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# Role of Major Histocompatibility Complex Class II in Resistance of Mice to Naturally Acquired Infection with *Syphacia obvelata*

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**Genetics plays a substantial role in host resistance in many host-parasite interactions. We examined the prevalence of naturally acquired infection with *Syphacia obvelata* in a number of mouse strains housed in a non-barrier facility. These mice, which included cross-bred and congenic, inbred strains on various genetic backgrounds, differ in the loci for the immune function genes—major histocompatibility complex class II (MHCII), toll-like receptor 4 (*Tlr4*), and solute carrier family 11, member 1 (*Slc11a1*)—which allowed comparisons of the impact of these genes on resistance to pinworm infection. Male and female mice of various ages were sampled over an 18-month period; infection was determined by use of the cellophane tape test. Results indicated that mice that were MHCII$^{+/+}$ had a significantly lower prevalence of infection than did mice that were MHCII$^{-/-}$. Differences were not seen between male and female mice. Although MHCII$^{+/+}$ mice had an age-associated decrease in infection prevalence, such decrease was not seen in MHCII$^{-/-}$ mice. In contrast, infection prevalence in mice with the normal *Tlr4* gene (*Tlr4*$^{LPS\text{-}n/LPS\text{-}n}$) gene did not differ significantly compared with that in mice that were homozygous for either the point mutation (*Tlr4*$^{LPS\text{-}d/LPS\text{-}d}$) or deletion (*Tlr4*$^{LPS\text{-}del/LPS\text{-}del}$) of that gene. Likewise, the presence (*Slc11a1*$^{r/r}$) or absence (*Slc11a1*$^{s/s}$) of functional alleles for *Slc11a1* had no effect on the prevalence of infection with *S. obvelata*. In conclusion, presence of MHCII, but not *Tlr4* or *Slc11a1* significantly influences prevalence of naturally acquired infection with *S. obvelata*. These data justify further comprehensive analyses of the immune components that are involved in pinworm resistance.**

Pinworm (*Syphacia obvelata* and *Aspiculuris tetraptera*) infection in laboratory mice is common and unwelcome. Although these oxyurids are generally thought to be nonpathogenic (1-3), infection with them has been associated with rectal prolapse, mucoid enteritis, and intestinal impaction/intussusception (2, 4-9). Pinworm infection alters the humoral immune response to nonparasitic antigenic stimuli (10) and the susceptibility of mice to infection with other intestinal nematodes (11). Infection with *S. obvelata*, but not *A. tetraptera* has been documented to decrease exploratory activity in C57BL/6NHsd mice (12).

There were early suggestions of a genetic basis for resistance to infection by pinworms. Wild mice were more susceptible to experimentally induced infection than were laboratory mice (13). Certain inbred mouse strains (DBA/2J, DBA/2An, AKR/LwNicr, and C3H/Cum) had higher prevalence of infection than did other strains (14-16). In experimental infections with *A. tetraptera*, the F1 cross of a susceptible strain (129/SV) with a resistant strain (C57BL/6) had an infection rate and parasite load significantly different from that of the susceptible parental strain, but not from the resistant parental strain (17). Athymic mice had higher incidence of infection, suggesting that T cells played a role in resistance (9).

Genetics has been documented to influence mouse susceptibility to other parasitic infections. Resistance to *Trichuris muris* infection has been measured in various inbred strains of mice (18). The F1 crosses between low- and high-resistance strains of mice had high resistance, indicating that resistance was inherited as a dominant trait. Strains with high resistance (faster worm expulsion) responded to a lower threshold of infection than did strains with low resistance, suggesting the possibility of genetically determined control of the level at which antigen recognition becomes effective (18). Studies in which mice were experimentally infected with *Nematospiroides dubius*, an intestinal nematode, supported the hypothesis that major histocompatibility complex (MHC) and non-MHC genes impact resistance to challenge infections with this parasite (19). Compared with H-2$^s$, H-2$^a$, and H-2$^d$ mice, H-2$^b$and H-2$^k$ mice were more susceptible to infection after multiple challenges.

