

Rapid full engraftment and successful immune reconstitution after allogeneic hematopoietic stem cell transplantation with reduced intensity conditioning in Omenn syndrome

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Abstract: OS is a variant of SCID characterized by generalized erythroderma, alopecia, eosinophilia, and elevated IgE levels. It is fatal unless treated with allogeneic HSCT, which is the only curative approach. However, treatment related complications and graft rejection are major obstacles to the success of treatment. In this report, we describe a patient with OS, complicated by prolonged cytomegalovirus infection, successfully treated by reduced intensity conditioning allogeneic HSCT from sibling donor.

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OS is a rare, autosomal recessive, SCID most often caused by mutations of the recombination activating genes *RAG1* or *RAG2*, typically associated with the triad of diffuse erythroderma, lymphadenopathy, hepatosplenomegaly, and increased risk of life-threatening infections (1–3). Other common symptoms include protracted

diarrhea, alopecia, and failure to thrive (4). Laboratory findings include lack of circulating B cells, defective antibody production followed by hypogammaglobulinemia, and high level of serum IgE, marked eosinophilia, and elevated or normal counts of activated oligoclonal T cells. These T lymphocytes, not of maternal origin, are often skewed toward a Th2 phenotype, which poorly responds to mitogens and infiltrates target organs like skin, intestine, liver, and spleen, causing a graft-vs.-host-like disease. Eosinophilia and high IgE levels probably result from the Th2-type cytokine (IL-4, IL-5) secretion. NK cell functions and absolute numbers are unaffected (4–6). Moreover, patients with OS have defective

Abbreviations: AIRE, autoimmune regulator; CsA, cyclosporin A; DLI, donor lymphocyte infusions; GvHD, graft-vs.-host disease; HSCT, hematopoietic stem cell transplantation; NK cells, natural killer cells; OS, Omenn syndrome; PHA, phytohemagglutinin; RIC, reduced intensity conditioning; SCID, severe combined immunodeficiency; TRM, transplant-related mortality.

thymic expression of AIRE transcription factor that controls expression of tissue-specific peptides by thymic medullary epithelial cells. This defect is likely to result in defective negative selection of autoreactive T cells in OS that can expand in the periphery in response to autoantigens (7). The clinical course of OS is inevitably fatal because of increased susceptibility to infections with a high mortality early in life. Despite intensive supportive care, 70% of patients die within the first year of life. Corticosteroids, cyclosporine, and interferon- γ have shown transient symptomatic efficacy (4).

Allogeneic HSCT is the only curative treatment for OS. Transplantation using marrow from genotypically identical sibling donor remains the treatment of choice in patients with OS (8, 9). However, only approximately 10% of infants with SCID have healthy, HLA-matched sibling bone marrow donors. Satisfactory results have been obtained mostly in transplantations from HLA-matched family donors, whereas a high rate of TRM, graft rejection, and long-term morbidity have been reported in the literature for transplantations from mismatched family donors or from matched unrelated donors (8, 10, 11). Although allogeneic HSCT is curative in OS, this procedure also carries significant risks of mortality and morbidity because of myeloablative conditioning regimens. The need to employ aggressive conditioning regimens to eliminate autoreactive T-lymphocytes and achieve engraftment has undoubtedly accounted for high TRM, particularly in patients suffering from organ damage as a consequence of severe/recurrent infections (12). RIC is associated with less toxicity from myeloablative conditioning agents and may be an alternative option for vulnerable patients considered ineligible to receive standard allograft (12, 13). However, RIC still carries significant risk of non-engraftment, rejection, and GvHD, particularly if DLI have to be used to ensure engraftment (13, 14). We report a case of successful allogeneic bone marrow transplantation with the use of RIC in a patient with OS complicated by CMV infection.

