Transplant International

Transplant International ISSN 0934-0874

REVIEW

Can immune monitoring help to minimize immunosuppression in kidney transplantation?

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Keywords
biomarker, immune, kidney, minimization, monitoring, transplantation.

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This work was supported in part by the Fondation Progreffe and the Centaure network.

Received: 13 May 2008
Revision requested: 10 June 2008
Accepted: 16 July 2008

doi:10.1111/j.1432-2277.2008.00748.x

Summary

The serious side-effects and complications related to the life-long use of immunosuppressors in transplantation have fuelled research into their possible minimization or even complete elimination. The field of transplantation is therefore tentatively moving from a phase of empiric immunosuppression towards individualized therapy. This process is highly dependent on the development of immune monitoring tests to detect an individual 'level of risk'. Immune monitoring is a way of measuring functional and molecular correlates of immune reactivity to provide clinically useful information for therapeutic decision-making. The technological breakthroughs over the last decade provide firm grounds for the achievement of this goal. Large, multicentric and prospective studies in the near future are now crucial if these tests are to achieve the necessary approval from the regulatory authorities and promptly enter the clinic for routine use.

Transplantation – advantages and limitations

Transplantation has made phenomenal progress over recent decades. The gradual introduction of newer immunosuppressive agents together with improved organ procurement techniques and patient care has led to a substantial reduction in the frequency of acute rejection episodes and improvements in long-term outcome [1]. Despite such improvements, thousands of patients have no access to transplantation and transplant recipients have a significantly lower life expectancy when compared with the general population. Moreover, improvement in the outcome of organ transplants has led to the use of extended criteria donors, which has undoubtedly slowed down the rate of overall improvement in transplantation. Thus, this is a field in constant evolution and a number of issues need to be urgently addressed for further improvement [2]. Some of these are well established such as the problem of donor vascular disease and cold ischaemia time [3]. Others have emerged as the result of progress or changes in the management of transplant patients such as the dilemmas posed by subclinical rejection [4] or isolated C4d diagnosed on protocol biopsies [5]. Perhaps, the most important issue, however, is that of the burden of life-long immunosuppression (IS) (see below).

The caveats of immunosuppression and their potential solutions: tolerance versus minimization

Despite having revolutionized the field in many ways, immunosuppressors are the Achilles heel of long-term outcome in transplantation. Currently, immunosuppressors have to be given as 'one-treatment suits all'. The problem with such empiric dosing is that it often leads to overimmunosuppression, resulting in serious side-effects. Specific examples include calcineurin inhibitors (CNI) that promote cancer progression [6] and induce nephrotoxicity,

with almost all CNI-treated patients displaying nephrotoxicity at 10 years post-transplantation [7]. Another example is that of corticosteroids (CS) which also increase the risk of infection and cancer and additionally have adverse effects on cardiovascular disease risk factors, such as diabetes, hypertension and hyperlipidaemia [8]. Thus, there is an urgent need to be able to more efficiently manage immunosuppression in transplant patients. One solution would be to minimize immunosuppression. Another would be to avoid it altogether by achieving a state of tolerance, both of which are discussed below. It is important to note that the ability to successfully minimize immunosuppression or induce tolerance is highly dependent on the type of organ transplant. For example, it is well known that liver transplant recipients are more receptive to tolerance induction protocols [9] and that weaning procedures are more successful after liver transplantation [10,11]. For the sake of simplicity, this review will focus primarily on kidney transplant recipients.

Avoidance of immunosuppression through tolerance

The purist ideology would be to induce transplant tolerance, a state of life-long graft acceptance whereby a patient could enjoy stable graft function without the need for immunosuppression and without being at an increased risk of developing cancer and infections. Strategies to induce tolerance in the clinic can be divided largely into two categories: one that is based on bone marrow transplantation and the other that is not. So far, only the first approach has been successful in small cohorts [12–14]. These approaches in humans are described in detail elsewhere [15–17] and will not be elaborated in detail here. Briefly, the former usually involves the consecutive or simultaneous administration of donor-derived haematopoietic cells, the aim being to create a robust form of 'central' tolerance through thymic deletion. The latter approach has only been successful in animal models and includes lymphocyte depletion, co-stimulatory blockade and donor antigen infusion and aims at creating 'peripheral' tolerance through immune deletion, deviation or suppression. Despite colossal research efforts, tolerance has proven difficult to achieve in humans (reviewed in [17]). Nevertheless, recent encouraging data would suggest that tolerance to allografts can be induced in specific patient groups through rationally designed protocols, at least in human leucocyte antigen (HLA)-matched or partially mismatched recipients [12,13]. Indeed, the degree of donor-recipient major histocompatibility complex matching is likely to be an important determining factor in tolerance protocols given their influence on transplant outcome [18]. Moreover, a state of spontaneous operational tolerance following immunosuppression withdrawal in patients with stable graft function has been reported both as anecdotal cases [19–21] and as series of recipients of HLA-mismatched grafts ([22] and reviewed in [23]), but its frequency remains unknown. These areas of research need to be explored further if tolerance is to be achievable on a larger scale. The search for biomarkers of tolerance has become a field in itself. There are so far no biomarkers of tolerance that have been sufficiently validated prospectively in large cohorts because of the rarity of this phenomenon. However, there are a number of phenotypic and molecular markers that have been associated with tolerance and are now being tested through international collaborations allowing for the study of larger cohorts. This subject has been addressed elsewhere [24]. This review will focus on biomarkers of immunosuppression weaning.

