Self-antigens and rejection: a proteomic analysis

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Purpose of review
Allo- and autoantibodies have been found to play important roles in both acute and chronic allograft rejection in organ transplantation, although only recently have non-human leukocyte antigen (non-HLA), nondonor-specific antibodies been given a more in-depth treatment. This review summarizes recent reports about investigations and proteomic approaches to identify self-antigens and corresponding autoantibodies that are associated with acute and chronic allograft rejection. Finally, we discuss the insights gained from these, challenges, and future prospects.

Recent findings
Significant discoveries have been made regarding the presence and role of autoantibodies and alloantibodies, both those formed pretransplant and posttransplant, in acute and chronic rejection. These discoveries are made possible because of the publication of the human genome and subsequent development in the ability of expression and analysis of human proteome.

Summary
Antibodies play a critical role in survival and dysfunction of a transplanted kidney. Even though HLA antibodies have been given the majority of the scientific community’s attention for the past few decades, antibodies against autoantigens and that of non-HLA origin are gaining attention. Recent publications have identified novel self-antigens that are associated with acute and chronic rejection that have added to our understanding of new players in immune-related transplant rejection.

Keywords
autoantibodies, proteomics, self-antigens, transplant rejection

INTRODUCTION
The deleterious role of antibodies, particularly those of donor-specific human leukocyte antigen (HLA) alloantibodies, has been well established in the scientific literature since the publication of the landmark study by Terasaki on the cross-match test in kidney transplantation [1–3]. Although ensuring HLA-compatibility has significantly reduced transplant rejection, recent advances in prevention, and treatment of acute rejection as well as the development of novel pharmaceuticals for immunosuppression have not prevented poor long-term outcome with regards to the survival of the transplanted organs. For example, the incidence of acute rejection of kidney transplants remains at 15% and the rate of graft survival has not improved significantly in recent decades [4].

As such, there still remains much be to understood regarding the key players of transplanted organ survival and failure. Recently, the role of autoantigens beyond the sole realm of autoimmunity has been investigated in transplantation. This review will summarize recent work investigating the development of autoantibodies to self-antigens and their roles in transplant rejection. Recent studies have provided initial evidence of a deleterious role of these autoantibodies. Several of these studies have used high-throughput proteomic approaches in the identification of their corresponding self-antigens and we highlight them accordingly.

RECENT ADVANCES IN UNDERSTANDING AUTOANTIBODIES IN THE REJECTION OF KIDNEY TRANSPLANTS
Research into non-HLA antibodies in kidney transplantation had identified the presence of antibodies, both donor-specific antibodies (DSA) and non-DSA,
Allo- and autoimmunity

**KEY POINTS**

- Self-antigens and corresponding autoantibodies have recently received increased scrutiny and play an important role in transplant survival.
- Proteomic approaches to identify autoantibodies and decipher mechanistic effects have been developed although are used infrequently.
- Many questions remain regarding the clinical utility of autoantibody detection with regards to transplant rejection and survival prognostic value.

against endothelial progenitor cells (EPC) but did not establish the clinical relevance of them [5,6]. Building off of these previous works, Daniel et al. [7] looked specifically at pretransplant IgG and IgM against human EPC in kidney transplant patients using the XM-ONE non-HLA cross-match kit, an assay that detects antibodies targeted generally against EPC but not to specific antigens. In contrast to studies from deceased donors that found a strong clinical impact of pretransplant EPC antibodies on the outcome of kidney transplants [8], this study found that the EPC antibodies had no association with acute rejection in transplants from living donors. Another study using the XM-ONE kit by Gäbel et al. [9] looked at both deceased and living donors and found no difference in their analyses and conclusions that pretransplant anti-EPC antibodies led to early graft rejection but not long-term function. These conflicting results may be a result of the small patient sample sizes in both the Daniel and Gäbel studies ($n = 71$ and $n = 53$ respectively).

