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Case Study

Severe Dermatitis Associated with Spontaneous *Staphylococcus xylosus* Infection in *Rag*$^{-/-}$*Tpl2*$^{-/-}$ Mice

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Staphylococcus xylosus **is a commensal bacterium found on the skin and mucosal surfaces of SPF mice.** ***S. xylosus*** **is rarely pathogenic, most often causing skin lesions and dermatitis in immunocompromised mice, particularly those with impaired NADPH oxidase function. Here we report spontaneous infection with** ***S. xylosus*** **in** ***Rag1*$^{-/-}$*Tpl2*$^{-/-}$** **mice. Infection was characterized by the presence of alopecia, crusts, and scaly skin.** ***S. xylosus*** **was detected in the feces, skin, lymph nodes, and lungs of** ***Rag1*$^{-/-}$*Tpl2*$^{-/-}$** **mice and led to mortality or euthanasia due to humane endpoints. C57BL/6 mice were culture-positive for** ***S. xylosus*** **on the skin, and** ***Rag1*$^{-/-}$** **and** ***Tpl2*$^{-/-}$** **mice were culture-positive on the skin and occasionally in the feces. However,** ***S. xylosus*** **did not cause clinical symptoms in C57BL/6,** ***Rag1*$^{-/-}$**, **or** ***Tpl2*$^{-/-}$** **mice. Compared with those in** ***Rag1*$^{-/-}$** **mice, relative concentrations of circulating monocytes, but not neutrophils or lymphocytes, were increased in** ***Rag1*$^{-/-}$*Tpl2*$^{-/-}$** **mice, consistent with their increased incidence of clinical symptoms. Overall, this case study suggests a novel role for Tpl2 in T-cell–independent host resistance to the otherwise commensal organism** ***S. xylosus*****.**

Abbreviation: Tpl2, tumor progression locus 2

Staphylococcus xylosus is a nonmotile coagulase-negative, gram-positive coccoid organism that was first identified in 1975.[31] *Staphylococci* are common bacteria in the environment and have been linked to opportunistic infections in both humans and animals. *S. xylosus* is generally considered a nonpathogenic bacterium and is commonly used in the production of meat and cheese products.[1,13,18,33,34,39] One reason *Staphylococcal* spp. are useful in food production is due to their expression of the *MprF* gene, which leads to the production of lantibiotics and protection against spoilage.[25] *S. xylosus* is a commensal bacterium found on the skin of SPF C57BL/6 mice.[35] All strains of *S. xylosus* isolated from laboratory animals have been susceptible to multiple antibiotics.[10] In addition, *S. xylosus* has been isolated from humans, dairy cows, ewes, gerbils, and poultry.[5,23]

Although large numbers of mice are used in research, few reports of spontaneous *S. xylosus* infection are available. Spontaneous infection of athymic nude mice with *S. xylosus*, characterized by extensive dermatitis on the neck, thorax, and shoulders has been reported;[4,29] however, *S. xylosus*-induced scalding dermatitis in athymic nude mice arose inconsistently, with significant dermal inflammation and the presence of gram-positive cocci in the lesions.[4,29] This presentation is consistent with the induction of the localized CD4 T-cell response after experimental colonization of germ-free C57BL/6 mice with *S. xylosus*.[24] Importantly, genetically modified mouse strains deficient in phagocyte superoxide production also show increased susceptibility to spontaneous *S. xylosus* infection, characterized by dermatitis and abscess formation.[10,14,15,26,38] The development of severe dermatitis, morbidity, and mortality in these strains suggests that both T cells and superoxide production contribute to host resistance. Superoxide is generated through activation and formation of the NADPH oxidase complex, which is composed of 5 subunits: p22^{phox} and gp91^{phox} (Cybb) on the cell membrane and p40^{phox}, p47^{phox}, and p67^{phox} in the cytoplasm. On activation, the 5 subunits combine at the cell membrane, generating the active NADPH oxidase and enabling superoxide production. Mice lacking the gp91^{phox} subunit (B6.129S6-*Cybb*^{tm1Din}/J) or p47^{phox} subunit (B6p47$^{phox-/-}$HLL and B6.129S2-Ncf1^{tm1shl}N14), which are commonly used as models for chronic granulomatous disease, have been reported to develop spontaneous *S. xylosus* infection.[4,14,26] Of note, infection in superoxide-deficient mice is also characterized by dissemination into other organs, including brain, lung, lymph nodes, and bone, whereas *S. xylosus* remains localized to the skin in superoxide-competent mice.[10,14]

