Attenuation of Antibody Response to Acute Pyelonephritis by Treatment with Antibiotics

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While acute pyelonephritis is known to elicit an antibody response, it is also known that a patient who has had pyelonephritis once is susceptible to recurrent renal infection. Using our experimental model of pyelonephritis in the monkey, we tested whether antibiotic therapy of the acute disease would affect the antibody response. We found that it did, because antibiotic therapy beginning 72 h after bacterial inoculation attenuated the antibody response so that rechallenge 3 months later produced acute pyelonephritis and prolonged bacteruria. The animals with untreated infection had an antibody response that lasted a sufficient period of time to prevent acute pyelonephritis after renal challenge. We have confirmed that antibody titers against P fimbriae are protective, and to a degree, this protective effect may be abrogated by antibiotic therapy.

Acute pyelonephritis is known to elicit an antibody response to a number of bacterial antigens (4, 7, 13, 16, 20). To one degree or another, this antibody response provides protection against reinfection. It is known, however, that a patient who has pyelonephritis is susceptible to reinfection in the future. At least one aspect of this susceptibility is the loss of systemic or local immunity to bacterial antigens. P-fimbriated Escherichia coli cause approximately 90% of the cases of uncomplicated pyelonephritis (1). Since antibodies to P fimbriae are elicited in experimental infections (3, 6) and since these antibodies have been shown to be protective against experimental infections (12, 14), it is logical that a decrease in the titer to this antigen might increase the susceptibility to infection, by allowing colonization and adherence of these E. coli to the urothelium.

The majority of all urinary tract infections are treated with antimicrobial chemotherapy. Although the time course from initiation of infection to antibiotic treatment is, of necessity, variable, an antibody response to the infection occurs nonetheless (13, 20). The degree of antibody response is dependent on a number of factors, including the amount of antigen exposure over a given period of time (5). Anything done to decrease the antigen exposure to the immune system logically would attenuate the antibody response.

We have previously shown that a single episode of acute pyelonephritis from a P-fimbriated E. coli strain elicits an antibody response to P fimbriae (11). We have also shown that immunization with P fimbriae elicits an antibody response which is protective against bacterial challenge (3, 8). We used the same model to study the antibody responses in monkeys with treated and untreated infections.

MATERIALS AND METHODS

Animals. Sixteen cynomolgus monkeys (Macaca fascicularis) were used in this study. They had free access to monkey chow and water. All experimental procedures were done after the monkeys were tranquilized with a mixture of ketamine and xylazine.

Bacterial characteristics. The strain of E. coli used in this study (JR1) was isolated from a patient with acute pyelonephritis and has been used extensively in our laboratory. It is serotype O4:H1, is K nontypeable, and has P fimbriae and type 1 fimbriae when it is grown in tryptose broth. The bacteria are susceptible to cefonicid. The bacteria were grown for 24 h on blood agar; this was followed by an 18-h culture in tryptose broth to produce the inoculum. The bacteria were centrifuged, washed with sterile saline, and diluted to 2 × 10⁸ bacteria per ml. This inoculum was combined with ¹³¹I-labeled hippuran and 10% sodium diatrizoate to achieve a final inoculum concentration of 10⁸ bacteria per ml.

Method of infection. All kidneys were infected by use of a cystoscopically introduced ureteral catheter. The inoculum volume of 0.6 ml was introduced under radiographic control to ensure that pyelotubular back flow occurred. In addition, blood samples taken at 1, 10, and 60 min were cultured and counted in a gamma counter. These samples showed that pyelovenous inoculation had not occurred.

Protocol. Animals were initially infected in one kidney and were randomly assigned to one of two treatment groups. In monkeys in group 1, the first kidney infection was allowed to run its course. In group 2 monkeys, the first kidney infection was treated 72 h after infection with cefonicid administered intramuscularly each day at a dose of 0.015 g/kg of body weight per day for 10 days. Cefonicid was supplied gratis by Smith Kline & French Laboratories, Philadelphia, Pa.

Three months after the first infection the animals in both groups were reinfected, and each group was subdivided into one of two groups so that the inoculum for the second infection was introduced into the same kidney or the opposite kidney as that of the first infection. Therefore, the study had four groups of monkeys, each of which had two renal bacterial inoculations, as follows: (i) nontreated, same kidney inoculated; (ii) nontreated, opposite kidney inoculated; (iii) cefonicid treated, same kidney inoculated; and (iv) cefonicid treated, opposite kidney inoculated.

For both infections, blood and urine were obtained at 0, 2, 24, 48, and 72 h and at 1, 2, 3, and 4 weeks after infection. The second infection was followed for 4 weeks, and the animal was then sacrificed humanely.

Bacteriology and immunology. Urine was obtained by suprapubic bladder puncture for culture at 0, 2, 24, 48, and

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72 h and at 1, 2, 3, and 4 weeks after infection. The undiluted urine was used for tube dilutions in tryptose broth; 1 ml was centrifuged at 1,800 × g for 10 min, and the sediment was cultured by using a 1-μl loop. Identification of organisms was accomplished by using plates from Analytab Products. Venous blood was taken at these same times for leukocyte, erythrocyte, and differential leukocyte counts; and serum was stored at −75°C for a later determination of anti-P-fimbriae and O-antibody titers by enzyme-linked immunosorbent assay.

