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ORIGINAL ARTICLE

FCGR3A-158V/F polymorphism may correlate with the levels of immunoglobulin in patients with non-Hodgkin's lymphoma after rituximab treatment as an adjuvant to autologous stem cell transplantation

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Abstract

Objectives: Recent studies have indicated that patients who receive stem cell transplantation (SCT) and rituximab demonstrate an increased risk of developing hypogammaglobulinemia. Such hypogammaglobulinemia has been found to be due to delayed recovery of memory B cells with an abnormal cell marker expression and impaired immunoglobulin production *in vitro*. However, no predictive factors for the levels of immunoglobulin after autologous SCT and rituximab therapy have been reported. The aim of this study is to clarify the relationships between the *FCGR3A*-158V/F genotype and the levels of serum immunoglobulin after SCT. *Methods:* A total of 24 non-Hodgkin's lymphoma (NHL) patients received autologous SCT with an adjuvant rituximab. The *FCGR3A*-158V/F genotype was determined in these patients. We also included ten NHL patients who received an identical conditioning regimen and autologous SCT but no rituximab as control patients. *Results:* The levels of IgG were significantly lower in *FCGR3A*-158F homozygous patients ($n = 9$) in comparison to those in *FCGR3A*-158V carriers ($n = 15$). Moreover, the levels of IgG and IgA of *FCGR3A*-158F homozygous patients, but not those of *FCGR3A*-158V carriers, were significantly lower than those of control patients. *Conclusions:* The genotype of *FCGR3A* determines not only the response to rituximab, but also the levels of immunoglobulin after SCT and an adjuvant rituximab.

Key words: rituximab; autologous peripheral blood stem cell transplantation; hypogammaglobulinemia; FcγRIIIa; polymorphism

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Rituximab is a chimeric monoclonal antibody directed toward CD20, a pan B-cell surface marker that has been proven effective in depleting normal and malignant B cells *in vivo*. Rituximab is widely used in the treatment of B-cell malignancies, especially non-Hodgkin's lymphoma (NHL) (1). Although rituximab induces almost a complete depletion of normal B lymphocytes in peripheral blood for an average of 6–9 months, hypogammaglobulinemia occurred in only 14% of all cases and it was not considered to be associated with any clinical morbidity (2).

However, there is increasing evidence that stem cell transplantation (SCT) and an adjuvant rituximab suppress the serum immunoglobulin for at least 12 months (3–6). Abnormal B-cell reconstitution was observed in patients who were treated with autologous peripheral blood stem cell transplantation (APBSCT) and an adjuvant rituximab by examining surface phenotype and isotype expressions (6). In addition, patients with hypogammaglobulinemia had significantly fewer memory B cells, especially $CD27^+IgD^-$ more mature switched populations. Furthermore, an impaired isotype expression was seen more frequently in patients with hypogammaglobulinemia. More recently, B cells reconstituted after APBSCT and an adjuvant rituximab were found to show a lower expression of CD27, CD40 and CD80 in

comparison to those of normal subjects. Moreover, the *in vitro* ability to generate immunoglobulin was also impaired in B cells from patients treated with APBSCT and rituximab (7). These data indicated that naive B cells may fail to differentiate into plasma cells, resulting in hypogammaglobulinemia after autologous SCT and rituximab therapy. However, no speculation has been made regarding what factor(s) determined the risk of developing hypogammaglobulinemia after APBSCT with the identical conditioning regimen and rituximab.

It has been reported that *FCGR3A* of valine (V) allele has a higher affinity to human IgG than the phenylalanine (F) allele, and that cells bearing the *FCGR3A* V allele mediate antibody dependent cellular cytotoxicity (ADCC) more effectively (8, 9). Compatibly, previous clinical studies that have examined single nucleotide polymorphisms (SNPs) of Fc receptor *(FcR)* genes demonstrated that *FCGR3A* gene SNPs are associated with the response to rituximab, as a single agent, in patients with follicular lymphoma (FL) (10, 11) or Waldenstrom's macroglobulinemia (12).

These findings suggest that *FCGR3A* SNPs may be related to the levels of immunoglobulin after SCT and an adjuvant rituximab. To clarify this hypothesis, the immunoglobulin levels of NHL patients treated with APBSCT and an adjuvant rituximab were compared according to the *FCGR3A* genotype. In addition, those patients who underwent SCT with the identical conditioning regimens, but no rituximab, were also investigated to compare the levels of immunoglobulin with those in patients who received rituximab as an adjuvant.

