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CD20 EXPRESSION IN THE TRANSPLANTED KIDNEY OF PATIENTS WITH GRAFT LOSS AND TRANSIENT ALLOGRAFT DYSFUNCTION

EKSPRESIJA CD20 U PRESAĐENOM BUBREGU KOD PACIJENATA SA GUBITKOM GRAFTA I PROLAZNOM DISFUNKCIJOM ALOGRAFTA

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Summary: This study aimed to explore the relationship between the infiltration of CD20+ B cells and the survival time of a renal allograft and to investigate the role of infiltrated B cells in the rejection of the renal allograft. A total of 40 patients with renal allograft loss due to refractory rejection and 20 patients with transient renal allograft dysfunction were recruited. Renal biopsy was done and CD20 expression was detected by immunohistochemistry. In addition, the survival time of the renal allograft was also obtained. The relationships between the CD20 expression and the survival time of the renal allograft and graft loss due to rejection were analyzed. The associations of gender, age and clinicopathogical types with the CD20 expression were also investigated. The proportion of patients positive for CD20 in the transplanted kidney was higher in patients receiving nephrectomy of the allograft due to rejection than in those with transient allograft dysfunction. The diffuse infiltration of CD20+ B cells was considered as positive staining. In 40 samples from patients with graft loss, 19 had diffuse infiltration of CD20+ B cells (47.5%). In 19 patients positive for CD20, hyperacute rejection was found in 1 patient, acute rejection in 5 and chronic rejection in 13. Statistical analysis showed the CD20 expression was not associated with the age and gender of donors and recipients, regimen for immunosuppressive treatment, cold/warm ischemia time and secondary transplantation. CD20+ B cell infiltration predicts a poor prognosis of patients with kidney transplantation and is one of the risk factors of graft loss.

Keywords: renal allograft, rejection, CD20, B cells

Kratak sadržaj: Cilj ove studije bio je da se ispita odnos između infiltracije CD20+ B ćelija i vremena preživljavanja bubrežnog alografta, kao i da se istraži uloga infiltriranih B ćelija u odbacivanju bubrežnog alografta. Obuhvaćeno je ukupno 40 pacijenata sa gubitkom bubrežnog alografta usled refraktornog odbacivanja i 20 pacijenata sa prolaznom disfunkcijom bubrežnog alografta. Izvršena je biopsija bubrega a ekspresija CD20 detektovana je imunohistohemijski. Pored toga, utvrđeno je vreme preživljavanja bubrežnog alografta. Analizirani su odnosi između ekspresije CD20 i vremena preživljavanja bubrežnog alografta i gubitka grafta usled odbacivanja. Takođe je ispitana povezanost pola, uzrasta i kliničko-patoloških tipova i ekspresije CD20. Proporcionalno više pacijenata kod kojih je nađena ekspresija CD20 u presađenom bubregu bilo je među pacijentima podvrgnutim nefrektomiji alografta usled odbacivanja nego među onima sa prolaznom disfunkcijom alografta. Difuzna infiltracija CD20+ B ćelija označena je kao pozitivno bojenje. Od 40 uzoraka od pacijenata sa gubitkom grafta, kod 19 je otkrivena difuzna infiltracija CD20+ B ćelija (47,5 %). Od 19 pacijenata pozitivnih na CD20, kod 1 je pronađeno hiperakutno odbacivanje, kod 5 akutno odbacivanje a hronično odbacivanje kod 13 subjekata. Statistička analiza je pokazala da ekspresija CD20 nije povezana sa uzrastom niti sa polom davalaca i primalaca, režimom imunosupresivne terapije, vremenom hladne/tople ishemije i sekundarnom transplantacijom. Infiltracija CD20+ B ćelija daje lošu prognozu kod pacijenata posle presađivanja bubrega i predstavlja jedan od faktora rizika za gubitak grafta.

