Invited Comment

Chronic allograft failure: a disease we don’t understand and can’t cure?

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During recent decades many new and successful strategies have been developed to improve 1-year renal allograft survival rates. It has become evident, however, that graft loss after the first year has not been reduced by the same magnitude. A high percentage of transplanted kidneys develop dysfunction in the first few months post-transplantation and ultimately fail, despite the use of modern immunosuppressive regimens. This type of graft failure has been termed chronic rejection. However, as both immune and non-immune mechanisms seem to contribute to its pathogenesis, the term currently used is chronic allograft nephropathy (CAN). The concept of chronic rejection slowly emerged in the 1950s and 1960s. Acute rejection was well known in the late 1950s, but even then only few kidneys survived for months. Initially Hume et al. [1], and later Porter et al. [2] and Jeannet et al. [3], reported arterial intimal fibrosis and obliteration in renal allografts in the late postoperative period, a process thought to be alloantibody mediated. This lesion with vascular predominance, already present in the very early stages, is now rare, even though the Hume–Porter–Jeannet syndrome remains the basis of the current animal models of chronic rejection, thus limiting their relevance for the human CAN problem [4]. It therefore remains questionable whether some findings obtained in animal models, such as the reversibility of chronic rejection as described by Tullius et al. [5] in the F344-Lewis model, can be reproduced in the human setting.

It is the major aim of this article to categorize (in the form of specific entities) the variety of factors that have been associated with human CAN. Finally, we will attempt to summarize the ideas of several authors on a potential common pathway that may link the diverse range of insults leading to functional and structural damage of kidney allografts.

Recipient factors

Recipient age

Meier-Kriesche et al. [6] recently analysed data collected by the U.S. Renal Transplant Scientific Registry and the U.S. Renal Data System from >59,000 subjects to determine whether recipient age is an independent risk factor for CAN (defined as graft loss after 6 months post-transplantation per 100 patient years). Not surprisingly, death with functioning graft was much more frequent in older subjects (4.1% at age 65+ years vs 1.1% in 18- to 49-year-old recipients). However, even when the outcome was censored for patient death, older individuals had a higher risk for graft failure (8.6% vs 3.9%), partly due to a higher incidence of CAN (3.9% vs 2.2%). When compared with the young reference population in a Cox proportional hazard model, the relative risk for developing CAN was 1.67 in subjects older than 65 years (with the exception of African-American patients), an risk increase surpassed only by the detrimental effects of an acute rejection within the first 6 months (relative risk for succeeding CAN, 2.3). The worst outcome is even more striking as the odds ratio (OR) for an acute rejection episode (0.66) is actually significantly lower in older recipients. The authors admitted that the pathogenesis of the increased allograft failure in older transplant recipients was not readily available from their database. They speculated that a more ‘atherogenic’ environment (more marked hypercholesterolemia or hyperhomocysteinemia, altered IGF, apo E and apo I levels, increased prevalence of hypertension or increased concentration of TGF-β) or a more chronic than acute immune injury (upregulation of HLA DR and increased IL-6 production with age) might explain their findings.

