Transplant Immunology 30 (2014) 159–167

Contents lists available at ScienceDirect

Transplant Immunology

journal homepage: www.elsevier.com/locate/trim

Decrease of immature B cell and interleukin-10 during early-post-transplant period in renal transplant recipients under tacrolimus based immunosuppression

Byung Ha Chung [a,b,c,1], Kyoung Woon Kim [a,1], Ji Hyun Yu [a,b,c], Bo-Mi Kim [a], Bum Soon Choi [b,c], Cheol Whee Park [b,c], Yong-Soo Kim [b,c], Mi-La Cho [a,*,2], Chul Woo Yang [a,b,c,**,2]

[a] Convergent Research Consortium for Immunologic disease, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea
[b] Transplant Research Center, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea
[c] Division of Nephrology, Department of Internal Medicine, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea

ARTICLE INFO

Article history:
Received 21 December 2013
Received in revised form 25 March 2014
Accepted 25 March 2014
Available online 5 April 2014

Keywords:
Kidney transplantation
Immature B cell
IL-10
Tacrolimus

ABSTRACT

B lymphocytes are known to play a role in kidney transplantation (KT) outcomes. Here, we evaluated the proportion of B cell subsets before and after KT. Twenty-one patients, who showed stable allograft function without acute rejection in the month following the KT, were included in this study. Peripheral blood samples were obtained from these patients before transplantation as well as 1 month after transplantation. Changes in the proportion of B cell subsets after transplantation were investigated using multi-color flow cytometry. The proportion of lymphocytes in the peripheral blood mononuclear cells (PBMCs) and of $CD19^+$ B cells in the total leukocyte population did not change after KT. Similarly, the proportions of $CD19^+CD24^+$ lymphocytes, mature B cells ($CD24^{Int}CD38^{Inter}/CD19^+$), and memory B cells ($CD24^+CD38^-/CD19^+$) did not change post-KT. However, the proportion of immature B cells ($CD24^+CD38^+/CD19^+$ B cells) decreased significantly after transplantation ($P < 0.01$). The levels of IL-10, and IL-21, and expression of the B cell marker BLNK also decreased significantly after transplantation. Incubation of PBMCs with tacrolimus (0.1, 1, and 10 ng/mL) and mycophenolate mofetil (200 μg/mL) an immunosuppressant, resulted in significant reduction in the percentage of immature B cells. In contrast, the proportion of memory and mature B cells was not affected. Taken together, these results show that while the total B lymphocyte count and the proportion of memory/mature B cell subsets do not change after KT, the proportion of immature B cells and the associated cytokines that they secrete decrease significantly.
© 2014 Elsevier B.V. All rights reserved.

1. Introduction

The early post-transplant period is the time when the most robust immune reaction to the allograft occurs in kidney transplantation (KT) and suppression of this reaction is important for preventing graft rejection. To date, most studies on KT have focused on the role of T cells in the early immune response because they are regarded as key players in allo-immune reactions [1–3]. In our previous study, we investigated the changes in T cell subsets during the early post-transplant period and showed that most effector T cells, except Th17 cells, were successfully suppressed by TAC [4].

B cells have recently been shown to play key roles in the immune response to KT. In fact, some recent findings have linked B cell-associated immune responses to worsened allograft outcome in KT [5,6]. For example, activation of B cells is associated with increased risk of DSA development and even allograft failure [7,8]. Another report showed the role of B cells in the progression of chronic rejection [9,10]. In addition, specific B cell signatures have been associated with donor-specific immune tolerance [11–13].

All of the above findings indicate that B cells have significant role in both activation and regulation of allo-immune responses in transplantation, hence a change in the compartment of B cell populations may have some impact on allograft outcome. However, few studies have been conducted to evaluate the changes in the proportion of B cell subsets during the early post-transplant period and the effect of tacrolimus (TAC) on B cell subtypes.

In this regard, we prospectively investigated the changes in the proportion of B cell subsets in renal transplant recipients during the early

Abbreviations: IL, interleukin; KT, kidney transplantation; PBMCs, peripheral blood mononuclear cells; RTR, renal transplant recipient; Scr, serum creatinine; TAC, tacrolimus.

