Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

Humoral immune response after kidney transplantation is enhanced by acute rejection and urological obstruction and is down-regulated by mycophenolate mofetil treatment

José G. G. Oliveira,[1,2] Manuela S. Monteiro,[3] José F. Teixeira,[3] Ermelinda Osório,[3] Susana M. S. Norton,[2] Helena Alves[3] and Manuel Pestana[1,2]

1 Unidade de Investigação e Desenvolvimento de Nefrologia, Faculty of Medicine of University of Porto, Portugal
2 Renal Department, Hospital S. João, Porto, Portugal
3 Tissue Typing Center, Rua Roberto Frias, Porto, Portugal

Keywords
donor-specific, HLA antibodies, kidney transplants, mycophenolate mofetil.

Correspondence
José Gerardo Gonçalves de Oliveira, Trav. Das Antas, 84, 5 °C, 4350-046, Porto, Portugal. Tel.: +351 225 106344; fax: +351 225 512228; e-mail: gerardooliveira@mail.telepac.pt

Received: 12 April 2005
Revision requested: 3 May 2005
Accepted: 14 July 2005

doi:10.1111/j.1432-2277.2005.00205.x

Summary

The anti-allograft immune response may have a cellular and a humoral component. Lymphocytotoxic antibodies (Ab) and anti-human leucocyte antigen (HLA) Ab present before kidney transplantation carry an enhanced risk of acute rejection. Current immunosuppressive drugs act predominantly upon the cellular immune pathway which may leave unopposed the humoral mechanisms of anti-allograft response. We studied the production of lymphocytotoxic Ab and anti-HLA Ab after kidney transplantation under different drug therapies. Two hundred and sixty-four *consecutive* kidney transplant recipients treated with different immunosuppressive drugs, either stable and or with previous acute rejection or acute urologic obstruction, entered this study. Lymphocytotoxic Ab and anti-HLA Ab were evaluated by complement-dependent cytotoxicity and by ELISA. Ab donor-specificity was determined by flow cytometry. Both lymphocytotoxic Ab and anti-HLA Ab were significantly increased in acute rejection whatever the immunosuppressive regimen and almost significantly in urologic obstruction treated with azathioprine (AZA) groups. The presence of antidonor-specific Ab was associated with a significantly higher rate of graft loss. Mycophenolate mofetil (MMF) therapy significantly down-regulated Ab synthesis in all patients groups when compared with AZA. The development of humoral antidonor response post-transplantation is associated with a dismal graft prognosis. This is the first report that acute urologic obstruction may be followed by unspecific lymphocytotoxic and anti-HLA Ab synthesis, surmising that a protracted obstruction may promote renal fibrosis through antibody mediation. The significant down-regulation of the humoral response by MMF when compared with AZA may herald a lower risk to mount a chronic rejection process.

Introduction

While the cellular theory of kidney graft rejection dominates, the ability of alloantibody to mediate graft injury is well established [1]. Furthermore, despite several attempts to detect the pretransplantation state of immunisation by testing T-lymphocyte function, only the humoral arm tests have been effective in measuring alloimmunisation, starting with the use of panel-reactive antibody (PRA) test [2]. Actually, the relevance of pretransplantation anti-HLA PRA is acknowledged by the transplant community and a positive high result portends a higher risk of acute

rejection and lower allograft survival [3,4] which turned the anti-HLA PRA test an universally accepted routine study pretransplantation. Until recently, post-transplantation monitoring of anti-HLA antibodies was not performed regularly. However, a substantial number of studies have reported a significant association between post-transplantation anti-HLA antibodies with adverse events [5], although their sensitivity and specificity has been questioned by others [6]. HLA antibodies are mainly found as a result of whole blood transfusions, pregnancies or earlier transplants [7,8,9]. It is quite uncommon to find HLA-specific antibodies in nonimmunised individuals, but such cases have been reported [10,11] and it has been speculated that the immunogens for these antibodies are cross-reactive microbial determinants.

