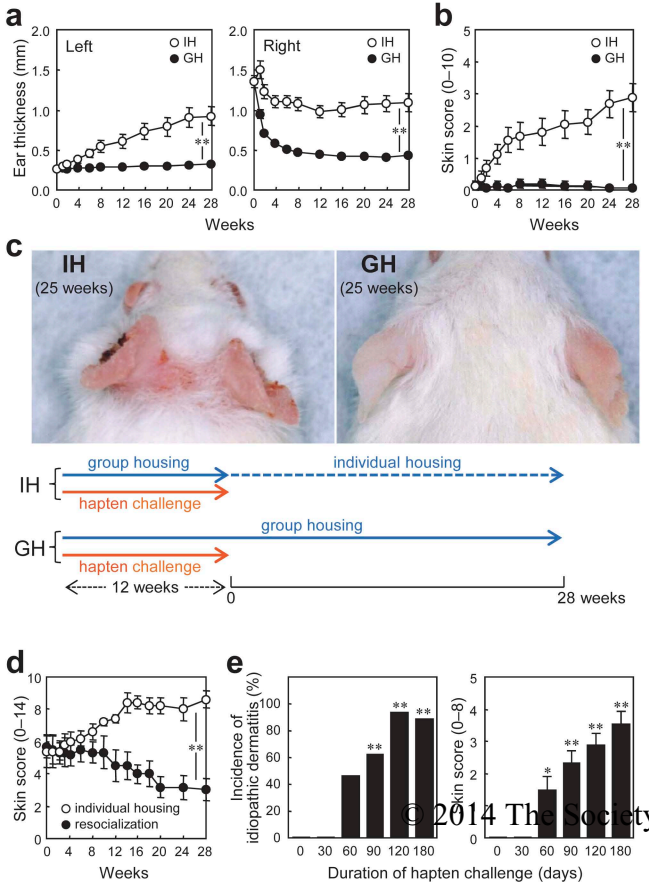
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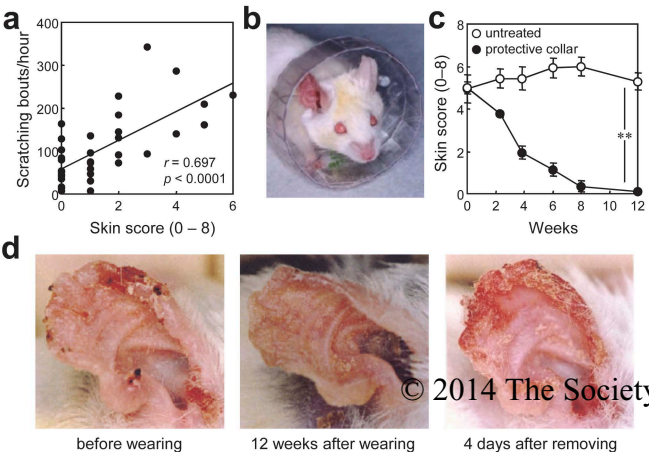
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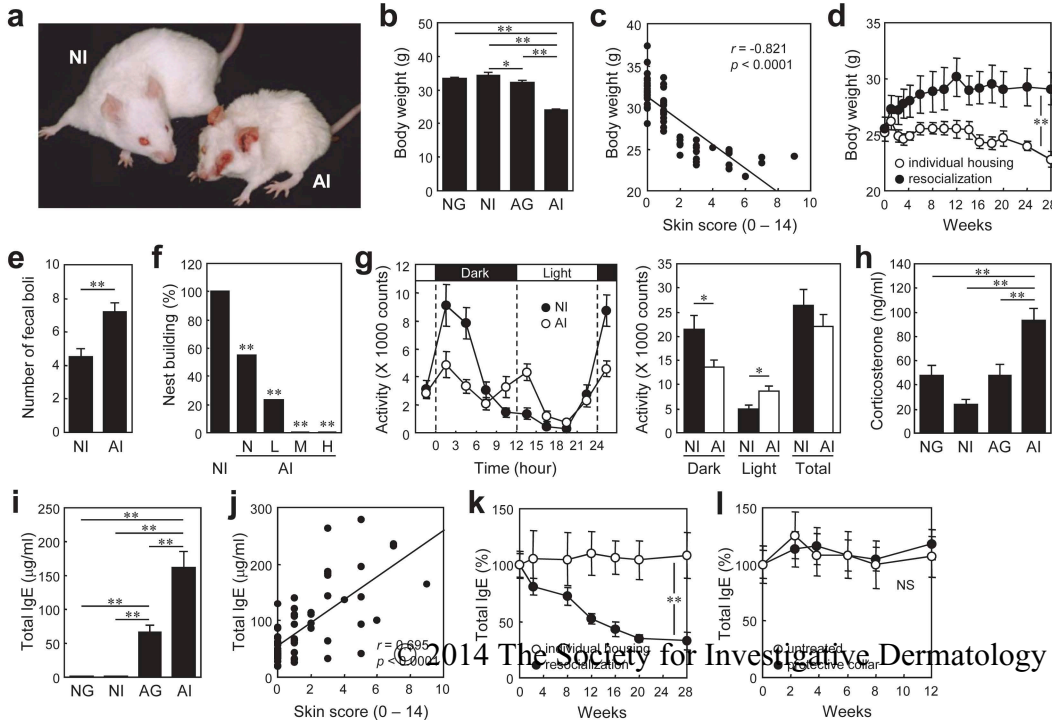
# Figure 2



# Figure 3



# Figure 4



# Figure 5

