


Effect of Cytokine Gene Polymorphisms on Eltrombopag Reactivity in Japanese Patients with Immune Thrombocytopenia

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Background: Immune thrombocytopenic purpura (ITP) is an autoimmune disease characterized by low platelet counts resulting from antiplatelet autoantibodies. Analysis of polymorphisms in cytokine-encoding genes is important for understanding the pathophysiology of ITP and selecting appropriate treatments. We investigated associations between polymorphisms in cytokine-encoding genes and responses to therapy in Japanese patients with ITP.

Methods: The participants in this study comprised 153 patients with ITP and 70 healthy controls. We extracted data on sex, age, platelet counts, bleeding symptoms, and therapeutic responses, including those to prednisolone (PSL) and eltrombopag. Genomic DNA was isolated from peripheral blood and polymorphisms in TNF- α , IL-10, TGF- β_1 , and IFN- γ genes were analyzed using the PCR-SSP method.

Results: Our results showed that the TGF- β_1 +869 C/C genotype might be related to ITP in Japanese patients. The IL-10 -592 C/C and A/A, -819 C/C and T/T, and -1082, -819, -592 ATA/ATA genotypes might be associated with reactivity to PSL. Furthermore, the IL-10 -592 C/A -819 C/T genotypes, IL-10 ACC/ATA genotype, and TGF- β_1 +869 T/T and T/C genotypes might be linked to the response to eltrombopag.

Conclusion: Our results indicate that analysis of polymorphisms in cytokine-encoding genes could aid in understanding PSL and eltrombopag responsiveness in Japanese patients with ITP.

Keywords: ITP, cytokine gene polymorphism, SNP, prednisolone, eltrombopag

Introduction

Immune thrombocytopenic purpura (ITP) is an autoimmune disease characterized by low platelet counts resulting from antiplatelet autoantibodies.^{1,2} The target of these antibodies is glycoprotein (GP), and anti-GP antibody is produced through actions of autoreactive B and T cells.³ In particular, GPIIb/IIIa proteins serve as major target antigens recognized by platelet-reactive CD4⁺ T cells (Figure 1).⁴⁻⁷ Helper T (Th) cells play an important role in the production of this antibody, and Th cells are classified into Th1, Th2, Th3 or Th17 populations based on their cytokine production pattern.⁷⁻⁹ The imbalance of Th1/Th2 participates in the production of antibody in ITP, similar to several other autoimmune diseases.^{3,7} Furthermore, regulatory T cells that regulate Th3 and Th17 are also important. Platelets that bind antiplatelet antibodies are destroyed by reticuloendothelial cells such as monocytes/macrophages in the spleen (Figure 1).¹⁻⁹ Additionally, antiplatelet

