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# Host immune status in uraemia

## III. HUMORAL RESPONSE TO SELECTED ANTIGENS IN THE RAT

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## SUMMARY

The humoral immune status of patients with uraemia is controversial. In the present experiments a carefully defined animal model, in which a chronic state of moderate and severe uraemia was induced, has been used to resolve conflicting views. The capacity of the uraemic host to respond to immunogenic stimulation was assessed by challenging uraemic animals with bacterial, viral, T cell-dependent and T cell-independent antigens. The experiments have shown that the immune responsiveness of animals with chronic severe uraemia, immunized with sheep red blood cells, *Escherichia coli* 075, keyhole limpet haemocyanin and ØX174 bacteriophage is comparable to those found in sham-operated and control groups. The results strongly suggest that uraemia *per se* does not affect the ability of the host to respond to a wide range of antigenic stimuli. This conclusion is important in that it provides a basis for assessing further the immune status of the uraemic host. If uraemic patients do have an immune deficit, one possible explanation is that uraemia potentiates the immunosuppressive activity of some drugs used in the clinical management of these patients.


## INTRODUCTION

The immune status of patients with uraemia has been studied in considerable detail over a number of years. Infection still remains a serious complication of renal failure and has been attributed to a number of changes in immune status, including depressed cell-mediated response (Kauffman, Manzler & Phair, 1975; Quadracci, Ringden & Krzymanski, 1976), impaired phagocytosis (Montgomerie, Kalmanson & Guze, 1972; McIntosh *et al.*, 1976), low concentrations of immunoglobulin and the complement component C3 (Weeke, Weeke & Bendixen, 1971; Dobbelstein, 1976) as well as depressed humoral immunity.

Studies of humoral immunity in uraemic patients, however, have produced conflicting results. Some authors (Stoloff *et al.*, 1958; Dammin, Couch & Murray, 1957; Balch, 1955) have reported normal responses to antigenic stimuli, while others (Boulton-Jones *et al.*, 1973; Byron, Mallick & Taylor, 1976; Wilson, Kirkpatrick & Talmage, 1965) found reduced antibody responses. The variables encountered in human studies are considerable and relate to the aetiology of renal disease and the therapeutic measures which include renal transplantation, haemodialysis and the use of immunosuppressive drugs. Ethical considerations limit the range of antigens with which a patient can be challenged, and it may be difficult to establish whether a challenge has initiated a primary or secondary response. One solution to these problems is to use an animal model which can be maintained in a stable uraemic state uncomplicated by drug administration or previous immuniza-

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tion. Some aspects of the effect of uraemia on immune status, using such a model, have already been reported (Miller & Stewart, 1980; Stewart & Miller, 1980). In the present experiments we have used the same carefully defined animal model of experimentally induced renal failure to determine the ability of the uraemic host to respond to antigenic stimulation. The results have shown that animals with severe and moderate chronic uraemia respond normally to a selection of immunogenic stimuli including bacterial, viral, T cell-dependent and -independent antigens.

## MATERIALS AND METHODS

*Animals.* Male animals, obtained from an inbred strain of DA rats, weighing 200–250 g were used.

*Induction of uraemia.* A standardized procedure (Miller & Stewart, 1980) involving the surgical resection of renal tissue was used to induce 'moderate' and 'severe' uraemia defined as a blood urea concentration of 100–200 mg/100 ml (17–34 mmol/l) and above 200 mg/100 ml (>34 mmol/l) respectively. The experimental model has been described in detail by Ormrod & Miller (1980). Blood urea levels of the animals were determined at the time of killing. Control groups of non-manipulated and sham-operated animals with blood urea levels in the normal range were included in all experiments.

*Collection and storage of blood samples.* Approximately 0·6 ml of blood was drawn from the ventral tail vein and serum samples were stored at −20°C until analysed.

*Immunizing antigens.* The antibody responses to sheep red blood cells (SRBC) (Dresser, 1972; Taylor & Wortis, 1968; Kindred, 1971), keyhole limpet haemocyanin (KLH) (Unanue, 1970) and ØX174 bacteriophage (Dresser & Tao, 1975) are accepted as being T cell-dependent. *E. coli* lipopolysaccharide has been used in these experiments as a T cell-independent antigen (Möller, Andersson & Sjöberg, 1972).

