表1　TPO受容体作動薬の懸念される副作用

1. 血栓症の誘発
2. 治療中止時の反撥性血小板減少および出血傾向の増悪
3. 骨髄線維症の誘発（reticulin, collagenの増加）
4. MDSにおける白血病の誘導
5. サイトカインとの相互作用
6. 長期使用時における幹細胞の枯渇

い．注意すべき副作用や可能性のある副作用を表1に示す．第一世代TPO製剤とは異なり，エルトロンボパグやロミプロスチム投与による内因性TPO阻害抗体の誘導は現在のところ観察されていない．しかしながらTPO受容体作動薬は血小板造血刺激剤であるため，血小板増多のみならず血栓症が誘導される可能性がある．さらにはTPO受容体作動薬の副作用として，骨髄レチクリンやコラーゲンの増加，長期使用による血液幹細胞の枯渇，骨髄異常の誘導の可能性などが懸念されている[17,27]．特に最近では，MDSなどに対して異常細胞の増殖を誘導する可能性が指摘されており，ITPとMDSの鑑別の重要性とともにMDSに対する使用は慎むべきである．また，小児ITPへの投与はいまだ承認されていないが，最近小規模臨床研究ではあるがロミプロスチムの小児ITPへの有効性が示された[28]．しかしながら，TPO受容体作動薬はITPを完全寛解させる薬剤ではなく，長期使用により病態をコントロールする薬剤であるため，上記副作用を考慮し小児への使用は慎重を期すべきと考える．

現時点ではTPO受容体作動薬の使用はステロイド療法無効例で，脾摘が無効あるいはなんらかの理由で脾摘が禁忌あるいは困難な症例に限定されるべきであり，第三選択との位置づけである．このTPO受容体作動薬のITP治療における位置づけは，最近発表されたアメリカ血液学会の2011年版ガイドラインでも同様の位置づけである[29]．実際，本邦においてはTPO受容体作動薬に関する使用上の注意として，1）他の治療にて十分な効果が得られない場合，または忍容性に問題があると考えられる場合に使用すること，2）血小板数，臨床症状からみて出血リスクが高いと考えられる場合に使用すること，と記載されている．この場合の「他の治療」とは，具体的には副腎皮質ステロイドおよび脾臓摘出術を意味している．さらに本薬剤使用時には血小板数が大きく変動することがしばしば経験されるため，使用開始時には，血小板数が安定するまで最低週1回は血小板数を測定する必要がある．またその用量も血小板数3万/μl以上を維持することを目標に必要最小限の使用とすべきである．

むすび

ITPの治療は，トロンボポエチン受容体作動薬の登場により大きく変化しようとしている．新規薬剤の長期安全性は今後の課題であり，その適正な使用が望まれる．

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Recognition of highly restricted regions in the β-propeller domain of αIIb by platelet-associated anti-αIIbβ3 autoantibodies in primary immune thrombocytopenia

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Platelet-associated (PA) IgG autoantibodies play an essential role in primary immune thrombocytopenia (ITP). However, little is known about the epitopes of these Abs. This study aimed to identify critical binding regions for PA anti-αIIbβ3 Abs. Because PA anti-αIIbβ3 Abs bound poorly to mouse αIIbβ3, we created human-mouse chimera constructs. We first examined 76 platelet eluates obtained from patients with primary ITP. Of these, 26 harbored PA anti-αIIbβ3 Abs (34%). Further analysis of 15 patients who provided sufficient materials showed that the epitopes of these Abs were mainly localized in the N-terminal half of the β-propeller domain in αIIb (L1-W235). We could identify 3 main recognition sites in the region; 2 eluates recognized a conformation formed by the W1:1-2 and W2:3-4 loops, 5 recognized W1:2-3, and 4 recognized W3:4-1. The remaining 4 eluates could not be defined by the binding sites. Within these regions, we identified residues critical for binding, including S29 and R32 in W1:1-2; G44 and P45 in W1:2-3; and P135, E136, and R139 in W2:3-4. Of 11 eluates whose recognition sites were identified, 5 clearly showed restricted κ/λ-chain usage. These results suggested that PA anti-αIIbβ3 Abs in primary ITP tended to recognize highly restricted regions of αIIb with clonality. (*Blood.* 2012;120(7):1499-1509)

Introduction

Primary immune thrombocytopenia (ITP) is an autoimmune disorder characterized by thrombocytopenia that results from immune-mediated platelet destruction and reduced platelet production.[1-4] In ITP, autoantibodies bound to platelets (platelet-associated antibodies; PA Abs) cause platelet destruction by Fcγ receptor–mediated phagocytosis.[1] Furthermore, autoantibodies binding to megakaryocytes led to decreased maturation and cell death.[5,6] Multiple targets of autoantibodies have been found in ITP. Among patients with chronic ITP, 43%-57% and 18%-50% harbored PA Abs that recognized the platelet membrane glycoprotein (GP) IIb/IIIa (integrin αIIbβ3) and the GPIb/IX/V complex, respectively.[7-10]

For more than 2 decades, efforts have been focused on identifying the target epitopes for PA Abs to improve our understanding of the pathogenesis of primary ITP and to pursue a therapeutic approach. We and others previously reported that, in chronic ITP, PA anti-αIIbβ3 Abs frequently bound to cation-dependent conformational antigens and did not react with αvβ3.[11-13] Those data suggested that, in primary ITP, the target epitopes of anti-αIIbβ3 Abs may be localized mainly on αIIb; in contrast, in HIV-associated ITP, the target epitopes appeared to be localized to the 49-66 residues of β3.[14] Moreover, we previously reported that, in one-third of patients with ITP (11 of 34), PA anti-αIIbβ3 Ab binding was markedly impaired against KO variant αIIbβ3, which had 2 amino acids inserted between residues 160 and 161 in the W3:4-1 loop of the β-propeller domain.[15] However, target epitopes of most PA anti-αIIbβ3 Abs remain to be elucidated.

PA anti-αIIbβ3 Abs typically recognize conformational epitopes, rather than linear epitopes.[16,17] Therefore, epitope mapping for PA Abs requires the retention of major conformations in αIIbβ3. Because we noticed that the PA anti-αIIbβ3 Abs from patients with ITP had markedly impaired reactivity to mouse αIIbβ3, we characterized target epitopes of PA anti-αIIbβ3 Abs by exploring their reactivity to the cells that expressed human-mouse chimeric αIIbβ3 in the present study. A thorough analysis of 15 eluates obtained from patients with primary ITP found that most of the PA anti-αIIbβ3 Abs recognized the N-terminal half of the β-propeller domain in αIIb. We identified 3 main Ab recognition sites in the region and residues that were critical for the binding of some Abs.

Methods

Patients

We first examined 76 eluates obtained from patients with primary ITP (21 men, 55 women). Diagnosis of primary ITP was based on a report from an international working group.[18] We obtained written, informed consent for blood sampling necessary for this study from all patients, in accordance with the Declaration of Helsinki. This study was approved by the Osaka University Institutional Review Board. With the use of flow cytometry with 293T cells expressing αIIbβ3, we detected anti-αIIbβ3 autoantibodies in 26 of 76 (34%) platelet eluates (6 and 20 eluates were obtained from men and women, respectively). Of these 26 eluates, we further analyzed 15 patients who provided sufficient quantity of platelet eluates for this study. The characteristics of these 15 patients (PTs) are shown in Table 1. Although 5 eluates were

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Table 1. Clinical profiles of 15 patients with ITP at blood sampling

PT no.	Age, y	Sex	Plt count, $\times 10^3/\mu L$	Medications	Splenectomy	Duration of ITP
1	71	F	189-311	P	No	1 mo
2	63	F	14-212	SB+P	Yes	> 10 y
3	37	F	5-199	SB+P	Yes	> 10 y
5	65	F	64-116	P	No	6-10 y
6	49	F	189	P	No	1 mo
7*	55	M	103-105	N	Yes	> 10 y
12*	56	F	22-116	SB+P	Yes	> 10 y
17	64	F	23-146	SB+P	Yes	> 10 y
23	75	F	41-97	N	No	1-5 y
34	67	M	17-144	P	No	4-8 mo
36	72	F	76-186	P+IV	No	1-3 mo
37	62	F	27	P	Yes	> 10 y
41	53	F	40-55	SB+P	Yes	> 10 y
42	42	F	128-271	SB	Yes	> 10 y
45	73	F	17-203	P+IV	No	3-7 mo

Plt indicates platelet; P, prednisolone; SB, eltrombopag; N, none, and IV, intravenous gamma globulin.

*PTs 7 and 12 were studied in our previous reports as nos. 2 and 6, respectively.[15]

obtained < 12 months after the ITP diagnosis, all (except PT 36) were classified as chronic ITP.[18] PT 7 and PT 12 were studied in our previous report.[15] The binding of PA anti-αIIbβ3 Ab of PT 12 was markedly impaired by the KO mutation in αIIb, whereas that of PT 7 was not affected by the mutation.

Platelet isolation and preparation of PA antibodies

Platelets and platelet eluates were prepared as previously described.[19] In brief, washed platelets were obtained by differential centrifugation and adjusted to a concentration of $200 \times 10^3/\mu L$ in PBS. PA Abs were eluted by vigorous mixing with an equal amount of diethyl ether. Platelet eluates were maintained at −80°C until use.

Construction of expression vectors

The N-terminal portion of αIIb, known as the β-propeller domain, contains 7 radially arranged β-sheets, termed "W" because of their topology. Each W structure has 4 anti-parallel β-strands and 4 connecting loops.[20] Figure 1A shows the human and mouse sequence alignment of the β-propeller domains. The boxes indicate the small loop structures of each β-sheet domain, and the asterisks indicate amino acid differences between the human and mouse sequences. Human *αIIb* and *β3* cDNAs cloned into the pcDNA3 vector were gifts from Dr Peter Newman and Dr Gilbert White (BloodCenter of Wisconsin), respectively. Mouse *αIIb* and *β3* cDNAs were obtained by reverse transcribing platelet RNA from C57BL/6 mice, amplifying by PCR, and subcloning into the pcDNA3 expression vector. αIIb expression vectors with swapped human and mouse cassettes were constructed with the megaprimer PCR method[21] or with a site-directed mutagenesis kit (Agilent Technologies). The entire mutated αIIb sequence was confirmed for each vector. The swapping mutants are shown in Figure 1B. The m(X)H expressed the human αIIb that carried the mouse sequences from the N-terminus to the X region; conversely, H(X)m expressed mouse αIIb that carried the human sequences from the N-terminus to the X region. Hm(X)H expressed human αIIb in which the only X region was swapped with the corresponding region from mouse αIIb. mH(X-Y)m expressed mouse αIIb with a large part of the N-terminal region (X to Y) swapped with the corresponding region from human αIIb. m(β-propeller)H expressed the entire β-propeller domain of mouse αIIb, attached to the (C-terminal) remainder of the protein from the human αIIb sequence. The human-mouse chimera plasmids are listed in supplemental Table 1 (available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article).

