five patients as serum was usually collected only every other day. C3 levels fell around the sixth day of the illness, when antigen was disappearing from the circulation and when free antibody could first be detected in the serum. Hence this transitory fall in serum C3 was probably due to the formation of immune complexes and these complexes were probably responsible for the patients' arthritis and cutaneous vasculitis. This view is supported by our demonstration in synovial fluid leucocytes from patients with meningococcal arthritis of deposits of meningococcal antigen and immunoglobulin that stained strongly for C3 and C4. Sera from patients with arthritis and vasculitis are currently being tested for the presence of immune complexes by more direct methods. Preliminary results suggest that circulating immune complexes are present when allergic complications appear. Possibly, however, immune complexes are also formed locally as a result of trapping of antigen by phagocytic cells during the phase of antigenaemia and the subsequent development of an Arthus reaction.

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T lymphocytes in kidney allograft recipients

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Summary

The spontaneous sheep erythrocyte (E) rosette test was used on 340 occasions to measure T cell levels in 28 renal allograft recipients. The E rosette counts were reduced by high-dose steroids but usually returned towards normal under maintenance treatment with azathioprine and low-dose prednisone. Unexpectedly, rejection crises were more often associated with a decrease in E rosettes before steroid treatment (12 cases) than with an increase in E rosettes (5 cases). Our results show the need for caution in using the E rosette test and more generally B and T cell markers for monitoring anti-graft immunity in renal allograft recipients.

Introduction

Immunological follow-up of kidney allograft recipients appears to be of great importance for the progress of renal transplantation. Immunosuppressive treatment may not only fail to suppress rejection crises but often induces undesirable side effects. Specific tests for adjusting the dose and duration of treatment according to the level of anti-graft immunity would be of great value. In the absence of such routine procedures, tests of the

non-specific immunity of allograft recipients might show whether azathioprine and steroids are immunosuppressive in a given patient. Furthermore, if it could be shown that rejection occurs when the minimum level of immunosuppression is lost higher doses could be prescribed before the onset of clinical or biological manifestations of rejection.

Serial measurements of T lymphocyte levels in peripheral blood have therefore been performed in 28 renal allograft recipients. T lymphocytes were characterised by their ability to form spontaneous rosettes with sheep erythrocytes.¹

Patients and methods

The 28 patients received their kidney allografts over a period of six months. Fifteen of them were followed up from the day of transplantation for two to six months. Twenty-four had been given cadaver kidneys, and four kidneys from living relatives.

Immunosuppressive treatment—All patients received azathioprine 5 mg/kg daily for the first three days, and then 3 mg/kg daily. Dosage was reduced if leucopenia or a severe infection developed. Steroids were given to the cadaver kidney recipients as follows: methylprednisolone (Solu-Medrol) 1 g intravenously on the day of transplantation, followed by betamethasone (Betnesol) 5 mg/kg (prednisone equivalent) daily by mouth from day 3 for five days, reducing thereafter to 0.25 mg/kg daily over 25 days. Maintenance treatment was with azathioprine 3 mg/kg and prednisone 0.25 mg/kg daily. Steroids were not given routinely to the recipients of kidneys from living donors. Three of these patients, however, had acute rejection crises in the days after transplantation and were therefore given betamethasone in a fashion similar to the cadaver kidney recipients. Thus only one patient received azathioprine without steroids.

Rejection crises—"Acute rejection crisis" was diagnosed when a significant rise in serum creatinine occurred without an obvious urological or infectious cause. In most cases the diagnosis was indicated by either hypocreatinuria or hyponatruuria or unexplained fever or an increase in the size or tenderness of the transplant. The diagnosis was in general only presumptive, which makes difficult the interpretation of individual results. Treatment included an intravenous "pulse" of methylprednisolone 1 g on days 1, 3, and 5. When this appeared to be

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ineffective a second course of betamethasone, identical with that given initially, was undertaken over 30 days.

