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Complement-mediated immune mechanisms in renal infection

II. EFFECT OF DECOMPLEMENTATION

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SUMMARY

Animals were depleted of complement using cobra venom factor and the influence of complement depletion on the course of renal infection was studied. Complement depletion markedly increased the susceptibility of renal tissue to a challenge with an *E. coli* strain sensitive to the bactericidal activity of normal serum, but did not influence the outcome of a challenge with a serum-resistant strain of *E. coli.* These observations are consistent with the hypothesis that complement-mediated host immune mechanisms do play a role in the biology of renal infection and are an important component of the host's immune response in pyelonephritis.

INTRODUCTION

Few studies have investigated the role of complement-mediated host immune mechanisms in the biology of renal infection, although it has been believed that the inactivation of the fourth component of complement by renal ammonia may be a factor contributing to the susceptibility of the kidney to infection (Beeson & Rowley, 1959).

During recent experiments we have confirmed that renal tissue does block the complement-mediated lysis of antibody-sensitized erythrocytes, but that the inactivation of complement by renal tissue was not due to ammonia production or restricted to renal tissue (Ormrod & Miller, 1978). In addition, it was found that an effective complement-mediated bactericidal activity was maintained, even in serum with a greatly reduced haemolytic complement titre as a result of exposure to renal tissue.

These results suggest that complement-mediated host immune mechanisms, rather than being inactivated, are likely to be intact and operative within the kidney. This assumption could be proven if the ablation of complement-mediated host immune mechanisms was found to have an adverse effect on the host's ability to eliminate complement-sensitive pathogens. In experiments using an infectious challenge with complement-sensitive and -resistant strains of *E. coli* and normocomplementaemic and decomplemented hosts, we have established that complement-mediated host immune mechanisms are operative in renal tissue and are an important component of the host's immune response to renal infection.

MATERIALS AND METHODS

Selection of complement-sensitive and resistant strains of E. coli. Cultures of *E. coli* isolated from individuals with urinary tract infections were used in these experiments (Miller, Fortune & Burnham, 1975). *E. coli* suspensions were examined in a serum bactericidal assay where the only selective criteria applied was that the isolates displayed clearly defined sensitivity or resistance to complement-mediated bactericidal activity in the assay system detailed below. On the basis of their growth characteristics in the assay system two strains were selected: *E. coli* 1 and *E. coli* 6, which were sensitive and resistant respectively to complement-mediated killing by DA rat serum.

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Serum bactericidal assay system. A 6 hr broth culture of the *E. coli* strain to be tested was washed twice with normal saline and once with rat Ringer's solution (Robinson, 1961), before being diluted in rat Ringer's to give a suspension containing 10^6 viable organisms per ml. The assay system consisted of 1·5 ml of rat serum, 1·5 ml of rat Ringer's and 1·0 ml of the bacterial suspension. Test samples and controls (rat Ringer's alone, serum inactivated at 56°C for 30 min and nutrient broth) were then incubated at 37°C. Aliquots of 0·1 ml were taken to determine bacterial numbers at the initiation of the assay, and subsequently at hourly intervals for 3 hr. The samples were incorporated into agar pour plates and bacterial numbers determined after incubation at 37°C overnight.

Cobra venom factor (CVF). CVF was isolated from *Naja naja* venom (Sigma Chemical Company, St Louis, Missouri), as previously described (Ballow & Cochrane, 1969). The fractions containing anti-complementary activity after DEAE chromatography were concentrated by gel filtration on Sephadex G-200. The active fractions were pooled and stored in aliquots at −70°C. Decomplementation of the experimental animals was achieved by the intraperitoneal administration of 100 μg of purified CVF 48 hr prior to infection in divided doses 8 hr apart.

Experimental animals. Female rats weighing 225–250 g obtained from an inbred strain of DA rat were used in these experiments.

Haematology. Blood was collected from the tail vein directly into heparinized syringes and diluted in an isotonic diluent. Samples were processed in a Coulter Counter, Model S and the total leucocyte count, haemoglobin, red blood cell count, packed cell volume and red blood cell indices determined. A blood film for differential leucocyte count and red blood cell morphology was also examined.

