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Combating Chronic Renal Allograft Dysfunction

Optimal Immunosuppressive Regimens

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Abstract

Kidney transplantation is the best treatment for patients with end-stage renal disease, both in terms of survival benefit and quality of life. The major limitation is the continuing shortage of kidneys suitable for transplantation, reinforcing the need to maximise graft survival. After the first year of transplantation, chronic renal allograft dysfunction (CRAD) is the first cause of late graft deterioration and failure. CRAD has been defined as a progressive renal dysfunction, independent of acute rejection, drug toxicity and recurrent or *de novo* nephropathy, with features on biopsy of chronic allograft nephropathy (CAN) characterised by vascular intimal hyperplasia, tubular atrophy, interstitial fibrosis and chronic transplant glomerulopathy. Protocol biopsy-based studies have demonstrated a high and early prevalence of CAN lesions during the first year in patients with

normal and stable renal function. Beyond 1 year, the injuries associated with calcineurin inhibitors (CNIs) appear to be very common. The physiopathology of CRAD is complex and multifactorial. Both alloantigen-dependent factors (acute rejection, HLA matching, donor-specific antibodies, inadequate immunosuppression) and alloantigen-independent factors (donor age, brain death, ischaemia/reperfusion injuries, hypertension, hyperlipidaemia, cytomegalovirus, CNI-related nephrotoxicity) are involved. Consequently, CRAD appears as a dynamic process, evolving with time, and immunosuppressive regimens need to be modulated in order to provide the most suitable treatment at the different phases of its natural history. On the basis of this scheme, the new paradigm would be the use of a CNI-based regimen during the period of maximal risk of (subclinical) acute rejection, followed by a conversion to a CNI-free regimen in order to avoid the long-term consequences of nephrotoxicity. Fortunately, new agents are being introduced in clinical practice providing a large range of combinations and allowing individualisation of immunosuppressive regimens. Large, prospective, multicentre trials are warranted, and the challenge is to define new endpoints of CRAD and to determine the best therapeutic strategy.

Renal transplantation is the gold treatment for most patients with end-stage renal disease, with a significant benefit both in terms of quality of life[1] and likelihood of survival.[2] Unfortunately, the number of cadaveric kidneys available does not match the increasing number of patients awaiting transplantation, underscoring the need to maximise graft survival.[3] With the introduction of ciclosporin (cyclosporine) and muromonab-CD3 (OKT 3) in the early 1980s, and consequently the reduction of the rate of acute rejection, the 1-year graft survival markedly improved. A recent report of the United Network for Organ Sharing (UNOS) Renal Transplant Registry indicated that between 1998 and 2001 the overall 1-year graft survival rates for 31 270 cadaveric renal transplants and for 14 162 living donor transplants were 89% and 95%, respectively.[4]

Nonetheless, until recently this improvement of the short-term graft survival did not seem to have a significant impact on the long-term graft survival. For instance, until the early 1990s, the half-life of cadaveric renal allografts remained inexorably constant at 7.5–9.5 years.[5] Fortunately, the picture has been changing, indicating a steady improvement of long-term graft survival since 1988.[4,6,7] For instance, the analysis of the 93 934 renal transplantations performed in the US during the period from 1988 to 1996 indicated that the half-life for grafts from living donors increased from 12.7 to 21.6 years and that for cadaveric grafts the half-life increased from 7.9 to 13.8 years (figure 1).[8] Furthermore, the slope of decline of glomerular filtration rate (GFR) beyond 6 months (slope GFR) has reversed in recent years; the mean slope since 1997 is actually positive with 3.5 mL/min/year, whereas it was −2.5 mL/min/year, in previous years.[9,10] However, this optimistic

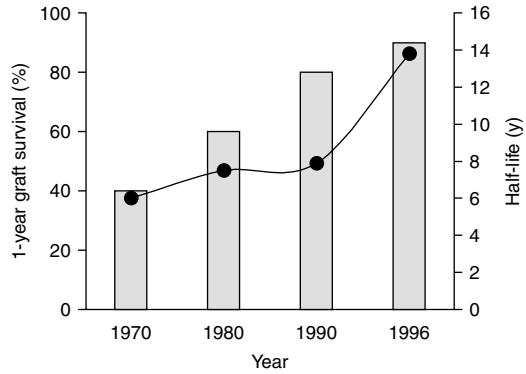

Fig. 1. The different ages of renal transplants. These data concern cadaveric renal transplants before censoring patients who die with a functioning graft. The data are adapted from the results of the United Network of Organ Sharing registry.[8] The 1-year graft survival is displayed on the bar graph and the graft half-life is displayed on the line graph.

view in renal transplant results has been recently moderated with a study analysing the data provided by the Scientific Registry of Transplant Recipients regarding more than 60 000 first renal transplants between 1995 and 2000. It showed no significant improvement in allograft survival in the recent years despite a marked increase in acute rejection rates.[11]

Causes of graft failure during the first year include acute rejection, surgical complications (arterial and/or venous thrombosis) and patient death. After the first year, causes of graft failure include chronic renal allograft dysfunction (CRAD), formerly called chronic rejection, patient death, late acute rejection, recurrent or *de novo* renal disease, and polyomavirus nephritis.[12] However, in death-censored graft survival studies, CRAD is the first cause of late graft deterioration and failure.[13] It has been estimated that 50% of the recipients who develop CRAD will lose graft function within 5 years of transplantation and that 50–80% of the recipients who return to dialysis beyond 2 years do so because of progressive CRAD.[14-16] These efforts of transplant units are now focused on determining optimal strategies to combat late renal graft failure through its more accessible component, which is the prevention and/or treatment of CRAD.