Mycophenolate mofetil (MMF) has substituted for azathioprine (AZA) in kidney transplantation following the demonstration of better graft survival, at least during the first years [12,13]. MMF is a potent, selective, noncompetitive inhibitor of the type 2 isoform of inosine monophosphate dehydroxygenase, a key enzyme in *de novo* pathway of purine synthesis. MMF seems to be endowed with better efficacy to down-regulate the humoral response than AZA. In a clinical study among kidney graft recipients, those treated with cyclosporine (CsA) and MMF displayed a poor humoral response to influenza vaccine when compared with those treated with CsA and AZA [14]. Furthermore, MMF has been associated with immunoglobulin deficiency after kidney transplantation [15] and with a reduced incidence of HLA antibodies post-transplantation [16].

According to what has been reported, we have seen a significant reduction of acute rejection episodes among our kidney transplant recipients as well as a significant improvement of the first-year graft survival following the substitution of MMF for AZA. Since 1995, we introduced in our transplant unit the routine evaluation HLA antibodies post-transplantation, that is, three years before we routinely used of MMF.

We hypothesised that MMF may be associated with significant changes of post-transplant HLA antibodies which may constitute one of the reasons behind improved transplant results.

Materials and methods

Two hundred and sixty-four consecutive first cadaver kidney grafts *performed between January 1995 and December 2000* entered this study. They were divided into six groups. Groups I, II and III were treated with CsA, AZA and prednisolone from the beginning and Groups IV, V and VI were treated with CsA, MMF and prednisolone from the outset. Groups I ($n = 83$) and IV ($n = 94$) remained free of acute rejection episodes during the first year postsurgery, at least, and they did not suffer any major complication. Groups II ($n = 21$) and V ($n = 11$) developed an acute rejection episode, confirmed by a renal biopsy, classified according to the Banff scale [17]. The severity of the acute rejections was not significantly different by comparing the two groups. Every acute rejection episode was treated with i.v. methylprednisolone pulses and in corticoresistant cases and whenever the Banff grade was II or higher, anti-thymocyte antibodies were used. Groups III ($n = 30$) and VI ($n = 25$) suffered an episode of urologic obstruction confirmed by echography, with a rise of serum creatinine (≥0.2 mg/dl) during the first 3-month postsurgery. Urologic obstruction was caused by either ureteral stricture or lymphocele and all cases resolved following either reconstructive surgery or lymphocele drainage with or without creation of a peritoneal window.

The aetiologies of renal failure were those commonly described among a Caucasoid European population and were not different when comparing the groups.

The analysis of antibodies was done by three methods. A complement-dependent cytotoxicity (CDC) test was performed according to the European Federation of Immunogenetics accreditation standards protocol [18]. The ELISA (LAT test) mixed Class I and II trays (LATM) and LAT Class I and II (88 antigens and 40 antigens panel) kits for analysis of anti-HLA specificity were purchased from OneLambda, Canoga Park, CA, USA, and tests were performed following to the manufacturer instructions. Positivity of ELISA results was defined by the LAT software by comparison of the optical density measured in sample wells with that of negative control wells. When either CDC or ELISA tests were positive, a flow cytometry crossmatch (FCXM) was performed as described previously [19]. Briefly, mononuclear cells were isolated from donor spleen cells and T lymphocytes were enriched by nylon wool separation, and selected by phycoerythrin-conjugated anti-CD2 monoclonal antibody from Becton-Dickinson, San Jose, CA, USA. Donor T lymphocytes ($0.5–1.10^6$ lymphocytes/test serum) were incubated with patient sera/or negative control for 30 min at 20 °C. Detection of patient IgG antibodies bound to respective donor cells was performed using fluorescein isothiocyanate-labelled $F(ab)_2$ goat anti-human IgG antibody, a 30 min, 20 °C incubation (Jackson ImmunoRes. Lab, West Grove, PA, USA). Samples were analysed after cell fixation on a FACScalibur flow cytometer from Becton Dickinson. Data analysis was performed using CellQuest software and the cutoff point was defined by the fluorescence of negative control samples plus two standard deviations.

