Biomarkers, 2011; 16(S1): S42–S50

ISSN 1354-750X print/ISSN 1366-5804 online
DOI: 10.3109/1354750X.2011.578754

informa
healthcare

REVIEW ARTICLE

Monitoring tolerance and rejection in organ transplant recipients

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Abstract

To avoid toxic side effects caused by permanent immunosuppressive treatment, research in transplantation focuses on new treatment strategies inducing tolerance or allowing drug weaning. Implementing drug minimization into clinical routine can be only safely achieved when guided by biomarkers reflecting the individual immune reactivity. We review recently described biomarkers and assays allowing identification of patients suitable for drug weaning or at risk of rejection. However, the majority of described biomarkers and assays have not been validated in prospective clinical trials. Thus, collaborative efforts are needed to design and perform prospective multicenter trials to validate the identified biomarkers across different laboratories.

Keywords: Kidney transplantation, Gene marker, T cell, B cell, HLA antibodies, Elispot

Introduction

In the past 20 years, major progress has been made in prolonging graft and patient survival after transplantation as a result of development of improved immunosuppressive drugs (IS). Long-term allograft survival has not significantly changed in particular because of the inherent toxicities of IS therapy. Thus, permanent IS treatment of transplant patients is associated with increased morbidity and mortality, and their long-term use should be minimized whenever possible. Furthermore, the occurrence of acute clinical and subclinical rejection episodes, which markedly influences long-term graft survival and function, could not be completely prevented with the current standard-of-care immunosuppressive protocols. It has become apparent that the immunologic status of the patient awaiting transplantation has a large impact on graft survival and function. Therefore, we have to identify biomarkers and develop assays that better reveal the ongoing antidonor immune responses and help to guide clinicians in therapeutic decision making pre- and posttransplant. Here, we summarize the recent results in identifying such biomarkers for an immune monitoring of transplant patients. Thereby, we will focus mainly on kidney graft recipients.

What do we need to monitor in transplant patients?

The immune response against allogeneic solid organ grafts is mainly based on the direct and indirect recognition of foreign donor major histocompatibility complex (MHC) I and II alleles by recipient effector $CD8^+$ and $CD4^+$ T cells, respectively. Therefore, monitoring the frequencies of $CD4^+$ and $CD8^+$ effector T-cell populations and their functional competence to produce inflammatory cytokines such as interferon-γ (IFN-γ) on stimulation with donor antigen is of enormous importance when determining antidonor immune responses in transplant patients. The activation and their functional capacity of effector T cells is controlled by regulatory T cells; thus, the balance between effector and regulatory T-cell populations is decisive for the outcome of an antidonor response. Apart from T cells, several other cell types of the innate and adaptive immune systems such as

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(Received 14 December 2010; revised 04 April 2011; accepted 04 April 2011)

dendritic cells, natural killer cells, and B cells are involved in the effector response. Their relative contribution to the rejection depends on the recipient's immune history, the immunogenicity of the donor organ, timing after transplantation, and the type of immunosuppressive therapy itself. For all above-mentioned leukocyte cell types, inflammatory and anti-inflammatory subpopulations have been described. The relative distribution of these subpopulations determines the quantity and quality of the alloresponse and thus also long-term graft outcome. An overview on the type of immune cells and their respective function known to take part in the antidonor immune response is shown in Figure 1.

By determining the quality and quantity of the patient's alloresponse, clinicians, in the future, could design personalized therapeutic regimens and therefore avoid unnecessary high immunosuppressive regimens but give sufficient drugs to control the alloresponse in case of highly reactive patients.

To predict graft outcome based on the ongoing antidonor immune response, the results of a pre- and posttransplant immune monitoring should help answering the following clinical relevant questions:

Before transplantation, it is important to know whether the patient is presensitized and thus likely to develop acute rejections. In contrast, clinicians will need to know whether a patient can be selected for early drug minimization protocols or tolerance-inducting protocols.

In the posttransplant period, immune monitoring should be able to predict acute rejection episodes and detect inflammatory processes leading to chronic graft dysfunction. Immune monitoring should also allow identification of operational tolerant (OT) patient: in whom IS can be safely minimized or completely withdrawn.

How can we monitor transplant patients?

When designing efficient pre- and posttransplant immune monitoring protocols, we have to first decide with which compartments and what kind of assays we can reveal ongoing antidonor immune responses. Table 1 summarizes the currently accessible tissue compartments and available assays that can be used for such an immune monitoring. Thereby, we have to discriminate between invasive procedures such as biopsy sampling and the broad spectrum of noninvasive procedures.

	Adaptive		Innate	
	T cells	B cells	Dendritic cells	NK cells
Balance between potentially anti-inflammatory and inflammatory subpopulations	- CD4+CD25+ CD127low Tregs - CD4+/CD8+ CD45RA/RO+ CCR7- T effector	- CD27-IgM+/IgD+ naive B cells - CD27++CD38++ plasma cells	- Lin-, CD123+, HLA-DR + plasmacytoid DCs - Lin-, CD11c+, BDCA1+ myeloid DCs	- CD56brightCD16- cytokine producing NK cells - CD56dimCD16+ cytotoxic NK cells
Function regulatory versus pathological	Cytokine: TGFß, IL-10 versus IFN-γ, IL-17	IL-10 secretion versus anti-donor-mAb secretion	Cytokine production: type I IFN, IL-27, IL-10 versus IL-12, IL-23, TNF-α, IL-6	Killing: APC, T_{eff} vs. tissue Cytokine: IL-10, TGFß vs. IFN-γ

Figure 1. Overview on the type of immune cells and their respective function, which could be monitored in patients to predict transplant outcome. For cells of the adaptive and the innate immune systems, regulatory functions have been ascribed. Therefore, it is important to capture the relative proportion of potentially anti-inflammatory and inflammatory subpopulations of T cells, B cells, dendritic cells, and natural killer cells. Furthermore, if no changes in relative or absolute numbers are detectable, differences in functional competence or the secreted cytokine profile on stimulation with donor antigen might be revealed.

Table 1. Overview on compartments and assays used to perform immune monitoring.

