DOI: 10.15825/1995-1191-2020-2-53-62

INDICATORS OF MONOCYTE-DERIVED COMPONENT OF THE IMMUNE SYSTEM IN PATIENTS WITH SATISFACTORY RENAL GRAFT FUNCTION

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Objective: to study the indicators of the monocyte-derived component of the immune system in kidney transplant recipients with satisfactory early and delayed renal transplant function. **Materials and methods.** The study involved 76 kidney transplant recipients. Concentrations of serum creatinine (sCr), serum urea (sUr) and serum cystatin C (sCysC) were measured. $CD14^{+mid/high}$ and $CD14^{+low}$ were isolated from $CD14^{+}$ monocytes. CD64- and CD86-expressing cell counts were determined for each subpopulation. Immunological examination was performed before surgery, as well as at days 1, 3, 7, 30, 90, 180 and 360 after surgery. **Results.** There was significant imbalance between the two monocyte subpopulations before transplantation and in the early post-transplant period (first 3 months). By the end of a 6-month follow-up period, the percentage of $CD14^{+}$ cells had normalized. The dynamics of the subclasses of CD86-expressing monocytes in the post-transplant period is somewhat different from the dynamics of the total count for these monocytes. However, by the end of a 6-month follow-up period, these biomarkers returned to normal for the group of healthy individuals ($CD14^{+mid/high}CD86^{+}$ $p_{180} = 0.079$; $CD14^{+low}CD86^{+}$ $p_{180} = 0.789$). $CD14^{+low}CD64^{+}$ level was significantly higher in the kidney transplant group than in the control group during the entire follow-up period ($p_0 = 0.0006$, $p_1 = 0.0001$, $p_7 = 0.005$, $p_{30} = 0.005$, $p_{90} = 0.007$, $p_{180} = 0.0002$, $p_{360} = 0.001$). On the other hand, $CD14^{+mid/high}CD64^{+}$ count for up to 180 days was not significantly different from that of the control group ($p_0 = 0.561$, $p_1 = 0.632$, $p_7 = 0.874$, $p_{30} = 0.926$, $p_{90} = 0.912$), with subsequent significant increase by day 360 of follow-up ($p_{180} = 0.01$, $p_{360} = 0.003$). We observed a negative correlation between $CD14^{+low}CD86^{+}$ level at day 0 and sCr levels at day 7 ($r = –0.4$; $p = 0.008$) and day 360 ($r = –0.34$; $p = 0.042$) and sCysC level at day 7 ($r = –0.57$; $p = 0.014$). A negative correlation was also found between $CD14^{+low}CD86^{+}$ at day 1 and sCr levels at day 7 ($r = –0.4$; $p = 0.005$) and day 360 ($r = –0.39$; $p = 0.02$). There was positive correlation between the $CD14^{+low}CD64^{+}$ subpopulation index at day 0 and sCr ($r = 0.54$; $p = 0.008$) and sCysC ($r = 0.6$; $p = 0.008$) levels at day 7, and also between the $CD14^{+low}CD64^{+}$ count at day 1 and sCr ($r = 0.55$; $p < 0.0001$) and sCysC ($r = 0.58$; $p = 0.004$) levels at day 7. $CD14^{+mid/high}CD64^{+}$ at day 0 negatively correlated with sCysC level at day 360 ($r = –0.85$; $p = 0.015$), while $CD14^{+mid/high}CD64^{+}$ at day 7 positively correlated with sCysC level at day 360 ($r = 0.50$; $p = 0.016$). **Conclusion.** Before transplant surgery, $CD14^{+mid/high}$, $CD14^{+mid/high}CD86^{+}$, and $CD14^{+low}CD86^{+}$ counts were reduced, while those of $CD14^{+low}$, $CD14^{+mid/high}CD64^{+}$ and $CD14^{+low}CD64^{+}$ were increased. By the 6-month follow-up, all these subpopulations except $CD14^{+mid/high}CD64^{+}$ had reached values for healthy people. Positive correlation between $CD14^{+mid/high}$, $CD14^{+low}CD64^{+}$, $CD14^{+mid/high}CD86^{+}$, $CD14^{+mid/high}CD64^{+}$ counts in the early post-transplant period and sCr/sCysC levels in long-term follow-up, as well as negative correlation between $CD14^{+low}$, $CD14^{+low}CD86^{+}$ counts in the early post-transplant period and sCr/sCysC levels in long-term follow-up can serve as a predictor of renal graft function.

Keywords: kidney transplantation, $CD14^{+}$ monocytes.

INTRODUCTION

Post-transplant immunologic monitoring in kidney transplant recipients is essential for improving transplant outcomes. However, many factors influencing the recipient's immune response make interpretation of immunological test results difficult. This problem is especially acute when evaluating the efficacy and toxicity of immunosuppressive therapy, predicting kidney transplant function, correcting secondary immunodeficiency in these patients with frequent and severe infectious complications and malignant tumors.

Patients are known to have post-transplant disorders in the basic functioning of both the acquired immune system and innate immune system associated with mononuclear phagocytic cells. In this regard, the study of the peculiarities of the subpopulation composition of peripheral blood monocytes in patients with uneventful post-transplant period seems to be quite logical and justified.

