著者名	論文タイトル名	発表誌名	巻号	ページ	出版年
小嶋哲人	血栓性素因としてのATとPC異常	日本検査血液学会雑誌	9(3)	365-370	2008
川崎富夫	学会セッションのテーマ変遷から見た医療水準-静脈血栓症における医療訴訟の検討-	日本血管外科学会雑誌	17(1)	7-12	2008
川崎富夫	(判例研究) 肺塞栓症予防対策における注意義務違反-医療水準とｶﾞｲﾄﾞﾗｲﾝ-	L&T	40	75-83	2008
辻　肇	抗凝固療法．未分画ヘパリンと低分子ヘパリン	CLINICIAN	55(3)	365-368	2008
辻　肇	血栓止血の臨床．ヘパリン類の適正使用	血栓止血誌	19(2)	187-190	2008
辻　肇	血小板・血栓止血の管理-薬物療法．ヘパリン・低分子ヘパリン	救急・集中治療	20(5-6)	747-752	2008
辻　肇	内科診療ガイドライン活用法．播種性血管内凝固症候群（ＤＩＣ）	内科	102(6)	1424-1429	2008
倉田義之	特発性血小板減少性紫斑病診断の進歩	日本検査血液学会雑誌	9	388-393	2008
倉田義之	特発性血小板減少性紫斑病	内科	102	1418-1423	2008
冨山佳昭	造血障害に対する免疫抑制療法 特発性血小板減少性紫斑病	血液フロンティア	18	87-93	2008
冨山佳昭	特発性血小板減少性紫斑病	臨床血液	49	14-21	2008
冨山佳昭	抗血小板薬の種類とその作用機序.	Clinician	567	18-22	2008
小林隆夫，中林正雄，石川睦男，池ノ上克，安達知子，前田真	産婦人科血栓症調査結果 2001－2005	日産婦新生児血会誌	18(1)	S3-S4	2008
左近賢人，塚本泰司，小林隆夫，藤田悟，川島隆之，門田守人	腹部手術後静脈血栓塞栓症予防に対するフォンダパリヌクスの臨床的評価－間欠的空気圧迫法をベンチマークとした無作為化オープン試験	臨床医薬	24(7)	679-689	2008
小林隆夫	肺血栓塞栓症/深部静	PTMガイドラ	10	1-2	2008

	脈血栓症(静脈血栓塞栓症)予防ガイドライン	インダイジェスト			
小林隆夫	産褥期深部静脈血栓症の予防対策は？	臨床婦人科産科	62(4)	428-433	2008
小林隆夫	周産期の静脈血栓塞栓症予防	International Review of Thrombosis	3 (S1)	143-149	2008
小林隆夫	静脈血栓塞栓症の予防および治療の進歩	臨床病理	56(7)	589-599	2008
小林隆夫	深部静脈血栓症の現状と問題点	Angiology Frontier	7(3)	138-144	2008
小林隆夫	静脈血栓症/肺塞栓症の予防ガイドライン：現状と展望	血栓と循環	16 (3)	228-232	2008
小林隆夫	妊産婦の薬物療法. 6. 抗凝固薬	臨床婦人科産科	62 (9)	1189-1193	2008
小林隆夫	婦人科手術における肺血栓塞栓症の予防	産婦人科治療	97(4)	412-419	2008
小林隆夫	婦人科癌と血栓症	血液フロンティア	18(10)	1575-1584	2008
小林隆夫	婦人科がん診療のリスクマネジメント，静脈血栓塞栓症	産婦人科の実際	57(11)	1794-1804	2008
小林隆夫	肺血栓塞栓症/深部静脈血栓症（静脈血栓塞栓症）の現況と予防対策の展望	県西部浜松医療センター学術誌	2(1)	6-15	2008
小林隆夫	妊娠中の静脈血栓塞栓症	綜合臨床	58(1)	147-148	2009
小林隆夫	静脈血栓塞栓症の治療戦略	Pharma Medica	27(1)	13-16	2009
小林隆夫	静脈血栓塞栓症（VTE）の現況	SRL 宝函	29(2)	20-27	2009
佐久間聖仁，中村真潮，山田典一，伊藤正明，中野赳，白土邦男，小林隆夫	静脈血栓塞栓症の頻度、臨床的特徴	Therapeutic Research	29(5)	639-640	2008
榛沢和彦	深部静脈血栓症. 新潟県中越地震における肺塞栓症と深部静脈血栓症-災害避難生活を考える	ASAHI medical	4	58-61	2008
冨山佳昭	特発性血小板減少性紫斑病.	内科　増大号：Emergency実戦ガイド	103(6)	1602-1606	2009
冨山佳昭	血小板に対する自己免	血液フロンテ	19(6)	41-48	2009

	疫応答.	ィア			
林悟、西山美保、末久悦次、柏木浩和、倉田義之、冨山佳昭	網状血小板測定法２法の比較検討と臨床的有用性の検討 -フロサイトメトリー(FCM)法と多項目自動血球分析装置 XE-2100 による自動測定(IPF)法-.	臨床病理	57(11)	1039-1044	2009
藤村欣吾	ＩＶ．後天性疾患の診断と治療 1．特発性血小板減少性紫斑病	日本内科学会雑誌	98	1619-1626	2009
藤村欣吾	ＩＴＰの治療総論	血液フロンティア	19	899-906	2009
佐藤隆司 桑名正隆	ITP のゲノム解析	血液フロンティア	19	873-881	2009
宮田敏之、岡田浩美、川崎富夫、辻肇、窓岩清治、坂田洋一、小嶋哲人、村田満、池田康夫	日本人の血栓性素因	臨床血液	50(5)	381-388	2009
小嶋哲人	血栓性疾患　先天性凝固阻止因子欠乏症（antithrombin, protein C, protein S 欠損症）	日本血栓止血学会誌	20(5)	484-486	2009
横山健次	「血栓症のリスク因子」高感度D-ダイマー。	臨床検査	53	1129-1132	2009
横山健次 池田康夫	日常診療で活かす最新のエビデンス」治す循環器疾患に関する大規模臨床試験　抗血小板療法。	Heart View	13	428-433	2009
横山健次	「抗血栓療法をめぐって」抗血小板薬。	MEDICO	40	79-82	2009
横山健次	「血栓症の疾患と治療（循環器系を中心に）」深部静脈血栓症/肺塞栓症の臨床。	Medical Science Digest	35	102-105	2009
横山健次	選択的抗トロンビン薬、抗Xa薬ではINRの計測は不要になる？	Heart View	13	1364-1367	2009
矢作かおり、村田満	偽性血小板減少症	最新医学･別冊新しい診断と治療のＡＢＣ63　血小板減少症・増加症第6		215-221	2009