Detection of PA anti-αIIbβ3 Ab binding

αIIb and β3 expression vectors were transiently transfected into 293T cells by lipofection (Lipofectamine2000; Invitrogen). Two days after transfection, the cells were detached from dishes with a solution of 0.02% ethylenediaminetetraacetic acid in PBS (Nakarai tesque); the cells were then washed once with PBS and resuspended at a concentration of 5×10^6/mL in Tyrode buffer with 1mM $CaCl_2$ and 1mM $MgCl_2$. Then, 50-μL aliquots of platelet eluates were incubated with equal amounts of cell suspension for 30 minutes on ice, followed by staining with Alexa Fluor 488–conjugated anti–human IgG (Invitrogen) and phycoerythrin-conjugated anti–human CD61 (Becton Dickinson [BD]). After washing, the cells were resuspended in Tyrode buffer that contained propidium iodine (1 μg/mL) to identify dead cells and then the cells were analyzed on a flow cytometer (FACScan; BD).

PA Ab binding (Alexa 488 staining) was analyzed in a subset of cells that were highly positive for CD61; this subset is denoted by the rectangle (R7) in the dot blots (Figure 1C). We confirmed that $CD61^+$ cells in this region were exclusively complexed with transfected αIIb but not with endogenous α_V by staining with anti-αIIbβ3–specific antibodies; an allophycocyanin (APC)–conjugated anti–human CD41a (clone HIP8; BD), an FITC-conjugated anti–human CD41 (clone P2; Beckman Coulter), and an FITC-conjugated anti–mouse CD41 (clone MWReg30; BD). PA Ab IgG binding 293T cells that expressed chimeric αIIb complexed with human β3 (chimera αIIbβ3) relative to the cells that expressed wild-type human αIIbβ3 (wt αIIbβ3) was calculated as follows: specific PA Ab binding to chimera αIIbβ3/specific PA Ab binding to wt αIIbβ3 × 100 (%).

We always used ≥ 2 eluates obtained from healthy subjects as control in each experiment, and specific IgG binding was calculated by subtracting the mean fluorescence intensity (MFI) of IgG binding in control eluates from the MFI of IgG binding in patient eluates. Expressions of the chimera αIIbβ3 and wt αIIbβ3 cells were monitored by anti-CD61 binding, and the specific PA Ab binding was compensated by CD61 expression.

PA Ab binding to platelets was determined as previously described.[22]

Detection of light chain usage by PA anti-αIIbβ3 Abs

Platelet eluates were incubated with 293T cells that transiently expressed human αIIbβ3. This was followed by incubation with FITC-conjugated anti-κ, PE-conjugated anti-λ Abs (Simultest Anti-Kappa/Anti-Lambda; BD), and APC-conjugated anti-CD61 (Invitrogen). After washing, cells were resuspended in Tyrode buffer that contained propidium iodine. Next, they were analyzed by flow cytometry to evaluate anti-κ and anti-λ Ab binding to cells highly positive for CD61.

Results

Target epitopes of PA anti-αIIbβ3 Abs are mainly localized in the N-terminus of the β-propeller domain in αIIb

We examined PA Ab reactivity to normal platelets and platelets obtained from a patient with type I Glanzmann thrombasthenia (GT), whose platelets completely lack surface αIIbβ3 expression as we previously reported.[23] In eluates from 9 of 10 patients with ITP, the PA Ab reactivity to GT platelets was markedly lower than their reactivity to normal platelets (Figure 2A). The marked reduction in the reactivity to GT platelets was also observed in other 3 eluates (supplemental Figure 1). These results indicated that most PA Abs in these eluates were directed to αIIbβ3. We then examined PA Ab reactivity to 293T cells that expressed mouse αIIbβ3. We found that, in the eluates examined, the PA Abs did not react with mouse αIIbβ3 (Figure 2B). To identify which subunit was essential for the binding, we examined the reactivity to mouse αIIb/human β3 (m2b/H3a) and human αIIb/mouse β3 (H2b/m3a). We found that the PA Abs reacted similarly to cells that expressed H2b/m3a and cells that expressed human αIIbβ3 (38.5%-100%). However, PA Ab reactivity to m2b/H3a was markedly reduced in all 9 eluates examined (1.3%-30.3%; Figure 2C). These results confirmed that the target epitopes of PA anti-αIIbβ3 Abs were mainly present in αIIb.

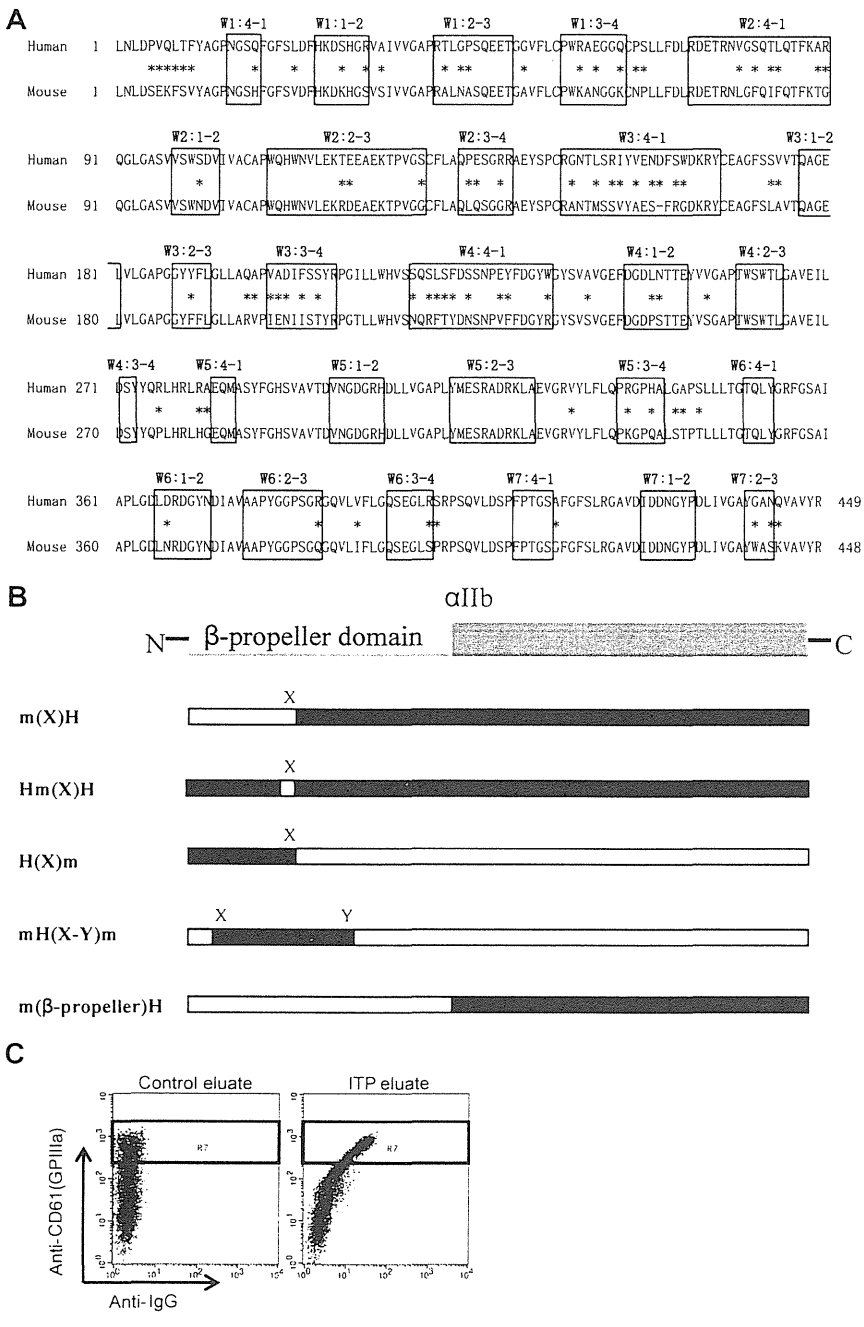

Figure 1. Structure and alignment of the β-propeller domain of αIIb. (A) Human and mouse sequence alignment of the β-propeller domain. The boxes indicate the small loop structures of each β-sheet domain, and the asterisks indicate amino acid difference between human and mouse sequences. (B) Abbreviation of each human-mouse chimeric αIIb/human β3. m(X)H; human αIIb (black) carrying mouse sequences (white) from the N-terminus to the X region. Hm(X)H; human αIIb was exchanged with the only X region for the corresponding sequence of mouse αIIb. H(X)m; mouse αIIb carrying human sequences from the N-terminus to the X region. mH(X-Y)m; mouse αIIb was exchanged with the region of X to Y for the corresponding human αIIb. m(β-propeller)H; human αIIb was exchanged with the entire β-propeller domain of αIIb for the corresponding mouse αIIb. (C) Assessment of PA Abs binding to mutated αIIbβ3-expressing cells. Binding of PA Abs (horizontal) was analyzed in a subset of CD61 highly positive cells denoted by the rectangle (R7) in the dot blots.

We next examined PA anti-αIIbβ3 Ab reactivity with several αIIb chimeras. All αIIb chimeras were expressed with the human β3 on 293T cells. The m(β-propeller)H and m(W4:4-1)H chimeras comprised the human αIIb with substitutions for the mouse β-propeller domain (L1-R449) and the N-terminal half of the β-propeller domain (L1-W235), respectively, because there are few amino acid differences between human and mouse in the C-terminal half of the β-propeller domain (Figure 1B). Compared with wt αIIbβ3, all 15 of the eluates showed markedly impaired binding to cells that expressed the m(β-propeller)H and those that expressed the m(W4:4-1)H chimera (Figure 2D). These results were confirmed by the antigen capture ELISA method in selected patients (supplemental Figure 2). In sharp contrast, most of the 15 eluates tested showed high PA Ab reactivity with the converse αIIb chimeras, H(β-propeller)m and H(W4:4-1)m, complexed with human β3. The PA Ab reactivity was comparable with that observed with wt human αIIbβ3 (Figure 2E). These results indicated that the epitopes of PA anti-αIIbβ3 Abs were mainly localized to the N-terminal half of the β-propeller domain (L1-W235) in αIIb.