Monocytes are an important cell type for studying aseptic inflammation occurring in kidney transplants during reperfusion. Depending on expression of the high-

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affinity receptor for lipopolysaccharide (LPS) (CD14), it is common to distinguish the classical subpopulations of $CD14^+$ monocytes, transient $CD14^+CD16^+$ and non-classical $CD14^{+high}CD16^+$. As is known, $CD14^{+high}$ monocytes are commonly referred to as "classical", representing a larger population of less mature cells, while $CD14^{+low}$ monocytes are usually referred to as "pro-inflammatory" [1]. Monocyte subpopulations differ in the expression of molecules mediating the recognition, phagocytosis and presentation of antigens, which determines their functional characteristics. Although the roles of various monocyte subpopulations are not clearly defined, non-classical monocytes have both pro-inflammatory and anti-inflammatory properties, while classical and intermediate monocytes play a major role in phagocytosis and inflammation [2–4].

Classical monocytes $CD14^+CD16^-$ constitute about 80–85% of the total monocyte population. These monocytes are characterized by increased phagocytic activity, including those opsonized by complement components, due to the expression of scavenger receptors and the CD11b/CD18 receptor (CR3) [5, 6]. Also, the $CD14^+CD16^-$ subpopulation expresses high affinity receptor CD64, which in non-activated cells is associated with immunoglobulin G. The CD64 receptor is also associated with interferon-gamma receptor (IFNγ). In turn, the CD64-IgG bond maintains signaling pathways in a preactivated state and provides rapid signal transduction when binding to the IFNγ receptor [1, 7, 8]. Due to the less pronounced expression of HLA-DR and co-stimulatory molecules CD80 and CD86, this monocyte has less important properties of antigen presentation compared to other subpopulations [*5*].

The other two monocyte groups belong to minor subpopulations. Transient monocytes $CD14^+CD16^+$ are formed as a result of activation and differentiation of classical monocytes [5]. Receptors CD64 and CD16, CD14 and TLR, HLA-DR and co-stimulatory molecules are expressed on the surface of these cells. Therefore, their main functions are IgG-mediated phagocytosis, antigen presentation, PAMP recognition and cytokine synthesis [5, 6, 9].

In turn, expression of scavenger receptors and CR3 on transient monocytes is less dense than on classical ones [6].

With minimum density, $CD14^+$ and scavenger receptors express non-classical monocytes, which reduces their role in recognition of the LPS + LPS-binding protein complex and elimination of apoptotic cells. However, nonclassical monocytes express HLA-DR and co-stimulatory molecules with maximum density. Therefore, antigen presentation is one of their main functions [5, 6].

In our study, we have investigated the features of the monocyte subpopulation phenotype in patients after kidney transplantation. We have determined the relationship between monocyte content in peripheral blood and indicators reflecting the kidney transplant function with a satisfactory post-transplant period.

MATERIALS AND METHODS

The study was performed at the Republican Research Centre for Radiation Medicine and Human Ecology, Gomel, Belarus. It involved kidney transplant recipients (KTR) who underwent kidney transplantation for stage 5 chronic kidney disease at the surgical department (transplantation, reconstructive and endocrine surgery) of the Republican Research and Practical Center of the Republic of Moldova. A group (main group) was formed consisting of 76 subjects that met the following criteria: primary renal transplantation, induction therapy with anti-CD25 monoclonal antibodies, triple immunosuppressive therapy during the first 12 months of follow-up; kidney transplant function was satisfactory at postoperative day 7 and during the first 12 months.

All patients were tested for serum concentrations of creatinine, urea, and cystatin C. Immunological tests were carried out before surgery, as well as at postoperative day 1, 3, 7, 30, 90, 180 and 360.

There were 49 (64.47%) male and 27 (35.53%) female patients in this group. The median age (Me) was 46.89 ± 1.37 [44.16; 49.63] years. Before transplantation, 76.32% of patients were on long-term hemodialysis, while 23.68% were on peritoneal dialysis. Average cold ischemia time was 11.87 ± 0.43 hours. A negative result of direct cross-match was observed in 100% of cases.

When the creatinine level at postoperative day 7 was below 300 μmol/L, the function was considered primary – primary graft function (PGF), with values equal to or exceeding 300 μmol/L, and if there were indications for dialysis in the first postoperative week, the state was classified as renal graft dysfunction (RGD) [10]. A satisfactory renal graft function after a year was characterized by a 150 μmol/L blood creatinine level, as well as absence of episodes of graft rejection and the need for dialysis in the first year of follow-up [11].

The comparison group included 90 apparently healthy patients. Post-transplant follow-up period lasted for 12 months. The clinical study was carried out in accordance with the 1975 Declaration of Helsinki and approved by the Ethics Committee of the Republican Research Centre for Radiation Medicine and Human Ecology (Protocol No. 5 dated December 2, 2013).

Patients received induction therapy with anti-CD25 monoclonal antibodies, calcineurin inhibitors in combination with mycophenolate (89.5%) or azathioprine (10.5%), as well as corticosteroids. Anti-CD25 monoclonal antibodies were administered twice at 20 mg, at day 0 and day 4. Calcineurin cyclosporine was used as an inhibitor in 73.7% of patients, while 26.3% received tacrolimus.

