

Pre-transplant CD200 and CD200R1 concentrations are associated with post-transplant events in kidney transplant recipients

Hani Oweira, MD^a, Elias Khajeh, MD^a, Sara Mohammadi, MD^a, Omid Ghamarnejad, MD^a, Volker Daniel, MD^b, Paul Schnitzler, MD^c, Mohammad Golriz, MD^a, Markus Mieth, MD^a, Christian Morath, MD^d, Martin Zeier, MD^d, Arianeb Mehrabi, MD^a, Mahmoud Sadeghi, MD^{a,*}

Abstract

CD200 is an immunoglobulin superfamily membrane protein that binds to a myeloid cell-specific receptor and induces inhibitory signaling. The aim of this study was to investigate the role of CD200 and its receptor (CD200R1) on kidney transplant (KTx) outcome. In a collective of 125 kidney recipients (University hospital, Heidelberg, Germany), CD200 and CD200R1 concentrations were evaluated immediately before transplantation. Recipient baseline and clinical characteristics and KTx outcome, including acute rejection (AR), acute tubular necrosis, delayed graft function, cytomegalovirus (CMV) and human polyomaviridae (BK) virus infections, and graft loss were evaluated during the first post-transplant year. The association of CD200 and CD200R1 concentrations and CD200R1/CD200 ratios with the outcome of KTx was investigated for the first time in a clinical setting in a prospective cohort. There was a positive association between pre-transplant CD200R1 concentrations and CMV (re)activation ($P = .041$). Also, increased CD200R1 concentration was associated with a longer duration of CMV infection ($P = .049$). Both the frequency of AR and levels of creatinine (3 and 6 months after KTx) were significantly higher in patients with an increased CD200R1/CD200 ratio (median: 126 vs 78, $P = .008$). Increased pre-transplant CD200R1/CD200 ratios predict immunocompetence and risk of AR, whereas high CD200R1 concentrations predict immunosuppression and high risk of severe CMV (re)activation after KTx.

Abbreviations: AR = acute rejection, ATN = acute tubular necrosis, BK virus = human polyomaviridae (BK) virus, CD200R = CD200 receptor, CI = confidence intervals, CMV = cytomegalovirus, CMV pp65+ = more than three CMV pp65-positive cells out of 500,000 cells (positive test result), DGF = delayed graft function, ESRD = end-stage renal disease, KTx = kidney transplantation, OR = odds ratio.

Keywords: CD200, CD200R1, cytomegalovirus, kidney, transplantation

1. Introduction

CD200 is an immunosuppressive protein that inhibits immune responses through its receptor. It is distributed on a broad range

of cell types (including B-cells, activated T-cells, and kidney cells, Table 1), while the CD200 receptor (CD200R1) is mainly expressed on myeloid cells (monocytes, granulocytes, and dendritic cells). The CD200R family has paired activating and inhibitory isoforms.^[1,2] CD200R on T-cells and B-cells inactivates leukocytes^[3] and CD200 expression is enhanced after immune system activation during the inflammatory response.^[1] Furthermore, CD200R1 signaling prohibits the expression of pro-inflammatory cascade components, including tumor necrosis factor, interferons, and inducible nitric oxide synthase in response to selected stimulation.^[4] CD200 also plays an important role in controlling autoimmunity and inflammation.

CD200 and CD200R molecules are involved in the down-regulation of myeloid and lymphoid cells. They have immunomodulatory effects, which regulate cell differentiation and cytokine release. The therapeutic potential of CD200/CD200R for the treatment of immune-associated diseases has been investigated.^[1] Previous studies have shown that overexpression of CD200 in transgenic mice decreases alloimmunity. These findings have potential clinical relevance, especially in transplantation studies.^[5] The strong inhibitory signals delivered to myeloid cells by CD200 represent interesting targets for therapeutic interventions.^[2,6]

Cytomegalovirus (CMV) and human polyomaviridae (BK) virus infections are the most common viral infections in patients undergoing kidney transplantation (KTx). These infections negatively affect graft function after transplantation.^[7] We reported previously that an up-regulation of immune responses,

Editor: Esaki M. Shankar.

The authors have no funding and conflicts of interest to disclose.

The data that support the findings of this study are available from the corresponding author [M.S.] upon reasonable request.

^a Department of General, Visceral and Transplant Surgery, ^b Transplantation-Immunology, Institute of Immunology, ^c Center for Infectious Diseases, Virology, ^d Division of Nephrology, Ruprecht Karls, University of Heidelberg, Heidelberg, Germany.

* Correspondence: Mahmoud Sadeghi, Department of General, Visceral and Transplantation Surgery, University of Heidelberg, Im Neuenheimer Feld 110, 69120 Heidelberg, Germany (e-mail: Mahmoud.Sadeghi@med.uni-heidelberg.de).

Copyright © 2019 the Author(s). Published by Wolters Kluwer Health, Inc. This is an open access article distributed under the terms of the Creative Commons Attribution-Non Commercial License 4.0 (CCBY-NC), where it is permissible to download, share, remix, transform, and buildup the work provided it is properly cited. The work cannot be used commercially without permission from the journal.

How to cite this article: Oweira H, Khajeh E, Mohammadi S, Ghamarnejad O, Daniel V, Schnitzler P, Golriz M, Mieth M, Morath C, Zeier M, Mehrabi A, Sadeghi M. Pre-transplant CD200 and CD200R1 concentrations are associated with post-transplant events in kidney transplant recipients. *Medicine* 2019;98:37 (e17006).

