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CASE REPORT ON PROLONGED KIDNEY GRAFT SURVIVAL WITHOUT IMMUNOSUPPRESSIVE THERAPY AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

N.V. Shmarina[1, 2], N.V. Borovkova[1, 2], E.S. Stolyarevich[3], V.A. Vasilyeva[4], R.V. Storozhev[1], I.V. Dmitriev[1, 2], A.G. Balkarov[1, 2], E.N. Parovichnikova[4]

[1] Sklifosovsky Research Institute of Emergency Care, Moscow, Russian Federation
[2] Pirogov Russian National Research Medical University, Moscow, Russian Federation
[3] Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow, Russian Federation
[4] National Medical Research Centre for Hematology, Moscow, Russian Federation

Background. The possibility of inducing immunological tolerance in allogeneic organ transplant recipients is a research goal of the transplantology community, as it will ensure the likelihood of complete engraftment of a foreign organ. However, such a task presently remains difficult to accomplish. **Objective:** to demonstrate long-term kidney graft survival without signs of acute rejection and without immunosuppressive therapy in a patient who underwent allogeneic hematopoietic stem cell transplantation (allo-HSCT) from a haploidentical donor for post-transplant lymphoproliferative disorder (PTLD). **Methods and materials.** Recipient's graft function was assessed using clinical, laboratory, instrumental and pathomorphological examination methods. **Results.** With no immunosuppressive therapy for more than four years, the kidney recipient showed stable, satisfactory graft function. **Conclusion.** The described clinical case demonstrates the development of immunological tolerance to a kidney graft in a recipient of allogeneic hematopoietic stem cells (HSCs).

Keywords: immunological tolerance, kidney transplantation, chimerism.

INTRODUCTION

The main challenge of organ transplantation is the body's immune response, manifested by acute or chronic rejection reactions. Immunosuppressive therapy (IST) in most cases suppresses the immune system and allows transplanted organs to function for a limited time. Currently, all known immunosuppressants have more or less significant side effects. This has encouraged researchers to develop new ways to suppress the immune system [1]. It is optimal to develop immune tolerance in the recipient, allowing the graft to function as long and efficiently as possible. Immune tolerance is understood as the absence of a specific response to certain foreign antigens, while retaining the ability to develop a full-fledged response to other antigens. The coexistence of cells of more than one genotype in the same individual is called a biological chimera. Chimerism is categorized into full and mixed chimerism. Full chimerism exists when all hematopoietic cells are of donor origin, while mixed chimerism is the coexistence of both donor and recipient cells in different proportions [2]. Mixed hematopoietic chimerism was first demonstrated by Ray Owen in 1945. He showed that bovine fraternal twins with common placental circulation are chimeric and tolerant to each other [3]. In 1953, Billingham, Brent, Medawar et al. described a state of "actively acquired tolerance" to skin allografts that developed after transplantation of viable allogeneic cells into embryos or newborn mice [4]. Studies of mixed chimerism and immunological tolerance in animals have led to the use of HSCs transplantation as a method of inducing tolerance in solid organ transplantation in humans [5–7].

Tolerance formation is a determining condition for long-term functioning of transplanted organs and tissues. Central and peripheral tolerance differ in terms of mechanism of development. Central tolerance is aimed at preventing the appearance of autoreactive T and B lymphocytes in the process of their maturation and occurs in the central organs of immunogenesis in the thymus and bone marrow [8, 9]. In this case, elimination of potentially dangerous T lymphocytes reacting to their own antigens is effected by inducing programmed cell death (apoptosis). This mechanism is called clonal deletion or negative selection. For B lymphocytes, another mechanism is also possible – receptor editing. With receptor editing, receptors can no longer bind to their own antigens. In allo-HSCT, central tolerance is a key mechanism and is determined by the presence and selection of donor immune cells in the thymus [10]. In

Corresponding author: Nonna Shmarina. Address: room 101, bldg. 2, 3, Bolshaya Sukharevskaya str., Moscow, Russian Federation. Phone/fax: (495) 625-28-80. E-mail: nonna_shm@mail.ru

allo-HSCT, immature T cells that are newly formed in the bone marrow for further maturation and proliferation, populate the thymus, where they actively proliferate and undergo positive selection by binding to short fragments of proteins on the major histocompatibility complex (MHC) molecules, which in humans are designated as HLA (human leukocyte antigens) classes I and II. As a result, the process of positive selection leads to the survival of mature CD8+ and CD4+ T cells that can recognize HLA molecules [11]. If T cell receptors bind too strongly to HLA molecules in the thymus, intracellular signaling is so strong that it ends in apoptotic cell death, thereby destroying cells with a high probability of autoreactivity (negative selection).

