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Local and Systemic Antibody Responses Accompany Spontaneous Resolution of Experimental Cystitis in Cynomolgus Monkeys

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Cynomolgus monkeys were infected intravesically with two different strains of uropathogenic *Escherichia coli*. A quantitative enzyme-linked immunosorbent assay method was used to monitor secretory and serum immune responses to the induced cystitis. Anti-*E. coli* secretory immunoglobulin A (sIgA) and IgG urinary antibodies were generated as a result of the cystitis. Urinary sIgA levels against the *E. coli* strains tested were highest at 25 to 31 days after cystitis induction, and urinary IgG levels were highest at 31 to 45 days after induction. Anti-*E. coli* IgM was not detected in the urine. The antibody response in serum was characterized by IgM, IgG, and IgA immunoglobulin production. IgM levels in serum rose at 14 days after cystitis induction and were sustained for another 1 to 2 weeks. Increases in serum IgG levels began at 7 to 21 days of infection and persisted in some cases for up to 70 days. Serum IgA to the infecting *E. coli* was produced within 1 week after cystitis induction and remained at elevated levels throughout the course of the cystitis. These results demonstrate the capacity of primates to spontaneously resolve a cystitis and to mount both local and systemic immune responses against the infecting bacteria.

The immune responses which occur during a urinary tract infection (UTI) are important because they may contribute to resolution of the infection and are potentially amenable to strengthening by immunization. The protective role of urinary immunoglobulins has been suggested by their increased levels following UTI (20, 22, 23) and their reported role in preventing bacterial adherence to uroepithelial cells in vitro and in vivo (15, 19, 26). Augmentation of the urinary antibody response by local immunization as a means of affording increased resistance to a subsequent UTI has been effective in rodents (10, 25) and has had some initial success in primates (24). These studies, as well as others in which immune responses following UTI in humans has been examined (20, 22), have demonstrated increased amounts of total secretory immunoglobulin A (sIgA) and IgG urinary immunoglobulins after infection; but they have neither directly quantitated antibodies specific for the infecting bacteria, nor monitored antibody responses before, during, and after development of the infection. Consequently, it is difficult to ascertain what levels of antigen-specific antibody are associated with bacterial clearance and when these antibodies are produced. The purpose of this investigation was to use a quantitative immunoglobulin assay to measure anti-*Escherichia coli* antibodies produced by primates over the course of an induced UTI and thereby more clearly define the local and systemic antibody responses which accompany resolution.

Cynomolgus monkeys were infected intravesically with two different uropathogenic *E. coli* strains and monitored over several weeks for bacteriuria and anti-*E. coli* immunoglobulins in urine and serum. The induced cystitis resolved without antibiotic intervention in 3 to 7 weeks, and levels of anti-*E. coli* antibodies increased significantly during resolution of the infection.

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MATERIALS AND METHODS

Bacteria. *E. coli* 1677 was isolated from the urine of a patient with a serious febrile UTI. Strain JR1 was provided by James Roberts, Tulane University (New Orleans, La.). Strain 1677 was characterized as type O6 with both type 1 and P fimbriae (8); strain JR1 was characterized as type O4 and expressed the same fimbrial types as strain 1677 when grown as described below. Frozen fractions of each bacterial strain were thawed and passaged two times in tryptose broth (Difco Laboratories, Detroit, Mich.); each incubation was for 48 h at 37°C. After the second growth cycle, the bacteria were streaked onto colonization factor antigen agar (4) plates and incubated for 18 h at 37°C. The bacteria were then harvested, washed with phosphate-buffered saline (PBS), concentrated by centrifugation, and suspended in PBS. Bacteria to be used in the enzyme-linked immunosorbent assay (ELISA) for *E. coli*-specific immunoglobulins were killed by using a 0.5% Formalin solution. Viable bacteria used to induce cystitis were suspended in PBS at a final concentration of 2×10^9/ml or 2×10^7/ml.

Animals. Female cynomolgus monkeys (*Macaca fascicularis*) were obtained from Charles River Research Primates, Port Washington, N.Y. The animals were housed at the University of Wisconsin Medical School Animal Care Unit, fed a standard diet, and provided with unrestricted access to water. The exact ages of the animals could not be determined because they were not born in captivity. Weights ranged from 2.0 to 3.0 kg. None of the monkeys had been used previously for any other experiments.

