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Large decrease of anti-tetanus anatoxin and anti-pneumococcal antibodies at one year after renal transplantation

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Key words
kidney transplantation – antibodies – pneumococcus – tetanus

Abstract. Aims: In kidney transplant recipients (KTR), antibody (Ab) synthesis is hampered by AZA and CsA. We here report in a prospective cohort study, the effects of mycophenolate mofetil (MMF) associated to a calcineurin inhibitor on plasma levels of anti-tetanus anatoxin Ab (TAnAb) and anti-pneumococcal Ab (PnPsAb). Methods: Serum titers of the TAnAb and the PnPsAb against serotypes 14, 19F and 23F were measured in 94 KTR on Day 0 (T0) and 1 year (T12) after renal transplantation and in 49 healthy controls. Results: 1) At T0, TAnAb were detected in only 71% of patients vs. 98% of controls ($p < 0.0001$) and the titers were significantly lower in KTR (1.46 UI/ml vs. 2.74 in controls, $p = 0.01$); they further decreased between T0 and T12 (1.46 UI/ml to 0.31, $p < 0.0001$). The calculated half-life ($t_{1/2}$) of TAnAb was 7.7 months, as compared to more than 10 years in a normal population. 2) In KTR, PnPsAb titers decreased significantly between T0 and T12 ($p < 0.005$); the $t_{1/2}$ of the different PnPsAb ranged from 9.2 to 11.9 months. Conclusions: In KTR treated by MMF and CNI, the TAnAbs and PnPsAbs titers decrease significantly and profoundly during the first year. Immunization pre-transplantation should be encouraged to maintain adequate post-transplant Abs levels.

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Introduction

Vaccinations are recommended before renal transplantation [1]. Proteic antigens like tetanus anatoxin (TAn) induce a T-cell-dependent B-cell response, whereas polysaccharidic antigens such as the pneumococcal polysaccharides (PnPs) induce a T-cell-independent pathway of B-cell activation [2]. Nevertheless, antibody titers decrease with time under both CNI and AZA [3, 4]. As compared to AZA, Mycophenolate Mofetil (MMF) more profoundly reduces B-cell counts [5] and B cell responses [6, 7, 8, 9, 10, 11]. To investigate the effect of the combination of MMF and a CNI on antibody (Ab) production, we prospectively measured serum levels of TAn and PnPs Ab titers at Day 0 (T0) and at 1 year (T12) after transplantation.

Methods

Patients and controls

94 consecutive adult KTR of our center who had a functional graft at 1 year were studied. The demographic characteristics were: gender: 57.4% males; mean age: 46 ± 13 years (Table 1a); 82 were recipients of their first renal allograft, 9 of their second, and 3 of their third graft. All patients received a CNI (tacrolimus (TRL), n = 78; CsA, n = 16) together with MMF and steroids. The proportion of patients free of steroids at 12 months was 28%. Induction therapy was given to 77 patients (82%): anti-IL2 receptor monoclonal antibodies, n = 43; anti-lymphocyte agents, n = 34. All patients were followed for 1 year. The controls were 49 healthy hospital workers (42 ± 10 years; $p = 0.039$ vs. patients) (Table 1a). Serum samples were collected just before transplantation (T0) and at 12 months (T12), and stored at –20 °C until analysis.

Table 1a. Demographic characteristics of patients and controls.

Population	Age (years)	Gender (F/M)	Ethnicity	Time on dialysis (months)*
KTR (n = 94)	46 ± 13	40/54	86 Caucasians, 4 North Africans, 4 Black Africans	28 (13 – 54)
Controls (n = 49)	42 ± 10	24/25	40 Caucasians, 5 North Africans, 4 black Africans	NA
p	0,039	0,46	0,45	

KTR = Kidney transplant recipients; NA = not applicable. Cause of ESRD: Glomerulonephritis: n = 22; interstitial nephritis: n = 15, unknown: n = 15, Nephroangiosclerosis: n = 14, Polycystic disease: n = 7, congenital: n = 6, Diabetes: n = 4, Analgesic nephropathy: n = 4, Chinese herb nephropathy: n = 4, Hemolytic-Uremic-Syndrome: n = 1, CsA nephropathy: n = 1 Lupus Erythematosus: n = 1. *Median (25 – 75%)

Table 1b. Number and proportion of kidney transplant recipients (at T0), patients with antibodies above treshold against pneumococcal antigens and tetanus anatoxin, and their median half-life estimates (T/2).