* Correspondence to: M.-L. Cho, Department of Internal Medicine, Seoul St. Mary's Hospital, 505 Banpo-Dong, Seocho-Ku, 137-040 Seoul, Republic of Korea. Tel.: +82 2 2258 7467; fax: +82 2 599 4287.
** Correspondence to: C. W. Yang, Department of Internal Medicine, Seoul St. Mary's Hospital, 505 Banpo-Dong, Seocho-Ku, 137-040 Seoul, Republic of Korea. Tel.: +82 2 2258 6037; fax: +82 2 536 0323.
E-mail addresses: iammila@catholic.ac.kr (M.-L. Cho), yangch@catholic.ac.kr (C.W. Yang).
[1] Byung Ha Chung and Kyoung Woon Kim contributed equally to this work.
[2] Mi-La Cho and Chul Woo Yang contributed equally to this work.

http://dx.doi.org/10.1016/j.trim.2014.03.003
0966-3274/© 2014 Elsevier B.V. All rights reserved.

post-transplant period and tested the effect of TAC on different B cell subsets in vitro.

2. Materials and methods

2.1. Patients and clinical information

The patient population comprised 21 living-donor renal transplant recipients. Second transplants, highly sensitized patients, and ABO-incompatible kidney transplant patients were excluded from this study because they were treated with modified or intensified immune suppressant protocols. For the included patients, the initial immunosuppressant regimen consisted of TAC in combination with mycophenolate mofetil (MMF) and prednisolone. Basiliximab was used as an additional induction therapy 2 h before transplantation as well as on day 4 after transplantation. The initial dose of TAC was 0.16 mg/kg per day orally; target trough levels were 8–12 ng/mL during the first 3 months and then 3–8 ng/mL. Methylprednisolone (1 g/day) was administered by intravenous infusion on the day of transplantation, and the dose was tapered to prednisolone at 30 mg/day on day 4 after transplantation. The initial dose of MMF was 1.5 g/day and it was modified to minimize adverse effects such as diarrhea or leukopenia. Peripheral blood mononuclear cells (PBMCs) were collected for analysis before the initiation of the immunosuppressants, and approximately 1 month after transplantation. This study was approved by the Institutional Review Board (KC10SISI0235) of the Seoul St. Mary's Hospital, and written informed consent was obtained from all patients.

2.2. Isolation of human cells

PBMCs were prepared from heparinized blood by Ficoll–Hypaque (GE Healthcare; PA) density-gradient centrifugation. Cells were cultured as described previously [14]. In brief, a cell suspension of 10^6 cells/mL was prepared in RPMI1640 medium supplemented with 10% fetal calf serum, 100 U/mL penicillin, 100 mg/mL streptomycin, and 2 mM L-glutamine. A 1-mL aliquot of the suspension was dispensed into 24-well plates (Nunc; Roskilde, Denmark) and incubated. For cytokine detection at the single-cell level, monolayer cultures of PBMCs were stimulated with 50 ng/mL phorbol myristate acetate (PMA) and 1 μg/mL ionomycin for 4 h.

2.3. Flow cytometric analysis of B cell subsets

For analysis of cytokine production, PBMCs were stimulated with PMA and ionomycin in the presence of GolgiStop (BD Biosciences, San Diego, CA) for 4 h. For surface staining, the cells were first immunostained with combinations of the following monoclonal antibodies (mAbs): CD38-PerCP Cy5.5 (HIT2; IgG1,κ; PharMingen); CD19-FITC (SJ25-C1; IgG1; SouthernBiotech; Birmingham, Alabama); and CD24-PE (ML5; IgG2a, κ; PharMingen). Next, the cells were washed, fixed, permeabilized, and the immunostained with mAbs to IL-10-APC (JES3-19F1; IgG2a, κ; PharMingen) to detect intracellular cytokines. Appropriate isotype controls were used for gate-setting for cytokine expression. The cells were analyzed using a FACS Calibur flow cytometry system (Becton Dickinson Systems).