Minimization of immunosuppression

Another means to reduce the complications of immunosuppression is to perform weaning towards achieving the minimal effective level. Knowledge of genetic factors affecting drug metabolism and effects, so-called pharmacogenetics, could, at least in theory, allow for individualized dosing of immunosuppressive agents and help to reach the target level at the outset of treatment or during switch. Research in this field has demonstrated that both pharmacokinetics as well as pharmacodynamics can be influenced by the genetic disposition of the transplant recipient (reviewed in [25]). For example, polymorphisms in the CYP3A5 gene have been shown to influence tacrolimus disposition and drug-related nephrotoxicity [26,27]. In this respect, genetic testing could help to avoid early toxic side-effects and optimize immunosuppression but would not have a major impact on minimization *per se*. A recent literature review on this subject [28] has shown that most weaning protocols concern CNI and CS, primarily because of the serious side effects inherent to their use as mentioned above. It is of key importance in such minimization trials to weigh up the benefits and risks posed by such a procedure. As in tolerance protocols, it is likely that HLA-matching will contribute to the potential success or failure of weaning procedures. This is supported by the fact that patients who undergo elective cyclosporin A (CsA)-weaning have a greater relative risk of acute rejection for each HLA-DR mismatch [29].

Early minimization

To date, most studies have reported on CNI- or CS-weaning early after transplantation (i.e. within the first year), because of the increased likelihood of a maximal

impact of CNI toxicity in the initial months following transplantation and the fact that toxic lesions may be less reversible at later stages. The risk of acute rejection and graft failure following IS minimization was well addressed in 2000 by Kasiske et al. [30] who reported on a meta-analysis of IS withdrawal in kidney transplantation including more than 1000 patients. According to their study, prednisone withdrawal led to a significantly increased rate of acute rejection and a significantly increased relative risk of graft failure [30]. CNI withdrawal seemed more advantageous in that it led to a significantly increased rate of acute rejection but no significant increase in relative risk of graft failure [30]. When compared within the same study, there was a trend for less graft failure upon CNI withdrawal versus prednisone withdrawal [30]. This study did not, however, report on any potential benefits in terms of reduction in adverse side effects of immunosuppressors or gain in renal function.

Since the latter study in 2000, several additional studies have been published in this field. Matas *et al.* [31] reported on a significant reduction in several CS-related complications such as post-transplant diabetes mellitus upon early CS withdrawal with a good five-year outcome in terms of graft and patient survival and renal function in a study of nearly 600 patients. Moreover, absence of CS or early CS withdrawal showed a similar benefit in a randomized study of type 1 diabetic recipients of a simultaneous kidney/pancreas graft [8]. However, a comparison of CNI versus CS withdrawal at 6 months post-transplant in approximately 200 patients showed that at 24-months follow-up, patients in the CNI-withdrawal arm had a higher acute rejection rate and incidence of chronic allograft nephropathy when compared with those undergoing CS minimization or those continuing on triple therapy whereas CS withdrawal had a positive impact on cardiovascular risk factors [32]. Moreover, in another study on a similar number of patients, CNI weaning from a triple regimen has more recently been shown to result in an increase in rejection episodes and graft loss and only a trend towards improved renal function, with no significant improvement in lipid profile, blood pressure or malignancy rate in the CNI-weaning group [33]. Thus, as recently emphasized [28], the literature provides mixed results with long-term follow-up being indispensable. The field of IS minimization thus remains open to much more intensive exploration with no general consensus as yet.

Late minimization

Weaning during the first year post-transplant has obvious benefits, as described above. However, patients with stable graft function several years post-transplant may also benefit from IS weaning to alleviate chronic nephrotoxicity and reduce the risk of the other side effects discussed above. It is indeed possible that certain patients displaying highly stable graft function several years post-transplant under IS may have become operationally tolerant, making the weaning procedure less risky and more logical. Evidence for this comes from studies of kidney transplant recipients who spontaneously tolerate their kidney grafts upon weaning of IS, years after transplantation [22]. The fact that these patients can enjoy stable graft function for years after having progressively weaned their IS of their own accord several years after transplantation further supports the idea of late weaning [22]. Moreover, in the case of liver transplantation, elective weaning is now feasible in some centers, with approximately 20% of patients selected for weaning successfully achieving operational tolerance upon IS withdrawal [10]. The percentage of kidney transplant recipients that could benefit from such an approach remains to be determined. The detection of operational tolerance may be hampered by a masking effect of immunosuppression and its induction may even be prevented by certain immunosuppressors. Nevertheless, in a recent study using micro-arrays, a signature of operational tolerance was detected in 1/12 patients under standard immunosuppression and in 5/10 patients under low-dose steroid monotherapy with stable long-term graft function, suggesting that it may indeed be feasible to detect operational tolerance despite ongoing immunosuppression [34]. However, chronic CNI treatment could also represent an intrinsic obstacle to safe minimization by preventing from taking place the natural mechanism of regulation in the recipient and engendering more severe rejection after weaning. A more precise calculation of this percentage would first require the clinical validation of biomarkers that are able to detect a state of operational tolerance to evaluate the immunological risk long term after transplantation. This would lead to the setting up of immune monitoring procedures and strategies for prospective, progressive weaning.

