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Original Research

Scratching Responses to Epidermal Injury in C57BL/6, DBA/2, BALB/c, and CD1 Mice

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Whereas early investigations into ulcerative dermatitis (UD) focused on the possibility of a primary dermatopathology, several recent studies have advocated scratching behavior as a primary driver for UD. The aim of this study was to assess whether B6 mice exhibit excessive scratching under resting conditions or when provoked by epidermal barrier disruption. We hypothesized that B6 mice would exhibit more spontaneous scratching behavior and that B6 mice would be more pruritic after mild epidermal barrier injury compared with the other strains and stock tested. The behavior of the retired breeder female C57BL/6J, DBA/2J, BALB/cByJ, and Crl:CD1 mice was videotaped for 60 min. Behavior filming occurred at 17:15 and at 07:00 the next morning prior to (baseline) and after tape-stripping to initiate epidermal barrier disruption. Scratching duration was recorded as brief (less than 3 s) or prolonged (3 s or longer), on the basis of observations during a pilot study. In contrast to the hypothesis, B6 mice did not scratch significantly more frequently, have more long-duration scratching events, nor have a higher median scratching duration of prolonged scratching as compared with the other types of mice tested. In fact, B6 mice showed the lowest average scratching frequency and duration under both conditions. B6 mice demonstrated increased scratching behavior after epidermal barrier disruption, but the increased scratching did not surpass the rate or duration of scratching in the other types of mice tested. These findings do not support the idea that a strain-related tendency toward exaggerated scratching behavior under resting or epidermal barrier disruption conditions predisposes B6 mice to UD.

Abbreviation: UD, ulcerative dermatitis

Ulcerative dermatitis (UD) is a condition characterized by pruritic open skin lesions in C57BL/6 inbred mice and related strains.[1,17,36] Affected mice typically are unsuitable research subjects, and the lesions raise significant concerns regarding animal wellbeing.[20,21] As a result, many mice with UD are euthanized, leading to significant economic losses and research interference. Although the cause is unknown, evidence suggests that self-inflicted trauma from hindlimb scratching has an important role in the progression of UD.[6,10,33]

B6 mice have high rates of barbering, which has led to speculation that UD may be caused by abnormal grooming behavior in the strain.[6,33] Although barbering behavior does not appear to lead to UD,[6,37] recent findings support a role for scratching behavior in UD lesion progression.[6,25,34,39] In one study,[6] a serotonin-upregulating diet was evaluated as a treatment for a barbering model of trichotillomania. Unexpectedly, mice that received the test diet showed increased scratching behavior and had a higher incidence of UD, and pretreatment scratching was predictive of later UD development.[6] In addition, interventions aimed at disrupting the itch–scratch cycle, such as substance P inhibition and nail trimming, have been reported to reduce UD lesion size and severity.[25,34,39]

Genetic factors influence scratching behavior in response to exogenous pruritogens among various mouse strains.[9,14] A study comparing the responses of different inbred and outbred mice to intradermal injection with serotonin and histamine found that histamine produced a profound increase in scratching frequency in ICR mice and a more modest but statistically significant increase in B6 mice.[14] Under the same conditions no significant increase in scratching was observed in most of the strains tested.[14] Another study found that peak scratching occurred at a lower chloroquine dose in B6 mice compared with other mouse strains.[9] However, neither spontaneous scratching behavior nor scratching behavior in response to epidermal barrier injury have been compared between mouse strains. Perhaps, compared with other strains or stocks, B6 mice demonstrate more spontaneous scratching behavior or scratch more in response to mild epidermal insults, such as those that occur during routine grooming or handling for research, thus potentially influencing the development of UD.

The aim of this study is to assess whether B6 mice exhibit excessive scratching under resting conditions or when provoked by epidermal barrier disruption. We hypothesized that B6 mice would exhibit more spontaneous scratching behavior than DBA/2, BALB/c, and ICR mice and that B6 mice would be more pruritic after mild epidermal barrier injury compared with the other mouse strains and stock.

Materials and Methods

Mice. Retired breeder female C57BL/6J (n = 15), DBA/2J (n = 10), and BALB/cByJ (n = 10) mice were purchased from Jackson Laboratories (JAX West, Sacramento, CA). Retired breeder female

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Crl:CD1 (ICR) mice (n = 10) were obtained inhouse from the Laboratory Animal Resources Center's foster and rederivation colony. Owing to concern that the B6 mice might develop spontaneous UD during the study and need to be removed from the experimental group for health reasons, 15 B6 mice in total were purchased so that 5 'replacement' mice would be available, if needed. All mice were 10 to 12 mo of age at the start of the experimental procedures. The inbred strains chosen are classic comparison strains for the B6. BALB/c mice have Th2-biased immune responses, which have been contrasted with the Th1-biased B6, and are common in infectious disease and allergy studies.[8,33] DBA/2 mice are a historic comparison strain that is frequently crossed with B6 for F1 hybrids, and a large number of B6 × DBA recombinant inbred strains exist.[8] These recombinant inbred strains potentially could be used to investigate genetic influences in UD.

