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Thomas F. Müller
Christine M. Neumann
Christoph Greb
Michael Kraus
Harald Lange

The anaphylatoxin C5a, a new parameter in the diagnosis of renal allograft rejection

T. F. Müller · C. M. Neumann · C. Greb · Harald Lange (✉)
Department of Nephrology/Centre of Internal Medicine,
Philipps-University of Marburg,
Baldinger Strasse 1,
D-35033 Marburg a. d. Lahn, Germany
Tel. 06421 28 27 09; Fax 06421 21 763

M. Kraus
Research Laboratories, Behringwerke AG,
Marburg, Germany

Abstract In the underlying study the diagnostic value of the anaphylatoxin C5a was evaluated in kidney transplantation. In 49 transplant patients the following parameters were measured daily for a mean period of 25.1 days: plasma C5a [P-C5a], urine C5a [U-C5a], serum amyloid A [SAA], serum neopterin [S-NEOP] and urine neopterin [U-NEOP]. Sensitivity, specificity and day of first significant parameter increase (exceeding a cut-off level of > 50 %) were evaluated retrospectively during 30 periods of rejection and 30 periods of stable graft function. U-C5a was the parameter with the highest sensitivity (84 %) and specificity (84 %), increasing in the mean 1.3 days before clinical diagnosis of rejection. Sensitivity and specificity of the other markers was lower: SAA 77 % and 77 %, U-NEOP 68 % and 65 %, S-NEOP 45 % and 77 %, and P-C5a 45 % and 48 %, respectively. During four instances of cytomegalovirus disease extremely high U-NEOP ($\geqslant$ = 1520 ± 518 μmol/mol creatinine) and slightly increased P-C5a levels ($\geqslant$ = 1.5 ± 1.4 ng/ml) occurred. Elevated urinary excretion of C5a seems to be a reliable and early marker of renal allograft rejection. In combination with SAA and U-NEOP, the daily assessment of U-C5a differentiates between viral infection and allograft rejection.

Key words Complement products · Anaphylatoxin C5a · Allograft rejection · Serum Amyloid A · Neopterin

Introduction

Parameters facilitating the differential diagnosis of a deterioration in graft function are needed [18]. The complement system is a very potent immune regulator [2, 17]. In the past, studies on complement activation in the field of organ transplantation were mainly focused on hyperacute rejections, especially after xenografting [20]. Early attempts to diagnose rejection episodes in human renal allografts by monitoring circulating, whole complement components failed [5]. Diagnostic approaches were primarily limited to the morphological analysis of complement deposits in tissues of grafted organs [1, 6, 8, 9]. Quite recently, complement products such as plasma C3d (28 Solling), C3a and the terminal complement complex TCC [12], and urine samples of C4d and TCC [3] were determined. Analysing these complement levels, which were not measured daily, no clear-cut correlation with the onset of acute rejection episodes was obtained.

In the following study, levels of the complement products C5a were measured in plasma (P) and urine (U) samples on a daily basis in patients following kidney transplantation. C5a is small molecule (MW: 11 kD) and a potent anaphylatoxin whose role has not yet been investigated in graft rejection [7, 14]. The diagnostic performance of P-C5a and U-C5a was compared with serum (S) and urine neopterin (S-NEOP and U-NEOP) and serum amyloid A (SAA). NEOP is a marker of the cellular immune response and elevated levels are pri-

marily seen during rejection episodes and viral infections [10, 15, 19]. SAA is a protein of the acute phase response and peaks are found during acute allograft rejections [16, 19].

Patients and methods

Forty-nine consecutive patients (17 female, 32 male) received a cadaveric kidney transplant between April 1990 and March 1992 at the Philipps University of Marburg. Their mean age was 51.1 years. The total period of observation on the ward was 1229 days, with a mean of 25.1 days per patient.

Immunosuppressive protocol

As basic immunosuppressive therapy, prednisolone started with 80 mg on day 1 and was tapered to 20 mg on day 4 after transplantation. Ciclosporine (Sandoz) was started intravenously with 5 mg/kg body weight on day 1, continued orally at 10 mg/kg body weight and was adapted to blood levels of 200 to 250 ng per ml. The whole blood levels of Ciclosporine were determined by ELISA based on monoclonal antibodies. In primary non-functioning grafts, polyclonal rabbit anti T cell antibodies ATG (Fresenius) were given, Ciclosporine was reinstituted after recovery of graft function. Acute rejection episodes were treated by steroid pulses and in steroid-resistant rejections the monoclonal antibody OKT3 (Orthoclone, Cilag) was used.