Production of renal infection. Pyelonephritis was induced by the direct inoculation of *E. coli* 075 into the surgically exposed kidney using a glass micro-capillary. Details of the method have been given previously (Miller & Robinson, 1973).

Determination of total serum haemolytic complement activity (CH_{50}) and C3 concentration. Details of the methods used have been given in an accompanying report (Ormrod & Miller, 1978).

Bacteriology. Nutrient agar pour plates of serial ten-fold dilutions of kidney homogenate were made to obtain a bacterial count per g of wet tissue.

Alternative pathway activation in normal rat serum. There is a critical difference between the alternative pathway and the classical pathway of complement activation with regard to divalent cation requirement. Complement activation in the presence of 10 mM Mg–ethyleneglycoltetracetic acid (Mg–EGTA) is taken as evidence of alternative pathway activity, but both the classical pathway of complement activation, which requires calcium ions, and the magnesium ion-dependent alternative pathways are inhibited by 10 mM ethylenediamine tetracetic acid (EDTA) (Sandberg & Osler, 1971; Fine *et al.*, 1972). Sheep red blood cells (SRBC) optimally sensitized with rabbit anti-SRBC serum (EA) were added to the assay system to give a final concentration of 2×10^6 cells per ml. Zymosan (Z) was suspended in normal saline (1·0 mg/ml) and boiled for 1 hr. After centrifugation at 2500 *g* for 15 min, the zymosan was washed three times in cold saline, resuspended in normal saline and stored at −70°C. The final concentration in the assay was 2·0 mg/ml. A culture of *E. coli* was grown overnight in nutrient broth to a concentration of 2×10^9 viable organisms per ml. The culture was then steamed for 1 hr and washed three times in phosphate-buffered saline. 10^6 organisms were used in the assay. 0·9 ml of undiluted serum was chelated with either EDTA or Mg–EGTA in a final concentration of 10 mM to a final volume of 1·0 ml. The chelated serum was then challenged with EA, Z or *E. coli* and incubated with frequent mixing at 37°C for 60 min. After incubation, the challenge substance was sedimented by centrifugation at 2500 *g* at 4°C for 10 min. The supernatant chelated serum was withdrawn and the chelator saturated by the addition of 0·1 ml of 10 mM $CaCl_2$/ml of serum. The CH_{50} level in the serum sample was then determined. Controls consisted of serum samples with saline substituted for the chelating agent and, where appropriate, the effect of the chelating agent on complement levels was determined.

RESULTS

Selection of the complement-sensitive and complement-resistant E. coli strains

Under the conditions of the assay, *E. coli* 1 (serum-resistant) replicated readily in the presence of serum and was resistant to the complement-mediated bactericidal activity of serum. In contrast, an inocula of the serum-sensitive strain (*E. coli* 6) was killed within 1 hr. Serum heated to 56°C for 30 min, however, did not inhibit the growth of the serum-sensitive strain (Fig. 1).

Effect of decomplementation on complement-mediated bactericidal activity

Complement-mediated killing of the serum-sensitive and resistant *E. coli* strains by normal serum and serum from animals decomplemented 48 hr previously with CVF was examined. Whereas the serum-sensitive strain was rapidly killed by normal serum in the assay system, serum from animals treated with CVF showed no bactericidal activity. Decomplementation did not affect the bactericidal activity of serum against the serum-resistant strain (Fig. 2).

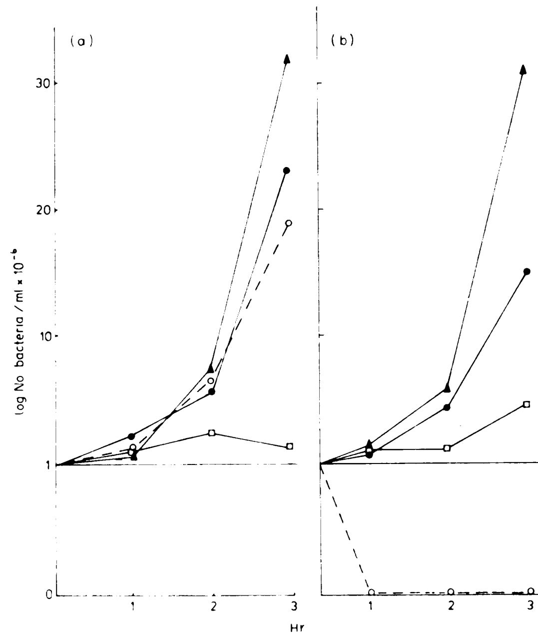

FIG. 1. Bacterial growth patterns in the bactericidal assay of the (a) serum-resistant and (b) serum-sensitive strains of *E. coli* used in these experiments. (▲) Nutrient broth; (○) serum; (●) serum 56°C, 30 min; (□) rat Ringer's.

