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Helicobacter pylori eradication shifts monocyte Fcγ receptor balance toward inhibitory FcγRIIB in immune thrombocytopenic purpura patients

Atsuko Asahi,[1,2] Tetsuya Nishimoto,[1] Yuka Okazaki,[1] Hidekazu Suzuki,[3] Tatsuhiro Masaoka,[3] Yutaka Kawakami,[4] Yasuo Ikeda,[2] and Masataka Kuwana[1]

[1]Division of Rheumatology, [2]Division of Hematology, and [3]Division of Gastroenterology and Hepatology, Department of Internal Medicine, and [4]Institute for Advanced Medical Research, Keio University School of Medicine, Tokyo, Japan.

Immune thrombocytopenia purpura (ITP) is a bleeding disorder in which platelet-specific autoantibodies cause a loss of platelets. In a subset of patients with ITP and infected with *Helicobacter pylori*, the number of platelets recovers after eradication of *H. pylori*. To examine the role of *H. pylori* infection in the pathogenesis of ITP, the response of 34 ITP patients to treatment with a standard *H. pylori* eradication regimen, irrespective of whether they were infected with *H. pylori*, was evaluated. Eradication of *H. pylori* was achieved in all *H. pylori*–positive patients, and a significant increase in platelets was observed in 61% of these patients. By contrast, none of the *H. pylori*–negative patients showed increased platelets. At baseline, monocytes from the *H. pylori*–positive patients exhibited an enhanced phagocytic capacity and low levels of the inhibitory Fcγ receptor IIB (FcγRIIB). One week after starting the *H. pylori* eradication regimen, this activated monocyte phenotype was suppressed and improvements in autoimmune and platelet kinetic parameters followed. Modulation of monocyte FcγR balance was also found in association with *H. pylori* infection in individuals who did not have ITP and in mice. Our findings strongly suggest that the recovery in platelet numbers observed in ITP patients after *H. pylori* eradication is mediated through a change in FcγR balance toward the inhibitory FcγRIIB.

Introduction

Immune thrombocytopenia purpura (ITP) is an autoimmune disorder caused by increased platelet clearance by anti-platelet autoantibodies (1). In 1998, Gasbarrini et al. reported increase in platelet count in ITP patients infected with *Helicobacter pylori* after successful eradication of this bacterium (2). Recent accumulating evidence in Italy and Japan indicates that the eradication of *H. pylori* is effective in increasing the platelet count in nearly half of *H. pylori*–infected patients with idiopathic ITP (3, 4). In addition, a recent report showed that this platelet response lasts for years and cases of relapse are few (5). Based on its efficacy, good safety profile, and low cost, *H. pylori* eradication therapy for adult ITP is becoming very popular in several countries. Some investigators have suggested that the efficacy of *H. pylori* eradication in ITP patients may be mediated by *H. pylori*–independent mechanisms, such as immunomodulatory effects of the drugs used for the regimen (3), but we recently reported its complete lack of efficacy in *H. pylori*–uninfected ITP patients in a prospective study in which the patients were treated with a standard *H. pylori* eradication regimen irrespective of their *H. pylori* infection status (6). This finding clearly indicates that the platelet recovery observed in ITP patients after the eradication regimen results from the disappearance of *H. pylori* itself.

Several hypotheses have been proposed regarding the mechanism by which *H. pylori* might induce the development of ITP. One is that Abs to *H. pylori* components cross-react with platelet surface antigens. In this regard, Takahashi et al. reported that platelet eluates from *H. pylori*–positive ITP patients recognized the cytotoxin-associated gene A (CagA), one of the *H. pylori*–derived proteins that determine bacterial virulence (7), although another group demonstrated that platelet eluates from *H. pylori*–positive ITP patients that reacted with glycoprotein IIb/IIIa (GPIIb/IIIa) or GPIb failed to recognize *H. pylori* antigens (8). Another potential mechanism is modulation of the host's immune system by *H. pylori* in a manner that promotes the emergence of autoreactive B cells (9). However, no significant difference between *H. pylori*–positive and *H. pylori*–negative individuals has been found for non-organ-specific autoantibody responses, such as anti-nuclear, anti-microsome, or anti-smooth muscle Abs (10). Despite these findings, the role of *H. pylori* infection in the pathogenesis of ITP remains obscure. These previous studies focused on anti-platelet Ab production in association with *H. pylori* infection, but potential effects of *H. pylori* infection on the platelet clearance process in ITP patients have not been assessed. In this study, to elucidate the mechanism responsible for platelet recovery in ITP patients after the successful eradication of *H. pylori*, we conducted a prospective study in which factors potentially associated with the pathogenic processes of ITP, i.e., autoimmune responses to the major platelet antigen GPIIb/IIIa, parameters associated with platelet turnover, and the phenotypic and functional properties of phagocytes, were serially measured in ITP patients who were treated with a standard eradication regimen, irrespective of their *H. pylori* infection status.

Nonstandard abbreviations used: CagA, cytotoxin-associated gene A; FcγRIIB, Fcγ receptor IIB; GPIIb/IIIa, glycoprotein IIb/IIIa; ITP, immune thrombocytopenia purpura; RES, reticuloendothelial system; TPO, thrombopoietin.

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Table 1
Clinical characteristics of 34 ITP patients according to the presence or absence of *H. pylori* infection

Demographics and laboratory findings	*H. pylori* positive ($n = 23$)	*H. pylori* negative ($n = 11$)	*P*
Sex (% female)	52	73	NS
Age at visit (yr)	59.7 ± 11.9	48.0 ± 17.8	NS
Disease duration (yr)	7.5 ± 6.7	8.3 ± 6.6	NS
Current treatment[A]			
Prednisolone[B]	35%	45%	NS
Danazol	0%	18%	NS
Splenectomy	4%	45%	0.01
Mean platelet count ($\times 10^9$/l)	42 ± 23	31 ± 13	NS

[A]Percentages indicate the percentage of patients who were currently using the treatment. [B]Mean daily dosage in *H. pylori*–positive and –negative patients was 4.8 mg (range 3–7.5) and 4.8 mg (range 4–5), respectively. Data are group means ± SD.

Our findings demonstrate that the platelet recovery observed in ITP patients after *H. pylori* eradication is associated with modulation of the monocyte Fcγ receptor balance toward the inhibitory Fcγ receptor IIB (FcγRIIB).

Results

Patient characteristics. Thirty-four consecutive patients with ITP (14 males and 20 females, aged 24 to 73 yr) were enrolled in this open-label, prospective study. *H. pylori* infection was detected in 23 patients (68%). Comparison of the pretreatment clinical characteristics of the *H. pylori*–positive and –negative patients (Table 1) showed the *H. pylori*–positive patients tended to be older than the *H. pylori*–negative patients ($P = 0.08$). Some patients took low-dose prednisolone (≤7.5 mg daily), but there was no difference in the frequency or the mean dosage between *H. pylori*–positive and –negative patients. A history of splenectomy was significantly less frequent in the *H. pylori*–positive patients compared with the *H. pylori*–negative patients, indicating that refractory cases were more common in the *H. pylori*–negative patients.

Immunologic and platelet turnover parameters before treatment in ITP patients with and without H. pylori infection. A total of 11 immunologic and platelet turnover parameters, including anti-GPIIb/IIIa Ab-producing B cells, platelet-associated anti-GPIIb/IIIa Abs, T cell responses induced by GPIIb/IIIa and tetanus toxoid, the proportion of reticulated platelets, the circulating thrombopoietin (TPO) level, the relative expression levels of FcγRI, FcγRII (FcγRIIA plus FcγRIIB), FcγRIII, and CD86 on circulating monocytes, and the nonspecific phagocytosis of circulating monocytes were compared between ITP patients with and without *H. pylori* infection (Figure 1). We evaluated the proportion of monocytes expressing FcγRIII instead of its expression level because only a small subset of the peripheral blood monocytes expressed this FcγR.

There was no difference in the frequency of anti-GPIIb/IIIa Ab-producing B cells or platelet-associated anti-GPIIb/IIIa Ab levels between the 2 groups. In contrast, *H. pylori*–positive patients showed a more prominent T cell response to GPIIb/IIIa compared with *H. pylori*–negative patients, and there was no difference in the degree of the response to tetanus toxoid, an irrelevant foreign recall antigen. The proportion of reticulated platelets was significantly greater in the *H. pylori*–positive patients than in the *H. pylori*–negative patients. In circulating monocytes, the expression of FcγRII, including both activating FcγRIIA and inhibitory FcγRIIB, was lower in the *H. pylori*–positive patients than the *H. pylori*–negative patients. Moreover, the expression of activating FcγRI tended to be upregulated in the *H. pylori*–positive patients ($P = 0.06$). Finally, monocytes from the *H. pylori*–positive patients exhibited an enhanced phagocytic capacity compared with those from the *H. pylori*–negative patients, suggesting an activated monocyte phenotype predominated in the *H. pylori*–positive patients.

Platelet response to the H. pylori eradication regimen. All 34 patients completed a 7-day standard eradication regimen that consisted of amoxicillin, clarithromycin, and lansoprazole regardless of whether they were positive for *H. pylori* infection. Adverse events potentially related to the therapy were observed in 10 patients (29%): abdominal pain and/or diarrhea in 9 and skin rash in 1. All the symptoms resolved quickly after the regimen ended. Eradication was successful in all 23 *H. pylori*–positive patients, and 14 (61%) of them were classified as responders. In contrast, none of the 11 *H. pylori*–negative patients showed an increased platelet count. These frequencies were significantly different ($P = 0.001$). The platelet counts 0, 12, and 24 wk after initiation of the eradication regimen in *H. pylori*–positive responders and nonresponders, and in *H. pylori*–negative patients are shown in Figure 2. Some *H. pylori*–positive nonresponders showed a slight increase in platelet count, but almost no fluctuation in platelet count was observed in the *H. pylori*–negative patients. During the 24-wk period, prednisolone dosage was reduced in 2 responders at 12 wk (7.5 to 5 mg and 5 to 2.5 mg), but the stable dosage was given in the remaining patients.

Serial changes in immunologic and platelet turnover parameters after H. pylori eradication. Immunologic and platelet turnover parameters measured prior to treatment were serially examined 12 and 24 wk after initiation of the eradication regimen in the *H. pylori*–positive responders and nonresponders and in the *H. pylori*–negative patients (Figure 3). The anti-GPIIb/IIIa Ab-producing B cells and platelet-associated anti-GPIIb/IIIa Abs were significantly reduced at 12 and 24 wk in the *H. pylori*–positive responders. The proportion of reticulated platelets was also significantly reduced in the *H. pylori*–positive responders. Similar but weak trends were observed in the *H. pylori*–positive nonresponders but not in the *H. pylori*–negative patients. The peripheral blood T cell response to GPIIb/IIIa tended to be suppressed in the *H. pylori*–positive responders ($P = 0.09$), while there was no fluctuation in the response to tetanus toxoid. In monocytes from *H. pylori*–positive responders, their baseline upregulated FcγRI expression decreased and the downregulated FcγRII (FcγRIIA plus FcγRIIB) expression increased to the levels of monocytes from *H. pylori*–negative patients after *H. pylori* eradication. In contrast, the proportion of FcγRIII-positive monocytes and CD86 expression levels on monocytes were stable in all 3 groups during the study period. The enhanced phagocytic capacity of monocytes in *H. pylori*–positive responders was significantly reduced after *H. pylori* eradication, reaching the level of *H. pylori*–negative patients. These changes in the monocyte phenotype were not observed in the *H. pylori*–positive nonresponders or in the *H. pylori*–negative patients. The IgG anti-CagA Ab level gradually reduced following the eradication regimen in the *H. pylori*–positive patients irrespective of the platelet response, but samples were still positive for anti-CagA Ab even at 12 and 24 wk, when the platelet count had nearly fully recovered.

