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遭遇することの多い止血異常の実地診療の実際

特発性血小板減少性紫斑病(ITP)

—病態と診断のすすめかた—

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はじめに—疾患名のゆくえ—

特発性血小板減少性紫斑病 idiopathic thrombocytopenic purpura(ITP)は，免疫学的な機序によって，血小板破壊の亢進あるいは血小板産生の障害が生じて，血小板数減少を呈する後天性疾患と定義される．最近，特に海外では，原因の特定ができない場合は原発性免疫性血小板減少症 primary immune thrombocytopenia(primary ITP)という名称で呼ぶことが提唱されている．

一方，わが国では，2012年に成人特発性血小板減少性紫斑病治療の参照ガイドが公表されているが[1]，特定疾患治療研究事業の疾患名が「特発性血小板減少性紫斑病」とされていることもあり，参照ガイドでもこの疾患名が用いられている．今後の検討課題と考えられる．

病　態

ITPの病態の中心はB細胞から産生される自己抗体による血小板の破壊亢進であると考えられていたが，近年，T細胞の異常，巨核球からの血小板産生の障害機序も関与することが明らかとなってきている[2,3]．

1. B細胞から産生される自己抗体

ITPの血小板に対する自己抗体(抗血小板抗体)がB細胞から産生されると，血液中の血小板に結合し，自己抗体が結合した血小板は網内系(特に脾臓)のマクロファージに貪食され，血小板破壊が亢進して血小板数が減少する．抗血小板抗体の標的のほとんどは，GPⅡb-Ⅲaおよび GPIb-Ⅸ-Ⅴなどの血小板膜糖蛋白とされている．

2. ITPの病態へのT細胞の関与

ITPの発症にT細胞が関与しているとの知見がいくつか報告されている．ITP症例においては，自己血小板に反応するT細胞クローンが存在し，自己抗体産生を促すだけでなく，直接血小板を障害している可能性が示唆されている．また，ITP症例においては調節性T細胞が抑制されるために自己抗原に対する免疫寛容が障害される可能性も示唆される．

3. 血小板産生の障害

ITP症例の骨髄所見では，巨核球数は正常ないしは増加していることが多いため，ITPの病態への血小板産生の障害の関与は重要視されてこなかった．しかし，詳細な形態学的な観察と，症例での検討などから巨核球のレベルで血小板産生が免疫学的に障害される可能性が示唆されている．

診断のすすめかた

ITPが疑われる症例が受診される契機としては，下記に示す典型的な臨床症状を呈する場合に加え，症状は比較的軽微であるが検診などの機会に血小板減少を指摘され受診される場合がある．このような症例について，血小板数の減少が確認され，ITPが疑われた症例では，血小板減少をきたすほかの疾患，病態(表1)を除外することによって診断をすすめていく[4,5]．

しかし，実際の臨床の現場では血小板減少をきたす多様な疾患の鑑別に苦慮することも多

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■ ITP は，免疫学的な機序によって，血小板破壊の亢進あるいは血小板産生の障害が生じて，血小板数減少を呈する．
■ B 細胞から産生される血小板に対する自己抗体が結合し，血小板破壊が亢進して血小板数が減少する．
■ 自己血小板に反応する T 細胞クローンが存在し，自己抗体産生を促すだけでなく，直接血小板を障害している．
■ 巨核球のレベルで血小板産生が障害される可能性も示唆されている．

表 1　血小板減少を認めた場合に鑑別が必要な疾患，病態

1. 偽性血小板減少症
2. 先天性血小板減少症
3. ほかの自己免疫疾患に合併する血小板減少症
4. 肝疾患に伴う血小板減少症
5. 血小板の消費亢進に伴う血小板減少症
播種性血管内凝固症候群(DIC)，血栓性血小板減少性紫斑病(TTP)
6. 悪性腫瘍に伴う血小板減少症
7. HIV 感染症に伴う血小板減少症
8. 薬剤性血小板減少症

表 2　新しい ITP 診断基準(案) (2007 年)

1. 血小板減少(10 万/μl 以下)
2. 末梢血塗抹標本で 3 系統すべてに明らかな形態異常を認めない
3. 以下の検査所見のうち，3)，4)，5)のいずれかを含む三つ以上を満たす
1)貧血がない
2)白血球数が正常
3)末梢血中の抗 GPⅡb/Ⅲa 抗体産生 B 細胞の増加
4)血小板関連抗 GPⅡb/Ⅲa 抗体の増加
5)網状血小板比率の増加
6)血漿トロンボポエチンは軽度上昇にとどまる(< 300 pg/ml)

・ITP の診断には上記の 3 項目すべてを満たすこと
・二次性 ITP をきたす疾患または病態(全身性エリテマトーデス，リンパ増殖性疾患，ヒト後天性免疫不全ウイルス感染症，肝硬変など)を欠如する場合は特発性 ITP と診断できる
・3 項目を満たしても ITP として非典型的な所見を認める場合は骨髄検査を行うことが望ましい．
(厚生労働省特発性凝固異常症研究班による)

い．この問題を解決するため，わが国の厚生労働省特発性凝固異常症研究班は，ITP の病態を反映し，特異性の比較的高い検査項目を組み合わせることによって，積極的に ITP を診断する「ITP の診断基準案」(表 2)を 2007 年に作成している．この基準では，特に 3 に含まれる 6 項目のうち 3 項目以上を初診時に満たした場合を ITP とすることによって，高い感度と特異度が得られることが明らかとなっている．しかし，この 6 項目のうち，ITP に特異性の高い 3)から 6)の項目は現時点でも保険収載されておらず，一般の施設での実施は困難であり，この診断基準は残念ながら実用化に至っていない．

以上の現状をふまえて，ITP の診断における，臨床症状，検査所見の見方，考え方を解説する．

臨床症状

診断基準においては，海外，わが国ともに一般的に $10 \times 10^4/\mu l$ 未満の血小板減少をきたすことが必須とされているが，通常，緩徐に血小板減少が進行した場合には $5 \times 10^4/\mu l$ 前後までは出血傾向は比較的軽微である．血小板数が $3 \times 10^4/\mu l$ 未満になると，紫斑，点状出血，鼻出血，歯肉出血，眼球結膜出血などの出血症状を認めるようになる．血小板数 $1 \times 10^4/\mu l$ 未満では頭蓋内出血，消化管出血のような重篤な出血症状を呈することがある．

検査所見

1. 血球数

通常の血液検査で著明な血小板減少を認めた場合には，EDTA による偽性血小板減少症を疑う必要がある．血液スメア標本上で血小板凝集塊がないかを確認するとともに，ほかの抗凝

- ITP を直接確認する検査法が確立されていないため，診断は主に除外が中心となる．
- わが国の「ITP の診断基準案(2007 年)」では特異性の比較的高い検査項目の利用を提案している．
- 血小板数が 3 万/μl 未満になると，紫斑，点状出血などの出血症状を認めるようになる．
- 通常の検査で著明な血小板減少を認めた場合には，偽性血小板減少症を疑う必要がある．
- ITP の鑑別診断として，まれではあるが先天性巨大血小板症の可能性も考慮する必要がある．

固剤(クエン酸ナトリウム，ヘパリン)を用いた採血により血小板数を確認することが必要である．

貧血は通常は認めないが，出血のため鉄欠乏性貧血をきたすことがある．また，ITP に自己免疫性溶血性貧血を合併することがあり，Evans 症候群と呼ばれる．

白血球数は感染の合併などがなければ，正常であることがほとんどである．白血球数減少などを認めた場合には膠原病の合併を考慮する必要がある．

2. 末梢血塗抹像

ITP では比較的大型の血小板が出現することがあるが，まれではあるが Bernard-Soulier 症候群などの先天性巨大血小板症の可能性も考慮する必要がある．鑑別の困難な症例もあるので，治療抵抗性の難治性 ITP 症例などではこれらの疾患も再検討する必要がある．