		章トピックス			
村田満	出血性疾患	内科学症例図説		489-495	2009
村田満	血栓傾向	改訂7版内科学書血液・造血器疾患神経疾患	6	189-193	2009
村田満	C．血栓・止血　3.ＤＩＣの病態と臨床	臨床検査Yearbook2009血液検査編	142	106-111	2009
松原由美子、村田満	抗血小板薬のモニタリング-抗血小板薬の薬効評価の基本的考え方と実際	医学のあゆみ	228	957-961	2009
村田満	アスピリンレジスタンス	特集抗血栓療法―最近の動向　日本医師会雑誌	138	492	2009
村田満	第1回動脈硬化/炎症と血小板 血小板の発見から血栓形成機序の解明へ	Mystery of Platelet Therapesearch	30	841-846	2009
村田満	第2回生活習慣病と血小板 血小板研究の主流となった血栓症学	Mystery of Platelet Therapesearch	30	1051-1061	2009
川崎富夫、小山信彌	第36回日本血管外科学会総会の新たな挑戦-総会記録集（司会者のまとめ）とコンセンサ	日本血管外科学会雑誌	18	425－430	2009
川崎富夫	訴訟に求められる「認識の統合」	年報医事法学	24	9－14	2009
川崎富夫	チーム医療の総責任者が手術説明について患者やその家族に対して負う義務　―チーム医療の措定―	年報医事法学	24	164－169	2009
川崎富夫	未熟児網膜症姫路日赤事件における医療水準の論考―医学的視点から・認識統合のために―	Law & Technology	46	36－44	2009
榛沢和彦、岡本竹司、佐藤浩一、林純一、山村修、伊倉真衣子、柴田宗一、小泉勝	岩手・宮城内陸地震のDVT頻度:避難環境との関連	Therapeutic Research	30	572-574	2009
榛沢和彦、林純一、田辺直仁、	新潟県中越地震における深部静脈血栓症-対	血栓と循環	17	121-124	2009

相澤義房、伊藤正一、鈴木幸雄、吉嶺文俊	照地域検査との比較				
榛沢和彦	震災と DVT	Heart View	13	89-99	2009
倉田義之	ITP の疫学	血液フロンティア	19	863-871	2009
猪狩敦子、村田満	<抗血栓療法のモニター>抗血小板療法の POCT(Point of care testing)	臨床検査　今月の特集血栓症と臨床検査	53	1175-1179	2009
村田満、Catherine P. Hayward	対談 Standardization of platelet function testing 血小板機能検査の標準化	International Review of Thrombosis	4	9-27	2009
冨山佳昭	ITP に関する用語の標準化：国際作業部会からの報告	日本血栓止血学会誌	21	278-282	2010
冨山佳昭	特発性血小板減少性紫斑病	小児科診療	73	2193-2196	2010
冨山佳昭	P2Y12 receptor occupancy	International review of thrombosis	5	34-38	2010
冨山佳昭	抗血小板薬の最近の進歩	最新医学	65	90-96	2010
藤村欣吾	*Helicobacter pylori* と特発性血小板減少性紫斑病	臨床検査	54	187－191	2010
藤村欣吾	難治性特発性血小板減少性紫斑病に対する治療：最近の動向	血液・腫瘍科	60	532-538	2010
藤村欣吾	プライマリ・ケア医に必要な血液疾患の知識「特発性（免疫性）血小板減少性紫斑病」	治療	92	2381-2386	2010
秋山正志，武田壮一，小亀浩市，高木淳一，宮田敏行	VWF切断酵素ADAMTS13のエキソサイト認識機構	生化学	82(10)	950-956	2010
小嶋哲人	注射・経口抗凝固薬（特集・血栓症）	最新医学	65(6)	1189-1194	2010
小嶋哲人	凝固制御因子	International Review of Thrombosis	5(2)	86-90	2010
小嶋哲人	門脈血行異常症と血液凝固関連遺伝子	肝胆膵	61(2)	167-173	2010
小嶋哲人	Q42 ヘパリン類似物質	救急・集中治療	22(11,12)	1603-1608	2010

小嶋哲人	経口抗Xa薬と経口抗トロンビン薬	Heart View	14(2)	271-274	2010
川崎富夫	未熟児網膜症姫路日赤事件における医療水準の論考 --医学的視点から・認識統合のために--	Law & Technology	46	36-44	2010
宮田敏行、川崎富夫、坂田洋一、村田満、小嶋哲人	日本人の血栓性素因、特にプロテインS欠損症を中心に(総説)	日本産婦人科・新生児血液学会誌	20		印刷中
横山健次	【内科疾患の診断基準病型分類・重症度】 診断メモ 血栓性血小板減少性紫斑病。	内科	105	1459	2010
横山健次	【内科疾患の診断基準病型分類・重症度】 血液 特発性血小板減少性紫斑病。	内科	105	1449-1452	2010
横山健次	【炎症性疾患・免疫疾患におけるγグロブリン大量静注療法】 血液疾患におけるγグロブリン大量静注療法。	炎症と免疫	18	157-161	2010
横山健次	【骨髄増殖性疾患】 骨髄増殖性疾患と血栓・止血。	臨床検査	54	304-308	2010
横山健次	抗血小板薬と抗凝固薬の副作用。	日本医事新報	4479	94-95	2010
佐久間聖仁、中村真潮、中西宣文、山田典一、白土邦男、伊藤正明、小林隆夫	院外発症静脈血栓塞栓症の危険因子	心臓	42(7)	908-909	2010
杉村基，平井久也，大橋涼太，金山尚裕	妊娠産褥期深部静脈血栓症診断マーカーとしての活性化プロテインC感受性の意義	心臓	42(7)	914-916	2010
榛沢和彦、佐藤浩一、中島孝、	新潟県中越沖地震2年目の被災者DVT検査結	心臓	42(7)	966-967	2010

伊倉真衣子	果				
榛沢和彦	震災と DVT	救急医療ジャーナル	102(18)	51-55	2010
笠松紀雄，小林隆夫，木倉睦人，岩瀬敏樹，岡田喜親，佐々木俊哉，内藤健助，金井俊和，小林正和，小澤享史，横井典子，平松みどり，遠藤裕子，山口幸子，中村直樹，松岡敏彦，神谷純子，鬼頭孝昌，石井良朋	当院における「静脈血栓塞栓症の予防・患者発生時対応への組織的取組み」導入後の肺塞栓症患者発生の推移	県西部浜松医療センター学術誌	4(1)	97-100	2010

研究成果の刊行物・別冊

Positive and Negative Regulation of Integrin Function

YOSHIAKI TOMIYAMA, MASAMICHI SHIRAGA, AND HIROKAZU KASHIWAGI

Summary. Platelet integrin $\alpha_{IIb}\beta_3$, a prototypic non-I domain integrin, plays an essential role in platelet aggregation. The structure and function of $\alpha_{IIb}\beta_3$ is dramatically changed during platelet plug formation and pathological thrombus formation. The function of this integrin is regulated by the balance of actions of positive and negative regulatory factors. Several novel regulators have emerged from recent studies. As a positive regulator, the $P2Y_{12}$ plays a critical role in thrombus stability; and continuous interaction between ADP and $P2Y_{12}$ is essential for sustained $\alpha_{IIb}\beta_3$ activation. Semaphorin 3A and SHPS-1 have been identified as negative regulators. These molecules are secreted from or expressed on endothelial cells and inhibit the function of platelets as well as $\alpha_{IIb}\beta_3$. Investigation on these positive and negative regulatory factors should provide a new insight into the treatment of pathological thrombosis.