Enzyme-linked immunosorbent assay. Ninety-six-well plates (Linbro) were coated with 10 μg of either lipopolysaccharide or fimbriae per ml and were incubated in the cold overnight. After washing four times in phosphate-buffered saline and 0.05% Tween 20, they were overlayed with 5% bovine serum albumin in phosphate-buffered saline and were incubated for 1 h at 37°C. Serially diluted serum was applied next, and the plates were incubated for 3 h at 37°C. After washing four times, anti-monkey immunoglobulin G conjugated to peroxidase was applied, and the plate was incubated for 45 min at room temperature. The plates were washed four times with saline-Tween before the o-phenylenediamine substrate solution was applied. After incubation at 37°C for 15 to 20 min, the plates were read for their A400 on an enzyme immunosassay reader. Control wells containing no serum were run on each plate simultaneously.

Quantitative renal scans. Renal functions were determined by using quantitative renal scans to allow longitudinal studies of the effect of infection without invasive techniques. Standard clearance studies to determine differential renal function would require obstruction of the ureteral catheters, which might have complicated the infection. Monkeys were positioned over the NaI crystal of a General Electric scintillation camera, and 50 μCi of 131I-labeled hippuran was given intravenously. An ADAC computer program provided information about isotope uptake, since renal regions of interest could be indicated and an appropriate area for background subtraction could be designated at the 1- to 2-min time interval (14). At that time, radionuclide would still be within the renal parenchyma but not in the collecting system. The accuracy and reproducibility of those individual renal function measurements have been reported previously (9).

Sacrifice. Animals were sacrificed by an overdose of barbiturate 4 weeks after the second infection. The kidneys were removed by a sterile technique and weighed. Half of each kidney was used for culture; the other half was fixed in 10% Formalin, embedded, and stained with hematoxylin-eosin for pathological evaluation.

Pathology. The standard section of tissue was taken transversely through the midportion of the kidney so that it contained the papilla, medulla, and cortex. Histological sections were examined by the pathologist in a double-blind manner by our established histologic parameters (10). Acute pyelonephritis is associated with a marked inflammatory exudate in areas of bacterial growth, tubular damage, and death with microabscess formation. The reparative response with fibrosis and scarring and a mononuclear cell infiltrate (especially in the subcapsular, pelvic, and periglomerular regions) were considered to be subacute to chronic pyelonephritis and are the typical late findings following an untreated infection in our experimental model (8). Severity of change was scored on a scale of 1 to 4, with 4 being the most severe. The sections were rated for the following parameters: tubular and/or interstitial neutrophils, tubular and/or interstitial mononuclear cells, fibrosis, scarring, pelvic infiltrate, glomerular change, subcapsular invasion, tubular dilatation, and tubular atrophy. In addition, the percentage of the kidney involved was estimated on the basis of the amount of kidney involved on the hematoxylin-eosin slide of the standard section.

Statistics. Data were subjected to a t test for either paired or independent samples and a two-way analysis of variance.

RESULTS

The first untreated infection lasted a mean of 10.8 ± 8.2 days in the eight untreated animals, whereas bacteriuria was absent after day 3 in the animals treated with cefonicid. Bacteriuria was defined as any bacterial growth of the same

FIG. 1. Kidney function studies (second infection in the opposite kidney from the first infection). The studies were done with a scintillation camera and ADAC computer by using 131I-labeled hippuran to determine effective renal plasma flow. Note that in previously treated animals, the second infection showed findings typical of acute pyelonephritis, including decrease in percent function and effective renal plasma flow, increase in peak time, and delayed clearing of the radionuclide. Previously untreated animals did not show the functional changes of acute pyelonephritis at the time of the second infection. • untreated monkeys, ○, monkeys treated with cefonicid. ANOVA, analysis of variance.
E. coli strain on culture, including culture of the spun sediment. The second infection in the group of animals who had received no treatment for the first infection lasted 3.9 ± 4.3 days, whereas the animals who had been treated with cefonicid at the time of the first infection had bacteriuria for 11.8 ± 5.3 days. Some local effect was apparent, since, when the animals were not treated, the second infection of the same kidney that was previously infected produced bacteriuria for 1.2 ± 1.1 days; however, if the opposite kidney was infected, bacteriuria lasted for 6.5 ± 4.7 days. The same local effect was found in the animals treated with cefonicid at the time of the first infection. If the same kidney was again infected, bacteriuria lasted for 9.5 ± 4.7 days; infection of the opposite kidney produced bacteriuria for 14 ± 4.9 days. The difference between treatment and no treatment showed a significant effect at the time of the first infection and the length of bacteriuria, since infection in all the treated animals cleared by day 4. At the time of the second bacterial challenge, significant protection against infection was found when the animals were not treated, as opposed to the findings in those that were treated with the antibiotic. The mean time difference between bacteriuria from the first and second infections in the untreated animals was −7.43 days \( (P = 0.035) \); for the treated animals it was +9 days \( (P = 0.0025) \). In other words, when the first infection is compared with the second infection, it took 7.43 days less for the infection to clear in untreated animals.