The recipients underwent immunological tests on a FacsCanto II flow cytometer (Becton Dickinson and

Company, BD Biosciences, USA) complete with a sample preparation station using CD14PC7, CD64 FITC, CD86PE monoclonal antibodies (Beckman Coulter, USA) by mono-, two- and six-parameter analysis according to the manufacturer's instructions using multiple translational gating.

Determination of the relative and absolute monocyte counts

Blood was taken from the median cubital vein into tubes with anticoagulant EDTA. To determine the expression of monocyte surface markers by flow cytometry, a sample was prepared using the no-wash technique. CD14PC7, CD64 FITC, and CD86 PE monoclonal antibodies (Beckman Coulter, USA) were added to 100 μL of blood in the quantity recommended by the manufacturer. The mixture was incubated for 15 minutes in the dark at room temperature. Lysis solution OptiLyse B was used for lysis of red blood cells. Samples were analyzed on a FACS CantoII flow cytometer (BD, USA). Up to 20,000 events were accumulated. The monocyte population was determined as CD14+ cells. Depending on the CD14 expression density, two subpopulations were identified among $CD14^+$ monocytes: $CD14^{+mid/high}$ (classical) and $CD14^{+low}$ (nonclassical). For each of the subpopulations, the relative CD64- and CD86-expressing cell count was determined. The absolute content of these subpopulations was calculated using the data obtained from a general blood test carried out from this tube on the same day.

The results were statistically processed using the Statistica 10.0 software package. Descriptive statistics of the qualitative features are presented by absolute and relative frequencies, and quantitative statistics in the format: mean (confidence interval) – M [Confidence –95%; +95%] and median (interquartile range) – Me [Q25; Q75]. To compare the values, we used a numerical characteristics method (Mann–Whitney U test, Wilcoxon Matched Pairs Test) with estimation of the distribution of variables. Correlation analysis of indicators was evaluated using Spearman rank-order correlations. The results were considered statistically significant with a less than 0.05 significance level.

RESULTS

Results presented in Table 1 were obtained during analysis of biochemical indicators of renal function.

When studying the indices of peripheral blood monocytes (of kidney transplant recipients) expressing the main differentiating marker, the LPS receptor CD14, on their surface, the monocytes were clearly divided into two subpopulations: $CD14^{+mid/high}$ and $CD14^{+low}$ (Tables 2 and 3).

In the KTR group before transplantation, the $CD14^{+low}$ level was significantly higher than in the comparison group ($p_{0Mann–Whitney\ U\ Test} = 0.0003$). There was a negative trend in the dynamics of this subpopulation at day 1 relative to the preoperative level ($p_{0.1Wilcoxon\ Matched\ Pairs\ Test} = 0.001$) and the achievement of the result of the comparison group ($p_{1Mann–Whitney\ U\ Test} = 0.289$), restoring and even significantly exceeding the control result by day 7 ($p_{7Mann–Whitney\ U\ Test} = 0.001$; $p_{30Mann–Whitney\ U\ Test} = 0.038$; $p_{90Mann–Whitney\ U\ Test} = 0.001$).

We noted a slight decrease in this subpopulation at day 180 and day 360, but not lower than that of the comparison group ($p_{180Mann–Whitney\ U\ Test} = 0.72$; $p_{360Mann–Whitney\ U\ Test} = 0.279$). As for the comparison group with the preoperative count of this subpopulation and its relative dynamics, we found decreased $CD14^{+low}$ monocytes at day 1 (p0.1Wilcoxon Matched Pairs Test = 0.001) and an increase in this subpopulation from day 7 ($p_{0.7Wilcoxon\ Matched\ Pairs\ Test} = 0.028$, $p_{0.30Wilcoxon\ Matched\ Pairs\ Test} = 0.028$, $p_{0.90Wilcoxon\ Matched\ Pairs\ Test} = 0.005$, $p_{0.180Wilcoxon\ Matched\ Pairs\ Test} = 0.001$, $p_{0.360Wilcoxon\ Matched\ Pairs\ Test} = 0.023$). The dynamics of the $CD14^{+low}$ monocyte subpopulation is shown in Fig. 1.

$CD14^{+low}$ monocytes are antigen-presenting, which are responsible for increased production of pro-inflammatory cytokines – interleukins-1, -6, tumor necrosis factor. An increase in their count can serve as a marker of acute and exacerbation of chronic infectious diseases [12]. The $CD14^{+low}$ monocyte count over time decreased

Table 1

Biochemical parameters of renal function in recipients of kidney transplant and comparison group (Me [Q25; Q75])

Group	Urea, mmol/l	Creatinine, μmol/l	Cystatin C, mg/l
RTR0	19.2 [16.8; 22.2]	649.50 [569.0; 927.5]	5.94 [3.71; 6.39]
RTR1	17.0 [15.0; 21.9]	466.5 [354.0; 616.5]*	2.51 [1.99; 3.77]*
RTR7	10.35 [7.8; 14.5]*	148.0 [114.0; 196.5]*	1.33 [1.19; 1.92]*
RTR30	10.7 [8.1; 13.4]*	115.0 [102.0; 136.0]*	1.37 [1.22; 1.75]*
RTR90	8.2 [6.5; 10.1]*	99.0 [86.0; 129.5]*	1.46 [1.23; 1.68]*
RTR180	8.2 [6.1; 10.0]*	106.0 [86.0; 125.0]*	1.49 [1.16; 1.77]*
RTR360	7.2 [5.9; 10.6]*	106.5 [84.0; 130.0]*	1.51 [1.26; 1.72]*

Note. * – $p < 0.05$ compared to preoperative level. ** – RTR = renal transplant recipient.

