Prevention of Experimental Hematogenous and Retrograde Pyelonephritis by Antibodies Against Enterobacterial Common Antigen

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Rabbits were immunized with common antigen (CA) derived from Salmonella typhimurium. Animals with CA hemagglutinin titers of 1-320 to 1-81,920 were injected with 10^9 viable Proteus mirabilis via the retrograde and hematogenous routes. Nonimmune control groups were challenged similarly. From the retrogradely challenged groups sacrificed at 4 weeks, pyelonephritis was found in 89% of the control animals but not in those immunized. Bacteriuria was present in 89% of the controls but in only 61% of the immunized group. Hematogenously challenged, immune animals sacrificed at 6 weeks did not show histological evidence of renal pathology, and only 6% had bacteriuria. Eighty-six per cent of the nonimmune controls showed both pyelonephritis and bacteriuria. The protective effect is specific because of the following. (i) Active and passive CA immunization did not prevent pyelonephritis due to Pseudomonas aeruginosa which does not produce this antigen. (ii) Passive immunization with a CA antiserum conferred protection against a P. mirabilis challenge. (iii) Passive administration of an antiserum from which CA antibodies had been differentially absorbed abolished the protective activity.

There is very limited information available on the immune response and the role of O antibodies in protection against urinary tract infections (1, 3, 9). Also, because of the persistence or recurrence of urinary tract infections with a strain of the same serological type in patients with high titers of homologous O antibodies, the value of host immunoglobulins versus the somatic O antigen of such bacteria has been questioned by many investigators. Numerous enteric bacteria, including Escherichia, Enterobacter, Proteus, Serratia, Salmonella, and Shigella, share a common antigen (CA), in addition to the well known O, H, and Vi antigens which they may have. The common antigen first described in 1962 by Kunin et al. (8) had probably escaped detection for many years because it could be demonstrated only by means of the hemagglutination and hemolysis tests and not by bacterial agglutination or precipitation procedures. Antibodies against the CA have been shown to opsonize enteric bacteria containing this antigen (4, 5). Since phagocytosis is one of the major defense mechanisms of the host, it is conceivable that such antibodies may play a protective role in infections including pyelonephritis of man and animals. In 1968, Gorzynski and Neter (E. A. Gorzynski and E. Neter, Bacteriol. Proc., p. 86, 1968) reported that C57BL/6Ha black mice immunized with the CA of Escherichia coli 014 had a death rate of 47 to 57% as compared with 72 to 80% for controls when challenged with Salmonella enteritidis. Swiss white mice, which produced CA antibodies less effectively than black mice, were not protected. The protective effects of antibodies against the enterobacterial CA in experimental hematogenous and retrograde pyelonephritis of rabbits are described in this paper.

MATERIAL AND METHODS

Common antigen preparation. CA was prepared by the previously described procedures of Suzuki et al. (10). The various bacterial strains were grown on Brain Veal Agar (Difco) in Kolle flasks for 18 hr at 37 C. The growth was suspended in hemagglutination buffer (Difco) and boiled for 1 hr. After centrifugation at 23,500 X g for 20 min, the supernatant fraction was exposed to ethyl alcohol (final concentration of 85%) for 18 hr. The mixture was centrifuged; the supernatant was air-dried for 24 hr and reconstituted in distilled water to the original volume.

Serum titrations: indirect hemagglutination (IHA).
Serum was obtained from all rabbits before immunization, before bacterial challenge, and at sacrifice. Sheep erythrocytes were exposed to the test CA preparation for 0.5 hr at 37 C. After three washings in hemagglutination buffer, 0.05 ml of 2.5% suspension of sensitized erythrocytes was added to 0.5 ml of test serum dilution from 1:5 to 1:81,920. After incubation for 0.5 hr at 37 C, the test mixtures were centrifuged at 2,000 rev/min for 2 min and hemagglutination was read as the sediments were shaken. The highest serum dilution producing visible hemagglutination was taken as the IHA titer.

**Immunization.** Rabbits (adult New Zealand whites) weighing 4 to 7 lb were actively immunized by intravenous injection on alternate days as follows: three injections (0.5-ml doses of *S. typhimurium* heat-killed supernatant) for priming to CA followed by two injections (0.5-ml doses of ethanol-soluble CA derived from *S. typhimurium*). Blood samples were obtained before and after immunization, and serum antibodies were determined by the IHA test. Three groups of rabbits were passively immunized by a single intravenous injection of immune serum. One group was injected intravenously with 0.5 ml of rabbit CA antiserum with an IHA titer of 1:10,240 and challenged 4 hr later with 10^7 viable *Proteus mirabilis* organisms; another was injected intravenously with 1 ml of rabbit CA antiserum with an IHA titer of 1:640 and challenged 4 hr later with 10^7 viable *P. mirabilis* organisms; and a third group was injected intravenously with 1 ml of rabbit CA antiserum with an IHA titer of 1:640 and challenged 4 hr later with 10^7 viable *Pseudomonas aeruginosa* organisms.

