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The complement system in renal homograft recipients

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The whole serum complement and its components were studied in 24 recipients of 27 renal homografts. In 12 of 13 instances in which homograft rejection was diagnosed, it was accompanied by significant declines in CH50, IA50, C4, and C3 levels, and to a lesser degree in C1 and C2 levels. Fourteen patients had normal graft function during the postoperative course of study, and in 13 of the 14 the complement levels were within the normal range throughout. In two recipients with systemic lupus erythematosus, very low initial complement levels increased to normal levels following removal of the native kidneys, splenectomy, and the provision of a well-functioning homograft. Anticomplement activity and elevated titers of C1 and C3 inactivators were observed in some patients, but these did not correlate with the changes in CH50. The findings confirm that the complement system participates in renal homograft rejection.

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THERE IS accumulating evidence that serum complement participates in the rejection of transplanted tissues and organs.[2, 4, 8, 9, 11] In human recipients of renal homografts, Guiney and associates[9] and Austen and Russell[2] described a drop in CH50 and particularly C2 levels coincident with rejection. Levine and associates[11] have confirmed falls in whole complement with graft repudiation. Austen and Russell[2] and Carpenter and associates[4] have demonstrated that decreases in either whole complement or its components may be delayed until sometime after clinically evident rejection.

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In the present study, we have analyzed the whole complement and its components after human renal transplantation in order to clarify further how various parts of the complement system are affected during convalescence, with and without rejection episodes.

MATERIAL AND METHODS

The case material. The ages of the 24 patients ranged from 11 to 46 years, and were in terminal renal failure due to chronic glomerulonephritis (16 instances), chronic pyelonephritis (2), polycystic kidney disease (2), systemic lupus erythematosus (SLE) (2), familial medullary cystic disease (1), and cystinosis (1). Immunosuppressive therapy was provided by a triple-drug regimen consisting of azathioprine, prednisone, and antilymphocyte globulin (ALG).[18]

There were 27 transplantations in the 24

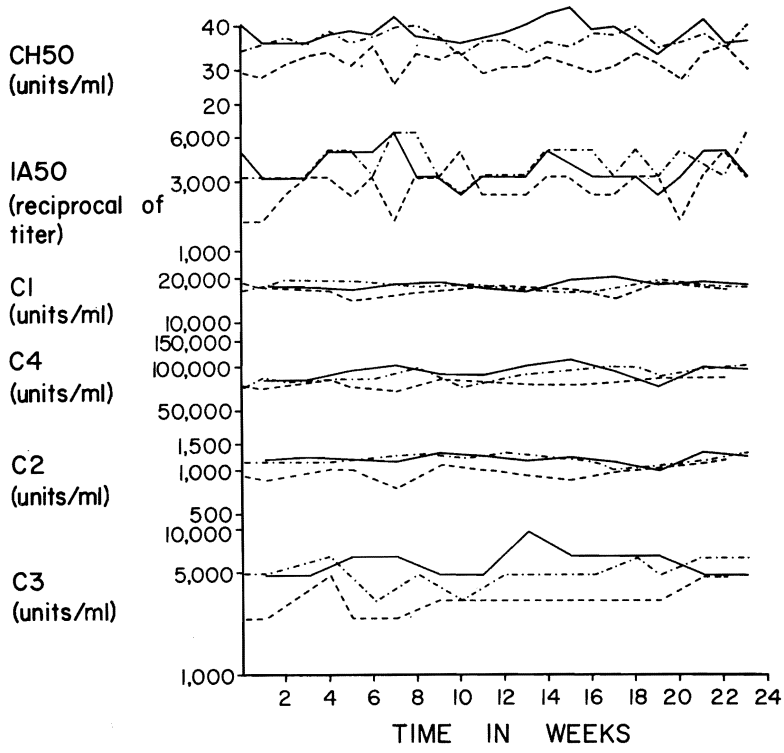

Fig. 1. Complement levels in three healthy volunteers studied at weekly intervals for six months. Only minor fluctuations occurred.

recipients. Fifteen of the grafts were from related donors, and the other 12 were from cadavers. The patients were a complicated group in that six were receiving second homografts after having rejected their first ones. One recipient underwent both first and second transplants during the period of study. Another underwent a second and third transplantation. A third recipient was given his third and fourth kidneys. In about two thirds of the cases, the native patients' kidneys were removed in conjunction with splenectomy, and in the other third, the diseased kidneys were not disturbed.

Serum complement assays. Blood samples were permitted to clot at room temperature for approximately one hour. Serum was separated by centrifugation at 3,000 rpm for 15 minutes at 0° to 4° C. and stored at –70° C. until used. All sera from an individual patient or control subject were analyzed simultaneously.