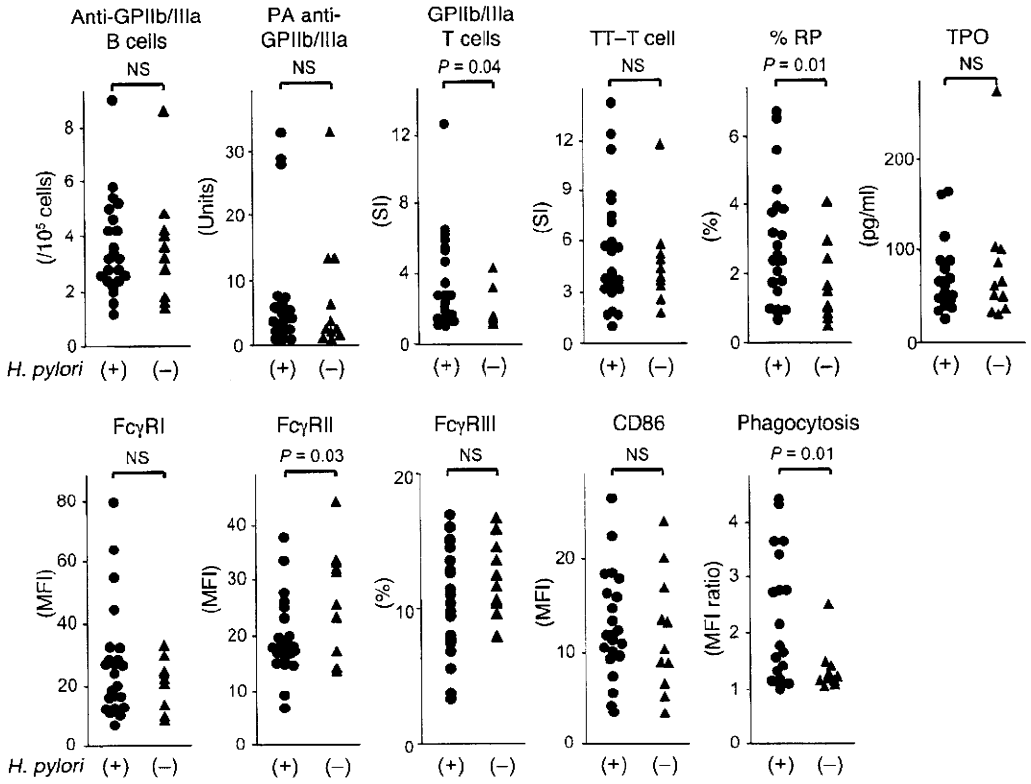

Figure 1

Immunologic and platelet turnover parameters prior to treatment in ITP patients with or without *H. pylori* infection. The anti-GPIIb/IIIa Ab–producing B cells, platelet-associated (PA) anti-GPIIb/IIIa Abs, GPIIb/IIIa-specific T cell response, tetanus toxoid–specific (TT-specific) T cell response, proportion of reticulated platelets (%RP), circulating TPO level, expression levels of FcγRI and FcγRII (FcγRIIA plus FcγRIIB) on monocytes, proportion of FcγRIII-positive monocytes, expression level of CD86 on monocytes, and nonspecific phagocytosis of monocytes were compared among 23 ITP patients infected with *H. pylori* and 11 ITP patients who were not infected with *H. pylori*. Expression of FcγRII was examined using mAb clone FLI8.26, which reacts with both FcγRIIA and FcγRIIB. The differences between the 2 groups were analyzed using the Mann-Whitney *U* test.

In the *H. pylori*–positive responders, after the eradication of *H. pylori*, the parameters for T and B cell responses to platelet antigens and platelet turnover improved, and the activated monocyte phenotype was suppressed, as the platelet count increased. To examine which parameter changed first, we further evaluated these parameters at 1 wk, when the eradication regimen had just ended, in the 14 *H. pylori*–positive responders (Table 2). The platelet count was significantly increased at 1 wk, consistent with a previous report (5). At this time point, the phagocytic capacity of monocytes was the only parameter that had changed from its pretreatment level.

In 3 *H. pylori*–positive patients who were untreated and whose platelet count was >50 × 10^9/l at study entry, 2 were responders and the remaining 1 was a nonresponder. When these 3 patients

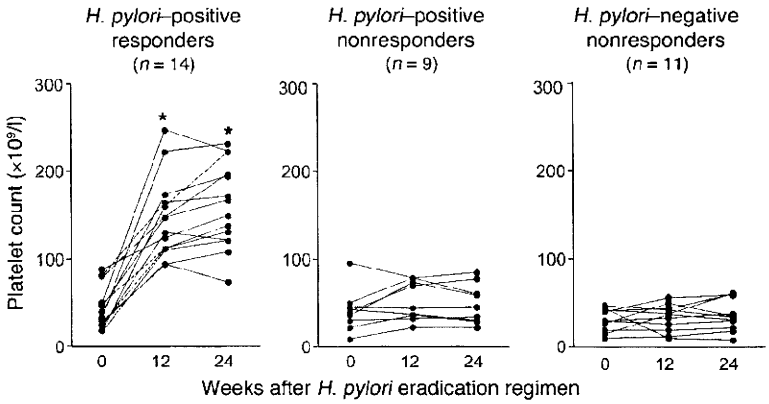

Figure 2

Serial platelet counts before and after initiation of the *H. pylori* eradication regimen in 14 *H. pylori*–positive ITP responders, 9 *H. pylori*–positive ITP nonresponders, and 11 *H. pylori*–negative ITP nonresponders. Changes in the absolute values 12 and 24 wk from the baseline value taken at wk 0 were assessed by paired *t* test. *$P < 0.01$ compared with wk 0.

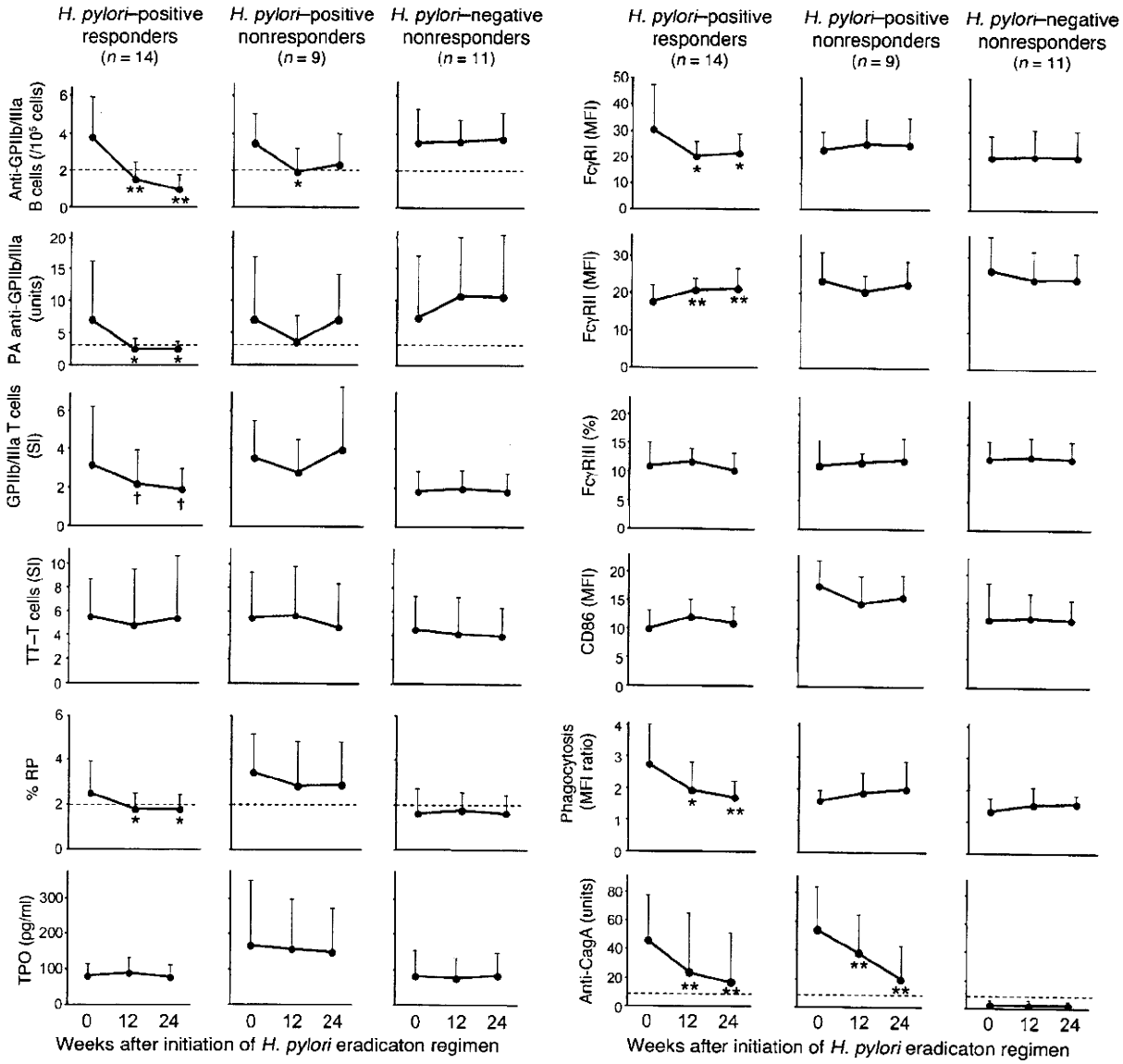

Figure 3

Serial measurements of immunologic and platelet turnover parameters before and after the *H. pylori* eradication in 14 *H. pylori*–positive ITP responders, 9 *H. pylori*–positive ITP nonresponders, and 11 *H. pylori*–negative ITP nonresponders. Anti-GPIIb/IIIa Ab–producing B cells, platelet-associated anti-GPIIb/IIIa Abs, GPIIb/IIIa-specific T cell response, tetanus toxoid–specific T cell response, proportion of reticulated platelets, circulating TPO level, expression levels of FcγRI and FcγRII (FcγRIIA plus FcγRIIB) on monocytes, proportion of FcγRIII-positive monocytes, expression level of CD86 on monocytes, nonspecific phagocytosis of circulating monocytes, and anti-CagA Ab levels were measured prior to treatment and 12 and 24 wk after initiation of the *H. pylori* eradication regimen. Expression of FcγRII was examined using mAb clone FLI8.26, which reacts with both FcγRIIA and FcγRIIB. Results are shown as the mean + SD. Changes in the values at 12 and 24 wk from the baseline value measured at wk 0 were assessed by paired *t* test. Dotted lines indicate the cut-off values: 2.0 for anti-GPIIb/IIIa Ab–producing B cells; 3.3 U for platelet-associated anti-GPIIb/IIIa Abs; 2.0% for % RP; and 7.5 U for anti-CagA Ab. †$P < 0.1$, *$P < 0.05$, and **$P < 0.01$ compared with wk 0.

were excluded from the analysis, concordant results regarding changes in the monocyte phenotype were obtained.