The timings for antibodies follow-up was as follows: previous to kidney transplantation, every patient PRA was

screened at a 3-month interval. After the transplant surgery, the follow-up was done by CDC, LAT test and FCXM when indicated on the 1st, 3rd, 6th, and 12th months.

Statistical analysis was done by Pearson's chi-squared testing and by odds ratio (OR) with 95% confidence intervals (CI), using Statistica from Statsoft, Tulsa, OK, USA.

Results

Anti-human leucocyte antigen match for the whole patients group was: A: 1.1 ± 0.2; B:0.9 ± 0.25 and DR:1 ± 0.1 and no significant differences were found when comparing either the HLA matching or mismatching between the six groups. Cold ischaemia time spanned from 6–30 h, however no significant differences were found between the groups.

Every rejection episode was diagnosed within the first 3-week post-transplantation among Groups II and V and neither significant differences were found for the Banff grading nor for immunological graft loss between both groups. Only two cases presented recurrent acute rejection episodes, one in Group II and one in V, both keep graft function more than 4 years post-transplant. The causes of urologic obstruction presented an equal distribution when comparing Groups III with VI. Every patient from Groups I, II, and III was followed by at least 6 years post-transplantation while patients from Groups IV, V, and VI were followed by at least 3-year post-transplantation, until the date of end of the study. In the whole studied population, the 1 and 5 years graft survival was 91% and 79%, respectively, and median/SD serum creatinine at the last outpatient visit was 1.4 ± 0.4 mg/dl. Furthermore, only 1.7% of cases have a serum creatinine higher than 2.5 mg/dl at 5-year postsurgery. However, while no significant difference was observed by comparing stable cases with those that developed acute rejection for 1- and 5-year graft survival, when donor-specific antibodies were superimposed on acute rejection, a significantly lower graft survival was observed, 52.3% at 5 years.

In Table 1, we present the CDC results pretransplant, both the maximum and the late presurgery test values. Only two patients presented a PRA >30% pretransplantation, one from Group I and one from Group V. Only one patient presented a donor-specific antibody pretransplant defined by FCXM, a young female with a record of several blood transfusions, included in Group IV. She did not develop any rejection episode and she continues to enjoy a good graft function more than 4-year post-transplantation. A positive but low (<10%) latest PRA pretransplant was found among 5.2% of the whole population and it was not different when comparing the six groups. However, when considering all the historical PRA pretransplant, a significantly lower prevalence of positive samples was found among the combined rejection-free Groups I, III, IV, and VI (14.6%) when compared with the combined rejection Groups II and V (37.8%), $P = 0.048$.

Table 1. Number and percentage of positive tests for CDC, among Group I, stable patients treated with AZA ($n = 83$) and Group II, acute rejection patients treated with AZA ($n = 21$), Group III, urologic obstruction cases treated with AZA ($n = 30$), Group IV, stable treated with MMF ($n = 94$), Group V, acute rejection cases treated with MMF ($n = 11$) and Group VI, urologic obstruction cases treated with MMF ($n = 25$).

	CDC maximum	CDC pretransplant
Group I	2.9 ± 2.1	1.7 ± 0.8
Group II	6.1 ± 3.8	4.2 ± 3.8
Group III	2.7 ± 1.9	1.8 ± 0.6
Group IV	2.8 ± 1.9	1.4 ± 0.8
Group V	6.8 ± 4.3	6.2 ± 4.9
Group VI	2.8 ± 1.5	1.9 ± 0.9

Table 2. Number and percentage of positive tests for CDC, LAT class I and LAT class II among Group I, stable patients treated with AZA ($n = 83$) and Group IV, stable patients treated with MMF ($n = 94$).