Patients, materials and methods

Patients and treatment

This study included 24 Japanese patients with NHL who had received treatment in Hokkaido University Hospital from August 2001 to April 2005 (Table 1). At presentation, all patients were considered to be at high risk according to the prognostic index, as established by Coiffier (13). These 24 patients were initially treated with three cycles of CHOP (14) every 2 wk, with G-CSF (Lenograstim, Chugai Pharmaceutical CO., Tokyo, Japan) support as induction chemotherapy. After a fourth cycle of CHOP, peripheral blood stem cells were mobilized using CHOP plus rituximab (Chugai Pharmaceutical CO.) intended as *in vivo* purging (15). After the fourth cycle of CHOP, patients' responses to the initial CHOP were evaluated. Patients who achieved tumour reduction rates of more than 50% were treated with two additional cycles of CHOP. Patients who showed less than a 50% response rate, however, were considered resistant to CHOP and were treated with other salvage regimens, including two cycles of DHAP (dexamethasone, cytarabine and cisplatin) (16) or FMD (fludarabine, mitoxantrone and dexamethasone) regimens (17). Pretransplant conditioning regimens consisted of ranimustine, carboplatin, etoposide and cyclophosphamide (MCVC regimen) (18). After engraftment, rituximab at a dose of 375 mg/m^2 was given weekly for up to 4 wk. All patients achieved a complete remission (CR) after APBSCT.

Table 1 Patients' characteristics according to *FCGR3A* alleles

	Low affinity	High affinity	Control
No. patients	9	15	10
Gender			
Male	4	6	6
Female	5	9	4
Age, median (range)	51 (34–65)	58 (35–64)	42 (31–58)
Histology			
FL	5	5	4
DL	3	10	4
MCL	1	0	0
LBL	0	0	1
NK	0	0	1
Disease status at presentation, No.			
Stage 3,4	6	14	7
Elevated LDH	5	7	3
PS>2	1	2	2
Bulky disease	5	7	5
No. of rituximab administration			
Total (mean ± SD)	5.9 ± 2.1	6.6 ± 2.0	-
Pre-APBST (mean ± SD)	3.2 ± 2.9	4.1 ± 2.8	-
Post-APBSCT (mean ± SD)	2.7 ± 1.1	2.5 ± 1.2	-
Salvage therapy other than CHOP			
Yes	3	6	4
No	6	9	6

FL, follicular lymphoma; DL, diffuse large cell lymphoma; MCL, mantle cell lymphoma; LBL, lymphoblastic lymphoma; NK, natural killer cell lymphoma; PS, performance status; SD, standard deviation; APBSCT, autologous peripheral blood stem cell transplantation; CHOP, cyclophosphamide, adriamycin, vincristine, predonine.

As a control, ten patients treated without rituximab before 2001, when rituximab was approved in Japan, were included. These patients received also CHOP therapy every 2 wk as an induction therapy. After the third cycle of CHOP, patients' responses to the initial CHOP were evaluated. Patients who achieved CR were treated with three additional cycles of CHOP. For patients who did not achieve CR, three cycles of VIP (etoposide, ifosfamide and cisplatin) regimen was subscribed as the salvage (19). Leukapheresis was performed after the fourth cycle of CHOP or the first cycle of VIP. These ten patients also received MCVC regimen as a conditioning prior to APBSCT (18).

In all patients, 6 months after APBSCT, the levels of serum immunoglobulin were measured. Approval to perform these studies was obtained from Hokkaido

University Hospital's institutional review board. Informed consent was obtained from all patients according to the Declaration of Helsinki protocol.

FCGR3A gene polymorphism

After the patients gave their informed consent, blood samples were collected before treatment at Hokkaido University Hospital. Peripheral blood mononuclear cells (PBMC) were isolated via density-gradient centrifugation with Ficoll-Hypaque (Pharmacia, Uppsala, Sweden). Two single-nucleotide polymorphisms of *FcR* genes were evaluated in the current study involving the *FCGR3A* gene as previously described (20). Briefly, genomic DNA was extracted from PBMC using a DNA extraction kit (Qiagen, Valencia, CA, USA). Genotyping of *FCGR3A* was performed by a polymerase chain reactions followed by allele-specific restriction enzyme digestion. All genotyping of *FCGR3A* polymorphism was confirmed by direct sequencing of the region of interest (data not shown).

