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Original Article

Increased expression of interleukin-17 is associated with macrophages in chronic immune thrombocytopenia

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Abstract: *Objectives:* Interleukin-(IL-)17-mediated cells contribute to the imbalance of cellular immunity in the pathogenesis of immune thrombocytopenia (ITP). We examined samples of bone marrow (BM) clots to determine if IL-17-mediated immunological changes involve the BM and to identify clinical predictors of treatment response. *Methods:* We enrolled 33 patients with chronic ITP. BM clots were obtained before treatment and stained with the following markers: CD3, CD4, CD8, CD20, CD25, CD68, CD163, and IL-17. Pathological findings and clinical information, including laboratory data, were compared between the patients and 11 control subjects and between IL-17-high and -low-expression groups. *Results:* Univariate analysis revealed increased cells expressing CD68, CD163, and IL-17 in the patients with ITP than in the control subjects (P = 0.02, 0.001, and 0.001, respectively). The expression of both CD68 and CD163 showed correlation with IL-17 expression (r = 0.60 and 0.48, respectively). Responses to Eltrombopag were better in the IL-17-low-expression group than in the IL-17-high-expression group (P = 0.056). *Conclusions:* Macrophages and monocytes were associated with IL-17 expression in patients with ITP. We demonstrated that ITP is associated with IL-17-expressing monocytes/macrophages and might be more difficult to treat.

Keywords: Immune thrombocytopenia, interleukin-17, CD68, CD163, macrophages, monocytes

Introduction

Immune thrombocytopenic purpura (ITP) is an acquired autoimmune disorder characterized by an isolated low platelet count in which platelets are destroyed mainly in the reticuloendothelial system [1]. The disorder is defined as primary ITP in the absence of other causes or disorders that may be associated with thrombocytopenia [1, 2]. Secondary ITP is diagnosed in the case of patients with chronic infections, including *Helicobacter pylori* (*H. pylori*), hepatitis C virus (HCV), human immunodeficiency virus (HIV), lymphoproliferative disorders (LPD), and other autoimmune disorders, such as systemic lupus erythematous (SLE), rheumatoid arthritis (RA), and antiphospholipid antibody syndrome (APS) [2, 3]. Classically, ITP is characterized by platelets that are destroyed by macrophages in the spleen and liver due to immunoglobulin (Ig) G autoantibodies opsonizing the individual's platelets. In addition, it has been reported that these autoantibodies can mediate megakaryocyte (MgK) inhibition/destruction both *in vitro* and *in vivo* [2, 4]. One study reported that abnormal MgKs in ITP were surrounded by neutrophils or macrophages, with some being in a state of phagocytosis [5] in the bone marrow (BM).

Recently, it has been demonstrated that T-cell-mediated MgK inhibition/destruction in the BM leads to thrombocytopenia. The imbalance of helper T (Th) cells, including a new subset of interleukin-(IL-)17-mediated cells is important in the pathogenesis of ITP [2, 6, 7]. For example, IL-17-secreting CD4-positive cells and CD8-positive cells are increased in patients with ITP

[8, 9]. A BM examination is not necessary in patients with typical ITP regardless of age because the diagnostic criteria according to the guidelines of the American Society of Hematology (ASH) [10] and BM findings are not considered to be specific other than a finding of normal or increasing numbers of MgK [5, 11]. Therefore, studies of pathological findings in BM using immunohistochemistry (IHC) are limited, and even though some flow cytometric studies on lymphocytes using peripheral blood (PB) or BM of patients with ITP have been conducted, the results have been inconsistent.

In this study, we examined samples of BM clot before treatments to test the hypothesis that IL-17-mediated immunological changes are involved in the BM of patients with ITP.

Materials and methods

Patients

We enrolled 33 patients (8 males and 25 females) with a median age of 61 years (range, 19-91 years) who were retrospectively selected at random and referred to our hospital between 2005 and 2016. All patients were diagnosed with chronic ITP on the basis of previously reported guidelines [3, 10] and underwent BM assessments before treatment. The patients received treatments, such as glucocorticosteroids (GC), cyclosporine A (CyA), azathioprine (AZA), danazol (DNZ), and intravenous immunoglobulin (IVIg) and/or splenectomy as first- or second-line conventional therapies [3]. Patients who were diagnosed as having *H. pylori* infection by using the ^{13}C-urea breath test and serum IgG antibody for *H. pylori* were included in this study. Before first-line therapy, all *H. pylori*-infected patients were administered eradication therapies consisting of a combination of antibiotics and lansoprazole. Those who did not respond to both the first- and second-line therapies with/without splenectomy received a thrombopoietin-receptor agonist, Eltrombopag. Eltrombopag treatment was initiated at a dose of 12.5 mg once daily taken between meals in all patients, as recommended by a previous Japanese study [12]. Dose adjustments and interruptions were performed on the basis of individual platelet counts to maintain counts between 50,000 and 200,000/μL. The use of rescue treatments, such as an increased dose of a previously used concomitant medication, and platelet transfusion were allowed. The maximal dose of Eltrombopag was 50 mg. All patients were required to avoid consuming dairy products and minerals simultaneously and maintain normal creatinine and liver enzyme concentrations. Patients with the following conditions were excluded: secondary ITP (e.g., patients with chronic infections, including hepatitis B virus or HCV and HIV, LPD, and other autoimmune disorders, such as SLE, RA, and APS), malignant tumors, congestive heart failure, arrhythmia, thrombosis ≤ 1 year before enrollment, and women who were nursing or pregnant.

