EDITORIAL

Progress in understanding humoral rejection in kidney transplantation: implications for patient management

M. A. Pascual (MD), M. Crespo (MD) y N. Tolkoff-Rubin (MD)
Renal and Transplantation Units. Departments of Medicine and Surgery. Massachusetts General Hospital and Harvard Medical School. Boston. Massachusetts.

Until recently, most studies on the mechanisms of renal allograft rejection have focused on the central role of T cells and of other cellular mechanisms of tissue injury. Over the years, it has been established that CD4 T cells are crucial in initiating most acute rejection episodes, and that alloactivated CD4T cells, cytotoxic CD8T cells, monocytes/macrophages and NK cells play a major role in cell-mediated mechanisms that eventually result in allograft destruction[1]. These research efforts in the cellular immunity of organ transplantation have been illustrated in a recent study by Li y cols., in with measurement of urinary mRNA encoding perforin and granzyme B was used as a noninvasive means of diagnosing acute renal allograft rejection[2]. Perforin and granzyme B are two proteins that are present in the cytoplasmic granules of cytotoxic T cells and NK cells which are an integral part of the effector mechanisms of cell-mediated allograft rejection.

In recent years, however, it has become increasingly appreciated that detection of anti-HLA donor specific antibodies (DSA) *de novo* after transplantation is associated with specific «humoral syndromes» which are due predominantly, or in part, to antibody-mediated effector mechanisms of tissue injury. The identification of the complement fragment C4d as a specific marker for humoral rejection in peritubular capillaries of renal allograft biopsies has helped to define and characterize these syndromes, which we have recently termed *acute humoral rejection* (AHR) and *chronic humoral rejection* (CHR)[3-7]. In this article, the three different clinical settings in which humoral immunity appears to play an important role in clinical kidney transplantation are reviewed. In addition, new therapeutic approaches to control the production or the detrimental consequences of alloantibodies are discussed in the light of our recent studies and those of others.

HYPERACUTE REJECTION

The rejection of an allograft within minutes to hours after transplantation is termed «hyperacute rejection» (HAR). HAR is generally mediated purely by humoral mechanisms, that is by the binding of recipient's DSA to the donor graft vasculature which triggers complement activation. Both preformed DSA to HLA antigens or anti-ABO isohemagglutinins can result in «hyperacute rejection»[8,9]. Preformed anti-HLA DSA have generally been induced by previous exposure to alloantigens through prior transplantations, pregnancies or multiple blood transfusions[10]. Anti-ABO blood group natural antibodies are present in recipients who receive a blood group-incompatible transplant[11].

The detrimental effect of preformed DSA became apparent in the 1960s. In the presence of pre-existing DSA («positive crossmatch» at the time of renal transplantation), hyperacute destruction of grafted kidneys occurred almost inevitably[8,9]. The histopathologic findings of HAR revealed intense neutro-

Correspondence: Dr. M. A. Pascual
Assistant Professor of Medicine, Renal Unit, Box MZ 70
Massachusetts General Hospital and Harvard Medical School
Boston, MA 02114
E-mail: mpascual@partners.org

M. Pascual is the recipient of the Helen and George Burr Endowed Research and Educational Award in Support of Transplantation. This work was also supported by the Yates Fund for Transplant Technology (M.P.), the Foundation Suisse de Bourses en Médecine et biologie (M.P.) and by the Fundación Lair, España (M.C.).
M. Crespo's current address is: Unidad de Trasplante Renal. Hospital Clínic de Barcelona.

philic infiltrate, edema, focal interstitial hemorrhage and thrombosis, and fibrin thrombi in capillaires associated in some cases with fibrinoid necrosis of small arteries. Importantly, the absence of a mononuclear cell infiltrate indicated that HAR was not due to cell-mediated immunity. This initial experience stimulated the requirement for demonstration of a pre-transplant «negative» crossmatch and, in most centers, ABO compatibility to perform kidney transplantation[12,13]. HAR has become a very rare clinical event in kidney transplantation. Renewed interest in HAR (and in humoral rejection in general) has originated from the field of discordant xenotransplantation, in which natural xenoantibodies are responsible for complement-mediated HAR that has proved difficult to overcome. Removal of circulating natural xenoantibodies and/or inhibition of complement activation are two methods that have been succesfully used to prevent HAR in pig to primate models[14].

