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Cellular basis of host defence in pyelonephritis. III. Deletion of individual components

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Summary. Hosts were depleted of individual cellular components to determine the effects of these manipulations on cellular defence mechanisms in acute and chronic pyelonephritis. T-lymphocytes were found to have little or no involvement in host protection but cyclosporin A administration had a dramatic effect on the gross pathology and bacteriological status of experimentally induced pyelonephritis. This change represented a major depression of host defence status. Cyclosporin A also activated resolved lesions in chronic pyelonephritis, associated with an increase in bacterial numbers. Administration of antineutrophil serum also led to a 1000-fold increase in bacterial numbers in the acute phase but had little effect on the host-parasite balance in chronic pyelonephritis. Macrophage blockade, on the other hand, did not affect the course of either acute or chronic infection. These studies have provided additional information on the immunobiology of experimental pyelonephritis and have focussed attention on the role of neutrophils, and an unidentified mechanism, affected by cyclosporin A, in host defence to renal infection.

Keywords: pyelonephritis, host defence, cellular defences

The host-parasite relationship in pyelonephritis has been studied for many years and excellent morphological descriptions of cellular aspects of the host's response to infection are available. A considerable amount of information has also been published detailing the local and systemic response to infection (Holmgren & Smith 1975). While an association between cellular components and host defences can be inferred from such studies, morphological observations can only provide a guide to their actual role *in vivo*. In the case of pyelonephritis, infection does not follow the course one would predict from observing the morphological and immunological data. For example, infection is readily established in the kidney in the face of a prompt inflammatory response and persists despite vigorous local and systemic responses of the immune system. In two recent papers in this series, we assessed the contribution of cellular components of the immune and inflammatory system to host defences in pyelonephritis by studying the effect of non-selective cytodepletion (Miller *et al.* 1986*a*,*b*). Cellular components of the host defence system were found to have a limited impact on the establishment of the infection

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but played an important role in containment. The present experiments were carried out to extend these initial studies by depleting the host of individual components of cellular defence mechanisms and to observe the effects of these manipulations on the course of acute and chronic pyelonephritis.

Materials and methods

Experimental host. Adult DA (Dark Agouti) rats from an inbred colony, each weighing between 180 and 250g, were used in the majority of these experiments.

Experimental infection. Pyelonephritis was induced by the direct inoculation of *Escherichia coli* 075 into the surgically exposed kidney using a glass microcapillary (Miller & Robinson 1973). The challenge of approximately 1500 viable organisms was delivered in two 5μl inocula into the poles of the kidney. To assess the effect on chronic infection, the various manipulations were carried out four weeks after challenge.

Quantitative evaluation of gross renal pathology. After removal of the kidney, the gross renal pathology was quantified using a paper profile of the kidney which was divided into 10 equal wedge shaped areas. Renal lesions and scar formation were shaded in on the kidney profile following direct visual inspection of the kidney. Where lesions existed on both sides of the kidney, two profiles were used. Each division of the profile therefore equalled 5% of the total kidney surface. Gross pathological changes were expressed as a percentage of the total kidney surface.

Bacterial content of renal tissue. Nutrient agar pour plates of serial 10-fold dilutions of homogenized, weighed kidneys were made to obtain the bacterial count per gram of wet renal tissue.

Peripheral blood leucocytes (PBL). Total blood leucocyte counts were carried out using a Coulter counter, on the venous blood (EDTA) of pyelonephritic animals, both cyclosporin A (CsA) and carrier treated. Differential leucocyte counts were performed on a Leishman stained blood film.

Neutrophil mobilization in vivo. The total number of neutrophils harvested from subcutaneously implanted polyurethane sponges were counted using a haemocytometer and a differential count was carried out on a Leishman stained Cytospin preparation. This method has been described fully elsewhere (Miller *et al.* 1986*a*).

Anti-neutrophil and anti-lymphocyte serum. Anti-neutrophil serum (ANS) was raised in rabbits by repeated immunization with rat polymorphonuclear leucocytes (PMN) extracted from subcutaneously implanted sponges. Lymphocytes from rat spleens and lymph nodes were injected into goats to produce anti-lymphocyte serum (ALS). After heat inactivation, unwanted antibodies were absorbed using lymphocytes for the ANS, PMNs for the ALS and red cells for both antisera. Titres were performed after the absorptions and were 1/160 and 1/320 for the ANS and ALS respectively. An immunoglobulin fraction of each antisera was prepared using the ammonium sulphate precipitation method. In the acute infection experiments, 0.4 ml of ANS or ALS was administered intraperitoneally, from 2 days before challenge until they were killed. With chronic infections, antisera was given daily for 4 days before killing.

