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Original Research

Ectoparasite Burden, Clinical Disease, and Immune Responses throughout Fur Mite (*Myocoptes musculinus*) Infestation in C57BL/6 and *Rag1*$^{-/-}$ Mice

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Immunocompetent weanling mice infested with *Myocoptes musculinus* harbor high mite loads, yet burdens decrease with age. The development of immunity to the parasite may explain this observation. In this study, we followed *M. musculinus* burdens in *Rag1*$^{-/-}$ mice and immunocompetent C57BL/6 controls from 4 to 36 wk of age and compared the clinical signs and body weights of noninfested and infested mice of both strains over time. In addition, histopathology of skin lesions and expression of cytokines and transcription factors associated with Th1- and Th2-type immune responses were assessed. *Myocoptes* burdens decreased and remained low in B6 mice over time, whereas *Rag1*$^{-/-}$ mice showed an initial decrease in burdens after 4 wk of age followed by an increase from 24 to 36 wk. In addition, *Rag1*$^{-/-}$ mice had higher burdens than B6 mice over time. Both strains of infested mice exhibited clinical signs of fur mite infestation—including alopecia, poor weight gain, mite-associated debris, and pruritus—and clinical signs positively correlated with the severity of the *Myocoptes* burden. Histopathology of skin from both strains of infested mice showed decreased lesion severity with age, likely a result of declining mite populations. Finally, compared with noninfested controls, infested B6 mice had increased expression of markers associated with the Th2-type immune response, which increased in magnitude with increasing age and duration of infestation. These results suggest that development of adaptive immunity plays a role in control of fur mite populations and that heavier infestations may result in more severe clinical signs and skin lesions.

Despite advances in diagnosis and treatment, control of murine fur mite infestations (*Myobia musculi*, *Myocoptes musculinus*, and *Radfordia affinis*) remains a challenge for many modern rodent facilities. Therefore, it is crucial define the potential effects of mite infestation on research, either as a result of their effects on host immune responses or development of clinical disease. Many reports describe the host immune response to fur mite infestation,[12,13,22,23] which may have pronounced effects on studies of immunology, infectious diseases, autoimmunity, or transplantation.[12,29] In addition, many studies report dermatologic pathology and other clinical signs of infestation, which may either confound experimental results or require early study removal.[6,12,13,22] Though fur mite infestations may directly affect rodent health and research outcomes, the relationships between clinical acariasis, magnitude of infestation, and host immunologic responses remain unclear. These relationships are of particular interest given that mite burdens vary substantially throughout the course of infestation.[25]

The clinical signs of fur mite infestation vary considerably. Although most infestations are subclinical, fur mite infestations may cause alopecia, pruritus, dermatitis, decreased weight gain, and poor reproductive performance.[3,10] One study reported no gross lesions in BALB/c and C57BL/6 (B6) mice infested with *M. musculinus* but overt atopic dermatitis in infested NC/Kuj mice.[22] Other reports described severe ulcerative dermatitis in *M. musculi*-infested B6 mice[6] and dermatitis and wasting in *M. musculinus*-infested BALB/c mice.[13] Conversely, enzootically infested mice on a mixed B6.129 background showed no clinical signs of *M. musculinus* infestation at any age.[19] Although disease manifestation might reflect strain susceptibility, changes in mite population levels may also explain variations in clinical severity.

Previous studies indicate that host fur mite burdens can decrease dramatically over the course of infestation. In one study, weanling mice enzootically infested with *M. musculinus* harbored heavy mite loads, yet by 28 wk of age, nearly a quarter of mice lacked any evidence of mites or eggs on microscopic exam.[25] In mice infested with *M. musculi*, mite levels rose initially but then declined to stable low levels.[8] Given recognized age-dependent decreases in the mite burden and well-documented immunologic changes, the development of adaptive immunity to the mite may play an important role in reducing populations over time.

Fur mite infestations may upregulate host adaptive immune responses, particularly Th2 CD4$^+$ T cell responses. Several studies of infested mice document this phenomenon, citing upregulation of the cytokine IL4, increased IgE concentration, and tissue mast cell infiltration and degranulation.[13,14,22,23,26] Effector functions of T cells and B cells via Th2-type responses and IgE production, respectively, lead to host clearance of and future protection from other parasites, such as helminths. For instance, the expulsion of various nematodes from experimentally infected mice is Th2-

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dependent, requiring Th2 cells and IL4 receptor signaling.[18,20] Additional evidence shows that host immunity to skin ectoparasites, stimulated by arthropod feeding or burrowing,[30] leads to host resistance.[4] For example, regulation of ectoparasite burdens and future immunity to *Sarcoptes* and *Demodex* mites is dependent on both humoral and cell-mediated immunity involving competent T cell responses and the expression of a variety of cytokines.[2,7,28] In addition, mice infested with the anopluran louse *Polyplax serrata* develop resistance to the parasite and a steady decline in louse burdens over time.[24] Although the specific mechanisms underlying the control of ectoparasite and helminth infections may differ, a functional, adaptive immune response is perhaps important for ectoparasite clearance and future host resistance.

The purpose of the current study is to more clearly define the relationships between host fur mite populations, clinical disease, and the immune response to infestation. To investigate these associations, we compared mite population levels and clinical signs of *M. musculinus* infestation in B6 mice and *Rag1*$^{-/-}$ mice, which lack mature T cells and B cells,[21] from weaning into adulthood. Our first goal was to assess the role of adaptive immunity on mite populations by evaluating *Myocoptes* burdens in infested B6 and *Rag1*$^{-/-}$ mice from 4 to 36 wk of age. We hypothesized that infested B6, but not *Rag1*$^{-/-}$ mice, would demonstrate age-related decreases in *Myocoptes* burdens and that B6 mice would have lower overall burdens compared with *Rag1*$^{-/-}$ mice. In addition, we compared clinical data (body weight and clinical signs) from noninfested and infested B6 and *Rag1*$^{-/-}$ mice over time to determine whether clinical severity correlated with host mite burdens. We hypothesized that clinical disease severity would positively correlate with higher mite burdens in both strains of mice. We also compared histopathologic skin lesion severity in both infested strains as they aged and hypothesized that lesion severity would decrease in B6 but not *Rag1*$^{-/-}$ mice over time, due to higher mite burdens in *Rag1*$^{-/-}$ mice. Finally, we evaluated cytokine and transcription factor markers of Th2-type and Th1-type responses in infested and uninfested B6 mice over time.