The molecular characterization of MHC class II (MHCII) in recent years has revealed the elegant way these molecules control responses to a number of antigens (20-22). Many of those studies were facilitated by the creation of MHCII knockout mice in 1991 (23, 24). Curiously, recent studies have not involved investigation of the ability of MHCII knockout mice to resist pinworm infection despite earlier suggestions that the MHCII may be important to resistance. Other genes are known to control immune responsiveness in mice. Among these are *Tlr4* and *Slc11a1* (formerly known as *Nramp1*), which control early host responses to infective agents by controlling responsiveness to gram- negative bacterial lipopolysaccharide, signal transduction, iron transport, and oxidative bursts (25-29). In the study reported here, we tested the hypothesis that mice which lack MHCII, *Tlr4*, and *Slc11a1* genes would be more susceptible to

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pinworm infection. To test this hypothesis, we compared the incidence of naturally acquired infection with *S. obvelata* in mice of various genetic backgrounds.

## Materials and Methods

**Mice and housing.** Mice were bred and housed in the vivarium of the Division of Biology at Kansas State University in accordance with the *Guide for the Care and Use of Laboratory Animals*. The Institutional Animal Care and Use Committee has approved protocols for the maintenance of the breeding colony and for the study reported here.

Mice were housed in two rooms with different types of caging. The immunocompetent strains (C57BL/6J, C57BL/10ScN, C3HeB/FeJ, and C3H/HeJ) plus two immunocompromised strains (FeJxC2D[S] and FeJxC2D[R]) were housed in one room, in polycarbonate cages with bonnet tops on shelves in non HEPA-filtered ventilated racks. The other immunocompromised strains (C2D, B10xC2D, HeJxC2D[(S] and HeJxC2D[R]) were housed in a different room in polycarbonate isolator cages in HEPA-filtered ventilated racks. Multiple strains of mice were housed in each rack. Only the immunocompromised strains received autoclaved water, cages, and bedding (Aspen Bed I, American Excelsior Co., Arlington, Tex.). All cage-changing procedures were done in the open without the use of changing stations. The mice were maintained on a 12:12-h light:dark cycle. A balanced rodent ration, ARF 3, produced by the Department of Grain Science at Kansas State University, was fed ad libitum. Recombinant MHCII$^{-/-}$ breeder mice were treated with Sulfatrim pediatric suspension (Alpharma USPD Inc., Baltimore, Md.) at a dosage of one milliliter (40 mg of sulfamethoxazole and eight milligrams of trimethoprim)/100 ml of $H_2O$ for one week each month to inhibit opportunistic bacterial infections. Weaned mice did not receive antibiotics.

**Determination of infection.** The cellophane tape test was used to determine infection with *S. obvelata*. This technique has a sensitivity of 67 to 88%, compared with 81% for examination of cecal contents (30, 31), and is routinely used to screen for the presence of *Syphacia* infection. Samples were collected from males and females of each strain, on the basis of the following age groups: less than two months, two to four months, and greater than four months of age. Actual age range of the mice was four weeks to nine months. Cecal and colonic contents were collected from some mice and examined microscopically to determine the species of *Syphacia* present. Concurrent infection with *A. tetraptera* was noted in approximately a third of the MHCII$^{-/-}$ mice, as determined by fecal flotation combined with the cellophane tape test.

Sentinel mice in these rooms were negative for mouse hepatitis virus, Sendai virus, pneumonia virus of mice, reovirus 3, Theiler's mouse encephalitis virus strain GDVII, ectromelia virus, *Mycoplasma pulmonis*, parvovirus, epizootic diarrhea of infant mice virus, and lymphocytic choriomeningitis virus.