Table 2

Indices of $CD14^{+low}$ monocyte subpopulations in recipients of kidney transplant and comparison group (Me [Q25; Q75])

Group	Unit	Monocyte subpopulations		
		$CD14^{+low}$	$CD14^{+low}CD86^{+}$	$CD14^{+low}CD64^{+}$
control	rel x %	3.7 [1.9; 5.5]	93.80 [88.2; 96.4]	82.00 [50.0; 91.3]
	10^9 cell/l	0.02 [0.01; 0.03]	0.02 [0.01; 0.02]	0.008 [0.006; 0.019]
RTR0	rel x %	7.50 [6.52; 8.04]*	78.27 [69.19; 90.36]*	96.14 [94.69; 97.59]*
	10^9 cell/l	0.02 [0.01; 0.02]	0.011 [0.011; 0.026]	0.016 [0.014; 0.032]
RTR1	rel x %	3.2 [2.28; 3.74]**	58.53 [48.7; 66.85]*	96.46 [94.69; 98.55]*
	10^9 cell/l	0.01 [0.01; 0.01]	0.004 [0.004; 0.005]	0.007 [0.006; 0.008]
RTR7	rel x %	5.57 [4.87; 6.39]*.**	81.43 [72.36; 90.51]*	93.94 [91.69; 95.39]*
	10^9 cell/l	0.03 [0.01; 0.04]	0.021 [0.01; 0.02]	0.024 [0.014; 0.031]*
RTR30	rel x %	6.08 [5.48; 7.13]*.**	71.27 [59.92; 75.80]*	96.94 [95.09; 98.39]*
	10^9 cell/l	0.04 [0.02; 0.05]	0.022 [0.017; 0.04]	0.037 [0.018; 0.051]
RTR90	rel x %	6.68 [6.21; 7.57]*.**	71.66 [66.36; 79.60]*	93.92 [92.39; 95.21]*
	10^9 cell/l	0.04 [0.03; 0.05]	0.032 [0.02; 0.04]	0.04 [0.028; 0.048]*
RTR180	rel x %	4.48 [3.43; 5.09]**	91.90 [88.31; 96.24]	97.13 [95.52; 98.18]*
	10^9 cell/l	0.02 [0.01; 0.03]	0.02 [0.016; 0.03]	0.022 [0.018; 0.034]*
RTR360	rel x %	4.79 [3.15; 7.0]**	85.03 [77.04; 90.12]*	94.96 [92.72; 96.64]*
	10^9 cell/l	0.03 [0.02; 0.04]	0.026 [0.018; 0.04]	0.029 [0.018; 0.043]*

Note. Here and in the Table 3: * – $p < 0.05$ relative to the matched control group; ** – $p < 0.05$ compared to preoperative level.

Table 3

Indices of $CD14^{+mid/high}$ monocyte subpopulations in recipients of kidney transplant and comparison group (Me [Q25; Q75])

Group	Unit	Monocyte subpopulations		
		$CD14^{+mid/high}$	$CD14^{+mid/high}CD86^{+}$	$CD14^{+mid/high}CD64^{+}$
control	rel x %	95.5 [93.6; 98.1]	98.6 [97.6; 99.6]	97.2 [96.3; 98.5]
	10^9 cell/l	0.42 [0.37; 0.56]	0.41 [0.37; 0.56]	0.41 [0.36; 0.54]
RTR0	rel x %	92.50 [91.96; 93.48]*	99.40 [99.02; 99.81]*	97.80 [96.22; 99.15]
	10^9 cell/l	0.18 [0.16; 0.37]	0.18 [0.16; 0.43]	0.172 [0.152; 0.43]
RTR1	rel x %	96.8 [96.26; 97.72]**	98.33 [98.02; 98.75]	97.80 [96.22; 99.15]
	10^9 cell/l	0.39 [0.28; 0.42]	0.36 [0.16; 0.19]	0.52 [0.39; 0.73]
RTR7	rel x %	94.43 [93.61; 95.13]**	98.83 [98.52; 99.20]	97.56 [96.22; 99.00]
	10^9 cell/l	0.18 [0.16; 0.39]	0.18 [0.16; 0.44]	0.155 [0.08; 0.45]
RTR30	rel x %	93.92 [92.87; 94.52]**	81.46 [76.42; 90.30]*	97.70 [96.00; 99.17]
	10^9 cell/l	0.29 [0.18; 0.39]**	0.28 [0.14; 0.49]	0.36 [0.16; 0.50]
RTR90	rel x %	93.32 [92.43; 93.79]*.**	94.63 [94.24; 95.0]*	97.38 [96.22; 98.95]
	10^9 cell/l	0.18 [0.16; 0.39]	0.17 [0.15; 0.43]	0.179 [0.15; 0.39]
RTR180	rel x %	95.52 [94.91; 96.58]**	97.19 [95.01; 98.69]	98.86 [98.19; 99.3]*
	10^9 cell/l	0.19 [0.16; 0.39]	0.18 [0.15; 0.45]	0.18 [0.15; 0.45]
RTR360	rel x %	95.21 [93.0; 96.85]**	97.80 [97.13; 98.57]	99.47 [99.13; 99.7]*
	10^9 cell/l	0.17 [0.15; 0.19]	0.15 [0.15; 0.19]	0.16 [0.16; 0.18]

relative to the preoperative level. However, during the entire follow-up period, we noted that the level in the comparison group was exceeded. This was due to the influence of a complex of factors, including surgical intervention, antigenic conflict, and immunosuppressive therapy.