Statistical analysis

The clinical characteristics of the patients were compared using Mann–Whitney *U*-tests according to the *FCGR3A* polymorphisms. For statistical comparisons among the three groups, the non-parametric Kruskal–Wallis test was used. A *P*-value of 0.05 was considered to be statistically significant. Analyses were performed using the Glanzman 'Primer of Biostatistics' software program (McGraw-Hill, New York, NY, USA).

Results

Of the 24 patients tested for the *FCGR3A*-158V/F polymorphism, nine patients (38%) had homozygous F/F (158 F/F), 12 (50%) had heterozygous V/F (158 V/F), and three (13%) had homozygous V/V (158 V/V). Since only three patient was found to have 158 V/V polymorphism in this study, we defined those patients who had 158 F/F as the low-affinity group, while those who had at least one 158 V allele were defined as the high-affinity group following the definition by Anolik *et al.* (21). In addition, we compared the levels of immunoglobulin whose APBSCT was underwent with the same conditioning regimen, but without rituximab (control group). The three groups were not different in terms of gender, age, the disease stage, bone marrow involvement, number of extranodal sites involved at diagnosis or the proportion of patients who required salvage therapy other than CHOP. In addition, the dose of rituximab pre- or post-APBSCT were not different between the high-affinity group and the low-affinity group (Table 1).

Before starting induction CHOP therapy, there was no significant difference in the levels of immunoglobulin among three groups. However, after SCT, the levels of IgG were significantly lower in the low-affinity group (6.21 ± 2.66 g/L) than those in the high-affinity group (10.43 ± 3.50 g/L) and control group (10.64 ± 3.04 g/L; both $P < 0.05$). In addition, a significant difference was seen in the levels of IgA between the low-affinity group (0.85 ± 0.64 g/L) and control group (1.63 ± 0.51 g/L) ($P < 0.05$). The levels of IgA in the high-affinity group (1.23 ± 0.64 g/L) were not significantly different from either those of control group or the low-affinity group. In contrast to the levels of IgG or IgA, no significant differences were observed in the levels of IgM among three groups (Fig. 1).

In our institute, the normal value of the serum IgG is more than 8.0 g/L. If we set this value as a normal range, then seven of the low-affinity group patients and only three of the high-affinity group were found to have hypogammaglobulinemia. Among these ten patients, the immunoglobulin levels were measured in eight patients serially after APBSCT. With a median follow up of 28 months (range 10–80 months), none of those patients once found to have the hypogammaglobulinemia recovered to have the normal IgG levels. Regarding the infectious complications, although the immunoglobulin levels were certainly low in those patients, only two patients in the low-affinity group suffered from repeated bacterial infections.

Discussions

This study demonstrated for the first time that the levels of immunoglobulin after APBSCT and an adjuvant rituximab were correlated with the *FCGR3A*-158V/F polymorphism. Those patients who had the F/F homozygotes had significantly reduced levels of IgG in comparison to those patients with the F/V or V/V alleles. In addition, only patients with the F/F allele had significantly lower IgG and IgA levels in comparison to those from NHL patients transplanted without rituximab.

Before performing this study, it was assumed that the levels of IgG from patients who had V allele, namely the high-affinity group, would be lower than those had F/F, as normal B cells from patients who had higher affinity might be sensitive to rituximab and be cleared with this immunotherapy more effectively. However, the results showed the high-affinity group of patients had the higher levels of IgG. From the clinical trials, it is evident that malignant B cells from patients with F/F are less sensitive to rituximab than those from patients with V allele (10, 11, 20). This might lead rituximab to be consumed

