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NONINVASIVE PREDICTION OF ORGAN GRAFT REJECTION AND OUTCOME USING GENE EXPRESSION PATTERNS

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Abstract

Development of predictive, diagnostic and prognostic biomarkers of allograft status and outcome is important and challenging, and may be rewarded with individualized therapy of the organ graft recipient. Herein, we summarize noninvasive mRNA profiling studies for ascertaining allograft status and outcome. Nucleic acid based biomarkers of allograft status have been developed by a number of laboratories but the studies have primarily been single center investigations. Ongoing multi-center trials including the Clinical Trials in Organ Transplantation (<https://www.ctotstudies.org>) should help further define the clinical utility of noninvasively developed mRNA profiles as biomarkers of allograft status and outcome.

Keywords

Transplantation; Biomarker; mRNA; Immune Monitoring; Diagnosis

Organ graft recipients treated with life-long immunosuppressive drugs have a heightened risk of infection and malignancy, whereas insufficient immunosuppressive drug exposure or interruption of drug therapy often increases rejection risk. The development and clinical application of mechanistically informative assays for the characterization of alloimmune responses remain formidable challenges in organ transplantation. Ideally, these tools should identify rejection without resorting to invasive procedures, as well as provide reliable indices of the host's immune status for customizing immunosuppressive drug therapy.

The current gold standard test for the diagnosis of allograft status remains histological examination of the allograft biopsy. The improved understanding of mechanisms of alloimmune response has led to hypothesis-driven candidate molecular markers for the evaluation of the transplant patient. Recent studies have also focused on noninvasive tests of easily accessible biological fluids such as urine and peripheral blood. Herein, we review recent developments in the field of molecular monitoring of the solid organ transplant recipient. This overview discusses mainly results obtained with hypothesis-driven candidate

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mRNA expression patterns ascertained in urine and blood; the clinical application of genome-wide analysis is also briefly addressed since interrogation of the whole transcriptome should yield finer texture to the mechanistic basis for graft dysfunction, rejection, and transplant tolerance. These complementary nucleic acid-based biomarker strategies should ultimately enable the development of personalized management for the organ graft recipient (1, 2).

RATIONALE FOR DEVELOPING NONINVASIVE BIOMARKERS

Post-transplant monitoring currently relies on surveillance of allograft function (e.g. serum creatinine levels in kidney transplantation). An acute alteration in the functional parameter suggests acute rejection occurrence, but confirmation requires the invasive procedure of allograft biopsy. Moreover, this approach recognizes rejection at a relatively advanced stage of immune process and tissue injury, and fails to diagnose subclinical acute rejection: histological rejection in the absence of graft dysfunction (Figure 1). Protocol or scheduled biopsies have been executed mainly in recipients of kidney (3) or heart allografts (4) to detect acute rejection prior to graft dysfunction. Moreover, the diagnosis of subclinical rejection requires multiple biopsies; the biopsy procedures are invasive, complications occur, and sampling errors may bias the histological diagnosis (5). The costs of this procedure need to be considered as well.

Gene/protein expression patterns of noninvasively collected biologic specimens have been investigated as biomarkers of allograft status (1, 2, 6–8). A noninvasive tool has a number of potential advantages including frequent and sequential assessments of recipient's immune status. Molecular perturbations may precede not only graft dysfunction but also histological changes (Figure 1). Importantly, molecular parameters may serve to guide minimization of immunosuppression and individualization of immunosuppressive therapy (Table 1). Clinicians with a more refined knowledge of the recipient's immune status may be able to adjust the immunosuppressive drug therapy that is often responsible for numerous side effects (7). A molecular-based approach that incorporates a set of markers, could function not only as a surrogate for the invasive biopsy procedure, but could also provide predictive, diagnostic, and prognostic information as well as provide mechanistic insights into the pathobiology of allograft dysfunction (Table 1).

