Graft rejection and hyperacute graft-versus-host disease in stem cell transplantation from non-inherited maternal antigen complementary HLA-mismatched siblings

Graft rejection and hyperacute graft-versus-host disease in stem cell transplantation from non-inherited maternal antigen complementary HLA-mismatched siblings

Hirokazu Okumura[1], Masaki Yamaguchi[2], Takeharu Kotani[2], Naomi Sugimori[1], Chiharu Sugimori[1], Jun Ozaki[1], Yukio Kondo[1], Hirohito Yamazaki[1], Tatsuya Chuhjo[3], Akiyoshi Takami[1], Mikio Ueda[3], Shigeki Ohtake[4], Shinji Nakao[1]

[1]Department of Cellular Transplantation Biology, Kanazawa University Graduate School of Medical Science

[2]Department of Internal Medicine, Ishikawa Prefectural Central Hospital

[3]Department of Internal Medicine, NTT West Japan Kanazawa Hospital

[4]Department of Laboratory Science, Kanazawa University Graduate School of Medical Science

Corresponding author: Hirokazu Okumura

Department of Cellular Transplantation Biology, Kanazawa University Graduate

School of Medical Science

Takara-machi 13-1, Kanazawa 920-8641, Japan

Tel +81-76-265-2273, Fax +81-76-234-4252

E-mail: hokumura@med3.m.kanazawa-u.ac.jp

Running title: Severe GVHD and graft failure in NIMA-mismatched SCT

Summary

HLA-mismatched stem cell transplantation from non-inherited maternal antigen (NIMA) complementary donors is known to produce stable engraftment without inducing severe GVHD. We treated two patients with AML and one patient with SAA with HLA-mismatched stem cell transplantation (SCT) from NIMA complementary donors (NIMA-mismatched SCT). The presence of donor and recipient-derived blood cells in the peripheral blood of recipient (donor microchimerism) and donor was documented respectively by amplifying NIMA-derived DNA in 2 of the 3 patients. Graft rejection occurred in the SAA patient who was conditioned with a fludarabine-based regimen. Grade III and grade IV acute GVHD developed in patients with AML on day 8 and day 11 acute GVHD respectively, and became a direct cause of death in one patient. The findings suggest that intensive conditioning and immunosuppression after stem cell transplantation are needed in NIMA-mismatched

SCT even if donor and recipient microchimerisms is detectable in the donor and recipient before SCT.

Key words: graft-versus-host disease (GVHD), rejection, graft failure, non-inherited maternal antigen (NIMA), fetomaternal microchimerism

Introduction

In allogeneic stem cell transplantation (SCT) from HLA-mismatched donors, severe acute GVHD and graft rejections occurs at he higher rate than SCT from HLA-matched donors (1, 2). Recently, allogeneic SCT from non-inherited maternal antigen (NIMA) complementary donors has received attentions as one of the methods that potentially overcome the barrier of HLA incompatibility. It is well known that a small number of maternal blood cells exist in the newborn's blood, and in turn, blood cells derived from children can be detected in the mother's blood long after labor. This phenomenon is referred to as fetomaternal microchimerism. The existence of fetomaternal microchimerism suggests that immunological tolerance of hematopoietic cells takes place both in mother and child. Van Rood et al (3) reported the incidence of chronic GVHD was significantly lower in mother-to-child SCTs than in father-to-child SCTs. Their findings also suggest that HLA-haploidentical NIMA complementary siblings

can be alternative donor candidates when there is no HLA-matched donor. Shimazaki et al (4) reported five patients treated with allogeneic SCT from two or three loci mismatched familiy donors who had a small number of recipient-derived cells in their blood before SCT. Engraftment occurred in all patients, and although acute GVHD developed in all five, their severity was grade I or II except for one patient who developed grade III acute GVHD. Ichinohe et al (5) reported that in HLA-haploidentical SCTs, NIMA mismatches in the graft-versus-host (GVH) direction, was associated with a lower risk of severe acute GVHD compared to IPA mismatches.

Based on these backgrounds, we treated two AML patients and one SAA patient with SCT from NIMA-mismatched sibling donors. Graft rejection and severe acute GVHD occurred despite the fact that donor of recipient-derived michrochimerisms were shown in donor and recipient.