1. Definitions of Chronic Renal Allograft Dysfunction (CRAD) and Chronic Allograft Nephropathy

CRAD is a nonspecific term describing a clinical syndrome which is the functional consequence of chronic allograft nephropathy (CAN). CAN is characterised by four main histopathological features: vascular intimal hyperplasia, tubular atrophy, interstitial fibrosis and chronic transplant glomerulopathy.[17,18]

CRAD has been defined as a progressive renal dysfunction occurring months to years after transplantation which is independent of acute rejection, drug toxicity and specific disease entities, with typical features on biopsy.[19]

Clinically, CRAD manifests as a gradually progressive decline in GFR, usually in combination with proteinuria and *de novo* or accelerated hypertension.[20] Methods have been validated to correlate these chronic alterations of renal function and late chronic allograft failure. For instance, it has been shown that the percentage of change in inverse creatinine (Δ1/Cr), and particularly a Δ1/Cr less than −30%, was a good predictor of graft failure.[21,22]

CAN refers to the histological changes of CRAD. As depicted in figure 2d, the vascular injuries are characterised by a thickening of the walls with a subintimal accumulation of connective tissue, an infiltration of mononuclear cells and a proliferation of myofibroblasts, leading to the narrowing of the vascular lumen.

In the interstitium, varying degrees of tubular atrophy and patchy fibrosis can be observed. This excessive fibrosis caused by accumulation of extracellular matrix proteins is a major pathological process and the degree of fibrosis probably offers the best correlation with renal outcome. However, these findings are nonspecific and are also found in calcineurin inhibitor (CNI)-related nephrotoxicity.

Glomerular lesions are less frequent but are considered the most specific finding for CAN.[18] Their pattern resembles that seen in membranoproliferative glomerulonephritis with a lobular aspect of the flocculus, a thickening of the capillary wall leading to a double-contour appearance (see figure 2c). Immunofluorescent examination shows a nonspecific pattern of immunoglobulin deposition but can be used to eliminate a recurrent nephropathy. Ultrastructural studies can eventually demonstrate multilayering of the peritubular capillary basement membranes.[24,25]

Protocol biopsy-based studies have convincingly established that these chronic histological lesions can appear in renal allograft recipients with normal and stable renal function. It has then been demonstrated that approximately 40% of renal allografts will develop CAN within the 2 years following transplant,[26] and a more recent study showed that CAN was present in 25% of patients as soon as 3 months after grafting.[27]

Recently, the natural history of CAN was evaluated in a prospective study based on 961 renal-transplant biopsies performed on 120 patients from

