Available online at www.sciencedirect.com
ScienceDirect
Transplant Immunology 17 (2007) 278–282
Transplant Immunology
www.elsevier.com/locate/trim

Pre- and post-transplant monitoring of soluble CD30 levels as predictor of acute renal allograft rejection

Dong Wang [a,c,1], Guo-Jun Wu [c,1], Wei-Zhen Wu [a], Shun-Liang Yang [a], Jin-Hua Chen [b], He Wang [c], Wen-Hong Lin [a], Qing-Hua Wang [a], Zhang-Xin Zeng [a], Jian-Ming Tan [a,*]

[a] *Organ Transplant Institute, Fuzhou General Hospital, No.156 Xi'erhuan North Road, Fuzhou, 350025, China*
[b] *Department of Statistics, Fuzhou General Hospital, Fuzhou 350025, China*
[c] *Department of Urology, Xijing Hospital, Fourth Military Medical University, Xi'an, 710032, China*

Received 20 December 2006; received in revised form 3 February 2007; accepted 12 February 2007

Abstract

Identification of renal graft candidates at high risk of impending acute rejection (AR) and graft loss may be helpful for patient-tailored immunosuppressive regimens and renal graft survival. To investigate the feasibility with soluble CD30 (sCD30) as predictor of AR, sCD30 levels of 70 patients were detected on day 0 pre-transplant and day 1, 3, 5, 7, 10, 14, 21, and 30 post-transplant. AR episodes in 6 months were recorded and then patients were divided into Group AR ($n=11$) and Group UC ($n=59$). Results showed that the patients had higher pre-transplant sCD30 levels than healthy people. A significant decrease of sCD30 was observed on the first day post-transplant and continued until day 14 post-transplant. Soluble CD30 presented a stable level from day 14 to 30 post-transplant. Pre-transplant sCD30 levels of Group AR were much higher than those of Group UC ($P<0.001$). Patients of Group AR also had higher sCD30 levels than those of Group UC on day 1, 3, 5, 7, 10 and 14 ($P<0.001$). The sCD30 level presented a significantly delayed decrease in the patients of Group AR. Statistical results showed that the highest value of area under ROC curve (0.95) was obtained on day 5 post-transplant, suggesting that sCD30 levels on day 5 are of high predictive value. Therefore, sCD30 level may be a good marker of increased alloreactivity and of significant predictive value. It's necessary to monitor the variation of sCD30 in the early period post-transplant.
© 2007 Elsevier B.V. All rights reserved.

Keywords: Soluble CD30; Acute rejection; ELISA; Renal transplantation; Immunosuppressive regimens

1. Introduction

Identification of renal graft candidates at high risk of graft loss is one important part of pre-transplant evaluation. Irreversible damage caused by rejections may be relieved and even avoided by recognizing impending rejections as early as possible. At present, panel reactive antibodies (PRA) are generally accepted as the indicators of immunological status of renal graft candidates and the predictors of renal graft outcome [1]. Some new monitoring tools, such as Immuknow and FoxP3, have been used in clinical practice [2,3]. However, it is urgent to establish more sensitive and specific tools for post-transplant monitoring of immunological status of renal graft recipients.

CD30 is a 120 KDa transmembrane glycoprotein and a member of the tumor necrosis factor receptor superfamilies [4]. In addition to its well-documented association with several lymphoid malignancies, CD30 can also be expressed on normal, healthy cells, which include T and B cells, natural killer cells, and some non-lymphoid cells [5]. Meanwhile, CD30 molecule is activation-dependent. A soluble form of CD30 (sCD30) is cleaved from the surface of activated $CD30^+$ cells via the action of the cell surface metalloproteinase TNF-α-converting enzyme (TACE). Soluble CD30 can be detected in the serum of most normal individuals, however, elevated serum sCD30 levels have been detected in patients with $CD30^+$ haematopoietic malignancies, certain viral infections, and several autoimmune disorders [6]. Recent studies have shown that renal graft

* Corresponding author. Tel.: +86 591 22859398.
E-mail address: wangdong1202@medmail.com.cn (J.-M. Tan).
[1] Contributed equally to this work.