Identification of 3 main recognition sites in the N-terminal half of the β-propeller domain in αIIb

Next, we aimed to identify critical target epitopes for PA anti-αIIbβ3 Abs in the N-terminal half of the β-propeller domain in αIIb. For this, we created human-mouse αIIb chimeras that had serial changes in which small sections of human αIIb were exchanged with the corresponding mouse sequences. The series extended from the N-terminus to the W4:4-1 loop (Table 2). We

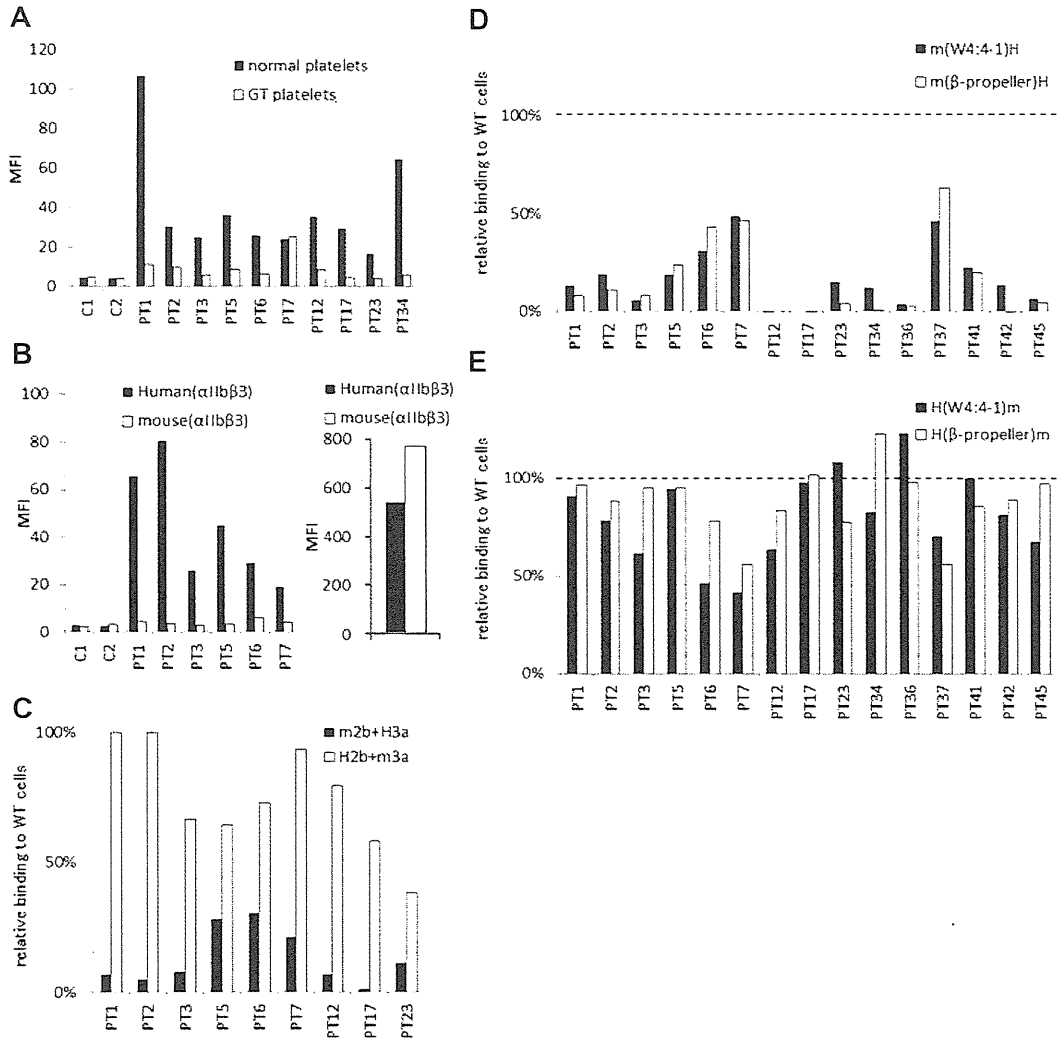

Figure 2. The epitopes of PA anti-αIIbβ3 Abs are mainly localized in the N-terminus of the β-propeller domain of αIIb. (A) Binding of PA Abs to normal (black) or αIIbβ3-deficient platelets (GT platelets; white). C1 and C2 show control eluates. (B) Binding of PA Abs to 293T cells that expressed human (black) or mouse αIIbβ3 (white; left). Expression levels of human and mouse αIIbβ3 were indicated in the right panel monitored by anti-CD61 Ab. (C) Relative binding of PA Abs to 293T cells that expressed mouse αIIb/human β3 (m2b/H3a; black) or human αIIb/mouse β3 (H2b/m3a; white) compared with human αIIbβ3-expressing cells. (D) Relative binding of PA Abs to 293T cells that expressed human αIIb replaced with whole β-propeller domain (white) or the N-terminus to W4:4-1 loop (black) of mouse αIIb with human β3. (E) Relative binding of PA Abs to 293T cells that expressed mouse αIIb replaced with whole β-propeller domain (white) or the N-terminus to W4:4-1 loop (black) of human αIIb with human β3. Shown were means of ≥ 2 independent experiments.

noticed that the reactivity of many PA Abs decreased markedly when a specific loop from human was exchanged for the corresponding mouse sequence. Consequently, we identified 3 groups (A, B, and C) on the basis of loops that appeared to be essential for PA Ab reactivity: the W1:1-2, W1:2-3, and W3:4-1 loops (Table 2). Then, we further characterized the autoantigenic epitopes in each group.

Group A: the W1:1-2 and W2:3-4 loops are essential for PA anti-αIIbβ3Ab binding. In 2 samples (from PTs 17 and 23), the PA Ab reactivity with m(W1:1-2)H, the human αIIb that carried the mouse sequence from the N-terminus to the W1:1-2 loop, was markedly impaired compared with PA Ab reactivity with m(W1:4-1)H (Table 2; Figure 3A). Moreover, these PA Abs showed impaired binding with Hm(W1:1-2)H, the human αIIb that carried only the mouse W1:1-2 loop (Figure 3A). These results suggested that the W1:1-2 loop was essential for PA Ab reactivity in these patients. Conversely, we tested whether we could restore PA Ab reactivity with the mouse αIIb sequence by replacing sections with a series of corresponding human sequences from the N-terminus. We found that PA Ab reactivity in these 2 samples was only restored when the mouse W2:3-4 loop was exchanged with the corresponding human sequence. This was confirmed by the observation that the PA Ab reactivity was markedly impaired for Hm(W2:3-4)H, the human αIIb that carried only the mouse W2:3-4 loop (Figure 3B). These results indicated that both the W1:1-2 and the W2:3-4 loops were essential for PA Ab reactivity. Moreover, the PA Ab reactivity with mH(W1:1-2–W2:3-4)m, the mouse αIIb that carried the human W1:1-2 to W2:3-4 sequence, was not reduced compared with the reactivity with the wt human αIIb. This supported our hypothesis that these PA Abs specifically recognized the W1:1-2 and W2:3-4 loops (Figure 3C).

The W1:1-2 and W2:3-4 loops harbor 2 and 3 amino acid differences between human and mouse, respectively. Therefore, we replaced each of these amino acids in the human sequence with that in the mouse sequence. The S29K and R32S mutations in the W1:1-2 loop and the E136Q and R139G mutations in the W2:3-4 loop almost completely abolished the reactivity of PA Abs from PT 17. The PA Abs from PT 23 also showed markedly impaired reactivity with the R32S and R139G mutations (Figure 3D-E). These results indicated that the S29, R32, E136, and R139 residues in αIIb, particularly the 2 arginines, were critical for the binding of PA Abs in these patients.

Table 2. Percentage of PA Ab binding to human-mouse chimeric αIIbs complexed with human β3 relative to PA Ab binding to wt αIIbβ3 in 293T cells

	m(W1:4-1)H	m(W1:1-2)H	m(W1:2-3)H	m(W2:2-3)H	m(W2:3-4)H	m(W3:4-1)H	m(W4:4-1)H
Group A							
PT 17	99.5*	−1.5*	−1.7	−4.7	NT	−2.9	0.3
PT 23	72.7*	18.9*	−25.0	−18.4	−6.6	−20.6	15.2
Group B							
PT 3	84.4	83.3*	19.6*	12.7	NT	2.9	7.9
PT 36	134.2	116.6*	1.5*	NT	1.7	2.6	3.8
PT 41	88.7	97.5*	50.3*	NT	51.0	44.6	22.3
PT 42	85.6	105.0*	47.5*	NT	41.3	20.5	13.5
PT 12	89.7	87.1	57.5	30.9	9.1	6.8	−1.7
Group C							
PT 1	75.2	NT	93.2	93.7	102.2*	27.3*	13.0
PT 6	109.1	NT	232.2	115.9	103.1*	30.7*	30.7
PT 34	94.0	91.7	108.5	NT	88.3*	−3.4*	12.1
PT 45	94.3	91.4	95.3	NT	73.5*	13.5*	6.5
Others							
PT 2	87.5	80.3	45.8	36.0	NT	19.6	18.9
PT 5	101.5	82.4	74.0	61.6	43.4	39.7	18.3
PT 7	104.9	NT	111.4	90.5	105.3	60.3	48.6
PT 37	123.5	128.5	102.8	NT	114.1	65.4	45.9

The PT no. designates the ITP sample that was tested for binding to cells that expressed the indicated chimeras. Values are percentages and represent the means of ≥ 2 independent experiments.

NT indicates not tested.

*Marked reductions (> 40%) in binding between 2 loops in addition to marked reduction (> 40%) in binding compared with binding with wt αIIbβ3.