ACUTE HUMORAL REJECTION

In the early 1990s, P. Halloran y cols., proposed that acute rejection associated with the development of *de novo* anti-HLA DSA in recipient's serum (i. e. AHR) is a clinico-pathologic entity carrying a poor prognosis[15,16]. In a subsequent report, these authors analyzed the histopathologic features of AHR. Neutrophils in peritubular capillaires, glomerulitis, fibrin thrombi, infarction, severe vasculitis and fibrinoid necrosis in vessels walls were found to correlate with circulating DSA against HLA class I antigens[17]. These findings are distinct from those of cell-mediated acute rejection (without circulating DSA), which is characterized predominantly by a mononuclear cellular infiltrate with tubulitis and/or endothelialitis[18]. In the past, the terms «accelerated rejection», «delayed hyperacute rejection» or «acute vascular rejection» have been used to describe what most likely was AHR in a majority of cases[5]. However, it should be noted that these other terms may be confusing. For example, «acute vascular rejection» or «accelerated rejection» are entities not necessarily restricted to an antibody-mediated process, as they can also reflect T cell-mediated injury[3,18]. In particular endarteritis or endothelialitis, a form of «vascular rejection», can be exclusively due to cell-mediated immunity[5]. For these reasons, we prefer to keep the term «AHR» which is based on pathogenic mechanisms.

In 1999, B. Collins y cols., demonstrated that staining of allograft biopsies for the fragment C4d, a split product of complement C4, is a reliable method to identify AHR[4]. It was found that widespread C4d deposits in cortical peritubular capillaries correlated with the detection of *de novo* anti-HLA DSA in recipient's serum *(de novo* positive crossmatch). After activation of the classical pathway of complement by antibodies, the fragment C4d is released and remains covalently bound to the nearby endothelium or basement membrane collagen, thereby providing *in situ* pathologic evidence of antibody-mediated injury. These observations extended prior work by H. Feucht y cols., in 1993. These authors found that allografts with early dysfunction whose biopsies demonstrated capillary deposition of C4d were at a singnificantly increased risk of failure when compared to allografts with no C4d[19]. In their study, however, repeat posttransplant crossmatches were not performed so that the presence of *de novo* circulating DSA was not assessed.

Clinically, AHR typically presents as early and severe allograft dysfunction[3,5,6]. The risk of allograft failure (50-80%) is particularly increased in the first three months posttransplant[17,20,21]. We have recently analyzed the Massachusetts General Hospital experience over a four-year period and found an incidence of AHR within the first three months after renal transplantation of 7.7%, that is 20-25% of all acute rejections had a humoral component[6]. Most often, DSA were IgG against HLA class I antigens, but in some cases specificities against HLA class II or IgM antibodies could be defined. In approximately half of the patients with AHR, the rejection was resistant to both steroid and antilymphocyte therapy. A higher level of pretransplantation sensitization and a history of a previous failed allograft were found to be significant risk factors for AHR, suggesting that a specific anamnestic humoral response against donor antigens plays an important role in its pathogenesis[6]. We have also diagnosed AHR in two patients receiving cyclosporine, prednisone and mycophenolate mofetil, in whom the dosage of cyclosporine was decreased to subtherapeutic levels because of cyclosporine toxicity as well as in two hepatitis C-infected recipients following the introduction of interferon therapy (M. Pascual, unpublished observations). Finally, it should be emphasized that not uncommonly, histopathologic findings of acute cellular rejection may be present in biopsies with AHR («mixed» cellular and humoral pattern). The identification of C4d deposits in peritubular capillaries can be the only pathologic feature indicating the humoral component of the rejection process.