0966-3274/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.trim.2007.02.001

candidates also have higher pre-transplant serum sCD30 levels than healthy people, which are predictive of early renal graft loss, and that post-transplant sCD30 level has also been evaluated as an early predictor of impending graft rejection [7,8]. Similar results have been found in our previous study [9]. However, there is still no detailed research of variation of sCD30 levels in the first month post-transplant.

In the present study, we monitored the variation of sCD30 in the first month post-transplant, analyzed the characteristics of variation of sCD30, and investigated the feasibility with sCD30 as predictor of acute rejection in 6 months post-transplant.

2. Methods

2.1. Patient demographics

Seventy patients were enrolled into this retrospective cohort study, who received their deceased donor renal allografts at Organ Transplant Institute of Fuzhou General Hospital in 2005. Informed consent was obtained from each patient. Exclusion criteria were PRA scores >10%, previous allograft, delayed graft function (DGF), and infection episodes in the first month post-transplant. Patients who needed dialysis during the first week without evidence of acute rejection were diagnosed as DGF. Patients enrolled were followed up for 6 months and were divided into two groups according to their clinical course. Eleven patients who experienced at least one episode of acute rejection (AR) were categorized as group AR. The other patients ($n=59$) who had primary graft function and an uncomplicated course (UC) without acute rejection were categorized as group UC. Donor–recipient blood group matching was identical in all patients. HLA crossmatch of patients was negative, which was determined by microdroplet assay of complement–dependent lymphocytotoxicity (CDC). PRA was tested using ELISA technology (LAT-M, One Lambda Inc., CA, USA). Both assays have limited sensitivity compared to other available tools [10]. Some of the patients may have had donor reactive antibodies by other methodologies. Pre-transplant CMV serostatus was determined by assessment of CMV-IgG antibodies in the donor (D) and recipient (R), respectively (CMV-IgG ELISA kit, Jingmei Biotech, Shanghai, China). The detailed data of patients' demographic characteristics and pre-transplant status are shown in Table 1, which were comparable in two groups.

2.2. Immunosuppressive regimens

All the renal graft recipients did not receive the induction therapy of biologic agents. They received 500 mg of intravenous methylprednisolone (MP) prior to revascularization of the graft during the operation and a 3-day bolus of intravenous MP therapy (8 mg/kg/day) post-transplant. Oral prednisone was prescribed on the fourth day at a daily dose of 20 mg and was tapered to a daily dose of 15 mg at the sixth month post-transplant. The patients all received standard triple therapy as maintenance immunosuppressive regimens, which consisted of calcineurin inhibitorin (CsA microemulsion or Tacrolimus), MMF and prednisone. MMF was administered immediately after operation and calcineurin inhibitorin was administered on the third day post-transplant. Three patients of group AR were converted from CsA to Tacrolimus because of rejection episodes (Table 1).

2.3. Measurement of serum sCD30

Blood samples of patients were obtained on day 0 before transplantation and on day 1, 3, 5, 7, 10, 14, 21, and 30 after transplantation. Then blood samples were centrifuged within 2 h and plasma was separated from cells, collected and stored at −70 °C until being tested. Human sCD30 instant ELISA kits were obtained from Bender MedSystems (Vienna, Austria). Serum levels of sCD30 were measured in a duplicate manner using ELISA kit according to manufacturers' instructions. Our previous study of healthy individuals was referred as normal control [9]. Compared with the patients enrolled into this study, they were sex and age matched.