**Mouse strains.** The following mouse strains were tested for the presence of *S. obvelata* infection: C57BL/6J, C2D, C57BL/10ScN, C3Heb/FeJ, CeH/HeJ, C57BL/10ScNxC2D, C3Heb/FeJxC2D(R), C3Heb/FeJxC2D(S), C3H/HeJxC2D(R), and C3H/HeJxC2D(S). Their corresponding genotypes for MHCII, *Tlr4*, and *Slc11a1* are summarized in Table 1. The C57BL/6J mice were originally obtained from the Jackson Laboratory (Bar Harbor, Maine). The C2D (B6.129-*Abb*$^{tm1}$ N5F20, MHCII$^{-/-}$, *Tlr4*$^{LPS\text{-}n/LPS\text{-}n}$, *Slc11a1*$^{s/s}$) mice used in this study have been brother-sister mated for over 20 generations during the past nine years at Kansas State University. The C2D mice lack functional MHCII genes due to a natural deletion of the $IE_\alpha$ gene and the targeted deletion of the $IA_\beta$ gene (23, 24). This strain has been backcrossed five times to the B6 background (32) and is considered an incipient congenic (32) to the B6 strain. The C57BL/6J (MHCII$^{+/+}$, *Tlr4*$^{LPS\text{-}n/LPS\text{-}n}$, B6) mouse strain is routinely used for comparisons with the C2D strain to determine the impact of MHCII genes (23, 33). The C57BL10/ScN (B10) mice, which were obtained from the animal resource facility at the NIH, have been bred in the vivarium at Kansas State University since 1995, and have served as founders for the B10xC2D (Stock-*Abb*$^{tm1}$, MHCII$^{-/-}$, *Tlr4*$^{LPS\text{-}del/LPS\text{-}del}$, *Slc11a1*$^{s/s}$) recombinant stock along with C2D mice. The B10xC2D mice were created at Kansas State University, and their characterization has been described (34, 35).

**Table 1.** Mouse strains used in the study

| Mouse | Abbreviation | Genotype* |
|---|---|---|
| C57BL/6J | B6 | MHCII$^{+/+}$, *Tlr4*$^{LPS\text{-}n/LPS\text{-}n}$, *Slc11a1*$^{s/s}$ |
| C3HeB/FeJ | FeJ | MHCII$^{+/+}$, *Tlr4*$^{LPS\text{-}n/LPS\text{-}n}$, *Slc11a1*$^{r/r}$ |
| C57BL/10ScN | B10 | MHCII$^{+/+}$, *Tlr4*$^{LPS\text{-}del/LPS\text{-}del}$, *Slc11a1*$^{s/s}$ |
| C3H/HeJ | HeJ | MHCII$^{+/+}$, *Tlr4*$^{LPS\text{-}d/LPS\text{-}d}$, *Slc11a1*$^{r/r}$ |
| C2D | C2D | MHCII$^{-/-}$, *Tlr4*$^{LPS\text{-}n/LPS\text{-}n}$, *Slc11a1*$^{s/s}$ |
| C3HeB/FeJxC2D(S) | FeJxC2D(S) | MHCII$^{-/-}$, *Tlr4*$^{LPS\text{-}n/LPS\text{-}n}$, *Slc11a1*$^{s/s}$ |
| C3HeB/FeJxC2D(R) | FeJxC2D(R) | MHCII$^{-/-}$, *Tlr4*$^{LPS\text{-}n/LPS\text{-}n}$, *Slc11a1*$^{r/r}$ |
| C3H/HeJxC2D(S) | HeJxC2D(S) | MHCII$^{-/-}$, *Tlr4*$^{LPS\text{-}d/LPS\text{-}d}$, *Slc11a1*$^{s/s}$ |
| C3H/HeJxC2D(R) | HeJxC2D(R) | MHCII$^{-/-}$, *Tlr4*$^{LPS\text{-}d/LPS\text{-}d}$, *Slc11a1*$^{r/r}$ |
| C57BL/10ScNxC2D | B10xC2D | MHCII$^{-/-}$, *Tlr4*$^{LPS\text{-}del/LPS\text{-}del}$, *Slc11a1*$^{s/s}$ |

*MHCII$^{+/+}$ has functional MHCII; MHCII$^{-/-}$ lacks a functional MHCII; *Tlr4*$^{LPS\text{-}n/LPS\text{-}n}$ has the normal *Tlr4* gene; *Tlr4*$^{LPS\text{-}d/LPS\text{-}d}$ has a point mutation, making the *Tlr4* gene nonfunctional; *Tlr4*$^{LPS\text{-}del/LPS\text{-}del}$ has a deletion of part of the *Tlr4* gene, making it nonfunctional; *Slc11a1*$^{r/r}$ has the normal, functional gene; and *Slc11a1*$^{s/s}$ has a mutant, nonfunctional gene.
MHC = major histocompatibility complex.