Recipient blood pressure at time of transplantation

Frei et al. [7] retrospectively analysed data from 639 kidney allograft recipients transplanted at the Medical
School of Hannover. Within a median follow-up period of 4.8 years, 106 subjects developed CAN, which was defined as a glomerular filtration rate (GFR) loss of >10 ml/min per year, regardless of the initial serum creatinine value. Patients with CAN were more likely to have an HLA-B and/or -DR mismatched graft (OR 1.86) and a minimum serum creatinine >1.7 mg/dl. Furthermore, a first acute rejection episode later than 60 days post-transplantation (but not earlier, OR 5.53) and the need to use more than two antihypertensive drugs 12 months after transplantation (OR 3.1) were more probable in the CAN group. Interestingly, hypertension at the time of transplantation was also associated with CAN (OR 3.42, if more than two antihypertensive drugs were needed to control blood pressure at the time of transplantation). From this intriguing observation Schindler et al. [8] later speculated that hypertension might initiate inflammatory pathways and thus act synergistically with alloantigen-dependent factors, leading to graft injury. In line with these observations are the results of a study by Fujihara et al., who described renal interstitial lymphocyte infiltration in Munich Wistar rats between 7 and 30 days after 5/6 nephrectomy [9]. While the infiltration of neutrophils and macrophages is part of a non-specific inflammatory reaction, the presence of lymphocytes and dendritic cells within lesions raises questions about their role in the maintenance and amplification of inflammatory response and whether or not their recruitment and activation are mediated by antigen-specific immune response. As T cells are effectors of the cellular immune response, their presence in association with evidence of cytokine production raises the possibility that they recognize and react to endogenous antigens. It is possible, as suggested by Nelson, that progressive damage causes loss of tolerance to parenchymal self and that, under the influence of cytokines, tubular epithelium expresses class II major histocompatibility complex (MHC) antigens, which may augment cell-mediated injury [10]. Renal infiltration with macrophages and lymphocytes is a well-known feature of not only immune, but also non-immune kidney disease such as acute and chronic ischaemia, protein overload hypercholesterolemia, obstructive uropathy, polycystic kidney disease, diabetes, aging and murine hypertension [11]. The same phenomenon has also been described in renal allografts with stable function, the pathological significance of which is unclear [12]. Interestingly, when Fujihara treated 5/6 nephrectomized Munich Wistar rats with mycophenolate mofetil (MMF), these cellular events were attenuated at all phases of the study, and the drug provided marked protection against the development of glomerulosclerosis and interstitial injury (although without affecting proteinuria). In line with these observations, Ojo et al. [13] reported that in human renal transplant recipients without acute rejection, MMF therapy decreased the risk of CAN by 20% compared with azathioprine. In contrast, however, Glicklich reported in a retrospective case control study [14] that substitution of azathioprine with MMF in patients with already ongoing CAN does not alter the clinical course of the disease. Therefore, it could be hypothesized that only early interventions are able to block the detrimental interaction between hypertension and alloantigen-dependent factors and thus protect against graft loss due to CAN.

Recipient renal function status

Using data from the U.S. Renal Data System, Mange et al. [15] recently identified >8400 subjects who had received a first kidney transplant from a living donor and had a known date of first treatment for end-stage renal disease. Approximately 1800 patients underwent transplantation without prior exposure to dialysis, and they experienced 52% and 86% reductions in the risk of allograft failure during the first year and during subsequent years, respectively. While a lower number of acute rejection episodes explained most of the difference early on, the beneficial effect of preemptive transplantation was subsequently found to be independent of biopsy-proven rejection. The authors speculated that alteration of the immune system in the more uraemic, non-dialysed subjects might protect the transplant [16,17], and a similar beneficial effect of uraemia has been described after ischaemia reperfusion injury by Vercauteren et al. [18] in 5/6 nephrectomized rats.

Recipient sex, race, body mass index and native kidney disease

In 1996, Gjertson analysed data from >80,000 renal transplant recipients from the UNOS registry with regard to multiple recipient input factors [19]. When 5-year transplant survival rates were calculated, only data from subjects with functioning grafts after 1 year post-transplantation were used. Females tended to do better than males (transplant survival rate 79.5% vs 72%, respectively; this trend being undetectable at 1 year after surgery), resulting in transplant half-lives of 8.8 and 12.0 years for male and female recipients, respectively. Black and Caucasian subjects had higher failure rates than Asian or Hispanic individuals (23% and 36% compared with 22% and 19%), a finding less relevant for the European situation. Recipients with a body mass index (BMI) >30 kg/m² had slightly lower 1- and 5-year graft survival rates (82.8% at 1 year vs 83.6–86% for BMIs of 5–15, 16–20 and 21–30 kg/m², and 74% vs 77.8% at 5 years, when recipients with an index <15 kg/m² also had an increased risk of 18.3% for graft loss). Patients with cystic inherited diseases as well as tubulointerstitial diseases did better than subjects with glomerular or systemic illness (80.3% and 78.5% vs 76.4% and 76.3% at 1 and 5 years, respectively). Even though (at least at the present time) a causal link between CAN and many of these factors cannot be defined, it is clear from this analysis that long-term graft damage is multifactorial.
**Number of previous transplants, pre-transplant pregnancies, pre-transplant transfusions and peak panel reactive antibodies**

Although the paper by Gjertson reported that at 1 year there was a steady trend of increasing allograft failure rates as the number of previous transplants increased, the long-term survival rate was only slightly lower in patients who had undergone more than three transplants (70.1% vs 77–80%). The same phenomenon was observed for peak panel reactivity (PRA). For 5-year graft survival only a PRA of >80% was detrimental (74.4% vs 76.8–77.5%). Both pre-transplant pregnancies and pre-transplant transfusions had no long-term effect.