CHRONIC HUMORAL REJECTION

In addition to non-immunological factors, both cellular and humoral immune mechanisms play a key

role in the pathogenesis of chronic rejection (CR), a condition also termed «chronic allograft nephropathy» (CAN)[22]. In particular, the presence in serum of alloantibodies to donor HLA class I or class II antigens has been associated with CR/CAN, possibly manifesting alloresponsiveness via the indirect pathway of allograft recognition[1,7,23-25]. Posttransplant production of alloantibodies can predate the clinical manifestations of CR/CAN, implicating humoral mechanisms as a possible cause of CR/CAN[23]. Recent studies indicate that C4d deposits in peritubular capillaries are not only found in patients with AHR but also in a subset of patients with CR/CAN[7]. Approximately 60% of biopsies with histologic criteria for CR/CAN (transplant arteriopathy and/or chronic transplant glomerulopathy) had C4d deposits in peritubular capillaires. In most cases, this was accompanied by detectable DSA in the patient's serum[7]. To determine the relative contribution of humoral mechanisms of rejection to late allograft failure, we studied the prevalence of CHR in patients with chronic renal allograft dysfunction of all causes. C4d deposits in PTC were found in 13% of renal recipients who received an allograft biopsy for chronic allograft dysfunction[26]. Contrary to AHR, it does not appear that pretransplant sensitization is a risk factor for the development of CHR. In our experience, non compliance or underimmunosuppression is often found to be a cause for the development of CHR, suggesting that clinical trials evaluating withdrawal of calcineurin inhibitors or steroids should monitor DSA production.

NEW THERAPEUTIC APPROACHES TO CONTROL HUMORAL REJECTION

Since 1995, we have evaluated a new therapeutic approach consisting of «Plasmapheresis combined with tacrolimus-mycophenolate rescue» (PPh-TAC-MMF rescue) for renal recipients suffering from AHR refractory to both steroid and antilymphocyte therapy[3,6]. During a 4-year period, 232 renal transplants were performed under cyclosporine-based immunosuppression. In 10/232 (4.4%) consecutively studied patients with «refractory» AHR, the protocol of PPh-TAC-MMF rescue was initiated. Ths therapeutic strategy significantly decreased circulating DSA over a period of 3 to 6 weeks with reversal of rejection in 9/10 patients. At the end of plasmapheresis, polyclonal immunoglobulin was administered intravenously to limit the risk of infectious complications. With a mean follow-up of 42 months, patient and graft survival are 100% and 80%, respectively. Long-term monitoring of DSA titers revealed persistently undetectable levels of DSA in all patients with functioning allografts. In contrast, DSA was demonstrated in both patients with failed allografts[27]. These data suggest that a therapeutic strategy using «PPh-TAC-MMF rescue» can consistently prevent allograft loss and improve the outcome of early refractory AHR.

These observations on the control of humoral responses in patients with refractory AHR have been recently extended to the treatment of CHR, i.e. CR/CAN associated with DSA and C4d deposits in PTC[24]. We found that in recipients with CHR, rescue therapy with TAC and MMF alone (i.e., without plasmapheresis) resulted in a progressive and sustained decrease in DSA titers, with stabilization of renal allograft function. In two patients, DSA became undetectable after six to nine months of therapy, and repeat biopsies performed at 12 months revealed a decrease or absence of C4d deposits in PTC[24]. These preliminary findings confirm that suppression of alloantibody production is possible with the combination of TAC-MMF, and this effect may be of value for the treatment or prevention of CR/CAN.

Similar therapeutic strategies may also be useful to prevent HAR and thus allow kidney transplantation in highly sensitized patients. In Spain and in the US, approximately 10-15% of kidney transplant recipients are highly sensitized[28,29]. A rapid «desensitization» protocol was recently evaluated by Montgomery y cols.[30]. Successful desensitization was achieved in four highly sensitized patients who had positive cross-matches against their potential living donors. The protocol consisted of PPh and intravenous immunoglobulin (IVIg) with concomitant administration of TAC-MMF-prednisone, initiated several days or weeks before the planned transplantation. This was continued until a negative cross-match was achieved. All four patients developed episodes of AHR in the first month posttransplant, but these were successfully reversed with additional PPh and IVIg. In another similar study, 11 out of 15 patients with a pretransplant positive cross-match against their living donor could be successfully desensitized[31]. Pretransplant, the patients received PPh three times weekly over two consecutive weeks, in combination with IVIg and TAC-MMF-prednisone. These patients underwent successful transplantation with OKT3 induction and continuation of TAC-MMF. Relatively low initial titers of DSA were predictive of successful attainment of a negative crossmatch. Suppression of HLA-specific antibodies by the administration of high-dose IVIg has also been proposed as a means to desensitize patients awaiting transplantation[32,33]. IVIg has well-known immunomodulatory properties, including inhibition of complement activation and a

decrease in antibody synthesis, and has also been shown to be beneficial in the treatment of AHR in renal and cardiac allograft recipients[34]. Thus, we believe that incorporating IVIg in protocols of combining PPh-TAC-MMF probably provides additive effects that may be useful to decrease DSA synthesis.

