Lipopolysaccharide-Responder and Nonresponder C3H Mouse Strains Are Equally Susceptible to an Induced Escherichia Coli Urinary Tract Infection

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Lipopolysaccharide-Responder and Nonresponder C3H Mouse Strains Are Equally Susceptible to an Induced Escherichia coli Urinary Tract Infection

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Host defense against bacterial urinary tract infections (UTI) includes both inflammatory and immune responses to infecting bacteria. The cellular events leading up to local inflammation are thought to be under genetic control and initiated by lipopolysaccharides (LPS) of gram-negative bacteria such as Escherichia coli. It has been previously reported that mice which lack functional Lps genes are more susceptible to induced E. coli UTI than mice with normal mitogenic responses to LPS. In contrast to these findings, data in this report demonstrate that LPS-responder and nonresponder C3H mouse strains are equally susceptible to E. coli UTI when C3H/OuJ (Lps+/Lps+) and C3H/HeJ (Lps+/Lps+) were intravesically inoculated with equal numbers of uropathogenic E. coli organisms, neither strain was able to effectively resolve the induced UTI. The inability of C3H/OuJ mice to combat the infection was not due to an impaired response to LPS, nor could a defect in the local inflammatory response be identified. The results indicate that factors other than LPS responsiveness are also important in determining host resistance to UTI.

Host defense against urinary tract infections (UTI) includes both local inflammation and antibody-mediated immune responses to uropathogenic bacteria causing these infections. Histologic studies of mice have shown that there is integration of these two protective responses during spontaneous resolution of an Escherichia coli UTI (12). Phagocytic cells were observed in the urine within 1 week after intravesical instillation of viable E. coli organisms, and the initial infiltrate consisted primarily of polymorphonuclear neutrophils followed by the appearance of mononuclear inflammatory cells. Opsonizing anti-E. coli antibody facilitates rapid clearance of bacteria by phagocytic cells as the infection is resolved over a 3-week infection period. Studies with nonhuman primates have shown that levels of both urinary and serum antibodies to infecting E. coli increase during the first 2 weeks following induction of E. coli cystitis and that this rise precedes spontaneous resolution of the infection (7). In addition, a high level of serum or urinary anti-E. coli immunoglobulin prior to induction of E. coli UTI in monkeys correlates with an accelerated resolution of the infection (6). Studies with humans have shown that pyuria indicative of a local inflammatory response occurs early in a UTI (3).

The role of lipopolysaccharide (LPS) in the cellular events leading up to local inflammatory responses in the infected bladder has been the focus of several recent investigations. Studies using a mouse model of E. coli UTI have indicated that cytokines induced in response to bacterial LPS act to potentiate the inflammatory response. Mice possessing the gene for LPS responsiveness (Lps), such as the C3H/HeN and C57BL/6J strains, have a high level of polymorphonuclear-neutrophil inflammatory response detectable in the urine within the first 24 h following intravesical infection with viable E. coli (1, 14) and can effectively resolve the induced UTI. In contrast, C3H/HeJ mice lacking the Lps gene (Lps+/Lps−) do not develop rapid inflammatory responses and have significantly higher bladder and kidney infection levels following similar bladder inoculations with viable E. coli (14).

The results reported here provide additional data on the course of an induced E. coli UTI in strains of C3H LPS-responder and nonresponder mice. In contrast to earlier reports, we have found that C3H mice with normal mitogenic responses to LPS and strong bladder and kidney inflammatory responses to E. coli UTI were unable to resolve their infections.

MATERIALS AND METHODS

Induction and assessment of UTI. E. coli 1677 was used to induce UTI in mice. This strain has been characterized as O6, type 1 and F fimbriated, hemolytic, and motile (16). Bacteria from frozen stock were passed twice in tryptose broth. Female C3H/HeJ and C3H/OuJ mice were purchased from the Jackson Laboratories (Bar Harbor, Maine), and female C3H/HeN mice were purchased from Harlan-Sprague Dawley (Indianapolis, Ind.). The C3H/HeN and C3H/OuJ strains are LPS responders, and C3H/HeJ mice are nonresponders. Animals were 8 to 10 weeks old when used in experiments. Mice were intravesically inoculated with 10⁹ E. coli organisms and assayed for bladder and kidney colonization on days 1, 3, 7, and 14 of infection. The inoculation and assay procedures have been described in detail elsewhere (5). At the time of sacrifice, one-half of the bladder and one-half of each kidney were minced and homogenized in sterile phosphate-buffered saline. The remaining portions of the bladder and kidneys were placed into 10% buffered formalin prior to histologic processing and evaluation of inflammation. Infection data for bladders and kidneys are expressed as E. coli CFU/mg of tissue.