Change in the FcγRIIA/IIB balance of monocytes after H. pylori eradication. In humans, 2 types of FcγRII are expressed on monocytes: an activating receptor, FcγRIIA, and an inhibitory receptor, FcγRIIB (11). Because the extracellular domains of FcγRIIA and FcγRIIB are highly homologous, we measured the mRNA expression levels of FcγRIIA and FcγRIIB on sorted monocytes independently, by quantitative PCR, using specific primers for the intracellular domains of these FcγRs. The results revealed that the FcγRIIA/IIB mRNA expression ratio was significantly higher in the *H. pylori*–positive than the *H. pylori*–negative patients prior to treatment (Figure 4A). In the *H. pylori*–positive responders, the FcγRIIA/IIB ratio was significantly decreased at 1 and 12 wk, but this change was not observed in the *H. pylori*–positive nonresponders or in the *H. pylori*–negative patients (Figure 4B). When the protein expression

Table 2
Serial measurement of ITP-related parameters before and 1 wk after the *H. pylori* eradication regimen in 14 *H. pylori*–positive responders

ITP-related parameter	Prior to treatment	1 wk after treatment	*P*
Platelet count (×10⁹/l)	42.9 ± 23.6	68.6 ± 32.8	0.003
Anti-GPIIb/IIIa Ab–producing B cells (/10⁵ PBMCs)	3.8 ± 2.1	3.6 ± 1.7	NS
Platelet-associated anti-GPIIb/IIIa Abs (U)	7.1 ± 9.2	7.1 ± 8.7	NS
GPIIb/IIIa-induced T cell response (SI)	3.4 ± 3.2	3.5 ± 3.4	NS
Tetanus toxoid–induced T cell response (SI)	5.7 ± 3.3	5.4 ± 4.1	NS
Reticulated platelets (%)	2.4 ± 1.6	2.6 ± 3.0	NS
Plasma TPO (pg/ml)	79.1 ± 40.5	78.2 ± 40.7	NS
FcγRI expression on monocytes (MFI)	31.0 ± 21.7	27.1 ± 20.2	NS
FcγRII expression on monocytes (MFI)[A]	17.2 ± 4.8	18.1 ± 3.7	NS
Proportion of FcγRIII-positive cells (%)	10.9 ± 4.0	11.0 ± 3.0	NS
CD86 expression on monocytes (MFI)	9.8 ± 3.5	10.1 ± 5.4	NS
Non-specific phagocytosis of monocytes (MFI ratio)	2.8 ± 1.5	1.8 ± 0.5	0.008

[A]Expression of FcγRII was examined using mAb clone FLI8.26, which reacts with both FcγRIIA and FcγRIIB. Results shown are the mean ± SD. SI, stimulation index.

of FcγRIIB on monocytes was examined by flow cytometry using mAbs specific to the intracellular domain of this molecule, FcγRIIB expression was increased at 12 wk in a representative *H. pylori*–positive responder but not in a nonresponder (Figure 4C). As a result, FcγRIIB expression was significantly upregulated at 12 wk in *H. pylori*–positive responders, but this change was not observed in the *H. pylori*–positive nonresponders or in the *H. pylori*–negative patients (Figure 4D). These findings indicate that a change in the FcγR balance toward the inhibitory FcγRIIB, together with a diminished phagocytic capacity of the monocytes, is the first event that occurs as a result of the *H. pylori* eradication in responders.

We further examined a potential role of Th1/Th2 balance in regulating FcγR expression profiles on monocytes in association with *H. pylori* infection (11), but there was no substantial change in the ratio of IFN-γ⁺CD4⁺ T cells to IL-4⁺CD4⁺ T cells in circulation prior to treatment and 12 wk after initiation of the eradication regimen in the *H. pylori*–positive responders (2.6 ± 1.3 and 2.7 ± 1.3, respectively), the *H. pylori*–positive nonresponders (3.1 ± 0.6 and 3.7 ± 1.0, respectively), or the *H. pylori*–negative patients (3.4 ± 1.1 and 3.5 ± 1.5, respectively).

Parameters that predict the platelet response to H. pylori eradication. To examine which parameters predict the platelet response to the eradication of *H. pylori* in ITP patients, patient characteristics, the immunologic and platelet turnover parameters listed in Figure 1, as well as the IgG anti-CagA Ab level and FcγRIIA/IIB mRNA expression ratio in monocytes at pretreatment were compared between the *H. pylori*–infected responders and nonresponders. The FcγRII expression level on monocytes was significantly lower in the responders than in nonresponders (17.2 ± 4.8 versus 23.4 ± 8.2 MFI, $P = 0.03$). In addition, the phagocytic capacity and FcγRIIA/IIB expression ratios of the monocytes were significantly higher in the responders than in the nonresponders (2.8 ± 1.5 versus 1.3 ± 0.3, $P = 0.02$; and 1.7 ± 0.6 versus 1.1 ± 0.4, $P = 0.04$).

Phenotypic and functional properties of monocytes before and after H. pylori eradication in non-ITP subjects. To evaluate whether changes in the phenotypic and functional properties of circulating monocytes after the eradication of *H. pylori* were specific to ITP patients, 9 non-thrombocytopenic volunteers infected with *H. pylori* were treated with the standard eradication regimen. Eradication was successful in 7 subjects, but not in 2. As expected, there was no substantial change in the platelet count during the observation period in any subject. In monocytes from the successfully treated individuals, there were trends toward downregulated FcγRI expression and phagocytic capacity and toward upregulated FcγRII (FcγRIIA plus FcγRIIB) expression 12 wk after the eradication, as observed in the *H. pylori*–infected ITP responders, but these changes were not statistically significant (Figure 5). The lack of significance appears to be due to the highly variable responses among individuals. It is of note that the subjects with an activated monocyte phenotype, i.e., high FcγRI expression, low FcγRII expression, and enhanced phagocytic capacity, at pretreatment were more likely to respond with improvements in these conditions upon the eradication of *H. pylori*.

FcγR phenotype on monocytes/macrophages in H. pylori–infected and uninfected mice. We prepared 4 groups of mice ($n = 5$ in each group): *H. pylori*–infected mice, uninfected mice, *H. pylori*–infected mice that had received eradication treatment, and uninfected mice that had received eradication treatment. In mice, there is only one FcγRII, which corresponds to human FcγRIIB. As shown in Table 3, the expression of FcγRII measured using mAb clone 2.4G2 on the monocytes/macrophages derived from peripheral blood, bone marrow, and spleen was significantly lower in mice infected with *H. pylori* than in uninfected mice, while the FcγRI expression tended to be higher in mice infected with *H. pylori* ($P < 0.1$). Both the infected and uninfected mice were given the triple *H. pylori* eradication regimen, and successful eradication was confirmed in all the infected mice by bacterial culture of the stomach at 6 wk. In the monocytes/macrophages derived from the peripheral blood, bone marrow, and spleen from these *H. pylori*–eradicated mice, the FcγRII expression was upregulated and FcγRI expression was downregulated after the eradication. Since mAb clone 2.4G2 cross-reacts with FcγRIII, peripheral blood samples were further examined using a mAb specific to FcγRII (clone Ly17.2) (12). The FcγRII expression level was again significantly lower in *H. pylori*–infected mice compared with mock-treated mice (3.2 ± 0.4 versus 3.9 ± 0.2 MFI, $P = 0.01$). In *H. pylori*–infected mice, the FcγRII expression level was increased after the eradication treatment (3.2 ± 0.4 versus 3.9 ± 0.3 MFI, $P = 0.02$).

Discussion

In this comprehensive analysis, we demonstrated that the platelet recovery observed in a subset of *H. pylori*–infected ITP patients after *H. pylori* eradication is likely to be mediated through a change in the FcγR balance on monocytes/macrophages. An expression level of FcγRII on monocytes was first identified as one of factors associated with *H. pylori* infection in ITP patients by flow cytometric analysis using a mAb that reacts with both FcγRIIA and FcγRIIB, but this association was later shown to be primarily attributable to inhibitory FcγRIIB based on 2 different assays: quantitative PCR

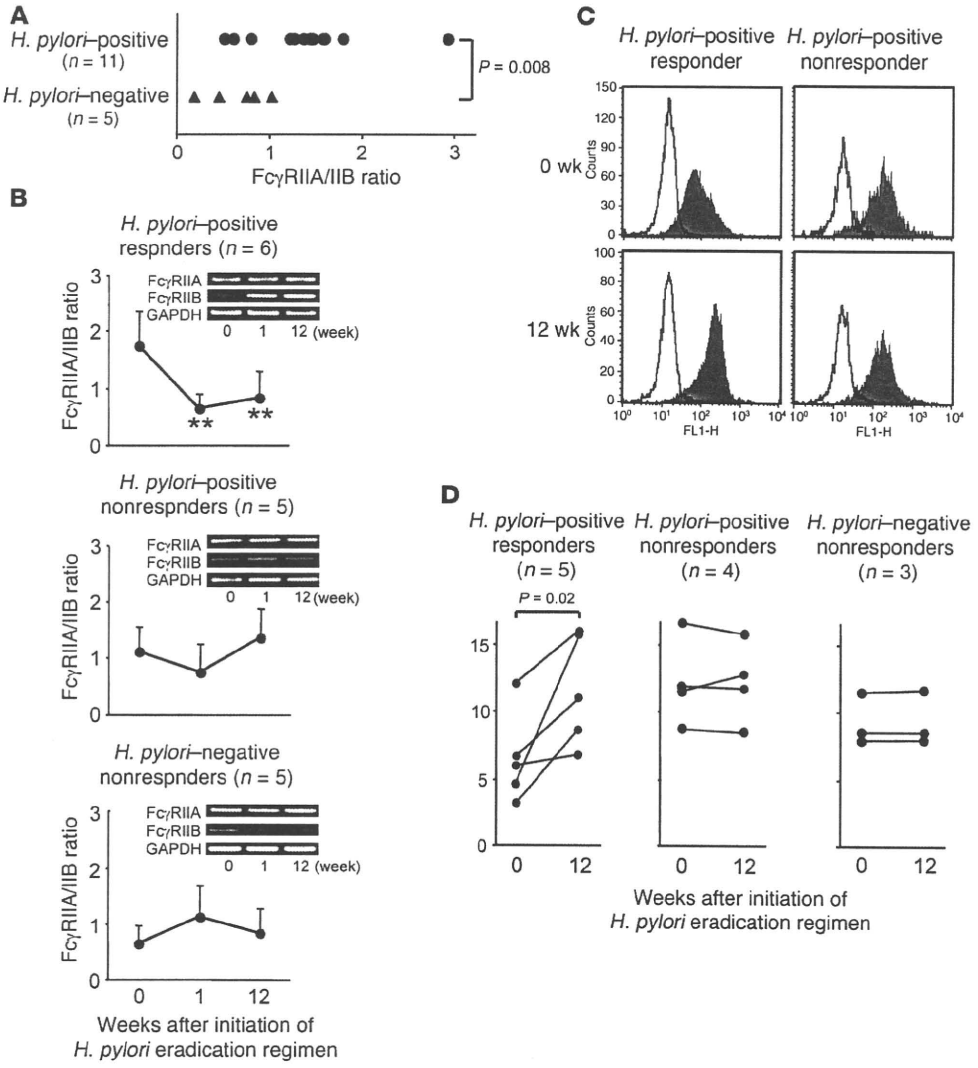

Figure 4

Change in FcγRIIB expression levels in circulating monocytes from ITP patients before and after initiation of the *H. pylori* eradication. (**A**) FcγRIIA/IIB mRNA expression ratio on monocytes prior to treatment was determined by quantitative TaqMan PCR in 11 ITP patients infected with *H. pylori* and 5 uninfected ITP patients. The difference between the 2 groups was analyzed using the Mann-Whitney *U* test. (**B**) The FcγRIIA/IIB expression ratio on monocytes was serially measured prior to treatment and at 1 and 12 wk after initiation of the *H. pylori* eradication regimen in 6 *H. pylori*–positive ITP responders, 5 *H. pylori*–positive ITP nonresponders, and 5 *H. pylori*–negative ITP nonresponders. Results are shown as the mean + SD. Changes in the values at 1 and 12 wk from the baseline value taken at wk 0 were assessed by paired *t* test. $^{**}P < 0.01$ compared with pretreatment. Representative RT-PCR results for the expression of FcγRIIA, FcγRIIB, and GAPDH are shown for each patient group. (**C**) The protein expression of FcγRIIB on monocytes prior to treatment and 12 wk after initiation of the *H. pylori* eradication regimen in a representative *H. pylori*–positive responder and nonresponder. Open histograms show the cells stained with isotype-matched control Ab, and closed histograms show anti-FcγRIIB mAb–treated cells. (**D**) Expression levels of FcγRIIB on monocytes were measured prior to treatment and 12 wk after initiation of the *H. pylori* eradication regimen in 5 *H. pylori*–positive ITP responders, 4 *H. pylori*–positive ITP nonresponders, and 3 *H. pylori*–negative ITP nonresponders.

for assessment of mRNA expression and flow cytometric analysis using a mAb specific to the intracellular domain of FcγRIIB. The functional properties of monocytes/macrophages, including phagocytosis and antigen presentation, are controlled by the balance between activating FcγRs and an inhibitory receptor, FcγRIIB (13). Circulating monocytes from *H. pylori*–infected ITP patients exhibited an activated phenotype with enhanced phagocytic capacity, which potentially resulted from downregulated FcγRIIB, and this phenotype reverted to that of *H. pylori*–uninfected ITP patients after the eradication of *H. pylori*, but only in the responders. In addition, this change in monocyte phenotype preceded the improvements in autoimmune and platelet kinetic parameters. Therefore, *H. pylori* infection plays an important role in ITP pathogenesis by altering the FcγR balance of monocytes/macrophages in favor of activating FcγRs, through downregulation of the inhibitory receptor FcγRIIB.