ITP においては，白血球，赤血球の形態は正常である．

3. 血液凝固検査

ITP では血液凝固検査の異常を伴うことはまれである．異常を認めた場合には，DIC，肝障害に伴う血小板減少などを疑い，検査をすすめることが必要である．

4. 臨床化学検査

肝機能障害を認めた場合は肝硬変など肝障害に伴う血小板減少の可能性を考え，凝固検査，肝炎ウイルス検査，画像検査などの検査が必要となる．

血小板減少に加え，貧血，総ビリルビン，LDH の上昇を認めた場合には自己免疫性溶血性貧血のほかに，血栓性血小板減少性紫斑病 thrombotic thrombocytopenic purpura(TTP)を鑑別疾患として考慮することが重要である．ITP の場合は著明な血小板減少がみられ，出血症状が強い場合には緊急時の処置のひとつとして血小板輸血も行われるが，TTP においては血小板輸血は病態を悪化させる可能性があり，禁忌とされている．

5. 骨髄検査

ITP の骨髄所見としては，巨核球数が正常ないしは増加しており，比較的未分化な巨核球が目立つことはあるが，骨髄異形性症候群(MDS)などにみられるような巨核球の異型性は通常認めない．また，赤芽球，顆粒球系の形態異常を認めない．

これまで骨髄検査は ITP の診断のために必須の検査とされてきたが，わが国の診断基準案(表 2)では，骨髄検査は診断上，必須の検査とはしておらず，ITP として非特異的である場合に，MDS などのほかの原因の鑑別の目的で実施することを推奨している．今後は，より慎重に骨髄検査の適応を検討し，鑑別診断上必要な症例に限って施行することが望ましい．

6. ITP の病態と関連すると思われる検査法

これまでに，ITP の診断に有用と考えられている検査法について解説する．これらの検査法のうち，わが国で保険適応のある検査法は a. の PA-IgG のみであるが，残念ながらその有用性は近年疑問視されている．また，ITP の診断基準案(表 2)で取り上げられた b. から e. の検査

■ 血小板減少に加え，貧血，総ビリルビン，LDHの上昇を認めた場合にはTTPも鑑別疾患として考慮する．
■ 骨髄検査は，ITPとして非特異的である症例に，MDSなどのほかの原因の鑑別の目的で実施する．
■ PA-IgG検査はITPにおける検出感度は約90%と高いが，特異度は低い．
■ 血小板関連抗GPⅡb/Ⅲa抗体を検出する検査法はITPにおける特異度は高いが，検出感度は低い．

法についても，方法，有用性が確立されておらず，保険適応も認められていない．

a. PA-IgG (platelet associated IgG：血小板関連IgG)

流血中の血小板に結合しているIgGを測定する検査である．ITPにおける検出感度は約90%と高い．わが国では，ITPの診断のために保険適応が認められていることもあり，筆者らは初診時に提出し，提示した症例のように著明高値を示す場合には診断の参考とすることとしている．しかし，血小板に非特異的に結合するIgGも検出するため，ITP以外の血小板減少患者の多くでも高値を呈し，特異度は低く，最近のわが国の診断基準案(表2)では必要な検査から除外されている．

b. 血小板関連抗GPⅡb/Ⅲa抗体

ITP患者にみられる特異的抗血小板抗体の多くは血小板膜糖蛋白であるGPⅡb/Ⅲaを認識することが明らかとなっている．この抗GPⅡb/Ⅲa抗体を検出する検査法が開発されている．本検査法はITPにおける特異度は高く，診断のために有用な検査ではあるが，検出感度が低いことが問題である．

c. 末梢血中の抗GPⅡb/Ⅲa抗体産生B細胞

抗GPⅡb/Ⅲa抗体を産生する末梢血中のB細胞を検出する方法がエリスポット法を応用して開発されている．本検査法によって，末梢血中の抗GPⅡb/Ⅲa抗体産生B細胞の増加はITP患者の91%で認められたが，血小板減少を示す非ITP患者では6%にとどまった．この結果からは，本検査は感度，特異度ともにすぐれた方法と考えられる．

d. 網状血小板比率

ITPでは血小板の破壊亢進状態があるため，多くの症例では骨髄での血小板産生がやや亢進し，その指標となる網状血小板数が増加するとされている．

e. 血漿トロンボポエチン(TPO)濃度

巨核球—血小板造血を促進する液性因子であるトロンボポエチンthrombopoietin (TPO)の血漿中濃度をELISA法にて測定することが可能となっており．ITP症例では軽度上昇にとどまることが知られている．

※d.とe.については，本誌の「トピックス」の項に詳しく解説があるので，そちらをご参照いただきたい．

おわりに

ITPの病態の中心は免疫の異常であることは明らかであり，近年T細胞の異常の関与も次々と明らかとなってきているが，その詳細についてはいまだに不明な点も多い．ITPの診断に関しては，現時点でも除外診断が重要な部分を占めているが，今後，病態がさらに明らかとなるにつれ，ITPの病態を理解したうえでの積極的な診断法の確立が期待される．

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■ 末梢血中の抗 GPⅡb/Ⅲa 抗体産生 B 細胞を検出する検査も開発されており，感度，特異度ともにすぐれている．

■ 網状血小板比率，血漿トロンボポエチン（TPO）濃度の測定も ITP の診断に有用であると考えられる．

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INTERNAL MEDICINE

□ CASE REPORT □

Effects of Immunosuppressive Therapy in a Patient with Aplastic Anemia-Paroxysmal Nocturnal Hemoglobinuria (AA-PNH) Syndrome during Ongoing Eculizumab Treatment

Jin Asano[1], Ryosuke Ueda[2], Yasuhiro Tanaka[1], Isaku Shinzato[1] and Toshiro Takafuta[1]

Abstract

A 65-year-old woman experienced a hemolytic attack triggered by sepsis. She presented with markedly increased $CD55^-$ $CD59^-$ erythrocytes and the signs of bone marrow failure, which led to a diagnosis of aplastic anemia-paroxysmal nocturnal hemoglobinuria (AA-PNH) syndrome. There was a possibility of increasing hemolysis, as large PNH clones remained after immunosuppressive therapy (IST). Accordingly, eculizumab was first used to control the hemolytic attack followed by IST with antithymocyte globulin and cyclosporine A. The patient was successfully weaned from blood transfusions and has been followed up without any recurrence of hemolytic attacks.

Key words: paroxysmal nocturnal hemoglobinuria, aplastic anemia, eculizumab, antithymocyte globulin

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Introduction

Paroxysmal nocturnal hemoglobinuria (PNH) is a hematopoietic stem cell disease characterized by the intravascular lysis of red blood cells that are abnormally sensitive to attacks by the complement system. The clonal expansion of a hematopoietic stem cell with an acquired mutation in the PIG-A gene is the pathobiological mechanism underlying the development of PNH (1-3).

Hematopoietic stem cell transplantation is the only curative therapy for PNH. However, due to the risks associated with transplantation, the three major pathophysiological features of the disease, including hemolysis, bone marrow failure (BMF) and thrombosis, are symptomatically managed primarily with blood transfusions, steroids and warfarin, with little success (4, 5). In recent years, eculizumab, a humanized monoclonal antibody that binds specifically to human complement protein C5 and inhibits the formation of the terminal complement complex, has been clinically used to prevent the hemolysis associated with PNH (6-8).

Aplastic anemia (AA)-PNH syndrome was initially reported as a case of aplastic anemia presenting with symptoms characteristic of PNH during the course of the disease (9). There have also been reports of patients with preexisting clinical symptoms of PNH at the onset of AA (10-14); in some cases, the size of the PNH clones may be significantly large. In these cases, there is a potential risk of inducing hemolysis during immunosuppressive therapy (IST) due to complement activation related to infection or xenoantibody-associated reactions (11, 12). These patients may benefit from eculizumab (13, 14). However, to date, no cases of AA-PNH syndrome treated with a combination of eculizumab and IST have been described in detail as a case report.