Cystitis induction. A total of 14 monkeys were randomly allocated to three groups of 4 or 5 animals each. Monkeys within each group were infected once with one of the dose-strain combinations used in this study. All animals were anesthetized with ketamine to facilitate handling. Prior to catheterization, the perineal area of each animal was cleansed with antiseptic as a precaution against the introduc-

tion of extraneous bacteria into the bladder along with the *E. coli* inoculum. To instill viable bacteria into the bladder, a no. 5 French feeding tube was introduced through the urethra, and any urine in the bladder was aspirated into a syringe. With the catheter still in place, 2 ml of viable *E. coli* (2×10^9/ml or 2×10^7/ml) was instilled into the bladder. The catheter was then removed. From our experience with other monkeys (24), it has been found that no further manipulations are required to maintain the inoculum in the bladder and induce a UTI.

Urine collection and processing. Urine samples were obtained from the monkeys in one of two ways, depending on the assays to be performed. To determine the numbers of viable bacteria present in bladder urine during the infection period, samples were obtained aseptically by suprapubic aspiration. Approximately 10 ml of urine per animal was obtained in this manner. Creatinine concentrations were determined for each sample by using a creatinine test kit and Unimeter obtained from Biodynamics Corp. (Indianapolis, Ind.). Leukocyte esterase levels in frozen samples were determined by using urine dipsticks (Uristix; Ames Division, Miles Laboratories, Elkhart, Ind.).

Urine for immunoglobulin determinations was collected from each monkey on a 24-h basis. Animals were kept in metabolic cages (Suburban Surgical, Wheeling, Ill.), in which urine drained into a small metal pan that was kept packed in dry ice, making it possible to freeze the urine immediately after collection and keep it frozen throughout the collection period. Frozen samples were thawed at room temperature (volume range, 100 to 300 ml per monkey) and filtered through a filter (pore size, 1.20 μm). Urine dipsticks (Bilistix, Ames Division, Miles Laboratories) were used to check for hematuria. Fractions of 2 ml of each 24-h urine sample were stored at −40°C.

Assays for erythrocyte sedimentation rate and C-reactive protein. Erythrocyte sedimentation rates (ESRs) for blood samples taken at weekly intervals were determined by using Wintrobe tubes (American Scientific Products, Evanston, Ill.). These blood samples were also tested for the presence of C-reactive protein (CRP) by using the RAPI/TEX-CRP test (Calbiochem-Behring, La Jolla, Calif.). Levels of >0.6 mg/dl could be detected by this method.

Immunoglobulin quantitation. Concentrations of antigen-specific immunoglobulins in urine or serum were determined by a modification of an ELISA described by Sorensen (17). Polystyrene microtest plates (Flow Laboratories, McLean, Va.) were used as a solid phase. All purified immunoglobulins and anti-immunoglobulin preparations were obtained from Cappel Laboratories (Malvern, Pa.). The first step in the ELISA consisted of coating wells with either class-specific anti-immunoglobulin or Formalin-killed *E. coli*. Each plate contained one section of wells for samples and another for IgM, IgG, or IgA standards. Wells in the immunoglobulin standards section were treated with anti-immunoglobulin antibody. A 0.1-ml sample of 1/4,000 goat anti-human sIgA, 0.1 ml of 1/2,000 goat anti-monkey IgG, or 0.1 ml of 1/3,000 goat anti-human IgM in carbonate buffer (pH 9) was added to appropriate wells on separate plates. Wells that were used to assay for anti-*E. coli* immunoglobulins in urine or serum samples were coated with killed *E. coli* by adding 0.1 ml of a suspension containing 4×10^7 bacteria per ml in carbonate buffer (pH 9) to each well. The *E. coli* strain used in the ELISA was the same as that used to induce cystitis. Plates were then incubated at room temperature for 45 min. The washing procedure employed after this coating step and the completion of each successive layer consisted of emptying the wells, adding 0.2 ml of PBS containing 0.05% Tween 20 to each well, incubating for 3 min at room temperature, and then emptying out the washing solution. This process was repeated two additional times, so that each well received a total of three washes.

The second step in the procedure was to add in triplicate each immunoglobulin standard or sample to appropriately coated wells. All samples and standards were prepared in PBS containing 0.5% bovine serum albumin. A 0.1-ml sample of purified human sIgA, monkey IgG, or human IgM, in twofold dilutions ranging from 1,000 to 31.25 ng/ml, was added to anti-immunoglobulin-coated wells of respective plates for later construction of a standard curve. A 0.1-ml sample of diluted urine or serum was added to *E. coli*-coated wells of each plate. All plates were then incubated for 30 min at room temperature and washed three times with PBS–0.05% Tween 20.