STRATEGIES FOR THE DEVELOPMENT OF NONINVASIVE BIOMARKERS

The advances in immunology have led to a more granular understanding of the anti-allograft repertory and have informed biomarker development. Gene products implicated in cellular traffic, physical contact between lymphocytes and targeted cells, target cell damage, and the counter-regulatory responses have been evaluated as biomarkers of allograft status. This 'candidate-gene' approach, allowing the evaluation of only a limited number of hypotheses, has been complemented by genome-wide technologies, which do not require *a priori* assumptions about gene expression patterns and facilitate interrogation of the whole transcriptome. For example, gene expression signatures of peripheral blood cells have been evaluated as noninvasive biomarkers of kidney transplant dysfunction, and were reported as correlates of transplant function and failure (9). The CARGO (Cardiac Allograft Rejection Gene Expression Observation) and the LARGO (Lung Allograft Rejection Gene Expression Observational study) studies have evaluated transcriptional profiles of allograft rejection in heart and lung transplant recipients, respectively (8). Noninvasive tools using proteomic and metabolomic analyses are also in development and appear promising (10–12).

NONINVASIVE URINE-BASED BIOMARKERS OF ALLOGRAFT STATUS

Data from gene expression analysis of renal allograft biopsies have generated candidate genes for evaluation in urine and blood specimens collected from allograft recipients (1, 2, 13). Noninvasive tests using urine is naturally appealing to nephrologists since the urine specimen provides a representative sampling of the entire kidney allograft (7). Table 2 summarizes the urinary cell gene expression patterns that have been evaluated as biomarkers of renal allograft status.

Perforin, a pore forming protein, and granzyme B, a serine peptidase, are integral components of cytotoxic T cell machinery. Li et al. investigated the diagnostic utility of measurement on mRNA encoding cytotoxic attack proteins granzyme B and perforin in urine specimens obtained from renal allograft recipients, and reported that mRNA levels of perforin and granzyme B were significantly higher in the urinary cells obtained from renal allograft recipients with a biopsy confirmed episode of acute rejection than in the patients without an episode of acute rejection. Analysis involving the receiver-operating-characteristic curve demonstrated that acute rejection can be predicted with a sensitivity of 83% and a specificity of 83% using perforin mRNA levels, and with a sensitivity of 79% and a specificity of 77% using granzyme B mRNA levels. Sequential urine specimens, collected during the first 9 days of transplantation, showed that patients who developed acute rejection within the first 10 days had higher levels of mRNA for perforin and granzyme B in samples obtained on days 4 through 6 and 7 through 9 compared to those who did not manifest an early episode of acute rejection. In addition, bacterial urinary tract infection was reported not to confound acute rejection diagnosis made by measurement of granzyme B mRNA in urine (15). However, Yannaraki et al. found that granzyme B mRNA levels are also increased in kidney transplant recipients with urinary tract infection, cytomegalovirus infection, and delayed graft function (16).

Serine proteinase inhibitor-9 (PI-9) is a natural endogenous antagonist of granzyme B/perforin cytolytic pathway. Muthukumar et al. demonstrated that mRNA for PI-9 was overexpressed in urinary cells during acute rejection of renal allograft and that a positive association exists between the levels of PI-9 mRNA and the levels of mRNA for perforin and granzyme B (18).

The activating cytotoxicity receptor NKG2D is expressed by all human NK cells and CD8⁺ T cells and functions as a co-stimulatory receptor for T-cell receptor-mediated signals in naive CD8⁺ T cells (19). Seiler et al. investigated the association between NKG2D mRNA levels and renal allograft rejection and reported that urinary cell NKG2D mRNA levels are high during acute rejection and that elevated levels in allograft biopsy samples were associated with reduced long-term graft function. Moreover, sequential urine samples collected within the first 8 postoperative weeks demonstrated significantly elevated levels of NKG2D mRNA in patients suffering from acute rejection compared to controls suggesting that NKG2D mRNA upregulation may precede clinical diagnosis of acute rejection.

Urinary cell levels of mRNA for granulysin, an effector molecule of cytotoxic cells, have also been reported to increase during acute rejection in kidney transplant patients (20). In the investigation by Kotsch et al., serial analyses of urinary cell levels of granulysin mRNA predicted the subsequent development of acute rejection and the increases in granulysin mRNA levels preceded the increases in serum creatinine levels by weeks.