SRBC, anticoagulated with Alsever's solution (Laboratory Services, New Zealand), were washed once in citrate saline, and three times in phosphate-buffered saline (PBS). A 5% solution was made in saline and adjusted to contain $1 \times 10^9$ SRBC per ml. The strain of *E. coli* 075 used in previous studies was also used in these experiments (Miller & Burnham, 1975; Miller *et al.*, 1978). A killed antigen suspension was prepared by growing *E. coli* in nutrient broth to a concentration of $1 \times 10^9$ organisms per ml. The culture was steamed for 2 hr, washed twice in PBS and resuspended in its original volume. Bacteriophage ØX174 (Miles Laboratories Inc., Elkhart, Indiana) was obtained as a suspension in 0·05 M sodium borate, pH 9·1, and 0·002 M ethylenediamine tetracetic acid. The bacteriophage was assayed by the conventional agar method to determine the number of plaque-forming units (PFU) per ml of suspension. Haemocyanin from the keyhole limpet (*Megathura crenulata*) was obtained as a slurry in 65% ammonium sulphate (Calbiochem, San Diego, California).

*Assay of antibody levels to SRBC.* Serum antibody responses to SRBC were measured by the haemagglutination assay using a semi-automated microtitre apparatus fitted with 50-μl diluters (Cook Microtitre Mini Diluters). Fifty-microlitre doubling dilutions of serum in PBS were prepared in duplicate. An equal volume of 0·05% SRBC was added to one set of dilutions and to the second set was added an equal volume of SRBC containing 2-mercaptoethanol (2-ME) in a final concentration of 0·1 M. The plates were sealed and incubated for 1 hr at 37°C and held overnight at 4°C. Titres were expressed as the reciprocal of the highest dilution showing gross agglutination.

*Assay of antibody levels to* E. coli *075.* A passive haemagglutination technique was used to determine antibody levels to *E. coli* 075. SRBC were sensitized with activated endotoxin prepared from bovine-type endotoxin which had been extracted with trichloroacetic acid from a culture of *E. coli* 075 (Webster *et al.*, 1955). To activate the endotoxin, a solution of 0·12 mg/ml in 0·02 M NaOH was heated for 5 min in a boiling water bath. The pH was adjusted on cooling to 7, and an equal volume of 0·3 M PBS was added. SRBC were sensitized by mixing equal volumes of washed SRBC and activated endotoxin and incubating the suspension of cells at 37°C for 2 hr. They were then washed three times in PBS. Sensitization of the SRBC was confirmed by carrying out passive haemagglutination tests with an anti-*E. coli* 075 serum that had been standardized against a bacterial antigen.

*Assay of bacteriophage.* The number of PFU of ØX174 bacteriophage were assayed using the agar layer method. The bottom layer of DIFCO trypticase soy agar contained thymine at a final concentration of 10 μg/ml. Three-millilitre aliquots of DIFCO trypticase soy broth (TSB) containing 0·8% Bacto-Agar were used to form the top layer. All dilutions of samples were made in TSB. TSB containing 10 μg/ml of thymine was used as the growth medium for the bacterial host, *E. coli* CR. The assay was carried out by adding 0·1 ml of an overnight culture of *E. coli* CR to 3 ml of top agar (46°C) followed by 0·1 ml of diluted phage sample. After gently vortex mixing, the contents of the tube were poured onto the hardened bottom agar. The agar plates were incubated at 37°C for 4–5 hr and the number of plaques were then counted.

*Assay of neutralizing antibody to bacteriophage.* A modification of the 50% neutralization method described by Stashak, Baker & Robertson (1970) was used to determine the amount of neutralizing antibody present in the serum samples. Dilutions of the test serum samples were made in TSB in sterile test tubes. An equal volume of viable bacteriophage containing approximately 200–250 PFU was added to tubes containing 0·1 ml of serum dilution. The tubes were then mixed and stored at 4°C for 24 hr. Two tubes containing equal volumes of TSB and bacteriophage were included as controls. After 24 hr, 0·1 ml of an overnight culture of *E. coli* CR and 3 ml of molten top agar (46°C) were added to each tube. The contents were mixed and layered onto hardened bottom agar. The number of plaques was counted after 4–5 hr of incubation at 37°C and the percentage of surviving bacteriophage calculated. The reciprocals of the dilutions were plotted against the percentage of surviving phage on probability–logarithmic graph paper and the dilution allowing 50% survival (SD50) was determined.

