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Gazing into a crystal ball to predict kidney transplant outcome

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Kidney transplantation is the optimal therapy for end-stage kidney disease but requires lifelong immunosuppression. Despite improvements in immunosuppression regimens that have reduced rates of acute transplant rejection, long-term allograft survival remains suboptimal. More than 50% of transplanted kidneys from deceased donors fail within 10 years. In order to improve long-term outcomes, physicians need to better understand mechanisms underlying transplant rejection and tolerance in humans. They also need biomarkers that differentiate patients likely to maintain excellent and stable allograft function from recipients at risk of losing their transplants. By studying kidney transplant recipients at high risk for graft loss and rare, spontaneously tolerant kidney transplant recipients, researchers reporting in 3 papers in this issue of the *JCI* shed new light on these topics.

Late graft failure after kidney transplantation

Kidney transplantation is the most common solid organ transplant procedure carried out in the US; more than 16,000 such transplants were performed in 2009 (1). Advances in immunosuppression over the past 2 decades have drastically reduced the incidence of acute T cell–mediated rejection episodes, but have not significantly improved long-term allograft survival (2). The actual half-life for a transplanted kidney from a deceased donor (the most common source of kidneys for transplantation) is only 8 years (2), and as few as

one-third of kidney allografts obtained from older donors remain functioning 10 years later (3). Allograft dysfunction can result in the need for a further transplant and is a strong independent predictor of recipient cardiovascular mortality (4). Late graft loss is commonly caused by chronic allograft nephropathy, characterized by tissue fibrosis and tubular atrophy (5). Among the factors known to contribute to its pathogenesis are uncontrolled alloimmune reactivity (T cell– and/or antibody-mediated injury), recurrent primary renal disease, hypertension, diabetes, and drug toxicity. With regard to the latter, kidney toxicity as a result of long-term treatment with the immunosuppressive agents tacrolimus or cyclosporine is detectable in virtually all transplanted kidneys (6).

Although research progress has improved our knowledge of the prevalence, clinical significance, and pathogenesis of chronic kidney allograft injury, research has not yet influenced therapeutic decision making to improve outcomes. Currently, posttransplant care, including immunosuppression, is protocol driven. Alterations in drug dosing are made based on center-derived protocols and physician experience. Because chronic injury is multifactorial, a one-size-fits-all approach is not ideal. To develop individual therapeutic approaches tailored to individual patients, transplant physicians need risk assessment tools that can discriminate specific pathogenic mechanisms, prospectively identify those transplant recipients destined to have excellent graft function, recognize those recipients at high risk for graft loss, and ultimately guide specific changes in therapy. In this issue of the *JCI*, 3 papers provide new data that move us closer to these goals (7–9).

An intragraft molecular signature can predict graft loss

To assess the risk of future graft loss, Einecke and colleagues (7) evaluated gene expression profiles in tissue from 105 kidney graft biopsies that were performed to determine the cause of allograft dysfunction and/or proteinuria using microarray
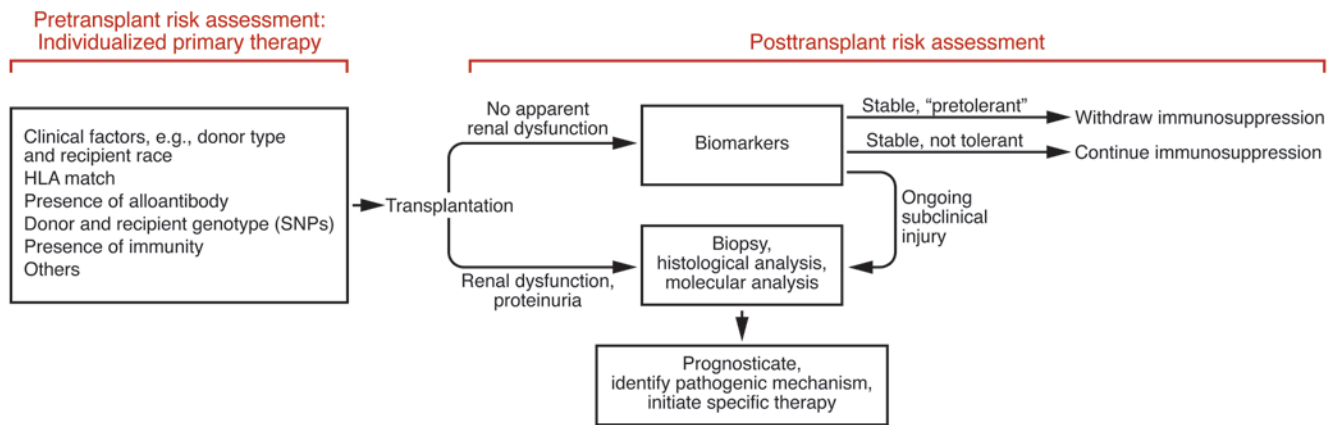
Figure 1
Theoretical approach to individualizing pre- and posttransplant therapy using clinical and biomarker risk assessment strategies. In this schema, the decision to use specific immunosuppression regimens is based on known risk factors, including those used currently (living or deceased donor type, recipient race, HLA match, alloantibodies) and emerging biomarkers (genetic polymorphisms and T cell memory) that may alter posttransplant risk of injury. If the recipient develops evidence of graft damage (e.g., proteinuria and/or elevated levels of creatinine), biomarker results and molecular analyses of graft tissue will supplement histopathology to guide specific alterations in therapy aimed at reversing the disease processes. In patients with stable kidney function, biomarker results will ideally differentiate patients with subclinical injury (need more therapy) from patients who need immunosuppression but are not tolerant and from patients who can be safely withdrawn from immunosuppression (operationally tolerant).