Materials and Methods

Animals. C57BL/6J and B6.129S7-*Rag1*^{tmMom}/J mice (age, 4 to 6 wk) were purchased from The Jackson Laboratory (Bar Harbor, ME). Mice were housed in compliance with the *Guide for the Care and Use of Laboratory Animals*,[11] and animal research was approved by the Johns Hopkins University IACUC. All animals were housed in ventilated racks within the Johns Hopkins rodent quarantine facility (Baltimore, MD) at an ambient temperature of 20 to 26 °C and relative humidity of 30% to 70%. Mice were fed autoclaved rodent chow (2018S, Harlan Teklad, Indianapolis, IN) and provided reverse-osmosis–purified water through an automated watering system (Rees Scientific, Trenton, NJ). Mice were maintained on autoclaved corncob bedding (Harlan) with a cotton enrichment square. Cages were changed every 2 wk in a dedicated change station, by using 100 ppm solution of chlorine dioxide for sanitation (Quip Laboratories, Wilmington, DE). A soiled-bedding sentinel system was used for infectious disease surveillance, and mice were considered negative for Sendai virus, pneumonia virus of mice, mouse hepatitis virus, mouse minute virus, mouse parvoviruses 1 and 2, Theiler mouse encephalomyelitis virus, reovirus, epizootic diarrhea of infant mice, lymphocytic choriomeningitis virus, ectromelia virus, murine adenoviruses 1 and 2, murine cytomegalovirus, *Mycoplasma pulmonis*, pinworms (*Aspiculuris* and *Syphacia* spp.), and fur mites (*Myobia* and *Radfordia* spp. only). After a 1-wk acclimation period in our facilities, breeding pairs or trios were established for each strain.

Mite infestation. B6.129 mixed background mice were used to infest breeders. These mice were previously donated from a research colony naturally infested with a monoinfection of mites (*M. musculinus*). For infestation of B6 and *Rag1*$^{-/-}$ breeders (used to produce offspring for mite burden and clinical disease studies), a single live, infested female mouse was placed in each breeding cage and removed (approximately 1 wk later) after infestation was confirmed by microscopic exam of fur pluck samples. For infestation of B6 and *Rag1*$^{-/-}$ breeders (used to produce offspring for tissue cytokine analysis and histology), a single, whole mouse pelt from a recently euthanized infested mouse was placed in each breeding cage of mice. Infestation was confirmed by microscopic exam of fur pluck samples, approximately 1 wk later. Hereafter, infestation occurred by direct contact from parents to offspring. Study animals from infested and noninfested breeding cages were weaned at approximately 21 to 28 d of age and placed in same-sex groups of 3 to 5 siblings for the remainder of the study. All noninfested B6 and *Rag1*$^{-/-}$ breeders and control mice were screened regularly through microscopic exam of fur plucks to ensure that negative status was maintained throughout the study.

Assessment of mite burden. Infested *Rag1*$^{-/-}$ and B6 mice at 4, 6, 8, 12, 16, 20, 24, 30, and 36 wk of age were assessed for *Myocoptes* burden by microscopic examination of fur plucks from 3 standard sites: the dorsal neck, posterior dorsum, and caudoventral abdomen. To collect fur samples, forceps were used to grasp and pluck approximately the same amount of fur from each site (one pluck of fur per site). Each of the 3 fur plucks was placed on a section of transparent cellophane tape (3M, St. Paul, MN) and adhered to a glass slide (Fisher Scientific, Waltham, MA). All samples were blinded prior to evaluation and examined by 1 of 2 experienced observers. Slides were examined under a microscope at 4× and 10× magnification, and the number of mites and eggs observed in the 3 fur samples were totaled to calculate a relative *Myocoptes* burden. The *Myocoptes* burden refers to the total number of eggs and mites observed in the 3 fur samples collected from each mouse at each time point. Whole mites of all life stages and mite parts were included in mite counts, and viable eggs and casings (nonviable eggs) were included in egg counts. If either the mite or egg count was 200 or greater, a maximal count of 200 was assigned for that category (egg or mite). Prior to initiation of the study, the 2 observers were assessed for interobserver similarity in counts. Both were assigned the same sets of slides and asked to count the number of mite and eggs per slide; sets of slides were reviewed until there was no significant difference in counts between observers.

Clinical disease evaluation. Mite-associated clinical disease was assessed at 4, 6, 8, 12, 16, 20, and 24 wk of age in 4 groups of mice: infested B6, noninfested B6, infested *Rag1*$^{-/-}$, and noninfested *Rag1*$^{-/-}$ mice. Each mouse was assigned a clinical disease score and weighed at each time point. The clinical disease score consisted of the sum of individual scores obtained by assessment of multiple clinical parameters (Figure 1). Total scores ranged from 0, indicating no clinical signs of mite infestation, to 4, indicating severe clinical disease associated with infestation.

Tissue collection. After euthanasia of mice by CO_2 asphyxiation, fur pluck samples were collected for microscopic exam to confirm infestation status prior to gene expression analysis and skin

Clinical sign	Score	Description
Pruritus	0	<3 scratches/2 minutes of observation
	1	≥3 scratches/2 minutes of observation
Alopecia (excludes barbering)	0	No alopecia
	1	Alopecia present; see Figure 4 for typical appearance of mite-associated alopecia
Mite debris	0	No mite debris
	1	Mite debris present; see Figure 4 for typical appearance of mite-related debris
Excoriation and/or ulceration (excludes fight wounds)	0	No gross lesions
	1	Gross lesions present
TOTAL SCORE	0-4	

Figure 1. System for scoring clinical disease.

sample histopathology. Skin was collected from 3 standard areas (dorsal neck, posterior dorsum, and caudoventral abdomen) and fixed in 10% neutral buffered formalin for histologic evaluation. Regional draining lymph nodes (inguinal and axillary) were harvested from each mouse, snap-frozen in liquid nitrogen, and stored at –80 °C.

Histopathology. Histologic changes in skin were assessed from the dorsal neck, posterior dorsum, and caudoventral abdomen in infested B6 and *Rag1*$^{-/-}$ mice at 4, 8, 16, and 24 wk of age. After fixation in 10% neutral buffered formalin, tissues were processed routinely, embedded in paraffin, sectioned at 7 to 10 µm, and stained with hematoxylin and eosin. A histopathology scoring system, modified from a published scheme,[12] was used by a blinded veterinary pathologist during histologic evaluation of the skin (Figure 2). This scoring system was based on epidermal, dermal, and subcutaneous changes. For each submission, the pathologist examined 6 different areas of skin under 40× magnification and assigned a score to each area, which then were averaged to the nearest whole number. Epidermal changes included acanthosis, hyperkeratosis, and inflammation. Dermal and subcutaneous inflammatory changes were also tabulated. Other changes, such as serocellular crusting, mites, and microscopic ulcerations, were recorded but not included in histopathology score.

