Inheritance of Susceptibility to Induced
Escherichia coli Bladder and Kidney Infections
in Female C3H/HeJ Mice

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In the present study, the inheritance of resistance and susceptibility to bladder and kidney infections in BALB/c, C3H/HeJ, F1, and backcross mice was investigated, and the number of genes contributing to the phenotypes was estimated. Infections were induced in female mice by intravesical inoculation with Escherichia coli, and the number of bacteria in bladder and kidneys was quantified at 10 days. The (BALB/c × C3H/HeJ) F1 mice had bladder and kidney infection intensities equivalent to those observed in the resistant BALB/c parents. Twelve percent of the (F1 × C3H/HeJ) backcross mice had severe bladder infections, similar to the susceptible C3H/HeJ parents. Kidney infections ranging in intensity between those observed in BALB/c and C3H/HeJ parents were present in one-half of the backcross mice. Statistical analyses indicated that 1 gene is responsible for the increased susceptibility of C3H/HeJ mice and that the trait appears to be recessive.

Animal models of urinary tract infection (UTI) have contributed to our understanding of host-parasite interactions during the infection process [1–3] and have provided a means of evaluating new treatment options [4–6]. Mouse models of induced, unobstructed UTI have proved to be especially important in demonstrating the influence of genetic factors on host susceptibility and resistance to bladder and kidney infections [1, 7, 8]. Studies of induced Escherichia coli UTI in C3H/HeN and C3H/HeJ female mice have shown that resistance to infection is diminished in C3H/HeJ mice, which are genetically unresponsive to the biological effects of E. coli lipopolysaccharide (LPS) [8, 9]. In more recent investigations of genetic influences on resistance to UTI, we tested the ability of different inbred mouse strains to resolve an induced E. coli UTI [1]. The results showed distinct differences among strains in their susceptibility to bladder and kidney infections. Mice from the BALB/c, DBA, C3H/HeN, and C57BL/6 strains effectively cleared bladder infections within 2 weeks after induction. These same mice were generally resistant to ascending kidney infections. In contrast, C3H/HeJ and C3H/OuJ mice developed bladder infections that were initially comparable in intensity with those observed in resistant strains but were not resolved over the course of a 2-week period. These 2 susceptible mouse strains also developed kidney infections that were initially comparable in intensity with those observed in resistant strains but were not resolved over the course of a 2-week period. Because inbred strains are genetically distinct, the ability to resolve a bladder infection and to resist an ascending infection must be attributable to differences in the genetic makeup of each strain.

The results of infection studies in C3H/HeJ and C3H/OuJ mice are particularly interesting, because they provide insight into the multigenic nature of increased susceptibility to UTI. These inbred strains were origi-
nally derived from a common ancestral line but diverged when each was propagated independently by different investigators and facilities. In the course of breeding of successive inbred generations, the C3H/HeJ strain developed a spontaneous mutation in the Toll-like receptor 4 gene (Tlr4) and became unresponsive to LPS [10]. The increased susceptibility of C3H/HeJ mice to an E. coli UTI has been attributed to their inability to develop an inflammatory response initiated by the interaction of LPS with its receptors on bladder epithelial cells [11]. This model provides an explanation for the absence of significant inflammation in the infected bladders and kidneys of C3H/HeJ mice; however, it does not fully account for the observation that LPS-responsive C3H/OuJ mice also are unable to resolve E. coli UTIs [7]. Because these 2 mouse strains are equally susceptible to infection but differ in their responsiveness to LPS, it is reasonable to conclude that 1 other gene is common to both strains and directly associated with decreased resistance to E. coli UTI.

An initial step in defining the genetic basis of susceptibility to a specific disease is to determine the inheritance pattern of the genetic trait, or phenotype. In the present model, there are 2 infection phenotypes in animals inoculated with the same uropathogenic E. coli strain. One is the ability to effectively resolve a bladder infection, which is determined by quantifying bacteria in the bladder at a time point when UTI-resistant mice, such as BALB/c mice, have essentially resolved their infections. The second phenotype is susceptibility to ascending infection and is exemplified by C3H/HeJ mice, which readily develop kidney infections after a minimal-dose inoculation and do not resolve them over an extended period of time. Thus, the differential, quantitative phenotypes of BALB/c and C3H/HeJ mice can be defined by determining the number of bacteria in the bladder and kidneys at a specific time point after inoculation.