In the current report, we describe spontaneous infection with *S. xylosus* that was associated with morbidity and mortality in *Rag1*$^{-/-}$*Tpl2*$^{-/-}$ mice. Affected mice presented with alopecia, scaly skin, crusts, and mortality or euthanasia according to humane endpoints. However, C57BL/6, *Rag1*$^{-/-}$, and *Tpl2*$^{-/-}$ mice did not develop clinical signs of infection even though all strains were culture-positive for *S. xylosus* on the skin. *Rag1*$^{-/-}$*Tpl2*$^{-/-}$ mice occasionally had disseminated bacteria in lymph nodes and

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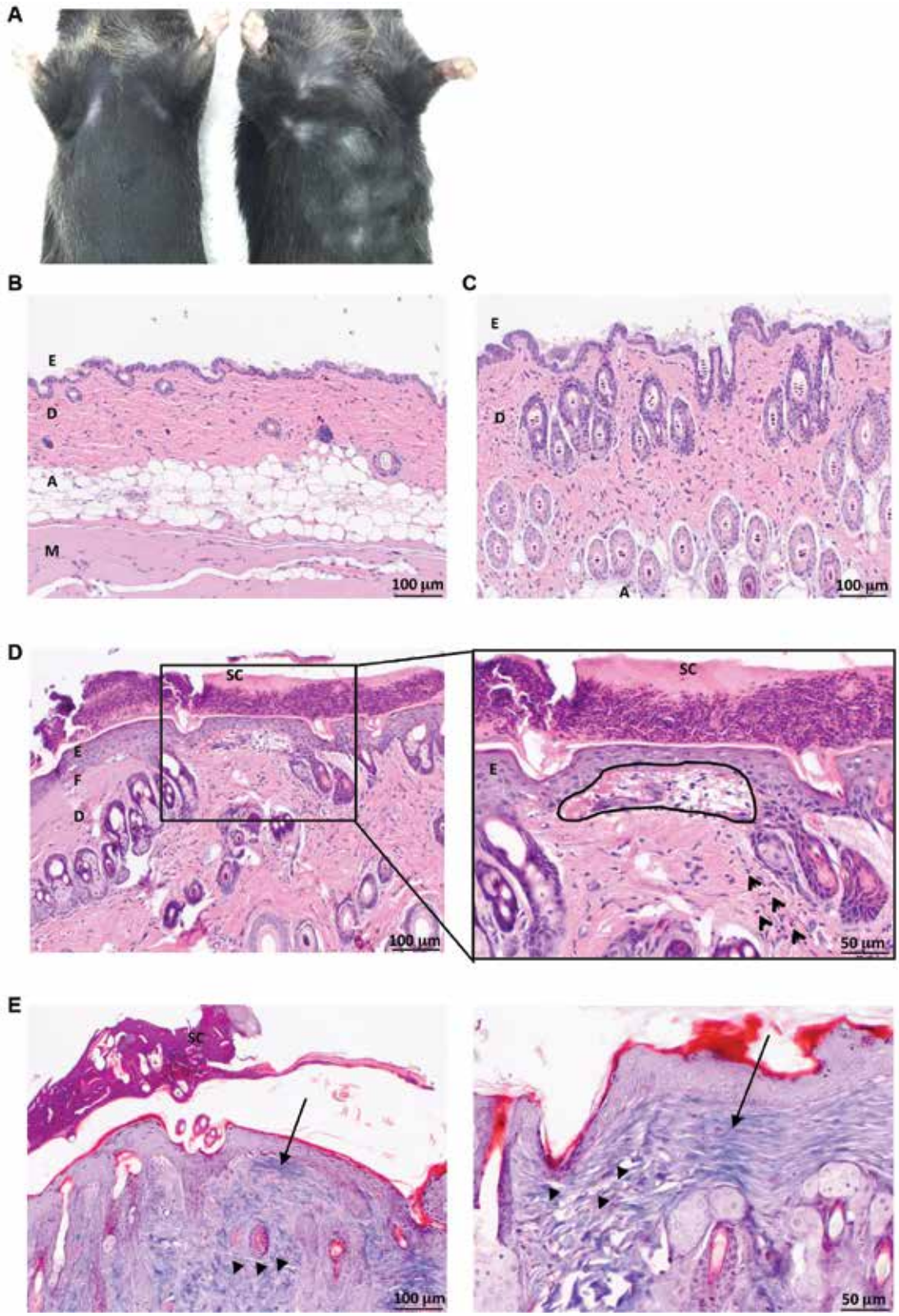

