まずヘリコバクターピロリ菌感染が関係しているITPか否かを検査し，陽性であればインフォームドコンセントの基にまず除菌療法を行う．ピロリ菌の検査は種々行われているが中でも簡便，感度，特異性が高い点で尿素呼気試験，或いは小児にも適応できる便中抗原検査，が有用である．内視鏡的に行う，迅速ウレアーゼ試験，検鏡法は特異性が高いが，血小板減少による出血傾向を考慮すると適応症例が限定される．

2）除菌療法に反応しない症例に関しては従来の標準的治療を行う，を大枠としている．紙面の都合上詳しくは難治性疾患克服事業報告書を参照していただきたい[20]．

なおHP陽性ITPに対する除菌療法については現在保険収載を目標に働きかけを行っている．

（本邦でのレトロスペクティブ研究は厚生労働省　難治性疾患克服事業　血液凝固異常症に関する調査研究班　班長　池田康夫教授（慶應義塾大学）のもと，大阪大学　倉田義之先生，慶應義塾大学　桑名正隆先生，並びに北海道大学，旭川赤十字病院，昭和大学藤が丘病院，東京都立駒込病院，東京女子医科大学，東京医科大学，関西医科大学，広島大学，広島赤十字原爆病院の各血液内科の先生方の協力を得て行われたもので，改めて深謝いたします．）

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総 説

ITPにおける*Helicobacter pylori*除菌療法について

藤村欣吾

Key words : ITP, *Helicobacter pylori*, Eradication, Molecular mimicry

はじめに

特発性血小板減少性紫斑病（ITP）の治療法は慢性ITPについては副腎皮質ステロイドを中心とした免疫抑制療法や血小板破壊場所，並びに抗体産生の中心的役割を果たす脾臓の除去，すなわち摘脾療法が定着している。しかしそれら一連による治療成績は大まかには約40％の完全寛解，所謂治癒といって良い症例と，日常生活には何ら支障がないが軽度の血小板数減少が持続する部分寛解と考えられる症例が40％，血小板減少に対する何らかの治療介入が必要な症例が約20％と推測される。この内治療介入症例，主として難治症例に対する新たな治療戦略が求められている。

1998年Gasbarriniらによって初めてヘリコバクターピロリ菌陽性（HP陽性）の特発性血小板減少性紫斑病（ITP）症例において除菌療法後に血小板数が増加した症例が報告された[1]。以来ヘリコバクタピロリ菌（以下HP菌）除菌療法は一部のHP陽性ITP症例においては血小板増加反応を手軽に期待させる治療法として脚光を浴びている。

ここではHP菌陽性ITP症例に対する除菌療法の世界的現状を述べ，本邦で行った厚生労働省難治性疾患克服事業「血液凝固異常症に関する調査研究」（班長　池田康夫慶応大学教授）における多施設共同研究の結果を示し，HP菌とITPの関連について考えるとともに除菌療法を組み込んだ，ITP治療ガイドラインを作成したので紹介する。

ヘリコバクターピロリ菌感染と全身性疾患

HP菌はグラム陰性桿菌で胃粘膜局所に感染し，胃炎，胃潰瘍の主たる原因として考えられ,さらには胃がんや胃のリンパ腫（MALTリンパ腫）の発症との関連が問題にされた。1994年には疫学的研究からType I（definit）のがん源因子として認定されている[2]。

最近では感染局所病変以外にHP感染と全身性疾患の関連，例えば甲状腺炎やシェグレン症候群，慢性関節リュウマチ，膜性腎症など自己免疫疾患との関連を始めとして血液疾患では悪性貧血，自己免疫性好中球減少症，シェーンライン・ヘノッホ紫斑，やHP除菌により経口鉄剤治療抵抗性の鉄欠乏性貧血の改善，MGUSの一部に単クローン性ガンマーグロブリン血症が改善する例が報告され，今後の展開が注目されている[3~6]。一方冠動脈の動脈硬化プラークにHP菌特異的DNAシークエンスが証明され，一般の動脈硬化症や冠動脈疾患との関連も示唆されている。このように多くの全身性疾患との関連性が報告されたが，中には疑問視されている疾患もありどのような機序で全身性疾患が生じるのか不明である[6]。これらの中にあってITPとHP感染については臨床的論文が最も多く，関連性を強く示唆する報告が多い[1,7~17]。

ITPにおけるヘリコバクターピロリ菌感染頻度と臨床病態

1）ピロリ菌感染の背景と感染頻度

HP菌感染症は広く全世界に認められるが，国，地域，人種，年齢によって感染率が異なる特徴がある。感染様式は経口感染で，感染源として，吐物，唾液，便，に加え水が伝播経路として重視されている。感染年齢としては胃酸分泌能や免疫能が十分に発達していない乳幼児期に感染し以後慢性に経過する。従って幼児期の衛生状態，社会経済的状況がHP感染率を規定すると言われている[2]。

一般人口の感染率は発展途上国では中高年の80％以上であるのに対し，先進国，工業国では40％前後に留まり，10歳代までの感染率は10％以下で，社会背景が感染率に影響すると考えられ，曳いてはそれが出生時期における感染率の違いを引き起こしている[18]。

ITPにおけるHP感染率は，イタリア，日本からの報告では，一般人口の感染率と同様に70～90％で，ITPに感染率が高いとする報告はない。

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世界的にITPを含めてHP陽性率に性差は認められていない。