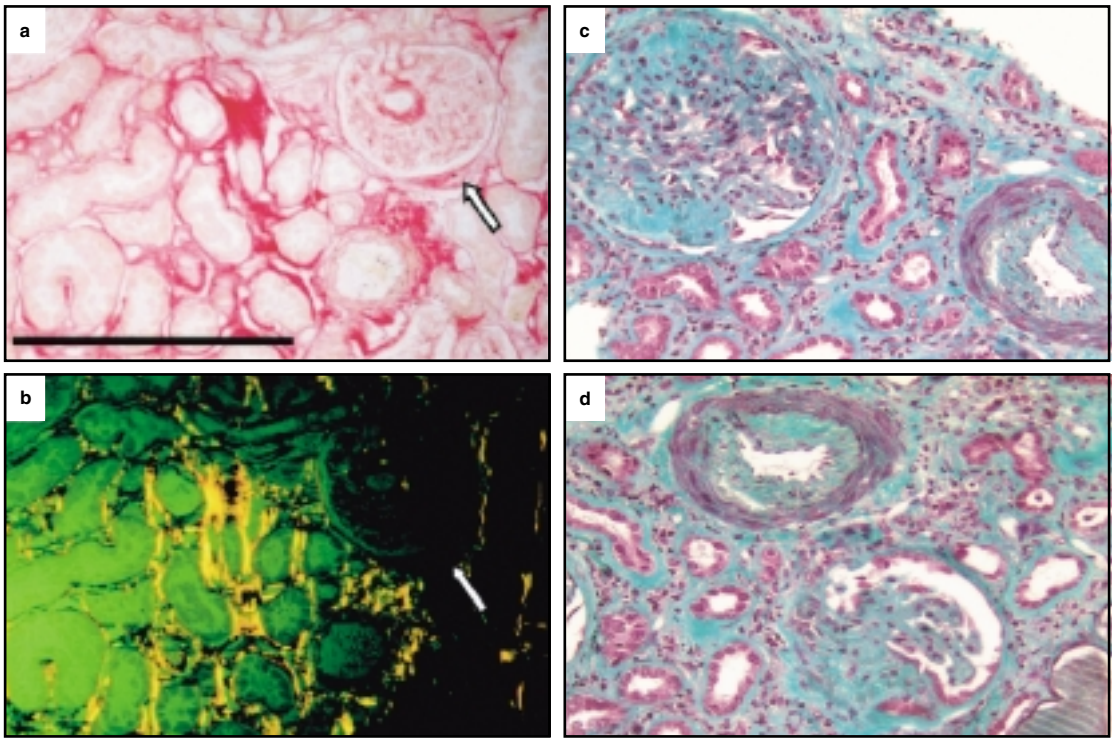

Fig. 2. Different features of chronic renal allograft dysfunction. Photomicrographs of Sirius Red-stained renal allograft biopsy observed under (**a**) white light and (**b**) polarised light with a birefringent tubulointerstitium. Some areas (indicated by arrows) are not birefringent because of the non-collagen types I or III of the matrix (reproduced from Grimm et al.,[23] with permission). (**c**) and (**d**) depict typical features of chronic allograft glomerulopathy and fibrointimal vascular thickening (Mallory trichrome stain). Notice also some degree of tubular atrophy (obtained by courtesy of Dr C. Deminière, Bordeaux University Hospital, Bordeaux, France).

the time of grafting to 10 years thereafter.[28] As depicted in figure 3, this first longitudinal description of CAN indicates two distinctive phases of injury. The first year is characterised by an exponential increase of Banff scores for interstitial fibrosis and tubular atrophy with Banff grade I and Banff grade II in 94.2% and 24.7% of patients, respectively, at the end of the year. Beyond 1 year, the injuries are characterised by progressive arteriolar hyalinosis, glomerulosclerosis and further tubulointerstitial damage associated with CNI-related nephrotoxicity, which is highly prevalent at 10 years. These interesting findings underline the importance of protocol biopsy as an indispensable tool to analyse prophylactic intervention in CRAD.

With the objective of testing new drugs as potential CRAD therapies, an important question now is: how to quantify CRAD?

The severity of CAN can be assessed by various scoring systems. To allow optimal inter-observer and intra-observer reproducibility, standardisation criteria were developed with the view of establishing objective endpoints for clinical trials. For instance, the Banff working classification grades the lesions of CAN according to the proportion of cortical area affected by the fibrosis and the tubular lesions.[18] Of note, in this classification, vascular changes and glomerular lesions are taken into account but not integrated within the grading system.

Other semi-quantitative scoring systems have been described, such as the Chronic Allograft Damage Index which scores the severity from 0 to 3 for interstitial inflammation, mesangial matrix expansion, interstitial fibrosis, glomerular sclerosis, tubular atrophy and vascular intimal proliferation.[26]

The major limitations of these scores are the inter-observer reproducibility[29] and their semi-quantitative approach, which precludes the use of some useful statistical analyses. Alternatively, promising results have been obtained using a precise measure of interstitial fibrosis by computerised analysis of Sirius Red stained allograft biopsies (see figure 2a and b), showing a strong correlation between this surrogate marker and the long-term allograft outcome.[23,30] Interestingly, it has also been shown that there was a correlation between the percentage of tubulointerstitial fibrosis assessed using quantitative image analysis and the GFR in CAN.[31]

2. Physiopathology of CRAD

Alloantigen-dependent and alloantigen-independent factors are involved in the development of CRAD,[32,33] both merging in a common pathway of progressive epithelial atrophy, endothelial deterioration and extensive fibrosis, and resulting in the replacement of normal tissues by fibrotic tissue and loss of function. A better understanding of these risk factors is of crucial importance since they represent potential targets to combat CRAD.

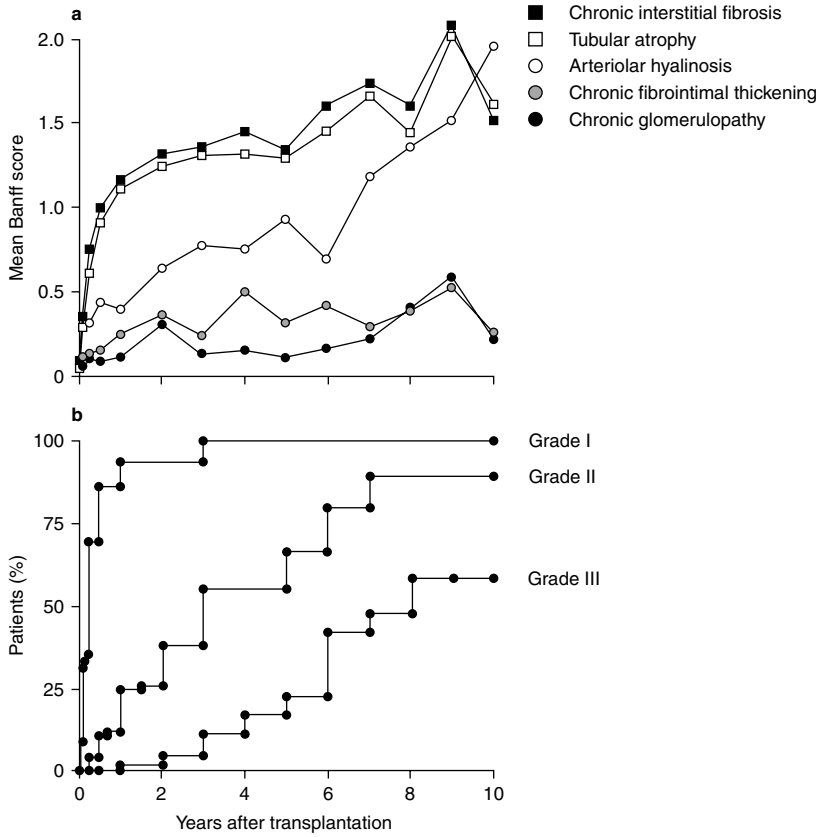

Fig. 3. Mean Banff scores for chronic interstitial fibrosis, tubular atrophy, arteriolar hyalinosis, chronic fibrointimal thickening and chronic glomerulopathy (**a**) and prevalence of mild (Grade I), moderate (Grade II) and severe (Grade III) chronic allograft nephropathy, according to the Banff criteria (**b**) [reproduced from Nankivell et al.,[28] with permission. Copyright © 2003 Massachusetts Medical Society].