**Hematogenous challenge.** Seven groups of rabbits were fed 1.2% oxamide mixed in their diet for 6 days (7) and on the sixth day were injected intravenously with 10^7 live *P. mirabilis* or *P. aeruginosa* organisms. These strains were obtained from patients with acute pyelonephritis. The next day, oxamide was discontinued from the diet. During the feeding of oxamide, microscopic intraductal calcifications are found. When the oxamide administration is stopped, the calcifications are soon absent. Pyelonephritis thus initiated, however, continues.

**Retrograde challenge.** Female rabbits were anesthetized with ether. The urinary bladders were emptied by needle aspiration and a sterile wound clip was sutured to the bladder wall near one of the ureteral junctions. To establish a chronic urinary tract infection, a foreign body must be applied; otherwise spontaneous cure of the infection occurs. Only those animals with intact wound clips at autopsy were included in the final analysis of the results. After closing the bladder, 1 ml of the bacterial suspension (containing ten million viable *P. mirabilis* organisms) was injected through the bladder wall. Refluxing urine was expected to carry bacteria to the kidneys for colonization.

**Absorption of antiserum.** Sheep erythrocytes were modified with ethanol-soluble CA derived from *E. coli* 04. Sediment of the CA-modified erythrocytes was mixed with *S. typhimurium* antiserum (containing O + CA antibodies) which had been inactivated at 56 C for 30 min, and absorption was allowed to proceed for 30 min in a water bath at 37 C followed by overnight incubation in a cold room. This antiserum had a heterologous hemagglutinin CA titer of 1:640 before absorption. The absorption process was repeated three times and the absorbed antiserum was shown to have a CA hemagglutinin titer of less than 1 to 2.

**Passive transfer of CA absorbed antiserum.** A 0.5-ml amount of absorbed antiserum (with O antibodies remaining) was injected intravenously into 10 rabbits followed by the iv administration of 5 × 10^5 viable *P. mirabilis* organisms. A 0.5-ml amount of unabsorbed serum (containing O + CA antibodies) was injected into nine control rabbits and the animals were likewise challenged.

**Postchallenge treatment.** Six weeks after hematogenous and four weeks after retrograde challenge with live bacteria, each rabbit was bled just before sacrifice. Both kidneys were removed aseptically, and urine was removed by needle puncture of the bladder for quantitative cultures.

The individual kidneys were weighed and examined grossly for abscesses. A section was cut for histopathology, and a weighed portion was then ground with Trypicase Soy Broth by using a motorized tissue homogenizer. Pour plates of the suspension were made by using Trypticase Soy Agar. The plates were incubated at 35 C for 18 to 24 hr, and the viable count was expressed per gram of kidney tissue. Representative bacterial colonies were chosen, and biochemical and drug susceptibility patterns and slide agglutination tests with homologous antiserum were used as criteria to insure identity of the organism with the original challenge strain. Thin sections of both kidneys of each rabbit were stained with hematoxylin-eosin and examined for evidence of renal pathology without knowledge by the pathologist as to identity of specimens.

**RESULTS**

Table 1 summarizes results of experiments on the effect of active immunization with CA of *S. typhimurium* on hematogenous and retrograde challenge with *P. mirabilis*. It is evident that CA immunization provided significant protection against renal infection in the hematogenously challenged group. The results also clearly show differences (significant at the 99.5% level) between the immune and nonimmune groups with respect to bacterial recovery rates from urines and kidneys as well as renal pathology. The data in Table 1 indicate that there was a difference (significant at the 95% level) between the immune and controls in the retrogradely challenged rabbits when comparing development of kidney pathology. As for the recovery of bacteria from the urine, there was no significant difference. Although immunization protected rabbits from developing renal pathology, bladder infection was found in the majority of these animals.
Table 1. Effect of active immunization with common antigen (CA) on hematogenous and retrograde pyelonephritis due to Proteus mirabilis