Invasive methods	Noninvasive methods			
Intragraft	Periphery			Fluids draining the graft
	Serum	Leukocytes		Urine
		Function	Phenotype	
Histology	ELISA	Elispot	Flow cytometry	ELISA
Transcriptomics	Antibodies	Cytokine release	Transcriptomics	Transcriptomics
Proteomics	Proteomics	Antibodies	Proteomics	Proteomics
Epigenetics		*Trans-vivo* DTH	Epigenetics	
			TCR profile	
			BCR profile	

Sampling intragraft material has one advantage because it can reveal more specifically the local ongoing antidonor immune responses. However, there is a big debate in the transplant community about the risk or benefit of protocol biopsies in revealing signs of continuous inflammation and fibrosis causing graft function deterioration at later time points (Rush, 2009). As outlined below, protocol biopsies can be informative revealing new mechanisms and cellular players of graft rejection and predicting long-term graft function. However, protocol biopsies have several limitations that include their morbidity, cost, and sampling error. Thus, their implementation in a posttransplant immune monitoring of transplant patients has to be carefully planned, so that the benefits outweigh the risks.

With regard to noninvasive methods, we can analyze peripheral blood samples, but in kidney transplant recipients, it might be also interesting to study fluids draining the graft such as the urine. Noninvasive methods analyzing peripheral blood samples can be performed more frequently but may lack a certain degree of specificity as they, depending on the assay used, also reflect other non–antidonor immune responses.

The techniques applied to detect differences in biomarker levels and functional competence of cells can vary from easy to perform enzyme-linked immunosorbent assay–based techniques on serum and urine samples (Li et al., 2001; Susal et al., 2002), whole genome–based transcription analysis (Sarwal et al., 2003), protein expression profiling (Quintana et al., 2009), profiling of T-cell and B-cell receptor distribution (Baeten et al., 2006) to functional assays such as Elispot and *Trans-vivo* delayed type hypersensivity (DTH) responses (Rodriguez et al., 2004). It has to be proven in future prospective multicenter clinical trials which of these assays can be routinely implemented into an efficient pre- and posttransplant immune monitoring.

Pretransplant identification of sensitized transplant patients

The importance of sensitization to nonself human leukocyte antigens (HLA) has been recognized since the beginning of transplantation medicine. Sensitization can occur through three main routes: blood transfusion, pregnancy, and previous transplantation. Terasaki et al. first described antibody-mediated hyperacute rejection episodes in the setting of a positive antibody crossmatch and lower graft survival rates in kidney transplant patients with cytotoxic HLA antibodies detected before transplantation (Terasaki et al., 1971). In the following years, the role of humoral immunity in causing antibody-mediated rejection (AMR) has been extensively studied and well documented. As a result, a negative complement-dependent cytotoxicity crossmatch test between recipient sera and donor T and B lymphocytes had become a mandatory requirement for cadaveric kidney transplantation. The test has been first implemented to detect donor HLA-specific antibodies, but later on, it has been shown that patients with circulating antibodies specific for a broad range of HLA antigens (panel reactive antibodies (PRA)) are also at increased risk of developing AMR (Opelz, 2005; Vasilescu et al., 2006). Implementation of newer techniques such as flow cytometric crossmatching and Luminex assay has improved sensitivity in determining anti-HLA I and HLA II antibodies. Although initially reported to improve pretransplant identification of patients at high risk (Patel et al., 2007), there are a growing number of reports indicating that not all antibodies detected by sensitive solid-phase assays are clinically relevant (Aubert et al., 2009; Opelz and Claas, 2009).

With the development of new cellular technologies, measurement of T-cell sensitization gained more attention. The IFN-γ Elispot assay represents a highly sensitive technique for detecting alloantigen-specific memory T cells (Heeger et al., 1999; Volk and Kern, 2001). Using IFN-γ Elispot, it has been recently demonstrated, that kidney transplant recipients with high frequencies of donor reactive memory T cells before transplantation are at risk of severe acute rejection episodes during the early posttransplant period and of poorer 1-year graft function independently of the occurrence of clinical rejection (Figure 2; Heeger et al., 1999; Nickel et al., 2004; Bellisola et al., 2006; Kim et al., 2007). Allo-specific memory is not necessarily a result of alloantigen exposure. Cross-reactivity is a common feature of the human T-cell repertoire, and the environmentally, e.g. by viral infections, primed recipient MHC-restricted T cells may cross-react with donor MHC–peptide complexes, leading to rapid T-cell activation and early graft injury in individuals who have not been directly immunized by alloantigens. It was further shown that panel reactive memory T-cell reactivity (PRT) might be an additional risk factor for dialysis patients awaiting a transplant (Andree et al., 2006; Augustine et al., 2007; Poggio et al., 2006, 2007). Interestingly, PRT can be independent on the presence of

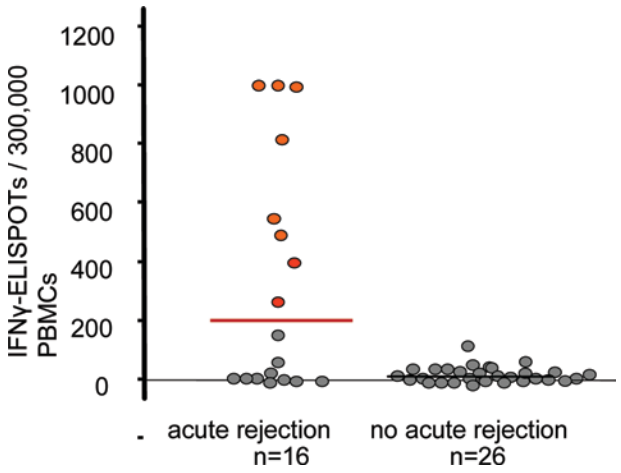

Figure 2. Frequency of donor-reactive IFNγ-producing T cells (ELISPOT) in peripheral blood mononuclear cells of renal transplant patients prior to transplantation experiencing acute rejection or no acute rejection.

humoral sensitization (PRA) because not all PRT-positive patients are PRA positive and *vice versa* (Andree et al., 2006).

The level of donor antigen nonspecific immune parameters can also correlate with graft outcome. These surrogate markers reflect the general activation of the adaptive or innate immunity (Reinsmoen et al., 2008). The CD30 molecule belongs to the tumor necrosis factor receptor (TNF-R) superfamily. In activated T cells, the membrane-bound CD30 molecule is proteolytically cleaved, thereby generating a soluble form (sCD30), which can be measured in serum (Saini et al., 2008; Spiridon et al., 2008). Low serum levels of soluble CD30 were found in healthy humans, whereas increased sCD30 serum concentrations can be detected under pathophysiologic situations (Schlaf et al., 2007). In addition, it has been suggested that pre- or posttransplant levels of sCD30 represent a biomarker for graft rejection associated with an impaired outcome for transplanted patients (Pelzl et al., 2002; Susal et al., 2002, 2003; Heinemann et al., 2007; Langan et al., 2007). Thus, sCD30 seems to reflect the pretransplant activation status of the T cells and thereby allowing the identification of high-risk recipients (Rodriguez et al., 2007; Spiridon et al., 2008). However, Altermann et al. (2007) detected a high interindividual alteration in serum sCD30 levels of potential kidney recipients when analyzed over time, aggravating its implementation as a pretransplant risk stratification marker. Indeed, studies by Platt and Slavcev revealed that, although low sCD30 pretransplant levels identify patients at low risk of developing acute cellular or humoral rejection, an accurate identification of patients at high risk was not possible (Slavcev et al., 2007; Platt et al., 2009).