Pathological findings and clinical information, including laboratory data, were compared between the patients with ITP and control subjects, which were obtained from 11 untreated malignant lymphoma patients without the infiltration of lymphoma cells in BM, and we also compared the findings and information between 2 groups: an IL-17-high-expression group (patients who showed IL-17-expressing cell staining above the mean) and an IL-17-low-expression group (patients who did not show IL-17-expressing cell staining above the mean). We also classified all patients who were administered Eltrombopag into 2 groups: responders and non-responders. Patients who attained platelet counts of > 50,000/μL for ≥ 12 weeks after the last dose of Eltrombopag without receiving any new or additional other ITP treatments were classified as responders. Conversely, the platelet count was not considered indicative of response within 6 weeks after the end of increased concomitant ITP drug use or platelet transfusion in accordance with previous studies [13-15].

This study was approved by the Research Ethics Committee of the Showa University School of Medicine (approval number 2205) and was conducted according to the Helsinki declaration of 1975, as revised in 2013.

Immunohistochemistry

BM clots were stained with hematoxylin and eosin (HE) stains of 3-μm sections for morphological examination. Formalin-fixed and paraffin-embedded specimens were made for IHC using antibodies for the following markers: CD3, CD4, CD8, CD20 (L26), CD25, CD68,

Table 1. Immunohistochemical antibodies and expression

Antibody	Animal species	Clone	Company	Epitope retrieval		Fold dilution	Expression
				Technique	Solution pH		
CD3	Mouse	PS1	Leica	HIER	9	100	
CD4	Mouse	1F6	Leica	HIER	9	40	
CD8	Mouse	C8/144B	Dako	HIER	7	50	
CD20	Mouse	L26	Leica	HIER	7	100	
CD25	Mouse	4C9	Leica	HIER	7	100	Interleukin-2 receptor
CD68	Mouse	KP-1	Dako	P	-	100	Strong in cytoplasmic granules
							Weak on the surface of Mφ, monocytes, neutrophils, basophils, and NK-cells
CD163	Mouse	10D6	Leica	HIER	7	100	Monocytes and Mφ
IL-17	Goat	Poly	LifeSpan BioSciences	HIER	9	500	

P, enzyme digestion (proteinase K). Incubate at room temperature for 5 min. HIER, heat-induced epitope retrieval. Incubate at 98°C for 40 min. Mφ, macrophages.

