Chronic renal allograft rejection can be predicted by area under the serum creatinine versus time curve (AUC$_{Cr}$)

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Chronic renal allograft rejection can be predicted by area under the serum creatinine versus time curve (AUC$_{Cr}$). Acute rejection is the most important single risk factor for chronic renal allograft rejection. Numerical quantitation of rejection episodes does not take into account the intensity and length of these episodes, both of which may contribute to the severity of chronic rejection. We propose a single numerical parameter for the frequency, intensity and length of acute rejections, the “Area Under the Serum Creatinine versus Time Curve” (AUC$_{Cr}$) using renal allografts between inbred rat strains. Twenty-seven renal transplantsations were performed from the DA to WF rat strains. The rats were immunosuppresed with 5 mg/kg body weight of CyA injected s.c. for 1, 2, 3 and 12 weeks, resulting in differing numbers (0–4) of biopsy-confirmed acute rejections of varying intensity (s-cre: 100–448 µmol/L) and length (3–24 days), all of which were reversed with additional CyA treatment. The intensity of chronic changes in graft histology was quantitated using the “Chronic Allograft Damage Index” (CADI). End-point transplant function was quantitated as level of serum creatinine at sacrifice. The AUCCr from 0 to 3 weeks (AUCCr03), encompassing the recovery period after operation, free of rejections, did not correlate with the CADI (r = 0.230, P = 0.249). All AUCCr, from 3 weeks onwards correlated with the CADI. The best correlation with the CADI was obtained with AUCCr from 3 to 12 weeks (AUCCr312) (r = 0.922, P = 0.0001). This interval coincides with the timing of all acute rejection episodes. AUCCr312 correlated equally well to end-point transplant function (r = 0.890, P = 0.0001). The correlations of AUCCr3–12 to CADI and to end-point transplant function were better than the correlations of the number of acute rejections (r = 0.876, P = 0.0001) and (r = 0.811, P = 0.0001), respectively, indicating that AUCCr3–12 is a more sensitive parameter than the number of acute rejections for predicting chronic rejection. Our results also indicate that there is a causal relationship between acute and chronic rejection, and that not only the frequency but also the intensity and length of acute rejections contribute. Reduction in the number of acute rejection episodes as well as minimizing their intensity with aggressive immunosuppressive therapy will both contribute to the reduction of chronic rejection.

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Chronic rejection is the major reason for the late loss of an allograft. In a recent consensus conference on chronic rejection (Fourth Alexis Carrel Conference, Kiruna, Sweden) [1], chronic renal allograft rejection was defined as “gradual deterioration in graft function (at least over a 3 month interval, after 3 months post-transplantation) in the absence of any other disease and necessary graft histology to confirm the diagnosis.” Transplant deterioration is usually quantitated as a decline in calculated creatinine clearance and/or an increase in the level of serum creatinine [2].

In another consensus conference (Banff classification of kidney transplant pathology) [3] agreement was reached on an internationally standardized criteria for renal allograft histology. The histological alterations of chronic rejection, agreed upon in the conference, were the same as suggested 40 years ago by Hume et al [4] and Porter et al [5], that is, persistent interstitial inflammation and fibrosis, glomerular mesangial matrix increase and sclerosis, vascular intimal proliferation and thickening, and tubular atrophy. We have recently shown that the histological alterations of chronic rejection may be quantitated and expressed as a single numerical figure, “The Chronic Allograft Damage Index” (CADI) [6]. In human renal allografts, incipient histological changes of chronic rejection, as quantitated with CADI at two years post-transplant are highly predictive to subsequent clinical chronic rejection at six years (r = 0.717, P = 0.0001) [7].

Risk factors leading to chronic renal allograft rejection focus largely on the perioperative period and the first post-transplant year. Acute rejection is probably the most important single risk factor for chronic rejection [8–11]. In addition, the following parameters may also contribute to rejection: histoincompatibility [12–14], preservation time [15–18], donor and recipient age [19–21], hyperlipidemia [22] and inadequate immunosuppression [8].