Mice were SPF for a wide range of common mouse pathogens, including mouse hepatitis virus, mouse minute virus, mouse norovirus, mouse parvovirus, Theiler mouse encephalomyelitis virus, rotavirus, Sendai virus, *Mycoplasma pulmonis*, pneumonia virus of mice, reovirus 3, lymphocytic choriomeningitis virus, ectromelia virus, *Helicobacter hepaticus*, *H. bilis*, *H. ganmani*, *H. rodentium*, *H. typhlonius*, *Aspiculuris tetraptera*, *Spironucleus muris*, *Syphcia obvelata*, *Myocoptes* fur mites, and *Myobia* and *Radfordia* fur mites, as assessed through quarterly sentinel testing. Mice were kept on a 12:12-h light:dark cycle, with lights on from 0630 to 1830. Mice were housed 4 per cage with one mouse of each strain/stock in each of the 10 cages to control for differences in microenvironment. They were housed in in standard mouse IVC (One Cage, Lab Products, Seaford, DE) on paper-based bedding (Biofresh, Absorption Corporation, Ferndale, WA). Rodent chow (5053, Purina Test Diets, Saint Louis, MO) and water (Hydropac, Lab Products, Seaford, DE) were provided without restriction. During the acclimation period, most of the DBA/2 and BALB/c mice appeared thin (body condition score of 2.0 to 2.5 out of 5.0), and supplemental gel diet (ClearH_2O, Portland, ME) was provided to all cages every 2 wk until the conclusion of the experiment. Nesting material (Cotton squares, Anacare, Bellmore, NY) and wheels (Anacare, Bellmore, NY) were provided for enrichment. All animal procedures were approved by the Oregon State University, Institutional Animal Care and Use Committee (IACUC). Oregon State University is fully AAALAC-accredited.

Scratching behavior. Mice were placed individually in observation chambers (10.5 in. × 14.5 in. × 8.5 in.). They were allowed to acclimate for 15 min and then their behavior was filmed for 1 h by using a video camera (Sony, Tokyo, Japan) mounted on the ceiling of the room. Four observation chambers were placed in the viewing area, and all of the mice in a cage had their behavior filmed at the same time. To control for minor variations in conditions between the observation chambers (light intensity, proximity to the door, spray test order, and so on), observation chamber assignment was randomized for each cage. This randomized observation chamber assignment number (1 to 4) also was used to determine the order for tape-stripping to induce epidermal barrier injury. To optimize lighting conditions and to film as close as possible to the active period of the mice, the first filming session started at 1715 (PM) and then was repeated the next morning starting at 0700 (AM).

Mice often scratch, pause to lick a hindpaw, and then continue scratching.[9,40] As in previous studies of pruritus and scratching behavior in mice, each scratching event was defined as ending when the hindpaw was placed on the floor.[40] In addition, scratching duration was recorded as brief (less than 3 s) or prolonged (3 s or longer). This cut-off was based on observations of scratching behavior during a pilot study (data not shown). The durations of prolonged scratching events were recorded. Each mouse was filmed 6 times: baseline PM, baseline AM, baseline spray test (AM), 5 h after tape-stripping injury (PM), 17 h after injury (AM), and 17 h after injury and spray test (AM). Due to phenotypic differences between strains, complete blinding of the observer to mouse strain was not possible. However, to minimize bias, a semiblinded approach was used, in which the observer could see only one pigmented mouse (DBA/2 or B6) and one albino mouse (BALB/c or ICR) on the screen at a time. Without the ability to compare coat color (lighter DBA/2 compared with the darker B6) or size (smaller BALB/c compared with the larger ICR) side-by-side, it was difficult to identify the strain of mouse observed. Although this arrangement did not achieve a truly blinded approach, it minimized bias in a situation where marked phenotypic differences made traditional blinding impossible.

Tape-stripping. Mice were anesthetized briefly with isoflurane; induction took place at 4% isoflurane in oxygen, and mice were maintained at 1.5% to 2% isoflurane concentration. The hair on the right dorsum was removed through an initial clipping of the hair followed by the application of a depilatory cream (Nair, Church and Dwight, Ewing, NJ). Then tape-stripping was performed as previously described.[24,30,31] Briefly, a strip of cellophane tape (Scotch, 3M, Hutchinson, MN) was adhered to the depilated skin and removed. This process was repeated 8 times by using a new piece of tape each time. The mice then recovered from anesthesia and were returned to their home cage. As mentioned earlier, the order for tape-stripping was randomized for each cage.

Spray test. At the end of the morning videotaping session, both at baseline and after tape-stripping, a spray test was performed as previously described.[6,23] This test was formerly demonstrated to be predictive of future UD development.[6] Briefly, each mouse was sprayed twice with a fine mist of water from a spray bottle. The subsequent behavior was filmed for an additional 15 min. The video was later reviewed, and the number of scratching events was recorded.

