Monitoring and Treating Post-Transplant Human Leukocyte Antigen Antibodies

Matthew J Everly, Paul I Terasaki

PII: S0198-8859(09)00108-6
DOI: 10.1016/j.humimm.2009.04.019
Reference: HIM 8236

To appear in: Human Immunology

Received date: 2 March 2009
Accepted date: 9 April 2009

Please cite this article as: Everly, M.J., Terasaki, P.I., Monitoring and Treating Post-Transplant Human Leukocyte Antigen Antibodies, Human Immunology (2009), doi: 10.1016/j.humimm.2009.04.019.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.
Monitoring and Treating Post-Transplant Human Leukocyte Antigen Antibodies

Matthew J Everly¹, Paul I Terasaki¹∗†.

¹Terasaki Foundation, Los Angeles, CA, USA.

∗Corresponding Author: Paul I. Terasaki, PhD, Terasaki Laboratory, 11570 W. Olympic Blvd, Los Angeles, CA 90064. Email: terasaki@terasakilab.org; Phone: (310) 479-6101; Fax: (310) 445-3381

†Reprint Author: Paul I. Terasaki, PhD, Terasaki Laboratory, 11570 W. Olympic Blvd, Los Angeles, CA 90064. Email: terasaki@terasakilab.org; Phone: (310) 479-6101; Fax: (310) 445-3381

Funding sources: None

Word count (total):
Abstract Word Count: 197
Main Text Word Count: 2989
Figures/Tables: 1/0

Running Title: Monitoring and Treating Post-Transplant Antibodies
Summary

The important role of preformed HLA antibodies in the outcome of organ transplants has been well demonstrated for the past 40 years. During this same period, the significance of HLA antibody formation after transplantation was largely ignored. It was only in the past 10 years, that it has become increasingly clear that HLA antibodies formed post-transplantation is the major cause of allograft failure. We provide here an updated review of the critical evidence implicating HLA antibodies in chronic rejection. It has now also been shown that antibodies formed soon after transplantation is much more toxic to the graft than antibodies formed after the first year. This is likely a result of adaptation of the graft after the first year, possibly through endothelial cell replacement. On the basis of this new finding, a policy of monitoring for antibody development after 3, 6, 9 and 12 months, with yearly checks thereafter are suggested. Also another important new development is the use of bortezomib, a fresh new agent for the removal of antibodies. Since this is the first agent to act on plasma cells, it use is promising. Early data on successful removal of antibodies is reviewed here.

Keywords: human leukocyte antigen, antibodies, allograft survival, rituximab, bortezomib, antibody mediated rejection, transplantation, transplant glomerulopathy.
Introduction

Thirty-three years ago, when we reviewed the survival of 6366 cadaver donor kidney transplants transplanted between 1966 and 1975, the one year graft survival of cadaver donor kidney transplants was 40% (1). This survival rate improved dramatically to the present rate of 90% in the ensuing 33 years (2). Many factors contributed to this success. One of the factors was the introduction of HLA antibody testing in the pre-transplant period (3). This led to the elimination of hyperacute rejection (4), and the reduction of acute rejections by the use of sensitive crossmatching methods (5-7). Thus, the current survival of second cadaver donor kidney transplants is now the same as the first transplant (8). In addition, patients who are highly sensitized now have a graft survival comparable to that of unsensitized patients (8). In all, the important role of preformed HLA antibodies was effectively identified and remedied over the past 40 years.

Unfortunately, during the same 40-year period, long-term allograft survival rates have remained unchanged. In 1976, deceased donor transplants performed between 1966 and 1975 were shown to have a half life of 7.5 years (1). In 2006, 30 years later when reviewed again, the half-life was 7.5 years for those deceased donor transplants performed between 1987 and 1995 (2). Even from the UNOS data of deceased donor transplants performed between 1996 and 2006, the half-life has improved only slightly to 8.1 years (2). This lack of change occurred despite the introduction of many new immunosuppressive agents and aggressive measures to reduce acute rejection rates and treat the T-cell arm of the immune system. It is clear now, though not...
generally appreciated, that cyclosporine, tacrolimus, sirolimus, mycophenolate mofetil (MMF), and other agents have had no effect on long term graft survival.

There are two reasons why these agents have not had an effect in the long run. First, the agents may have been applied in the incorrect way. All protocols used today, give a constant dose and sometimes declining dosages during the maintenance period. If a monitoring system had existed, the drugs might have been given only when the recipient mounts an immunological response, say in the second year after transplantation. New evidence has been provided by Rebellato and colleagues that MMF, given in higher dosages when HLA antibodies appeared, had the effect of reducing antibodies (9). It is thus possible that MMF, when given at the right time, might be more effective, than when given continuously without any knowledge of when to increase its dosage. Secondly, since the usual maintenance drugs have been shown to prevent antibody formation in about 75% of the organ transplant patients post-transplantation (10), with proper monitoring, the dosages needed per patient could have been adjusted. A comparison of the various drugs showed that they were almost equivalent in efficacy in preventing antibody formation (11).