One limitation of the aforementioned Daniel study [7] and use of the cross-match kit is that although the presence of antibodies was detected, the antigenic targets from these endothelial cells were not identified. We have used novel proteomic approaches to determine the specific endothelial cell antigen targets present in kidney transplant patients [10]. Using the ProtoArray Human Protein Microarrays from Life Technologies, anti-endothelial cell antibodies (AECAs) isolated from kidney transplant patient sera were profiled against approximately 9500 human proteins. Autoantibodies against endoglin, epidermal growth factor-like repeats (EGF-like repeats) and discoidin I-like domains 3, intercellular adhesion molecule 4, and Fms-like tyrosine kinase-3 were found in all samples who had previously tested positive for AECAs. Significantly, Jackson et al. found that antibody-mediated rejection (AMR) was higher in recipients who tested positive for these AECAs. Although recipients who had higher levels of AECAs also had greater DSAs, looking at biopsies of patients with high AECAs and no or low-level HLA-DSAs still showed greater microvasculature injury than negative recipients. The authors suggest that AECA-induced endothelial cell activation may be responsible for immune cell recruitment and injury and that further studies of AECAs on graft survival are warranted.

Subramanian et al. [11] found a role of ABO incompatibility in the development of autoantibodies against kidney self-antigens. Patients with ABO incompatible (ABOi) kidney transplant following antibody depletion had less rejections than those with ABO compatible kidney transplant. They found that ABOi kidney transplant recipients did not develop antibodies against collagen IV and fibronectin. Although ABO compatible kidney transplant lymphocytes responded in a proinflammatory manner to antigen stimulation, those from ABOi kidney transplant actually produced more IL-10 and less IFN-$\gamma$ and IL-17. Clinically, they showed that ABOi kidney transplant recipients had better glomerular filtration rate and they proposed that the reduced immune response to self-antigens may be partially responsible to the reduction in acute rejection seen.

Hesemann et al. [12] looked at the development of de-novo autoantibodies against angiotensin II receptor type I (ATR1), collagen IV, and fibronectin in pediatric kidney transplant recipients. In their cohort, none of the patients had these autoantibodies pretransplant but half of them developed antibodies to one of more of these at 1-year posttransplant. However, the authors found no correlation with graft function or to the occurrence of acute rejection, although the sample size was small ($n = 20$). ATR1 antibodies were also assessed in patients with AMR by Lee et al. [13] both pretransplant and at time of biopsy ($n = 12$). They find a correlation between ATR1 autoantibodies and AMR in patients without DSAs, in line with other current literature, although a causal linkage was not identified [14–17].

How alloimmune and autoimmune responses are related to one another in transplantation is still under examination, but a review by Subramanian et al. [18] suggests that alloimmune responses may facilitate induction of responses against self-antigens, ultimately leading to chronic rejection. How exactly this de-novo antibody response is generated is an area of active investigation. One potential contributing factor is through polyreactivity of antibodies against both HLA and self-antigens. The Zorn lab of Mass General originally found that specific B-cell clones in a kidney transplant patient experiencing AMR produced antibodies that
reacted against multiple self-antigens as well as HLA class I molecules [19]. These polyreactive antibodies bound apoptotic cells specifically, activated complement, and led to C4d deposition, a hallmark of AMR. In a follow-up publication [20], they extended this analysis to additional clones as well as the evaluation of 300 pretransplant serum samples from kidney transplant recipients. Four clones from two patients bound to numerous HLA class I molecules, including at least one donor HLA antigen. All of these clones additionally reacted to apoptotic cells and other autoantigens. Looking at the pretransplant serum samples, samples with reactivity to HLA class I, II, or MHC Class I Chain-Related Protein A also had increased reactivity to apoptotic cells. This is significant because, in another publication, Gao et al. [21] reported that the presence of these polyreactive antibodies were largely of the IgG1 and IgG3 subtypes, which can induce complement activation, and highly correlated with late kidney graft loss. Although a causal link was not established, the results warrant further investigation into the role of these polyreactive antibodies in kidney allograft rejection.