*Determination of antibody levels to KLH.* The response was measured by the passive haemagglutination technique using glutaraldehyde-treated SRBC conjugated with KLH as an antigen suspension. SRBC were washed three times in complement fixation diluent (CFD) and added to 1% glutaraldehyde solution in CFD (pH 7·2) to give a final cell concentration of 1%. The suspension was incubated at 4°C with stirring for 30 min. The cells were then washed five times with distilled water and stored at 4°C as a 50% suspension in distilled water until required. Twenty-five millilitres of a 0·5% solution was made by washing 0·3 ml of the treated SRBC twice in distilled water and resuspending in 1 ml of a 10 mg/ml solution of 1-ethyl-3 (3-dimethyl-amino-propyl) carbodiimide HCl (EDCI). After thorough mixing, 7 mg of KLH suspension was added and the mixture was held on ice for 60 min with occasional mixing. The conjugated cells were washed three times in PBS containing 1·5% inactivated and SRBC-absorbed rabbit serum and finally suspended in 25 ml of this medium.

*Experimental protocol.* A standardized procedure was used to assess the immune response to all antigens. Four groups of ten animals were used in each experiment. Surgically manipulated animals in two of the groups were defined as moderately or severely uraemic according to the surgical procedure. This designation was confirmed by the determination of blood urea concentration in individual animals. Sham-operated and non-manipulated groups were included in each experiment as controls. Animals were challenged with antigen at the commencement of the experiment and a secondary challenge was given 28 days later.

## RESULTS

*Immune response to SRBC*

Four groups of ten animals (control, sham-operated, moderate and severe uraemia) were challenged with an intraperitoneal injection of $1 \times 10^9$ SRBC in saline. Serum antibody titres were determined at intervals over a 28-day period. Some statistically significant differences in immune responsiveness were found. For example, peak titres of antibody in sham-operated and uraemic animals 7 days after challenge were all lower than the non-manipulated controls ($P=0{\cdot}05$, Fig. 1). More significant differences were found after a secondary challenge with $1 \times 10^9$ SRBC ($P=0{\cdot}01$). These experiments were then repeated using a further four groups of ten animals and a lesser challenge of $2 \times 10^7$ SRBC. The antibody titres of all four groups were similar to each other (mean peak antibody titre for control group 1:773 and 1:1,408 for primary and secondary responses respectively) and in this respect differed from the results obtained with the greater challenge.

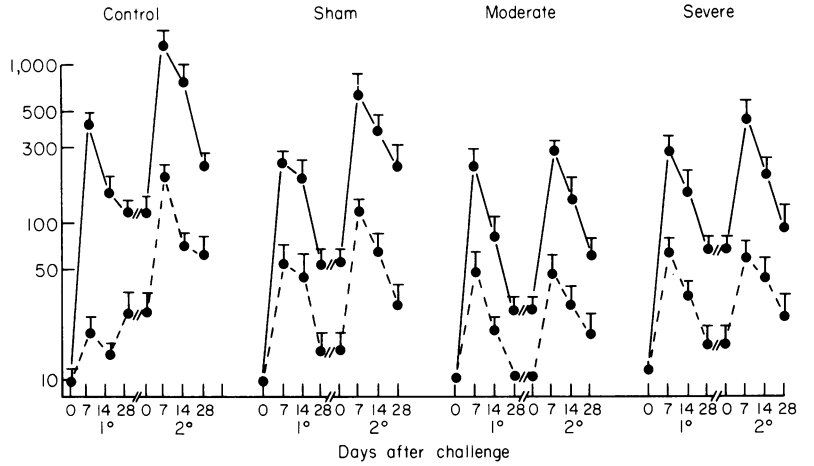


**Fig. 1.** Effect of uraemia on immune response to sheep red blood cells. Four groups of animals were injected i.p. with a primary challenge of $1 \times 10^9$ SRBC and a secondary challenge of $1 \times 10^9$ SRBC 28 days later. (●——●) Total antibody titre, (● - - - ●) IgG component. The ordinate is the reciprocal of the highest dilution of serum giving macroscopic agglutination.

*Bacterial antigen*

Four groups of ten animals were immunized with an intramuscular injection of $1 \times 10^9$ killed *E. coli* 075. The ability of the moderately and severely uraemic animals to respond to a challenge with bacterial antigen was unimpaired and remarkably similar antibody levels were found in all groups during the primary and secondary responses (Fig. 2). Four additional groups, each of ten animals, were immunized with a lesser challenge of $1 \times 10^7$ killed *E. coli* 075. Similar responses were again found in all groups.

