HOST RESISTANCE TO *CANDIDA ALBICANS* IN URAEMIA

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SUMMARY. In renal failure, infection is a frequent complication and cause of death, which suggests that uraemia impairs immunity. In a comparison of *Candida albicans* fungaemia, peritonitis and subcutaneous infection in normal and severely uraemic rats, uraemia did not affect the course of these infections. *C. albicans* is an important pathogen in immunocompromised hosts and we conclude that uraemia is unlikely to be the primary factor that increases host susceptibility to infection with this fungus.

INTRODUCTION

Infection is a frequent complication and cause of death in renal failure (Montgomerie, Kalmanson and Guze, 1968). Although it is widely believed that uraemia impairs immunity and predisposes the host to infection, the association of uraemia and depressed immune status with susceptibility to infection is far from established. Conflicting results have emerged from analysis of the immune status of patients with renal failure. Most workers have not commented specifically on the immunomodulatory effects of uraemia and the contribution of uraemia to the observed reduction in immune status is difficult to assess. Furthermore, the laboratory analysis of immune cell competence is an indirect measure of host resistance and, in practice, this can best be assessed by studying the course of infection after an experimental challenge. *Candida albicans* was selected for such a challenge in a rat model because this organism is a common commensal that readily invades the immunocompromised host (Young *et al.*, 1974).

MATERIALS AND METHODS

Animals. Male and female rats obtained from the inbred Dark Agouti strain and weighing 200–250 g were divided into control and uraemic groups.

Induction of uraemia. Severe stable uraemia was induced by removing $88\% \pm 2\%$ of the renal tissue as previously described (Ormrod and Miller, 1980).

Yeast strain. A culture of *C. albicans* from a clinical specimen was obtained from the Diagnostic Laboratory, Auckland Hospital. Cultures of the yeast phase of this organism were maintained in Sabouraud's dextrose broth (Gibco peptone 180, 1%; dextrose, 4%) at -20°C and subcultured monthly on to Sabouraud's dextrose agar for storage at 4°C . Cultures used for these experiments were grown overnight in nutrient broth at 37°C and washed three times in sterile normal saline. The concentration was then adjusted to the required concentration by

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visual counting in an improved Neubauer counting chamber. The number of viable organisms was confirmed by serial ten-fold dilutions in Sabouraud's agar pour plates.

Experimental infections. (a) *Fungaemia* was induced by injection of 10^6 viable yeast cells in 500 μ l of saline into the ventral tail vein of each lightly anaesthetised rat.

(b) *Peritonitis* was induced by intraperitoneal injection of 10^8 viable yeast cells in 2 ml of saline into the lower right quadrant of the abdomen whilst the rat was lightly anaesthetised.

(c) *Infection with subcutaneously-implanted sponges.* Cylindrical polyurethane sponges (2.5×1 cm, 40 ± 1 mg) were loaded with 10^6 yeast cells in 10 μ l of saline and implanted subcutaneously, as previously described (Clarke, Ormrod and Miller, 1983). After the selected time period, the sponges were removed aseptically and weighed, and the yeast cells removed by squeezing the sponge in 9 ml of saline with a rotating teflon pestle in a heavy-walled glass tube. Sabouraud's dextrose agar pour plates were made from serial ten-fold dilutions to obtain the yeast-cell count per sponge after correcting for the amount of inflammatory fluid in the sponge. The yeast-cell count was expressed as the log number of cells per sponge; two sponges were placed in each rat.

(d) *Footpad challenge.* Viable yeast cells (10^7) suspended in 20 μ l of sterile normal saline were injected into the plantar tissue of the hind foot, but not subcutaneously, with a 26-gauge needle and a 0.1-ml Hamilton syringe (Gray and Jennings, 1955). Injections were made into the footpads of both hind feet in all rats whilst they were lightly anaesthetised. The thickness of the footpad was measured in its centre, between the dorsal and plantar surfaces, with a dial micrometer (model 7309, range 0.01–9.00 mm $\pm$ 0.01 mm, Mitutoyo Manufacturing Co. Ltd, Tokyo, Japan), modified to avoid pressure on the swollen tissue by reducing the spring tension. Before the yeasts were injected into the footpads, a base-line thickness for each animal was measured. After injection, the footpads were measured daily and the percentage increase in thickness calculated by the formula:

$$\text{Percentage change} = \frac{\text{present thickness} - \text{base-line thickness}}{\text{base-line thickness}} \times 100$$

The footpad thicknesses of 10 normal untreated rats were measured over 10 days to establish the error of the procedure. The variation from the base-line footpad thickness was $\pm 1.8\%$.

Determination of numbers of yeast cells in tissues. After the selected time periods the rats were anaesthetised for the collection of heparinised blood samples to be used for the measurement of urea and numbers of yeast cells. The animals were then killed for assessment of the number of yeast cells in the lungs or peritoneal cavity, spleen and the median lobe of the liver. For each sample, Sabouraud's dextrose agar pour plates seeded with serial ten-fold dilutions were used to obtain yeast-cell numbers/g of tissue or ml of blood or rinse fluid. (a) *Blood* (2 ml) was collected by aseptic heart puncture in a heparinised syringe; 1 ml was diluted in 5 ml of sterile normal saline for culture, and from the remainder the plasma was separated for urea analysis by an autoanalyser (Technicon Inc., Tarrytown, NY). (b) *Peritoneal cavity.* Sterile normal saline (10 ml) was injected into the peritoneal cavity and the abdomen was then massaged to ensure even distribution of the fluid. The saline was aspirated into a sterile syringe through an adapted pediatric trochar and a 0.1-ml sample was spread on blood agar to check for contamination. (c) *Lungs, liver and spleen.* To expose the thoracic and abdominal organs, the abdominal wall and ribcage were resected aseptically. The tissues required were removed aseptically and weighed. The main thoracic blood vessels were clamped if the lungs were to be removed. Where peritonitis had been induced, the spleen and liver lobe were each rinsed twice in sterile normal saline and blotted to remove cells adhering to their outer surfaces. Tissue samples were homogenised in normal saline by a rotating teflon pestle in a heavily walled glass tube (Tri-R Instruments, Long Island, NY); spleens and lungs were chopped coarsely with fine scissors to facilitate homogenisation.