Peripheral (postthymic) tolerance is aimed at identifying and controlling autoreactive cell clones that have escaped central tolerance mechanisms. Peripheral tolerance is ensured by various mechanisms (1) by ignoring the antigen when there is insufficient or excess quantity of it, and when antigen presentation is impaired, T cell anergy occurs due to insufficient expression of T cell receptor or coreceptor molecules; (2) there is negative activation of lymphocytes leading them to apoptosis; (3) under the action of regulatory T cells. Given the toxicity of IST, the search for approaches that are aimed at tolerance induction after solid organ transplantation continues. These approaches include costimulatory blockade, lymphodepletion, induction of regulatory T cell formation, and mixed chimerism [12]. Despite the progress made in research on induction of immune tolerance in animal models, transferring the proposed strategies to humans is a challenging task.

Attempts to avoid or minimize IST in patients after kidney transplantation lead to rapid graft rejection and graft loss [13]. In this clinical case report, we can speak about the formation of tolerance to a transplanted organ in the patient.

Objective: to demonstrate long-term kidney graft survival without signs of acute rejection and without IST in a patient who underwent allo-HSCT from a haploidentical donor for PTLD.

CLINICAL CASE REPORT

Patient B., female, born in 1985, was diagnosed with mesangial proliferative glomerulonephritis in adolescence after suffering from pharyngitis, and received pathogenetic therapy involving prednisolone and mycophenolates. By adulthood, the girl had progression of chronic kidney disease to the end stage. As a result, long-term hemodialysis therapy was initiated. In 2011, at the age of 25, she underwent kidney allotransplantation from a deceased donor at Sklifosovsky Research Institute of Emergency Care. Mismatches in MHC antigens in the donor-recipient pair consisted of four antigens: A3, B7, 35, and Dr10. The patient received standard IST: tacrolimus, mycophenolates, prednisolone and basiliximab induction on postoperative days 0 and 4. Graft function was delayed, diuresis was restored from postoperative day 38, blood creatinine levels (121 μmol/l) normalized on day 57. In 2013, she suffered acute graft pyelonephritis, then a graft biopsy was performed, which revealed grade 1 chronic allograft nephropathy. In 2016, due to a planned pregnancy, mycophenolates were converted to azathioprine. She was in a satisfactory condition until April 2017; blood nitrogen metabolism parameters were creatinine 110–130 μmol/L and urea 7–8 mmol/L.

In May 2017, her condition deteriorated, which was clinically manifested by food-borne toxicoinfection, blood creatinine levels increased to 170 μmol/l. From June to August 2017, her condition worsened: weakness, fever episodes, stomach heaviness and increase in stomach size, dry cough; she did not seek medical help. In August 2017, she was hospitalized at the Transplanted Kidney Pathology ward of the Municipal Clinical Hospital No. 52 with leukopenia (2.8×10^9), proteinuria (2.3 g/day), elevation of C-reactive protein (to 115 mg/dL) and blood creatinine level (214 μmol/L). Azathioprine was discontinued due to leukopenia. PTLD was suspected; trepanobiopsy and sternal puncture were performed, and paraproteinemia tests were taken. On August 23, 2017, she was transferred to the National Medical Research Center for Hematology, where the final diagnosis was established: diffuse large B cell lymphoma, DLBCL (post-transplant lymphoproliferative disorder, PTLD) with liver, spleen, stomach and bone marrow involvement, probably associated with IST. Given the severity of the condition, sepsis and immunodeficiency, the remaining IST (tacrolimus and prednisolone) was discontinued. For the treatment of DLBCL, the patient received 6 cycles of CHOD (total doses: cyclophosphamide 1200 mg, doxorubicin 75 mg, vincristine 2 mg, dexamethasone 80 mg) from August 26, 2017 to January 11, 2018, in combination with rituximab (600 mg); the first cycle was performed with a prephase dexamethasone and cyclophosphamide. After that, the patient was in remission of the lymphoproliferative disorder for a year.