Tissue collection. At 24 h after tape-stripping, mice were euthanized by CO_2 exposure. The left dorsum was clipped. The entire dorsal pelt was then removed and sectioned for fixation in 10% buffered formalin. The long, thin skin sections created were rolled from the cranial end to the caudal end around the wooden end of a cotton-tipped applicator prior to being placed in formalin. After standard processing, 5-µm slices were stained with hematoxylin and eosin. The prepared slides were labeled with new, randomly generated ID numbers to enable blinded analysis. The tape-stripped tissue was evaluated for degree of barrier disruption, qualitative assessment of inflammation, inflammatory infiltrate depth, percentage of the surface area of the skin section that was affected, and the number of mast cells per 10 high-power (400×) fields. The overall severity of inflammation was scored semiobjectively by using a 5-point scale (5, most severe). Mast cell counts were performed in 10 adjacent fields encompassing the most inflamed areas of dermatitis. The tape-stripped skin was compared with skin samples from the contralateral side of the dorsum, which had not been tape-stripped.

Statistics. Comparisons within strains between time points were performed by using a paired t test. ANOVA was used to

identify significant differences (defined as P value of 0.05 or less) in scratching frequency, severity of inflammation score, and mast cell count among the 4 strains; when differences were present, an unpaired t test was used to compare between B6 mice and the other 3 strains, with correction for multiple comparisons (adjusted significance level, $P \leq 0.017$). Scratching frequency was normally distributed and presented graphically as an average, with error bars representing 1 SD. Because scratching duration for long bouts of scratching was skewed due to a small number of greatly prolonged scratching events, the data are represented as median durations.

Results

Animals. During the acclimation period, 2 DBA/2 mice died. In both cases, the remains were in poor condition (marked autolysis and partially cannibalized), so diagnostic necropsy was unrewarding. No premonitory signs had been noted, and no procedures had been performed on these animals prior to death. Replacement B6 mice were added to the cages to maintain a density of 4 mice per cage; these B6 mice underwent behavioral observations and tape-stripping with the other mice in the cage. Two additional DBA/2 mice found dead without premonitory signs during the interval between baseline and epidermal barrier injury. Again the remains were in poor condition, thus precluding diagnosing a cause of death. These mice were also replaced with B6 mice to maintain the cage density. However, because corresponding baseline behavioral observations for these mice were unavailable, they did not undergo tape-stripping, postinjury behavioral observations, or tissue collection. All new social housing groups were allowed at least 1 wk of acclimation prior to behavioral observations.

Owing to the described issues, the final analysis includes data from 12 B6 mice, 10 BALB/c, 10 ICR, and 8 DBA/2 mice for baseline behavioral observations, with 6 DBA/2 mice available for behavioral observation and tissue collection after epithelial barrier injury.

Scratching frequency at baseline and after injury. Scratching behavior prior to skin barrier disruption was recorded during two 60-min sessions, one starting at 1715 (PM) and one starting the next morning at 0700 (AM). The ICR mice had significantly ($P = 0.004$) more scratching events during the PM session compared with the AM; no other mouse strain had a significant difference between baseline recording events. Under baseline conditions, there was no evidence that B6 mice scratched more than the other mice. In fact, B6 mice had the fewest scratching events among the 4 strains evaluated (Figure 1). Specifically, B6 mice scratched significantly less than DBA/2 mice at both time points (PM, $P = 0.0068$; AM, $P = 0.0023$) and significantly less than the BALB/c ($P = 0.0042$) and ICR ($P = 0.00080$) mice at the PM time point. The BALB/c mice scratched more than the B6 at the AM time point, but the difference was not significant after correcting for multiple comparisons ($P = 0.021$; corrected level of significance, $P \leq 0.017$).

After barrier disruption with tape-stripping, filming of scratching behavior was repeated at the same time points, leading to the observation of scratching at 5 and 17 h after epidermal injury. At 5 and 17 h after injury, the BALB/c and DBA/2 mice showed no statistically significant increase in scratching behavior compared with baseline. Scratching behavior increased significantly after barrier disruption as compared with baseline scratching during the same period (that is, PM baseline compared with 5 h after injury; and AM baseline compared with 17 h after injury) for both the B6 ($P = 0.00037$ and $P = 0.0042$, respectively) and ICR ($P = 0.00032$ and $P = 0.0086$, respectively) mice. As with the baseline scratching behavior, the ICR mice showed a significant ($P < 0.0001$) difference in scratching frequency between the PM and AM time points after epidermal barrier injury. Again there was no evidence that B6 mice scratched more frequently than did the other strains or stock of mice, and during baseline behavior recordings, B6 mice scratched the least. This lower frequency of scratching events in B6 mice was statistically significant compared with ICR mice at the 5-h postinjury time point ($P = 0.0004$), and at 17 h after injury the B6 scratched significantly ($P = 0.0009$) less than the DBA/2 mice.