Ključne reči: bubrežni alograft, odbacivanje, CD20, B ćelije

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Introduction

Kidney transplantation has undergone development for more than 50 years. Currently, it is an effective strategy for the treatment of renal failure. Thus, to improve the long-term survival rate of the renal allograft and patients as well as the quality of life is a hot topic in the researches on renal transplantation. In 2005, Hippen et al. (1) found the CD20 expression aggregated in the transplanted kidney showing the infiltration of B cells in the allograft which may be a cause of the poor prognosis of patients undergoing kidney transplantation. In 2006, Doria et al. (2) reported the infiltration of B cells was unlikely related to the poor prognosis of patients with kidney transplantation. It will be seen from this paper that there is still controversy on the role of B cell infiltration in the allograft dysfunction. In the present study, 40 patients undergoing nephrectomy of the transplanted kidney due to rejection and 20 patients with renal biopsy who had transient renal dysfunction post-transplantation were recruited, and immunohistochemistry was performed to detect the CD20 expression in the kidney tissues. Our study aimed to investigate the role of B cell infiltration in the allograft survival and loss.

Materials and Methods

Patients

A total of 40 patients undergoing nephrectomy of the transplanted kidney due to refractory rejection and 20 patients with renal biopsy who had transient renal dysfunction post-transplantation were recruited from the Center of Kidney Transplantation from January 1989 to December 2006. Infection was excluded among these patients before study.

Materials

The paraffin-embedded kidney tissues were provided by the Department of Pathology. Mouse anti-human CD20 monoclonal antibody and EliVisionTM-plus kit were purchased from Fuzhou Maixin Biotech Co., Ltd.

Review of medical history

The medical history of 40 patients undergoing rejection was reviewed. There were 5 with hyperacute rejection, 8 with acute rejection and 27 with chronic rejection. The age and gender of recipients, regimen for immunosuppressive treatment, time of cold/warm ischemia, post-transplantation hypertension and secondary transplantation were recorded.

Immunohistochemistry for CD20

The immunohistochemistry was performed according to manufacturer's instructions. In brief, the paraffin-embedded sections underwent de-paraffinization, hydration, inactivation of endogenous peroxidase, washing with PBS and antigen retrieval. Then, these sections were treated with goat serum and then with mouse anti-human CD20 monoclonal antibody at 37 °C for 1 h. After washing with PBS, biotin conjugated goat anti-rabbit IgG was added followed by incubation at 37 °C for 20 min. Following washing in PBS, the sections were incubated with SABC at 37 °C for 20 min followed by washing in PBS and color development with DAB. Counterstaining was performed with hematoxylin followed by dehydration, transparentization and mounting. Diffuse, intensive and aggregated staining was regarded as positive staining, and the scattered and light staining as negative staining.

Statistical analysis

Statistical analysis was done with the SPSS version 10.0 statistic software package. The factors influencing the survival of the transplanted kidney were analyzed with t test and chi square test, and the survival time of the transplanted kidney was compared using log-Rank test.

Results

Immunohistochemistry

In 40 patients with graft loss, 19 were positive for CD20 (47.5%), and 2 (10.0%) were positive for CD20 in 20 patients with transient dysfunction of the transplanted kidney. Of 40 patients undergoing graft loss whose survival time was less than 5 years, 13 were positive for CD20, accounting for 68.4% of the patients positive for CD20. Moreover, the number of patients with survival time of <5 years was higher than that of patients with survival time of >5 years ($n=6$; 31.6%) among patients positive for CD20. CD20 positive B cells aggregated in the renal interstitium (*Figure 1*).

Relationship between CD20 expression and pathological types

According to the Banff 97 working classification of renal allograft pathology, 5 patients had hyperacute rejection (one was positive for CD20), 12 had acute rejection (5 were positive for CD20) and 13 had chronic rejection (10 were positive for CD20). Chi square test showed there was no marked difference in the CD20 expression between any two groups ($P>0.05$) (*Table I*).