2.1 Alloantigen-Dependent Factors:

Alloantigen-dependent factors have a major impact on graft survival and include:

- acute rejection episodes
- HLA matching
- donor-specific antibodies and
- inadequate immunosuppression/noncompliance.

2.1.1 Acute Rejection Episodes

T-cell recognition of the alloantigen is the central immunological event leading to the process of rejection. Two distinct but not mutually exclusive pathways of allorecognition have been described, both of them requiring appropriate costimulatory signals. In the direct pathway, recipient T cells are able to recognise intact major histocompatibility complex (MHC) molecules on the surface of antigen-presenting cells of donor origin. In contrast, in the indirect pathway recipient T cells are able to recognise processed MHC peptides of donor origin presented by self antigen-presenting cells. It has been suggested that the direct pathway may mediate early acute cellular rejection. Its role tends to become less important when passenger leukocytes have disappeared within a few months after transplantation. On the other hand, in the indirect pathway the continuous supply of donor allopeptides may chronically stimulate self-MHC-restricted T cells, leading to long-term alloimmunological injuries such as the production of alloantibodies.[34,35]

Numerous studies have demonstrated that patients with a history of multiple rejection episodes are more likely to develop CRAD than those without such a history.[14,16,36,37] The risk of graft loss during the first year is closely associated with the number of acute rejection episodes. For instance, it has been reported that this risk was 9% with a first rejection, 38% with a second rejection and 50% with a third rejection.[38] However, prompt and efficient treatment of the first episode of acute rejection, combined with a complete functional recovery, does not have any deleterious effect on long-term graft survival.[39]

More importantly, the spreading use of sequential protocol biopsies underlines the impact of subclinical acute rejection on CRAD progression.[27,40] A recent study reported that the prevalence of subclinical acute rejection was high during the first year, concerning 41.8% of biopsy specimens during the first 3 months, 36.8% from 6 to 12 months then falling to 12–20% thereafter.[28] The detection of early subclinical rejection could be an important issue since it has been suggested that the treatment of such episodes may lead to better histological and functional outcomes.[41]

2.1.2 HLA Matching

Data from the best transplant registries indicate long-term graft survival is correlated with the degree of histocompatibility matching between the donor and the recipient.[4,42-44]

Schematically in cadaveric transplants, it appears that the graft half-life is 12–20 years for patients transplanted with no HLA-A, -B or -DR mismatch, 10–12 years for patients transplanted with one HLA-A, -B or -DR mismatch, and 7–10 years for patients transplanted with greater degrees of mismatching.

However, the precise effect of HLA matching on CRAD has yet to be clarified, especially in its relationship with acute rejection.

2.1.3 Donor-Specific Antibodies

Some investigators have reported an association between the presence of donor-specific anti-HLA antibodies and a reduction of half-life of various transplanted organs.[45,46] Interestingly, the post-transplant production of anti-B-cell antibodies was significantly associated with CRAD.[47] Moreover, elution studies from CRAD allografts demonstrate the presence of antibodies directed against HLA and non-HLA determinants.[48,49] Recently, a new subset of antibody-mediated CRAD has been identified. It is characterised by C4d deposits in peritubular capillaries and a high percentage of anti-donor HLA antibodies.[50] Interestingly, a close link has been reported between endothelial C4d deposition and transplant glomerulopathy and multilayering of basement membranes in peritubular capillaries[51] which are commonly considered as signs of chronic rejection. This finding favours an active process of alloantibody production in a subset of CRAD, likely to be modulated by therapeutic intervention.[52]

2.1.4 Inadequate Immunosuppression/ Noncompliance

Inadequate immunosuppression has been previously identified as a risk factor for CRAD. For example, a ciclosporin regimen below 5 mg/kg/day at 5 years increases the risk of CRAD.[37] However, the long-term exposure of high doses of CNIs results in important adverse effects, especially nephrotoxicity, which is the hallmark of the late ongoing parenchymal injuries at 10 years after transplant.[28] Attempts have been made by some investigators either to completely discontinue ciclosporin, with a non-negligible rate of acute rejection,[53,54] or to more safely reduce ciclosporin in order to alleviate nephrotoxicity.[55] In this context, the main challenge is to resolve the multiparametric equation allowing the tailoring of an 'optimal' immnosuppressive regimen for a particular individual.

As a matter of evidence, noncompliant patients or even patients with highly variable ciclosporin exposure are predisposed to developing CRAD and late deterioration of graft function, reinforcing CNI toxicity in the long term.[56-58]

2.2 Alloantigen-Independent Factors

As a common denominator, alloantigen-independent factors represent a stress placed upon the allograft leading to an acceleration of the aging process.[19,59] These can be separated into input factors, referring to pre-existing conditions, donor age, brain death, ischaemia/reperfusion injury, and load factors, corresponding to post-transplant stresses such as hypertension, lipid abnormalities, cytomegalovirus (CMV) infection, and CNI-related nephrotoxicity.