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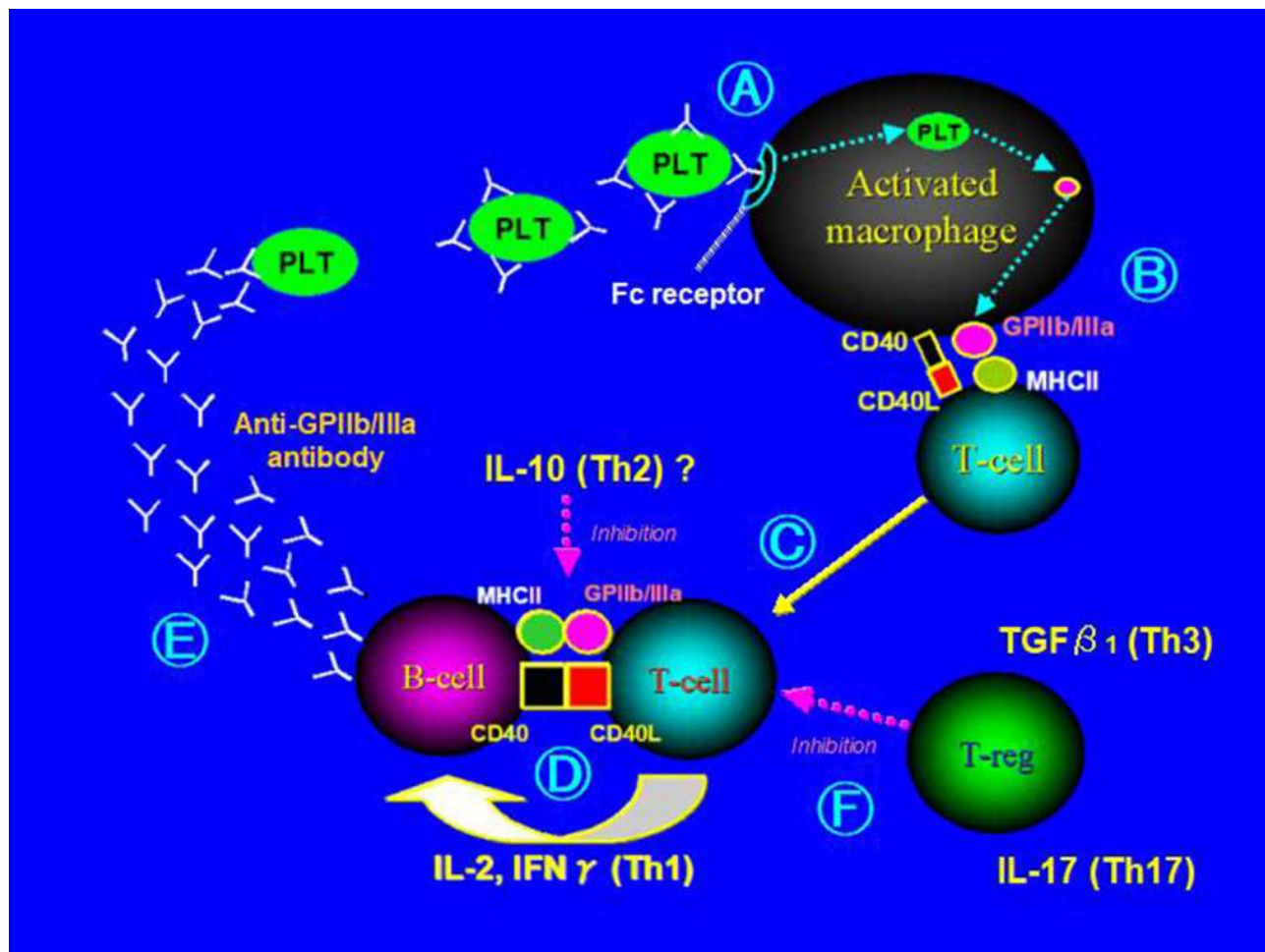


Figure 1 Presumed mechanism of anti-platelet antibody and platelet destruction in ITP. A. Anti-GPIIb/IIIa antibody-coated platelets are trapped by the Fc receptors of activated macrophages, following which the platelets are destroyed. B. Activated macrophages present GPIIb/IIIa-derived antigen to T cells. C. These T cells interact with autoreactive B cells and present the GPIIb/IIIa-derived antigen. D. Th1 cytokines such as IL-2 and IFN γ promote the activation of B cells. E. Anti-GPIIb/IIIa antibodies are produced by activated B cells. F. T-reg's control is weakened.

Abbreviations: PLT, platelet; CD40L, CD40 ligand; MHCII, major histocompatibility complex II.

autoantibodies induce megakaryocytic maturity disorder and apoptosis, which affects platelet production.⁹

The management of ITP has changed substantially over the last 50 years as understanding of the disease has increased.^{10,11} The standard therapy for ITP comprises corticosteroids.^{1,2} Recently, physiologic therapeutics have been developed as viable alternatives to immunosuppressive therapy.^{11,12} These drugs include thrombopoietin receptor agonists (TRAs) such as romiplostim and eltrombopag.^{11,13–16} TRAs are largely regarded as a third-line therapy, a position which has remained unchanged in recent years, because they are effective for the improvement of maturation disorders in megakaryocytes.^{11,12} Trials are underway to test several new drugs for their ability to treat ITP.¹⁰ These drugs contribute to improvements in the prognosis of refractory ITP.^{17–19}