Key words. Inside-out signaling · Outside-in signaling · $P2Y_{12}$ · Semaphorin 3A · SHPS-1

Introduction

Platelets play a crucial role not only in hemostatic plug formation but also in a pathological thrombus formation, particularly in atherosclerotic arteries subjected to high shear stress [1, 2]. Moreover, recent studies have revealed that the platelet is a major player in the initiation of vascular remodeling as well as atherosclerotic lesion formation [3, 4]. As an initial step in thrombogenesis, platelets adhere to altered vascular surfaces or exposed subendothelial matrices and then become activated and aggregate with each other. As summarized in Fig. 1, it has been well documented that these processes are primarily mediated by platelet surface glycoproteins: GPIb-IX-V, integrin $\alpha_2\beta_1$ (also known as GPIa-IIa), GPVI, and integrin $\alpha_{IIb}\beta_3$ (GPIIb-IIIa) [5, 6].

Integrins comprise a family of heterodimeric adhesion receptors that mediate cellular attachment to the extracellular matrix and cell cohesion [7–9]. Platelets express at least five integrins on their surface: $\alpha_2\beta_1$(GPIa-IIa); $\alpha_5\beta_1$(GPIc-IIa); $\alpha_6\beta_1$(GPIc′-IIa); $\alpha_{IIb}\beta_3$(GPIIb-IIIa); $\alpha_v\beta_3$. Platelet integrin $\alpha_{IIb}\beta_3$ is a prototypic non-I domain integrin and plays an essential role in platelet aggregation as a physiological receptor for fibrinogen and von Willebrand factor. The importance of this integrin has been well documented by the clinical features of a congenital bleeding disorder, Glanzmann

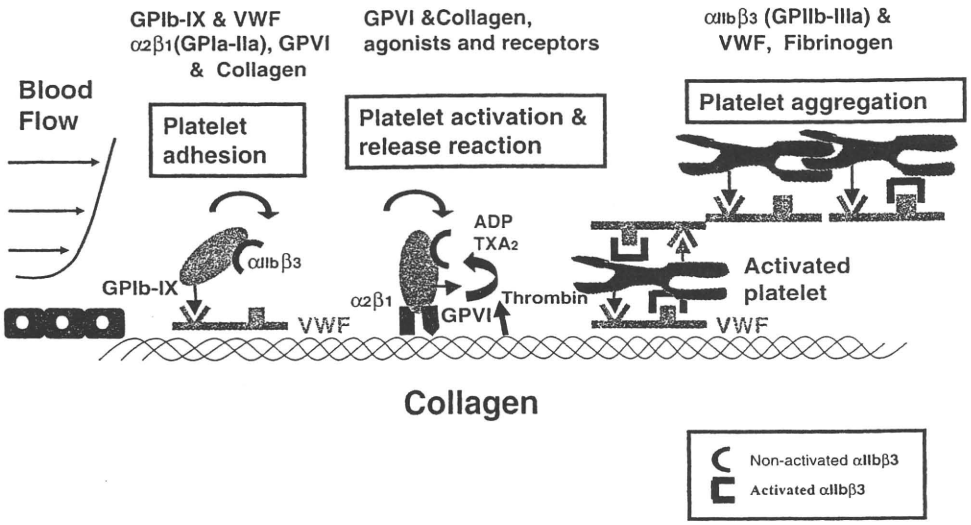

FIG. 1. Mechanisms of platelet plug formation and pathological thrombus formation. These processes depend primarily on platelet adhesive interactions with both platelet surface glycoproteins including integrins and extracellular matrix proteins. Platelet adhesion (or tethering) is mainly mediated by glycoprotein (*GP*)Ib-IX and von Willebrand factor (*VWF*) especially under high shear rates; and platelet aggregation is mediated by $\alpha_{IIb}\beta_3$ and VWF and fibrinogen. Platelet activation and released factors such as adenosine diphosphate (ADP) play a critical role in thrombus stability. *TXA_2*, thromboxane A_2

thrombasthenia (GT) [10, 11]. The crystal structure of $\alpha_{IIb}\beta_3$ revealed that the ligand-binding head is formed by a seven-bladed β-propeller domain from α_{IIb} and a β I-domain from β_3 [12, 13]. Despite the presence of integrin $\alpha_{IIb}\beta_3$ and its ligands, single platelets circulate freely within the vascular tree that is lined by an intact monolayer of endothelial cells. Thus, the function of integrin $\alpha_{IIb}\beta_3$ is regulated by the balance of actions of positive and negative regulatory factors. During thrombogenesis, the affinity of $\alpha_{IIb}\beta_3$ for macromolecular ligands is dynamically changed [8, 9]. In resting platelets, $\alpha_{IIb}\beta_3$ is in a low-affinity state and does not bind soluble macromolecular ligands. However, after exposure to subendothelial matrix and several mediators such as adenosine 5′-diphosphate (ADP), thromboxane A_2, and thrombin, platelets become activated, and activation signals (inside-out signaling) that induce a high-affinity state of $\alpha_{IIb}\beta_3$ for soluble ligands ($\alpha_{IIb}\beta_3$ activation) are generated. After ligand binding to $\alpha_{IIb}\beta_3$, postligand-binding signals (outside-in signaling) that induce tyrosine phosphorylation and cytoskeletal reorganization are further generated, leading to full expression of $\alpha_{IIb}\beta_3$ function. Molecular characterization of GT due to a dysfunctional $\alpha_{IIb}\beta_3$ (referred as variant GT) provides strong evidence that the cytoplasmic domain of β_3 is involved in inside-out signaling [14, 15]. Indeed, specific binding of the cytoskeletal protein talin to integrin β subunit cytoplasmic tails leads to $\alpha_{IIb}\beta_3$ activation as a final common step in integrin activation [16]. Major advances have been made regarding the structural basis of $\alpha_{IIb}\beta_3$ activation, resulting in the proposal of the "switchblade" model [17]. However, much remains to be elucidated about factors (or molecules) surrounding platelets that positively or negatively regulate $\alpha_{IIb}\beta_3$ function. In this review, we focus on recently identified factors and/or mechanisms that regulate $\alpha_{IIb}\beta_3$ function.