LPS-induced mitogenesis. Spleens were aseptically removed from two to four mice per experiment. Single-cell suspensions of spleen cells from each mouse were prepared (2), and 10⁵ cells in 0.1 ml were placed into triplicate wells of a 96-well microtiter plate. LPS isolated from E. coli 1677 (17) was added to each well at a concentration of 1 or 10 μg/ml. Concanavalin A (ConA) was added at a concentration of 5 μg/ml. Cells were incubated for a total of 72 h, with [3H]thymidine added for the last 8 h of culture. Cultures were harvested onto glass filter paper, dried, and counted for 4 min in a liquid scintillation counter. Data from similar groups of mice from separate experiments were pooled, and the results were expressed as mean counts per minute.

Histopathology. Bladder and kidney tissues were embedded in paraffin, and sections were cut to a thickness of 5 μm. Four to two sections from each tissue sample were cut and stained with hematoxylin and eosin. Most kidney
sections included the renal pelvis. The degree of inflammation over the total area of each section (both bladder and kidney) was graded by the same veterinary pathologist (A. Gendron-Fitzpatrick) according to the scale shown in Table 1. Slides of tissue sections from untreated animals were identified and used to establish a baseline for uninfected tissue. Bladder and kidney sections from infected animals were read without knowledge of the level of bacterial infection or time point.

Statistical analysis. Data were analyzed by statistical methods appropriate to the type and structure of the data set by using SAS statistical software (13). Bladder and kidney CFU levels at each time point were compared between strains by analysis of variance followed by Fisher’s protected least-significant-difference procedure (15). The data were first log transformed to better meet the assumption of homogeneous variances. Time patterns of CFU levels within strains were examined by linear regression. The dependent variable was log CFU, and the independent variable was log time. Tests for nonlinearity were performed by including a quadratic term (log squared) in the model.

Data for mitogenic responses, which were recorded as counts per minute, were analyzed by analysis of variance followed by Fisher’s protected least-significant-difference procedure. As is appropriate for scintillation count data following a Poisson distribution, the data were first square-root transformed (15).

Bladder and kidney inflammation scores at each time point were compared in the same manner as that used for CFU-level data, by performing analysis of variance on log-transformed scores. Time trends in the inflammation scores were determined by ordinal logistic regression, since score data represent ordinal measurements (11).

RESULTS

Urinary tract infections in LPS-responder and nonresponder mice. At 1 day after infection, C3H/HeN (Lps+/Lps−), C3H/HeJ (Lps−/Lps−), and C3H/OuJ (Lps−/Lps−) mice had equivalent numbers of E. coli CFU in their bladders (P = 0.60) (Fig. 1). Thereafter, C3H/HeN mice had continually decreasing numbers of CFU (P < 0.0001; R² = 0.54) while C3H/HeJ and C3H/OuJ mice did not. Moreover, the C3H/OuJ mice had bladder infections that increased in severity over time (P = 0.04; R² = 0.19). The C3H/HeJ and C3H/OuJ mice had significantly higher numbers of CFU in their bladders than did C3H/HeN mice at day 7 (P = 0.024 and P = 0.0004, respectively) and day 14 (P = 0.001 and P = 0.0001, respectively). Bladder CFU levels were not statistically different between C3H/HeJ and C3H/OuJ mice at day 7 or 14.

Dissimilar patterns to the course of kidney infections in these C3H strains were also noted (Fig. 2). The C3H/HeN mice had progressively decreasing CFU levels over the 14-day period. The nonresponder C3H/HeJ mice had significantly higher infection levels on day 1 (P = 0.0009), and these levels remained unchanged for the next 2 weeks. Although C3H/OuJ mice initially had CFU levels that were similar to those in C3H/HeN mice, their infection levels increased to a maximum at day 3 and then decreased by the end of the study period (P = 0.015; R² = 0.28).

LPS-induced mitogenesis in C3H mice. To verify LPS responsiveness in the three strains of mice, spleen cells were stimulated in vitro with LPS isolated from E. coli 1677. As shown in Table 2, C3H/HeN and C3H/OuJ spleen cells had equivalent mitogenic responses to LPS at both 1 and 10 μg/ml. Cells from nonresponder C3H/HeJ mice did not undergo mitogenesis in the presence of either dose of LPS, as the counts per minute were similar to those in cultures incubated in culture medium alone. ConA induced significant mitogenesis in

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**TABLE 1. Histopathological grading scale for degree of inflammation**