It is known that autoimmune diseases have genetic factors.²⁰ As previously mentioned, several cytokines (interleukin [IL]-1, IL-2, IL-3 and IL-17) are very important in the pathophysiology of ITP.^{7–9} Therefore, the analysis of polymorphisms in cytokine-encoding genes is important for clinical setting of ITP.^{21–23} The distribution of single-nucleotide polymorphisms varies among different ethnic groups, which is also true specifically for polymorphisms in the cytokine-encoding genes related to ITP.^{24–38} However, there are few reports on the association of cytokine-related polymorphisms with responsiveness to TRAs in Japanese patients with ITP. Therefore, the associations between polymorphisms in cytokine-encoding genes and therapeutic responses to steroids and TRA in Japanese patients with ITP were investigated in the present study.

Materials and Methods

Study Design and Participants

This study was approved by the ethics committee of Kansai Medical University (Osaka, Japan). All participants, or a parent or legal guardian for those under 20 years of age, provided informed consent to participate in this study, which was conducted in accordance with the Declaration of Helsinki. The participants in this study included 153 patients with ITP and 70 healthy controls. Because there are no disease-specific tests for ITP, the diagnosis of ITP was based on excluding other diagnoses.^{1,2,39} We diagnosed ITP according to several international reports.^{40,41}

Disease Grade by Assessment of Platelet Count and Bleeding Symptoms

Disease grade was established according to platelet count (PC) and bleeding symptoms (BS), as follows: grade 0 = PC of $81 \times 10^9/L$ to $100 \times 10^9/L$ and/or no BS; grade 1 = PC of $51 \times 10^9/L$ to $80 \times 10^9/L$, and/or no BS or purpura 0–2; grade 2 = PC of $31 \times 10^9/L$ to $50 \times 10^9/L$, and/or purpura 3–10 or mucosal bleeding; grade 3 = PC of $11 \times 10^9/L$ to $30 \times 10^9/L$, and/or many purpura or moderate bleeding, with hemoglobin of 7.1–10.0 g/dL; grade 4 = PC, 0 to $10 \times 10^9/L$, and/or severe or organ bleeding, with hemoglobin of <7.0 g/dL.

Therapeutic Responses

Therapeutic responses to steroid (prednisolone; PSL) and TRA (eltrombopag) were determined using the following criteria after 3 to 12 months of therapy.

PSL start: grade 0, observation grade 1, PSL 5–10 mg/d grade 2, 3, and 4, PSL 0.5–1 mg/kg of body weight per day.

Eltrombopag start: at the time PSL ineffective. Eltrombopag 12.5–37.5 mg/d.

Responders and nonresponders to PSL or eltrombopag were defined according to the following criteria: (1) No response: PC $<30 \times 10^9/L$ or the absence of a twofold increase in baseline PC or hemorrhage; (2) partial response: PC between $30 \times 10^9/L$ and $100 \times 10^9/L$, a minimum twofold increase in baseline PC, and the absence of hemorrhage; and (3) complete response: PC $>100 \times 10^9/L$ and the absence of hemorrhage. Patients who met (2) or (3) criteria were considered responders, and those meeting (1) were considered nonresponders.

The effectiveness of PSL was assessed 3 to 6 months after the start of treatment, and effectiveness of

eltrombopag was assessed at 12 months. We extracted data on sex, age, PC, BS, and therapeutic responses to PSL and eltrombopag.

Genotyping

The details have already been reported.⁴² Polymorphisms in the TNF- α , TGF- β_1 , IL-10, and IFN- γ genes were analyzed using a PCR-SSP method using the Cytokine Genotyping Tray (One Lambda Inc., Los Angeles, CA, USA). For the cytokines under investigation, the following polymorphisms were analyzed. For TNF- α , the polymorphism in the promoter region at position -308 (G/A; rs1800629); for TGF- β_1 , a polymorphism in the coding region, that is, codon 10 +869 (T/C; rs1982073); for IL-10, three polymorphisms in the promoter region, -1082 (G/A; rs1800896), -819 (C/T; rs1800871), and -592 (C/A; rs1800872); for IFN- γ , a polymorphism in the coding region at position +874 (T/A; rs2430561). DNA fragments corresponding to each cytokine were amplified in accordance with the manufacturer's instructions.