technology. The tested biopsies were obtained 1–31 years (median, 4.7 years) after transplantation. The authors identified a molecular risk score, composed of approximately 600 genes, that was predictive of incipient graft failure. Many of the detected genes behaved similarly, such that prediction of graft failure could be accomplished with a subset of 30 genes. Histology of those biopsies with scores indicative of a high risk of graft failure often revealed either chronic antibody-mediated graft injury or recurrent primary renal disease. The molecular risk score was associated with incipient graft loss, independent of histological features associated with chronic injury (including C4d staining), serum alloantibodies, presence or absence of proteinuria (dipstick rather than exact quantification), and estimated GFR. The risk score was successfully validated independently in a transplant cohort from a different institution.

It is notable that the authors detected the same high-risk signature in a subset of biopsies performed within the first year after transplant, but in these early biopsies, the pattern did not predict graft loss. Histological diagnoses in these early biopsies with high-risk scores were different from those found after 1 year and included acute kidney injury and immune-mediated rejection, which can be self-limited or highly responsive to therapy.

The authors suggest that the intragraft gene expression pattern they identified (7), which is composed of genes encoding proteins involved in tissue injury, matrix remodeling, and epithelial dedifferentiation, is a stereotypical response induced by many disease processes within the graft (and likely primary renal disease as well). If the disease process is reversible, as T cell–mediated rejection is typically reversible with steroid therapy and/or T cell–specific antibodies, then the data suggest the profile has little predictive value for graft loss. In contrast, if the cause of the profile is unknown and/or if there is no effective therapy (e.g., for antibody-mediated injury), then the profile may be highly predictive of incipient graft loss. The authors contend that the molecular profile is not a disease-specific signature of incipient graft failure, but rather an indicator of ongoing severe allograft damage that, if left untreated, will result in failure (7). Whether this contention is correct will require prospective testing and the development of specific therapies, including those capable of reversing antibody-mediated injury and fibrosis, which are not yet available. Additional studies that include molecular analysis of biopsies performed on stably functioning allografts (i.e., protocol biopsies) are needed to determine the value of this molecular signature as a predictor of graft loss in kidney transplant

patients without clinically overt disease. Developing and testing noninvasive biomarkers — including gene sets from blood and/or urine samples — that have similar predictive ability would ultimately be preferable, so that disease could be detected earlier and biopsy-associated morbidity could be avoided.