Quantitative PCR analysis of lymph node homogenates. Frozen regional lymph nodes (inguinal and axillary) from 4 or 5 infested B6 mice at 4, 8, 16, and 24 wk of age and 2 noninfested mice at 8 and 16 wk of age were used for gene expression analyses. Samples were placed in Lysing Matrix D tubes (MP Biomedicals, Santa Ana, CA); one mL QIAzol (Qiagen, Valencia, CA) was added, and samples were homogenized by using a FastPrep-24 homogenizer (MP Biomedicals) at 6.0 m/s twice for 40 s each. RNA was isolated using the Qiagen RNeasy Lipid Mini kit according to manufacturer's instructions and quantified by spectrophotometry (NanoDrop, Thermo Scientific, Waltham, MA). cDNA was synthesized from 2 µg RNA by using the High Capacity cDNA Reverse Transcriptase kit with random primers and RNaseOut (Life Technologies, Grand Island, NY). Quantitative real-time PCR was performed on a 7500 Fast Real-Time qPCR System (Applied Biosystems, Foster City, CA) with TaqMan Universal PCR Master Mix (Roche, Indianapolis, IN) and commercially available TaqMan gene expression assays (Integrated DNA Technologies, Coralville, IA). Data were processed by using Sequence Detector software (version 1.4, Applied Biosystems), and results were analyzed using the ΔΔCt method, by using endogenous mouse glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*) mRNA for normalization and mRNA from noninfested mice for calibration. Data from 3 noninfested mice were excluded from calculations due to markedly disparate Ct values and unacceptable sample quality, leaving a sample size of $n = 4$ for calibration.

Statistical analysis. All results were evaluated using a commercial statistical software package (Prism 5.0, GraphPad Software, San Diego, CA). Statistical tests included 2-way ANOVA, Kruskal–Wallis test, Spearman rank correlation, and appropriate posthoc tests as necessary. Results were considered statistically significant when the P value was less than 0.05.

Results

Mite burdens of B6 and *Rag1*$^{-/-}$ mice over time. *Myocoptes* burdens were assessed in infested B6 and *Rag1*$^{-/-}$ mice by examining fur samples collected from 4 to 36 wk of age. Burdens differed significantly (2 way ANOVA, $P < 0.0001$) between strains over the 36 wk examined (Figure 3). Burdens were lower in B6 mice than *Rag1*$^{-/-}$ mice at 4, 30, and 36 wk (Bonferroni multiple-comparisons posthoc test; 4 wk, $P < 0.01$; 30 wk, $P < 0.05$; and 36 wk, $P < 0.001$) and trended nonsignificantly lower at 20 and 24 wk.

Age-dependent decreases in mite burdens in B6 and *Rag1*$^{-/-}$ mice. In B6 mice, *Myocoptes* burdens peaked at 4 wk of age, decreased from 6 to 16 wk of age, and remained at a steady low level from 16 wk of age until the completion of the study at 36 wk. Burdens from 16 to 36 wk of age were significantly lower than at earlier time points (Kruskal–Wallis test, $P < 0.0001$; Dunn multiple-comparison posthoc test, $P < 0.05$ for all time points from 16 to 36 wk of age; data not shown). In *Rag1*$^{-/-}$ mice, burdens decreased after 4 wk of age such that they were statistically lower from 8 to 30 wk than at 4 wk (Kruskal–Wallis test, $P < 0.0001$; Dunn multiple-comparison posthoc test, $P < 0.05$ for all time points from 8 to 30 wk of age; data not shown). However, *Rag1*$^{-/-}$ mite burdens began to rise from 20 to 36 wk of age such that by 36 wk of age, they were not significantly different from those at 4 wk of age (Dunn multiple comparison posthoc test, $P > 0.05$).

Sex-associated differences between *Myocoptes* burdens were assessed subsequently in each study group. Burdens were significantly higher in male than female B6 mice over time (2-way ANOVA, $P = 0.004$; data not shown). However, no significant sex-associated differences in mite counts were noted between

Feature	Score	Description	Subjective quantification
			x normal width
Hyperkeratosis	0 or 1	Normal to minimal	n/a
	2	Mild	2 x
	3	Moderate	3 x
	4	Severe	≥4 x
			Cell layers
Acanthosis	0 or 1	Normal to minimal	n/a
	2	Mild	3-4 cell layers
	3	Moderate	5-6 cell layers
	4	Severe	≥7 cell layers
			Cells/hpf
Epidermal Inflammation	0 or 1	Normal to minimal	≤ 5
	2	Mild	5-25
	3	Moderate	25-50
	4	Severe	>50
			Cells/hpf
Dermal Inflammation	0 or 1	Normal to minimal	≤ 5
	2	Mild	5-25
	3	Moderate	25-50
	4	Severe	>50
			Cells/hpf
Subcutaneous Inflammation	0 or 1	Normal to minimal	≤ 5
	2	Mild	5-25
	3	Moderate	25-50
	4	Severe	>50
TOTAL SCORE	0-20		

Figure 2. Scoring system for histologic evaluation of skin. Hpf (high-power field) evaluated at 40 × .

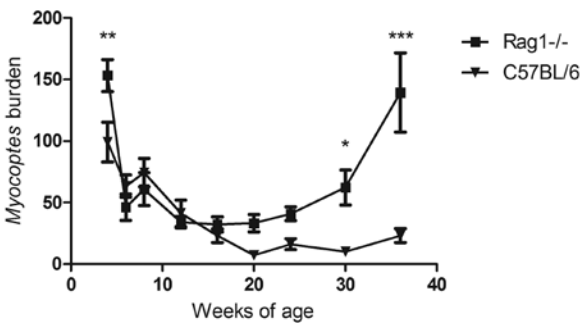

Figure 3. Comparison of *Myocoptes* burden in aging C57BL/6 and *Rag1*$^{-/-}$ mice (n = 17 per strain at 4 wk; n = 12 per strain at all other time points). There was a significant difference in *Myocoptes* burden between strains over time (2 way ANOVA: $P < 0.0001$). In wild type mice, *Myocoptes* burdens decreased over time and were significantly lower than *Rag1*$^{-/-}$ mice at 4, 30 and 36 wk of age. (Bonferroni multiple comparisons post hoc: **, $P < 0.01$ at 4 wk; **, $P < 0.01$ at 30 wk; ***, $P < 0.001$ at 36 wk). Error bars indicate standard error of the mean (SEM).

male and female *Rag1*$^{-/-}$ mice (2 way ANOVA: $P = 0.1397$; data not shown).

Correlation between clinical disease severity of mite infestation and mite burden. Clinical signs noted in infested study mice included alopecia, pruritus, and mite-associated debris. These clinical signs were noted in nearly all mice at 4 wk but diminished as mice aged. Mite-associated debris appeared as multifocal, pinpoint tan to white foci on the epidermal surface at the base of the hair shaft (Figure 4 C). On microscopic exam, these foci were composed of mites, eggs, and epidermal crusts. Alopecia was characterized by locally extensive thinning of the hair coat and was most common on the ventral abdomen, ventral thorax, axillary region, and inguinal region (Figure 4 B). However, more severe alopecia involved diffuse thinning of the entire hair coat (Figure 4 A). Pruritus was noted in nearly all 4-wk-old mice, which were the most heavily infested groups of animals, with as many as 17 scratches in a 2-min period. No study mice showed gross evidence of skin excoriation or ulceration at any point during the study, nor did any die or exhibit severe illness. However, subjective assessment of infested study mice included observations of cutaneous erythema during clinical scoring and enlargement of inguinal and axillary lymph nodes during tissue harvest.

