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Review

Transcriptomic studies in tolerance: Lessons learned and the path forward

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ABSTRACT

Immunosuppression after solid organ transplantation is a delicate balance of the immune response and is a complex phenomenon with many factors involved. Despite advances in the care of patients receiving organ transplants the adverse effects associated with immunosuppressive agents and the risks of long-term immunosuppression present a series of challenges and the need to weigh the risks and benefits of either over or under-immunosuppression. Ideally, if all transplant recipients could develop donor-specific immunological tolerance, it could drastically improve long-term graft survival without the need for immunosuppressive agents. In the absence of this ideal situation, the next best approach would be to develop tools to determine the adequacy of immunosuppression in each patient, in a manner that would individualize or personalize therapy. Despite current genomics-based studies of tolerance biomarkers in transplantation there are currently, no clinically validated tools to safely increase or decrease the level of IS that is beneficial to the patient. However, the successful identification of biomarkers and/or mechanisms of tolerance that have implications on long-term graft survival and outcomes depend on proper integration of study design, experimental protocols, and data-driven hypotheses. The objective of this article is to first, discuss the progress made on genomic biomarkers of immunological tolerance and the future avenues for the development of such biomarkers specifically in kidney transplantation. Secondly, we provide a set of guiding principles and identify the pitfalls, advantages, and drawbacks of studies that generate genomic data aimed at understanding transplant tolerance that is applicable to all solid transplants.

1. Introduction

Immunosuppression after solid organ transplantation is a complex phenomenon. Over the past 5 decades, the transplant community has witnessed great advances in the care of patients receiving organ transplants in the form of increasing success rates as evidenced by better patient and graft survival rates. On the flip side, the adverse effects associated with these immunosuppressive agents and the risks of long-term immunosuppression present a series of challenges for clinicians. This necessitates the need to weigh the risks and benefits of either over or under-immunosuppression. Continuous immunosuppression (IS) after transplantation has prevented renal transplant (RT) rejection in most kidney transplant recipients in general and even between HLA identical siblings [1–3]. If every transplant recipient could develop donor-specific immunological tolerance, this would be an ideal scenario that could drastically improve long-term graft survival without the need for any immunosuppressive agents. However, since this is more of wishful thinking the next best approach would be to develop tools to determine the adequacy of immunosuppression in each patient, in a manner that would individualize or personalize therapy. This clinical need hassled to a search for biomarkers of clinical use in transplantation. Despite studies on biomarkers of tolerance in transplantation, there are currently, no clinically validated tools to safely increase or decrease the level of IS that is beneficial to the patient without over or under immunosuppressing them. In this review, we will discuss the progress made to date and the future avenues in the development of biomarkers of immunological tolerance specifically in kidney transplantation.

Abbreviations: RT, Renal Transplant; NGS, Next Generation Sequencing; GEP, Gene Expression Profile; LDA, Linear Discriminant Analysis; PPV, Positive Predictive Value; NPV, Negative Predictive Value; Tregs, Regulatory T-cells; MHC, Major Histocompatibility Complex; DHSC, Donor Hematopoietic Stem Cells; SRL, Sirolimus; TAC, Tacrolimus

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1.1. Genomics

While there are many techniques to profile the genome at the DNA, RNA, protein and metabolome level, in this article we will concentrate on profiling RNA and the transcriptome which have been the focus of our studies of tolerance. The sequencing of the human genome, which started in the early 2000 s, brought about a rapid expansion of high throughput profiling technologies to measure the mRNA transcription of the cell's genome. One of the most successful global expression profiling platforms has been DNA Microarrays, which are a powerful discovery tool for diagnosis and can be used to establish the genomic and biological basis of many diseases. Microarray technology made it possible to survey the whole human genome on a single chip making it very appealing for biomarker discovery, drug discovery, and pharmacogenomics. Microarrays were primarily used in the cancer field to classify tumors, then rapidly expanded to immunology, and are currently providing useful information about the transcriptional machinery and complex immunological networks involved in T-cell, B-cell and innate immune biology [4–6]. DNA microarrays have also been used to decipher the immune and inflammatory pathways involved in kidney [7–12], liver [13–15], heart [16–18], and lung transplant rejection [19–21]. Microarray platforms have the added advantage of a proven track record that dates back to the late 1990 s. Many researchers have become comfortable with the technology, and also with analyzing the results since data analysis tools for microarrays have become more reliable and user-friendly. Microarrays were generally considered easier to use with less complicated and less labor-intensive protocols than newer technologies such as Next Generation Sequencing (NGS). However, despite its advantages and the ability to provide an accurate profile of what is being transcribed at the mRNA level, microarray technology still has its pitfalls. For example, even though the new generation of microarrays promises whole genome coverage, this is a misnomer. They are limited to the use of probe sequences representing only known transcripts, thereby excluding the potential for discovering novel transcripts as well as transcript variants. The advent of NGS has by and large changed the whole landscape of transcriptional profiling because of its many advantages. These include providing direct access to gene-specific intronic and exonic definitions and sample-specific sequence data, even when not identical to the established reference. This can be extremely useful when it comes to discovering novel features in a gene or sample. The use of NGS in transplantation is still in its infancy and there have been very limited studies using this technology. It has the obvious potential to answer fundamental mechanistic questions of transplant immunology, particularly in the transplant tolerance field. One important aspect of transplantation is HLA typing. HLA typing is currently being performed primarily by using Luminex bead-based assays and to a lesser extent by molecular methods. HLA typing is an ideal candidate for the use of sequencing-based technologies especially due to the high throughput and low cost when it comes to molecular typing. Sequencing also holds a lot of promise in the field of transplant tolerance where, despite good HLA matching, there are still grafts that are not tolerated well. Some of these mismatches may be explained by the granular molecular heterogeneity of the HLA molecules which can be resolved accurately using sequencing-based technologies.