Case report

Case 1: A 27-year-old man was diagnosed as having chronic myloid leukemia (CML) in myeloid crisis. He underwent an HLA-matched unrelated bone marrow transplantation in September 1999. However, he relapsed with CML in blastic crisis in October 2000. He received allogeneic peripheral blood hematopoietic stem cell transplantation from a NIMA complementary dizygotic sibling in November 2002. A fever of 38℃ occurred on day 3 after transplantation and erythema developed in upper and lower extremities on day 8. A diagnosis of acute GVHD, which met the criteria of hyperacute GVHD (6, 7) was made through skin biopsy findings. The patient's GVHD responded to the treatment and both erythema and icterus disappeared on day 26. The complete donor chimerism was confirmed on day 17 by microsatellite marker analysis. Imatinib mesylate was administered on day 21 and he was in molecular remission on day 58. However, CML recurred as subcutaneous nodules on day 153 and the patient

died of CML on day 203.

Case 2: A 15-year-old woman was diagnosed as having SAA in 2000. She did not respond to all kinds of therapy including ATG and anabolic steroids, and required frequent transfusions. An HLA-matched donor was absent either in relatives or in the bone marrow banks. Allogeneic bone marrow transplantation from the NIMA complementary sister was performed in September 2003. Michrochimerism was revealed in both the patient and donor (8). Her neutrophil count rose to 750 /μl on day 25, but it became 0/μl following high fever associated with hyperferritinemia (24490 ng/dl). Virus-associated hemophagocytic syndrome was suspected and foscarnet was administered without any effect. A chimerism analysis performed on day 34 revealed the absence of donor-derived cells in both the peripheral blood and bone marrow, thus leading to the diagnosis of secondary graft failure. She received an infusion of 1.65 x 10^6 /kg of peripheral blood $CD34^+$ cells collected from the marrow donor without

conditioning due to the deteriorating clinical condition, but no hematological recovery occurred. She underwent a cord blood cell transplantation (CBT) following conditioning with Flu, 125 mg/m^2; melphalan, 160 mg/m^2 and TBI at 4 Gy on day 89 after the first transplantation. She achieved a complete reconstitution of hematopoiesis after CBT and remains well 33 months after CBT.

Case 3: In January 2002, a 32-year-old man was diagnosed to have acute myeloid leukemia (AML) with a normal karyotype. He achieved a complete remission following standard chemotherapy. A year later, he relapsed with acute lymphocytic leukaemia with the Philadelphia chromosome (Ph$^+$ALL). He was treated with chemotherapy consisting of daunorubicin, vincristine, L-asparaginase, and prednisolone, followed by the administration of imatinib mesylate, but did not achieve a complete remission. There was no HLA-matched family member. The michrochimerism by NIMAs possessed by the patient was documented in the blood of

one brother. He received allogeneic SCT from this NIMA complementary brother. He became febrile from day 2 and erythema appeared diffusely on the generalized skin. He was diagnosed to have hyperacute GVHD. His skin GVHD deteriorated thus leading to a diagnosis of grade III acute GVHD. Bohrus methylprednisolone therapy could not improve the symptoms of acute GVHD. As a result, the patient died of thrombotic microangiopathy associated with acute GVHD on day 47.

Results and discussion

This study is observational. The incidence of grade II to IV acute GVHD and graft failure in patients who were transplanted from HLA haploidentical NIMA complemetary siblings has been reported to be 40-50% and 0-18% respectively (3, 5). Based on the results of these studies, HLA haploidentical siblings whose NIMA is complementary to that of a patient are thought to be a possible donor candidate when

HLA matched donors are unavailable. However, our experience of hyperacute GVHD and graft rejection in the present report raises a concern about the efficacy of HLA-mismatched SCT from NIMA complementary siblings.

Tables 1 and 2 summarize the patient characteristics and outcome of SCT for the three patients. Although the HLA disparity was one locus in the GVH direction in case 1, acute GVHD appeared on day 8 before neutrophil engraftment and rapidly progressed to grade III. Case 3 also developed hyperacute GVHD despite the fact that michrochimerism was documented in the donor's blood. Acute GVHD is known to occur frequently before engraftment of neutrophil in recipient of HLA-mismatched SCT (6, 7). In the analysis of SCTs between NIMA-complementary family members described by Ichinohe et al (5), the presence of acute GVHD was observed from day 10. The presence of the recipient-specific microchimerism did not necessary predict low incidence of acute GVHD in this study, in line with our experience. Our findings

suggest the necessity of intensive immunosuppressive therapy to prevent acute GVHD such as ATG (9, 10) or alemtuzumab (11) even when a donor shows recipient-specific microchimerism. Since case 1 had received HLA-matched unrelated bone marrow transplantation before undergoing the second SCT from a NIMA complementary sibling, recipient dendritic cells were probably replaced by the cells of the unrelated donor. The dendritic cells of a recipient play an important role in the development of acute GVHD (12, 13). The absence of the patient-derived dendritic cells, which were educated to tolerate donor T cells, may be responsible for hyperacute GVHD of the patients.