A number of genes implicated in cellular traffic to the allograft have been studied as biomarkers of allograft status. Chemokines are small peptides that provide signals for the recruitment of different subsets of T cells. Pre-clinical studies have shown that the interaction between the chemokine receptor CXCR3, expressed by activated T cells, and its

ligand interferon-inducible protein-10 (IP-10), leads to the recruitment of activated T cells to sites of inflammation, and plays an important role in acute cardiac allograft rejection (21). Clinical investigations have extended these observations; urinary cell levels of IP-10 mRNA and CXCR3 mRNA were evaluated by Tatapudi et al. as a noninvasive means of elucidating cellular traffic associated with acute rejection of renal allografts (22). Both IP-10 and CXCR3 mRNA levels were highly associated with acute rejection. Acute rejection could be predicted with a sensitivity of 100% and a specificity of 78% using urinary cell IP-10 mRNA levels and with a sensitivity of 63% and a specificity of 83% using urinary cell CXCR3 mRNA levels. These results were extended by Matz et al. who reported that urinary IP-10 protein levels were correlates of both acute rejection episodes and graft function and that elevated levels of urinary IP-10 protein within the first month post-transplantation were predictive of graft function at 6 months even in the absence of acute rejection (23).

The cell surface protein CD103 is a component of the integrin $\alpha E\beta 7$ responsible for binding to E-cadherin expressed by epithelial cells and is critical for intraepithelial homing of T cells (24). Tubulitis, a histologic hallmark of acute rejection of renal allografts, is intratubular localization of graft infiltrating alloreactive cells. CD103 is critical for intraepithelial localization of T cells. Ding et al. therefore investigated the hypothesis that urinary cell levels of CD103 mRNA are a correlate of acute rejection (25). Their study showed that CD103 mRNA levels are higher in urinary cells from the renal allograft recipients with a biopsy confirmed episode of acute rejection than in the patients without acute rejection. A noninvasive assessment of the presence of tubulitis appears feasible by measurement of mRNA for CD103 in urinary cells of renal allograft recipients.

Urinary cell mRNA profiling may help evaluate the regulatory responses associated with acute rejection, as well as prognosticate outcome of renal transplants following an episode of acute rejection. The fork-head box transcription factor FOXP3 is a specification and functional transcription factor for regulatory T cells. Muthukumar et al. measured urinary cell levels of FOXP3 mRNA in 36 renal allograft recipients with acute rejection, 18 subjects with chronic allograft nephropathy (CAN), and 29 subjects with normal (protocol-defined) biopsy results (17). Levels of mRNA for CD25, CD3 ϵ -chain and perforin were also measured in this investigation. Whereas mRNA for FOXP3, CD25, CD3 ϵ -chain and perforin were all higher during an episode of acute rejection than in the group with CAN or normal biopsy group, levels of mRNA for FOXP3 alone: (a) inversely correlated with serum creatinine levels measured at the time of biopsy in the acute rejection group; (b) predicted the reversal of acute rejection, and (c) identified subjects at risk for graft failure within six months after the incident episode of acute rejection (17). Because FOXP3 expression by human T cells may reflect their activation status rather than their regulatory potential, the heightened expression of FOXP3 mRNA in urinary cells, reported by Muthukumar et al., may potentially be from activated T cells without regulatory properties rather than from regulatory T cells. However, the finding that acute rejection reversal was associated with high levels of FOXP3 mRNA in urinary cells support the hypothesis that FOXP3 mRNA was derived, at least in part, from T cells with regulatory activity.

Renesto et al. studied urinary cell mRNA expression of Th1-related molecules, IFN- γ and TIM-3 (26). TIM-3 expression was strongly associated with acute rejection, supporting the critical role of Th1 activation in human alloimmune response.

NONINVASIVE BLOOD-BASED BIOMARKERS OF ALLOGRAFT STATUS

Peripheral blood-based gene expression patterns have been investigated as biomarkers of allograft status. Table 3 lists peripheral blood cell mRNA expression patterns examined as correlates of allograft rejection.

