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Immune Subsets From Ficoll Density Gradient Separation in Kidney Transplant Recipients

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Many studies in transplantation have used peripheral blood mononuclear cells (PBMCs) to assess immunological risk and to unravel the mechanisms of rejection and tolerance.[1-3] Examples include functional T- and B-lymphocyte studies by ELISPOT,[1,4,5] T-lymphocyte proliferation studies,[2] gene expression profiling,[3] and intracellular tacrolimus concentration measurements.[6] However, the PBMC fraction consists of various leukocyte subpopulations, including T, B, and NK lymphocytes and monocytes, with potential contamination by granulocytes. By using Ficoll density gradient separation to isolate the PBMCs, the proportion of low-density granulocytes is increased in inflammatory conditions such as sepsis,[7] burn wounds,[8] or in autoimmune disease.[9] The biological validity of these studies could therefore be affected if based on faulty methodological assumptions.[7,10]

Surprisingly, no studies report on whether the proportion of PBMC subpopulations from transplant recipients differ from healthy controls, or if rejection affects cellular PBMC composition. We compared the proportions of PBMC subsets in kidney transplant recipients during a biopsy-proven acute T cell–mediated rejection (TCMR) episode (n = 11) with those from kidney transplant recipients without acute rejection (n = 12) and healthy controls (kidney transplant donors before transplantation) (n = 10). All kidney transplant recipients received tacrolimus (targeted predose concentration 7–14 ng/mL), mycophenolate mofetil (fixed-dose 2 g/d), and prednisolone (total daily dose 5–20 mg/d). PBMCs were obtained from heparinized blood using the standard Ficoll density gradient procedure (Ficoll-Paque, GE Healthcare, Uppsala, Sweden) and were frozen in RPMI-1640 with glutamax (Life Technologies/Gibco BRL, Paisley, Scotland) with 15% heat-inactivated human serum and 10% dimethyl sulfoxide (Merck, Darmstadt, Germany).[11] PBMCs were stored in liquid nitrogen at –190 °C until use.[1,12] All participants gave written informed consent. The study was performed in accordance with the declaration of Helsinki (2013) and approved by the institutional review board of the Erasmus MC (No. 2018-035).[13]

PBMCs were thawed using RPMI medium with DNase (DNase I, Roche, Germany) and stained with anti-CD3-BV510, anti-CD19-PE-Cy7, anti-CD16-PE, anti-CD56-PerCP, anti-CD45-APC, anti-CD15-BV421, and anti-CD14-FITC. All antibodies were from Biolegend (San Diego, CA) except anti-CD14 (BD Bioscience, San Jose, CA). PBMC subsets were measured on a FACS Canto II flow cytometer (BD Biosciences). Flow cytometric data were analyzed by Kaluza Analysis Software version 2.1 (Beckman Coulter Life Sciences, Indianapolis, IN).

Figure 1 illustrates the gating strategy, and Table 1 summarizes the composition of the PBMCs from the patients and healthy controls. Overall, T lymphocytes predominated in the PBMC fraction (mean: 52.4% ± 12.9%), followed by monocytes (28.3% ± 12.3%), and B lymphocytes (9.1% ± 5.2%). There was no significant difference between kidney transplant recipients (with or without acute TCMR) and healthy controls regarding the proportion of T, B, and NK lymphocytes, monocytes, and granulocytes in PBMCs after the Ficoll procedure.

In contrast to previous studies that showed a significant increase in the proportion of granulocytes in patients suffering from inflammation compared with controls,[7,10] there was no statistically significant difference in the cellular make up of the PBMC fraction of kidney transplant recipients with acute TCMR compared with nonrejecting kidney transplant recipients. The results in our study show that acute rejection might therefore be considered as a relatively "milder" state of inflammation in the peripheral