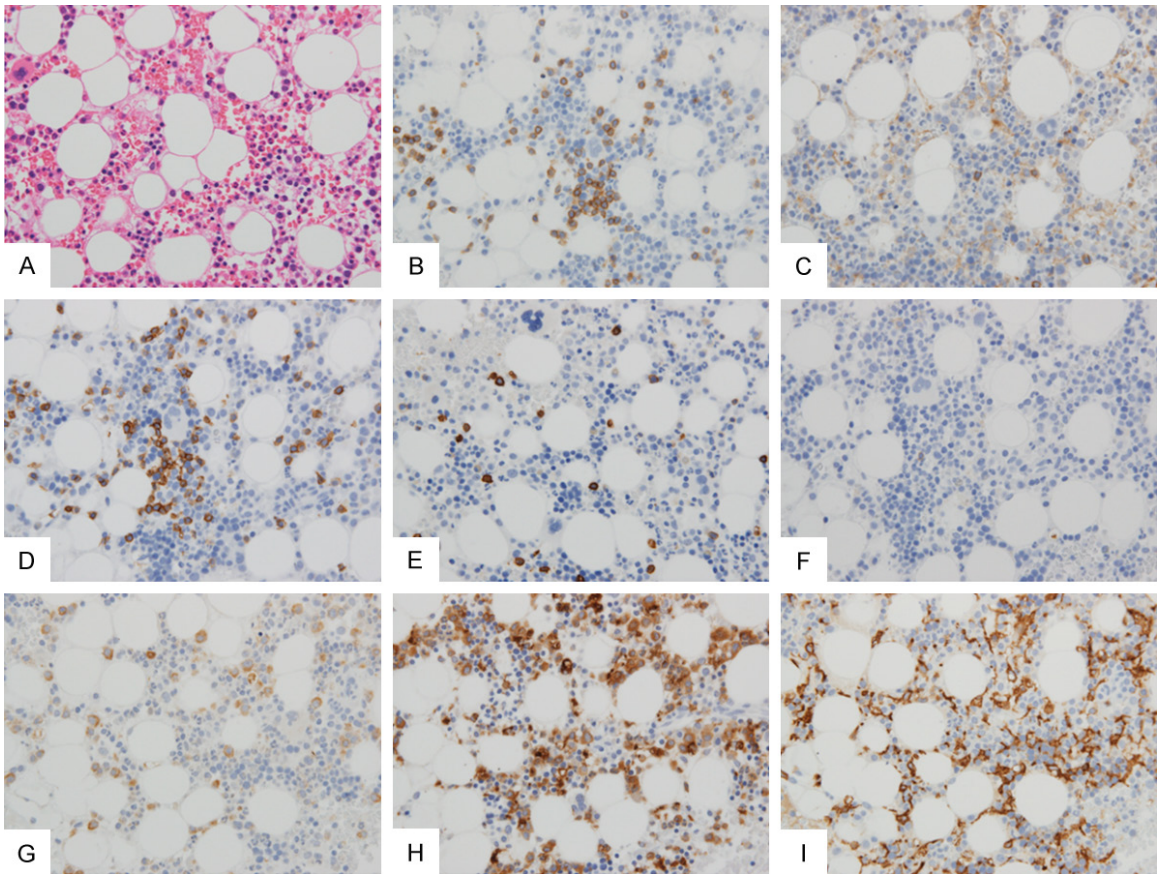

Figure 1. Hematoxylin and eosin (HE) section and immunohistochemical staining for CD3, CD4, CD8, CD20, CD25, IL-17, CD68, and CD163 in patients with ITP. HE and immunohistochemical staining of a typical case are shown: (A) HE, (B) CD3, (C) CD4, (D) CD8, (E) CD20, (F) CD25, (G) IL-17, (H) CD68, and (I) CD163 (objective, 40 ×), and the percentages of positively stained cells by immunohistochemistry were 10%, 1.8%, 7.8%, 3.1%, 0.2%, 4.5%, 19.5%, and 9.7%, respectively.

CD163, and IL-17 (**Table 1**). We defined cells as positively expressing when the CD3, CD4, CD8, CD20 (L26), and CD25 markers for membranes of lymphocytes and the CD163 marker for

Table 2. Clinical characteristics of the patients

			N = 33 n (%)
Age at ITP diagnosis, year	Median	61	
	Range	19-91	
Sex	Male	8	(24.2)
	Female	25	(75.8)
Eltrombopag use, Yes		21	(63.6)
Prior therapies*		20	
GC		17	
CyA		6	
AZA		1	
DNZ		3	
IVIg		7	
Number of prior therapies			
1		10	
2		8	
≥ 3		2	
Eltrombopag only		1	
H. pylori eradication therapy		6	
Splenectomy		3	
Concomitant therapies		10	
Eltrombopag use, No		12	(36.4)
Other therapies*		4	(12.1)
GC		4	
DNZ		2	
IVIg		3	
H. pylori eradication therapy		1	
H. pylori eradication therapy only		4	(12.1)
No therapy		4	(12.1)

GC, glucocorticosteroids; CyA, cyclosporine A; AZA, azathioprine; DNZ, danazol; IVIg intravenous immunoglobulin. *The therapies are duplicated for same patients.

monocytes/macrophages were entirely stained. The CD68 marker was defined as positively expressing when the membranes of the cells were entirely stained, and then we excluded neutrophils and basophils morphologically. The IL-17 marker was defined as positively expressing when the entire cytoplasm, including membranes of nucleated cells, were stained (**Figure 1**). The numbers of cells expressing each marker were enumerated under high-power microscopic field magnification (objective, 40 ×). We chose three fields, and three pathologists counted the cells in each field three times with no knowledge of patient background. The mean percentages of the cells expressing each marker divided by all nucleated cells in the 3 high-power fields of hot spots were calculated.

Statistical analysis

Statistical analysis was performed by using JMP 12 Pro (SAS Institute Inc., Cary, NC, USA). Student's *t*-test was used to compare ages, pathological findings, and laboratory data between the patients with ITP and control subjects and between the IL-17-high-expression group and IL-17-low-expression group. Fisher's exact test was performed to compare clinical information except for age between these groups. Correlations between pathological findings were calculated by using Spearman's rank correlation test. A p value of < 0.05 was considered as indicating statistical significance in all analyses.

Results

Characteristics of the patients

The study cohort included 33 patients with a median age of 61 years (range, 19-91 years). Among them, four (12.1%) patients achieved complete responses (CRs; platelet count ≥ 100,000/μL) after receiving treatments such as GC, DNZ, and IVIg. Four (12.1%) patients exhibited increased platelet counts in the absence of treatment. Of the entire study cohort, 11 (33.3%) patients received *H. pylori* eradication therapy, four (12.1%) of whom achieved CRs. In total, 12 patients did not receive Eltrombopag. Among 21 (63.6%) patients who were prescribed Eltrombopag, 20 received prior treatments with or without *H. pylori* eradication therapies, with the remaining patient electing to receive Eltrombopag alone. Overall, 17, 6, 1, 3, and 7 patients received GC, CyA, AZA, DNZ, and IVIg, respectively, and three (9.1%) patients underwent splenectomy. Ten patients received one prior therapy, whereas eight and two patients received two and three or more prior therapies, respectively. Ten patients received other treatments concomitantly with Eltrombopag therapy (**Table 2**). Fourteen patients responded to Eltrombopag, and the mean duration of response was 97.2 (range, 16.0-288) weeks. The mean duration of maximum-dose Eltrombopag administration before a non-response was declared was 69.3 (range, 6-208) weeks.