Positive Regulators for $\alpha_{IIb}\beta_3$ Function

In vivo fluorescence microscopy reveals that a few platelets are tethered to the intact vascular wall even under physiological conditions [18]. However, ~100% of these platelets were displaced from the vascular wall without firm arrest. Thus, a threshold for further platelet activation and the initiation of thrombus formation seems to exist, and $\alpha_{IIb}\beta_3$ function should be dynamically controlled by the balance of positive and negative regulators. A number of factors have been identified as a positive regulator for $\alpha_{IIb}\beta_3$ function (Table 1). These factors contribute to stabilize the platelet thrombus as well as initiate thrombus formation. ADP, collagen, and thrombin are classic, well-known factors that initiate thrombus formation by inducing $\alpha_{IIb}\beta_3$ activation. In contrast, serotonin acts as a potentiator, rather than an initiator, for $\alpha_{IIb}\beta_3$ activation. Recently, several factors that contribute to stabilize platelet thrombus have been identified: CD40L and $\alpha_{IIb}\beta_3$, Eph kinases and ephrins, Gas6 and its receptors, and ADP and $P2Y_{12}$ receptor (for review see ref. 19). CD40L, a member of the tumor necrosis factor (TNF) family, is expressed on the platelet surface after platelet activation, and a soluble form of CD40L (sCD40L) is generated by the activation as well. Although CD40 is known to be a receptor for CD40L, the effect of CD40L (and sCD40L) on platelets is mediated by $\alpha_{IIb}\beta_3$ but not by CD40. The interaction of CD40L and $\alpha_{IIb}\beta_3$ contributes to thrombus stability, probably via augmentation of $\alpha_{IIb}\beta_3$-mediated outside-in signaling [20, 21]. Eph kinases and ephrins also augment $\alpha_{IIb}\beta_3$ outside-in signaling [22, 23]. Platelets express the Eph receptor kinase (EphA4 and EphB1) and the Eph kinase ligand, ephrinB1; and blockade of the Eph/Ephrin interactions causes platelet disaggregation induced by low concentrations of ADP and decreased platelet thrombus volume on a collagen-coated surface at high shear rates. Gas6 is a secreted protein localized in α-granules; and its receptors Axl, Sky, and Mer are also expressed

TABLE 1. Regulators for $\alpha_{IIb}\beta_3$ function

Positive regulators
ADP
Collagen
Thrombin
Epinephrine
PAF
Serotonin
CD40L
Eph kinases/ephrins
Gas6
Leptin
Negative regulators
Prostacyclin
Nitric oxide
CD39 (NTPDase1)
PECAM-1
Semaphorin 3A
SHPS-1 (SIRPα1)

on platelets. It has been demonstrated that secreted Gas6 binds to its receptors, leading to the promotion and stabilization of platelet plug formation via $\alpha_{IIb}\beta_3$ outside-in signaling [24]. Thus, these newly identified factors may play a role in the stability of platelet aggregation in vivo. However, recent studies have revealed that the interaction between ADP and its receptor $P2Y_{12}$ play a critical role in the stability of platelet thrombus.

Role of the Interaction Between ADP and $P2Y_{12}$ in the Maintenance of $\alpha_{IIb}\beta_3$ Activation

ADP is stored within platelet dense granules and actively secreted upon platelet activation; approximately 2.5 μmol ADP exists in 10^{11} platelets [25]. Platelets have at least two major G protein-coupled ADP receptors: $P2Y_1$ is a G_q-coupled receptor responsible for mediating platelet shape change and reversible platelet aggregation through intracellular calcium mobilization, whereas $P2Y_{12}$ is a G_i-coupled receptor responsible for mediating the inhibition of adenylyl cyclase and sustained platelet aggregation [26]. $P2Y_{12}$ consists of 342 amino acid residues with seven transmembrane domains. The importance of $P2Y_{12}$ is well documented by the clinical feature of congenital bleeding disorder due to $P2Y_{12}$ deficiency [27–29]. We have identified a Japanese patient with $P2Y_{12}$ deficiency, OSP-1, caused by a point mutation in the translation initiation codon (ATG to AGG) [30]. $P2Y_{12}$-mediated signaling evoked by endogenous ADP plays a major role in platelet aggregation induced by low concentrations of collagen, U46619, and PAR1 TRAP in vitro. We and others have demonstrated impaired thrombus stability under flow conditions [29, 30]. Employing whole blood obtained from OSP-1, real-time analysis of thrombogenesis on a type I collagen-coated surface under a high shear rate ($2000\,s^{-1}$) revealed that $P2Y_{12}$ deficiency led to loosely packed thrombus and impaired thrombus growth with enhancing adhesion to collagen. The increase in platelet adhesion to collagen was probably due to the impaired platelet consumption by the growing thrombi. Moreover, our real-time observation indicated that the loosely packed aggregates were unable to resist against high shear stress, and most of the aggregates at the apex of the thrombi came off the thrombi [30]. In a mesenteric artery injury model $P2Y_{12}$-knockout mice also demonstrated the instability of thrombus formation [31]. Thus, the ADP-$P2Y_{12}$ interaction plays a major role in the stability of thrombus.

We assessed the $\alpha_{IIb}\beta_3$ activation on OPS-1 platelets in vitro by the binding of ligand-mimetic monoclonal antibody, PAC-1. Interestingly, $\alpha_{IIb}\beta_3$ activation is markedly impaired by stimulation with PAR1-TRAP, PAR4-TRAP, or U46619 in the absence of $P2Y_{12}$ [30]. On the other hand, PAR1-TRAP and U46619 are able to induce transient aggregation of OSP-1 platelets, indicating that $\alpha_{IIb}\beta_3$ could be transiently activated with these agonists. Based on these findings, we assume that $\alpha_{IIb}\beta_3$ activation may be too short and unstable to be detected by the PAC1 binding assay on OSP-1 platelets and that released ADP and $P2Y_{12}$-mediated signaling may play a critical role in the maintenance of $\alpha_{IIb}\beta_3$ activation. Employing modified ligand-binding assays, we have analyzed the mechanism of sustained $\alpha_{IIb}\beta_3$ activation induced by thrombin. After completion of $\alpha_{IIb}\beta_3$ activation and induction of α-granule secretion, a $P2Y_{12}$ antagonist (AR-C69931MX) was added to the activated platelets [32]. Under these conditions, the stimulated platelets showed long-lasting $\alpha_{IIb}\beta_3$ activation. However, the addition

