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Noninvasive Diagnosis of Acute Rejection of Renal Allografts

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Abstract

Purpose of review—Acute rejection is an immune process that begins with the recognition of the allograft as non-self and ends in graft destruction. Histological features of the allograft biopsy are currently used for the differential diagnosis of allograft dysfunction. In view of the safety and the opportunity for repetitive sampling, development of noninvasive biomarkers of allograft status is an important objective in transplantation. Herein we review some of the progress towards the development of noninvasive biomarkers of human allograft status.

Recent findings—Urinary cell and peripheral blood cell messenger RNA profiles have been associated with acute rejection of human renal allografts. Emerging data support the idea that development of noninvasive biomarkers predictive of antibody-mediated rejection is feasible. The demonstration that intragraft micro RNA expression predicts renal allograft status suggests that noninvasively ascertained miRNA profiles may be of value.

Summary—We are pleased with the progress to date, and anticipate clinical trials investigating the hypotheses that noninvasively ascertained mRNA profiles: (a) will minimize the need for invasive biopsy procedures; (b) predict the development of acute rejection and chronic allograft nephropathy; (c) facilitate preemptive therapy capable of preserving graft function; and (d) facilitate personalization of immunosuppressive therapy for the allograft recipient.

Keywords

Transplantation; Acute Rejection; mRNA Profiling

INTRODUCTION

Acute rejection is an allograft destructive immune response that may occur at any time during the life-span of an organ transplant. The mechanistic pathways of acute rejection are being resolved, and the consequences of immune rejection are evidenced by graft dysfunction and classified by histological features of the allograft biopsy specimen [1••]. Percutaneous needle biopsy of the renal allograft is currently used to diagnose acute rejection of renal allografts. Besides being an invasive procedure, sampling errors and inter-observer variations are additional concerns [2,3].

We have hypothesized a time-line model to illustrate the development of acute rejection as a continuum, with initial events identified by molecular perturbations, and histological changes

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and clinical manifestations being relatively latter events ([4••], Figure 1). In this conceptualization, biomarkers may serve not only as diagnostic parameters but also as predictive biomarkers that anticipate the subsequent development of sub-clinical and clinical acute rejection. Towards these objectives, we and others have investigated the hypotheses that urine and peripheral blood cell profiles offer a noninvasive means of predicting the development of acute rejection and are diagnostic of biopsy confirmed acute rejection [reviewed in (4)]. In our laboratory, we have also tested the postulate that urinary cell mRNA profiles that include measurement of mRNA for FOXP3 predicts the outcome of an episode of acute rejection [5]. Whereas our studies have been hypothesis driven and focused on mechanistically related genes, others have cast a wider net by the clinical application of "omics" technology and have developed biomarkers predictive of allograft status [6,7•].

PLATFORMS FOR BIOMARKER DISCOVERY AND VALIDATION

Existing technologies to investigate gene expression patterns can be considered as those that are hypothesis testing (candidate-gene approach) or hypothesis generating (genome-wide sweep). Each approach has its own merits and limitations, and we believe that the two strategies are complementary rather than competitive. In addition to nucleic acid based assays, proteomics and metabolomics based approaches represent additional avenues for the development of biomarkers of allograft status.

Techniques have evolved to assess cell functions as well. Enzyme-linked immunosorbent spot assay (ELISPOT) testing detects the production of cytokines such as interferon-gamma (IFN-γ) in response to alloantigens, whereas the Immunknow® assay exploits ATP production in response to polyclonal mitogen phytohaemagglutinin as a measure of immune competence.

Flow cytometry and Luminex platform serve well to detect clinically relevant anti-allograft antibodies. Some of the tools used for biomarker discovery are summarized in Table 1, and reviewed by Hartono *et al.* [8].

NONINVASIVE BIOMARKERS OF ALLOGRAFT STATUS

A number of laboratories have investigated whether urinary cell and peripheral blood cell mRNA profiles are diagnostic of acute rejection in renal allografts [4]. A summary of data from the published studies is provided in Table 2.

URINARY CELL MESSENGER RNA PROFILES DIAGNOSTIC OF ACUTE REJECTION OF RENAL ALLOGRAFTS

Cytotoxic T lymphocytes (CTL) have been implicated in allograft rejection, and data exist that both perforin and granzyme B contribute to the cytotoxic T cell machinery. Li *et al.* investigated the hypothesis that measurement of urinary cell levels of mRNA for perforin and granzyme B offers a noninvasive means of diagnosing acute rejection of renal allografts, and reported that acute rejection is predicted with a high degree of accuracy by urinary cell levels of perforin mRNA and granzyme B mRNA [9]. Muthukumar *et al.* and Dadhania *et al.* have confirmed that the levels of mRNAs encoding cytotoxic attack molecules are diagnostic of acute rejection of renal allografts [10,11], and showed further that mRNA for serine proteinase inhibitor-9 (PI-9), an endogenous antagonist of granzyme B, is increased in urine of patients with acute rejection [10], and bacterial urinary tract infection in renal allograft recipients is not associated with an increase in the levels of granzyme B in urine [11].