Received: 29 May 2019 / Received in final form: 11 July 2019 / Accepted: 9 August 2019

<http://dx.doi.org/10.1097/MD.00000000000017006>

Table 1
Tissue distribution of CD200 and CD200R1.

Cell type	Membrane protein	
	CD200	CD200R1
Thymocyte	+	–
T-cell	+/–	–
Activated T-cell	+	–
B-cell	+	–
Activated B-cell	+/–	–
Dendritic cell	+/–	+
Monocyte/macrophage	–	+
Endothelium	+	–
Neuron	+	–
Platelets	–	ND
Erythrocyte	–	–

– = no labeling, + = Some or all cells are labeled, +/- = weak labeling, ND = not determined.
 Source: Minas and Liversidge [2006] 2.

especially inflammatory cytokine production, occurs in allograft recipients with CMV and BK virus infections.^[8,9] Because of the regulatory role of CD200R1 in multiple inflammatory pathways, many bacterial and viral pathogens use this pathway to suppress host defenses.^[4] Helminthic and bacterial infections induce CD200 receptor 1 (CD200R1) expression,^[10,11] and inhibiting CD200/CD200R interactions has been suggested as a method to enhance immunotherapy.^[3] Interaction of CD200 with CD200R1 has an immunosuppressive effect and is an important factor in the hyporesponsive innate immune state after viral infection.^[12–18]

The effect of CD200 and CD200R1 on organ transplantation outcome has been studied in animal experiments,^[15,19–22] but has not been thoroughly explored in humans. This study investigated the impact of pre-operative CD200 and CD200R1 concentrations on post-operative outcomes in KTx recipients for the first time.

2. Materials and methods

2.1. Study population

We investigated 125 consecutive patients (between January 2010 and December 2011) with end-stage renal disease (ESRD) who underwent KTx in our center (Department of General, Visceral and Transplantation Surgery, University of Heidelberg). To determine whether serum CD200 and serum CD200R1 concentrations can predict post-transplant events, pre-transplant serum CD200 and serum CD200R1 concentrations were evaluated in all recipients (patient data were extracted from the university hospital database). CMV pp65 antigen in recipients who received organs from CMV-negative donors was evaluated during the first 6 post-operative months and once every year after that. Patients who received kidneys from CMV-positive donors were given CMV prophylaxis with 900 mg valganciclovir every day for 3 months. These patients were evaluated for CMV pp65 antigen once every week during the first 3 months after transplantation, twice per month between 4 and 6 months after transplantation, once every month between 7 and 12 months after transplantation, and 1 to 4 times every year after that. Post-operative immunosuppressive therapy varied according to the date of transplantation. Triple-drug therapy, including calcineurin inhibitor (cyclosporine or tacrolimus), methylprednisolone, and mycophenolate mofetil, was administered. The recipients were followed-up for at least 1 year, and post-transplant events

including acute rejection (AR), delayed graft function (DGF), acute tubular necrosis (ATN), graft loss, and viral infections (CMV and BK virus) were evaluated. The study was carried out in accordance with the code of ethics of the world medical association (*Declaration of Helsinki*) for experiments involving humans. Informed consent was obtained from all patients enrolled in the study. Participation in the study did not affect the management or treatment plan. The study was approved by the Ethical Committee of the University of Heidelberg (S-225/2014).

2.2. Blood sample preparation and determination of CD200 and CD200R1 plasma concentrations

Blood samples were drawn from all recipients 1 day before the operation. Within 2 hours of blood sample collection, plasma was separated from cells by centrifugation at 1550×g for 10 minutes. Plasma was snap-frozen and stored at –30°C until testing. When 80 plasma samples were collected, CD200 and CD200R1 plasma concentrations were determined using ELISA kits (Cloud-Clone Corp., Houston, TX). All assays were performed according to the manufacturer's instructions.

2.3. Determining CMV (re)activation by CMV pp65 antigen detection

To isolate leukocytes, approximately 8 ml of blood was collected in an EDTA tube. In a cytospin centrifuge, 500,000 leukocytes were carefully spun down on a slide. Cells were fixed and stained with an anti-CMV pp65 mouse monoclonal antibody, then washed and further incubated with an anti-mouse immunoglobulin G FITC-labeled antibody.^[23] Finally, slides were analyzed using ultraviolet microscopy, and CMV pp65 antigen-positive cells were counted. More than three CMV pp65-positive cells out of 500,000 cells were denoted as a positive CMV test result (CMV pp65+).^[24]

2.4. Detection of active BK virus infection by a real-time polymerase chain reaction

Nucleic acids were isolated from 200 µl of untreated plasma using a QIAamp kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The large T-antigen in the BK virus genome was amplified and quantified using TaqMan real-time polymerase chain reaction. To quantify BK virus DNA, 5 µl of extracted nucleic acids were amplified as described previously.^[25] The detection limit was 50 copies/ml. A BK virus load of more than 10,000 copies/ml was considered an active infection.^[26]

2.5. Quantification of post-transplant events

AR was defined as a Banff 97 classification of grade IA or greater and was confirmed by renal biopsy.^[27] ATN was diagnosed by the presence of necrotic renal tubules in histological specimens. DGF was defined as the temporary need for one or more dialysis treatments in the first post-operative week. One-year graft loss refers to re-transplantation, transplant nephrectomy, or permanent dialysis during the first year after transplantation.