Prevalence of immunization					Antibodies half-lives		
Antibody specificities		Controls n = 49 (%)	Patients T0 n = 94 (%)	p	Median T/2 (months)	IQR[a]	N° of measures[b]
Anti-tetanus anatoxin ab		48 (98%)	67 (71%)	< 0.0001	7.7	12.0	56
Anti-PnPs Ab positive for	≥ 1 serotype	44 (90%)	74 (79%)	0.11	–	–	–
	Serotype 14	28 (57%)	53 (56%)	0.89	10.0	21.4	38
	Serotype 19	41 (84%)	57 (61%)	0.0047	11.9	23.0	42
	Serotype 23F	31 (63%)	50 (53%)	0.28	9.2	20.1	36

[a]IQR = interquartile range 25 – 75%. [b]Half-life is measurable if Ab levels are decreasing with time (T12 < T0) and Ab levels at T0 were above treshold values.

TAnAb IgG titers

They were determined by Elisa (enzyme-linked immunosorbant assay) as previously described [12]. The threshold for immunization was 0.1 IU/ml [3, 13, 14, 15].

Specific PnPsAb titers (IgG)

Specific PnPsAb titers (IgG) were measured by Elisa against serotypes (S) 14, 19F and 23F as previously described with slight modifications [16]. These serotypes were chosen because of their high prevalence among invasive diseases [17, 18] and in antibiotic resistance [18,19], and of their different immunogenicities (high for S14, intermediate for S19F, low for S23F) [20]. The threshold level of immunization was defined as 1000 ng/ml (S14), 750 ng/ml (S19F), and 500 ng/ml (S23F).

Statistical analysis

Categorical data are presented as proportions. Continuous data for antibody titers are presented as geometric means (TAnAb and specific PnPsAb). Differences between patient categories were analyzed by the Mann-Whitney test, and for paired data by the Wilcoxon signed rank test. Estimation of the median half-life of TAnAb and PnPsAb ($t_{1/2}$) were calculated by the neperian logarithmic model: $t_{1/2}$ (months) = $\ln_2 \times 12 / (\ln T0 - \ln T12)$.

Results

TAnAb

The proportion of immunized patients on the day of transplantation (T0) was lower than in controls (71% vs. 98%, $p < 0.0001$) (Table 1b). The geometric mean level was lower at T0 than among control population ($p = 0.01$). Among immunized KTR, the geometric means of TAnAb decreased more than 4-fold between T0 and T12 ($p < 0.0001$) (Figure 1). During the first transplant year, the proportion of KTR with detectable TAnAb decreased from 71% to 55%. The median half-life estimate of TAnAb was 7.7 months (Table 1b).

