Major Histocompatibility Complex-Dependent Susceptibility to Cryptococcus neoformans in Mice

Erin E. McClelland,¹* Donald L. Granger,² and Wayne K. Potts¹

Biology Department, University of Utah, Salt Lake City, Utah 84112,¹ and Division of Infectious Diseases, University of Utah and Veterans Affairs Medical Center, Salt Lake City, Utah 84132²

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To evaluate the role of major histocompatibility complex (MHC) genes in the resistance to Cryptococcus neoformans, we conducted infection experiments in MHC-congenic strains of mice. Significant MHC-dependent susceptibility differences were found among homozygotes and heterozygotes. This study is the first experimental demonstration of MHC-dependent susceptibility to C. neoformans infections in mice and indicates that MHC genes can be important in host resistance.

Major histocompatibility complex (MHC) genes are unique in their general importance for conferring susceptibility or resistance to infectious and autoimmune diseases (5). It is unclear what keeps these demonstrably harmful genes from being eliminated by natural selection unless they provide some advantage, presumably against some other disease. The only way to unravel these interactions is to characterize the effects of MHC genes on a variety of pathogens and autoimmune diseases. The goal of this study was to experimentally test for an MHC-dependent susceptibility pattern during chronic Cryptococcus neoformans infections.

C. neoformans is a common, opportunistic pathogen that causes disease in immunocompromised individuals. Previous studies have found that various components of immunity are important in clearing a C. neoformans infection. These components include interleukin-12 (11), interleukin-18 (17), inducible nitric oxide synthase (2), gamma interferon (16), B cells (3), and T cells (7). Previous attempts to determine if MHC genes can influence C. neoformans infections in mice (22) and humans (20) have had conflicting results.

MHC-congenic mice (C57BL/10SnJ-H-², B10.D2-H-², B10.M-H-², B10.BR-H-², B10.Q-H-²) and BALB/c mice were obtained from Jackson Laboratories and bred thereafter under specific-pathogen-free conditions. The inclusion of BALB/c mice allowed a comparison between strains that have identical MHC genes but differ for other genes that may affect resistance to C. neoformans. All animals used for the infection experiments were either F₂ segregants or first-generation progeny of F₁ segregants. MHC F₂ segregants were created by intercrossing the F₁ heterozygotes (b/q, d/q, d/k, f/k) in order to randomize any genetic mutations that might have become differentially fixed in the backgrounds of these strains of mice. The differential accumulation of mutations in congeneric strains separated by a substantial period of time (for example, over 30 years for these MHC B10 congenics) is a serious problem that can lead to erroneous conclusions (reference 8 and references therein). Generating F₂ segregants randomizes any background mutations and therefore solves this problem in a much simpler way than rederiving the lines (8, 13, 25). Mice were MHC genotyped by PCR by means of two different microsatellite loci within the MHC region (a tetranucleotide repeat [23] and d17Mit34 [6]).

Infection experiments were conducted five times, with five of the seven genotypes being tested three or more times. (BALB/c and d/k mice were tested twice.) Mice were infected via intraperitoneal injection with 2 × 10⁷ CFU of the wild-type H99 strain of C. neoformans (21) per ml. An error caused inocula for experiments 3 and 4 to be 4 × 10⁶ and 3 × 10⁶ CFU/ml, respectively. These dosage differences did not result in any significant load differences for female mice and were not correlated with final loads in males. Initially, the spleen, liver, and brain were collected to determine which organ had the most consistent loads. Thus, the liver was chosen as the optimal organ for collection because it is a site of primary infection. Mice were sacrificed 39 days postinfection. This end date was chosen because the chronic infection needed to proceed long enough for the MHC genes to have an effect but not long enough to risk death of the mice. At the time of sacrifice, there were no obvious signs of clinical disease, as measured by significant weight loss and neurological symptoms, though some of the mice had ruffled fur and many had lost 5% of their body weight. C. neoformans loads were determined from platings of homogenized livers. Briefly, the livers were collected under antiseptic conditions and then homogenized in 10 ml of phosphate-buffered saline. Ten microliters of the homogenate was diluted in 90 µl of phosphate-buffered saline in serial dilutions.
The loads from female mice across the five infection experiments were similar, so the data were pooled. Figure 1 depicts the C. neoformans loads for five different strains of mice. The MHC-congenic strains with haplotypes b/b, d/d, and q/q and BALB/c mice were relatively resistant, while k/k mice were relatively susceptible (P < 0.028). Though male mice were also infected, the loads were significantly different between experiments (analysis of variance, P = 0.001) and relative susceptibilities between genotypes were sometimes reversed, making interpretation difficult (data not shown).