The cell surface protein CD103 is a natural ligand for E-cadherin, and Ding *et al.* reported that CD103 mRNA levels in urine are higher in patients with acute rejection compared to those without acute rejection [12]. Urinary cell levels of mRNA for granulysin, an effector molecule

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of expressed on cytotoxic T cells, were found to be diagnostic of acute rejection by Kotsch *et al*. [13]. Urinary cell levels of mRNA for chemokines and chemokine receptors have also been associated with acute rejection of renal allografts. Tatapudi *et al.* reported that levels of IP-10 mRNA and CXCR3 mRNA are diagnostic of acute rejection [14], and Matz *et al.* observed that levels of IP-10 mRNA as well as levels of IP-10 protein are diagnostic of acute rejection [15].

The role of FOXP3 expressing T regulatory cells in transplantation is being explored in a number of laboratories. Muthukumar *et al.* measured urinary cell levels of FOXP3 mRNA in renal allograft recipients and reported that levels of mRNA for FOXP3 in urine is increased during an episode of acute rejection, and the levels were inversely correlated with serum creatinine levels measured at the time of biopsy in the acute rejection group [5].

NKG2D, the activating cytotoxicity receptor, is expressed by all human NK cells and was reported by Seiler et al. that acute rejection of renal allografts is associated with an increase in urinary cell levels of NKG2D mRNA [17].

Urinary cell levels of mRNA for TIM-3, a type 1 membrane protein selectively expressed on the surface of terminally differentiated T-helper 1 cells, have been associated with acute rejection by Renesto *et al.* [18] and by Manfro *et al.* [19••]. Importantly, in the investigation by Manfro *et al.* urinary cell TIM-3 mRNA levels distinguished the 28 renal allograft recipients with delayed graft function (DGF) and biopsy diagnosis of acute rejection and acute tubular necrosis (ATN) from the 22 the recipients with DGF and biopsy diagnosis of ATN with a sensitivity of 100% and specificity of 100% [19]. Aqiuno-Dias *et al.* have extended the diagnostic accuracy of mRNA profiles in recipients with DGF, and reported that urinary cell levels of mRNA for perforin, granzyme B, FasL, PI-9 and FOXP3 predict acute rejection with a very high degree of accuracy [20•]. In this study, urinary cell FOXP3 mRNA levels were the most accurate and predicted acute rejection with a sensitivity of 100% and a specificity of 100%.

Immune response directed at infections could confound the diagnostic utility of inflammatory gene based signatures of acute rejection, and a number of laboratories have addressed this important concern. Whereas Dadhania *et al.* found that granzyme B mRNA levels are not increased in renal allograft recipients with bacterial urinary tract infection [11], Yannaraki *et al.* reported that mRNAs were not only increased during acute rejection but also in patients diagnosed with complications such as UTI, CMV infection, and DGF [16]. In accord with findings of Dadhania *et al.* that UTI is not associated with an increase in granzyme B mRNA in urine, Ozbay *et al.* found that granzyme B mRNA levels in urine are not increased in renal allograft recipients with bacteriuria and that both granzyme B and granulysin levels in urine distinguish acute rejection from bacteriuria [21]. It was also found in this study that perforin mRNA levels distinguish acute rejection from CMV infection.

PERIPHERAL BLOOD CELL MESSENGER RNA PROFILES DIAGNOSTIC OF ACUTE REJECTION OF ALLOGRAFTS

Vasconcellos *et al.* investigated expression patterns of mRNA for perforin, granzyme B, and Fas ligand (FasL) in peripheral blood mononuclear cells (PBMCs) collected from renal transplant recipients and reported that 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% [22]. In this study, the up-regulated expression of 2 or more genes was diagnostic of acute rejection with a positive predictive value of 100%, and a lack of up-regulation of any gene ruled out rejection with a negative predictive value of 95%.

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The expression patterns of mRNA for cytokines have also been found to be informative of allograft status. Dugre *et al.* reported that levels of mRNA for IL-4, IL-5, IL-6, IFN-γ, as well as perforin, and granzyme B mRNA levels were correlates of acute rejection of renal allografts [23]. Levels of mRNAs for proteins central to costimulation have been associated with acute rejection. Shoker *et al.* have reported that peripheral blood cell CD40L mRNA levels are higher in kidney allograft recipients with acute rejection and/or chronic allograft nephropathy, and their levels were also predictive of acute rejection severity [24].

The original observations of Vasconcellos *et al.* that peripheral blood cell levels of perforin and granzyme are informative of renal allograft status [22] have been confirmed and extended by Sabek *et al.* [25], Netto *et al.* [26], Simon *et al.* [27], Shin et al. [28] and Veale *et al.* [29]. In contrast, Graziotto *et al.* 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 biopsy diagnosis [30].