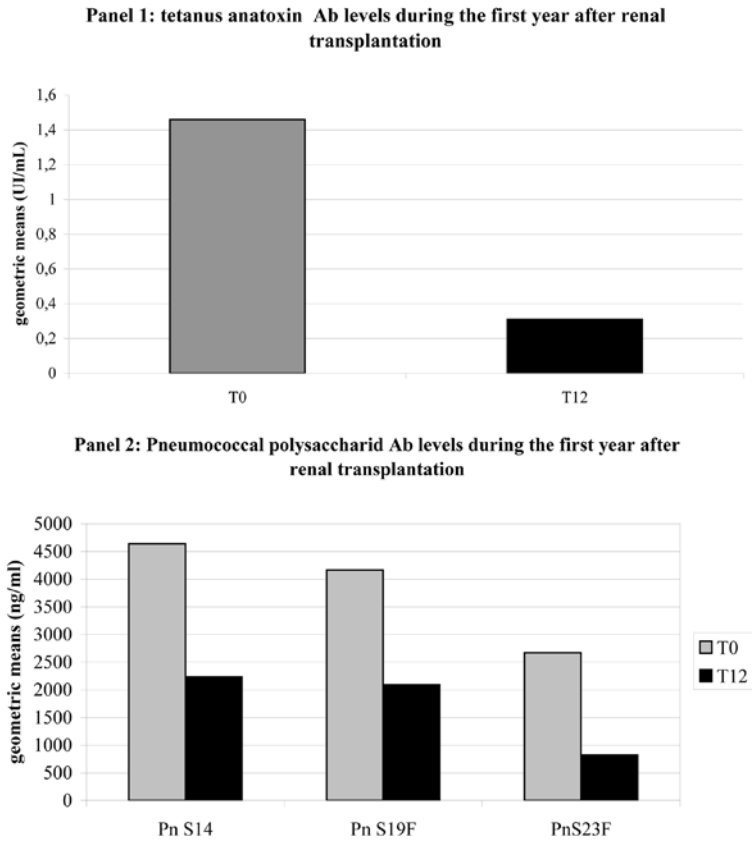

Figure 1. Geometric means of antibody levels against TAn and PnPs serotypes at T0 and 12 months. $p < 0.0001$ for TAn, and $p < 0.005$ for PnPs serotypes.

PnPsAb

The proportion of KTR immunized against at least 1 out of 3 serotypes of pneumococcus was not statistically lower at T0 than among controls (Table 1b). But, when single PnPs Ags were considered, it was significantly lower for serotypes 19F but not for the two other serotypes. Among patients who were immunized against PnPs at T0, the geometric means of all PnPsAbs decreased significantly between T0 and T12 (Figure 1). For 16 to 19% of patients, Ab titers decreased below the immunization threshold at T12. The median half-life estimates were less than 12 months for all serotypes (Table 1b).

Impact of steroid treatment for rejection

Among the transplanted patients, 8/94 (9%) experienced an acute rejection during the first year. They were all treated with steroid pulses. We then compared rejectors and non-rejectors for the proportion of patients who lost Abs. With respect to TAnAb, the proportion who lost detectable Ab was 1/5 among rejectors, as compared to 14/62 among non-rejectors ($p = 0.69$). With respect to PnPsAbs, the proportion was among rejectors, of 3/6, 2/6,and 5/6 for serotypes 14, 19, and 23, respectively, as compared to a 7/47, 8/51, and 3/44, among non-rejectors ($p = 0.07$, $p = 0.28$, and < 0.001, respectively).

Impact of calcineurin inhibitors blood levels

We compared serum levels of tacrolimus and CsA at both 3 months and 6 months, between patients who, at 12 months, lost initially detectable Ab and those who did not. We performed this analysis for both TAnAb and PnPsAb. There was no significant difference in either tacrolimus or CsA levels between groups (data not shown).

Impact of induction treatment

We compared, between the patients treated either with ATG, basiliximab, or no induction, the proportion who, at 12 months, lost initially detectable Ab and those who did not. We performed this analysis for both TAnAb and PnPsAb. With respect to TAnAb, the proportion who lost detectable Ab was 5/26 among ATG-treated patients, as compared to 7/32 among basiliximab-treated patients, and 3/9 among patients who received no induction ($p = 0.6$). With respect to PnPsAb, the proportion who lost ≥ 1 PnPsAb serotype detection was, among ATG-treated patients: 5/30, as compared to 12/33 among basiliximab-treated patients and 5/11 KTR who received no induction ($p = 0.09$).

Discussion

TAnAb and PnPsAb titers were lower among our cohort of CKD stage 5D patients than in matched controls, in accordance with the impairment of antibody synthesis observed in renal failure [21, 22]. We must acknowledge, however, that we do not have

adequate vaccination records, neither for tetanus toxoid nor for pneumococcus. Therefore, these results must be taken with caution. In addition, the mean age of the control group was 4 years younger than the patients. While this reaches statistical significance, the biological meaning of this minor difference is probably small. The lower prevalence of TAnAb positivity among kidney transplant recipients at T0, the day of transplantation, might therefore be due to the combination of a higher propensity of vaccine booster among hospital workers, as well as to increased rate of Ab clearance in CKD Stage 5D. The situation is the reverse with regard to pneumococcus, where there is no recommendation to vaccinate the control population, whereas patients with end-stage renal disease should be vaccinated. Therefore, the lower prevalence of anti-pneumococcal 19F serotype Ab in KTR at T0 than controls is likely to reflect higher Ab clearance. With regard to transplanted patients (CKD Stage 5T), the combination of a CNI and MMF profoundly suppressed antibody production [11], which parallels our previous findings of reduced gammaglobulin levels after renal transplantation [23].