<table>
<thead>
<tr>
<th>Challenge route</th>
<th>Immune status</th>
<th>No. of animals</th>
<th>Rabbits with recovery of <em>P. mirabilis</em> at sacrifice from</th>
<th>Rabbits with renal histopathology</th>
<th>Hemagglutinin titer range</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. positive</td>
<td>Range of organisms/m³</td>
<td>No. positive</td>
<td>Range of organisms/g³</td>
<td>Gross abscesses</td>
</tr>
<tr>
<td>Hematogenous</td>
<td>Immune</td>
<td>15</td>
<td>1 (20)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Nonimmune</td>
<td>7</td>
<td>6 (10^3 \times 10^4) (1.5 (10^6))</td>
<td>6</td>
<td>7 (10^4 \times 10^4) (5.2 (10^6))</td>
</tr>
<tr>
<td>Retrograde</td>
<td>Immune</td>
<td>13</td>
<td>8 (10^3 \times 10^4) (10⁶)</td>
<td>4</td>
<td>10–10^4 (2.5 (10^3))</td>
</tr>
<tr>
<td></td>
<td>Nonimmune</td>
<td>9</td>
<td>8 (10^3 \times 10^4) (1.5 (10^6))</td>
<td>7</td>
<td>10^4–4 (10^4) (2.5 (10^3))</td>
</tr>
</tbody>
</table>

* Value for mean given in parentheses.
Table 2. Effect of active immunization with common antigen (CA) on hematogenous pyelonephritis due to Pseudomonas aeruginosa

<table>
<thead>
<tr>
<th>Immune status</th>
<th>Rabbits with recovery of P. aeruginosa at sacrifice from</th>
<th>Hemagglutinin titer range</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Urine</td>
<td>Kidneys</td>
</tr>
<tr>
<td></td>
<td>No. positive</td>
<td>Range of organisms/µl</td>
</tr>
<tr>
<td>Immune</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>Nonimmune</td>
<td>5</td>
<td>1</td>
</tr>
</tbody>
</table>

* Value for mean given in parentheses.

These characteristics were also noted occasionally in the nonimmune control groups as follows: (i) Calciﬁc deposits as well as focal abscess formation were found in several instances; (ii) yeasts were common to a large proportion of cases; and (iii) vacuolar nephropathy was observed in the kidneys of control animals with low-titered antiserum administered at 4 weeks postchallenge. Animals injected with unabsorbed antiserum containing O + CA antigens demonstrated no protective effect. To assess the specificity of the CA antibody protective effect, the following experiments were performed. The first concerned the effect of preimmunization with CA on homologous pyelonephritis and the occurrence of renal pathology and Hemagglutinin titer range. The second series of experiments were transferred to prove speciﬁcally involved passive transfer in animals which were challenged in the fourth week with P. aeruginosa. In the second series of experiments, animals which were challenged in the fourth week with P. aeruginosa were not protected by antibody from the presence or absence of bacteria. The second series of experiments were transferred to prove speciﬁcally involved passive transfer in animals which were challenged in the fourth week with P. aeruginosa.
TABLE 3. Comparison of pyelonephritis in passively immunized rabbits challenged with Proteus mirabilis or Pseudomonas aeruginosa

<table>
<thead>
<tr>
<th>Passive immunization and challenge bacteria</th>
<th>Rabbits with recovery of bacteria at sacrifice from</th>
<th>Rabbits with renal histopathologya (ratio of no. positive per total)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Urine</td>
<td>Kidneys</td>
</tr>
<tr>
<td></td>
<td>Ratio of no. positive per total</td>
<td>Range of organisms/mlb</td>
</tr>
<tr>
<td>High-titered CA serumc and Proteus</td>
<td>1/7</td>
<td>(14 X 10³)</td>
</tr>
<tr>
<td>Low-titered CA serumd and Proteus</td>
<td>1/7</td>
<td>(10⁴)</td>
</tr>
<tr>
<td>None and Proteus</td>
<td>6/7</td>
<td>10¹-3 X 10⁶</td>
</tr>
<tr>
<td>Low-titered CA serum and Pseudomonas</td>
<td>1/5</td>
<td>(1.5 X 10⁴)</td>
</tr>
<tr>
<td>None and Pseudomonas</td>
<td>1/5</td>
<td>(3 X 10⁶)</td>
</tr>
</tbody>
</table>

a Histopathology—acute pyelonephritis, focal scar formation, pyelitis.
b Value for mean given in parentheses.
c Group given high-titered (1:10,240) common antigen (CA) antiserum.
d Group given low-titered (1:640) CA antiserum.