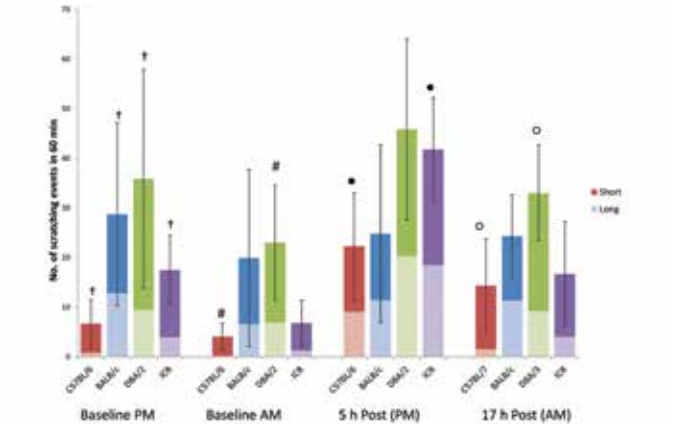

Figure 1. Scratching frequency according to strain or stock and time point. Mean scratching behavior (error bars, 1 SD) at the baseline time points and at 5 and 17 h after tape-stripping is displayed. B6 mice scratched less than the BALB/c, DBA/2, and ICR mice at the PM time point (†, $P < 0.01$) and less than the DBA/2 at the AM time point (#, $P = 0.0068$). At 5 and 17 h post injury, the B6 mice scratched the least, with the B6 scratching less than the ICR mice at the 5-h postinjury time point (●, $P = 0.0004$) and less than the DBA/2 at 17 h after injury (○, $P = 0.0009$). The lighter region in each bar represents the proportion of scratching bouts that were longer than 3 s in duration. The B6 mice did not have more prolonged scratching bouts than did the BALB/c, DBA/2, and ICR mice.

Scratching duration at baseline and after injury. Most scratching bouts under baseline conditions were brief (less than 3 s each; Figure 1), and B6 mice showed no evidence of having longer scratching events than those in the other strains and stock evaluated. The B6 mice had the fewest prolonged (3 s or more) scratching bouts, with 11% (PM) and 4% (AM) long-duration scratching events at the 2 baseline time points. At 5 h after injury, the percentage of short-duration scratching bouts was remarkably similar among all 4 strains and ranged between 54% and 59%. By 17 h after injury, the percentage of short-duration scratching bouts was similar to baseline values, with B6 mice having the highest percentage of brief scratching events (90%). Long-duration scratching events ranged from 3 to 39 s, with a median of 5 s (Figure 2). In examining maximal scratching duration by strain, B6 mice had the shortest maximum during baseline testing, and B6 and ICR mice had similarly short maximal scratching durations compared with DBA/2 and BALB/c mice (Table 1).

Spray test. Scratching frequency at baseline and 17 h after injury is depicted in Figure 3. As in the previous analyses, there is no evidence that the B6 mice scratched more frequently than did mice of the 3 other strains tested, although B6 mice did scratch