*Viral challenge (ØX174 bacteriophage)*

Control and uraemic animals were challenged with an intraperitoneal injection of $1 \times 10^{11}$ PFU of

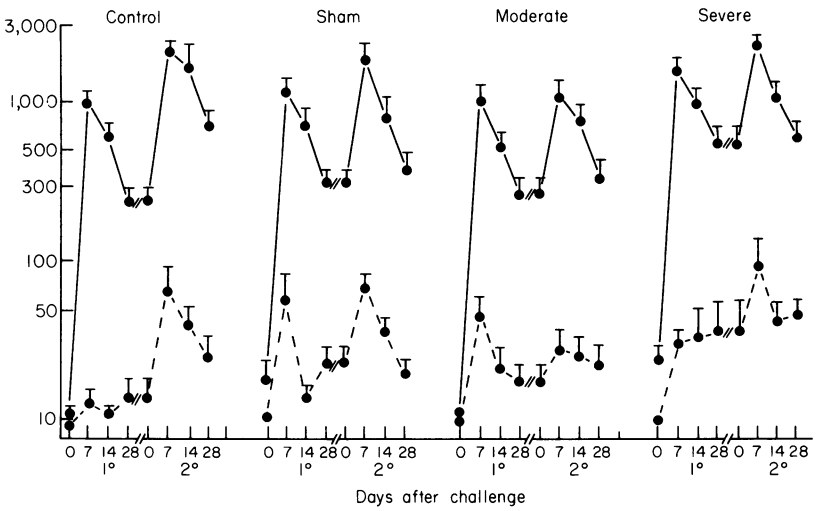


**Fig. 2.** Effect of uraemia on immune responses to *E. coli* 075. Four groups of animals were injected i.p. with a primary challenge of $1 \times 10^9$ *E. coli* 075 and a secondary challenge of $1 \times 10^8$ *E. coli* 075. (●——●) Total antibody titre, (● - - - ●) IgG component. The ordinate is the reciprocal of the highest dilution of serum giving macroscopic agglutination.

the bacterial virus ØX174. Peak titres of neutralizing antibody in excess of 1:3,000 were found in all groups tested 28 days later (Fig. 3). All animals were then challenged with a further $1 \times 10^{11}$ PFU of ØX174. Mean peak serum antibody titres of between 1:20,000 and 1:30,000 were found in the control and sham-operated groups. Titres in excess of 1:30,000 were also obtained for animals in both the moderately and severely uraemic groups.

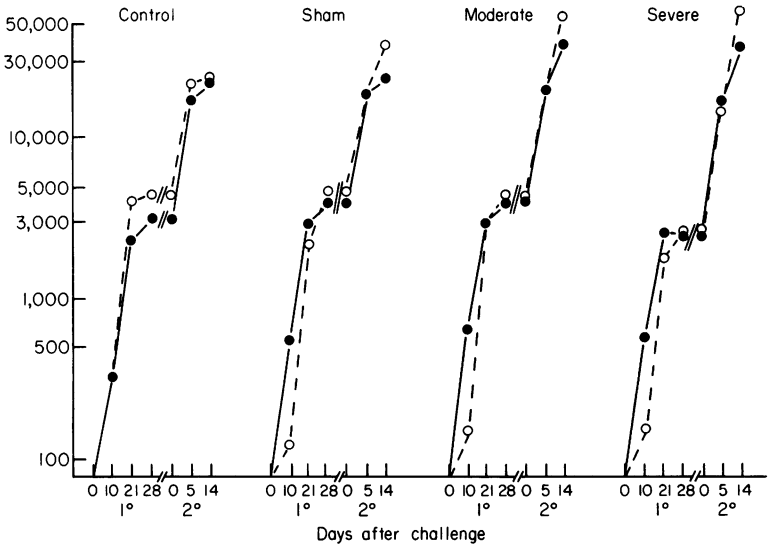


**Fig. 3.** Effect of uraemia on immune response to ØX174. Four groups of animals were injected intraperitoneally with $1 \times 10^{11}$ PFU of ØX174 bacteriophage. A secondary challenge of $1 \times 10^{11}$ PFU was given 28 days later. (●——●) Total antibody titre, (o - - - o) IgG component. The ordinate is the reciprocal of the dilution of serum giving 50% neutralization of virus.