We herein report the case of a patient with AA-PNH who developed a hemolytic attack triggered by an infection. IST with antithymocyte globulin (ATG) and cyclosporine A (CsA) was initiated during ongoing eculizumab treatment, which allowed the patient to be successfully weaned from blood transfusions.

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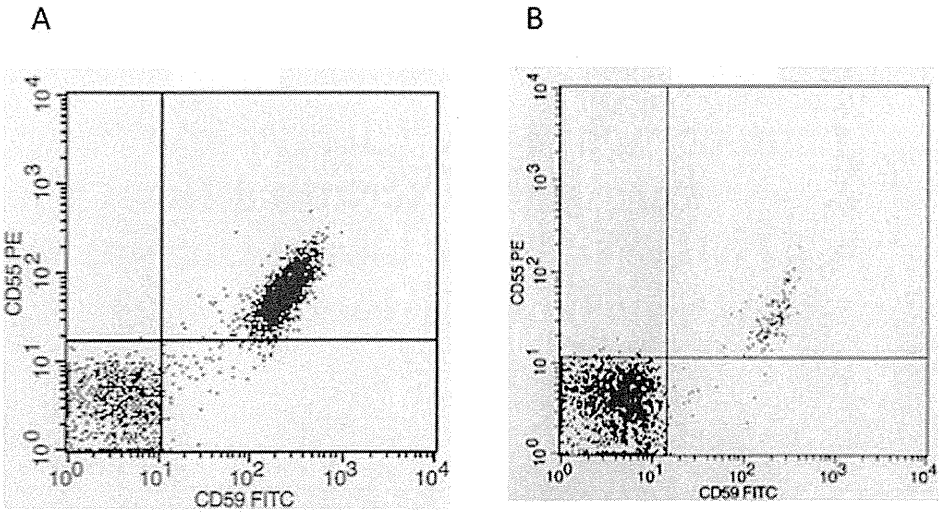

Figure 1. Immunophenotypic analysis of red blood cells using flow cytometry. A: The size of the PNH clone ($CD55^-$ $CD59^-$) was 27% at the onset of the hemolysis attack. B: The size of the PNH clone was 90% 19 months after the eculizumab treatment. PNH: paroxysmal nocturnal hemoglobinuria

Case Report

A 65-year-old Japanese woman developed a fever, malaise, dorsal pain and chills four days before her first visit to our facility in October 2010. These symptoms gradually worsened, and she was admitted to our hospital. The physical examination findings revealed a body temperature of 38.9℃ and an anemic and icteric conjunctiva. The patient's urine was "port wine" in color. She also presented with petechiae and purpura on her extremities. A peripheral blood examination indicated pancytopenia: WBC count, 0.50×10^9/L (neutrophils, 18.0%; lymphocytes, 70.0%); RBC count, 960×10^9/L; Hb, 3.7 g/dL; Ht, 10.8%; platelet count, 9×10^9/L; and reticulocytes, 2.3%. No morphological abnormalities were observed upon examination of a peripheral blood smear. A bone marrow aspiration showed hypocellular marrow with a predominance of plasma cells and reticulum cells, suggesting AA or hemophagocytic syndrome. The bone marrow cells displayed no morphological abnormalities, and a karyotypic analysis indicated normal 46,XX, which made a diagnosis of myelodysplastic syndrome unlikely. Other laboratory findings were as follows: C-reactive protein (CRP), 29.2 mg/dL; T-Bil, 5.9 mg/dL; D-Bil, 2.7 mg/dL; asparate aminotransferase (AST), 89 IU/L; alanine aminotransferase (ALT), 30 IU/L; lactate dehydrogenase (LDH), 1,118 IU/L (normal range: 120-230); blood urea nitrogen (BUN), 42 mg/dL; Cr, 1.5 mg/dL; and haptoglobin, <10 mg/dL, which indicated inflammation, hemolysis and renal dysfunction. Of the tests performed for hemolysis, the Coombs test was negative and the Ham test was positive. Consequently, a test for the surface markers of peripheral blood erythrocytes was performed, which revealed 27% $CD55^-$ $CD59^-$ erythrocytes (Fig. 1A). Based on these findings, the patient was diagnosed with PNH. As *Escherichia coli* was isolated from both urine and blood culture samples, we concluded that the elevated PNH-type blood cells had previously existed and that sepsis induced the PNH-associated hemolytic attack and hemophagocytic syndrome.

Since the patient was under septic shock and exhibited marked pancytopenia with hemolysis, systemic management with intravenous fluids and catecholamines was initiated accompanied by treatment with antibiotics, granulocyte colony-stimulating factor (G-CSF), erythrocyte transfusions, platelet transfusions, methylprednisolone (mPSL) pulse therapy and haptoglobin. The urinary tract infection and sepsis subsided on the 13th hospital day, and the patient's general condition stabilized. A bone marrow aspiration and biopsy were performed due to prolonged pancytopenia. Hypoplastic marrow and the absence of atypical cells were observed. The patient was diagnosed with AA-PNH syndrome based on the clinical course and test findings.

After vaccination against *Neisseria meningitidis*, treatment with eculizumab was started on the 77th day (eculizumab at a dose of 600 mg via an intravenous infusion every seven days for four doses, followed by a maintenance dose of a 900 mg eculizumab infusion every 14 days). The LDH level decreased from 400 to 100 IU/L three weeks after the start of the treatment accompanied by an increase in the Hb level from 5-6 g/dL to 6-7 g/dL (Fig. 2). The neutrophil and platelet counts remained below 0.50×10^9/L and 20×10^9/L, respectively, even after the start of the eculizumab treatment. Since the BMF was not markedly alleviated (Fig. 2), IST using ATG and CsA for BMF was considered. Due to the persistent neutropenia, a subcutaneous injection of G-CSF (lenograstim 100 μg/day) was administered on the 118th day followed by ATG treatment (rabbit ATG: thymoglobulin 100 mg/day, five days) on the 124th day. Because the sixth administration of eculizumab was given five days before the start of the ATG treatment, we considered the possibility of weakened ATG activity. However, in practice, the patient's lymphocyte count decreased sharply from 1.46×10^9/L imme-

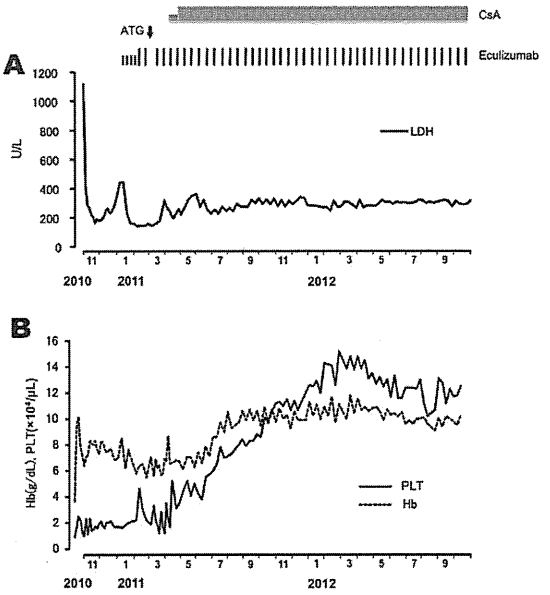

Figure 2. The patient's clinical course. A: Lactate dehydrogenase (LDH), B: Platelets (solid line) and hemoglobin (Hb, dotted line).

