Tubular Histuria and Serumproteinuria Following Human Kidney Allotransplantation

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Summary

The urinary excretion of brush-border membrane antigens and the tubular indicator enzymes alanine-aminopeptidase and gamma-glutamyl-transpeptidase, as well as the pattern of serumproteinuria, were studied in 34 patients following kidney allotransplantation by immunological and biochemical methods. The clinically defined situation of normally functioning graft, the recovery period of acute tubular necrosis and acute rejection episodes each showed specific patterns of the parameters investigated. There was a good correlation between positive laboratory findings and clinical criteria of acute rejection episodes, demonstrating the diagnostic value of the parameters studied.

Introduction

The early phase of acute renal graft rejection is often very hard to diagnose. Since successful treatment of an acute rejection episode (RE) may be endangered by delay in starting therapy, the availability of diagnostic parameters in addition to clinical and functional criteria is of considerable importance. Recently, it has been suggested that study of the pattern of serumproteinuria has possible value in the diagnosis of acute RE (Boesken et al, 1974). In addition, current interest has been focused on the significance of the urinary excretion of human kidney brush-border membrane antigens (Mondorf et al, 1972) and enzymes of tubular origin (Mondorf et al, 1973; Sandman et al, 1973) as indicators of rejection episodes.

In the present study serumproteinuria, tubular histuria as well as enzymuria
were correlated with clinical data of transplanted patients in order to evaluate further the importance of these parameters for the detection of acute RE.

PATIENTS AND METHODS

A total of 34 patients was studied following kidney transplantation, 14 having received cadaveric kidneys and 20 kidneys from living related donors. Histuria, enzymuria and serumproteiunuria were followed over a period of approximately two months after grafting.

Urine was collected daily over a 24-hour period. Aliquots were used for enzymatic and part of the immunological studies, the remaining volume was concentrated in an Amicon-DC 2 ultrafiltration system. Histuria and serumproteiunuria were studied by methods described below.

Tubular indicator enzymes gamma-glutamyltranspeptidase (gGTP) and alanineaminopeptidase (AAP) were assayed with the modified test combinations of Boehringer, Mannheim.

Antisera against brush border (BB) membranes, that have been isolated from human kidney cortex as previously described (Mondorf et al, 1972; Scherberich et al, 1974) as well as against soluble BB antigens (Scherberich et al, 1974, 1975) were raised in rabbits and goats. Antibodies against serum-proteins were absorbed by plasma proteins covalently linked to cyanogen bromide activated sepharose. Gammaglobulin fractions were prepared by fractional ammonium-sulphate precipitation.

For reverse radial immunodiffusion aliquots of native urine were mixed with agarose solution (0.8% final concentration). Gel plates were prepared, and antisera were pipetted in six different concentrations into wells of 3.5 mm diameter. Anti-BB antisera, as well as commercial antisera that had been raised against the following antigens were chosen: beta-2-microglobulin (Nordic), kappa- and lambda chains, erythrocyte membrane antigens (Medac-Dacopatts); retinol-binding protein, alpha-1-glycoprotein, prealbumin, albumin, IgG, fibrinogen, fibrinogen degradation products (FDP) D and E, alpha-2-macroglobulin, IgM (Behring-Werke, Marburg). The slides were processed as in radial immunodiffusion.

Radial immunodiffusion was carried out according to Mancini et al (1965). Three per cent anti-BB antisera were used.

One and two-dimensional as well as tandem crossed electro-immunodiffusion was performed as described by Axelsen et al (1973). Five per cent anti-BB antisera were used. Purified AAP, solubilised from BB membranes (Scherberich et al, 1974) was used as a standard.

Specific staining of precipitates was performed as reported elsewhere.

Polyacrylamide disc electrophoresis (7.5% gels, pH 8.6) was done according to Maurer (1971).

Polyacrylamide gel isoelectric focusing (Ampholine pH gradient 3.5—9.5) was carried out using an automatic LKB Multiphor system as described in the producer’s instruction manual.
Figure 1. Mean daily excretion of tubular indicator enzymes alanine-aminopeptidase (AAP) and gamma glutamyltranspeptidase (gGTP) in healthy individuals, in patients with a normally functioning graft and in patients with allotransplants undergoing acute rejection episode.
RESULTS

Observations in Uncomplicated Cases

Five of the recipients of living related donor kidneys showed no stimulation in mixed lymphocyte cultures prior to transplantation. Their clinical course following grafting was characterised by immediate onset of diuresis and rapid recovery of renal function. During the period of observation no clinical signs of graft rejection were observed.