Group B: the W1:2-3 loop is essential for PA anti-αIIbβ3Ab binding. In 5 samples (from PTs 3, 12, 36, 41, and 42), the PA Ab reactivity was impaired with m(W1:2-3)H compared with m(W1:1-2)H (Table 2; Figure 4A). The sample from PT 36 showed complete loss of PA Ab reactivity with m(W1:2-3)H. The reactivity with Hm(W1:2-3)H was essentially the same as the reactivity with

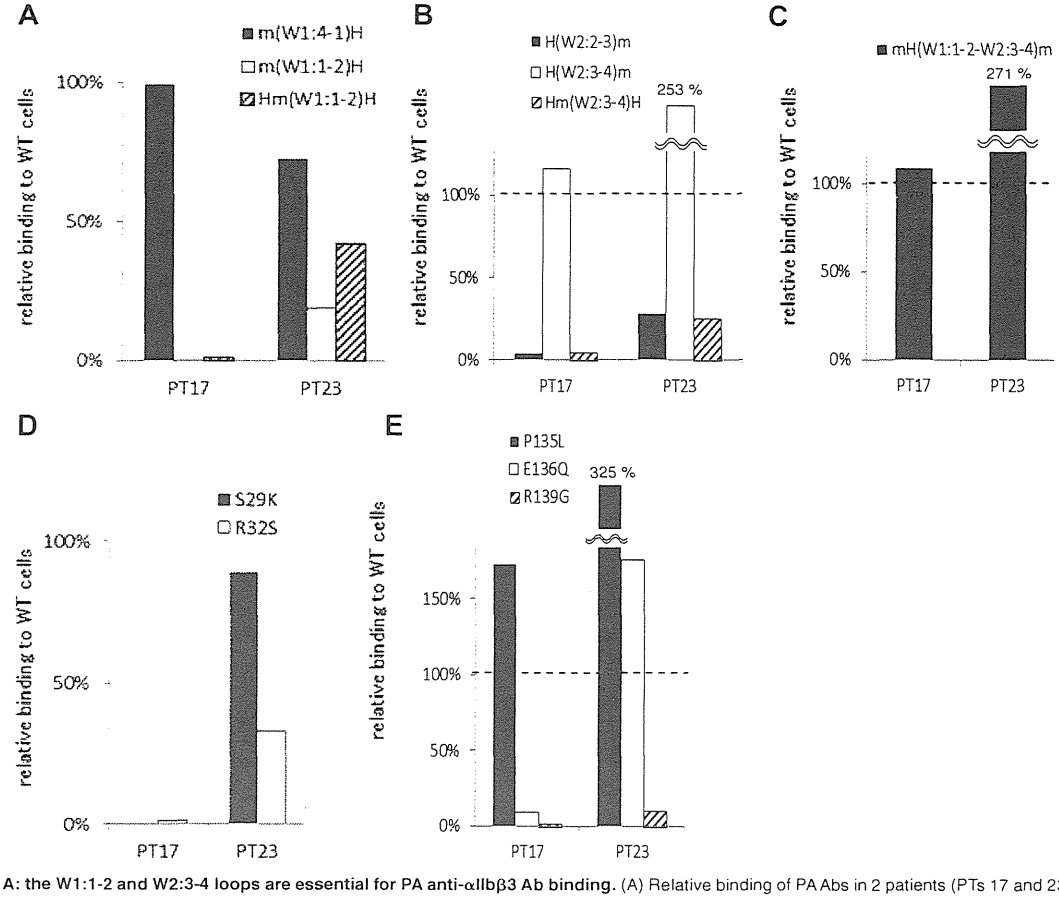

Figure 3. Group A: the W1:1-2 and W2:3-4 loops are essential for PA anti-αIIbβ3 Ab binding. (A) Relative binding of PA Abs in 2 patients (PTs 17 and 23) to m(W1:4-1)H (black), m(W1:1-2)H (white), and Hm(W1:1-2)H (shaded) compared with wt αIIbβ3. (B) Relative binding of PA Abs to H(W2:2-3)m (black), H(W2:3-4)m (white), and Hm(W2:3-4)H (shaded). (C) Relative binding of PA Abs to mH(W1:1-2–W2:3-4)m that the mouse αIIb carried the human W1:1-2 to W2:3-4 sequences. (D) Relative binding of PA Abs to S29K (black) and R32S (white) mutants. (E) Relative binding of PA Abs to P135L (black), E136Q (white) and R139G (shaded) mutants. Shown were means of ≥ 2 independent experiments.

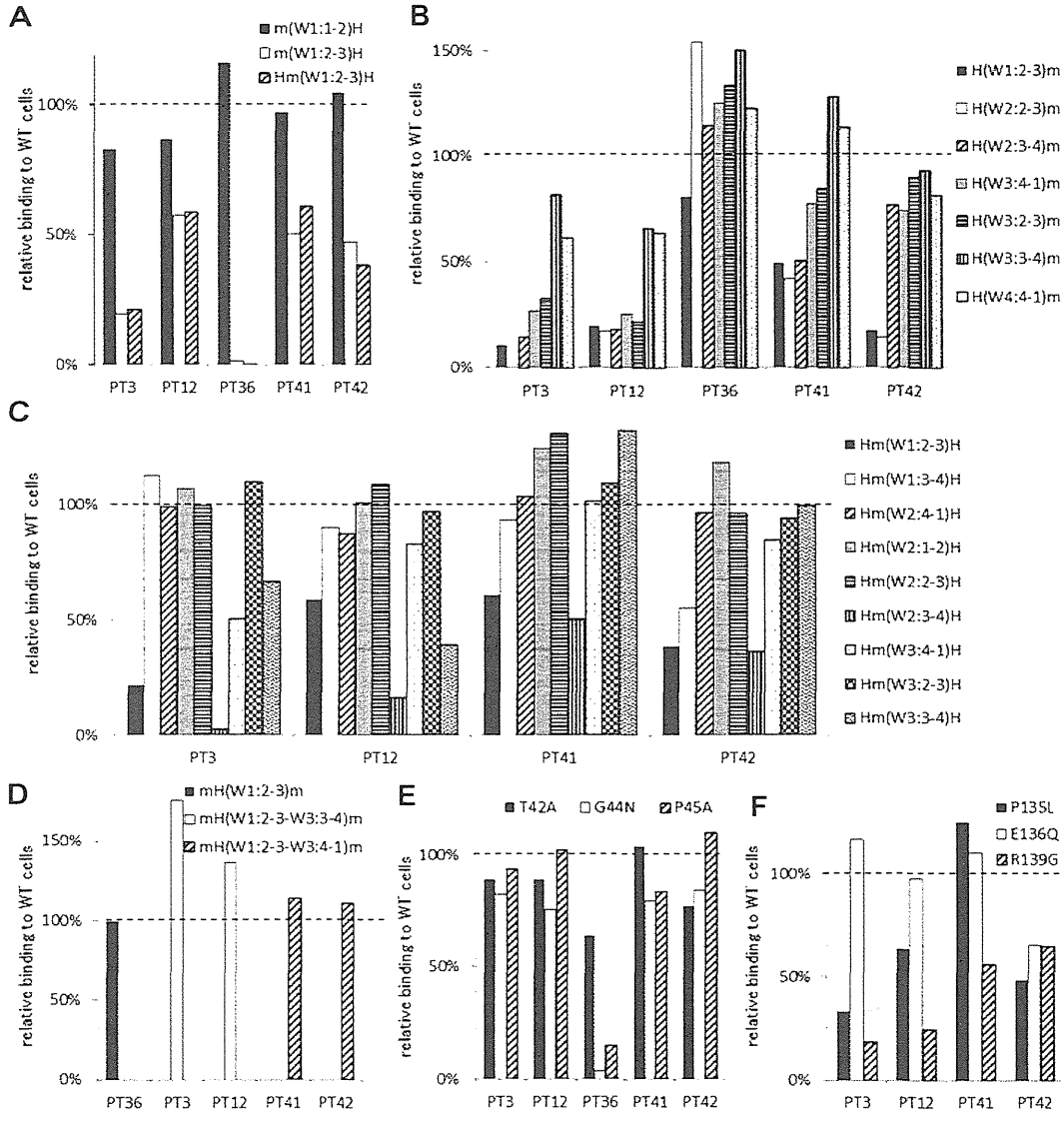

Figure 4. Group B: the W1:2-3 loop is essential for PA anti-αIIbβ3 Ab binding. (A) Relative binding of PA Abs in 5 patients (PTs 3, 12, 36, 41 and 42) to m(W1:1-2)H (black), m(W1:2-3)H (white), and Hm(W1:2-3)H (shaded) compared with wt αIIbβ3. (B) Relative binding of PA Abs to mouse αIIb replaced from the N-terminus to the indicated loops with the corresponding human sequences. (C) Relative binding of PA Abs to human αIIb replaced the indicated loop with the corresponding mouse sequences. (D) Relative binding of PA Abs to mH(W1:2-3)m (black), mH(W1:2-3–W3:3-4)m (white), and mH(W1:2-3–W3:4-1)m (shaded). (E) Relative binding of PA Abs to T42A (black), G44N (white), and P45A (shaded) mutants. (F) Relative binding of PA Abs to P135L (black), E136G (white), and R139G (shaded) mutants. Shown were means of ≥ 2 independent experiments.

m(W1:2-3)H (Figure 4A). These results suggested that the W1:2-3 loop was essential for PA Ab reactivity in these samples. Again, we tested whether we could restore PA Ab reactivity with the mouse αIIb sequence by replacing sections with a series of corresponding human sequences from the N-terminus (Figure 4B). We found that the PA Ab reactivity in the sample from PT 36 was almost fully restored with H(W1:2-3)m, which had the human amino acid sequence from the N-terminus to the W1:2-3 loop. The sample reactivity from PT 36 with mH(W1:2-3)m was the same as that observed with the wt human αIIbβ3 sequence (Figure 4D). These results suggested that the W1:2-3 loop contained an epitope(s) for PA anti-αIIbβ3 Abs in the sample from PT 36.

In contrast, the PA Abs in the samples from PTs 3 and 12 showed restored reactivities with H(W3:3-4)m, and the samples from PT 41 and PT 42 showed restored reactivities with H(W3:4-1)m and H(W2:3-4)m, respectively (Figure 4B). These results suggested that loop(s) other than the W1:2-3 may be important for PA Ab reactivity in these patients. To localize the epitope(s) important for PA Ab reactivity in these patients, we constructed human αIIb expression vectors in which each surface loop from W1:2-3 to W3:3-4 was replaced with the corresponding mouse sequence (Figure 4C). We found that the PA Abs from these 4 patients showed impaired reactivity with Hm(W1:2-3)H and Hm(W2:3-4)H. This indicated that both the W1:2-3 and the W2:3-4 loops were involved in PA Ab binding efficiency. In addition, the W3:3-4 loop also appeared to be involved in PA Ab reactivity in samples from PTs 3 and 12 (Figure 4C). Furthermore, PA Ab binding was fully restored to levels observed with wt αIIb, when the samples from PTs 3 and 12 reacted with mH(W1:2-3-W3:3-4)m and the samples from PTs 41 and 42 reacted with mH(W1:2-3-W3:4-1)m (Figure 4D). These results suggested that the PA Abs from PTs 3 and 12 mainly recognized the W1:2-3, W2:3-4, and W3:3-4 loops and that the PA Abs from PTs 41 and 42 recognized the W1:2-3 and W2:3-4 loops.