Our recent studies on T cells that were autoreactive to GPIIb/IIIa in ITP patients revealed that the pathogenic process of ITP can be explained by a continuous loop in which B cells produce IgG anti-platelet autoantibodies, macrophages in the reticuloendothelial system (RES) phagocytose opsonized platelets via FcγRs and present GPIIb/IIIa-derived antigenic peptides, and GPIIb/IIIa-reactive T cells are activated and exert their helper activity (14, 15). It has been shown that anti-GPIIb/IIIa Abs in ITP patients are predominant of the IgG1 subclass (16), which has the highest affinity for FcγRII (13). Thus, FcγRs expressed on macrophages in the RES apparently play a central role in 2 aspects of the pathogenic process: platelet destruction and sustained autoimmune responses to the platelet antigens. It is still debated which activating FcγR is predominantly involved in ITP pathogenesis (17), but the increase in the expression of inhibitory FcγRIIB relative to the activating FcγRs could eventually result in the attenuation of the pathogenic continuous loop. Therefore, platelet recovery mediated through the upregulated FcγRIIB expression on monocytes after the eradication of *H. pylori* in ITP patients involves 2 consequent

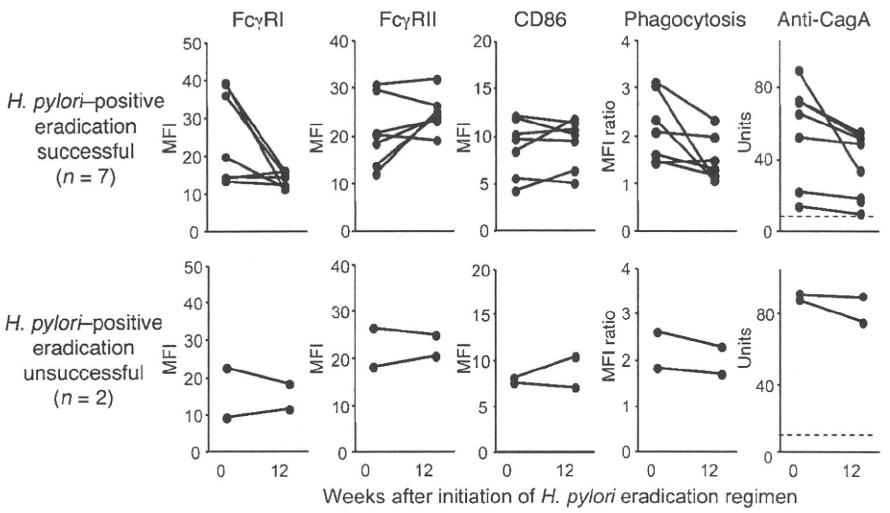

Figure 5
Serial measurements of the phenotype of circulating monocytes and the anti-CagA Ab level before and after initiation of the *H. pylori* eradication regimen in 9 non-ITP individuals infected with *H. pylori* according to the outcome of the regimen. Expression levels of FcγRI, FcγRII (FcγRIIA plus FcγRIIB), and CD86, non-specific phagocytosis of circulating monocytes, and anti-CagA Ab level were measured prior to treatment and 12 wk after initiation of the *H. pylori* eradication regimen. Expression of FcγRII was examined using mAb clone FLI8.26, which reacts with both FcγRIIA and FcγRIIB. Results for 7 individuals in which *H. pylori* was successfully eradicated and for 2 individuals in whom the eradication failed are shown separately. A dotted line indicates the cut-off for anti-CagA Abs (7.5 U).

steps: the rapid platelet increase observed at 1 wk is the result of the blockade of platelet clearance by macrophages in the RES, while the sustained platelet increase results from the suppression of antigen presentation by macrophages and the subsequent inhibition of T and B cell responses to platelet antigens. To assess the role of *H. pylori* infection on platelet clearance, it is interesting to evaluate whether *H. pylori* infection boosts platelet destruction in mice treated with platelet-depleting mAbs. A similar suppressive effect on the FcγRIIB expression on macrophages is reported for intravenous immunoglobulin (IVIG). Samuelsson et al. demonstrated that IVIG requires the presence of FcγRIIB to prevent thrombocytopenia in a murine model of passive ITP (18). In this model, FcγRIIB expression on macrophages was upregulated upon IVIG treatment.

The pathogenesis of ITP in association with *H. pylori* infection is most likely multifactorial. Our mechanism does not exclude other proposed mechanisms for platelet recovery after eradication of *H. pylori* in ITP, such as molecular mimicry between CagA and platelet surface antigens (7). Moreover, it has been shown that some strains of *H. pylori* induce platelet aggregation that is dependent on von Willebrand factor and IgG Abs specific for *H. pylori* interacting with their corresponding receptors GPIb and FcγRIIA on platelets (19). In this model, Abs specific for *H. pylori* are capable of opsonizing platelets through binding to *H. pylori*, von Willebrand factor, and GPIb, as are anti-platelet autoantibodies. Thus, decrease in *H. pylori*–specific Ab levels after the eradication treatment may also suppress presentation of platelet antigens by macrophages. These Ab-mediated mechanisms appear to play a role in the later phase of platelet recovery after the *H. pylori* eradication, since the change in monocyte phenotype preceded the improvements in the Ab responses to GPIIb/IIIa and CagA.

Platelet recovery after the eradication of *H. pylori* is observed only in a subgroup of *H. pylori*–infected ITP patients (3, 4). Our results here indicate that ITP patients with an activated monocyte phenotype, such as an enhanced phagocytic capacity and reduced FcγRIIB expression, are likely to respond to *H. pylori* eradication, suggesting that the activated monocyte/macrophage phenotype contributes to driving the pathogenic loop that maintains the anti-platelet autoimmune condition in such responders. This finding is clinically important, since an activated monocyte phenotype can predict platelet recovery after the eradication of *H. pylori* in ITP patients. The activation status of circulating monocytes was also heterogeneous in non-ITP individuals infected with *H. pylori*, and subjects with an activated monocyte phenotype were responsive to *H. pylori* eradication, which changed the FcγR balance. Thus,

Table 3
Expression levels of FcγRI and FcγRII on monocytes/macrophages in uninfected mice and mice infected with *H. pylori* after the eradication regimen

		FcγRI			FcγRII[A]		
Source of monocytes	***H. pylori* infection**	**Mice without eradication treatment**	**Mice receiving eradication treatment**	***P***	**Mice without eradication treatment**	**Mice receiving eradication treatment**	***P***
Peripheral blood	–	234.7 ± 42.9	227.4 ± 13.7	NS	241.8 ± 46.1[B]	241.0 ± 78.5	NS
	+	270.3 ± 29.6	194.4 ± 35.5	0.05	106.6 ± 32.4[B]	195.0 ± 26.8	0.006
Bone marrow	–	79.4 ± 26.3	74.8 ± 28.3	NS	343.4 ± 37.1[C]	361.9 ± 37.0	NS
	+	116.9 ± 28.4	66.6 ± 13.5	0.04	228.6 ± 64.0[C]	306.0 ± 18.2	0.03
Spleen	–	54.1 ± 17.0	47.2 ± 9.1	NS	451.5 ± 10.9[C]	483.5 ± 140.7	NS
	+	101.1 ± 39.2	42.0 ± 7.7	0.04	245.5 ± 35.4[C]	369.2 ± 45.0	0.001

[A]Measured using mAb clone 2.4G2, which cross-reacts with FcγRIII. [B]$P < 0.05$ and [C]$P < 0.01$ for the comparison between *H. pylori*–infected and –uninfected groups. –, not infected; +, infected.

whether *H. pylori* infection activates the host's monocytes/macrophages differs among individuals independently of the presence or absence of ITP. There is great variability in the efficacy of the *H. pylori* eradication therapy in ITP patients among ethnic groups. Cohorts from Japan and Italy report response rates of 39%–100%, while those from Spain and the United States document little to no platelet response (20). The reason for such variability among countries is not clear, but this could be explained by differences in epidemic *H. pylori* strains or genetic backgrounds among populations. In this regard, *H. pylori* genotypes were different between the eastern Asian strain and the European strain (21). In addition, genetic factors that regulate the expression of FcγRs may contribute to this difference. Several polymorphisms exist within the human FcγR genes that exhibit altered affinities for IgG, potentially resulting in specific allele-dependent clearance rates of immune complexes (22). In particular, one SNP within the FcγRIIB gene alters its receptor signaling (23, 24) and predicts the development of chronic disease in children with acute ITP (25). Another polymorphism in the promoter region of the FcγRIIB gene downregulates its transcriptional activity (26).

Potential patient selection bias is one of the limitations of this study. Because our hospital is a referral center, many patients had relatively long disease duration and had been treated with prednisolone and/or splenectomy. In addition, our patients were a relatively mild subset, since we excluded patients with active bleeding to minimize dropouts during the study period. We have to consider this potential bias upon interpreting the results, but there was no apparent difference in patient characteristics between *H. pylori*–infected responders and nonresponders. In addition, the high frequency of splenectomized patients in the *H. pylori*–negative group might affect the phenotypic and functional properties of circulating monocytes, although there was no apparent difference in the FcγR expression levels and the phagocytic capacity of monocytes among the *H. pylori*–negative ITP patients regardless of their history of splenectomy (data not shown). Another limitation is the use of peripheral blood monocytes instead of macrophages in the RES in the analysis. However, in mice, *H. pylori* infection changed the FcγR balance similarly in monocytes/macrophages derived from peripheral blood, bone marrow, and spleen, suggesting that circulating monocytes potentially represent the whole monocyte/macrophage system.

The modulation of the monocyte FcγR balance associated with *H. pylori* infection was detected in ITP and non-ITP individuals as well as in mice, indicating that this response might be a normal host immune response against microorganisms that establish chronic infection lasting several decades. It is known that *H. pylori* colonizes the mucous layer of the stomach and does not invade the gastric epithelium, but *H. pylori* infection induces local and systemic responses through stimulation of the innate and adaptive immune systems (27). The molecular events that induce the change in the properties of monocytes/macrophages still remain unclear. We failed to show the role of Th1 response related with *H. pylori* infection in the monocyte phenotype change. On the other hand, it is reported that components of *H. pylori* are released and are responsible for the activation of dendritic cells and macrophages through toll-like receptor signaling (28, 29). In this regard, high frequencies of some specific *H. pylori* genotypes are reported in ITP patients compared with non-ITP subjects (5), suggesting potential roles for the bacterium's structure and virulence profiles in the pathogenesis of ITP.

Previous studies demonstrated a causal link between infection and autoimmune diseases, such as intestinal infections by *Campylobacter jejuni* and Guillian-Barré syndrome. In this case, Abs cross-reacting with lipooligosaccharides present in *C. jejuni* and in ganglioside GM1 are responsible for development of this disease (30). On the other hand, in ITP patients, *H. pylori* infection enhanced immunogenicity of platelet-specific autoantigens secondary to infection-mediated FcγR balance change of macrophages. A similar mechanism is shown in the animal model for Coxsackie B4 virus–induced type I diabetes (31), in which the virus is thought to act by increasing immunogenicity of autoantigens secondary to local inflammation. In addition, infections may also protect from autoimmune diseases. Onset of type I diabetes was inhibited by helminth parasite infection in animal models (32). A recent randomized, double-blind, placebo-controlled trial demonstrated improvement of active ulcerative colitis by infection with *Trichuris suis* (33). Effects of infectious agents on the pathogenesis of autoimmunity appear to be variable among diseases, and underlying mechanisms are multiple and complex, probably different according to pathogens.