Non specific cytoreduction

Irradiation. Graded levels of cytodepletion were achieved by irradiating groups of animals, 2 days before challenge, with 1, 3, 5, 7 or 9 Grays (Gy) of total body irradiation using a Cobalt-60 source.

Cyclophosphamide. Varying numbers of subcutaneously implanted bone cement discs containing 75 mg of cyclosphosphamide (Endoxan-Asta, Asta-Werke AG, Bielefeld,

FRG), were implanted into animals to achieve graded cytodepletion (Ormrod *et al.* 1985). Doses ranged from 75 to 375 mg, and the discs were implanted 6 days before challenge. Control animals had drug free discs implanted.

Specific deletion

Cell-mediated immunity. An athymic nude mutant rat strain was obtained from an outbred colony, maintained by the Department of Medicine, University of Auckland. These homozygous (rnu^{nz}/rnu^{nz}) rats are totally hairless and have a non functional thymic anlage (Douglas-Jones *et al.* 1981; Marshall & Miller 1981). Euthymic rats of the wild-type Wistar strain, from which the rnu^{nz} mutant was derived, were included in the experiments as controls. These animals had normal hair and immune function.

T helper (T_H) lymphocyte function. Cyclosporin A, (Sandoz Ltd., Basle, Switzerland), was provided in powder form and dissolved in warm Cremaphor EL (BASF, Aktiengesellschaft, D-6700, Ludwigshafen). The appropriate dose was administered in 0.1 ml amounts, by intramuscular injection into the thigh muscle *alte die.* During studies on the effects of CsA on acute infection, the drug, or carrier material for controls, was administered from 7 days prior to challenge and throughout the course of the experiment. Preliminary experiments with acute infection have shown that a 5–7 day course of CsA, before challenge, was necessary in order to affect host defences. Treatment for chronically infected animals was started 4 weeks after the initiation of infection.

Antineutrophil serum. The normal host has considerable neutrophil reserves. During preliminary experiments it was established that ANS alone was not able to totally ablate mobilizable neutrophil numbers without loss of specificity. Neutrophil depletion was therefore accomplished by first exposing the host to 5 Grays of total body irradiation. Neutrophil numbers were reduced without affecting host defences or the bacteriological status of the kidney in the irradiated animals. In protocols that involved acute experiments, animals were first irradiated, then 0.4 ml of ANS (ALS and normal rat serum (NRS) as controls) was administered intraperitoneally from 2 days before challenge and continued daily until autopsy. Pyelonephritis was established 5 days after irradiation. In chronic infections, animals were also irradiated before the administration of antisera which was given as a daily dose commencing 4 days before autopsy.

Macrophage and reticuloendothelial system (RES) blockade. Carrageenan type II (Sigma Chemical Co., St Louis, MO) was injected intraperitoneally at a dose of 200 mg/kg in 7.5 ml saline and silica (1.5 μm, Sigma) was given intravenously at a dose of 200 mg/kg in 0.4 ml saline. The silica treated group and their controls, were given oral Bart's solution to prevent lower urinary tract obstruction associated with silica administration. In acute experiments, the agents were administered 24 h before challenge.

Results

Manipulation of thymus derived (T) cells

Ablation of cell-mediated immunity. A recently described mutant (rnu^{nz}/rnu^{nz}) rat (Douglas-Jones *et al.* 1981; Marshall & Miller 1981) has been shown to lack cell-mediated immune capability. This cellular deficiency did not affect host defence mechanisms as judged by the bacteriological status of the kidneys of the athymic (nude) and heterozygous (euthymic) animals with experimentally induced renal infection (Fig. 1).