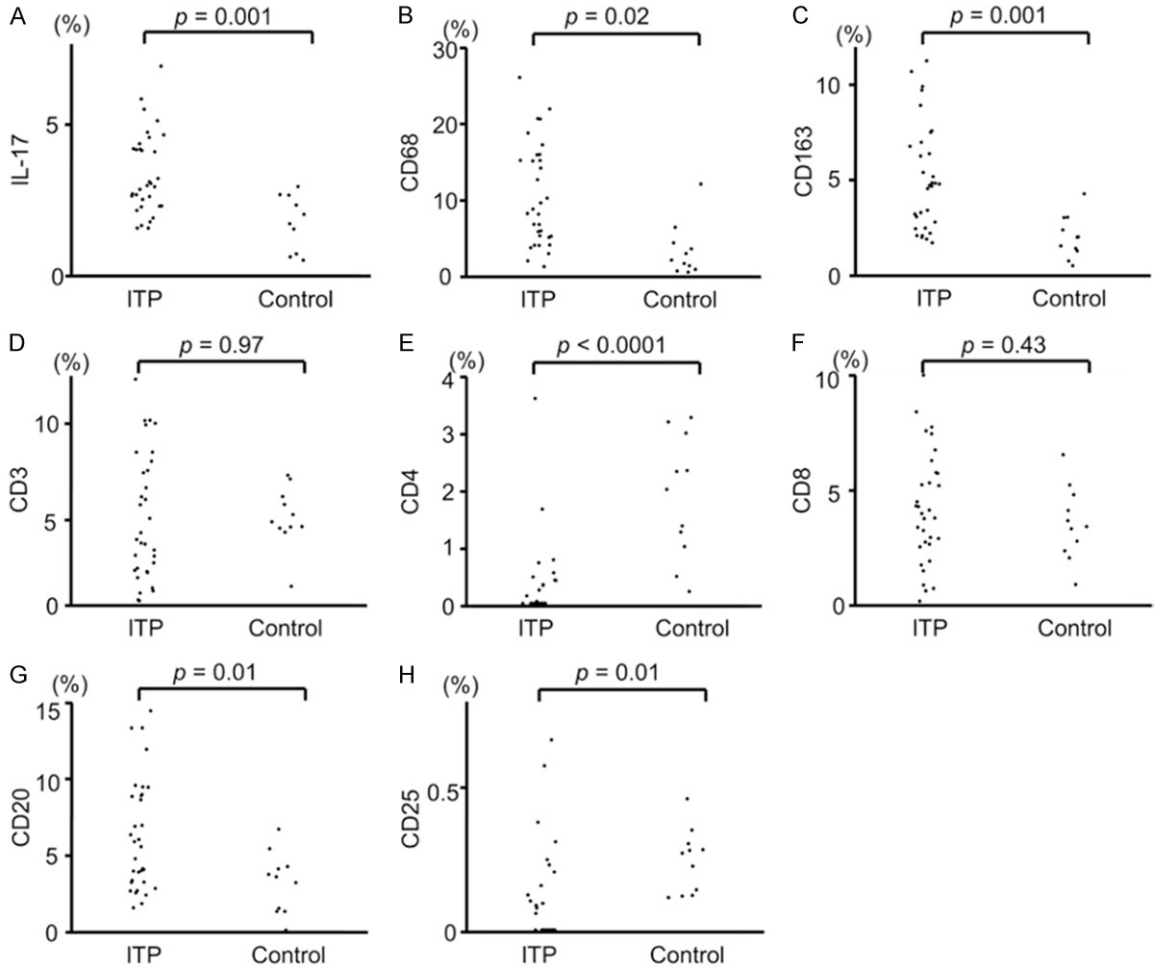

Figure 2. Comparison of patients with ITP and control subjects by immunohistochemical analysis. Analyses of (A) IL-17, (B) CD68, (C) CD163, (D) CD3, (E) CD4, (F) CD8, (G) CD20, and (H) CD25 are shown between ITP patients and control subjects. There was increased expression in the patients with ITP relative to those of the control subjects for (A) IL-17 (3.18 ± 1.50% vs. 1.49 ± 1.00%), (B) CD68 (11.6 ± 11.3% vs. 3.14 ± 3.23%), (C) CD163 (5.16 ± 2.71% vs. 2.13 ± 1.10%), and (G) CD20 (6.00 ± 3.49% vs. 3.06 ± 1.90%). The expression of (E) CD4 (0.31 ± 0.73% vs. 1.96 ± 1.13%) and (H) CD25 (0.09 ± 0.16% vs. 0.23 ± 0.10%) was decreased in the patients with ITP. (D) CD3 and (F) CD8 expression was not statistically significant.

Comparison of pathological features between patients and controls

Univariate analysis revealed an increase in cells expressing the following markers in patients with ITP than in the control subjects: CD20 (6.00 ± 3.49% vs. 3.06 ± 1.90%; P = 0.01), CD68 (11.6 ± 11.3% vs. 3.14 ± 3.23%; P = 0.02), CD163 (5.16 ± 2.71% vs. 2.13 ± 1.10%; P = 0.001); and IL-17 (3.18 ± 1.50% vs. 1.49 ± 1.00%; P = 0.001); additionally, a decrease in cells expressing the following markers was found: CD4 (0.31 ± 0.73% vs. 1.96 ± 1.13%; P < 0.0001) and CD25 (0.09% ± 0.16% vs. 0.23% ± 0.10%; P = 0.01). The differences between the cells expressing CD3 (P = 0.97) and CD8 (P = 0.43) were not statistically significant (**Figure 2**). There was significant correlation between the CD68 and CD163 expression (r = 0.57). The expression of both CD68 and CD163 showed correlation with IL-17 expression (r = 0.60 and 0.48, respectively) (**Figure 3**).