Rejection episodes

Acute rejection episodes were diagnosed by a declining creatinine clearance when other non-immunological causes of graft failure were ruled out. In addition, a positive response to the immunosuppressive antirejection therapy was obligatory. Facultatively, fine needle as well as core needle biopsies were performed to support the clinical diagnosis. To allow comparisons between patients, the first day of clinically manifest rejection and the start of antirejection therapy was labelled as day 0. Subsequently, the days before and after day 0 were labelled, starting with day – 3 (i.e. three days before day 0) and ending with day + 2 (i.e. two days after day 0).

Stable graft function

Periods of stable graft function were defined as time intervals of at least 6 days duration where complications such as rejection episodes, viral infections, acute tubular necrosis, surgical interventions and therapies with mono- or polyclonal antibodies were ruled out by clinical and laboratory findings. The first 3 postoperative days were never considered for evaluation.

Blood collection

Daily, morning blood samples of 10 ml were drawn into EDTA tubes (Sarstedt) to which 0.25 ml aprotinin (Trasylol, Bayer; 20000 KIE/ml) were added. The samples were immediately cooled and centrifuged at 3000 *g* for 10 min at 4 °C. Supernatants were separated into 300 µl fractions, frozen immediately and stored at – 70 °C.

Urine collection

Daily samples of spontaneously voided morning urine (between 6 and 7 a.m.) were taken into sterile containers (Sarstedt) and again immediately cooled. In the laboratory, these samples wee separated into 300 µl fractions, frozen immediately and stored at – 70 °C. In cases of pyuria or haematuria the urine samples were centrifuged beforehand.

Measurement of the Parameters

Anaphylatoxin (C5a)

Concentrations of C5a in plasma and urine were measured by a new enzyme immunoassay (EIA, Enzygnost C5a, Behringwerke AG, Marburg, Germany). For adjustment with a reference curve, known concentrations of purified C5 and C5a (Behringwerke AG, Marburg, FRG) were used as standards. In the C5a EIA, an anti-C5a monoclonal antibody (mAb) (561) was used as capture antibody and a horseradish-peroxidase labelled anti-C5a mAb (557) for detection as described by Klos et al. [13]. The intra- (within run) and interassay (between runs) coefficients of variance were 5.3 % and 6.5 %, respectively. The P-C5a levels were expressed in ng/ml. The U-C5a values were both measured in ng/ml as well as related to the daily urine volume and expressed in ng/d.

Neopterin (NEOP)

S-NEOP and U-NEOP levels were measured by radioimmunoassay (RIAcid, Henning/Brahms, Berlin, FRG). U-NEOP was related to creatinine excretion and expressed as µmol/mol creatinine. S-NEOP was measured in nmol/l.

Serum amyloid A (SAA)

A rapid immunonephelometric assay was developed to measure SAA levels, as previously described [11]. Basis of the test are highly specific antibodies raised against purified SAA, the antigen-antibody complexes being measured by laser nephelometer (Behringwerke AG, Marburg, FRG). The concentrations are expressed as mg/dl.

Analysis of the parameters

All markers investigated were measured on a daily basis. Clinical data as well as parameter values were displayed graphically for retrospective analysis. The postoperative courses were analysed and 6-day periods of interest were defined (day – 3 to day + 2) for rejection episodes and stable graft function. Each rejection episode was matched with a corresponding 6-day period of stable graft function of another patient. For each pair, the time of occurrence in the postoperative course as well as the number of HLA mismatches was matched. During the defined periods of interest (rejection/stable function) the individual parameter behaviour was analysed according to the criteria of a diagnostic test [4]. Changes in P-C5a, U-C5a, S-NEOP, U-NEOP and SAA were assessed as true positive (TP), false positive (FP), true negative (TN) or false negative (FN). Each parameter could show one TP or FN (during each rejection) and one FP or TN behaviour (during each period of stable function), respectively. The cut-off level for TP/FP and TN/FN parameter behaviour was a parameter increase of greater

than 100 % from the pervious day. Sensitivity was calculated as TP/(FN + TP) and specificity as TN/(FP + TN). The day of first TP parameter increase related to day 0, i.e. the day of clinical diagnosis and start of rejection therapy, was calculated. Using the SAS statistical analysing system a k-sample median test (Brown-Mood) was performed to analyse the median parameter values during periods of stable graft function, rejection and viral infection.