Statistical Analysis

All statistical analyses were performed using StatFlex v7 software (Artech, Osaka, Japan). Comparison of age between patient and control groups was done using an unpaired *t*-test. Analyses for the studied polymorphisms were done using Pearson's chi-squared test and Fisher's exact probability test. In addition, genotype distribution was analyzed for the studied polymorphisms in two groups of patients with ITP divided by whether treatment (with PSL and/or eltrombopag) had been undertaken. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated for disease susceptibility and whether PSL or eltrombopag had been administered (discriminated analysis).

Results

Patient Characteristics

Table 1 exhibits the characteristics of ITP and controls. The mean age of the ITP patients was 49.2 ± 13.1 (range 19–86) years, which was significantly higher than the age of the control groups (49.2 ± 13.1 , $p=0.00054$). In terms of sex, the frequency of females was not significantly different between patient and control groups (64.1% vs 55.7%, $p=0.23517$). Platelet counts ranged from $3 \times 10^9/L$ to $91 \times 10^9/L$, with a mean of $26 \times 10^9/L$ at the initial diagnosis. Additionally, 54 patients (35.3%) had severe thrombocytopenia. Steroid

Table 1 Baseline Characteristics of the Patients with ITP and Healthy Controls

	ITP	Control
Number	153	70
Age, years, mean±SD	49.2±13.1	41.3±7.8
Age range	19–86	24–62
Gender, F/M	98/55	39/31
First platelet count ×10 ⁹ /L (range)	26 (3–91)	
Severe thrombocytopenia ^{*,a} No. of cases (%)	54 (35.3)	
Bleeding symptoms No. of cases (%), Yes No	94 (61.4) 59 (38.6)	
Treatment, No. of cases (%) Steroid, prednisolone TRA, eltrombopag	135 (88.2) 62 (40.5)	
Eradication of <i>H. pylori</i> Splenectomy IVIG Other, rituximab etc. Observation	19 (12.4) 5 (3.3) 18 (11.8) 22 (14.4) 18 (11.8)	
Response to steroid treatment Responsive, PR & CR (%) Unresponsive (%)	73/135 (54.1) 62/135 (45.9)	
Complications Cardiovascular, no. of cases (%) Diabetes, no. of cases (%) Malignancy, no. of cases (%)	14 (9.2) 8 (5.2) 3 (2.0)	

Notes: *Platelet count <30 ×10⁹/L. Bold letters/numbers represent statistical significance ($p < 0.05$).

Abbreviations: ITP, immune thrombocytopenia; TRA, thrombopoietin receptor agonist; IVIG, intravenous-immunoglobulin therapy; PR, partial response; CR, complete response.

treatment was given to 135 patients (88.2%); 73 (54.1%) were responsive and 62 (45.9%) were unresponsive. Therefore, TRA (eltrombopag) was administered in 62 patients (40.5%). Eighteen patients (11.8%) were untreated.

Association of ITP Susceptibility with Polymorphisms in Cytokine-Encoding Genes

Three of the 153 ITP patients had malignancies. Therefore, we removed these three malignancy cases from the analysis, resulting in 150 ITP patients. We detected significantly lower frequencies of the TGF- β_1 +869 C/C genotype in ITP patients compared with controls (9.3% vs 22.9%; $\chi^2 =$

5.4154; $p = 0.01996$; OR = 0.408) (Table 2). However, no differences in IFN- γ , TNF- α , or IL-10 genotype frequencies were observed between patients with ITP and controls (Table 2).

Association of Therapeutic Responses in ITP Patients with Polymorphisms in Cytokine-Encoding Genes

PSL

A total of 135 patients with ITP began PSL treatment; 73 patients responded to PSL, whereas 62 did not. Responders had higher frequencies of the IL-10 -819 CC and -592 CC genotypes than did nonresponders (26.0% vs 3.2%; $\chi^2 = 9.9532$, $p = 0.00161$, OR = 0.124) (Table 3). In contrast, responders had a lower frequency of the IL-10 -819 TT and -592 AA genotypes than did nonresponders (31.5% vs 59.7%; $\chi^2 = 4.1178$, $p = 0.04243$, OR = 1.894) (Table 3).