Ablation of T_H cell function using CsA. The dose of CsA selected for administration to the experimental host had been previously shown to be the equivalent of a 'clinical' dose of the drug (Thomson *et al.* 1981). CsA administration did not affect bacterial

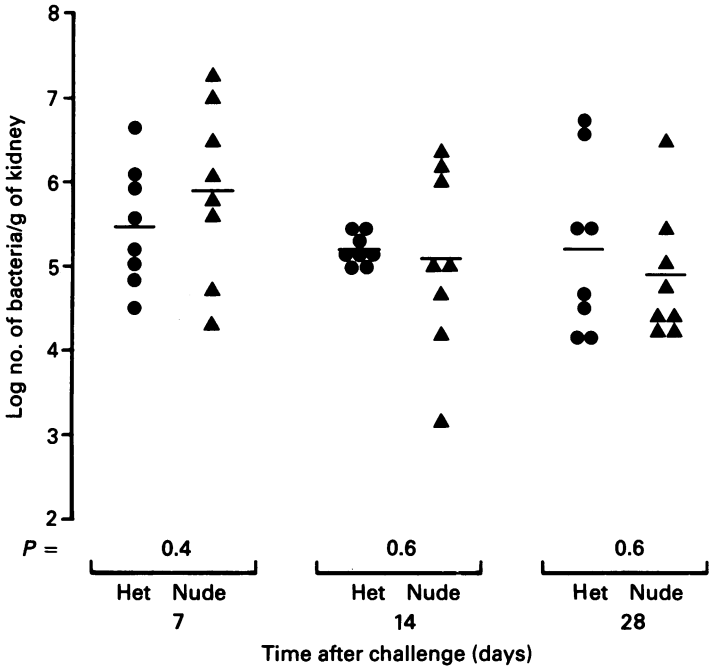

Fig. 1. Bacteriological status in the kidney of nude (athymic) hosts compared with heterozygous (euthymic) controls.

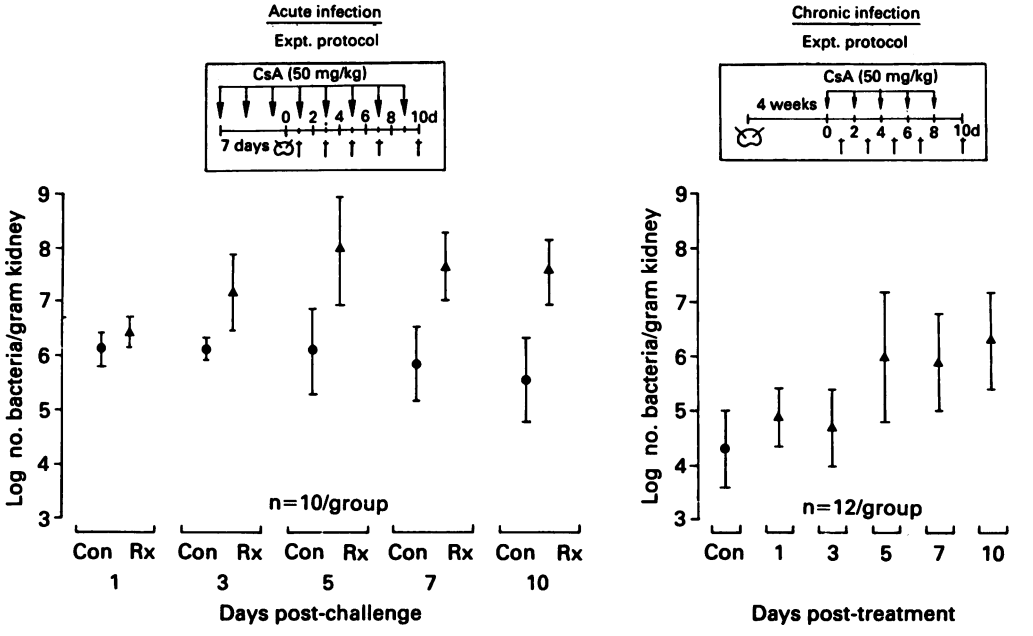

Fig. 2. The effect of CsA (50mg/kg) on acute and chronic pyelonephritis. (challenge; † killed). Range = s.d.