The need for immune monitoring

If there is indeed to be a consensus within the transplant community in terms of IS minimization with minimum risk and maximum benefit, methods for effective monitoring of the response of patients or for potentially predicting such a response to minimization will be essential. Immune monitoring would be key to clinical decision-making during IS minimization procedures (see Fig. 1). Currently, there is a lack of reliable markers for the early monitoring of the degree of immunosuppression. Moreover, given that the patients respond individually to

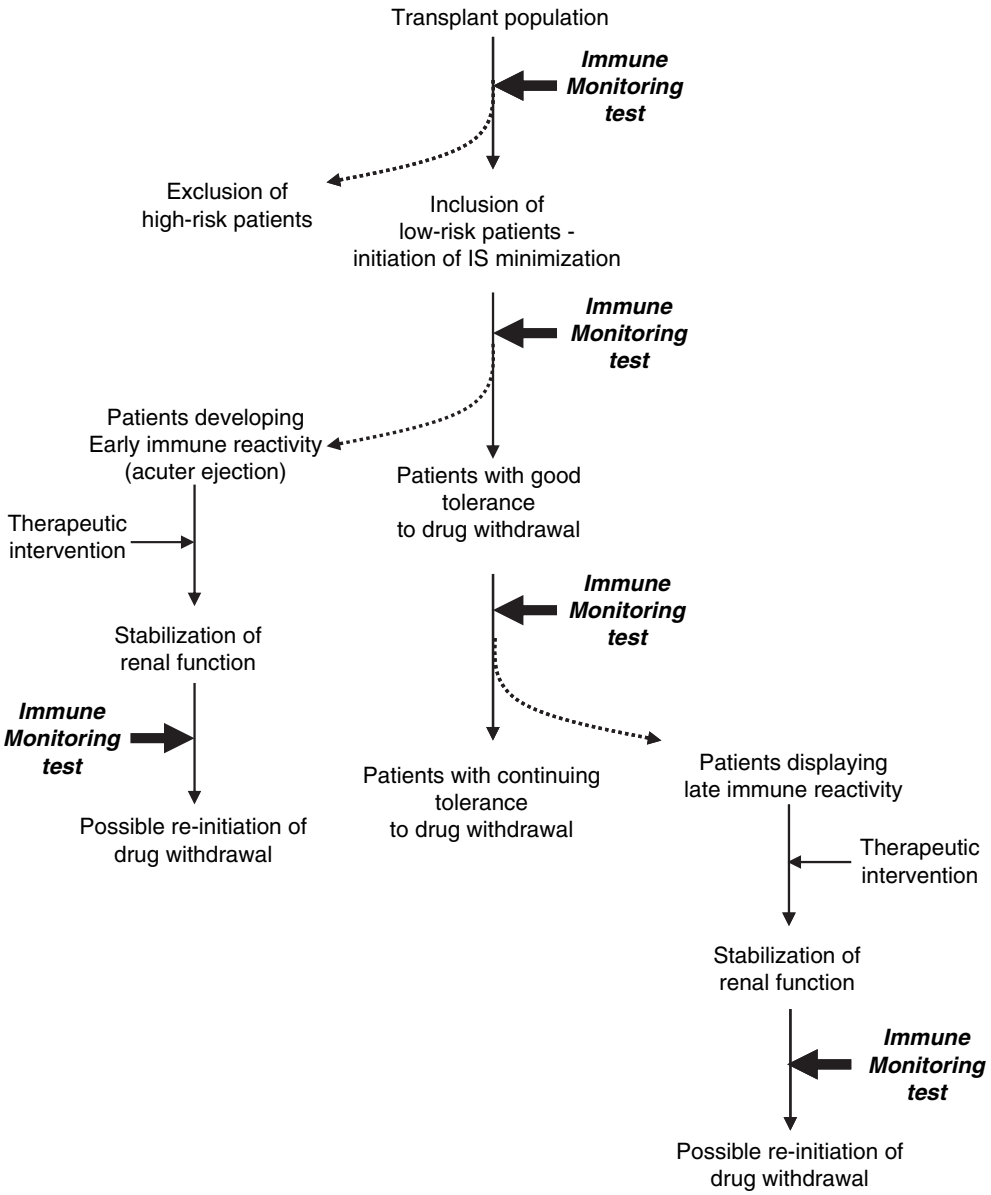

Figure 1 Immune monitoring for decision-making during IS minimization procedures.

treatments, even though pharmacogenetic approaches would make it possible to place every patient in the correct therapeutic window, empiric minimization may put all patients at risk of rejection. Immune monitoring would be necessary prior to the minimization procedure and would increase its safety. Immune monitoring would also be essential for identifying patients who increase their risk of acute rejection once the minimization has been initiated, or to detect acute rejection prior to clinical symptoms so as to be able to perform pre-emptive intervention to reduce/prevent damage to the graft. Likewise, there would be a need to identify patients whose grafts are developing other types of lesions of alloimmune and nonalloimmune causes such as interstitial fibrosis and tubular atrophy or chronic antibody-mediated rejection, respectively, without accompanying clinical signs. Although protocol biopsies are likely to help in this process, they cannot be performed serially in patients who are considered 'at risk' over long time-periods. Thus, the identification of biomarkers is fundamental to achieving an effective means of immune monitoring in the context of IS minimization. Let us first look at the definitions of immune monitoring and biomarkers.

Table 1. Examples of tests/markers already established in clinical transplantation as well as those that are in the pipeline of clinical validation that could be applicable to weaning procedures.