Vasconcellos et al. published the first study on peripheral blood gene expression as a noninvasive screening tool for kidney transplant dysfunction (27). The investigators investigated expression of mRNA for perforin, granzyme B and Fas ligand (FasL) mRNA in peripheral blood mononuclear cells (PBMCs) obtained from renal transplant recipients. Perforin mRNA predicted acute rejection with a sensitivity of 82% and a specificity of 85%; granzyme B mRNA predicted with a sensitivity of 55% and a specificity of 85%, and FasL with a sensitivity of 100% and a specificity of 75%. The upregulated expression of 2 or more genes was diagnostic of acute rejection with a positive predictive value of 100%, and lack of upregulation of 1 or less gene ruled out rejection with a negative predictive value of 95%.

Subsequent studies have confirmed and extended the observation of Vasconcellos et al. that peripheral blood cell levels of mRNA for perforin and granzyme B are useful for the noninvasive diagnosis of acute rejection of kidney allografts (28–33). Simon et al. quantified expression levels of mRNA for perforin and granzyme B mRNA in 364 peripheral blood samples obtained sequentially from 67 patients during the first month post-transplantation (32). In this longitudinal study, the renal allograft recipients were identified as rejecters at a median time interval of 11 days prior to the clinical diagnosis using peripheral blood gene expression patterns. In contrast to the majority of studies supporting the diagnostic utility of levels of mRNA cytotoxic attack proteins, Graziotto et al., have reported that peripheral blood cell levels of mRNA for perforin, granzyme-B, and FasL are not significantly higher during acute rejection compared to no acute rejection and that the mRNA levels are not informative of allograft status (34). In this study, perforin, granzyme B and FasL expression were analyzed in peripheral blood cell collected at the time of a renal dysfunction suggesting acute rejection (26 samples/11 acute rejections) and “per protocol” 2 months post transplant (24 samples/4 acute rejections) in patients with stable renal function, without any suspicion of acute rejection. The authors report that when samples were divided according to the presence/absence of rejection in the “diagnosis” and “protocol” groups, there was a greater, although not significant transcription of the three genes in those with acute rejection. The small sample size of each group of patients may be responsible for the lack of significant association.

Cytokine gene expression patterns have been explored as biomarkers of allograft status. Dugre et al. evaluated levels of mRNA in mitogen-stimulated PBMCs, and reported that levels of mRNA for IL-4, IL-5, IL-6, IFN- γ , as well as perforin, and granzyme B mRNA levels are associated with acute rejection of renal allografts (28). Also, when at least two of these cytokine markers were upregulated in a given patient, 75% of the rejecting recipients were identified against 15% of the non-rejecting patients.

Full T cell activation is contingent upon antigenic signals as well as costimulatory signals generated by the physical contacts between T cell surface proteins and their counter-receptors on antigen presenting cells. In view of the contribution of costimulatory signals to the anti-allograft response, Shoker et al. evaluated CD40L gene expression in peripheral blood CD4⁺ T cells from 20 renal allograft recipients with adequate renal function, 25 patients with acute rejection and/or chronic allograft nephropathy, and 12 normal controls (35). Peripheral blood cell CD40L mRNA levels were significantly higher in kidney transplant recipients with acute rejection and/or chronic allograft nephropathy; the peripheral blood cell CD40L mRNA levels were also correlated also with the acute rejection severity.

Peripheral blood cell gene expression patterns may also be informative of allograft status in recipients of non-renal organ recipients. Cashion et al. determined whether levels of mRNA for granzyme B, perforin, and HLA-DRA in PBMCs are correlates of histologically

confirmed acute rejection of the pancreas allograft (36). In this pilot study, patients with biopsy-proven pancreas rejection (n=7) had higher levels of mRNA for granzyme B, perforin, and HLA-DRA than patients who did not have acute rejection (n=7), although the difference was not statistically significant. Also, patients with biopsy-proven pancreas rejection had higher granzyme B mRNA levels than control subjects with type 1 diabetes (n=6).