There were several objectives in the current studies: (1) to further define the quantitative UTI phenotypes of BALB/c and C3H/HeJ mice; (2) to determine the inheritance pattern of the traits by defining the phenotypes of F1 mice derived from the resistant and susceptible parental strains; and (3) to use the results to estimate the number of genes contributing to each phenotype using data from backcross mice. Results from these studies have indicated that resistance to bladder and kidney infections is a multigenic trait in mice.

**MATERIALS AND METHODS**

**Animals.** Mice used in the present studies were obtained from commercial vendors or bred in our animal care facility. Male and female BALB/c mice were purchased from Harlan Sprague Dawley, and C3H/HeJ mice of both sexes were purchased from Jackson Laboratories. Males or females of either strain were used to produce (BALB/c × C3H/HeJ) F1 mice, and either male or female C3H/HeJ mice were backcrossed to F1 males or females.

**Infection induction and quantitation.** Female BALB/c, C3H/HeJ, F1, or backcross mice were inoculated intravesically with uropathogenic E. coli, according to a minimal inoculum protocol that greatly reduces the likelihood of reflux-associated inoculation of the kidneys and induces infections in all animals inoculated [12]. The E. coli strain 1677 used in these experiments was isolated from the urine of a woman with a febrile UTI. This strain is O6 and has genes for hemolysin, aerobactin, P fimbriae, and type 1 fimbriae, but not for afimbrial adhesin I, cytotoxic necrotizing factor 1, or S fimbriae [13]. To prepare the inoculum, bacteria were grown from frozen stock by 2 passages in tryptose broth (Difco Laboratories), washed with PBS, and resuspended to a concentration of 2 × 10^7 bacteria/mL. Mice were deprived of water for 1 h and had urine expressed from their bladders immediately before inoculation. Ten microliters of bacterial inoculum were instilled into the bladder by urethral catheterization under isoflurane anesthesia, resulting in a dose of 2 × 10^8 E. coli per mouse. The animals were allowed to recover from anesthesia and were given water 1 h later.

Mice were killed 10 days after inoculation to assess the intensities of bladder and kidney infections. The bladder and both kidneys of each animal were removed, weighed, and homogenized in sterile PBS, after which the homogenates were serially plated onto Levine’s eosin-methylene blue agar (Difco Laboratories). The number of E. coli colonies on each plate was counted after overnight incubation at 37°C and was used to calculate the total number of bacteria in each bladder or pair of kidneys. The infection intensity was used as the quantitative phenotype.

**Statistical analysis.** Bladder and kidney infection phenotypes were defined using a data transformation for colony-forming unit values: cfu_t = log_{10} [(cfu + 100)/tissue weight], where cfu_t was the total number of colony-forming units calculated per tissue sample and the weight was measured in milligrams. This transformation was necessary because tissue samples with a colony-forming unit count of zero were not uncommon and the data were not normally distributed. Although no transformation will achieve near-normality, the above transformation was the best among the several that were evaluated for tissue infection data sets.

Analysis of variance (ANOVA), followed by Fisher’s protected least-significant-difference test, was used to determine significant differences between group cfu_t mean values. The Wright-Castle formula was used to estimate the number of genes potentially associated with resistance to bladder or kidney infection [14].
Kidney cfu

prepared histograms of the number of animals with bladder and determined the infection phenotypes of 109 backcross mice and we inoculated mice from these 2 strains, as well as F1 and back-

their susceptibility to ascending infections. For the present study, in their ability to successfully resolve a bladder infection and in have shown elsewhere [1] that BALB/c and C3H/HeJ mice differ kidney infection phenotypes that were significantly different. We

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values for backcross mice were equivalent to those observed in BALB/c mice.

2, respectively). These graphs compare infection intensities in parental, backcross, and F1 mice and clearly show differences in the distribution of cfu values for the 2 different urinary organs in backcross mice.

If susceptibility to severe bladder infection was determined by a single recessive gene in C3H/HeJ mice, the numbers of backcross animals with infection phenotypes similar to those of the resistant and susceptible parental strains should be equal. However, the data that we obtained showed a very skewed distribution of the phenotypes, with 97 mice having low cfu values, similar to the BALB/c parent, and only 12 mice with severe infections similar to the C3H/HeJ parent. Thus, the ratio of resistant to susceptible animals in this backcross was ~8:1.