Comparison between the IL-17 expression groups

We classified patients into the IL-17-low-expression group (20 patients) and IL-17-high-expression group (13 patients). The clinical information between the IL-17-low-expression group and IL-17-high-expression group did not significantly differ for age, sex, Eltrombopag

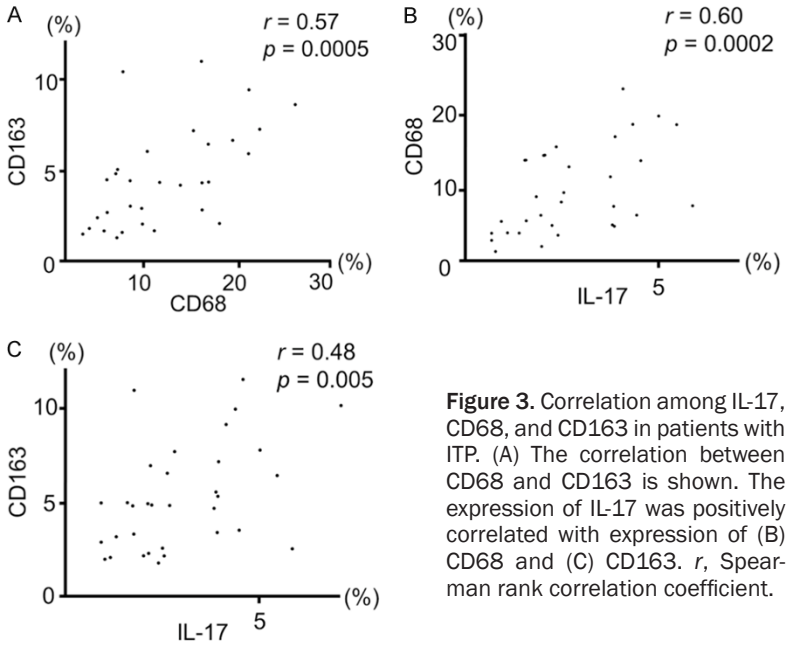

Figure 3. Correlation among IL-17, CD68, and CD163 in patients with ITP. (A) The correlation between CD68 and CD163 is shown. The expression of IL-17 was positively correlated with expression of (B) CD68 and (C) CD163. *r*, Spearman rank correlation coefficient.

Table 3. Comparison of clinical and pathological findings between the low and high IL-17 expression groups

Data	Low IL-17 expression (N = 20)	High IL-17 expression (N = 13)	*p*-value
Age, years	60.3 ± 19.8	53.6 ± 22.4	0.38
Male, % (n)	30.0 (6)	15.4 (2)	0.43*
Eltrombopag use No, % (n)	35.0 (7)	38.5 (5)	1.00*
Yes, % (n)	65.0 (13)	61.5 (8)	
Eltrombopag responder, % (n)	84.6 (11)	37.5 (3)	0.06*
Concomitant treatments, % (n)	25.0 (5)[a]	38.5 (5)[b]	0.39*
H. pylori infection, % (n)	30.0 (6)	38.5 (5)	0.71*
Splenectomy, % (n)	15.0 (3)	0 (0)	0.26*
Platelet counts, × 10^4/μl	2.3 ± 2.0	3.3 ± 1.9	0.16
PAIgG, ng/10^7 cells	220.9 ± 315.8	267.8 ± 369.7	0.70
Megakaryocyte count, /μl	50.7 ± 41.8	34.9 ± 23.5	0.23
CD3-positive cells, %	3.92 ± 3.09	6.37 ± 3.33	0.04
CD4-positive cells, %	0.29 ± 0.85	0.33 ± 0.52	0.90
CD8-positive cells, %	3.79 ± 2.39	4.31 ± 2.21	0.53
CD20-positive cells, %	6.18 ± 4.02	5.64 ± 2.58	0.68
CD25-positive cells, %	0.05 ± 0.07	0.17 ± 0.22	0.03
CD68-positive cells, %	7.94 ± 5.08	17.3 ± 15.6	0.02
CD163-positive cells, %	4.24 ± 2.29	6.57 ± 2.77	0.01

*indicate that statistical analyses were performed using Fisher's exact test. Others were performed using Student's *t*-test. Results are shown as the mean ± SD. PAIgG, platelet-associated immunoglobulin G. a; Three patients received glucocorticosteroids (GC), one patient received GC and cyclosporine A (CyA), and one patient received danazol. b; Four patients received GC, and one patient received GC and CyA.

counts, and platelet-associated IgG levels at baseline. Responses to Eltrombopag were better in the IL-17-low-expression group than in the IL-17-high-expression group (*P* = 0.056). The mean response duration in the low and high IL-17 expression groups was 114.3 (range, 24.0-288) and 34.7 (range, 16.0-48) weeks, respectively. Of the 12 patients who did not receive Eltrombopag, seven (58.3%) and five (41.7%) exhibited low and high IL-17 expression, respectively.