What antigens primed the immune system to produce these polyreactive antibodies remains unclear, but one possible mechanism may be class-switching from IgM to IgG of B cells producing natural antibodies (NAbs). NAbs are typically of the IgM or IgG3 subtype, are polyreactive, and generally low affinity. NAbs have been shown to react toward HLA and apoptotic cells independently [22,23]. Although natural IgM antibodies against apoptotic cells have been shown to be anti-inflammatory in a normal environment, they can act in a proinflammatory manner in tissues undergoing stress [24] and natural antibodies have previously been linked to autoimmune responses [25]. Further studies will need to be done to evaluate the contributions of natural antibodies against HLA and/or apoptotic cells in transplant rejection.

**RECENT ADVANCES IN UNDERSTANDING OTHER SOLID ORGAN TRANSPLANT AUTOANTIBODIES IN REJECTION**

With regards to other types of solid organ transplants, the role of autoantibodies in rejection is mixed. In liver transplantation, anti-nuclear, anti-smooth muscle (ASMA), and anti-liver–kidney microsomal (ALKM) type 1 antibodies have been found in patients with varying correlations to graft dysfunction but their clinical contributions, if any, were varied. Foschi et al. [26] retrospectively looked at a cohort of 100 liver transplantation patients and found that patients with graft dysfunction had greater levels of autoantibodies. However, after sorting these patients into three groups based on clinical and histological data, they found that all groups had detectable autoantibodies and the authors conclude that the presence was not necessarily causal, but reflected nonspecific activation of the immune system after liver injury. Stanca et al. [27] also investigated autoantibodies against anti-nuclear, ASMA, ALKM, anti-liver cytosol, and anti-soluble liver antigen in liver transplantation and found no particular pattern between autoantibodies and acute cellular rejection or chronic rejection. This is in accordance with studies done in pediatric liver transplantation recipients, where autoantibody presence had no correlation with rejection [28,29].

In pancreatic transplantation, Mujtaba et al. [30] assessed the clinical utility of beta cell autoantibodies such as those against GAD65, insulinoma-associated antigen 2, insulin, and islet-specific zine transporter isoform-8, both pre- and posttransplant concomitantly with DSAs. However, they found little correlation between preexisting or de-novo beta cell autoantibodies and pancreatic allograft dysfunction in short- and medium-term outcomes. For reasons unknown, this is in contrast to findings by Lorenzo et al. [31] where in islet transplantation alone, in contrast to pancreatic transplantation, increases in posttransplant serum autoantibodies against these same antigens predicted islet pancreatic transplant failure.

In cardiac transplantation, posttransplant antivimentin antibodies (AVAs) have previously been shown to be correlated with rejection and negative transplant outcomes [32,33]. However, the role of pretransplant antibodies had not been established. Young et al. [34] investigated the presence and role of pretransplant AVAs and found that, at least in the short-term, the presence of AVAs could not predict rejection within the first year posttransplant or graft survival at a follow-up period of 26 months suggesting that AVAs may be simply an epiphenomenon.

In a thorough study using large-scale proteomic analyses, Dieudé et al. [35*] determined a role of apoptotic cells in the creation of self-antigens and subsequent aortic graft rejection using murine models. They found that apoptotic cells released exosome-like vesicles that contained perlecand specifically the laminin-like globular (LG3) fragment. As LG3 antibodies had been shown to be associated with rejection previously [36], they subsequently showed that apoptotic exosome-like vesicles induced LG3 autoantibody formation and aggravate vascular rejection in an immune complex and proteasome-dependent manner. What was particularly noteworthy was the use of unbiased
proteomic approaches to identify apoptotic exosome-like vesicles specifically containing LG3 and the full 20S proteasome machinery. This allowed the group to identify mechanistically the contributions of LG3 and corresponding autoantibodies to allograft rejection.

**PROTEOMIC APPROACHES TO SELF-ANTIGEN IDENTIFICATION**

Advances in proteomic analyses both in the laboratory and in the clinic have been achieved in recent years, although their use has still yet to become widespread. Although assay platforms such as Luminex™ (Austin, TX, USA) and other bead-based modalities will undoubtedly remain the gold standard for years to come in the clinic [37], recent identification of non-HLA-dependent rejection methods will necessitate high-throughput screens to assess the risks of various types of rejection and immune-mediated transplant injury.