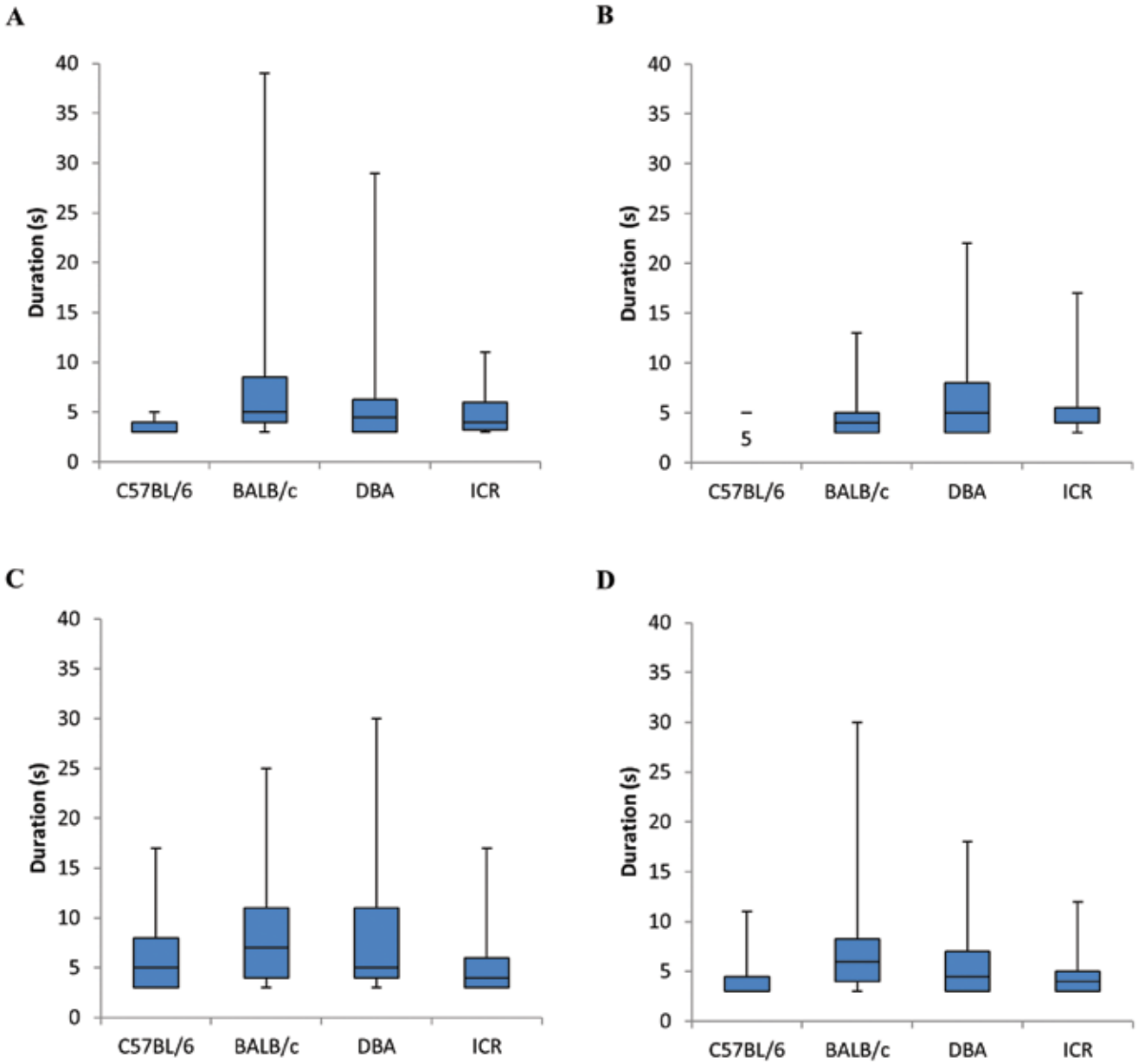

Figure 2. Median duration (s) of prolonged scratching events according to strain or stock and time point. Median duration was very similar between strains, with no significant difference between B6 mice and other genotypes. At the baseline AM time point, only one scratching event for the B6 mice had a duration of greater than 3 s, so the value is reported on the graph rather than being illustrated in a plot. (A) PM baseline; (B) AM baseline; (C) 5 h after tape-stripping; and (D) 17 h after tape-stripping.

significantly less than DBA/2 mice at baseline ($P = 0.008$) and 17 h ($P = 0.0009$). In addition, the postinjury scratching frequency was significantly ($P = 0.0429$) different from baseline in the ICR mice. There was no significant difference in scratching frequency before compared with after epidermal barrier injury in the B6, BALB/c, or DBA/2 mice, and 53% to 60% of the scratching events in all genotypes evaluated were brief (Figure 3). After epidermal barrier injury, BALB/c had the largest proportion of long-duration scratching events (67%), and B6 did not show evidence have of having longer scratching events than other strains.

Histology. The tape-stripped skin had gross lesions ranging from mild erythema to locally extensive dermatitis with associated crusts. Histology confirmed that tape-stripping successfully disrupted the epidermal barrier, resulting in the removal of the outermost cornified layer of skin when compared with control skin, which had not been manipulated prior to tissue harvest. Consistently the tape-stripped skin had suppurative dermatitis with multifocal to coalescing pustules, crusts, and erosions (Figure 4). The percentage of surface area affected by this pustular dermatitis (1% to 70%) and depth of inflammatory infiltrate (from very superficial to inflammation extending in to the subcutis) varied. The overall inflammation severity score did not differ significantly (ANOVA, $P = 0.256$) between strains. The number of mast cells per 10 high-power (400×) fields (range, 11 to 60) did not differ by strain (ANOVA, $P = 0.259$).

Table 1. Maximal duration (s) of scratching according to genotype and time point

	Baseline PM	Baseline AM	After tape-stripping 5 h	After tape-stripping 17 h
C57BL/6	5	5	17	11
BALB/c	39	13	25	30
DBA/2	29	22	30	18
ICR	11	17	17	12

The single scratching event with the longest duration at each time point is reported for each genotype. B6 mice did not have the longest scratching event at any time point.

Discussion

UD is a frustrating condition to manage, and efforts to improve clinical outcomes have been undermined by the poorly understood etiology of the disease. Several publications have suggested that behavior, especially hindlimb scratching, is an important driver of UD. Therefore, we hypothesized that B6 mice would demonstrate a tendency to scratch more, either spontaneously or when provoked by epidermal barrier disruption or the spray test. In contrast with this hypothesis, there was no evidence that B6 scratched more frequently or for longer durations than the other commonly used strains and stock tested. In fact, under some conditions, the B6 mice scratched significantly less than did the other mice. These findings do not support the idea that a strain-related tendency toward exaggerated scratching behavior under resting or epidermal barrier disruption conditions predisposes B6 mice to UD.