Eltrombopag

A total of 62 patients were treated with eltrombopag; 32 responded to eltrombopag, whereas 30 did not. The responders had a higher frequency of the IL-10 -819 C/T and -592 C/A genotypes than did nonresponders (56.3% vs 16.7%; $\chi^2 = 4.9185$, $p = 0.02657$, OR = 0.296) (Table 4). Additionally, responders had a higher frequency of the TGF- β_1 T/C genotype than did nonresponders (68.7% vs 13.3%; $\chi^2 = 8.4153$, $p = 0.00372$, OR = 0.194) (Table 4). In contrast, eltrombopag responders had a lower frequency of the TGF- β_1 T/T genotype than nonresponders (18.8% vs 70.0%; $\chi^2 = 6.6407$, $p = 0.00997$, OR = 3.733) (Table 4).

IL10 Haplotype Frequencies and Genotypes in Drug Responders and Nonresponders

The frequency of the IL-10 ACC (-1082, -819, -592) haplotype was significantly increased in PSL and eltrombopag responders (PSL: 44.5% vs 20.2%; eltrombopag: 31.2% vs 8.3%) (Table 5). In contrast, the IL-10 ATA haplotype was decreased in PSL responders (52.8% vs 78.2%). The frequency of the ACC/ACC genotype was higher (26.0% vs 3.2%), whereas the frequency of the ATA/ATA genotype was lower in PSL responders (31.5% vs 59.7%) (Table 5). In contrast, the frequency of the ACC/ATA genotype was higher in eltrombopag responders than in nonresponders (56.3% vs 10.0%) (Table 5).

Table 2 Frequencies of Cytokine Gene Polymorphisms in ITP and Normal Controls

Polymorphism	Genotype	150 Patients N (%)	70 Controls N (%)	χ^2 Value	P value	OR	95% CI
IL-10-592	C/C	24 (16.0)	17 (24.3)	1.4468	0.22904	1.518	0.767–3.005
	A/A	66 (44.0)	28 (40.0)	0.1265	0.72206	0.909	0.538–1.537
	C/A	60 (40.0)	25 (35.7)	0.1656	0.68409	0.893	0.517–1.541
IL-10-819	C/C	24 (16.0)	17 (24.3)	1.4468	0.22904	1.518	0.767–3.005
	T/T	66 (44.0)	28 (40.0)	0.1265	0.72206	0.909	0.538–1.537
	C/T	60 (40.0)	25 (35.7)	0.1656	0.68409	0.893	0.517–1.541
IL-10-1082	G/G	0 (0)	0 (0)	–	–	–	–
	A/A	143 (95.3)	66 (94.3)	0.0028	0.95755	0.989	0.658–1.486
	G/A	7 (4.7)	4 (5.7)	0.0994	0.75253	1.224	0.347–4.320
TNF- α -308	G/G	149 (99.3)	70 (100)	0.0011	0.97395	1.007	0.674–1.504
	A/A	0 (0)	0 (0)	–	–	–	–
	G/A	1 (0.7)	0 (0)	0.4657	0.49498	–	–
TGF- β 1 +869	T/T	56 (37.3)	18 (25.7)	1.4827	0.22335	0.689	0.377–1.258
	C/C	14 (9.3)	16 (22.9)	5.4154	0.01996	0.408	0.189–0.883
	T/C	80 (53.4)	36 (51.4)	0.0216	0.88315	0.964	0.594–1.566
IFN- γ +874	T/T	7 (4.7)	4 (5.7)	0.0994	0.75253	1.224	0.347–4.320
	A/A	121 (80.6)	55 (78.6)	0.0146	0.90378	0.974	0.636–1.492
	T/A	22 (14.7)	11 (15.7)	0.0303	0.86189	1.071	0.492–2.331

Note: Bold letters/numbers represent statistical significance ($p < 0.05$).

Abbreviations: N, absolute number; OR, odds ratio; CI, confidence interval; TNF, tumor necrosis factor; TGF, transforming growth factor; IL-10, interleukin-10; IFN, interferon.