Posttransplant monitoring of inflammatory processes leading to acute and chronic rejections

The ultimate goal of the posttransplant phase is to avoid not only a state of over-immunosuppression but also under-immunosuppression. Protocol biopsies have revealed inflammation below the Banff threshold of rejection, which was, if persistent, associated with poor allograft function at 1 and 2 years (Mengel et al., 2007). Furthermore, *de novo* expression of CD44 and vimentin on tubular cells in 6-month protocol biopsies can function as a surrogate marker for interstitial fibrosis and tubular atrophy and graft function at 12 months (Kers et al., 2010). In addition, the quality of intragraft cell infiltrate may determine transplant outcome. Brown et al. (2007) showed in a murine kidney allograft model that mononuclear cell infiltration can occur in both rejecting and tolerance-developing grafts, but tolerant kidney grafts demonstrated a higher and sustained level of Foxp3$^+$ regulatory T cells. In contrast, on human graft biopsies, Foxp3$^+$ cells were detected in biopsies with acute cellular rejection type I and II, and the degree of Foxp3$^+$ infiltrates was associated with worse outcome (Veronese et al., 2007). Recently, Yapici et al. (2009) demonstrated that presence of Foxp3$^+$ T cells in infiltrates during acute cellular rejection does not correlate with a response to antirejection therapy but rather predicts renal fibrosis. Thus, protocol biopsies can be informative revealing new mechanisms and cellular players of graft rejection and predicting long-term graft function.

Several groups have studied the urine mRNA and protein profile to detect signs of allograft damage. Following the pioneering work performed by the group of Suthanthiran describing the possibility of a noninvasive diagnosis of renal-allograft rejection by measurement of mRNA for perforin and granzyme B in urine (Li et al., 2001), many other groups could validate the usefulness of this analysis not only to diagnose rejection but also to predict long-term graft function (Kotsch et al., 2004; Muthukumar, et al., 2005; Seiler et al., 2007; Ho et al., 2010). In particular, increase of urinary CXCL10 (IP-10) and its receptor at transcription and protein level is associated with impaired short-term and long-term graft function (Tatapudi et al., 2004; Matz et al., 2006; Hu et al., 2009; Schaub et al., 2009).

As outlined earlier, peripheral blood transcription analysis of biomarkers can also be used to reveal ongoing antidonor immune responses. Following our earlier work, where we could show that peripheral and intragraft Toag-1 expression correlates with transplant outcome in experimental kidney, heart, and liver transplant models (Sawitzki et al., 2007), we now revealed that pre- and posttransplant whole blood expression levels correlate with graft function and histologic signs of acute or chronic rejection (unpublished observation) in transplant patients.

T-cell immune activation status can be also measured by quantifying the stimulation-dependent adenosine triphosphate (ATP) release (iATP) of CD4$^+$ T cells (Sottong et al., 2000; Kowalski et al., 2006, 2007). The so-called ImmuKnow assay (Cylex, Inc., Columbia, MD) allows a direct and standardized analysis of T-cell function and can discriminate between under- and over-immunosuppression (Israeli et al., 2007). The assay has been recently approved by the Food and Drug Administration as a tool to measure lymphocyte reactivity in immune-suppressed patients. High iATP levels are associated with occurrence of acute rejections (Kowalski et al., 2006), whereas patients characterized by low iATP levels have a high risk of developing posttransplant infectious complications and increased risk of short-term mortality (Sanchez-Velasco et al., 2008; Berglund et al., 2011). However, recent results have also shown that iATP levels defining a stable (no rejection + infection), high (rejection), or low-risk (infection) immune response much depends on patient age and immunosuppressive regimen, with transplant recipients younger than 12 years showing lower immune response levels (Hooper et al., 2005; Israeli et al., 2008; Rossano et al., 2009; Serban et al., 2009). Huskey et al. (2011) have shown that a single time point analysis of iATP levels does not allow accurate prediction of future

opportunistic infections or acute rejections, highlighting the importance of longitudinal immune monitoring of transplant patients.

Codarri et al. (2007) have investigated the distribution of two CD4 T-cell populations ($CD4^+CD25^{high}CD127^{low}$Tregs and $CD4^+CD25^+CD127^{high}$ effector T cells) in 36 subjects after liver and kidney transplantation and in 45 healthy subjects using a flow cytometry-based approach. They observed that the Treg population was reduced in transplant recipients. Interestingly, the effector T-cell population was significantly increased in stable transplant recipients compared with healthy subjects, and the expansion of this cell population was even greater in patients with documented humoral chronic rejection compared with stable transplant recipients. The expanded effector T cell contained allo-specific $CD4^+$ T cells secreting effector cytokines such as TNF-α and IFN-γ and were part of the T-cell population infiltrating the allograft of patients with a documented diagnosis of chronic humoral rejection. These results indicate that the $CD4^+CD25^+CD127^{high}$ effector T-cell population may represent a valuable biomarker to monitor antidonor $CD4^+$ T-cell responses both in blood and in tissues after organ transplantation.