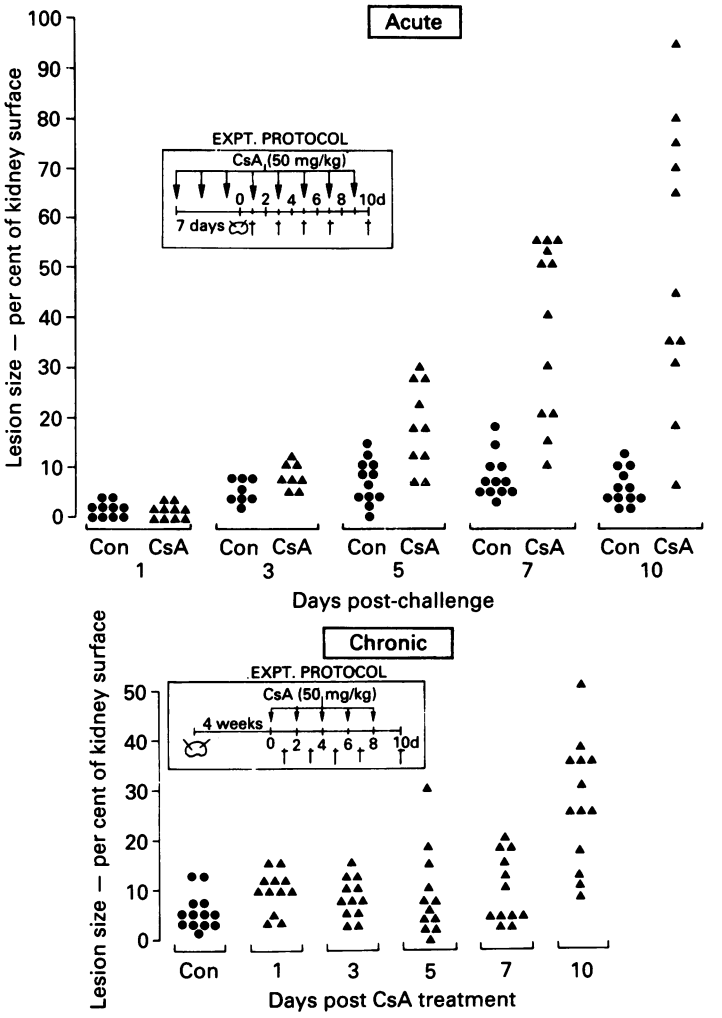

Fig. 3. Cyclosporin A administration (50mg/kg) markedly affects lesion formation in acute pyelonephritis. Gross reactivation of the lesion occurs in chronic infection (challenge; † killed).

numbers in the kidney 24 h after challenge but up to a 100-fold increase in bacterial numbers was demonstrable over a 10 day period (Fig. 2). Chronically infected kidneys showed a 100-fold increase in bacteriological numbers, 10 days after the commencement of treatment with CsA. The lesion size in acute pyelonephritis markedly increased in CsA treated animals and by 10 days involved over 65% of the surface in half of the kidneys examined, compared with less than 10% for the controls. Ten days after CsA treatment, gross reactivation of the renal lesion involving up to 50% of the kidney surface, was seen in chronically infected kidneys (Fig. 3).

Peripheral blood leucocytes in CsA treated animals. Haematological parameters were determined in CsA and carrier treated pyelo-

Table 1. The effect of CsA (50mg/kg) on PBL and neutrophil mobilization in animals with acute pyelonephritis

Days post-challenge	Total PBL ($\times 10^9$/l)		Neutrophils ($\times 10^9$/l)		Lymphocytes ($\times 10^9$/l		Mobilization Cells/sponge ($\times 10^6$)	
	Con	CsA	Con	CsA	Con	CsA	Con	CsA
1	11.9±2.0	19.6±1.9	4.1±1.1	6.4±2.8	7.7±1.3	12.8±2.5	—	—
5	17.4±3.8	28.6±2.9	5.6±2.2	10.8±3.0	11.5±1.9	16.9±2.6	—	—
7	17.2±3.9	31.6±2.9	4.7±1.9	12.2±2.9	12.4±2.7	17.2±1.6	11.0±2.0	4.4±1.6
10	16.7±1.0	27.4±4.1	4.5±1.0	9.6±3.0	10.2±3.3	14.6±4.0	—	—

nephritic animals in view of the possibility that provocation of infection resulted from CsA induced leucopenia. In fact, CsA administration led to a considerable increase in total peripheral blood leucocytes in pyelonephritic animals compared with infected control animals (32×10^9/l *vs* 17×10^9/l). An increase in both neutrophils and lymphocytes accounted for the rise in circulating PBL (Table 1).