The $CD14^{+high}$ monocytes are considered to be "classical", representing a larger population of less mature cells that provide antimicrobial protection as a result of phagocytic activity [13]. In our study, a reduced pre-transplantation level of the $CD14^{+mid/high}$ subpopulation ($p_{0Mann–Whitney\ U\ Test} = 0.0005$) was noted (Table 3, Fig. 2).

Starting from postoperative day 1, the level of this subpopulation reached that of the comparison group and remained so until 3 months of follow-up, briefly decreased at day 90 of follow-up and restored by day 180 ($p_{1Mann–Whitney\ U\ Test} = 0.207$, $p_{7Mann–Whitney\ U\ Test} = 0.528$, $p_{30Mann–Whitney\ U\ Test} = 0.077$, $p_{90Mann–Whitney\ U\ Test} = 0.003$, $p_{180Mann–Whitney\ U\ Test} = 0.858$, $p_{360Mann–Whitney\ U\ Test} = 0.426$). Although the entire follow-up period showed a signi-

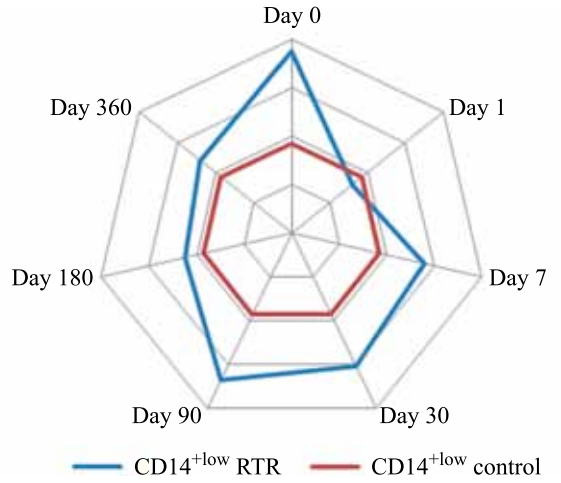

Fig. 1. Dynamics of $CD14^{+low}$ monocytes in recipients of kidney allograft during the first year of observation (CG)

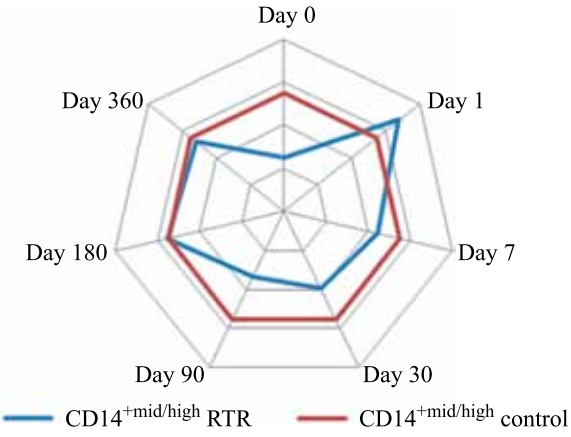

Fig. 2. Dynamics of $CD14^{+mid/high}$ monocytes in recipients of kidney allograft during the first year of observation

ficant upward trend relative to the preoperative level ($p_{0.1Wilcoxon\ Matched\ Pairs\ Test} = 0.001$, $p_{0.7Wilcoxon\ Matched\ Pairs\ Test} = 0.0003$, $p_{0.30Wilcoxon\ Matched\ Pairs\ Test} = 0.003$, $p_{0.90Wilcoxon\ Matched\ Pairs\ Test} = 0.028$, $p_{0.180Wilcoxon\ Matched\ Pairs\ Test} = 0.005$, $p_{0.360Wilcoxon\ Matched\ Pairs\ Test} = 0.001$).

Based on results obtained, it can be stated that before transplantation and in the early post-transplant period (first 3 months), two monocyte subpopulations were found to have a significant imbalance. By month 6 of follow-up, the percentage of $CD14^{+}$ cells had normalized.

In performing correlation analysis of the level of subpopulations $CD14^{+low}$ and $CD14^{+mid/high}$ with indicators characterizing kidney function, we found a negative correlation between the relative count of $CD14^{+low}$ before transplantation and creatinine level at day 1 ($r = –0.60$; $p = 0.008$) and between the absolute count at day 7 with creatinine level at year 1 ($r = –0.85$; $p = 0.016$). Also, a high level of $CD14^{+low}$ at day 30 of follow-up negatively correlated with serum cystatin C levels at year 1 ($r = –0.81$; $p = 0.015$). In turn, the preoperative $CD14^{+mid/high}$ level and the serum creatinine level at day 1 had a positive correlation ($r = 0.60$; $p = 0.008$), while the $CD14^{+mid/high}$ level after 1 month of follow-up and serum cystatin C levels at year 1 had a positive correlation ($r = 0.8$; $p = 0.015$). Thus, the negative correlation between the $CD14^{+low}$ level at the early stages of examination and the creatinine/cystatin C levels at 12 months of follow-up, and the positive correlation between the $CD14^{+mid/high}$ level at the early stages of examination and the creatinine/cystatin C levels at 12 months of follow-up suggest that these indicators could be used for predictive purposes.