TABLE 4. Effects of common antigen (CA) antibody absorption on passive immunization in animals challenged with Proteus mirabilis

<table>
<thead>
<tr>
<th>Serum administered</th>
<th>Recovery of P. mirabilis from kidneys at sacrifice</th>
<th>Rabbits with renal histopathology (ratio of no. positive per total)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ratio of no. positive per total</td>
<td>Range organisms/gb</td>
</tr>
<tr>
<td>CA absorbed serumc</td>
<td>10/10</td>
<td>10¹-3 X 10⁶</td>
</tr>
<tr>
<td>Unabsorbed serumd</td>
<td>0/9</td>
<td>(2.3 X 10⁶)</td>
</tr>
<tr>
<td>None</td>
<td>6/7</td>
<td>7 X 10⁴-10⁶</td>
</tr>
</tbody>
</table>

a Histopathology: acute pyelonephritis, focal scar formation, pyelitis.
b Value for mean given in parentheses.
c Heterologous CA hemagglutinin titer, <1 to 2; homologous O titer before absorption, 1:640; homologous O titer after absorption, 1:640.
d Containing heterologous O antibodies; heterologous CA hemagglutinin titer, 1:640.

After challenge and absent in the kidneys of immune animals.

Calcific deposition in renal tissue was characterized by marked variation in degree from animal to animal. In most animals, the degree of involvement of the two kidneys was approximately equal. The material was localized largely in the convoluted tubules and, in many cases, was grossly visible as rather discrete white areas in the cortex.

The foci of early scar formation were composed of chronic inflammation, largely lymphocytic, and early fibroblastic proliferation. In some cases, calcific depositions and foci of material appearing to represent hemosiderin were also found. Loss of parenchymal tissue with collapse of remaining stroma, as well as the immediate subcapsular location of many of these areas, caused flat broad-based depressions on the subcapsular cortical surfaces of the kidneys in...
several instances. There appeared to be no consistent relationship between these cicatrizing areas and calcific deposits, as each phenomenon was seen to occur independently. The destruction of parenchyma, chronic inflammation, and scarring were considered to constitute evidence of healing acute pyelonephritis.

In the hematogenously challenged animals demonstrating acute pyelonephritis histologically, the lesions were largely of the "cannonball" type. They were, for the most part, rounded discrete masses of inflammatory infiltrate. Destruction of preexisting tissue and abscess formation were a common finding. The rounded discrete nature of the lesion was suggestive of hematogenous pyelonephritis, rather than "ascending" infection.

Pyelitis, when present, was sometimes unilateral, but when bilateral, differences in severity, such as between the two kidneys, were frequently demonstrated. When acute inflammatory cells were present, sections of the renal parenchyma usually showed acute pyelonephritis as well.

An inconstant finding was a focal vacuolar nephropathy, the significance of which was not determined. It was frequently associated with areas of scarring. In some instances, the cell membranes of the involved tubular epithelium appeared to be calcified.

**DISCUSSION**

Evidence has been obtained suggesting that immunization with the enterobacterial common antigen derived from *S. typhimurium* protects rabbits against pyelonephritis after hematogenous and retrograde challenge with unrelated enteric bacteria containing CA.

The following lines of evidence indicate that this protective effect is specific. (i) CA immunization did not prevent pyelonephritis due to *P. aeruginosa* which does not produce this antigen. (ii) Animals passively immunized with CA antiserum did not develop pyelonephritis due to *P. mirabilis*. (iii) Passive immunization did not prevent a *P. aeruginosa* pyelonephritis. (iv) Differential absorption of CA antibodies from an antiserum containing O + CA antibodies abolished the protective activity of the antiserum when passively administered.

Since the vaccines utilized in priming injections for immunizing the animals contained endotoxin, the nonspecific protective activity of endotoxin might be suggested as a possible explanation for our findings. In addition to the afore mentioned evidence which serves to argue against this hypothesis, it must be emphasized that a completely heterologous O system was employed.

There is no known cross-reaction between *S. typhimurium* O antibodies and *P. mirabilis*. Also, if antitrough antibodies, anti-O antibodies, or nonspecific endotoxin protection were playing a role, one would have expected to observe this phenomenon with the animals challenged with *P. aeruginosa*. *Pseudomonas* and *Proteus* both contain endotoxin, yet nonspecific protection was not evident in any of the controls. The protective capacity of passively transferred antibody excludes not only tolerance but also delayed hypersensitivity as the sole mechanism of protection. Since a variety of *Enterobacteriaceae* have not been employed as challenge microorganisms, we have not eliminated the possibility that the protective effect may be due to antibodies directed against an antigen shared by *Salmonella* and *Proteus* only, and capable of being absorbed from the serum by an ethanol-soluble fraction derived from *E. coli* O4.

