COMPLEMENT COMPONENTS, DEGRADATION PRODUCTS AND IMMUNE COMPLEXES AFTER KIDNEY TRANSPLANTATION

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Summary

Levels of complement components and the presence of immune complexes were determined in blood samples from 23 patients as a function of time after kidney transplantation. During the first three post-transplantation weeks a decrease in the concentration of plasma C₃ with a simultaneous increase of one of its breakdown products (C₃d) was generally observed. This pattern often accompanied acute rejection episodes beyond 4 weeks after transplantation, while in the absence of complications normal and stable levels prevailed. In contrast, the presence of circulating immune complexes appeared not to correlate with rejection reactions. All 7 cases with detectable immune complexes presented with various concomitant neoplastic (renal carcinoma, Kaposi sarcoma) or infectious diseases (pneumonia, septicaemia, Herpes zoster or Cytomegalovirus infection). Thus, monitoring of plasma C₃ and C₃d may represent a helpful additional criterion for the assessment of acute rejection in recipients of kidney allografts; the presence of circulating immune complexes, although not correlating with graft rejection, may be taken as a sign of complicating additional disease.

Introduction

Most recipients of renal allografts experience one or more clinical episodes of acute rejection. The pathogenesis of the rejection reaction comprises a complex mixture of immunological and non-immunological mechanisms. With regard to immunological graft rejection, there is abundant evidence for the involvement of both cell-mediated and humoral immune reactions. In particular, the latter may induce the activation of the complement system by antigen-antibody complexes or various other means. Different investigators have noted a decreased activity of total complement or of isolated complement components after kidney transplantation [1–6]. Turnover studies with radiolabelled C₃, which were performed in order to clarify whether the low concentration
was due either to decreased synthesis and/or to hypercatabolism, led to variable results. In some allografted patients hypercatabolism of C₃ was predominant, while in others normal catabolism and even increased synthesis prevailed [7]. Studies on the presence of circulating immune complexes after transplantation also led to controversial results. In a recent report a correlation between increased Clq-binding activity (Clq-BA) and acute rejection episodes, characterised by fibrin deposition in the grafted kidneys, was described [8].

In an effort to study the effects of renal transplantation on the complement system, components C₃, C₄, C₃PA, Clq, properdin and breakdown products of C₃ (C₃d) as well as of C₃PA (Ba), were sequentially measured in plasma samples of patients carrying recently implanted kidney allografts. In addition, the sera were checked for the presence of circulating immune complexes to test possible correlations between their appearance and complement levels.

Material and Methods

A total of 23 patients, 13 females and 10 males, were transplanted with cadaver kidneys. End stage renal failure was caused by chronic interstitial nephritis in 10, chronic glomerulonephritis in 9 and diabetic glomerulosclerosis in 2 of these patients; one allograft recipient had congenital malformation of the kidney and in another the renal disease was of undetermined aetiology. All patients were treated by standard immunosuppression, consisting of a combination of prednisone 50–100 mg/day (pulses of 15 mg/kg/day for rejection episodes), azathioprine 1.5–2.5 mg/kg/day and antilymphocyte globulin (ALG, 250 mg IgG/day). The ALG injections were restricted to the first 3 weeks after transplantation. Diagnostic signs of rejection were considered to be tenderness with enlargement of the graft, oliguria, hypertension, fever, increase of plasma creatinine and decrease of urinary sodium. The rejection episodes were graded (type 1 to type 5 reactions) according to severity [14].

Plasma and serum samples from all patients were collected at frequent intervals during the first 3 months after transplantation. Blood was sampled at least twice per week, in some cases daily; plasma samples were drawn on EDTA (3.5 mg/ml). Both plasma and sera samples were stored at -90°C to be thawed only before use. Plasma concentrations of C₃, C₄, C₃PA, Clq and properdin were measured by radial immunodiffusion [9]. Monospecific antisera against C₃, C₄ and Clq were obtained from Hoechst Pharma AG, Zürich (Behring), those against C₃PA and properdin from Dr O Goetze, Scripps Clinic and Research Foundation, La Jolla, California. The breakdown products of C₃, C₃d and of C₃PA, Ba, were first freed from denatured molecules by differential precipitation with polyethylene glycol and then quantitated by immunodiffusion, using antisera specific for the D-antigen of C₃ and the Ba-antigen of properdin factor B (C₃PA) [10]. These antisera were kindly provided by Prof. PH Lambert, WHO, Geneva. Circulating immune complexes were assayed by the radiolabelled Clq-binding technique [11].