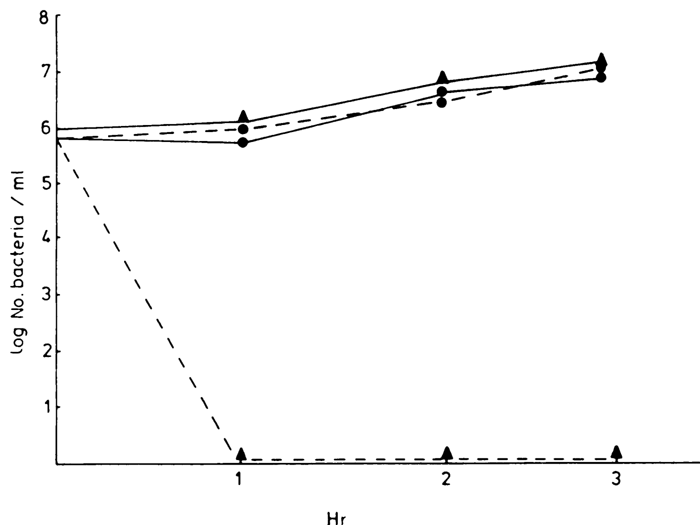

FIG. 2. The effect of decomplementation on the bactericidal activity of DA serum against complement-sensitive (▲) and complement-resistant (●) *E. coli*. Bacterial growth patterns in the serum bactericidal assay. Normal serum, (– – –); CVF serum, (– – –).

Biological effects of decomplementation

The level of functional serum complement fell to below detectable levels (less than 0·5% of normal) within 24 hr of CVF administration, and remained at this level for at least 5 days (Table 1). A moderate neutrophilic leucocytosis (20–30% increase) was found within 24 hr after CVF administration and was the only other significant haematological change demonstrated.

TABLE 1. Haemolytic complement activity and C3 concentration in animals treated with cobra venom factor (CVF)

Days after CVF administration	Haemolytic complement	C3 concentration
0	100	103±12*
2	0	1·5±0·3
4	0	1·4±0·3
5	0	2·3±1·2
8	74±30	84±17

* Mean±s.d., the results from an analysis of eight animals are percentages of normal.

Complement depletion and renal infection

In this experiment, CVF-treated (complement-depleted) and saline-treated (normocomplementaemic) animals were challenged 24 and 48 hr after CVF administration with the serum-resistant strain (*E. coli* 1) and the serum-sensitive strain (*E. coli* 6). Control and CVF-treated animals challenged with the serum-resistant strain showed similar bacterial numbers in the kidney 24 and 48 hr after challenge (Fig. 3). Complement depletion, however, markedly enhanced the ability of the serum-sensitive strain to establish infection. 24 hr after challenge there was a significant increase in the capacity of the serum-sensitive strain to establish an infection in the kidney of the decomplemented animals ($P = 0{\cdot}01$). 48 hr after challenge, the differences were even more marked, and whereas the kidneys of fourteen out of nineteen normocomplementaemic animals challenged with the serum-sensitive strain of *E. coli* were either sterile or had low bacterial counts, substantial bacterial numbers were found in the kidneys of all twenty decomplemented animals.