Test category	Marker	Utility	Reference
Protocol biopsy	Histological lesions	Gold standard for analysing graft damage	Reviewed in [68]
Anti-donor response	Anti-HLA antibodies	Antibodies known to have a negative impact on graft outcome	[35,36]
Pharmacogenomics	CYP3A5	Influence of tacrolimus disposition and drug-related nephrotoxicity	[26]
ELISA	Soluble CD30	Indicator of graft outcome	[46–48]
Quantitative PCR	AlloMap	Diagnoses acute rejection of heart transplants	[60]

ELISA, enzyme-linked immunosorbent assay; HLA, human leucocyte antigen.

A definition of immune monitoring

Immune monitoring is a way of measuring functional and molecular correlates of immune reactivity to provide clinically useful information for therapeutic decision-making. Monitoring requires the implementation of advanced assays that are standardized on a technical level, enabling large-scale, multi-centric application. In the transplant setting, monitoring would be useful for measuring both alloimmune (e.g. donor reactivity) and nonalloimmune reactivity to the graft. Even though logically the withdrawal of IS would lead to increased immune reactivity, it would nevertheless be useful to distinguish this alloimmune reactivity from other types of graft insults that occur during the post-transplant course.

Biomarkers as tools for immune monitoring

A biological biomarker, as defined by the Biomarkers Definitions Working Group, is '*a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to therapeutic intervention*' [22]. In order to be useful, biomarkers need to be accurate, specific and widely adoptable with a rapidity of data interpretation. To fulfill these criteria, biomarkers need to be tested in large patient cohorts, over adequate observation periods and over a range of disease severities and types. Ideally, they should be detectable in a noninvasive manner, for example, in the blood or urine. In transplantation probably the most obvious but most basic biomarkers are serum creatinine and proteinuria that are used to monitor graft function. However, the latter are biomarkers of clinical function and cannot provide specific information concerning the state of the recipient's immune system. Indeed, histological biomarkers of graft biopsies according to the Banff classification system [5] are currently the gold standard for diagnosing the status of organ transplants, indirectly reflecting immune reactivity towards the organ transplant. Nevertheless, the invasive (and therefore risky) and expensive nature of biopsies are major constraints to their repetitive use as would be required for immune monitoring. Newer, more sophisticated and potentially less invasive methods are therefore indispensable to immune monitoring. Examples of methods that would be applicable in the context of IS minimization are outlined below.

Methods for immune monitoring in transplantation with examples

The field of immune monitoring has seen an explosion in recent years, with more and more sophisticated and high-throughput techniques being developed. Outlined below and in Table 1 are some conventional as well as more innovative techniques used in the field of transplantation and applicable to immune monitoring in the context of IS minimization.

Conventional monitoring tests

The most conventional techniques routinely used in kidney transplantation are the measurement of blood creatinine, creatinine clearance and proteinuria to evaluate renal function. Although useful for detecting potential episodes of rejection, besides giving a crude estimation of renal function these measurements cannot accurately detect risk of rejection or provide any sort of specific indication of immune reactivity.

Biopsies, on the other hand, are the gold standard in transplantation for diagnosis. Biopsies indirectly provide evidence of immune reactivity and are therefore crucial for immune monitoring. More recently, there has been a further implementation of protocol biopsies by many centers. Such protocol biopsies have revealed that rejection can occur in the absence of clinical features (subclinical rejection; [4]) no doubt making them key to minimization strategies in the immediate future. Nonetheless, there are considerable efforts to find less invasive techniques to obviate the need for repetitive biopsies.

The analysis of anti-HLA, including donor-specific antibodies by enzyme-linked immunosorbent assay (ELISA) and more recently by Luminex has also been introduced as a systematic method of monitoring renal transplant

recipients. Measuring anti-HLA is useful given that circulating anti-donor antibodies or even those that are nondonor-specific have been shown to have a negative impact on graft outcome [35,36] and may therefore be used as an exclusion criterion when patients are being selected for weaning. They may also signal the need for re-introduction of IS if they appear during or after the weaning procedure. However, they are not highly accurate predictors of graft failure given that they can persist for years before any apparent deterioration of graft function [37], they appear late following transplantation (up to several years) [38] and they can also be present in patients displaying operational tolerance [22]. In future, the measurement of other types of antibodies such as anti-major histocompatibility complex class I-related molecule A (MICA) [39] and anti-endothelial cell antibodies using the recently developed XM-One endothelial cross-match test [40] may also be introduced routinely and could contribute to identifying at-risk patients involved in weaning.

Various cellular assays have also been used in the past, such as the mixed leucocyte reaction, cytotoxic T lymphocyte (CTL) assay and the limiting dilution assay. Although they can be informative in certain settings, as was demonstrated for the CTL assay [41], they have generally proved time-consuming and have therefore not reached routine use but have been overtaken my more sophisticated or reliable tests.

Innovative monitoring tests

There are now a variety of novel *in vitro* tests that appear promising and could prove useful for monitoring transplant recipients undergoing weaning, this list seems endless and will not be discussed exhaustively here. A more in-depth description of these methods can be found in [42] and [43].

One of these is the *transvivo* delayed-type hypersensitivity test, which is perhaps the only *in vivo* assay used to gauge an immune response. This test measures T-cell reactivity according to a swelling response induced following T-cell or peripheral blood mononuclear cell co-injection with donor antigen into the footpads of immunodeficient mice. The test has been frequently used to assess the anti-donor response in human tolerant transplant recipients [44,45]. However, the *in vivo* nature of this test is a major drawback, precluding its routine clinical use and making it less likely to be used for immune monitoring during weaning procedures.

