to that of the 12 patients who had pretransplant splenectomy (91.6 ± 7.9% and 66.1 ± 13.8% respectively). Posttransplant splenectomy produced the desired effects in all 11 patients in that the leukopenia was permanently reversed, significantly higher doses of cytotoxic drugs were tolerated, and serum creatinine levels declined. Although the numbers are small, this preliminary study is encouraging and indicates that selective posttransplant splenectomy may be a reasonable alternative to routine prophylactic pretransplant splenectomy in recipients of cadaveric renal allografts.

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PERIPHERAL BLOOD T LYMPHOCYTES FOUND IN RENAL ALLOGRAFT RECIPIENTS TREATED WITH CYCLOSPORINE[1]

Improved allograft survival has been observed in patients treated with cyclosporine, a new immunosuppressive agent (*1*, *2*). One problem in the management of patients treated with cyclosporine is a drug-associated nephrotoxicity (*2*, *3*) that can be difficult to distinguish from rejection. Recently, it has been noted that changes in T lymphocyte subpopulations in the peripheral blood may indicate rejection in renal allograft recipients immunosuppressed with azathioprine, steroids, and antilymphocyte serum (ALS) (*4*, *5*). To evaluate this technique for its application in detecting rejection episodes and to see if specific changes in the T cell subpopulations occur that may account for the immunosuppressive properties of cyclosporine early in the posttransplant period, we followed the peripheral blood T lymphocyte subpopulations sequentially after transplantation in cadaveric renal allograft recipients treated with cyclosporine.

Samples of blood were taken from 59 uremic patients visiting the Urology Clinic for transplant evaluation, and from 20 patients at intervals before and after cadaveric renal transplantation. The twenty transplant recipients received cyclosporine (Sandoz) at a dose of 17.5 mg/kg by mouth 5 hr before transplantation, along with intravenous administration of 1 g of methylprednisolone. The cyclosporine was given daily postoperatively and the dose was tapered to 10–12 mg/kg from 2 weeks to 2 months after the operation, depending on the serum creatinine. Oral prednisone was begun at 200 mg on the first postoperative day and tapered over the next 5 days to a maintenance dose of 20 mg.

Rejection was diagnosed when serum creatinine rose at least 0.5 mg/100 ml over baseline, together with a decrease in the urine output and the appearance of graft tenderness. In addition, other causes of the decreased renal function were excluded by physical examination, ultrasound, renal flow studies, and intravenous pyelogram (IVP). Rejection was treated with an i.v. bolus of 1 g hydrocortisone, graft irradiation, and an increase in the oral prednisone to 200 mg on the first day, tapered to a 20-mg maintenance dose over the next five days. Cyclosporine toxicity, which clinically appears similar to rejection with a rising serum creatinine, was suspected when urine output was maintained (1.5–2 L/day), the patient felt well, and renal flow studies were normal or nearly normal. Cyclosporine toxicity was presumed when the patient failed to respond to radiation and increased steroid treatment but did respond with a decrease in serum creatinine when the cyclosporine dose was diminished.

Of the 20 transplanted kidneys with cyclosporine treatment, four were lost; two kidneys were rejected despite immunosuppression; one kidney was lost when a patient with good renal function died of myocardial infarction; and one kidney was lost to rejection when immunosuppression was discontinued because of medical complications.

Automated leukocyte counts were performed on the blood samples using a Coulter Counter (Coulter Electronics, Inc., Hialeah, FL) and a differential blood count was obtained manually from smears stained with Wright's stain. The absolute number of lymphocytes was calculated from the leukocyte count and the percentage of lymphocytes. Lymphocytes were separated from the blood samples on a Ficoll-Hypaque gradient and aliquots of the cells were treated with fluorescein isothiocyanate-conjugated monoclonal antibodies Leu 4 (reacting with the total T lymphocyte population), Leu 2a (reacting with the T cytotoxic/suppressor population), or Leu 3a (reacting with the T helper population; Becton-Dickinson, Mountain View, CA) (*6*). The cells were washed, and the fluorescent populations were counted using the fluorescence-activated cell sorter (FACS IV, Becton-Dickinson, Mountain View, CA). The lymphocyte subpopulation was expressed as a percentage of the total number of lymphocytes. The helper/suppressor ratio (H/S) was calculated by dividing the percentage of Leu-3a-positive cells by the percentage of Leu-2a-positive cells. The values were compared statistically using the nonparametric Mann-Whitney

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U and Kruskal-Wallis tests. Approximately 70% of the lymphocytes from normal subjects were Leu-4-positive; 50% reacted with Leu 3a, and 20% with Leu 2a. The normal H/S ratio ranged from 1.1 to 2.0.

