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Reports

A Retrospective Study of Idiopathic Ulcerative Dermatitis in Mice with a C57BL/6 Background

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Idiopathic ulcerative dermatitis is a well-recognized disease in C57BL mice and related strains. This disease manifests as a pruritic dermatitis with resulting self-mutilation, dermal ulceration, necrosis, and fibrosis. Ulcerative dermatitis has the ability to confound ongoing research by causing systemic pathologic changes, such as lymphadenopathy and splenomegaly. Although various treatments have been described, none has been curative consistently; therefore, minimizing negative effects on research through prevention of disease is ideal. To identify etiologic factors, we conducted a 2-y retrospective study of 1352 mice with a C57BL/6 genetic background; these mice demonstrated an overall prevalence of 4.1% and a seasonal effect with a peak incidence during midsummer. Corroborating previous studies, our study revealed a disease predilection for female mice. In contrast to prior reports, the disease prevalence was greatest in 10- to 16-mo-old mice. In addition, mice with a C57BL/6 background that were deficient in the gene for inducible nitric oxide synthase had a 50% disease incidence, suggesting a potential animal model for further characterizing the pathogenesis, prevention, and treatment of ulcerative dermatitis.

Abbreviations: iNOS, inducible nitric oxide synthase; UD, ulcerative dermatitis

Idiopathic ulcerative dermatitis (UD) with pruritus is a common condition in C57BL mice, especially C57BL/6J mice. The disease has been characterized histologically as a chronic ulceration with adherent serocellular crust and adjacent pseudoepitheliomatous hyperplasia.[1,9,10] Profound inflammation with neutrophils, lymphocytes, macrophages, and mast cells can occur at the site of ulceration.[1] The ulceration may heal with fibrosis and resulting skin contracture or progress to a secondary bacterial infection.[9] The disease can be differentiated from other causes of ulcerative dermatitis by the characteristic distribution on the thorax and head and the failure to respond to treatments. Fight wounds and infectious agents are other common causes of ulcerative dermatitis in mice but frequently respond to treatment.

Although a 1971 publication describing UD in C57BL/10Sn mice may be the first report of this condition, the disease did not become widely recognized until the mid-1990s, with numerous reports of the disease in mice from commercial vendors.[1,9] Numerous studies have addressed the epidemiology of this disease, but none have definitively identified the cause or proposed a consistently curative treatment, despite multiple attempts (Table 1).[1,2,5,8,15] The disease likely is multi-factorial with an epigenetic component.[2]

To advance epidemiologic knowledge about UD, we undertook a 2-y retrospective study of an isolated subsection of our colony to identify disease trends and lines predisposed to this chronic disease.

Materials and Methods

Housing. Mice were housed within a single room in sterile, static, microisolation caging (Allentown Caging Equipment, Allentown, NJ) with corncob bedding (Harlan Teklad, Madison, WI) and nesting material (Nestlet, Ancare, Bellmore, NY). Mice were housed in groups of as many as 4 mice per cage. Cages were changed twice a week. Mice received an autoclaved pelleted rodent diet (NIH-31 Open Formula, Zeigler Brothers, Gardners, PA) and acidified water (pH = 2.9). Room temperature was maintained between 20.0 and 23.3 °C, and relative humidity was maintained between 30% and 50%. Rarely, deviations from these ranges occurred and lasted 1 h or more before being restored to the usual range. Temperature and humidity were recorded every 15 min (Building Automation System, Siemens, Buffalo Grove, IL) and verified weekly with a calibrated thermometer and hygrometer (model HM34, Vaisala, Woburn, MA). The mice were housed on a 14:10-h light:dark cycle; the light intensity at cage level was less than 325 lux (confirmed quarterly).