Table 1
Demographic characteristic, pre-transplant status and maintenance immunosuppressive regimens of patients

	AR	UC	*P* value
Number of patients	11	59	–
Gender distribution (M: F)	8:3	41:18	NS
Recipient age ($X\pm$S.D.)	39±9	36±10	NS
Donor age	32±6	34±7	NS
Cold ischemia time (h)	9.1±2.6	8.4±2.2	NS
Waiting time (m)	9.2±7.0	8.8±8.7	NS
HLA mismatches	2.4±0.9	2.5±0.8	NS
Immunosuppressive regimen			
CsA+MMF+MP	4	31	NS
FK+MMF+MP	4	28	
Conversion (CsA to FK506)	3	0	–
CMV serostatus			
D−/R−	1	7	
D+/R−	2	6	NS
D+/R+	6	36	
D−/R+	2	10	

2.4. Diagnosis of AR

Percutaneous kidney biopsy was carried out in cases of graft function deterioration, and kidney pathology was classified using the definitions given by the Banff 97 [11]. A rabbit polyclonal antibody (Biomedica, Vienna, Austria) was used to assess C4d status on frozen sections according to manufacturers' instructions.

2.5. Statistical analysis

EXCEL2003 and SPSS 13.0 were used for statistical analysis. The methods used in our study included chi-squared, Fisher's exact test, repeated measures ANOVA and receiver operating characteristic (ROC) curves. P values <0.05 were considered significant.

3. Results

3.1. Main outcome

Six-month follow-up data showed 100% patients and 98.6% renal grafts survival. There was one graft loss following irreversible biopsy-proven acute vascular rejection (AVR). Within the 6 months post-transplant, 11 out of 70 patients (15.7%) experienced biopsy-proven AR episodes. Mean time of AR diagnosis was 25±30 days post-transplant (5–102 days; median time: 12 days). Pathological results showed that 11 AR episodes included 9 tubulointerstitial rejections (TIR) and 2 vascular rejections (VR). Two patients were positive for C4d staining (VR and TIR each one). Nine TIR episodes were all reversed by the treatment of 3-day bolus of MP (8 mg/kg/d). The two patients with AVR were treated with ATG (Fresenius, 2 mg/kg/d×10 d). The C4d− AVR was reversed by the treatment of ATG, and the patient with C4d+ AVR was not sensitive to the treatment of ATG and suffered graft loss.

3.2. Characteristics of soluble CD30

Compared with healthy control [9], the patients had much higher serum sCD30 levels before transplantation (147±77 U/ml *vs*. 41±13 U/ml, $P<0.001$). Although compared with pre-transplant levels, slightly elevated sCD30 levels (no more than 12 U/ml) were detected in four patients, the sCD30 levels of most patients presented a significant decrease on the first day post-transplant ($P<0.001$). As shown in

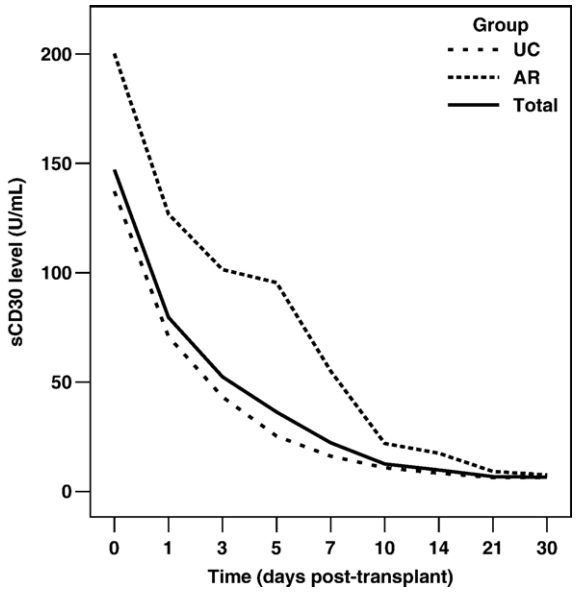

Fig. 1. Variation of average sCD30 levels of Group AR, Group UC and the total patients.