2.2.1 Input Factors

Donor Age

The fact that increased donor age correlates with reduced renal allograft survival has been repeatedly confirmed by the UNOS registries during the past years,[4,60,61] underlying the hypothesis of the age-related loss of functional glomeruli. However, other studies, using multivariate analysis, have demonstrated that donor age *per se* was not always associated with poor long-term graft survival,[62,63] suggesting that other donor factors were important to consider, such as hypertension history, aortorenal arteriosclerosis or renal function.[64]

Brain Death

Brain death is a very complex phenomenon with profound haemodynamic, neuro-hormonal and immunological alterations, and this partly explains the better graft survival observed in living related donors, when compared with cadaveric donors. On the basis of experimental studies in rats, it has become clear that brain death has a deleterious effect on kidney viability, increases the likelihood of CRAD development and equates to lower graft survival.[65,66] However, one has to consider that the rat may not be a pertinent model on which to analyse brain death and its effects, since it develops only focal segmental glomerulosclerosis and not CAN. As a corollary, it is then recommended that the state of brain death should be kept as short as possible.[67]

Delayed Graft Function and Ischaemia/ Reperfusion Injuries

Delayed graft function (DGF) is a clinical term used to describe a form of post-transplant acute renal failure, resulting in an increased risk of acute rejection and possibly a reduction of graft survival.[68] Ischaemia/reperfusion injuries play a pivotal role in the development of DGF, with their histological consequences, mainly characterised by lesions of acute tubular necrosis. Ischaemia/reperfusion injuries are also multifactorial and can be considered as the sum of lesions resulting from brain death, cold preservation, rewarming during anastomosis and reperfusion. DGF, usually defined as the need for dialysis during the first week, reflects these different events and can be used as a surrogate marker. The impact of DGF on graft survival is a matter of controversy in renal transplantation. For example, data from the large transplant registries, such as UNOS registry, indicate a significant impact of DGF on graft survival,[69,70] whereas other studies did not.[71] This discrepancy could originate from the role of silent acute rejection, which is common (as high as 50%) during prolonged DGF,[72] underlining the need for sequential protocol biopsies in this setting. Consequently, the relationship between

CRAD and DGF remains unclear because prospective studies are lacking and available retrospective studies do not monitor for acute rejection. In fact, when DGF was defined by more stringent functional criteria, independent from the need for dialysis, it was found to be an independent factor to acute rejection, but not to graft survival.[73]

2.2.2 Load Factors

Hypertension

Hypertension is highly prevalent among renal transplant recipients, and the vast majority (approximately 75%) needs antihypertensive treatment.[74,75] The causes of hypertension are numerous: pre-transplant hypertension, renal artery stenosis, recurrence of renal diseases, high renin plasma levels in output from native kidneys and immunosuppressive agents, such as CNIs and corticosteroids. In most studies, hypertension is associated with CRAD and graft failure, either as a cause or a consequence,[76,77] since hypertension before transplantation has also been associated with CRAD.[78] On the basis of the well established role of hypertension in the development and progression of chronic renal disease, it has been suggested that glomerular capillary hypertension and proteinuria may act as mediators of CRAD.[74,79] Large prospective trials are needed to establish which level of blood pressure is suitable to reduce the rate of CRAD, and which antihypertensive drugs should be used.

Hyperlipidaemia

The parallel between vascular lesions of CRAD and atherosclerosis led to the hypothesis of a role for lipid disorders in allograft failure associated with CRAD. Lipid abnormalities are common in the renal transplant population and probably contribute, together with hypertension, to cardiovascular mortality, which is the leading cause of death in patients with a functioning graft.[80-82] Both increased levels of total cholesterol, particularly low-density lipoprotein (LDL)-cholesterol, and hypertriglyceridaemia have been reported to be associated with development of CRAD.[83-85] Moreover, it has been shown that the level of cholesterol at 1 year after transplant was a predictor of graft and patient survival.[86] Immunosuppressive agents, such as ciclosporin, sirolimus or corticosteroids, play an important role in lipid disorders observed in renal transplant recipients.[87,88] The beneficial effect of HMG-Co-A reductase inhibitors (statins) on coronary heart disease of cardiac recipients has been demonstrated in two randomised trials.[89,90] Recently, a randomised, placebo-controlled trial (ALERT study) in 2102 renal transplant recipients treated or not with fluvastatin showed no significant benefit of lipid-lowering treatment for the primary endpoint, which was the reduction of major cardiac adverse events, i.e. cardiac death, non-fatal myocardial infarction and intervention procedures. The renal composite endpoint of graft loss or doubling serum creatinine was not different between the two groups.[91]

Cytomegalovirus Infection

The impact of CMV infection on long-term outcome and CRAD remains controversial.[92] CMV infection alone does not seem to be a major risk factor for the development of CRAD.[93] Nevertheless, the association of CMV infection with another event, such as acute rejection,[94,95] certain HLA mismatches,[96,97] combination with human herpesvirus 6 infection[98] or an elevated renal arterial resistance index,[99] could represent favourable conditions for the emergence of CRAD. On the other hand, it has been shown that prophylactic treatment of CMV infection reduces the incidence of acute rejection in the high-risk group of seronegative patients, although there was no difference in the rate of chronic graft rejection in the first 6 months.[100] There is probably a need for future prospective studies with a larger number of patients to examine the effects of CMV on CRAD.

