Serum amyloid A protein is a clinically useful indicator of acute renal allograft rejection

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Abstract

Background. Early diagnosis of acute rejection after renal transplantation is important. There is evidence that measurement of the acute phase proteins, C-reactive protein (CRP) and serum amyloid A protein (SAA) is helpful.

Methods. In 64 consecutive patients, CRP was measured in a routine clinical system (Technicon RA1000, Bayer) and SAA in a new sensitive automated immunoassay on the Abbott IMx instrument, daily or on alternate days for 30 days after renal transplantation.

Results. Patients all received triple immunosuppression with cyclosporin, azathioprine, and prednisolone and all mounted a post-surgical acute phase response of SAA, but the CRP response was reduced or absent. Serum creatinine rose significantly in 36 patients, leading to treatment for first rejection. Thirty of these episodes were confirmed rejection, three were definitely not and three were uncertain. SAA, normally <10 mg/l, rose to more than 100 mg/l in all episodes except when rejection was definitely absent. In six cases SAA rose above 100 mg/l 1–3 days before the rise in creatinine leading to antirejection therapy, and only twice did creatinine rise 1 day before SAA. In contrast, CRP responses to rejection were modest or absent. In four patients there were SAA and CRP responses unrelated to rejection, three associated with intercurrent infection and one with administration of antilymphocyte globulin. There were also two unexplained isolated spikes of SAA.

Conclusions. SAA is a sensitive marker of acute renal allograft rejection. It is not specific, but the differential behaviour of CRP in patients receiving cyclosporin helps to distinguish infection from rejection. Availability of rapid assays for these analytes should facilitate management of renal allograft recipients.

Key words: acute phase protein; C-reactive protein; diagnosis; rejection; renal transplantation; serum amyloid A protein

Introduction

Effective management of patients receiving renal allografts requires early recognition and adequate treatment of rejection episodes. Apart from direct monitoring of renal function, a variety of putative markers of rejection have been evaluated in serum and urine, but none is both highly specific and highly sensitive. Nevertheless there is substantial evidence that the serum concentrations of the highly sensitive major acute phase proteins [1], C-reactive protein (CRP) [2–13] and serum amyloid A protein (SAA) [14–21], can provide useful information in this context. These observations were first made with CRP before the widespread introduction of cyclosporin. Subsequently it has become clear that standard immunosuppressive therapy with cyclosporin and prednisolone markedly suppresses the acute phase response of this protein to transplantation surgery and to acute rejection [11,22], although not the response to intercurrent infection. In contrast the SAA response to transplant surgery still occurs during cyclosporin and steroid treatment, and the responses to rejection and infection are apparently unimpaired [14–21].

The acute phase response is a non-specific phenomenon induced by almost all forms of tissue damage, inflammation and infection, and although both CRP and SAA are exquisitely sensitive and rapid reactants covering extremely wide dynamic ranges, their serum levels can never be diagnostic on their own [1,23]. They must be interpreted in the context of the full clinical picture and can then provide invaluable information for both diagnosis and management. In the present study we have used a new, rapid, automated immunoassay [24] to confirm and extend earlier observations of the sensitive and frequently predictive SAA...
response to episodes of renal allograft rejection. The contrast between specific failure of the CRP response to rejection in these cyclosporin-treated patients and preservation of the response of both proteins to infection enhances the value of frequent routine measurement of both SAA and CRP.

Subjects and methods

Serum samples (n = 1021) obtained daily or on alternate days for the first 30 days after transplantation from 64 consecutive patients receiving renal allografts during 5 months in 1992 were stored frozen at −70°C and assayed in single batch at the end of the study. SAA was determined by monoclonal–polyclonal microparticle enzyme immunoassay on the Abbott IMx instrument [24]. Using this method in 105 healthy normal adults, the mean (SD) SAA value was 3.7 (3.6) mg/l, the median (range) was 3.0 (0.7–26.4) mg/l; 82% of values were below 5 mg/l and 96% below 10 mg/l [24]. CRP was measured by the Technicon RA1000 immunoturbidimetric method, which has a lower limit of sensitivity of 2 mg/l. However, the median normal level of CRP in healthy adults is 0.8 mg/l with 90% of values being less than 3 mg/l and 99% less than 10 mg/l [25]. In serial studies of healthy subjects it is clear that low levels of both CRP and SAA, close to the medians cited above, are actually normal, whilst higher values occur rarely and sporadically in most individuals (M. B. Pepys, J. R. Gallimore and J. Stuart, unpublished observations) and almost certainly reflect the sensitivity of the response of these reactants to minor or subclinical intercurrent events.