Markers of transplant tolerance
Also in this issue, 2 additional papers from the Immune Tolerance Network (8) and from the Indices of Tolerance European Union consortium (9) focus on the other end of the transplant spectrum. These groups attempted to define mechanisms and biomarkers associated with outstanding kidney transplant outcomes. Ideally, clues about mechanisms could be exploited therapeutically to induce tolerance, while identifying reliable biomarkers of excellent outcome could facilitate safe drug withdrawal to prevent the long-term consequences of immunosuppression. To do this, the 2 research groups independently identified and studied rare, operationally tolerant kidney transplant recipients (i.e., recipients who had excellent and stable graft function despite the absence of immunosuppression). These patients stopped taking the medications unilaterally and voluntarily (against physician advice) or because the immunosuppression resulted in severe infection or malignancy. It should

be noted that the overwhelming majority of transplant recipients who stop taking immunosuppression lose their grafts due to rejection — spontaneous tolerance is extremely rare.

In the paper by Newell and colleagues (8), the authors examined laboratory parameters in 25 operationally tolerant kidney transplant recipients and compared them with those obtained from kidney transplant recipients on immunosuppression and those of healthy volunteers. Flow cytometric analysis of lymphocyte subpopulations, and blood and urine gene expression using arrays and PCR, indicated that B cell–related genes were strongly expressed in the tolerant patients. A subset of 3 B cell–related genes discriminated the tolerant patients from the stable recipients taking immunosuppression, with reasonable positive and negative predictive values. The initial findings were validated in an independent cohort, confirming and extending a previous report associating B cells with human allograft tolerance (10).

In the paper by Sagoo and colleagues (9), the authors analogously screened multiple biomarkers and bioassays in 11 operationally tolerant kidney transplant recipients and in cohorts of immunosuppressed recipients exhibiting chronic allograft injury and healthy controls. Several of the assay results that strongly correlated with the tolerant state were validated in an independent test set of 24 tolerant kidney transplant recipients. Sagoo and colleagues observed that tolerant patients had expanded populations of peripheral blood B cells and NK cells, but fewer activated CD4+ T cells, and that patients' peripheral blood contained higher quantities of B cell–related genes, similar to the findings of Newell and colleagues (8).

Samples were shared between the 2 consortia, and cross-validation studies confirmed a strong association between B cell–related genes/markers and the tolerant state, despite differing cell preparations and assay protocols. Neither group performed transplant kidney biopsies, making it impossible to directly correlate intragraft molecular patterns with peripheral blood gene expression profiles. However, RNA from cells in the urine in the cohort of tolerant patients studied by Newell and colleagues (8) contained higher quantities of CD20 transcripts (which encode a cell surface marker expressed on B cells), suggesting that B cells in the graft may be

relevant. Together, the data generated by Newell and colleagues (8) and Sagoo and colleagues (9) indicate that a B cell signature is associated with the rare occurrence of spontaneous kidney graft tolerance, raising the speculative possibility that future approaches aimed at manipulating B cells might facilitate tolerance. A recent study by others described a population of IL-10–producing regulatory B cells (11), but whether such B cells are functionally involved in inducing and/or maintaining allograft tolerance cannot be discerned from the present work.

Although the findings of Newell and colleagues (8) and Sagoo and colleagues (9) are intriguing and important, it is premature to conclude that these B cell signatures can be applied clinically. Standardized assay protocols need to be adopted so that results can be compared among laboratories. Prospective studies must be performed to determine the prevalence of the molecular profile in cohorts of stable kidney transplant recipients taking immunosuppressants and whether the profile can be used to safely guide drug withdrawal. More studies will be needed to determine whether the pattern occurs in patients intentionally treated to become tolerant, for example, through cotransplantation with donor bone marrow (12–14). Previously published work has revealed that tolerant liver transplant recipients express a distinctly different profile (15), which suggests that the B cell signature may be specific for kidney transplant tolerance. Moreover, additional factors are likely to influence the ability of an individual to develop kidney graft tolerance, regardless of the presence of this B cell signature. For example, genetic polymorphisms (16) as well as preexisting donor-specific alloantibodies and donor-reactive memory T cells may be significant barriers to transplant tolerance (17, 18).

Concluding remarks

Together, the papers from Einecke and colleagues (7), Newell and colleagues (8), and Sagoo and colleagues (9) provide proof of principle that it might be possible to develop biomarkers capable of predicting outcomes after transplantation. One can envision future scenarios in which clinical and histological manifestations of allograft injury are supplemented with molecular and functional biomarkers, including a molecular risk score as proposed by Einecke and colleagues (7), to prognosticate out-

come and guide therapy in patients at high risk for graft loss (Figure 1). Analogously, biomarkers, including the B cell signatures developed by Newell and colleagues (8) and Sagoo and colleagues (9), may help to guide drug withdrawal in selected stable patients, limiting toxicity caused by long-term treatment with immunosuppressive agents. Only appropriately designed, prospective, randomized trials will determine whether the promise of a molecular crystal ball that predicts transplant outcome will evolve into reality.