Infested and noninfested B6 and *Rag1*$^{-/-}$ mice were assigned clinical disease scores according to physical signs of fur mite in-

Figure 4. Clinical signs of mite infestation. A 4-wk-old C57BL/6 mouse with diffuse alopecia on the (A) dorsum and (B) ventrum typical of mite infestation at this age. (C) B6 mice at postnatal day 6, with gross evidence of mite-associated debris as hair begins to appear.

festation at time points from 4 to 24 wk of age. *Myocoptes* burden and clinical score showed positive correlation in both infested B6 and *Rag1*$^{-/-}$ mice (Spearman rank correlation: B6, r = 0.9550, P = 0.0028; *Rag1*$^{-/-}$, r = 0.8829, P = 0.0123; Figure 5). Clinical score and age showed negative correlation in infested B6 mice (Spearman rank correlation, r = –0.9910, $P < 0.001$; Figure 6) but not *Rag1*$^{-/-}$ mice. All noninfested mice had clinical scores of 0 throughout the study, except for two 4-wk-old B6 mice. One of these mice had a clinical score of 1 due to presence of unexplained alopecia, and the other had a clinical score of 1 due to evidence of pruritus.

All mice were weighed at each time point, and the weights of noninfested and infested animals were compared. *Rag1*$^{-/-}$ and B6 mice were combined for weight analysis because weight did not differ between strains in mice of the same infection status over time (2-way ANOVA: noninfested B6 compared with *Rag1*$^{-/-}$ male mice, P = 0.7544; infested B6 compared with *Rag1*$^{-/-}$ male mice, P = 0.4798; noninfested B6 compared with *Rag1*$^{-/-}$ female mice, P = 0.3433; infested B6 compared with *Rag1*$^{-/-}$ female mice, P = 0.1965). Uninfested male mice weighed significantly more than infested male mice at all time points (Bonferroni multiple-comparison posthoc test, $P < 0.001$ for all; Figure 7). However, although weights were significantly higher in noninfested compared with infested female mice at 4 wk of age (Bonferroni multiple-comparisons posthoc test, $P < 0.001$; Figure 7), their weights did not differ at any other time point.

Many of the infested B6 and *Rag1*$^{-/-}$ female mice used to provide offspring for the study exhibited more severe clinical signs, including diffuse alopecia, cutaneous erythema, pruritus, excoriations, and mite-associated ulcerative dermatitis (data not shown). These clinical signs appeared to worsen with age and parity. In fact, one infested *Rag1*$^{-/-}$ female breeder exhibited such severe ulcerative dermatitis that she was euthanized. Mite and egg burdens were tabulated from 3 older female breeders (9-mo-old B6, 7-mo-old B6, and 6-mo-old *Rag1*$^{-/-}$) with noticeable signs of *Myo-*

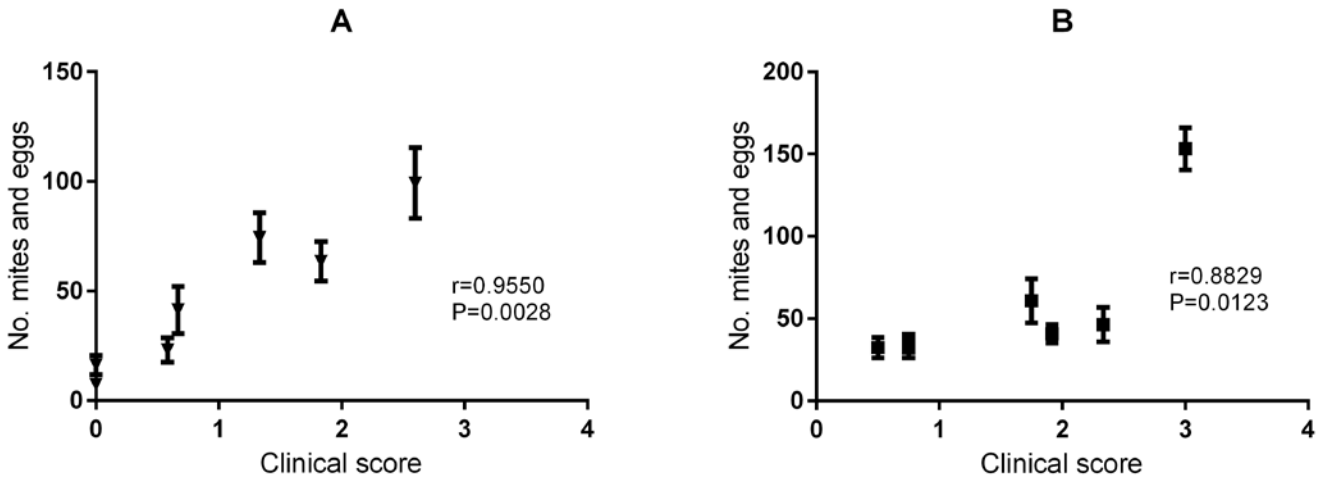

Figure 5. Correlation of average clinical score and *Myocoptes* burden in infested (A) C57BL/6 and (B) *Rag1*$^{-/-}$ mice (n = 17 per group at age 4 wk; n = 12 per group at all other time points). There was a positive correlation between *Myocoptes* burdens and average clinical scores in both B6 and *Rag1*$^{-/-}$ mice (Spearman rank correlation). Error bars indicate SEM.