The resistance to pyelonephritis as reported by Sanford et al. (9) was specific, acquired immunity due to circulating antibodies. This resistance was type-specific. Both early nonspecific resistance and tolerance are classically not type-specific but are shared between various species of gram-negative bacteria. In their study, significant reduction in gross renal abscesses was not observed with any of the four heterotypic strains of *E. coli* or with a strain of *Klebsiella pneumoniae* type C when injected into animals which had been previously infected with a heterologous strain of *E. coli* and allowed to heal. Nonspecific protection of endotoxin or antitrough antibodies, or both, did not seem to play a role in protection in these studies. Furthermore, animals so injected would not be expected to develop CA antibodies to any appreciable titers. It is therefore difficult to implicate any protective activity due to CA antibodies in this study in view of the experimental design.

The frequent absence of bacteria in the urines and kidneys of the immunized groups challenged hematogenously lends support to our interpretation of contrary findings (absence of pyelonephritis but presence of bacteriuria) in the urine of animals in the experiments on retrograde pyelonephritis: serum, rather than secretory, CA antibodies neutralize infection depending on the method of bacterial seeding. We are therefore proposing that multiplication of bacteria may be markedly retarded within the blood or kidneys, or both. It remains to be determined whether there is more rapid removal of bacteria from the circulation in the immune versus nonimmune animals, thereby diminishing the bacterial inoculation to which the renal tissue is exposed.
Two possibilities must be considered in interpreting the observation that *Pseudomonas* in the control and immune groups caused renal pathology, but was eliminated from the kidneys in the process. The microorganisms may have converted to persisting L-forms and were, therefore, not detectable on the routine media employed. The microorganisms may have been rapidly destroyed by the animals' normal defense mechanisms and extracellular products released after their destruction may have caused tissue damage. It must be emphasized that the majority of these animals were sacrificed at six weeks. Those few animals sacrificed at 1 and 2 weeks showed the presence of the challenging microorganism. In any event, active chronic pyelonephritis without bacteriuria is known to occur in man (2).

An explanation for the occurrence of obvious protection from development of renal pathology, but not from a urinary bladder infection, after challenge with *P. mirabilis* in the retrogradely challenged immune group may well be found in the observation that the anti-CA globulins are of the 19S type, which, as far as is known, have not been reported in the urine. The presence of bacteria in low count in the renal tissues of 4 of the 13 immunized animals could be the result of continual reflux of infected urine into the kidneys which is known to occur in the presence of cystitis. This then may be interpreted as internal contamination since no apparent histological changes were observed. The CA antibody titers by IHA for three of these animals closely paralleled those of the other immunized animals whose kidneys were negative on culture. Only one of the four had a CA antibody titer of less than 1:320 at sacrifice.

Unfortunately, the immune mechanisms of the urinary tract which may be highly significant in terms of defense against infection are incompletely explored. Although the protective mechanism of CA antibody appears to be humoral, attention deserves to be focused also on cellular immune mechanisms which are obviously of considerable importance in bacterial infections. It has been previously shown that intravenous injection of CA leads to the formation of CA antibody-forming cells in the spleen and administration of CA into a foot pad results in a substantial increase in antibody-forming cells in the corresponding lymph node, as tested by means of the hemolytic plaque technique of Jerne (6). It will be interesting to learn whether cell-bound antibodies with CA specificity are found in the kidney and urinary bladder.

The finding that the O antigen (lipopolysaccharide) and its lipoid A component interfere with the immunogenicity of the common antigen is of probable significance here (11, 13). Individuals with various enterobacterial infections have been shown to develop very low hemagglutinating titers to CA antibodies with the probable exception of *E. coli* O14 (12). As has been experimentally documented, it is only when CA is separated from the O antigen that high hemagglutinating antibody titers develop (10). In a natural infection, CA antibodies may, therefore, seldom reach levels which are protective because of the immunosuppressive action of the bacterial lipopolysaccharide.

In addition to the reported protective effects of CA antibodies, this paper further substantiates the suitability of the oxamide-feeding experimental model for studying hemagglutogenous pyelonephritis. Oxamide forms obstructive oxalate microdeposits in the collecting tubules, thus allowing for implantation of bacteria in the kidneys. These deposits disappear soon after the oxamide diet is discontinued. The initial feeding of oxamide allows the study of the disease in an essentially normal urinary tract and the variables of surgical or traumatic damage to the urinary tract are avoided, thus mimicking human disease. This model undoubtedly lends itself well to varied manipulation in experimental pyelonephritis.

The dynamics of the CA antibody protection are being further elucidated in our laboratory with a view to shedding further light on the following: duration of protective effect and long-term effectiveness of the CA antibodies unrelated to chemotherapy; booster effects on the antibody levels with relation to protective activity; the relationship between protective effects and various infective agents containing CA; and effects of passively administered CA antibody on the course of an already established infection. Further studies may eventually lead to clinical application.

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LITERATURE CITED