Numerous studies have been conducted to determine the prevalence and significance of *de novo* development of HLA antibodies after transplantation (Seveso et al., 2009). From the obtained data, it seems that *de novo* development of anti-HLA antibodies being donor specific or nonspecific is associated with poor long-term graft outcome. However, with the development of new solid-phase antibody detection methods, this general assumption was challenged as the number of antibody-positive patients was markedly increasing. This started a discussion whether these new techniques are too sensitive and whether the obtained results are always clinically relevant. Recently, Lachmann et al. (2009) using the Luminex platform could show that anti-HLA antibodies being either donor-specific or nonspecific serve as biomarkers for chronic rejection. They collected serum samples of 1014 deceased kidney transplant recipients, and the patients were followed up for 5.5 years on serum collection. Especially the presence of donor-specific antibodies was associated with a significant lower graft survival compared with the HLA antibody negative group (49% vs. 83%). These and other reports clearly show that the presence of HLA antibodies correlates with graft failure. In some cases, detection of donor-specific antibodies may give false-negative results because the antibodies can be absorbed by the graft and therefore sequestered from the circulation. Recently, expression analysis of critical factors regulating B-cell maturation and activation such as B-cell activating factor (BAFF) has gained attention. Zhang and coworkers could show that BAFF staining of kidney biopsies was strongly associated with C4d positivity (Xu et al., 2009b) and that peripheral BAFF expression correlated with poorer graft outcome (Xu et al., 2009a). Furthermore, Bloom et al. (2009) showed that BAFF serum levels are increased in renal transplant patients after treatment with alemtuzumab. Because patients treated with alemtuzumab have propensity to develop alloantibodies and may undergo AMR, these data suggested an association between BAFF/BAFF-R and AMR in such patients. Interestingly, Sarwal and coworkers have recently shown that BAFF seems to support the repopulation of circulating B cells after rituximab treatment (Zarkhin et al., 2009). In this trial, patients with CD20+ renal rejections were treated with four doses of rituximab. BAFF levels significantly increased during B-cell depletion. Interestingly, BAFF levels after B-cell depletion positively correlated with higher levels of donor-specific HLA-I antibodies and negatively with creatinine clearance. Thus, BAFF expression levels may allow not only early monitoring of B-cell repopulation but also activation and therefore a valuable tool in revealing developing humoral antidonor immune responses.

Immune reactivity of patients undergoing protolerogenic protocols

In a clinical trial performed by Kawai et al. (2008), complete IS withdrawal could be successfully achieved in HLA-mismatched renal transplantation by a combined bone marrow and kidney transplant with nonmyeloablative regimen. In four of five patients, it was possible to discontinue all IS therapy 9–14 months after transplantation.

However, further analysis revealed that three of the four patients developed *de novo* antibodies to donor antigens and/or C4d deposition in the graft (Porcheray et al., 2009). Two different types of antibody responses could be distinguished in these patients, an early transient response versus a late persistent response. The antidonor antibody response in the two patients showing persistency was accompanied by development of autoantibodies preceded by increased BAFF serum levels. The clinical significance is not clear yet because there are no other evidences for a humoral rejection. This seems to be contradictory to the findings described above. Whether the B-cell responses and antibodies produced in the presence of T-cell unresponsiveness are qualitatively different or whether the patients will develop evidences for chronic humoral rejection has to be investigated in future studies.

Recently, Mathew et al. (2010) described tolerance profiles in donor bone marrow–infused kidney transplant patients using *ex vivo* functional assays. Here, donor bone marrow cells (DBMC) were infused in a setting of living-related haploidentical transplantation in combination with an induction regimen and standard IS maintenance. Results of DBMC-treated recipients were compared with those of noninfused haploidentical control group and HLA identical sibling recipients. The patient samples were tested for presence of donor-specific antibodies, proliferative responses (mixed lymphocyte reaction), killing activity (cell-mediated lympholysis), granzyme B, and IFN-γ

production (Elispot) and trans-vivo DTH responses. The authors conclude that although individual assay reveal signs of unresponsiveness, only a cluster analysis combining these assays will reveal tolerant recipients in whom IS minimization may be safely tested. However, none of the tested patients was off all IS, and the combinatorial analysis has not been prospectively validated yet.

Identification of OT patients

Identifying the so-called OT patients applying biomarkers is crucial for a safe drug withdrawal in a cohort of stable transplant recipients. In recent years, there has been tremendous effort put into profiling of OT patients. As a result of two multinational collaborative networks "Indices of Tolerance" and "Reprogramming the Immune System for Reestablishment of Tolerance RISET" funded by the FP5 and FP6 program of the European Commission and the National Institutes of Health funded "Immune Tolerance Network (ITN)", last year, two reports characterizing the immune profile of OT kidney transplant patients have been published (Newell et al., 2010; Sagoo et al., 2010). Within the European network, OT renal transplant patients, recipients with stable graft function receiving different IS maintenance therapy, recipients undergoing chronic rejection, and healthy subjects were screened for several biomarkers and functional assays to identify a signature of tolerance. The study performed by the ITN aimed to identify immune parameters that would discriminate tolerant individuals from recipients with stable graft function while on IS maintenance and healthy subjects; thus, in contrast to the European approach, specifically excluding patients undergoing chronic rejection. In both cases, the collaborative nature of the project ensured recruitment of a relatively large cohort of OT patients, who represent a rare population among kidney recipients. Furthermore, sample exchange between the European and ITN network allowed validation of identified biomarkers and assays in a large test set on both sides. There were important differences between the European and American OT patient cohort because the European patients were characterized by a high degree of HLA mismatches, mainly received cadaveric transplants, and most were male patients. In both OT patient cohorts, a significantly increased frequencies and absolute numbers of total $CD19^+$ B cells were detected in comparison to patients on maintenance IS therapy or undergoing chronic rejection. Although OT patients of both cohorts were characterized by increased peripheral B-cell frequencies, they did not have detectable antidonor HLA-specific antibodies. Further investigation revealed a particular increase in $CD19^+CD27^-IgD^+$ naïve and $CD19^+CD27^-CD24^+CD38^+IgD^+$ transitional B cells. Increased numbers of B cells have been previously reported in OT kidney recipients (Louis et al., 2006). Determining the B-cell subset expanded in OT patients is of extreme importance because this may help to reveal the underlying mechanism.

The increase in B-cell numbers was accompanied by an enrichment of B-cell–specific genes in peripheral blood samples of OT patients. Although the list of most specifically enriched genes was not identical between the Indices of Tolerance (IOT) and ITN cohort, several B-cell–related genes (e.g. TCL1A, CD20, and CD79b) were common to both lists.

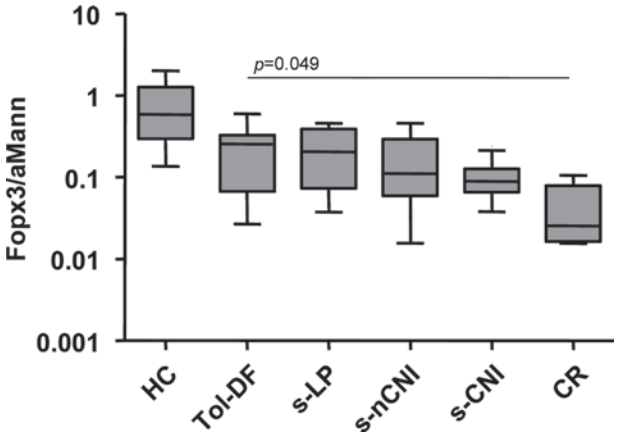

Figure 3. Combined qRT-PCR gene expression analysis of Foxp3 and α-1,2-mannosidase in whole blood samples of different kidney transplant patient groups of the "Indices of tolerance" consortium. HC, healthy controls; Tol-DF, tolerant drug free; s-LP, stale on low dose prednisolone; s-nCNI, stable on non-Calcineurin-inhibitor-based drugs; s-CNI, stable on Calcineurin inhibitors; CR, chronically rejecting patients.