Demographic and clinical details of the patients studied are shown in Table 1. All recipients had primary renal graft function. The immunosuppression regimen consisted of cyclosporin, azathioprine and prednisolone. Cyclosporin dosage was adjusted in order to obtain whole blood levels of about 300–400 ng/ml in the first month after transplantation. On the day of transplantation all patients received 0.5 g methylprednisolone intravenously. After transplantation the oral daily dose of prednisolone was 80 mg, reducing by 10 mg/day down to 20 mg/day during the first month. Initial azathioprine dosage was 2 mg/kg/day tapered to 1 mg/kg/day over a few weeks. All patients received co-trimoxazole prophylaxis. Induction therapy with anti-T-cell antibody (OKT3) was given to one HLA-sensitized patient. Rejection episodes requiring treatment were identified clinically by a rise of 20% or more in serum creatinine compared to the preceding one or two measurements, usually combined with a fall in urine output. The grafts were examined by ultrasound to exclude urinary obstruction and duplex Doppler did not reveal any renal transplant artery stenosis. Exposure to nephrotoxic medications was evaluated and infection excluded by clinical and microbiological investigation. Cyclosporin nephrotoxicity was not suspected on clinical grounds to be the cause of increased serum creatinine in any case, and in no patient was cyclosporin dosage reduced during antirejection treatment. Despite this, and despite even increased doses of cyclosporin in some cases, the rise in serum creatinine was always reversed by antirejection therapy. This is strong clinical evidence against acute cyclosporin toxicity as the cause of rising serum creatinine in those rejection episodes that were not confirmed by biopsy. Fifteen episodes of rejection were also verified by biopsy, taken with an ultrasound guided automatic gun biopsy system using 18-G needles (Biopry, Bard, Covington, GA, USA) and immediately fixed, embedded, and stained by standard techniques before independent evaluation by at least two renal pathologists. Results were reported as either negative or positive for rejection; the Banff classification was not used at that time. First rejection episodes presented here were treated with divided daily doses of methylprednisolone (0.5 g, 0.25 g, 0.25 g, and 0.25 g) for 4 or 5 days. Rejection unresponsive to steroids was treated with monoclonal anti-T-cell antibody (OKT3) or polyclonal antilymphocyte globulin (ATG).

Results

A typical postoperative acute-phase response of SAA is shown in Figure 1. The peak was usually on day 2 but there was enormous variation between individuals in the values attained (45–1829 mg/l). In marked contrast, although some patients mounted an acute-phase response of CRP, in many there was only a modest or even no rise at all in CRP concentration.

Rising serum creatinine considered to represent a first rejection episode, and consequently treated as

Table 1. Demographic and clinical data in 64 renal allograft recipients

<table>
<thead>
<tr>
<th>Patient and transplantation characteristics</th>
<th>Number of patients, median and range values</th>
</tr>
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<tbody>
<tr>
<td>Age at transplantation (years)</td>
<td>43 (10–77)</td>
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<tr>
<td>Male/female (n)</td>
<td>44/20</td>
</tr>
<tr>
<td>Previous CAPD (n)</td>
<td>5</td>
</tr>
<tr>
<td>Previous haemodialysis (n)</td>
<td>35</td>
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<tr>
<td>Predialysis transplantation (n)</td>
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</tr>
<tr>
<td>Retransplants (n)</td>
<td>7</td>
</tr>
<tr>
<td>Combined pancreas/kidney graft (n)</td>
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</tr>
<tr>
<td>Simultaneous nephrectomy (n)</td>
<td>10</td>
</tr>
<tr>
<td>Living donor graft (n)</td>
<td>29</td>
</tr>
<tr>
<td>Cadaver donor graft (n)</td>
<td>35</td>
</tr>
<tr>
<td>Cold ischaemia time (cadaver donor grafts) (h)</td>
<td>14 (4–28)</td>
</tr>
</tbody>
</table>

Fig. 1. Time of peak postoperative SAA value after renal transplantation surgery in the 44 patients for whom data were available.
Serum amyloid A protein is a useful marker of renal allograft rejection such, was observed in 36 patients (56%). In three of these cases it subsequently became clear that there had been no rejection and in none of these was there any increase in SAA. However, there was an acute phase response of SAA in association with all the other episodes (Figure 2a–c). Measurements of SAA and serum creatinine were available the day before and on the day of rejection treatment in 15 episodes of first rejection in 15 patients. The median (range) SAA value rose from 72 (19–1049) mg/l to 201 (135–1686) mg/l, whereas the serum creatinine levels in the same samples increased from 171 (131–182) mmol/l to 232 (151–411) mmol/l. The relative increase was significantly higher for SAA than for creatinine ($P < 0.001$).

Although there was sometimes a CRP acute-phase response related to rejection, it was often minimal and/or entirely absent (Figure 2a–c). In six cases the rise in SAA preceded the rise in serum creatinine by 1–4 days, and thus predicted the rejection episode (Figure 3a,b). Also in five of these cases the peak of the SAA acute phase response to rejection occurred 1 or 2 days before rather than on the day of starting antirejection therapy (Figure 3a,b). Only twice did the rise in serum creatinine precede that of SAA.

When acute rejection occurred very soon after transplantation the corresponding SAA acute-phase response was masked by the normal postoperative peak as shown in Figure 4. This same case also illustrates the acute-phase response that was observed in all patients treated with antilymphocyte globulin. Furthermore, although dwarfed in magnitude by the enormous SAA response, this individual did mount a CRP response, at least to surgery and to the injection of antithymocyte globulin.