Case report

We describe a male infant (birth weight 3660 g) presenting with phenotypic features of OS. First symptoms (generalized erythematous rash with skin desquamation, lymphadenopathy, and organomegaly) appeared at 3 wk of age. Over the next two months, he developed failure to thrive, weight loss, and progressive hair loss from scalp, eyebrows, and eyelashes. Moreover, he

developed cytopenia and interstitial pneumonia requiring mechanical ventilation. Because of poor general condition of the patient, the interstitial pneumonia was not confirmed by biopsy. Management included antibiotics, antifungal, antiviral, and immunoglobulin infusion. Fever and clinical as well as radiological signs and symptoms of pneumonia subsided following this treatment. The diagnosis of OS was established at the age of 13 wk and immunosuppressive therapy with dexamethasone 0.25 mg/kg i.v. and CsA 2–4 mg/kg i.v. was initiated to counterbalance the autoimmune manifestations. Slight improvement of skin lesions was recorded within 1 wk. The treatment was continued at this dosage for five wk, resulting in occurrence of blood CsA levels between 50 and 100 ng/mL. As a matched sibling donor (sister – without any signs and symptoms of OS) was available, the child was referred for bone marrow transplantation at 22 wk of age. Because of the medical history of serious CMV infection (Fig. 1) and CMV-negative status of the donor, there were major concerns that myeloablative conditioning regimen would not be tolerated by the patient. Therefore, RIC consisting of fludarabine 30 mg/m² i.v. once daily on days from –7 to –3 (total dose 150 mg/m²) and melphalan 70 mg/m² i.v. once daily on days –3 and –2 (total dose 140 mg/m²) were introduced. Moreover, the patient had been continuously treated for CMV with foscarnet 180 mg/kg/day in two divided doses from the day of transplantation until engraftment, and he received a combination of gancyclovir 10 mg/kg/day and foscarnet 120 mg/kg/day in two divided doses. Nevertheless, high level of CMV in the blood was still observed (Fig. 1). For GvHD prophylaxis, CsA was introduced in standard i.v. doses from day –1, to maintain CsA blood concentration between 100 and 200 ng/mL. On day 0, the patient received 7×10^6 CD34⁺ cells/kg harvested from the donor's bone marrow. Although the RIC was anticipated to be non-myeloablative, he developed absolute neutropenia and thrombocytopenia. Recovery of neutrophils to over 1.0×10^9 /L on day +9 and of platelets to over 20.0×10^9 /L on day +19 were observed. During the entire peritransplant period, the patient received only filtered and irradiated blood products. The early clinical post-transplant course was unremarkable, complicated only by mild mucositis, fever, and mild to moderate transient systemic skin rash interpreted as acute GvHD (grade I–II). Repeated fungal culture tests of blood, urine, and stool were negative. Moreover, the results of

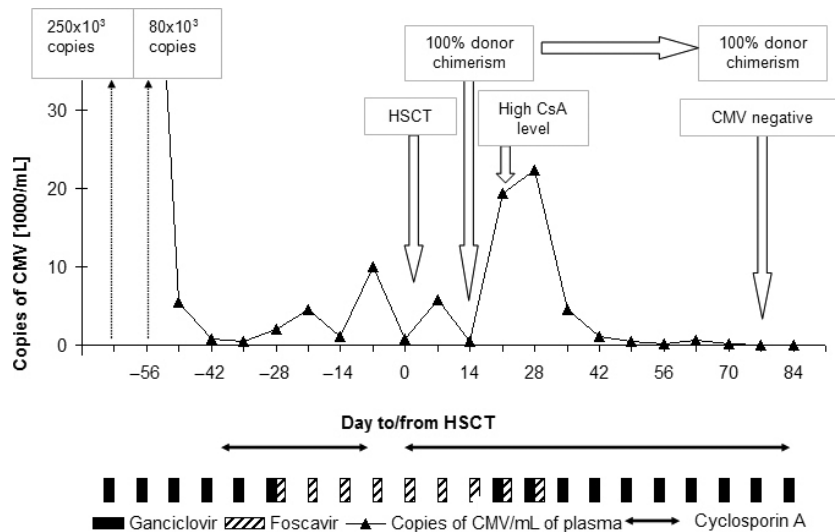


Fig. 1. The course of CMV infection, immunosuppressive therapy and engraftment.