Table 2. Examples of biomarkers and assays that may be used for an immune monitoring of transplant patients.

Pretransplant period	Donor-/HLA-specific antibodies	Presensitization
	Soluble CD30	Presensitization
	IFN-γ Elispot	Presensitization
Posttransplant period	Urinary IP-10	Acute cellular rejection
	Serum BAFF	Humoral rejection
	Cylex ImmunoKnow assay	Acute rejection and infectious complications
	Intragraft vimentin/CD44	Chronic rejection
	TOAG-1 transcription	Acute rejection
	Frequency of $CD4^+CD25^+CD127^{high}$	Chronic humoral rejection
	Ratio Foxp3/aMann	Identification of operational tolerant patients
	Frequency of naïve/transitional B cells	Identification of operational tolerant patients
	Trans-vivo DTH	Regulation of antidonor responses

HLA, human leukocyte antigen; IFN, interferon.

However, analysis of the microarray data resulted in two distinct sets of genes most predictive for tolerance. An OT gene signature was identified using different sample collection (Paxgene vs. Tempus), RNA purification and microarray platforms (Affymetrix vs. Agilent), and data analysis. Such methodologic variations and the apparent different clinical characteristics might explain the observed differences in the OT gene signatures. The B-cell gene signatures obtained by the ITN and IOT trial correlate only in part with findings of Brouard et al. (2007), who used cDNA microarrays to identify tolerance-specific genes. Here, peripheral blood mononuclear cells were used instead of whole blood analyzed within the ITN and IOT studies. Whether the methodologic differences account for the missing overlap has to be investigated in future clinical trials.

Sagoo et al. (2010) also studied direct antidonor T-cell responses of the IOT and ITN OT patients. The IOT cohort was characterized by significantly reduced frequencies of IFN-γ–producing $CD4^+$ T cells when stimulated with donor antigen in comparison to third-party antigen. Although the same tendency was detectable for the ITN trial samples, this was not significant and only low frequencies of cytokine-producing T cells could be detected. This was probably due to high proportion of completely HLA-matched donor grafts within the ITN tolerant patients. Thus, results of functional assays, which are likely influenced by the degree of HLA matching, such as the IFN-γ Elispot, have to be interpreted with caution when screening stable transplant patients for signs of operational tolerance in future prospective clinical trials. Such assays are much more likely to be used for a pretransplant risk assessment (Sawitzki et al., 2009) or when studying longitudinal responses.

The diminished direct T-cell responses indicate an altered balance of regulatory or anti-inflammatory T cells and effector T cells in tolerant patients in comparison to nontolerant stable or chronically rejecting patients. Indeed, although Foxp3 transcription *per se* was not significantly increased in tolerant patients, a combinatorial analysis calculating a ratio of Foxp3 and MAN1A1 transcription (Sawitzki et al., 2007) could discriminate between drug-free patients, stable kidney recipients, or recipients undergoing chronic rejection (Figure 3). This was shown for the IOT training set and the ITN test set. Such findings highlight the importance of analyzing a set of biomarkers reflecting different aspects of the immune response when performing diagnostic trial on transplant patients. However, similar to the results obtained by Mathew et al. (2010), the IOT and ITN tolerance signature have not been prospectively validated yet and thus need to be interpreted with caution.

Conclusions

The results summarized in this review are encouraging. The described biomarkers and functional assays may help us to design comprehensive pre- and posttransplant immune monitoring schemes. A summary of currently described biomarkers and assays that may be used for an immune monitoring of transplant patients is shown in Table 2. However, most of the biomarkers have been identified based on a retrospective analysis of transplant patients. Thus, for future clinical implementation, the biomarker performance needs to be validated in large prospective clinical trials also comparing the sensitivity and specificity of several biomarkers side by side. Thus, for these clinical trials, new monitoring profiles comprising a specifically adapted set of biomarkers and assays at crucial time points need to be designed based on the existing data.

Declaration of interest

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the article.

References

Altermann W, Schlaf G, Rothhoff A, Seliger B. (2007). High variation of individual soluble serum CD30 levels of pre-transplantation patients: sCD30 a feasible marker for prediction of kidney allograft rejection? *Nephrol Dial Transplant* 22:2795–2799.

Andree H, Nickel P, Nasiadko C, Hammer MH, Schönemann C, Pruss A, Volk HD, Reinke P. (2006). Identification of dialysis patients with panel-reactive memory T cells before kidney transplantation using an allogeneic cell bank. *J Am Soc Nephrol* 17:573–580.

Aubert V, Venetz JP, Pantaleo G, Pascual M. (2009). Low levels of human leukocyte antigen donor-specific antibodies detected by solid phase assay before transplantation are frequently clinically irrelevant. *Hum Immunol* 70:580–583.

Augustine JJ, Poggio ED, Clemente M, Aeder MI, Bodziak KA, Schulak JA, Heeger PS, Hricik DE. (2007). Hemodialysis vintage, black ethnicity, and pretransplantation antidonor cellular immunity in kidney transplant recipients. *J Am Soc Nephrol* 18:1602–1606.

Baeten D, Louis S, Braud C, Braudeau C, Ballet C, Moizant F, Pallier A, Giral M, Brouard S, Soulillou JP. (2006). Phenotypically and functionally distinct CD8+ lymphocyte populations in long-term drug-free tolerance and chronic rejection in human kidney graft recipients. *J Am Soc Nephrol* 17:294–304.

Bellisola G, Tridente G, Nacchia F, Fior F, Boschiero L. (2006). Monitoring of cellular immunity by interferon-γ enzyme-linked immunosorbent spot assay in kidney allograft recipients: preliminary results of a longitudinal study. *Transplant Proc* 38:1014–1017.

Berglund D, Bengtsson M, Biglarnia A, Berglund E, Yamamoto S, von Zur-Mühlen B, Lorant T, Tufveson G. (2011). Screening of mortality in transplant patients using an assay for immune function. *Transpl Immunol (In Press)*.