Colony health status. Outbred NIH Swiss Webster sentinels were exposed to dirty bedding weekly for 18 wk before being submitted for serology, parasitology, and necropsy (maximum of 24 cages/sentinel cage). The oldest sentinel mouse from each sentinel cage was submitted, and a new young mouse added, every 6 to 8 wk. All sentinels from this facility have been free from ectoparasites since 1991 and endoparasites since 1999. With the exception of mouse adenovirus 2 in 2000, all animal rooms in the building have been negative for tested adventitious viral agents since 1991. The viruses tested for are mouse hepatitis virus, pneumonia virus of mice, Sendai virus, Theiler murine encephalomyelitis virus, mouse rotavirus, lymphocytic choriomeningitis virus, ectromelia virus, mouse cytomegalovirus, minute virus of mice, polyoma virus, reovirus 3, mouse adenovirus, Hantaan virus, and rodent parvoviruses. In addition, the following murine pathogenic bacteria were not found in the facility: cilia-associated respiratory bacillus, *Citrobacter rodentium*, *Clostridium piliforme*, *Corynebacterium kutscheri*, *Mycoplasma* spp., *Salmonella* spp., and *Streptobacillus moniliformis*. All mice were

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Table 1. Anecdotally described treatments for murine idiopathic ulcerative dermatitis, based upon CompMed query results (January 2003, November 2004)

Topical treatments	Systemic treatments	Environmental treatments
Corticosteroids	Oral pediatric acetaminophen	Paper-based enrichment
Hypertonic saline	Oral vitamin E	Delayed weaning
Chlorhexidine covered with aerosol bandage material	Oral vitamin E and essential fatty acids	Clipping of toenails
10% povidone iodine ointment	High-fat feed	
EMLA cream	Oral antibiotics	
Calamine lotion	Oral analgesics	
Antibiotic ointments		
70% ethanol		

Table 2. C57BL/6 background strains analyzed

Strain	Source
B6129P2F1/J	Commercial vendor
C57BL/6NAi – [KO]IL10 -[KO]IL12	Proprietary contract
C57BL/6- [KO]IL10 – [KO] IL5	In-house breeding
B6.129-[KO]IL12p40 N10	Proprietary contract
B6.129P2-IL10^{tm1Cgn}	In-house breeding
C57BL/6-IL5^{tm1Kopf}	In-house breeding
C57BL/6-IL4^{tm1Cgn}	Proprietary contract
C57BL/6NTac – [KO]iNOS	Proprietary contract
C57BL/6- (Tg)IL5 (NJ1638)	Extramural institution
B6.129P2-Fcgr3^{tm1Sjv}	In-house breeding
B6.129P2-Fcer1g^{tm1Rav}	In-house breeding
C57BL/6	Commercial vendors
B6.129S4-C3^{tm1Crr}	In-house breeding

on protocols approved by the animal care and use committee in accordance with applicable federal regulations.

Disease tracking. Mice in the room were examined by the animal caretaker at least once a day for general condition. Any mice that were ill or displayed skin lesions were brought to the attention of the facility technicians, who performed a thorough evaluation; noted the sex, strain, and age of the mouse; and wrote a description of the initial lesion. Mice then were examined by the facility veterinarian, who evaluated the distribution and extent of the skin lesion, presence of pruritus or lymphadenopathy, and provided the diagnosis of UD when appropriate. Mice were euthanized if more than 10% of the total body surface area was affected.

Pathology and bacteriology. Over the 2-y period, 34 mice with lesions typical of UD were submitted alive for euthanasia by carbon dioxide and a comprehensive necropsy including examination for external parasites, bacterial culture of the lesion, and histopathology of the dermal lesion and other abnormal tissues. Tissues were preserved in 10% neutral buffered formalin prior to dehydration in an alcohol series followed by paraffin embedding. Tissue sections were stained with hematoxylin and eosin and evaluated by a veterinary pathologist. Bacterial cultures were taken from tissues that had been surface-sterilized by heat application. Lesions then were incised with a sterile scalpel, and a sterile swab was used to obtain a culture from the cut surface. This swab was cultured on 5% sheep blood agar, MacConkey agar, phenylethyl alcohol agar with 5% sheep blood, Sabouraud dextrose agar, and thioglyocollate broth. Bacteria were identified by Gram stain and through use of automated classification equipment (Vitek II, bioMérieux, Balmes-les-Grottes, France).

Statistics. Using Microsoft Excel (Microsoft Office Professional Edition, 2003, Microsoft Corp, Redmond, WA), the chi square test for independence and the binomial test was performed. Statistical significance for these tests was set at $P < 0.01$.

Figure 1. Typical presentation of chronic murine UD in a C57BL/6 mouse, showing intrascapular ulceration with fibrosis.