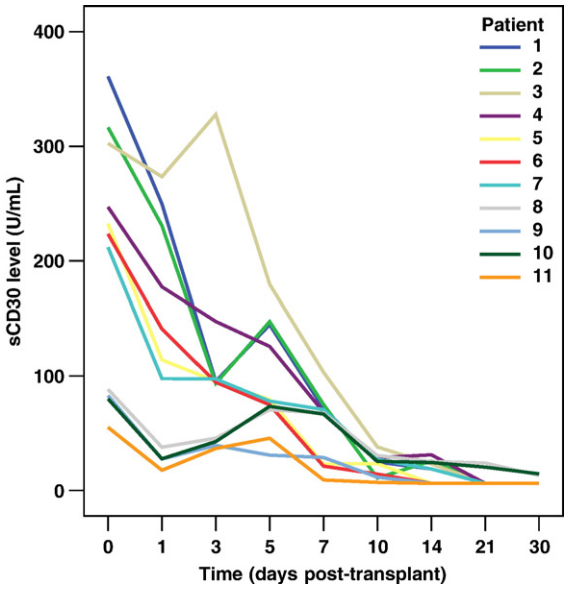

Fig. 2. Respective variation of sCD30 levels of 11 patients with AR.

Fig. 1, the sCD30 levels also recorded a series of significant decrease in the following days ($P<0.001$). ANOVA results showed that the difference of sCD30 levels between different time points reached statistical significance except for that between day 10 and 14 post-transplant ($P<0.001$), suggesting that the sCD30 shows a significant decrease in the 10 days post-transplant and then presents a stable level after day 10 post-transplant. Detailed data was shown in Table 2.

As shown in Table 2, patients of Group AR had higher sCD30 levels than those of Group UC before and after transplantation. Results of repeated measures ANOVA showed that the difference of sCD30 levels between Group AR and UC reached a statistical significance ($P<0.001$). When the sCD30 levels of two groups at the same time point were compared independently, results showed that patients with AR had much higher sCD30 levels than those without AR before transplantation and on day 1, 3, 5, 7,10 and 14 post-transplant (Table 2). This is to say, the sCD30 levels of patients with AR presented a delayed decrease within the first two weeks post-transplant. This phenomenon was also shown in Fig. 1. When the patients with AR were investigated respectively on the variation of sCD30 level, marked rebound of sCD30 level occurred in 7 patients with AR on day 3 or 5 post-transplant compared with that of the first or third day (Fig. 2). As shown in Fig. 2, the patients with AR were numbered from 1 to11 according to their pre-transplant sCD30 levels. The AR time of the patients from No.1 to 11 was day 10, 9, 13, 102, 23, 54, 36, 12, 8, 5, and 6 post-transplant respectively.

Table 2
sCD30 plasma levels (U/ml) of patients of two groups at different time

Time	Group AR	Group UC	Total
Pre-transplant	200±108[a]	137±66[a]	147±77
Day 1 post-transplant	127±95[b]	71±37[b]	80±54
Day 3 post-transplant	101±83[c]	43±32[c]	52±48
Day 5 post-transplant	95±43[d]	25±20[d]	36±36
Day 7 post-transplant	55±29[e]	16±14[e]	22±22
Day 10 post-transplant	20±10[f]	11±10[f]	13±11
Day 14 post-transplant	18±10[g]	8±10[g]	10±10
Day 21 post-transplant	9±7	6±1	7±3
Day 30 post-transplant	8±3	6±1	7±1

P values for pairwise comparisons: [a, b, c, d, e, f, g] $P<0.001$. All other pairwise comparisons: P=NS.

Receiver operating characteristic (ROC) curve was used to determine the feasibility of sCD30 levels at different time points as predictors of AR. Results showed that area under ROC curve were 0.63, 0.65, 0.82, 0.95, 0.89, 0.87, and 0.78 before transplantation and on day 1, 3, 5, 7, 10, and 14 post-transplant respectively, suggesting that the fifth day is the most suitable time to use sCD30 level to predict AR episodes with the 6 months post-transplant.