Bloom D, Chang Z, Pauly K, Kwun J, Fechner J, Hayes C, Samaniego M, Knechtle S. (2009). BAFF is increased in renal transplant patients following treatment with alemtuzumab. *Am J Transplant* 9:1835–1845.

Brouard S, Mansfield E, Braud C, Li L, Giral M, Hsieh SC, Baeten D, Zhang M, Ashton-Chess J, Braudeau C, Hsieh F, Dupont A, Pallier A, Moreau A, Louis S, Ruiz C, Salvatierra O, Soulillou JP, Sarwal M. (2007). Identification of a peripheral blood transcriptional biomarker panel associated with operational renal allograft tolerance. *Proc Natl Acad Sci usa* 104:15448–15453.

Brown K, Moxham V, Karegli J, Phillips R, Sacks SH, Wong W. (2007). Ultra-localization of Foxp3+ T cells within renal allografts shows infiltration of tubules mimicking rejection. *Am J Pathol* 171:1915–1922.

Codarri L, Vallotton L, Ciuffreda D, Venetz JP, Garcia M, Hadaya K, Buhler L, Rotman S, Pascual M, Pantaleo G. (2007). Expansion and tissue infiltration of an allospecific CD4+CD25+CD45RO+IL-7Rα high cell population in solid organ transplant recipients. *J Exp Med* 204:1533–1541.

Heeger PS, Greenspan NS, Kuhlenschmidt S, Dejelo C, Hricik DE, Schulak JA, Tary-Lehmann M. (1999). Pretransplant frequency of donor-specific, IFN-γ-producing lymphocytes is a manifestation of immunologic memory and correlates with the risk of posttransplant rejection episodes. *J Immunol* 163:2267–2275.

Heinemann FM, Rebmann V, Witzke O, Philipp T, Broelsch CE, Grosse-Wilde H. (2007). Association of elevated pretransplant sCD30 levels with graft loss in 206 patients treated with modern immunosuppressive therapies after renal transplantation. *Transplantation* 83:706–711.

Ho J, Rush DN, Gibson IW, Karpinski M, Storsley L, Bestland J, Stefura W, HayGlass KT, Nickerson PW. (2010). Early urinary CCL2 is associated with the later development of interstitial fibrosis and tubular atrophy in renal allografts. *Transplantation* 90:394–400.

Hooper E, Hawkins DM, Kowalski RJ, Post DR, Britz JA, Brooks KC, Turman MA. (2005). Establishing pediatric immune response zones using the Cylex ImmuKnow assay. *Clin Transplant* 19:834–839.

Hu H, Kwun J, Aizenstein BD, Knechtle SJ. (2009). Noninvasive detection of acute and chronic injuries in human renal transplant by elevation of multiple cytokines/chemokines in urine. *Transplantation* 87:1814–1820.

Huskey J, Gralla J, Wiseman AC. (2011). Single time point immune function assay (ImmuKnow) testing does not aid in the prediction of future opportunistic infections or acute rejection. *Clin J Am Soc Nephrol* 6:423–429.

Israeli M, Klein T, Sredni B, Avitzur Y, Mor E, Bar-Nathen N, Steinberg R, Dinari G, Shapiro R. (2008). ImmuKnow: a new parameter in immune monitoring of pediatric liver transplantation recipients. *Liver Transpl* 14:893–898.

Israeli M, Yussim A, Mor E, Sredni B, Klein T. (2007). Preceeding the rejection: in search for a comprehensive post-transplant immune monitoring platform. *Transpl Immunol* 18:7–12.

Kawai T, Cosimi AB, Spitzer TR, Tolkoff-Rubin N, Suthanthiran M, Saidman SL, Shaffer J, Preffer FI, Ding R, Sharma V, Fishman JA, Dey B, Ko DS, Hertl M, Goes NB, Wong W, Williams WW Jr, Colvin RB, Sykes M, Sachs DH. (2008). HLA-mismatched renal transplantation without maintenance immunosuppression. *N Engl J Med* 358:353–361.

Kers J, Xu-Dubois YC, Rondeau E, Claessen N, Idu MM, Roelofs JJ, Bemelman FJ, ten Berge IJ, Florquin S. (2010). Intragraft tubular vimentin and CD44 expression correlate with long-term renal allograft function and interstitial fibrosis and tubular atrophy. *Transplantation* 90:502–509.

Kim SH, Oh EJ, Kim MJ, Park YJ, Han K, Yang HJ, Kim JY, Choi BS, Yang CW, Kim YS, Bang BK. (2007). Pretransplant donor-specific interferon-γ ELISPOT assay predicts acute rejection episodes in renal transplant recipients. *Transplant Proc* 39:3057–3060.

Kotsch K, Mashreghi MF, Bold G, Tretow P, Beyer J, Matz M, Hoerstrup J, Pratschke J, Ding R, Suthanthiran M, Volk HD, Reinke P. (2004). Enhanced granulysin mRNA expression in urinary sediment in early and delayed acute renal allograft rejection. *Transplantation* 77:1866–1875.

Kowalski RJ, Post DR, Mannon RB, Sebastian A, Wright HI, Sigle G, Burdick J, Elmagd KA, Zeevi A, Lopez-Cepero M, Daller JA, Gritsch HA, Reed EF, Jonsson J, Hawkins D, Britz JA. (2006). Assessing relative risks of infection and rejection: a meta-analysis using an immune function assay. *Transplantation* 82:663–668.

Kowalski RJ, Zeevi A, Mannon RB, Britz JA, Carruth LM. (2007). Immunodiagnostics: evaluation of functional T-cell immunocompetence in whole blood independent of circulating cell numbers. *J Immunotoxicol* 4:225–232.

Lachmann N, Terasaki PI, Budde K, Liefeldt L, Kahl A, Reinke P, Pratschke J, Rudolph B, Schmidt D, Salama A, Schönemann C. (2009). Anti-human leukocyte antigen and donor-specific antibodies detected by luminex posttransplant serve as biomarkers for chronic rejection of renal allografts. *Transplantation* 87:1505–1513.

Langan LL, Park LP, Hughes TL, Irish A, Luxton G, Witt CS, Christiansen FT. (2007). Post-transplant HLA class II antibodies and high soluble CD30 levels are independently associated with poor kidney graft survival. *Am J Transplant* 7:847–856.

Li B, Hartono C, Ding R, Sharma VK, Ramaswamy R, Qian B, Serur D, Mouradian J, Schwartz JE, Suthanthiran M. (2001). Noninvasive diagnosis of renal-allograft rejection by measurement of messenger RNA for perforin and granzyme B in urine. *N Engl J Med* 344:947–954.