The final steps in the assay were the addition of horseradish peroxidase-conjugated anti-immunoglobulin and substrate. Samples of 0.1 ml of peroxidase-conjugated IgG fractions of goat anti-human sIgA, goat anti-monkey IgG, or goat anti-human IgM diluted 1/1,000, 1/2,000, or 1/3,000, respectively, in PBS-bovine serum albumin were added to both standard and test wells of appropriate plates. The plates were incubated for 45 min at room temperature and then washed with PBS–0.05% Tween 20. A 0.1-ml sample of substrate-chromogen (0.01% H_2O_2 and 5 mM *o*-phenylenediamine in citrate buffer) was added to all wells, and the plates were incubated for 5 min at room temperature. The reaction was stopped by adding 0.15 ml of 1 N H_2SO_4 to each well, and the optical density at 490 nm of each well was determined by using a plate reader (Microelisa; Dynatech Laboratories, Inc., Alexandria, Va.).

Processing of data and statistics. All samples and standards were assayed in triplicate. The optical density of wells that were coated with either anti-immunoglobulin or *E. coli* and treated with peroxidase-conjugated anti-immunoglobulin were subtracted from all values as the mean background at an optical density of 490 nm. Data that came directly from the plate reader were processed by using a software program (Immunosoft; Dynatech). A standard curve relating the optical density at 490 nm and immunoglobulin concentration was constructed from the absorbance values that were obtained from wells containing known amounts of immunoglobulin. The correlation coefficient of the regression line fitted to these datum points was most often >0.990. Estimates of *E. coli*-specific IgA, IgM, and IgG in urine or serum samples were made from this standard curve.

Statistical comparisons between pre- and postinfection values were made by using a two-tailed, paired *t* test. Differences between means of independent groups were analyzed by a two-tailed Student's *t* test. A *P* value of <0.05 was considered to be statistically significant.

RESULTS

Localization of induced UTI. We observed no postinfection increases in ESR, CRP, or total leukocyte counts for any group of infected monkeys. No animals exhibited hematuria. A retrospective study for pyuria based on the presence of leukocyte esterase (6) revealed transient increases in leukocyte numbers in urine at 1 to 2 weeks following induction of UTI. Histopathology of similarly infected monkeys several weeks after infection resolution revealed no abnormal renal pathology (unpublished data).

Time course for cystitis resolution. Untreated cynomolgus monkeys were infected intravesically with one of the follow-

ing *E. coli* dose and strain combinations: 4×10^7 strain 1677, 4×10^9 strain 1677, or 4×10^9 strain JR1. Suprapubic urine samples obtained at weekly intervals were assayed for viable *E. coli* to establish that an infection had been induced and to measure its duration. The course of UTI for each experimental group is shown in Fig. 1. The instillation of live bacteria into the bladder on day 0 led to a high level of bacteriuria in all animals by day 2. The infection tended to increase for each group until day 7, after which the numbers of bacteria recovered in the urine steadily decreased. When either 4×10^7 or 4×10^9 *E. coli* 1677 were used to induce cystitis, the infections resolved at approximately the same rate until they reached an endpoint of ≤1 CFU/mg of creatinine at 21 to 28 days postinfection (comparison of mean resolution times: $P = 0.32$). The cystitis induced by 4×10^9 *E. coli* JR1 was slower to clear, and all animals in this group were not completely free of bacteria in the urine until day 42. Mean resolution time for JR1-infected monkeys was 35 days, and this was significantly longer than for monkeys infected with either 4×10^7 1677 (18.8 days; $P < 0.01$) or 4×10^9 1677 (21.0 days; $P = 0.01$).

Urinary antibody response. The anti-*E. coli* antibody response in urine was monitored for each experimental group. The kinetics of anti-*E. coli* urinary sIgA and IgG responses in monkeys infected with different *E. coli* strains and doses is shown in Fig. 2. A pattern of increasing, maximal, and decreasing sIgA antibody production was observed following infection with 4×10^9 *E. coli* 1677 or 4×10^9 *E. coli* JR1. Antibody levels at day 23 (strain 1677) and days 10 to 31 (strain JR1) were significantly elevated ($P < 0.01$ to 0.04) over preinfection values. The highest antibody responses to strains 1677 and JR1 occurred at 25 and 31 days, respectively, both of which coincided with rapidly declining numbers of bacteria in the urine. An inoculum of 4×10^7 1677 did not appear to stimulate significant sIgA production in urine.