Figure 1. Gross morphology and histopathology of *Rag1*$^{-/-}$*Tpl2*$^{-/-}$ mice. (A) *Rag1*$^{-/-}$*Tpl2*$^{-/-}$ mouse (right) showing ruffled fur, alopecia, crusts, and scabbing next to a *Rag1*$^{-/-}$ mouse (left). (B–D) Representative histology of skin from (B) *Tpl2*$^{-/-}$, (C) *Rag1*$^{-/-}$, and (D) *Rag1*$^{-/-}$*Tpl2*$^{-/-}$ mice stained with hematoxylin and eosin. The outline indicates focal edema with fibrin in the superficial dermis immediately beneath the epidermis; the small arrows

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lung. Dissemination into internal organs is indicative of defects in NADPH oxidase[10] and implicates Tpl2 in the production of superoxide by phagocytes *in vivo*.

Materials and Methods

Mice. Wild-type (C57BL/6J) and *Rag1*$^{-/-}$ mice were obtained from the Jackson Laboratory (Bar Harbor, ME). *Tpl2*$^{-/-}$ mice backcrossed for more than 10 generations onto the C57BL/6 genetic background were kindly provided by Dr Philip Tsichlis and Thomas Jefferson University (Philadelphia, PA). *Rag1*$^{-/-}$ mice were crossed with *Tpl2*$^{-/-}$ mice to generate *Rag1*$^{-/-}$*Tpl2*$^{-/-}$ mice. C57BL/6 and *Tpl2*$^{-/-}$ mice were crossed to generate *Tpl2*$^{+/-}$ mice; the wild-type and *Tpl2*$^{-/-}$ mice used in this report were bred from heterozygous matings. Genotyping of mice as *Tpl2*$^{+/+}$, *Tpl2*$^{+/-}$, or *Tpl2*$^{-/-}$ was performed on tissue samples harvested by either tail snip or ear punch. DNA was extracted by using the E.Z.N.A Tissue DNA Kit (Omega Bio-Tek, Norcross, GA) and evaluated by PCR analysis using the following primer sequences: shared forward primer, 5′ CTT CAG TCA TCT TAA CAC TCA GGC 3′; WT reverse primer, 5′ CTG CTT GGA ACT TGC TGT TCT AGA TG 3′; and *Tpl2*$^{-/-}$ reverse primer, 5′ CTG CAC GAG ACT AGT GAG ACG TGC 3′. Mice were bred and maintained in sterile microisolation cages at the University of Georgia (Athens, GA) according to IACUC-approved guidelines for laboratory animals. The central animal facility at the University of Georgia monitors all mouse cages daily and routinely tests for the presence of pathogenic infection in female sentinel cages. Throughout this study, sentinels consistently tested negative for various endoparasites, ectoparasites, and viral infections including mouse parvovirus, mouse hepatitis virus, Sendai virus, pneumonia virus of mice, *Mycoplasma pulmonis*, and lymphocytic chroiomeningitis virus. *Rag1*$^{-/-}$*Tpl2*$^{-/-}$ mice were euthanized when they met criteria for humane endpoints including ruffled fur, hunched posture, and evidence of infection. All euthanized mice in this case report were male and ranged in age from 6 wk to 4 mo old. All mice were euthanized with CO_2 gas followed by cervical dislocation to confirm death.