Experimental protocol. Pilot experiments were performed to establish the pattern of yeast cell elimination in normal animals challenged with different numbers of *C. albicans*. Our objective was to determine the size of an inoculum of *C. albicans* that presented a "severe" challenge to the normal host, the criterion adopted being the dose that lead to the persistence of fungus for several days after challenge. From the results, a suitable inoculum and representative time interval was selected for each experiment. All subsequent experimental

Fig. 1.—Clearance of *C. albicans* from various tissues after induction of fungaemia by i.v. injection of 10^6 yeast cells. left. Clearance rates in normal animals ($n = 8$, range = SD). right. Comparison of numbers of yeast cells isolated from samples of tissues from control animals (C) that had undergone sham operations and uraemic rats (U) 24 h after inoculation.

FIG. 3.—Increase in footpad thickness after intraplantar inoculation of 10^7 yeast cells. Footpad oedema was measured daily and the percentage increase over the base-line calculated for control (C) and uraemic (U) rats.

infections were induced 4 weeks after the second surgical operation (see Ormrod and Miller, 1980) to reduce renal mass (unilateral nephrectomy) or a sham operation.

RESULTS

Renal function in test animals. The mean blood urea levels in normal and severely uraemic rats were 7.5 ± 1.0 mmol/L (44.8 ± 6.1 mg/dl) and 24.5 ± 7.3 mmol/L (146.6 ± 43.4 mg/dl) respectively.

Fungaemia. Within 48 h after intravenous inoculation of 10^6 yeast cells, normal animals had eliminated the organisms from their blood. The lungs were also rapidly cleared of yeast cells, but the liver and spleen retained large numbers (fig. 1 left). The time interval chosen for the comparative study was 24 h. No difference between the persistence of fungaemia in normal and uraemic animals was found (fig. 1 right).

Peritonitis. After inoculation of 10^8 yeast cells into the peritoneal cavity, normal rats almost totally cleared the cells from their blood within 4 h and the numbers in the cavity were reduced to 2.5×10^2 /ml of rinse fluid by 72 h. The liver and spleen again retained high numbers of cells (fig. 2 left). The time interval chosen for the comparative study was 72 h. Again no difference between the results obtained with normal and uraemic animals was found (fig. 2 right).

Subcutaneous infection with implanted sponges. Yeast cell numbers in sponges implanted into normal rats were constant throughout a 72-h period at $(1.23 \pm 0.44) \times 10^5$ cells/sponge after a challenge of 10^6 cells even though the volume of inflammatory fluid gradually increased. Moreover, a comparative study of this in normal and uraemic animals 72 h after implantation showed no differences.

Footpad challenge. The footpad inflammatory response was used as an index of

fungal replication because it has been shown to be of value in demonstrating a reduced immune status (T.E. Miller and G. Findon, unpublished observation). Apart from a slightly enhanced response in uraemic animals 1 day after challenge, similar increases in footpad oedema were found in the footpads of control and uraemic rats during a 10-day period (fig. 3).

DISCUSSION

Experimentally induced infections in uraemia seem to provide the most direct method of assessing the effect of uraemia on immune capability. With our model (Ormrod and Miller, 1980), stable mean blood-urea concentrations of 25 mmol/L (147 mg/100 ml) and glomerular filtration rates that were 10% of normal ensured that the animals were in a stable state of chronic renal failure. Although it is well recognised that the kidney is one of the target organs for *Candida* localisation in experimental infection (Seelig, 1966), our requirement was that the model should predict the effect of uraemia on infection once an inoculum of yeast cells gained access to otherwise sterile sites. In this respect, the inoculum size was chosen carefully to allow a comparison of the immune capabilities of the uraemic and normal hosts. As judged by the persistence of yeast cells in the tissues for several days, the selected inocula established severe infection in normal animals. There was adequate opportunity, therefore, for evidence of a deficit in immune capability in the uraemic animals to be manifest.

Uraemia did not affect the course of *C. albicans* fungaemia, peritonitis or subcutaneous infection. *Candida* infection is a frequent complication in the management of the immunocompromised host and this organism may be considered as representative of the pathogens that cause problems in these patients (Young *et al.*, 1974; Bodey, 1975). In view of the belief that uraemia predisposes patients to infection, some indication of this might have been expected in our animal experiments. Clinical studies, however, are complicated by many variables including haemodialysis, drug therapy, malnutrition and surgical manipulations, all of which may alter the immune response. Uraemia is only one of several physiological and metabolic disturbances found in renal failure, but, in our opinion, there is no clear clinical or experimental evidence to show that uraemia itself is the primary factor that increases host susceptibility to infection. This is not to say that uraemia might not have affected the immune capability of the rat if a different endpoint (e.g. an LD₅₀) had been used. However, overwhelming the rat with an extremely high challenge dose is not a realistic model of the human disease and our objective was to investigate the effect of uraemia on host immunity with a clinically relevant challenge.

We conclude that factors other than uraemia, either individually or collectively, are more likely to provide an explanation for the increased infection rate found in uraemic hosts.

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