Total complement activity was measured as hemolytic complement activity (CH50) according to Mayer[12] and with the immune adherence hemagglutination (IA50) method of Nishioka.[14] The C1, C4, and C2 components were assayed by stoichiometric tube titration with the use of $EAC4^{hu}$, $EAC1^{gp}$, and $EAC1^{hu}$ $C4^{hu}$ cells at a concentration of 1.5×10^8 cells per milliliter, respectively.[2, 13] The C3 activity was assayed by immune adherence[15] with the use of $EAC1^{hu}$ 4^{hu} 2^{hu} cells.

The anticomplement activity (ACA) in the serum[16, 17, 20] and the presence of inactivators to C1[10] and C3[19] were assayed with previously described methods.

Sera from the two patients with SLE were examined for complement-fixing antibody to DNA and heat-denatured DNA by microtechnique.[21] The LE test was performed with latex fixation.*

Control studies. Complement (CH50 and IA50) was measured in 250 normal people; the C1, C4, C2 and C3 were analyzed in 100 of them. In addition, three normal volun-

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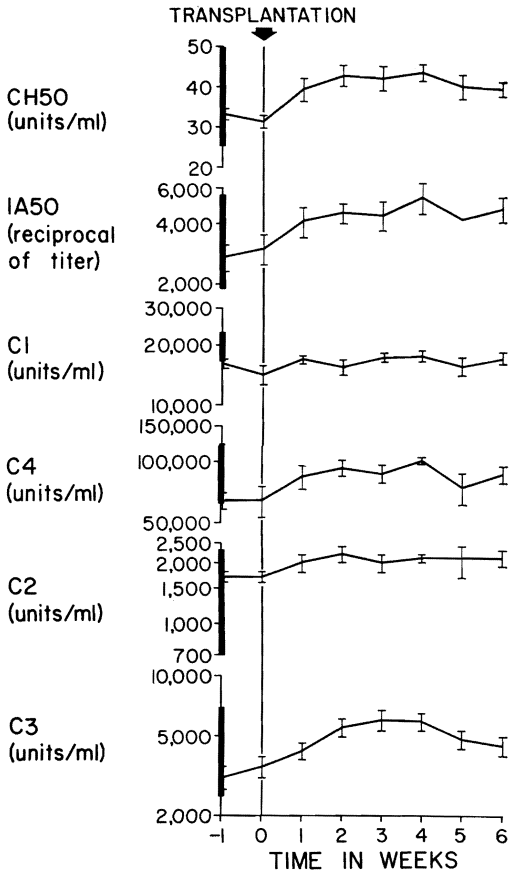

Fig. 2. Complement levels in 14 transplant recipients whose renal homografts displayed normal function throughout the time of postoperative study. The mean ± S.E. are given. The heavy line along the ordinate indicates the normal range.

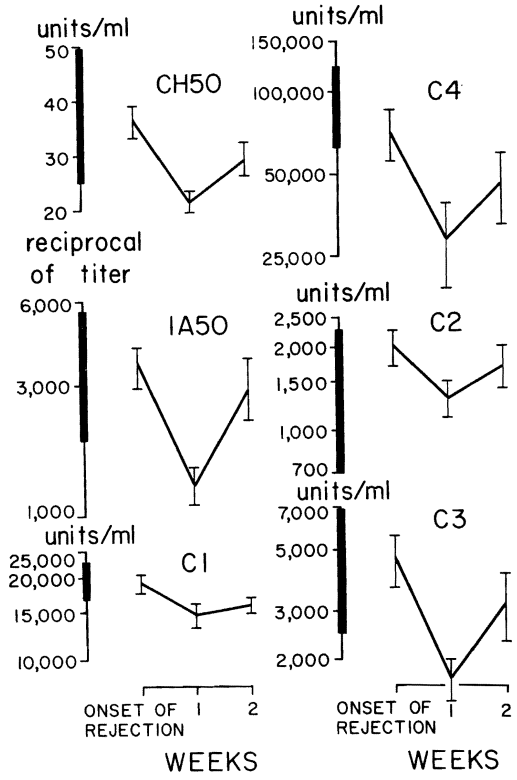

Fig. 3. Complement levels during homograft rejection in seven patients. The CH50, IA50, C4, and C3 values all displayed marked decreases. The C1 and C2 levels also fell but to a lesser degree. The mean ± S.E. are given. The heavy line along the ordinate indicates the normal range.

teers were studied weekly at the same time of day for six months, as it was demonstrated by Arata[1] that daily fluctuations within 8 CH50 units might occur in an individual. The results are presented in Table I and Fig. 1.