Comparative effects of CsA and non-specific cytoreductive agents. Previous experiments have shown that before host defences are compromised, cellular competence, as indicated by PBL numbers and confirmed by leucocyte mobilization studies, needs to be markedly reduced (Miller *et al.* 1986a,*b*). CsA administration did not reduce PBL numbers although host defences were depressed. These results suggested a different relationship to those previously observed. In this experiment, the effect of two cytoreductive procedures (irradiation and cyclophosphamide) on cellular competence and host defence were compared with CsA. Graded levels of depletion of cellular mechanisms were achieved using increasing doses of irradiation or cyclophosphamide. In the case of the cytodepletive agents, substantial reductions of cellular competence had a minimal effect on host defences as shown by changes to the bacteriological status of the kidney. Increasing doses of CsA on the other hand, induced changes in host defence capability at quite modest reductions in cellular competence, as judged by the neutrophil mobilization test (Fig. 4).

Anti-neutrophil serum

Neutrophil mobilization. Evidence for the specificity of the ANS was sought by implanting polyurethane sponges subcutaneously in several groups of animals. The effect of the various antisera on neutrophil mobilization was then determined. Irradiation reduced the inflammatory response to 30% of normal and the additional administration of ALS and NRS had no additional effect on cell mobilization. ANS was the only agent to completely ablate neutrophil response and affect the accumulation of inflammatory fluid in the sponge (Fig. 5).

Acute and chronic infection. Animals were irradiated (5 Gy) before the administration of ANS, ALS or NRS. At autopsy, 48 h after challenge, the bacteriological status of renal tissue in animals with acute pyelonephritis, was not affected by irradiation, ALS or NRS. Neutrophil depleted animals (ANS) however, showed a 500-fold increase in bacterial numbers. The effect of ANS on chronic

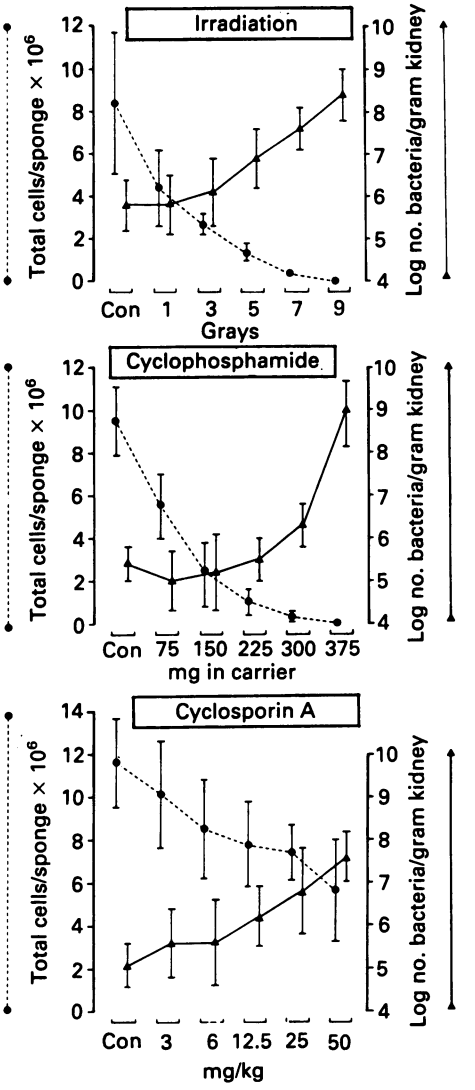

Fig. 4. The relationship between the cellular response, (leucocyte mobilization), and the bacteriological status in early renal infection, using varying amounts of irradiation, cyclophosphamide and cyclosporin A. Range = s.d.

infection was also evaluated. Animals with chronically infected kidneys (4 weeks), were irradiated and treated with ALS or ANS. Although a trend was evident, these agents did not have a significant effect on bacterial numbers in established infection (Fig. 6).

Macrophage blockade. Two agents were used to inhibit macrophage and RES function. Administration of silica particles or carrageenan at doses known to depress host resistance, did not alter the course of acute or chronic pyelonephritis (Fig. 7).