We analyzed the count of $CD14^{+mid/high}$ and $CD14^{+low}$ subpopulations expressing CD86, which is a costimulatory ligand of the CD28 and CD152 molecules. The interaction between these molecules contributes to either positive or negative regulation of the immune response [14].

In the KTR group, almost the entire follow-up period, with the exception of day 180, revealed a significantly lower $CD14^{+low}CD86^{+}$ monocyte subpopulation than in the comparison group ($p_{0Mann–Whitney\ U\ Test} = 0.0004$, $p_{1Mann–Whitney\ U\ Test} < 0.0001$, $p_{7Mann–Whitney\ U\ Test} = 0.001$, $p_{30Mann–Whitney\ U\ Test} = 0.0001$, $p_{90Mann–Whitney\ U\ Test} < 0.0001$, $p_{180Mann–Whitney\ U\ Test} = 0.789$, $p_{360Mann–Whitney\ U\ Test} = 0.0006$). As for the dynamics relative to the preoperative level, the trend towards a reduced $CD14^{+low}CD86^{+}$ count persisted up to day 360, when there were no differences with the preoperative period ($p_{0.1Wilcoxon\ Matched\ Pairs\ Test} = 0.001$, $p_{0.7Wilcoxon\ Matched\ Pairs\ Test} = 0.0003$, $p_{0.30Wilcoxon\ Matched\ Pairs\ Test} = 0.028$, $p_{0.90Wilcoxon\ Matched\ Pairs\ Test} = 0.005$, $p_{0.180Wilcoxon\ Matched\ Pairs\ Test} = 0.001$, $p_{0.360Wilcoxon\ Matched\ Pairs\ Test} = 0.307$) (Fig. 3).

In turn, the $CD14^{+mid/high}CD86^{+}$ monocyte subpopulation had less pronounced dynamics. It decreased significantly only at day 30 and 90 relative to the preoperative level ($p_{0.30Wilcoxon\ Matched\ Pairs\ Test} = 0.028$,

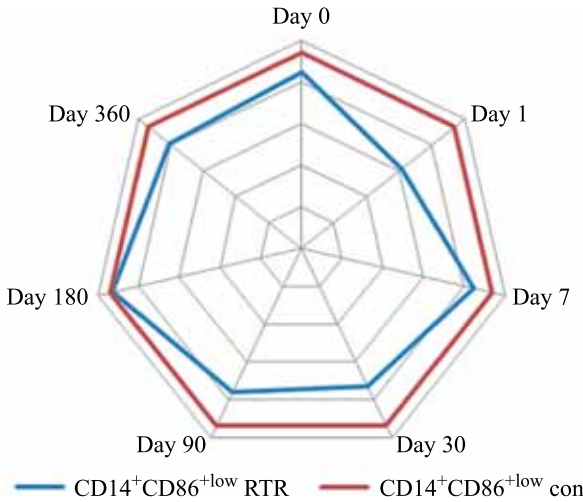

Fig. 3. Dynamics of $CD14^{+low}CD86^{+}$ monocytes in recipients of kidney allograft during the first year of observation

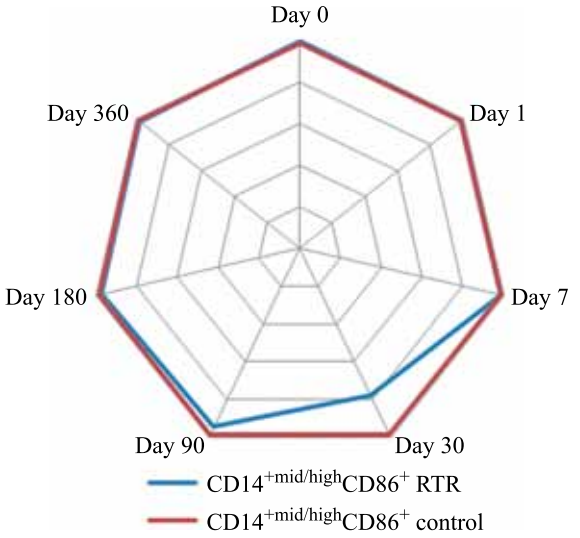

Fig. 4. Dynamics of $CD14^{+mid/high}CD86^{+}$ monocytes in recipients of kidney allograft during the first year of observation

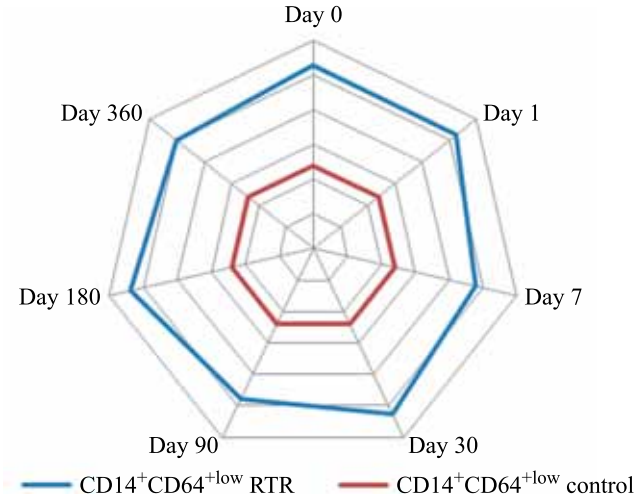

Fig. 5. Dynamics of $CD14^{+low}CD64^{+}$ monocytes in recipients of kidney allograft during the first year of observation