Criteria of rejection. Rejection was defined by elevations in blood urea nitrogen and serum creatinine or by falls in creatinine clearance or urine sodium concentration. Three of these findings on two consecutive days were required to establish the diagnosis.

RESULTS

Renal recipients with normal homograft function. Fourteen patients including one with SLE always had good renal function after transplantation with no sign of rejection. In this group, the pre- and postoperative complement levels were essentially within normal limits (Fig. 2), and in all but one patient, the fluctuations were no larger than in the normal volunteers. In the single exceptional case, transient moderate decreases in complement occurred two and six weeks after transplantation.

Renal recipients with typical rejection episodes. Seven of the patients including one with SLE had initially well-functioning grafts that suffered from rejection episodes. During these times, there was always a significant decrease in CH50 and IA50 titers (Fig. 3). The first four complement components showed different behavior patterns. The C1, C4, and C3 levels all dropped to subnormal values during the rejection episode with the most pronounced changes occurring for C4 and C3. The C2 also decreased but did not

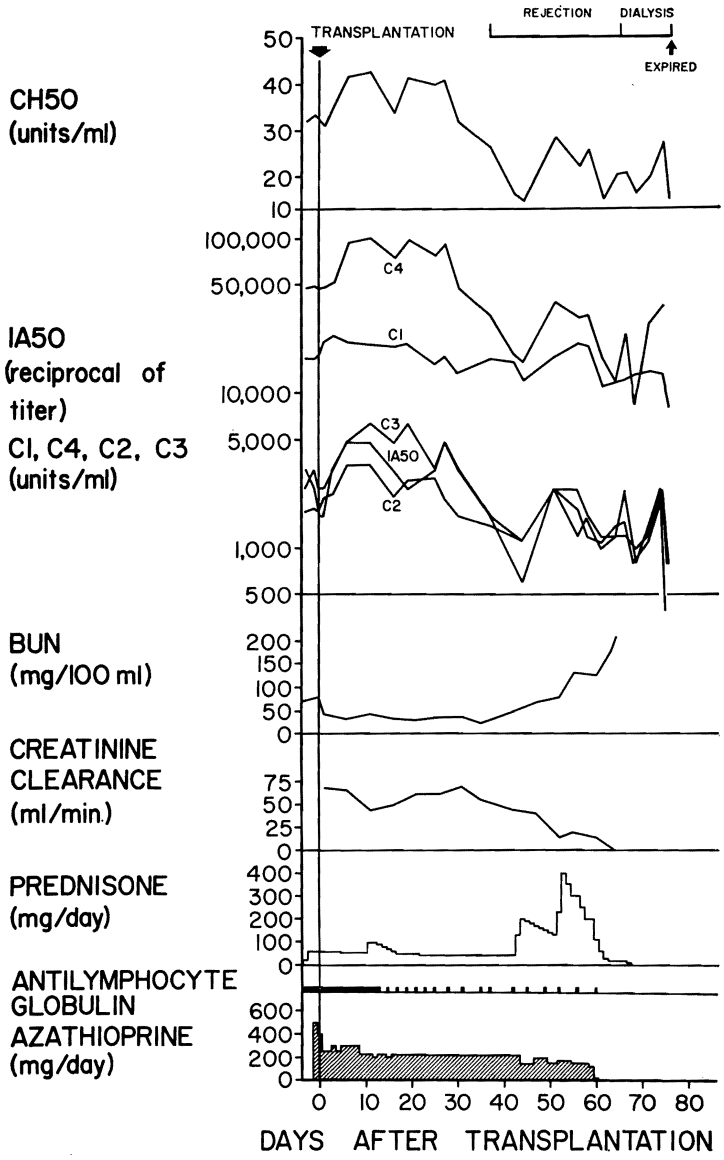

Fig. 4. The course in a patient who developed severe rejection six weeks after transplantation. Note marked drop in all the complement assays except the C1. Following massive prednisone treatment encephalomyelitis developed, causing death 2 weeks later.

Table I. *Normal range of values for the various complement assays (mean ± 2 S.D.)*

Complement	*Range*	*Measurement*
CH50	25.3–49.7	Units per milliliter
IA50	1,900– 5,500	Reciprocal of titer
C1	16,800– 22,800	Units per milliliter
C4	62,000–122,400	Units per milliliter
C2	700– 2,300	Units per milliliter
C3	2,500– 6,900	Units per milliliter

reach values below the normal range (Fig. 3).