To evaluate resistance patterns in heterozygotes, four MHC heterozygotes (b/d, b/q, d/k, and d/q) were also infected. Unfortunately, three heterozygote combinations (b/d, b/q, and d/q) were uninformative with respect to dominant or recessive patterns because the loads among the b/b, d/d, and q/q homozygotes were not significantly different (Fig. 1). In the case where the loads of the d/d and k/k homozygotes were significantly different (Fig. 1), the d/k heterozygote inherited resistance in a dominant fashion (Fig. 2a). In addition, the d/q heterozygote displayed a pattern of underdominance where the heterozygote did worse than either homozygote (heterozygote disadvantage) (Fig. 2b).

C. neoformans loads in female MHC-congenic mice showed that the k/k genotype was 4- to 10-fold more susceptible than three other MHC genotypes and the BALB/c strain (H-2d) (P < 0.028). Previous studies have disagreed on the role of MHC in C. neoformans infections. Two studies which assayed humoral responses found by Dromer et al. (12). Possible explanations for this inconsistency include the following factors: (i) survival was assayed rather than pathogen loads, (ii) a different strain of C. neoformans was used, and (iii) B10 k/k mice were not infected, and B10/k/k is the only MHC genotype that showed significant susceptibility according to our data. However, two recombinant strains [B10.A and B10.A (2R)] which express k alleles at the class I K locus and both class II loci were infected. These two strains showed no significant pattern of susceptibility. These data combined with our own suggest that the Dk allele may confer susceptibility to C. neoformans infection. This involvement of a class I gene is consistent with evidence that Th1 and cellular immunity are important in clearing C. neoformans infections (1).

Our results might seem inconsistent with the results of Droemer et al. (12), where the k haplotype was a high antibody responder. However, high antibody titers are difficult to interpret because they can have opposite implications with regard to immunocompetence: they may indicate either high responsiveness or trouble clearing the infection. A Th2 response to a C. neoformans infection often results in high antibody titers and a lack of clearance, leading to a chronic infection (18).

How resistance is inherited (dominant or recessive) appears to be haplotype dependent (Fig. 2). These different allele-specific dominant or recessive patterns are not unique as similar findings have been seen for both Streptococcus pyogenes (9) and Salmonella enterica serovar Typhimurium (19). The molecular basis of these differences is presumably due to the influence of the MHC genotype on which T-cell epitope becomes immunodominant. The relatively rare pattern of under-
dominance seen in the d/q heterozygotes might arise if the important immunodominant T cells of the homozygotes are deleted during thymic education of the heterozygote. T cells used by one MHC allele can be deleted during negative selection in the thymus when another MHC allele is present (15). Alternatively, the addition of new MHC specificities can alter which T-cell epitope becomes immunodominant (26).

MHC molecules are important in disease resistance for many infectious agents, including other fungal pathogens such as Candida albicans (4, 10). This study is the first demonstration that MHC can have a role in the clearance of C. neoformans infections and suggests that MHC-mediated immune recognition can be an important variable in susceptibility to C. neoformans infections. However, it must be noted that, as is the case with all studies using MHC-congenic strains, an observed effect may be due to other genes linked to the MHC.

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