Renal function studies also showed marked protection from the second infection of the opposite kidney in untreated as opposed to treated animals, as shown in Fig. 1. There was very little effect on peak time, clearance time, or percentage of function in the animals whose first infections were not treated, whereas the animals in the treated group showed functional changes on the quantitative renal camera study at the second infection that were almost as severe as those at the time of the first untreated infection.

At the time of autopsy, the weights of the two kidneys were compared. When the second infection was in the same kidney as that of the first infection, there was a significant decrease in total renal weight in the untreated animals, which would be expected after two infections; however, there was no significant difference in kidney weights in the animals who had received cefonicid. When the second infection was in the opposite kidney from that of the first infection, there was no difference in the weights of the kidneys in the untreated and treated animals, as would be expected, since both kidneys had been infected.

Pathology scores showed differences only when a comparison of the second infection in the opposite kidney was made to determine whether antibiotic treatment as opposed to no treatment had an effect. There was no significant difference between the pathology scores of the two kidneys when the second infection was in the same kidney. When the second infection was in the opposite kidney of the treated animals, there was a significant increase in interstitial mononuclear cells, subcapsular involvement, and tubular atrophy and a marked increase in the percentage of kidney involved, as shown in Table 1. This shows that antibiotic treatment of the first infection was followed by an increase in the pathologic changes from the second infection if the opposite kidney was infected.

Antibody titers were significantly different in the two groups whether the same or opposite kidney had been infected (Table 2). Titers to P fimbriae in the untreated animals were at a level that was expected to be protective against repeat bacterial challenge, as opposed to the low titers in animals whose treatment began at 72 h after infection during the first infection. The same elevation in titers to the O antigen was not seen. No significant difference was seen in the treated versus the nontreated group during the second infection, either preinfection or at any other measured interval, when monkeys were followed up to 4 weeks.
DISCUSSION

In considering this study, a number of points should be emphasized. The first is that antibiotic therapy attenuates the protective antibody response in monkeys with experimental pyelonephritis. This is evident when the infection is treated within 3 days of initiation of the renal infection. Pathological effects were not significant, in general, because each group (treated and nontreated) was subjected to infection with little available protection (e.g., antibodies or specific antibody). It is not known what other effects would be seen if the treatment time interval were altered or what the effect of this phenomenon would be in the clinical situation, because the exact time of initiation of infection is never known. It has been suggested that the length of the incubation period, i.e., the time from exposure to clinical illness, affects the immune response (5). Results of this study indicate that the immune response may be altered by medical therapy, which may, in turn, alter the body's ability to attain an immune status that is protective. We assume that this is because of a decrease in exposure time to the bacterial antigens. Since it is known that immunization with P fimbriae is efficacious in producing an immune response (15), vaccination with P fimbriae might be used to augment immunity in antibiotic-treated patients.

The second point is that there is a local effect in the initially infected kidney, in addition to the humoral (serum) antibody response. This may be a local effect of secretory immunoglobulin A produced in the renal unit itself, as suggested by those who studied urinary excretion of antibody classes after acute infection (2, 17, 19). Specific or nonspecific activation of resident macrophages as well as other elements of the cellular immune response in the reticuloendothelial system may occur in the infected organ to limit the damaging effects of reinfection. A mechanism of local immunity in investigators offering protection against certain infections has been implicated by some (2, 18). Therefore, it seems that this mechanism would be operative whether there is antimicrobial therapy or not, and thus, it may not be as dependent on the time of exposure to a particular antigen.

Urinary tract infections occur because of a number of factors both in the infecting organism and in the host. Humoral immunity is just another element to be considered when studying the pathogenesis of this disease. As stated above, P fimbriae antibody titers were protective, at least in our model. It is difficult to draw an exact analogy with human pyelonephritis, however.

The next logical step in the investigation of this phenomenon would be to stimulate active immunity to P fimbriae or perhaps other antigens at the time of treatment of acute pyelonephritis with antibiotics, since it has already been shown that active immunization elicits protective antibody. Whether this will diminish the degree of renal damage or whether lasting protective immunity will be achieved remains unclear. For certain high-risk individuals, this could potentially prove to be beneficial.

REFERENCES


TABLE 2. Anti-P fimbriae titers

<table>
<thead>
<tr>
<th>First infection:</th>
<th>Mean titer at the following times after second infection*</th>
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<tr>
<td></td>
<td>0 h*  48 h  72 h  1 wk  2 wk  3 wk  4 wk</td>
</tr>
<tr>
<td>Not treated</td>
<td>132,125 7,250 7,000 7,375 19,000 53,750 48,370</td>
</tr>
<tr>
<td>Treated</td>
<td>7,875 2,200 1,813 4,778 6,571 9,500 19,750</td>
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* The significance of treatment (P = 0.0062), time (P = 0.211), and the interaction of treatment × time (P = 0.0509) was calculated by two-way analysis of variance.

* Significance was P = 0.0362 by the t test for independent samples.