Regarding pathological findings, there were statistically significant differences between the IL-17-low-expression group and IL-17-high-expression group (**Table 3** and **Figure 4**). We found significantly increased expression of CD3 (3.92 ± 3.09% vs. 6.37% ± 3.33%; *P* = 0.04), CD25 (0.05 ± 0.07% vs. 0.17 ± 0.22%; *P* = 0.03), CD68 (7.94 ± 5.08% vs. 17.3 ± 15.6%; *P* = 0.02), and CD163 (4.24 ± 2.29% vs. 6.57 ± 2.77%; *P* = 0.01) in the IL-17-high-expression group relative to those in the IL-17-low-expression group. The differences in expression of CD4 (*P* = 0.90), CD8 (*P* = 0.53), CD20 (*P* = 0.68), and the counts of MgKs (*P* = 0.23) were not statistically significant. Morphological findings, such as phagocytosis of MgKs, or abnormalities in the distribution of other cells around MgKs were not found.

Discussions

usage, *H. pylori* infection (patients who were administered eradication therapy), splenectomy, use of concomitant therapies, platelet

In this study, we retrospectively analyzed patients with chronic ITP to determine if IL-17-mediated immunological changes are involved

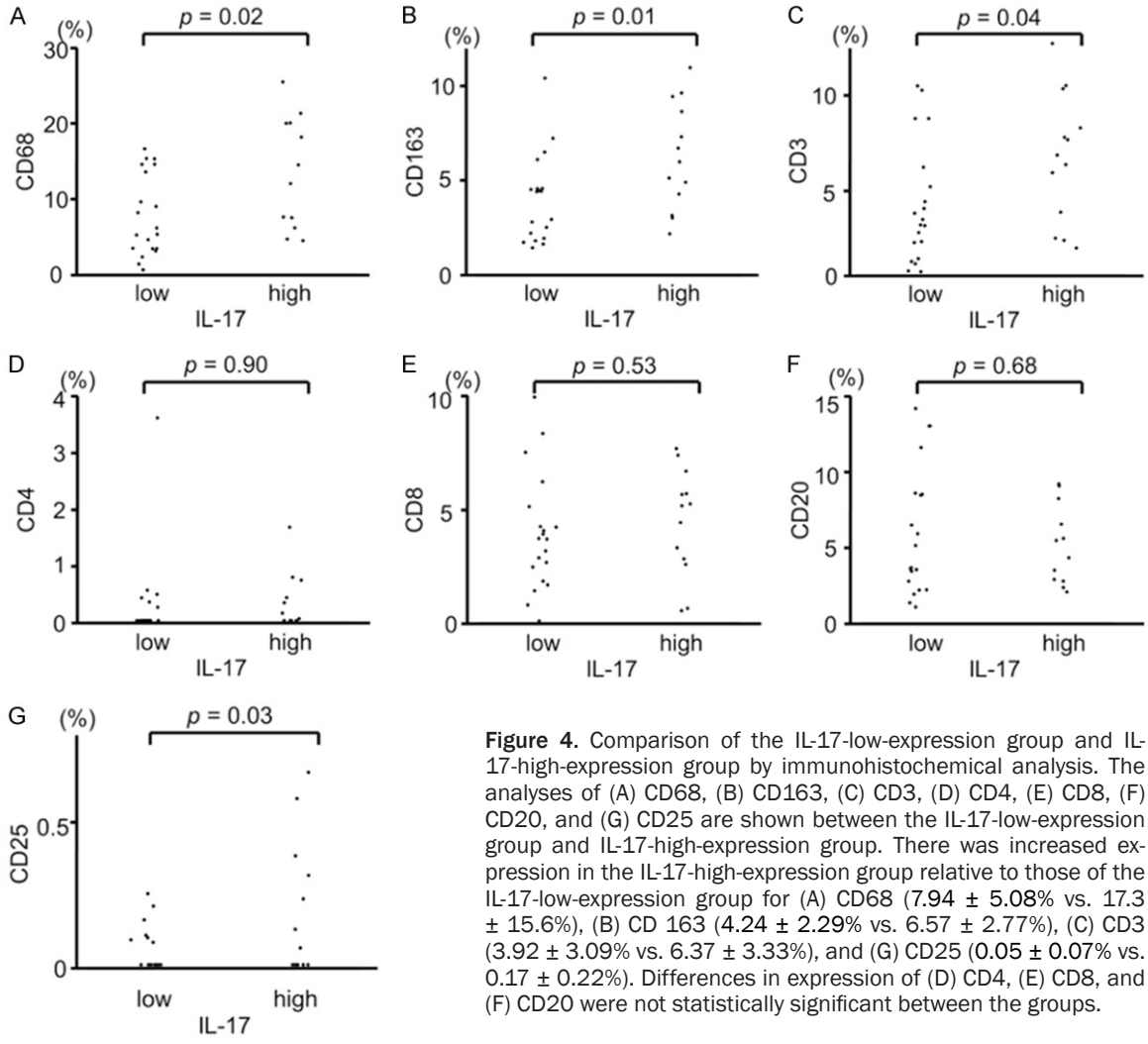

Figure 4. Comparison of the IL-17-low-expression group and IL-17-high-expression group by immunohistochemical analysis. The analyses of (A) CD68, (B) CD163, (C) CD3, (D) CD4, (E) CD8, (F) CD20, and (G) CD25 are shown between the IL-17-low-expression group and IL-17-high-expression group. There was increased expression in the IL-17-high-expression group relative to those of the IL-17-low-expression group for (A) CD68 (7.94 ± 5.08% vs. 17.3 ± 15.6%), (B) CD 163 (4.24 ± 2.29% vs. 6.57 ± 2.77%), (C) CD3 (3.92 ± 3.09% vs. 6.37 ± 3.33%), and (G) CD25 (0.05 ± 0.07% vs. 0.17 ± 0.22%). Differences in expression of (D) CD4, (E) CD8, and (F) CD20 were not statistically significant between the groups.