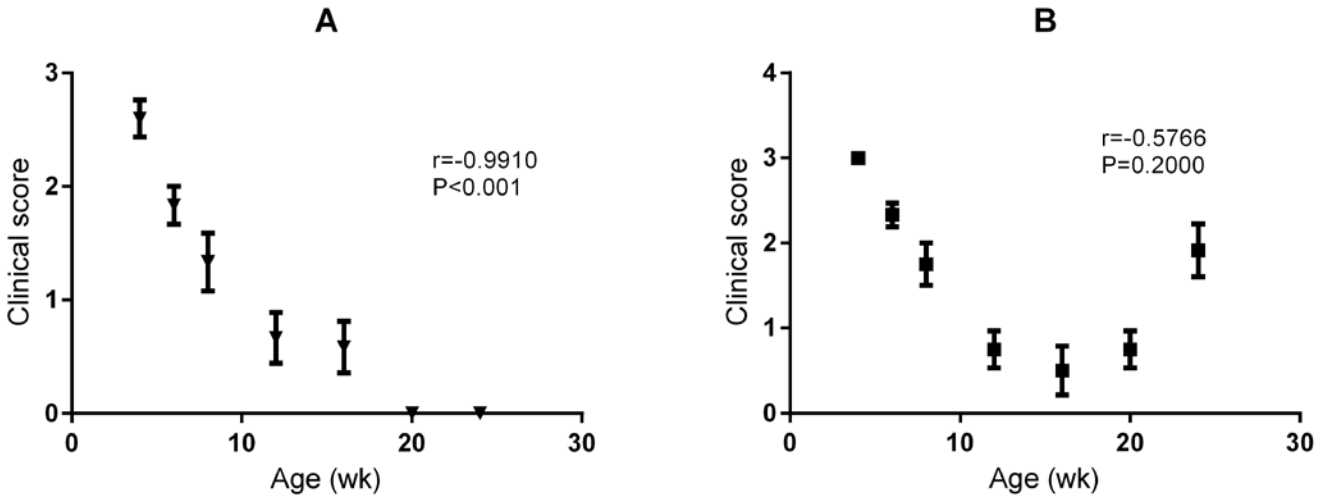

Figure 6. Correlation of clinical score and age in infested (A) C57BL/6 and (B) *Rag1*$^{-/-}$ mice (n = 17 per group at age 4 wk; n = 12 per group at all other time points). There was a negative correlation between age and clinical score in B6 mice; however, there was no correlation of age and clinical score in infested *Rag1*$^{-/-}$ mice (Spearman rank correlation). Error bars indicate SEM.

coptes infestation. Combined mite and egg burdens for these animals were 226, 192, and 129, respectively.

Correlation of histopathologic severity of mite infestation with mouse age. Histopathologic changes of skin were assessed in infested B6 and *Rag1*$^{-/-}$ mice at 4, 8, 16, and 24 wk of age. Pathologic changes were evident in the skin samples of all infested mice and included hyperkeratosis, acanthosis, and inflammation of the epidermis, dermis, and subcutaneous tissues (Figure 8). Inflammatory infiltrates in both strains of infested mice consisted of predominantly mononuclear cell types, with fewer mast and granulocytic cells (data not shown). Other histopathologic observations included dermal thickening, serocellular crusting, multifocal ulceration, and folliculitis. In addition, mites were noted on skin histology of nearly all mice sampled.

Each skin slide was scored according to previously described assessments (Figure 2). Lower scores indicated little to no microscopic pathology, and higher scores indicated more severe pathology. The histopathology scores differed significantly between B6 and *Rag1*$^{-/-}$ mice at the 4 time points examined, with B6 mice having lower scores at 16 wk of age (2-way ANOVA, $P < 0.001$; Bonferroni multiple-comparison posthoc test, $P < 0.001$ at 16 wk; Figure 9) and nonsignificantly lower scores at 24 wk. In addition, scores of both strains differed significantly over time (2-way ANOVA: $P < 0.001$), with lower scores observed as mice aged.

Th2 and Th1 responses over time. Axillary and inguinal lymph nodes from infested B6 mice at 4, 8, 16, and 24 wk of age were used for gene expression analyses (n = 4 or 5 mice per time point) and normalized to that of noninfested B6 controls (n = 4). The relative mRNA expression of Th1-associated genes *Tnf*, *Il2*, *Ifng*, and *Tbx21* and of Th2-associated genes *Il4*, *Gata3*, and *Il5* were assessed by quantitative real-time PCR (Figure 10). The expression of *Il4*, *Il5*, and *Gata3* increased over the course of infestation, as did that of *Il2* and *Tbx21*, although to a lesser extent. The expression of *Tnf* remained relatively constant, and *Ifng* expression was downregulated over time.

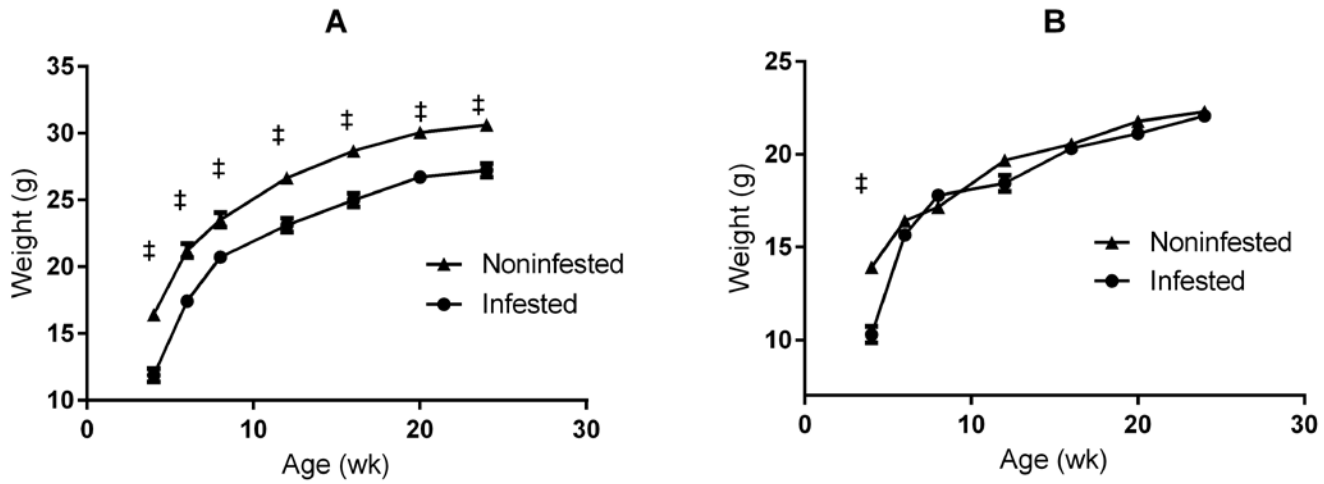

Figure 7. Weights (g) of infested and noninfested (A) male and (B) female mice from 4 to 24 wk of age (infested females $n = 22$, noninfested females $n = 17$, infested males $n = 14$, noninfested males $n = 12$ at 4 wk; $n = 12$ for all groups at all other time points). Uninfested males had significantly higher body weights than did infested males at all time points (Bonferroni multiple-comparison posthoc test, $P < 0.001$ [‡] at all time points). Noninfested female mice had significantly higher body weights than infested females at 4 wk of age (Bonferroni multiple-comparison posthoc test, $P < 0.001$ [‡]) but not at other time points. Error bars indicate SEM.