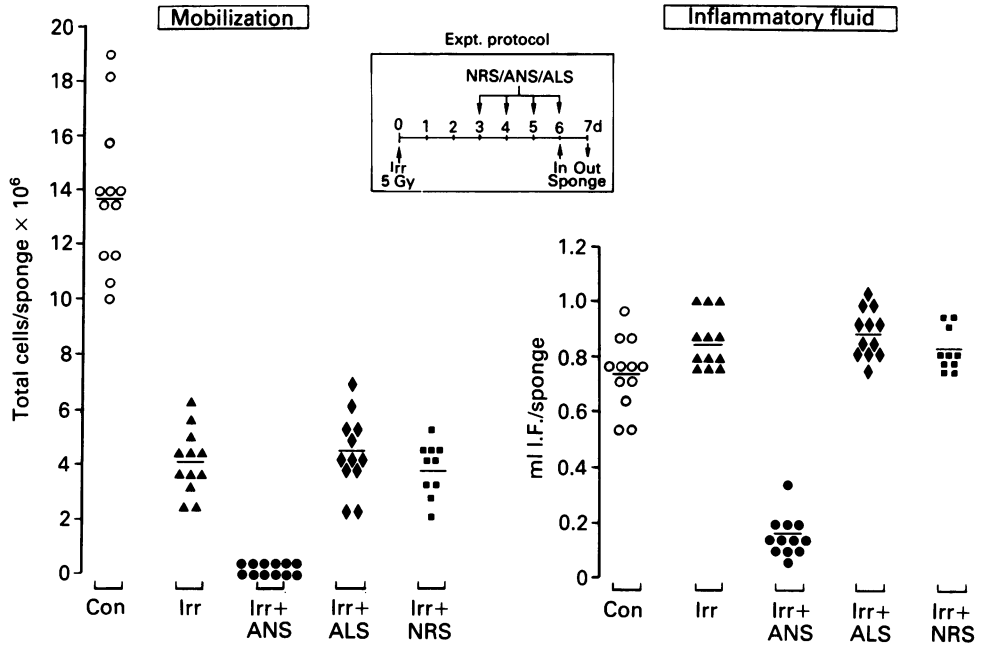

Fig. 5. Effect of anti-neutrophil and anti-lymphocyte serum, plus total body irradiation (5 Grays), on the inflammatory response, using leucocyte mobilization and the volume of inflammatory fluid as indicators.

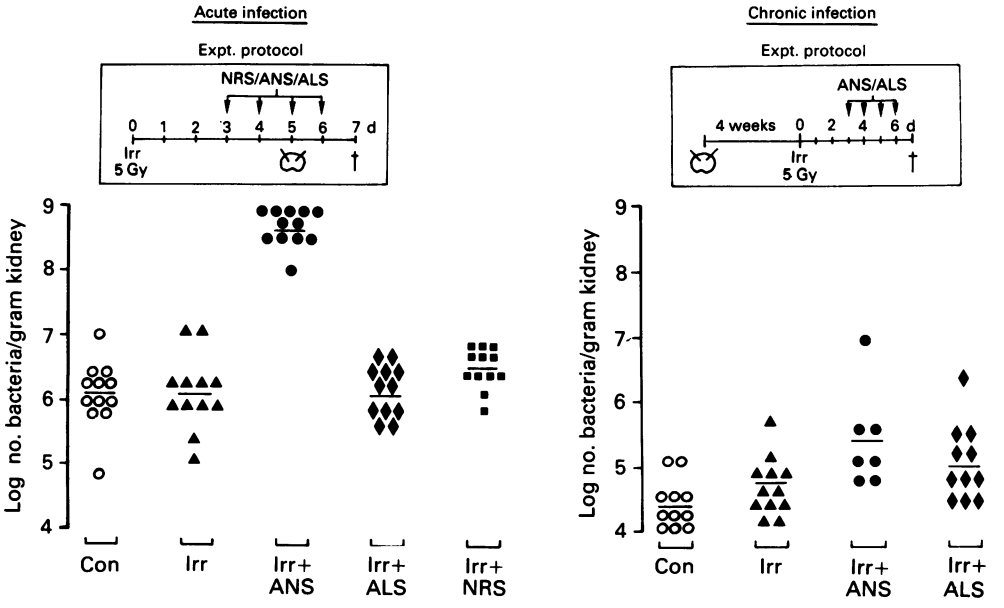

Fig. 6. Anti-neutrophil serum withirradiation (5 Grays), affects host defences in acute pyelonephritis. Chronic pyelonephritis shows no significant change. (ᘓ challenge; † killed).