The initial efficacy of pneumococcal vaccination in KTR is well established (seroconversion rate: 91 – 100% of patients mainly treated with AZA/PDS, with or without a CNI) [24, 25, 26]. However, pneumococcal vaccination is less efficient among patients treated with MMF (seroconversion rate of 53 – 73%, depending on the vaccine: 23-valent or heptavalent-protein-conjugated) [27, 28, 29]. Other vaccination studies also suggest that MMF appears to more profoundly impair the response to vaccines as compared to AZA [8, 9, 30]. With regard to the maintenance of the Ab titers, we found a reduction in the proportion of immunized patients of ~ 16 – 19% at 1 year. While steroid treatment for antibody rejection seems to impair anti-polysaccharide antibody maintenance, the loss of TAnAb or PnPsAb detection could not be ascribed to differences in either CNI blood levels at 3 and 6 months nor in the differences in induction treatments. Of note, patients who received depleting ATG preparations did not show a higher prevalence of Ab disappearance at 1year. The fact that the doses of ATG used in human are unlikely to affect splenic and lymph node T and B cells might explain this observation [31]. In our cohort study, the antibody half-life after transplantation was less than one year, as compared to more than 10 years in a normal population [32]. Along this line, long-term studies in KTR are limited. Lindemann shows that the PnPsAb level declines by 23% after 15 months [33]. In another study, 21% of KTR loose their immunization at 3 years [28]. Pourfarziani [29] showed that in newly vaccinated KTR, the serum IgG PnPsAb levels rapidly decreased as early as 6 months after vaccination, more rapidly among KTR than in the dialyzed population. Based on our present observation of the rapid clearance of antibodies in MMF-treated transplanted patients, we feel that recent guidelines that propose to monitor the PnPsAb levels and to repeat the pneumococcal vaccination every 3 – 5 years after initial vaccination [1] are sound and should be enforced.

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References

[1] *Danzinger-Isakov L, Kumar D*; *AST Infectious Diseases Community of Practice*. Guidelines for vaccination of solid organ transplant candidates and recipients. Am J Transplant. 2009; *9* (*Suppl 4*): S258-S262. doi:10.1111/j.1600-6143.2009.02917.x PubMed

[2] *Mond JJ, Lees A, Snapper CM*. T cell-independent antigens type 2. Annu Rev Immunol. 1995; *13:* 655-692. doi:10.1146/annurev.iy.13.040195.003255 PubMed

[3] *Huzly D, Neifer S, Reinke P, Schröder K, Schönfeld C, Hofmann T, Bienzle U*. Routine immunizations in adult renal transplant recipients. Transplantation. 1997; *63:* 839-845. PubMed doi:10.1097/00007890-199703270-00008

[4] *Cosio FG, Giebink GS, Le CT, Schiffman G*. Pneumococcal vaccination in patients with chronic renal disease and renal allograft recipients. Kidney Int. 1981; *20:* 254-258. doi:10.1038/ki.1981.128 PubMed

[5] *Weigel G, Griesmacher A, Karimi A, Zuckermann AO, Grimm M, Mueller MM*. Effect of mycophenolate mofetil therapy on lymphocyte activation in heart transplant recipients. J Heart Lung Transplant. 2002; *21:* 1074-1079. doi:10.1016/S1053-2498(02)00440-0 PubMed

[6] *Eugui EM, Almquist SJ, Muller CD, Allison AC*. Lymphocyte-selective cytostatic and immunosup-

pressive effects of mycophenolic acid in vitro: role of deoxyguanosine nucleotide depletion. Scand J Immunol. 1991; *33:* 161-173. doi:10.1111/j.1365-3083.1991.tb03746.x PubMed