Results

Example of an acute rejection episode and reactivated cytomegalovirus (CMV) infection

Figure 1 gives an example of an acute, steroid-resistant, reversible rejection episode, which was clinically diagnosed on postoperative day 28. The deterioration in graft function is shown by the increasing creatinine levels and accompanied by TP peaks, i.e. increases exceeding the 50 % cut-off level, of the parameters SAA, P-C5a and U-C5a. The U-NEOP levels are FN, a peak occurring on day 31 is due to the cytokine release induced by the OKT3 therapy. On day 45, CMV DNA was detected by PCR testing. Together with clinical symptoms a CMV disease could be verified. The viral infection is reflected in the increasing U-NEOP levels, exceeding levels of 1000 µmol/mol creatinine. In addition, a marked P-C5a peak is shown, quickly returning to baseline levels.

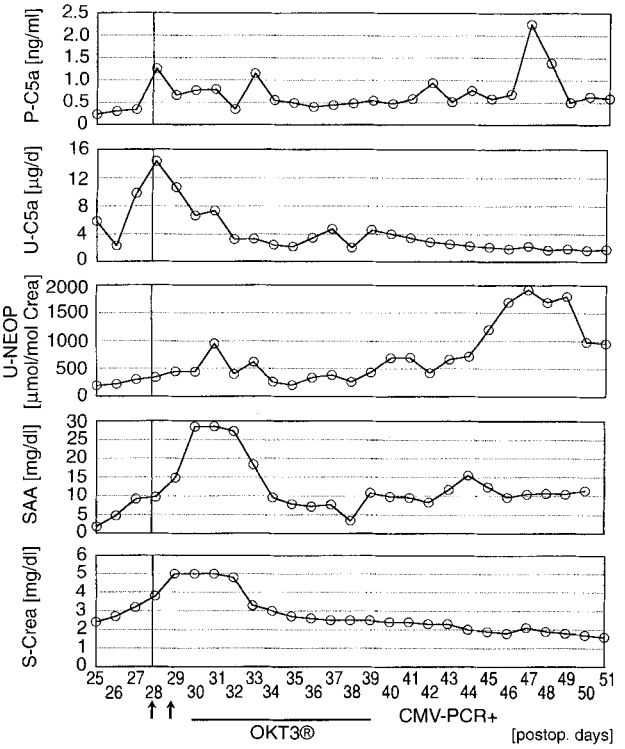

Fig. 1 Example of an acute rejection episode and reactivated cytomegalovirus (CMV) infection. Postoperative (day 25 to day 51) plasma C5a *(P-C5a)*, urine C5a *(U-C5a)*, urine neopterin *(U-NEOP)*, serum amyloid A *(SAA)* and serum creatinine *(S-Crea)* levels are shown in a patient with an acute, steroid-resistant rejection episode (↑ steroid pulse therapy on day 28 and day 29, OKT3® therapy from day 30 to day 39) and a reactivated CMV infection, diagnosed by a positive CMV-PCR on day 45

Median parameter values during periods of stable graft function, acute rejection and viral infection

Following the retrospective analysis of the parameter curves in the 49 patients, 30 acute, reversible rejection episodes were diagnosed. In 4 patients severe, clinically manifest CMV diseases occurred. These CMV infections were monitored for a total period of 53 days. Figure 2 shows the median values of the parameters SAA, U-C5a and U-NEOP obtained from the 6-day periods during the 30 rejections (n = 180), the 30 corresponding periods of stable graft function (n = 180) and 53 measurements during the four CMV infections. It is shown that the U-C5a median levels are significantly elevated during the rejection episodes in comparison to the stable graft function and infection periods. On the other hand, the viral infections are associated with significant elevations of the U-NEOP median values. The SAA median values did not discriminate significantly between the three clinical settings. The same applies for the parameters S-NEOP and P-C5as, the values are not shown.

Diagnostic qualities of the rejection parameters

Figure 3 summarises the diagnostic test qualities of the five markers by analysing the parameter behaviour during the 30 acute rejection episodes and 30 periods of stable graft function. U-C5a is the parameter with the highest sensitivity and specificity (84 %, respectively), followed by SAA with 77 % sensitivity and specificity. The diagnostic accuracy of U-NEOP, S-NEOP and P-C5a do not reach that level. In addition it is shown, that U-C5a is also the earliest predictor of an impending allograft rejection, increasing significantly in the mean 1.3 days before the clinical diagnosis of rejection is made. U-NEOP and SAA peaks occurred in the mean 0.5 days before the day of clinical diagnosis and start of antirejection therapy. Both, P-C5a and S-NEOP respond later in the rejection process.