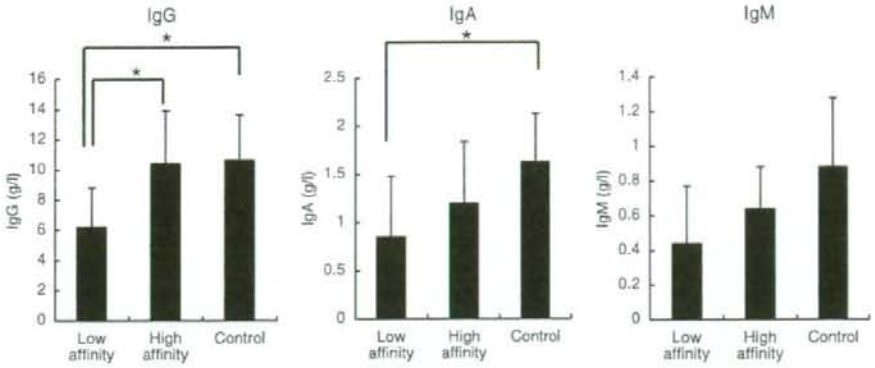

Figure 1 Immunoglobulin levels of NHL patients who underwent APBSCT and an adjuvant rituximab according to the *FCGR3A* allele. Each histogram shows the mean and SD results obtained in each group. According to *FCGR3A*-158V/F polymorphism, Low affinity (n = 9) is defined as patients who had 158F/F and high affinity (n = 15) is V allele carrier. Control (n = 10) is patients who underwent APBSCT without rituximab. (*P < 0.05).

by lymphoma B cells preferentially in the high-affinity group. Then the low residual rituximab could have a lower affect on normal B cells. However, at this moment, the precise mechanisms that cause low-affinity group patients to have the lower IgG and IgA is unknown. Recently, Cabanillas *et al.* reported that when rituximab was combined with conventional chemotherapy, the use of fludarabine and female gender were found to be predictive factors of non-neutropenic infection and hypogammaglobulinemia (22). It appears to be reasonable that the use of fludarabine correlated to hypogammaglobulinemia as it could put patients into the immunosuppressive status like APBSCT. However, the precise mechanisms regarding how a female gender may influence the immunoglobulin levels were not discussed in their study. There were no differences in the absolute number of lymphocytes between the polymorphic subgroups (data not shown). There was no correlation between the total dose of Rituximab (pre-SCT) and the level of immunoglobulin.

It is intriguing that no significant difference in the levels of IgM were observed among the three groups. This seems to be compatible to the previously reported our data (6, 7). First, no deficiency of IgM isotype transcripts was seen in PBMC from all patients treated with APBSCT and rituximab. In addition, even patients who had lower IgG or IgA had the comparable levels of IgM (6). Second, when B cells from patients treated APBSCT and rituximab were stimulated to differentiate B cells into plasma cells *in vitro*, recovery of the impaired IgG and IgA, but not IgM production was observed in comparison to those from healthy volunteers (7). All data suggested that even after APBSCT combined with rituximab, the B-cell differentiation into IgM producing steps might not be disturbed, rather the maturation arrest into the step at class switching occurred.

In conclusion, the *FCGR3A* genotype may influence not only the outcome of rituximab therapy, but also the levels of IgG and IgA after APBSCT and rituximab. Studies with more patients in the future might explain why the low-affinity group patients demonstrate lower levels of IgG and IgA.

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Autoimmune disease after autologous hematopoietic stem cell transplantation

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Abstract

Hematopoietic stem cell transplantation (HSCT) is an effective treatment for refractory autoimmune diseases. The safety and long-term outcome have been also acceptable. Infectious diseases under immune suppressive state after autologous HSCT are common transplantation related complications whereas autoimmune diseases are uncommon. Organ specific autoimmune diseases, such as immune mediated thrombocytopenia and thyroid dysfunction, are the most common after autologous HSCT. Systemic autoimmune diseases can also develop after autologous HSCT in patients with hematological disorders with genetic predisposition to autoimmune diseases. Although the mechanism of autoimmunity after HSCT is not well-known, long-term follow-up is essential in patients with autoimmune diseases treated with autologous HSCT.