Allograft failure in the presence of HLA antibodies

In the quest to fully appreciate the risk associated with post-transplant anti-HLA antibodies, we have analyzed data from numerous centers worldwide over the past 10 years. In 2000 (12), 2003 (13), 2005 (14, 15), and 2006 (16), we reviewed evidence associating HLA antibodies post-transplantation with chronic rejection. This then led to our consolidation of the evidence to the conclusion that HLA antibodies causes chronic rejection (17).
In the intervening period since our last review(17), further evidence for the role of HLA antibodies in chronic rejection has appeared. Details on the injury produced by antibody mediated rejection especially regarding transplant glomerulopathy was reported by Gloor (18) and Issa et al.(19). Additionally, post-transplant tests for HLA antibodies were found to be useful in predicting renal allograft dysfunction (20-22). Even blood group Lewis antibodies in 3 patients were noted to produce severe kidney allograft dysfunction resulting in one graft loss(23).

Similarly for lung transplants, pulmonary capillaritis was associated with acute humoral rejection and donor specific HLA antibodies (22, 24). Convincing evidence for presence of antibodies in broncho-alveolar lavage of lung transplants was found in conjunction with soluble C4d (25). Heart transplant failure could be predicted with the use of C4d fixing and HLA antibodies found by Luminex testing (26). Donor specific antibodies were noted to have a long term effect in cardiac allograft vasculopathy (27). Even in liver transplants, new evidence of lower graft survival in patients with preformed antibodies was noted (28).

Despite a large body of evidence that links HLA-antibodies to allograft dysfunction and loss, some doubt remains about the strength of evidence for a cause-and effect relationship. Two questions still remain: 1) Why do patients have good function despite the presence of HLA antibodies; and 2) Does treating antibodies improve allograft survival? The new research developments above begin to shed light on the answer to this question, but continued research is needed.

This review will discuss the current research with a specific focus on the following areas: 1) how should the presence of antibodies during good function be interpreted? 2) What is the appropriate means and timing to monitor patients? 3) Under what circumstances and at what
level/intensity for antibody should treatment be started? 4) What therapeutic agents should be
used to remove antibodies? and 5) How much antibody removal is enough?

**Good graft function despite the presence of HLA antibodies.**

One of the most frequently heard criticisms of the antibody theory of chronic rejection is
that many patients survive well despite the presence of HLA antibodies. This finding was
apparent since the time when 4,763 patients with functioning transplants were studied as part of
the International Histocompatibility Workshop (29). It was noted that among patients with
functioning grafts, 21% of kidney, 19% of liver, 23% of heart and 14% of lung transplant
patients had HLA antibodies. Thus almost 1,000 patients with well functioning grafts of various
types were surviving while having HLA antibodies. Subsequent longitudinal studies, with serial
serum samples tested from the time of transplantation, have shown that many patients develop
HLA antibodies well before failure of the graft (30-32). Bartel et al. have recently published an
interesting study in which they had selected 34 patients with uneventful first year course and
with excellent graft function and a mean follow up time of 68 months (33). Serum samples taken
2, 6 and 12 months after transplant were tested for HLA antibodies. Nine recipients (27%) with
excellent 1 year graft function were found to be positive by flow PRA screening. Since these
patients continued to have good function for a mean of 68 months, the authors conclude that
antibody monitoring results should be interpreted cautiously. As noted above, we had also seen
many patients with antibodies who have survived with good function. Unfortunately, the study
design did not include patients who failed within the same time period. We believe if they had
been included, the authors would have found that a much higher proportion of patients would
have had antibodies, as we had seen in a similar retrospective study (34).
Another criticism of the antibody theory of chronic rejection is the theory of accommodation. Accommodation is clearly seen in ABO incompatible transplants (35), and is suggested as a phenomenon responsible for good function in the presence of antibodies. One such study by Higgins et al. analyzed twenty-four patients who had HLA antibodies pre-transplant, were treated with plasmapheresis ± intravenous immune globulin (IVIG), and had daily monitoring for HLA antibodies until post-operative day 14 (36). Of these patients, donor-specific antibodies (DSA) either were found to cause no rejection (12 patients) or rejection (10 patients) that resolved despite persistence of DSA. From this, the authors suggest that accommodation to DSA is the dominant mechanism of graft acceptance. Because the follow-up period in this study was 3 months, we believe accommodation unlikely, for it is common that antibodies require a much longer time to actually damage a whole kidney. In some instances many years had elapsed before the graft was rejected in the above cited serial longitudinal studies. Additionally, it was recently shown that although DSA does persist in many acute rejection cases despite histologic resolution of rejection, reduction of the antibody within 2 weeks of rejection onset drastically improves survival (37). Finally, in the experimental animal model, the duration of time after the appearance of antibody in the circulation until C4d deposits, glomerular changes and final increase in serum creatinine occurred between 6 months and 28 months (38). Thus, we do not see a need to postulate accommodation.