Apart from three unexplained isolated spikes of SAA, there were four acute-phase responses unrelated to rejection episodes. Three of these were clearly caused by intercurrent microbial infections and the other by induction therapy with OKT3 in a patient receiving a second transplant. In all of these four cases there was a vigorous response of CRP as well as SAA.

In the study as a whole, SAA concentrations above 100 mg/l were observed in 37 patients associated with 33 rejection episodes, giving a positive predictive value for the test at this level of 0.89. Among 27 patient episodes in which there was no rejection, there were no elevations of SAA, giving a negative predictive value of 1.0. The negative predictive value of a <20% rise in serum creatinine was, obviously, also 1.0, as this was the primary criterion used to identify rejection, whilst the positive predictive value was 0.92 based on 33 rejection episodes among 36 increases in creatinine.

**Discussion**

In this study, episodes of rejection were diagnosed and treated primarily on clinical grounds and, when impairment of renal function was reversed by antirejection therapy, biopsies were not routinely taken. This was
Fig. 4. SAA and CRP responses following renal transplantation. A first rejection episode occurred very early after transplantation and the associated SAA response was masked by the postoperative peak. Subsequently the SAA rose well before a second episode of rejection, and there was then a further SAA response to antirejection therapy with antithymocyte globulin. There was a major CRP response in relation to surgery and/or the first rejection episode, no CRP response to the second rejection episode, and a further response to administration of antithymocyte globulin.

greater than that of the serum creatinine concentration. In contrast the response of CRP, which in all other clinical circumstances is an exquisitely sensitive acute-phase reactant [23], was blunted and frequently completely absent, not only in relation to rejection but also following the usually potent stimulus of surgical trauma. This suppression of the CRP response reflects inclusion of cyclosporin in the standard immunosuppression regime [11,22], and it demonstrates important differences between control of production of SAA and CRP.

There have in the past been claims that the SAA response in general is more sensitive than that of CRP, but these have largely been based on the fact that sensitive immunoassays for SAA covering its whole range have been compared with routine clinical assays for CRP with lower detection limits of 5 mg/l or greater. This is very misleading because the normal range for CRP is actually 0.05–3.0 mg/l [25], and if suitably sensitive CRP assays are used similar or even more useful clinical information can be derived from the CRP values [26]. This may in part be because the high sensitivity and dramatic dynamic range of SAA production lead to more ‘noise’ in the system. Nevertheless the original observation of Maury et al. that renal allograft rejection is a particularly powerful stimulus for SAA production [14–18] has been abundantly confirmed, and the distinction from CRP has been enhanced since the introduction of cyclosporin that so effectively suppresses CRP production in this context. Indeed this enables measurement of these two acute phase proteins to provide improved discrimination, since both of them respond sensitively to infec-
Serum amyloid A protein is a useful marker of renal allograft rejection, the major differential diagnosis, but only SAA responds well to graft rejection.

The acute-phase response is non-specific and measurements of acute-phase proteins can therefore never be diagnostic on their own [1,23]. However the universal association of significantly increased SAA production with acute graft rejection, the speed and range of the SAA response, and the availability of rapid, precise and robust assay technology combine to provide a test that will be useful in routine transplant practice by facilitating early diagnosis and efficient management. The wide variation between individuals in the absolute levels of SAA attained means that setting 'diagnostic' cut-off points is not biologically meaningful. Nevertheless, the value did exceed 100 mg/l in all of the 30 confirmed rejection episodes in this study, giving a sensitivity of 100%, and if single sporadic SAA peaks and the response to antilymphocyte globulin are excluded the specificity is 80–90% at this level.

The positive predictive value of an SAA level above 100 mg/l was 0.89 in this study, the same as the value for a 20% rise in creatinine (0.92) which was the primary criterion for diagnosing rejection. However, in four patients with infection, CRP rose in parallel with SAA, whereas this did not occur during rejection, indicating that an isolated increase in SAA would have a much higher positive predictive value. The negative predictive value for both SAA and creatinine was 1.0, but this is, of course, arbitrary for the latter as the start of a rejection episode must go unrecognized until the creatinine rises, when this is the primary diagnostic criterion. Interestingly, SAA increased significantly 1 or more days before the creatinine in six patients, whilst the reverse occurred in only two individuals. Together with the wider dynamic range of SAA these observations further support the use of regular measurements of SAA in addition to creatinine for diagnosis of rejection.

In conclusion, any rise in SAA value above 10 mg/l, the 96th centile of the normal range [24], should be viewed as abnormal and its significance considered in the light of all clinical information and other investigations available in the patient, especially with respect to rejection or infection. A doubling or more in the SAA level should always arouse suspicion and prompt further investigation. It supports the diagnosis of rejection in a patient with a simultaneously rising creatinine, especially if the CRP does not rise, whilst absence of an SAA response in such a situation argues strongly against rejection.

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References


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