The distribution of kidney infection intensities in backcross mice was markedly different. Approximately one-half of the animals developed no or very mild upper tract infections (cfu > 0.5), as observed in UTI-resistant BALB/c mice. The remaining animals had kidney infections with cfu values ranging from 0.5 to 5.5. This distribution was a somewhat lower range of values than that observed in UTI-susceptible C3H/HeJ mice, which had cfu values of 3.5–6.0.

**Estimate of number of genes contributing to bladder and kidney infection phenotypes.** The chromosomal site containing a gene associated with a quantitative phenotype such as cfu, is regarded as a quantitative trait locus (QTL), and it is possible to estimate the number of QTL for a given phenotype. A derivation of the method used to estimate the number of QTL governing susceptibility to bladder and kidney infections is given in the Appendix. This method is that of Wright-Castle [14], and it provided estimates for the number of bladder and kidney QTLs of 1.3 and 1.2, respectively. Thus,

<table>
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<tr>
<th>Table 1. Infection phenotypes of parental, F1, and backcross mice used in linkage analysis.</th>
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<td>Mice tested</td>
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a Parental strains tested were BALB/c and C3H/HeJ. F1 mice were bred from BALB/c and C3H/HeJ mice. Backcross mice were bred from F1 and C3H/HeJ mice.

b log10[(cfu/1000)/mg of tissue] 10 days after bladder inoculation.

c Pooled variance estimates (see Appendix) for BALB/c, C3H/HeJ, and F1 mice for bladder and kidney infections were 0.753 and 0.489, respectively.

d Comparison of means for BALB/c and C3H/HeJ mice by Fisher’s protected least-significant-difference test; P < .001 for bladder and kidney values.

e Comparison of means for BALB/c and F1 mice by Fisher’s protected least-significant-difference test; P = .94 and P = .80 for bladder and kidney values, respectively.

f Comparison of means for C3H/HeJ and F1 mice by Fisher’s protected least-significant-difference test; P < .001 for bladder and kidney values.

RESULTS

**Infection phenotypes of parental, F1, and backcross mice.** The initial step in evaluating the inheritance of UTI resistance and susceptibility was to establish that mice with distinct genetic backgrounds and their F1 offspring had quantitative bladder and kidney infection phenotypes that were significantly different. We have shown elsewhere [1] that BALB/c and C3H/HeJ mice differ in their ability to successfully resolve a bladder infection and in their susceptibility to ascending infections. For the present study, we inoculated mice from these 2 strains, as well as F1 and backcross strains, and used the number of colony-forming units in their bladders and kidneys as a quantitative phenotype.

The infection phenotypes for parental, F1, and backcross mice are summarized in table 1. The number of bacteria in the bladders of BALB/c mice was ~4 orders of magnitude less than that in C3H/HeJ mice at 10 days after inoculation. In addition, BALB/c mice did not develop kidney infections, whereas C3H/HeJ mice developed severe kidney infections. Both bladder and kidney infection intensities were significantly different in BALB/c and C3H/HeJ mice. The F1 mice had low numbers of bacteria in their bladders and kidneys and thus were similar in phenotype to the UTI-resistant BALB/c parent. The mean bladder and kidney cfu values for backcross mice were equivalent to those observed in the BALB/c strain.

**Distribution of bladder and kidney infection phenotypes among backcross mice.** To further define the quantitative nature of infection phenotypes and to determine whether the resistance to bladder and kidney infections observed in BALB/c mice was partial or complete, backcross animals were produced by breeding UTI-susceptible C3H/HeJ mice with F1 mice. We determined the infection phenotypes of 109 backcross mice and prepared histograms of the number of animals with bladder and kidney infection intensities within specific ranges (figures 1 and 2, respectively). These graphs compare infection intensities in parental, backcross, and F1 mice and clearly show differences in the distribution of cfuT values for the 2 different urinary organs in backcross mice.

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we can conclude that there is at least 1 segregating QTL for both the bladder and kidney infection phenotypes. The QTLs could be the same or different for each phenotype.

DISCUSSION

The results of the present study provide new insights into genetic factors associated with decreased resistance to \textit{E. coli} bladder and kidney infections in C3H/HeJ female mice. We have shown that inheritance of resistance and susceptibility to these infections can be elucidated by evaluating UTI frequency and severity in mice derived by selective breeding. Most importantly, the data demonstrate that the increased UTI susceptibility of C3H/HeJ mice is a complex, heritable trait.