The frequency (or number) of acute rejection episodes [8–11] as a numerical parameter for chronic rejection does not take into account the intensity of and/or the length of the episodes, neither does it take into account whether the episodes have been completely treated and the transplant has been returned to optimal level of function. In this study we investigated, under controlled laboratory conditions, whether and to what extent the intensity and length of acute rejection episodes contribute to the development of chronic renal allograft rejection. Using rat renal transplants with differing levels of immunosuppression and varying frequencies and intensities of acute rejection episodes, we demonstrated that the “area under the serum creatinine versus time curve” (AUC$_{Cr}$) correlates better than the number of acute rejection episodes both to the CADI and to late graft function. Furthermore, we established a causal relationship between acute and chronic rejection, suggesting that the AUC$_{Cr}$ may be used as an early predictive parameter for chronic renal allograft rejection.
Methods

Animals

Male inbred WF (RT1\(^{b}\)) and DA (RT1\(^{a}\)) rats weighing 250 to 300 g and two to three months of age were used for transplantation. The animals were purchased from The Laboratory Animal Centre, University of Helsinki, Helsinki, Finland. They were fed with regular rat food (althrorn, Standart Dioet, Chr. Petersen A/S, Ringsted, Denmark), and tap water ad libitum and maintained on a 12 hour light/dark cycle. The animals received humane care in compliance with the “Principles of Laboratory Animal Care” and the “Guide for the Care and Use of Laboratory Animal Resources” published by the National Institutes of Health (NIH Publication No. 86–23, revised 1985).

Transplantations

Kidney transplants were performed from the DA to the WF strain using a modified technique of Fisher and Lee [23]. The donor kidney was transplanted heterotopically by end-to-side anastomosis of donor renal artery and recipient aorta, donor renal vein and recipient inferior vena cava below the left renal artery under intraperitoneal chloral hydrate anaesthesia. Ureteral anastomosis was performed end-to-end close to the renal pelvis. The right native kidney was removed during transplantation. Left nephrectomy was delayed for seven days to allow recovery from acute tubular necrosis of the transplanted kidney. Buprenorphinum was used for postoperative pain relief (Temgesic, Reckitt & Colman, Hull, UK). Total perioperative ischemia time was standardized between 25 and 30 minutes, beginning with exanguination and cold perfusion of the graft, and ending with revascularization of the graft.

Medication

The recipient rats were divided in four groups. Cyclosporine (CsA; Sandimmun, Sandoz, Basle, Switzerland) at a concentration of 50 mg/ml was dissolved in intralipid (Kabi Vitrum, Stockholm, Sweden) and administered subcutaneously once a day. Subcutaneous administration of CsA was begun immediately after completion of the operation using a dose of 5 mg/kg of body weight for 12 (N = 5), 3 (N = 7), 2 (N = 7) and 1 (N = 8) weeks.

Follow-up

The diagnosis of acute allograft rejection was made if there was a rise in serum creatinine to over 200 μmol/liter during or more than seven days after the last drug administration and if other causes were excluded. On such occasions, additional CsA was given s.c. at a dose of 5 mg/kg of body weight during the period when serum creatinine stayed over 200 μmol/liter to counteract acute rejection. The first rejection episode was always verified by core needle biopsy taken with a Biopsy instrument (Radioplast, Upplusa, Sweden) with a 18 G Biopsy-Cut needle. The needle was placed parallel to the long axis of the graft and two cores of tissue were obtained. No grafts were lost due the biopsy.

Serum creatinine and CsA concentrations were measured twice a week, until the rats were sacrificed. Blood was drawn from the tail tip.

Definition of chronic rejection

Chronic renal allograft rejection was defined according to the recent consensus conference on chronic rejection [1]. In this study we applied these criteria to the rat. In addition to the descriptive histopathologic diagnosis of chronic rejection, we quantitated biopsy histology to determine the intensity of histological changes of chronic rejection [6, 24].

Quantitation of histopathology

The grafts were harvested 12 weeks after transplantation. They were bisected horizontally and fixed in 10% buffered formalin. The specimens were examined histologically following sectioning and staining with hematoxylin-eosin, Masson’s trichrome, periodic acid-Schiff and methyl-green-pyronin. For each specimen, 31 histologic features [24] were evaluated and scored blindly by two independent investigators using an arbitrary scale from 0 to 3, 0 indicating no pathological alterations and 3 extreme changes. The final score was reached by consensus agreement. The “Chronic Allograft Damage Index” (CADI) [6] was calculated from the sum of inflammation and fibrosis in interstitium, mesangial matrix increase and sclerosis of glomeruli, intimal proliferation of vessels and tubular atrophy. The CADI is expressed as a single numerical figure to show the intensity of chronic changes in the transplant.

AUC\(_{Cr}\) calculation

Area under the postoperative serum creatinine versus time curve (AUC\(_{Cr}\)) over 100 μmol/liter of serum creatinine was calculated using a Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) program.