Discussion

Recent studies suggest the major role of complement activation in allograft rejection. Substantial deposits of various complement components were found in renal allograft tissues during rejection processes [6, 8, 9]. Donor-specific, local complement synthesis could be dem-

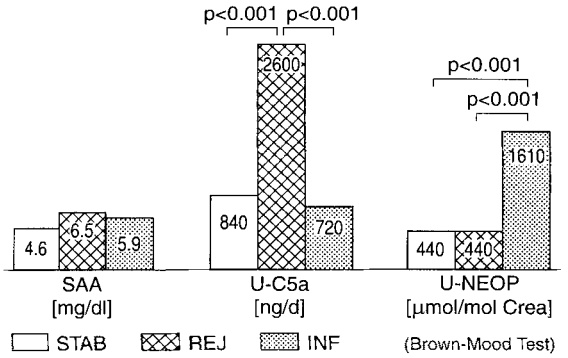

Fig. 2 Median parameter values during periods of stable graft function, acute rejection and viral infection. The median values of each parameter obtained from daily measurements during 30 periods of acute rejection (n = 180), 30 corresponding periods of stable graft function (n = 180) and 4 periods of clinically manifest CMV infection (n = 53) are given. The significance probability across the three different clinical situations is indicated

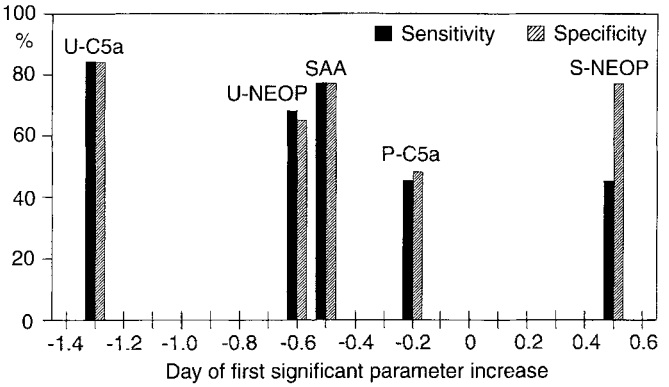

Fig. 3 Diagnostic qualities of the rejection parameters. Sensitivity, specificity and day of first significant increase (i.e. increase of > 50 % from level of the previous day) of the parameters obtained during 30 periods of acute allograft rejection and 30 corresponding periods of stable graft function are shown

onstrated in kidney transplantation [1, 2, 21]. Despite this strong morphological evidence, conflicting results are provided by several studies concerning the diagnostic value of circulating complement levels and split products. Once or twice weekly C3a, C3d, C1rsC1INH, C3b(Bb)P and sC5b-9 were measured. A clear-cut relationship to renal allograft rejections could not be established [12, 22]. In one study sC4d and sC5b-9 were measured in a few selected urine samples. Increased sC4d levels were found in steroid-resistant rejections and a further evaluation by longitudinal monitoring was suggested [3].

Therefore, daily measurements of the chemoattractant C5a were performed in plasma as well as urine samples of 49 renal transplant patients in the immediate postoperative course. The diagnostic value of P-C5a and U-C5a was compared with markers of the immune response in organ transplantation. SAA is a parameter of the acute phase reaction [16, 19]. S-NEOP and U-NEOP are markers of the cellular immune response [10, 15]. Analysing 30 periods of rejection and stable graft function, U-C5a was the parameter with the highest diagnostic accuracy in the detection of rejection episodes. In addition, it was the earliest predictor of an impending rejection. As a rejection marker, U-C5a was superior to SAA and U-NEOP. The circulating P-C5a levels showed a low sensitivity and specificity, and increases were seen predominantly during CMV infections. Viral infections were best detected by increases in the U-NEOP levels. There was no correlation between plasma and urine C5a levels.

These findings support the relevance of the complement system as an immune regulator [2]. The disproportionately high U-C5a levels in relation to the P-C5a levels favour the concept of the local immune synthesis in the allograft and agree with the intrarenal distribution of complement products, mainly located in the tubules [1, 21]. Therefore, the urine compartment is probably a better window than the blood compartment to look at complement activation associated with an immune response targeted against the renal allograft. This might, on the contrary, explain the increased P-C5a levels during systemic CMV diseases, which were not reflected by changes in the U-C5a excretion. Concerning the kinetics of the complement cascade and the quick turnover of complement components, short intervals between the determinations seem to be essential [23]. Probably the lack of diagnostic value in the detection of rejection episodes described for different complement products is partly due to the long intervals between the determinations.

In conclusion, U-C5a seems to be a reliable and early rejection parameter in kidney transplantation. In combination with SAA and U-NEOP, the daily monitoring of U-C5a facilitates the differential diagnosis between viral infection and acute rejection.

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