A significant percentage of recipients of deceased donor grafts suffer from DGF and noninvasive diagnosis of acute rejection in this setting is of considerable significance. Manfro *et al.* have reported that peripheral blood cell levels of TIM-3 mRNA predict acute rejection in renal allograft recipients with DGF with a sensitivity of 100% and a specificity of 100% [19]. Aquino-Dias *et al.* have reported that peripheral blood cell levels of perforin, granzyme B, FasL, PI-9 and FOXP3 predict acute rejection in renal allograft recipients with DGF with a high degree of precision [20].

BIOMARKERS PREDICTIVE OF SUBSEQUENT DEVELOPMENT OF ACUTE REJECTION

Table 3 provides a summary of published biomarker studies that have been reported to predict the development of an episode of acute rejection allograft status.

EMERGING BIOMARKERS OF RENAL ALLOGRAFT REJECTION

The association between the presence of donor specific antibodies (DSA) and acute rejection has been noted in the late 1970s [40]. With the effective control of T cell mediated acute rejection with the current immunosuppressive regimens, antibody-mediated rejection (AMR) of renal allograft has re-emerged as an important post-transplant complication.

Ashton-Chess *et al.* investigated mRNA for Tribbles-1 (TRIB1) as a biomarker for chronic AMR [41••]. TRIB1, an intracellular human homolog of Drosophila tribbles is involved in toll-like receptor-mediated response and in the regulation of nuclear factor κB and mitogen-activated protein kinases. Intragraft expression of TRIB1 mRNA was higher in biopsies with chronic AMR compared to normal one. PBMC levels of TRIB1 mRNA were also higher during chronic AMR. Levels of TRIB1 mRNA in biopsy samples and PBMC distinguished transplant glomerulopathy with positive C4d staining and anti-HLA antibodies from transplant glomerulopathy without positive C4d staining and anti-HLA antibodies. The authors did not find urinary cell levels TRIB1 mRNA to be informative of chronic AMR. Further studies examining the clinical utility of TRIB1 as a biomarker for antibody mediated rejection are worthy of pursuit.

Sis *et al.* investigated 173 renal allograft biopsies for-cause for intragraft expression of endothelial-associated transcripts (ENDATs) with the use of Affymetrix microarrays [42••]. The mean ENDAT scores were higher in biopsies showing rejection, and was also higher in AMR compared to T cell-mediated rejection. Death censored graft survival rates were inferior in those with antibodies, C4d and a high ENDAT score (ACE group) compared to no ACE

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group. However, a high ENDAT score alone or the presence of antibodies alone did not impact graft survival.

MICRO RNAs AS BIOMARKERS OF ALLOGRAFT STATUS

Micro RNAs (miRNAs) are small non-coding RNAs approximately 22 nucleotides long that regulate gene expression by inducing translational repression, mRNA degradation, and/or transcriptional inhibition [43]. A single miRNA has the ability to regulate the expression of hundreds of mRNAs. miRNAs have been shown to control processes such as cellular survival, development, differentiation, proliferation [44] as well as modulate both innate and adaptive immunity [45]. Anglicheau *et al*. recently identified several miRNAs predictive of acute rejection of human renal allografts [46••]. The hypothesis that urinary cell and/or peripheral blood cell miRNA expression profiles are predictive, diagnostic and/or prognostic biomarkers of allografts is worthy of investigation.

POTENTIAL CONFOUNDING FACTORS

Issues related to bacterial infections confounding the diagnostic utility of inflammatory gene based signatures of acute rejection have been addressed in the earlier section. Herein we discuss the issues related to polyomavirus BK- associated nephropathy (PVAN) [47]. An existing challenge is to distinguish allograft dysfunction due to PVAN from acute rejection. Rogers *et al.* investigated whether immunophenotyping of renal allograft infiltrates help in the differential diagnosis of PVAN versus acute rejection [48]. The investigators performed immunohistochemical analysis of 10 biopsy samples from 10 renal allograft recipients with PVAN and 20 biopsy samples from 20 patients with acute rejection. They found that the percentage of perforin positive cells were significantly different between the PVAN and acute rejection biopsies; they also found that the percentages of CD20-stained cells and granzyme B positive cells were not different between the PVAN and acute rejection biopsies [48]. Mannon *et al.* investigated mRNA profiles of renal allograft biopsies from 10 patients with PVAN and from 17 patients with acute rejection [49]. Banff inflammation and tubulitis scores were not different between the two groups whereas transcripts for CD8, CXCR3, Perforin, HLA-DR, and IFN-γ were significantly higher in the PVAN biopsies compared to the acute rejection group. Moreover, mRNA for TGFβ, MMP2 and 9, collagen I and IV, fibronectin were higher in the PVAN group compared to the acute rejection group [49]. An important goal in this area is to investigate whether acute rejection could be distinguished from PVAN noninvasively. In this regard, a major challenge is to exclude the possibility that the inflammatory signal associated with PVAN is due to PVAN alone and not due to co-existing acute rejection.