<table>
<thead>
<tr>
<th>Grade</th>
<th>Bladder</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>1</td>
<td>Subepithelial cell inflammatory infiltration (focal and multifocal)</td>
<td>Focal inflammation (cellular infiltration and/or edema in the pelvis)</td>
</tr>
<tr>
<td>2</td>
<td>Edema and subepithelial inflammatory cell infiltration (diffuse); marked subepithelial inflammatory cells with necrosis and neutrophils in and on bladder mucosal epithelium</td>
<td>Focal inflammation (more severe than that for grade 1) from pelvis to medulla with and without moderate edema; multifocal inflammation and inflammatory cells from pelvis to medulla</td>
</tr>
<tr>
<td>3</td>
<td>Inflammatory cell infiltrate extends into muscle, in addition to criteria for grade 3</td>
<td>Extensive segmental inflammation and necrosis evident from pelvis to cortex</td>
</tr>
<tr>
<td>4</td>
<td>Loss of surface epithelium (necrosis with full-thickness inflammatory cell infiltration)</td>
<td>Diffuse tissue necrosis and inflammatory cell infiltration extending from pelvis to cortex</td>
</tr>
</tbody>
</table>

FIG. 1. Bladder infection levels in LPS-responder and nonresponder mice during the course of induced E. coli UTI. Animals were intravesically inoculated with E. coli and assayed at 1, 3, 7, and 14 days to determine the E. coli CFU levels in the bladder. Values are means (error bars show standard errors of the means [SEMs]) for groups of five or six mice.

FIG. 2. Kidney infection levels in LPS-responder and nonresponder mice over the course of induced E. coli UTI. Animals were intravesically inoculated with E. coli and assayed at 1, 3, 7, and 14 days to determine the E. coli CFU levels in the kidneys. Values are the means (error bars show SEMs) for groups of five or six mice.


TABLE 2. Mitogenic responses of stimulated mouse spleen cells

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>Genotype</th>
<th>[3H]thymidine incorporation (cpm) into cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>LPS stimulated (1 µg/ml) (P)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C3H/HeN</td>
<td>Lps&lt;sup&gt;b&lt;/sup&gt;/Lps&lt;sup&gt;b&lt;/sup&gt;</td>
<td>521 ± 36</td>
</tr>
<tr>
<td>C3H/HeJ</td>
<td>Lps&lt;sup&gt;a&lt;/sup&gt;/Lps&lt;sup&gt;d&lt;/sup&gt;</td>
<td>524 ± 105</td>
</tr>
<tr>
<td>C3H/OuJ</td>
<td>Lps&lt;sup&gt;a&lt;/sup&gt;/Lps&lt;sup&gt;d&lt;/sup&gt;</td>
<td>390 ± 16</td>
</tr>
</tbody>
</table>

<sup>a</sup> Spleen cells were obtained from two or four mice and placed into tissue culture for 72 h. Values are mean counts per minute and SEMs for [3H]thymidine incorporation into cells during the last 8 h of culture. Control cultures contained medium only. Mitogenesis was induced by adding LPS isolated from E. coli 1677 (1 or 10 µg/ml) or ConA (5 µg/ml).

<sup>b</sup> P values are for comparisons between means of control and mitogen-stimulated cultures. Data were analyzed by analysis of variance followed by Fisher's protected least-significant-difference procedure.

spleen cells from all three C3H strains. The response to ConA was greatest in spleen cells from C3H/HeJ mice.

**Inflammatory responses in bladders and kidneys during UTI.** We next investigated whether there were histologic differences in the inflammatory responses occurring in infected bladders and kidneys in LPS-responder and nonresponder mice. The LPS-responder C3H/HeN mice had moderate bladder inflammation (grade 2 to 3) on day 1 that gradually decreased over time as the infections cleared (P = 0.026) (Fig. 3). In contrast, C3H/HeJ nonresponder mice had minimal inflammation (grade 0 to 1) with no evidence of change (P = 0.17) over 14 days even though the intensity of bladder infection remained very high during this same period. The C3H/OuJ mice (Lps<sup>a</sup>/Lps<sup>d</sup>) had grade-2 to -4 inflammation at both the beginning and end of the course of the infection (P = 0.59). The bladder inflammation scores for C3H/HeJ mice were statistically different from those of C3H/OuJ mice at all time points (P values ranged from 0.0001 to 0.0085). Significant differences between scores for C3H/HeJ and C3H/HeN mice were seen on days 1 and 3 (P < 0.001 and P = 0.006, respectively).

Kidney inflammation by histologic measure in C3H/HeN mice was highest on day 1 and decreased thereafter (P = 0.008) (Fig. 4). Continuous levels of very mild inflammation in C3H/HeJ (Lps<sup>a</sup>/Lps<sup>d</sup>) mice were observed over the 14-day infection period. Inflammation increased steadily from grade 0 to 1 to grade 2 to 3 between days 1 and 14 in LPS-responder C3H/OuJ mice (P = 0.003).