Two *Tlr4* gene congenic mouse sets were used to assess the role of *Tlr4* on *S. obvelata* infection. The C3HeB/FeJ (MHCII$^{+/+}$, *Tlr4*$^{LPS\text{-}n/LPS\text{-}n}$, FeJ) mice were directly compared with the C3H/HeJ (MHCII$^{+/+}$, *Tlr4*$^{LPS\text{-}d/LPS\text{-}d}$, HeJ) mice. In addition, C57BL/10ScN (MHCII$^{+/+}$, *Tlr4*$^{LPS\text{-}del/LPS\text{-}del}$, B10) mice were compared with B6 mice (MHCII$^{+/+}$, *Tlr4*$^{LPS\text{-}n/LPS\text{-}n}$). The FeJ mice were embryo-derived from HeJ mice, and have the same genetic background (36). The subsequent spontaneous mutation of the *Tlr4* gene (37, 38) at the Jackson Laboratories between 1960 and1965 allows congenic comparisons between mice that express functional *Tlr4* genes (FeJ mice) and mice that do not carry functional *Tlr4* gene alleles (HeJ mice). The B6 and B10 mice differ only at the *H9*, *Igh2*, and *Lv* loci (36). In addition, the C57BL/10ScN mice carry the additional deletion of the *Tlr4* gene, but do not carry the IL-12 defect recently reported in C57BL/10 ScCr mice (39). Since *Tlr4* functions normally in B6 mice, comparison between B6 and B10 mice allowed comparisons of *S. obvelata* infection in mice with essentially the same "black" background.

The hybrid mouse strains FeJxC2D (MHCII$^{-/-}$, *Tlr4*$^{LPS\text{-}n/LPS\text{-}n}$) and HeJxC2D (MHCII$^{-/-}$, *Tlr4*$^{LPS\text{-}d/LPS\text{-}d}$) (34, 35) also carry mutations for MHCII genes; additionally, the HeJxC2D strain is a mutant for the *Tlr4* gene. These mice were used as comparative controls for HeJ and FeJ mice.

The FeJxC2D(R) (MHCII$^{-/-}$, *Tlr4*$^{LPS\text{-}n/LPS\text{-}n}$, *Slc11a1*$^{r/r}$), FeJxC2D(S) (MHCII$^{-/-}$, *Tlr4*$^{LPS\text{-}n/LPS\text{-}n}$, *Slc11a1*$^{s/s}$), HeJxC2D(R) (MHCII$^{-/-}$, *Tlr4*$^{LPS\text{-}d/LPS\text{-}d}$, *Slc11a1*$^{r/r}$), and HeJxC2D(S) (MHCII$^{-/-}$, *Tlr4*$^{LPS\text{-}d/LPS\text{-}d}$, *Slc11a1*$^{s/s}$) mice were selected for expression of functional and nonfunctional alleles of *Slc11a1* genes, commonly

**Table 2.** Effects of MHCII and *Tlr4* genes on *Syphacia obvelata* infection in H-$2^b$ mice

| Mouse strain | Genotype | No. of mice infected/total mice screened | % of population infected |
|---|---|---|---|
| C57BL/6J | MHCII$^{+/+}$ *Tlr4*$^{LPS\text{-}n/LPS\text{-}n}$ | 1/86[1] | 1.2 |
| C57BL/10ScN | MHCII$^{+/+}$, *Tlr4*$^{LPS\text{-}del/LPS\text{-}del}$ | 6/33[2] | 18.2 |
| C2D | MHCII$^{-/-}$, *Tlr4*$^{LPS\text{-}n/LPS\text{-}n}$ | 71/105[3] | 67.6 |
| B10xC2D | MHCII$^{-/-}$, *Tlr4*$^{LPS\text{-}del/LPS\text{-}del}$ | 18/32[3] | 56.3 |

*Samples were collected using cellophane tape, transferred to glass slides, and screened for the presence of eggs, using light microscopy. Numbers represent number of mice scored positive/total number of mice screened. Differences in superscript numbers indicate a significant ($P < 0.01$) difference between mouse strains, as determined by use of a Fisher's exact test.
*See* Table 1 for key.

referred to as *Nramp1* (34, 35). These mice were included in our final comprehensive screening to determine the impact of MHCII, *Tlr4*, and *Slc11a1* genes on *S. obvelata* infection.