In the upcoming years, it is likely that the effects of other newer immunosuppresive drugs on *in vivo* alloantibody synthesis will be clarified. Blocking the interleukin-2 receptor with daclizumab has already been shown to reduce the formation of anti-HLA antibodies posttransplant in cardiac transplant patients[35]. Rapamycin (sirolimus) suppresses immunoglobulin synthesis *in vitro*[36], thus it can be speculated that combining tacrolimus with sirolimus may offer an interesting alternative to tacrolimus-MMF regimen to control humoral responses in humans[37]. Finally, the role of complement inhibitors remains to be defined in solid organ transplantation. Indeed, specific inhibition of the complement system may allow preventing or treating the deleterious consequences of DSA without the need of antibody removal. Soluble CR1 (sCR1 or TP10), a recombinant form of endogenous soluble complement receptor 1 (CR1, CD35, C3b/C4b receptor), is a very efficient inhibitor of both the classical and alternative pathways of complement[14, 38, 39]. The presence of C4d in peritubular capillaries of renal recipients with humoral rejection indicates that the classical pathway is activated due to the binding of DSA to the graft endothelial cells. In the near future, induction therapy or posttransplant administration of complement inhibitors may open new avenues in transplantation. This new class of drugs, already undergoing phase I clinical trials in allograft recipients may become an important addition to the pharmacologic armamentarium used in organ transplantation and other immune-mediated diseases[40].

SUMMARY AND CONCLUSION

After more than 40 years of clinical renal transplantation, the contribution of humoral immunity to the pathogenesis of allograft rejection is progressively being clarified. With the advent of a new generation of immunosuppressive agents, the production and consequences of anti-donor alloantibodies can now be controlled. In the upcoming years, immunosuppressive regimens that will specifically control both T- and B-cell responses may further improve long-term allograft survival, if the immunosuppresive efficacy of such regimens is not hampered by an increase in infectious, neoplastic or cardio-vascular complications.

ACKNOWLEDGMENTS

We thank Prof. Jürg A. Schifferli (Kantonsspital Basel, Basel, Switzerland) for his review of the manuscript. We also would like to thank many members of the Renal and Transplantation Units, and of the Department of Pathology of Massachusetts General Hospital who contributed to the studies on acute and chronic humoral rejection.