The use of standard laboratory assays such as the ELISA has been implemented for the easy measurement of potentially interesting candidates in the serum of transplant patients. One specific example of this that holds promise for the future is soluble CD30 (sCD30). This molecule has been shown to be an indicator of graft outcome in a number of studies, when measured both pre- and post-transplant [46–48]. As such, measuring sCD30 could be useful in the context of weaning to assess risk prior to inclusion and throughout the follow up.

Another promising test, which was adapted from the ELISA technique, is the enzyme-linked immunosorbent spot assay. This assay quantifies the frequency of T cells responding to a given antigen through the measurement of a given cytokine. When used among renal transplant recipients, this test was able to detect patients at risk of rejection [49,50]. In a manner similar to the measurement of sCD30 and anti-HLA, this test could also be useful to monitor patients in the context of weaning, serving initially to exclude high-risk patients.

Another method of monitoring the T cells of transplant recipients is the so-called TcLandscape, which gives a global appraisal of the T-cell repertoire, with the potential of revealing perturbations that may be specific to a particular immunological status [51,52]. Refinement of this technique has shown that it can distinguish the T-cell repertoires in the peripheral blood of operationally tolerant patients from patients with chronic rejection on a statistical basis [53]. Efforts to screen a large cohort of highly stable kidney transplant recipients as a means to determine high or low-risk patients according to their resemblance to tolerant or chronic rejection patients are currently underway in our laboratory [54].

Immune monitoring could also benefit from the technical advances made over recent years in flow cytometry. These days, numerous markers can be analysed simultaneously using multiple fluorochromes. Such polychromatic flow cytometry has become the standard in preclinical research and is now moving rapidly towards routine clinical application in the field of transplantation, with the setting up of platforms specific for this purpose. In the context of weaning, so far this technique has been used primarily in IS weaning protocols involving liver transplant recipients. There have been several reports on particular peripheral blood cell phenotypes developing upon weaning in liver transplant patients, indicating whether a patient is tolerant or requires re-introduction of IS. Examples include potentially regulatory T-cell subsets such as CD4 + CD25 + T-cells and Vdelta1 + T cells [55]. Similar exhaustive phenotypic studies have also been performed in kidney transplantation in the context of operational tolerance [56], although not yet in the context of weaning. Thus, blood phenotyping is likely to be a key tool in the immune monitoring arsenal.

Besides these several small-scale techniques, several medium-to-large-scale techniques also have been and continue to be developed. Among these, quantitative PCR has taken its place as a reliable and reproducible test for the

measurement of specific genes or gene sets. Like polychromatic flow cytometry, quantitative PCR is now well on the road towards routine clinical use in the transplant setting. In the context of kidney transplantation, measuring certain molecules in the urine such as forkhead-winged helix transcription factor P3 (FOXP3) or interferon-gamma-inducible protein-10 (IP-10) has been shown to be useful for predicting acute rejection episodes [57,58] whereas measuring other molecules in peripheral blood such as the recently identified Tribbles-1 has been shown to be useful for diagnosing chronic antibody-mediated rejection [59]. The noninvasive nature of these types of analyses makes them ideal for immune monitoring during weaning procedures.

Along the same lines, other groups including our own are working on the identification of gene sets in addition to individual genes for diagnostic or prognostic purposes in transplantation. These studies have focused on the use of highly innovative micro-array technology to measure thousands of genes simultaneously. Since their introduction in the 1990s, micro-arrays have become more and more sophisticated and refined, so that today, several tens of thousands of genes can be measured in a single quasi-exhaustive gene transcription test. In most cases, the goal is to titrate down from micro-arrays to quantitative PCR for the measurement of a minimal gene set. To date, the only test that has achieved Food and Drug Administration (FDA) approval is the AlloMap test based on the expression of 11 genes in the peripheral blood for the diagnosis of acute rejection of heart transplants [60]. In kidney transplantation, the majority of studies have focused on micro-array analysis of graft biopsies to detect signatures of chronic allograft nephropathy [61,62]. Such signatures are probably of limited use in weaning studies given the obvious need for invasive biopsies. However, there was one report focusing in peripheral blood where the authors showed that micro-array analysis of peripheral blood mononuclear cells could be used to diagnose acute kidney graft rejection [63]. If this test proves to be able to predict acute rejection before the appearance of clinical signs it could be of use during weaning protocols. Our group recently reported on the use of this technology for the identification of a 'signature' in the blood of patients displaying operational tolerance [34,64]. There are hopes that this signature may help to identify highly stable patients under IS, years after transplantation who may in fact be operationally tolerant and may thus benefit from IS weaning. Thus, we are coming closer to having blood tests for diagnosis and/or prognosis using gene signatures, but clinical validation is still urgently needed in kidney transplantation.

Proteomics is another innovative technology that is relatively new in transplantation, and has not yet been exploited to the same extent as transcriptomics. This technique, which uses chromatography and mass spectrometry to analyse protein fractions or fragments, has been applied to urine samples to diagnose acute rejection [65,66]. More recently, high throughput proteomics assays have been developed for the simultaneous measurement of thousands of proteins in a given sample. These assays hold great potential for the identification of pertinent urine markers that could subsequently be measured by more simple techniques such as ELISA mentioned above in the context of routine clinical monitoring during weaning.