In previous studies, B6 mice exhibited robust scratching behavior compared with other strains in response to low doses of intradermal pruritogens.[9,14] The B6 mice were relatively susceptible to low doses of the exogenous pruritogen, but the maximal time that B6 mice spent scratching at the peak of the dose–response curve was less than that for other strains.[14] The low maximal scratching frequency and duration seen in the current study is similar to previous observations.[9] In contrast with this study, scratching behavior was either spontaneous or induced by epidermal damage associated endogenous pruritogens, which may account for some of the differences seen. In addition, the previous studies used 6- to 10-wk-old mice for their experiments, but we used mice that were about 1 y old because of our interest in UD, which occurs most commonly in mice older than 6 mo.[1,17] However, this difference in the age of experimental subjects may account for some of the differences seen in prior studies. Furthermore, although increased pruritus as assessed by the spray test was previously associated with later UD development,[9,14] our study did not reveal a significant increase in scratching in UD-predisposed B6 mice compared with the other strains and stock tested. One previous study[6] used only B6 mice, so perhaps the spray test has predictive value for individual animals within the strain, but the response to this test is not associated with the B6 strain's predisposition to UD development. None of the B6 mice in this study developed UD during the acclimation period or the interval between baseline and after injury to the epithelial barrier, so an association between UD development and scratching behavior after the spray test could not be assessed.

Two DBA/2 mice died during the initial acclimation period, and 2 more died in the 3-wk interval between initial filming and the tape-stripping procedure. None of the mice died after experimental procedures (behavioral observation, anesthesia, and so forth). In all cases, the mice had no premonitory signs and were found dead during routine morning health checks by the husbandry staff. Diagnostic necropsy of the dead mice was unrewarding because of marked autolysis and partial cannibalism in all cases. Gross necropsies of the DBA/2 mice that survived to the study endpoint revealed cardiac calcinosis. Epicardial and myocardial calcification is considered a common finding in the strain, especially in female mice.[33] Perhaps cardiac dysfunction, such as arrhythmia, secondary to myocardial calcification was the cause of death for these 4 female DBA/2 mice, although a definitive cause of death could not be determined.

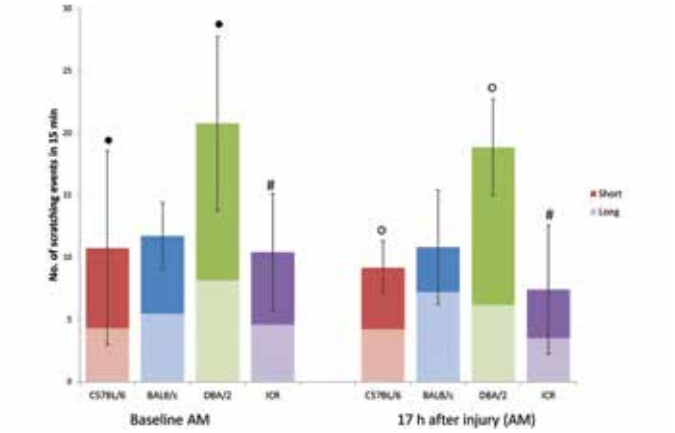

Figure 3. Scratching frequency during the 15-min spray test. Scratching frequency is displayed as mean scratching frequency by strain (error bars, 1 SD). The B6 mice scratched significantly less than the DBA/2 mice at baseline (●, $P = 0.008$) and 17 h afterward (○, $P = 0.0009$). In addition, the postinjury scratching frequency differed from baseline in ICR mice (#, $P = 0.0429$). Scratching frequency did not differ between pre- and postinjury in B6, BALB/c, or DBA/2 mice. The lighter region of each bar represents the proportion of scratching bouts that were over 3 s in duration. Similar to the findings shown in Figure 1, the B6 mice did not have a larger proportion of prolonged scratching bouts compared with BALB/c, DBA/2, and ICR mice.

In this study the ICR mice—but not the inbred strains—showed a highly significant difference in scratching frequency between morning and evening. A similar tendency was seen in the B6 and DBA/2 mice, although the difference was not statistically significant. The frequency, duration, and character of grooming behavior that rodents display can be affected by anxiety, such as that potentially experienced in novel environments,[15,16,35,38] and scratching was often observed to occur at the beginning or end of grooming sessions. The baseline and postinjury tests occurred several weeks apart, and the differences in scratching behavior between the first and second filming sessions for baseline and after injury might be related to familiarity with the testing set up (and less anxiety-related grooming behavior) rather than the time of day at which filming occurred. Alternatively, this mouse stock might be more pruritic or scratch more in the evening rather than in the morning. Although some studies have controlled for time of day by performing behavior testing during a specified window of time on each day,[6,29] we found no previous studies that compared morning and evening scratching behavior in mice.