T cell evaluation—Erythrocyte (E) rosette tests were usually performed weekly. After patients had been discharged, however, the frequency was variable. Extra tests were performed when rejection crises occurred, particularly before beginning treatment and in the first days after it had been begun. Altogether 340 tests were performed over eight months. Persons looking for the rosettes were not aware of the source of the samples, and samples from patients not included in the study were mixed blindly with sera from the patients with transplants.

Sheep E rosette technique—The technique has been described elsewhere¹ and is only summarised here. Lymphocytes were isolated on a Ficoll-Hypaque gradient. Untreated sheep red blood cells (SRBCs) were added in a ratio of 30:1. The mixture was incubated for 30 minutes at 37°C in Hanks's medium with 25% fetal calf serum added, centrifuged at 200 g for five minutes, and then left overnight at 4°C. The cells were then gently resuspended for one to two minutes. Rosettes were defined as lymphocytes with at least three SRBCs attached. Results were expressed (1) as the percentage of lymphocytes forming rosettes, and (2) in absolute numbers of such lymphocytes in peripheral blood (number of peripheral blood lymphocytes $\times$ percentage of E rosettes).

Results

Effect of high-dose steroids (fig 1)—An appreciable decrease in E rosette counts occurred in all cases during the first 15 days of steroid treatment, when the dosage was 3 mg/kg or more (prednisone equivalent) daily. This decrease was progressive, several subnormal values being noted with steroid doses of 4–5 mg/kg daily. When the dosage was reduced to 1 mg/kg daily the E rosette counts increased appreciably in all patients. In two patients this rise occurred when the dosage was reduced to 2 mg/kg daily. Two other patients whose E rosette counts remained low even when the steroid dosage was reduced to 0.25 mg/kg daily died with severe infections within a few days.

Effect of azathioprine—The action of azathioprine on E rosette counts could not be tested since only one patient received azathioprine without steroids. In this patient, however, the E rosette counts were only slightly, though definitely, depressed, the proportion of lymphocytes forming rosettes ranging from 32% to 58% (normal absolute values 515×10^6 – $940 \times 10^6/l$).

FIG 1—Effect of betamethasone on E rosette counts in renal allograft recipients. Initial dosage of 5 mg/kg daily was progressively decreased to 0.25 mg/kg daily over the 30 days of treatment. Shaded areas represent mean values.

Effect of maintenance treatment without rejection—Sixteen patients on maintenance treatment and showing no signs of rejection were followed up for one to six months (115 laboratory data). E rosette counts remained normal (over $500 \times 10^6/l$ and 50%) and remarkably steady in 12 of them. In four, however, a fall in E rosettes to under $300 \times 10^6/l$ and 30% was noted. Two of these patients had serum creatinine levels over $133 \mu\text{mol/l}$ (1.5 mg/100 ml) and were thought to have chronic rejection. In the other two patients the decrease in E rosettes was not associated with any clinical or biological evidence of rejection.

REJECTION CRISES

Seventeen rejection crises occurred in 14 patients. Five were associated with an increase in the E rosette count, but, unexpectedly, 12 were characterised by a decrease in E rosettes at the onset of rejection.

Rejection with decrease in E rosettes (fig 2)—Eight of the 12 patients who showed a decrease in E rosettes had been examined 5 to 10 days before rejection was diagnosed and had then had normal or subnormal E rosette counts (415×10^6 – $1150 \times 10^6/l$). In nine of the 12 patients the three days of treatment with methylprednisolone as described above was attempted. Although in normal people this treatment significantly decreases the E rosette count,² in eight of these nine patients an increase occurred as the serum creatinine levels fell to normal. In only one case were steroid injections not associated with an increase in E rosettes. Interestingly this was the only case in which treatment failed to reduce the serum creatinine levels to normal.

FIG 2—Rejection crises with decreased E rosette counts. Steroid treatment was begun on day 0.