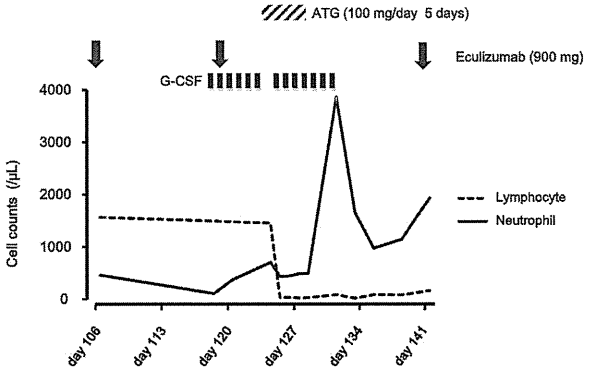

Figure 3. Changes in the neutrophil and lymphocyte counts with ATG treatment. The solid and dashed lines indicate neutrophils and lymphocytes, respectively.

diately before the ATG treatment to 0.03×10^9/L on day 2 of the ATG treatment. On the other hand, the neutrophil count did not decrease markedly after the start of the ATG treatment, which may have been due to the influence of the G-CSF treatment. On day 8 of the ATG treatment (the 131st day), the administration of G-CSF was suspended because the neutrophil count increased (>3.0×10^9/L) (Fig. 3). Starting on the 156th day, CsA was administered at a dose that would maintain its trough level at 150 to 250 ng/mL. The patient subsequently followed an uneventful course without developing any severe infections. From the 183rd day, the Hb level was maintained over 6 g/dL and the platelet count was maintained over 30×10^9/L without blood transfusions. Prednisolone (PSL) therapy at a dose of 5 mg/day was started on the 196th day.

The patient was discharged from the hospital approximately seven months after being admitted. Since then, she has been managed as an outpatient and is receiving continued treatment with eculizumab, CsA and PSL. Her peripheral blood neutrophil count remains low (0.3×10^9/L to 0.7×10^9/L), and G-CSF continues to be administered when the count falls below 0.5×10^9/L. The RBC and platelet counts have been gradually increasing; the Hb level has been maintained at approximately 10 g/dL and the platelet count has been maintained over 100×10^9/L since one year after the disease onset. The patient has been followed without any events to date (two years and two months after disease onset at the time of writing). When the levels of peripheral blood erythrocyte surface markers were analyzed 19 months after the start of the eculizumab treatment, the percentage of CD 55^- $CD59^-$ cells was 90%, which was higher than the percentage (27%) recorded during the first examination (Fig. 1B). Therefore, in the present case, normal hematopoiesis was not sufficiently restored, even after the administration of IST with ATG/CsA, and PNH-associated blood cells that had escaped hemolysis remained predominant after the eculizumab treatment.

Discussion

Patients with AA-PNH syndrome are commonly treated using IST in a manner similar to that for AA, and there is no evidence that treatment with IST influences clonal expansion either positively or negatively (14). Most patients with AA-PNH syndrome have small PNH clones and require no specific PNH therapy prior to IST. In rare cases, significant amounts of the PNH clone remain even after IST and treatment, including eculizumab therapy, for the complications of PNH is required (11-17). Scheinberg et al. reported that seven of 14 patients with a PNH clone greater than 50% exhibited hemolysis or thrombosis after IST and required treatment using eculizumab (two patients) and/or oral anticoagulation (six patients) (13). Nakasone et al. administered ATG therapy in four PNH patients with cytopenia, and the anemia improved in all cases; however, three patients demonstrated hemolytic exacerbation and thrombocytopenia during ATG administration, which may have been due to xenoantibody-associated complement activation (11). In a conference presentation, five cases were reported in which IST was initiated during ongoing eculizumab treatment, and three of the five patients demonstrated a partial response to IST added to eculizumab (17).

In the present case, the results of a flow cytometry analysis (Fig. 1A), the high LDH level and the patient's history of a severe hemolytic attack induced by infection suggested that large PNH clones may have remained and marked hemolysis may have recurred if IST for BMF had been applied without reducing the hemolysis. The potential risk of intravenous ATG in inducing xenoantibody-associated complement activation must be considered, especially in this patient (11, 12). As IST induces a severe decline in immune competence, infection-associated complement activation was also considered in this case. To prevent these problems, eculizumab was first administered, followed by ATG/CsA therapy after achieving a reduction in the LDH level and fre-

quency of blood transfusions. In this manner, the BMF was alleviated without inducing a hemolytic attack.

Furthermore, since eculizumab is known to have immunosuppressive effects, such as reducing the immune function against *N. meningitidis*, we anticipated that eculizumab therapy with ATG and CsA might increase the risk of severe infection due to immunodeficiency. CsA treatment was then initiated approximately one month after the start of the ATG treatment, rather than starting both drugs simultaneously. This management may have allowed the patient to remain free of severe infection.

Eculizumab does not suppress PNH clone formation per se. In a phase III study of eculizumab, treatment of PNH with eculizumab resulted in a significant increase in the percentage of $CD55^-$ $CD59^-$ erythrocytes and escape from hemolysis (18). In another study, when patients with AA-PNH syndrome received ATG/CsA therapy without concomitant eculizumab treatment, most cases showed no increases in PNH clones (13). In the present case, the anemia decreased in response to preceding eculizumab treatment and the subsequent concomitant use of ATG/CsA; however, the percentage of $CD55^-$ $CD59^-$ erythrocytes in the peripheral blood markedly increased. Furthermore, $CD55^-$ $CD59^-$ granulocytes were also predominant (>90%, data not shown) after the eculizumab treatment. Therefore, the hematopoiesis appeared to greatly depend on the presence of PNH-type hematopoietic stem cells, even after the disease was alleviated in response to IST.

In summary, we herein reported a case of AA-PNH syndrome in which eculizumab treatment combined with IST was effective and well tolerated. The findings obtained in this case are valuable for clarifying the features of AA-PNH syndrome.

The authors state that they have no Conflict of Interest (COI).

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TOPIC HIGHLIGHT

WJG 20th Anniversary Special Issues (6): *Helicobacter pylori*

Helicobacter pylori-associated immune thrombocytopenia: Clinical features and pathogenic mechanisms

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Abstract

Immune thrombocytopenia (ITP) is an autoimmune disease mediated by anti-platelet autoantibodies. There is growing evidence that the eradication of *Helicobacter pylori* (*H. pylori*) effectively increases platelet count in a considerable proportion of ITP patients infected with this bacterium. In the majority of ITP patients responding to *H. pylori* eradication therapy, the anti-platelet autoantibody response is completely resolved with no relapse for more than 7 years, indicating that the disease is cured. Therefore, adult patients with suspected ITP should be examined for *H. pylori* infection, and eradication therapy is recommended if the infection is present. Notably, however, the efficacy of *H. pylori* eradication therapy in ITP patients varies widely among countries, with a higher response rate in Japan compared with the United States and European countries other than Italy. The pathogenesis of *H. pylori*-associated ITP is still uncertain, although the mechanisms are known to involve multiple factors. *H. pylori* may modulate the Fcγ-receptor balance of monocytes/macrophages in favor of activating Fcγ receptors, and *H. pylori* components may mimic the molecular makeup of platelet antigens. Further studies of the pathogenic process of *H. pylori*-associated ITP may be useful for the development of new therapeutic strategies for ITP.

Key words: Autoantibody; Childhood; *Helicobacter pylori*; Fcγ receptor; Immune thrombocytopenia; Idiopathic thrombocytopenic purpura; Systemic lupus erythematosus

Core tip: In this review, we summarize recent updates on basic and clinical aspects of *Helicobacter pylori* (*H. pylori*)-associated immune thrombocytopenia (ITP). We highlight the efficacy of *H. pylori* eradication in adult and childhood ITP as well as in secondary ITP, variability in the efficacy of eradication in various countries, factors predicting the eradication-related platelet response, and the mechanisms responsible for the development of ITP in association with *H. pylori* infection. It is apparent that in a distinct subgroup of *H. pylori*-associated ITP, this bacterial infection is central to the ITP pathogenesis.