Technical issues may also confound biomarker discovery. Excessive globin mRNA in red blood cells has been reported to influence expression profiling of whole blood specimens. Field *et al.* reported that globin reduction resulted in the detection of additional 2652±395 genes when the Affymetrix HU133A 2.0 arrays was used to profile whole blood collected using the PAXgene blood RNA system [50]. Tian *et al.* found a lower than expected present call rates and high degree of sample-to-sample variability when whole blood samples were profiled using Affymetrix microarrays for biomarker discovery, and recommended both globin gene reduction and hybridization on Illuminia BeadChips [51]. Li *et al.* recommended the use of isolated leukocytes instead of whole blood, globin reduction, and mathematical depletion to alleviate the confounding globin molecular signatures [52].

CONCLUSIONS

Noninvasive strategies by allowing repetitive sampling should facilitate detection of immune rejection prior to fixed tissue injury. Importantly, noninvasive ascertained parameters may

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inform the timing of the biopsy procedure, and the information gleaned from both strategies may complement one another. We are optimistic of the progress to date, and anticipate clinical trials investigating the hypotheses that noninvasively ascertained mRNA profiles will minimize the need for an invasive allograft biopsy procedure; predict the development of acute rejection and chronic allograft nephropathy and facilitate preemptive therapy capable of preserving graft function; and bring about personalization of immunosuppressive therapy for the organ graft recipient.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as: • of special interest •• of outstanding interest

1. 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. [PubMed: 18294345] An update on the collaborative international effort to refine the classification and interpretation of allograft biopsies.
2. Sorof JM, Vartanian RK, Olson JL, et al. Histopathological concordance of paired renal allograft biopsy cores. Effect on the diagnosis and management of acute rejection. Transplantation 1995;60:1215. [PubMed: 8525513]
3. Furness, PN.; Taub, N. International variation in the interpretation of renal transplant biopsies: report of the CERTRAP project; Kidney Int. 2001. p. 1998
4. Anglicheau D, Suthanthiran M. Noninvasive prediction of organ graft rejection and outcome using gene expression patterns. Transplantation 2008;86:192. [PubMed: 18645476] A comprehensive review of data regarding mRNA profiles in organ graft recipients
5. Muthukumar T, Dadhania D, Ding RC, et al. Messenger RNA for FOXP3 in the urine of renal-allograft recipients. N Engl J Med 2005;353:2342. [PubMed: 16319383]
6. 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. [PubMed: 15307835]
7. Khatri P, Sarwal MM. Using gene arrays in diagnosis of rejection. Curr Opin Organ Transplant 2009;14:34. [PubMed: 19337144] An update on the use of microarray technology in organ transplantation.
8. Hartono C, Dadhania D, Suthanthiran M. Noninvasive diagnosis of acute rejection of solid organ transplants. Frontiers in Bioscience 2004;9:145. [PubMed: 14766354]
9. Li B, Hartono C, Ding RC, et al. Noninvasive diagnosis of renal-allograft rejection by measurement of messenger RNA for perforin and granzyme B in urine. N Engl J Med 2001;344:947. [PubMed: 11274620]
10. Muthukumar T, Ding RC, Dadhania D, et al. Serine proteinase inhibitor-9, an endogenous blocker of granzyme B/perforin lytic pathway, is hyperexpressed during acute rejection of renal allografts. Transplantation 2003;75:1565. [PubMed: 12792516]
11. Dadhania D, Muthukumar T, Ding R, et al. Molecular signatures of urinary cells distinguish acute rejection of renal allografts from urinary tract infection. Transplantation 2003;75:1752. [PubMed: 12777869]
12. Ding R, Li B, Muthukumar T, et al. CD103 mRNA levels in urinary cells predict acute rejection of renal allografts. Transplantation 2003;75:1307. [PubMed: 12717221]
13. Kotsch K, Mashreghi MF, Bold G, et al. Enhanced granulysin mRNA expression in urinary sediment in early and delayed acute renal allograft rejection. Transplantation 2004;77:1866. [PubMed: 15223905]