3.3. Effects of immunosuppressive regimens on post-transplant sCD30 levels

Immunosuppressive regimens of three patients were converted from CsA to FK-506 due to AR episodes on day 6, 8 and 54 post-transplant respectively. Therefore, sCD30 data of two patients with conversion in the first month was excluded for the evaluation of the effects of immunosuppressive regimens on post-transplant sCD30 levels. The rest patients were divided into Group CsA and Group FK according to their initial immunosuppressive regimens. Statistical results showed that there was no significant difference of sCD30 levels between two groups before the administration of CsA or FK-506 (day 3 post-transplant), and difference of sCD30 levels between two groups after day 3 post-transplant did not reach a statistical significance, too (P=NS).

4. Discussion

After Susal et al first documented that increased sCD30 levels occur in renal graft candidates and are detrimental for renal graft survival in his studies in 2002 [7,12], some other researchers also investigated the feasibility of serum sCD30 levels as predictor of impending acute rejection and graft outcome [9,10,13–18]. Although different pre-transplant average levels of sCD30 have been reported, previous studies have shown that renal graft recipients have significantly higher serum sCD30 levels before transplantation than adult healthy persons,

and serum sCD30 levels may be a good predictor of impending acute rejection and graft outcome. Some of them also detected post-transplant sCD30 levels of patients. They documented that higher post-transplant sCD30 levels may be predictive of impending acute allograft rejection post-transplant. However, there was no identical post-transplant sampling time in their studies. Pelzl et al found that sCD30 measured on post-transplantation days 3 to 5 can offer a noninvasive means for recognizing patients with impending acute allograft rejection [8]. Slavcev et al [10] and Sengul et al [16] detected sCD30 levels of patients 2 weeks and 15 days after transplantation respectively and found that sCD30 was significantly elevated in patients suffering AR compared with those without AR. The similar results as Pelzl's were observed in our previous study [9].

Therefore, to investigate the characteristics of variation of sCD30 and determine the best monitoring time post-transplant, sCD30 levels were detected before transplantation and on day 1, 3, 5, 7, 10, 14, 21 and 30 post-transplant in our study. This is the first study of variation of sCD30 levels in the first month after transplantation. As shown in previous studies, our study also documented that renal graft recipients have significantly higher serum sCD30 levels before transplantation than adult healthy persons. Results also showed that patients with AR had a higher pre-transplant sCD30 levels than those without AR in 6 months post-transplant, which is in agreement with the results of others [7], but contrary to our previous study [9]. An explanation to this discrepancy might be that some patients with positive PRA or previous renal graft were enrolled into our previous study, and that the follow-up time was 6 months and 27.3% (3/11) AR episodes occurred beyond the first month post-transplant in the present study. Additionally, our results showed that sCD30 levels of most patients recorded a significant decrease on the first day post-transplant. It is not clear why sCD30 levels changed so greatly within short time. An inverse correlation has been reported previously between sCD30 and GFR in children with chronic renal failure [19]. Therefore, marked reduction of sCD30 levels may be caused by increased renal excretion of sCD30 though functioning graft. Although precise mechanism needs further investigation, the changing of sCD30 level may partially reflects the change of patients' immune status because CD30 molecule is an important costimulator molecule in the regulation of the balance between TH1/TH2 responses [20]. More importantly, the changing went on in the following days and significant difference of the changing was observed between patients with AR and those without AR on day 1, 3, 5, 7, 10, and 14 post-transplant. The patients with AR experienced a significantly delayed decrease of sCD30 levels within first two weeks after transplantation. Furthermore, marked rebound of sCD30 level occurred in some patients with AR on day 3 or 5 post-transplant compared with that of the first or third day. On *in vitro* activated human and mouse T and B cells, CD30 is a relatively late activation-induced antigen, with maximal expression observed 4–5 day poststimulation [21,22]. These results may partially explain the phenomenon of delayed decrease of sCD30 levels in our study. We presume that allogeneic immune response occurred at the time of grafting and CD30 expression induced by foreign antigen reached the peak on 3–5 days post-transplant. Correlation of AR episode and high sCD30 levels post-transplant has been documented in our and others' studies [8–10,16], however, high expression of CD30 on 3–5 days post-transplant was not accompanied by AR episode. According to our results, we think that high expression of CD30 probably reflects increased alloreactivity of the immune system, and patients with increased alloreactivity are at high risk of early (within 6 months) AR episodes.