	CDC	LAT class I	LAT class II
Group I, month 1	17 (20.4)	15 (18.0)	8 (9.6)
Group IV, month 1	19 (20.2)	16 (17.0)	5 (5.3)
Group I, month 3	23 (29.1)	21 (26.6)	14 (17.8)
Group IV, month 3	10 (11.1)	9 (10.0)	6 (6.7)
Group I, month 6	4 (5.1)	4 (5.1)	2 (2.6)
Group IV, month 6	2 (2.2)	2 (2.2)	2 (2.2)

In Table 2, we present the results of CDC and LAT tests for stable Groups, I plus IV. No significant differences were found when comparing the evolution of serum creatinine and whole blood CsA levels between both groups during the study period. No significant difference was observed when comparing Group I versus Group IV for either CDC or LAT results on the first month post-transplantation. However, on the third month post-transplantation, a significantly up-regulated humoral response was observed among Group I when compared with Group IV, specifically for CDC an OR of 3.22 (CI 1.428–7.26) was observed. A significant correlation for CDC was observed between pre- and post-transplantation positivity, $P < 0.001$, which suggests that although the low CDC values pretransplant (always <10%, Table 1) these antibodies did not disappear following the conventional post-transplant immunosuppression. Of interest, three cases presented donor-specific antibodies, two from Group I and one from Group IV. They never developed acute

Table 3. Number and percentage of positive tests for CDC, LAT class I and LAT class II among Group II, patients with acute rejection treated with AZA ($n = 21$) and Group V, patients with acute rejection treated with MMF ($n = 11$).

	CDC	LAT class I	LAT class II	FCXM
Group II, postrejection	17 (81.0)	17 (81.0)	12 (57.1)	11 (52.4)
Group V, postrejection	8 (72.7)	8 (72.7)	7 (63.6)	6 (54.5)
Group II, month 3	15 (88.2)	15 (88.2)	12 (70.5)	9 (52.9)
Group V, month 3	3 (33.3)	3 (33.3)	3 (33.3)	2 (22.2)
Group II, month 6	6 (40.0)	6 (40.0)	4 (26.6)	2 (13.3)
Group V, month 6	0	0	0	0

rejection and they go on to enjoy a good graft function. By 12-month post-transplant, these antibodies were not detectable.

In Table 3, we present the data concerning the cases who developed acute rejection, Groups II and V. While no significant difference was found concerning the development of antibodies postrejection, whatever its class or specificity when comparing both groups, a significant difference was observed at follow-up in a way that by the 6-month post-transplantation, only cases treated with AZA were still presenting antibodies.

When we compared Groups I versus II, a significantly higher humoral reactivity was found among II, and acute rejection was associated with an OR of 11.08 (CI 3.3–36.5) for CDC positivity; also, when comparing Groups IV with V a significantly higher humoral reactivity was observed among V and acute rejection was associated with an OR of 22.4 (CI 5.1–98.4) for CDC positivity. Of special interest, by combining the results observed for rejection-free (I and IV) and acute rejection groups (II and V), the presence of a positive FCXM at the third month was associated with an OR 85.7 (CI 15.6–463.7) for immune graft loss. On the contrary, no significant increase of immune graft loss was observed when the antibodies were not donor-specific.

In Table 4, we present the data for groups III and VI, the cases that developed urologic obstruction. Within the groups receiving AZA, when comparing stable cases of I with urologic obstruction of III, an enhanced humoral response with urologic obstruction was seen, OR 1.74 (CI 0.72–4.17) although it did not reach a level of significance ($P = 0.21$). Under an MMF, this up-regulation was not observed. Of interest, urologic obstruction cases treated with AZA displayed a significantly higher rate of humoral sensitisation than when treated with MMF, OR 4.9 (CI 1.2–20.0), $P = 0.02$. However, in both groups the antibodies were not donor-specific and they were not any more present by the 6-month post-transplantation.

Whatever the group at 1 year post-transplantation, we almost never observed HLA ab, with the interesting exception of cases from Group II which showed positivity in 6/21 cases.