There are 3 amino acid differences between human and mouse in each W1:2-3 and W2:3-4 loop. These are the T42A, G44N, and P45A in the W1:2-3 loop and P135L, E136Q, and R139G in the

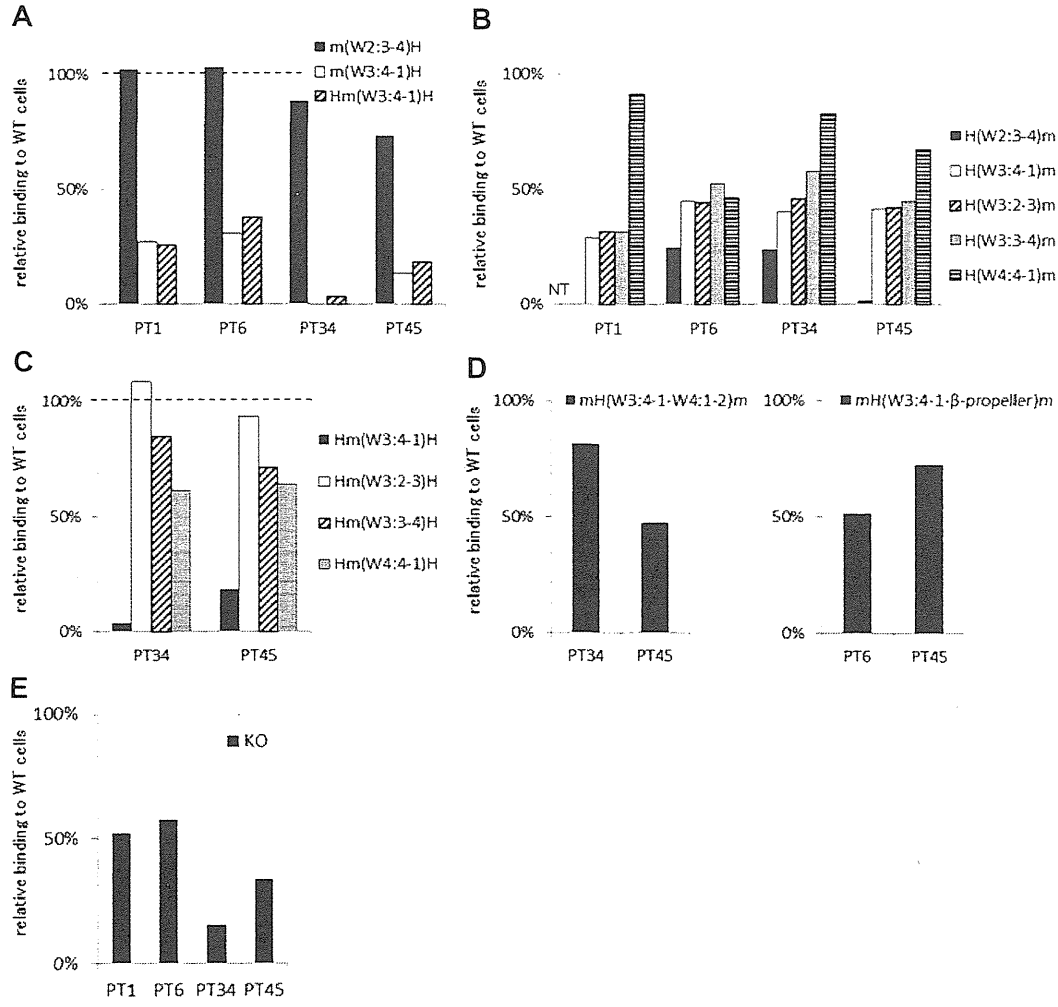

Figure 5. Group C: the W3:4-1 loop is essential for PA anti-αIIbβ3 Ab binding. (A) Relative binding of PA Abs to m(W2:3-4)H (black), m(W3:4-1)H (white), and Hm(W3:4-1)H (shaded) compared with wt αIIbβ3. (B) Relative binding of PA Abs to mouse αIIb replaced from the N-terminus to the indicated loops with the human corresponding sequences. (C) Relative binding of PA Abs to human αIIb replaced the indicated loop with the mouse corresponding sequences. (D) Relative binding of PA Abs to mH(W3:4-1–W4:1-2)m and mH(W3:4-1–β-propeller)m that the mouse αIIb carried the human sequences from W3:4-1 to the C-terminal of the β-propeller domain. (E) Relative binding of PA Abs to 2 amino acids insertion mutant in W3:4-1 loop (KO variant αIIbβ3). Shown were means of ≥ 2 independent experiments.

W2:3-4 loop. Again, we examined the effects of substituting these single amino acids on PA Ab reactivity. We found that the PA Ab reactivity in the sample from PT 36 remained markedly impaired with either the G44N or the P45A mutation in the W1:2-3 loop. However, no mutation in the W1:2-3 loop significantly impaired the PA Ab reactivities in samples from the other 4 patients (Figure 4E). In contrast, the R139G mutation in the W2:3-4 loop impaired reactivity in the samples from all 4 of these patients. In addition, samples from PTs 3 and 12 showed a similarly impaired pattern in the PA Ab reactivities with the P135L, E136Q, and R139G mutations (Figure 4F).

Group C: the W3:4-1 loop is essential for PA anti-αIIbβ3 Ab binding. In 4 samples (from PTs 1, 6, 34, and 45) the PA Ab reactivity was markedly impaired with m(W3:4-1)H compared with m(W2:3-4)H. In addition, the PA Ab reactivity was similarly impaired with Hm(W3:4-1)H (Table 2; Figure 5A). These results suggested that the W3:4-1 loop was essential for PA Ab reactivity in these samples. Interestingly, in samples from PTs 1, 34, and 45, the PA Abs showed nearly fully restored reactivity with H(W4:4-1)m (Figure 5B). This suggested that the W4:4-1 loop may also affect PA Ab reactivity. In fact, in PTs 34 and 45, the PA Ab reactivity was markedly impaired with Hm(W3:4-1)H and moderately impaired with Hm(W4:4-1)H (Figure 5C). The sample from PT 1 was not tested because of insufficient sample. The sample from PT 34 showed PA Ab reactivity with mH(W3:4-1–W4:1-2)m that was nearly comparable with its reactivity with wt αIIb (Figure 5D). This supported the notion that both W3:4-1 and W4:4-1 loops were important for PA Ab reactivity in this patient. In contrast, in the sample from PT 45, PA Ab reactivity was not restored with mH(W3:4-1–W4:1-2)m (Figure 5D). Finally, we tested the sample from PT 45 with the mouse αIIb that carried the human sequence from W3:4-1 to the C-terminal of the β-propeller domain, mH(W3:4-1–β-propeller)m. We found that the sample from PT 45 showed reactivity with the mH(W3:4-1–β-propeller)m comparable with reactivity with the wt αIIb. This suggested that the C-terminal half of the β-propeller domain may also contribute to PA Ab binding in the sample from PT 45 (Figure 5D). In the sample from PT 6, PA Ab reactivity was not fully restored with H(W4:4-1)m but was almost fully restored with H(β-propeller)m (Figures 5B and 2E). However, the PA Ab reactivity was impaired with mH(W3:4-1–β-propeller)m (Figure 5D). These results suggested that the N-terminus and the C-terminal portion of the β-propeller domain were important for the PA Ab reactivity in the sample from PT 6.

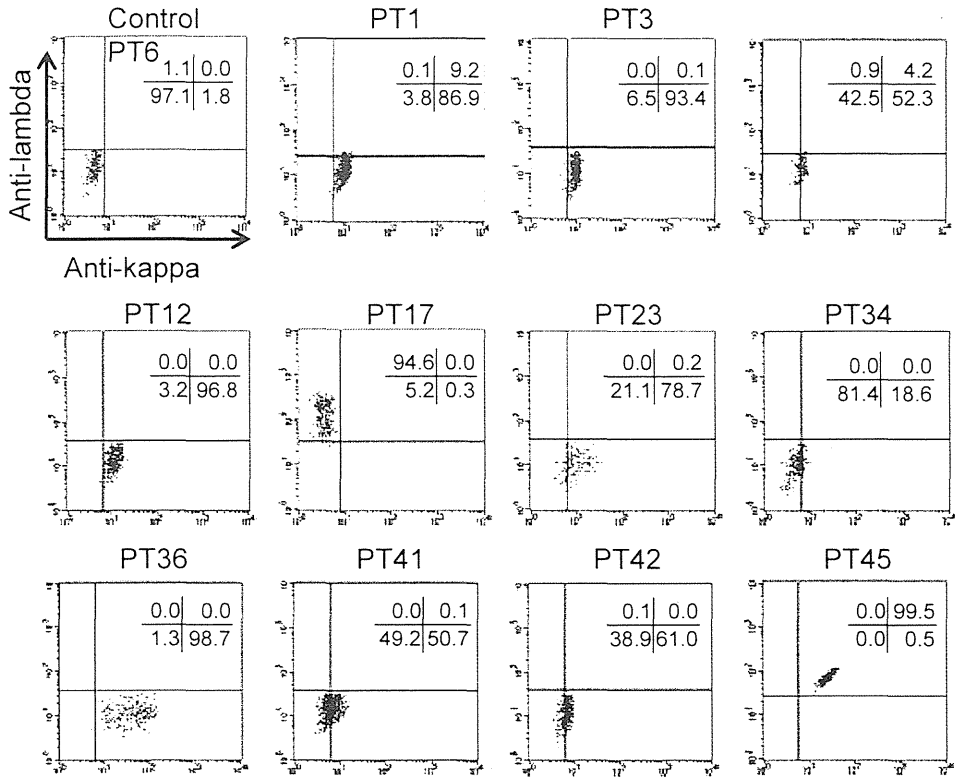

Figure 6. Light-chain usage of PA anti-αIIbβ3 Abs. Platelet eluates were reacted with wt αIIbβ3-expressing 293T cells, followed by the incubation with FITC–anti-κ, PE–anti-λ, and APC–anti-CD61 Abs. Anti-κ (horizontal) and anti-λ (vertical) Abs bindings were analyzed in a subset of cells that were highly positive for CD61. Representative results of ≥ 2 independent experiments are shown.

There are many amino acid differences between human and mouse in the W3:4-1 and W4:4-1 loops (Figure 1A). Therefore, it was difficult to identify critical residues in these regions. However, we previously studied a KO variant of αIIbβ3 with a 2-amino acid (Arg-Thr) insertion between F160 and S161 in the W3:4-1 loop. In that study, we found that this insertion affected PA Ab reactivity as well as ligand binding capacity.[15] In the present study, samples from all 4 patients of group C showed impaired PA Ab reactivity with this mutation (KO; Figure 5E).

Samples from the remaining 4 patients (PTs 2, 5, 7, and 37) could not be classified in the 3 groups described. However, all 4 had PA anti-αIIbβ3 Abs that mainly recognized the N-terminal half of the β-propeller domain of αIIb. In these patients, we did not detect any unique characteristics that might identify an epitope on any specific loop(s).