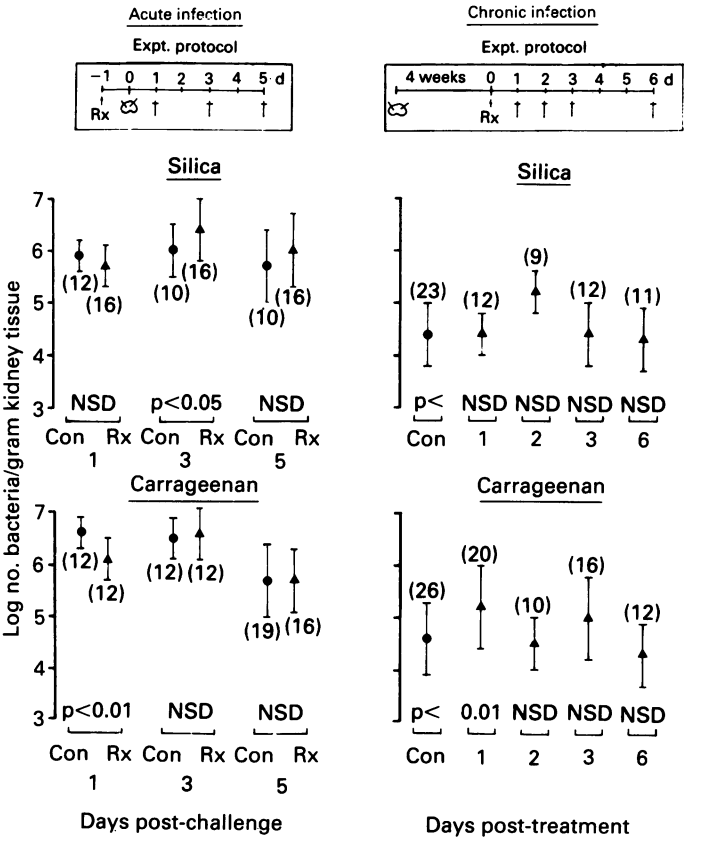

Fig. 7. Silica and carrageenan, 200 mg/kg, and their effect on acute and chronic pyelonephritis. (ᘓ challenge; † killed). Numbers in brackets = number of kidneys assayed. Range = s.d.

Discussion

There is an adequate description of the host response to pyelonephritis but the influence of the cellular response in renal infection has only recently been investigated. In two previous publications we have studied the quantitative relationship between cellular competence and host defences (Miller *et al.* 1986*a*,*b*). We concluded that cellular components participate in host protective mechanisms in the kidney in both acute and chronic renal infection but although they are quantitatively adequate, they are not effective at pathogen eradication. In the above experiments, cytodepletive agents were used to deplete the host of cellular reserves, but because the effect was non-selective, it was not possible to attribute any particular host protective capability to an individual cellular component. The present studies set out to determine the effect of selective depletion of cellular mechanisms on the course of acute and chronic renal infection.

The experiments first established that thymus-derived T lymphocytes have little or no involvement in host protection, and confirm earlier observations which would have predicted such a result (Miller 1984; Coles *et al.* 1974). Studies with the athymic mutant however were essential to establish this fact with certainty. With this finding in mind, the results obtained following the administration of CsA were surprising. This immunomodulator blocks cell-mediated immunity through it's effect on lymphokine production

by T cells (Klaus & Chisholm 1986) but because the absence of T cells did not affect host defences, CsA was not expected to alter the course of renal infection. CsA administration however, had a dramatic effect on the gross pathology and bacteriological status of experimentally infected kidneys, which represented a major change in defence status. Bacterial numbers in renal tissue increased up to 100-fold in infected animals receiving CsA and 10 days after challenge, at a time when lesions in the control group were resolving, CsA treated animals exhibited unresolving lesions, some involving over 50% of the surface area of the kidney. CsA administration also activated the resolved lesions of chronic pyelonephritis. Scarred areas with no evidence of an active infection were provoked, and over a 10 day period, were found at autopsy to have undergone a change from quiescent, healed lesions, to renal abscesses, compared with unmanipulated controls. One point that needs emphasizing is that the dose used did not induce leucopenia and was considered to be the equivalent in the rodent host to a 'clincally effective dose' in man (Thomson *et al.* 1981). This dose does not cause any haematological, biochemical or histopathological changes in normal animal hosts, apart from a small increase in circulating leucocyte numbers. Non-specific cytoreductive activity by CsA therefore does not explain the results obtained. When the effects of CsA and the cytodepletive agents, cyclophosphamide and irradiation were compared, two totally different relationships were found. The explanation for the depression of host resistance is not clear at this stage but two possibilities come to mind. The first is that cellular mechanisms, not affected by cytoreductive procedures, could be depressed by CsA. The other more likely possibility is that CsA induces a qualitative change in a critical cellular component that renders it ineffective. Such a change however is not immediately obvious from the quantitative and qualitative analyses carried out so far (Miller *et al.* 1986*a*,*b*). Our present findings in renal infection are supported by a number of other recent reports describing the provocation of infection by CsA and although some of these could be explained by an effect on T lymphocytes, others clearly cannot (Loliger & Lehmann-Grube 1985; Moffat *et al.* 1985; Schaffner *et al.* 1983; Perfect & Durack 1985).