2.6. Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp., 2013, Armonk, NY).

Categorical data are presented as proportions and percentages, and continuous data are presented as means \pm standard deviations or as medians (range). Categorical data were compared using the chi-square test of association or Fisher exact test. The Mann-Whitney *U* test was used to compare continuous variables between groups. Univariate logistic regression analysis was performed to determine the association of CD200 concentration, CD200R1 concentration, CD200R1/CD200 ratio, with CMV (re)activation, duration of CMV infection, and with AR. Results of logistic regression are reported as odds ratios (OR) with 95% confidence intervals (CI). A 2-tailed *P* value of less than .05 was considered statistically significant.

3. Results

3.1. Patient demographics and clinical data

As shown in Table 2, the mean age of recipients was 54.5 ± 12.7 years, and 39.2% of recipients were female. Glomerulonephritis was the most common cause for ESRD (33.6% of recipients), and 92.8% of recipients underwent pre-operative dialysis for a mean duration of 70.9 ± 37.6 months. Multiple-organ transplantation was performed in 10.4% of recipients and re-transplantation in 12.0% of recipients. Biopsy-proven AR was detected in 63 (50.4%) recipients. Of them, 54 (43.2%) recipients experienced borderline rejection. Two (1.6%) recipients had humoral rejection, 3 (2.4%) recipients grade IA rejection, and four (3.2%) recipients grade IIA rejection. The rate of patients with CMV serostatus of donor positive/recipient negative was 20.8% ($n=26$) in our collective. The rate of post-transplant CMV infection was not significantly higher in donor positive/recipient negative KTx patients ($P=.321$). CMV (re)activation was detected in 20.0% of recipients, and BK virus (re)activation in 10.4% of recipients after KTx. Detailed demographics and clinical data and outcome of KTx are presented in Table 2.

3.2. Predictive value of pre-operative CD200, CD200R1 concentrations, and CD200R1/CD200 ratio

CD200 concentrations were not significantly associated with post-transplant CMV pp65+ ($P=.33$) and duration of CMV infection ($P=.99$) (Fig. 1A). However, a significant association was shown between pre-transplant CD200R1 concentrations and post-transplant CMV pp65+ ($P=.041$) (Fig. 1B). No association was shown between duration of CMV infection and CD200R1 concentrations ($P=.87$). Moreover, there was a significant association between CD200R1 concentrations and (re)activation of CMV infection in seropositive KTx recipients (CD200R1 (pg/ml) 638,126 (10,167–2,100,000) vs 33,127 (6,419–92,952), $P=.027$). There was no association between the CD200R1/CD200 ratio and CMV (re)activation ($P=.37$) (Fig. 1C) or duration of infection ($P=.097$) (Table 3).

Furthermore, CD200 concentrations, CD200R1 concentrations, and CD200R1/CD200 ratios were not significantly associated with other post-transplant outcomes, including BK virus (re)activation, DGF, ATN, and graft loss.

To investigate the impact of pre-operative CD200 and CD200R1 concentrations, and CD200R1/CD200 ratios on post-transplant outcomes, univariate logistic regression analyses were performed. A cut-off of 320 (the median CD200 concentration) was set for CD200. High CD200 concentrations

Table 2

Kidney transplant recipients' baseline and clinical characteristics and outcome after Kidney transplant.

Variables	Total (n=125)
Baseline and clinical characteristics	
Age (yr)	54.5 ± 12.7 (24–76)
Sex (Female/male)	49 (39.2%)/76 (60.8%)
Blood group	
A	62 (50.0%)
B	14 (11.3%)
AB	10 (8.0%)
O	38 (30.6%)
Indication for transplant	
Glomerulonephritis	42 (33.6%)
Diabetes mellitus	27 (21.6%)
Autoimmune/polycystic disease	38 (3.4%)
Pyelonephritis	2 (1.6%)
Hypertension	7 (5.6%)
Other/unknown	9 (7.2%)
Pre-operative dialysis	
Hemodialysis	101 (80.8%)
Peritonealdialyse	15 (12.8%)
Duration (months)	70.9 ± 37.6 (4.1–162.7)
Number of total HLA MM	
≤ 2	47 (37.6%)
> 2	78 (62.4%)
CD200 (pg/ml)	387 ± 457 (44–4547)
CD200R1 (pg/ml)	81188 ± 267849 (1558–2100000)
CD200R1/CD200	230 ± 591 (8–5319)
Cold ischemia time (hours)	13.5 ± 4.4
Multiple-organ transplantation	13 (10.4%)
Re-transplantations	15 (12.0%)
Outcome of KTx	
Acute allograft rejection	63 (50.4%)
Acute tubular necrosis	10 (8.0%)
Delayed graft function	25 (20.0%)
CMV (re)activation	25 (20.0%)
BK virus (re)activation	13 (10.4%)
Graft loss	4 (3.2%)

CMV=cytomegalovirus, HLA=human leukocyte antigen, KTx=Kidney transplantation, MM=mismatch.