Light chain–restricted usage of PA anti-αIIbβ3 Abs

Our findings indicated that autoantigenic epitopes may be located on highly restricted regions of αIIb, which also suggested that many PA anti-αIIbβ3 Abs might exhibit clonality. Therefore, we next determined whether PA Abs exhibited restricted κ/λ light chain usage in the 11 eluates that had been classified in 1 of the 3 groups described in the previous paragraphs. We determined that samples from PTs 1, 3, 12, and 36 clearly showed restricted κ-chain usage, and the sample from PT 17 showed restricted λ-chain usage. In contrast, the PA Abs in samples from PT 45 were polyclonal. The samples from the remaining 5 patients also showed κ-chain preference, although the positivity was weak (Figure 6). These results suggested that the PA anti-αIIbβ3 Abs were clonal in many patients with primary ITP.

Discussion

Previous reports have found the importance of the β-propeller domain in αIIb, particularly the W3:4-1 loop, for PA anti-αIIbβ3Ab binding in patients with chronic ITP.[13,15] In this study, we found that samples from 15 patients with primary ITP harbored PA anti-αIIbβ3 Abs that mainly recognized the N-terminal half of the β-propeller domain (L1-W235) of αIIb. A systematic examination with human-mouse αIIb chimeras found 3 main recognition sites: (1) a conformational epitope composed of W1:1-2 and W2:3-4 loops, (2) a region containing the W1:2-3 loop, and (3) a region containing the W3:4-1 loop. We further identified some single residues in these loops that were critical for PA Ab reactivity. Moreover, PA anti-αIIbβ3 Abs in many patients showed restricted κ/λ light chain usage. Our findings indicated that major epitopes of PA anti-αIIbβ3 Abs were localized in highly restricted regions in the β-propeller domain of αIIb and that PA anti-αIIbβ3 Abs may be monoclonal or oligoclonal in many patients with ITP.

It was surprising that PA anti-αIIbβ3 Abs did not bind to mouse αIIbβ3. αIIb and β3 showed 82% and 85% nucleotide sequence homology, respectively, between human and mouse. However, one report suggested that anti–human platelet Abs produced from splenocytes obtained from patients with ITP exhibited low cross-reactivity with mouse platelets.[24] Another report showed that an anti–human αIIb antibody generated in mice exhibited significantly diminished binding to ITP platelets compared with normal platelets.[25] Those results suggested that the reactivity of PA anti-αIIbβ3 Abs may be affected by subtle conformational differences between human and mouse αIIbβ3.

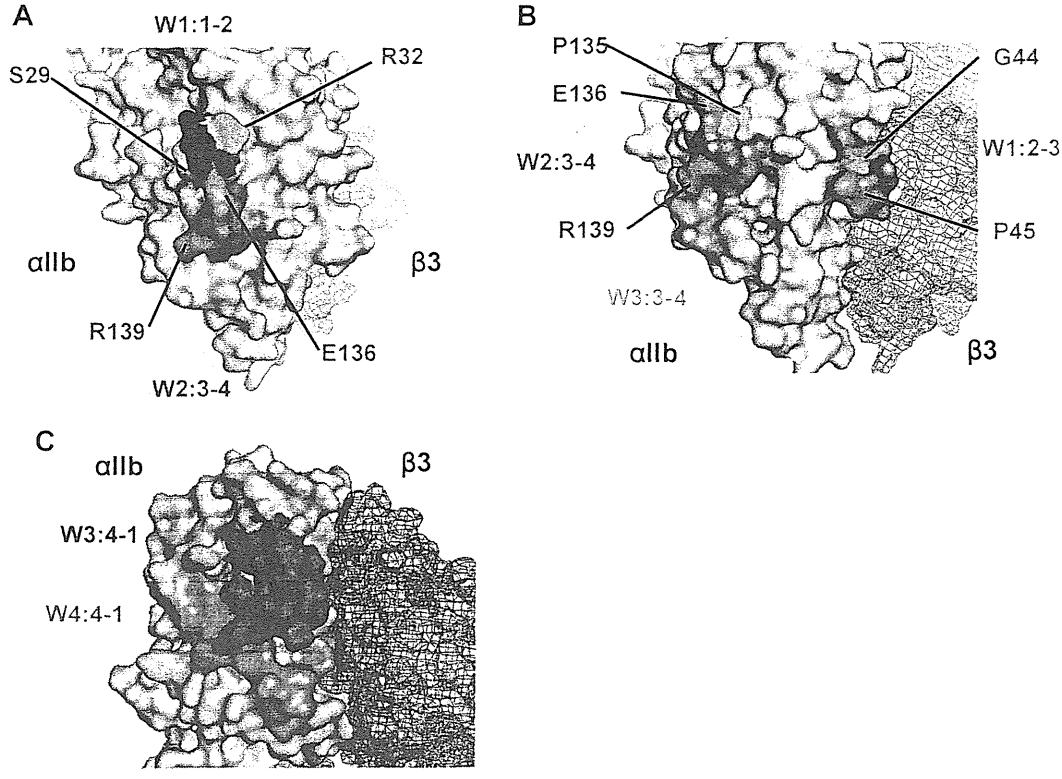

Figure 7. Crystal structure of the recognition sites for the PA Abs. (A) Crystal structure of the recognition sites of group A made by PyMOL Version 1.4 software (DeLano Scientific LLC). W1:1-2 (blue) and W2:3-4 (green) loops and 4 critical residues (S29, R32, E136, R139) for the binding of PA Abs were indicated. (B) Crystal structure of the recognition sites of group B. W1:2-3 loop (orange) is located in the upper surface of αIIbβ3 interface, and W2:3-4 and W3:3-4 loops (green) are in the lower surface. Five critical residues (G44, P45, P135, E136, R139) for the binding of PA Abs were also indicated. (C) Crystal structure of the recognition sites of group C. W3:4-1 (red) and W4:4-1 (pink) loops were indicated. Both loops are located in the upper surface of αIIbβ3 interface, which is near the ligand binding site.

Two patients of group A had PA Abs that recognized the W1:1-2 and W2:3-4 loops. Although these 2 loops are separated by ~ 100 amino acids in the primary αIIb sequence, the crystal structure showed that the W1:1-2 loop was close to the W2:3-4 loop on the lower face of the β-propeller domain (Figure 7A). Furthermore, the binding of PA Abs from PT 17 to wt αIIbβ3 could not be inhibited by linear peptides that corresponded to W1:1-2, W2:3-4, or a mixture of these peptides; this also suggested that the PA Abs recognized a conformational epitope composed of these 2 loops (data not shown). Moreover, the reactivity of Abs was highly affected by single amino acid substitutions (S29K, R32S, E136Q, and R139G) in the W1:1-2 and W2:3-4 loops (Figure 3D-E). Because arginine (R), lysine (K), and glutamic acid (E) are charged amino acids, these substitutions may affect ionic bonds between αIIb and the complementarity determining regions of the PA Abs.

We found that the W1:2-3 loop was critical for the reactivity of PA Abs in 5 patients of group B. However, when epitopes of the mouse αIIb were swapped with the corresponding human N-terminus sequences, recovery of PA Ab binding was heterogeneous among the patient samples (Figure 4B). In particular, the PA Abs from PT 36 appeared to exclusively recognize the W1:2-3 loop, and we further found that the PA Ab reactivity was markedly impaired with a single G44N or P45A substitution in the loop (Figure 4E). Because asparagine (N) has an amino group and proline (P) has a cyclic structure, the presence or absence of these amino acids may have highly affected hydrogen bonding between the Abs in PT 36 and αIIb, and/or they may have disrupted the conformation of the W1:2-3 loop. In addition, swapping human and mouse loop sequences showed that W2:3-4 loop was also important for the reactivity of PA Abs in the eluates of the remaining 4 patients. Finally, the W3:3-4 loop appeared to contribute to PA Ab binding in the eluates from 2 patients, PTs 3 and 12 (Figure 4C). Interestingly, the R139G mutation in the W2:3-4 loop had a profound effect on PA Ab binding in the eluates of the 4 patients in group B and the 2 patients in group A (Figures 3E and 4F). This indicated that R139 may be a critical epitope for many PA Abs. The crystal structure of αIIb showed that the W1:2-3 loop was located on the upper face, and the W2:3-4 and W3:3-4 loops were on the lower face of the β-propeller domain (Figure 7B). Although we could not rule out the possibility that PA Abs may be polyclonal and recognize different epitopes, we hypothesized that PA Abs recognize conformational epitope(s) composed of these multiple loops, based on our findings that PA Ab binding was markedly impaired with a single loop substitution. The crystal structure showed that the W1:2-3 and W2:3-4/W3:3-4 loops were located ~ 30 Å apart; this circumscribes an area consistent with the typical contact areas between protein antigens and their cognate Abs.[26,27] The 3-dimensional structure suggested that these loops formed a relatively flat surface (Figure 7B), also consistent with the observation that Abs typically interact with protein antigens on relatively flat complementarity determining regions.[28]

In the 4 patients of group C, we confirmed our previous finding that the W3:4-1 loop was one of the main target epitopes for PA anti-αIIbβ3 Abs. The PA Abs from these 4 eluates showed impaired reactivity with the KO variant αIIbβ3.[20,29] We also showed that the W4:4-1 loop was important for the binding of 3 of 4 sample eluates. Again, the 3-dimensional structure (Figure 7C) indicated that the W3:4-1 loop was immediately adjacent to the W4:4-1 loop on the upper face of the β-propeller domain; this suggested that the PA Abs recognized an epitope composed of these 2 loops. Moreover,

the C-terminal half of the β-propeller domain was important for efficient binding of the PA Abs in the samples from PTs 6 and 45. This suggested that the C-terminal region might maintain the proper conformation of the W3:4-1 loop region for the binding of PA Abs.

Our findings that many PA anti-αIIbβ3 Abs in patients with ITP recognized restricted regions of the β-propeller domain in αIIb have some interesting implications. The cause of primary ITP remains obscure; however, it has been suggested that molecular mimicry may trigger an immune response against platelet antigens in some secondary forms of ITP.[14,30] A recent study reported that many bacterial proteins contained the human integrin-type β-propeller domain[31]; this suggested that conformational mimicry between these bacterial proteins and the β-propeller domain in αIIb might be involved in the production of PA anti-αIIbβ3 Abs. Consistent with other reports,[32,33] we observed that many of the PA anti-αIIbβ3 Abs exhibited restricted κ/λ light chain usage, which further suggested that PA Abs might arise from an antigen-derived clonal expansion rather than from polyclonal B-cell activation triggered by nonspecific stimuli.