Significant IgG responses in urine, relative to preinfection levels ($P < 0.05$), were observed by days 38 and 45 in animals infected with 4×10^7 *E. coli* 1677. Monkeys infected with 4×10^9 JR1 had significant IgG responses on day 3 and days 31 to 45. No increases in anti-*E. coli* IgG were detected in urine in response to an infection with 4×10^9 *E. coli* 1677.

No significant increases in postinfection anti-*E. coli* IgM were noted in urine for any dose and strain combination. IgM levels in urine before and after cystitis induction were ordinarily less than or equal to 0.1 μg/24 h, which is at the limit of detectability of this ELISA. Therefore, we could not reliably infer increases or decreases in IgM at or below this level of sensitivity.

Serum antibody response. The cystitis caused by strains 1677 or JR1 elicited significant increases in anti-*E. coli* immunoglobulins in serum. Serum samples were not collected from animals infected with 4×10^9 *E. coli* 1677. Following cystitis induced by 4×10^7 1677, there were significant increases in IgM in serum at weeks 2 ($P = 0.04$) and 3 ($P = 0.03$) of the infection (Fig. 3). An overall increase in IgG anti-*E. coli* antibody was also evident during the course of the infection, and statistically significant increases were observed from weeks 5 through 10 after induction (P values ranged from 0.01 to 0.02). Peak IgG values occurred at weeks 5 and 6. Anti-*E. coli* IgA in serum appeared to increase as a result of the cystitis, but the elevations were not significant because of large variability between individual animals (Fig. 3). As with urinary immunoglobulins, levels of serum antibody to the infecting bacteria were greatest as urinary bacterial numbers were declining.

Increases in *E. coli*-specific antibody were also seen in monkeys that developed cystitis after infection with 4×10^9 *E. coli* JR1 (Fig. 4). Serum IgM antibodies to the infecting bacteria tended to increase over the course of the infection, but none of the time points had mean immunoglobulin levels that were statistically different from preinfection values. Anti-*E. coli* IgG and IgA levels rose significantly after cystitis induction. An IgG response was first observed 1 week after cystitis was induced, after which it increased to its highest level over the next 6 weeks and then declined (P values ranged from <0.01 to 0.05). Production of anti-*E. coli* IgA began during week 1 of the infection, was maximum at 3 weeks, and persisted at all later time points measured ($P <$ 0.01 to 0.03). It can again be noted (Fig. 4) that CFU in urine decreased as the levels of immunoglobulins in serum increased.

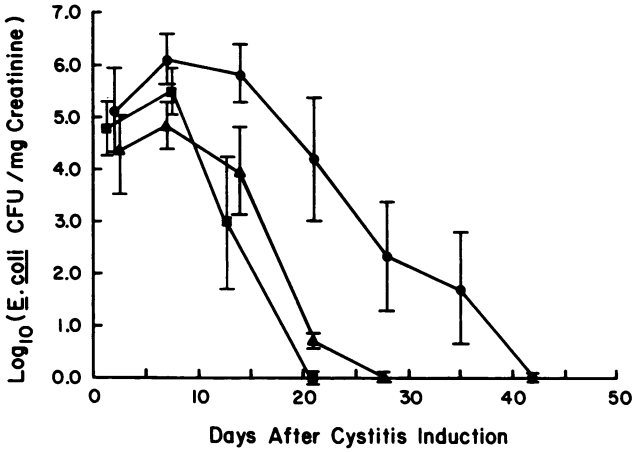

FIG. 1. Resolution of induced cystitis in monkeys infected with *E. coli* 1677 or JR1. Viable *E. coli* isolates were administered intravesically to three groups of animals on day 0. Doses of 4×10^7 *E. coli* 1677 (■), 4×10^9 1677 (▲), or 4×10^9 JR1 (●) were administered. The level of bacteriuria, determined as the number of CFU present in a urine aspirate, was monitored in each monkey. Each value represents the mean ± standard error of the mean for four to five animals.

DISCUSSION

In this study we examined the natural resolution of a UTI induced in primates by uropathogenic *E. coli* and the antibody responses elicited by that infection. Because it is possible that the antibody classes produced and their distribution in serum or secretions might vary with the location of infecting bacteria, it is important to localize the induced infection. In the absence of direct microbiological assessment of the kidney, other criteria such as ESR, levels of CRP in serum, and leukocytosis can be used to evaluate the level of a UTI in primates (11, 14, 28). Increases in ESR, CRP, and peripheral leukocyte numbers suggest upper tract involvement; bacteriuria and pyuria without leukocytosis are characteristic of cystitis in monkeys (14). The animals that we studied showed evidence of transient pyuria during resolution of their infections but did not have accelerated ESRs, CRP levels greater than 6 μg/ml, or leukocytosis. Thus, within the limitations of the localization tests employed, the *E. coli* strains tested most likely induced a cystitis, but we cannot totally exclude the possibility of mild upper tract involvement in some animals.