Han et al. studied 13 type 1 diabetic recipients of sequential allogeneic islet cell infusions (37). All 13 recipients became insulin independent initially, but 8 of 13 experienced deterioration in their islet graft function and required re-initiation of insulin therapy for glycemic control. Sequential peripheral blood samples from the islet graft recipients showed an elevation in the levels of mRNA for granzyme B, perforin, and FasL 25 to 203 days before the onset of frequent hyperglycemia in their eight back-to-insulin patients. Among the mRNA profiled, granzyme B mRNA levels were the most reliable indicator of impending islet graft failure.

T-cell immune response gene C7 (TIRC7) encodes a T-cell co-stimulatory molecule central to T-cell activation. Shulzhenko et al. investigated the association between cardiac allograft rejection and intragraft and PBMCs TIRC7 mRNA levels (38). Endomyocardial biopsies collected during acute rejection or up to 15 days before rejection showed increased TIRC7 mRNA expression whereas levels of TIRC7 mRNA in PBMCs were lower during acute rejection compared to rejection-free periods.

The expression level of CD27, another co-stimulatory molecule, has been investigated in peripheral blood of cardiac transplant patients (39). Higher levels of CD27 transcripts were observed in biopsies with rejection than in samples without rejection. In accord with the study of Shulzhenko et al., PBMCs collected during rejection showed significantly lower levels of CD27 mRNA than PBMCs collected during rejection-free periods.

Morgun et al. have investigated the relationship between acute cardiac rejection and PBMC levels of mRNA for IL-8, TNF- α , IFN- γ , granzyme B, perforin, and TIRC7 (40). Whereas levels of TIRC7 mRNA were increased in the endomyocardial biopsies showing rejection, there was a decrease in the PBMC levels of mRNAs for TIRC7, perforin, granzyme B, and IFN- γ during rejection. On the other hand, TNF- α and IL-8 transcript levels were augmented in PBMCs collected during rejection (40).

Shoels et al. investigated whether noninvasive diagnosis of cardiac allograft rejection is feasible by measurement of mRNA in peripheral blood cells collected on the day of endomyocardial biopsy (41). Perforin, CD95 ligand, granzyme B, RANTES, CXCR3, COX2, ENA 78 and TGF- β 1 mRNA levels were significantly different in PBMCs of patients with mild to moderate degrees of allograft rejection compared with patients exhibiting no or minor forms of rejection. Using Discriminant analysis, 5 parameters were found to discriminate grade 2 or greater rejection from less than grade 2 rejection with a sensitivity of 84% and a specificity of 82%, as assessed by receiver operating characteristic curve analysis suggesting that quantitative analysis of gene expression in PBMCs may be a valuable tool for noninvasive diagnosis of heart allograft rejection.

CHALLENGES AND PERSPECTIVES

The complexity of the anti-allograft repertory, investigated mostly by profiling a limited set of gene products implicated in cell activation, effector pathways, cell traffic, target cell damage, and regulatory responses, has been complemented using a genome-wide approach to garner mechanistic insights on the type and severity of rejection. DNA microarrays have already been evaluated to develop minimally invasive blood biomarkers for acute and

chronic rejection in kidney graft recipients (reviewed in 42,43) and in heart transplant recipients (reviewed in 8), and to study operational tolerance in kidney (44, 45) and in liver transplant patients (46). We believe that a hypothesis-driven and a mechanistically informed, focused mRNA target panel, and a hypothesis-generating and global gene expression pattern complement each other rather than represent an either-or strategy for gene expression analysis. Moreover, these complementary tools differ in their sensitivity and precision with PCR based approaches being more precise in detecting quantitative differences. Also, PCR based measurements that use the standard curve method allow absolute quantification with respect to mRNA copy number, whereas microarray based gene expression patterns provide relative levels of gene expression rather than absolute levels. Microarray based gene expression profiling, however, allow interrogation of the entire transcriptome, and facilitate the creation of an informative mRNA panel for further confirmation by more sensitive technique such as quantitative PCR assays.