Of note, all 4 eluates that did not show clear epitopes (PTs 2, 5, 7, and 37) were collected > 6 years after the diagnosis; in contrast, all 5 eluates collected within 1 year after diagnosis were categorized as group B or C (PTs 1, 6, 34, 36, and 45; Table 1). These results suggested that restricted epitopes tended to be more common in the early stages of ITP and that epitopes may spread out in the later, chronic phase of the disease, although in some cases, such as PTs 17 and 23, highly specific epitopes were identified in patients with a long ITP history. This time dependence was also found in other autoimmune diseases.[34,35] In this context, it is intriguing that PA Abs which were categorized as group C were only found in patients with a diagnosis made < 1 year ago in this study. In fact, the PA Abs from PT 12, which showed highly impaired reactivity with the substituted epitopes in the KO mutation in our previous study (PT 6 [patient no. 6] in the previous study[15]), appeared to react with extended or changed epitopes in the W1:2-3, W2:3-4, and W3:3-4 loops in the present study, with eluates collected > 10 years later. These results suggest that W3:4-1 loop may be the target epitope of the early phase of ITP. Clearly, concrete evidence of epitope spreading will require long-term studies of patients with ITP.

This study had several limitations. First, we used human-mouse αIIb chimeras for epitope mapping; thus, we could not evaluate whether identical residues between human and mouse were significant. Second, there was an inevitable bias for patient and sample selection, because patients with severe thrombocytopenia could not provide sufficient platelet eluates for the study. Finally, we analyzed only PA anti-αIIbβ3 Abs; thus, we could not rule out the possibility that PA Abs that recognized other GPs (such as GPIb/IX/V) might play a role in the pathogenesis of ITP in our patients.

In summary, we have shown that PA anti-αIIbβ3 Abs tend to recognize highly restricted regions in the N-terminal half of the β-propeller domain of αIIb with clonality. These results may contribute to a better understanding of the pathogenesis of chronic ITP.

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Authorship

Contribution: K.K., H.K., and Y.T. designed the study; K.K. performed most of the experiments and wrote the paper; H.K. and Y.T. edited the paper; T.N. and S.T. performed the transfection studies; and S.H. and Y.K. supervised several aspects of the projects and helped with manuscript preparation.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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1．特発性血小板減少性紫斑病（ITP）治療の参照ガイド

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Summary　ITPの治療に関して，その治療目標は血小板数を正常に戻すことではなく，重篤な出血を予防し得る血小板数に維持することである。このことは過剰な薬剤の長期投与による患者QOLの低下を憂慮しての判断である。2010年にヘリコバクター・ピロリ除菌療法が保険適用となり，さらに2010年末から2011年に新薬であるトロンボポエチン受容体作動薬が登場してきた。これら治療法の進歩に対応するとともに，それらの適正使用に関しての参照として作成した成人ITP治療の参照ガイド2012版を概説する。

以前のガイドラインからの変更点

① 治療目標を定め，漫然とした治療を避けることを示した。② 血小板数と臨床症状から，治療の必要なITP症例を層別化した。③ ピロリ菌陽性ITP症例の取り扱いを定めた。④ トロンボポエチン受容体作動薬の位置づけを定めた。⑤ 保険適用と適用外使用を明示した。

はじめに

ITPという用語が広く日常臨床で用いられているが，本疾患の正式な名称に関してはいまだ統一されていないのが現状である。具体的には，厚生労働省の特定疾患であるITPは，本邦ではidiopathic thrombocytopenic purpura（特発性血小板減少性紫斑病）と呼ばれており，その名称変更には多大な労力を要するため，本稿でも特発性血小板減少性紫斑病の病名を使用した。一方，その自己免疫機序が明らかになるに従い，海外ではautoimmune thrombocytopenic purpura（自己免疫性血小板減少性紫斑病），さらにはchronic immune thrombocytopenic purpura（慢性免疫性血小板減少性紫斑病）などの用語も用いられている。最近，トロンボポエチン（TPO）受容体作動薬が開発され，難治性ITPを対象として前向き無作為臨床試験が行われ，その有効性がエビデンスとして示されるようになってきた。この進歩を踏まえ，ITPに関する用語や治療効果の判定を標準化する必要性が叫ばれるようになり，国際作業

ITP（idiopathic thrombocytopenic purpura；特発性血小板減少性紫斑病）　TPO（トロンボポエチン）

部会 (International Working Group：IWG) が編成され，本疾患の病名として primary immune thrombocytopenia (primary ITP) との名称が提唱されている[1, 2]。この世界的な潮流に対応し，さらに本邦にてITPに対して保険適用となったヘリコバクター・ピロリ除菌療法やTPO受容体作動薬の適正使用に関して指標が必要と考え，厚生労働省難治性疾患克服研究事業「血液凝固異常症に関する調査研究」班において2004年に発表したITP治療ガイドライン(案)を改訂し，成人特発性血小板減少性紫斑病治療の参照ガイド2012版を作成した[3]。治療の参照ガイドの全文は「臨床血液」誌に公開予定であるため(2012年5月予定)，本稿ではその概要を紹介する。

1．治療の参照ガイド2012版の特徴

ITP治療の参照ガイド2012版の改訂内容のポイントは以下の点である。

① 治療目標を定め漫然とした治療を避けることを示した，② 血小板数と臨床症状から治療の必要なITP症例を層別化した，③ ピロリ菌陽性ITP症例の取り扱いを定めた，④ トロンボポエチン受容体作動薬の位置づけを定めた，⑤ 保険適用と適用外使用を明示した。

2．ITP治療の基本的な考え方（図1）

治療の目標は血小板数を正常に戻すことではなく，重篤な出血を予防し得る血小板数に維持することである。一般に，高齢になるほど出血の危険や出血死のリスクは増大する。成人において致死的な出血の大部分は血小板数3万/μL未満で起こっている。ステロイドなど，使用する治療薬の副作用は無視できないため，出血症状のない血小板減少患者に対して無用の治療を行わないようにするよう努めなければならない。

1）治療開始時期

治療開始時期に関しては，現行の欧米のガイドライン[4~6]においては，出血症状を伴う場合に治療を開始するとされている。血小板数で治療開始を決定するのは議論の分かれるところであるが，成人においてはガイドラインの多くは血小板数3万/μL未満で治療を開始するとしており，今回の参照ガイドでもこの数値を採用した。以下に具体的に示す。

(A)血小板数3万/μL以上で重篤な出血症状がない場合には無治療で経過を観察する。

(B)血小板数2～3万/μLで出血症状がなければ，1カ月に1回程度の注意深い経過観察を行い，治療がいつでも行える状態にあることが必要である。

(C)以下の症例では，積極的治療対象となる。

① 重篤な出血症状(脳内出血，下血，吐血，血尿，多量の性器出血，止血困難な鼻出血，口腔内出血，外傷部位の止血困難など)，多発する紫斑，点状出血を伴う症例。

② 血小板数2万/μL以下の症例。

③ 血小板数2～3万/μLであっても出血傾向の有無にかかわらず，60歳以上，高血圧症，あるいは活動性の高い症例（肉体労働者，激しい運動をする方など)。

2）治療目標血小板数

当然，血小板数10万/μL以上を維持でき，出血症状がない完全寛解(CR)状態が理想であるが，CR到達が困難な症例には，治療中止（休薬)，あるいは維持量で血小板数3万/μL以上で，かつ出血症状がない状態への改善を目指すこととした。

IWG (International Working Group；国際作業部会)　CR (完全寛解)

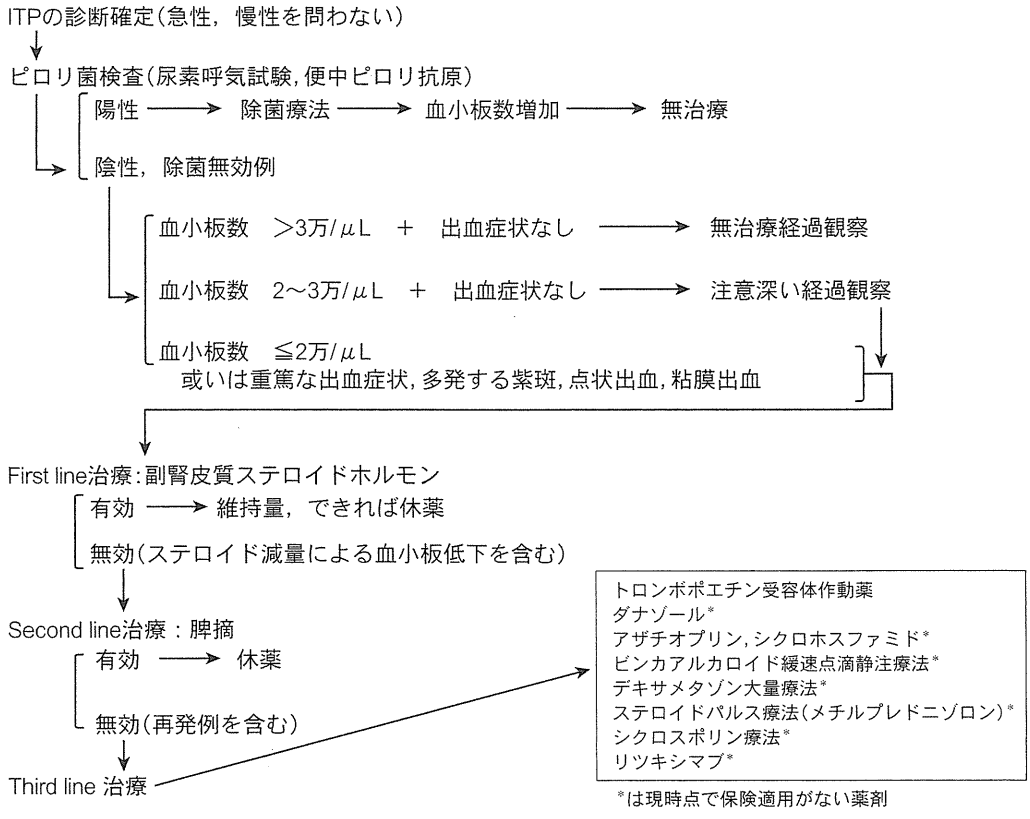

図1　成人ITP治療の参照ガイド

成人ITP治療の参照ガイドをフローチャートで示す。治療の目標は血小板数を正常に戻すことではなく，重篤な出血を予防し得る血小板数に維持することである。本邦において，ヘリコバクター・ピロリ感染ITP患者における除菌療法は，緊急時や妊娠合併ITP症例を除くすべての症例において，最優先される治療である。TPO受容体作動薬は，Third line治療の位置づけとした。（筆者作成）

3．各治療の推奨度

ITPの治療薬に関しては，無作為比較試験にて評価されている薬剤がほとんどないため，各治療の推奨度はGRADE system（Grading of Recommendations, Assessment, Development and Evaluation）を用いることとし，その具体的な内容は**表1**に示している[7]。

4．ヘリコバクター・ピロリ除菌療法の位置づけ（推奨度：1B）

ITPにおけるピロリ除菌療法は2010年6月より保険適用となったことを受け，今回の改訂ではITPにおいてピロリ菌感染陽性の場合，緊急時を除き血小板数に関係なく，除菌療法を最優先治療として推奨した。後方視的解析ではあるが，本邦では除菌療法奏効例のうち，約60～70%において血小板増加が認められる。興味深いことに，本邦およびイタリアではITPにおけるピロリ除菌療法の有効性は高いが，アメリカやスペインでは