Within the first week following transplantation a pattern of serumproteinurina, best described as 'glomerulo-tubular', was observed. Later on, serumproteinurina adopted more and more a 'tubular' pattern. Proteins excreted included a significant proportion of albumin and relatively large amounts of plasmaproteins of a smaller molecular size such as alpha-1-glycoprotein, lambda- and kappa light chains, retinol-binding protein and beta-2-microglobulin. Fibrinogen, fibrinogen degradation products D and E, alpha-2-macroglobulin, and IgM could not be detected with sensitive reverse radial immunodiffusion technique. Tests for erythrocyte membrane antigens were only positive in phases of haematuria.

Excretion of tubular indicator enzymes gGTP and AAP during the time of observation was significantly higher when compared to concentrations found in healthy individuals, as demonstrated in Figure 1. In all five patients only small amounts of brush-border membrane antigens could be demonstrated, not measurable quantitatively with the techniques applied.

Observations in Complicated Cases

Initial Acute Tubular Necrosis (ATN)

In 8 cases (all of them recipients of cadaveric grafts) the initial phase was complicated by oligo-anuria lasting 7 to 30 days. During the recovery phase of these cases, which were retrospectively diagnosed to have had acute tubular necrosis, the urine always showed a tubular protein excretion pattern. In all 8 cases in the recovery period a direct and linear correlation between daily urine volume and daily total enzyme excretion (AAP and gGTP) could be demonstrated (r-values ranging from 0.88 to 0.97, p < 0.01 - P < 0.005). This positive correlation was found to exist up to urine volumes of approximately 3500 ml/day. Figure 2 gives a demonstration of a typical case. Brush-border membrane antigens did not exceed the basic level found in normally functioning transplanted kidneys.

Acute Rejection Episode

Within the observation period a total of 26 rejection episodes were diagnosed based on a constellation of some of the criteria listed as follows: sudden, otherwise unexplained, decrease of creatinine clearance or increase of serum creatinine
Figure 2. Selected case of a patient demonstrating (a) typical correlation of enzymuria and urine volume during recovery period of ATN, and (b) rapid increase of elimination of tubular indicator enzyme AAP accompanied with loss of correlation with diuresis during RE.

concentration, decrease of urine volume in spite of increasing body weight, hypertension, decrease of urinary sodium excretion, proteinuria, haematuria, lymphocyturia, enlargement of the graft, fever without sign of infection, local pain.

In 24 of 26 RE a change in serumproteinuria pattern from the ‘tubular’ to the ‘glomerular’ type could be demonstrated, but in two cases ‘tubular’ proteinuria persisted. In addition to increased urinary excretion of high molecular weight substances including fibrinogen, FDP D and E, alpha-2-macroglobulin and IgM, a marked output of proteins below 69,000 Daltons was found. In 25 of the 26 RE a rapid increase in the daily excretion of AAP and gGTP, exceeding the levels found in normal functioning grafts, was observed. This is shown in Figure 1, where the means of the highest values of the daily excretion of AAP and gGTP during RE are given. When RE occurred during the recovery phase of ATN the correlation between urine volume and enzyme excretion, characteristic for this period, was lost (Figure 2). The increase of gGTP excretion was usually greater than that of AAP. Retrospectively in some cases increased enzymuria was noticed two days prior to clinical diagnosis of RE. In addition to increase of enzymuria and a change of serumproteinuria, considerable amounts of brush-border membrane
antigens were detected in all cases of RE (as in the example given in Figure 3). In two dimensional electroimmunodiffusion (EID) two of the precipitates could be specifically stained for gGTP and AAP activity.

In tandem crossed EID kidney brush-border antigens, artificially cleaved off from the membranes by proteolysis, gave complete immunological identity with such antigens found in urine during RE.

In one case with acute tubular necrosis in a previously normally functioning
kidney, a precipitation pattern differing from that of RE was observed: instead of gGTP, AAP was the predominant immunoreactive antigen.