Discussion

In this study, aging mice deficient in adaptive immunity (*Rag1*$^{-/-}$) harbored higher *M. musculinus* burdens than did aging immunocompetent (B6) mice. We also observed different patterns of mite population dynamics in *Rag1*$^{-/-}$ and B6 mouse strains. In B6 mice, *Myocoptes* burdens were initially high at 4 wk of age, decreased over time, and remained low, consistent with previous reports of mite population dynamics.[9,25] *Rag1*$^{-/-}$ mice showed an initial decrease in burdens followed by a steady rise from 24 to 36 wk of age. In addition, we determined that more heavily infested mice had increased clinical signs associated with infestation, such as poor weight gain, pruritus, alopecia, and dermatitis, and histopathologic severity of skin lesions in infested mice decreased with age. Finally, infested B6 mice exhibited upregulation of markers associated with the Th2-type immune response over the course of infestation.

Myocoptes burdens were higher in aging *Rag1*$^{-/-}$ than B6 mice, with burdens significantly higher in *Rag1*$^{-/-}$ mice at 4, 30, and 36 wk of age. This finding suggests that the development of adaptive immunity in response to mite infestation plays a role controlling mite populations over the course of infestation. Similar findings were documented in immunodeficient mice with *S. obvelata* infections, in which higher parasite burdens were observed in athymic nude mice, MHC II$^{-/-}$ mice, and mice deficient in Th2 responses (IL4, IL4 receptor, and Il13) compared with immunocompetent mice.[5,20,27] In the present study, a deficiency of mature B and T cells may have compromised *Myocoptes* population control in *Rag1*$^{-/-}$ mice, with higher burdens persisting into adulthood. That mice develop an immune response to surface-dwelling parasites and possibly control them through this mechanism is surprising, given that fur mites live on the surface of the epidermis and do not penetrate the deeper layers of the skin.[23] Therefore, mite antigen exposure of the host may occur through scratch-induced skin injury, and exposure of the mite to the host immune system may occur when mites feed on extracellular fluids and epidermal debris.

In this study, we examined Th1- and Th2-type cytokine and transcription factor expression in infested B6 mice over time. Markers of the Th1-type response included expression of the genes *Ifng*, *Tnf*, *Il2*, and *Tbx21.* Elevations of the cytokines TNFα and IL2 are associated with upregulated Th1 responses, and the cytokine IFNγ promotes Th1 differentiation by upregulating T-bet (encoded by *Tbx21*), the transcription factor responsible for the differentiation of naïve T cells into Th1 cells.[1] Markers of the Th2-type response included expression of the genes *Il4*, *Il5*, and *Gata3*. IL4 and IL5 are the primary cytokines produced by Th2 cells during Th2-type responses, and IL4 promotes Th2 differentiation by upregulation of Gata3, the transcription factor responsible for the differentiation of naïve T cells into Th2 cells.[1] As they aged, infested B6 mice demonstrated a progressively pronounced Th2-type response, as indicated by increased regional lymph node expression levels of *Il4*, *Il5,* and *Gata3* over the 4 time points examined. Conversely, infested B6 mice showed less prominent Th1-type responses. Collectively, these findings support the induction of adaptive immunity, specifically Th2-type immune responses, during fur mite infestation.

For some ectoparasite infections, such as scabies, Th1- rather than Th2-type responses appear to be important for host immunity and parasite clearance. For instance, the proposed mechanism for *Sarcoptes* resistance is the development of an IFNγ-driven, Th1-biased immune response.[28] However, Th2-type immune reactions also appear to be associated with protection and reductions in ectoparasite burdens. A recent study demonstrated that effective Th2-type responses are important for the control of *Demodex musculi* in laboratory mice.[17] In that study, mice deficient in both STAT6, an important IL4 receptor signaling molecule, and CD28, a T cell costimulatory molecule important in a number of Th2-type immune reactions, developed chronic dermatitis and high numbers of *Demodex* mites. The author hypothesized that both CD28 and STAT6 signaling are important for the development of the Th2 response and that mice with deficient Th2 responses develop ineffective immune reactions and excessive dermatitis in response to infestation.[17] However, excessive Th2-type responses can have detrimental effects on the host. In another