In summary, *H. pylori* infection plays an important role in the pathogenesis of ITP by altering the FcγR balance of monocytes/macrophages in favor of activating FcγRs, through the downregulation of FcγRIIB. The efficacy and safety profiles of the *H. pylori* eradication make this regimen an attractive option, but it is indicated only for *H. pylori*–infected patients in certain ethnic groups. The mechanism that induced platelet recovery after *H. pylori* eradication reported here indicates that the FcγR balance on monocytes/macrophages may be a reasonable therapeutic target for ITP. In this case, small molecules that inhibit the downstream signal of activating FcγR could have beneficial effects in ITP patients (34). Further studies to evaluate how *H. pylori* infection modulates monocyte/macrophage function may be useful for understanding the pathogenesis of ITP and for developing new therapeutic strategies for ITP.

Methods

Patients and controls. This was a single-center, open-label, prospective study involving consecutive 34 patients with ITP. The diagnosis of ITP was based on thrombocytopenia persisting longer than 6 mo, normal or increased bone marrow megakaryocytes without morphologic evidence of dysplasia, and no secondary immune or non-immune disease that could account for the thrombocytopenic state (35). All the patients were referred to Keio University Hospital, Tokyo, Japan, and had been treated for at least 6 mo. Platelet counts were $<100 \times 10^9$/l during the preceding 3 mo. The exclusion criteria included age (< 18 yr), active bleeding, pregnancy or lactation, a history of potential adverse effects associated with penicillins, current or previous treatment with proton pump inhibitors, or other debilitating illness (e.g., cancer). Nine healthy volunteers infected with *H. pylori* served as control subjects. The study protocol conformed to the ethical principles of the World Medical Association Declaration of Helsinki as reflected in a priori approval from the Keio University Institutional Review Board, and written informed consent was obtained from each participant.

Assessment of H. pylori infection. To evaluate *H. pylori* infection, we performed a ^{13}C urea breath test using a UBiT tablet (Otsuka Assay), analyzed serum IgG *H. pylori*–specific Abs using a commercially available kit (Kyowa Medex Company), and analyzed *H. pylori* antigen in stool samples using ImmunoCard STAT! HpSA (Meridian Bioscience Inc.), in all patients (36). Patients positive for the urea breath test plus at least one additional test were regarded as *H. pylori* positive, whereas those negative for all 3 tests

were considered *H. pylori* negative. To minimize false-negative results, patients had not received antacids or antibiotics for at least 2 wk before the tests. Successful eradication was defined as a negative result for the urea breath test 12 wk after the eradication regimen.

H. pylori eradication regimen and follow-up. All patients and controls were given amoxicillin (750 mg twice daily), clarithromycin (400 mg twice daily), and lansoprazole (30 mg twice daily) for 7 d regardless of their *H. pylori* infection status and were then followed for the subsequent 24 wk. The patients were allowed to continue their other therapies during the study period, provided the dosages of drugs were maintained at a constant level until the study was completed, except for prednisolone, which could be decreased or discontinued. A therapeutic response was defined as a platelet count higher than 50×10^9/l and doubling of the baseline at 24 wk after initiation of the eradication regimen.

Cell preparation. Heparinized peripheral blood samples were obtained from all subjects at 0 (pre-treatment), 12, and 24 wk, and from some patients at 1 wk, after initiation of the eradication regimen. After the platelet-rich plasma was isolated, the residual cell components were subjected to Lymphoprep (Nycomed Pharma AS) density gradient centrifugation to isolate the PBMCs. Freshly isolated PBMCs were resuspended in RPMI 1640 containing 10% heat-inactivated FBS, 2 mmol/l L-glutamine, 50 U/ml penicillin, and 50 μg/ml streptomycin and were immediately used in the following experiments. Platelet-poor plasma was isolated from platelet-rich plasma by centrifugation.

Evaluation of anti-GPIIb/IIIa Ab response. B cells producing IgG anti-GPIIb/IIIa Abs were detected using the enzyme-linked immunospot assay, as previously described (37). Each experiment was conducted in 5 independent wells, and the results represent the mean of the 5 values. The frequency of circulating anti-GPIIb/IIIa Ab–producing B cells was calculated as the number per 10^5 PBMCs, and the cut-off value was defined as 2.0 cells. IgG anti-GPIIb/IIIa Abs in platelet eluates (from 5×10^7 platelets) were measured by ELISA using purified human GPIIb/IIIa as the antigen (38). Ab units were calculated from the optical density at 450 nm (OD_{450}) results, based on a standard curve obtained from serial concentrations of pooled plasma with high-titer IgG anti-GPIIb/IIIa Abs. All samples were examined in duplicate, and the results were calculated as the mean of the 2 values. The cut-off for platelet-associated anti-GPIIb/IIIa Abs was 3.3 U.

Evaluation of T cell response to GPIIb/IIIa. The antigenic specificity of T cells was determined by antigen-induced T cell proliferation as previously described (39). Briefly, PBMCs were cultured in the presence or absence of antigen for 7 d. After a final 16-h incubation with 0.5 μCi/well ^{3}H-thymidine, the cells were harvested, and the ^{3}H-thymidine incorporation was determined in a TopCount microplate scintillation counter (Packard). Antigens used were trypsin-digested GPIIb/IIIa, mock-treated PBS, and tetanus toxoid (List Biological Laboratories) used at a concentration of 5 μg/ml. All the cultures were prepared in triplicate, and all the values represent the mean of triplicate determinations. Antigen-specific T cell response was expressed as the stimulation index, which was calculated as the cpm incorporated into cultures with trypsin-digested GPIIb/IIIa or tetanus toxoid divided by the cpm incorporated into cultures with mock treatment with PBS.

Evaluation of platelet turnover. Reticulated platelets were detected by staining freshly isolated platelets with thiazole orange (Retic-COUNT; Becton Dickinson) followed by flow cytometric analysis, as described previously (40). The cut-off for the percentage of reticulated platelets was 2.0%. The plasma TPO level was measured using a commercially available ELISA kit (Quantikine; R&D Systems) according to the manufacturer's protocol.

Evaluation of phenotypic and functional properties of circulating monocytes. Unfixed PBMCs were stained with FITC-conjugated anti-FcγRI/CD64 (clone 10.1), anti-FcγRII/CD32 (clone FLI8.26), anti-FcγRIII/CD16 (clone 3G8) (BD Biosciences), or anti-CD86 mAbs (clone BU63; Ancell), in combination with PC5-conjugated anti-CD14 mAb (clone RMO52; Beckman Coulter). For FcγRIIB staining, the cells were fixed and permeabilized using a buffer containing paraformaldehyde and saponin (BD Biosciences) and subsequently stained with mAbs to the C-terminal portion of FcγRIIB (clone EP888Y; Epitomics), followed by incubation with FITC-labeled anti-rabbit IgG $F(ab')_2$ (Beckman Coulter) and PC5-conjugated anti-CD14 mAb. The negative controls were cells incubated with fluorescent-labeled isotype-matched mouse mAbs against an irrelevant antigen. The cells were analyzed on a FACSCalibur flow cytometer (BD Biosciences) using CellQuest software. Viable cells were selected by the exclusion of apoptotic cells stained with propidium iodide (Sigma-Aldrich). Relative expression levels of FcγRI, FcγRII (FcγRIIA plus FcγRIIB), FcγRIIB, FcγRIII, and CD86 on gated $CD14^+$ monocytes were expressed as MFI, which was calculated based on the intensity of the cells incubated with appropriate isotype-matched control mAb as a reference. The proportion of cells positive for FcγRIII in $CD14^+$ monocytes was also determined. Each assay included a quality control PBMC sample derived from a single healthy donor, which had been stored in aliquots at –80°C. The nonspecific phagocytic capacity of monocytes was examined as described previously (41). In brief, PBMCs were cultured with neutral FITC-dextran with a molecular weight of 2,000 kDa (Sigma-Aldrich) for 30 min at 0°C or 37°C and analyzed on a flow cytometer. The uptake of FITC-labeled dextran was evaluated by MFI on gated $CD14^+$ monocytes. The phagocytic capacity was expressed as an MFI ratio, which was calculated as the MFI obtained at 37°C divided by the MFI at 0°C. Consistent settings for detector sensitivity, compensation, and scatter gating were used in the analyses of all the samples.

Th1/Th2 balance. Proportions of IFN-γ^+ cells and IL-4^+ cells in peripheral blood $CD4^+$ T cells were evaluated using flow cytometry–based Intracellular Cytokine Staining Kit Human (BD Biosciences) according to the manufacturer's protocol. The results were expressed as the ratio of IFN-γ^+CD4^+ T cells to IL-4^+CD4^+ T cells.

Anti-CagA Abs. Anti-CagA Abs of the IgG isotype were measured in duplicate in plasma samples using a commercially available ELISA kit (ravo Diagnostika). The cut-off of 7.5 U was based on the manufacturer's information.

Gene expression of FcγRIIA and FcγRIIB on monocytes. Circulating $CD14^+$ monocytes were isolated from PBMCs using an anti-CD14 mAb coupled to magnetic beads (Miltenyi Biotech) followed by MACS column separation. Flow cytometric analysis revealed that the sorted fractions contained >95% $CD14^+$ cells. Total RNA was extracted from monocytes using the RNeasy kit (Qiagen), and first-strand cDNA synthesized from the total RNA was subjected to PCR using a panel of specific primers (FcγRIIA: sense primer, 5′-CTGACTGTGCTTTCCGAATG-3′, and antisense primer, 5′-TGGATGAGAACAGCGTGTAG-3′; FcγRIIB: sense primer, 5′-ACAAGCCTCTGGTCAAGGTC-3′, and antisense primer, 5′-TTCCCTGCACTCAGGGTATC-3′; and GAPDH: sense primer, 5′-TGAACGGGAAGCTCACTGG -3′, and antisense primer, 5′-TCCACCACCCTGTTGCTGTA-3′). The PCR products were resolved by electrophoresis on 2% agarose gels and visualized by ethidium bromide staining. In addition, mRNA expression levels were quantitatively assessed using the TaqMan real-time PCR system (Applied Biosystems). A combination of primers and a probe specific for human FcγRIIA and FcγRIIB was purchased from Applied Biosystems. The relative expression levels were calculated from a standard curve generated by plotting the amount of PCR product against the serial amount of input PBMC cDNA, and the FcγRIIA/IIB ratio was calculated from the relative expression levels in the same sample.

Infection and eradication of H. pylori in mice. Six-week-old, specific pathogen–free male mice (C57BL/6; Sankyo Lab Service) were given irradiated food and autoclaved distilled water ad libitum. The Sydney strain of *H. pylori* SS1, which was grown at 37°C under microaerobic conditions, was used for oral inoculation, as described previously (42, 43). Suspensions of

H. pylori (2×10^7 CFU/ml, 15 ml/kg) were administered to mice after overnight starvation, twice with a 2-wk interval, while control mice received suspensions of buffer solution alone (mock infection). Twelve weeks later, half of the mice in the *H. pylori*–inoculated group and the control group were sacrificed for examination. The remaining *H. pylori*–inoculated and control mice were treated with the *H. pylori* eradication treatment consisting of lansoprazole (10 mg/kg), amoxicillin (3 mg/kg), and clarithromycin (30 mg/kg) suspended in 0.5% carboxymethyl cellulose sodium salt solution, once a day for 2 d (44). Six weeks later, all the mice were sacrificed for examination. *H. pylori* infection in the excised stomach was evaluated by microaerobic bacterial culture, as previously described (45). Mononuclear cells were isolated from the peripheral blood, spleen, and bone marrow and stored at −80 °C, and all samples were subjected to flow cytometric analysis on the same day. Cells were incubated with a Cy5-labeled anti-mouse CD11b mAb (clone M1/70; Beckman-Coulter) in combination with a biotin-labeled anti-mouse FcγRI polyclonal Ab (R&D Systems) or a rat mAb reactive with both mouse FcγRII (clone 2.4G2; BD Biosciences – Pharmingen) and biotin-labeled anti-rat IgG (Jackson ImmunoResearch Laboratories Inc.), followed by treatment with FITC-streptavidin (Beckman Coulter). In some instances, we used a mouse mAb specific to FcγRII (clone Ly17.2) (Sloan-Kettering Cancer Institute) in combination with FITC-labeled anti-mouse IgG $F(ab')_2$. All mouse procedures were approved by the Keio University Animal Research Committee.