[7] *Rose ML, Smith J, Dureau G, Keogh A, Kobashigowa J*. Mycophenolate mofetil decreases antibody production after cardiac transplantation. J Heart Lung Transplant. 2002; *21:* 282-285. doi:10.1016/S1053-2498(01)00335-7 PubMed

[8] *Rentenaar RJ, van Diepen FN, Meijer RT, Surachno S, Wilmink JM, Schellekens PT, Pals ST, van Lier RA, ten Berge IJ*. Immune responsiveness in renal transplant recipients: mycophenolic acid severely depresses humoral immunity in vivo. Kidney Int. 2002; *62:* 319-328. doi:10.1046/j.1523-1755.2002.00425.x PubMed

[9] *Smith KG, Isbel NM, Catton MG, Leydon JA, Becker GJ, Walker RG*. Suppression of the humoral immune response by mycophenolate mofetil. Nephrol Dial Transplant. 1998; *13:* 160-164. doi:10.1093/ndt/13.1.160 PubMed

[10] *Kimball JA, Pescovitz MD, Book BK, Norman DJ*. Reduced human IgG anti-ATGAM antibody formation in renal transplant recipients receiving mycophenolate mofetil. Transplantation. 1995; *60:* 1379-1383. doi:10.1097/00007890-199560120-00001 PubMed

[11] *Broeders N, Wissing KM, Crusiaux A, Kinnaert P, Vereerstraeten P, Abramowicz D*. Mycophenolate mofetil, together with cyclosporin A, prevents anti-OKT3 antibody response in kidney transplant recipients. J Am Soc Nephrol. 1998; *9:* 1521-1525. PubMed

[12] *Stubbe M, Swinnen R, Crusiaux A, Mascart F, Lheureux PE*. Seroprotection against tetanus in patients attending an emergency department in Belgium and evaluation of a bedside immunotest. Eur J Emerg Med. 2007; *14:* 14-24. doi:10.1097/01.mej.0000228449.37974.7e PubMed

[13] *Matousková I, Matlerová S, Janoutová G, Janout V*. Persistence of antibodies against tetanus upon revaccination. Cent Eur J Public Health. 2005; *13:* 99-102. PubMed

[14] *Janout V, Matouskova I, Machova L, Cizek L, Janoutova G, Hoskova J*. Protection against tetanus in the aged people in the Czech Republic--cross-sectional study. Arch Gerontol Geriatr. 2005; *40:* 123-128. doi:10.1016/j.archger.2004.07.002 PubMed

[15] *Simonsen O, Bentzon MW, Heron I*. ELISA for the routine determination of antitoxic immunity to tetanus. J Biol Stand. 1986; *14:* 231-239. PubMed doi:10.1016/0092-1157(86)90008-9

[16] *Quataert SA, Kirch CS, Wiedl LJ, Phipps DC, Strohmeyer S, Cimino CO, Skuse J, Madore DV*. Assignment of weight-based antibody units to a human antipneumococcal standard reference serum, lot 89-S. Clin Diagn Lab Immunol. 1995; *2:* 590-597. PubMed

[17] *Smart LE, Platt DJ, Timbury MC*. A comparison of the distribution of pneumococcal types in systemic disease and the upper respiratory tract in adults and children. Epidemiol Infect. 1987; *98:* 203-209. doi:10.1017/S0950268800061926 PubMed

[18] *Lee HJ, Park JY, Jang SH, Kim JH, Kim EC, Choi KW*. High incidence of resistance to multiple antimicrobials in clinical isolates of Streptococcus pneumoniae from a university hospital in Korea. Clin Infect Dis. 1995; *20:* 826-835. doi:10.1093/clinids/20.4.826 PubMed

[19] *Fenoll A, Asensio G, Jado I et al.* Antimicrobial susceptibility and pneumococcal serotypes. J Antimicrob Chemother 2002; *50 [Suppl S2]:* 13-19.