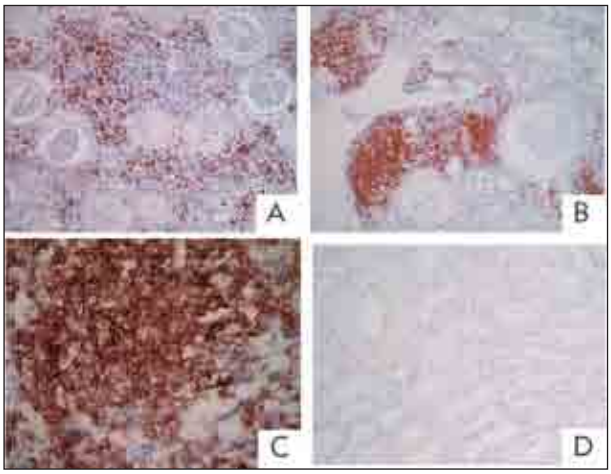

Figure 1 The expression of CD20 on a paraffin section of renal graft tissue. A and B: CD20+ B cells infiltrated in the interstitium of transplanted kidney (×200); C: Tissues negative for CD20 (×200); D: Negative control of CD20.

Table I CD20 expression in tissues of different pathological types.

Disordered two-way chi-square test			
	Positive	Negative	Total
Acute rejection	5	7	12
Hyperacute rejection	1	6	7
Chronic rejection	13	10	23
Total	19	23	41

Note: X^2=3.9510; λ=2; P=0.1387

CD20 expression in patients undergoing nephrectomy of transplanted kidney and those with transient dysfunction of transplanted kidney

Of 40 patients with nephrectomy of the transplanted kidney, 19 were positive for CD20 and 21 negative for CD20. In 20 patients experiencing renal biopsy due to transient dysfunction of the allograft, 2 were positive for CD20 and 18 negative for CD20. Chi square test revealed a marked difference in the proportion of patients positive for CD20 between the two groups (P<0.05) (*Table II*).

Relationship between factors of graft loss and CD20 expression

The transplanted kidneys in patients undergoing nephrectomy were from deceased donors. The age of the donors was 26.8±7.6 years (range: 19–42 years) and that of recipients was 38.8±10.0 years (range: 27–67 years). The time of cold ischemia was 6–12 min and that of warm ischemia was 170–720 min. The regimens for immunohistochemistry included mycophenolate mofetil + cyclosporine + glucocorticoids (n=19), mycophenolate mofetil + tacrolimus + glucocorticoids (n=7), and azathioprine + cyclosporine + glucocorticoids (n=14). The regimens were not altered during the immunosuppressive treatment. The gender of recipients, post-transplant hypertension and secondary transplantation are displayed in *Table III*.

Table II CD20 expression in the transplanted kidney of different groups.

Disordered two-way chi-square test			
	Positive	Negative	Total
Nephrectomy	19	21	40
Renal biopsy	2	18	20
Total	21	39	60

Note: X^2=8.2418; λ=1; P=0.0041

Table III Factors influencing the CD20 expression.

Contributing factors	CD20+(19)	CD20-(21)	P
Age (year)			
Recipient, mean ± SD	40.1±11.1	36.6±8.7	0.32
Donor, mean ± SD	22.1±3.4	28.3±5.9	0.44
Gender			
Male, n (%)	13 (68.4)	16 (76.2)	0.78
Female, n (%)	6 (31.6)	5 (23.8)	
Immunosuppressants			0.09
Azathioprine+ cyclosporine +glucocorticoid, n (%)	7 (36.8)	9 (42.9)	>0.05
Mycophenolate mofetil+cyclosporine + glucocorticoid, n (%)	9 (47.4)	10 (47.6)	>0.05
Mycophenolate mofetil + tacrolimus + glucocorticoid, n (%)	3 (15.8)	2 (9.5)	>0.05
Time of cold ischemia (min), mean ± SD	8.1±3.3	6.9±4.9	0.24
Time of warm ischemia (min), mean ± SD	514.3±219.8	499.1±432.7	0.32
Secondary transplantation, n (%)	2 (10.5)	1 (4.7)	0.76
Post-transplant hypertension, n (%)	8 (42.1)	12 (57.1)	0.20

The t test and chi square test showed the CD20+ B cell infiltration in the transplanted kidney was not related to the factors above ($P>0.05$).

Discussion

CD20 is a phosphorylated protein with a molecular weight of 33–37 kDa. It is predominantly expressed on the surface of B lymphocytes and has been regarded as a surface differentiation antigen of B lymphocytes. Studies have demonstrated CD20 is involved in the proliferation and differentiation of B lymphocytes and plays an important role in the immune regulation. Our results showed 47.5% of patients with graft loss had diffuse infiltration of CD20+ B cells which may be a cause of poor prognosis in kidney transplantation.