Rejection with increase in E rosettes (fig 3)—The five rejection crises with high E rosette counts occurred at the end of the initial high-dose steroid treatment. In four instances when methylprednisolone pulses were given the E rosette counts rapidly decreased, as is the case in normal people. These steroid pulses had a prolonged clinical effect in one case, but long-term treatment had to be undertaken in the other three patients.

Discussion

In evaluating the variations in E rosette counts in kidney allograft recipients one must first take into account the effect of immunosuppressive treatment on the E rosette phenomenon. Our results show a depressive effect of prolonged high-dose steroid treatment on E rosettes. This effect disappears when the dosage is reduced to under 1 mg/kg daily and sometimes when it is reduced to under 2 mg/kg daily. This relative resistance of

FIG 3—Rejection crises with increased E rosette counts. Steroid treatment begun on day 0.

circulating E rosettes to steroids seen in these allograft recipients compared with the high sensitivity of E rosettes to steroids in normal people² may be related to the graft itself, subclinical rejection possibly leading to an abnormally high T cell level. Possibly the association of azathioprine with steroids modifies steroid sensitivity of T cells in selecting a steroid-resistant T cell subset.

The somewhat paradoxical results concerning rejection crises are still more difficult to interpret. One might have thought that immunosuppressive treatment would have produced a T cell depression and that rejection would occur when this depression became insufficient. We, however, found that maintenance of immunosuppression only slightly altered the E rosette counts and that they could not be used as a reliable measure of the efficacy of treatment. More paradoxical was that most crises were associated with a rapid decrease in E rosettes before steroid

treatment and that clinically successful steroid pulses, which decrease the number of E rosettes in normal people,² induced in these patients an increase in E rosettes. This phenomenon may be specific for rejection, since it was observed in only two cases in the absence of clinical evidence of rejection.

One may wonder whether in such cases there was merely a loss of the marker expression under steroid influence or whether T cells were indeed depleted at the onset of rejection and replaced by another cell population. In the latter hypothesis the loss of T cells might eventually be the result of a suppressor T cell population having a favourable action on graft survival, acting either on B cells or on other T cells including those involved in cytolytic processes.³ The nature of the cells replacing the E rosette forming cells, however, remains unknown. Preliminary results in our laboratory using B cell evaluations by the EAC rosette technique⁴ show that decreases in E rosette counts during rejection are not associated with an increase in B cells but are associated with a large excess of "null" cells, as defined by the absence of the two markers used here, which include the newly defined "K" cells.⁵ Interestingly, K cells are highly sensitive to steroids.⁶

Finally, our results remain impossible to explain in relation to current concepts of the mechanisms of organ transplant rejection. Nevertheless, they show the need for caution in judging the efficacy of immunosuppressive treatment in allograft recipients on the basis of available B and T cell markers. Thus one cannot use E rosettes for monitoring anti-rejection treatment except for the detection of the most severe states of immunosuppression, which may expose the patient to severe infections, as indicated by the persistent low E rosette counts in patients with lethal infections.

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Response and survival in advanced breast cancer after two non-cross-resistant combinations

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Summary

A prospective study with two cytotoxic combinations (cyclophosphamide, methotrexate, and fluorouracil (CMF), and adriamycin plus vincristine (AV)) was carried out in 110 patients with advanced breast cancer. There was

no significant difference between the treatment groups in the response rate after primary treatment, the median duration of response, and the median survival. In both groups responders survived for longer than non-responders. Secondary treatment after crossover for progression or relapse resulted in response rates of 35% for AV and 20% for CMF. Toxicity was mainly represented by reversible haemosuppression. These results are comparable with those obtained with other multiple-drug regimens, and combination chemotherapy alone seems to have reached a plateau in its capacity to control disseminated breast cancer.

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Introduction

The control of advanced breast cancer at any stage of the disease presents many challenging problems. The advent of cyclical