## Results

We investigated the impact of MHCII and *Tlr4* deletion on pinworm infection in mice on the C57BL background (H-$2^b$) by testing strains which carried various combinations of functional alleles of these two genes (Tables 1 and 2). To determine the impact of MHCII, C57BL/6J mice were compared with C2D mice and C57BL/10ScN mice were compared with B10xC2D mice. To determine the impact of *Tlr4*, C57BL/6J mice were compared with C57BL/10ScN mice and C2D mice were compared with B10xC2D mice. Mice lacking a functional MHCII had a significantly ($P < 0.01$) higher prevalence of pinworm infection than did mice with a functional MHCII (Table 2). An effect of the *Tlr4* gene was not seen when C2D mice were compared with B10xC2D mice. However, there was a significant ($P < 0.01$) difference in infection prevalence in the B6 versus B10 mice (1.2% versus 18.2%), both of which are MHCII$^{+/+}$, but differ at the *Tlr4* locus.

To ascertain whether strain background had an impact on the susceptibility of mice to infection in the absence of MHCII, we screened mice which carried all or part of the C3H mouse background (H-$2^k$ and H-$2^{k/b}$). Use of recombinant, heterozygous mice was necessary because MHCII knockout mice do not exist on the C3H mouse background (Tables 1 and 3). Again, when functional MHCII genes were absent, there was a significantly higher prevalence of *Syphacia* infection regardless of the presence or absence of a functional *Tlr4* gene (Table 3). The C3Heb/FeJ and C3H/HeJ mice, which are congenic for *Tlr4* and carry inbred C3H backgrounds, had similar infection rates, indicating that *Tlr4* did not influence susceptibility to infection (23.9% versus 22.9%). Likewise, comparison of FeJxC2D and HeJxC2D, which also differ at *Tlr4*, did not reveal a significant effect of this gene (68.9% versus 73.0%).

Our analyses of mice carrying C57BL and C3H backgrounds indicated that MHCII was important to *Syphacia* resistance. However, the impact of *Tlr4* expression was not as obvious. The mouse colony at our institution had a substantial number of mice on various backgrounds carrying different combinations of functional genes for MHCII and *Tlr4* (Table 1). In addition, the mice carried different combinations of functional alleles for *Slc11a1*, another gene involved in innate host resistance that controls macrophage responses and iron transport (27-29) (Table 1). Therefore, to determine the impact of these three gene mutations on *Syphacia* infection, mice were analyzed according

**Table 3.** Effects of MHCII and *Tlr4* genes on *S. obvelata* infection H-$2^k$ and H-$2^{k/b}$ mice

| Mouse strain | Genotype | No. of mice infected/total mice screened | % of population infected |
|---|---|---|---|
| C3Heb/FeJ | MHCII$^{+/+}$, *Tlr4*$^{LPS\text{-}n/LPS\text{-}n}$ | 11/46[1] | 23.9 |
| C3H/HeJ | MHCII$^{+/+}$, *Tlr4*$^{LPS\text{-}d/LPS\text{-}d}$ | 11/48[1] | 22.9 |
| FeJxC2D | MHCII$^{-/-}$, *Tlr4*$^{LPS\text{-}n/LPS\text{-}n}$ | 42/61[2] | 68.9 |
| HeJxC2D | MHCII$^{-/-}$, *Tlr4*$^{LPS\text{-}d/LPS\text{-}d}$ | 46/63[2] | 73.0 |

*See* Tables 1 and 2 for key.

to their individual MHCII, *Tlr4*, or *Slc11a1* genotypes independently of other gene expression (Table 4). When the mice were segregated by sex, there were no differences between males and females in susceptibility to infection with *S. obvelata*. Therefore, data were compiled without regard to sex. Neither *Tlr4* nor *Slc11a1* (*Nramp1*) had a significant impact on *S. obvelata* infection ($P > 0.1$). In contrast, there was a highly significant ($P < 0.001$) difference between mice carrying functional MHCII genes and mice that had deleted MHCII genes. When the mice were segregated by age into three groups, less than two months old, two to four months old, and greater than four months old, an age-related decrease in infection prevalence was noted in the MHCII $^{+/+}$ mice (21% versus 11% versus 4%, respectively). Infection prevalence in the MHCII$^{-/-}$ mice remained high in all ages of mice tested (62% versus 70% versus 69%, respectively) (Table 5).