Statistics

Statistical analyses including linear regression and the Mann-Whitney U-test were performed using the StatView statistical package (Abacus Concepts, Inc., Berkeley, CA, USA). The results are expressed as the mean ± sd, and a probability of < 0.05 was accepted as significant.

Results

The clinical course

Renal transplants were performed from the DA to the WF strain of rats and they were immunosuppressed with 5 mg/kg/day s.c. of CsA. We have previously [24] demonstrated that this level of immunosuppression brings CsA to the level of 1250 mg/liter in seven days, where it then stabilizes.

The rats were divided into four groups according to the length of immunosuppression: the first group (N = 5) received CsA for 12 weeks, the second (N = 7) for 3 weeks, the third (N = 7) for 2 weeks and the fourth (N = 8) for 1 week. After discontinuing of CsA the blood CsA levels returned to zero within two to three days.

Serum creatinine determinations were performed on alternate days. When the serum creatinine (SCr) rose above 200 mmol/liter, a core needle biopsy was performed and additional CsA at a dose of 5 mg/kg/day was administered until the serum creatinine level fell below 200 mmol/liter; on most occasions it returned to normal. The acute rejection episodes were of different intensities (SCr 100 mmol/liter to 448 mmol/liter) and length (3 to 24 days). All early elevations in serum creatinine at this time period coincided with biopsy histology consistent with acute rejection [25].
The clinical course of eight representative recipient rats is given in Figure 1, where serum creatinine and duration of immunosuppression are depicted. Despite the fact that inbred animals were used as donors and recipients and that the same dose of immunosuppression was used, the clinical course was astonishingly variable. The postoperative downward slope of the serum creatinine was more or less uniform in these rats; however, the animals experienced none to several rejection episodes. In addition the intensity and length of rejection episodes was variable.

Regardless of the variation between individual rats, the length of immunosuppressive treatment still correlated significantly with the frequency of acute rejection episodes within each group: there were no rejection episodes in the group receiving 12-week immunosuppression, on the average one rejection episode per rat in the 3-week treatment group, 2.3 rejections per rat in the 2-week treatment group and 2.6 rejections per rat in the 1-week treatment group (data not shown).

The largest cumulative dose of CsA per rat, which was 84 mg/kg, was observed in the 12-week treatment group. In the groups receiving 1-, 2-, and 3-week treatments, the total CsA dose was approximately about 1/3 of the above. Because each individual acute rejection episode was treated by CsA, the total cumulative dose of CsA in the remaining three groups (1-, 2-, and 3-weeks) was the same: 32 ± 8, 33 ± 12, and 30 ± 11 mg/kg body wt (P = NS), respectively. As the adequacy of immunosuppression will be the subject of a separate report, it will not be commented further on in this communication.

To quantitate the intensity of chronic rejection, we used the quantitative histological CADI and end-point transplant function (serum creatinine). The Chronic Allograft Damage Index, CADI, at sacrifice almost linearly correlated to end-point transplant function \( r = 0.874, P = 0.0001 \) as depicted in Figure 2.

**Correlation of AUC\(_{Cr}\) to CADI**

The best correlation of the AUC\(_{Cr}\) to the CADI was determined by calculating the AUC\(_{Cr}\) over different postoperative time periods, and by quantitating the significance of regression. This is shown in Table 1.

If we calculated the AUC\(_{Cr}\) throughout most of observation period, from 0 to 12 weeks (AUC\(_{Cr}\),0–12), correlation to the CADI was highly significant \( r = 0.877, r^2 = 0.769, P = 0.0001 \). If we calculated only the initial time period from 0 to 3 weeks (AUC\(_{Cr}\),0–3), which encompasses primarily the recovery period from operation with no or very few rejections, the correlation was not significant \( r = 0.230, r^2 = 0.053, P = 0.249; \) Fig. 3a).

We considered this observation to reflect the fact that the
operative procedure and the subsequent recovery period were well standardized and homogeneous among this population of animals, and that the high correlation observed between the

Table 1. Correlations between AUC_{Cr} in different time periods and histopathological end-point CADI

<table>
<thead>
<tr>
<th>Time period of AUC_{Cr}</th>
<th>Correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r$</td>
</tr>
<tr>
<td>0–12 weeks</td>
<td>0.877</td>
</tr>
<tr>
<td>0–3 weeks</td>
<td>0.230</td>
</tr>
<tr>
<td>3–8 weeks</td>
<td>0.748</td>
</tr>
<tr>
<td>8–12 weeks</td>
<td>0.889</td>
</tr>
<tr>
<td>3–12 weeks</td>
<td>0.922</td>
</tr>
</tbody>
</table>

AUC_{Cr}0–12 and the CADI was mainly because both indices reflect subsequent immunologic events occurring within the graft.