Blood samples from 50 healthy voluntary blood donors served to establish control values.
Results

Renal Recipients with Normal Allograft Function, First Three Weeks

Nine patients with good renal function exhibited no signs of rejection during the first three postoperative weeks, i.e. during treatment with ALG. Plasma concentrations of C₃ were significantly below control values in 7/9 individuals.

![Graph showing plasma concentrations of creatinine, C₃, C₃d, and C₁q-BA](image)

**Figure 1.** Plasma concentration of creatinine, C₃, C₃d and serum Clq-BA during the first 3 weeks after kidney transplantation. Example of a patient free of rejection or infectious episodes.
An example of a patient is shown in Figure 1. C₃ usually reached subnormal levels (normal range 100 ± 32%) in the second or third week after transplantation. The two remaining patients started with elevated C₃ concentrations before transplantation; therefore their C₃ values, although decreasing after transplantation, remained within the normal range.

In contrast to the concentration of C₃, C₃d (normal range 0–20%) was elevated in five of the seven C₃-hypocomplementaemic patients. However Clq-BA of these patients remained normal.

Renal Recipients with Rejections During the First Three Weeks

In the first three weeks after transplantation ten patients experienced a total of 11 rejection reactions. In all of them a significant decrease of the C₃ levels occurred at the onset of the rejection. This decrease coincided with increased C₃d values in nine rejection episodes.

During the course of three weeks the mean values of the plasma concentration of C₃ from the patients with rejections (78.6 ± 15.3%) and from those without rejections (89.9 ± 27.6%) did not differ significantly. However there was a significant difference for the C₃d levels (19.6 ± 8.2% with rejection; 13.2 ± 4.8% without rejection; 2p<0.05). The Clq-BA of the patients sera with a rejection reaction was normal, except in two cases, where it was elevated immediately after transplantation and remained so during an early rejection crisis within the first week.

Further Evolution after Withdrawal of ALG Treatment During the Second and Third Month after Transplantation

Five patients remained without any complication during this observation period. Their levels of complement components and breakdown products remained stable and normal, and no increase of immune complexes could be discovered by the Clq-binding test in their sera.

However 11 patients experienced a total of 16 rejection reactions in the second and third month after transplantation (Figure 2). In 10 of these 16 rejection episodes the plasma concentration of C₃ was decreased simultaneously with the onset of the rejection. In 8 rejection reactions the C₃d values began to rise at the same time, thus indicating some hypercatabolism of C₃. The level of circulating immune complexes did not seem to correlate with the rejection crisis. There were two exceptions however. The first concerned one patient with an irreversible type 5 rejection, which required nephrectomy; his Clq-BA started to rise a few days before the allograft was removed. The other patient, who displayed increased immune complexes after the rejection, suffered from a severe cytomegalovirus infection. Abnormal levels of circulating immune complexes, not correlated with any rejection episode, were determined in 7 further patients. All of them suffered from either neoplastic (renal carcinoma, Kaposi sarcoma) or infectious (pneumonia, septicaemia, Herpes zoster, 2 cytomegalovirus infections) concomitant diseases. In the latter the
circulating immune complexes were elevated during the acute infection. In the two patients with malignant metastatic diseases however, the immune complexes remained high until death.

After the initial period of ALG treatment the $C_3$ values were pathological in 62.5% of acute rejections, compared with the pathological $C_3d$ values in 50% (Figure 3). A significant correlation between the changes of $C_3$ and $C_3d$ could not be detected. The mean plasma concentrations of $C_3$ and $C_3d$ at the onset of the rejections were significantly different from those obtained before and after these episodes (Figure 4). The concentration of other complement components as $C_4$, $C_3PA$, Clq, properdin and the Ba fragment was variable, although a decrease in the concentration of $C_3$ was occasionally associated with a decrease of some of the other components. In some further cases increased values of $C_4$ and properdin were noted.
Figure 3. Plasma concentration of C3, C3d and serum Clq-BA of patients at the onset of a rejection reaction during the 2nd and 3rd month after renal transplantation.
Figure 4. Mean plasma concentration ± 1 SD of C₃ and C₃d before, at the onset and after acute rejection reactions.
Discussion