Louis S, Braudeau C, Giral M, Dupont A, Moizant F, Robillard N, Moreau A, Soulillou JP, Brouard S. (2006). Contrasting CD25hiCD4+T cells/FOXP3 patterns in chronic rejection and operational drug-free tolerance. *Transplantation* 81:398–407.

Mathew JM, Ciancio G, Burke GW, Garcia-Morales RO, Rosen A, Wang E, Gomez CI, Blomberg BB, Fuller L, Esquenazi V, Ricordi C, Miller J. (2010). Immune "tolerance profiles" in donor bone marrow infused kidney transplant patients using multiple ex vivo functional assays. *Hum Immunol* 71:566–576.

Matz M, Beyer J, Wunsch D, Mashreghi MF, Seiler M, Pratschke J, Babel N, Volk HD, Reinke P, Kotsch K. (2006). Early post-transplant urinary IP-10 expression after kidney transplantation is predictive of short- and long-term graft function. *Kidney Int* 69:1683–1690.

Mengel M, Gwinner W, Schwarz A, Bajeski R, Franz I, Bröcker V, Becker T, Neipp M, Klempnauer J, Haller H, Kreipe H. (2007). Infiltrates in protocol biopsies from renal allografts. *Am J Transplant* 7:356–365.

Muthukumar T, Dadhania D, Ding R, Snopkowski C, Naqvi R, Lee JB, Hartono C, Li B, Sharma VK, Seshan SV, Kapur S, Hancock WW, Schwartz JE, Suthanthiran M. (2005). Messenger RNA for FOXP3 in the urine of renal-allograft recipients. *N Engl J Med* 353:2342–2351.

Newell KA, Asare A, Kirk AD, Gisler TD, Bourcier K, Suthanthiran M, Burlingham WJ, Marks WH, Sanz I, Lechler RI, Hernandez-Fuentes MP, Turka LA, Seyfert-Margolis VL; Immune Tolerance Network ST507 Study Group. (2010). Identification of a B cell signature associated with renal transplant tolerance in humans. *J Clin Invest* 120:1836–1847.

Nickel P, Presber F, Bold G, Biti D, Schönemann C, Tullius SG, Volk HD, Reinke P. (2004). Enzyme-linked immunosorbent spot assay for donor-reactive interferon-γ-producing cells identifies T-cell presensitization and correlates with graft function at 6 and 12 months in renal-transplant recipients. *Transplantation* 78:1640–1646.

Opelz G; Collaborative Transplant Study. (2005). Non-HLA transplantation immunity revealed by lymphocytotoxic antibodies. *Lancet* 365:1570–1576.

Opelz G, Claas FH. (2009). Which human leukocyte antigen antibodies are really clinically relevant? *Hum Immunol* 70:561–562.

Patel AM, Pancoska C, Mulgaonkar S, Weng FL. (2007). Renal transplantation in patients with pre-transplant donor-specific antibodies and negative flow cytometry crossmatches. *Am J Transplant* 7:2371–2377.

Pelzl S, Opelz G, Wiesel M, Schnülle P, Schönemann C, Döhler B, Süsal C. (2002). Soluble CD30 as a predictor of kidney graft outcome. *Transplantation* 73:3–6.

Platt RE, Wu KS, Poole K, Newstead CG, Clark B. (2009). Soluble CD30 as a prognostic factor for outcome following renal transplantation. *J Clin Pathol* 62:662–663.

Poggio ED, Augustine JJ, Clemente M, Danzig JM, Volokh N, Zand MS, Hricik DE, Heeger PS. (2007). Pretransplant cellular alloimmunity as assessed by a panel of reactive T cells assay correlates with acute renal graft rejection. *Transplantation* 83:847–852.

Poggio ED, Clemente M, Hricik DE, Heeger PS. (2006). Panel of reactive T cells as a measurement of primed cellular alloimmunity in kidney transplant candidates. *J Am Soc Nephrol* 17:564–572.

Porcheray F, Wong W, Saidman SL, De Vito J, Girouard TC, Chittenden M, Shaffer J, Tolkoff-Rubin N, Dey BR, Spitzer TR, Colvin RB, Cosimi AB, Kawai T, Sachs DH, Sykes M, Zorn E. (2009). B-cell immunity in the context of T-cell tolerance after combined kidney and bone marrow transplantation in humans. *Am J Transplant* 9:2126–2135.

Quintana LF, Solé-Gonzalez A, Kalko SG, Bañon-Maneus E, Solé M, Diekmann F, Gutierrez-Dalmau A, Abian J, Campistol JM. (2009).

Urine proteomics to detect biomarkers for chronic allograft dysfunction. *J Am Soc Nephrol* 20:428–435.

Reinsmoen NL, Cornett KM, Kloehn R, Burnette AD, McHugh L, Flewellen BK, Matas A, Savik K. (2008). Pretransplant donor-specific and non-specific immune parameters associated with early acute rejection. *Transplantation* 85:462–470.

Rodriguez DS, Jankowska-Gan E, Haynes LD, Leverson G, Munoz A, Heisey D, Sollinger HW, Burlingham WJ. (2004). Immune regulation and graft survival in kidney transplant recipients are both enhanced by human leukocyte antigen matching. *Am J Transplant* 4:537–543.

Rodríguez LM, París SC, Arbeláez M, Cotes JM, Süsal C, Torres Y, García LF. (2007). Kidney graft recipients with pretransplantation HLA CLASS I antibodies and high soluble CD30 are at high risk for graft loss. *Hum Immunol* 68:652–660.

Rossano JW, Denfield SW, Kim JJ, Price JF, Jefferies JL, Decker JA, Smith EO, Clunie SK, Towbin JA, Dreyer WJ. (2009). Assessment of the Cylex ImmuKnow cell function assay in pediatric heart transplant patients. *J Heart Lung Transplant* 28:26–31.

Rush D. (2009). Can protocol biopsy better inform our choices in renal transplantation? *Transplant Proc* 41:S6–S8.

Sagoo P, Perucha E, Sawitzki B, Tomiuk S, Stephens DA, Miqueu P, Chapman S, Craciun L, Sergeant R, Brouard S, Rovis F, Jimenez E, Ballow A, Giral M, Rebollo-Mesa I, Le Moine A, Braudeau C, Hilton R, Gerstmayer B, Bourcier K, Sharif A, Krajewska M, Lord GM, Roberts I, Goldman M, Wood KJ, Newell K, Seyfert-Margolis V, Warrens AN, Janssen U, Volk HD, Soulillou JP, Hernandez-Fuentes MP, Lechler RI. (2010). Development of a cross-platform biomarker signature to detect renal transplant tolerance in humans. *J Clin Invest* 120:1848–1861.