APPLICATION TO OTHER RELEVANT SIDE EFFECTS

The vast majority of the available studies have focused on acute rejection, a major determinant of long-term allograft survival. However, a number of other pathological conditions may also significantly alter long-term allograft function. In kidney transplantation, the progressive scarring process of the allograft, formerly called “chronic allograft nephropathy”, which is secondary to a number of contributory factors such as immunological insults, hemodynamic changes, and calcineurin inhibitor nephrotoxicity, remains the chief cause of kidney transplant failure (47). Recent data suggest that differential expression of a number of transcripts in urinary cells could be a valuable biomarker of chronic allograft nephropathy (48–50). Several laboratories are developing noninvasive tools to molecularly define subgroups of chronic allograft nephropathies (e.g. chronic rejection, calcineurin inhibitor nephrotoxicity), and to detect fibrogenic activity before the advent of fixed renal injury.. Also, differentiation between chronic rejection and chronic cyclosporine toxicity remains a significant challenge in renal transplant monitoring. Koop et al. have reported that intragraft expression of mRNA for laminin beta2 and TGF-beta1 is a distinguishing feature (51). Should noninvasively ascertained gene expression patterns help distinguish chronic rejection from chronic calcineurin inhibitor toxicity, clinical management would be refined.

TRANSLATION FROM EXPLORATORY STUDIES TO CLINICAL PRACTICE

The clinical utility of nucleic acid based biomarkers of allograft status has primarily been demonstrated in single center investigations. A number of multi-center trials including the Clinical Trials in Organ Transplantation (<https://www.ctotstudies.org>), a cooperative research program sponsored by the National Institute of Allergy and Infectious Diseases (NIAID), and with co-sponsorship by the National Heart, Lung and Blood Institute (NHLBI) and the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), should help further define the predictive, diagnostic and prognostic usefulness of urinary cell mRNA profiles and peripheral blood cell mRNA profiles as biomarkers of allograft status and outcome. Such independent and robust testing of molecular monitoring of allograft recipients may also serve as a model for translating proteomic and metabolomic based diagnostic strategies to the clinic. It is also important that future investigations include cost-effectiveness analyses.

Recent reviews have suggested strategies for progressing from biomarker discovery, primarily a scientific exercise, to biomarker “validation” and “qualification”, processes primarily under the aegis of regulatory agencies (53, 54). Although it is beyond the scope of this overview to proffer a roadmap for this fast evolving process, it is worth emphasizing what biomarkers are, and what purposes they may serve. We subscribe to the following

definition proposed by NIH Biomarker Definition Working Group (55): “A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic responses, or pharmacological responses to a therapeutic intervention”. In this light, mRNA profiles, measured at the time of allograft biopsy and predicting acute rejection with a high specificity and sensitivity (14) serve as a diagnostic biomarker; mRNA levels, measured during an episode of acute rejection and predicting responsiveness to anti-rejection therapy (17) serve as a prognostic biomarker. We have listed some of the significant objectives for mRNA profiling of organ graft recipients in Table 1. We believe that the biomarker discovery phase has been more or less accomplished regarding objectives 1, 2, 3 and 4. The ongoing NIH sponsored Cooperative Clinical Trials in Transplantation that are currently in the recruitment phase should validate or refute the hypothesis that urinary cell and peripheral blood mRNA profiles as predictive biomarkers (mRNA levels in sequential samples predicting the subsequent development of acute rejection or maintain stable graft function) and noninvasive diagnostic biomarkers (mRNA levels measured at the time of diagnostic allograft biopsies predictive of allograft histopathology) of acute rejection of renal allografts. These trials are also designed to test the hypothesis that sequential urinary cell/peripheral blood cell mRNA profiles predict renal allograft status (stable graft function vs. acute rejection) and function. Should future studies further elucidate that the measured biomarker is causatively involved in the biologic process studied (e.g., acute rejection), the biomarker can be designated as a mechanistic biomarker as well, and help accomplish the objective of developing mechanism based therapy (Objective 5, Table 1).