REFERENCES

1. Sayegh MH: Why do we reject a graft? Role of indirect allorecognition in graft rejection. *Kidney Int* 56: 1967-1979, 1999.
2. Li B, Hartono C, Ding R y cols.: Noninvasive diagnosis of renal allograft rejection by measurement of messenger RNA for perforin and granzyme B in urine. *N Engl J Med* 344: 947-954, 2001.
3. Pascual M, Saidman S, Tolkoff-Rubin N, Williams WW, Mauiyyedi S, Duan JM, Farrell ML, Colvin RB, Cosimi AB, Delmonico FL: Plasma exchange and tacrolimus-mycophenolate rescue for acute humoral rejection in kidney transplantation. *Transplantation* 66: 1460-1464, 1998.
4. Collins AB, Schneeberger E, Pascual M, Saidman S, Williams W, Tolkoff-Rubin N, Cosimi AB, Colvin RB: Complement activation in acute humoral renal allograft rejection: diagnostic significance of C4d deposits in peritubular capillaries. *J Am Soc Nephrol* 10: 2208-2214, 1999.
5. Crespo M, Delmonico F, Saidman S, Tolkoff-Rubin N, Williams W, Colvin RB, Cosimi AB, Pascual M: Acute humoral rejection in kidney transplantation. *Graft* 3: 12-17, 2000.
6. Crespo M, Pascual M, Tolkoff-Rubin N, Mauiyyedi S, Collins AB, Fitzpatrick D, Delmonico FL, Cosimi AB, Colvil RB, Saidman S: Acute humoral rejection in renal allograft recipients: incidence, serology and clinical characteristics. *Transplantation* 71: 652-658, 2001.
7. Mauiyyedi S, Pelle P, Saidman S, Collins AB, Pascual M, Tolkoff-Rubin N, Williams W, Cosimi AB, Schneeberger E, Colvin RB: Chronic humoral rejection: identification of antibody mediated chronic renal allograft rejection by C4d deposits in peritubular capillaries. *J Am Soc Nephrol* 12: 574-582, 2001.
8. Kissmeyer-Nielsen F, Olsen S, Petersen VP, Fjeldborg O: hyperacute rejection of kidney allografts, associated with preexisting humoral antibodies against donor cells. *Lancet* 1: 662-665, 1966.
9. Williams GM, Hume DM, Hudson RP Jr., Morris PJ, Kano K, Milgram F: «Hyperacute» renal-homograft rejection in man. *N Engl J Med* 279: 611-618, 1968.
10. Braun WE: Laboratory and clinical management of the highly sensitized organ transplant recipient. *Hum Immunol* 26: 245-260, 1989.
11. Tanabe K, Takahashi K, Sonda T y cols.: Long-term results of ABO-incompatible living kidney transplantation: a single center experience. *Transplantation* 65: 224-228, 1998.
12. Patel R, Terasaki PI: Significance of the positive crossmatch test in kidney transplantation. *N Engl J Med* 280: 735-739, 1969.
13. Murray JE, Merrill JP, Dammin GJ y cols.: Kidney transplantation in modified recipients. *Ann Surgery* 156: 337-355, 1962.
14. Gianello P: Preventing hyperacute rejection. *Graft* 4: 18-21, 2001.