Table 4. Number and percentage of positive tests for CDC, LAT class I and LAT class II among Group III, patients with urologic obstruction treated with AZA ($n = 30$) and Group VI, patients with urologic obstruction treated with MMF ($n = 25$).

	CDC	LAT class I	LAT class II
Group III, postobstruction	12 (40.0)	10 (33.3)	6 (20.0)
Group VI, postobstruction	3 (12.5)	3 (12.5)	2 (8.3)
Group III, month 6	3 (10.3)	3 (10.3)	2 (6.9)
Group VI, month 6	0	0	0

Discussion

We performed a longitudinal study of the humoral response pre- and post-transplantation among a large group of first cadaver kidney recipients treated either with AZA or MMF which produced the following major findings:

1 MMF therapy is associated with a significant down-regulation of antibody production on the third month post-transplantation among stable cases;

2 MMF is associated with a significantly shorter period of antibody positivity when compared with AZA among cases that suffered acute rejection;

3 A close to significant upregulation of the humoral response observed within AZA cases with urologic obstruction is not observed within the MMF group, and MMF is associated with a significant down-regulation of humoral response postobstruction when compared with AZA;

4 The presence of donor-specific antibodies post-transplantation among acute rejection cases portends a very poor graft prognosis.

MMF has largely replaced AZA in the immunosuppressive therapy for kidney transplantation as the first trials have suggested a better graft survival, at least during the first years post-transplant [12,13]. Its superior efficacy is (causally?) associated with a more profound suppression of antibody production [14] and in some cases with immunoglobulin deficiency [15]. Our patients presented low levels of PRA pretransplant which could be anticipated as they were recipients of a first graft and as a rule they did not receive blood transfusions during the pretransplant period. In agreement with others, patients with a positive PRA pretransplant displayed a significantly higher incidence of acute rejection than cases persistently PRA negative pretransplant. The number of positive cases for antibodies post-transplantation among stable cases treated with either AZA or MMF is within the values

previously reported by others [16, 20]. While no significant difference was found when comparing antibody production at the first month postsurgery, we observed a significant down-regulation of the humoral response at the third month post-transplantation with MMF when compared with AZA. Moreover, while antibody positivity was progressively declining along with the time interval since the transplantation among MMF cases, an unexpected rise of antibody positivity was seen at the third month when compared with first month postsurgery among AZA cases, for which we have no reasonable explication. By sixth month postsurgery, the positive cases were rather low in both groups precluding a meaningful statistical analysis. In agreement with data from Terasaki and Ozawa [16], no significant difference is found when comparing data generated by CDC analysis with ELISA testing. However, our data differ from that reported by these authors as the number of positive cases for either anti-HLA class I or class II with MMF therapy is clearly higher (21/94) among our patients when compared with their figure, 9.8%. Of interest, the rather low number of positive cases among our patients at the sixth month post-transplantation does not suggest that the humoral response is causally related to long-term loss of transplants among rejection-free cases. Actually, the four positive cases among Group I keep a good graft function more than 6-year post-transplant. Our data seem to suggest that either CDC or ELISA positivity among stable cases does not seem to carry important clinical consequences, at least as far as first kidney transplants concerns, and even donor-specific antibodies may not be harmful within this patient population.

We did not observe a significant difference of antibody positivity following acute rejection between AZA and MMF. In agreement with others [5], a significantly higher number of Ab positive cases were found among acute rejection cases when compared with rejection-free transplants. However, a positive unspecific Ab response did not confer a significantly higher risk for graft loss while a donor-specific antibody reaction was a very powerful predictor of graft loss among both groups. We have not a clear explanation for this wide significant difference concerning the clinical implications of donor-specific antibodies among rejection-free and acute rejection cases, however, we surmise that the presence of Ab without other inflammatory/immune responses may underlie this discrepancy. Alternatively, one may speculate that the quantity of Ab may be significantly lower within rejection-free cases than among acute rejection transplants and some evidence has been presented that low doses of anti-HLA antibodies may be associated with induction of cell survival genes and endothelial cell accommodation [21]. Supon *et al.* [6] observed that donor-specific antibodies presented a significant risk for acute rejection but they reported a significantly lower of positive cases when compared with our figures. We believe that our finding of a significantly higher rate of antibody disappearance among acute rejection cases treated with MMF when compared with AZA may be of clinical importance signalling a lower risk to go on to develop chronic rejection following acute rejection under MMF therapy.