One of the most important recent findings that indicates why patients may have good function despite circulating antibodies was the observation that antibodies developed early after transplantation may be more damaging to the allograft than antibodies developed after a year post-transplant as shown by Lee et al (39) (Figure 1). This marked difference in effect of antibodies which had developed early or late after transplantation had not been shown in earlier
studies. The strong effect of antibodies developed in the acute phase as noted by Burns et al (40), may reinforce the above findings of a greater effect of early antibodies. To explain this effect, we postulated that the kidney may be more resistant to damage after it had been in place over one year, possibly due to some replacement of graft endothelium lining by host cells during this period.

Methods for Monitoring Post-Transplant Patients for HLA Antibodies

As stated above, most patients post-transplantation will develop antibodies at some point (41-44). Once alloantibodies appear, progression to transplant glomerulopathy and rising creatinine/renal failure are eminent (29, 34, 38, 41, 44-48). Given this, what then are the appropriate times to measure for antibodies? The answer to this question is unfortunately not as clear. Time to first detection of antibodies differs vastly between patients. In a retrospectively examined serial sampling study from 93 kidney patients who had failed allografts, some antibodies were formed in as little as 1 month after transplantation. The mean time to antibody formation was 11 months and once appeared a mean of 29 months elapsed before allograft loss (16, 49). Similar to these finding, a recent study of evaluating time to HLA antibody development and its association with allograft loss showed that patients with early antibodies (within 1st year) post-transplant, specifically class I, were at 8 times higher risk of allograft loss (39). On the basis of these results, antibody monitoring in the first year is essential. Adopting a monitoring frequency of 7, 14, 30, 60, 90, 180 and 360 days that has been suggested by some may be excessive. When pre-transplant antibody testing is negative, testing in the first month post-transplant may reveal a high level of HLA antibody that is elevated secondary to inflammatory response of kidney transplant rather than as a result of immune activation.
Therefore, in low risk (unsensitized) patients, pre-transplant and every 3 month monitoring for the first year may be a reasonable approach. High-risk (presensitized and re-transplants) may benefit for more testing in the first 3 months post-transplants to detect early immune reactivity as a result of preformed antibody. Following the first year bi-annual or annual measurements seem reasonable. Testing corresponding with a biopsy for allograft dysfunction is a necessity. Testing following immunosuppression reduction or changing has also been shown to have benefit (11, 50, 51).

**Methods for Removal of Antibody**

Once antibodies are detected removal with follow-up monitoring is necessary. A number of treatment modalities have historically been used to treat antibodies in the settings of pre-transplant highly sensitized patients and post-transplant acute rejection cases. The modalities include plasmapheresis, intravenous immune globulin (IVIG), low-dose cyclophosphamide, and anti-thymocyte globulin. These agents and their effect on HLA antibodies was previously reviewed (14). In sum, although each of these therapies has had some effect on ABO incompatible patients and highly sensitized patients or even in treating antibody-mediated rejection, their effect on HLA antibodies is inconsistent and at best transient (52-54). Even rituximab, although it has been shown to lead to histological reversal of acute humoral rejection multiple times (55-60), fails to reduce antibody levels (61).

Of the currently available agents, none mentioned above have the adequate ability to inhibit mature plasma cells and therefore has little effect on reducing antibody production (62). A study by Perry et al. recently evaluated plasma cell depleting of anti-thymocyte globulin and rituximab (63). On bone marrow biopsy of highly sensitized transplant patients treated with
rituximab, as well as anti-thymocyte globulin, produced only minimal apoptosis of plasma cells. However, a new agent, bortezomib has been shown to have significant apoptotic effect on plasma cell(63, 64). Proteasome inhibition, via bortezomib, represents a novel treatment strategy because it provides a means for depleting plasma cells (64). It is FDA approved for the treatment of multiple myeloma, but recently bortezomib has been used as a rescue strategy for the treatment of refractory acute humoral rejection(52). In addition, it has also been used prior to allograft dysfunction in an attempt to improve survival (65). Its ability to reduce anti-HLA antibody levels can be mechanistically explained by its ability to regulate a number of immunomodulatory processes (64, 66-75). In brief, bortezomib acts directly on short- and long-lived plasma cells, although it does not affect all plasma cells equally. Those hyper-producing antibodies to HLA are most sensitive to effects of bortezomib. In the end, bortezomib places these plasma cells under great stress and induces apoptosis (76-82). Bortezomib is given at a dose of 1.3 mg/m² on days 1, 4, 8, and 11. This dosing constitutes 1 cycle. In a recent analysis of 13 patients with DSA treated with bortezomib, it was found that those patients (n=9) with DSA levels <10,000 MFI responded well to one cycle leading to complete removal below 1000 MFI (83). However, those patients with MFI >10,000 only partially responded (n=4). This indicates the need for multiple (2-3) cycles in cases of high intensity DSA. Overall, bortezomib is safe and effective, making it a refreshing new addition to transplantation.