Our results demonstrate that cystitis caused by *E. coli* 1677 or JR1 can be resolved solely by host defenses without

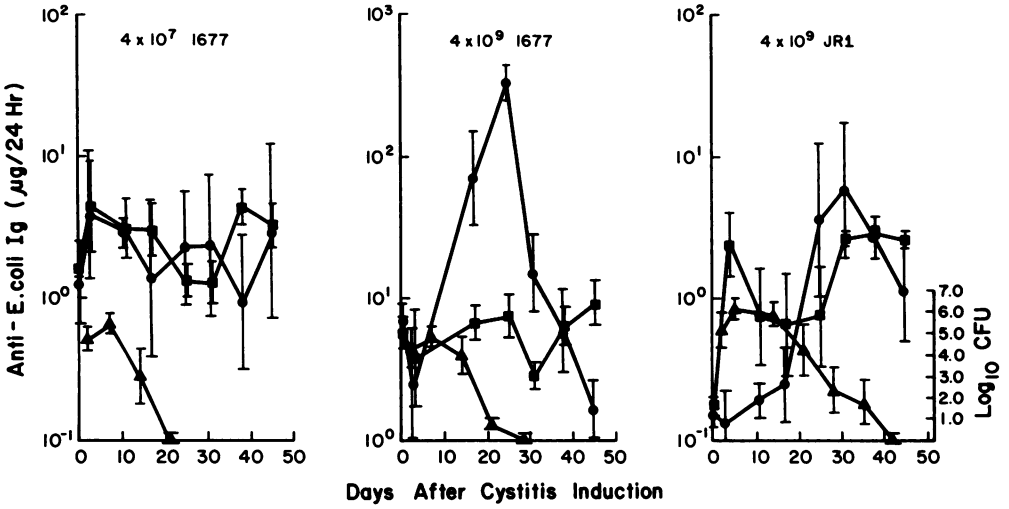

FIG. 2. Urinary antibody response of cynomolgus monkeys during resolution of cystitis induced by *E. coli* 1677 or JR1. Cystitis was initiated on day 0 by administering the indicated dose and strain of viable *E. coli*. Anti-*E. coli* sIgA (●) and IgG (■) antibody levels were determined on 24-h urine samples obtained during cystitis resolution. *E. coli* CFU (▲) in urine aspirates were measured at regular intervals until the infection resolved. Log_{10} CFU is the log_{10} (CFU of *E. coli*/mg of creatinine). Each immunoglobulin or CFU value represents the geometric mean ± standard error of the mean for groups of four to five monkeys.

the use of antibiotics. When any of three dose and strain combinations were tested in our primate model, similar levels of bacteriuria were induced within 1 week. Each animal cleared its infection over the next several weeks, but it was apparent that resolution rates for the two strains differed, because the JR1-induced cystitis persisted 2 weeks longer than that caused by either infecting dose of strain 1677. The reason for these apparent differences in resolution rates could be attributed to any of the myriad factors that comprise host-parasite interactions: bladder emptying (2), phagocytosis by polymorphonuclear neutrophils (6), antibody production, variations in suspectibility caused by endocrine factors (12, 15), the number of *E. coli* receptors on uroepithelial cells (16), possible immunologic unresponsiveness to *E. coli* (18), or *E. coli* fimbrial phase variation in vivo (9).

In addition to delineating the time frame for resolution of cystitis in primates, we have also specifically addressed the questions of when antibody is produced in response to a predominantly lower tract infection and which antibody classes are induced. In studies in which a similar ELISA method was used for immunoglobulin measurement, Rene et al. (13) found that anti-fimbrial IgM and IgG antibody titers in serum were elevated in some women with cystitis; however, a urinary antibody response to fimbrial antigens was not observed. The differences in findings between the two studies may relate to the fact that we measured antibody to whole bacteria rather than to purified pili and to the fact that the bacteriuria in this monkey model lasted longer than the bacteriuria in patients studied by Rene et al. (13). Increases in total sIgA and IgG in urine have been reported to occur in children and adults with UTIs (20, 22), but these studies did