1.2. Renal transplant tolerance and genomic studies

Tolerance can be "operational" where there are no manipulations to induce a tolerogenic state, but rather dependent on an innate ability to tolerate an allograft. Such operational tolerance is believed to be the result of either nonadherence or a specific physician directed termination of immunosuppression. Studies of tolerant individuals have identified molecular signatures that are specific to these individuals, such as a 33 gene peripheral blood gene expression signature from a discovery cohort of kidney transplant recipients and healthy normal individuals without a transplant [7]. This signature was used to predict a tolerant state in a validation cohort of transplant patients. The biological significance of this signature was underlined by reduced costimulatory signaling, apoptosis, and immune quiescence in memory T-cell responses coupled with increased numbers of regulatory Foxp3 T cells. There were also attempts to apply diagnostic metrics to this signature to test its ability to distinguish tolerant patients. The results showed that this 33 gene signature distinguished operationally tolerant patients from patients with chronic rejection with 99% specificity and 86% sensitivity. In a later study from this same group using a 40 gene PCR panel on a discovery set of samples, 20 genes were selected to form a potentially useful gene set to identify patients at 'minimal risk' of rejection, whereby the clinicians could objectively reduce immunosuppression [22]. Testing of this gene set in a cohort of 144 patients who were at least 5 years post-transplant with stable graft function revealed that only 3.5% displayed a tolerance profile in the peripheral blood-based. The obvious limitation of the study was that the peripheral blood findings were not "benchmarked" to kidney biopsies to histologically verify the absence of rejection nor is there very good estimates of the prevalence of operational tolerance.

A major finding of two other studies of operationally tolerant patients was an enhanced B cell response in the tolerant state. The first study was the largest ever reported cohort of tolerant renal transplant recipients, defined as having stable graft function and receiving no immunosuppression for more than 1 year [23]. Gene expression profiles (GEP) and peripheral blood lymphocyte subsets from this group of patients were compared with a group combining subjects with stable graft function and receiving immunosuppressive drugs as well as healthy controls. In the 19 operationally tolerant patients, the study identified a set of 30 genes that were upregulated by at least two-fold relative to patients with stable graft function who were on triple immunosuppression. While an interesting observation was that 22 of the 30 genes were B-cell specific, this GEP was unable to perform as a biomarker in differentiating between tolerant patients and healthy (non-transplanted) controls. Based on the training set data and using a leave-one-out cross-validation method with a Linear Discriminant Analysis (LDA) model, a 3-gene signature was found to be the most predictive. It had a positive predictive value (PPV) of 83% and a negative predictive value (NPV) of 84%. The study also concluded that their observation of increased total B cell numbers and naive B cells in the peripheral blood of TOL suggested that these cells may be important regulators of the anti-donor immune response. The second study using microarray analysis again revealed a bias toward the differential expression of B cell-related genes and their pathways in tolerant recipients [24]. However, it has been suggested that this predominance of B-cell related genes may actually reflect the absence of immunosuppression, rather than the state of tolerance [25]. Even though both these studies were used to cross-validate the findings, none of the three genes in the predictive set found in the first study validated in the second.

In contrast to operational tolerance, there have been studies of induced tolerance that have looked at genomic correlates of expression in the peripheral blood. Some of these studies have been performed in HLA-identical recipients and donors which has the unique advantage of eliminating the variability of immune response genes associated with donor/recipient specific HLA polymorphisms. Additionally, immunoregulation driven by regulatory T-cells (Tregs) may be aided by self-recognition by the recipient of both donor major histocompatibility complexes (MHC) [26,27]. There are at least three major United States centers which are conducting RT tolerance protocols in HLA identical and disparate living donor/recipient pairs [28–35]. Our collaborators at Northwestern University, have successfully implemented human tolerance induction protocols using a cell-based protocol with T cell depletion and two doses of alemtuzumab (anti-CD52). Our first study described the 3-year results of a tolerance protocol in 10 renal transplant recipients who were HLA identical with their living donor siblings [31]. All recipients received four infusions of donor hematopoietic stem cells