Rejection episodes occurred in two patients at the same time as liver dysfunction, which was so severe that it ultimately caused death. Drops in the complement measures occurred to an extent not seen during rejection episodes without liver complications. Another patient developed severe rejection with marked complement changes six weeks after

transplantation (Fig. 4). Following massive prednisone treatment, death ensued from encephalomyelitis two weeks later. At autopsy, it was interesting that the swollen homograft had virtually no histopathologic findings of rejection. In the remaining four patients, recovery of homograft function was accompanied by the complement levels returning to normal.

Renal recipients with unusual rejection episodes. Three kidneys never functioned and probably underwent hyperacute rejection. Two of these grafts were transplanted consecutively to the same patient. Both recipients had preformed antibodies, although not against their donors. Two more transplants functioned well for a few hours but then shut down for 10 to 14 days before resuming significant urine excretion. A sixth graft became anuric two weeks after transplantation and was removed; the organ was edematous and had tubular necrosis but with little cellular infiltration.

After five of the foregoing six transplantations, there were definite and fluctuating decreases in CH50, IA50, C4, C3, and, to a lesser extent, C2 values. The C1 level remained essentially unchanged, sometimes with small variations. In one of the patients who experienced a hyperacute rejection episode, the complement levels remained unchanged.

Two renal recipients with pre-existing SLE. The native kidneys and spleen were removed prior to renal transplantation. The lowest titers of CH50, IA50, C4, C3, and C2 were recorded before the bilateral nephrectomy. These titers slightly increased in the anephric state, and following successful transplantation, they rose to normal levels. Antibodies to DNA and heat-denatured DNA were detectable both before and after the bilateral nephrectomies. However, they disappeared within a few days after transplantation. At the same time, the LE test turned from slightly positive to negative.

After operation, one of the recipients had neither rejection nor complement depression (Fig. 5). The other patient suffered a rejection episode during which the CH50, IA50,

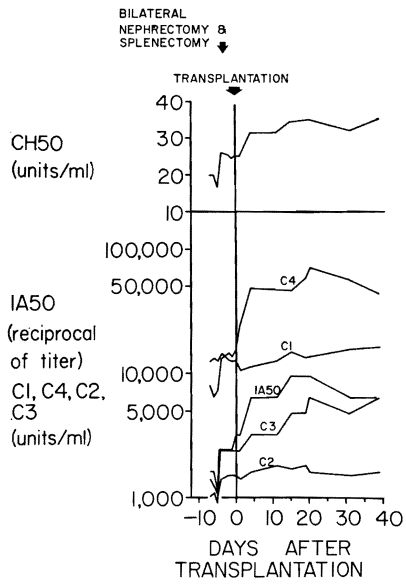

Fig. 5. Complement levels in a patient whose original disease was systemic lupus erythematosus. Very low complement values were slightly improved by bilateral nephrectomy and splenectomy. After successful cadaveric renal transplantation was performed, complement levels rose to normal.

and C4 levels decreased significantly and returned to normal concomitantly with reversal of the rejection; this course is included in Fig. 4.

Correlation between CH50 and anticomplement activity (ACA) and C1 and C3 inactivators. Thirteen of the 24 patients became Australia (Au)-antigen positive after transplantation, and in 11, an increased ACA was demonstrated. The data of ACA were plotted against CH50 titers in Fig. 6. There was no correlation, suggesting that the ACA had no measurable effect on the in vivo complement system in these cases.

C1 and C3 inactivators were measured in six patients during normal renal homograft function and rejection. There was no correlation between CH50 and C1 and C3 inactivators under either circumstance (Fig. 6).

DISCUSSION

Complement and its components in the 14 homograft recipients who maintained good renal function remained continuously within normal range and were comparable to that