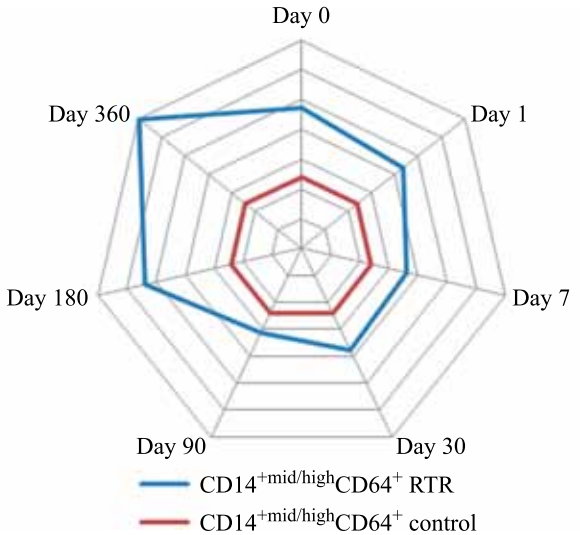

Fig. 6. Dynamics of $CD14^{+mid/high}CD64^{+}$ monocytes in recipients of kidney allograft during the first year of observation

$p_{0.90Wilcoxon\ Matched\ Pairs\ Test} = 0.005$). In the comparison group, the $CD14^{+mid/high}CD86^{+}$ cell count before transplantation was slightly higher ($p = 0.02$). But later there was a decrease in this subpopulation and recovery at month 6 of follow-up ($p_{1Mann–Whitney\ U\ Test} = 0.528$, $p_{7Mann–Whitney\ U\ Test} = 0.479$, $p_{30Mann–Whitney\ U\ Test} < 0.0001$, $p_{90Mann–Whitney\ U\ Test} = 0.002$, $p_{180Mann–Whitney\ U\ Test} = 0.079$, $p_{360Mann–Whitney\ U\ Test} = 0.209$) (Fig. 4).

Based on the observed results, it can be stated that the dynamics of the two studied subclasses of CD86-expressing monocytes in the post-transplantation period slightly differs from the dynamics of the total count of these monocytes. But at month 6 of follow-up, not only was the preoperative level restored, but these indicators normalized relative to the groups of healthy individuals.

With a stable constancy, the count of minor subpopulation of monocytes expressing $CD14^{+low}CD64^{+}$, the high-affinity receptor for IgG, prevailed in the KTR group relative to the comparison group throughout the follow-up period ($p_{0Mann–Whitney\ U\ Test} = 0.0006$, $p_{1Mann–Whitney\ U\ Test} = 0.0001$, $p_{7Mann–Whitney\ U\ Test} = 0.005$, $p_{30Mann–Whitney\ U\ Test} = 0.005$, $p_{90Mann–Whitney\ U\ Test} = 0.007$, $p_{180Mann–Whitney\ U\ Test} = 0.0002$, $p_{360Mann–Whitney\ U\ Test} = 0.001$) (Fig. 5).

Some differences were revealed in the dynamics of classical monocytes expressing the IgG receptor. Before day 180, the $CD14^{+mid/high}CD64^{+}$ count did not significantly differ from the comparison group ($p_{0Mann–Whitney\ U\ Test} = 0.561$, $p_{1Mann–Whitney\ U\ Test} = 0.632$, $p_{7Mann–Whitney\ U\ Test} = 0.874$, $p_{30Mann–Whitney\ U\ Test} = 0.926$, $p_{90Mann–Whitney\ U\ Test} = 0.912$). Then, a progressive increase in the level of this subpopulation led to maximum count of the cells at day 360 of follow-up ($p_{180Mann–Whitney\ U\ Test} = 0.01$, $p_{360Mann–Whitney\ U\ Test} = 0.003$) (Fig. 6).

Correlation analysis of the above subpopulations with renal graft function indicators revealed the following. The $CD14^{+low}CD86^{+}$ count at day 0 was negatively correlated with creatinine level at day 7 and day 360 ($r = –0.4$; $p = 0.008$ and $r = –0.34$; $p = 0.042$, respectively) and with cystatin C level at day 7 ($r = –0.57$; $p = 0.014$). The $CD14^{+low}CD86^{+}$ count at day 1 was also found to be negatively correlated with creatinine levels at day 7 and day 360 ($r = –0.4$; $p = 0.005$ and $r = –0.39$; $p = 0.02$, respectively). There was a positive correlation between the $CD14^{+low}CD64^{+}$ count at day 0 and creatinine and cystatin C levels at day 7 ($r = 0.54$; $p = 0.008$ and $r = 0.6$; $p = 0.008$, respectively). The $CD14^{+low}CD64^{+}$ count at day 1 and creatinine and cystatin C levels at day 7 were also positively correlated ($r = 0.55$; $p < 0.0001$ and $r = 0.58$; $p = 0.004$, respectively).