Statistics. All continuous values are shown as the mean ± SD. Comparisons to determine the statistical significance between 2 groups were performed using the Fisher's Exact test or Mann-Whitney *U* test, as appropriate. Changes in the absolute values at different time points from the baseline value taken at week 0 were compared by paired *t* test.

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Address correspondence to: Masataka Kuwana, Division of Rheumatology, Department of Internal Medicine, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan. Phone: 81-3-3350-3567; Fax: 81-3-3350-3567; E-mail: kuwanam@sc.itc.keio.ac.jp.

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Clinical utility of anti-signal recognition particle antibody in the differential diagnosis of myopathies

S. Suzuki[1], T. Satoh[2], S. Sato[2], M. Otomo[3], Y. Hirayama[4], H. Sato[1], M. Kawai[3], T. Ishihara[5], N. Suzuki[1] and M. Kuwana[2]

Objective. Auto-antibodies to signal recognition particle (SRP) are known to be specific to PM among rheumatic disorders, but the specificity in myopathic diseases remains unclear. The clinical utility of anti-SRP antibody in the differential diagnosis of myopathies has not been studied. The aim of the present study was to elucidate whether detection of anti-SRP antibody can discriminate of PM from muscular dystrophy (MD).

Methods. We report a patient with a childhood onset myopathy, in whom it was clinically difficult to make a differential diagnosis of PM or MD for 21 yrs, despite repeated muscle biopsies. Myositis-specific auto-antibodies to RNA-associated antigens were screened in this particular case as well as in 105 serum samples from various types of MD and 84 from PM patients using RNA immunoprecipitation. The MD and PM serum samples were obtained from different institutions. The presence of anti-SRP antibody was confirmed by RNA immunoprecipitation combined with immunodepletion of SRP from the antigen.

Results. Anti-SRP antibody was positive in the present patient, supporting the diagnosis of PM. Anti-SRP antibody was detected in seven (8.3%) patients with PM, but in none of the patients with MD. Myositis-specific auto-antibodies were not detected in any of the patients with MD.

Conclusion. Anti-SRP antibody is useful for discriminating PM from MD among patients with myopathies.

KEY WORDS: Anti-signal recognition particle antibody, Polymyositis, Muscular dystrophy.

Introduction

The inflammatory myopathies are a heterogeneous group of systemic diseases characterized by muscle weakness, elevated serum creatine kinase (CK) values, electromyographic abnormalities and inflammatory infiltrates in skeletal muscle [1]. Myositis-specific auto-antibodies include those directed against aminoacyl-tRNA synthetases (ARS), signal recognition particle (SRP) and nuclear helicase Mi-2. Anti-Jo-1 antibody, one of the anti-ARS antibodies, is closely related to PM and DM with a high frequency of intestinal lung disease (ILD) [2]. On the other hand, anti-SRP antibody is clinically associated with pure PM [3–6]. SRP, one of the most abundant and best characterized RNP particles, regulates the translocation of proteins across the endoplasmic reticulum during protein synthesis [3, 4]. Patients with anti-SRP antibodies most often present with severe muscle involvement characterized by rapidly developing proximal weakness that culminates in severe disability, and often by a poor response to steroid therapy [6].

Muscular dystrophy (MD), a group of hereditary and sporadic progressive diseases, each with unique phenotypic and genetic features, is the most common and representative myopathy [7]. It is sometimes difficult to distinguish anti-SRP-positive PM patients from MD patients for the following reasons [8]. First, since anti-SRP-positive PM patients have a low incidence of pulmonary fibrosis, skin rash, arthritis and RP, it is difficult to differentiate them from MD patients based on the clinical manifestations alone. Second, severe necrosis of skeletal muscle without lymphocytic infiltration is a histological finding common to both anti-SRP-positive PM and MD [9, 10]. In similar clinical settings, it has not been elucidated whether the detection of auto-antibodies using RNA immunoprecipitation is reliable for the differential diagnosis of myopathies, especially between MD and PM. To address this question, we screened the serum of MD patients and PM patients for myositis-specific auto-antibodies, including anti-SRP antibody.

Patients, materials and methods

Case report

A 32-yr-old Japanese man was admitted to Keio University Hospital in 2004 for evaluation of long-standing myopathy. He had a 21-yr history of severe weakness in the trunk, arms and legs, but no familial history of neuromuscular disorders. He was normal until the age of 10 yrs of age, when he had difficulty in running fast and hanging from a horizontal bar. When he first visited the other hospital at age 11 yrs, he showed scapulohumeral dominant muscle atrophy without facial muscle involvement. Serum CK was 4180 IU/l (normal, <198 IU/l), and the electromyography showed myopathic features. The first muscle biopsy of the left biceps brachii muscle revealed a prominent variation in muscle fibre size, but no perifascicular atrophy. Necrotic muscle fibres were observed with evidence of regeneration, but there was no lymphocytic infiltration in the perimysial or perivascular region. Endomysial connective tissue was increased (Fig. 1A and B). Under the tentative diagnosis of scapulohumeral MD, oral predonisolone (1 mg/kg/day) therapy was prescribed for 3 months, but the patient did not respond to the treatment. His weakness worsened over the next 2 yrs, and he eventually lost ambulation and had difficulty in blowing, swallowing and eating at the age of 13 yrs. After the bulbar symptoms continued for ~6 months, his symptoms gradually began to improve. However, he could not walk and required a wheelchair. The serum CK levels decreased to 1640 IU/l at the age of 14 yrs and 614 IU/l at the age of 16 yrs. The second muscle biopsy of the quadriceps femoris muscle was performed at 16 yrs of age. The muscle fibres had largely been

[1]Department of Neurology, [2]Division of Rheumatology, Department of Internal Medicine, Keio University School of Medicine, Tokyo, [3]Department of Neurology, Higashisaitama National Hospital, Saitama, [4]Department of Pediatric Neurology, Tokyo Metropolitan Higashiyamato Medical Center for the Severely Disabled, Tokyo and [5]Department of Neurology, Hakone National Hospital, Kanagawa, Japan.

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Correspondence to: S. Suzuki, Department of Neurology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan. E-mail: shigeaki@sc.itc.keio.ac.jp

replaced by adipose tissue, but there still was no lymphocytic infiltration (Fig. 1C). There have been no changes in his muscle weakness since then.

The neurological examination on admission revealed severe symmetrical proximal-dominant weakness, with Medical Research Council scale grade at 3/5 in the lower extremities and 4/5 in the upper extremities, but there was no facial muscle involvement. Muscle atrophy was remarkable in the lower trunk and proximal muscles of the legs. Deep tendon reflex was absent. The CK levels had retuned to the normal range. T_1-weighted muscle magnetic resonance images of the thighs showed evidence of severe diffuse muscle atrophy.

Patients and sera

Serum samples were obtained from *the patient* described above, and from 105 Japanese patients with MD (82 males and 23 females) who were seen consecutively between January 2005 and June 2006 at Higashisaitama National Hospital. The mean age of the MD patients at the time of serum collection was 30.3 ± 17.2 yrs. The MD cases included those with Duchenne MD ($n = 58$), Becker MD ($n = 6$), myotonic MD ($n = 19$), limb-girdle MD ($n = 6$), facioscapulohumeral MD ($n = 4$), Fukuyama-type congenital MD ($n = 7$) and unclassified types of MD ($n = 5$), and the diagnosis of each was based on clinical, pathological and genetic features [7]. In contrast, stored serum for a cohort of 84 Japanese patients with PM (25 males and 59 females) was available at Keio University Hospital. All sera were obtained at diagnosis before initiation of immunosuppresive therapy. The mean age of the PM patients at the time of serum collection was 51.4 ± 13.4 yrs. The diagnosis of probable or definite PM was based on the criteria of Bohan and Peter [11]. Patients with DM, IBM and overlap syndrome were excluded from study entry. We obtained MD and PM serum samples from different institutions because most MD patients, especially those with Duchenne MD, stayed at the national hospital and sanatorium for the severely disabled. All blood samples and clinical information were obtained after patients provided informed consent and ethical approval. The study was approved by the institutional review boards.

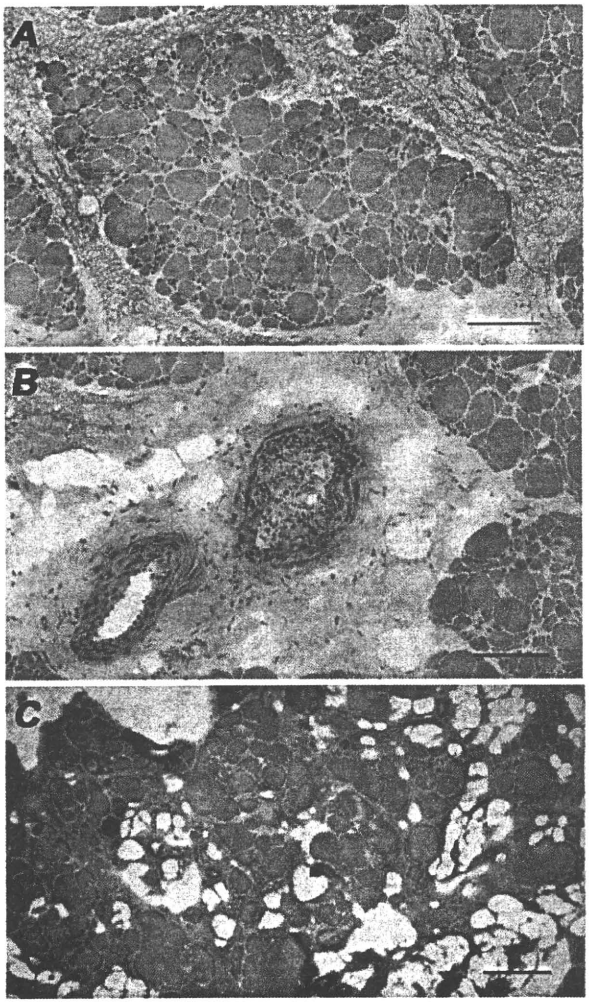

FIG. 1. Histological findings of the present patient. The histological appearance of muscle biopsy specimens obtained at 11 yrs of age (**A** and **B**) and 16 yrs of age (**C**). Haematoxylin–eosin staining. Bar = 100 μm.

RNA immunoprecipitaion

RNA immunoprecipitation assay was performed using extracts from leukaemia cell line K562, as previously described [12]. Briefly, a 10 μl volume of patient serum was mixed with 2 mg of protein A-Sepharose CL-4B (Pharmacia Biotech AB, Uppsala, Sweden) in 500 μl of immunoprecipitation buffer (10 mM Tris–HCl, pH 8.0, 500 mM NaCl, 0.1% Nonidet P40), and after incubation for 2 h, was washed three times with immunoprecipitation buffer. Antibody-bound Sepharose beads were mixed with 100 μl of K562 cell extract (6×10^6 cell equivalents per sample) for 2 h, and 30 μl of 3 M sodium acetate, 30 μl of 10% SDS and 300 μl of phenol:chloroform:isoamyl alcohol (50:50:1, containing 0.1% 8-hydroxyquinoline) were added to extract bound RNA. After ethanol precipitation, the RNA was resolved on a 7 M urea–10% polyacrylamide gel, and the gel was silver-stained (Bio-Rad, Hercules, CA, USA).

Immunodepletion experiments

The immunodepletion studies were undertaken using prototype sera obtained from two anti-SRP-positive PM patients (patients #1 and #2). A 50 μl volume of the prototype serum #2 and normal serum as a negative control was mixed with 10 mg of protein A-Sepharose CL-4B in 500 μl of immunoprecipitation buffer, and incubated for 2 h. After washing three times with immunoprecipitation buffer, the antibody-bound Sepharose beads were mixed with 100 μl of K562 cell extract for 2 h to fully the deplete antigens recognized by prototype serum #2 or normal serum. Then, the supernatant was further incubated with Sepharose beads preconjugated with the present patient serum and the prototype serum #1. After washing for five times, the RNA immunoprecipitation assay was analysed as described above.