The percentage of cases suffering from urologic obstruction was 22.4% (30/134) among AZA and 19.2% (25/130) among MMF group which is not very different from the 17.6% incidence of lymphoceles reported among CsA-Pred-treated kidney transplants [22]. More than 80% of our obstruction cases were secondary to lymphocele in both groups. Lymphoceles have been associated with rejection episodes [23], but we excluded acute rejection from the two urologic obstruction groups. We observed a close to significant higher donor-unspecific Ab response following urologic obstruction among AZA group which was not seen among MMF group. Moreover, MMF cases displayed a significantly lower Ab response than AZA cases. As far as we know, a rise of humoral response following urologic obstruction has not been previously reported. Although the clinical importance of our finding is not clear, we believe that this different behaviour may constitute another indirect indication of a greater immunosuppressive effect by MMF which may abrogate the powerful inflammatory reaction described in animal models of ureteral obstruction [24]. Furthermore, our findings may raise the hypothesis that the unspecific inflammation brought about by urologic obstruction may under certain circumstances promote the synthesis of HLA and non-HLA Ab (Table 4).

A note of caution is warranted about our data. Our transplant population was restricted to first graft recipients and we have preliminary data showing that Ab synthesis is a frequent finding in second and third graft recipients whatever the clinical course may be. Furthermore, the interpretation of our data may improve with Ab isotype classification and Ab dilution studies.

In summary, we report a significant down-regulation of the humoral response when MMF replaces AZA in the post-transplant immunosuppressive regimens both among rejection-free and urologic obstruction cases, and the powerful predictive value for graft survival of donor-specific antibodies. Furthermore, the significant different pattern of disappearance of both donor-specific and unspecific antibodies among the cases who suffered an acute rejection episode treated with MMF when compared with those treated with AZA strongly suggests a decreased risk to develop a chronic rejection under MMF coverage, in agreement with the report by Theruvath *et al.* [25] who showed that MMF with tacrolimus as rescue therapy

promoted a sustained decrease in antidonor antibodies. The beneficial effects of MMF by down-regulation of the unspecific humoral response following a transient urologic obstruction episode compared with AZA may also be of clinical significance.