Results

Mice. A total population of 3011 mice (2035 female and 976 male) were housed in the room between 1 January 2003 and 31 December 2004. This census comprised 1368 mice that were purchased from 4 commercial vendors and 1617 mice that were bred within the room. After quarantine and testing, 26 mice (including 5 with C57BL/6 backgrounds) were brought into the room from outside institutions. Of these 3011 mice, 1352 had C57BL/6 genetic backgrounds (13 strains; 840 female and 512 male mice), were housed in the room for at least 1 mo, and were included in this study (Table 2). During this time period, 55 mice were diagnosed by the veterinarian as having UD, for an overall prevalence of 4.1%. The incidence of UD in mice purchased directly from vendors was not statistically different compared with that of mice bred in-house ($P > 0.1$). The diagnosis of UD was made if the skin was erythematous or ulcerated, the lesion was cranial to the abdomen or involved the pinna of the ear, and the mice were pruritic, singly housed, or lacked wounds on the flanks and rump. Typically, UD was seen as focal, bilaterally symmetric, interscapular erythema with mild pruritus that progressed to ulceration within 1 wk (Figure 1). The lesion frequently extended unilaterally to the ears, face, and portions of the remaining thoracic regions.

Epidemiologic assessment. The date of onset of UD for each mouse was determined, and a dramatic increase was seen in midsummer for both years. When the percentage of the total population of C57BL/6 mice that developed UD was evaluated, the increase during the summer was still evident, confirming that this summer spike was not due to an increase in the mouse population (Figure 2). The analysis of incident cases and environmental temperature extremes did not show a correlation with the onset of cases (data not shown). More cases occurred when

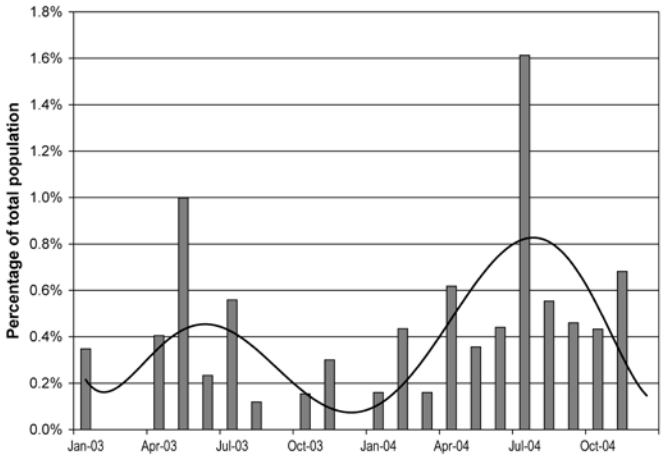

Figure 2. The monthly incidence of murine UD, expressed as a percentage of the total number of C57BL/6 background mice present during that month, has an obvious seasonal effect. The trendline demonstrates the increased incidence in midsummer.

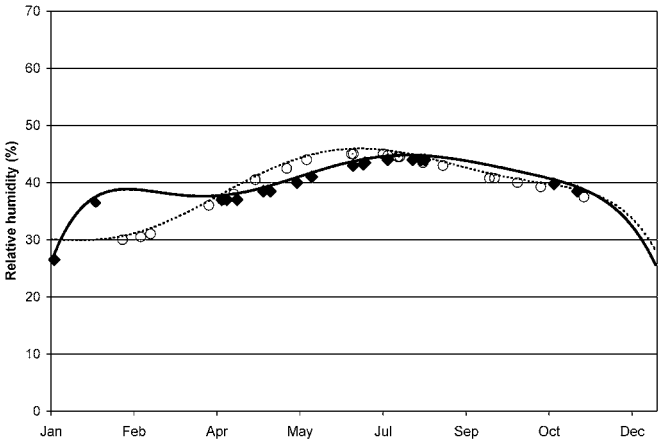

Figure 3. Daily average relative humidity (trend line) was plotted against incidents of UD (diamonds or circles). Data from 2003 (solid trendline with diamonds for cases) and 2004 (dashed trendline with circles for cases) are shown.

humidity was from 35% to 45% (Figure 3), with an increase in cases occurring both during times of increasing humidity (2003) and decreasing humidity (2004). Although the average age of onset in this colony was 13 mo, approximately 13% of the affected mice developed UD when younger than 6 mo (data not shown). The number of mice developing UD, as a percentage of the population of C57BL/6 background mice of the same age, tended to increase as mice aged (Figure 4). Female mice were affected disproportionately; 47 female mice, 6% of the total population of female C57BL/6 mice, developed UD ($P < 0.001$). This figure is in comparison to the 8 male mice, 2% of the total population of male C57BL/6 mice, that developed UD.