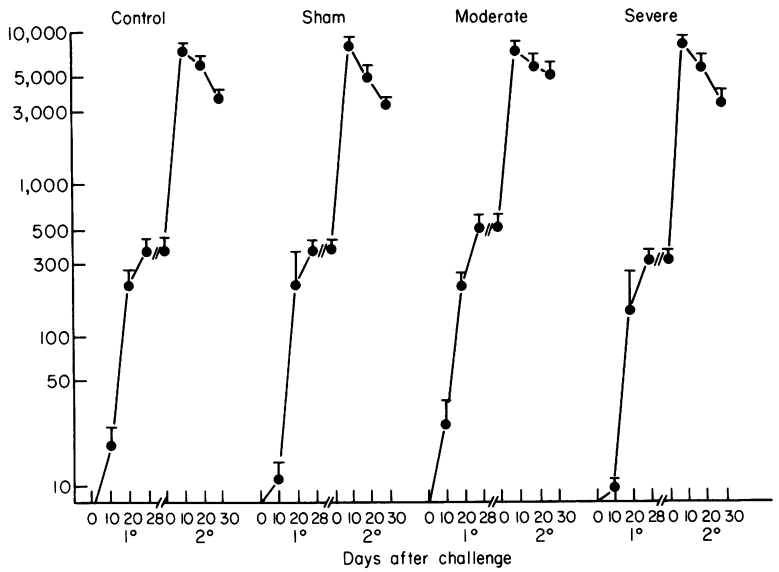


**Fig. 4.** Effect of uraemia on immune responses to KLH. Four groups of animals were injected subcutaneously with 0·5 mg of KLH and a secondary challenge of 0·05 mg of KLH injected intravenously 28 days later. (●——●) Total antibody titre. The ordinate is the reciprocal of the highest dilution of serum giving macroscopic agglutination.

*Keyhole limpet haemocyanin*

The four groups of control and uraemic animals were injected subcutaneously with 0·5 mg of KLH in Freund's incomplete adjuvant. The profile of the serum antibody response over a 28-day period was comparable in all four groups of animals studied (Fig. 4). A secondary challenge of 0·05 mg of KLH was administered intravenously after 28 days. Once again, the results from all four groups were similar and peak antibody titres approaching 1 : 10,000 were attained by both the uraemic and control groups of animals.

## DISCUSSION

The role of uraemia as a factor contributing to the depression of humoral immune responses is controversial. Clinical studies indicate that some individuals have reduced antibody responses to a limited range of antigens (Boulton-Jones *et al.*, 1973; Byron *et al.*, 1976; Wilson *et al.*, 1965), but the evidence for impaired humoral immunity caused by uraemia *per se* is unconvincing. Varying aetiology and management factors associated with renal failure such as multiple drug therapy, haemodialysis, surgery and renal transplantation make it difficult to assess the effect of uraemia on the immune status in these patients. In the present experiments a carefully defined model has been used to investigate the humoral immune status of animals with varying degrees of uraemia. The ability of chronically uraemic animals to respond to antigenic challenge was determined and compared with sham-operated and control groups. The experiments have shown that animals with chronic, severe uraemia respond normally to challenges with SRBC, KLH, *E. coli* 075 and the bacteriophage ØX174. Uraemic and sham-operated animals did show a slightly reduced antibody response to the higher challenge of SRBC ($1 \times 10^9$) but were still able to produce a normal response to a lesser challenge of $2 \times 10^7$ SRBC. Thus it appears that the capacity of the severely uraemic host to respond to a variety of antigenic stimuli is relatively unimpaired.

Previous studies employing animal models have indicated that humoral immunity may be impaired. Gowland & Smiddy (1962) reported a reduced primary response to bovine serum albumin (BSA) in severely uraemic rabbits, while Souhami (1973) found that uraemic mice produced slightly lowered primary and secondary responses to SRBC and BSA. The humoral immune status of patients with uraemia has also been assessed but the results, on critical examination, have been inconclusive. Stoloff *et al.* (1958) found that a group of Schick-negative uraemic patients were able to produce diphtheria anti-toxin after a booster dose of diphtheria toxoid. Other workers have found reduced antibody responses to typhoid vaccine (Wilson *et al.*, 1965), TABT vaccine (Byron *et al.*, 1976) and KLH (Boulton-Jones *et al.*, 1973) in patients with chronic renal failure.

There is no evidence from the present experiments to suggest that chronic renal failure affected the ability of the antigen-stimulated host to produce antibody. Rather the results have shown clearly that the immune responsiveness of the uraemic host is normal. This conclusion is important in that it provides a basis for the further assessment of the immune status of the uraemic host. If indeed it can be established that these patients do have an immune deficit predisposing them to infection, it seems likely that the pharmacological activity of some drugs depressing immune mechanisms may be potentiated in uraemia. This possibility is currently under investigation.

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