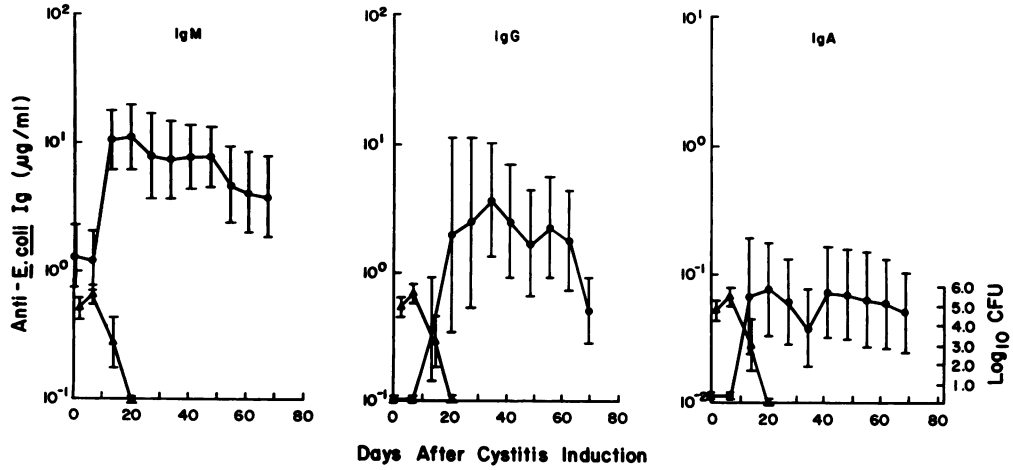

FIG. 3. Serum antibody response of cynomolgus monkeys during resolution of cystitis induced by 4×10^7 *E. coli* 1677. Anti-*E. coli* IgM, IgG, and IgA levels (●) were determined at 7-day intervals following induction of cystitis on day 0. *E. coli* CFU (▲) in urine aspirates were measured at regular intervals until the infection resolved. Log_{10} CFU is the log_{10} (CFU of *E. coli*/mg of creatinine). Each immunoglobulin or CFU value represents the geometric mean ± standard error of the mean for groups of five animals.

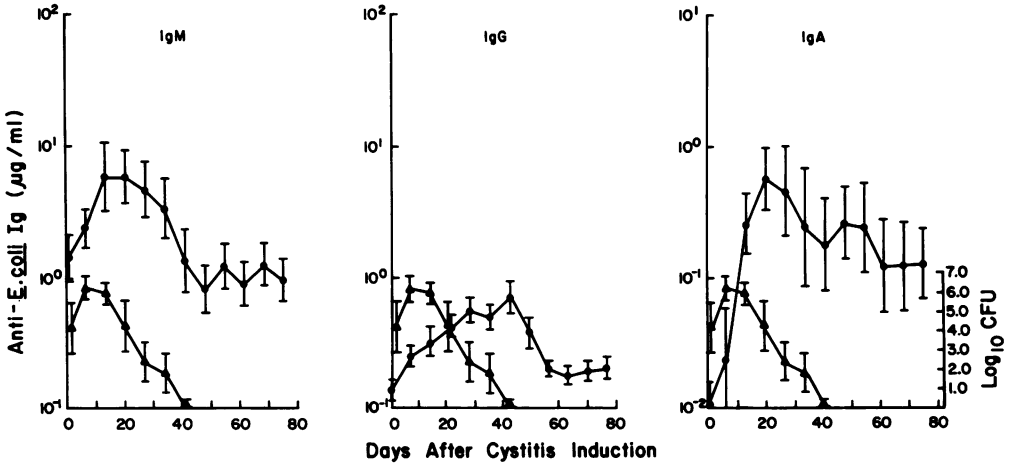

FIG. 4. Serum antibody response of cynomolgus monkeys during resolution of cystitis induced by 4×10^9 *E. coli* JR1. Anti-*E. coli* IgM, IgG, and IgA levels (●) were determined at 7-day intervals following induction of cystitis on day 0. *E. coli* CFU (▲) in urine aspirates were measured at regular intervals until the infection resolved. Log_{10} CFU is the log_{10} (CFU of *E. coli*/mg of creatinine). Each immunoglobulin or CFU value represent the geometric mean ± standard error of the mean for groups of five animals.

not quantitate antibody levels against the infecting bacteria or delineate the time course of local and systemic antibody production. Using a sensitive, quantitative ELISA, we have been able to measure both urinary and serum anti-*E. coli* antibodies induced by a lower tract UTI. Urinary antibodies arising in untreated, nonhuman primates during resolution of the cystitis were primarily sIgA and IgG. Both *E. coli* 1677 and JR1 stimulated production of these two immunoglobulin types. For strain 1677, however, significant increases in both immunoglobulin classes were not seen at each infecting dose tested. The local immune response was accompanied by systemic increases in IgM, IgG, and IgA against the infecting bacteria. Again, both strains elicited anti-*E. coli* antibodies of each class, but significant increases in all classes were not observed for each strain. From these results we believe that in the normal course of cystitis resolution, the urinary antibody response in primates is comprised of IgA and IgG antibodies, and the systemic response includes these classes as well as IgM.