Statistic analysis

The frequencies of MD patients and PM patients who were positive for each auto-antibody were compared. Categorical variables were compared by the χ^2-test.

Results

Our patient's serum immunoprecipitated RNA located in the 7S RNA lesion, as did the prototype serum #1 obtained from an anti-SRP-positive PM patient (Fig. 2, lanes 2 and 5). Immunodepletion treatment with the other prototype #2 serum obtained from an anti-SRP-positive PM patient successfully depleted the SRP-antigen complex from the extract (lane 3), but normal serum did not (lane 4). Immunoprecipitation of 7S RNA in our patient was cancelled when SRP-antigen depleted extract was used (lane 6). These findings confirmed that our patient's serum was positive for anti-SRP antibody.

We screened auto-antibodies to RNA-associated auto-antigens in sera from 105 MD patients and 84 PM patients by the RNA

immunoprecipitation assay. The frequencies of myositis-specific auto-antibodies detected by RNA immunoprecipitation in MD patients and PM patients are summarized in Table 1. Anti-SRP antibodies were detected in 7 PM patients (8.3%), but in none of the MD patients ($P=0.008$). Similarly, auto-antibodies to ARS, such as those to Jo-1, PL-7, PL-12, EJ and OJ, were detected in PM patients, but not in MD patients. Myositis-specific auto-antigens were not detected in MD patients.

Table 2 shows the clinical and histological findings of the present case and for seven PM patients with anti-SRP antibodies. The eight PM patients with anti-SRP antibodies had common clinical features including muscle weakness, levels of serum CK > 3000 IU/l (except for one case) and myopathic findings in the EMG. Although three patients had ILD, none had skin rash, arthritis or RP. Malignancy was found in two patients. Response to high-dose steroid therapy was generally poor. Most patients showed refractory muscle weakness and required other immunosuppressive agents. Histological findings disclosed severe necrosis with regeneration of skeletal muscle in all patients, and lymphocytic infiltration in half.

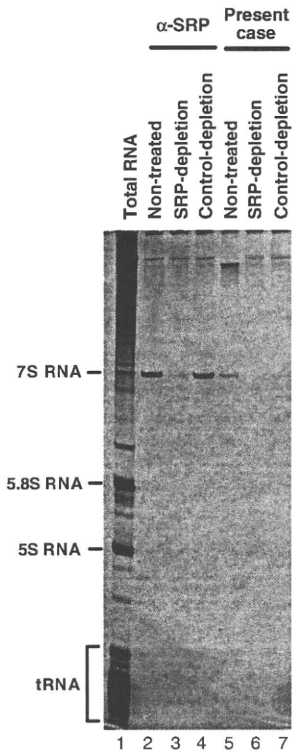

FIG. 2. Analysis of immunoprecipitates from K562 cell extracts by 7 M urea–10% PAGE and silver staining. The results of the prototype serum #1 of the anti-signal recognition particle (SRP)-positive PM patient (lanes 2–4) and the present patient serum (lanes 5–7) are compared. Immunoprecipitates were obtained from no treatment (lanes 2 and 5), after immunodepletion using prototype serum #2 from an anti-SRP-positive patient (lanes 3 and 6), and serum from a healthy control (lanes 4 and 7).

Discussion

In this article, we describe a case of myopathy with childhood onset, in whom it was difficult to make a differential diagnosis between PM and MD despite repeated muscle biopsy. The patient was found to be positive for anti-SRP antibody 21 yrs after onset. Screening the serum of MD patients and PM patients by RNA immunoprecipitation assay for auto-antibodies revealed anti-SRP antibodies in seven (8.3%) PM patients, but not in MD patients. However, the comparison of frequencies of these auto-antibodies may not be appropriate, since the MD and PM patients were selected from medical centres with different settings. Anti-SRP antibody was found to be specific to PM and useful in excluding MD in patients with myopathies.

Anti-SRP antibody was detected in 8.3% in our cohort of Japanese PM patients, similar to the rate in European patients [13]. Although anti-SRP antibody is known to be PM-specific, it has also been found in patients with DM, IBM and SSc [6, 13]. However, the specificity of anti-SRP antibody in myopathic diseases is not fully elucidated. Perurena *et al.* [14] and Hengstman *et al.* [15] reported that anti-SRP antibody was negative in 17 and 48 patients with MD, respectively. Combining these results with our findings, anti-SRP antibody is not exclusively present in a total of 170 patients with MD. These findings suggest that production of anti-SRP antibody does not result from non-specific muscle injuries.

Although muscle biopsy is the most accurate method of establishing a diagnosis of PM, but its interpretation is sometimes difficult [1]. van der Meulen *et al.* [16] have reported that PM is an overdiagnosed entity using strict diagnostic criteria of muscle biopsy. To avoid misdiagnosis of PM, Dalakas and Hohlfeld [1]

TABLE 1. Frequencies of myositis-specific auto-antibody detection by RNA immunoprecipitation in patients with MD and those with PM

Auto-antibodies	MD ($n=105$)	PM ($n=84$)	*P*-value
Anti-SRP, *n* (%)	0	7 (8.3)	0.008
Anti-ARS (anti-Jo-1), *n* (%)	0	17 (20.2)	<0.0001
Anti-ARS (non-anti-Jo-1), *n* (%)	0	10 (11.9)[a]	0.0009

[a]Auto-antibodies to PL-7 were found in three, PL-12 in two, EJ in four, and OJ in one.

TABLE 2. Clinical and histological findings from PM patients with anti-SRP antibodies

Findings	Presen case	#1	#2	#3	#4	#5	#6	#7
Onset age/gender	11/M	39/M	41/F	48/F	53/F	55/M	66/M	82/M
Muscle weakness	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Muscle atrophy	(+)	(−)	(−)	(−)	(−)	(−)	(−)	(+)
ILD	(−)	(+)	(+)	(−)	(−)	(−)	(+)	(−)
DM rash	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)
Arthritis	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)
RP	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)
CK (IU/l)	4180	6400	569	3670	6471	3477	8594	15 880
Myopathic change in EMG	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Malignancy	(−)	(−)	(−)	(−)	(+)	(−)	(−)	(+)
Treatment response	None	Partial	Partial	None	Partial	Partial	Partial	Partial
Muscle biopsy								
Small size of myofibre	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Lymphocyte infiltration	(−)	(+)	(−)	(+)	(+)	(−)	(+)	(−)
Necrosis	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Regeneration	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Perifascular atrophy	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)

have advocated the use of new histological criteria based on $CD8^+$ and MHC-I immunohistochemistry. Examination of the muscle biopsy specimens obtained from anti-SRP-positive patients have usually shown active necrosis and little or no inflammation [9, 10], and the same histological findings were observed in our patient. Based on the histological findings alone, anti-SRP-positive myopathy may be included in a different category from 'histological' PM. In fact, some investigators have considered that anti-SRP antibody is associated with a 'necrotizing myopathy' that differs from 'histological' PM [8–10]. The clinical diagnosis of PM, however, is not based on histological findings alone. The diagnostic criteria proposed by Bohan and Peter [11] are known to be clinically practical, sensitive, and specific, and they have served the community well for nearly three decades [17]. We therefore conclude that 'anti-SRP-positive' should be classified as a particular form of PM.

We concluded that our patient had anti-SRP-positive 'probable PM'. Anti-SRP-positive PM usually affects middle-aged people, however, and our patient is the youngest reported in the literature [5, 6, 8–10]. More importantly, anti-SRP antibody is associated with treatment-resistant and refractory PM, although the clinical features of anti-SRP-positive PM patients show heterogeneity and some of them have a favourable prognosis [9]. It should be realized that anti-SRP-positive PM has sometimes been misdiagnosed as other types of myopathies [8].

Rheumatology key messages

- Anti-SRP antibody is useful for differential diagnosis of myopathies.
- Anti-SRP-positive PM has sometimes been misdiagnosed as other types of myopathies.

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Review Article

Autoantibodies to platelets: Roles in thrombocytopenia

Masataka Kuwana

Division of Rheumatology, Department of Internal Medicine, Keio University School of Medicine, Tokyo, Japan

Circulating platelets are targeted by autoantibodies in various pathologic conditions, such as immune thrombocytopenic purpura (ITP). Anti-platelet antibodies cause thrombocytopenia through enhanced platelet clearance via Fcγ receptor-mediated platelet destruction by the reticuloendothelial system and impaired platelet production. Moreover, functional blockade of platelet surface receptors by autoantibodies may further promote bleeding tendency. The major targets of these autoantibodies are platelet membrane glycoproteins, including GPIIb/IIIa and GPIb/IX, receptors for fibrinogen and other platelet-activating ligands, but some patients with ITP have antibodies to a receptor for thrombopoietin, which is a growth factor required for megakaryocytogenesis and platelet production. Several antigen-specific assays have been developed to measure anti-glycoprotein antibodies, whereas we have recently established an enzyme-linked immunospot assay for the detection of circulating B cells secreting IgG anti-GPIIb/IIIa antibodies, which is a sensitive, specific, and convenient method for evaluating the presence or absence of ITP. Production of pathogenic anti-platelet antibodies is maintained by a continuous loop, in which B cells produce anti-platelet antibodies, antibody-coated platelets are phagocytosed and GPIIb/IIIa-derived cryptic peptides presented by splenic macrophages, and GPIIb/IIIa-reactive $CD4^+$ T cells exert their helper activity. Important discoveries on cellular and molecular mechanisms for anti-platelet autoantibody production contribute to development of diagnostic assays and therapeutic strategies for ITP.

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*Correspondence should be addressed to:
Masataka Kuwana, MD, PhD, Division of Rheumatology, Department of Internal Medicine, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan. Phone: +81-3-3350-3567, Fax: +81-3-3350-3567, e-mail address: kuwanam@sc.itc.keio.ac.jp

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Introduction

Platelets are a common target of autoantibody responses in a variety of pathologic conditions, including immune thrombocytopenic purpura (ITP), bacterial and viral infections, and drug-induced thrombocytopenia. ITP is an acquired hemorrhagic condition of accelerated platelet consumption caused by anti-platelet autoantibodies[1]. This condition is seen in patients with various diseases, such as systemic lupus erythematosus (SLE) and

human immunodeficiency virus infection, and can also occur without an underlying disease, in which case it is known as idiopathic form of ITP. The classic studies of Harrington and coworkers provided the first evidence for the existence of a serum anti-platelet factor in ITP patients in 1951.These investigators infused plasma from ITP patients into healthy volunteers or patients with inoperable malignant neoplasms, resulting in a marked and transient thrombocytopenia in many recipients[2]. The factor responsible for platelet destruction was subsequently shown to be present within the immunoglobulin fraction, and this activity was cancelled by preincubation of plasma with normal platelets. These early studies demonstrated the role of anti-platelet autoantibodies in the thrombocytopenic state[3]. Until now, a variety of platelet membrane glycoproteins (GPs), including GPIIb/IIIa and GPIb/IX, have been shown to be targets recognized by anti-platelet autoantibodies[4]. In addition, we have recently found that some patients with ITP have autoantibody to thrombopoietin (TPO) receptor, which suppresses megakaryocyte differentiation and platelet production[5]. This review summarizes autoantibodies to a series of platelet autoantigens and their roles in pathophysiologies of the autoimmune thrombocytopenic state.

Pathogenic roles of anti-platelet autoantibodies

1)Enhanced platelet clearance

Anti-platelet antibodies bind to circulating platelets, resulting in Fcγ receptor-mediated platelet destruction by the reticuloendothelial system[1]. Antibody-coated platelets may be destroyed by complement-mediated lysis, but the clinical response of most ITP patients to a monoclonal antibody to the Fcγ receptor[6] suggests that the Fcγ receptor-mediated mechanism is more important. Both *in vitro* and clinical studies have shown that the spleen is the dominant organ for the clearance of antibody-coated platelets, while hepatic clearance predominates in a minority of patients[7].