Although significant difference of sCD30 levels was recorded between patients with AR and those without AR before transplantation and on day 1, 3, 5, 7, 10, and 14 post-transplant, results of ROC curves showed that the highest area under the ROC curve was obtained on day 5 post-transplant, suggesting that measurement of sCD30 levels on day 5 post-transplant may be a promising method to predict acute graft rejection and may identify the patients at the risk of impending AR episode within 6 months post-transplant as early as possible. This result is identical with that of our previous study [9].

Factors influencing sCD30 levels have been investigated in detail recently [23]. There were only CMV diseases and immunosuppressive regimens to be able to influence sCD30 levels among many factors mentioned by authors. However, our results showed that there was no significant difference between patients receiving different immunosuppressive regimens. An explanation to this discrepancy might be that our monitoring time of sCD30 was too short.

Our results referred to a limited number of patients, and whether CD30 molecule is involved in acute allograft rejection and its role in allogeneic immune response need further investigation. However, our present results showed that renal graft recipients with AR have a higher pre-transplant sCD30 levels and a delayed decrease of post-transplant sCD30 levels compared with those without AR in 6 months after transplantation. Soluble CD30 is a good marker of increased alloreactivity and of significant predictive value. Sequentially monitoring of post-transplant sCD30 level may identify the patients at the risk of impending AR episodes within 6 months after transplantation. Soluble CD30 level on day 5 post-transplant may be of the highest predictive value.

Acknowledgments

We wish to thank Ting-Zhao Xu, Jin-Quan Cai, Xiao-Qin Tao, Xiao-Ling Yang, Jun Hu, and other staff members at our laboratory and clinical unit for supplying us with blood samples and clinical follow-up data.

References

[1] Gebel HM, Bray RA, Nickerson P. Pre-transplant assessment of donor-reactive, HLA-specific antibodies in renal transplantation: contraindication vs. risk. Am J Transplant 2003;3(12):1488–500.

[2] Banham AH, Powrie FM, Suri-Payer E. FOXP3+ regulatory T cells: current controversies and future perspectives. Eur J Immunol 2006;36 (11):2832–6.