In his summary analysis, Gjertson determined that of all recipient-related factors analysed, race explained 22% of the variation in outcome after 5 years, gender 6.2%, age 2.2%, native kidney disease 1.3%, PRA 0.6%, transfusions and pregnancies 0.4% and graft number 0.2%, while BMI was no longer of significance.

**Donor factors**

**Donor age and donor type**

Donor age is currently the single most important factor determining long-term graft outcome, accounting for almost 30% of the total variability observed [19]. This is especially true for cadaver donation. Terasaki et al. [20], who used data from the UNOS registry, found 3-year graft survival rates for spousal or other living unrelated donors to be consistently higher than those for cadaver donors, regardless of the age group analysed. (The oldest group analysed being the one >50 years, it is still possible that the situation is different among very old donors.) However, the difference in outcome between older and younger donors was much more prominent in cadaver grafts, signalling an interaction between donor source and age [20]. The aging kidney becomes functionally impaired, particularly in men, most likely due to age [20]. The aging kidney becomes functionally impaired, particularly in men, most likely due to age [20]. The aging kidney becomes functionally impaired, particularly in men, most likely due to age [20]. The aging kidney becomes functionally impaired, particularly in men, most likely due to age [20]. The aging kidney becomes functionally impaired, particularly in men, most likely due to age [20]. The aging kidney becomes functionally impaired, particularly in men, most likely due to age [20]. The aging kidney becomes functionally impaired, particularly in men, most likely due to age [20]. The aging kidney becomes functionally impaired, particularly in men, most likely due to age [20]. The aging kidney becomes functionally impaired, particularly in men, most likely due to age [20]. The aging kidney becomes functionally impaired, particularly in men, most likely due to age [20]. The aging kidney becomes functionally impaired, particularly in men, most likely due to age [20]. The aging kidney becomes functionally impaired, particularly in men, most likely due to age [20]. The aging kidney becomes functionally impaired, particularly in men, most likely due to age [20]. The aging kidney becomes functionally impaired, particularly in men, most likely due to age [20]. The aging kidney becomes functionally impaired, particularly in men, most likely due to age [20]. The aging kidney becomes functionally impaired, particularly in men, most likely due to age [20].

**Type of donor death and medical history of the donor**

The finding of superior success rates for living kidney transplantation when compared with cadaver donation is consistent throughout the literature, and many factors have been cited as an explanation. One obvious difference is certainly the fact that brain death is a conditio sine qua non for the latter procedure to be performed. As has recently been shown, however, brain death is not purely a local problem, but affects the whole organism. Takada et al. [28] increased the intracranial pressure of rats by inflating a Fogarty arterial embolectomy catheter in the subdural space, causing herniation of the brain stem within 20 min. Following this procedure, mRNA expression of T-cell- and macrophage-associated products (IL-1, IL-6, TNFα, IL-2, IFNγ and IL-4) increased in all tissues investigated, including the kidney. This was accompanied by an upregulation of MHC class I and II antigens and the co-stimulatory molecule B7, suggesting an increased immunogenicity of the peripheral organs. These changes were not observed in the organs of control animals or rats dying of severe acute ischemic injury or haemorrhagic shock. When animals were treated, simultaneously to brain death induction, with a soluble form of P-selectin glycoprotein ligand (which inhibits both P and E selectin activity) or CTLA4Ig (which interferes with the B7-CD28 co-stimulatory pathway of T-cell activation), these changes were almost completely suppressed. Using immunohistochemistry we were able to show that tubular epithelial cells and peritubular endothelial cells of cadaver kidneys obtained in biopsies performed at...
the time of explantation express ICAM and VCAM 1 at significantly higher levels than when obtained in biopsies performed in organs from living donors [29]. Hypotension of the donor during the stay in the intensive care unit has also been claimed to negatively affect transplant outcome, at least in part by causing an increase in the incidence of delayed graft function. The need for catecholamine administration was long thought to be a bad prognostic marker. Recently, Schnuelle et al. [30] studied 152 consecutive cadaver renal transplantations performed in Heidelberg and Mannheim. Indeed, the use of noradrenaline or dobutamine was associated with an increased rate of delayed graft function. However, graft survival after 8 years was significantly improved in recipients of a graft, whose donors had received catecholamines, mostly via a reduced incidence of acute rejection episodes (OR 0.22 for dopamine and 0.24 for noradrenaline). The authors argued that a downregulatory effect of adrenergic substances on the expression of adhesion molecules in the vessel walls of the graft takes place, which might explain their findings. Whether a donor history of hypertension affects outcome after renal transplantation remains to be determined. Smith et al. [31] reported a higher kidney discard rate and increased incidence of acute tubular necrosis in kidneys from donors with a history of hypertension. One-year graft function, however, was similar to that achieved in recipients of kidneys from normotensive donors. Guidi et al. [32] came to similar conclusions as far as graft function after 2 years is concerned. However, mean arterial blood pressure was higher in recipients of kidneys from hypertensive donors.