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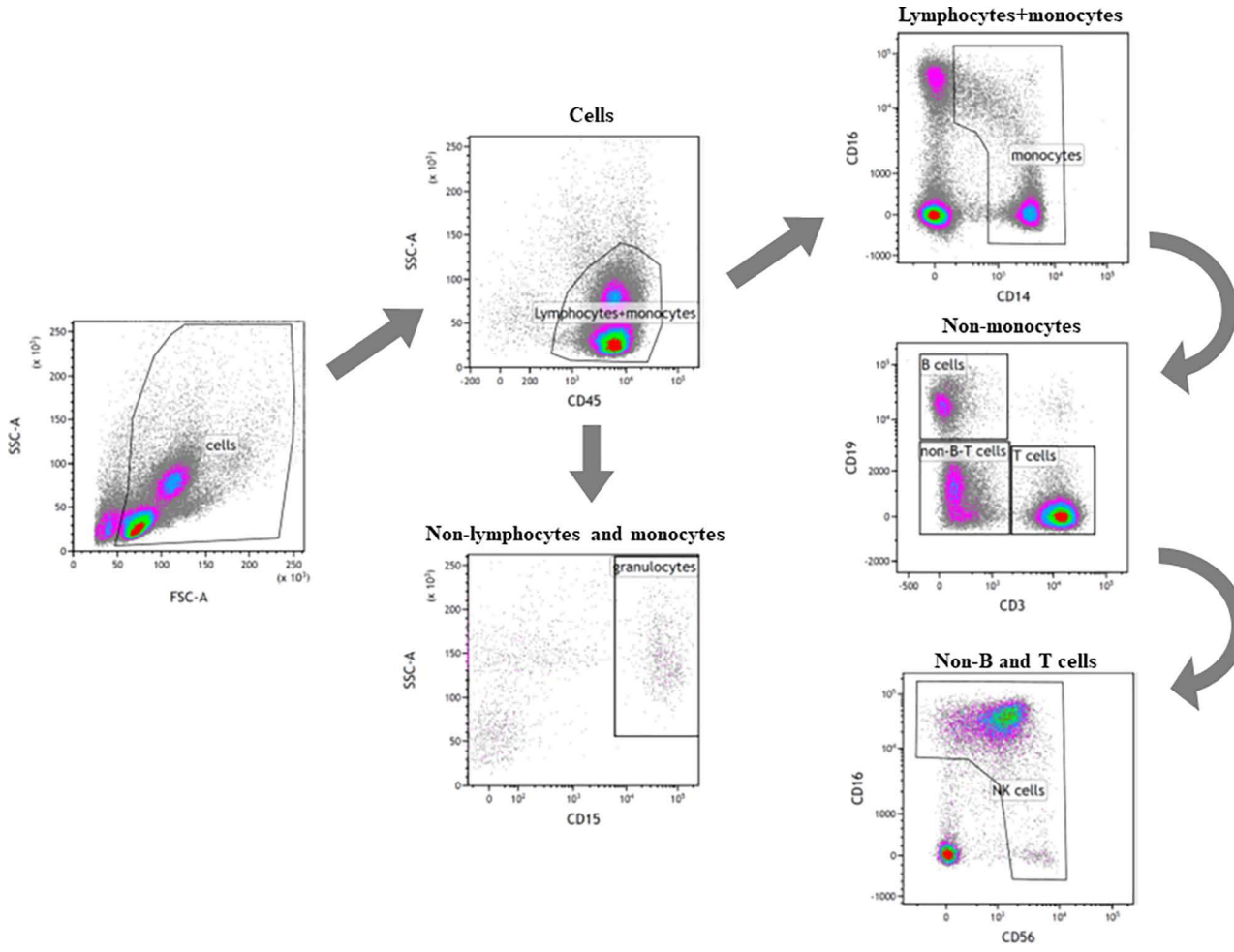

FIGURE 1. Representative data of the flowcytometric analysis.

blood compartment compared with septicemia or other systemic inflammatory diseases.[8-10]

Interestingly, a trend toward a lower proportion of NK lymphocytes in the PBMCs fraction of recipients with acute TCMR was observed. Previous studies have shown a significantly higher number of infiltrating NK lymphocytes in the kidney allografts with TCMR compared with kidneys affected by antibody-mediated rejection or kidneys without signs of rejection.[14-16] Moreover, the number of infiltrating NK lymphocytes was positively correlated with a higher

TABLE 1.

PBMC subsets in healthy controls and kidney transplant recipients with and without acute T cell–mediated rejection

Variable	All subjects	Healthy controls	Recipients without rejection	Recipients with acute TCMR	*P* value for nonrejection recipients vs acute TCMR recipients	*P* value for healthy controls vs acute TCMR recipients	*P* value across all 3 groups
Number of subjects	33	10	12	11	–	–	–
Time after transplantation, days (median, Q1–Q3)	–	–	19.5 (9.5–48.0)	12.5 (7.0–42.5)	0.38	–	–
Age, y (mean ± SD)	54.5 ± 11.3	53.4 ± 7.1	56.0 ± 13.4	54.0 ± 12.7	0.63	0.96	0.80
Male, n (%)	25 (76%)	7 (70%)	9 (75%)	9 (82%)	0.69	0.40	0.82
T lymphocytes, % (mean ± SD)	52.4 ± 12.9	55.4 ± 12.6	52.6 ± 13.3	49.4 ± 13.3	0.57	0.30	0.58
Monocytes, % (mean ± SD)	28.3 ± 12.3	24.1 ± 9.0	28.3 ± 11.4	32.0 ± 15.2	0.51	0.17	0.34
B lymphocytes, % (mean ± SD)	9.1 ± 5.2	7.0 ± 2.2	10.6 ± 6.0	9.3 ± 6.1	0.62	0.26	0.27
NK lymphocytes, % (mean ± SD)	7.7 ± 5.3	10.8 ± 5.5	7.2 ± 4.4	5.5 ± 5.3	0.42	0.09	0.06
Granulocytes, % (mean ± SD)	2.6 ± 4.4	2.7 ± 5.0	1.4 ± 2.0	3.8 ± 5.8	0.20	0.67	0.46

PBMC, peripheral blood mononuclear cell; TCMR, T cell–mediated rejection.