Platelia Candida Antigen and Aspergillus EIA (a sandwich immuno-enzymatic assay performed on microplates allowing for quantitative and qualitative detection of the circulating Candida mannan and Aspergillus galactomannan antigens in human serum) were negative. Nevertheless, in view of the high risk of fungal infection voriconazole was used as preemptive therapy. Intravenous methylprednisolone 2 mg/kg/day was started at day +6 and continued until day +30. A dramatic improvement of skin lesions was noted within two wk after transplantation. No signs or symptoms of other transplant-related organ toxicity and chronic GvHD were observed. CsA had been continued at 2–3 mg/kg/day for three months, then slowly tapered, and stopped five months after HSCT. Persistent hematopoietic full donor chimerism was demonstrated in the blood cells determined by genetic polymorphism of variable tandem repeat short DNA sequences, from day +14 and maintained since then. Low level of CMV in blood (< 500 copies/mL) was observed from day +64, clinically regarded as negative results. Since day +84, CMV was undetectable. Immunological tests revealed the presence of B and NK cells as early as one month after transplantation, and significant decrease in activated T lymphocytes had been observed. Vigorous lymphoproliferative response to PHA was noted at one month and remained steady, while the response to PWM and anti-C3 mAb substantially increased at the sixth month. IgG level became normal at one month and then increased steadily together with the number of B cells. IgA and IgM levels also increased. Importantly, IgE remained undetectable. CD8⁺ cells decreased from four

months, while CD4⁺ remained at the same level. Details of immunological reconstitution are summarized in Table 1. Fourteen months after transplantation, the child is in good clinical condition without any symptoms of OS.

Discussion

In this report, we describe a five-month-old boy with OS who was referred to our department for allogeneic transplantation from sibling donor. OS was diagnosed at the age of 13 wk. Before transplantation, his clinical status was poor because of a history of various recurrent bacterial, fungal, and viral infections. Moreover, he developed cytopenia and respiratory failure, which necessitated mechanically supported oxygen therapy.

Therapy for OS syndrome has two goals: immunosuppression to stop autoimmune organ damage and establishment of long-term immunological reconstitution to avoid serious infections. So far, allogeneic HSCT is the only treatment available proved to be potentially curative, which should be considered as soon as possible after the diagnosis is established. However, the procedure carries considerable risk to the recipient, especially when the patient lacks a histocompatible family donor, because of a high rate of TRM or graft rejection (15). Poor results of this therapy can often be directly or indirectly attributed to problems associated with peritransplant autoimmunity-mediated organ damage, infections, and side effects of drugs. To eliminate autoreactive T cells and to allow engraftment, conditioning chemotherapy and specific

Table 1. Assessment of immunological status before and after transplantation

Study	Normal value	Pre-HSCT	Post-HSCT			
			1 month	4 months	6 months	8 months
Immunoglobulin levels						
IgG (g/L)	3.30–8.72	1.99	10.6	13.7	15.8	11.0
IgA (g/L)	0.14–0.84	<0.66	0.64	0.15	0.33	0.26
IgM (g/L)	0.27–1.45	0.79	1.96	0.61	1.11	0.52
IgE (IU/mL)	0.0–20	>1000	–	<20	<20	<17.3
Lymphocyte count (×10 ⁶ /L)	5900 (3900–9000)	3432	2964	2304	2492	7740
Lymphocyte subset (×10 ⁶ /L)						
T cells CD3 ⁺	3930 (1900–5900)	2951	2756	1589	1619	5688
CD4 ⁺	2670 (1400–4300)	652	622	552	623	1975
CD8 ⁺	1040 (500–1700)	1784	2015	898	772	3397
CD3 ⁺ /HLA-DR ⁺	181 (135–950)	2436	2637	875	1021	2291
NK cell (CD3 ⁺ CD16 ⁺ CD56 ⁺)	400 (160–950)	34	148	115	74	474
B cells (CD19 ⁺)	1520 (610–2600)	<34	15	529	872	1580
Proliferative response (cpm)						
NIL		813	604	847	2246	1714
PHA		2113	48 308	58 212	65 362	147 295
α-CD3		3002	20 429	18 172	69 443	51 560
PWM		3415	17 667	6660	37787	14 381

Pre-HSCT, before hematopoietic stem cell transplantation; post-HSCT, after hematopoietic stem cell transplantation; NIL, unstimulated; antiCD3, antibody; PWM, pokeweed mitogen.

anti-T-cell agents are considered necessary before and after HSCT. However, the need to employ aggressive (myeloablative) conditioning regimens to eliminate autoreactive T lymphocytes and to achieve engraftment has undoubtedly accounted for the high TRM. Such regimens may also lead to long-term sequelae, including a lower intelligence quotient, cataract, infertility, and growth and developmental retardation (12, 16). In particular, experience with myeloablative transplantation from related donors in OS in a single center has resulted in almost 70% survival (8). Lower survival rate (17%) has been reported in another study focusing on mismatched related or matched unrelated transplantations (11).