Blood and tissue collection. Blood was collected through terminal cardiocentesis into microvette tubes containing EDTA (Starstedt, Nümbrecht, Germany). Total WBC (reported as number of cells per microliter) were measured automatically (HemaTrue Veterinary Hematology Analyzer, Heska, Loveland, CO). Skin (including subcutaneous areas), spleen, lymph nodes (mesenteric and inguinal), lung, brain, and fecal samples were collected into sterile PBS by using aseptic technique, homogenized in BHI broth (Becton Dickenson, Franklin Lanes, NJ), and plated on blood agar (Becton Dickenson), MacConkey agar, and phenylethyl alcohol agar (Thermo Fisher Scientific, Waltham, MA) at 35 °C with 5% CO_2. *S. xylosus* was identified by matrix-assisted laser desorption/ionization–time of flight mass spectrometry[7] by the University of Georgia Veterinary Diagnostic Laboratories using the Vitek system (bioMérieux, Durham, NC). Additional skin samples were fixed in 10% neutral buffered formalin for 24 h at room temperature. Complete cross-sections of formalin-fixed tissue were placed in cassettes, embedded in paraffin, sectioned at 4 µm, mounted on glass slides, and stained with hematoxylin and eosin or Masson trichrome and periodic acid–Schiff–hematoxylin. Histologic sections were evaluated by a veterinary pathologist (TN).

Table 1. Detection of S. *xylosus* in murine organs

Genotype	Feces	Skin	Spleen	LN	Lung	Brain
C57BL/6	0/3[a]	3/3	0/3	0/3	0/3	0/3
Tpl2$^{-/-}$	2/4[b]	4/4	0/4	0/4	0/4	0/4
Rag1$^{-/-}$	1/6[a]	5/5	0/5	0/5	0/5	0/5
Rag1$^{-/-}$ *Tpl2*$^{-/-}$	10/10	8/8	0/8	1/4	1/3	0/3

Tissue samples collected from age-matched mice were cultured for *S. xylosus*. Mice were sampled randomly; not all mice were evaluated for bacterial dissemination in all organs. Data are given as 'no. mice positive/total no. of mice sampled'.
Values significantly ([a]$P < 0.005$, [b]$P < 0.06$ [Fisher exact test]) different from those of *Rag1*$^{-/-}$ *Tpl2*$^{-/-}$ mice are indicated.

Statistics. Data were compared relative to *Rag1*$^{-/-}$ *Tpl2*$^{-/-}$ mice by using the Fisher exact test or one-way ANOVA with Tukey posthoc testing by using Prism software (GraphPad Software, La Jolla, CA). Differences were considered significant when the *P* value was 0.05 or less (0.016 or less with multiple comparisons).

Case Report

Over a 1-y period, we noted thickened scaly skin that required euthanasia or led to mortality in several male naive *Rag1*$^{-/-}$*Tpl2*$^{-/-}$ mice in our mouse colony. The skin of affected *Rag1*$^{-/-}$*Tpl2*$^{-/-}$ mice was characterized by ruffled fur, mild alopecia, scabs, and scattered discrete crusts (Figure 1 A). Microscopic analysis revealed a markedly hyperplastic epidermis focally covered with a serocellular crust composed of neutrophils, cellular debris, and bacteria. Regions of fibrosis beneath the epidermis indicated chronic infection, and acute hemorrhage between the epidermis and superficial dermis signified a severe inflammatory condition. Mononuclear inflammatory cell infiltrates were present also (Figure 1 B). Masson trichrome and periodic acid Schiff–hematoxylin staining revealed regions of fibrosis in *Rag1*$^{-/-}$*Tpl2*$^{-/-}$ mice (Figure 1 C). Necropsy and diagnostic testing, consisting of differential culture followed by mass spectrometry, revealed that all mice demonstrating clinical symptoms were positive for *S. xylosus*. Additional bacterial species identified included *Enterococcus gallinarum*, *Leuconostoc* spp., and *Corynebacterium mastitidis*, but the presence of these bacterial species did not correlate with the incidence of skin lesions. Collectively, these findings strongly suggested that skin lesions were due to spontaneous *S. xylosus* infection, which occurred at different time points throughout the 1-y period in 4 separate cages of male mice among a total of 29 naive *Rag1*$^{-/-}$*Tpl2*$^{-/-}$ cages, corresponding to an infection incidence rate of 13.8%. None of the 23 cages of naïve *Rag1*$^{-/-}$ mice or 152 cages of naïve *Tpl2*$^{+/-}$ × *Tpl2*$^{+/-}$ mice (comprising *Tpl2*$^{+/+}$ [C57BL/6], *Tpl2*$^{+/-}$, and *Tpl2*$^{-/-}$ mice) showed clinical signs of infection or required euthanasia during the same time period.