Calcineurin Inhibitor-Related Nephrotoxicity

There is a complex (and somewhat paradoxical) relationship between CRAD and CNIs.[101] On one hand, the use of CNIs in the current immunosuppressive regimens, either ciclosporin or tacrolimus, allows for a significant decrease of acute rejection and an improvement in the short-term renal graft survival. On the other hand, the prolonged use of CNIs may result in an irreversible nephrotoxicity,

with a histopathological pattern which is quite indistinguishable from the CRAD pattern. Whether or not CNI-related nephrotoxicity belongs to CRAD is possibly of secondary importance. However, it should be emphasised that CNIs act on alloantigen-independent factors which are clearly associated with CRAD, such as hypertension,[102] hyperlipidaemia[87] and renal ischaemia via vasoconstriction of renal arterioles.[103] Moreover, it has been shown that CNIs induce the expression of cytokines with profibrotic properties such as tumour growth factor-β.[104,105] Finally, it has been recently demonstrated in a longitudinal study of the natural history of CAN that the prevalence of CNI-related nephrotoxicity progressively increases in biopsies beyond the first year, to play the major role in damages noted in biopsies 3 years after transplantation.[28]

3. Impact of Immunosuppressive Drugs on Long-Term Outcome

In the absence of specific randomised trials designed with respect to long-term outcome, it is very difficult to assess the impact of various immunosuppressive drugs on the development of CRAD. In fact, most of the large randomised trials in transplantation have focused on early endpoints, such as acute rejection episodes during the first 6–12 months after transplantation. The problem in designing a clinical trial aimed at modifying the natural history of CRAD is the choice of a definitive endpoint that could be related to graft survival. The minimal sample size required for such a clinical trial could be very large. For instance, it has been calculated that a primary prevention trial would require the inclusion of 1500 patients over 3 years.[106] Another way of analysing the role of immunosuppressive therapy on long-term graft survival is to focus on some meta-analyses performed in the field of renal transplantation. Table I summarises the main meta-analyses aimed at determining the effect of currently used immunosuppressive drugs on renal graft survival. Whereas the role of immunosuppressive drugs on the prevention of acute rejection is evident, their impact on the prevention of late graft loss seems more elusive. However, these meta-analyses do not reflect the impact of new immunosuppressants, such as tacrolimus, mycophenolate mofetil (MMF), in-

Table I. Meta-analyses of randomised trials aimed at determining the effect of immunosuppressive regimens on graft survival

Meta-analysis (year)	Aim of the meta-analysis	Number of randomised trials included	Results
Kunz and Neumayer[108] (1997)	To compare the effects of triple (CsA, AZA, CS) vs double (CsA, CS) immunosuppressive therapy	5	No significant difference between the two regimens
Szczech et al.[109] (1998)	To assess the effectiveness of induction therapy in prolonging allograft survival	7	A 6% greater 2-year graft survival rate in the induction group No significant benefit of induction therapy at 5 years except for a subgroup of sensitised patients
Hricik et al.[110] (1993)	To determine the effect of CS-free regimen on allograft survival	7	An increased risk of acute graft rejection
Kasiske et al.[111] (2000)	To determine the effect of CS withdrawal	9	An increased risk of acute graft rejection and graft failure
	To determine the effect of CsA withdrawal	10	An increased risk of acute rejection but less risk of graft failure
Kasiske et al.[112] (1993)	To determine the effect of CsA withdrawal	10	The increased incidence of acute rejection does not affect short-term graft or patient survival
Adu et al.[113] (2003)	To study the effect of anti-IL-2R antibodies in renal transplants	8	Reduction by 49% of acute rejection rate No difference between the different antibodies Long-term effect on graft failure unknown
Keown et al.[114] (2003)	To examine the clinical benefit of the anti-IL-2R antibody basiliximab	4	A significant reduction of the risk of acute rejection Long-term effect on graft failure unknown

AZA = azathioprine; **CS** = corticosteroids; **CsA** = ciclosporin (cyclosporine); **IL-2R** = interleukin 2 receptor.

Table II. Impact of new immunosuppressive molecules on long-term outcome: analysis of calcineurin inhibitors

Immunosuppressive drugs	Type of study	Results	Reference
TAC vs CsA old formulation	Analysis of UNOS registry (TAC: 544 pts vs CsA: >35 000 pts)	An increase in the projected half-life of pts treated with TAC	115
	5-year follow-up of the US Multicentre Trial of Tacrolimus (412 pts)	Equivalent pt/graft survival between the two treatment arms (ITT analysis) Improvement of graft survival in the TAC arm when cross-over due to rejection was counted as graft failure	116
	5-year follow-up of the European Multicentre Trial of Tacrolimus (451 pts)	Equivalent pt and graft survival between the two treatment arms A trend toward lower incidence of CRAD in the TAC arm	117
TAC or CsA microemulsion formulation vs CsA old formulation	Analysis of USRDS database	CsA microemulsion and TAC are associated with an improved graft survival and decreased relative risk of CRAD	118
CsA microemulsion formulation vs CsA old formulation	Analysis of USRDS database	CsA microemulsion is associated with an improved graft survival and decreased relative risk of CRAD	118
TAC vs CsA microemulsion formulation	5-year graft survival using a paired kidney analysis	Equivalent graft survival Superior renal function in the TAC group No difference in the slope of 1/Cr	119
	2- and 3-year results of a randomised trial comparing TAC + MMF or AZA vs CsA + MMF	Equivalent pt/graft survival across the three groups Superior renal function in the TAC groups Better graft survival in TAC groups in pts who experienced DGF	120,121