in the BM. We found increased expression of IL-17 in patients with ITP than in the control subjects. There was significant correlation between CD68 and CD163 expression. Both CD68 and CD163 were respectively correlated with IL-17 expression [16]. In the IL-17-high-expression group, the CD68- and CD163-expressing cells were expectedly elevated. These results suggested that CD68- and/or CD163-expressing histiocytes, such as macrophages and monocytes, would also mediate IL-17 in chronic ITP. We used BM samples from patients with malignant lymphoma without infiltration of lymphoma cells as controls because BM samples were not obtained from healthy donors. Handa et al. used BM cells from patients with lymphoma without infiltration of lymphoma cells as controls in their study. They compared gene expression between the multiple myeloma and control groups. Favorable results were obtained, confirming that BM cells from patients with malignant lymphoma without infiltration of lymphoma cells could be used as control samples [17].

Generally, IL-17 is secreted by Th17 cells and CD8-positive T cells, which are known as new T-cell subsets, and these cells have been found to be increased in patients with ITP [8, 9]. Additionally, imbalances in T cells, mainly Th1-dominant shift in the Th1/Th2 subsets, and bias of cytokines have been reported to be the pathogenesis of chronic ITP [18, 19]. Some studies using flow cytometry of PB in patients with chronic ITP have demonstrated that CD4-positive cells, including Th1 and Th17, were increased [8, 20, 21]. According to the other report, the percentage of CD3-positive cells was found to be increased in BM by both flow cytometry and IHC [22]. Regarding PB, some

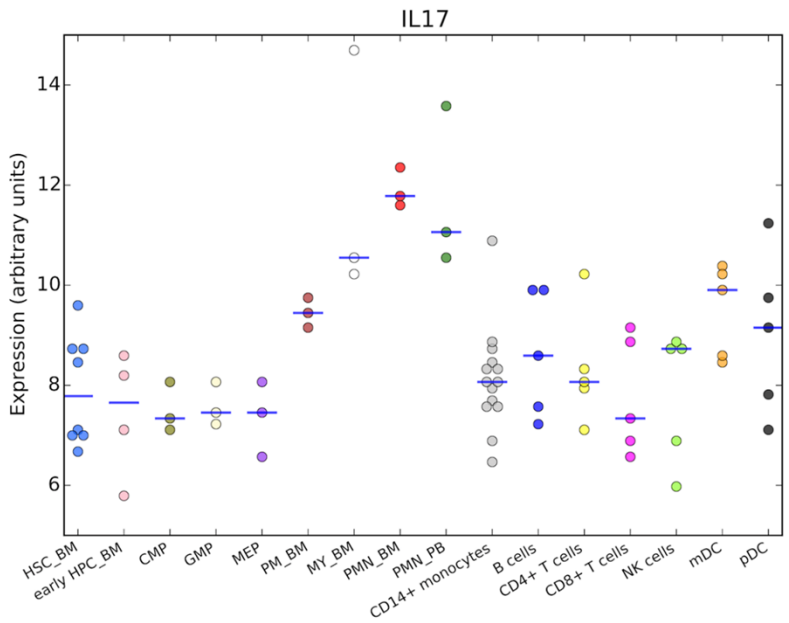

Figure 5. IL-17 mRNA gene expression profile. Each dot in the plot corresponds to expression of IL-17 in a microarray. Horizontal lines represent the median expression for each class of cells. HSC_BM, Hematopoietic stem cells from bone marrow (BM); early HPC_BM, Hematopoietic progenitor cells from BM; CMP, Common myeloid progenitor cell; GMP, Granulocyte monocyte progenitors; MEP, Megakaryocyte-erythroid progenitor cell; PM_BM, Promyelocyte from BM; MY_BM, Myelocyte from BM; PMN_BM, Polymorphonuclear cells from BM; PMN_PB, Polymorphonuclear cells from peripheral blood; CD14+ monocytes, CD14+ Monocytes; B cells, CD19+ B cells; CD4+ T cells, CD4+ T cells; CD8+ T cells, CD8+ T cells; NK cells, CD56+ natural killer cells; mDC, CD11c+ myeloid dendritic cells; pDC, CD123+ plasmacytoid dendritic cells.

reports have shown that the percentage of CD8-positive cells was elevated [8, 23], but another mentioned no changes in any kinds of lymphocytes [22]. These previous reports show that the idea of recruitment of T cells for platelet distraction in chronic ITP can be controversial. However, in our study, the expression of CD4 was decreased and those of CD3 and CD8 were not statistically different between the patients with ITP and control subjects in BM.