Research methods to evaluate these have largely turned to high density autoantigen microarrays from which antibodies in sera or urine can be detected. Recently developed bioinformatics analyses and software kits have enabled these tools to be widely available. In Fig. 1, we highlight two proteomic approaches used by two separate groups for both identification and determination of mechanism.

These proteomic approaches hold much promise in advancing clinical therapies in organ transplantation, although limitations remain. Although protein arrays that can assess a large number of proteins have been used with increasing frequency in the identification of specific antigenic targets [10*,35*], they have not alone been able to separate pathogenic and nonpathogenic autoantibodies and responses. Use of these approaches in conjunction with complementary, well designed functional assays will be needed to fully determine the prognostic value and clinical validity of newly identified self-antigens and corresponding autoantibodies. Antibodies identified to non-HLA targets by the above mentioned discovery approaches can often be supplemented by further validation of these newly discovered antibodies in independent samples not used in the original discovery set, evaluation of these antibody titers by ELISA or Luminex™, and interrogation of the distribution of their corresponding antigens in the inflamed tissue. When and if these different autoantibodies prove clinically relevant, commercial assays will then need to be developed for clinical use.

**CLINICAL IMPLICATIONS**

Mechanisms controlling the functions of antibodies, both DSA and non-HLA, are complicated in nature. Numerous pathways are upstream and act downstream from autoantibody production and binding. Several significant, actionable ideas to answer questions regarding autoantibodies in transplantation follow from the current data and state of the transplantation field.

Why are there differences in the prognostic value of pretransplant and postransplant antibodies between pediatric and adult transplant recipients? A thorough review of antibody-mediated rejection in pediatric transplantation has been published, which also highlighted the need for greater understanding of this divergence [38]. Many of these conflicting studies have small sample and effect sizes; as such, larger, more robust studies will need to be conducted to resolve these differences. If these differences persist, studies will need to be done to evaluate the mechanisms behind these differences.

What contribution or prognostic value do autoantibodies have in the different long-term survival of deceased and living transplants? Some initial literature supports a differential role of de-novo DSAs in living vs. deceased donor transplant rejection [39] and recent publications have shown potential differences regarding the presence of autoantibodies [7,8]. It has been shown using whole genome expression microarrays that there are significant increase in complement expression in deceased donor kidneys [40] and that inflammation can lead to presentation of cryptic self-antigens [18]. Further work will need to be done to evaluate what phenomena are responsible for differences.

For autoantibodies against particular cell types, such as ASMA or ALKm, what are the specific cellular or surface targets that they recognize? Use of high-throughput proteomic approaches has resulted in the determination of the self-antigens targets by AECAs in kidney transplantation [10*] but such technologies have yet to be applied to other types of transplants or cells.

Which autoantibodies are an epiphenomenon and which are pathogenic? Most clinical studies have only established a correlation between the levels of an autoantibody and incidence of rejection. If an antibody is found to be causal to rejection, what is the mechanism of the pathogenicity? Proteomic approaches such as that used by Dieudé et al. [35*] will be vital to decipher the complex interactions between autoantibody responses, self-antigens, and rejection.
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CONCLUSION

Although recent work has contributed significantly to the identification of specific antigen/autoantibody pairs, the mechanistic and clinical contributions of many remain undetermined. Future studies into nondonor, non-HLA antibodies will need to separate epiphenomenal autoantibodies produced nonspecifically as a result of general injury from those that causally serve to induce immune damage and increase the risk of rejection. Proteomic approaches that lead to rapid identification of autoantibodies will be the key to shifting research efforts toward mechanistic studies. We are optimistic that as mechanisms behind the contributions of autoantibodies become better defined, novel approaches to the treatment and prevention of transplant rejection will be developed to help transplant recipients.

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Conflicts of interest

There are no conflicts of interest.

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


One of the earliest publications to use high density protein microarrays and bioinformatics analyses in the identification of specific antigen-autoantibody pairs in kidney transplantation.

FIGURE 1. Example workflows highlighting (a) proteomic approaches to identification of antigen-specific autoantibodies [adapted from Jackson et al. [10*]] and (b) the determination of self-antigen pathogenicity [adapted from Dieude et al. [35*]].
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