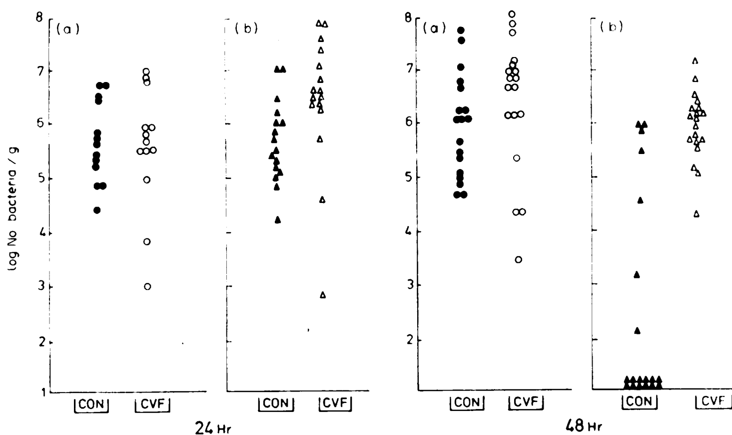

FIG. 3. Bacterial numbers in the kidney 24 and 48 hr after the initiation of infection in complement-depleted and control animals with (a) serum-resistant and (b) serum-sensitive *E. coli*.

Pathways of complement activation in DA rat serum

The preceding experiment provided strong evidence that complement-mediated host immune mechanisms were effective in renal tissue, but the protective mechanism was not established. One factor for consideration was that the classical pathway of complement activation was blocked by renal tissue, but that components of bacterial cell walls activated the alternative pathway to produce a C3 convertase independent of the early complement components. Experiments were carried out to confirm that *E. coli* and zymosan (activators of the alternative pathway) were capable of producing complement consumption in the DA rat serum *in vitro*. The results of experiments comparing complement consumption by *E. coli*,

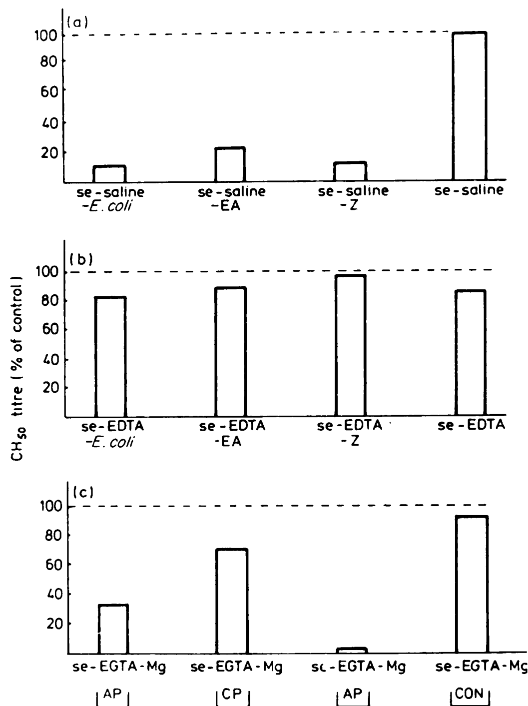

FIG. 4.

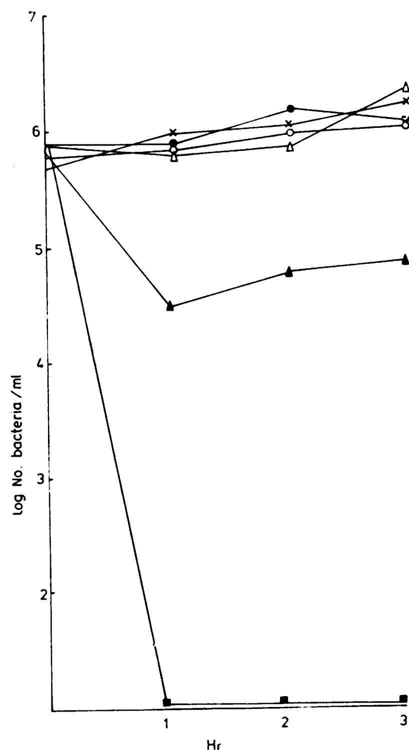

FIG. 5.

FIG. 4. Residual complement titres in normal rat serum and EDTA and Mg-EGTA-chelated serum after incubation with sensitized erythrocytes (EA), *E. coli* cells or zymosan. The challenge substances were incubated in the sera for 1 hr at 37°C, after which CH_{50} titres were determined in the supernatant sera. CP and AP indicate the classical and alternative pathway activating agents. (se) Serum.