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14. Tatapudi RR, Muthukumar T, Dadhania D, et al. Noninvasive detection of renal allograft inflammation by measurements of mRNA for IP-10 and CXCR3 in urine. Kidney Int 2004;65:2390. [PubMed: 15149352]
15. 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. [PubMed: 16572110]
16. Yannaraki M, Rebibou J-M, Ducloux D, et al. Urinary cytotoxic molecular markers for a noninvasive diagnosis in acute renal transplant rejection. Transplant International 2006;19:759. [PubMed: 16918537]
17. Seiler M, Brabcova I, Viklicky O, et al. Heightened expression of the cytotoxicity receptor NKG2D correlates with acute and chronic nephropathy after kidney transplantation. Am J Transplant 2007;7:423. [PubMed: 17173658]
18. Renesto PG, Ponciano VC, Cenedeze MA, et al. High expression of Tim-3 mRNA in urinary cells from kidney transplant recipients with acute rejection. Am J Transplant 2007;7:1661. [PubMed: 17430399]
19. Manfro RC, Aquino-Dias EC, Joelsons G, et al. Noninvasive Tim-3 messenger RNA evaluation in renal transplant recipients with graft dysfunction. Transplantation 2008;86:1869. [PubMed: 19104436] The clinically important issue of noninvasively diagnosing acute rejection in patients with DGF is reported in this manuscript.
20. Aquino-Dias EC, Joelsons G, da Silva DM, et al. Non-invasive diagnosis of acute rejection in kidney transplants with delayed graft function. Kidney Int 2008;73:877. [PubMed: 18216781] The report extends the findings in the report of Manfro et al. that noninvasive diagnosis acute rejection is feasible in patients with DGF.
21. Ǿzbay A, Torring C, Olsen R, et al. Transcriptional profiles in urine during acute rejection, bacteriuria, CMV infection and stable graft function after renal transplantation. Scandinavian Journal of Immunology 2009;69:357. [PubMed: 19284501]
22. Vasconcellos LM, Schachter AD, Zheng XX, et al. Cytotoxic lymphocyte gene expression in peripheral blood leukocytes correlates with rejecting renal allografts. Transplantation 1998;66:562. Erratum in: Transplantation 1998;66:1264. [PubMed: 9753332]
23. Dugre FJ, Gaudreau S, Belles-Isles M, et al. Cytokine and cytotoxic molecule gene expression determined in peripheral blood mononuclear cells in the diagnosis of acute renal rejection. Transplantation 2000;70:1074. [PubMed: 11045645]
24. Shoker A, George D, Yang H, et al. Heightened CD40 ligand gene expression in peripheral CD4+ T cells from patients with kidney allograft rejection. Transplantation 2000;70:497. [PubMed: 10949194]
25. Sabek O, Dorak MT, Kotb M, et al. Quantitative detection of T-cell activation markers by real-time PCR in renal transplant rejection and correlation with histopathologic evaluation. Transplantation 2002;74:701. [PubMed: 12352889]
26. Netto MV, Fonseca BA, Dantas M, et al. Granzyme B, FAS-ligand and perforin expression during acute cellular rejection episodes after kidney transplantation: comparison between blood and renal aspirates. Transplant Proc 2002;34:476. [PubMed: 12009596]
27. Simon T, Opelz G, Wiesel M, et al. Serial peripheral blood perforin and granzyme B gene expression measurements for prediction of acute rejection in kidney graft recipients. Am J Transplant 2003;3:1121. [PubMed: 12919092]
28. Shin GT, Kim SJ, Lee TS, et al. Gene expression of perforin by peripheral blood lymphocytes as a marker of acute rejection. Nephron Clin Pract 2005;100:c63. [PubMed: 15824509]
29. Veale JL, Liang LW, Zhang Q, et al. Noninvasive diagnosis of cellular and antibody-mediated rejection by perforin and granzyme B in renal allografts. Human Immunol 2006;67:777. [PubMed: 17055354]
30. Graziotto R, Del Prete D, Rigotti P, et al. Perforin, granzyme B, and Fas ligand for molecular diagnosis of acute renal-allograft rejection: analyses on serial biopsies suggest methodological issues. Transplantation 2006;81:1125. [PubMed: 16641597]