Several possible interpretations might explain the relatively low scratching frequency and duration seen in the B6 mice in this study. Perhaps scratching behavior is not an initiating factor but rather a secondary consequence of UD. In one study where

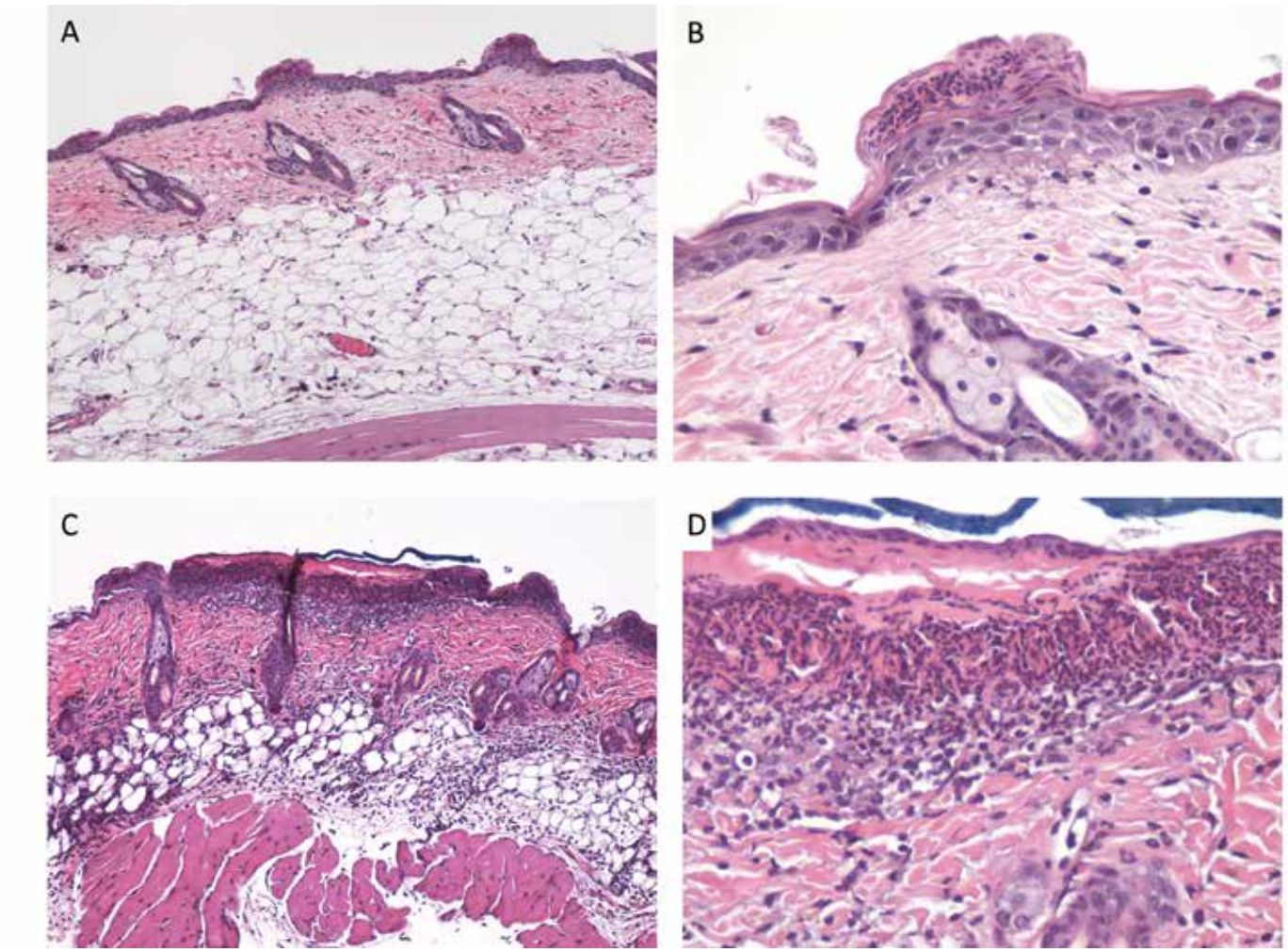

Figure 4. Histology of tape-stripped skin. Representative histology images of the suppurative dermatitis with pustules and/or crusts and erosions seen during evaluation of the tissues. (A and B) ICR mouse, skin. Hematoxylin and eosin stain; magnification, 100× (A), 400× (B). Small discrete pustules seen on the epidermal surface. (C and D) BALB/c mouse, skin. Hematoxylin and eosin stain; magnification, 100× (C), 400× (D). Larger coalescing pustules resulting in erosions and serocellular crusts accompanied by a mixed inflammatory infiltrate that extends from the dermis into the subcutaneous adipose tissue. Blue tissue dye used to mark the tape stripped region is apparent on the surface of the sectioned tissue.