(>320 pg/ml) were not significantly associated with CMV pp65+ and duration of CMV infection according to univariate logistic regression analysis (OR 1.691, 95% CI 0.694–4.124, $P=.24$, OR 0.643, 95% CI 0.117–3.526, $P=.61$, respectively). A cut-off of 35,000 (the median CD200R1 concentration) was set for CD200R1. High CD200R1 concentrations (>35,000 pg/ml) were also not significantly associated with CMV pp65+ (OR 1.909, 95% CI 0.782–4.659, $P=.15$). CD200R1 concentrations more than 35,000 were associated with a longer duration of CMV infection (OR 6.000, 95% CI 1.003–35.908, $P=.050$). For CD200R1/CD200 ratios, a cut-off of 90 was set based on the results of previous studies. No association between CD200R1/CD200 ratios >90 and CMV reactivation or duration of CMV infection was reported (OR 1.577, 95% CI 0.637–3.902, $P=.32$; OR 3.750, 95% CI 0.662–21.252, $P=.13$, respectively). Univariate analysis of association of CD200 concentrations, CD200R1 concentrations, and CD200R1/CD200 ratios with AR showed no significant association between AR and CD200 concentration (OR 1.000, 95% CI 0.999–1.001, $P=.84$) or CD200R1 concentration (OR 1.000, 95% CI 1.000–1.001, $P=.23$) (Fig. 1D and E). However, KTx patients with AR had higher CD200R1/CD200 ratios (Fig. 1F) than recipients without

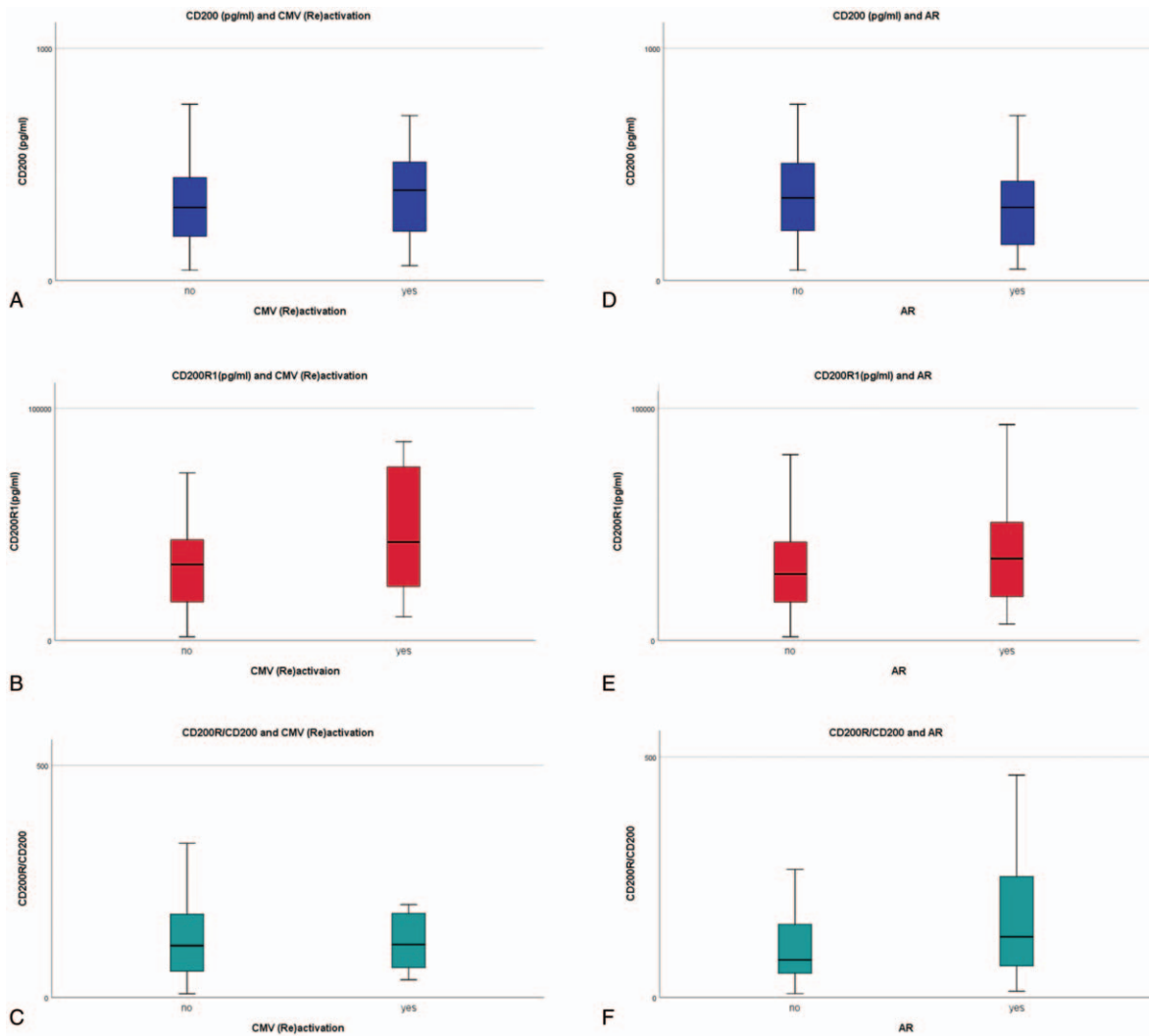


Figure 1. Box plot of CD200 (A) and CD200R1 (B) concentrations and CD200R1/CD200 ratios (C) in KTx recipients with and without CMV (re)activation. Box plots of CD200 (D) and CD200R1 (E) concentrations and CD200R1/CD200 ratios (F) in KTx recipients with and without acute rejection (AR).