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31. Simon T, Opelz G, Wiesel M, et al. Serial peripheral blood interleukin-18 and perforin gene expression measurements for prediction of acute kidney graft rejection. Transplantation 2004;77:1589. [PubMed: 15239627]
32. Heeger PS, Greenspan NS, Kuhlenschmidt S, et al. 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 1999;163:2267. [PubMed: 10438971]
33. Nickel P, Presber F, Bold G, et al. Enzyme-linked immunosorbent spot assay for donor-reactive interferon-gamma-producing cells identifies T-cell presensitization and correlates with graft function at 6 and 12 months in renal-transplant recipients. Transplantation 2004;78:1640. [PubMed: 15591953]
34. Augustine JJ, Siu DS, Clemente MJ, et al. Pre-transplant IFN-gamma ELISPOTs are associated with post-transplant renal function in African-American renal transplant recipients. Am J Transplant 2005;8:1971. [PubMed: 15996247]
35. Nather BJ, Nickel P, Bold G, et al. Modified ELISPOT technique – highly significant inverse correlation of post-Tx donor-reactive IFNγ-producing cell frequencies with 6 and 12 months graft function in kidney transplant recipients. Transpl Immunol 2006;16:232. [PubMed: 17138059]
36. Hauser IA, Spiegler S, Kiss E, et al. Prediction of acute renal allograft rejection by urinary monokine induced by IFN-γ (MIG). J Am Soc Nephrol 2005;16:1849. [PubMed: 15857922]
37. Sengul S, Keven K, Gormez U, et al. Identification of patients at risk of acute rejection by pretransplantation and posttransplantation monitoring of soluble CD30 levels in kidney transplantation. Transplantation 2006;81:1216. [PubMed: 16641611]
38. Cadillo-Chavez R, de Echegaray S, Santiago-Delpin EA, et al. Assessing the risk of infection and rejection in Hispanic transplant recipients by means of an adenosine triphosphate release assay. Transplant Proc 2006;38:918. [PubMed: 16647510]
39. Breimer ME, Rydberg L, Jackson AM, et al. Multicenter evaluation of a novel endothelial cell crossmatch test in kidney transplantation. Transplantation 2009;87:549. [PubMed: 19307793] This article introduced a novel flow-cytometry crossmatch technique to predict acute rejection.
40. Suthanthiran M, Garovoy MR. Immunologic monitoring of the renal transplant recipient. Urol Clin North Am 1983;10:315. [PubMed: 6222530]
41. Ashton-Chess J, Giral M, Mengel M, et al. Tribbles-1 as a novel biomarker of chronic antibody-mediated rejection. J Am Soc of Nephrol 2008;19:1116. [PubMed: 18369086] This article identified a potential biomarker for antibody-mediated rejection in kidney transplant.
42. Sis B, Jhangri GS, Bunnag S, et al. Endothelial gene expression in kidney transplants with alloantibody indicates antibody-mediated damage despite lack of C4d staining. Am J Transplant 2009;9:1. [PubMed: 19133925] This article identified novel endothelial related molecular biomarkers for antibody-mediated rejection in kidney transplant.
43. Hoefig KP, Heissmeyer. MicroRNAs grow up in the immune system. Curr Opin in Immunology 2008;20:281.
44. Lodish HF, Zhou B, Liu G, et al. Micromanagement of the immune system by microRNAs. Nat Rev Immunol 2008;8:120. [PubMed: 18204468]
45. Valencia-Sanchez MA, Liu J, Hannon GJ, et al. Control of translational and mRNA degradation by miRNAs and siRNAs. Genes Dev 2006;20:515. [PubMed: 16510870]
46. Anglicheau D, Sharma VK, Ding RC, et al. MicroRNA expression profiles predictive of human renal allograft status. Proc Natl Acad Sci USA 2009;106:5330. [PubMed: 19289845] This article demonstrated miRNA expression patterns to be biomarkers of acute rejection of renal allografts.
47. Ramos E, Drachenberg CB, Wali R, et al. The decade of polyomavirus BK-associated nephropathy: state of affairs. Transplantation 2009;87:621. [PubMed: 19295303]
48. Rogers NM, Russ GR, Cooper J, et al. Immunophenotyping of interstitial infiltrate does not distinguish between BK virus nephropathy and acute cellular rejection. Nephrology 2009;14:118. [PubMed: 19143944]
49. Mannon RB, Hoffmann SC, Kampen RL, et al. Molecular evaluation of BK polyomavirus nephropathy. Am J Transplant 2005;5:2883. [PubMed: 16303001]

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50. Field LA, Jordan RM, Hadix JA, et al. Functional identity of genes detectable in expression profiling assays following globin mRNA reduction of peripheral blood samples. Clin Biochem 2007;40:499. [PubMed: 17303101]
51. Tian Z, Palmer N, Schmid P, et al. A practical platform for blood biomarker study by using global gene expression profiling of peripheral whole blood. PLoS One 2009;4:5157.
52. Li L, Ying L, Naesens M, et al. Interference of globin genes with biomarker discovery for allograft rejection in peripheral blood samples. Physiol Genomics 2008;32:190. [PubMed: 17971501]