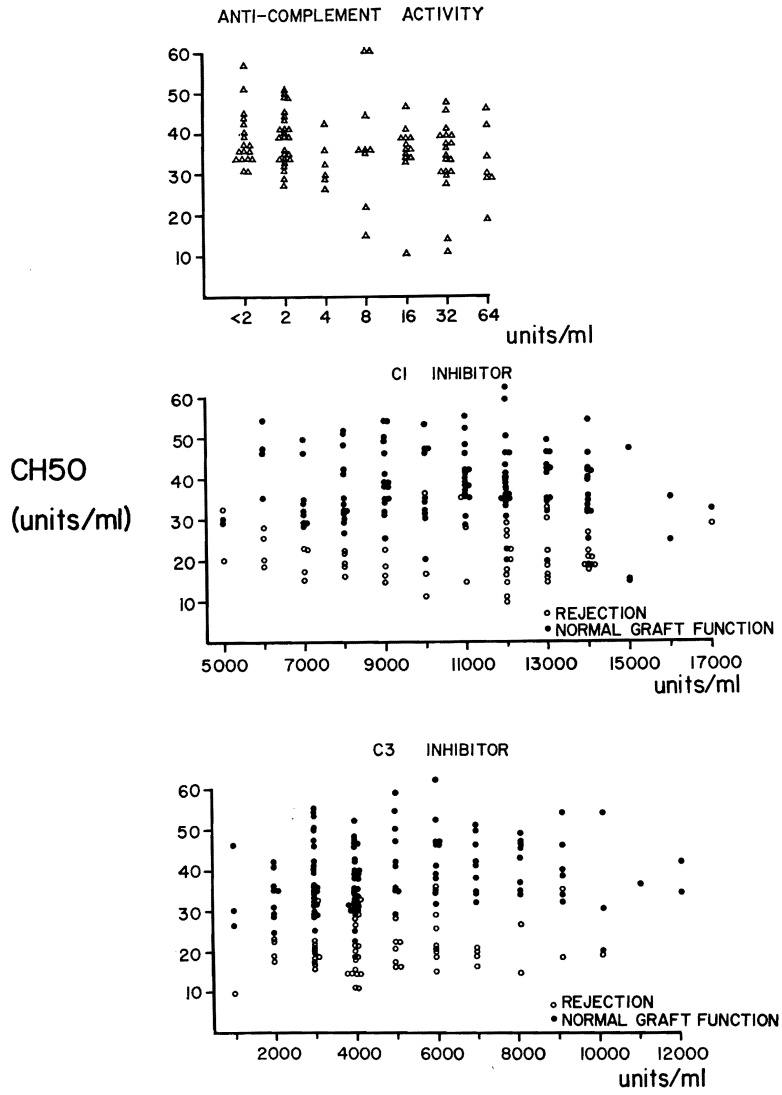

Fig. 6. Anticomplement activity (in 11 patients) and C1 and C3 inactivators (in six patients) related to the CH50 levels. No correlation was apparent.

of the normal volunteers. This stability, which has been noted in renal recipients by Austen and Russell[2] and Gewurz and associates[8] indicates that immunosuppressive therapy as such has no influence on the complement system.

In contrast, the development of postoperative rejection was invariably reflected by changes within the complement system. These occurred simultaneously with the development of the rejection rather than afterward, as has been reported by others.[2, 4] There were depressions of CH50, IA50, C4, C3, and, to a lesser degree, C1 and C2. With reversal of rejection, the CH50 and the decreased components returned to normal levels.

It was previously reported by Austen and Russell[2] that the C2 titers fell to half their preoperative values during rejection, Gewurz and co-workers,[8] Carpenter and associates,[4] and Levine and colleagues[11] demonstrated a similar decrease of C2 β1C globulin and whole complement, respectively. None of these authors stressed the most impressive decrease of serum C4 complement component, although Carpenter and associates[5] de-

scribed a moderate hypercatabolism of radioactive C4 and C3 intravenously injected into renal allograft recipients undergoing rejection.

In addition to those instances in which the diagnosis of classical rejection was unequivocal, there were six cases of transient or permanent homograft failure in which the role of immunologic mechanisms was less clear as the result of the features of the clinical course. In five of the latter recipients, there were decreased levels of CH50, IA50, C4, and C3 during the period of graft nonfunction. In addition to an accelerated cell-mediated immunologic reaction, other possibilities for the complement depletion could be either by deposition in the glomeruli of antibodies to glomerular basement membrane or by deposition of circulating antigen-antibody complexes. Both of the latter reactions are complement-consuming.[6, 7]

The two patients with SLE were of special interest because the disease that destroyed their native kidneys was accompanied by complement changes similar to those of rejection. When first evaluated, both potential recipients had extremely low levels of CH50, IA50, C4, C2, and C3. Following bilateral nephrectomy, there was a slight elevation of complement but by no means to normal level. After provision of well-functioning renal homografts, the complement returned to normal level, at which it remained in one instance and became secondarily and transiently depressed in the other with the onset of rejection. The explanation for complement restoration by virtue of the homotransplantation is unclear.

An additional group of interesting patients was made up of the 13 who developed evidence of Australia antigenemia after transplantation. In 11 of these recipients, increases of anticomplementary activity (ACA) was found. In spite of the significant titers of ACA, these had no correlation with CH50. In addition, the C1 and C3 inactivators which were measured in six patients during normal graft function and rejection did not have any correlation with the complement levels.

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