2.4. Real-time polymerase chain reaction (PCR)

After incubation for 4 h with PMA and ionomycin, mRNA was extracted from PBMCs using RNAzol B (Biotex Laboratories; Houston, TX) according to the manufacturer's instructions. Reverse transcription of 2 μg of total mRNA was performed at 42 °C using the Superscript™ reverse transcription system (Takara; Shiga, Japan). PCR was performed in a 20-μL final volume in capillary tubes in a LightCycler instrument (Roche Diagnostics; Mannheim, Germany). The reaction mixture contained 2 μL of LightCycler Fast Start DNA Master Mix for SYBR® Green I (Roche Diagnostics), 0.5 μM of each primer, 4 mM $MgCl_2$, and 2 μL of template DNA. All capillaries were sealed and centrifuged at 500 × *g* for 5 s, and then the DNA was amplified in a LightCycler instrument. The amplification reaction involved polymerase activation at 95 °C for 10 min, followed by 45 cycles of 10 s at 95 °C, 10 s at 60 °C (β-actin) or 57 °C (IL-1β, HMGB-1), and 10 s at 72 °C. The temperature transition rate was 20 °C/s for all steps. The double-stranded PCR product was quantified during the 72 °C extension step by evaluating the fluorescence associated with the binding of SYBR Green I to the product. Fluorescence curves were analyzed with LightCycler software v. 3.0 (Roche Diagnostics). The LightCycler was also used to quantify BLNK, MS4A1, and TCL1A mRNA. The relative expression level of each sample was normalized to the level of *β-actin*, an endogenously expressed housekeeping gene. Melting curve analysis was performed immediately after amplification under the following conditions: 0 s (hold time) at 95 °C, 15 s at 71 °C, and 0 s (hold time) at 95 °C. The rate of temperature change was 20 °C/s for all steps, except for 0.1 °C/s in the final step. The crossing point (C_p) was defined as the maximum of the second derivative from the fluorescence curve. Negative controls were also included, which contained all the elements of the reaction mixture except for template DNA. All samples were processed in duplicate.

2.5. Enzyme-linked immunosorbent assay (ELISA)

In brief, a 96-well plate (Nunc) was coated with 4 μg/mL mAbs against IL-21 and IL-10 (R&D Systems) at 4 °C overnight. After blocking with a solution of phosphate-buffered saline (PBS), 1% bovine serum albumin (BSA), and 0.05% Tween 20 for 2 h at room temperature (22–25 °C), test samples and standard recombinant IL-21 and IL-10 were added to the 96-well plate, and the plates were incubated at room temperature for 2 h. After incubation, the plates were washed four times with PBS/Tween 20 and then incubated with 500 ng/mL biotinylated mouse monoclonal antibodies against IL-21 and IL-10 for 2 h at room temperature. After washing, the streptavidin–alkaline phosphate–horseradish peroxidase conjugate (Sigma) was added, and the plate was incubated for 2 h. The plate was then washed and incubated again with 1 mg/mL *p*-nitrophenyl phosphate (Sigma) dissolved in diethanolamine (Sigma) to allow color development. The reaction was stopped by adding 1 M NaOH, and the optical density for each well was read at 405 nm. The lower limit of IL-21 and IL-10 detection was 10 pg/mL. Recombinant human IL-21 and IL-10 diluted in culture medium were used as the calibration standards (concentration, 10–2000 pg/mL). A standard curve was generated by plotting the optical density values against the log of the concentration of recombinant cytokines and was used to calculate the IL-21 and IL-10 concentrations in the test samples.

Table 1
Baseline characteristics of patient population in prospective observation group.

	N = 21
Age (year)	42.6 ± 11.6
Male (n, %)	16 (69.2)
HLA mismatch number	3.4 ± 2.1
Dialysis modality before KT (n, %)	
Hemodialysis	11 (52)
Peritoneal dialysis	4 (19)
Preemptive KT	6 (29)
Dialysis duration (month)	13.7 ± 18.8
Primary renal disease (n, %)	
Chronic GN	10 (47)
Diabetes mellitus	5 (24)
Hypertension	3 (14)
Others	3 (14)
Donor type (n, %)	
Living related donor	15 (71)
Living unrelated donor	6 (29)

KT, kidney transplantation, GN, glomerulonephritis.

Download English Version:

https://daneshyari.com/en/article/3392075

Download Persian Version:

https://daneshyari.com/article/3392075

Daneshyari.com