Keywords: Hematopoietic stem cell transplantation; Immune reconstitution; Autoimmune disease

Contents

1. Introduction

Autologous hematopoietic stem cell transplantation (HSCT) has been an effective treatment for refractory autoimmune diseases. Many patients with multiple sclerosis, systemic sclerosis or systemic lupus erythematosus have benefited from this treatment [1–3]. Autologus HSCT has been well tolerated in patients

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Table 1
Organ specific autoimmune diseases after autologous hematopoietic stem cell transplantation

Patient (Age Sex)	Primary disease	Mobilization, Conditioning	Graft	Autoimmune disease after autologous HSCT				Remarks	Reference
				Diagnosis	Onset	Therapy	Outcome		
61 Male	DLBL	ETP+G, MCVC	PBSC	AITP	49 day	Nothing	CR	PAIgG(+)	[15]
45 Male	Germ cell tumor	CY+G, CEC	PBSC	AITP	70 day	PSL, IVIG, splenectomy	CR		[16]
45 Male	FL	CY+G, CY+ETP+TBI	PBSC	AITP	8 month	PSL	CR	Viral infection	[5]
42 Female	FL	CY+G, CY+ETP+TBI	PBSC	AITP	6 month	PSL, IVIG, splenectomy	CR		[5]
51 Female	Anap T	CY+G, BEAM	PBSC	AITP	14 month	PSL, IVIG, splenectomy	CR	Viral infection	[5]
50 Male	T in B	CY+G, CY+ETP+TBI	PBSC	AITP	21 month	PSL	CR		[5]
23 Female	Burkitt	CY+G, BEAM	PBSC	AITP	1 month	PSL, IVIG	CR		[5]
56 Male	Mantle	CY+G, CY+ETP+TBI	PBSC	AITP	5 month	PSL, IVIG, splenectomy	CR	Influenza virus vaccine	[5]
19 Male	AML	?, Bus+CY	PBSC	AITP	76 day	PSL	CR	Autoantibody (−), infection (−)	[17]
58 Female	Breast	ETP+CY+G, CTCb	PBSC	AITP	41 day	PSL, IVIG	CR	Sinus fullness, VZV	[18]
22 Female	AML	?, Bus+CY	PBSC	AITP	50 day	PSL	CR		[19]
8 Male	Ewing's	(−), Melphalan	BM	AITP	1 month	?	?	PAIgG (+)	[20]
36 Female	MDS	(−), AraC+CY+TBI	BM	AITP AIHA	35 month	PSL, IVIG, CyA	CR	IIb/IIIa (+), LAC (+)	[21]
36 Female	SLE, APS	CY+G, CY+ATG	CD34+	Hemop	30 month	aVII, PSL, Rx	CR	VIII inhibitor (+)	[6]
28 Female	SLE	CY+G, CY+ATG	CD34+	Hemop	9 month	aVII, PSL, Rx, IVCY, MMF	CR	VIII inhibitor (+)	[6]
46 Female	MS	CY+G, CY+ alemtuzumab	PBSC	AITP	8 month	PSL, IVIG, Rx, CY	CR		[6]
45 Female	SSc	CY+G, CY+ alemtuzumab	PBSC	AIHA	5 month	PSL, IVIG, Rx, MMF, IVCY	PR		[6]
45 Female	SLE	CY+G, CY+ alemtuzumab	PBSC	ANeutro AIHA	2 month	G, PSL, IVIG, ATG, CY, Rx, MMF, tacrolium, splenectomy	CR	anti-Neutro (+)	[6]
51 Female	MS	CY+G, CY+ alemtuzumab	PBSC	AITP	14 month	PSL, IVIG, Rx, MMF			[6]
34 Female	B-NHL	ETP+G, ETP+ Carbo+CY	PBSC	Evans'	49 day	PSL, pepleo, VCR	PR	dCoombs' (+), PAIgG (+), CMV	[22]
3 Female	Neuro	?, Bus+melphalan	PBSC	Evans'	20 day	PSL, IVIG	CR	dCoombs' (+), anti-Plt (+)	[23]
28 Female	HD	(−), CY+ETP+ carmustine	BM	Evans'	42 month	PSL, IVIG, splenectomy, CY, CVR, CyA, AZP, PE	PR	dCoombs' (+), iCoombs' (+)	[24]
35 Female	MM	CY+G, TBI+melphalan	PBSC	Hyper Thyroid	34 day	Nothing	CR	PAIgG (+), ANA (+)	[25]

(continued on next page)

Table 1 (*continued*)

Patient (Age Sex)	Primary disease	Mobilization, Conditioning	Graft	Autoimmune disease after autologous HSCT				Remarks	Reference
				Diagnosis	Onset	Therapy	Outcome		
46 Male	MultP	CY+G, Melphalan	BM	Hyper Thyroid	28 month	Carbimazole	CR	Mobilization failure	[26]
54 Female	Peri-T	AraC+G, CY+ETP+ ranimustine	PBSC	UC	110 day	PSL, CyA, mesalazine	CR	anti-trop (+)	[27]