Several occurrences of spontaneous *S. xylosus* infection in *Rag1*$^{-/-}$*Tpl2*$^{-/-}$ mice prompted the investigative staff to euthanize a cohort of male C57BL/6, *Rag1*$^{-/-}$, *Tpl2*$^{-/-}$, and *Rag1*$^{-/-}$*Tpl2*$^{-/-}$ mice and examine them for the presence of *S. xylosus* on the skin as

indicate mononuclear inflammatory cells. (E) Representative histology from skin of *Rag1*$^{-/-}$*Tpl2*$^{-/-}$ mice stained with Masson trichrome and periodic acid–Schiff–hematoxylin. The arrow indicates a region of fibrosis, and the arrowhead indicates normal collagen. A, adipose; D, dermis; E, epidermis; F, fibrosis; M, cutaneous muscle; SC, serocellular crust.

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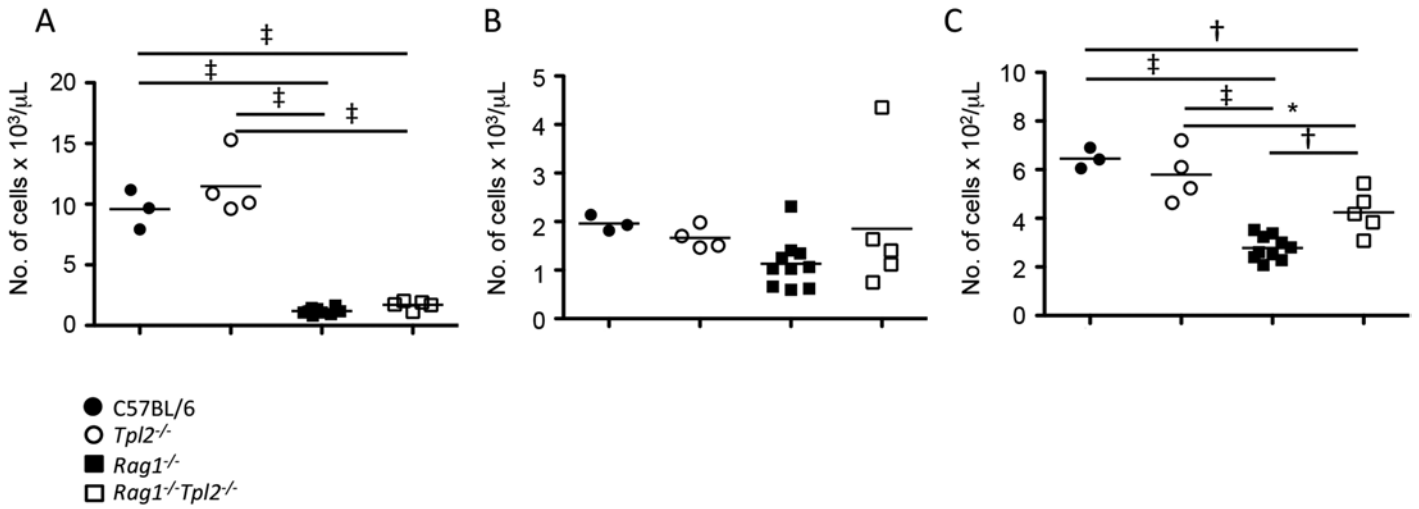