**Combined recipient/donor factors**

**HLA matching**

Several large registries have reported a significant effect of HLA-matching on the risk of allograft failure. Held et al. [33] showed that HLA-identical transplants had the highest success rate and that even a single mismatch increased the relative risk of failure at 5 years by ~20%. Further incompatibilities in the HLA system had less effect, increasing the risk by only ~6% per mismatch. Five-year graft survival in HLA-identical transplantations was 65.2% and dropped to 47.7% in completely mismatched grafts; ~50% of this difference could already be observed at 1 year post-transplantation. In the Gjertson [19] analysis, HLA matching also affected 1-year graft survival rates, but thereafter a beneficial effect was only observed with complete HLA-A and -B compatibility, whereas HLA-DR-matching no longer mattered.

**Organ preservation time**

Besides studying the effect of HLA matching, Held et al. [33] were also interested in whether prolonged organ preservation time has an effect on graft outcome. They determined that the relative risk of graft failure increased by 8% per 12 h cold ischaemia time, which translated into a 2.4% decrease in absolute 5-year graft survival. This continuous effect was not observed by Gjertson [19], who found a threshold level of 24 h for cold ischaemia time, beyond which allograft survival was compromised.

**Delayed graft function**

Prolonged cold ischaemia time might influence graft survival via an increased incidence of acute renal failure in the postoperative period. Indeed, Teresaki et al. [20] showed that regardless of HLA matching, cadaver grafts with diuresis on day 1 had a better 3-year graft survival than did those with postoperative oligo-anuria. In fact, completely HLA-compatible grafts without diuresis had a worse 3-year graft survival rate than full-house mismatched grafts with initial diuresis. The exact mechanism of how acute tubular necrosis affects long-term outcome is still the subject of discussion. Troppmann et al. [34], for example, found that 5-year graft (and patient) survival was affected by delayed graft function only when the postoperative course was also complicated by rejection. Interestingly, however, patients with delayed function in their sample were much more prone to rejection than subjects with immediate postoperative diuresis. In a mouse model of renal ischaemia, Shoskes et al. [35] reported enhanced early MHC class I antigen expression in interstitial and tubular cells after 3 days, and these phenomena were associated with an accumulation of inflammatory cells and cytokine release. A bi-directional interaction between the immune system and delayed graft function was also suggested by Boom et al. [36], who found a significantly increased relative risk of delayed graft function in recipients with a peak panel reactivity of >50%.

**Acute rejection**

Acute allograft rejection has always had a major effect on long-term outcome. Recently it has become clear, however, that a more subtle analysis of this relationship might be needed. In 1994, Matas et al. [37] analysed 653 patients after primary kidney transplantation with at least 1-year allograft function, the majority being living donor transplant recipients. Transplant half-life was 46 years if no rejection occurred during the first year. This decreased to 25 and 5 years if one or more rejection had to be treated and to 3 years if rejection episodes occurred after the first year.

In light of these figures, the question arises of why new immunosuppressive drugs, all of which in studies are very effective in reducing acute rejection episodes, do not significantly prolong transplant half-life. In an analysis of the USRDS database, Meier-Kriesche et al. [38] tried to evaluate whether the time of...
transplantation and rejection influences the development of chronic allograft failure. If the relative risk for chronic rejection in patients transplanted in 1988/1989 without acute rejection was taken as 1, an acute rejection at that time increased the risk of chronic failure by 67%. For a patient transplanted in 1996/1997, the corresponding numbers were 1.5 and 5.2. Thus, even if the total number of acute rejection episodes had declined, the overall impact of each episode was dramatically increased. This observation could also explain why aggressive induction therapy may well reduce acute rejection but does not prevent chronic failure [39].