[20] *Tuerlinckx D, Vermeulen F, Pékus V, de Bilderling G, Glupczynski Y, Collet S, Jamart J, Bodart E, Mascart F*. Optimal assessment of the ability of children with recurrent respiratory tract infections to produce anti-polysaccharide antibodies. Clin Exp Immunol. 2007; *149:* 295-302. doi:10.1111/j.1365-2249.2007.03409.x PubMed

[21] *Chatenoud L, Herbelin A, Beaurain G, Descamps-Latscha B*. Immune deficiency of the uremic patient. Adv Nephrol Necker Hosp. 1990; *19:* 259-274. PubMed

[22] *Girndt M, Sester M, Sester U, Kaul H, Köhler H*. Molecular aspects of T- and B-cell function in uremia. Kidney Int Suppl. 2001; *78:* S206-S211. PubMed

[23] *Broeders EN, Wissing KM, Hazzan M, Ghisdal L, Hoang AD, Noel C, Mascart F, Abramowicz D*. Evolution of immunoglobulin and mannose binding protein levels after renal transplantation: association with infectious complications. Transpl Int. 2008; *21:* 57-64. PubMed

[24] *Linnemann CC Jr, First MR, Schiffman G*. Response to pneumococcal vaccine in renal transplant and hemodialysis patients. Arch Intern Med. 1981; *141:* 1637-1640. doi:10.1001/archinte.1981.00340130081018 PubMed

[25] *Kazancioğlu R, Sever MS, Yüksel-Onel D, Eraksoy H, Yildiz A, Celik AV, Kayacan SM, Badur S*. Immunization of renal transplant recipients with pneumococcal polysaccharide vaccine. Clin Transplant. 2000; *14:* 61-65. doi:10.1034/j.1399-0012.2000.140111.x PubMed

[26] *Lindemann M, Heinemann FM, Horn PA, Witzke O*. Immunity to pneumococcal antigens in kidney transplant recipients. Transplantation. 2010; *90:* 1463-1467. doi:10.1097/TP.0b013e3181f5d878 PubMed

[27] *Kumar D, Rotstein C, Miyata G, Arlen D, Humar A*. Randomized, double-blind, controlled trial of pneumococcal vaccination in renal transplant recipients. J Infect Dis. 2003; *187:* 1639-1645. doi:10.1086/374784 PubMed

[28] *Kumar D, Welsh B, Siegal D, Chen MH, Humar A*. Immunogenicity of pneumococcal vaccine in renal transplant recipients – three year follow-up of a randomized trial. Am J Transplant. 2007; *7:* 633-638. doi:10.1111/j.1600-6143.2007.01668.x PubMed

[29] *Pourfarziani V, Ramezani MB, Taheri S, Izadi M, Einollahi B*. Immunogenicity of pneumococcal vaccination in renal transplant recipients and hemodialysis patients: a comparative controlled trial. Ann Transplant. 2008; *13:* 43-47. PubMed

[30] *Scharpé J, Evenepoel P, Maes B, Bammens B, Claes K, Osterhaus AD, Vanrenterghem Y, Peetermans WE*. Influenza vaccination is efficacious and safe in renal transplant recipients. Am J Transplant. 2008; *8:* 332-337. doi:10.1111/j.1600-6143.2007.02066.x PubMed

[31] *Préville X, Flacher M, LeMauff B, Beauchard S, Davelu P, Tiollier J, Revillard JP*. Mechanisms involved in antithymocyte globulin immunosuppressive activity in a nonhuman primate model. Transplantation. 2001; *71:* 460-468. PubMed doi:10.1097/00007890-200102150-00021

[32] *Amanna IJ, Carlson NE, Slifka MK*. Duration of humoral immunity to common viral and vaccine antigens. N Engl J Med. 2007; *357:* 1903-1915. doi:10.1056/NEJMoa066092 PubMed

[33] *Lindemann M, Heinemann FM, Horn PA, Witzke O*. Long-term response to vaccination against pneumococcal antigens in kidney transplant recipients. Transplantation. 2012; *94:* 50-56. doi:10.1097/TP.0b013e318250fc8c PubMed