Figure 2. *Rag1*$^{-/-}$*Tpl2*$^{-/-}$ mice had increased numbers of circulating monocytes. Blood collected from age-matched C57BL/6 (n = 3), *Tpl2*$^{-/-}$ (n = 4), *Rag1*$^{-/-}$ (n = 10), and *Rag1*$^{-/-}$*Tpl2*$^{-/-}$ (n = 5) mice was analyzed for (A) lymphocytes, (B) neutrophils, and (C) monocytes. Horizontal lines indicate mean values; data were analyzed by one-way ANOVA with Tukey posthoc testing (*, $P < 0.05$; †, $P < 0.01$; ‡, $P < 0.001$)

well as in the feces, spleen, lymph nodes, lung, and brain. The organism was detected on the skin of all mice and in the feces of 2 *Tpl2*$^{-/-}$ mice, a single *Rag1*$^{-/-}$ mouse, and all *Rag1*$^{-/-}$*Tpl2*$^{-/-}$ mice (Table 1). These data are consistent with reports of *S. xylosus* on the skin of SPF C57BL/6 mice.[35] Fecal testing of *Rag1*$^{-/-}$*Tpl2*$^{-/-}$ mice included female cages and confirmed that colonization with *S. xylosus* in *Rag1*$^{-/-}$*Tpl2*$^{-/-}$ mice was not sex-specific, although dermatitis, morbidity, and mortality were restricted to male mice. In addition, aerobic culture of *S. xylosus* documented dissemination to lymph nodes in one *Rag1*$^{-/-}$*Tpl2*$^{-/-}$ mouse and lung in another (Table 1). These data further implicate *S. xylosus* as the causative agent of dermatitis in the current outbreak and suggest that the additional absence of Tpl2 predisposes in immunocompromised *Rag1*$^{-/-}$ mice to *S. xylosus* infection, thus indicating the importance of Tpl2 in innate immune cells.

Given the presence of infection, we evaluated WBC concentrations in the circulation to evaluate immune responses generated to infection. As expected, *Rag1*$^{-/-}$ and *Rag1*$^{-/-}$*Tpl2*$^{-/-}$ mice had fewer circulating lymphocytes, compared with C57BL/6 and *Tpl2*$^{-/-}$ mice[22,32] (Figure 2). In addition, C57BL/6, *Rag1*$^{-/-}$, *Tpl2*$^{-/-}$, and *Rag1*$^{-/-}$*Tpl2*$^{-/-}$ mice all had similar concentrations of circulating neutrophils (Figure 2). Both *Rag1*$^{-/-}$ and *Rag1*$^{-/-}$*Tpl2*$^{-/-}$ mice had fewer circulating monocytes than C57BL/6 and *Tpl2*$^{-/-}$ mice, but *Rag1*$^{-/-}$*Tpl2*$^{-/-}$ mice had more monocytes than *Rag1*$^{-/-}$ mice (Figure 2).

Discussion

Tumor progression locus 2 (Tpl2, also known as MAP3K8) is a serine–threonine protein kinase that is expressed in both innate and adaptive immune cells. Due to their reduced production of TNF, *Tpl2*$^{-/-}$ mice were originally described as resistant to endotoxin-induced septic shock.[8] Because Tpl2 promotes TNF processing and secretion,[8,28] it is being investigated as a therapeutic target for treating autoimmune diseases, especially those exacerbated by TNF, such as rheumatoid arthritis.[9,11,12] The roles for Tpl2 in immune responses during autoimmune and infectious diseases are currently being investigated. *Tpl2*$^{-/-}$ mice are more susceptible to infection with *Toxoplasma gondii*,[37] *Listeria monocytogenes*,[19,21] *Mycobacterium tuberculosis*,[19] and influenza,[17] compared with wild-type mice. Similarly, *Rag1*$^{-/-}$*Tpl2*$^{-/-}$ mice develop larger bacterial burdens in response to *M. tuberculosis* and *L. monocytogenes* infections than do *Rag1*$^{-/-}$ mice,[19] indicating the important role of Tpl2 in regulating innate immune responses to infection.

