Synergism Between Immunosuppressive Agents and Uremia?

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The susceptibility of uremic patients to infectious disease has been widely reported, but the host immune factors associated with the increased incidence of infection have not been clearly defined. In this study, the possibility of synergism between biologically active components that accumulate in uremia and immunosuppressive drugs used in the course of management was investigated. An animal model of chronic stable uremia was used in these experiments to assess the effect of cyclophosphamide, methotrexate, and azathioprine on antibody response in the uremic host. Chronic uremia did not affect the immunosuppressive activity of cyclophosphamide, methotrexate, or azathioprine, and synergism between these agents and uremic components is unlikely to complicate further the immune status of the host in renal failure.

The effect of uremia on host immune responses has been examined in an earlier series of experiments (5, 7, 11; J. Nelson, D. J. Omrod, and T. E. Miller, Kidney Int., in press). These studies have shown that no single component of the cellular immune mechanism is compromised to a degree that could account for the increased incidence of infectious disease found in uremic patients. Thus, there is a disparity between experimental findings and clinical observations. One explanation is that drugs commonly used in the management of renal failure become immunomodulatory in the uremic host. Immunosuppressive drugs are frequently given to patients in renal failure, and in a case report it was postulated that synergism between these agents and biologically active components that accumulate in uremia might lead to an increased susceptibility to infection (4). Others have stated that such a combination predisposes patients to serious infection (3, 6, 9, 10), but to date no experimental evidence for or against this postulate has been presented. A model of chronic stable uremia has been developed recently in our laboratory (8). In the present experiments, this carefully defined model was used to evaluate the combined effect of uremia and the administration of the immunosuppressive drugs cyclophosphamide, methotrexate, and azathioprine on the antibody response to sheep erythrocytes (SRBC).

MATERIALS AND METHODS

Animals. Female animals, obtained from an inbred strain of Dark Agouti rats, weighing 200 to 220 g, were used.

Induction of uremia. A standardized procedure involving the surgical resection of renal tissue was used to induce severe uremia, defined by a blood urea concentration greater than 150 mg/100 ml (>25 mmol/liter). Control groups of "sham"-operated animals were included in each experiment. The sham operation duplicated the surgical trauma associated with the induction of uremia but did not affect renal function. The experimental model has been described in detail elsewhere (8).

Collection and storage of blood samples. All blood samples were obtained from the ventral tail vein with a 1-ml syringe and a 26-gauge needle. Serum was separated within 30 min and stored at −20°C until analyses for serum antibody or blood urea were carried out.

Measurement of blood urea. Blood urea was measured by using an autoanalyzer (Technicon Corp., Inc., Tarrytown, N.Y.).

Determination of antibody response. Animals were challenged intraperitoneally with 10⁶ washed SRBC in 1 ml of complement fixation diluent. Twenty-eight days after primary challenge, a secondary response was elicited with an intraperitoneal injection of 10⁶ SRBC. Serum antibody responses were measured by the hemagglutination assay. Fifty-microliter doubling dilutions of serum in complement fixation diluent were prepared in U-well microtiter plates with a semiautomated microtiter apparatus (Dynatech Laboratories, Alexandria, Va.). An equal volume of 0.05% SRBC was added, and the plates were sealed and incubated for 1 h at 37°C and then overnight at 4°C. The antibody response was expressed as the reciprocal of the highest dilution showing gross agglutination.

Experimental protocol. The degree of immunosuppression is influenced by the interval between the administration of drug and antigen (1, 2; G. W. Santos, Fed. Proc. 26:907, 1967). A series of preliminary experiments was therefore carried out to establish a regimen for each drug which would reduce the antibody response to SRBC by 50%. Cyclophosphamide was found to fulfill this criteria when given 2 and 4 days after primary challenge (5 mg/kg) and 2 and 4 days after secondary challenge at 2.5 mg/kg. The activity of methotrexate was optimum when the drug
RESULTS

Experimental uremia. Blood samples were collected from all animals at the termination of each experiment. Analysis of blood urea confirmed the severe degree of uremia in animals with resected kidneys. Renal function was normal in the sham-operated animals. Blood urea levels of animals in the three experiments, determined at the time of immunization, are shown in Table 1.

Effect of cyclophosphamide on the antibody response in uremia. Uremia per se did not affect the ability of the host to respond to antigenic challenge with SRBC. Similar antibody titers were found in the sham-operated controls and in the uremic group during both primary and secondary responses. In cyclophosphamide-treated animals, the antibody titers of sham-operated and uremic rats were both depressed to about 20% of their appropriate control levels 7 days after challenge. At 28 days, the responses of the cyclophosphamide-treated group had risen to approximately half that of the control group. After a secondary challenge with SRBC, the responses of the two cyclophosphamide-treated groups were similar, approximately 40% of the control values (Fig. 1). The experiment shows that the immunosuppressive activity of cyclophosphamide was not significantly increased in the uremic group.