scratching behavior was assessed every 2 wk until the animals were harvested for tissue collection, mice had a marked increase in scratching frequency when UD was present, but there was no increase at the time point 2 wk preceding the diagnosis of UD.[6] Alternatively, a dysfunction of the nerves innervating the epidermis could lead to a combination a paresthesia and loss of protective pain during scratching events. In human medicine, the condition known as trigeminal tropic syndrome that combines intractable neuropathic itch and the profound loss of cutaneous sensation, leading to painless self-injurious scratching.[27] Similarly, aged B6 mice with cutaneous neuropathy could have hypoesthesia that manifests as a relatively infrequent scratching but, once provoked to scratch by secondary bacterial infection or other compounding factors, mice may lack the necessary feedback to stop scratching before they harm themselves. Intraepidermal nerved-fiber density analysis has been used to assess for neuropathy secondary to diabetes and experimental models of neuropathy in rodents and could be used in the study of UD.[2,12,18] We are currently performing this technique on frozen tissue sections collected from the various strains during this experiment to evaluate the possibility of abnormal cutaneous innervation in B6 mice.

Histologic evaluation revealed a consistent pattern of pustular suppurative dermatitis following tape-stripping. The dermatitis ranged in severity from mild lesions, with superficial keratinocytes removed and very rare inflammatory cells, to severe lesions, with coalescing pustules, inflammatory cells in deeper layers of skin, and edema affecting large portions of the tissue section. One potential refinement for future studies would be to incorporate a means of assessing the uniformity of epidermal barrier disruption created. For example, transepidermal water loss could be used to assess the degree of barrier disruption. This method has been described previously and, in some cases, was the end point for tape-stripping rather than a specific number of tape application–removal cycles.[24,28,30] In addition, it was difficult to differentiate which changes were due to the tape-stripping procedure or due to the scratching and grooming behavior that occurred afterward.

One important limitation is that this study addressed scratching behavior after acute injury only. Differences in scratching

behavior due to aberrant feedback mechanisms or wound repair that occur more than 24 h after epidermal barrier disruption would not have been apparent. In addition, the intensity of scratching could be strain-related and of significance to UD development but was not assessed in this study. Both of these areas are promising avenues for further study.

For this study, mice of different strains were cohoused, and behavioral observations took place in novel chambers with no other mice present during the light phase. This set-up allowed us to control for microenvironment factors and allowed observers to accurately record individual scratching events. However, this arrangement has its limitations. Scratching behavior during the dark phase (when mice are active) may be different from what we were able to observe by using these methods. In addition, the scratching behavior when mice are alone in the relatively barren environment may differ from that when they are left undisturbed in their home cage with conspecifics, bedding, and other enrichment. In addition, mice of different strains may acclimate to a novel environment, such as the observation chambers, at different rates, thus potentially influencing scratching behavior. The use of different methods and materials, such as automated scratch counters, might enable scratching behavior to be monitored in the home cage and during the dark cycle and is an important consideration for future studies in this area. Housing mice with other mice of different strains is unusual for most conventional housing arrangements. This feature of the study design controlled for cage factors among the strains tested but is not representative of typical mouse husbandry and, in fact, may result in stress for some strains under certain conditions.[19] Single housing could have eliminated shared-cage factors for mice of the same strain as a confounding variable, but this arrangement would have carried additional wellbeing considerations; we therefore elected social housing in mixed groups for this study.

Another interesting consideration is the potential role for gut and epidermal microbiota in scratching behavior. Previous research has shown that gut and epidermal microbiota influence inflammation.[22,26,32] Furthermore mice from different genetic backgrounds and different vendors may have significant differences in their microbiota.[7,11,13] If these differences in microbiota modulate pruritus-associated cytokines and immune responses to epidermal injury, then they might influence scratching behavior. In this study, mice of different strains were cohoused for the prolonged period prior to the start of the experiment, thereby likely mitigating differences in microbiota between mice of different genetic backgrounds. Although the role of microbiota was beyond the scope of this study, future investigations into this topic are warranted, especially given the reported link between UD and diet,[6,20,21] an important modulator of gut microbiota.[3-5]

The inbred strains used in the current study were chosen because they are commonly used and are classic comparison strains for B6 mice. Although these strains represent reasonable comparison strains for the B6 strain, they should not be considered representative of inbred strains as a whole. In addition, only female mice were used in this study, and this practice made it possible to recombine social housing groups on arrival from the vendor to control for microenvironmental factors between strains. In addition, a female sex predilection for UD has been reported,[6,17] making these mice a clinically important group to study. However, our current findings may not be representative of the scratching behavior that male mice would demonstrate under the same conditions. Therefore, the findings from this study should be generalized with due consideration for these limitations.

In the current report, we demonstrated a relatively low frequency and duration of scratching behavior in UD-prone B6 mice as compared with BALB/c, DBA/2, and ICR mice. This outcome is contrary to what would be expected if excessive scratching during typical grooming behavior or after mild skin insults were the underlying cause of UD in this strain. Understanding the etiopathogensis of this condition will ultimately be important for improving the clinical management of UD, and further study of potential behavioral, metabolic, and neuropathic mechanisms is critical.

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