表1　GRADE system による推奨度

推奨度の強さ
　1：強い推奨
　　殆どの患者において，良好な結果が不良な結果より明らかに勝っており，その信頼度が高い
　2：弱い推奨
　　良好な結果が不良な結果より勝っているが，その信頼度は低い

推奨の基になったエビデンスの質
　A：複数の RCTs において確立したエビデンス，あるいは観察研究による極めて強いエビデンス
　B：RCTs による限定的なエビデンス，あるいは観察研究による強いエビデンス
　C：重大な弱点のある RCTs によるエビデンス，観察研究による弱いエビデンス，あるいは間接的エビデンス

各治療の推奨度に関しては，GRADE system を用いた。
（文献7より）

除菌療法の ITP への有効性は低く，除菌療法の効果は一定ではない。このため，本邦におけるヘリコバクター・ピロリ除菌療法の位置づけはユニークであると言える。ピロリ除菌により完全寛解となる症例は，厳密には特発性血小板減少性紫斑病ではなく，ピロリ菌感染に伴う二次性の ITP であるが，両者を除菌前に区別することは現時点では不可能である。

5．First line 治療としての副腎皮質ステロイド（推奨度：2B）

ピロリ菌陰性症例あるいは除菌無効症例に対する第一選択薬としては，副腎皮質ステロイドが推奨される。副腎皮質ステロイドは網内系における血小板の貪食および血小板自己抗体の産生を抑制する。本治療を行うにあたっては事前に高血圧，糖尿病，活動性感染症，慢性感染症，骨粗鬆症，高脂血症，免疫能低下状態，消化性潰瘍などの合併症の有無を把握し，また副腎皮質ステロイド治療による副作用の発症に注意する。合併症のある場合には，合併症のコントロール下で副腎皮質ステロイド療法を行う。

上記合併症のために本治療が選択できない場合は，脾摘（Second line 治療）や難治例に対する治療（Third line 治療）を選択し，出血の危険性の回避に努め，QOL の向上を目指す。

1）投与量

初回使用量は，プレドニゾロン換算 0.5 ～ 1 mg/kg/day を2～4週間用いる。その後，血小板数の増加の有無にかかわらず，8～12 週かけてプレドニゾロン 10 mg/day 以下にまで漸減し，維持量とする。維持療法中あるいは治療中止後に出血傾向や症状が増悪する場合には，ステロイドの増量や再投与，緊急時の治療を行う。骨粗鬆症，コントロール不良な高血圧症，糖尿病予備群，肥満，慢性感染症を合併している症例や 60 歳以上の高齢者では，初回副腎ステロイド投与量は 0.5 mg/kg/day から開始することを推奨する。

2）治療目標

副腎皮質ステロイド投与により 50 ～ 75%において血小板が増加するが，多くは副腎皮質ステロイド減量に伴い血小板が減少する。副腎皮質ステロイド療法により CR となった症例に対しては，副腎皮質ステロイドを減量し，可能であれば中止する。CR に到達しない症例については，生命予後に影響を与えない血小板数3万/μL以上を維持できる状態に副腎皮質ステロイドを減量する。すなわち，最低の維持量で経過を観察し，薬剤による副作用の軽減を含めた QOL を良好に保つことを目標にする。ステロイド無効例（NR）では，出血症状の軽減が維持できれば，副作用の点から休薬してもよい。部分寛解（PR）および NR の定義は，**表2**を参照されたい。

NR（無効例）　PR（部分寛解）

表2　治療効果の判定基準

- 完全寛解（CR）：血小板数が10万/μL以上で出血症状がない。
- 部分寛解（PR）：血小板数3万/μL以上かつ治療前値の2倍以上で出血症状がない。
- 無効（NR）：血小板数3万/μL未満または治療前値の2倍未満の増加，あるいは出血症状がある。

血小板数は少なくとも1週間以上あけて2回以上測定した値を用いる（2回の測定値がいずれも基準を満たす必要がある）。（筆者作成）

6．Second line 治療としての脾臓摘出療法（脾摘）（推奨度：1B）

副腎皮質ステロイド治療効果がNRないしPRの一部で無治療，維持量で血小板数3万/μL未満の症例や，副腎皮質ステロイドによる副作用が強く，充分な治療が行えない症例（副腎皮質ステロイド不耐容症例）が対象となる。

脾摘により約80%に血小板増加反応が認められるが，約20%が再発し，寛解率は約60%である。Vianelliらは，ITPでの摘脾施行402例に関して長期間の有効性と安全性を検証している。彼らは，摘脾により約86%の症例は一時的に血小板が5万/μL以上に増加するが，その内の23%が再発したとし，再発例の多くは摘脾後4年以内に起こるとの成績を示している。しかしながら，摘脾後再発ITPにおいては，治療に対する反応性も改善していることが多く，再発例の約68%が副腎皮質ステロイド投与などにより血小板数3万/μL以上を維持でき，さらに摘脾が無効であった57例においても，約50%の症例が治療に反応していると報告している[8]。同様の成績は，McMillanらによっても報告されている[9]。脾摘を行う時期に関しては，診断から6～12カ月以上経過した症例が望ましい。

術前には，血小板数を5万/μL以上に増加させることが望ましい。具体的には，術前5～7日前より免疫グロブリン大量療法（IVIg）を開始し，血小板数増加が不充分の症例に対しては血小板輸血10～20単位を手術直前あるいは手術中に輸血する。

脾摘の合併症としては，消化管癒着によるイレウス，血栓症（門脈血栓など），感染症などが挙げられる。また，脾摘により肺炎球菌，髄膜炎菌，インフルエンザ菌などに対して防御能が低下する。したがって，予防的にこれらに対するワクチン接種を脾摘4週間前までに行うことが望ましい。本邦では肺炎球菌ワクチンのみが保険適用となっている。脾摘後の感染症は重篤化することがあり，早期の対応が必要である。発熱など感染症が疑われる場合には早めに充分なペニシリン系，ニューキノロン系などの抗生剤の投与を行い，重症化に注意し経過観察を行う。

7．Third line 治療

First lineおよびSecond line治療がNRの症例，脾摘の了解が得られない症例もしくは合併症により脾摘が困難な症例，副腎皮質ステロイド不耐用症例が対象となる。Third lineの治療に使用される薬剤の多くは，健康保険未収載あるいは治験段階の薬剤が多く，特異な副作用が出現する可能性がある。中にはエビデンスレベルが低いものもあり，1～2クール後，あるいは1～2カ月で効果と副作用を評価し，効果がなければ他の薬剤を選択する。

1）トロンボポエチン受容体作動薬（推奨度：1B）

ロミプロスチム：1～10μg/kg，毎週1回，皮下注射

エルトロンボパグ：12.5～50 mg/day，1日1回，毎日内服（空腹時服用）

巨核球・血小板産生刺激因子であるトロンボポエチンの受容体に結合し，巨核球の成熟を促進し血小板産生を亢進させる薬剤である[10]。いずれも

用量依存的に血小板増加反応を示す。一定用量投与により５～７日目から血小板数が増加し始め，12～16日目くらいに最大の血小板数となる。継続使用により血小板数の増加効果を維持することができる。難治症例の80%以上が血小板数５万/μL以上に増加し，出血が回避される。

Third line治療の中では唯一保険適用のある薬剤であるが，作用機序からITPを治癒させる根本治療でなく，出血症状をコントロールすることに主眼を置いた治療薬剤で，長期に使用し続ける必要がある。使用に際しては，以下の基本的注意事項が掲載されている。① 血液疾患の治療に充分な経験をもつ医師のもとで使用する。② 血小板数が正常範囲以下であっても血栓症，血栓塞栓症を起こすことがある。③ 脳梗塞，心筋梗塞，肺塞栓などの血栓症の既往のある症例や抗リン脂質抗体を有する症例には慎重投与する。④ ITP以外の血小板減少症には使用が認められていない（急性ITPには適用はない）。⑤ 腎機能障害あるいは肝機能障害のある症例に対しては慎重投与が必要。これらの点を考慮すると共に，長期の安全性が確立されていない現状で本薬剤の有用性を高めるためには，以下の使用法が推奨される。

投与量

出血症状の軽減や，血小板数が３～５万/μL以上に維持される最小量が適切な投与量となる。日常生活においては，血小板数を正常化させる必要はない。エルトロンボパグに関しては，血中濃度が併用薬や食事の影響を受けやすく，使用については服薬時間，併用薬などの注意点を確認し処方する。

現時点では，一年以上の長期投与においても比較的安全に用いられている。しかし，一般的な副作用として，① 使用中止後に血小板数は治療前値よりも低下する可能性，② 骨髄でレチクリン（細網）線維が増加する可能性（投与を中止すれば回復するとの報告がある），③ 白血病細胞の増殖を刺激する可能性，④ 重篤な血栓症の発症などが挙げられる。長期投与を余儀なくされる難治性ITPに関しては，長期使用による安全性の検証が必要である。

２）ダナゾール療法（保険適用外）

ITPの血小板増加反応に対する詳細な作用機序は不明である。通常は200～400 mg/dayで治療するが，副作用を勘案し200 mg/dayが推奨される。最近ではダナゾール少量療法も試みられており（50～100 mg/day），反応には時間を要するが（６～８週），副作用が少ない利点がある。

副作用として肝障害，男性化作用による嗄声，多毛，血栓症，低エストロゲン症状などが起こる。

３）リツキシマブ療法（保険適用外）

CD20陽性Ｂリンパ球に対するヒトマウスキメラ抗体で，主としてＢ細胞を傷害し抗体産生を低下させる目的で使用される。リツキシマブ375 mg/m^{2}を週１回，４週間点滴静注する。

欧米における後方視的解析では，48%に完全寛解（血小板数15万/μL以上），60%に部分寛解以上（５万/μL以上）の効果を誘導し得るとされている。しかしながら，ウイルスの再活性化や進行性多巣性白質脳症の発症などが問題となっている。本邦においては，医師主導型治験において難治性ITPに対するリツキシマブの有効性と安全性を検討する第Ⅲ相試験が2011年10月より進行中である。

４）シクロスポリン（保険適用外）

３mg/kg/dayを経口投与し，血中トラフが100～200 ng/mLになるように調節する。血小板増加反応が80%以上（12症例中10例）に見られ，また60%以上が治療を中止しても血小板増加反応を維持しているとの報告がある。副作用として腎障害，高血圧，全身倦怠，肝障害，多毛，易感染性などがあり，腎障害例や高齢者には不向きである。