There is considerable interest in developing biomarkers for drug discovery, development, and personalized treatment. There is also interest in utilizing biomarkers as surrogate end points for clinical events (e.g., cholesterol for cardiovascular events). We are not aware any molecular biomarkers that are clinically utilized to titrate immunosuppressive therapy in an individualized fashion. The data required to accomplish this important goal (Objective 6, Table 1), in our opinion, can be accomplished by designing appropriate clinical trials in which immunosuppressive drug therapy is titrated to maintaining mRNA levels within a pre-determined levels in a fashion similar to maintaining blood pressure levels with the use of anti-hypertensive drugs or blood glucose levels with hypoglycemic agents. Such “interventional” trials are contingent upon robust data from observational studies demonstrating that mRNA levels in sequential samples are predictive of allograft status. It is self evident that interventional trials have adequate safety parameters (e.g., protocol biopsy, monitoring for donor specific antibodies) embedded in them as well as be supervised by an independent Data Safety Monitored Board.

CONCLUSION

mRNA profiling of urinary cells and peripheral blood cells is fast evolving as a noninvasive approach for predicting the development of an episode of acute rejection, as a substitute for the invasive allograft biopsy procedure and for prognosticating allograft outcome. Large scale and prospective multi-center studies are ongoing to investigate the clinical utility of molecular biomarkers identified in single center investigations. We hypothesize that gene expression patterns would facilitate in the near future individualization of immunosuppressive therapy in organ graft recipients in a fashion similar to blood pressure monitoring guiding antihypertensive therapy and plasma glucose levels and hemoglobin A1c measurements guiding diabetes management.

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Abbreviations

CTL	Cytotoxic T lymphocytes
mRNA	messenger RNA
PCR	Polymerase chain reaction

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Rejection: A Time-Line Model

Figure 1. A Time-Line Model for Allograft Rejection

In this formulation, rejection defined by molecular markers precedes histologically defined rejection and this precedes clinically defined rejection. The hypothesis that early intervention is highly efficacious is an important rationale for the development of molecular surveillance strategies to anticipate histologic and clinical rejection.

Table 1**Objectives of mRNA Profiling of Organ Graft Recipients**

1	Diagnose rejection by noninvasive means and obviate the need for the invasive procedure of allograft biopsy
2	Anticipate the subsequent development of rejection prior to the development of tissue injury
3	Prognosticate the outcome of an episode of rejection, and responsiveness to anti-rejection therapy
4	Predict subsequent allograft function
5	Help develop mechanism-based therapy
6	Facilitate individualization/optimization of immunosuppressive drug therapy including weaning or reintroduction of therapy

Urinary Cell Biomarkers of Renal Allograft Rejection and Outcome.

Table 2

Author (year)	N (specimens/patients)	mRNA markers during acute rejection	Diagnosis value	Predictive value	Ref.
Li <i>et al</i> (2001)	151/85	Perforin (se=83%; sp=83%) and GB (se=79%; sp=77%) mRNAs increased; mRNA upregulation preceded acute rejection diagnosis			(14)
Dadhania <i>et al</i> (2003)	99/99	GB mRNA increased; UIT did not increase GB mRNA levels			(15)
Ding <i>et al</i> (2003)	89/79	CD 103 mRNA increased (se=59%; sp=75%)			(25)
Muthukumar <i>et al</i> (2003)	95/87	PI-9 (se=76%; sp=79%), GB, and perforin mRNAs increased; PI-9 predicted acute rejection histology grade and graft function 6 months later			(18)
Kotsch <i>et al</i> (2004)	221/26	Granulysin mRNA increased; Granulysin upregulation preceded acute rejection diagnosis			(20)
Tatapudi <i>et al</i> (2004)	63/58	IP-10 (se=100%; sp=78%) and CXCR3 (se=63%; sp=83%) mRNAs increased			(22)
Muthukumar <i>et al</i> (2005)	83/83	FOXP3 mRNA increased, inversely correlated with serum creatinine level, and predictive of reversibility of acute rejection (se=90% and sp=73% for reversal prediction)			(17)
Yannaraki <i>et al</i> (2006)	162/37	Perforin, GB, and FasL mRNAs increased in acute rejection, urinary tract infection, cytomegalovirus infection, and delayed graft function			(16)
Matz <i>et al</i> (2006)	-/96	IP-10 mRNA increased; urinary IP-10 protein was predictive of graft function			(23)
Renesto <i>et al</i> (2007)	72/72	Tim-3 and IFN- γ mRNAs increased			(26)
Seiler <i>et al</i> (2007)	-/117	NKG2D mRNA increased (se=57%; sp=67%); mRNA upregulation preceded acute rejection diagnosis			(19)