Finally, a novel approach that our group is undertaking is to perform statistical modelling of clinical data collected at the time of transplant or at various increments thereafter to calculate a 'score' to predict the risk of subsequent graft failure (Y. Foucher *et al.*, unpublished data). This is a very attractive approach as it does not require specific sampling, making it relatively simple and cost-effective. Moreover, if validated, this approach would be extremely useful as an inclusion criterion in a weaning protocol as well as for monitoring change in risk upon IS minimization.

From discovery to application – getting immune monitoring into routine clinical use

As seen from the above, there is certainly no lack of tests that are potentially useful for immune monitoring during drug minimization protocols. What is lacking, however, is the necessary clinical validation and approval from the regulatory authorities. The current problem is that the majority of studies tend to be relatively small and thereby statistically lacking in power and monocentric. Large, multi-centric and prospective studies in the near future are crucial if these tests are to achieve the necessary approval from the regulatory authorities and promptly enter the clinic for routine use. Biocollections such as the DIVAT database [67] as well as collaborations through research networks will be fundamental. It is highly likely that a battery of tests will be necessary during weaning procedures such that patients are not put under unnecessary risk.

Conclusion

The field of transplantation is making a tentative move from a phase of empiric IS towards pre-emptive individualized therapy. This process is highly dependent on the development of immune monitoring tests to detect an individual level of 'risk'. Being able to measure such 'risk' in transplant recipients is the key to the implementation of across-the-board IS minimization. The goal of such minimization is to achieve either a minimal effective dose, or a state of operational tolerance. The task of

translating immune monitoring assays to the clinical arena for routine use is not a simple one but much progress has already been made in this direction. There is certainly reason for optimism that immune monitoring will, in the near future, be part of the standard of care for transplant recipients.

References

1. Hariharan S, Stablein DE. Improvements in long-term renal transplant graft survival. *Am J Transplant* 2005; **5**: 630; author reply 632–3.
2. Chapman JR. Introduction: targets for improving outcomes in renal allografts transplanted during the next ten years. *Transplantation* 2008; **85**: S1.
3. Opelz G, Dohler B. Multicenter analysis of kidney preservation. *Transplantation* 2007; **83**: 247.
4. Nankivell BJ, Chapman JR. The significance of subclinical rejection and the value of protocol biopsies. *Am J Transplant* 2006; **6**: 2006.
5. Solez K, Colvin RB, Racusen LC, *et al.* Banff 07 classification of renal allograft pathology: updates and future directions. *Am J Transplant* 2008; **8**: 753.
6. Dantal J, Hourmant M, Cantarovich D, *et al.* Effect of long-term immunosuppression in kidney-graft recipients on cancer incidence: randomised comparison of two cyclosporin regimens. *Lancet* 1998; **351**: 623.
7. Nankivell BJ, Borrows RJ, Fung CL, O'Connell PJ, Chapman JR, Allen RD. Calcineurin inhibitor nephrotoxicity: longitudinal assessment by protocol histology. *Transplantation* 2004; **78**: 557.
8. Cantarovich D, Karam G, Hourmant M, *et al.* Steroid avoidance versus steroid withdrawal after simultaneous pancreas-kidney transplantation. *Am J Transplant* 2005; **5**: 1332.
9. Calne RY. Immunological tolerance – the liver effect. *Immunol Rev* 2000; **174**: 280.
10. Lerut J, Sanchez-Fueyo A. An appraisal of tolerance in liver transplantation. *Am J Transplant* 2006; **6**: 1774.
11. Martinez-Llordella M, Lozano J, Puig-Pey I, *et al.* Liver transplantation employing transcriptional profiling. *J Clin Invest* 2008 [E-pub ahead of Print].
12. Fudaba Y, Spitzer TR, Shaffer J, *et al.* Myeloma responses and tolerance following combined kidney and non-myeloablative marrow transplantation: in vivo and in vitro analyses. *Am J Transplant* 2006; **6**: 2121.
13. Kawai T, Cosimi AB, Spitzer TR, *et al.* HLA-mismatched renal transplantation without maintenance immunosuppression. *N Engl J Med* 2008; **358**: 353.
14. Millan MT, Shizuru JA, Hoffmann P, *et al.* Mixed chimerism and immunosuppressive drug withdrawal after HLA-mismatched kidney and hematopoietic progenitor transplantation. *Transplantation* 2002; **73**(9): 1386.
15. Sykes M. Immune tolerance: mechanisms and application in clinical transplantation. *J Intern Med* 2007; **262**: 288.
16. Cosimi AB, Sachs DH. Mixed chimerism and transplantation tolerance. *Transplantation* 2004; **77**: 943.
17. Ashton-Chess J, Brouard S, Soulillou JP. Is clinical tolerance realistic in the next decade? *Transpl Int* 2006; **19**: 539.
18. Opelz G, Dohler B. Effect of human leukocyte antigen compatibility on kidney graft survival: comparative analysis of two decades. *Transplantation* 2007; **84**: 137.
19. Uehling DT, Hussey JL, Weinstein AB, Wank R, Bach FH. Cessation of immunosuppression after renal transplantation. *Surgery* 1976; **79**: 278.
20. Burlingham WJ, Grailer AP, Fechner JH Jr, *et al.* Microchimerism linked to cytotoxic T lymphocyte functional unresponsiveness (clonal anergy) in a tolerant renal transplant recipient. *Transplantation* 1995; **59**: 1147.
21. Cai J, Lee J, Jankowska-Gan E, *et al.* Minor H antigen HA-1-specific regulator and effector CD8+ T cells and HA-1 microchimerism in allograft tolerance. *J Exp Med* 2004; **199**: 1017.
22. Roussey-Kesler G, Giral M, Moreau A, *et al.* Clinical operational tolerance after kidney transplantation. *Am J Transplant* 2006; **6**: 736.
23. Ashton-Chess J, Giral M, Brouard S, Soulillou JP. Spontaneous operational tolerance after immunosuppressive drug withdrawal in clinical renal allotransplantation. *Transplantation* 2007; **84**: 1215.
24. Ashton-Chess J, Soulillou JP, Brouard S. The use of biomarkers in clinical transplant tolerance. *Trends Transpl* 2007; **1**: 46.
25. Cattaneo D, Baldelli S, Perico N. Pharmacogenetics of immunosuppressants: progress, pitfalls and promises. *Am J Transplant* 2008; **8**: 1374.
26. Kuypers DR, de Jonge H, Naesens M, Lerut E, Verbeke K, Vanrenterghem Y. CYP3A5 and CYP3A4 but not MDR1 single nucleotide polymorphisms determine long-term tacrolimus disposition and drug-related nephrotoxicity in renal recipients. *Clin Pharmacol Ther* 2007; **82**: 711.
27. Anglicheau D, Legendre C, Beaune P, Thervet E. Cytochrome P450 3A polymorphisms and immunosuppressive drugs: an update. *Pharmacogenomics* 2007; **8**: 835.
28. Cantarovich D, Vistoli F, Soulillou JP. Immunosuppression minimization in kidney transplantation. *Front Biosci* 2008; **13**: 1413.
29. Heim-Duthoy KL, Chitwood KK, Tortorice KL, Massy ZA, Kasiske BL. Elective cyclosporine withdrawal 1 year after renal transplantation. *Am J Kidney Dis* 1994; **24**: 846.
30. Kasiske BL, Chakkera HA, Louis TA, Ma JZ. A meta-analysis of immunosuppression withdrawal trials in renal transplantation. *J Am Soc Nephrol* 2000; **11**: 1910.
31. Matas AJ, Kandaswamy R, Gillingham KJ, *et al.* Prednisone-free maintenance immunosuppression-a 5-year experience. *Am J Transplant* 2005; **5**: 2473.
32. Smak Gregoor PJ, de Sevaux RG, Ligtenberg G, *et al.* Withdrawal of cyclosporine or prednisone six months after kidney transplantation in patients on triple drug therapy:

a randomized, prospective, multicenter study. *J Am Soc Nephrol* 2002; **13**: 1365.
33. Abramowicz D, Del Carmen Rial M, Vitko S, *et al.* Cyclosporine withdrawal from a mycophenolate mofetil-containing immunosuppressive regimen: results of a five-year, prospective, randomized study. *J Am Soc Nephrol* 2005; **16**: 2234.
34. Brouard S, Mansfield E, Braud C, *et al.* Identification of a peripheral blood transcriptional biomarker panel associated with operational renal allograft tolerance. *Proc Natl Acad Sci USA* 2007; **104**: 15448.
35. Langan LL, Park LP, Hughes TL, *et al.* Post-transplant hla class II antibodies and high soluble CD30 levels are independently associated with poor kidney graft survival. *Am J Transplant* 2007; **7**: 847.
36. Terasaki PI, Ozawa M. Predicting kidney graft failure by HLA antibodies: a prospective trial. *Am J Transplant* 2004; **4**: 438.
37. Lee PC, Terasaki PI, Takemoto SK, *et al.* All chronic rejection failures of kidney transplants were preceded by the development of HLA antibodies. *Transplantation* 2002; **74**: 1192.
38. Hourmant M, Cesbron-Gautier A, Terasaki PI, *et al.* Frequency and clinical implications of development of donor-specific and non-donor-specific HLA antibodies after kidney transplantation. *J Am Soc Nephrol* 2005; **16**: 2804.
39. Zou Y, Stastny P, Susal C, Dohler B, Opelz G. Antibodies against MICA antigens and kidney transplant rejection. *N Engl J Med* 2007; **357**: 1293.
40. Holgersson J, Elsheikh E, Grufman P, Sumitran-Holgersson S, Tyden G. A case of acute vascular rejection caused by endothelial-reactive non-HLA antibodies. *Clin Transpl* 2006; **00**: 535.
41. Weimar W, Rischen-Vos J, de Kuiper P, *et al.* Tapering immunosuppression in recipients of living donor kidney transplants. *Nephrol Dial Transpl* 2004; **19**(Suppl. 4): iv61.
42. Lau A, Turnquist H, Tokita D, Zeevi A, Mazariegos G, Thomon A. ''Tolerance'' assays: the physician's guide to safe weaning of immunosuppression? *Transpl Rev* 2006; **20**: 208.
43. Rodrigo E, Arias M. A practical approach to immune monitoring in kidney transplantation. *Minerva Urol Nefrol* 2007; **59**: 337.
44. VanBuskirk AM, Burlingham WJ, Jankowska-Gan E, *et al.* Human allograft acceptance is associated with immune regulation. *J Clin Invest* 2000; **106**: 145.
45. Xu Q, Lee J, Jankowska-Gan E, *et al.* Human CD4 + CD25 low adaptive T regulatory cells suppress delayed-type hypersensitivity during transplant tolerance. *J Immunol* 2007; **178**: 3983.
46. Vaidya S, Partlow D, Barnes T, Thomas P, Gugliuzza K. Soluble CD30 concentrations in ESRD patients with and without panel reactive HLA antibodies. *Clin Transpl* 2006; **20**: 461.
47. Sengul S, Keven K, Gormez U, Kutlay S, Erturk S, Erbay B. Identification of patients at risk of acute rejection by pretransplantation and posttransplantation monitoring of soluble CD30 levels in kidney transplantation. *Transplantation* 2006; **81**: 1216.
48. Weimer R, Susal C, Yildiz S, *et al.* Post-transplants CD30 and neopterin as predictors of chronic allograft nephropathy: impact of different immunosuppressive regimens. *Am J Transplant* 2006; **6**: 1865.
49. Najafian N, Salama AD, Fedoseyeva EV, Benichou G, Sayegh MH. Enzyme-linked immunosorbent spot assay analysis of peripheral blood lymphocyte reactivity to donor HLA-DR peptides: potential novel assay for prediction of outcomes for renal transplant recipients. *J Am Soc Nephrol* 2002; **13**: 252.
50. Nather BJ, Nickel P, Bold G, *et al.* Modified ELISPOT technique – shighly significant inverse correlation of post-Tx donor-reactive IFNgamma-producing cell frequencies with 6 and 12 months graft function in kidney transplant recipients. *Transpl Immunol* 2006; **16**: 232.
51. Sebille F, Vanhove B, Soulillou JP. Mechanisms of tolerance induction: blockade of co-stimulation. *Philos Trans R Soc Lond B Biol Sci* 2001; **356**: 649.
52. Miqueu P, Guillet M, Degauque N, Dore JC, Soulillou JP, Brouard S. Statistical analysis of CDR3 length distributions for the assessment of T and B cell repertoire biases. *Mol Immunol* 2007; **44**: 1057.
53. Miqueu P, Guillet M, Dore JC, Soulillou JP, Brouard S. Discrimination of kidney transplant recipients presenting operational tolerance or chronic rejection based on recognition of T cell receptor cdr3 length distribution patterns. *Transplantation* 2006; **1**(Suppl. 2): 403.
54. Miqueu P, Guillet M, Giral M, *et al.* T cell repertoire analysis of stable kidney graft recipients for detection of low and high risk of chronic rejection. 8th International Conference on new trends in immunosuppression and immunotherapy. Berlin, Germany, 2008.
55. Martinez-Llordella M, Puig-Pey I, Orlando G, *et al.* Multiparameter immune profiling of operational tolerance in liver transplantation. *Am J Transplant* 2007; **7**: 309.
56. Louis S, Braudeau C, Giral M, *et al.* Contrasting CD25hiCD4 + T cells/FOXP3 patterns in chronic rejection and operational drug-free tolerance. *Transplantation* 2006; **81**: 398.
57. Muthukumar T, Dadhania D, Ding R, *et al.* Messenger RNA for FOXP3 in the urine of renal allograft recipients. *N Engl J Med* 2005; **353**: 2342.
58. Matz M, Beyer J, Wunsch D, *et al.* Early post-transplant urinary IP-10 expression after kidney transplantation is predictive of short- and long-term graft function. *Kidney Int* 2006; **69**: 1683.
59. Ashton-Chess J, Giral M, Mengel M, *et al.* Tribbles-1 as a novel biomarker of chronic antibody-mediated rejection. *J Am Soc Nephrol* 2008; **19**: 1116.