Table 3 Distribution of Cytokine Genotypes in Prednisolone (PSL) Responder and Nonresponder Patients with ITP

Polymorphism	Genotype	PSL-res. 73 Patients N (%)	PSL-non. 62 Patients N (%)	χ^2 Value	P value	OR	95% CI
IL-10-592	C/C	19 (26.0)	2 (3.2)	9.9532	0.00161	0.124	0.028–0.553
	A/A	23 (31.5)	37 (59.7)	4.1178	0.04243	1.894	1.018–3.524
	C/A	31 (42.5)	23 (37.1)	0.1732	0.67730	0.874	0.462–1.651
IL-10-819	C/C	19 (26.0)	2 (3.2)	9.9532	0.00161	0.124	0.028–0.553
	T/T	23 (31.5)	10 (59.7)	4.1178	0.04243	1.894	1.018–3.524
	C/T	31 (42.5)	11 (37.1)	0.1732	0.67730	0.874	0.462–1.651
IL-10-1082	G/G	0 (0)	0 (0)	–	–	–	–
	A/A	69 (94.5)	60 (96.8)	0.00091	0.92399	1.024	0.631–1.661
	G/A	4 (5.5)	2 (3.2)	0.3675	0.54437	0.589	0.104–3.323
TNF- α -308	G/G	73 (100)	61 (98.4)	0.0044	0.94704	0.984	0.609–1.590
	A/A	0 (0)	0 (0)	–	–	–	–
	G/A	0 (0)	1 (1.6)	1.1673	0.28000	–	–
TGF- β 1 +869	T/T	23 (31.5)	27 (43.6)	0.9528	0.32901	1.382	0.721–2.650
	C/C	5 (6.8)	9 (14.5)	1.7141	0.19051	2.119	0.688–6.524
	T/C	45 (61.7)	26 (41.9)	1.6468	0.19942	0.680	0.378–1.225
IFN- γ +874	T/T	4 (5.5)	1 (1.6)	1.3094	0.25251	0.294	0.036–2.391
	A/A	58 (79.5)	52 (83.9)	0.0442	0.83354	1.056	0.637–1.749
	T/A	11 (15.0)	9 (14.5)	0.0060	0.93820	0.963	0.375–2.475

Note: Bold letters/numbers represent statistical significance ($p < 0.05$).

Abbreviations: PSL-res., prednisolone responder; PSL-non., prednisolone nonresponder; other abbreviations: see Table 2.

Table 4 Distribution of Cytokine Genotypes in Eltrombopag Responder and Nonresponder Patients with ITP

Polymorphism	Genotype	Elt-res. 32 Patients N (%)	Elt-non. 30 Patients N (%)	χ^2 Value	P value	OR	95% CI
IL-10-592	C/C	1 (3.1)	1 (3.3)	0.7381	0.96421	1.067	0.064–17.820
	A/A	13 (40.6)	24 (80.0)	2.5376	0.11122	1.970	0.855–4.533
	C/A	18 (56.3)	5 (16.7)	4.9185	0.02657	0.296	0.098–0.898
IL-10-819	C/C	1 (3.1)	1 (3.3)	0.7381	0.96421	1.067	0.064–17.820
	T/T	13 (40.6)	24 (80.0)	2.5376	0.11122	1.970	0.855–4.533
	C/T	18 (56.3)	5 (16.7)	4.9185	0.02657	0.296	0.098–0.898
IL-10-1082	G/G	0 (0)	0 (0)	–	–	–	–
	A/A	32 (100)	28 (93.3)	0.0362	0.84913	0.933	0.458–1.900
	G/A	0 (0)	2 (6.7)	2.0645	0.15080	–	–
TNF- α -308	G/G	32 (100)	29 (96.7)	0.0088	0.92521	0.967	0.476–1.961
	A/A	0 (0)	0 (0)	–	–	–	–
	G/A	0 (0)	1 (3.3)	1.0489	0.49212	–	–
TGF- β 1 +869	T/T	6 (18.8)	21 (70.0)	6.6407	0.00997	3.733	1.326–10.510
	C/C	4 (12.5)	5 (16.7)	0.1616	0.68772	1.333	0.328–5.422
	T/C	22 (68.7)	4 (13.3)	8.4153	0.00372	0.194	0.060–0.629
IFN- γ +874	T/T	0 (0)	1 (3.3)	1.0489	0.49212	–	–
	A/A	28 (87.5)	24 (80.0)	0.0566	0.81203	0.914	0.437–1.913
	T/A	4 (12.5)	5 (16.7)	0.1616	0.68772	1.333	0.328–5.422

Note: Bold letters/numbers represent statistical significance ($p < 0.05$).