**DISCUSSION**

Results of this study demonstrate that LPS-responder mice can be as susceptible to severe E. coli UTI as LPS nonresponders. C3H/HeN mice, one strain of LPS-responder mice, are able to mount an effective host response to an induced UTI and can reduce bladder and kidney infection levels by more than 100-fold over a 14-day period. However, both the C3H/HeJ (LPS-nonresponder) and the C3H/OuJ (LPS-responder) mice were unable to clear their UTI; bladder infections in the C3H/OuJ strain actually became more severe over time. The lack of correlation between Lps<sup>a</sup>/Lps<sup>d</sup> genotype and UTI resistance for the C3H/OuJ strain raised the question of whether these mice were typical LPS responders. The finding that spleen cells from C3H/OuJ mice had LPS-induced mitogenic responses that were equivalent to those of C3H/HeN mice at two LPS dose levels confirmed that C3H/OuJ mice were phenotypically LPS responders and, therefore, would be expected to successfully resolve an E. coli UTI.

It is clear that, in contrast to C3H/HeN mice, both C3H/HeJ and C3H/OuJ mice have significantly decreased abilities to combat infections in the upper and lower urinary tracts. We do not, however, know whether these two strains share a common defect in their defense mechanism(s) against UTI. The fact that LPS-responder C3H/OuJ mice have UTI that are equivalent to those seen in nonresponder C3H/HeJ mice indicates that LPS responsiveness is not an exclusive determinant of UTI resistance. In fact, bladder and kidney inflammation scores for C3H/OuJ mice were equivalent to those observed for C3H/HeN mice.
HeN mice having infections of approximately the same intensity.

The possibility that C3H/HeJ and C3H/OuJ mice have a limited deficiency in their antibody-mediated immune responses to E. coli antigens associated with urovirulence could explain our findings. Such a limitation could lower the resistance of these mouse strains to E. coli UTI. Studies on antibody responses to E. coli UTI in experimental animals and data on the antibody profiles of UTI-susceptible women support this concept. Data from induced UTI in monkeys have shown that serum and urinary anti-E. coli immunoglobulin levels increased as the infection spontaneously resolved (7) and that those animals with the highest serum and urinary anti-E. coli immunoglobulin levels prior to infection induction resolved their infections most rapidly (9). These findings demonstrate the positive effect of antibody-mediated immunity as a component of host resistance to UTI. We have also shown that women who have never had a clinical UTI have serum immunoglobulin G antibodies to E. coli antigens more often than do UTI patients (8). Taken together, the monkey and human studies indicate that successful UTI resolution and resistance to recurrent infections are closely correlated with the host’s level of antibody-mediated immunity to E. coli. More specifically, it appears that a diminished capacity to produce antibodies to a set of antigens found on uropathogenic E. coli increases UTI susceptibility.

One plausible explanation of the current data is that C3H/HeN mice have the innate ability to produce UTI-protective antibodies against E. coli whereas C3H/OuJ and C3H/HeJ do not. The fact that both of the Jackson Laboratory C3H strains have difficulty in resolving E. coli UTI is significant. These two strains are more likely to share a similar immunogenetic background with each other than with C3H/HeN mice and thus are also more likely to have similar anti-E. coli antibody repertoires; consequently, their antibody responses to E. coli UTI might be much different than those of C3H/HeN mice. Historically, the C3H/HeJ strain originated from a cross between a Bragg albino female and a DBA/2 male in 1920 (4). Progeny of these mice were transferred to Heston at the National Institutes of Health (NIH) (C3H/HeN) and from Heston to Jackson in 1948 (C3H/HeJ) (4). The C3H/OuJ strain resulted from a transfer of C3H/HeJ mice to Outzen from the Jackson Laboratories in 1952, and these mice were then transferred back to Jackson in 1981. The ancestors of the Jackson and NIH strains of C3H mice have thus been separated for several decades, and it is conceivable that during this time there has been genetic drift between the Jackson and NIH Harlan strains. Evidence supporting development of genetic dissimilarity between C3H/HeJ and C3H/HeN mice comes from DNA fingerprinting of several C3H/He substrains and sublines (10). These studies clearly showed that the C3H/He substrains divided into clusters which originated from either Jackson or NIH animals. While C3H/OuJ mice were not included in this study, they would be part of the Jackson cluster. The genetic drift in C3H/HeN mice could have led to changes in one or more immunoglobulin genes that enable these mice to produce anti-E. coli antibodies which afford protection against UTI. It is also possible that both of the Jackson strains lost the ability to make UTI-protective antibodies; however, the changes would need to have occurred in the strains’ common ancestor or in both strains independently.

The data presented here indicate that resistance to UTI is very likely determined by many host factors. While inflammatory responses initiated by cellular reactions to LPS are one aspect of overall host defense, other defense mechanisms, such as the ability to produce UTI-protective antibodies, are also very likely important components in the resolution of E. coli UTI. Further investigations will be necessary to define the role of immunogenetics in host defense against UTI.

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