To evaluate this further, we calculated the period from 3 to 8 weeks (AUC_{Cr}3–8) post-transplant and correlated this to CADI; again the correlation was significant ($r = 0.748$, $r^2 = 0.560$, $P = 0.0001$). We performed the same analysis to the very end of the observation period, from 8 to 12 weeks (AUC_{Cr}8–12), and again AUC_{Cr}8–12 correlated with the CADI significantly ($r = 0.889$, $r^2 = 0.79$, $P = 0.0001$). However, analysis of the 3 to 12 weeks period (AUC_{Cr}3–12) correlated best with the CADI ($r = 0.922$, $r^2 = 0.851$, $P = 0.0001$; Fig. 3b). Thus the extent of the histological changes of chronic rejection completely depended on the sum of the intensity and the length of the acute rejection episodes as determined by AUC_{Cr} from 3 to 12 weeks (Fig. 4).
Clinical and experimental evidence indicates that the frequency of acute rejection episodes is the most important single risk factor for chronic renal allograft rejection. We therefore considered it important to separately compare both the rejection frequency and the AUCCr3-12 to CADI and to end-point serum creatinine. These are depicted in Table 2. AUCCr3-12 correlated better than the number of rejection episodes both to CADI \((r = 0.922, r^2 = 0.845, P = 0.0001, vs. r = 0.876, r^2 = 0.767, P = 0.0001)\) and to end-point S\(_{\text{cre}}\) \((r = 0.890, r^2 = 0.793, P = 0.0001, vs. r = 0.811, r^2 = 0.657, P = 0.0001)\). The result suggests that AUCCr3-12 is superior to rejection frequency in the prediction of chronic rejection.

**Discussion**

Chronic rejection is a complex metabolic disorder. It represents an allograft response to injury which is accompanied by physiologic changes in the graft. Chronic rejection is defined (Kiruna and Palo Alto consensus conferences) [1, 26] as a permanent decline in graft function, with other causes excluded and with compatible histology on biopsy.

The histological hallmarks of chronic rejection are interstitial inflammation and fibrosis, glomerular sclerosis, sclerosis of intragraft arteries (allograft arteriosclerosis) and tubular atrophy [27, 28], all of which are nonspecific [27, 29–32]. We and others have previously demonstrated that the chronic histological changes in the allograft can be expressed as a single numerical figure, the Chronic Allograft Damage Index, CADI [6, 33, 34].
The etiology is multifactorial where both immunologically non-specific and specific factors contribute [35]. The major immunologically non-specific etiological factors contributing to chronic allograft injury are infection (particularly CMV), advanced donor age, small graft size, longer cold ischemic times, procurement and reperfusion injury, and factors contributing to atherosclerosis in general. The major immunological factors include histoincompatibility, suboptimal immunosuppression, intensity of the anti-allograft response, etc. Previous retrospective human studies [8—11] and our own experimental data [25] clearly indicate that acute rejection episodes may be the single most important etiological factor in chronic rejection, and may both initiate and/or exacerbate chronic allograft rejection.

Once trauma has been initiated by multiple pathways, chronic rejection may become independent of the initiating event and the metabolic changes progress via the mechanisms described under “response to injury” [36]. When the loss of renal mass reaches a critical point, adaptive responses to the loss of functioning nephron mass, such as hyperfiltration of in the remaining nephrons as originally postulated by Brenner, Meyer and Hostetter in 1982 [37], will result in an accelerated cycle of mesangial injury with progressive glomerular sclerosis following initial damage [38—41].

Considering the various etiologic factors, both immunologically specific and non-specific, it is important to determine the relative significance of each of them and how they are related. As this is virtually impossible to determine in humans, we have resorted to a standardized experimental rat model.

Assuming that the (retrospective) human studies [8—11] are correct, acute rejection episodes are the primary risk factor for chronic rejection in human renal transplant recipients. However, between acute rejection and chronic rejection there might be a third factor or confounding variable. Such a variable may be associated with the acute rejection and/or may independently affect the risk of developing chronic rejection. While the confounding factor(s) must be predictive of the occurrence of chronic rejection, the association need not be causal. Most frequently the known confounding variables are correlates of another causal factor, such as acute rejection. For example, tissue incompatibility, delayed graft function or long cold ischemia time, regrafting, low dose immunosuppression, low CsA bioavailability and high CsA clearance rates are associated with chronic rejection. Thus they should be considered as potential confounders of association. These variables, however, may not be causally related to chronic rejection but rather act as surrogates of other immunological factors, particularly acute rejection. Alternatively, these potential confounding factors may be predictive of chronic rejection independently of their association with acute rejection.