Table 3

Comparison of kidney transplant recipients' characteristics and outcome after Kidney transplant in Patients with CMV (re)activation and no CMV (re)activation and between patients with acute allograft rejection and without acute allograft rejection.

Variables	CMV (re)activation (n=25)	No CMV (re)activation (n=100)	P	Acute allograft rejection (n=63)	No Acute allograft rejection (n=62)	P
Age (yr)	53.0 (28–76)	54.0 (24–76)	.49	53.0 (29–76)	54.5 (24–76)	.90
Sex (Female/male)	10 (40.0%)/15 (60.0%)	39 (39.0%)/61 (61.0%)	.92	21 (33.3%)/42 (66.7%)	28 (45.2%)/34 (54.8%)	.17
Pre-operative dialysis			.70			.09
Hemodialysis	21 (91.3%)	80 (79.2%)		54 (93.1%)	47 (79.7%)	
Peritonealdialyse	2 (8.7%)	13 (13.8%)		4 (6.9%)	11 (18.6%)	
Duration (months)	69.9 (7.2–130.3)	72.2 (4.1–162.7)	.57	72.7 (10.9–162.7)	71.0 (4.1–157.7)	.34
Number of total HLA MM			.51			.90
≤2	8 (32.0%)	39 (39.0%)		24 (38.1%)	23 (37.1%)	
>2	17 (68.0%)	61 (61.0%)		39 (61.9%)	39 (62.9%)	
CD200 (pg/ml)	389 (64–4547)	315 (44–2493)	.33	315 (49–4547)	355 (44–2493)	0.21
CD200R1 (pg/ml)	42342 (10167–2100000)	32682 (1558–1100000)	.041	35239 (7081–2100000)	28567 (1558–92673)	.12
CD200R1/CD200	114 (38–3625)	112 (8–5319)	.37	126 (13–5319)	78 (8–988)	.008
Cold ischemia time (hours)	13.0 (6.0–18.7)	14.0 (2.0–25.0)	.45	14.0 (2.0–25.0)	13.0 (5.5–23.5)	.044

CMV = cytomegalovirus, HLA = human leukocyte antigen, KTx = Kidney transplantation, MM = mismatch.

AR (median: 126 vs 78) (Table 3); the univariate regression analysis revealed an association between CD200R/CD200 ratio and AR (OR 1.003, 95% CI 1.000–1.006, $P=.028$).

3.3. Effect of CD200 and CD200R concentrations and CD200R1/CD200 ratio on long-term graft function

High CD200 concentrations (> 320 pg/ml) were not associated with 3- (1.9 ± 0.9 mg/dl vs 1.7 ± 0.8 , $p=0.18$) and 6-month (1.9 ± 0.8 mg/dl vs 1.7 ± 0.7 , $P=.10$) serum creatinine levels. Similarly, no significant associations were seen between high CD200R concentrations ($>35,000$ pg/ml) and 3- (1.8 ± 0.7 mg/dl vs 1.9 ± 1.0 , $P=.55$) and 6-month (1.7 ± 0.8 mg/dl vs 1.8 ± 0.8 , $P=.74$) serum creatinine levels. However, recipients with CD200R1/CD200 ratios >90 had higher 3- (1.9 ± 0.9 mg/dl vs 1.7 ± 0.8 , $P=.021$) and 6-month (1.9 ± 0.8 mg/dl vs 1.6 ± 0.6 , $P=.011$) serum creatinine levels than patients with CD200R1/CD200 ratios ≤ 90 .

4. Discussion

The CD200R1/CD200 pathway down-regulates the inflammatory reaction. The interaction of CD200 with CD200R1 has an immunosuppressive effect, which has an important impact on hyporesponsive innate immunity after viral infections.^[12,13] Previous experimental studies have shown that anti-CD200 antibodies have a positive impact on allograft survival after transplantation.^[17,18] In the present study, the association between CD200 concentration, CD200R concentration, and the CD200R1/CD200 ratio with KTx outcome was investigated in a clinical setting for the first time. Increased CD200R1 concentrations were significantly higher in KTx patients with CMV infection. Furthermore, higher pre-transplant CD200R1/CD200 ratios were associated with the risk of AR and increased 3-month and 6-month creatinine levels after KTx.

CMV (re)activation is generally accompanied by CMV antigenemia/DNAemia, which suggests that bone marrow precursor cells are an essential reservoir of the latent virus.^[28] Despite anti-CMV prophylaxis in donor seropositive/recipient seronegative KTx recipients, the rate of CMV reactivation remains high.^[29] CMV infection is associated with monocyte/macrophage activation,^[30,31] and we have previously shown that monocyte/T-helper cell 2 activation and a T-helper cell 1 blockade play a role in CMV reactivation.^[8,9,30,32]