Abbreviations: diffuse large B-cell lymphoma (DLBL), follicular lymphoma (FL), anaplastic T cell lymphoma (Anap T), T cell rich B-cell lymphoma (T in B), Burkitt lymphoma (Burkitt), Mantle cell lymphoma (Mantle), acute myelogeneous leukemia (AML), breast cancer (Breast), Ewing's sarcoma (Ewing's), myelodysplastic syndrome (MDS), systemic lupus erythematosus (SLE), antiphospholipid syndrome (APS), multiple sclerosis (MS), systemic sclerosis (SSc), B-cell non-Hodgkin lymphoma (B-NHL), neuroblastoma (Neuro), Hodgkin disease (HD), multiple myeloma (MM), multiple plasmacytome (MultiP), peripheral T cell lymphoma (Peri-T), etoposide (ETP), granulocyte-colony-stimulating factor (G), ranimustine + carboplatin + ETP + cyclophosphamide (CY) (MCVC), carboplatin + ETP + CY (CEC), total body irradiation (TBI), carmustine + ETP + cytarabine + melphalan (BEAM), busulphan (Bus), CY + thiotepa + carboplatin (Carbo) (CTCb), cytarabine (AraC), anti-thymocyte globulin (ATG), peripheral blood stem cell (PBSC), bone marrow (BM), autoimmune thrombocytopenia (AITP), autoimmune hemolytic anemia (AIHA), Evans' syndrome (Evans'), autoimmune neutropenia (ANeutro)hyperthyroidism (Hyper Thyroid), ulcerative colitis (UC), prednisolone (PSL), intravenous immunoglobulin (IVIG), cyclosporine A (CyA), activated factor VII (aVII), rituximab (Rx), mycophenolate mofetil (MMF), intravenous CY (IVCY), pepleomycin (pepleo), vincristine (VCR), azathioprine (AZP), plasma exchange (PE), partial response (PR), complete response (CR), platelet associated immunoglobulin G (PAIgG), herpes zoster virus infection (VZV), anti-IIb/IIIa autoantibody (IIb/IIIa), lupus anticoagulant (LAC), anti-neutrophil autoantibody (anti-Neutro), direct Coombs' test (dCoombs'), cytomegalovirus antigenemia (CMV), antiplatelet autoantibody (anti-Plt), indirect Coombs' test (iCoombs'), antinuclear autoantibody (ANA), anti-tropomyosin autoantibody (anti-trop).

with autoimmune diseases and common transplantation related complications are infectious diseases during immune suppressive state at the early stage after autologous HSCT [4]. Although the role of HSCT in autoimmune diseases is not well-known, immune modulation with severe immune suppressive treatment can contribute disease outcome. Autoimmune disease after autologous HSCT is relatively-uncommon transplantation related complication and 2% of patients treated autologous HSCT develop autoimmune thrombocytopenia which is the most common organ specific autoimmune diseases after autologous HSCT [5]. Secondary autoimmune diseases after autologous HSCT for autoimmune diseases have been reported [6], but systemic autoimmune diseases have been rarely reported [7,8]. We reported a patient with systemic sclerosis who developed systemic lupus erythematosus after $CD34^+$-selected autologous hematopoietic stem cell transplantation [9]. We described clinical findings about autoimmune diseases after autologus HSCT from our experience and the review of literature.

2. HSCT for autoimmune diseases

HSCT is categorized into several procedures depending on the graft condition and conditioning regimen. Among them autologous HSCT has been usually performed in autoimmune diseases based on the safety and clinical efficacy. Clinical benefit has been obtained in two third patients with autoimmune diseases. About 5% of transplantation related mortality has been also reported in phase I–II studies [1–3]. Clinical effect including stabilization of autoimmune diseases has persisted for up to 7 years after autologous HSCT [1]. Although it is still unclear how autologous HSCT improves autoimmune diseases it is believed that lymphocytes, progenitor cells and hematopoietic stem cells in the graft modify the disease condition and/or the immune system. In peripheral immunity, regulatory T cells can play a role in disease improvement in patients with adult onset juvenile idiopathic arthritis and cytokine balance may influence the disease development [10,11]. In addition, thymic function can contribute to immune modulation leading to clinical benefit in patients with systemic lupus erythematosus or multiple sclerosis [12,13]. In allogenic HSCT, immune reconstitution against autoimmunity may also play a role in improvement of autoimmune diseases [14].