Generation of a local antibody response to infecting *E. coli* may be helpful to the host through the mechanism by which antibodies in urine coat bacteria and subsequently lessen bacterial adherence to bladder epithelial cells (21). Our observation that the infections caused by 4×10^9 *E. coli* 1677 or JR1 cleared most rapidly (days 15 to 27 for strain 1677; days 16 to 24 for strain JR1) when the urinary sIgA response began and increased to its maximum (days 10 to 23 for strain 1677; days 17 to 30 for strain JR1) is consistent with this model of cystitis resolution because antibody production precedes or parallels decreasing bacterial numbers. Additional studies that would monitor the presence of antibody-coated bacteria in urine might be helpful in elucidating the exact role of sIgA in resolving the infection. IgG against infecting bacteria in urine may have been somewhat less important than sIgA because IgG tended to be produced somewhat later in the infection. Also, in the one group of monkeys in which IgG levels increased within the first week (those given 4×10^9 JR1), the cystitis did not resolve more rapidly than in animals which gave no evidence of an early IgG response to the infection (those given either dose of strain 1677).

Systemic anti-*E. coli* antibody production paralleled urinary antibody responses to an induced cystitis in primates. The role of these serum antibodies in resolution of the infection is speculative. Serum immunoglobulins, particularly IgA, could become directly involved by entering the urine collecting system (1) or by being secreted by mucosal epithelial cells into the bladder, as described previously for sIgA in bile (3). These processes conceivably could be enhanced during an infection (1) to increase the normally low transfer of IgA from blood to secretions (27). IgG in serum is apparently fragmented before glomerular filtration and accumulates in the urine as lower molecular weight breakdown products (5). It is thus unlikely that IgG in serum would be directly involved in cystitis resolution unless the infection process were to alter filtration so that intact molecules could pass through glomeruli. Because we did not observe increases in IgM in urine, IgM in serum probably does not play a role in resolving the infection; however, systemic IgM and IgG production may indicate that local immune responses are taking place. Detailed analyses of the origins and locations of immunoglobulin-producing cells will be necessary to define the exact relationship between urinary and serum antibodies that are produced during cystitis resolution.

In summary, we used a nonhuman primate model to study naturally occurring cystitis resolution and generation of immune responses to infecting bacteria. Antibody production in both serum and urine followed an induced *E. coli* cystitis and appeared to correlate with declining bacteriuria. These data quantitatively define primate antibody responses during and after cystitis.

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LITERATURE CITED

1. **Abramowsky, C. R., and G. L. Swinehart.** 1986. Secretory immune responses in human kidneys. Am. J. Pathol. **125**:571–577.
2. **Cox, C. E., and F. Hinman.** 1961. Experiments with induced bacteriuria, vesical emptying and growth on the mechanism of bladder defense to infection. J. Urol. **86**:739–748.
3. **Delacroix, D. L., H. J. F. Hodgson, A. McPherson, C. Dive, and J. P. Vaerman.** 1982. Selective transport of polymeric IgA in