Immunocompromised breeders (MHCII$^{-/-}$) that received Sulfatrim pediatric suspension did not have significantly different infection prevalence than did non-treated immunocompromised mice (61% versus 67%). This antibiotic was chosen because it is considered safe even in species, such as the hamster and guinea pig, that are known to be susceptible to antibiotic-induced enterotoxemia (40).

## Discussion

We examined several hundred mice maintained in a non-barrier mouse colony for the presence of pinworms (*Syphacia* sp.). Microscopic analysis of intestinal contents from mice which initially screened positive by use of cellophane tape testing confirmed that the pinworm detected was *S. obvelata*. Although the mice were housed in two rooms with different caging systems, we believe that the difference in infection prevalence was not due to environmental factors, exposure levels, or animal location in the mouse facility. The majority of mice with the highest prevalence of *Syphacia* infection were housed under more stringent holding conditions (isolators in HEPA-filtered ventilated racks) than were mice that had minimal infections. Moreover, some of the MHCII$^{-/-}$ mice (FeJxC2D) with the highest infection rates were housed adjacent to mice with minimal to no *Syphacia* infection.

The high prevalence of pinworm infection in MHCII$^{-/-}$ mice confirms earlier suggestions that the MHC regulates host resistance to *Syphacia* sp. (19). Recent studies with *T. spiralis* found that MHCII knockout mice are less able to expel that intestinal parasite; the increased susceptibility of these MHCII$^{-/-}$ mice has been associated with the absence of T cells and decreased intestinal muscle contractions (33). Because MHCII$^{-/-}$ mice lack CD4$^+$ T cells (24), and because athymic, T cell-deficient mice had a higher incidence of infection (9), it appears that T cells may be a necessary component for efficient elimination of *Syphacia* sp. The decreased prevalence with age of *Syphacia* infection in MHCII$^{+/+}$ mice also suggests that, as these mice progress to-

**Table 4.** Influence of MHCII, *Slc11a1*, and *Tlr4* genes on *S. obvelata* infection in mice

| MHCII | | | *Slc11a1* (*Nramp1*)* | | | *Tlr4*† | | |
|---|---|---|---|---|---|---|---|---|
| +/+ | -/- | Stat. | r/r | s/s | Stat. | n/n | d/d | Stat. |
| 29/213 (13.6) | 177/261(67.8) | $P < 0.001$ | 60/150(40.0) | 146/324(45.1) | $P > 0.1$ | 125/298(41.9) | 81/176(46.0) | $P > 0.1$ |

*_Slc11a1_: r/r has the normal, functional gene, and s/s has a mutant, nonfunctional gene.
†_Tlr4_: n/n has the normal _Tlr4_ gene and d/d combines $Tlr4^{LPS\text{-}d/LPS\text{-}d}$ and $Tlr4^{LPS\text{-}del/LPS\text{-}del}$, both of which have nonfunctional _Tlr4_ genes.
Stat. = Statistical significance.

**Table 5.** Effect of age at time of screening on prevalence of infection with *S. obvelata*

| Variable | Age | | |
|---|---|---|---|
| | < 2 months | 2-4 months | > 4 months |
| $MHCII^{+/+}$ mice positive for *Syphacia* | 21% | 11% | 4% |
| Total $MHCII^{+/+}$ mice screened | 54% | 30% | 16% |
| $MHCII^{-/-}$ mice positive for *Syphacia* | 62% | 70% | 69% |
| Total $MHCII^{-/-}$ mice screened | 39% | 42% | 19% |

ward full T-cell immunocompetency (41), they become more resistant. However, because we did not do an experimental infection with mice specifically lacking $CD4^+$ T cells, additional experiments will be necessary to document this conclusively.