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INTRODUCTION

Helicobacter pylori (*H. pylori*), a gram-negative spiral bacterium, is the causative agent in chronic gastritis, gastric and duodenal ulcer disease, and gastric cancer. *H. pylori*

has also been implicated in the pathogenesis of extra-digestive disorders, including cardiovascular, hematologic, and autoimmune diseases[1]. The strongest evidence has been reported for immune thrombocytopenia (ITP), with high-quality studies showing that the disease improved after *H. pylori* was successfully eradicated.

ITP is a typical organ-specific autoimmune disease; it is mediated by anti-platelet autoantibodies that bind to platelets and megakaryocytes, accelerating platelet destruction by the reticuloendothelial system and suppressing platelet production[2]. The autoantibody response primarily targets platelet-surface glycoproteins such as GP II b/III a and GP I b. This condition is known as primary ITP when it occurs without an underlying disease, but it is also seen in patients with various diseases, including systemic lupus erythematosus (SLE). Although the etiology of ITP is obscure, microorganisms such as human immunodeficiency virus and hepatitis C virus are known to contribute to its development[3], indicating that in a particular subset of ITP, infectious agents play a significant role in the pathogenesis of the autoimmune response.

First observed in 1988[4], the increase in platelet counts in ITP patients after eradicating *H. pylori* has since been confirmed by several studies. Consequently, *H. pylori* eradication therapy is now a treatment option for ITP[5]. However, a number of questions regarding the relationship between *H. pylori* infection and ITP remained unsolved, including the great variability in the efficacy of *H. pylori* eradication therapy among countries, factors predicting the platelet response after *H. pylori* eradication, and mechanisms responsible for the platelet response associated with *H. pylori* eradication[6]. Some of these questions have been answered in the past few years. This review summarizes recent updates on clinical and therapeutic aspects of *H. pylori*-associated ITP, as well as the pathogenesis of this disease.

EFFICACY OF *H. PYLORI* ERADICATION IN ITP

Adult ITP

In 1988, Gasbarrini and colleagues reported that platelet counts increased in all of 8 *H. pylori*-infected patients with ITP who were treated with a regimen to eradicate *H. pylori*, while the platelet counts were unchanged in 3 *H. pylori*-infected patients who did not receive the regimen[4]. Reports followed of partial or complete platelet responses observed in a large proportion of ITP patients treated with an *H. pylori* eradication regimen consisting of a 1- to 2-wk standard triple therapy with a proton pump inhibitor, clarithromycin, and either amoxicillin or metronidazole[6-8]. A nation-wide survey in Japan involved 207 *H. pylori*-infected adult patients with ITP, making it the largest study on the efficacy of eradicating *H. pylori* in ITP patients[9]. In that study, after the successful eradication of *H. pylori*, 63% of the patients achieved some degree of platelet recovery, and within this group, 23% showed complete remission at 12 mo after the eradication. Although most early studies excluded patients with severe thrombocytopenia, who were at high risk of bleeding, several case series have reported the efficacy of eradicating *H. pylori* even in patients with refractory ITP, including patients with severe thrombocytopenia that resisted multiple therapeutic regimens including splenectomy[9-11]. Although most studies began assessing the platelet counts one month after starting eradication therapy, we observed platelet recovery after just a week in almost half of those responding[12]. Long-term follow-up studies showed that this platelet response lasted 7 or more years after *H. pylori* was eradicated, with very few cases of relapse[13,14]. An assessment of circulating B cells producing anti-GP II b/III a antibodies before and after eradication treatment indicated that the anti-platelet autoantibodies disappeared after platelet recovery when *H. pylori* had been successfully eradicated[12]. Thus, in certain patients, ITP appears to be clinically and immunologically cured by eradicating *H. pylori*.

In the first systematic review with meta-analysis, Franchini *et al*[15] reviewed 788 patients with ITP, including 494 *H pylori*-infected patients collected from 17 studies, in 2007. Platelet counts increased in ITP patients who received eradication treatment, compared with untreated patients, and the weighted mean difference (WMD) in platelet count was 34.0×10^9/L regardless of the outcome of *H. pylori* eradication. The platelet counts increased significantly in *H. pylori*-infected patients after successful *H. pylori* eradication, compared with the following groups: untreated *H. pylori*-infected patients (WMD of 40.8×10^9/L), *H. pylori*-infected patients who failed eradication (WMD of 52.2×10^9/L), and *H. pylori*-uninfected patients (WMD of 46.4×10^9/L). Another systematic literature review involving 1555 patients revealed a weighted mean complete response (platelet count $\geqslant 100 \times 10^9$/L) after successful *H. pylori* eradication of 42.7%, and an overall response (platelet count $\geqslant 30 \times 10^9$/L, and at least doubling of the basal count) of 50.3%[16]. Even in patients with a low baseline platelet count ($< 30 \times 10^9$/L), the overall response rate was 35.2%, including 20.1% with a complete response. These findings indicate that *H. pylori* eradication is closely related to platelet recovery in adult ITP patients. Finally, another systematic review evaluated the efficacy of the *H. pylori* eradication regimen in patients with ITP by comparing the platelet response in patients with or without an *H. pylori* infection[17]. The odds of achieving platelet recovery following the eradication regimen were 14.5 times higher in 205 *H. pylori*-infected patients than in 77 *H. pylori*-uninfected patients. This clearly indicates that platelet recovery after the eradication regimen results from the eradication of *H. pylori* itself, rather than from *H. pylori*-independent mechanisms such as immune-modulatory effects of the drugs themselves, or the eradication of bacteria other than *H. pylori*.

The clear linkage between platelet recovery and the disappearance of *H. pylori* suggests a direct role for *H. py-*

lori infection in the ITP pathogenesis. In addition, in the majority of ITP patients who achieved a complete platelet response after *H. pylori* eradication, the anti-platelet autoantibody response was also eliminated. Thus, patients who are infected with *H. pylori* and who respond to the eradication therapy fall into a distinct, widely recognized ITP subgroup, termed *H. pylori*-associated ITP[18,19], that is considered a type of secondary ITP[5]. Given the relatively high efficacy, safety, and economy of *H. pylori* eradication therapy, *H. pylori* detection should be considered when examining adult patients who are suspected to have ITP, and eradication therapy is recommended if *H. pylori* infection is present[5,8].

Variability among countries

Although the efficacy of *H. pylori* eradication therapy in infected adults with ITP has been confirmed by high-quality systematic reviews, some studies have reported little to no platelet response after *H. pylori* eradication therapy. For example, Jarque *et al*[20] and Ahn *et al*[21] observed platelet recovery was in only 13% and 7%, respectively, of adult patients with ITP after successfully eradicating *H. pylori*. Michel *et al*[22] found no platelet response in 14 *H. pylori*-infected ITP patients even after successfully eradicating the *H. pylori*. These widely varying reports of the efficacy of *H. pylori* eradication therapy in ITP patients are explained, in part, by different eligibility criteria and different definitions of platelet response among the various studies. However, response rates differed between the 57.9% reported by studies in Japan, and the 38.3% rate reported by studies from other countries[16]. Studies from Japan and Italy tend toward better response rates, ranging from 28% to 100%, than studies from the United States and Spain (< 13).

Thus, variability in the efficacy of *H. pylori* eradication in different populations should be considered to establish recommendations for screening and eradicating *H. pylori*. For example, *H. pylori* screening is certainly worthwhile in Japan, a country with a high prevalence of infection and a high response rate to eradication treatment. In fact, in a recently developed reference guide for managing adult ITP in Japan, *H. pylori* eradication is a prominent strategy for managing ITP in adult patients[23]. Namely, the guide states that all patients diagnosed with ITP should be screened for *H. pylori* infection, and eradication therapy is recommended as a first line of treatment, regardless of platelet count, if *H. pylori* infection is present. These recommendations may not be appropriate in the United States or in European countries (other than Italy), in which both the prevalence of infection and the response rates to eradication therapy are low. Indeed, *H. pylori* eradication is not mentioned in the international consensus report on the management of primary ITP[24], while the eradication therapy is recommended in patients who are found to have *H. pylori* infection in the American Society of Hematology 2011 evidence-based practice guideline for ITP[5].