It is not only the time when rejection occurs that has an impact on long-term outcome. An exact histological differential diagnosis also permits conclusions to be drawn. Interstitial infiltration yields a much better prognosis than vascular rejection, which reduces 9-year graft survival by >50% [40]. Early recognition and treatment are also necessary. In an animal model, Tullius et al. [41] transplanted kidneys of Brown (Norway) rats into Lewis rats. When the kidneys were retransplanted into their native environment within 3 days, animal survival was excellent. However, animals retransplanted after 5 days all died of uraemia [41]. Additional treatment of subclinical rejection might also improve long-term outcome. Rush et al. [42] performed protocol biopsies in one group of allograft recipients, whereas other patients underwent this procedure only when clinically indicated. Not surprisingly, the steroid dose to be used was much higher in the protocol biopsy group. However, serum creatinine 24 months after transplantation was significantly lower [42].

Type of maintenance immunosuppression
A complete review of the strategies proposed to prevent chronic failure is beyond the scope of this article. When Opelz analysed his database, he found that patients treated with cyclosporine A and azathioprine immunosuppression had the best long-term graft survival rate, followed by subjects on cyclosporine monotherapy. However, retrospective data analysis like this can always be criticized, because patient selection bias is a critical problem [43]. Even though cyclosporine has been in use now for several years, many questions concerning its optimal use are still far from being answered. Only recently, Kahan et al. [44] reported that the incidence of chronic rejection was 24% after 5 years for renal transplant recipients with a relatively stable pharmacokinetic cyclosporine A profile, but 40% in unstable patients. Hence, cyclosporine A drug treatment based on 2 h post-intake blood sample measurements might lessen toxicity and optimize immunosuppressive potency [45]. There is also evidence that cyclosporine A absorption profiling in the immediate post-transplantation period reduces the incidence and severity of acute rejection [46].

Miscellaneous factors

Blood pressure post-transplantation
High systolic blood pressure is among the most important variables associated with long-term graft outcome [47]. This effect is clearly present even in subjects without an acute rejection episode in their first year post-transplantation. Therefore, blood pressure per se appears to affect allograft outcome, even though renovascular hypertension from other damage still cannot be ruled out. Interestingly, however, studies on the effect of blood pressure-lowering therapy on graft outcome in renal transplant recipients are still rare. Rahn et al. [48] treated transplant recipients with a calcium antagonist and were able to show after 2 years of follow-up that renal function was better preserved in the treated group. Nevertheless, the difference was quite small, and thus further studies on this topic are urgently needed, as are investigations into the treatment of hyperlipidemia. Guijarro et al. [49] reported that increased serum triglyceride levels appear to be consistently associated with chronic allograft failure. However, as renal dysfunction and proteinuria contribute to lipoprotein abnormalities, it is still unclear whether hypertriglyceridemia is not merely a marker for chronic damage.

The search for a unifying hypothesis
As can be appreciated from the above discussion, a broad variety of factors have been associated with the clinical pathway of chronic renal allograft failure. Hence the search for a unifying hypothesis, which may result in such diverse factors as donor age or acute rejection damaging a graft, is still ongoing. In addition to its academic challenge, such a hypothesis could pave the way for a greatly desired ‘magic bullet’ treatment option.

Halloran and co-workers [4] summarized the available clinical and experimental evidence to determine several basic theories defining a common pathogenetic pathway. In the chronic cytokine excess concept, a prolonged inflammatory and fibrogenic situation in the graft leads to progressive vascular damage and scarring. As one possible causal treatment option, Ziai et al. [50] recently used the angiotensin II receptor antagonist losartan to demonstrate the downregulation of a whole array of cytokines such as RANTES, IL-1, -2 and -6, MCP-1, IFN-γ, iNOS or TGF-β by this agent. Another concept elaborated by Halloran [51] is based on the fact that many forms of tissue injury result in irreversible disruption of the three-dimensional extracellular matrix structure. As a consequence, tubular epithelial cells lose their contact with the basement membrane and undergo either apoptosis or transformation to fibroblasts. Another fascinating theory draws from an observation made by Hayflick et al. in 1961 [52], who reported that cells in tissue culture ultimately die after a finite number of cell
chronic allograft failure