We also screened for the prevalence of pinworms in mice lacking functional *Tlr4* genes. There was a significant ($P < 0.01$) difference between wild-type B6 ($Tlr4^{LPS\text{-}n/LPS\text{-}n}$) mice and B10 ($Tlr4^{LPS\text{-}del/LPS\text{-}del}$) mice. However, the prevalence of infection in the $MHCII^{+/+}$, $Tlr4^{LPS\text{-}del/LPS\text{-}del}$ mice was still relatively low, compared with that in C2D ($MHCII^{-/-}$, $Tlr4^{LPS\text{-}n/LPS\text{-}n)}$ mice (18.2% versus 67.6%) and B10xC2D ($MHCII^{-/-}$, $Tlr4^{LPS\text{-}del/LPS\text{-}del}$) mice (18.2% versus 56.3%). Lack of an increased prevalence in $Tlr4^{LPS\text{-}d/LPS\text{-}d}$ congenic mice on the C3H background ($H\text{-}2^k$, Table 3) or in a larger group that contained mice with heterogenous backgrounds (Table 4), compared with normal mice, suggests that if *Tlr4* plays a role in pinworm resistance, it may be a minor role, and/or one that is only apparent in mice carrying the $H\text{-}2^b$ haplotype, C57BL mouse background. Enriquez and co-workers (19) did not see a difference in the clearance of *Syphacia* sp. between HeJ ($H\text{-}2^k$, $Tlr4^{LPS\text{-}d/LPS\text{-}d}$) mice and AKR/J mice ($H\text{-}2^k$, $Tlr4^{LPS\text{-}n/LPS\text{-}n}$). Our results confirm their observation. Interestingly, several groups (17, 19, 42) did not see a difference in pinworm (*A. tetraptera* or *S. obvelata*) clearance between $H\text{-}2^b$ and $H\text{-}2^k$ mice carrying normal MHCII and *Tlr4* alleles. However, Derothe and co-workers (13) observed higher *A. tetraptera* egg loads in C3H/OuJIco mice ($H\text{-}2^k$), compared with C57BL/6JIco ($H\text{-}2^b$) or C57BL/10/Ola/Hsd ($H\text{-}2^b$) mice. Therefore, additional controlled *S. obvelata* infection experiments will need to be done in congenic mice to determine whether *Tlr4* has a minor role in pinworm resistance and whether MHCII haplotype makes a difference.

The assessment of mice on the C57BL background strongly implicated an important role for the MHCII in murine resistance to *S. obvelata* when comparing congenic strains B6 versus C2D. The MHCII knockout mice do not exist on the C3H background for the $H\text{-}2^k$ haplotype. However, our group has created heterozygote, MHCII knockout mice that are $H\text{-}2^{b/k}$ at MHCI (34, 35). Although the analysis cannot be done using congenics, our data suggest that MHCII genes play a role in resistance even in mice that carry C3H mouse alleles (Table 2). In fact, when we did a large, comprehensive screening and grouped mice according to their individual MHCII, *Tlr4*, and *Slc11a1* (formerly referred to as *Nramp1*) genotypes independently of other gene expression (Table 4), there was a clear association between the presence of *S. obvelata* and the absence of MHCII. Moreover, there was no impact of either *Tlr4* or *Slc11a1* after doing similar analyses. It should be noted that analysis of *Slc11a1* was not done using congenic mice, and the results for this gene should not be considered conclusive until congenic studies are done.

In contrast to earlier suggestions that males were more susceptible than females to pinworm *(A. tetraptera)* infection (16, 43), we did not find that sex played a role in susceptibility to *Syphacia* infection. These results are consistent with the findings of Derothe and co-workers (13) that genetics was more important than sex in determining susceptibility. Also, on the basis of these studies, the mouse background on which the MHCII deletion occurs probably does not have an impact on host resistance to pinworms, although we cannot rule out that there are subtle MHC haplotype differences that will only be discernable in experimental challenge studies using inbred mice.

In conclusion, we have completed a large screening for the prevalence of pinworms in a non-barrier mouse colony containing several mouse strains of various genetic backgrounds. The data implicate the MHCII as an important component in host resistance. There was higher pinworm prevalence in $MHCII^{-/-}$ mice, regardless of the background with which the MHCII deletion was associated. Given that the MHCII plays a role in host resistance to other parasites (44-48), this is not unexpected. On the basis of these results, future studies using mice with homogeneous B6 and C3H backgrounds are justified to more comprehensively analyze the immune components involved in pinworm resistance.

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