Previous studies have suggested that some pathogens use the CD200/CD200R1 signaling pathway. Antiviral immunity comprises both humoral and cell-mediated immunity. Viruses such as CMV encode and express a viral CD200 homologue on the surface of host cells, and this viral CD200 homologue contributes to inhibition of the host immune system. However, the viral CD200 homologue has another effect on the host immune system at the onset of viral infections which can persist in their host.^[6,33] Previous studies have described this reaction as viral escape through cytokine-mediated neoangiogenesis.^[1,6] The initial activation of leukocytes at the site of infection in reaction to the virus enhances the dissemination of the pathogen throughout the infected host. The excessive inflammation observed in response to the infectious organism can be a great threat to the host. Vaine et al^[4] reported that anti-pathogen gene subgroups, which are suppressed through CD200R1 activation, may promote survival of the pathogen at the expense of the host.^[4] In agreement with this, the present study showed that

higher CD200R1 plasma concentrations are associated with CMV (re)activation in KTx recipients. Previous studies have revealed that CD200R is involved in regulating lymphocyte activity, as well as regulating myeloid cell function.^[34] Rijkers et al studied both human and mice lymphocytes and reported highest CD200R expression in memory B-cells and plasma cells in peripheral blood.^[34] They have suggested that CD200R is involved in restraining the reactivation of effector or memory B-cells rather than affecting the activation of naïve B-cells. In the present study, higher CD200R1 concentrations were associated with increased (re)activation of CMV and longer duration of CMV infection.

Renal biopsy is the gold standard diagnostic tool for AR.^[35] However, the management of AR in KTx patients can be improved by rapid non-invasive predictive factors. The influence of the CD200/CD200R interaction on graft acceptance and graft rejection has been studied in animal models. CD200 and CD200R may play a critical role in kidney rejection and post-transplant outcomes. In animal models, CD200/CD200R signaling prevents allograft rejection.^[36] Yu et al^[5] showed that CD200R positively stimulated DCs, modulated alloimmune tolerance, and reduced natural killer cell activity in transgenic mice overexpressing CD200. Moreover, enhanced interleukin 10 and TGF- β production through CD200R increased the chance of graft acceptance. Gorczynski et al reported that CD200 can promote graft acceptance after binding to CD200R. They concluded that an intense inflammatory stimulus may disrupt immunoregulatory balance and graft loss in an accepted tissue allograft.^[37] Monocytes and macrophages have both pro- and anti-inflammatory roles. M1 and M2 monocytes/macrophages modulate the immune system through cytokine production.^[38] CD200R is mainly expressed on monocytes, granulocytes, and dendritic cells. Monocyte infiltration in kidney allografts is associated with a higher risk of rejection. Acute interstitial rejection is detected histologically by infiltration of leucocytes into the transplanted kidney.^[35,39,40] CD200 suppresses alloimmune and autoimmune responses through CD200R, which activates myeloid-derived suppressor cells.^[41–44] Contradictory to previous studies,^[18,20,21,45,46] Rygiel et al showed that an anti-CD200 antibody has an immunosuppressive effect in a mouse allograft transplantation model, which improved renal and cardiac graft survival.^[47] However, to the best of our knowledge, the association between the outcome of KTx and CD200R1/CD200 plasma concentrations has not been studied in a clinical setting. Our findings have revealed that higher pre-transplant CD200R1/CD200 ratios are associated with AR and increased serum creatinine levels after KTx.

The small sample size meant the present study did not have enough power to allow a multivariable analysis. As a result, residual confounding may have biased the results. Therefore, our novel observations on the association of CD200 and CD200R concentrations with KTx outcome should be validated in future prospective studies with large sample sizes.

5. Conclusion

Higher pre-transplant CD200R1/CD200 ratios predict immunocompetence and AR, whereas high CD200R1 concentrations predict immunosuppression and severe post-transplant CMV infection. These results indicate that CD200 and CD200R are non-invasive predictors of post-transplantation outcome and can be used to modify patient monitoring, including renal biopsies,

after KTx. This improved monitoring may reduce the rate of graft biopsies, thereby reducing the risk of biopsy-related complications. Adjusting the dose of immunosuppressants based on pre-transplant CD200 and CD200R1 concentrations may also reduce the risk of AR and CMV infection after KTx.

Author contributions

Conceptualization: Hani Oweira, Arianeb Mehrabi, Mahmoud Sadeghi.

Data curation: Sara Mohammadi, Volker Daniel, Paul Schnitzler.

Formal analysis: Elias Khajeh, Omid Ghamarnejad, Mohammad Golriz.

Methodology: Mahmoud Sadeghi.

Project administration: Mahmoud Sadeghi.

Supervision: Mohammad Golriz, Markus Mieth, Christian Morath, Martin Zeier, Arianeb Mehrabi, Mahmoud Sadeghi.

Writing – original draft: Hani Oweira, Elias Khajeh, Sara Mohammadi, Omid Ghamarnejad, Mohammad Golriz.

Writing – review & editing: Volker Daniel, Paul Schnitzler, Markus Mieth, Christian Morath, Martin Zeier, Arianeb Mehrabi.