1/Cr = 1/creatinine; **AZA** = azathioprine; **CRAD** = chronic renal allograft dysfunction; **CsA** = ciclosporin; **DGF** = delayed graft function; **ITT** = intent-to-treat; **MMF** = mycophenolate mofetil; **pt(s)** = patient(s); **TAC** = tacrolimus; **UNOS** = United Network of Organ Sharing; **USRDS** = US Renal Data System.

terleukin (IL)-2 receptor antibodies and sirolimus, approved in the US by the FDA in 1995, 1996, 1998 and 1999, respectively. Furthermore, in 1995, a new microemulsion formulation of ciclosporin, providing more consistent drug exposure, was also introduced. The implementation of these new molecules has been accompanied by an impressive improvement of acute rejection rates, but it is probably too early to see final demonstration that this trend will translate into better long-term outcomes. A recent study identified two periods: an improvement of both short-term and long-term graft survival in the period of 1988–96,[8] followed by a lack of improvement in renal allograft survival from 1995, despite decreased rejection rates.[11] Table II (CNI) and table III (MMF and sirolimus) summarise some recent studies aimed at determining the impact of the new immunosuppressive drugs on long-term outcome. In our department, we have conducted a prospective, open-label, multicentre study to examine the possibility that MMF could prevent the emergence of CAN. The incidence of biopsy-proven CAN at 1 year was compared between two ciclosporin-based regimens comprising either MMF (n = 37) or azathioprine (n = 34). On the basis of both an intent-to-treat analysis and observed data, the number of patients with CAN at 1 year after transplantation was significantly reduced in the MMF group compared with the azathioprine group.[107]

4. Strategies to Combat CRAD Progression

In the battle against CRAD, weapons and active strategies are mandatory.

4.1 Weapons Against CRAD

Two categories of weapons are useful for the physician to prevent and/or treat CRAD, those used for the diagnosis of CRAD and those used for its treatment.

In terms of diagnosis, an ideal tool should allow the non-invasive detection and quantification of incipient CRAD. Such a diagnostic tool is not yet

available in renal transplantation, but recent promising studies have addressed this question and have identified some possible molecular biomarkers. For instance, microarray gene expression analysis probably represents an interesting approach to characterise different molecular patterns of chronic allograft pathology,[129] and to correlate these transcriptome changes with different immunosuppressive regimens.[130] In the meantime, renal biopsy is considered as the standard method for evaluating graft dysfunction, and protocol biopsies could be used to diagnose and semi-quantify CAN, with a strong predictive value for graft survival[27,131-133] and a low risk for the patient.[134] Besides the possibility of being able to detect and monitor the progression of CRAD, the protocol biopsies may also help to diagnose subclinical acute rejection. Indeed, it has been demonstrated that low-grade acute rejection superimposed on CAN is correlated with progression of histological lesions and that repeated subclinical acute rejection has a detrimental impact on graft function and survival.[40] More importantly, it has been suggested in a randomised study that the treatment of early subclinical rejection resulted in histological and functional benefits.[41] Finally, a biopsy will also allow for the diagnosis of others cases of renal deterioration, such as CNI nephrotoxicity, recurrent or *de novo* nephropathy or polyoma virus nephropathy. In fact, based on sequential biopsy studies, it becomes clearer that such a drug will have beneficial effect at one point of the graft's life, but may have detrimental consequences later.

In terms of treatment, the arsenal of immunosuppressants has been steadily expanding during the last 10 years. Different CNIs (ciclosporin, tacrolimus), anti-proliferative agents (azathioprine, MMF), target of rapamycin inhibitors (sirolimus, everolimus), and polyclonal and humanised monoclonal anti-IL-2 receptor antibodies (basiliximab, daclizumab) are currently available. New immunosuppressive molecules are at different phases of clinical or preclinical studies and may be available in the near future. These include FTY 720, a sphingosine-derived immune modulator forcing T-cell homing from spleen and blood to lymph nodes,[135,136] Janus kinase 3 inhibitors,[137] alemtuzumab (Campath 1H), a humanised monoclonal antibody directed against CD52 used as an induction therapy,[138,139] or leflunomide, a synthetic isoxazole derivative with anti-inflammatory and anti-viral properties.[140]

Table III. Impact of new immunosuppressive molecules on long-term outcome: analysis of mycophenolate mofetil (MMF) and sirolimus (SIR)

Immunosuppressive drug	Type of study	Results	Reference
MMF vs AZA	Results at year 3 of the Tricontinental prospective study	A nonsignificant trend towards an advantage for MMF	122
	Analysis of USRTS registry (>66 000 pts)	MMF decreases the relative risk to develop CRAD by 27%. This effect is partly independent of its outcome on acute rejection	123
	Analysis of USRDS registry (>49 000 pts)	Protective effect of MMF against declining renal function beyond 1 year post-transplant	124
	Analysis of USRDS registry (>47 000 pts)	Long-term us of MMF is associated with a reduced incidence of late acute rejection	125
	Single-centre retrospective analysis (>1500 pts)	Nonsignificant trend towards better graft survival with MMF	126
Sirolimus	2-year results of a study comparing SIR + CsA + CS vs SIR + CS	No difference in terms of pt/graft survival and incidence of acute rejection Superior renal function and slope of 1/Cr in the SIR + CS group	127
	Pts were randomly assigned at month 3 to either continue CsA + SIR + CS or to withdraw CsA and pursue SIR + CS	Lower incidence of CRAD lesions at 1 year in the SIR + CS group with a superior graft function	128

1/Cr = 1/creatinine; **AZA** = azathioprine; **CS** = corticosteroid; **CsA** = ciclosporin; **pt(s)** = patient(s); **USRDS** = US Renal Data System; **USRTS** = US Renal Transplant Scientific.