FIG. 5. The effect of Mg-EGTA on the serum bactericidal activity of DA rat serum. Bacterial growth patterns of the serum-sensitive *E. coli* strain in the serum bactericidal assay. (×) Rat Ringer's; (△) Mg-EGTA-rat Ringer's; (●) EDTA-serum; (○) EDTA-rat Ringer's; (▲) Mg-EGTA-se (■) saline-serum.

zymosan and sensitized erythrocytes in the presence of EDTA and Mg–EGTA are given in Fig. 4. EDTA blocked both systems while Mg–EGTA allowed alternative pathway complement consumption. The Mg–EGTA did not inhibit complement consumption by EA completely, and this finding remains unexplained. Whether this represented some escape of the calcium-dependent classical pathway from Mg-EGTA chelation or activation of the alternative pathway by EA is not certain.

Biological role of the alternative pathway

The potential role of alternative pathway-mediated bacterial killing was examined in experiments where the bactericidal capacity of normal DA rat serum and Mg–EDTA-treated serum (classical pathway-mediated bactericidal activity destroyed) was compared. Normal serum killed an inocula of 10^6 serum-sensitive *E. coli* within 1 hr, whereas Mg–EGTA-treated serum failed to eradicate the same inocula over a 3 hr period, although a reduction in numbers was demonstrable (Fig. 5).

DISCUSSION

Recent advances in complement technology have contributed to an understanding of the role of the complement system in infectious disease. CVF has allowed the use of complement-depleted animals as experimental models, and the understanding of the alternative pathway has disclosed a complement-mediated host immune mechanism that is largely independent of antibody. In the present studies we

have recognized these factors and have demonstrated that complement-mediated host immune mechanisms are as important and effective components of the host's immune response in pyelonephritis, despite the ability of renal tissue to inactivate complement *in vitro*.

Two strains or *E. coli*, sensitive and resistant to the bactericidal activity of DA rat serum, were characterized with a view to inducing infection in normocomplementaemic and complement-depleted animals. It was reasoned that if complement-mediated immune mechanisms were inactivated by renal tissue, then complement depletion with CVF would have little effect on the course of the disease. On the other hand, if the complement system was an important component or host defence in renal infection, then complement depletion with CVF could be expected to enhance the susceptibility of the kidney to infection with the complement-sensitive strain but not the serum-resistant strain. The latter was found to be the case, and the results of the experiment provided strong evidence supporting the active role of the complement-mediated host immune mechanisms in the kidney. One further factor needed careful consideration, in view of the ability of the alternative pathway of complement activation to initiate complement-mediated bacteriolysis. It could have been argued that early components of the classical pathway were blocked by the anti-complementary activity of the renal tissue, but that the alternative pathway of complement activation provided a substitute mechanism for the production of the C3 convertase and the subsequent participation of the complement system in local immune mechanisms. In the present experiments limited complement-mediated bactericidal activity was demonstrable in 10 mM Mg–EGTA, which could have been interpreted as alternative pathway-mediated bacterial killing. However, EA was able to produce some complement consumption in the presence of Mg–EGTA and could represent calcium-independent activation of the classical pathway.

The role of the complement-mediated serum bactericidal activity in the pathogenesis of renal infection has also been investigated in a number of clinical studies. Kimball, Garcia & Petersdorf (1964) studied the serum sensitivity of strains of *E. coli* from patients with urinary tract infections, and concluded that strains more frequently isolated from bacteriuric patient were more resistant to the bactericidal activity of normal serum than the less frequently isolated strains. Gower *et al.* (1972) found that a significantly higher number of patients with upper compared to lower urinary tract infection had either a specific defect in bactericidal activity against the homologous organisms or were infected with organisms inherently resistant to normal serum bactericidal activity. In related experiments, Waisbren & Brown (1966) and Taylor (1972) have reported a defect in the bactericidal activity of serum from patients with renal infection that may have contributed to the establishment of the renal infection.

When the clinical and experimental evidence are considered together, there is convincing evidence that complement-mediated host immune mechanisms are, in fact, effective in renal tissue and not inactivated, as had been previously proposed. One relevant question is why the latter belief prevailed unchallenged for so long. The answer is simply that until recently, the appropriate technology has not been available to assess the role of the complement system in renal infection by direct experimentation. CVF-induced complement depletion, however, has allowed the examination of host immune mechanisms in decomplemented animals and the demonstration that the complement system in an important determinant in the immunobiology of renal infection.

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