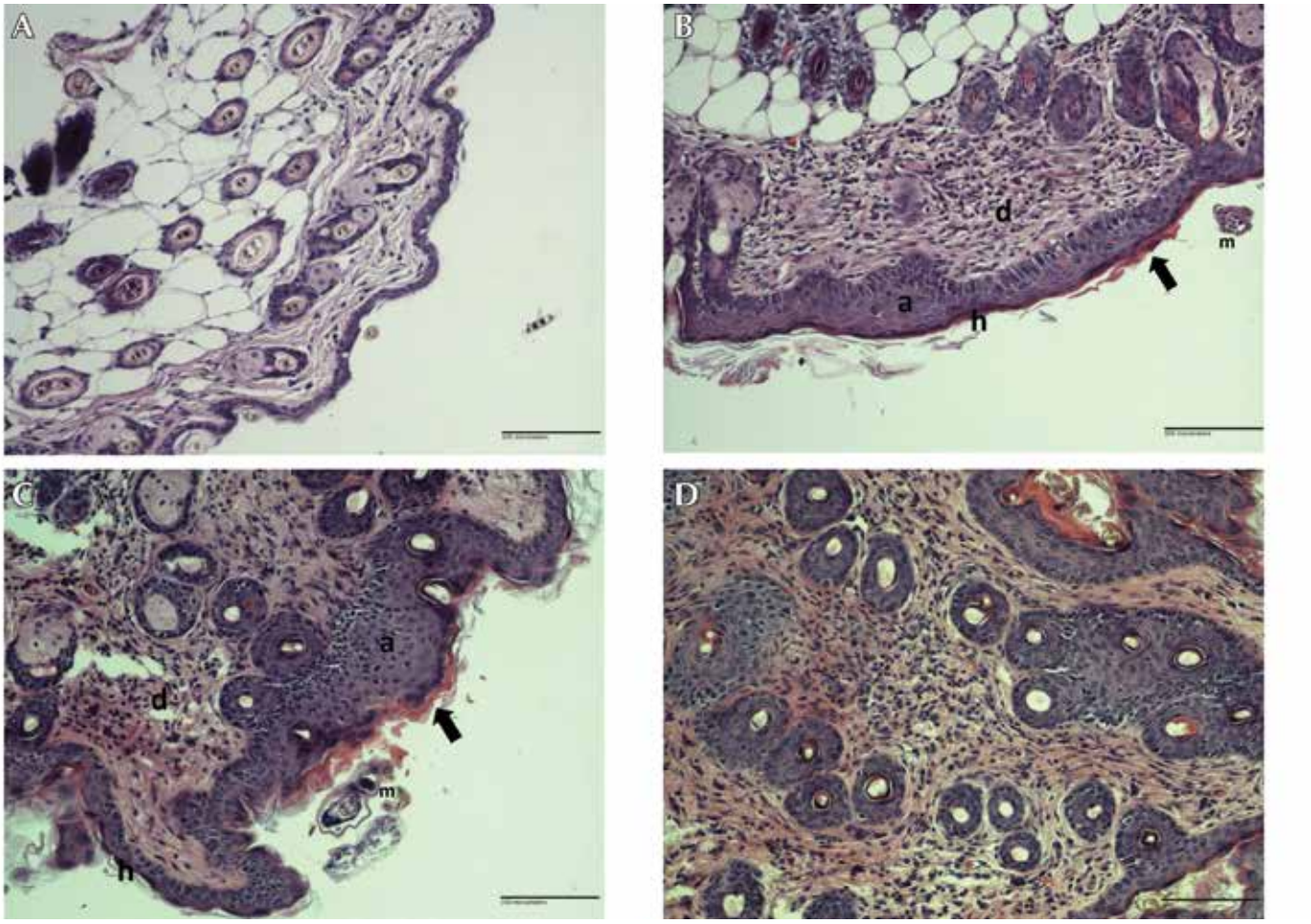

Figure 8. Representative histopathology of skin samples from (A) a noninfested 4-wk-old B6 mouse, (B) an infested 4-wk-old B6 mouse, and (C and D) an infested 4-wk-old *Rag1*$^{-/-}$ mouse. *Myocoptes* mites (m) were noted in the majority of infested samples. Infested mice exhibited signs of hyperkeratosis (h), acanthosis (a), serocellular crusting (arrow), dermatitis and dermal thickening (d), and inflammation of the subcutis. Inflammatory infiltrates consisted of primarily mononuclear cells with a smaller percentage of granulocytes and mast cells. Hematoxylin and eosin stain; magnification, 40×.

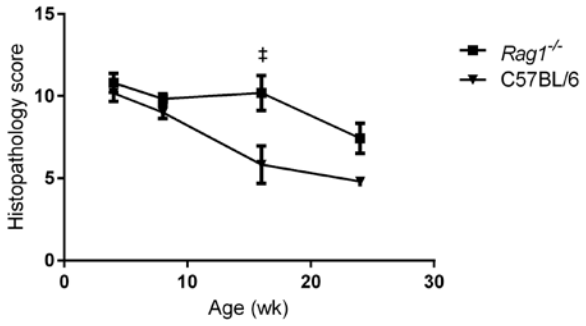

Figure 9. Comparison of histopathology scores in aging C57BL/6 and *Rag1*$^{-/-}$ mice (n = 5 or 6 mice per strain for each time point). There was a significant difference in scores between B6 and *Rag1*$^{-/-}$ mice (2-way ANOVA: $P < 0.001$), with B6 mice having significantly lower scores at 16 wk of age (Bonferroni multiple-comparison posthoc test, $P < 0.001$ [‡] at 16 wk). In addition, there was a significant difference in scores over time (2-way ANOVA: $P < 0.001$), with lower scores observed as mice aged.

study, infestation of BALB/c mice with *M. musculinus* resulted in a chronic allergic state, characterized by excessive Th2-type immune responses, mite-associated ulcerative dermatitis, wasting, and mortality.[13] Collectively, these findings indicate a complex interplay between various arms of the adaptive immune response, which are important for control of ectoparasite burdens and regulation of excessive, chronic host immune responses. In our study, B6 mice showed effective control of parasite burdens, decreased clinical signs of infestation, and increased expression of Th2 markers with age and duration of infestation. These findings suggests that Th2 responses influence the control of mouse fur mite populations over the course of infestation. However, further investigation is required to elucidate the exact mechanism of host immunologic control.

Although mite and egg burdens were higher in *Rag1*$^{-/-}$ than B6 mice over the course of the study, *Rag1*$^{-/-}$ and B6 mice showed similar decreases in mite burdens from 4 to 20 wk of age. This finding suggests that other factors contribute to reduction of *Mycoptes* burdens during this time. Grooming behaviors play an important role in controlling ectoparasite populations in mice[9,24] and may explain the decreased burdens noted in both strains. Another explanation for the initial decrease in *Rag1*$^{-/-}$ *Mycoptes* burdens was a change in housing conditions. In our study, mice were initially housed in breeding cages consisting of a single male, 1 or 2 females, and 1 or 2 litters that were weaned at 21 to 28 d of age.