60. Deng MC, Eisen HJ, Mehra MR, *et al.* Noninvasive discrimination of rejection in cardiac allograft recipients using gene expression profiling. *Am J Transplant* 2006; **6**: 150.
61. Scherer A, Krause A, Walker JR, Korn A, Niese D, Raulf F. Early prognosis of the development of renal chronic allograft rejection by gene expression profiling of human protocol biopsies. *Transplantation* 2003; **75**: 1323.
62. Donauer J, Rumberger B, Klein M, *et al.* Expression profiling on chronically rejected transplant kidneys. *Transplantation* 2003; **76**: 539.
63. Flechner SM, Kurian SM, Head SR, *et al.* Kidney transplant rejection and tissue injury by gene profiling of biopsies and peripheral blood lymphocytes. *Am J Transplant* 2004; **4**: 1475.
64. Braud C, Baeten D, Giral M, *et al.* Immunosuppressive drug-free operational immune tolerance in human kidney transplant recipients. Part I. Blood gene expression statistical analysis. *J Cell Biochem* 2008; **103**: 1681.
65. O'Riordan E, Orlova TN, Mei JJ, *et al.* Bioinformatic analysis of the urine proteome of acute allograft rejection. *J Am Soc Nephrol* 2004; **15**: 3240.
66. Schaub S, Rush D, Wilkins J, *et al.* Proteomic-based detection of urine proteins associated with acute renal allograft rejection. *J Am Soc Nephrol* 2004; **15**: 219.
67. Foucher Y, Giral M, Soulillou JP, Daurès J-P. A flexible semi-Markov model for interval-censored data and goodness-of-fit testing. *Stat Methods Med Res* (in press).
68. Rush D. Protocol transplant biopsies: an underutilized tool in kidney transplantation. *Clin J Am Soc Nephrol* 2006; **1**: 138.