After completion of chemotherapy (6 courses), due to the risk of kidney graft rejection, tacrolimus-based IST (2 mg/day) was resumed from April 2018.

In January 2019, PTLD-DLBCL reoccurred and 3 polychemotherapy courses (cytarabine (Σ = 4 g) + etoposide (Σ = 400 mg) combined with lenalidomide (Σ = 30 mg)) were administered. The first course was given with a prephase cyclophosphamide and dexamethasone. A second PET-negative remission of PTLD-DBCCL was achieved. There was still a need to resume IST to prevent graft rejection, which would entail another relapse of the disease. Therefore, on May 14, 2019, the patient underwent HSCs transplantation from a related haploidentical donor (mother) using the TCRαβ+/CD19+ graft depletion technique. The choice of this transplantation approach was based on the fact that it does not invol-

ve long-term (6 months) IST. Graft-versus-host disease prophylaxis was performed according to the scheme: rituximab + bortezomib + tocilizumab + abatacept, and it was completed 1 month after haploidentical allo-HSCT. It was assumed that the donor immune system would "accept" the kidney graft as a host tissue and that tolerance to the graft would also be formed.

Due to not taking immunosuppressive drugs within three months from the moment of haploidentical allo-HSCT, the patient was examined at Sklifosovsky Research Institute of Emergency Care in August 2019 to assess the state of the kidney graft. No evidence of kidney allograft (KA) rejection was found – blood creatinine 120 μmol/L, glomerular filtration rate (GFR) 34 ml/min, no anti-HLA antibodies were detected. Considering the non-standard clinical situation and the patient's informed consent, a kidney transplant biopsy was carried out. The biopsy revealed no signs of rejection; moderate focal global glomerulosclerosis, arteriosclerosis, interstitial fibrosis and tubular atrophy were observed (Fig. 1).

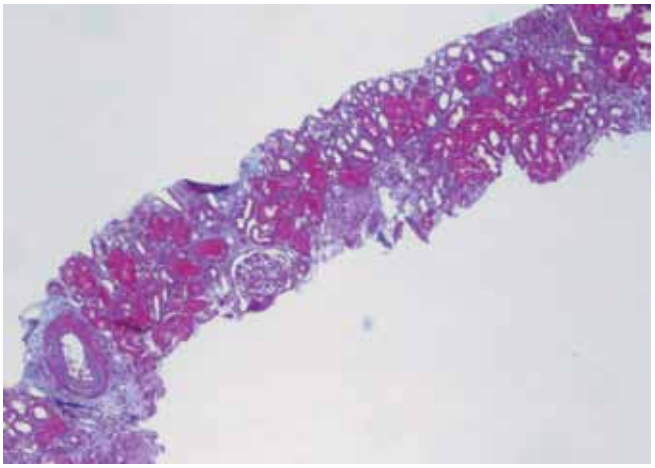

Fig. 1. Micrograph. Kidney transplant specimen: focal global glomerulosclerosis, arteriolosclerosis, interstitial fibrosis and tubular atrophy grade 2-3

Transplantologists and hematologists jointly decided to continue monitoring the patient without prescribing IST due to the high risk of recurrent DLBCL, as well as due to the high probability of developing immunological tolerance as a result of the functioning of the new donor immune system.

At patient B.'s repeated follow-up hospitalization in April 202: the patient's condition was satisfactory and stable. During hospitalization, blood creatinine levels fluctuated in the 162–178 μmol/L range, GFR was 54 ml/min, daily proteinuria 1 g. Ultrasound examination found no evidence of graft dysfunction: graft dimensions were within normal values, 112 × 49 mm, there was arterial blood flow in the entire kidney up to the capsule, resistivity indices were 0.56–0.66 (Fig. 2).

Given the moderate increase in blood creatinine levels and proteinuria, kidney graft biopsy was performed. The histologic findings showed chronic inactive rejection, moderate interstitial fibrosis and tubular atrophy (Fig. 3). Deposition of complement component C4d in the renal graft tissues was not detected by immunofluorescence. When blood plasma was examined for the presence of anti-HLA antibodies, including donor-specific antibodies, the test returned negative.

Given the absence of active kidney graft rejection, no anti-crisis therapy was administered to the patient. In the hospital, conservative treatment was performed to improve microcirculation in the KA, and blood creatinine decreased to 150 μmol/L. The patient was discharged with recommendations to continue follow-up without taking immunosuppressants.