A recent systematic review demonstrated a correlation in ITP patients between the prevalence of *H. pylori* infection and the platelet response rates to eradication therapy[16]. The reason for such variability among countries is not clear, but differences in the epidemic *H. pylori* strains according to geographical area could account for differences in the clinical response. In this regard, *H. pylori* strains that possess cytotoxin-associated gene A (CagA) have been proposed to have a role in the pathogenesis of *H. pylori*-associated ITP based on the potential cross-reactivity between CagA and platelet glycoproteins[25,26]. The frequency of CagA-positive strains varies by geographic location: the majority of the *H. pylori* strains found in Eastern Asia, including Japan, express CagA, whereas the proportion of CagA-positive strains in European countries and North America is much lower[27]. However, genetic and other environmental factors are also likely to contribute to the variability in platelet recovery after *H. pylori* eradication among different populations.

Table 1 Prevalence of *Helicobacter pylori* infection and platelet response after *Helicobacter pylori* eradication in children with immune thrombocytopenia *n* (%)

Author, yr	Country	Prevalence of *Helicobacter pylori* infection	Platelet response
Rajantie *et al*[29]	Finland	0/17 (0)	ND
Jaing *et al*[30]	Taiwan	9/22 (41)	5/9 (50)
Hayashi *et al*[31]	Japan	2/10 (20)	1/1 (100)
Yetgin *et al*[32]	Turkey	11/35 (31)	0/9 (0)
Jaing *et al*[33]	Taiwan	10/63 (16)	ND
Loffredo *et al*[34]	Italy	8/39 (21)	0/8 (0)
Neefjes *et al*[35]	Netherlands	3/47 (6)	3/3 (100)
Wu *et al*[36]	Taiwan	6/32 (19)	ND
Bisogno *et al*[37]	Italy	8/24 (33)	1/8 (13)
Hamidieh *et al*[38]	Iran	4/31 (13)	0/4 (0)
Treepongkaruna *et al*[39]	Thailand	16/55 (29)	0/7 (0)
Ferrara *et al*[40]	Italy	8/24 (33)	8/8 (100)
Maghbool *et al*[41]	Iran	5/30 (17)	5/5 (100)
Russo *et al*[42]	Italy	50/244 (20)	13/33 (39)

ND: Not described.

Childhood ITP

The clinical course of ITP is quite different in children than in adult patients. ITP in children is usually an acute form with spontaneous recovery within 6 mo, although thrombocytopenia lasts more than 6 mo in about 20% of children with ITP[28]. There are only a few pediatric studies evaluating the role of *H. pylori* infection in chronic ITP in children. Table 1 summarizes the studies assessing the prevalence of *H. pylori* infection and platelet response after eradicating *H. pylori* in children with ITP. Although a Finnish study failed to detect *H. pylori* infection in any of 17 children with chronic ITP[29], other studies detected *H. pylori* infection in a small proportion of children with chronic ITP[30-42]. The highest prevalence was reported in a study conducted in Taiwan, which showed an infection rate of 41%[29]. A recent multicenter study in Italy revealed that 50 (20) of 244 children with ITP were in-

fected with *H. pylori*[42]. In general, the prevalence of *H. pylori* infection is lower in children than in adults with ITP in a given population.

Reports of platelet recovery after *H. pylori* eradication therapy in children are highly variable and inconsistent. In the first study in Taiwan, platelet counts increased in 5 (56) of 9 *H. pylori*-infected children with ITP after eradication therapy[29]. In studies from the Netherland, Italy, and Iran, platelet counts increased measurably in all *H. pylori*-infected children after eradication treatment, although the number of subjects analyzed was very small (3, 8, and 5, respectively)[35,40,41]. However, other studies from Turkey, Italy, Iran, and Thailand showed that none of the patients who underwent treatment to eradicate *H. pylori* infection responded to it[32,34,38,39]. Even studies from the same country gave diametrically opposite accounts of the efficacy of *H. pylori* eradication. These contradictory results may be partly due to the small number of *H. pylori*-infected children with ITP. An Italian study, which had the largest number of patients, showed that 13 (39) of 33 children with *H. pylori* infection responded to the eradication treatment[42]. The prevalence of *H. pylori* infection in children with chronic ITP is generally low, suggesting that *H. pylori* infection plays only a minor role in the development of childhood ITP. However, we should recognize that some children infected with *H. pylori* can recover if the *H. pylori* is successfully eliminated. A large-scale study is necessary to confirm the relationship between *H. pylori* infection and childhood ITP.

Other forms of secondary ITP

ITP may develop in the context of other disorders or conditions, including lymphoproliferative, autoimmune, and infectious diseases[43]. Although *H. pylori*-associated ITP is now categorized as a secondary ITP condition, the efficacy of *H. pylori* eradication therapy has not been deeply examined in patients with other types of secondary ITP. To evaluate the role of *H. pylori* infection in the pathogenesis of secondary ITP, we conducted an open-label prospective study involving 34 consecutive *H. pylori*-infected patients with ITP, including 16 with primary ITP, 8 with secondary ITP associated with SLE, and 10 with secondary ITP associated with liver cirrhosis. All the patients received a standard *H. pylori* eradication regimen consisting of amoxicillin, clarithromycin, and lansoprazole, and the *H. pylori* was successfully eradicated in all except one patient with primary ITP, and another with liver cirrhosis. As shown in Figure 1, the platelet count had increased 3 mo after eradication treatment in nearly all the patients with primary ITP, but was virtually unchanged in patients with SLE or liver cirrhosis. In addition, a decrease in circulating anti-GP IIb/IIIa antibody-producing B cells was observed in patients with primary ITP, but not in ITP patients with SLE or liver cirrhosis. These findings clearly indicate that the eradication of *H. pylori* fails to improve the pathogenic process in patients with some forms of secondary ITP. Thus, the efficacy of *H. pylori* eradication therapy is likely to be restricted to patients in a subgroup of seemingly primary ITP (*H. pylori*-associated ITP), and *H. pylori* infection appears to be uninvolved in the pathogenic process of other secondary ITPs.

CHARACTERISTICS OF *H. PYLORI*-ASSOCIATED ITP

Features of ITP patients infected with H. pylori

Several authors have tried to identify characteristics of *H. pylori*-associated ITP by comparing the clinical features of adult ITP patients with or without *H. pylori* infection. ITP patients infected with *H. pylori* were significantly older than uninfected patients[9], but this is predictable because the prevalence of *H. pylori* infection increases with age in the general population[44]. Multiple studies have failed to detect significant differences in any other demographic or clinical characteristic, including sex, platelet count, or response to therapy.

Several studies have looked for differences in genetic factors in ITP patients with or without *H. pylori*. Veneri and colleagues examined the human leukocyte antigen (HLA)-DRB1 and *DQB1* alleles in Italian patients with ITP, and found that *H. pylori*-positive patients had a lower frequency of *DRB1*03*, and higher frequencies of *DRB1*11*, *DRB1*14*, and *DQB1*03*, compared with *H. pylori*-negative patients[45]. However, we failed to detect any association between *H. pylori* infection and HLA-DRB1 or DQB1 alleles in Japanese patients with ITP[46]. Instead, we found that gene polymorphism within the loci for interleukin (IL)-1β was associated with *H. pylori* infection in patients diagnosed before age 50. While these observations suggest an involvement of genetic background in *H. pylori*-related ITP, the associations should be confirmed by replication studies enrolling patients from other populations.