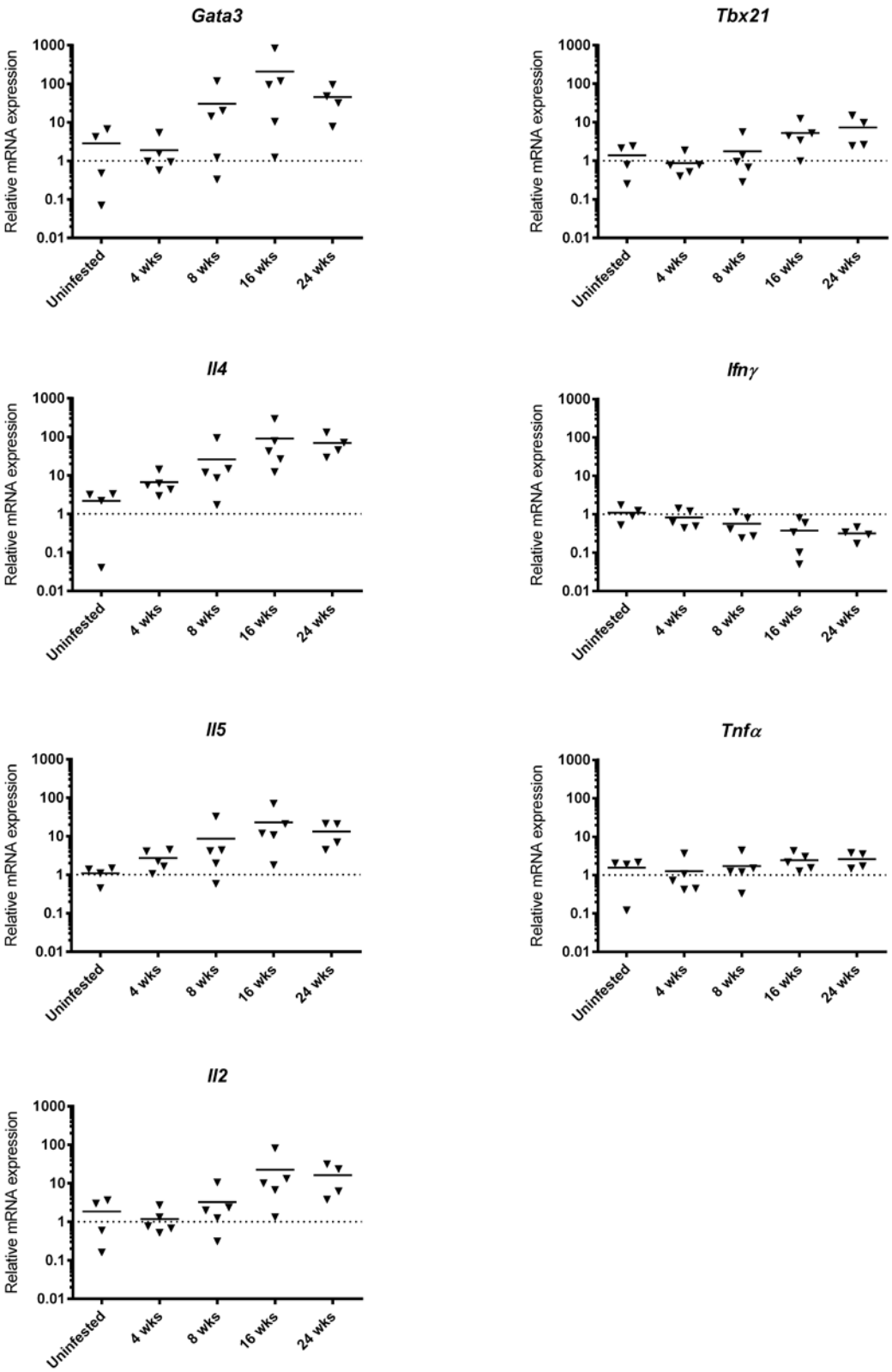

Figure 10. Relative mRNA expression of cytokines and transcription factors from regional lymph nodes of infested mice at 4, 8, 16, and 24 wk of age (n = 4 or 5 animals per time point), normalized to noninfested controls (n = 4).

At weaning, the number of mice decreased from over 10 mice to 3 to 5 mice per cage. A reduction in housing density and change in social circumstance (breeding cage to a smaller single-sex group) may have affected fur mite levels. Changes in mouse population density can affect cage microenvironment, including cage CO_2 levels, humidity, and temperature, which may in turn affect ectoparasite populations.[11,19] In addition, breeding provides mites with a continuous source of naïve hosts, which likely results in an overall increase in total fur mite population within a group of socially housed mice. As a result, host mite burdens may have declined in response to decreased housing density and lack of naïve host availability. Finally, innate immune responses may have contributed to host ectoparasite control. The release of cytokines from local inflammatory cells within the dermis and subcutis can recruit natural killer cells, neutrophils, eosinophils, mast cells, and other inflammatory cells to sites of infestation; these cells may play a role in the control of host parasite burdens.[28]

In the current study, heavily infested 4-wk-old mice clearly showed clinical signs of acariasis, which included pruritus, epidermal mite-associated debris, and a distinct appearance and distribution of alopecia. Clinical signs diminished as *Myocoptes* burdens fell in both B6 and *Rag1*$^{-/-}$ strains, such that mice with low parasite burdens showed little to no clinical signs of infestation. Waning clinical score correlated with increased age in B6 mice but not *Rag1*$^{-/-}$ mice, possibly because older *Rag1*$^{-/-}$ mice had higher mite burdens than B6 mice at later time points. These findings indicate that, in immunocompetent hosts, signs of infestation may occur in young or recently infested mice (when ectoparasite burdens are higher), and these signs are likely to diminish over time. However, in immunodeficient hosts, mite burdens are more likely to remain elevated, and clinical signs may remain obvious in older animals. Finally, alopecia, noted as thinning of the haircoat (especially on the ventrum), appears to be a distinctive finding of mite infestation. This appearance may be an important clinical sign of infestation within large colonies of mice, particularly given that traditional methods of pathogen screening (such as dirty-bedding sentinels) may be unreliable for detection.[16]

Infested male mice weighed less than noninfested male mice at all time points examined. Weight was lower in infested female mice than noninfested females at 4 wk of age but not thereafter. Growth curves are steeper for male mice than female mice, so heavy infestations during growth and development may simply have a more pronounced effect on weight gain in male mice. However, differences in mite and egg burdens between sexes might have affected weight. Burdens were higher in male B6 than female B6 mice over time, and the heavier mite burdens noted in male B6 mice might have decreased their growth rates. Studies have documented the potential effects of sex hormones on immune responses to parasitic and other types of infections,[15] and sex hormones might also affect immune responses to fur mites, influencing parasite load. Although sex-associated differences in *Myocoptes* burdens were noted in B6 mice, no significant differences were noted between male and female *Rag1*$^{-/-}$ mice. The reasons for this finding are unclear but may be related to this strain's adaptive immune system deficiency. If sex hormones can affect immune responses, the effect of sex on mite burdens may not be as prominent in an immunodeficient host.

Interestingly, older breeding female mice of both strains developed pronounced signs of fur mite infestation including alopecia, cutaneous erythema, pruritus, skin excoriations, and mite-associated ulcerative dermatitis. In contrast to those in study mice, these clinical signs appeared to worsen with age and parity. Several explanations for these findings are possible. Breeding females had constant, direct exposure to naïve offspring with high mite burdens, perhaps allowing the dams to become repeatedly reinfested by their offspring. Indeed, *Myocoptes* burdens from 3 older female breeders (2 B6 and one *Rag1*-/-) were high, comparable to those seen at 4 wk of age. In addition, these female mice were chronically exposed to mite antigen on pups or in nest material. Chronically high mite burdens due to constant reinfestation, in addition to continuous exposure to antigens in the environment, possibly resulted in chronic dermatitis in these animals.

In this study, histopathologic changes noted during mite infestation were consistent with previous observations and included dermatitis, dermal thickening, hyperkeratosis, and acanthosis.[12] Histopathologic severity decreased in both *Rag1*$^{-/-}$ and B6 infested mice with age, as evidenced by decreased histopathology scores over time. This finding is consistent with observations of decreasing clinical signs of infestation as mice age and mite burdens decrease. In addition, scores were higher in *Rag1*$^{-/-}$ mice than in B6 mice at 16 wk (significant) and 24 wk (nonsignificant) of age. Higher mite burdens over time may explain this finding, as chronic antigenic stimulation and irritation from the parasite could result in chronic inflammation and associated epidermal changes. Alternatively, the chronic dermatitis noted may have resulted from development of an ineffective immune response that was continuously stimulated by *Myocoptes* infestation, similar to that noted in STAT6/CD28−/− mice infested with *Demodex* mites.[17]

In conclusion, this study suggests the importance of adaptive immune responses in the control of murine fur mite burdens. We demonstrated that although mite populations were higher in *Rag1*$^{-/-}$ than B6 mice over time, mite populations decreased in both strains over the course of the study. Therefore, other factors likely influence mite populations, such as grooming behaviors, housing density, the availability of naïve hosts, host sex, and innate immune responses. In addition, mite burdens appear to affect clinical signs and skin lesion severity, with higher fur mite burdens associated with more severe lesions and clinical signs. Finally, we demonstrated increased expression of Th2 response-associated genes in B6 mice over the course of infestation. Collectively, these findings reveal complex interactions controlling host ectoparasite burdens and highlight the correlation between changing ectoparasite burdens and host clinical disease.

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