During follow-up examination in March 2022, patient B. remained without IST, was in good health, quality of life was high, and was socially adapted – she worked as a programmer. Laboratory tests: blood creatinine was

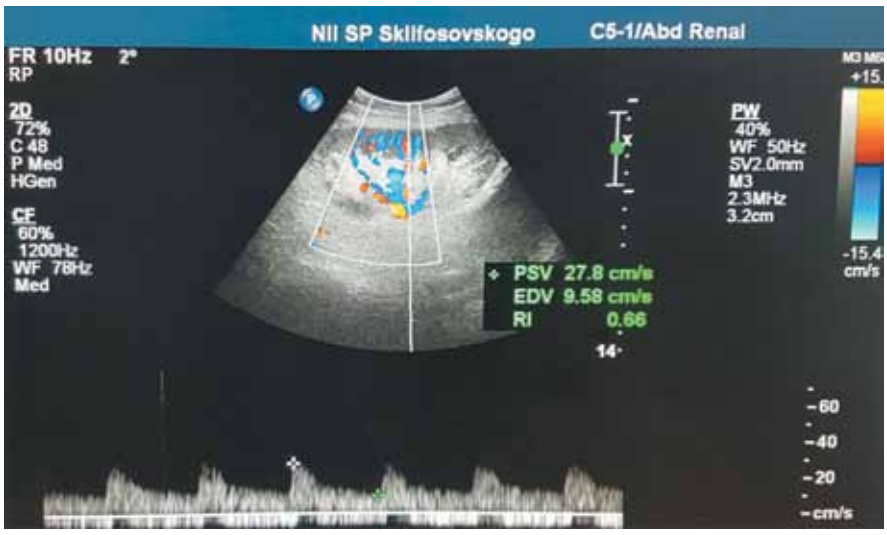

Fig. 2. Ultrasonogram of the kidney graft of patient B. April 2021

168 μmol/L, urea 13.6 mmol/L, GFR 44 l/min, and daily proteinuria 0.86 g/day. Ultrasound examination found no evidence of graft dysfunction. The doctors decided not to perform a biopsy due to stable graft function.

In January 2023, the patient was called for a routine checkup. She did not take immunosuppressive drugs. Her health was satisfactory, and she continued to work. Her blood creatinine level was 154 μmol/L, urea was 17 mmol/L, GFR was 44 ml/min, and daily proteinuria 0.87 g. No anti-MHC antigen antibodies were detected in the blood. Ultrasound examination found no signs of KA dysfunction (Fig. 4).

Thus, evidence obtained suggests a stable satisfactory renal graft function in the patient who has not received IST for more than 4 years. At the time of writing this paper, graft function lasted for 12 years. Control examinations showed that PET-negative remission of PTLD-DLBCL, on the background of 100% donor chimerism, with no signs of graft-versus-host reaction, persisted within 4 years after haploidentical allo-HSCT.

DISCUSSION

The presented clinical case is a unique example of the development of tolerance to a transplanted solid organ against the background of formation of full hematopoietic donor chimerism due to transplantation of HSCs. It is worth noting that the approach in which immunological tolerance was observed when using allo-HSCT in solid organ transplantation, is considered the most promising direction, making IST unnecessary. Thus, the results of three pilot studies on induction of tolerance to renal allografts from living donors, performed at Stanford, Northwestern, and Massachusetts General Hospitals have been published [14, 15]. Patients underwent combined kidney and bone marrow transplantation from an HLA-mismatched donor. All subjects received non-