These conclusions are strongly supported by data on bladder and kidney infection phenotypes of F1 and backcross mice. When the F1 offspring of C3H/HeJ and BALB/c mice were inoculated with \textit{E. coli}, they were able to resolve their bladder infections within 10 days, and there was little evidence of kidney infections in the animals tested. This finding indicates that resistance to UTI, which is characteristic of BALB/c parents, is a dominant trait. Evidence for the multigenic nature of UTI susceptibility in C3H/HeJ mice is provided by analysis of bladder and kidney phenotype distributions in backcross mice. If severe bladder or kidney infections were each due to a recessive allele at a single locus, there would be approximately equal numbers of mice with infection intensities equivalent to those of each parental strain. We did not observe this distribution for infections of either organ. The number of backcross mice with intense bladder infections at 10 days after inoculation was ~12% of the total, rather than 50%. For kidney infections, the numbers of mice with and without infections were nearly equal; however, the infection intensity values were widely distributed rather than clustered near those of the C3H/HeJ parent, which again indicates the activity of several genes in determining the phenotype.

The results obtained from infection studies of backcross mice are particularly interesting, because they provide some insights into host susceptibility and resistance for the 2 different organs. The most important implications are that susceptibility and resistance to infection are due to multiple host factors in both the bladder and kidney, yet the exact host traits and ways in which defense mechanisms function are potentially different. The bladder infection results suggest that defense mechanisms are redundant, because most animals are able to resolve infections and only those mice with combined deficiencies in several mechanisms develop severe infections. Alternatively, there may be a combination of a host trait that increases bacterial infectivity and other factors that lower immune responses to the pathogen. Results of kidney infection studies also strongly suggest that multiple mechanisms are involved in susceptibility and resistance; however, there appears to be less redundancy. If this is the case, as the number of genes associated with susceptibility increases within individual mice, the severity of kidney infections should increase proportionately. This model is supported by the current data, in which infection intensities ranged between those observed in the resistant and susceptible parents, and it is conceivable that animals with a single sus-
ceptibility factor have the lowest infection intensities and that those with multiple factors have the severest infections.

At this point, it is difficult to provide an accurate estimate of the number of genes determining susceptibility of C3H/HeJ mice to severe bladder or kidney infections. The Wright-Castle analysis of the current data confirmed that there is ≥1 gene involved; however, it is important to note that, because this estimator uses strict assumptions regarding codominance, independence, and equality of loci, its primary value is qualitative and not quantitative. Thus, it is only possible to establish the multigenic nature of the infection phenotypes.

The overall goal of the present study was to elucidate the genetic differences between C3H/HeJ and BALB/c mice that make C3H/HeJ mice extremely susceptible to both bladder and kidney infections. The current results strongly support a multigenic model and are consistent with our earlier study on induced E. coli UTIs in BALB/c, C3H/HeJ, C3H/HeN, and C3H/OuJ mice [1]. It has been proposed that the C3H/HeN and BALB/c strains are resistant to UTIs because they are immunologically responsive to LPS and that the C3H/HeJ strain is susceptible because it is unresponsive to LPS [9]. However, we have demonstrated elsewhere that C3H/OuJ mice had normal responses to LPS and thus concluded that the increased UTI susceptibility of these mice must be attributable to ≥1 gene other than Thr4 [7]. The present findings reinforce this model and, in addition, demonstrate that susceptibility is very likely a recessive trait for both bladder and kidney infections.

The eventual identification of mouse genes associated with resistance and susceptibility to bladder and kidney infections has direct implications for our understanding of genetic factors that increase the risk of recurrent UTIs in women and female children. In a previous study [15], we reported the increased incidence of UTIs in the immediate female family members of women with recurrent UTIs and suggested that this finding supported a model of genetic predisposition to disease. According to the results thus far in our mouse studies and given the multigenic nature of susceptibility to other human diseases [16], it is very likely that several genes contribute to the UTI-susceptible phenotype in women. It is also probable that these genes are directly involved in the complex interactions that take place between host and pathogen during the induction and eventual resolution of an infection.