References

- Holmannova D, Kolackova M, Kondelkova K, et al. CD200/CD200R paired potent inhibitory molecules regulating immune and inflammatory responses; Part I: CD200/CD200R structure, activation, and function. *Acta Medica* 2012;55:12–7. 10.14712/18059694.2015.68.
- Minas K, Liversidge J. Is the CD200/CD200 receptor interaction more than just a myeloid cell inhibitory signal? *Crit Rev Immunol* 2006;26:213–30.
- Xiong Z, Ampudia-Mesias E, Shaver R, et al. Tumor-derived vaccines containing CD200 inhibit immune activation: implications for immunotherapy. *Immunotherapy* 2016;8:1059–71. 10.2217/imt-2016-0033.
- Vaine CA, Soberman RJ. The CD200-CD200R1 inhibitory signaling pathway: immune regulation and host-pathogen interactions. *Adv Immunol* 2014;121:191–211. 10.1016/B978-0-12-800100-4.00005-2.
- Yu K, Chen Z, Wang S, et al. Decreased alloreactivity using donor cells from mice expressing a CD200 transgene under control of a tetracycline-inducible promoter. *Transplantation* 2005;80:394–401.
- Holmannova D, Kolackova M, Kondelkova K, et al. CD200/CD200R paired potent inhibitory molecules regulating immune and inflammatory responses; Part II: CD200/CD200R potential clinical applications. *Acta Medica* 2012;55:59–65. 10.14712/18059694.2015.56.
- Rostaing L, Weclawiak H, Mengelle C, et al. Viral infections after kidney transplantation. *Minerva Urol Nefrol* 2011;63:59–71.
- Sadeghi M, Lahdou I, Daniel V, et al. Strong association of phenylalanine and tryptophan metabolites with activated cytomegalovirus infection in kidney transplant recipients. *Hum Immunol* 2012;73:186–92. 10.1016/j.humimm.2011.11.002.
- Sadeghi M, Lahdou I, Opelz G, et al. IL-23 plasma level is strongly associated with CMV status and reactivation of CMV in renal transplant recipients. *BMC Immunol* 2016;17:3510.1186/s12865-016-0175-7.
- Vicetti Miguel RD, Harvey SA, LaFramboise WA, et al. Human female genital tract infection by the obligate intracellular bacterium *Chlamydia trachomatis* elicits robust Type 2 immunity. *PLoS One* 2013;8:e58565. 10.1371/journal.pone.0058565.
- Caserta S, Nausch N, Sawtell A, et al. Chronic infection drives expression of the inhibitory receptor CD200R, and its ligand CD200, by mouse and human CD4 T cells. *PLoS One* 2012;7:e35466. 10.1371/journal.pone.0035466.
- Soberman RJ, MacKay CR, Vaine CA, et al. CD200R1 supports HSV-1 viral replication and licenses pro-inflammatory signaling functions of TLR2. *PLoS One* 2012;7:e47740. 10.1371/journal.pone.0047740.
- Goulding J, Godlee A, Vekaria S, et al. Lowering the threshold of lung innate immune cell activation alters susceptibility to secondary bacterial superinfection. *J Infect Dis* 2011;204:1086–94. 10.1093/infdis/jir467.
- Rygiel TP, Rijkers ES, de Ruiter T, et al. Lack of CD200 enhances pathological T cell responses during influenza infection. *J Immunol* 2009;183:1990–6. 10.4049/jimmunol.0900252.
- Boudakov I, Liu J, Fan N, et al. Mice lacking CD200R1 show absence of suppression of lipopolysaccharide-induced tumor necrosis factor- α and mixed leukocyte culture responses by CD200. *Transplantation* 2007;84:251–7. 10.1097/01.tp.0000269795.04592.cc.
- Fallarino F, Asselin-Paturel C, Vacca C, et al. Murine plasmacytoid dendritic cells initiate the immunosuppressive pathway of tryptophan catabolism in response to CD200 receptor engagement. *J Immunol* 2004;173:3748–54.
- Gorczynski R, Khatri I, Lee L, et al. An interaction between CD200 and monoclonal antibody agonists to CD200R2 in development of dendritic cells that preferentially induce populations of CD4+CD25+ T regulatory cells. *J Immunol* 2008;180:5946–55.
- Gorczynski RM, Lee L, Boudakov I. Augmented Induction of CD4+CD25+ Treg using monoclonal antibodies to CD200R. *Transplantation* 2005;79:1180–3.
- Clark DA, Gorczynski RM, Blajchman MA. Transfusion-related immunomodulation due to peripheral blood dendritic cells expressing the CD200 tolerance signaling molecule and alloantigen. *Transfusion* 2008;48:814–21. 10.1111/j.1537-2995.2008.01654.x.
- Gorczynski R, Chen Z, Khatri I, et al. sCD200 present in mice receiving cardiac and skin allografts causes immunosuppression in vitro and induces Tregs. *Transplantation* 2013;95:442–7. 10.1097/TP.0b013e3182754c30.
- Gorczynski RM, Chen Z, Khatri I, et al. Graft-infiltrating cells expressing a CD200 transgene prolong allogeneic skin graft survival in association with local increases in Foxp3(+)Treg and mast cells. *Transpl Immunol* 2011;25:187–93. 10.1016/j.trim.2011.07.006.
- Yan JJ, Koo TY, Lee HS, et al. Role of human CD200 overexpression in pig-to-human xenogeneic immune response compared with human CD47 overexpression. *Transplantation* 2018;102:406–16.
- Landry ML, Ferguson D. Comparison of quantitative cytomegalovirus antigenemia assay with culture methods and correlation with clinical disease. *J Clin Microbiol* 1993;31:2851–6.
- Asadullah K, Prosch S, Audring H, et al. A high prevalence of cytomegalovirus antigenaemia in patients with moderate to severe chronic plaque psoriasis: an association with systemic tumour necrosis factor α overexpression. *Br J Dermatol* 1999;141:94–102.
- Sadeghi M, Daniel V, Schnitzler P, et al. Urinary proinflammatory cytokine response in renal transplant recipients with polyomavirus BK viraemia. *Transplantation* 2009;88:1109–16. 10.1097/TP.0b013e3181ba0e17.
- Bechert CJ, Schnadig VJ, Payne DA, et al. Monitoring of BK viral load in renal allograft recipients by real-time PCR assays. *Am J Clin Pathol* 2010;133:242–50. 10.1309/AJCP63VDFCKCRUUL.
- Racusen LC, Solez K, Colvin RB, et al. The Banff 97 working classification of renal allograft pathology. *Kidney Int* 1999;55:713–23.
- Smirnov SV, Harbacheuski R, Lewis-Antes A, et al. Bone-marrow-derived mesenchymal stem cells as a target for cytomegalovirus infection: implications for hematopoiesis, self-renewal and differentiation potential. *Virology* 2007;360:6–16. 10.1016/j.virol.2006.09.017.
- Fallatah SM, Marquez MA, Bazerbachi F, et al. Cytomegalovirus infection post-pancreas-kidney transplantation—results of antiviral prophylaxis in high-risk patients. *Clin Transplant* 2013;27:503–9. 10.1111/ctr.12138.
- Sadeghi M, Daniel V, Naujokat C, et al. Dysregulated cytokine responses during cytomegalovirus infection in renal transplant recipients. *Transplantation* 2008;86:275–85. 10.1097/TP.0b013e31817b063d.
- Weimer R, Susal C, Yildiz S, et al. sCD30 and neopterin as risk factors of chronic renal transplant rejection: impact of cyclosporine A, tacrolimus, and mycophenolate mofetil. *Transplant Proc* 2005;37:1776–8. 10.1016/j.transproceed.2005.02.088.
- Weimer R, Susal C, Yildiz S, et al. Post-transplant sCD30 and neopterin as predictors of chronic allograft nephropathy: impact of different immunosuppressive regimens. *Am J Transplant* 2006;6:1865–74. 10.1111/j.1600-6143.2006.01407.x.
- Chung YH, Means RE, Choi JK, et al. Kaposi's sarcoma-associated herpesvirus OX2 glycoprotein activates myeloid-lineage cells to induce inflammatory cytokine production. *J Virol* 2002;76:4688–98. 10.1128/jvi.76.10.4688-4698.2002.

