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Letter to the Editor
Reflux in the Mouse Model of Urinary Tract Infection

Two studies recently published in *Infection and Immunity* involved a mouse model of ascending urinary tract infection (UTI) (1, 6). The inoculation conditions used in both studies, i.e., 20 to 50 μl of bacterial suspension inoculated into the bladder, had been found in a previous study from the same laboratory to produce immediate vesicoureteral reflux (VUR) in a substantial proportion of mice (2).

As recognized by these and other investigators, VUR should be avoided in the mouse model of UTI because it introduces bacteria directly into the upper urinary tract, thereby bypassing important proximal steps in pathogenesis (but in an uncontrolled fashion), and because it can cause hydrostatic injury to the renal parenchyma, thereby rendering the model unrepresentative of uncomplicated ascending UTI in humans (2, 3). The investigators' previous study, in which VUR could be avoided only by reduction of the inoculum volume to 10 μl and inoculation into the urethra rather than the bladder (2), was cited for the methods used in the recent studies despite the use of larger volumes and inoculation into the bladder in the recent studies (1, 6).

High levels of kidney infection were observed in the recent studies (1, 6), whereas negligible levels were achieved in the investigators' previous study with the same bacterial strain (strain 1677) when VUR-free inoculation conditions were used (2). This finding suggests that inoculation-induced VUR may have accounted for some or most of the kidney infections encountered in the recent studies, a possibility which calls into question the physiological relevance of the studies' findings. Interpretation of results thus would be aided by knowledge of whether experiments were done to confirm that the inoculation conditions used did not induce immediate VUR. In addition, since others have documented that 25 to 50 μl can be inoculated into the mouse bladder without causing VUR (4, 5), whereas Hopkins et al. previously found this not to be the case (2), it would be of considerable value to current or future users of the mouse model to know what VUR-avoidance measures Hopkins et al. may have introduced since their previous study (2).

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Author's Reply

My colleagues and I appreciate the observations and comments of Dr. Johnson regarding two recent articles on susceptibility and resistance of mice to an induced *Escherichia coli* urinary tract infection (UTI) (2, 3). Of particular interest were the inoculation procedures used in these studies and the effect of inoculation on development of kidney infections.

The objectives of the first study (1) were to demonstrate possible differences in the susceptibility and immune responses of different inbred mouse strains to an induced *E. coli* UTI. These experiments examined the UTI resolution patterns and host inflammatory and antibody-forming cell responses of 10 mouse strains inoculated with the same bacterial strain and were conducted over an extended period of time. During the course of these and other studies (3) of induced UTIs in mice, we examined the effects of inoculum volume and route on vesicoureteral reflux and induction of kidney infections. Our results indicated that large inoculum volumes delivered into the bladder increased the incidence of kidney infections. A majority of the 10 mouse strains in the susceptibility/host response study had been screened by using a 50-μl bladder inoculum prior to our examination of inoculation conditions. Consequently, the remaining mice were also inoculated with a 50-μl volume to maintain consistency. It is thus possible that kidney infections observed in some mouse strains could be attributed to inoculation-associated reflux. It is important to note, however, that even under these conditions, not all mouse strains developed kidney infections and there were differences in severity among strains both early and late in the course of infection. We also noted that inflammatory and splenic antibody-forming cell responses were positively correlated with kidney infection intensity and believe this would be the case regardless of how the kidney infections were induced.

The objective of the second study (4) was to examine the effect of superantigen exposure on the resolution of bladder infection and induction of cytokines. Mice in these experiments were inoculated with *E. coli* instilled into the bladder in a 20-μl volume. We did not evaluate kidney infections in these mice and, therefore, do not have information on infection intensities or cytokine production in the kidneys. The reported effects of staphylococcal enterotoxin B on bladder infection resolution and cytokine induction are not likely to be affected by inoculation conditions.

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