bile. J. Clin. Invest. **70**:230–241.
4. **Evans, D. G., D. J. Evans, and W. Tjoa.** 1977. Hemagglutination of human group A erythrocytes by enterotoxigenic *Escherichia coli* isolated from adults with diarrhea: correlation with colonization factor. Infect. Immun. **18**:330–337.
5. **Franklin, E. C.** 1959. Physiochemical and immunologic studies of gamma globulins of normal human urine. J. Clin. Invest. **38**: 2159–2167.
6. **Gillenwater, J. Y.** 1981. Detection of urinary leukocytes by chemstrip-L. J. Urol. **125**:383–384.
7. **Gillon, G., M. Small, O. Medalia, and M. Aronson.** 1984. Sequential study of bacterial clearance in experimental cystitis. J. Med. Microbiol. **18**:319–326.
8. **Hopkins, W. J., J. Jensen, D. T. Uehling, and E. Balish.** 1986. *In vitro* and *in vivo* adherence of uropathogenic *Escherichia coli* strains. J. Urol. **135**:1319–1321.
9. **Hultgren, S. J., T. N. Porter, A. J. Schaeffer, and J. L. Duncan.** 1985. Role of type 1 pili and effects of phase variation on lower urinary tract infections produced by *Escherichia coli*. Infect. Immun. **22**:247–254.
10. **Jensen, J., D. T. Uehling, K. Kim, K. Seagren-Rasmussen, and E. Balish.** 1984. Enhanced immune response in the urinary tract of the rat following vaginal immunization. J. Urol. **132**:164–166.
11. **Jodal, V., V. Lindberg, and K. Lincoln.** 1975. Level diagnosis of symptomatic urinary tract infections in childhood. Acta Pediatr. Scand. **64**:201–208.
12. **Reid, G., H. J. L. Brooks, and D. F. Bacon.** 1983. In vitro attachment of *Escherichia coli* to human uroepithelial cells: variation in receptivity during the menstrual cycle and pregnancy. J. Infect. Dis. **148**:412–421.
13. **Rene, P., M. Dinolfo, and F. J. Silverblatt.** 1982. Serum and urogenital antibody response to *Escherichia coli* pili in cystitis. Infect. Immun. **38**:542–547.
14. **Roberts, J. A., G. M. Suarez, B. Kaack, G. Kallenius, and S. B. Svenson.** 1985. Experimental pyelonephritis in the monkey. VII. Ascending pyelonephritis in the absence of vesicoureteral reflux. J. Urol. **133**:1068–1075.
15. **Schaeffer, A. J., S. K. Amundson, and L. N. Schmidt.** 1979. Adherence of *Escherichia coli* to human urinary tract epithelial cells. Infect. Immun. **24**:753–759.
16. **Sobel, J. D., and D. Kaye.** 1986. Enhancement of *Escherichia coli* adherence to epithelial cells derived from estrogen-stimulated rats. Infect. Immun. **53**:53–56.
17. **Sorensen, C. H.** 1982. Secretory IgA enzyme immunoassay. Application of a model for computation of the standard curve. Scand. J. Clin. Lab. Invest. **42**:577–538.
18. **Stamey, T. A., N. Wehner, G. Mahara, and M. Condy.** 1978. The immunological basis of recurrent bacteriuria: role of cervicovaginal antibody in enterobacterial colonization of the introital mucosa. Medicine **57**:47–56.
19. **Svanborg-Eden, C., B. Erickson, and L. A. Hanson.** 1977. Adhesion of *Escherichia coli* to human uroepithelial cells in vitro. Infect. Immun. **18**:797–774.
20. **Svanborg-Eden, C., R. Kulhavy, S., Marild, S. J. Prince, and J. Mestecky.** 1985. Urinary immunoglobulins in healthy individuals and children with acute pyelonephritis. Scand. J. Immunol. **21**: 305–313.
21. **Svanborg-Eden, C., and A.-M. Svennerholm.** 1978. Secretory immunoglobulin A and G antibodies prevent adhesion of *Escherichia coli* to human urinary tract epithelial cells. Infect. Immun. **33**:790–797.
22. **Uehling, D., and R. Hong.** 1977. Quantitiation of urinary immunoglobulins by a double antibody technique. Invest. Urol. **15**: 39–41.
23. **Uehling, D. T., D. D. Barnhart, and C. V. Seastone.** 1968. Antibody production in urinary bladder infection. Invest. Urol. **6**:211–222.
24. **Uehling, D. T., W. J. Hopkins, J. Jensen, and E. Balish.** 1987. Vaginal immunization against induced cystitis in monkeys. J. Urol. **137**:327–329.
25. **Uehling, D. T., J. Jensen, and E. Balish.** 1982. Vaginal immunization against urinary tract infection. J. Urol. **128**:1382–1384.
26. **Uehling, D. T., K. Mitzutani, and E. Balish.** 1978. Effect of immunization on bacterial adherence to urothelium. Invest. Urol. **16**:145–147.
27. **Underdown, B. J., and J. M. Schiff.** 1986. Immunoglobulin A: strategic denfense initiative at the mucosal surface. Annu. Rev. Immunol. **4**:389–417.
28. **Wientzen, R. L., G. H. McCracken, M. L. Petruska, S. G. Swinson, B. Kaijser, and L. Hanson.** 1979. Localization and therapy of urinary tract infections in childhood. Pediatrics **63**:467–474.