In our animal model all known risk factors for chronic rejection are constant, while we control for our study objects by noting the frequency, intensity and length of acute rejection episodes. Thus, in our experimental setting potential confounding variables which may influence transplant function and/or histology, such as infections (as CMV), donor age, size incompatibility, cold ischemia time, technical problems, extent of atherosclerotic disease, etc., can be excluded.

Regardless of these highly standardized conditions, there was still a considerable variation between individual animals in their renal function during acute rejection episodes, and their response to intensified immunosuppression. Because of the variability in the onset, intensity and course of acute rejection episodes and in their response to treatment, we considered it likely that the impact on histopathological lesions in chronic rejection might be related not only to the frequency but also to the intensity and length of acute rejections. We therefore looked upon a single numerical parameter, the area under the serum creatinine versus time curve (AUCcr), to also cover these variables.

We thought that the duration of each episode (in days), the level of elevation in serum creatinine over normal values (in µmol/liter) and the efficacy of treatment may all be quantitated by AUCcr, and that the AUCcr is a more precise measure of the severity of transplant injury than the rejection frequency. Experimentally this could be tested by using different lengths of prophylactic immunosuppression, thus creating rejections of differing intensities and by postponing the first rejection episode to varying time points after transplantation. By treating rejections only when creatinine levels rose above 200 µmol/liter, we also permitted some on-going acute rejections to continue for several days before treatment was begun. As a quantitative end-point for chronic rejection we used the histological CADI in addition to end-point transplant function (Scr). The perfect positive linear correlation (r = 0.874, P = 0.0001) between CADI and serum creatinine measurement (made on the same rats) at three months increased the reliability of the CADI to quantitate the intensity of chronic histologic changes relevant to chronic rejection.

There was a perfect positive linear relationship (r = 0.922) between the AUCcr—3—12 and CADI. The power of statistical tests (P = 0.0001) was too strong to detect an association of the specified magnitude. As a simultaneously strong (r = 0.890) and statistically significant (P = 0.0001) positive correlation exists between AUCcr—3—12 and end-point transplant function, a possible association is suggested between the severity of acute rejection and chronic rejection. Since the relationship between variables for chronic rejection was a positive linear relationship, we did not need to use alternate of transformation such as logarithmic, reciprocal or square root.

We are aware that the statistically significant correlation between AUCcr—3—12 and CADI does not yet prove that a cause-and-effect relationship exists between these two parameters. Our second objective was to judge whether the association between these two parameters is, in fact, causal. In addition to the demonstrated association between AUCcr (risk factor; represents the severity of rejection) and CADI (disease; represents the chronic rejection) has been identified, several questions must be answered to strengthen the argument of causality [42].

First, our study is experimental and less subject to bias than observational (retrospective) study designs (for example, increases in creatinine level caused by reasons other than acute rejection episodes, such as CsA toxicity, can be excluded) and favors a causal relationship.

Second, if the causal association is valid, a change in the frequency and/or intensity of acute rejections should result in a corresponding change in the intensity of chronic changes as described by the CADI. In other words there is a dose-response relationship between the variables. This was indeed the case: the respective regression relationship was: CADI = 0.45 + [0.001 X (AUCcr—3—12)] (r = 0.922, P = 0.0001).

Third, our strong and statistically significant (P = 0.0001) association is likely to represent a cause-and-effect relationship.
Fourth, evidence for causality is strengthened by the fact that several clinical investigators using different study designs, which were mostly case-control and employed genetically outbred populations of subjects and different immunosuppressive regimens, reached the same conclusion [8–11]. The intensity and duration of acute rejection episodes have been shown to be associated with chronic graft loss. In a study by Gold et al. [8], patients with high acute rejection scores had a reduced graft survival, with a resultant decrease in chronic rejection.

Taken together, our results suggest that AUC_c, correlates, even better than the frequency of acute rejection episodes, to chronic allograft rejection. We believe that the improved correlation is related to the fact that AUC_c also takes into account the intensity and length of acute rejection episodes. We suggest that if the intensity and length of acute rejection episodes is reduced to a minimum by aggressive treatment, the long-term graft survival will be improved. However, this new finding must still be clinically validated.

**References**