Methotrexate in uremia. Methotrexate treatment produced a similar depression of the antibody response to SRBC in both control and uremic animals. At 7 and 28 days after primary challenge, the responses of both treated groups were reduced to 30% of their appropriate control levels. After a secondary challenge, the antibody response of the two treated groups was 10% of the control titers (Fig. 2). No biological significance can be attached to the small differences observed in the effect of methotrexate on

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Blood urea (mg/100 ml)</th>
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<tbody>
<tr>
<td>Sham-operated</td>
<td>200 ± 25 (60.3 ± 42.2)</td>
</tr>
<tr>
<td>Cyclophosphamide +</td>
<td>90 ± 1.3 (66.9 ± 6.3)</td>
</tr>
<tr>
<td>Methotrexate +</td>
<td>50 ± 5.6 (80.0 ± 0.8)</td>
</tr>
<tr>
<td>Azathioprine +</td>
<td>4.1 ± 1.5 (7.4 ± 0.3)</td>
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| Table 1. Blood urea levels in sham-operated and uremic animals treated with immunosuppressive agents or not treated at the time of antigen challenge. Each value is the mean of 10 to 15 animals. Numbers in parentheses are blood urea values in millimoles per liter. |
FIG. 1. Effect of cyclophosphamide on the antibody response to SRBC in uremic and sham-operated rats. The response is expressed as the reciprocal of the highest dilution of serum showing gross agglutination (y axis). Each point is the mean titer ± standard error from at least 10 animals. Symbols: ●, saline treated; ○, cyclophosphamide treated. 1*, First challenge; 2*, second challenge.

FIG. 2. Effect of methotrexate on the antibody response to SRBC in uremic and sham-operated rats. The response is expressed as the reciprocal of the highest dilution of serum showing gross agglutination (y axis). Each point is the mean titer ± standard error from at least 10 animals. Symbols: ●, saline treated; ○, methotrexate treated. 1*, First challenge; 2*, second challenge.
antibody response in normal and uremic animals.

Azathioprine in uremia. A similar protocol was followed with control and uremic animals treated with azathioprine. Administration of the drug reduced both the primary and secondary immune response to SRBC to 50% of the response found in untreated animals (Fig. 3). Uremia did not increase the degree of depression of immune responsiveness by azathioprine.

DISCUSSION

The immune status in uremia is difficult to assess in patients subjected to stress, surgical procedures, multiple drug therapy, and rapid changes in renal function. It can be said, therefore, that in many clinical studies the immune status of a compromised host, rather than the influence of uremia on immune mechanisms, has been evaluated. These problems can be overcome by studying immune mechanisms in a clearly defined animal model of chronic uremia free from the therapeutic variables associated with patient management.

In a series of related experiments utilizing this model, cell-mediated immune mechanisms, antibody responses, neutrophil function, and reticuloendothelial function were examined and shown to be normal despite chronic uremia (5, 7, 11; Nelson et al., in press). Nonetheless, an increased incidence of infection in patients with renal failure has been reported (6, 12). Many incidents of infection can be attributed to manipulations affecting host resistance. These include surgical manipulations, catheterization, damage to the mucous membranes, and poor drainage of secretions. Uremia, however, has been shown to be associated with a significant increase in the incidence of infection in immunosuppressed individuals (3), but the effect of individual factors on immune status was not determined. Our thesis was that, even if uremia alone could not explain the increased susceptibility of the host to infection, immune mechanisms might be suppressed as a result of an interaction between a factor associated with uremia and immunosuppressive drugs used in patient management. Many substances found in vivo, such as the corticosteroids, the prostaglandins, certain α globulins, and fibrin degradation products, have immunosuppressive effects, and the possibility that some of these substances might act synergistically with administered immunosuppressive agents to enhance the degree of immunosuppression needed evaluation.

In the first instance, we examined the combined effect of uremia and each of the three immunosuppressive drugs cyclophosphamide,
methotrexate, and azathioprine. A protocol was devised whereby the drugs were given in doses that significantly reduced but did not abrogate the immune response. This allowed any synergism between uremia and immunosuppressive agents to be readily assessed. The results confirmed previous findings that uremia per se did not affect the ability of the host to respond to an antigenic challenge (7). In addition, it was established that the administration of immunosuppressive agents depressed the immune response as anticipated, but the degree of suppression was not enhanced in animals with chronic uremia. One anticipated criticism of the protocol used in these experiments is that the immunosuppressive agents were administered as a “pulse” rather than on a regular basis, as would be the case in clinical practice. Drug accumulation after chronic administration, however, presents a major obstacle to the interpretation of the results of such an experiment, and a protocol during which the minimum dose capable of reducing the immune responsiveness of the host by 50% was selected as the most appropriate regimen. Under these conditions, drug accumulation was reduced to a minimum so that any observed effects of uremia on the activity of the immunosuppressive agents could be ascribed to synergism rather than drug accumulation. Although there does not appear to be any synergism between uremic components and immunosuppressive agents in relation to antigenic responsiveness, an effect on other facets of the immune response cannot be ruled out. We are currently developing several models of infectious disease to resolve this problem. The effect of uremia, immunosuppressive therapy, and a combination of these two on the initiation and course of experimentally induced infections will then be studied.

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LITERATURE CITED