Abbreviations: Elt-res., eltrombopag responder; Elt-non., eltrombopag nonresponder; other abbreviations: see Table 2.

Discussion

Cellular immunity and cytokine-related responses are very important in the pathophysiology of ITP.^{1,3,8,12} Cytokine

gene polymorphisms have been associated with various autoimmune diseases.^{22,27} In the present study, we analyzed the frequencies of some cytokine polymorphisms of

Table 5 Individual IL-10 Haplotype Frequencies and Genotypes in Prednisolone (PSL) or Eltrombopag (Elt) Responders and Nonresponder Patients with ITP

IL-10 HT & GT	PSL-res. 73 Patients N (%)	PSL-non. 62 Patients N (%)	χ^2 , p value	Elt-res. 32 Patients N (%)	Elt-non. 30 Patients N (%)	χ^2 , p value
Haplotype						
ACC	65 (44.5)	25 (20.2)	0.00247	20 (31.2)	5 (8.3)	0.00903
ATA	77 (52.8)	97 (78.2)	0.04333	44 (68.8)	53 (88.4)	0.35616
GCC	4 (2.7)	2 (1.6)	0.54022	0 (0)	2 (3.3)	0.14751
Genotype						
ACC/ACC	19 (26.0)	2 (3.2)	0.00161	1 (3.1)	1 (3.3)	0.96417
ACC/ATA	27 (37.0)	21 (33.9)	0.79480	18 (56.3)	3 (10.0)	0.00578
ATA/ATA	23 (31.5)	37 (59.7)	0.04243	13 (40.6)	24 (80.0)	0.11117
GCC/ACC	0 (0)	0 (0)	–	0 (0)	0 (0)	–
GCC/ATA	4 (5.5)	2 (2.2)	0.54437	0 (0)	2 (6.7)	0.15076
GCC/GCC	0 (0)	0 (0)	–	0 (0)	0 (0)	–

Note: Bold letters/numbers represent statistical significance ($p < 0.05$).

Abbreviations: PSL-res., prednisolone responder; PSL-non., prednisolone nonresponders; Elt-res., eltrombopag responder; Elt-non., eltrombopag nonresponder; other abbreviations: see Table 2.

patients with ITP and healthy controls. The frequency of the TGF- β_1 +869 C/C genotype was significantly lower in patients with ITP than in healthy controls. TGF- β_1 is produced by dendritic cells and T-cells, and it plays important roles in regulating immune function.⁴³ Serum TGF- β_1 concentration is decreased in systemic lupus erythematosus (SLE).^{44,45} Moreover, mice deficient in TGF- β_1 exhibit enhanced production of autoantibodies, which could be related to the pathology of SLE.⁴⁶ However, the relationship between ITP and polymorphisms in TGF- β_1 is not well defined, because there are few studies in the literature assessing TGF- β_1 in ITP patients.^{24,28,30} The TGF- β_1 polymorphism might be useful as a predictor of radiographic progression but further investigation is needed into the feasibility of this.

IL-10 is an anti-inflammatory cytokine produced mainly by Th2 cells and a variety of other cells, including Th1 and Th17 cells, B cells, and macrophages.^{47,48} Serum concentrations of IL-10 are elevated in the serum of patients with ITP compared with those in healthy individuals.³² Furthermore, IL-10 has been reported to affect immunological modulation in ITP^{49,50} and might, therefore, be involved in the pathophysiology of ITP.^{26,32,38} In this study, however, we observed no significant differences in IL-10 genotypes in ITP patients and healthy controls. This discrepancy between studies may be related to ethnic factors. Saitoh et al³¹ reported that Japanese patients with ITP did not differ significantly from healthy controls with respect to polymorphisms in IL-10.

