PGRN blockade of TNF signaling to the inhibition of NOD2 expression and attenuation of ischemic AKI was not addressed by Zhou *et al.*[1] and deserves further investigation. Studies in a variety of forms of kidney injury point to a causal relation between TNF and tissue injury. TNF is an inducer of NOD2 in epithelial cells. Therefore, it is possible that PGRN binding to TNF receptors and attenuating TNF signaling leads to a reduction in NOD2 expression and ischemic kidney injury (Figure 1). PGRN has also been shown to promote the expansion of regulatory T cells.[7] Since regulatory T cells reduce both ischemic and cisplatin AKI,[8] this constitutes another plausible mechanism of action for PGRN. Additional studies in mouse models deficient in PGRN, TNF, TNF receptors, and/or T regulatory cells would provide more insight into the mechanism behind PGRN protection from inflammation and AKI. Nonetheless, with the development of a PGRN-derived protein, Atsttrin, that binds TNF receptors and exhibits potent anti-inflammatory activity with less adverse effects and far greater half-life than PGRN, the findings of Zhou *et al.*[1] in AKI are very significant and perhaps can be exploited for therapeutic intervention against AKI.

DISCLOSURE
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Telling the tiger by its stripes: mapping the genomics of kidney graft tolerance in real time

Daniel L. Roden[1,2] and Shane T. Grey[2,3]

Though the majority of kidney allografts are eventually lost to the process of chronic rejection, there are instances when kidney function is maintained after patients have stopped their immunosuppression. Baron and colleagues have examined the blood gene signature of patients with spontaneous kidney tolerance and identified a series of genes that they suggest define kidney graft acceptance. This exciting development provides a potential list of biomarkers defining immunological tolerance in humans.

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Kidney transplantation is the treatment of choice for patients with end-stage renal disease, but its success, like that of all organ transplants, is limited by the requirement for heavy immunosuppression. Further, despite immunosuppression the majority of kidney allografts are eventually lost to the process of chronic rejection; thus the majority of transplant patients eventually require an additional kidney transplant or transition to dialysis. The loss of kidney grafts, whether acute or chronic, has a significant immune-mediated component; however, in rare cases, something different happens and kidney function can be sustained in the absence of immunosuppression. Thus kidney transplantation tolerance is defined as stable kidney allograft function in the absence of continuing immunosuppression. While rare, clinical kidney transplant tolerance has been reported in a number of different centers, with the majority of patients showing stable kidney allograft function with normal serum creatinine for more than 1 year off immunosuppression.[1] In 1953 Billingham and colleagues[2] demonstrated that one could make 'tissue homografts immunologically acceptable' to a recipient, suggesting the existence of 'actively acquired tolerance.' If it were possible to engineer such a state clinically, this would render the requirement for heavy, and perhaps lifelong, immunosuppression obsolete and potentially reverse the insidious loss of otherwise stable kidney allografts to chronic rejection. Though the identification of patients who are tolerant of their renal transplants is more often serendipitous, the result of medical

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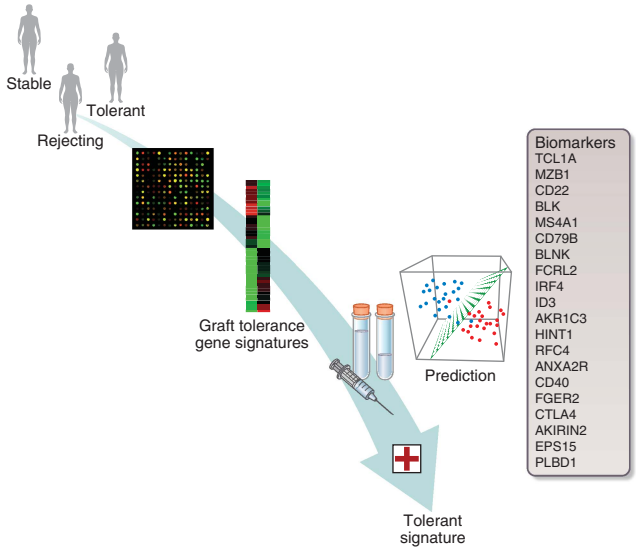

Figure 1 | Schematic overview of the approach taken by Baron *et al.* to identify predictive biomarkers for kidney graft tolerance. Gene expression microarrays from stable, tolerant, and rejecting patients were collated to identify gene signatures associated with graft tolerance. A core set of 20 gene biomarkers were identified and shown to be predictive of tolerance. Future use of these biomarkers could lead to clinical prediction of the presence of a graft-tolerant signature.

nonadherence with regard to taking their medication, their existence is no less exciting and provides a strong clinical proof to Billingham's line of questioning as to whether acquired tolerance is only an artificial phenomenon or a clinical hope.