2)Defective platelet function

Since platelet GPs targeted by autoantibodies are involved in platelet activation, anti-platelet antibodies may affect platelet function and, rarely, mimic thombasthenia or Bernard-Soulier syndrome. In fact, there was a case report of the patient with a normal platelet count who had clinically significant bleeding with defective platelet aggregation[8]. This patient had a high titer anti-GPIIb/IIIa antibody, which blocked the binding of fibrinogen to this complex. The predominant autoantibody subclass was IgG4 with low affinity to Fcγ receptors and complement. This functional impairment may promote bleeding tendency in patients with ITP.

3)Impaired platelet production

Recent studies suggest that some anti-platelet autoantibodies also affect platelet production. In the 1980s, it was shown that platelet turnover in the majority of ITP patients was either normal or reduced rather than increased as would be expected, suggesting either inhibition of megakaryocytopoiesis or destruction of megakaryocytes in bone marrow[9]. Circulating TPO levels, which is regulated by a 'sponge effect', meaning it is controlled solely by binding to its receptor mainly expressed on bone marrow megakaryocytes and their precursors[10], are normal or slightly elevated in ITP patients, suggesting a normal or decreased megakaryocyte mass[11]. In recent *in vitro* study, plasma antibody containing anti-GPIIb/IIIa antibodies from 12 of 18 adults with severe ITP inhibited maturation of hematopoietic stem cells into megakaryocytes[12]. An ultrastructural study of bone marrow from ITP patients showed that 78% were morphologically abnormal, manifesting mitochondrial swelling with cytoplasmic vacuolization, distention of the demarcation membranes, and chromatin condensation within the nucleus, all of which are features of paraapoptosis[13]. These findings together indicate that impaired platelet production induced by anti-platelet autoantibodies in some ITP patients is mediated through two distinct processes: suppression of megakaryocytopoiesis and megakaryocyte damage.

Platelet autoantigens

In 1975, Dixon and colleagues showed that platelets from ITP patients had an elevated level of platelet-associated IgG (PAIgG)[14]. Subsequent studies, however, showed that PAIgG was also increased to some extent in many patients with non-immune thrombocytopenia and was therefore too non-specific for its measurement to be clinically useful. This is because normal platelets contain two distinct pools of IgG, one located on the surface as a form complexed with Fcγ receptors and the other located in the intracellular α-granules[15]. Later, IgG eluted from ITP platelets was shown to bind to normal platelets, but not to platelets from patients with Granzmann thrombasthenia, who genetically lack GPIIb/IIIa on platelet surface[16]. This was the first evidence for autoantibodies to the platelet surface GP. Table 1 lists platelet surface autoantigens, most of which are platelet membrane GPs. Anti-GP antibodies induce thrombocytopenia, primarily by enhancing platelet clearance through opsonization of circulating platelets. Since platelet GPs are not only expressed on platelets, but also present on the surface of magakaryocytes and their precursors, anti-GP antibodies also suppress platelet production.

Table 1 Targets of anti-platelet autoantibodies

Platelet antigen	CD nomenclature	Molecular weight (kDa)	Ligand	Frequency in ITP patients (%)
GPIIb/IIIa	CD41/61	145/110	Fibrinogen, collagen (I), fibronectin, vitronectin	50-90
GPIb/IX	CD42abc	170/17	vWF	30-80
GPV	CD42d	82	vWF	~20
GPIa/IIa	CD49b/29	160/138	Collagen (I)	~20
GPIV	CD36	88	Collagen (I), thrombospondin	~10
TPO receptor	CD110	71	TPO	10-20

1)GPIIb/IIIa

GPIIb/IIIa, also designated CD41/CD61or αIIbβ3 integrin, is specific to the magakaryocyte lineage, including platelets. GPIIb and GPIIIa are major platelet membrane proteins and make up of ~17% of the total platelet membrane protein mass. These two subunits form a calcium-dependent, non-covalently bound complex. In resting platelets, GPIIb/IIIa exists in a low-affinity state and does not bind its ligands. During platelet activation, a conformational change results in the exposure of the binding site for a variety of ligands, most notably fibrinogen, which allows firm adhesion to the extracellular matrix and aggregation[17]. GPIIb/IIIa is the most common target recognized by anti-platelet autoantibodies in ITP patients; the frequency ranged from 50 to 90%, while GPIIb/IIIa is also targeted by antibodies found in alloimmune thrombocytopenia and drug-induced thrombocytopenia. Anti-GPIIb/IIIa antibodies in ITP patients mainly recognize cation-dependent conformational epitopes located at extracellular structure of the complex. These epitopes are localized to the region close to the ligand-binding site in GPIIb and/or the structure that requires discontinuous amino acids from both GPIIb and GPIIIa[18]. On the other hand, plasma samples from some ITP patients have antibodies reactive with intracellular epitopes of GPIIIa, which are considered non-pathogenic and produced secondary in response to massive platelet destruction[19].

Several antigen-specific assays have been developed to measure autoantibodies that recognize one or more platelet surface GPs[4]. These monoclonal antibody-based assays include immunobead assay and monoclonal antibody-specific immobilization of platelet antigens (MAIPA) assay. Using these assays, platelet-associated anti-GPIIb/IIIa antibodies can be demonstrated in about 50-60% of ITP patients, but specificity was relatively high (78-93%) when patients suspected of having ITP are compared with healthy individuals or patients with non-immune thrombocytopenia. Thus, a positive antigen-specific assay provides confirmatory evidence for the diagnosis in patients suspected of having ITP while a negative test does not rule it out. Moreover, the presence or absence of platelet-associated anti-GPIIb/IIIa antibodies has prognostic significance[20]. However, inter-laboratory standardization of platelet antigen-specific assays has been difficult to achieve[21]. In these assays, it is necessary to use platelets, instead of serum or plasma, as the source of the antibodies, because the majority of pathogenic anti-platelet antibodies are present as platelet-associated antibodies. For this reason, these assays require complicated procedures, such as platelet solubilization, and a relatively large blood sample, especially from patients with a low platelet count. These limitations have prevented the assays to be routinely used in clinical laboratories. We have recently established an enzyme-linked immunospot (ELISPOT) assay for detection of circulating B cells secreting IgG anti-GPIIb/IIIa antibodies[22]. This assay is shown to be a sensitive, specific, and convenient method for evaluating the presence or absence of ITP. We have recently conducted a prospective study to identify initial laboratory findings that are useful for predicting a diagnosis of ITP, and identified increased anti-GPIIb/IIIa antibody-producing B cells and platelet-associated anti-GPIIb/IIIa antibody, elevated proportion of reticulated platelets, and normal or slightly increased circulating TPO[23]. Based on these findings, we have proposed diagnostic criteria for ITP that depend solely on non-invasive laboratory tests using peripheral blood samples[24].

2)GPIb/IX and GPV

GPIb/IX, a receptor for von Willebrand factor (vWF), is the second major GP complex on platelets, and unique to platelets and megakaryocytes. GPIb, which is composed of a heavy chain

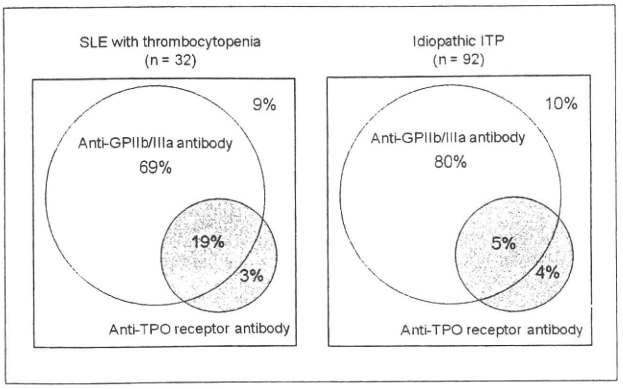

Fig.1 Distribution of anti-GPIIb/IIIa and anti-TPO receptor antibodies in patients with SLE and thrombocytopenia (left) and in those with idiopathic ITP (right).

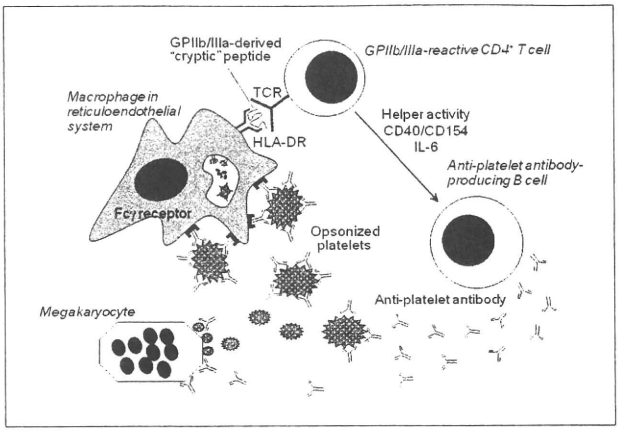

Fig.2 Schematic representation of pathophysiology of ITP and a continuous pathogenic loop carried out by macrophages in the reticuloendothelial system, GPIIb/IIIa-reactive CD4+ T cells, and anti-platelet antibody-producing B cells that maintains anti-platelet antibody production. TCR = T-cell receptor.

Ibα and a light chain Ibβ, is non-covalently associated with GPIX. The majority of GPIb/IX molecules on the platelet surface are present in association with GPV. All four subunits belong to the leucine-rich GP family. An extracellular domain of GPIbα, named glycocalicin, is cleaved off by a Ca^{2+}-dependent protease calpain, and present in circulation. Thrombus formation mediated by high hemodynamic shear stress is mediated primarily by the binding of GPIb/IX/V to immobilized vWF, resulting in a complex series of events that include platelet adhesion, activation, and aggregation. GPIb/IX is the second common target recognized by anti-platelet autoantibodies in ITP patients; the frequency ranged from 30 to 80%, while anti-GPV antibodies occur in 10% to 20% of patients with ITP[4]. Platelet-associated and plasma antibodies to GPIb/IX and GPV are detectable by monoclonal antibody-based assays. The majority of sera and platelet eluates positive for anti-GPIb/IX antibodies reacted with glycocalicin[25]. The epitopes on this extracellular region are thought to be conformational, but one of them has been mapped at a linear amino acid sequence of GPIbα. Platelet-associated antibodies reactive with GPV, but not with GPIb/IX, were frequently detected in rheumatoid arthritis patients with gold-induced thrombocytopenia[26].

3)Other platelet GPs

A small proportion of anti-platelet antibodies are shown to recognize GPIa/IIa or GPIV, which are highly expressed on platelets, but also in several other cell types. In a cohort of adult ITP patients, 93% of sera reacted with more than one GP, but GPIa/IIa and GPIV were never the sole targets[27].

4)TPO receptor

TPO receptor, also named as c-Mpl, is a type I transmembrane protein and expressed specifically on hematopietic stem cells and cells in the megakaryocyte lineage. We have demonstrated the presence of autoantibodies to TPO receptor in SLE patients with thrombocytopenia by enzyme-linked immunosorbent assay using recombinant TPO receptor as an antigen[5]. This antibody specificity was clinically associated with thrombocytopenia with megakaryocytic hypoplasia, and interfered with TPO function by blocking its ligation to the receptor *in vitro*. The involvement of the anti-TPO receptor antibody in impaired thrombopoiesis was further supported by the clinical course of a patient with amegakaryocytic thrombocytopenia, in whom the platelet count was negatively correlated with circulating anti-TPO receptor antibody titer and TPO concentration[28]. Anti-TPO receptor antibody was detected in 22% of patients with SLE and thrombocytopenia and in 10% of patients diagnosed as having idiopathic ITP[29]. More than 90% of patients with ITP had either anti-GPIIb/IIIa or anti-TPO receptor antibodies, independent of the idiopathic or secondary form (Fig.1). In addition, the majority of patients with anti-TPO receptor antibody had concomitant anti-GPIIb/IIIa antibody. In SLE patients with thrombocytopenia, patients with anti-TPO receptor antibody had significantly higher frequencies of megakaryocytic hypoplasia and poor therapeutic responses to corticosteroids and intravenous immunoglobulin than did the patients without this antibody, most of whom had the anti-GPIIb/IIIa antibody alone.