

Expansion of Tregs after HSCT in an IPEX patient

consistently free from symptoms of enteropathy and any other autoimmune diseases. Laboratory findings showed improvement in hypoalbuminemia and anemia caused by severe enteropathy on day 21. Colonoscopy examination on day 60 revealed disappearance of mucosal inflammation, multiple ulcerations and hemorrhage that were observed before the HSCT.

After the discharge on day 120, we had followed the patient every two wk. He had no episodes of autoimmune disorders and infection, and we could taper and stop immunosuppressive agents at six months. Unfortunately, he suffered from MLL gene-rearranged ALL at 24 months after transplantation. The origin of precursor B lymphoblasts was recipient cells. We treated him with chemotherapy and allogeneic PBSCT from the same donor. We used myeloablative conditioning regimen consisted of busulfan at a dose of 4 mg/kg for four days and melphalan at a dose of 90 mg/m² for two days for the second transplant from the same sibling donor to cure this secondary ALL. He has been in complete remission for more than two yr. Chimerism completely changed to donor-type and the number of Tregs increased to normal after the second transplant.

Chimerism and immunological evaluation after first allogeneic HSCT

Because the ratio of donor T cells, total lymphocytes, and granulocytes in bone marrow was 74%, 48%, and 48%, respectively, on day 22

after HSCT, we reduced the dose of CyA immediately. The ratio of donor cells, however, was further declined to 5% on day 50, and the donor bone marrow was assumed to be rejected (Fig. 1). At that point, flow cytometric analysis of peripheral blood showed that 17.8% of PBMCs were CD4+CD25+Foxp3+ Tregs (Fig. 2a,b). This discordant result on day 50 was explained by selective expansion of donor-derived Tregs. After discontinuation of CyA on day 50, the ratio of donor T cells, total lymphocytes, and granulocytes was transiently increased up to 40% and then gradually decreased (Fig. 1). At 24 months after HSCT, donor cells were around 20% and CD4+CD25+Foxp3+ Tregs were at the range of 1.2–3.0% of PBMCs, which were comparable to healthy controls (Fig. 2a). We did not perform DLI because the ratio of donor cells was <50% and supposed that the patient was in high risk of bone marrow aplasia after repeated DLI.

The anti-villin autoantibody was detected by immunoblot analysis when the disease was active before HSCT. The antibody was under detectable levels both in clinical remission by immunosuppressive therapy before HSCT and after engraftment was achieved following HSCT even when immunosuppressive agents were not administrated (Table 1).

Discussion

The defect of Tregs in patients with IPEX syndrome causes symptoms related to

Fig. 1. Frequency of donor cells after allogeneic HSCT. Percentages of donor cells in each fraction of T cells, total lymphocytes, and granulocytes after HSCT are shown.

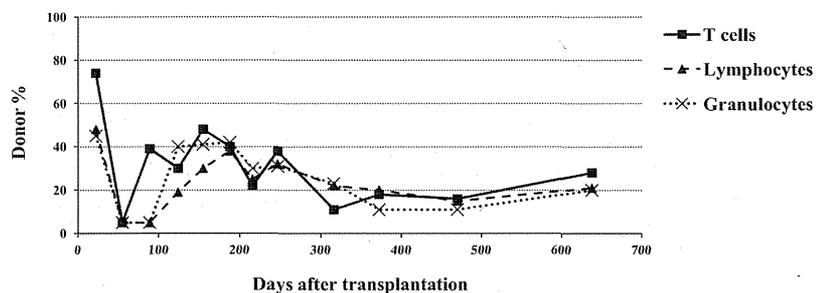
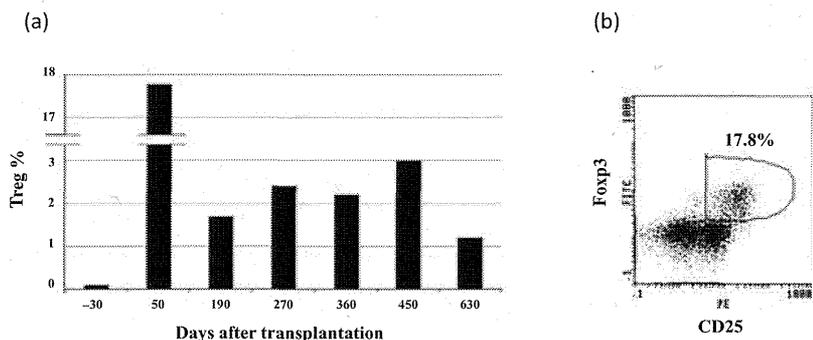