In this issue, *Kidney International* carries a paper[3] that looks to have identified a specific blood-borne gene signature, within patients who are tolerant of their kidney allotransplant, that indicates the establishment of tolerance (Figure 1). Baron and colleagues carried out an extensive meta-analysis of previous gene expression microarray studies that have looked at the link between transcriptional signatures and allograft tolerance and rejection. Surprisingly, they found minimal concordance between the gene signatures in the five individual studies that they investigated. In an attempt to understand this and boost the power of the individual studies, they combined these into a single data set containing 96 graft-tolerant patients. Although an effort was made to minimize batch effects from this data integration, they are always a concern that should be highlighted when expression data are combined from multiple studies across different technology platforms as was done here.[4]

Despite this caveat, from this meta-analysis clusters of gene expression were identified that highlighted a role in tolerance for early markers of proliferating T and B cells; B-cell and T-cell modules; and proinflammatory-related monocyte modules. In an effort to further refine these gene signatures, a virtual microdissection approach was used to identify immune cell-specific transcriptional profiles present in the graft-tolerant cases. From this the authors showed that the previously derived immune cell-specific signatures were maintained, adding additional robustness to the analysis. This method, pioneered in the area of cancer research, leverages the continually expanding online genome-wide expression database resources that are available to computationally analyze cell lineage-specific responses in heterogeneous tissue samples. It is, however, reasonable to expect that the rapid improvements in cell purification, coupled with the potential power of single-cell transcriptomics[5] and genomics, will make this an area that can be directly addressed in future clinical and mechanistic studies and ultimately translated into personalized treatments.

In addition to the characterization of a B-cell and other specific immune-cell gene signatures associated with tolerance, the most differentially expressed genes were identified and further investigated for their predictive power. Independent experimental validation, coupled with machine learning approaches, showed that the top 20 gene biomarkers were predictive of graft tolerance. Although the wider utility of this type of classification approach in the clinic is still unclear, it provides a valuable first step for gene biomarker-based prognostic identification of graft-tolerant patients.

A defining genetic feature of this current study,[3] and of others that have analyzed the gene signature of patients tolerant of their kidney allografts,[1] is the identification of genes that indicate B-cell involvement. B cells are more typically associated with immune effector function, such as in the case of presensitized kidney transplant patients who harbor human leukocyte antigen antibodies directed against allogeneic major histocompatibility complex determinants; however, emerging evidence indicates that B cells can play both immune effector and immune regulatory roles.[6] The concept that B cells are associated with kidney allograft tolerance therefore raises very interesting questions regarding whether the B-cell signature represents the aftermath of processes that resulted in tolerance toward the kidney graft or whether it represents an active and ongoing B cell-dependent process. In the latter case, B cells may be intimately required for tolerance induction and/or maintenance. The idea that B cells can modulate immune responses toward tolerance is gaining acceptance, and work from a number of experimental systems suggests different possible mechanisms by which B cells could affect kidney tolerance. B cells could indirectly provide immune tolerance by engendering Foxp3$^+$ regulatory T cells (Tregs).[6] These Tregs are master regulators of immune homeostasis, and their lack is associated with autoimmunity in animal models and human studies. The development of Tregs is

critically dependent on interactions with antigen-presenting cells such as dendritic cells but also B cells.[7] In that scenario, B cells could potentially capture peripheral antigens, such as kidney alloantigens, and shuttle these to the thymus to engender development of Tregs that provide kidney graft tolerance, as has been shown for experimental allogeneic islet transplantation.[7] Further, tissue-involved B cells could secrete pleiotropic factors including interluekin-10, which on the one hand would act to promote Tregs that could provide transplant tolerance,[8] but also could act locally to suppress tissue inflammation.[9] B cells can also directly kill effector T cells through expression of immune molecules such as Fas ligand and secreted transforming growth factor-β[6] suggesting they could play a role in 'pruning' the alloreactive T-cell repertoire. As B cells have been shown to promote tolerance in experimental transplantation and inflammatory settings, the possibility exists that the B-cell signature represents their role in an active process.

This study raises further questions. The most pressing relates to understanding the functional significance of the B-cell signature: what is the nature of the B cells represented—are B cells the cause or secondary to kidney tolerance? Further, models that can better mimic the clinical data to explore the causal relationship between this signature and the observed operational tolerance would be of benefit. Also, the study raises interesting questions as to the relationship between tolerance achieved under the conditions of kidney transplantation and immunosuppression and that observed in the situation of a normal individual with no transplant. Is the emerging tolerant state related to that of a subject with normal immune homeostasis? In relation to this line of reasoning, a critique regarding the current study is that stable patients on immunosuppression were used as the control to identify the tolerant genes. There is significant rationalization about why this is valid, using patients with chronic rejection of immunosuppression and kidney transplant to show they do not have the signature. However, the study raises interesting questions as to the relationship between tolerance achieved under the conditions of kidney transplantation with immunosuppression and the situation of a normal individual with no transplant. Future comparative analyses that include normal subjects would illuminate this line of questioning.

The realization that a sub-population of kidney transplant recipients show stable kidney graft function after immunosuppression withdrawal is an exciting experiment of nature, providing proof that clinical transplant tolerance is not an experimental artifact as Billingham once wondered.[2] A better understanding of the mechanism of these phenomena, and the development of assays and biomarkers to allow selective minimization of immunosuppression, would constitute a significant advancement to our management of patients receiving a kidney transplant as a renal replacement therapy.

DISCLOSURE
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Is it time to tip your glass to prevent CKD?

John W. Kusek[1]

Chronic kidney disease (CKD) is a major global medical and public health challenge. On the basis primarily of a modest number of prospective epidemiological studies, it appears that alcohol consumption reduces the risk of CKD in the general population. Our understanding of the potential benefits of alcohol consumption with regard to CKD is likely to evolve in the future and will be informed primarily by observational epidemiological studies.

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Chronic kidney disease (CKD) is a global medical and public health problem. Studies to determine risk factors at the population level, especially those that are modifiable, are needed to stem the burden of these diseases. Koning and colleagues[1] (this issue) add to the relatively modest literature (compared with the substantial numbers of studies for