On the other hand, there was no difference in the IL-2, IL-4, or IL-6 serum levels between patients with and without *H. pylori* infection[47]. The serum levels of chemokines, including monocyte chemoattractant protein-1, regulated upon activation normally T-cell expressed and secreted, and epithelial cell-derived neutrophil attractant-78, were significantly higher in patients with *H. pylori* infection than in those without[48], although increased levels of these chemokines were also observed in individuals that had *H. pylori*-related gastrointestinal disorders but did not have ITP. Thus, studies have failed to identify demographic, clinical, genetic, or immunologic characteristics unique to ITP patients infected with *H. pylori*. This is probably because there are at least two distinct subgroups of *H. pylori*-infected ITP patients: those with *H. pylori*-associated secondary ITP, who respond to eradication therapy, and those with primary ITP and a coincidental *H. pylori* infection.

Factors predicting a positive response to H. pylori eradication therapy

Parameters that predict the platelet response to *H. pylori*

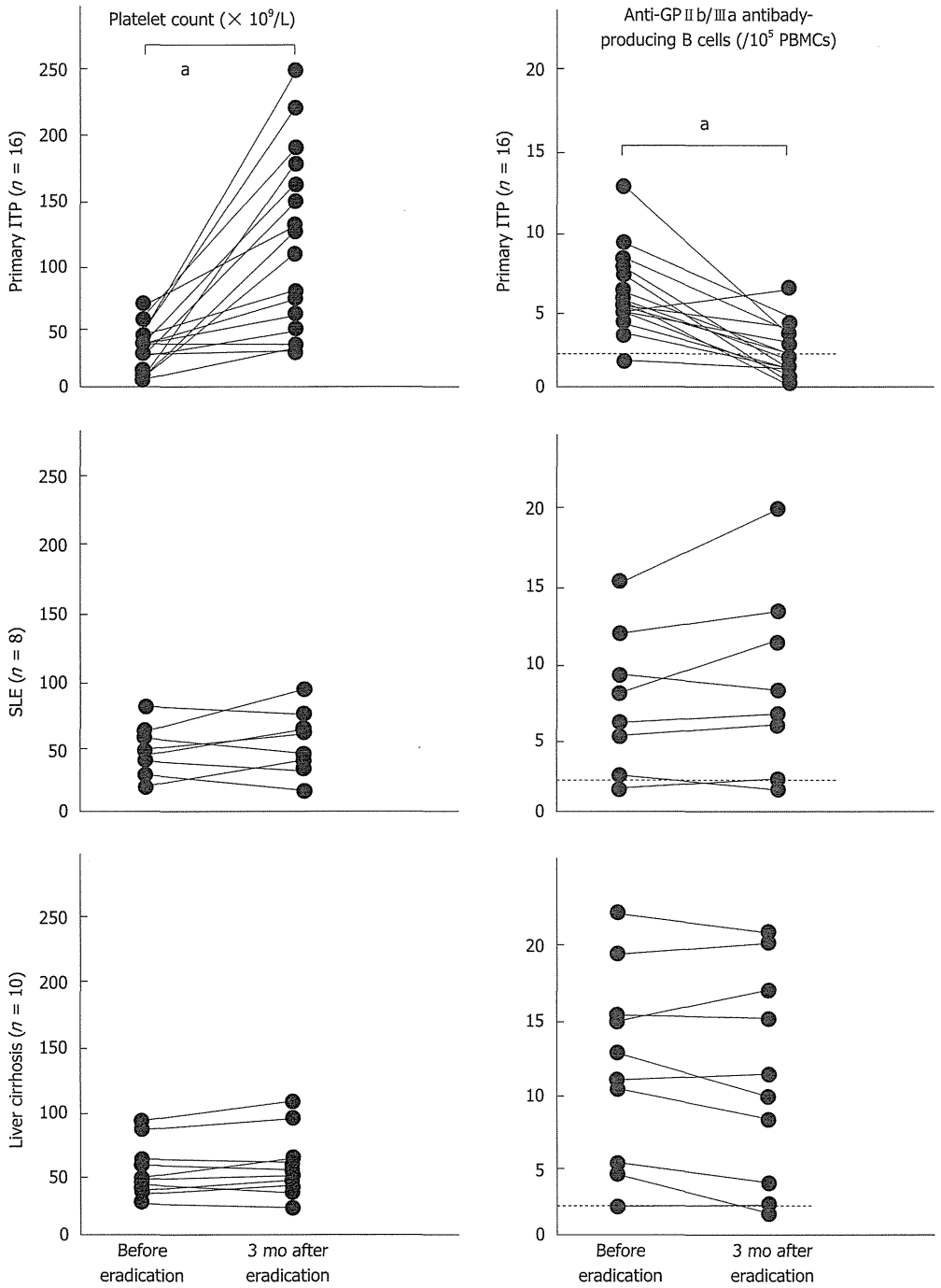

Figure 1 Changes in platelet count and in anti-GPIIb/IIIa antibody-producing circulating B cells before and 3 mo after an *Helicobacter pylori* eradication regimen in *Helicobacter pylori*-positive immune thrombocytopenia patients with no additional disease, or with systemic lupus erythematosus or liver cirrhosis. Changes in absolute values were compared by paired *t* test. $^{a}P < 0.05$. A dotted line indicates the cut-off for circulating anti-GP II b/III a antibody-producing B cells, which was 2/10^{5} peripheral blood mononuclear cells (PBMCs). ITP: Immune thrombocytopenia; SLE: Systemic lupus erythematosus.

eradication therapy have been extensively analyzed in *H. pylori*-infected ITP patients. The most consistently reported feature that predicts a favorable response is a shorter duration of ITP[9,49,50], but other studies have not found this association[51-53]. Other clinical characteristics, including an age less than 65 when diagnosed with ITP[49], a higher baseline platelet count[49], no prior corticosteroid therapy[51], no concomitant corticosteroid therapy[54,55], and no prior therapy for ITP[49], have been reported as factors predicting the platelet response, but other studies have reported conflicting results.

Several studies have examined whether there is a genetic predisposition to the platelet response. An association was shown between the *HLA-DQB1*03* haplotypes and a higher probability of the platelet response[45]. Moreover, single nucleotide polymorphisms within the genes for tumor necrosis factor-β and an inhibitory Fcγ receptor II B (FcγR II B) were found to be useful for predicting the response to the eradication treatment[55,56]. In terms of the anti-platelet autoantibody specificity, we found

that the presence of an anti-GPIb autoantibody response predicts resistance to *H. pylori* eradication therapy. Interestingly, a study from Italy reported that ITP patients with antibodies to CagA were more likely to respond to eradication therapy than patients without these antibodies[57]; although a study conducted in Japan failed to confirm this observation[50], we should recognize that CagA-positive strains are more common in Japan than in Italy.

Finally, Sato and colleagues assessed potential associations of the platelet response to *H. pylori* eradication with upper gastrointestinal endoscopic findings and histologic features of stomach tissue obtained by biopsy[58]. A severe degree of gastric atrophy on endoscopy and intense inflammation and atrophy in the gastric corpus detected by biopsy were predictors for a favorable response. These findings together indicate that both genetic background and bacterial factors, which collaboratively regulate the host inflammatory response to the bacterium, can account, at least in part, for the variable response to *H. pylori* eradication therapy.

MECHANISMS OF *H. PYLORI*-ASSOCIATED ITP

It has become clear that *H. pylori*-associated ITP is a subset of ITP in which *H. pylori* infection is actively involved in the pathogenic process. In patients with *H. pylori*-associated ITP, *H. pylori* eradication increases the platelet count in parallel with a suppression of anti-platelet autoantibody production, and results in the remission or even cure of the disease in many patients. Since eradicating *H. pylori* does not increase the platelet count in non-ITP subjects[59], the platelet recovery after successful *H. pylori* eradication is specific to ITP patients, and is likely to be mediated through the inhibition of an ongoing autoimmune response to platelets.