Abbreviations: GB: granzyme B; se: sensitivity; sp: specificity; UIT: urinary tract infection; PI-9: serine proteinase inhibitor-9; IP-10: interferon-inducible protein-10.

Table 3

Peripheral Blood Cell Biomarkers of Allograft Rejection.

Organ Author (year)	N (specimens/patient s)	mRNA markers during acute rejection	Diagnosis value	Predictive value	Ref.
Kidney transplantation					
Vasconcellos <i>et al</i> (1998)	31/25	GB (se:55%; sp:85%), perforin (se:82%; sp:85%), and FasL (se:100%; sp:75%) mRNAs increased			(27)
Shoker <i>et al</i> (2000)	-/57	CD40L mRNA increased in patients with acute rejection and/or CAN			(35)
Dugre <i>et al</i> (2000)	-/21	IL-4, IL-5, IL-6, IFN- γ , GB, and perforin mRNAs increased			(28)
Sabek <i>et al</i> (2002)	27/27	GB (se: 63%; sp:95%), perforin (se: 63%; sp:74%), and HLA-DRA (se:88%; sp:79%) mRNAs increased. Combination of HLA-DRA and GB showed a PPV of 100% and a NPV of 83%			(30)
Netto <i>et al</i> (2002)	206/29	GB (se: 85%; sp:100%), perforin (se: 85%; sp:100%), and FasL (se:100%; sp:100%) mRNAs increased			(29)
Simon <i>et al</i> (2003)	364/67	GB (se: 87%; sp:72%), and perforin (se: 90%; sp:82%) mRNAs increased; rejection diagnosis was possible 2–30 days before traditional diagnosis (median 11 days)			(32)
Shin <i>et al</i> (2005)	88/15	Perforin (but not GB and FasL) mRNA increased			(31)
Veale <i>et al</i> (2006)	268/46	GB and perforin mRNAs increased (se: 50%; sp: 95%)			(33)
Graziotto <i>et al</i> (2006)	64/-	GB, perforin, and FasL mRNAs increased (not significantly)			(34)
Islet transplantation					
Han <i>et al</i> (2006)	-/13	GB, perforin, and FasL mRNAs upregulation preceded the onset of frequent hyperglycemia			(37)
Pancreas transplantation					
Cashion <i>et al</i> (2006)	-/31	GB, perforin, and HLA-DRA mRNAs increased (not significantly)			(36)
Heart transplantation					
Shulzenko <i>et al</i> (2001)	80/16	TIRC7 mRNA increased in the biopsy, and significantly decreased in the blood			(38)
Morgun <i>et al</i> (2001)	16/9	TIRC7, perforin, GB, and IFN- γ mRNAs decreased, and TNF- α and IL-8 mRNAs increased			(40)
Morgun <i>et al</i> (2003)	89/31	CD27 mRNA increased in the biopsy and significantly decreased in the blood			(39)
Schoels <i>et al</i> (2004)	58/44	Perforin, CD95 ligand, GB, RANTES, CXCR3, COX2, ENA 78 and TGF- β 1 mRNAs discriminate patients with mild to moderate degrees of allograft rejection compared with patients exhibiting no or minor forms of rejection			(41)

Abbreviations: GB: granzyme B; se: sensibility; sp: specificity; CAN: chronic allograft nephropathy; PPV: positive predictive value; NPV: negative predictive value; TIRC7: T-cell immune response cDNA