The 59 uremic patients had normal values for the lymphocyte subpopulations studied (Table 1). No statistical difference was observed between values obtained from male and female patients, as had been reported (*7, 8*).

Blood samples were taken from renal allograft recipients preoperatively, within the first 24 hr, and then at weekly intervals up to four months following transplantation. Although a sharp decrease in the absolute numbers of lymphocytes was observed immediately after transplantation in cyclosporine-treated patients, this value rebounded to nearly normal levels by one week after transplantation (Table 2). The peripheral blood T lymphocyte subpopulations were determined daily in two cyclosporine-treated patients for the first week after transplantation, and the values showed a drop in the number of lymphocytes on the first postoperative day, followed by gradual recovery to normal values at one week postoperatively (data not shown). The lower number of lymphocytes in the peripheral blood of cyclosporine-treated patients may be due to the high initial steroid dosage that was diminished early in these patients.

A comparison between lymphocyte population values obtained from cyclosporine-immunosuppressed patients with good renal function and from those with various disturbances in renal function is shown in Table 3. The absolute number of blood lymphocytes and the percentage of the cytotoxic/suppressor subpopulation (Leu-2a-positive lymphocytes) were sig-

TABLE 1. Peripheral blood T lymphocyte subpopulations in uremic patients

Patients	N	Lymphocytes[a]	Total T cells[a,b]	SUP/CYT T Cells[a,c]	Helper T cells[a,d]	H/S ratio[e]
Male	36	21 (1472 ± 618)	65 (983 ± 506)	23 (364 ± 271)	44 (664 ± 389)	2.2 (0.4–4.1)
Female	23	22 (1510 ± 694)	71 (1095 ± 552)	27 (412 ± 249)	46 (694 ± 375)	2.1 (0.6–5.2)
Total	59	22 (1487 ± 643)	68 (1026 ± 523)	24 (383 ± 262)	44 (676 ± 381)	2.2 (0.4–5.2)

[a] The first number is the percentage of the leukocytes; the number in parentheses is the mean absolute cell count/mm^3 ± one standard deviation.

[b] Leu-4-positive lymphocytes; the first number is the percentage of positive lymphocytes; the number in parentheses is the mean absolute cell count/mm^3 ± one standard deviation.

[c] Leu-2a-positive lymphocytes.

[d] Leu-3a-positive lymphocytes.

[e] The numbers in parentheses are the range.

TABLE 2. Peripheral blood T lymphocyte subpopulations in cyclosporine-treated renal allograft recipients

Time	N	Lymphocytes[a]	Total T cells[a,b]	SUP/CYT T Cells[a,c]	Helper T cells[a,d]	H/S ratio[e]
Preoperative	9	30 (1872 ± 605)	66 (1259 ± 475)	25 (482 ± 317)	39 (731 ± 335)	1.3 (0.6–2.0)
Postoperative	20	9 (756 ± 469)	45 (335 ± 252)	20 (153 ± 104)	30 (241 ± 192)	1.7 (0.4–3.6)
1 Week	12	18 (2297 ± 1448)	53 (1327 ± 1036)	17 (403 ± 283)	37 (911 ± 671)	2.2 (1.1–4.1)
2 Weeks	15	19 (1887 ± 1031)	57 (1157 ± 839)	19 (391 ± 287)	38 (737 ± 602)	2.1 (0.7–4.4)
3 Weeks	8	18 (1335 ± 1210)	56 (825 ± 923)[f]	22 (293 ± 256)	29 (316 ± 160)	1.4 (0.8–3.0)
4 Weeks	8	25 (1576 ± 1058)	57 (1028 ± 992)	26 (420 ± 456)	38 (694 ± 690)	2.1 (0.3–3.5)
2 Months	14	17 (1205 ± 741)	70 (841 ± 576)	27 (312 ± 200)	42 (511 ± 364)	1.8 (0.5–3.8)
3 Months	3	21 (1231 ± 1227)	81 (978 ± 925)	25 (314 ± 168)	52 (644 ± 636)	2.1 (1.7–2.5)
4 Months	6	24 (1974 ± 1031)	77 (1384 ± 800)	28 (538 ± 413)	43 (727 ± 360)	1.9 (0.6–3.3)

[a] Number is the percentage of the leukocytes; the number in parentheses is the mean absolute number of cells, ± one standard deviation.