Successful treatment of RE was accompanied by a decrease of enzymuria and histuria to baseline levels established for normally functioning grafts. In accordance, brush-border membrane antigens disappeared from urine and serumproteinuria returned to the preceding 'tubular' pattern. In five cases where enzymuria, excretion of brush-border antigens, and the pattern of serumproteinuria was not affected by repeated rejection therapy, the grafts had to be removed because of spontaneous rupture or insufficient function.

DISCUSSION

It is well established that interstitial and tubular rather than glomerular changes are associated with acute RE. Biopsy specimens obtained from renal allografts at the time of acute RE show major histological abnormalities in the interstitium (Porter, 1967). It appeared desirable therefore to evaluate the importance of parameters indicating tubular damage in the diagnosis of RE.

When the data were correlated with the clinically defined situations of normally functioning grafts, the recovery period of acute tubular necrosis and rejection episodes, a distinct pattern emerged.

Normally functioning grafts were characterised by consistent tubular proteinuria, moderate elevation of excretion of AAP and gGTP and only traces of brush-border membrane antigens in the urine, probably due to circulatory and adaptive events resulting in tubular dysfunction. The outstanding feature in the recovery period of acute tubular necrosis, besides tubular proteinuria, was a pronounced increase in excretion of tubular indicator enzymes correlated with urine volume. In normally functioning grafts only small amounts of brush-border membrane antigens were found in the urine. In RE however excretion of kidney tissue antigens was significant and high urinary excretion of AAP and especially of gGTP was observed. The coincidence of enzymuria and histuria may be explained by the fact that AAP and gGTP, which were shown to be an integral part of the BB membrane surface, are released at a rather high rate from epithelial cell fragments (Scherberich et al, 1974; Scherberich & Mondorf, 1975). In addition, in RE serumproteinuria changed from 'tubular' to 'glomerulo-tubular' pattern.

Thus the most frequent clinical situations in the early post-transplantation period appear to be well defined by the parameters investigated. This applies especially to the correlation of positive laboratory findings with the clinical diagnosis of acute RE. However the possibility of false positive tests has to be considered, since a variety of other factors may be responsible for increased urinary excretion of kidney related enzymes and kidney tissue antigens (Mondorf et al, 1972–75; Scherberich et al, 1974–76). In our experience out of eight cases with unexplained enzymuria, retrospectively four could be related to preceding angio- graphy and urography.
CONCLUSIONS

1. Normally functioning grafts, the recovery phase of ATN and acute RE show marked differences in histuria, enzymuria (AAP and gGTP) and the pattern of serumproteinuria.

2. Urinary excretion of BB membrane antigens, AAP and gGTP, as well as glomerular proteinuria are closely correlated with the clinical diagnosis of acute RE.

3. Close observation of histuria, enzymuria and the pattern of serumproteinuria may contribute to early diagnosis of acute RE.

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References

Axelsen, NH, Kröll, J and Weeke, B [Eds] (1973) Scandinavian Journal of Immunology, 2, Suppt. 1
Mancini, G, Carbonaro, AO and Heremans, JF (1965) Immunochemistry, 2, 235
Maurer, HR (1971) Disc Electrophoresis. de Gruyter, Berlin
Mondorf, AW, Carpenter, CB, Scherberich, JE and Merrill, JP (1972) Fifth International Congress of Nephrology, Mexico
Mondorf, AW, Carpenter, CB, Scherberich, JE and Merrill, JP (1973) In Protides of the Biological Fluids, 21, 493
Mondorf, AW, Scherberich, JE and Reitinger, W (1975) Contributo to Nephrology, 1, 119
Scherberich, JE and Mondorf, AW (1975) Protides of the Biological Fluids, 23, 575

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Open Discussion

PIERIDES (Newcastle upon Tyne) You have shown us the changes of gamma-glutamyltranspeptidase. Have you got any information on plasma changes please?

SCHERBERICH We expected this question and therefore we have followed all the sera obtained from these patients through the rejection episode. There was no elevation of serum levels of those enzymes studied.

PIERIDES In the long-term, do you find that sera of patients after transplantation have higher activity of gamma-glutamyltranspeptidase than those of dialysed or uraemic patients?

SCHERBERICH In these patients we have not found significantly higher serum levels of gamma-glutamyltranspeptidase than in ordinary dialysed patients or uraemic patients.