- [34] Rijkers ES, de Ruiter T, Baridi A, et al. The inhibitory CD200R is differentially expressed on human and mouse T and B lymphocytes. *Mol Immunol* 2008;45:1126–35. 10.1016/j.molimm.2007.07.013.
- [35] Sadeghi M, Daniel V, Weimer R, et al. Pre-transplant Th1 and post-transplant Th2 cytokine patterns are associated with early acute rejection in renal transplant recipients. *Clin Transplant* 2003;17:151–7.
- [36] Sakai R, Maeda A, Choi TV, et al. Human CD200 suppresses macrophage-mediated xenogeneic cytotoxicity and phagocytosis. *Surg Today* 2018;48:119–26. 10.1007/s00595-017-1546-2.
- [37] Gorczynski RM, Chen Z, He W, et al. Expression of a CD200 transgene is necessary for induction but not maintenance of tolerance to cardiac and skin allografts. *J Immunol* 2009;183:1560–8. 10.4049/jimmunol.0900200.
- [38] Mills CD. M1 and M2 macrophages: oracles of health and disease. *Crit Rev Immunol* 2012;32:463–88.
- [39] Hayry P, von Willebrand E. Monitoring of human renal allograft rejection with fine-needle aspiration cytology. *Scand J Immunol* 1981;13:87–97.
- [40] Rowshani AT, Vereyken EJ. The role of macrophage lineage cells in kidney graft rejection and survival. *Transplantation* 2012;94:309–18. 10.1097/TP.0b013e318250c10f.
- [41] Stumpfova M, Ratner D, Desciak EB, et al. The immunosuppressive surface ligand CD200 augments the metastatic capacity of squamous cell carcinoma. *Cancer Res* 2010;70:2962–72. 10.1158/0008-5472.CAN-09-4380.
- [42] Moertel CL, Xia J, LaRue R, et al. CD200 in CNS tumor-induced immunosuppression: the role for CD200 pathway blockade in targeted immunotherapy. *J Immunother Cancer* 2014;2:4610.1186/s40425-014-0046-9.
- [43] Banerjee D, Dick AD. Blocking CD200-CD200 receptor axis augments NOS-2 expression and aggravates experimental autoimmune uveoretinitis in Lewis rats. *Ocul Immunol Inflamm* 2004;12:115–25. 10.1080/09273940490895326.
- [44] Talebian F, Bai XF. The role of tumor expression of CD200 in tumor formation, metastasis and susceptibility to T lymphocyte adoptive transfer therapy. *Oncoimmunology* 2012;1:971–3. 10.4161/onci.20034.
- [45] Gorczynski RM, Chen Z, Hu J, et al. Evidence of a role for CD200 in regulation of immune rejection of leukaemic tumour cells in C57BL/6 mice. *Clin Exp Immunol* 2001;126:220–9.
- [46] Gorczynski RM, Hu J, Chen Z, et al. A CD200FC immunoadhesin prolongs rat islet xenograft survival in mice. *Transplantation* 2002;73:1948–53.
- [47] Rygiel TP, Luijk B, Meyaard L. Use of an anti-CD200 antibody for prolonging the survival of allografts: a patent evaluation of WO2012106634A1. *Expert Opin Ther Pat* 2013;23:389–92. 10.1517/13543776.2013.765406.