Reduced-intensity conditioning regimens have been increasingly used as an alternative to conventional myeloablative conditioning regimens for elderly patients, for patients medically unfit to qualify for conventional allogeneic stem cell transplantation, and for disorders in which traditional transplantation is associated with high rates of non-relapse mortality. The use of RIC prevents graft rejection and establishes stable donor-derived hematopoiesis at a level sufficient for curing the underlying disease, while decreasing the short- and long-term complications associated with myeloablative conditioning therapy (17). Theoretical advantages of RIC HSCT are as follows: (i) it might lead to better immune reconstitution after transplantation because of less damage of the thymus, (ii) regeneration of naive T cells derived from prethymic

donor stem cells or from immunologically competent host cells that survive the conditioning regimen (18). Currently, sufficient data exist to support routine use of RIC in patients with primary immunodeficiencies without comorbidity (14, 19). However, to our knowledge, only a few cases of an RIC regimen used before HSCT in patients with OS were reported (9, 12).

CMV infection is also an important cause of major complications and death in patients with SCID. Moreover, in allogeneic graft recipients CMV infection is mainly a consequence of latent-virus reactivation, in the absence of adequate immune control, especially when a seropositive recipient is transplanted with hematopoietic stem cells from a seronegative donor (20, 21). However, further progression of CMV infection depends not only on the patient but also on the virus-dependent immune evasion mechanisms. CMV avoids immune response by interfering with the process of antigen recognition by T lymphocytes and NK cells (22). The early detection of CMV infection is warranted to prevent the progression to clinically manifested disease. The real-time PCR assay has increased sensitivity of CMV detection. This assay is of particular value in the neutropenic phase after HSCT. The result of this assay is a basis for preemptive therapy management strategy. However, the threshold of viral load to start the therapy has not been unequivocally determined (23). Moreover, the treatment of CMV infection after allogeneic HSCT is limited by toxicities of

current antiviral drugs and the occurrence of drug-resistant strains. Resistance to antiviral drugs in immunocompromised patients can develop very quickly and was detected as early as after 6–12 wk of antiviral treatment (24).

In this report we describe a patient with OS who presented with persistent CMV infection before transplantation, treated with prolonged antiviral therapy (gancyclovir and/or foscavir). Because of CMV infection resistant to standard therapy and the CMV-seronegative donor, we decided to perform RIC allogeneic transplantation in the active phase of infection using preemptive therapy with foscavir and CMV hyperimmune intravenous immunoglobulin infusions repeated every 2 wk. CMV detection using real-time PCR was performed every week. To discriminate positive and negative results, the threshold of the CMV load has been determined in our department at the level of 500 copies/mL of plasma. Negative results of CMV detection in blood in our patient had been obtained from day +42.

The patient was analyzed for the quality of immune reconstitution (Table 1). His lymphocyte counts were slightly lower than normal. The number of circulating CD3⁺/HLADR lymphocytes slowly decreased and distribution of CD4⁺ and CD8⁺ T lymphocytes was skewed at diagnosis to slowly normalize after HSCT. Normal lymphoproliferative response to mitogens and anti-C3 mAb was detected as early as one month after transplantation. While he had no significant amount of circulating B lymphocytes at diagnosis, he showed reconstitution of B cell compartment at six months after HSCT. Intravenous immunoglobulin administration was discontinued on day +13 after HSCT, and from then on, his immunoglobulin values were normal.

Our case confirms that RIC should be considered an alternative to standard myeloablative conditioning in OS, particularly in patients with active infection at the time of transplantation. This highly immunosuppressive RIC regimen based on fludarabine and melphalan is able to suppress autoreactive T lymphocytes and establish host tolerance to the donor immune cells responsible for normal immune reconstitution (25).

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