of 1 μM AR-C69931MX at any time tested after thrombin stimulation disrupted the sustained $\alpha_{IIb}\beta_3$ activation without inhibiting CD62P expression (Fig. 2). Neither yohimbine (an adrenergic receptor antagonist), MIC-9042 (a 5-HT_2 receptor antagonist), nor SQ-29548 (a thromboxane A_2 receptor antagonist) inhibited sustained $\alpha_{IIb}\beta_3$ activation. Dilution of platelet concentrations from 50 000 platelets/μl to 500 platelets/μl also abolished sustained $\alpha_{IIb}\beta_3$ activation, and disruption of $\alpha_{IIb}\beta_3$ activation by the dilution was abrogated by the addition of small amounts of "exogenous" ADP. Thus, the continuous interaction between secreted ADP with $P2Y_{12}$ is necessary for sustained $\alpha_{IIb}\beta_3$ activation induced by thrombin; and substantial amounts of ADP (= substantial platelets) are needed to maintain $\alpha_{IIb}\beta_3$ activation. The critical role of the interaction between ADP and $P2Y_{12}$ is also evident in the sustained $\alpha_{IIb}\beta_3$ activation induced by U46619 (TXA_2 analogue) [32]. Even in the absence of $P2Y_{12}$, platelets can transiently aggregate with each other. However, platelets lacking G_q and G_{13} are completely unresponsive to thrombin, and the activation of G_i-mediated signaling alone is not sufficient to induce platelet aggregation [33]. Thus, it is likely that once $\alpha_{IIb}\beta_3$ is activated by G_q and/or G_{13}-mediated signaling the ADP-$P2Y_{12}$ may prevent the shift from the activated $\alpha_{IIb}\beta_3$ to the resting $\alpha_{IIb}\beta_3$ (Fig. 2).

Recent in vivo observations demonstrated that during platelet thrombus formation circulating platelets were tethered to the luminal surface of growing thrombi by VWF–GPIb interaction. However, more than 95% of tethered platelets were subsequently translocated and/or detached [18]. Activated $\alpha_{IIb}\beta_3$ on the detached platelets should become inactivated because the released ADP is immediately diluted by the

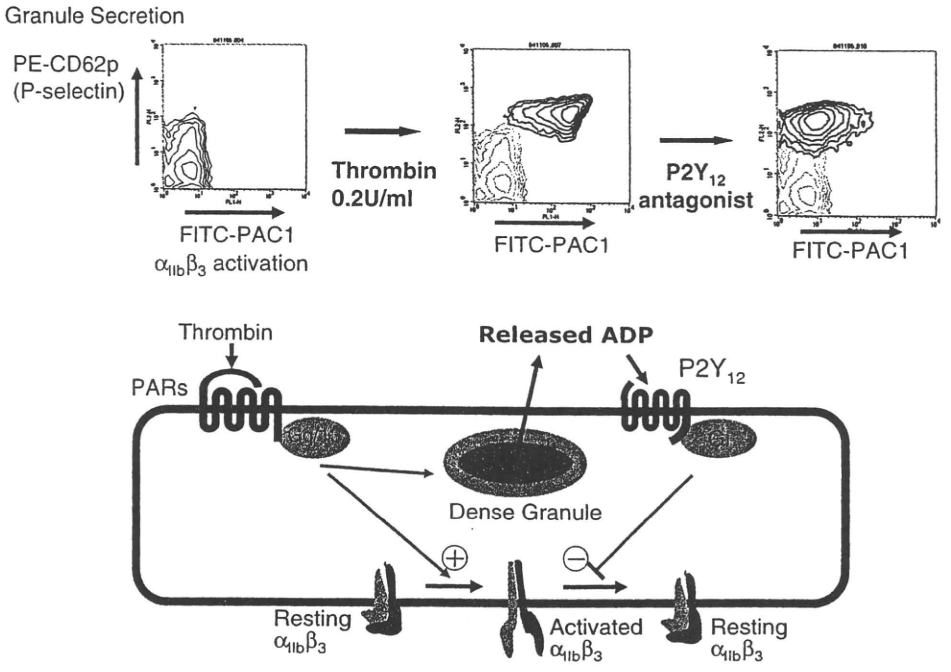

FIG. 2. Critical role of ADP–$P2Y_{12}$ interaction in the maintenance of $\alpha_{IIb}\beta_3$ activation. Blockade of ADP–$P2Y_{12}$ interaction at any time after thrombin stimulation disrupts $\alpha_{IIb}\beta_3$ activation. Once $\alpha_{IIb}\beta_3$ is activated by G_q- and/or G_{13}-mediated signaling, the ADP–$P2Y_{12}$ may prevent the shift from activated $\alpha_{IIb}\beta_3$ to resting $\alpha_{IIb}\beta_3$. *FITC-PAC1*, fluorescein isothiocyanate-conjugated PAC1

blood flow. At the luminal surface, activated $\alpha_{IIb}\beta_3$ on the tethered platelets would be maintained only when the platelets are continuously exposed to ADP released from adjacent activated platelets. At the inside of growing thrombi, it appears that platelets are constantly exposed to such high concentrations of released ADP that $\alpha_{IIb}\beta_3$ can be maintained in its high-affinity state in concert with the effects of thrombin and TXA_2. It is possible that ADP concentrations surrounding platelets may largely influence whether platelets participate in thrombus formation. Thus, $P2Y_{12}$ may serve as a sensor for thrombogenic status surrounding individual platelets, and the interaction between ADP and $P2Y_{12}$ likely determines thrombus size.

Negative Regulators for $\alpha_{IIb}\beta_3$ Function

Prostacyclin and nitric oxide produced by endothelial cells are well-known negative regulators for the platelet function [34]. In addition to these molecules several negative regulators have been emerged in recent studies (Table 1). We have identified that semaphorin 3A and SHPS-1 act as negative regulators for $\alpha_{IIb}\beta_3$ function [35, 36].

Semaphorin 3A as a Negative Regulator for Platelet Function

The semaphorin family comprises soluble and membrane-bound proteins that are defined by the presence of a conserved 500-amino-acid semaphorin domain at their amino termini. Class 3 semaphorins are secreted disulfide-bound homodimeric molecules; and Sema3A, a prototypic class 3 semaphorin, causes growth cone collapse and provides chemorepulsive guidance for migrating axons. Cell surface receptor for Sema3A consists of a complex of two distinct transmembrane receptors, neuropilin-1 and plexin A (A1-A3). It has been demonstrated that Sema3A is produced by endothelial cells and inhibits integrin function on endothelial cells in an autocrine manner [37]. Employing two distinct Sema3A chimera proteins, we have demonstrated that Sema3A has extensive inhibitory effects on platelet function [35]. Sema3A inhibited agonist-induced $\alpha_{IIb}\beta_3$ activation dose-dependently. Moreover, Sema3A inhibited granular secretion as well as platelet spreading on immobilized fibrinogen. However, Sema3A did not show any effects on the levels of cAMP or cGMP or thrombin-induced increase in intracellular Ca^{2+} concentrations. It is likely that Sema3A inhibits cytoskeletal reorganization in activated platelets as Sema3A inhibits platelet spreading and granule secretion.