Molecular mimicry

Several hypotheses have been proposed regarding the mechanism by which *H. pylori* induces the development of ITP. One intriguing theory is that cross-reactive antibodies are produced that react with both *H. pylori* components and platelet surface antigens through molecular mimicry. Michel *et al*[22] investigated this molecular-mimicry hypothesis by testing platelet eluates derived from *H. pylori*-infected ITP patients, and found that platelet eluates with the capacity to react with GP Ⅱb/Ⅲa or GPIb failed to recognize any *H. pylori* antigens. On the other hand, Takahashi *et al*[25] reported that platelet eluates from *H. pylori*-infected ITP patients recognized CagA in immunoblots, but those from *H. pylori*-infected non-thrombocytopenic individuals did not. Unfortunately, since the IgG concentrations in the eluates were not adjusted in these studies, the intensity of individual bands was not quantitative. In addition, since platelets are known to take up and concentrate circulating IgG in intracellular granules, it is not clear whether the anti-CagA antibodies detected truly cross-react with platelet-surface antigens. Although it was recently reported that monoclonal antibodies generated against recombinant *H. pylori* urease B react with GP Ⅱb/Ⅲa expressed on the platelet surface, this study failed to show the presence of this cross-reactive antibody repertoire in patients with *H. pylori*-associated ITP[60]. While these findings suggest that cross-reacting antibodies against *H. pylori* may be present in patients with ITP, their pathogenic role remains obscure.

Non-specific activation of the immune system

In another potential mechanism, chronic *H. pylori* infection may act on the host's immune system to stimulate acquired immune responses, causing autoreactive T and B cells to emerge. Yamanishi *et al*[61] showed that *H. pylori* components are able to initiate autoimmune responses *via* autoantibodies that are produced through the activation of B-1 cells. However, this non-specific mechanism alone does not explain how an autoimmune response specific to platelet glycoproteins, such as that observed in ITP patients, would develop. In fact, there is no difference in the production of non-specific autoantibodies, including anti-nuclear, anti-microsome, and anti-smooth muscle antibodies, in individuals with and without *H. pylori*[62].

Modulation of monocyte/macrophage function

We have been evaluating mechanisms that elicit and maintain autoantibody responses to platelet glycoproteins in patients with ITP[63-65]. After detailed analyses of the GP Ⅱb/Ⅲa-reactive $CD4^+$ T cells and B cells in ITP patients, we proposed a "pathogenic loop" model for the ongoing IgG anti-platelet autoantibody response in ITP patients[66]. Specifically, macrophages in the reticuloendothelial system capture opsonized platelets *via* Fcγ receptors, and present antigenic platelet glycoprotein-derived peptides to T cells. Autoreactive $CD4^+$ T cells are then activated by their recognition of the antigenic peptides and exert helper activity to stimulate B cells to produce IgG anti-platelet autoantibodies, which in turn bind to circulating platelets. Theoretically, once this pathogenic loop is established, the production of IgG anti-platelet autoantibodies continues endlessly. Since anti-platelet autoantibodies are eliminated after eradicating *H. pylori* in patients with *H. pylori*-associated ITP[12], this pathogenic loop would consequently be disrupted and blocked. To elucidate the mechanism by which *H. pylori* eradication changes the ongoing pathogenic loop in ITP patients, we conducted a prospective study in which the phenotype and function of the autoreactive T and B cells and of the monocytes/macrophages involved in the pathogenic loop were serially measured in *H. pylori*-infected and -uninfected ITP patients treated with a standard eradication regimen[67]. At baseline, we found enhanced phagocytic capacity and low expression levels of inhibitory FcγR Ⅱ B in the circulating monocytes from *H. pylori*-infected patients, but not in those from uninfected patients. Suppression of this activated-monocyte phenotype was observed one week after starting the *H. pylori* eradication regimen,

when eradication was successful. The anti-platelet autoantibody responses and platelet kinetic parameters subsequently improved, indicating that suppression of the activated-monocyte function precedes the improvement in the autoantibody response.

Interestingly, modulation of the Fcγ-receptor balance toward an activating phenotype has also been observed in *H. pylori*-infected mice, through a downregulation of inhibitory FcγR II B in splenic and circulating monocytes/macrophages. A study in China recently confirmed that the FcγR II B expression on circulating monocytes is down-regulated in *H. pylori*-infected ITP patients[68]. Therefore, *H. pylori* infection plays an important role in ITP pathogenesis by altering the Fcγ receptor balance of monocytes/macrophages in favor of activating Fcγ receptors, through downregulation of the inhibitory receptor FcγR II B. These findings indicate that the platelet recovery after *H. pylori* eradication in ITP patients is mediated, at least in part, through a change in Fcγ receptor balance toward the inhibitory FcγR II B. The molecular events that induce this change in monocytes/macrophage properties are unclear. Although *H. pylori* does not invade the gastric epithelium, it can induce both the secretion of soluble inflammatory mediators and cellular apoptosis in the host, leading to local inflammation in the epithelium and the subepithelial layers. Released *H. pylori* components have been reported to be responsible for activating dendritic cells and macrophages through toll-like receptor signaling[69,70].

Interestingly, a change in Fcγ receptor balance toward the inhibitory FcγR II B in monocytes/macrophages has also reported in the therapeutic action of other established treatment regimens for ITP, such as intravenous immunoglobulin and high-dose dexamethasone. Samuelsson et al. demonstrated that intravenous immunoglobulin requires the presence of FcγR II B to prevent antibody-induced thrombocytopenia in a murine model of passive ITP[71], in which the FcγR II B expression on splenic macrophages was upregulated upon intravenous immunoglobulin treatment. The upregulation of inhibitory FcγR II B expression on circulating monocytes was also reported in ITP patients successfully treated with high-dose dexamethasone[72]. These findings point to the Fcγ receptor balance of monocytes/macrophages as an attractive therapeutic target for ITP.

A multifactorial mechanism

The pathogenesis of *H. pylori*-associated ITP most likely involves several factors. The mechanism of Fcγ-receptor-balance modulation in monocytes/macrophages does not exclude other proposed mechanisms for platelet recovery in ITP after *H. pylori* eradication, such as molecular mimicry between CagA and platelet-surface antigens. Moreover, some *H. pylori* strains induce platelet aggregation that is dependent on the interaction of von Willebrand factor and IgG antibodies against *H. pylori* with their corresponding receptors, GPIb and FcγR II A, on platelets[73]. In this model, anti-*H. pylori* antibodies are capable of opsonizing platelets by binding to *H. pylori*, von Willebrand factor, and GP I b, like anti-platelet autoantibodies. However, we should recognize that *H. pylori* infection is usually established in infants with an immature immune system, and that the prevalence of ITP among *H. pylori*-infected individuals is extremely low. Therefore, it is apparent that *H. pylori* infection alone is insufficient to induce the onset of ITP. Additional triggers would be necessary to elicit the anti-platelet autoimmune response observed in *H. pylori*-associated ITP.

CONCLUSION

The past few years have witnessed important advances in our understanding of the relationship between *H. pylori* infection and ITP. The sustained efficacy of the *H. pylori* eradication regimen in adults with ITP has affected decision-making in clinical practice. The importance of detecting and eradicating *H. pylori* in patients with seemingly typical ITP is now recognized, especially where *H. pylori* infection is prevalent, such as in Japan. A growing body of evidence indicates that *H. pylori*-associated ITP is a unique ITP subset in which the bacterium is central to the pathogenic process. The development of *H. pylori*-associated ITP appears to depend on multiple factors. Among these, modulation of the Fcγ receptor balance of monocytes/macrophages through inhibition of the immunosuppressive FcγR II B signal, which is a host immune response associated with *H. pylori* infection, is a key mechanism for initiating and maintaining the anti-platelet autoantibody response. These insights provide valuable clues that may assist in the development of new therapeutic strategies for ITP.

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