The distribution of UD among strains was not proportional (Figure 5), because mice that lacked the gene for inducible nitric oxide synthase (iNOS) developed significantly more UD than did other strains ($P < 0.00001$). The onset of disease in the iNOS mice occurred between 16 and 20 mo of age. We performed a chi-square analysis to determine whether inclusion of the iNOS mice biased the gender predisposition. The chi-square analysis revealed that the statistical significance for the predominance of females remained when the iNOS female mice were discounted ($P < 0.005$).

Pathology. Mice were submitted for gross necropsy and histopathologic assessment to confirm that the lesions seen were

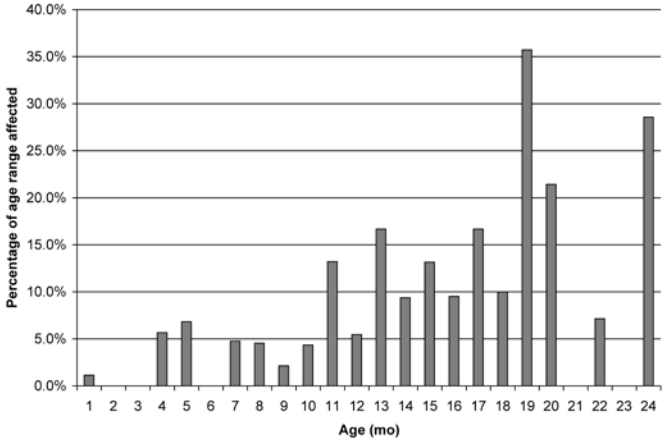

Figure 4. The age of onset of UD, expressed as a percentage of the age range of the total C57BL/6 background. Although the average and median age at onset was 13 mo, the cases of UD proportionally increased as the mice aged.

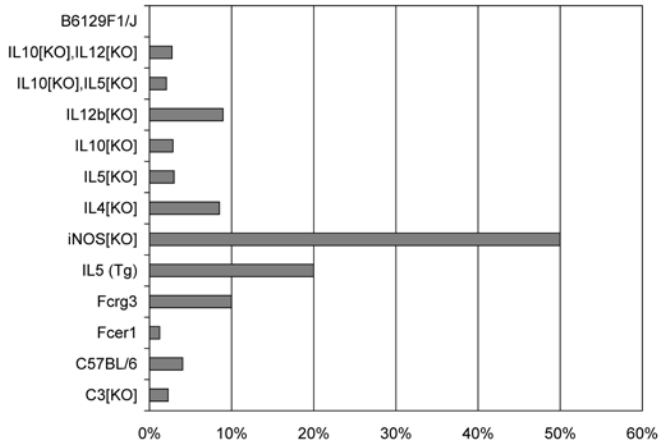

Figure 5. The incidence of UD in the 13 strains analyzed.

attributable to UD. The histopathologic findings (Figure 6) were consistent with classic UD lesions: multifocal to coalescing regions of ulceration extending to the subcutis, with fibrosis, neutrophils, and lymphocytes present. The adjacent epidermis was frequently hyperplastic. Splenomegaly frequently was present, with extramedulary hematopoiesis and a reactive lymphadenopathy of the axillary and cervical lymph nodes. Ectoparasites were not seen during the gross examination or upon histopathologic evaluation. When the surface-sterilized lesions were cultured for bacterial growth, *Staphylococcus xylosus* was the bacterium most frequently isolated. *Enterococcus faecalis*, *Enterobacter cloacae*, *Candida* spp., and streptococcal and other staphylococcal species, including *S. aureus*, also were isolated from the lesions.