Indeed, Sema3A inhibited agonist-induced elevation of filamentous actin (F-actin) contents and Rac1 activation. Rac1 activation is necessary for platelet actin assembly and lamellipodia formation after agonist stimulation. Therefore, marked impairment of Rac1 activation is likely to account for the Sema3A-induced impairment of actin rearrangement and spreading in platelets. There were two major downstream effectors of Rac1 identified: PAK and WAVEs [Wiskott-Aldrich syndrome protein (WASP) family verprolin-homologous proteins]. Several PAK substrates or binding partners have been implicated in the effects of PAK, including filamin, LIM kinase, myosin, and paxillin. Among them, LIM kinase phosphorylates and inactivates cofilin, a protein that promotes severing and depolymerization of F actin. Consistent with the inhibition of Rac1 activation, Sema3A inhibited phosphorylation of cofilin in both resting

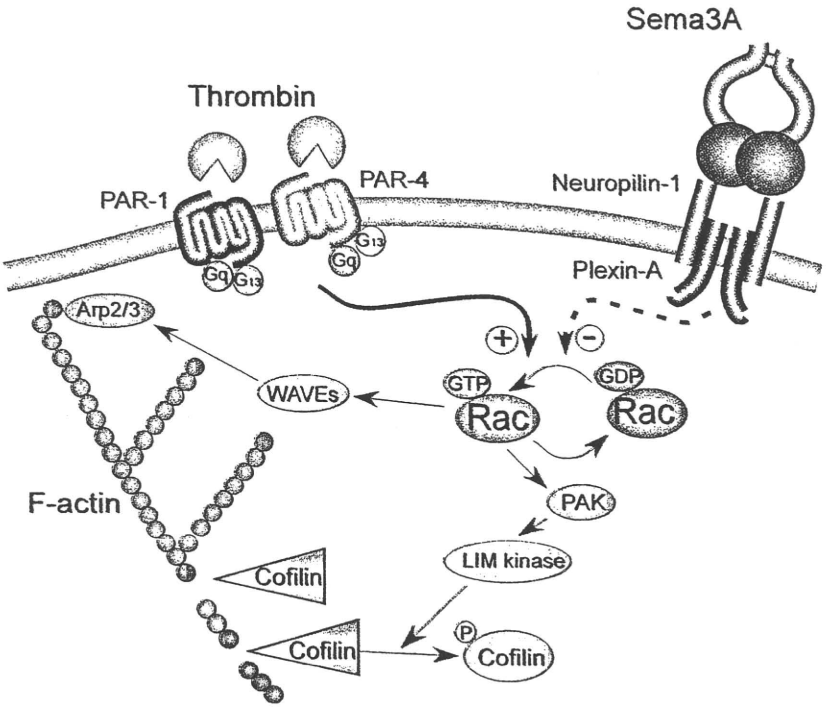

FIG. 3. Inhibitory mechanisms of platelet function by Sema 3A. Sema 3A inhibits platelet spreading and granular secretion as well as $\alpha_{IIb}\beta_3$ activation. The inhibitory effects are mediated in part by the inhibition of agonist-induced Rac1 activation and phosphorylation of cofilin. This inhibition leads to the inhibition of F-actin elevation and cytoskeleton rearrangement

and activated platelets, suggesting that Sema3A increases severing and depolymerization of F-actin by keeping cofilin in the activated state (Fig. 3). In addition to Rac1 inactivation, our recent data showed that Sema3A inhibited the PI3 kinase pathway, including Rap1B, which may account for the inhibition of $\alpha_{IIb}\beta_3$ activation (unpublished data).

SHPS-1 as a Negative Regulator for Platelet Function

SHPS-1 (Src homology 2 domain-containing protein tyrosine phosphatase substrate-1), also known as signal regulatory protein α1 (SIRP α1), is a membrane glycoprotein with three extracellular immunoglobulin (Ig)-like domains, a single transmembrane domain, and an intracellular domain containing two immunoreceptor tyrosine-based inhibitory motifs (ITIM) and expressed on endothelial cells and leukocytes. CD47 (integrin-associated protein, or IAP) is a ubiquitously expressed 50-kDa membrane glycoprotein with an extracellular Ig domain, five membrane-spanning domains, and a short cytoplasmic tail. CD47 physically associates with $\alpha_{IIb}\beta_3$, $\alpha_v\beta_3$, and $\alpha_2\beta_1$ and modulates a variety of cell functions [38]. Two ligands are known to bind to CD47: thrombospondin-1 (TSP-1) and SHPS-1. The TSP-1–CD47 interaction has been believed to augment integrin-mediated platelet function. On the other hand, SHPS-1–Ig, a fusion protein consisting of the extracellular domain of SHPS-1 and human Ig Fc domain, impaired secondary platelet aggregation induced by a low concentration

of ADP (2.5 μM). Moreover, SHPS-1–Ig markedly impaired $\alpha_{IIb}\beta_3$-mediated platelet spreading onto immobilized fibrinogen. The inhibition of platelet spreading is CD47-specific because it was not observed in CD47-deficient ($CD47^{-/-}$) murine platelets. Of particular interest is that SHPS-1 inhibits $\alpha_{IIb}\beta_3$-mediated platelet spreading without disturbing Syk and FAK tyrosine phosphorylation. SHPS-1 did inhibit tyrosin phosphorylation of α-actinin, a downstream effector of FAK. Thus, SHPS-1 negatively regulates platelet function through CD47, especially $\alpha_{IIb}\beta_3$-mediated outside-in signaling, by interfering with the downstream pathway of FAK.

Conclusion

Thrombogenesis is a complex process regulated by the balance of positive and negative regulatory proteins (or molecules). Further investigations of these regulatory molecules would provide a new insight into the more effective prevention of pathological thrombosis.

Acknowledgments. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology in Japan; the Ministry of Health, Labor, and Welfare in Japan; and the "Academic Frontier" Project in Japan; and Mitsubishi Pharma Research Foundation.