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Revisiting an old acquaintance:
role for eIF5A in diabetes

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Dysfunction of pancreatic islet β cells underlies both type 1 and type 2 diabetes and appears to result in part from the local release of proinflammatory cytokines. An improved understanding of the mechanisms that mediate islet responsiveness to proinflammatory cytokines may therefore expand our knowledge of the role of cytokine signaling in the development of diabetes, providing potential new targets for the development of therapeutics to protect pancreatic islets from inflammation. In this issue of the *JCI*, Maier and colleagues identify eukaryotic translation initiation factor 5A (eIF5A) as a critical regulator of the inflammatory response in mouse pancreatic islets. I believe these data provide new and important insights into the regulatory pathways that contribute to the development of diabetes and deepen our understanding of the function of the, so far, rather enigmatic cellular protein eIF5A.

Diabetes is a highly prevalent condition characterized by high blood glucose levels. Dysfunction and/or destruction of insulin-producing pancreatic islet β cells underlies all forms of diabetes: in type 1 diabetes, β cells are destroyed by an autoimmune response, while in type 2 diabetes, β cell dysfunction and/or destruction is thought to arise because β cells are unable to meet the increased demands for insulin. Despite these 2 distinct causes of β cell dysfunction and/or destruction, emerging data suggest that in both cases local release of proinflammatory cytokines, such as IL-1β, TNF-α, and IFN-γ, is a central event. One pathway that contributes to the early pathogenesis of β cell dysfunction in response to proinflammatory cytokines is NF-κB–mediated induction of the *Nos2* gene, which encodes iNOS (1, 2). However, proinflammatory cytokine signaling ultimately leads to β cell dysfunc-

tion and death via both iNOS-dependent and -independent effects. Understanding the molecular mechanisms underlying the responsiveness of β cells to proinflammatory cytokines will not only provide more insight into the pathogenesis of diabetes but also provide potential new targets for therapeutics to preserve pancreatic function. In this context, in this issue of the *JCI*, Maier et al. (3) identify eukaryotic translation initiation factor 5A (eIF5A, formerly known as either IF-M2Bα or eIF-4D) as a critical regulator of the response of mouse β cells to proinflammatory cytokines.

An initial career as translation initiation factor

Functional characterization of eIF5A, purified by extraction of ribosomes from rabbit reticulocytes with buffers containing a high salt concentration, indicated that it had a stimulatory effect on the synthesis of methionyl-puromycin, an in vitro model reaction that mimics the formation of the first peptide bond during protein synthesis (4). Although it had, at that time, already been demonstrated that eIF5A had no

effect on the translation of native rabbit globin mRNA (5), which actually argued against a role for eIF5A as an initiation factor in vivo, its activity in the methionyl-puromycin assay led to this protein being considered a genuine translation initiation factor. For many years this notion seemed to be carved in stone, since the methionyl-puromycin assay was the only available in vitro test system for analyzing eIF5A activity. However, the assumption that eIF5A directs translation initiation was challenged in the 1990s, when studies in the yeast *Saccharomyces cerevisiae* demonstrated that protein synthesis is only mildly affected by complete depletion of cellular eIF5A (6). This finding triggered the careful reevaluation of the reaction parameters of the methionyl-puromycin in vitro assay, and it became obvious that large amounts of eIF5A were required to obtain stimulation of methionyl-puromycin synthesis (7). In fact, routinely, 50–200 picomoles of eIF5A were used to obtain only 1–2 picomoles of reaction product, suggesting that this effect of eIF5A is an artifact of the assay system (7). More recent studies, employing polysome profiling in yeast and *Drosophila* cells, provided evidence that eIF5A participates in the elongation step of translation, rather than regulating the initiation of protein synthesis (8, 9). Whether this eIF5A activity affects the translation of all cellular mRNAs or occurs only in certain cell types and/or at specific physiological conditions remains to be elucidated.

Posttranslational hypusine formation in eIF5A

eIF5A is unique because it is the only known cellular protein to contain the