Discussion

Murine UD is an ever-present disease in most laboratory animal facilities. The lack of a curative treatment and the systemic effects of the disease are not conducive to research using the affected mice, so most animals that develop this disease are euthanized. We undertook this retrospective study with the hope of identifying and removing any etiologic agents that might predispose mice to this disease. Most likely UD is a multifactorial disease with an underlying genetic component.[2]

The literature indicates that C57BL/6 mice predominantly are affected by this disease. Our study confirmed this trend: none

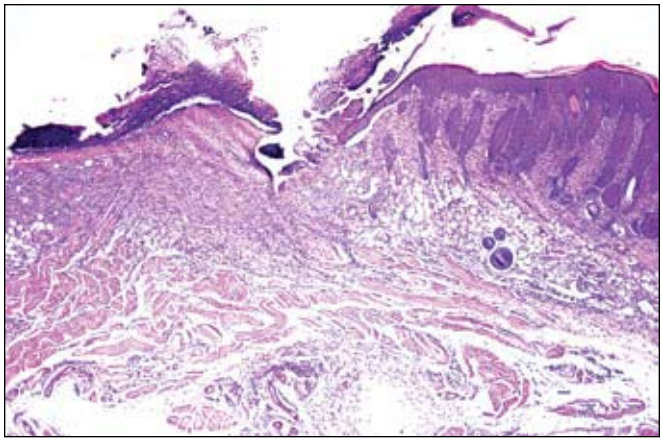

Figure 6. The typical histopathologic findings for UD included ulceration extending to the subcutis with inflammatory cells and fibrosis. The adjacent dermis was often hyperplastic.

of the 1659 non-C57BL/6 mice housed in this room developed UD. In addition, our study concurred with prior reports that female mice were significantly more likely to develop disease than were male mice, with 47 of the 840 C57BL/6 background female mice on this study developing UD.[1,9]

Some of the findings generated in our study do not agree with prior studies. Previous studies had suggested a predominance of disease during the late fall, perhaps during a time of decreasing humidity.[9] In contrast, our retrospective study found a dramatic increase during summer and times of higher humidity. The significance of the increased cases is unknown and may be associated with an absolute intracage level relative humidity or the time of the year. Perhaps the increase in the number of cases was due to an increase in an environmental allergen, such as pollen or fungal spores that were brought into the room on personnel clothing or in the feed or bedding. This correlation, of environmental allergen peaks with increased UD incidence, warrants a prospective study with environmental sampling to determine the concentration of fungal spores or other allergens in the animal's cage. The affected mice in this study developed UD over a wide range of ages, and the incidence increased as the mice aged.

Although the etiology of UD remains unknown, our study yields some insights into possible factors for the disease. *S. xylosus* was cultured from each case submitted for necropsy and bacterial isolation. This organism can be a primary pathogen in mice and can induce an extensive dermatitis when inoculated into the skin.[12,16] Perhaps the mice self-inoculate *S. xylosus* during the initial erythematous and pruritic stage, and self-trauma results in progression to ulceration. *S. xylosus* is known to be sensitive to trimethoprim–sulfamethoxazole, treating UD with selected systemic antibiotics might be beneficial, although in our experience antibiotics alone have not resulted in a definitive cure.[12] The high prevalence of disease in iNOS-deficient C57BL/6 mice hints at another possible contributory factor. iNOS has been shown to play a role in skin repair: iNOS-deficient mice have delayed wound closure and may be more prone to wound infections by bacteria.[7] In addition, iNOS-deficient mice develop a robust Th1 immune response.[14] C57BL/6 mice, in contrast to BALB/c mice, preferentially produce a Th1 response through the production of IL-12 by macrophages.[3,13] Thus the innate T-cell response in the C57BL/6 background may be part of the underlying cause of UD.

As models of murine UD, mice with or without iNOS deficiency but inoculated with *S. xylosus* could help ascertain a reliably effective treatment. Although multiple treatment options have been proposed, none is recognized as consistently effective or curative. Once a predictive model with *S. xylosus* has been established, the efficacy of systemic trimethoprim-sulfamethoxazole treatment can be evaluated. Studies involving the spontaneously atopic dermatitis seen in NC/Nga mice have suggested that inhibitors of substance P or tachykinin agonists might decrease the scratching behavior in mice.[6] In addition, substance P has been shown to play an important role in the induction of iNOS in macrophages.[4] Another group using the same mouse model has shown that administration of royal jelly, collected from worker honeybees, stimulates iNOS and inhibits the development of atopic dermatitis.[11]

Although our retrospective study did not focus on curative or preventative treatments for murine UD, it has suggested unique models for further study. Ideally, our findings will stimulate discussions between the laboratory animal community and researchers in dermatitis that will eventually lead to the resolution of this common, chronic, and devastating disease.

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