References

1. Kissmeyer-Nielsen F, Olsen S, Peterson VP, Fjeldborg O. Hyperacute rejection of kidney allografts associated with pre-existing humoral antibodies against donor cells. *Lancet* 1966; **2**: 662.
2. Patel R, Terasaki PI. Significance of the positive crossmatch test in kidney transplantation. *N Eng J Med* 1969; **280:** 735.
3. Sumitran S. The clinical importance of choosing the right assay for detection of HLA-specific donor-reactive antibodies. *Transplantation* 1991; **68**: 502.
4. Feucht HE, Opelz G. The humoral immune response towards HLA class II determinants in renal transplantation [Editorial]. *Kidney Int* 1996; **50**: 1464.
5. McKenna RM, Takemoto SK, Terasaki PI. Anti-HLA antibodies after solid organ transplantation. *Transplantation* 2000; **69**: 319.
6. Supon P, Constantino D, Hao P, *et al*. Prevalence of donor-specific anti-HLA antibodies during episodes of renal allograft rejection. *Transplantation* 2001; **71**: 577.
7. Dausset J, Leuco-agglutinins IV. Leuco-agglutinins and blood transfusion. *Vox Sang* 1954; **4**: 190.
8. Payne R, Rolfs MR. Fetomaternal leukocyte incompatibility. *J Clin Invest* 1958; **37**: 1756.
9. Walford RL, Carter PK, Anderson RE. Leukocyte antibodies following skin homografting in the human. *Transplant Bull* 1964; **29**: 106.
10. Lepage V, Degos L, Dasset J. A natural anti-HLA-A2 antibody reacting with homozygous cells. *Tissue Antigens* 1976; **8**: 139.
11. Sumitran-Karuppan S, Lindholm A, Möller E. Characterization and significance of donor-reactive B cell antibodies in current sera of kidney transplant patients. *Transplantation* 1990; **49**: 510.
12. The Tricontinental Mycophenolate Mofetil Renal Transplantation Study Group. A blinded, randomized clinical trial of mycophenolate mofetil for the prevention of acute rejection in cadaveric renal transplantation. *Transplantation* 1996; **61**: 1029.
13. Mathew TH., for the Tricontinental Mycophenolate Mofetil Renal Transplantation Study Group. A blinded, long-term, randomized multicenter study of mycophenolate mofetil in cadaveric renal transplantation. *Transplantation* 1998; **65**: 1450.
14. Smith KG, Isbel NM, Catton MG, Leydon JÁ, Becker GJ, Walker RG. Suppression of the humoral response by mycophenolate mofetil. *Nephrol Dial Transplant* 1998; **13**: 160.
15. Keven K, Sahin M, Kutlay S, *et al*. Immunoglobulin deficiency in kidney allograft recipients. Comparative effects of mycophenolate mofetil and azathioprine. *Transpl Infec Dis* 2003; **5**: 18.
16. Terasaki PI, Ozawa M. Predicting kidney graft failure by HLA antibodies. A prospective trial. *Am J Transplant* 2004; **4**: 438.
17. Racusen LC, Solez K, Colvin RB, *et al.* The Banff 97 working classification of renal allograft pathology. *Kidney Int* 1999; **55**: 713.
18. Martin S, Claas F. Antibodies and crossmatching for transplantation. In: Dyer PA, Middleton D eds. *Histocompatibility Testing*. Oxford: Oxford University Press, 1993: 81.
19. Slavcev A, Ivaskova E, Kalanin J, *et al.* Flow cytometry cross-match before kidney transplantation in relation to early posttransplant rejection. *Bone Marrow Transplant* 1998; **22**: S114.
20. Pelletier RP, Henessy PK, Adams PW, VanBuskirk A, Ferguson RM, Orosz CG. Clinical significance of MHC-reactive alloantibodies that develop after kidney or kidney-pancreas transplantation. *Am J Transplant* 2002; **2**: 134.
21. Narayanan K, Phelan D, Mohanakumar T. Effects of subsaturating anti-HLA antibodies on endothelial cells. Phosphatidylinositol 3-kinase/AKT mediated phosphorylation of bad in human aortic endothelial cells exposed to subsaturating concentrations of anti-HLA class I antibodies confers resistance against antibody-complement mediated cell death. *Hum Immunol* 2003; **64**: S39.
22. Langer RM, Kahan BD. Incidence, therapy, and consequences of lymphocele after sirolimus-cyclosporine-prednisone immunosuppression in renal transplant recipients. *Transplantation* 2002; **74**: 804.
23. Khauli RB, Stoff JS, Lovewell T, Ghavamian R, Baker S. Post-transplant lymphoceles: a critical look into the risk factors, pathophysiology and management. *J Urol* 1993; **150**: 22.
24. Vielhauer V, Anders HJ, Mack M, *et al.* Obstructive nephropathy in the mouse: progressive fibrosis correlates with tubulointerstitial chemokyne expression and accumulation of CC-chemokine receptor-2 and -5 positive leukocytes. *J Am Soc Nephrol* 2001; **12**: 1173.
25. Theruvath TP, Saidman SL, Mauiyyedi S, *et al.* Control of antidonor antibody production with tacrolimus and mycophenolate mofetil in renal allograft recipients with chronic rejection. *Transplantation* 2001; **72**: 77.