Banff TCMR grade.[14] The present findings suggest that NK lymphocytes migrate from the periphery to the transplanted kidney. Since NK lymphocyte make up the smallest PBMC subfraction, the infiltration of the kidney transplant may have an observable effect in the peripheral compartment. However, further studies are needed to confirm this hypothesis.

Our study has limitations. First, the sample size was relatively small and might be underpowered to detect significant differences, although most of the measured immune cell subsets (except NK lymphocytes) show a large overlap in their proportion of PBMCs across all groups. Second, this study was conducted in kidney transplant recipients, and the results might be different in recipients of a nonrenal organ transplant.

In conclusion, our study shows that PBMC fractions of immunosuppressed kidney transplant recipients, with or without rejection, are not noticeably influenced by the Ficoll separation procedure. These results may be useful for future immune monitoring studies of transplant recipients involving human PBMCs.

REFERENCES

1. Mendoza Rojas A, van Gelder T, de Kuiper R, et al. Pre-transplant donor-reactive IL-21 producing T cells as a tool to identify an increased risk for acute rejection. *Sci Rep.* 2021;11:12445.
2. Ezzelarab MB, Zhang H, Sasaki K, et al. Ex vivo expanded donor alloreactive regulatory T cells lose immunoregulatory, proliferation, and antiapoptotic markers after infusion into ATG-lymphodepleted, nonhuman primate heart allograft recipients. *Transplantation.* 2021;105:1965–1979.
3. Luan D, Dadhania DM, Ding R, et al. FOXP3 mRNA profile prognostic of acute T cell–mediated rejection and human kidney allograft survival. *Transplantation.* 2021;105:1825–1839.
4. Udomkarnjananun S, Kerr SJ, Townamchai N, et al. Donor-specific ELISPOT assay for predicting acute rejection and allograft function after kidney transplantation: a systematic review and meta-analysis. *Clin Biochem.* 2021;94:1–11.
5. Udomkarnjananun S, Kerr SJ, Francke MI, et al. A systematic review and meta-analysis of enzyme-linked immunosorbent spot (ELISPOT) assay for BK polyomavirus immune response monitoring after kidney transplantation. *J Clin Virol.* 2021;140:104848.
6. Francke MI, Andrews LM, Lan Le H, et al. Monitoring intracellular tacrolimus concentrations and its relationship with rejection in the early phase after renal transplantation. *Clin Biochem.* 2022;101:9–15.
7. Schenz J, Obermaier M, Uhle S, et al. Low-density granulocyte contamination from peripheral blood mononuclear cells of patients with sepsis and how to remove it—a technical report. *Front Immunol.* 2021;12:684119.
8. Calvano SE, Greenlee PG, Reid AM, et al. Granulocyte contamination of Ficoll-Hypaque preparations of mononuclear cells following thermal injury may lead to substantial overestimation of lymphocyte recovery. *J Trauma.* 1988;28:353–361.
9. Carmona-Rivera C, Kaplan MJ. Low-density granulocytes: a distinct class of neutrophils in systemic autoimmunity. *Semin Immunopathol.* 2013;35:455–463.
10. van den Akker EL, Baan CC, van den Berg B, et al. Ficoll-separated mononuclear cells from sepsis patients are contaminated with granulocytes. *Intensive Care Med.* 2008;34:912–916.
11. Hønge BL, Petersen MS, Olesen R, et al. Optimizing recovery of frozen human peripheral blood mononuclear cells for flow cytometry. *PLoS One.* 2017;12:e0187440.
12. van Besouw NM, Yan L, de Kuiper R, et al. The number of donor-specific IL-21 producing cells before and after transplantation predicts kidney graft rejection. *Front Immunol.* 2019;10:748.
13. Verhoeven JGHP, Boer K, Peeters AMA, et al. A novel high-throughput droplet digital PCR-based INDEL quantification method for the detection of circulating donor-derived cell-free DNA after kidney transplantation. *Transplantation.* [Epub ahead of print. March 10, 2022]. doi:10.1097/TP.0000000000004078.
14. Shin S, Kim YH, Cho YM, et al. Interpreting CD56+ and CD163+ infiltrates in early versus late renal transplant biopsies. *Am J Nephrol.* 2015;41:362–369.
15. Dos Santos DC, Campos EF, Saraiva Câmara NO, et al. Compartment-specific expression of natural killer cell markers in renal transplantation: immune profile in acute rejection. *Transpl Int.* 2016;29:443–452.
16. Dos Santos DC, Saraiva Camara NO, David DSR, et al. Expression patterns of CD56+ and CD16+ cells in renal transplant biopsies with acute rejection: associations with microcirculation injuries and graft survival. *Nephrology (Carlton).* 2017;22:993–1001.