Case 2 was conditioned with fludarabine-based regimen which was known to ensure engraftment of bone marrow from HLA-matched unrelated donors in AA patients (14). Microchimerism by donor cells was documented in the patient. Nevertheless, SCT from the donor ended up with secondary graft rejection. Although

the number of of $CD34^+$ cells infused (1.4×10^6 /kg) was relatively low, the minimal number of $CD34^+$ cells in the successful SCT was 1.26×10^6 /kg in the report by Shimazaki et al (4) and 1.3×10^6 /kg in the report by Ichinohe et al (5). It is therefore necessary to intensify the conditioning regimen to prevent graft rejection when HLA-mismatched SCTs from NIMA complementary siblings are administered to patients with AA even if microchimerism by donor cells is documented in the recipient.

Rreference

1. Anasetti C, Amos D, Beatty PG, Appelbaum FR, Bensinger W, Buckner CD, Clift R, Doney K, Martin PJ, Mickelson E, et al. Effect of HLA compatibility on engraftment of bone marrow transplants in patients with leukemia or lymphoma. N Engl J Med 1989;**320**: 197-204.

2. Beatty PG, Clift RA, Mickelson EM, Nisperos BB, Flournoy N, Martin PJ, Sanders JE, Stewart P, Buckner CD, Storb R, et al. Marrow transplantation from related donors other than HLA-identical siblings. N Engl J Med 1985;**313**: 765-71.

3. van Rood JJ, Loberiza FR, Jr., Zhang MJ, Oudshoorn M, Claas F, Cairo MS, Champlin RE, Gale RP, Ringden O, Hows JM, Horowitz MH. Effect of tolerance to noninherited maternal antigens

on the occurrence of graft-versus-host disease after bone marrow transplantation from a parent or an HLA-haploidentical sibling. Blood 2002;**99**: 1572-7.

4. Shimazaki C, Ochiai N, Uchida R, Okano A, Fuchida S, Ashihara E, Inaba T, Fujita N, Maruya E, Nakagawa M. Non-T-cell-depleted HLA haploidentical stem cell transplantation in advanced hematologic malignancies based on the feto-maternal michrochimerism. Blood 2003;**101**: 3334-6.

5. Ichinohe T, Uchiyama T, Shimazaki C, Matsuo K, Tamaki S, Hino M, Watanabe A, Hamaguchi M, Adachi S, Gondo H, Uoshima N, Yoshihara T, Hatanaka K, Fujii H, Kawa K, Kawanishi K, Oka K, Kimura H, Itoh M, Inukai T, Maruya E, Saji H, Kodera Y. Feasibility of HLA-haploidentical hematopoietic stem cell transplantation between noninherited maternal antigen (NIMA)-mismatched family members linked with long-term fetomaternal microchimerism. Blood 2004;**104**: 3821-8.

6. Sullivan KM, Deeg HJ, Sanders J, Klosterman A, Amos D, Shulman H, Sale G, Martin P, Witherspoon R, Appelbaum F, et al. Hyperacute graft-v-host disease in patients not given immunosuppression after allogeneic marrow transplantation. Blood 1986;**67**: 1172-5.

7. Kim DH, Sohn SK, Kim JG, Suh JS, Lee KS, Lee KB. Clinical impact of hyperacute graft-versus-host disease on results of allogeneic stem cell transplantation. Bone Marrow Transplant 2004;**33**: 1025-30.

8. Tokita K, Terasaki P, Maruya E, Saji H. Tumour regression following stem cell infusion from daughter to microchimeric mother. Lancet 2001;**358**: 2047-8.

9. Lu DP, Dong L, Wu T, Huang XJ, Zhang MJ, Han W, Chen H, Liu DH, Gao ZY, Chen YH, Xu LP, Zhang YC, Ren HY, Li D, Liu KY. Conditioning including antithymocyte globulin followed by unmanipulated HLA-mismatched/haploidentical blood and marrow transplantation can achieve comparable outcomes with HLA-identical sibling transplantation. Blood 2006;**107**: 3065-73.