Our observations confirm that a decrease in the plasma concentration of C₃ during the first three weeks after transplantation and following rejection reactions is a rather constant phenomenon. The decrease in the first three weeks after the operation, unrelated to rejection or infectious episodes, may be either a consequence of the postoperative metabolic condition or of the particularly active immunosuppressive therapy, especially ALG administration. It has been suggested that ALG has an anticomplement activity [12]. The simultaneous elevation of the C₃d fragment may suggest that C₃ has been activated even without rejection episodes and thus would indicate a certain degree of hypercatabolism. Therefore decreased synthesis does not seem to be the only cause of the observed hypocomplementaemia. In view of the fact that a decrease in synthesis as well as an increase in catabolism may be contributing to the genesis of C₃-hypocomplementaemia in the first weeks after kidney transplantation, the determination of C₃ and C₃d does not permit diagnosis of a rejection during this particular period. From the fourth week on, however, the level of C₃ and C₃d seems to be a useful parameter in the diagnosis of acute rejections, since almost two-thirds of acute rejection episodes could be detected by this criterion. These findings are consistent with the activation of the complement system during graft rejection. No correlation existed between the severity of rejection and the decrease of C₃, which occurred — as well as the elevation of C₃d — almost coincidentally with the beginning of the rejection reaction. During the rejection crisis the plasma levels of both components were frequently variable. Thus only repeated determinations seem to be of diagnostic value.

In contrast to recent reports [8,13] an association between the presence of circulating immune complexes and rejection reactions could not be observed in our patients.

In the 27 rejection episodes an increase of Clq-BA, possibly related to a rejection reaction, was found in only two cases. Elevated immune complexes however were detected in seven different patients suffering from malignant or infectious diseases. There is no evidence to suggest that drugs were involved in the formation of these immune complexes. The role of ALG as an immunogen, which could have produced antigen-antibody complexes and of the other immunosuppressive agents, can probably be excluded. Thus elevated Clq-BA in allografted patients may indicate severe complications, not related to rejection phenomena. Its determination could be of interest in the monitoring of kidney-grafted patients in order to detect additional concomitant diseases. Further studies are required to elucidate the nature of the possible antigens which could initiate elevation of circulating immune complexes.

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Open Discussion

HECKING (Mainz) Is it possible that C3 concentration is dependent upon renal function? Rejection must increase creatinine and it is possible that therefore the C3 level decreases.

WEGMÜLLER No, I do not think so. The C3 concentration is not dependent upon small alterations of creatinine levels. That is, the difference of 2–3 mg/100 ml during a rejection is not sufficient. However, from other studies measuring C3 levels in uraemic and dialysis patients we know of course that the C3 levels are usually decreased.

HALL (Birmingham) We have used the Raji Cell Assay to follow immune complex levels in patients receiving kidney allografts. Our data confirms yours almost entirely; when complexes are found they are not related to episodes of rejection and they indicate other pathological processes, in particular infective ones. In a group of patients receiving antilymphocyte globulin as part of the immunosuppressive regime we found a much higher incidence of immune complexes, and we wondered whether in fact we were detecting immune complexes in which the antigen was the ALG and the patient was forming antibodies to the ALG accounting for these raised levels of complexes. I wonder if you have any observations about that?

WEGMÜLLER We have seen in our experiments that there were two cases in which the immune complexes were initially high so these might possibly have been induced by ALG administration. I am very happy that you had similar experiences to ours. Traeger reported similar observations to yours. He found
in many cases initially high levels of immune complexes in the first 48 hours after transplantation attributable to ALG administration. We could not confirm this observation.

HALL In patients on chronic haemodialysis we find about 40% of patients have raised levels of complexes. There are a lot of reasons why these patients should have complexes; chronic infection, aggregations of gammaglobulin on dialysis membranes and so on. Is this your experience also?

WEGMÜLLER We have not yet checked immune complexes in chronic haemodialysis patients. But let me come back to the other problem you mentioned before. I think it is extremely important to confirm and state that the circulating immune complexes are usually not correlated with rejection reactions. This is in contradiction to a recent paper by Ooi and others from Cincinnati where such a correlation has been observed.

HALL I think the other thing one must point out is that a lot of these assays in fact measure not only immune complexes but a number of things like complement aggregation and AHG, so what we are hopefully calling complexes in a great majority of cases may not be true immune complexes.