3. Autoimmune disease after autologous hematopoietic stem cell transplantation

Several autoimmune diseases have been reported. Among them, autoimmune thrombocytopenia is the most common organ specific autoimmune diseases after autologous HSCT. Autoimmune thrombocytopenia with the platelet counts drop following an apparent normal initial recovery after autologous HSCT (secondary

Table 2
Systemic autoimmune diseases after autologous hematopoietic stem cell transplantation

Patient (Age Sex)	Primary disease	Mobilization, Conditioning	Graft	Autoimmune disease after autologous HSCT				Remarks	Reference
				Diagnosis	Onset	Therapy	Outcome		
51 Male	DLBL	ETP+G, MCVC	PBSC	RA	40 day	NSAIDs, PSL	PR	RF (+), HLA-DRB1 0405 (+)	[7]
40 Male	FL	Dexa-BEAM, BEAM	PBSC	AS	3 month	NSAIDs, SASP	PR	HLA-B27 (+)	[8]
39 Male	DLBL	Dexa-BEAM, BEAM	PBSC	AS	4 month	NSAIDs	PR	HLA-B27 (+)	[8]
29 Female	AML	(−), CY+TBI	BM	AS	1 year	NSAIDs	PR	HLA-B27 (+), *Yersinia enterocolitica* (+)	[8]
19 Female	SSc	CY+G, CY	CD34+	SLE	4 year	PSL, CyA	CR		[9]

Abbreviations: diffuse large B-cell lymphoma (DLBL), follicular lymphoma (FL), acute myelogeneous leukemia (AML), systemic sclerosis (SSc), etoposide (ETP), granulocyte-colony-stimulating factor (G), ranimustine+carboplatin+ETP+cyclophosphamide (CY) (MCVC), dexamethasone (Dexa), carmustine+ETP+cytarabine+melphalan (BEAM), total body irradiation (TBI), peripheral blood stem cell (PBSC), bone marrow (BM), selected CD34+ cells (CD34+), rheumatoid arthritis (RA), spondylarthropathy (AS), systemic lupus erythematosus (SLE), non steroidal anti-inflammatory drugs (NSAIDs), prednisolone (PSL), salazosulfapyridine (SASP), cyclosporine A (CyA), partial response (PR), complete response (CR), rheumatoid factor (RF).

autoimmune thrombocytopenia) seems to be rare [15]. More than 15 cases of secondary autoimmune thrombocytopenia have been reported (Table 1) [5,6,16–22]. Nine of 15 cases occurred within 6 months after autologous HSCT. Some cases might relate to infectious diseases after autologous HSCT. Most patients with autoimmune thrombocytopenia were treated with corticosteroid with feasible clinical response. Acquired hemophilia, autoimmune hemolytic anemia, Evans' syndrome, hyperthyroidism and ulcerative colitis have been also reported (Table 1) [6,23–28]. Intensive immunosuppressive treatments were sometimes needed for treatment with these conditions.

Rheumatoid arthritis and spondylarthropathy have been reported as systemic autoimmune diseases after autologous HSCT (Table 2) [7,8]. Although their primary diseases were hematological malignancy without autoimmune diseases before autologous HSCT, positive for rheumatoid factor, HLA-DRB1 0405 or HLA-B27 before treatment suggested their genetic predisposition to autoimmune diseases. They had suffered from systemic autoimmune diseases within one year after autologous HSCT during incomplete immune reconstitution. One of them, infectious disease might contribute to the onset of systemic autoimmune disease. These findings suggest that the transient immune suppression following autologous HSCT and infectious disease post-transplant period may play a role in the development of systemic autoimmune diseases in susceptible patients.