CD20 is initially expressed in pre B cells and mature B cells also express CD20. The activation of B cells may lead to over-expression of CD20. B cells during the growth and differentiation stages have high expression of CD20. When the B cells transform into plasma cells, the CD20 expression is decreasing and plasma cells do not express CD20. Thus, the rejection induced by CD20+ B cells is unlikely dependent on the secreted antibody. Evidence shows CD20+ B cells have potent functions of antigen-presenting cells (3, 4). Traditionally, the antigen-presenting cells include dendritic cells and macrophages. Under pathological conditions, such as in rheumatoid arthritis, the activation of T cells in the synovial fluid depends on B cells in the lymphoid follicles in the synovial fluid, but the mechanism is still unknown (3). B cells in the transplanted kidney can act as antigen presenting cells mediating the cellular rejection or these cells participate in the humoral rejection through inducing the infiltration and differentiation of plasma cells. However, the exact mechanisms should be clarified in future studies (5, 6).

Mechanisms of acute rejection of solid organs may be roughly divided into the so-called acute cellular rejection characterized by cytotoxic T-cell infiltrates at the graft level and the antibody-mediated (or humoral) rejection which is believed to be a consequence of donor-specific antibody production by plasma cells. Currently, immunosuppressants are to inhibit the activation and proliferation of T cells and are less effective on B cells, which may be the main cause of the insensitivity of patients with B cell infiltration in the transplanted kidney to traditional immunosuppressants. Our results showed that the infiltration of CD20+ B cells in the transplanted kidney could shorten the survival time of the allograft and decrease the 5-year survival rate. This finding suggests B cell infiltration may be one of the risk factors of allograft loss. The role of B cells in acute allograft rejection has recently been reappreciated. Some studies report an association between B-cell aggregates in the graft and poor allograft outcome (1). Martins et al (7) also detected the CD20 expression in the normal kidney and kidneys with acute rejection or chronic allograft nephropathy. They found that CD20+ cells occurred in the infiltrate in 2 distinct patterns: scattered or nodular. Their results demonstrated B lymphocytes in cases of renal allograft dysfunction, which were more pronounced in acute allograft rejection, and the presence of B-cell clusters tended to be associated with a higher level of serum creatinine in the acute rejection group. Scheepstra et al (8) investigated if B-cell infiltrates are present during rejection; they occur with T-cell infiltrates in a concurrent fashion, but no relation was found between the number of CD20+ cells, in aggregates or in a scattered interstitial pattern, and response to conventional therapy. In the study of Bagnasco et al, they examined rejection outcome and graft survival in 58 adult patients with acute cellular rejection Banff type I (ARI) or II (ARII), within 1 year after transplantation, with or without CD20-positive infiltrates (9). Their results did not support the association of B cell-rich infiltrates in allograft biopsies with worse outcome in acute rejection type I or II, but did not exclude the possible contribution of B cells to allograft rejection.

Moreover, isolated case reports have described the successful treatment of steroid-resistant episodes of acute rejection with specific anti-CD20 monoclonal antibody (Rituximab) (10). Rituximab has been clinically applied in the treatment of B cells related diseases including multiple myeloma (11). Tyden et al (12) found the effectiveness of Rituximab in patients with renal transplant rejection due to mismatched ABO blood type. In a recent study of Faguer et al (13) Rituximab together with plasma exchange, glucocorticoids, mycophenolate ethyl phenol and tacrolimus were used in patients with acute humoral rejection. Findings in the follow up revealed this strategy was beneficial for the acute humoral rejection. Treatment with anti-CD20 monoclonal antibody provides a new strategy of immunotherapy for patients undergoing kidney transplantation, especially for patients with rejection due to high anti-panel antibody or with antibody mediated humoral rejection. Taken together, CD20+ B cell infiltration may be an important cause of the poor prognosis of kidney transplantation patients and a critical risk factor of allograft loss. Renal biopsy followed by immunohistochemistry for CD20 may be helpful for the determination of prognosis and can provide a basis for the determination of a therapeutic regimen.

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Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

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