Saini D, Ramachandran S, Nataraju A, Benshoff N, Liu W, Desai N, Chapman W, Mohanakumar T. (2008). Activated effector and memory T cells contribute to circulating sCD30: potential marker for islet allograft rejection. *Am J Transplant* 8:1798–1808.

Sánchez-Velasco P, Rodrigo E, Valero R, Ruiz JC, Fernández-Fresnedo G, López-Hoyos M, Piñera C, Palomar R, Leyva-Cobián F, Arias M. (2008). Intracellular ATP concentrations of CD4 cells in kidney transplant patients with and without infection. *Clin Transplant* 22:55–60.

Sarwal M, Chua MS, Kambham N, Hsieh SC, Satterwhite T, Masek M, Salvatierra O Jr. (2003). Molecular heterogeneity in acute renal allograft rejection identified by DNA microarray profiling. *N Engl J Med* 349:125–138.

Sawitzki B, Bushell A, Steger U, Jones N, Risch K, Siepert A, Lehmann M, Schmitt-Knosalla I, Vogt K, Gebuhr I, Wood K, Volk HD. (2007). Identification of gene markers for the prediction of allograft rejection or permanent acceptance. *Am J Transplant* 7:1091–1102.

Sawitzki B, Schlickeiser S, Reinke P, Volk HD. (2009). Pretransplant immune risk assessment. *Curr Opin Organ Transplant* 14:650–655.

Schaub S, Nickerson P, Rush D, Mayr M, Hess C, Golian M, Stefura W, Hayglass K. (2009). Urinary CXCL9 and CXCL10 levels correlate with the extent of subclinical tubulitis. *Am J Transplant* 9:1347–1353.

Schlaf G, Altermann WW, Rothhoff A, Seliger B. (2007). Soluble CD30 serum level–an adequate marker for allograft rejection of solid organs? *Histol Histopathol* 22:1269–1279.

Seiler M, Brabcova I, Viklicky O, Hribova P, Rosenberger C, Pratschke J, Lodererova A, Matz M, Schönemann C, Reinke P, Volk HD, Kotsch K. (2007). Heightened expression of the cytotoxicity receptor NKG2D correlates with acute and chronic nephropathy after kidney transplantation. *Am J Transplant* 7:423–433.

Serban G, Whittaker V, Fan J, Liu Z, Manga K, Khan M, Kontogianni K, Padmanabhan A, Cohen D, Suciu-Foca N, Ratner L, Colovai AI. (2009). Significance of immune cell function monitoring in renal transplantation after Thymoglobulin induction therapy. *Hum Immunol* 70:882–890.

Seveso M, Bosio E, Ancona E, Cozzi E. (2009). De novo anti-HLA antibody responses after renal transplantation: detection and clinical impact. *Contrib Nephrol* 162:87–98.

Slavcev A, Honsova E, Lodererova A, Pavlova Y, Sajdlova H, Vitko S, Skibova J, Striz I, Viklicky O. (2007). Soluble CD30 in patients with antibody-mediated rejection of the kidney allograft. *Transpl Immunol* 18:22–27.

Sottong PR, Rosebrock JA, Britz JA, Kramer TR. (2000). Measurement of T-lymphocyte responses in whole-blood cultures using newly synthesized DNA and ATP. *Clin Diagn Lab Immunol* 7:307–311.

Spiridon C, Nikaein A, Lerman M, Hunt J, Dickerman R, Mack M. (2008). CD30, a marker to detect the high-risk kidney transplant recipients. *Clin Transplant* 22:765–769.

Süsal C, Pelzl S, Döhler B, Opelz G. (2002). Identification of highly responsive kidney transplant recipients using pretransplant soluble CD30. *J Am Soc Nephrol* 13:1650–1656.

Süsal C, Pelzl S, Opelz G. (2003). Strong human leukocyte antigen matching effect in nonsensitized kidney recipients with high pretransplant soluble CD30. *Transplantation* 76:1231–1232.

Tatapudi RR, Muthukumar T, Dadhania D, Ding R, Li B, Sharma VK, Lozada-Pastorio E, Seetharamu N, Hartono C, Serur D, Seshan SV, Kapur S, Hancock WW, Suthanthiran M. (2004). Noninvasive detection of renal allograft inflammation by measurements of mRNA for IP-10 and CXCR3 in urine. *Kidney Int* 65:2390–2397.

Terasaki PI, Kreisler M, Mickey RM. (1971). Presensitization and kidney transplant failures. *Postgrad Med J* 47:89–100.

Vasilescu ER, Ho EK, Colovai AI, Vlad G, Foca-Rodi A, Markowitz GS, D'Agati V, Hardy MA, Ratner LE, Suciu-Foca N. (2006). Alloantibodies and the outcome of cadaver kidney allografts. *Hum Immunol* 67:597–604.

Veronese F, Rotman S, Smith RN, Pelle TD, Farrell ML, Kawai T, Benedict Cosimi A, Colvin RB. (2007). Pathological and clinical correlates of FOXP3+ cells in renal allografts during acute rejection. *Am J Transplant* 7:914–922.

Volk HD, Kern F. (2001). Insights into the specificity and function of (allo)antigen-reactive T cells. *Am J Transplant* 1:109–114.

Xu H, He X, Liu Q, Shi D, Chen Y, Zhu Y, Zhang X. (2009a). Abnormal high expression of B-cell activating factor belonging to the TNF superfamily (BAFF) associated with long-term outcome in kidney transplant recipients. *Transplant Proc* 41:1552–1556.

Xu H, He X, Sun J, Shi D, Zhu Y, Zhang X. (2009b). The expression of B-cell activating factor belonging to tumor necrosis factor superfamily (BAFF) significantly correlated with C4D in kidney allograft rejection. *Transplant Proc* 41:112–116.

Yapici U, Bemelman FJ, Scheepstra CG, Roelofs JJ, Claessen N, van der Loos C, van Donselaar-van der Pant K, Bouts AH, Idu MM, Rowshani AT, ten Berge IJ, Florquin S. (2009). Intragraft FOXP3 protein or mRNA during acute renal allograft rejection correlates with inflammation, fibrosis, and poor renal outcome. *Transplantation* 87:1377–1380.

Zarkhin V, Li L, Sarwal MM. (2009). BAFF may modulate the rate of B-cell repopulation after rituximab therapy for acute renal transplant rejection. *Transplantation* 88:1229–1230.