4. Discussion

Autoimmune diseases such as sicca syndrome or scleroderma occasionally develop after allogenic HSCT while autoimmunity after autologous HSCT is uncommon [29]. Especially, systemic autoimmune diseases have been rarely reported [7,8]. Although the exact mechanism of autoimmunity after autologous HSCT is not known, several possibilities have been proposed, which include the impairment of peripheral T cell reconstitution as typified by markedly delayed regeneration of the $CD4^+$ subset, clonal expansion of peripheral T cell, stem cell damage during clinical procedure, homeostatic proliferation in patients with autoimmune background or an impaired development of regulatory T cells and generation of interleukin-17-producing helper T cells under the instability of immune system after autologous HSCT [9,16,19,30]. The genetic predisposition to autoimmune disease can also play a role in the development of autoimmune disease after autologous HSCT [7,8,31]. Autoimmune diseases can be treated only high-dose chemotherapy without stem cell rescue and the importance of conditioning regimen has been also reported in patients with secondary autoimmune diseases after autologous HSCT [6,32]. The necessity of stem cell rescue and its impact on immune reconstitution is obscure. The efficacy and safety of autologous HSCT for autoimmune diseases has been accepted, while long-term prognosis is not concluded. Systemic autoimmune diseases developed within one year after autologous

HSCT in patients with hematological disorders unlike our patient. Previous chemotherapies in patients with hematological disorders might result in profound immune suppression leading to the early appearance of systemic autoimmune diseases. Although autoimmune diseases are uncommon condition in patients treated with autologous HSCT, secondary autoimmune diseases can develop after autologous HSCT in patients with autoimmune diseases.

Take-home messages

- Hematopoietic stem cell transplantation is effective for refractory autoimmune diseases and well tolerated in patients with autoimmune diseases.
- Secondary autoimmune diseases can develop after autologous hematopoietic stem cell transplantation.
- Immune instability after autologous hematopoietic stem cell transplantation may lead to development of autoimmunity in patients with immune prone background.
- Long-term follow-up is essential for patients with autoimmune diseases treated with autologous hematopoietic stem cell transplantation.

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BAFF and MyD88 signals promote a lupus-like disease independent of T cells

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterized by the production of autoantibodies. However, the underlying cause of disease appears to relate to defects in T cell tolerance or T cell help to B cells. Transgenic (Tg) mice over-expressing the cytokine B cell-activating factor (BAFF) develop an autoimmune disorder similar to SLE and show impaired B cell tolerance and altered T cell differentiation. In this study, Groom JR, et al. (J Exp Med 2007; 204: 1959-71) generated BAFF Tg mice that were completely deficient in T cells, and surprisingly, these mice developed an SLE-like disease indistinguishable from that of BAFF Tg mice. Autoimmunity in BAFF Tg mice did, however, require B cell-intrinsic signals through the Toll-like receptor (TLR)-associated signaling adaptor MyD88, which controlled the production of pro-inflammatory autoantibody isotypes. TLR7/9 activation strongly up-regulated expression of transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI), which is a receptor for BAFF involved in B cell responses to T cell-independent antigens. Moreover, BAFF enhanced TLR7/9 expression on B cells and TLR-mediated production of autoantibodies. Therefore, autoimmunity in BAFF Tg mice results from altered B cell tolerance, but requires TLR signaling and is independent of T cell help.

The opposite-direction modulation of CD4+CD25+ Tregs and T helper 1 cells in acute coronary syndromes

Different subsets of T lymphocytes have different functions in atherosclerosis advancement. T helper 1 cells and T regulatory 1 cells have been demonstrated to play opposite roles in rupture of atherosclerotic lesions. However, the role of novel subsets of T regulatory cells, known as CD4+CD25+foxp3+ T cells remains largely unknown in coronary artery disease (CAD). In this study, **Shu-fang H, et al (Clin Immunol 2007; 124: 90-7)** investigated the peripheral CD4+CD25+Foxp3+ T cells of patients with CAD and controls. The patients submitted were divided into three groups: stable angina pectoris (SA) group, unstable angina pectoris (UA) group and acute myocardial infarction (AMI) group. The authors analyzed the frequencies of peripheral CD4+CD25+Foxp3+ T cells and T helper 1/T helper2 cells, expression of Foxp3 in CD4+CD25+ T subsets and cytokines pattern in patients and controls. It was found that the reduction of CD4+CD25+Foxp3+ T lymphocytes was consistent with the expansion of Th1 cells in patients with unstable CAD. The reversed development between CD4+CD25+ Tregs and Th1 cells might contribute to plaque destabilization.