Taken together, a large range of immunosuppressant combinations is currently available and the main issue is to determine the best strategies to modify the natural history of CRAD.

4.2 Strategies Against CRAD

4.2.1 Non-Immune Strategy

Although the non-immune strategy is beyond the scope of this review, it remains of crucial importance and should act in concert with the immune approach. In the non-immune strategy, the clinician will take care to properly handle donor/recipient age matching, minimisation of cold ischaemia time, optimisation of brain donor, reduction of proteinuria using angiotensin-converting enzyme inhibitors or angiotensin II receptor antagonists, optimisation of hypertension treatment, treatment of CMV infection, control of lipid disorders preferentially through the use of statins, and careful screening and treatment of post-transplant diabetes mellitus.[141]

4.2.2 Immune Strategies

Immune Strategy Based on a Time-Dependent Modulation of the Immunosuppressive Regimen

The studies based on sequential protocol biopsies[28,132,142] suggest that CAN develops in three distinctive periods. The first 3 months is a period characterised by a high prevalence of both lesions of tubular necrosis (as a result of DGF) and subclinical acute rejection. During this first period, the initiation of CAN lesions is characterised by a very rapid increase of Banff scores for interstitial and tubular atrophy, which culminates in the second period, from month 3 to the end of the first year after transplant. The third period, beyond 1 year, is mainly characterised by lesions of CNI-related nephrotoxicity.

On the basis of this scheme, the new paradigm for a time-dependent modulation of immunosuppression would be the use of a CNI-based regimen during the first period, so that a short and powerful burst of immunosuppression is given during the period of maximal risk of subclinical acute rejection. The second period is probably the right time to assess the possibility of a withdrawal of CNIs. For each patient, a number of individual parameters are important to consider before withdrawing CNIs. These include cardiovascular risk factors, renal function, prior acute rejection, results of protocol biopsy and CMV infection. It is probably also important to consider that while one might remove the CNI toxicity, other immunological lesions, such as subclinical acute rejection may be produced.

The conversion of a CNI-based therapy to a non-nephrotoxic regimen, and the choice of an adequate immunosuppressant (MMF, sirolimus or other) will require additional controlled trials. For instance, a recently published, prospective, randomised trial has demonstrated a beneficial effect on renal function (slope of 1/serum creatinine) of a regimen where CNIs were replaced by MMF, in addition to cortocosteroids, in patients with histologically proven CAN.[143] Along the same lines, it has been demonstrated that early ciclosporin withdrawal followed by sirolimus-based therapy provides significantly improved renal histology and function at 3 years.[144] However, immunosuppressive drugs that are candidates to replace CNIs also display their own toxicity profile and an important aim for future studies is to identify patients who are likely to benefit from conversion from CNI to a non-CNI regimen. A recent study analysed the predictors of successful conversion from CNI-based to sirolimus-based immunosuppressive treatment and found that low proteinuria (<800 mg/day) at time of conversion was the most important predictive factor for a beneficial outcome.[145]

The main therapeutic issue during the third period, which is characterised by CNI-related nephrotoxicity, is the hypothetical reversibility of the lesions, even in the case of withdrawal of the toxic agent. Consequently, one can speculate on the existence of a 'point of no return', beyond which any modification of immunosuppressive treatment would be inefficient and even deleterious.

Immune Strategy to Achieve Transplantation Tolerance

The ultimate goal in transplantation is to achieve long-term drug-free graft acceptance with normal organ function, which means the complete absence

of CRAD, in an immunocompetent recipient.[146] Different successful strategies have been described in rodents, based for instance on the blockade of T-cell costimulatory signals, but these have consistently failed when applied to nonhuman primate models.[147-149] The control of the central or peripheral T-cell alloreactive repertoire is of crucial importance and some immunosuppressive drugs may play a beneficial role, underlining the fact that the contribution of conventional treatment requires investigation. Moreover, the institutional means for developing tolerance induction strategies are not comparable with those of pharmaceutical companies manufacturing and developing a new immunosuppressive agent. However, the success of such a strategy will require collaboration between transplant biologists, physicians and the biotechnology industry.

5. Conclusion

CRAD continues to be the main challenge in the field of organ transplantation and the limiting factor for the prolongation of graft survival. However, during the past few years, a new paradigm has emerged in renal transplantation with a progressive improvement of renal allograft half-lives, suggesting that the progress in immunosuppression (with a reduction of acute rejection episodes) translates into beneficial effect on the natural history of CRAD. With a better understanding of the complex and multifactorial pathogenesis of CRAD, some therapeutic strategies may be identified. This hypothesis needs to be urgently confirmed by large, randomised, multicentre trials involving all factors of renal transplantation.

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