[b] Leu-4-positive lymphocytes; the first number is the percentage of positive lymphocytes; the number in parentheses is the mean absolute cell count/mm^3 ± one standard deviation.

[c] Leu-2a-positive lymphocytes.

[d] Leu-3a-positive lymphocytes.

[e] Numbers in parentheses are the range.

TABLE 3. Peripheral blood T lymphocyte subpopulations in cyclosporine-treated patients compared by graft status

Graft status	No. Patients	No. Observations	Lymphocytes[a]	Total T cells[a,b]	SUP/CUT cells[a,c]	Helper cells[a,d]	H/S ratio[e]
Good	20	61	19 (1606 ± 1155)	63 (1071 ± 831)	24 (378 ± 294)	39 (652 ± 526)	1.9 (0.3–4.4)
Rejection	3	10	13 (905 ± 412)[f]	42 (337 ± 186)[f]	14[f] (116 ± 67)[f]	32 (267 ± 134)[f]	2.5 (0.7–2.9)
ATN	8	13	12 (688 ± 816)	43 (1441 ± 1179)	18 (265 ± 264)	27 (442 ± 545)	1.6 (1–1.7)
Cyclosporine Toxicity	2	2	10 (481 ± 70)	58 (282 ± 57)	32 (149 ± 69)	39 (186 ± 16)	1.4 (1–1.7)

[a] The first number is the percentage of the leukocytes; the number in parentheses is the mean absolute number of cells ± one standard deviation.

[b] Leu-4-positive lymphocytes; the first number is the percentage of positive lymphocytes; the number in parentheses is the mean absolute cell count/mm^3 ± one standard deviation.

[c] Leu-2a-positive lymphocytes.

[d] Leu-3a-positive lymphocytes.

[e] The numbers in parentheses are the range.

[f] Significantly different ($P < 0.05$) from values during good graft function.

nificantly lower in samples taken during rejection. Although the number of observations is small, the lower absolute lymphocyte count and percentage of the cytotoxic/suppressor subpopulation may be a result of the increased steroid therapy given these patients. Too few observations during cyclosporine toxicity were made for a useful statistical comparison. No correlation was observed between rejection and any of the other values, including the H/S ratio.

Cyclosporine has profound immunosuppressive effects, but the mechanism of its action is not known (*1*). Cyclosporine has been reported to spare suppressor T lymphocyte subpopulations and to prevent the secretion of, or response to, interleukin 2 (*9–11*). In this study, we found no specific changes in T lymphocyte subpopulations in response to immunosuppression with cyclosporine. These results may indicate that cyclosporine immunosuppression causes functional changes in lymphocytes that are not reflected by numerical alteration in the peripheral blood, or that the population changes occurring during cyclosporine therapy were too subtle to be detected with the methods we used. Other antisera reacting with specific functional subpopulations of T lymphocytes are currently under investigation (*12*) and these may prove more useful in following patients treated with cyclosporine. The significantly lower percentage of Leu-2a-positive lymphocytes observed in the cyclosporine-treated patients during rejection may indicate the response to increased steroid therapy . These changes are statistically significant, but they are not useful in monitoring individual patients because there is great individual variation and overlap with results from other patient populations (Table 3).

These results differ from those of other reports that have shown specific changes in peripheral blood T lymphocyte subpopulations during rejection in azathioprine, steroid, and ALS-immunosuppressed patients (*4, 5*). Sweny and Tidman examined peripheral blood T lymphocyte subpopulations in patients treated with cyclosporine alone; they observed a specific increase in the T suppressor/cytotoxic subpopulation in patients after more than two months of cyclosporine immunosuppression, but not early in treatment with cyclosporine alone or in treatment with cyclosporine plus prednisolone (*13*). However, Morris et al., in a study of patients treated with cyclosporine alone from 4–8 weeks following transplantation, did not report an increase in the suppressor population, and found no correlation with rejection and changes in the H/S ratio (*14*). Differences in the immunosuppressive protocol may affect the observations, especially in the combined use of ALS with azathioprine and steroids (*4, 5*), in the use of steroids with cyclosporine (*13*); the time of sampling (*13*) or the presence of viral or fungal infections (*15*) may also have an effect. Thus, further work in this area is necessary. However, in this study, the determination of peripheral blood T lymphocyte subpopulations was not helpful in detecting rejection or in discriminating between rejection and cyclosporine toxicity.

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