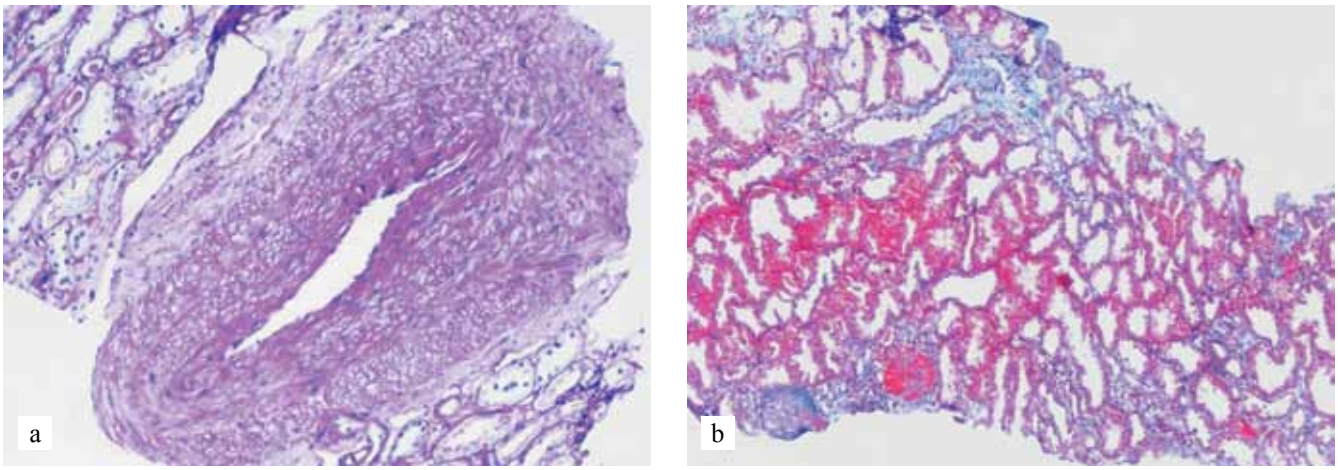

Fig. 3. Micrograph. Kidney graft specimens: a, transplant vasculopathy (arterial wall thickening due to myointimal proliferation and intimal fibrosis, inflammatory cells in the intima thickness); b, focal global glomerulosclerosis, interstitial fibrosis and tubular atrophy grade 2

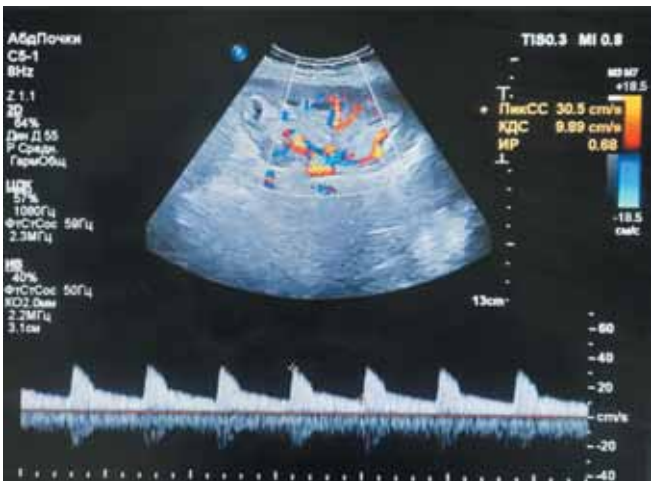

Fig. 4. Ultrasonogram of the kidney graft (12 years after kidney transplantation). Resistive index in the segmental artery of the renal graft is within normal range

myeloablative conditioning at the stages of preparation for transplantation. Patients achieved stable chimerism in 38.5% of cases and transient chimerism in 26% of cases, which allowed complete withdrawal of IST in 63% of cases. Despite good renal graft engraftment, this approach is extremely limited due to the high risk of graft-versus-host disease [12, 14, 15].

The uniqueness of this case of tolerance to a kidney allograft is due to a number of major differences from the strategies of induction of hematopoietic chimerism described in the literature. An aggressive B-cell lymphoma emerging six years after kidney transplantation was a complication of long-term IST in the patient. PTLD treatment included the first and repeated stages of long-term chemotherapy, and subsequently, transplantation of allogeneic HSCs from a related haploidentical donor. We can assume that the formation of immunological

tolerance to the kidney allograft is most likely due to the fact that the cells of the donor immune system, during the process of engraftment and expansion, saw the kidney graft as a host tissue via a universal mechanism. Since all antigens in the recipient's body are foreign to the donor immune system, the donor alloreactive T cells were restricted against the kidney allograft as well.

Thus, because after allo-HSCT, immunological tolerance was induced not only to all host tissues but also to the tissue functioning in the kidney recipient's body from another donor, there was no need for lifelong immunosuppressants.

CONCLUSION

The clinical case described represents the only successful case of formation of immunological tolerance in our practice, which allows a kidney transplant recipient to get along without IST for more than 4 years.

The authors declare no conflict of interest.

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