Administration of anti-neutrophil serum led to a 500-fold increase in bacterial numbers in the acute phase but had little effect on the host-parasite balance in chronic pyelonephritis. The specific ablation of neutrophil numbers and functional capability by the antiserum and the lack of effect by anti-lymphocyte serum was a particularly clear cut result. Obviously the neutrophilic component of the inflammatory response does represent an effective host defence. That infection is established in the face of a vigorous inflammatory response can be explained by the rapid bacterial growth once microorganisms have penetrated the renal parenchyma. This outstrips the ability of the host to mount an effective response. Total depletion of cellular competence makes little difference to the microbial growth curve during the first 16 h after challenge. Our results parallel recent observations involving experimentally induced endocarditis and meningitis (Ernst *et al.* 1983; Meddens *et al.* 1982). In those experiments, cellular depletion with nitrogen mustard had no measurable effect on the induction of endocarditis but did enhance the level of infection subsequently. Similarly, during experimental pneumococcal meningitis, leucocyte depletion did not affect the level of infection in the CSF in the early stages but did limit host defences concerned with bacteraemia. In these examples, as in the kidney, infection is established before inflammatory cells can intervene. This clearly limits the host-protective role of these cells in the early stages of infection so that cellular defence mechanisms have a limited impact on the establishment of infection but play an important role in containment.

Although the role of inflammatory cells in

limiting bacterial growth in the kidney seems obvious in retrospect, few studies of this aspect of the host response have been reported in the literature. Experiments similar to ours, in which nitrogen mustard was used to deplete the host of neutrophils, were carried out by Jackson *et al.* in 1965. We disagree with their conclusion that granulocytes did not play a major role in the initiation or progression of experimentally induced pyelonephritis. Two factors need to be taken into account by way of an explanation: first, from our own studies, it is evident that host defence competence may be maintained in the face of a severe reduction in the number of circulating neutrophils and it is likely that the cytoreduction, achieved by Jackson *et al.* (1965), was not sufficient to affect host defences. Secondly, our studies have concentrated on the early phase of infection, which is more likely to reveal the effect of neutrophil depletion, than the 2 and 6 week intervals used by Jackson *et al.* (1965). In related studies, Rocha and Fekety (1964) showed that granulocytes were not found in the medulla until 12 h after thermal injury whereas they appeared in the cortex within 2 h. Although they concluded that this explained the comparative susceptibility of the medulla to infection, a delay in neutrophil mobilization, *per se*, does not explain this finding, as cellular components are not effective in eradicating invasive microorganisms in the first 8 to 16 h.

Macrophage blockade using the selectively toxic agents, silica and carrageenan, did not affect the course of either acute or chronic infection. We could be criticized for not determining that the dose of silica used was capable of depressing mononuclear-macrophage function. There is however, considerable literature on this topic, showing the ability of silica to deplete macrophages from lymphoid cell suspensions *in vitro* (Levy & Wheelock 1975) and to depress host resistance to a number of infectious diseases. These include *Salmonella typhimurium* (O'Brien *et al.* 1979), *Cryptococcus neoformans* (Monga 1981), *Entamoeba histolytica* (Ghadirian & Kongshavn 1984), *Listeria monocytogenes* (Takeya *et al.* 1977) and some tumours (Keller 1977). We regard these reports as adequate confirmation of the efficacy of silica to deplete the host of mononuclear macrophages and support for our view that this system is not a critical component of the cellular defence system in pyelonephritis.

There is little doubt that renal infection initiates a vigorous immune and inflammatory response. However, we do need to know what contribution the cellular involvement makes to host defences but the question has not received a lot of attention. The current studies have helped to define the immunobiology of pyelonephritis and have shown that the inflammatory, rather than the immune system, is the dominant host defence component in this disease. Additionally, we have identified cyclosporin A as a useful biological probe that will allow the role of cellular components to be determined.

Acknowledgements

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