Despite the substantial effects and promise of bortezomib, antibody removal cannot be looked at as a static process. Use of one cycle of bortezomib, with the expectations that a patient will never again produce anti-HLA or non-HLA antibodies, is unrealistic. The transplanted kidney is a constant antigenic stimulus; therefore the approach to treatment must focus on monitoring, suppressing, and occasionally ablating the antibody response. Currently, at the
University of Cincinnati bortezomib treatments have primarily been used in acute humoral rejection cases and at IKDRC-ITS in India in patients without dysfunction. Many of these patients also received therapy with rituximab in order to delete precursor b-cells and plasmapheresis to aid in removal of antibodies. This combination had profound effect on antibody production beyond the completion of treatment with bortezomib. In Cincinnati, most cases have at least 6 months of antibody reduction following one cycle of bortezomib. Therefore based on the result of these studies the treatment of antibody is based on 5 elements:

1. Depletion of plasma cells (bortezomib);
2. Inhibition of naive and memory b-cells progressing to plasma cells (rituximab);
3. Suppression of the cell cycle and proliferation of both B and T-cells (this can be done with current baseline immunosuppression such as tacrolimus and MMF);
4. Elimination of circulating antibodies with plasmapheresis (after treatment with bortezomib) – this is also helpful in determining the effectiveness of bortezomib on plasma cell depletion.
5. Monitoring: This process is only effective if serial monitoring of anti-HLA/MICA antibodies occurs.

Beyond treatment, a preventative approach to antibodies is the ultimate goal. Serial antibody monitoring is the beginning of prevention, but appropriate ways to use immunosuppression to suppress antibodies may test the current standard of care protocols. As all protocols today give a constant dose and sometimes declining dosages during the maintenance period a more patient specific approach using HLA single antigen monitoring may provide means to adjust immunosuppression to the immune system. This concept has currently been
tested in by the use of spaced weaning of immunosuppression (50) as well in a clonal deletion protocol (84).

**Antibody Removal: How much is enough?**

Once a patient has persistence of antibody, treatment is essential because it is clear that antibodies will progress to allograft loss at some point post-transplant. With a decision to treat antibody endpoints need to be well described. Recent results from Cincinnati and Greenville indicate that in acute rejection a 50% reduction in the highest antibody is a possible breakpoint (37). In the Cincinnati study, patients were evaluated retrospective for prospective treated patients. All patients were treated. All patients achieved histologic reversal of rejection. However, those patients with a 50% reduction in their highest level donor-specific antibody had no episodes of allograft loss. This study showed a new endpoint in therapy – treat that antibody, not just the histology, however, the 50% reduction point was chose arbitrarily because it was thought to be a significant decrease. To confirm these findings a similar retrospective analysis of prospectively treated patients was conducted (85). Again a 50% reduction in antibody intensity was shown to be the point necessary to improve allograft survival. The findings from these two studies give the initial proof that antibody reduction will have a strong influence on long-term survival. Further studies with this focus will be needed to determine exactly what level of antibody is to be targeted or if removal of antibody to below detectable levels should be the goal.

**Conclusions**

Data on post-transplant HLA antibodies as a major cause of allograft loss is now substantial. It appears that almost all patients following transplantation will develop antibodies at
some point although it may take many years for the antibody to destroy the kidney graft (16, 43, 44, 46, 86) and the theory of accommodation is becoming less likely, rather some patient’s allograft is damaged by antibodies faster than others. New data suggesting reduction of antibody improves survival and the use of a new agent, bortezomib to remove antibody producing cells is promising. Moving forward, we must focus monitoring and treating at first detection of persistent antibodies. This may be the only reasonable way to have significant impact on long-term allograft survival.
FIGURE LEGEND

Figure 1. Death-Censored Allograft Survival Stratified by the Presence of Antibody within 1 Year. All patients who developed HLA antibodies had a significant lower survival rate than in patients without de novo HLA antibodies (log rank p<0.001). Moreover, patients developed HLA antibodies within 1 year had even worse survival rate than in patients developed antibodies beyond 1 year.
References


Monitoring and Treating Post-Transplant Antibodies


Figure 1

The figure shows the percent allograft survival over years posttransplant for patients with and without HLA Abs (+) before or after 1 year. The Log-rank test indicates a significant difference (p<0.0001).