15. Halloran PF, Wadgymar A, Ritchie S, Falk J, Solez K, Srinivasa NS: The significance of anti-class I antibody response. Clinical and pathologic features of anti-class I mediated rejection. *Transplantation* 49: 85-91, 1990.
16. Halloran PF, Schlaut J, Solez K, Srinivasa NS: The significance of anti-class I antibody response II. Clinical and pathologic features or renal transplants with anti-class I-like antibody. *Transplantation* 53: 550-555, 1992.
17. Trpkov K, Campbell P, Pazderka F, Cockfield S, Solez K, Halloran PF: Pathologic features of acute renal allograft rejection associated with donor-specific antibody. *Transplantation* 61: 1586-1592, 1996.
18. Colvin RB: The renal allograft biopsy. *Kidney Int* 50: 1069-1082, 1996.
19. Feucht HE, Schneeberger H, Hillebrand G, Burkhardt K, Weiss M, Land W, Albert E: Capillary deposition of C4d complement fragment and early graft loss. *Kidney Int* 43: 1333-1338, 1993.
20. Lobo PI, Spencer CE, Stevenson WC, Pruett TL: Evidence demonstrating poor kidney graft survival when acute rejections are associated with IgG donor-specific lymphocytotoxin. *Transplantation* 59: 357-360, 1995.
21. Martin S, Dyer PA, Mallick NP, Gokal R, Harris R, Johnson RWG: Posttransplant antidonor lymphocytotoxic antibody production in relation to graft outcome. *Transplantation* 44: 50-53, 1987.
22. Pascual M, Swinford RD, Ingelfinger JR, Williams WW, Cosimi AB, Tolkoff-Rubin N: Chronic rejection and chronic cyclosporine toxicity in renal allografts. *Immunol Today* 19: 514-519, 1998.
23. Davenport A, Younie ME, Parsons JE, Klouda PT: Development of cytotoxic antibodies following renal allograft transplantation is associated with reduced graft survival due to chronic vascular rejection. *Nephrol Dial Transplant* 9: 1351-1319, 1994.
24. Theruvath TP, Saidman SL, Mauiyyedi S, Delmonico FL, Williams WW, Tolkoff-Rubin N, Collins AB, Colvin RB, Cosimi AB, Pascual M: Control of antidonor antibody production with tacrolimus and mycophenolate mofetil in renal allograft recipients with chronic rejection. *Transplantation* 2001 (in press).
25. Abe M, Kawai T, Futatsuyama K, Tanabe K, Fuchinoue S, Teraoka S, Toma H, Ota K: Postoperative production of antidonor antibody and chronic rejection in renal transplantation. *Transplantation* 63: 1616-1619, 1997.
26. Theruvath TP, Saidman S, Mauiyyedi M, Rubin N, Williams WW, Collins B, Colvin RB, Delmonico F, Cosimi AB, Pascual M: Prevalence of chronic humoral rejection in chronic renal allograft dysfunction. *Am J Transpl* 1 (S1): 360 (A896), 2001.
27. Theruvath TP, Delmonico FL, Saidman S, Rubin N, Mauiyyedi Williams WW, Collins B, Colvin RB, Cosimi AB, Pascual M: Long-term outcome after treatment of early refractory acute humoral rejection in kidney transplantation. *Am J Transpl* 1 (S1): 151 (A61), 2001.
28. Miranda B, Cañón J, Nava MT, Cuende N: Donación y trasplante renal en España 1989-1999. *Nefrología* 20 (Supl. 5): 45-54, 2000.
29. Hariharan S, Johnson CP, Bresnahan BA, Taranto Se, McIntosh MJ, Stablein D: Improved graft survival after renal transplantation in the united states, 1988 to 1996. *N Engl J Med* 342: 605-612, 2000.
30. Montgomery RA, Zachary AA, Racusen LC, Leffell MS, King KE, Burdick J, Maley WR, Ratner LE: Plasmapheresis and intravenous immune globulin provides effective rescue therapy for refractory humoral rejection and allows kidneys to be sucessfully transplanted into cross-match positive recipients. *Transplantation* 70: 887-895, 2000.
31. Schweitzer EJ, Wilson JS, Fernández-Vina M, Fox M, Gutiérrez M, Wiland A, Hunter J, Farney A, Philosophe B, Colonna J, Jarrell B, Bartlett ST: A high panel-reactive antibody rescue protocol for cross-match-positive live donor kidney transplants. *Transplantation* 70: 1531-1536, 2000.
32. Glotz D, Hayman J, Naiudet P, Lang P, Druet P, Bairety J: Sucessful kidney transplantation of immunized patients after desensitization with normal human polyclonal immunoglobulins. *Transplant Proc* 27: 1038-1039, 1995.
33. Tyan D, Li Va, Czer L, Trento A, Jordan SC: Intravenous immunoglobulin suppression of HLA antibody in highly sensitized transplant candidates and transplantation with a histoincompatible organ. *Transplantation* 57: 553-562, 1994.
34. Jordan SC, Quartel AW, Czer LS, Adman D, Chen G, Fishbein MC, Schwieger J, Steiner RW, Davis C, Tyan DB: Posttransplant therapy using high-dose human immunoglobulin (intravenous gammaglobulin) to control acute humoral rejection in renal and cardiac allograft recipients and potential mechanism of action. *Transplantation* 66: 800-805, 1998.
35. Beniaminovitz A, Itescu S, Lietz K, Donovan M, Burke EM, Groff BD, Edwards N, Mancini DM. *N Engl J Med* 342: 613-619, 2000.
36. Sunders RN, Matcalfe MS, Nicholsan ML: Rapamycin in transplantation: a review of the evidence. *Kidney Int* 59: 3-16, 2001.
37. McDonald AS, McAlister VC: Sirolimus-tacrolimus combination. *Graft* 3: 245-247, 2000.
38. Weisman HF, Bartow T, Leppo MK y cols.: Soluble human complement receptor type 1: in vivo inhibitor of complement suppressing post-ischemic myocardial inflammation and necrosis. *Science* 249: 146-51, 1990.
39. Pascual M, Duchosal MA, Steiger G, Giostra E, Pechere A, Paccaud JP, Schifferli JA: Circulating soluble CR1 (CD35). Serum levels in diseases and evidence for its release by human leukocytes. *J Immunol* 151: 1702-11, 1993.
40. Zimmerman JL, Dellinger RP, Straube RC , Levin JL: Phase I trial of the recombinant soluble complement receptor 1 in acute lung injury and acute respiratory distress syndrome. *Crit Care Med* 3149-54, 2000.