CD68 and CD163 expression was markedly elevated, and IL-17 was detected in these cells. This finding might be explained by a shift to a Th1-dominant state, which involved Th17. Previous studies revealed that Th17 and IL-17 positivity is positively correlated with Th1 and cytokine expression. Th1 cells are mainly involved in macrophage activation and interferon gamma (INF-γ) production [8, 20, 24]. In line with this finding, Wang et al. reported that IL-17 levels were elevated in patients with chronic ITP and positively correlated with INF-γ expression [24].

Previous studies on the involvement of monocytes or macrophages in chronic ITP are limited and not consistent. Olsson et al. reported that no statistically significant differences were found in the number of CD14+ monocytes/macrophages by flow cytometry between the BM of patients with chronic ITP and the controls [22]. On the other hand, Zhong et al. showed that a CD16+ monocyte subset was expanded, CD4+IL-17+ cell expansion was inhibited, and CD4+IFN-γ+ cell proliferation was promoted in PB of chronic ITP [25]. This result for IL-17 was not consistent with our findings. However, monocytes can differentiate into various macrophages with different potentials to influence Th differentiation [25]. In both cases involvement of CD68- or CD163-positive monocytes/macrophages was not demonstrated. Interestingly, in patients with RA, which is known as an IL-17-mediated inflammatory disease [26], CD14+ monocytes spontaneously arise from inflamed joints and specifically promote Th17 responses, but monocytes from PB did not induce Th17 responses [27]. Hence, our results showing increased expression of IL-17, which correlated with CD68 and CD163 but not with lymphocytes, suggested that mechanisms other than T-cell-mediated immunity might also be involved in the IL-17-related pathophysiology of ITP, specifically in BM, in which MgKs/platelets are produced.

Although we could not find any previous reports on IL-17-secreting CD68 or CD163-expressing cells in ITP, there is a report that shows a relationship between IL-17 and CD68-positive monocytes/macrophages by IHC. The report on inflammatory bowel disease, which is also known as a Th1- and Th17-related disease [26], found that IL-17 expression was clearly detectable in CD3-positive T cells or CD68-positive monocytes/macrophages, and IL-17 mRNA ex-

pression was also detected in those cells. It shows that monocytes/macrophages are the local source of IL-17 [28]. In addition to the findings in diseased states, human mRNA gene profiling revealed that normal monocytes/macrophages also express IL-17 *in vitro* (**Figure 5**) [29].

It remains difficult to identify which cells secrete or express IL-17 in the BM of patients with chronic ITP because we did not examine mRNA or perform double staining. However, the presence of IL-17 protein in the cytoplasm of cells with increased CD68 and CD163 expression may indicate an effect of IL-17, which would have been mediated by Th17- and/or Th1-mediated autoimmunity and direct IL-17 secretion by monocytes/macrophages. Although the shift to a Th1-dominant state may also reflect chronic inflammation as well as the involvement of inflammatory cytokines and increased numbers of monocytes/macrophages, there were no differences in the baseline data, which reflected signs of inflammation such as CRP levels and monocyte counts in the PB of patients with chronic ITP (data not shown).

In our study, the percentage of responders to Eltrombopag was lower in the IL-17-high-expression group, but the ratio of administration of Eltrombopag and response to other ITP treatment therapies were not differed. This result is similar to that of Zhong et al. that non-responders continued with increased levels of CD16+ monocytes in PB despite treatment [25]. These findings indicate that increased levels of monocytes or macrophages are one of the causes for non-response to Eltrombopag.

Moreover, we suggest a new category called macrophage-associated ITP of patients who show CD68 and/or CD163 high expressions and in whom macrophages and/or monocytes produce cytokines, including IL-17. Between the IL-17-low-expression group and IL-17-high-expression group, there was no difference in the percentages of patients who used Eltrombopag, which is administered to patients who failed in immunosuppressive treatments primarily targeting lymphocytes. In addition, steroid treatment can also suppress monocyte phagocytosis in patients with ITP [30]. This finding suggests that mechanisms other than lymphocyte-mediated immunity and phagocytosis act in the BM of patients with macrophage-associated ITP. This is where Eltrombopag regulates MgK/platelet induction. However, we could not identify any reports on the relationship between high IL-17 expression and/or monocytes/macrophages and the effectiveness of Eltrombopag. This result may also reflect that ITP associated with IL-17 mediated macrophages is more difficult to treat. Although more research is needed on the pathogenesis of ITP, our results suggest that therapies targeted for IL-17 have potential as further treatment.

This study has some limitations. First, we did not analyze other lymphoid organs, such as the spleen, in which T-cell development and proliferation occur. Second, this was a single-center retrospective study with the inherent possibility of selection bias. Third, we could not collect data from sufficient numbers of patients because not all patients with chronic ITP underwent BM examinations as recommended by the guidelines.

Few previous studies have assessed the relationship between the effectiveness of treatments and immunohistochemical changes in the BM of patients with ITP before treatment. Even though BM examination is not necessary to diagnose ITP according to the guidelines of ASH [10], we propose that BM examination and immunohistochemical staining in patients with ITP may lead to better understanding of the mechanism of ITP and identify prognostic factors associated with various treatments.

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Disclosure of conflict of interest

None.

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