Fig. 2. Sustained expansion of donor Tregs after allogeneic HSCT. (a) Percentages of Tregs are evaluated as CD4+CD25+Foxp3+ cells in PBMCs. (b) Flow cytometric demonstration of CD25+Foxp3+ cells in CD4 gated PBMCs on day 50 after HSCT.



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Table 1. Anti-villin autoantibody is correlated with clinical condition of enteropathy

Clinical condition of enteropathy	Anti-villin autoantibody
Disease onset	+
Remission before HSCT	-
Relapse before HSCT	+
After HSCT	-

GST-villin protein (121 kD) was transferred to the membrane, and immunoblot was performed with 1:160 diluted patient's serum at different disease condition of enteropathy as indicated. + indicates the presence of anti-villin antibody that recognizes GST-villin protein.

autoimmunity. However, clinical benefit of immunosuppressive therapy is often limited by its adverse effects and increased susceptibility to infection. At present, allogeneic HSCT is recognized as the curative therapy for patients with IPEX syndrome. We summarized all reported cases treated with HSCT in Table 2. Myeloablative regimen resulted in high fatality due to regimen-related toxicity or lethal infection (2, 5, 6). On the other hand, Rao et al. (7) first reported four patients who were successfully treated with non-myeloablative conditioning regimen consisted of fludarabine, melphalan, and alemtuzumab, and achieved high rate of donor chimerism above 84.6%. Non-myeloablative regimens with anti-T-lymphocyte antibody such as

alemtuzumab or ATG have been used, and all patients are alive (7-11). However, it is known that alemtuzumab and ATG induce profound depletion of T cells and increase the risk of viral reactivation and fungal infection after HSCT. Therefore, we used low-dose TBI instead of anti-T-lymphocyte antibodies. The combination of low-dose TBI, fludarabine, and cyclophosphamide was well tolerated, and the patient was free from infections and severe regimen-related toxicities. Burroughs et al. (12) also reported that RIC regimen including low-dose TBI for IPEX syndrome resulted in stable engraftment of Tregs and better clinical outcome, proposing that this regimen was preferable for patients with IPEX syndrome.

The patient developed MLL-related secondary ALL in recipient cells. Although the dose of TBI was less than used in myeloablative conditioning, radiation and alkylating agents might cause DNA damage and increased the risk of secondary leukemia in recipient cells. Alternatively, the use of anti-T-lymphocyte antibody instead of low-dose TBI and/or dose reduction in alkylating agents should be carefully considered in IPEX syndrome.

Selected and sustained expansion of Tregs resulted in clinical improvement even though the patient was in mixed chimera after HSCT. Seidel et al. (11) reported a patient with IPEX

Table 2. Summary of IPEX patients treated with allogeneic HSCT reported in the literature