There was a positive correlation between the $CD14^{+mid/high}CD86^{+}$ count at day 0 and day 7 and the cystatin C level at day 360 ($r = 0.48$; $p = 0.019$ and $r = 0.36$; $p = 0.033$, respectively).

As for the $CD14^{+mid/high}CD64^{+}$ subpopulation, there were no significant correlations with the creatinine level over the entire follow-up period, but correlations

were found with cystatin C level. The $CD14^{+mid/high}CD64^{+}$ count was negatively correlated at day 0 and positively correlated at day 7 with cystatin C level at day 360 (r = 0.-85; p = 0.015 and r = 0.50; p = 0.016, respectively.

DISCUSSION

At the pre-transplantation stage of comparing monocyte subpopulations with the group of healthy individuals, it was revealed that the classical $CD14^{+mid/high}$ monocyte count reduced, while the non-classical $CD14^{+low}$ count increased in the KTR group. Similar changes were found in studies conducted earlier to investigate the dynamics of these subpopulations in other types of surgical interventions, particularly in patients with coronary heart disease who underwent coronary artery bypass grafting under artificial blood circulation [15].

The most pronounced changes in the two main monocyte subpopulations – $CD14^{+mid/high}$ and $CD14^{+low}$ – were observed at day 1. The sharp increase in the $CD14^{+mid/high}$ subpopulation may be due to the positive correlation between the content of classical monocytes and the concentration of interleukin-6 (IL-6) [2]. According to the authors, an increase in the absolute count of classical monocytes and IL-6 is an indirect criterion for assessing the degree of activation of endothelium, an active producer of growth factors myeloid germ and IL-6 [2]. With regard to the secretion of IL-6 in the early post-transplant period, a number of researchers noted that during transplantation of brain-dead donor kidneys, an IL-6 release can be expected within the first 4–6 hours after reperfusion. The peak time of IL-6 release depends on the influence of many factors, such as the warm and cold ischemia time, type of donor, and characteristics of the initial graft function. Moreover, the authors found that the absence of such a reaction is a poor prognostic sign [16]. Therefore, the peak of increase in classical monocytes at day 1 and restoration of their preoperative level at day 7 is prognostically favorable for the patient.

The peculiarities of the dynamics of monocyte subpopulations $CD14^{+mid/high}$, $CD14^{+low}$, $CD14^{+mid/high}CD86^{+}$, $CD14^{+low}CD86^{+}$, $CD14^{+mid/high}CD64^{+}$, and $CD14^{+low}CD64^{+}$ revealed in our study were that all significant differences with the comparison group were erased at month 6 after transplantation, except for the minor subpopulation $CD14^{+mid/high}CD64^{+}$, whose count increased maximally by year 1 of follow-up. At various stages of the study, the $CD14^{+mid/high}$, $CD14^{+low}CD64^{+}$, $CD14^{+mid/high}CD86^{+}$, $CD14^{+mid/high}CD64^{+}$ counts in the early post-transplant period were found to be positively correlated with creatinine and cystatin levels at long-term follow-up, and the $CD14^{+low}$, $CD14^{+low}CD86$ counts in the early post-transplant period were negatively correlated with creatinine and cystatin levels at long-term follow-up. The exception was the minor subpopulation $CD14^{+mid/high}$, which expresses the high-affinity IgG receptor. There were no correlations between this monocyte subpopulation and creatinine levels. However, there was a positive correlation between this subpopulation, which is a precursor of inflammatory macrophages [17], at day 0, day 1, and day 7 with serum cystatin C level at day 90, day 180 and day 360 of follow-up.

Considering that $CD14^{+mid/high}CD64^{+}$ are an important link in the innate immune system, particularly in implementation of phagocytic function, the results obtained in our study should be further explored.

So, the revealed relationships between minor monocyte subpopulations and laboratory indicators of kidney graft function indicate the possibility of using indicators of these subpopulations on postoperative day 1 in order to predict the functional state of the graft. Considering that the study was conducted in patients with satisfactory renal graft function at year 1 of follow-up and a comparable immunosuppressive therapy, the described dynamics of monocyte subpopulations can be used for immunological monitoring in the post-transplant period.

CONCLUSION

The revealed features of the monocytic immunity link in kidney recipients with satisfactory early and delayed graft function included reduced pre-transplant count of classical monocytes $CD14^{+mid/high}$, $CD14^{+mid/high}CD86^{+}$, $CD14^{+low}CD86$ and increased count of $CD14^{+low}$, $CD14^{+mid/high}CD64^{+}$, $CD14^{+low}CD64^{+}$ relative to those of healthy individuals. In the post-transplant period, all indicated subpopulations, with the exception of $CD14^{+mid/high}CD64^{+}$, reached normal values by month 6 of follow-up. The presence of negative correlations between the $CD14^{+low}$, $CD14^{+low}CD86^{+}$ count in the early post-transplant period and the creatinine/cystatin C levels in the long-term follow-up, and the positive correlations between the $CD14^{+mid/high}$, $CD14^{+low}CD64^{+}$, $CD14^{+mid/high}CD86^{+}$, $CD14^{+mid/high}CD64^{+}$ count in the early post-transplant period and the creatinine/cystatin C levels in the long-term follow-up can be used as prognostic factors for delayed kidney graft function.

The authors declare no conflict of interest.

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The article was submitted to the journal on October 4, 2019