In this case study, we describe spontaneous infection of *Rag1*$^{-/-}$*Tpl2*$^{-/-}$ mice with *S. xylosus*, an environmental contaminant and common commensal bacterium of barrier surfaces of mammals, including laboratory mice.[23] This organism has been detected on the skin of C57BL/6 mice housed under SPF conditions.[35] All of the mice in this study were culture-positive for *S. xylosus* on the skin, and all *Rag1*$^{-/-}$*Tpl2*$^{-/-}$ mice, one *Rag1*$^{-/-}$ mouse, and 2 *Tpl2*$^{-/-}$ mice also cultured positive for *S. xylosus* in their feces. Clinical disease was never noted in *Tpl2*$^{-/-}$ or *Rag1*$^{-/-}$ mice, despite skin and fecal cultures that were positive for *S. xylosus*. In addition, dermatitis, morbidity, and mortality associated with *S. xylosus* infection were noted only in male mice, even though both male and female *Rag1*$^{-/-}$*Tpl2*$^{-/-}$ mice were culture-positive for *S. xylosus*. Spontaneous *S. xylosus* infection in athymic nude mice reportedly occurs with increased frequency in male mice,[4] although a second study noted similar frequencies in male and female mice.[29] In addition, male and female mice deficient in NADPH oxidase showed similar susceptibility to opportunistic infections by *S. xylosus*.[21] The apparent restriction of dermatitis, morbidity, and mortality due to *S. xylosus* to male *Rag1*$^{-/-}$*Tpl2*$^{-/-}$ mice in the current study may reflect variability due to the small number of severe dermatitis cases or may correlate with the increased intracage aggression generally observed with male mice.[20,27,36] The resultant bite wounds or skin abrasions may facilitate bacterial entry into the skin and the establishment of infection.[4]

Although *S. xylosus* is generally considered a commensal bacterium, the organism has caused opportunistic infections in immunocompromised animals. Spontaneous *S. xylosus* infection has previously been reported in athymic nude mice,[4,29] indicating that T cells contribute to protection against spontaneous *S. xylosus* infection. Like athymic nude mice, *Rag1*$^{-/-}$ mice lack mature T cells,[6,22,32] but they failed to develop clinical symptoms in the current study. The apparent difference in susceptibility to infection

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between these 2 strains is unclear. Because athymic nude mice lack a protective layer of hair, they may be more susceptible to dermal injuries that predispose to bacterial infections of the skin. *S. xylosus* was not detected in internal organs but was localized to the skin in nude mice.[4] Dissemination into internal organs has only been reported in mice deficient in NADPH oxidase and superoxide production.[10,14,26] Tpl2 is required for superoxide production in macrophages[16] and peritoneal exudate cells,[30] possibly through defects in formation of the NADPH oxidase complex.[16] The combined defects in mature T cells and superoxide production in *Rag1*$^{-/-}$*Tpl2*$^{-/-}$ mice is consistent with their increased susceptibility to *S. xylosus* infection.

The reduced numbers of circulating monocytes in *Rag1*$^{-/-}$ and *Rag1*$^{-/-}$*Tpl2*$^{-/-}$ mice compared with C57BL/6 and *Tpl2*$^{-/-}$ mice indicate that defects in lymphocyte production affect peripheral monocyte concentrations. Interestingly, *Rag1*$^{-/-}$*Tpl2*$^{-/-}$ mice had more circulating monocytes compared with *Rag1*$^{-/-}$ mice. The increased monocyte levels in *Rag1*$^{-/-}$*Tpl2*$^{-/-}$ mice might indicate clinical infection with *S. xylosus*, given that a similar increase in monocytes occurs with *S. aureus* infection.[2] However, experimental colonization of germ-free C57BL/6 mice with *S. xylosus*, among other commensals, did not influence relative numbers of blood myeloid cells.[3] Perhaps clinical *S. xylosus* infection induces more prominent perturbations in leukocyte populations in immunocompromised hosts such as *Rag1*$^{-/-}$*Tpl2*$^{-/-}$ mice than are induced by the same microorganism in immunocompetent hosts, where it exists as a nonpathogenic commensal.

In conclusion, we describe spontaneous *S. xylosus* infection in a genetically modified murine model. *S. xylosus* infection in *Rag1*$^{-/-}$*Tpl2*$^{-/-}$ mice correlated with disseminated bacteria and elevated numbers of circulating monocytes compared with those in *Rag1*$^{-/-}$ mice. Overall, these data may represent an important consideration for future research using *Rag*$^{-/-}$*Tpl2*$^{-/-}$ mice, in which underlying *S. xylosus* infection might alter immune status and obscure experimental interpretations.

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