Case	Age	Donor	Conditioning regimen	Complications after HSCT	Outcome	% Donor after HSCT	Reference
1	13 yr	HLA-matched sibling	TBI 12 Gy + CY + ATG	Adenovirus infection, pneumonia	Dead	50%	2
2	9 yr	HLA-matched unrelated	TBI 12 Gy + CY + ATG	Cytomegalovirus infection, hemorrhagic cystitis, lymphoproliferative disorder	Dead	70%	2
3	4 months	HLA-matched sibling	BU + CY + ALG	Hemophagocytic syndrome	Dead	30% in T cell	5
4	1 yr	HLA-matched sibling	BU + CY + Flu + ATG		Alive	70% in T cell	6
5	7 yr	HLA-matched unrelated	Flu + L-PAM + alemtuzumab	Cytomegalovirus infection	Alive	100%	7
6	1 yr	HLA-matched unrelated	Flu + L-PAM + alemtuzumab	Acute respiratory distress syndrome	Alive	100%	7
7	4 yr	HLA-matched sibling	Flu + L-PAM + alemtuzumab	Histoplasma infection	Alive	89%	7
8	5 months	HLA-matched unrelated	Flu + L-PAM + alemtuzumab		Alive	84.6%	7
9	7 yr	HLA 5/6-matched cord blood	Flu + BU + ATG	Lymphoproliferative disorder	Alive	81 ~ 98%	8
10	7 months	HLA-matched unrelated	Flu + L-PAM + alemtuzumab	Sepsis of <i>Enterobacter cloacae</i>	Alive	100%	9
11	5 months	HLA-matched unrelated	Flu + L-PAM + alemtuzumab + anti-CD 45 monoclonal antibody		Alive	100%	10
12	11 months	HLA-matched unrelated	Flu + L-PAM + alemtuzumab		Alive	<10%	11
13	9 months	HLA-matched unrelated	TBI 4 Gy + Flu	Bacteremia	Alive	100%	12
14	16 yr	HLA-matched related	TBI 4 Gy + Flu	Bacteremia	Alive	20 ~ 60% in T cell	12

Case series transplanted with RIC regimens were highlighted.

CY, cyclophosphamide; ALG, antilymphocyte globulin; BU, busulfan; Flu, fludarabine; L-PAM, melphalan.

syndrome who showed selective engraftment of Tregs for six yr after non-myeloablative transplantation. It has been reported that partial BMT or injection of T-enriched splenocytes resulted in the rescue of autoimmunity in Scurfy mice, a mouse model for IPEX syndrome in which *FOXP3* gene is naturally mutated. Sustained engraftment of relatively high frequency of CD4⁺CD25⁺Foxp3⁺ Tregs was observed even though the frequency of donor cells in whole peripheral blood ranged from 1.7% to 50% (18). These observations illustrate that the paradigm in the generation of Tregs is reinforced by the requirement and growth advantage regardless of chimerism of other hematopoietic cells in IPEX syndrome. However, we should still consider the possibility that mixed chimerism may result in subsequent development of autoimmune diseases observed in other primary immunodeficiency, as previously reported in some patients with Wisnott–Aldrich syndrome (19).

Intractable diarrhea is a major symptom in patients with IPEX syndrome. Villin, an actin-binding protein, is expressed as the 95 kD antigen in the small intestine, which is frequently targeted by autoantibodies in patients with IPEX syndrome (17). Anti-villin antibody was clearly correlated with the severity of clinical symptoms in our patient. Therefore, monitoring of anti-villin antibody might serve as a useful examination for evaluating gastrointestinal complications in patients with IPEX syndrome.

We reported here a unique phenomenon of selective growth advantage of Tregs in a patient with IPEX syndrome who was in mixed chimera after RIC and allogeneic HSCT. Sustained expansion of donor-derived Tregs resulted in the significant improvement in enteropathy. To determine optimal RIC regimen to achieve complete chimera and avoid secondary malignancy in residual recipient cells, further analysis in more patients and long-term follow-up study after HSCT are required to conclude this issue.

Authors' contributions

Horino S and Sasahara Y designed the study, interpreted the data, wrote the paper, and treated the patient. Sato M, Kanegane H, Kamachi Y, and Kobayashi I performed experiments. All other authors treated the patient and collected clinical data.

Acknowledgments

We thank all of our colleagues in the Department of Pediatrics, Tohoku University, who contributed to the patient's care. This work was supported by the grants-in-aid from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (23591528 to YS), a grant for research on

intractable diseases from the Ministry of Health, Labour and Welfare of Japan (to YS), grants from Japan Leukemia Research Fund and SENSHIN Medical Research Foundation (to YS).

Conflict of interest disclosure

The authors declare no conflict of interest.

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厚生労働科学研究費補助金
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原発性免疫不全症に対する造血幹細胞移植法の
確立に関する研究

平成25年度総括・分担研究報告書

発行日 平成26年3月31日
発行者 野々山 恵章
発行所 厚生労働省難治性疾患等克服研究事業
原発性免疫不全症に対する造血幹細胞移植法の
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