References

1. Fuster V, Badimon L, Badimon JJ, et al (1992) The pathogenesis of coronary artery disease and the acute coronary syndromes. N Engl J Med 326:242–250
2. Antithrombotic Trialists' Collaboration (2002) Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. BMJ 324:71–86
3. Massberg S, Brand K, Grüner S, et al (2002) A critical role of platelet adhesion in the initiation of atherosclerotic lesion formation. J Exp Med 196:887–896
4. Massberg S, Konrad I, Schürzinger K, et al (2006) Platelets secrete stromal cell-derived factor 1α and recruit bone marrow-derived progenitor cells to arterial thrombi in vivo. J Exp Med 203:1221–1233
5. Tomiyama Y, Shiraga M, Shattil SJ (2002) Platelet membrane proteins as adhesion receptors. In: Gresele P, Page C, Fuster V, et al (eds) Platelets in thrombotic and non-thrombotic disorders: pathophysiology, pharmacology and therapeutics. Cambridge University Press, Cambridge, UK, pp 80–92
6. Savage B, Almus-Jacobs F, Ruggeri ZM (1998) Specific synergy of multiple substrate-receptor interactions in platelet thrombus formation under flow. Cell 94:657–666
7. Hynes RO (2002) Integrins: bidirectional, allosteric signaling machines. Cell 110:673–687
8. Phillips DR, Charo IF, Scarborough RM (1991) GPIIb-IIIa: the responsive integrin. Cell 65:359–362
9. Shattil SJ, Newman PJ (2004) Integrins: dynamic scaffolds for adhesion and signaling in platelets. Blood 104:1606–1615
10. George JN, Caen JP, Nurden AT (1990) Glanzmann's thrombasthenia: the spectrum of clinical disease. Blood 75:1383–1395
11. Tomiyama Y (2000) Glanzmann thrombasthenia: integrin $\alpha_{IIb}\beta_3$ deficiency. Int J Hematol 72:448–454

12. Xiong JP, Stehle T, Diefenbach B, et al (2001) Crystal structure of the extracellular segment of integrin $\alpha_v\beta_3$. Science 294:339–345
13. Xiao T, Takagi J, Coller BS, et al (2004) Structural basis for allostery in integrins and binding to fibrinogen-mimetic therapeutics. Nature 432:59–67
14. Chen Y-P, Djaffar I, Pidard D, et al (1992) Ser-752 → Pro mutation in the cytoplasmic domain of integrin β_3 subunit and defective activation of platelet integrin $\alpha_{IIb}\beta_3$ (glycoprotein IIb-IIIa) in a variant of Glanzmann thrombasthenia. Proc Natl Acad Sci U S A 89:10169–10173
15. Wang R, Shattil SJ, Ambruso DR, et al (1997) Truncation of the cytoplasmic domain of β_3 in a variant form of Glanzmann thrombasthenia abrogates signaling through the integrin $\alpha_{IIb}\beta_3$. J Clin Invest 100:2393–2403
16. Tadokoro S, Shattil SJ, Eto K, et al (2003) Talin binding to integrin beta tails: a final common step in integrin activation. Science 302:103–106
17. Takagi J, Petre BM, Walz T, et al (2002) Global conformational rearrangements in integrin extracellular domains in outside-in and inside-out signaling. Cell 110:599–611
18. Massberg S, Gawaz M, Gruner S, et al (2003) A crucial role of glycoprotein VI for platelet recruitment to the injured arterial wall in vivo. J Exp Med 197:41–49
19. Brass LF, Zhu L, Stalker TJ (2005) Minding the gaps to promote thrombus growth and stability. J Clin Invest 115:3385–3392
20. André P, Prasad KS, Denis CV, et al (2002) CD40L stabilizes arterial thrombi by a β_3 integrin-dependent mechanism. Nat Med 8:247–252
21. Prasad KS, André P, He M, et al (2003) Soluble CD40 ligand induces β_3 integrin tyrosine phosphorylation and triggers platelet activation by outside-in signaling. Proc Natl Acad Sci U S A 100:12367–12371
22. Prévost N, Woulfe D, Tanaka T, et al (2002) Interactions between Eph kinases and ephrins provide a mechanism to support platelet aggregation once cell-to-cell contact has occurred. Proc Natl Acad Sci U S A 99:9219–9224
23. Prévost N, Woulfe DS, Jiang H (2005) Eph kinases and ephrins support thrombus growth and stability by regulating integrin outside-in signaling in platelets. Proc Natl Acad Sci USA 102:9820–9825
24. Angelillo-Scherrer A, de Frutos P, Aparicio C, et al (2001) Deficiency or inhibition of Gas6 causes platelet dysfunction and protects mice against thrombosis. Nat Med 7:215–221
25. D'Souza L, Glueck HI (1997) Measurement of nucleotide pools in platelets using high pressure liquid chromatography. Thromb Haemost 38:990–1001
26. Gachet C (2001) ADP receptors of platelets and their inhibition. Thromb Haemost 86:222–232
27. Cattaneo M, Lecchi A, Randi AM, et al (1992) Identification of a new congenital defect of platelet function characterized by severe impairment of platelet responses to adenosine diphosphate. Blood 80:2787–2796
28. Nurden P, Savi P, Heilmann E, et al (1995) An inherited bleeding disorder linked to a defective interaction between ADP and its receptor on platelets: its influence on glycoprotein IIb-IIIa complex function. J Clin Invest 95:1612–1622
29. Remijn JA, Wu YP, Jeninga EH, et al (2002) Role of ADP receptor $P2Y_{12}$ in platelet adhesion and thrombus formation in flowing blood. Arterioscler Thromb Vasc Biol 22:686–691
30. Shiraga M, Miyata S, Kato H, et al (2005) Impaired platelet function in a patient with $P2Y_{12}$ deficiency caused by a mutation in the translation initiation codon. J Thromb Haemost 3:2315–2323
31. Andre P, Delaney SM, LaRocca T, et al (2003) $P2Y_{12}$ regulates platelet adhesion/activation, thrombus growth, and thrombus stability in injured arteries. J Clin Invest 112:398–406

32. Kamae T, Shiraga M, Kashiwagi H, et al (2006) Critical role of ADP interaction with $P2Y_{12}$ receptor in the maintenance of $\alpha_{IIb}\beta_3$ activation: association with Rap1B activation. J Thromb Haemost 4:1379–1387
33. Moers A, Wettschureck N, Grüner S, et al (2004) Unresponsiveness of platelets lacking both $G\alpha_q$ and $G\alpha_{13}$: implications for collagen-induced platelet activation. J Biol Chem 279:45354–45359
34. Wu KK, Thiagarajan P (1996) Role of endothelium in thrombosis and hemostasis. Annu Rev Med 47:315–331
35. Kashiwagi H, Shiraga M, Kato H, et al (2005) Negative regulation of platelet function by a secreted cell repulsive protein, semaphorin 3A. Blood 106:913–921
36. Kato H, Honda S, Yoshida H, et al (2005) SHPS-1 negatively regulates integrin $\alpha_{IIb}\beta_3$ function through CD47 without disturbing FAK phosphorylation. J Thromb Haemost 3:763–774
37. Serini G, Valdembri D, Zanivan S, et al (2003) Class 3 semaphorins control vascular morphogenesis by inhibiting integrin function. Nature 424:391–397
38. Brown EJ, Frazier WA (2001) Integrin-associated protein (CD47) and its ligands. Trends in Cell Biol 11:130–135

2. ITP における *H. pylori* 除菌療法の治療成績は？

1 序論

特発性血小板減少性紫斑病 idiopathic thrombocytopenic purpura（ITP）は血小板破壊の亢進により血小板減少を来たし，皮膚や粘膜に紫斑を主体とする出血症状を来す基礎疾患や原因の明らかでない後天性の出血性疾患である．