[3] Zeevi A, Britz JA, Bentlejewski CA, Guaspari D, Tong W, Bond G, et al. Monitoring immune function during tacrolimus tapering in small bowel transplant recipients. Transpl Immunol 2005;15(1):17–24.
[4] Smith CA, Gruss HJ, Davis T, Anderson D, Farrah T, Baker E, et al. CD30 antigen, a marker for Hodgkin's lymphoma, is a receptor whose ligand defines an emerging family of cytokines with homology to TNF. Cell 1993;73(7):1349–60.
[5] Tarkowski M. Expression and a role of CD30 in regulation of T-cell activity. Curr Opin Hematol 2003;10(4):267–71.
[6] Kennedy MK, Willis CR, Armitage RJ. Deciphering CD30 ligand biology and its role in humoral immunity. Immunology 2006;118(2):143–52.
[7] Susal C, Pelzl S, Dohler B, Opelz G. Identification of highly responsive kidney transplant recipients using pretransplant soluble CD30. J Am Soc Nephrol 2002;13(6):1650–6.
[8] Pelzl S, Opelz G, Daniel V, Wiesel M, Susal C. Evaluation of posttransplantation soluble CD30 for diagnosis of acute renal allograft rejection. Transplantation 2003;75(3):421–3.
[9] Dong W, Shunliang Y, Weizhen W, Qinghua W, Zhangxin Z, Jianming T, et al. Prediction of acute renal allograft rejection in early post-transplantation period by soluble CD30. Transpl Immunol 2006;16(1):41–5.
[10] Slavcev A, Lacha J, Honsova E, Sajdlova H, Lodererova A, Vitko S, et al. Soluble CD30 and HLA antibodies as potential risk factors for kidney transplant rejection. Transpl Immunol 2005;14(2):117–21.
[11] Racusen LC, Solez K, Colvin RB, Bonsib SM, Castro MC, Cavallo T, et al. The Banff 97 working classification of renal allograft pathology. Kidney Int Feb 1999;55(2):713–23.
[12] Pelzl S, Opelz G, Wiesel M, Schnulle P, Schonemann C, Dohler B, et al. Soluble CD30 as a predictor of kidney graft outcome. Transplantation 2002;73(1):3–6.
[13] Ayed K, Abdallah TB, Bardi R, Abderrahim E, Kheder A. Plasma levels of soluble CD30 in kidney graft recipients as predictors of acute allograft rejection. Transplant Proc 2006;38(7):2300–2.
[14] Vaidya S, Partlow D, Barnes T, Thomas P, Gugliuzza K. Soluble CD30 concentrations in ESRD patients with and without panel reactive HLA antibodies. Clin Transplant 2006;20(4):461–4.
[15] Kim KH, Oh EJ, Jung ES, Park YJ, Choi JY, Kim DG, et al. Evaluation of pre-and posttransplantation serum interferon-gamma and soluble CD30 for predicting liver allograft rejection. Transplant Proc 2006;38 (5):1429–31.
[16] Sengul S, Keven K, Gormez U, Kutlay S, Erturk S, Erbay B. Identification of patients at risk of acute rejection by pretransplantation and post-transplantation monitoring of soluble CD30 levels in kidney transplantation. Transplantation 2006;81(8):1216–9.
[17] Rajakariar R, Jivanji N, Varagunam M, Rafiq M, Gupta A, Sheaff M, et al. High pre-transplant soluble CD30 levels are predictive of the grade of rejection. Am J Transplant 2005;5(8):1922–5.
[18] Cinti P, Pretagostini R, Arpino A, Tamburro ML, Mengasini S, Lattanzi R, et al. Evaluation of pretransplant immunologic status in kidney-transplant recipients by panel reactive antibody and soluble CD30 determinations. Transplantation 2005;79(9):1154–6.
[19] Barbano G, Cappa F, Prigione I, Pistoia V, Cohen A, Chiesa S, et al. Plasma levels of soluble CD30 are increased in children with chronic renal failure and with primary growth deficiency and decrease during treatment with recombination human growth hormone. Nephrol Dial Transplant 2001;16(9):1807–13.
[20] Pellegrini P, Berghella AM, Contasta I, Adorno D. CD30 antigen: not a physiological marker for TH2 cells but an important costimulator molecule in the regulation of the balance between TH1/TH2 response. Transpl Immunol 2003;12(1):49–61.
[21] Nakamura T, Lee RK, Nam SY, Al-Ramadi BK, Koni PA, Bottomly K, et al. Reciprocal regulation of CD30 expression on CD4+ T cells by IL-4 and IFN-gamma. J Immunol 1997;158(5):2090–8.
[22] Bowen MA, Lee RK, Miragliotta G, Nam SY, Podack ER. Structure and expression of murine CD30 and its role in cytokine production. J Immunol 1996;156(2):442–9.
[23] Weimer R, Susal C, Yildiz S, Staak A, Pelzl S, Renner F, et al. Post-transplant sCD30 and neopterin as predictors of chronic allograft nephropathy: impact of different immunosuppressive regimens. Am J Transplant 2006;6(8):1865–74.