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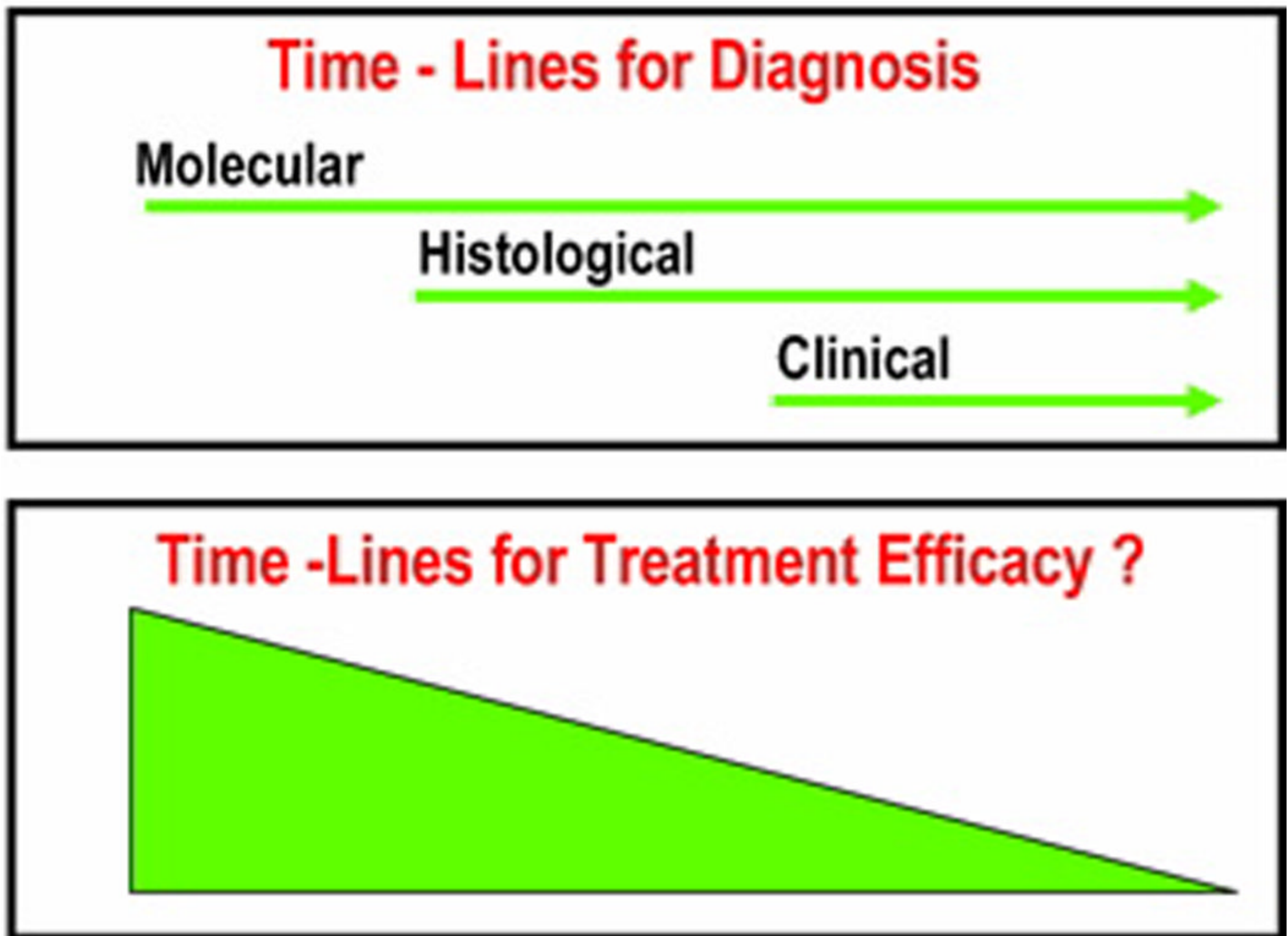

Figure 1.
A time-line model for allograft rejection. In this formulation, rejection defined by molecular markers precedes histologically defined rejection and this precedes clinically defined rejection. The hypothesis that early intervention is efficacious is an important rationale for the development of molecular surveillance strategies to anticipate histologic and clinical rejection.

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Table 1

Platforms for Biomarker Discovery and Validation

TEST		PLATFORM	EXAMPLES OF POTENTIAL BIOMARKERS
Gene Transcripts	Single gene	RT-PCR	mRNA: Granzyme B, Perforin, FoxP3; miRNA: miR155, miR223
	Multiple Genes	DNA Microarray	
Proteins	Single Protein	Enzyme-linked immunosorbent assay (ELISA)	Fractalkine, Amyloid A, β2 microglobulin
	Multiple Proteins	Protein Microarray	
Lymphocyte Function	Cytokine Producing Cells	Enzyme-linked immunosorbent spot assay (ELISPOT)	IFN-γ
	ATP levels in Activated T cells	Immuknow®	ATP
Alloantibodies	Single or Multiple Antibodies	Luminex xMAP®	Anti-HLA Antibody, Anti-MICA Antibody

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Table 2

mRNA Profiles Diagnostic of Acute Rejection of Renal Allografts.