10. Aversa F, Terenzi A, Tabilio A, Falzetti F, Carotti A, Ballanti S, Felicini R, Falcinelli F, Velardi A, Ruggeri L, Aloisi T, Saab JP, Santucci A, Perruccio K, Martelli MP, Mecucci C, Reisner Y, Martelli MF. Full haplotype-mismatched hematopoietic stem-cell transplantation: a phase II study in patients with acute leukemia at high risk of relapse. J Clin Oncol 2005;**23**: 3447-54.

11. Kanda Y, Oshima K, Asano-Mori Y, Kandabashi K, Nakagawa M, Sakata-Yanagimoto M, Izutsu K, Hangaishi A, Tsujino S, Ogawa S, Motokura T, Chiba S, Hirai H. In vivo alemtuzumab enables haploidentical human leukocyte antigen-mismatched hematopoietic stem-cell transplantation without ex vivo graft manipulation. Transplantation 2005;**79**: 1351-7.

12. Wright-Browne V, McClain KL, Talpaz M, Ordonez N, Estrov Z. Physiology and pathophysiology of dendritic cells. Hum Pathol 1997;**28**: 563-79.

13. Shlomchik WD, Couzens MS, Tang CB, McNiff J, Robert ME, Liu J, Shlomchik MJ, Emerson SG. Prevention of graft versus host disease by inactivation of host antigen-presenting cells. Science 1999;**285**: 412-5.

14. Bacigalupo A, Locatelli F, Lanino E, Marsh J, Socie G, Maury S, Prete A, Locasciulli A, Cesaro S, Passweg J. Fludarabine, cyclophosphamide and anti-thymocyte globulin for alternative donor transplants in acquired severe aplastic anemia: a report from the EBMT-SAA Working Party.

Bone Marrow Transplant 2005;**36**: 947-50.

Table 1. Patient Characteristics

Case	Age	Sex	Diagnosis	Status at SCT	Preconditioning regimen	GVHD prophylaxis	CD34+ cells ($\times 10^6$/kg)	HLA (A, B, DR)		Microchimerism	
								recipient	donor	Patinet	Donor
1	27	M	CML	BC, relapse after UR-BMT	Flu 150 mg/m^2 + BU 8 mg/kg + ATG 40 mg/kg	CSA	12.5	2/–,51/38, 4/8	2/33, 51/44, 4/8	ND	ND
2	15	F	AA	refractory to immunosuppresive therapy	Flu 150 mg/m2 + CY 120 mg/kg + ATG 25 mg/kg + TBI 2Gy	CSA+sMTX	1.4	11/–, 55/67, 4/–	11/24, 52/67, 4/15	+	+
3	32	M	Ph+ALL	resistant	TBI 12 Gy + CY 120 mg/kg	FK506+sMTX	3.1	2/–, 52/51, 9/8	2/11, 51/60, 8/14	–	+

CML=chronic myeloid leukemia; AA=aplastic anemia; Ph+ALL=Philadelphia chromosome positive acute lymphoblastic leukemia; BC=blastic crisis; UR-BMT=unrelated bone marrow transplantation; Flu=fludarabine; BU=busulfan; ATG=antithymocyte globulin; TBI=total body irradiation; CY=cyclophosphamide; CSA=cyclosporine; FK506=tacrolimus; sMTX=short term methotrexate; NIMA=non-inherited mathernal antigens; HVG=host versus graft; ND=not done

Table 2.Clinical outcome

Case	Engraftment		aGVHD		GVHD stage	Treatment of GVHD	Complications	Outcome	Survival after SCT (days)
	Neu (day)	Plt (day)	Onset (day)	Grade					
1	9	9	8	3	skin 2, liver 2	2 mg/kg of mPSL started on day 9, 15 mg/m^2 of MTX on day11 and 1000 mg/day of MMF started on day15,	–	Relapse on day131	203
2	22	NR	–	–	–	–	HPS on day23, graft rejection on day27	CBT on day89	993+
3	13	15	11	4	skin 4, liver 4, gut 3	1 g/day of mPSL for 3 days	TMA, convulsion	Death by GVHD	47

Neu=neutrophil; Plt=platelet; G=granulocyte; T=T lymphocyte; aGVHD=acute graft-vessus-host disease; HPS=hemophagocytic syndrome; SCT=stem cell transplantation; mPSL=methylprednisolone; MTX=methotrexate; MMF=mycophenolate mofetil; TMA=thrombotic microangiopathy; CMV=cytomegalovirus