Uropathogenic E. coli possess several virulence factors that increase their ability to colonize and persist in the urogenital tract [17], and it is the host’s susceptibility and response to these factors that limit the colonization and persistence of pathogens. For example, uropathogenic E. coli adhere in greater numbers to vaginal and bladder epithelial cells of UTI-susceptible women than to similar cells from healthy control subjects [18]. This adherence is an essential first step in vaginal colonization and precedes sequential colonization of the urethra and bladder. In addition, the observation that women who are genetically nonsecretors of blood-group antigens have a higher incidence of recurrent UTIs than women with the secretor phenotype provides direct evidence of genetic predisposition to UTI [19]. The physiological basis of the increased susceptibility of nonsecretors may be attributed to structural interference with epithelial–cell surface receptors for E. coli or the presence of E. coli–binding glycolipids on their vaginal cells [19]. Another physiological difference between UTI-susceptible and -nonsusceptible women is the increased binding of E. coli by vaginal fluid from women with recurrent UTIs [20]. It is conceivable that phenotypic variants of glycoproteins or oligosaccharides in cervicovaginal secretions account for more avid binding of bacterial adhesins.

It should also be noted that various aspects of both innate and adaptive immune responses to pathogens are genetically determined and, therefore, offer the potential for genetic variation between UTI-susceptible and -nonsusceptible individuals. In humans, variations in tumor necrosis factor (TNF)-α production are attributable to polymorphisms in its promoter [21], and it is conceivable that differences in the amounts of TNF-α synthesized in response to a UTI could affect host resistance to the infection. Furthermore, recent studies have identified genetic polymorphisms of the Tlr4 molecule [22]. Such genetically determined structural variations could affect innate immune responses to uropathogenic E. coli by increasing or decreasing the affinity of Tlr4 for LPS. The antigenic specificities of host adaptive immune responses to bacterial antigens might also be expected to vary because of polymorphisms in genes specifying polypeptides contributing to the 3-dimensional structures of major histocompatibility antigens, antigen-binding sites of antibodies, and T cell receptors [23]. A limited repertoire of B or T cell responses could decrease resistance to E. coli or other bacteria. Thus, there are numerous examples of phenotypic variations in humans that could potentially increase susceptibility to UTI, and it will be possible to apply results from mouse genetic studies to more specifically identify these risk factors.

APPENDIX

To define the relationships needed to estimate the number of QTL in our genetic model, it is possible to start by considering a single QTL and later add relevant quantities over other loci, because we assume the independence of loci. The phenotypic effect, δ, of a QTL will be defined as the increase observed in the measured phenotype after substituting both A alleles with B alleles at the QTL. We assume codominance, so each B allele increases the phenotype by δ/2. The F1 genotype is AB, for which the phenotypic mean value over the AA genotype is δ/2. The genotypes AB and BB, with mean values of
δ/2 and δ, respectively, appear with equal frequency in the backcross. Thus, the mean value for the backcross is 3δ/4, and the average deviation from this mean is δ/4. The genetic variance is the average squared deviation, or $\delta^2/16$. If there are k segregating QTL, each with independent additive effects of size δ, the total genetic variance for the backcross should be $\sigma^2_G = k\delta^2/16$. The mean phenotypic difference between the parental strains will be $D = k\delta$, which can be squared to give $D^2 = k^2\delta^2$. Combining these results shows that the number of QTL could be estimated from the relationship $k = D^2/16\sigma^2_G$, which is the Wright-Castle formula [14].

An estimate of D is obtained as the difference of the mean phenotypes for the parental strains, $\hat{D}$. The total phenotypic variance for the backcross is then $\sigma^2_{bc} = \sigma^2_{G} + \sigma^2_{E}$, where $\sigma^2_{E}$ is the environmental variance. Because the 2 parental strains and the F1 strain should be genetically identical within each strain, their genetic variance should be zero, and the observed phenotypic variance should be due solely to environmental variance $\sigma^2_{E}$. The environmental variance is estimated by the pooled variance estimate of the parental strains and F1 animals, estimated as the mean squared error from a 1-way ANOVA, $\hat{\sigma}^2_{E}$. We can estimate the genetic variance as $\hat{\sigma}^2_{G} = \hat{\sigma}^2_{bc} - \hat{\sigma}^2_{E}$, where $\hat{\sigma}^2_{bc}$ is the estimated phenotypic variance of the backcross animals, which gives us the quantities we need to estimate k from the Wright-Castle equation.

References