AUTHOR, YEAR [REF]	N*	URINARY CELL mRNA LEVELS DURING ACUTE REJECTION COMPARED TO NO ACUTE REJECTION
Li et al, 2001 [9]	151/85	Perforin and granzyme B higher
Muthukumar et al, 2003 [10]	95/87	PI-9, Granzyme B, and perforin mRNAs higher
Dadhania et al, 2003 [11]	99/99	Granzyme B higher; UTI did not increase granzyme B levels
Ding et al, 2003 [12]	89/79	CD103 higher
Kotsch et al, 2004 [13]	221/26	Granulysin higher.
Tatapudi et al, 2004 [14]	63/58	IP-10 and CXCR3 mRNAs higher
Muthukumar et al, 2005 [5]	83/83	FOXP3 mRNA higher
Matz et al, 2006 [15]	−/76	IP-10 mRNA higher
Yannaraki et al, 2006 [16]	162 /37	Perforin, Granzyme B, and FasL mRNAs high during AR, UTI, CMV and DGF
Seiler et al, 2007 [17]	−/117	NKG2D mRNA higher
Renesto et al, 2007 [18]	72/72	Tim-3 and IFN-γ mRNAs higher
Manfro et al, 2008 [19]	165/115	Tim-3 higher
Aquino-Dias et al, 2008 [20]	48/35	Perforin, granzyme B, FasL, Pi-9, FOXP3 higher
Ozbay et al, 2009 [21]	64/64	Perforin, granzyme B granulysin higher; granzyme B, granulysin but not perforin higher in AR compared to bacteriuria; perforin but not granzyme B and granulysin higher in AR compared to CMV
Vasconcellos et al, 1998 [22]	31/25	Granzyme B, perforin and FasL mRNAs increased
Dugre et al, 2000 [23]	−/21	IL-4, IL-5, IL-6, IFN-γ, Granzyme B, and perforin mRNAs increased
Shoker et al, 2000 [24]	−/57	CD40L mRNA increased in AR and/or CAN
Sabek et al, 2002 [25]	27/27	Granzyme B, perforin and HLA-DRA mRNAs increased
Netto et al, 2002 [26]	206/29	Granzyme B, perforin and FasL mRNAs increased
Simon et al, 2003 [27]	364/67	Granzyme B and perforin mRNAs increased
Shin et al, 2005 [28]	88/15	Perforin mRNA increased
Veale et al, 2006 [29]	268/46	Granzyme B and perforin mRNAs increased
Graziotto et al 2006 [30]	64/−	Granzyme B, perforin, and FasL mRNAs increased
Manfro et al, 2008 [19]	165/115	Tim-3 higher
Aquino-Dias et al, 2008 [20]	48/35	Perforin, granzyme B, FasL, PI-9, FOXP3 higher

*number of samples/ number of patients

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Table 3

Biomarkers Predictive of Subsequent Development of Acute Rejection of Renal Allografts

SAMPLE	BIOMARKERS DETECTED	N*	END-POINT	AUTHOR, YEAR [REF]
COMPETITIVE QUANTITATIVE PCR				
Urine	Granzyme B mRNA and Perforin mRNA, 1–9 days post-transplant	37	Development of AR within 10 days of transplant	Li et al, 2001 [9]
REAL TIME QUANTITATIVE PCR				
Urine	Serine Proteinase Inhibitor -9 mRNA during acute rejection	29	Serum creatinine at 6 months following AR	Muthukumar et al, 2003 [10]
Urine	Granulysin mRNA 1–90 days post-transplant	26	Development of AR	Kotsch et al, 2004 [13]
Urine	FOXP3 mRNA during AR	36	Graft loss within 6 months following AR	Muthukumar et al, 2005 [5]
Urine	IP-10 mRNA and protein post-transplant	mRNA: 58 Protein: 70	Development of AR	Matz et al, 2006 [15]
Urine	IP-10 mRNA and protein post-transplant	mRNA: 58 Protein: 70	Development of AR	Matz et al, 2006 [15]
Urine	NKG2D mRNA post-transplant	94	Development of AR	Seiler et al, 2007 [17]
Blood	Granzyme B mRNA and Perforin mRNA 5–29 days post-transplant	67	Development of AR	Simon T et al, 2003 [27]
Blood	Granzyme B and Perforin mRNA 1–65 weeks post-transplant	46	Development of AR	Veale et al, 2006 [29]
Blood	Perforin mRNA and IL-18 mRNA 1–16 days post-transplant	54	Development of AR	Simon et al, 2004, [31]
ELISPOT				
Blood	Pre-transplant donor-specific IFN-γ producing cells	19	Development of AR	Heeger et al, 1999 [32]
Blood	Pre-transplant donor-specific IFN-γ producing cells	42	Development of AR	Nickel et al, 2004 [33]
Blood	Pre-transplant donor-specific IFN-γ producing cells	37	Development of AR	Augustine et al, 2005 [34]
Blood	Pre-transplant donor-specific IFN-γ producing cells	22	Development of AR	Nather et al, 2006 [35]
ELISA				
Urine	MIG protein 5–90 days post-transplant	69	Development of AR	Hauser et al, 2005 [36]
Serum	sCD30 protein pre and day15-post transplant	50	Development of AR	Sengul et al, 2006 [37]
ATP Release Assay				
Blood	ATP levels pre and day 14 post-transplant	58	Development of AR	Cadillo-Chávez et al, 2006 [38]
Flow Cytometry				
Blood	Anti-endothelial cell (anti-Tie-2) antibodies pre-transplant	147	Development of AR ≥3 months after transplant.	Breimer et al, 2009 [39•]

*number of patients