In the present study, we investigated the relationship between such polymorphisms and therapeutic responses to PSL and eltrombopag in patients with ITP. Patients who responded to PSL had higher frequencies of IL-10 -592 C/C and -819 C/C genotypes compared with nonresponders. Similarly, PSL responders had a lower frequency of the IL-10 -592 A/A and -819 T/T genotypes than responders. Other reports have described a relationship between IL-10 -592 A/A genotype and ITP severity.^{31,35} Our results partly support these previous reports. In the present study, we analyzed the IL-10 promoter polymorphism (-1082, -819, -592) haplotypes ACC, ATA, and GCC (Table 5). PSL nonresponders had a lower frequency of the ACC/ATA genotype and a higher frequency of the ATA/ATA genotype. These results suggested that presence of the ATA haplotype and absence of the ACC haplotype were associated with response to PSL or ITP severity, which is similar to some previously reported

studies.^{31,32,35} It is reported that IL-10 itself participates in reactivity to steroid in a murine model of ITP.⁴⁷

Eltrombopag is a well-established treatment for ITP and is increasingly used in second-line management.^{17,19,51,52} However, the relationship between eltrombopag and cytokine gene polymorphisms has yet been reported, which we addressed in the present study. A total of 62 patients were treated with eltrombopag; 32 patients responded and 30 did not. Eltrombopag responders had higher frequencies of the IL-10 -819 C/T and -592 C/A genotypes than the nonresponders. Furthermore, the IL-10 ACC/ATA genotype was significantly more common in eltrombopag responders. Thus, the results of IL-10 haplotype and genotype analysis did not always match in terms of responses to PSL and eltrombopag therapy. Eltrombopag is a second-line therapy used in patients who do not respond to PSL.¹⁷⁻¹⁹ However, the differences in IL-10 haplotypes and genotypes between eltrombopag responders and nonresponders are very interesting. It will be useful to determine the eltrombopag effect ITP patients. In addition, eltrombopag responders exhibited a higher frequency of the TGF- β_1 T/C genotype than nonresponders and a lower frequency of the T/T genotype. TGF- β_1 plays important roles in regulating immune function.⁴³ We previously reported that TGF- β_1 and soluble cytotoxic T-lymphocyte-associated antigen 4 (sCTLA-4) levels are increased in eltrombopag-exposed patients with ITP.⁵³ Our previous results suggested that eltrombopag partly modulates some immune responses through TGF- β_1 and sCTLA-4. TGF- β_1 plays an important role in the induction of tolerance by regulatory T cells in ITP.^{43,53,54} Further studies are needed to determine the clinical significance of polymorphisms in TGF- β_1 and immune modulation of eltrombopag.

This study had some limitations. First, the samples were obtained from a single facility and thus our data are probably not representative of the Japanese ITP population. Second, eltrombopag is only a second-line treatment. Finally, we did not define the relationship between polymorphisms in cytokine-encoding genes and TRAs other than eltrombopag in this study. Therefore, we do not know whether all TRAs are directly linked to such polymorphisms in patients with ITP. Prospective studies are needed to confirm the observations from this study.

Conclusions

In this study, we showed that the TGF- β_1 +869 C/C genotype might be related to ITP in Japanese patients with ITP. The IL-10 -592 C/C and A/A, -819 C/C and T/T, and (-1082, -819, -592) ATA/ATA genotypes may be

associated with the response to PSL. Furthermore, the IL-10 -592 C/A, -819 C/T, and -1082, -819, -592 ACC/ATA genotypes and the TGF- β_1 +869 T/T and T/C genotypes might be linked to the response to eltrombopag. These results indicate that polymorphism analysis of the cytokine-encoding genes could be useful in understanding PSL and eltrombopag responsiveness in Japanese patients with ITP.

Acknowledgments

This study was partly supported by a Grant (19K07948 to S.N.) from the Ministry of Education, Science or Culture of Japan. We thank Louise Adam, ELS (D) and Gillian Campbell, from Edanz Group (<https://en-author-services.edanz.com/ac>) for editing a draft of this manuscript.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors do not have any conflicts of interest to report in this work.

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