急性型は小児に多く，ウイルス感染など感染症の後に急激に出血症状を伴って発症し 6 カ月以内に治癒するのに対し，慢性型は成人に多く，緩慢に出血傾向を繰り返す．慢性 ITP は出血性疾患の中で日常最も遭遇する機会が多く，最近では中高年齢者に多い傾向がある[1]．

慢性 ITP の血小板破壊については，種々の原因で血小板膜特異糖蛋白（GPIIb/IIIa，GPIb/IX/V，GPVI，GPV など）に対する自己抗体（血小板抗体）が産生され[2]，この血小板抗体によって感作された血小板は脾臓を中心とする網内系細胞の Fc レセプターを介して貪食され，或いは補体成分の活性化による血小板溶解，さらにはリンパ球を介して血小板が破壊され（ADCC）血小板減少が生じる．

またこの抗体は巨核球にも作用して巨核球の成熟を抑制する結果血小板産生が低下するとの報告も成されている[3]．いずれにしても免疫学的に末梢における血小板の貪食・破壊，さらには血小板産生抑制などが加わって血小板数の減少がもたらされる病態と考えられている．従って特発性 idiopathic と言うよりむしろ免疫性血小板減少性紫斑病 immune thrombocytopenic purpura としての概念が主流である．免疫性血小板減少性紫斑病には続発性免疫性血小板減少性紫斑病と，原因が不明の原発性免疫性血小板減少性紫斑病に分けられている．

2 指針

基礎疾患や薬剤の関与がなく，原因の明らかでない ITP に対して従来は，副腎皮質ステロイド，摘脾療法が標準的治療として主流を成していた．1998 年イタリアの Gasbarrini A らによって *Helicobacter pylori*（以下 *HP*）菌陽性の ITP 症例に対し *HP* 除菌療法成功後に血小板数が増加した 8 症例が報告された[4]．これを契機に特にイタリア，本邦において多くの *HP* 陽性 ITP 症例に対して除菌療法による血小板増加効果が報告された．2004 年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」では，これら本邦のエビデンスに基づいた ITP 治療ガイドラインを提案している（図 1）[5]．

このガイドラインの特徴は ITP と診断されればまず *HP* 検査を行い，*HP* 陽性症例に対しては血小板数にかかわらず除菌療法を行う点にある．一方 *HP* 陰性例や，除菌成功後も血小板増加効果のない症例に対しては，血小板数と出血傾向に応じて治療を層別化し，従来の治療指針に準じて治療を行う．すなわち出血傾向を認めたり血小板数 2 万以下では積極的に従来

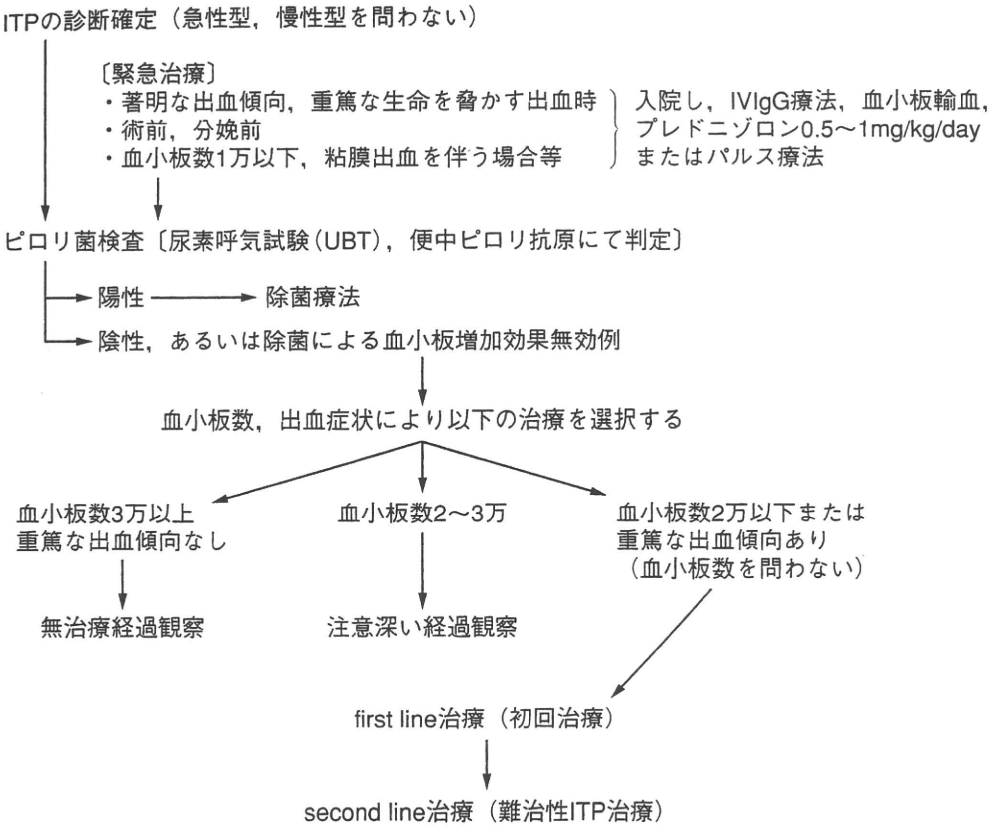

図1　ITP 治療の流れ（2004 年ガイドライン試案より）[5]

注：ガイドライン案にある付記はすべて除いてある．

の副腎皮質ステロイド療法を開始し，それ以外であれば無治療で経過観察する．本邦では慢性 ITP は中高年齢者が最も多くを占め，また約 7～8 割がピロリ菌陽性であることから除菌対象症例が多い点，除菌療法成功後の血小板増加頻度が格段に高い点，再発がごく少数例である点，除菌療法が簡単に行えること，またその副作用が副腎皮質ステロイドに比し圧倒的に少なく，治療期間も短く，総合的に見れば治療費が安価である，など本邦の ITP 症例にとって除菌療法は画期的な治療法である．

3　エビデンス

1　Fujimura K, et al（Int J Hematol. 2005; 81: 162–8）（retrospective study）[6]

目的：本邦 ITP における *HP* 感染の頻度，除菌療法の血小板増加効果と増加に関する予知因子について検討した．

方法：日本の主要 11 施設に対し 2002 年 7 月から 2003 年 12 月の間に受診した成人 ITP 症例についてアンケート調査を行い解析した．

結果：435 症例を検索対象として選択した．このうち *HP* 陽性 ITP 症例は 300 例（69%）で陰性例に比し中高年齢者に頻度が高かった．*HP* 陽性率には性差はなく，また日本の一般人口における陽性率と差はなかった．228 例に除菌が行われ，除菌結果の明らかな 207 例中 161 例（78%）が除菌成功，46 例が除菌不成