小児慢性ITPにおいては北欧（フィンランド）では17症例について検索されたがHP菌陽性例は認められなかった[19]。しかし台湾においては22例の小児慢性ITPの内9例（40%）にHP陽性で除菌による血小板増加も5例に認められている[20]。本邦においても小児慢性ITP10例の検索では2例に糞便中のHP抗原陽性が認められ，除菌によりHP菌陰性化し血小板数が上昇した報告がある[21]。このことから小児のHP感染率は成人に比し低いがその感染率は成人と同様に社会背景，環境によって左右され，また治療効果も変わらないと考えられる。小児に多い急性のITPにおいてはHP感染との関連は有意ではなく，急性ITPの発症には関わっていないとの報告がある[22]。

以上より慢性ITPにおけるHP感染は若年症例に比し中高年症例に頻度が高いが，年齢をマッチさせた一般人口との間では感染率に差はなく，ITPに感染率が高い訳ではない。急性ITPにおいては報告が少ないがHP菌感染が急性ITPの原因になっているとは考えにくく，HP菌が関与するITPは慢性型のITPと考えて良さそうである。

2）ピロリ感染とITPの臨床病態

世界的にいずれの報告においてもHP菌陽性ITP症例の年齢はHP菌陰性ITPに比し高く，前述の一般人口において中高齢に伴って感染率が上昇する傾向を反映している[7,9~11]。

しかし，性差，ITP罹病期間，臨床症状，初診時血小板数，において両者間では差は認められないとする報告がほとんどで，HP陽性ITPに特徴的な臨床像は認められていない[16]。またHP菌感染による胃腸障害の頻度や程度も非HP感染ITPと変わりはなくHP菌陽性ITPは消化器症状の面からも差を見いだすことが出来ない。

3）ITP症例におけるピロリ菌株

HP菌陽性ITP症例の胃病変についての報告では殆どpangastritis或いは胃体部に著明な胃炎の所見を示している。また本邦からの報告では感染菌株は血小板減少を伴わない単なる胃，十二指腸潰瘍症例と差は無く，Cag A, Vac A, ice A, IL-8の発現なども他のHP感染消化器疾患と差は無いとされている[17]。従って本邦では血小板減少を引き起こす特有の菌株や菌の性状は見当たっていない[23]。

ヘリコバクターピロリ菌感染ITPにおける除菌による血小板増加について

1998年のGasbarriniらの報告以来多くの除菌療法による血小板増加効果を検討した報告がなされている。その一覧をTable 1に示す。

除菌による血小板増加効果は国によって有効率に差があり，スペイン，フランス，米国からの報告では除菌による血小板増加効果はほとんど認められない。スペインの報告では13%がPRとなっているに過ぎない[12~14]。一方イタリアや日本からの報告では総じて50%以上に血小板の増加反応が認められている[7~11,15,16]。

最近イギリスとイタリアの共同研究によると半数以上英国人からなる集団に対する臨床研究では33%の症例に除菌による血小板増加反応が認められた[24]。

今までの傾向を考えると英国人とイタリア人の混合対象であったために有効率がこのような中間的値になった可能性がある。

このように除菌による血小板増加効果について大まかに2極化した報告になっているのが現状である。この原因は不明であるが1）対象者の免疫学的背景に差がある，2）地域的に感染したピロリ菌株によって発現している抗原性の程度が異なる（Cag, Vag, がコードする蛋白抗原，Lewis（Le）抗原など）等が推測されている。

本邦のレトロスペクティブ共同研究によるHP菌陽性ITPに対するHP除菌の血小板増加反応について

本邦において2002年7月から2003年12月の間に11施設の協力の下にレトロスペクティブ共同研究を行った[16]。その結果慢性ITP435例のうち300例（69%）にHP陽性であった。年齢別HP陽性ITPの頻度は，20歳代は40%以下で，40歳代では約50%であるが以後高齢に伴い上昇し，50歳代では70%以上が，60歳以上では80%以上がHP陽性である。これは本邦における一般人口に見られる感染率と近似している[16,25]（Fig. 1）。

1）除菌による血小板増加効果（Table 2）

HP陽性ITP300例の内228例に除菌療法が行われ，除菌効果判定が可能であった207例中161例78%に除菌が成功している。この時の除菌療法は殆どが通常のアモキシシリン，クラリスロマイシン，プロトンポンプインヒビターの3剤，7日間併用療法である。

この内12ヶ月以上経過観察された155例（除菌成功群122例，除菌不成功群33例）の長期予後は，除菌成功群の内79例は血小板数増加を維持し，このうち28例は除菌後無治療で血小板数15万以上となりCRの症例である（23%）。残り51例の多くは血小板数5万以上となり殆どが無治療観察（PR）となっている（42%）。残り43例は除菌に成功したものの血小板数の増加が認められない症例で（35%），除菌後の血小板数は除菌前値と変わりなく軽微な増減に留まっている（Table 2）。すなわち除菌成功群では65%の症例が12ヶ月以上の間何らか

Table 1 *Helicobacter pylori* positive rate and eradication effect on platelet counts in adult ITP
Many reports from Italy and Japan showed the good response rate of platelet counts after eradication, however reports from Spanish and USA did not support the platelet elevation by eradication. Overall *Helicobacter pylori* positive rate was 64% and platelet response rate was 49%.

Adult cases Authors	Reported Year	cases	*H.pylori* infected rate	Eradication effective rate	Platelet response cases & (%) in eradication effective cases	Median follow up duration (M)
Gasbarrini et al. (Italy)	1998	18	11 (61%)	8/11 (73%)	8 (100%)	4
Emilia et al. (Italy)	2001	30	13 (43%)	12/13 (92%)	6 (50%)	8.3
Jarque et al. (Spain)	2001	56	40 (71%)	23/32 (72%)	3 (13%)	24
Veneri et al. (Italy)	2002	35	25 (71%)	15/16 (94%)	11 (73%)	11.7
Kohda et al. (Japan)	2002	48	27 (56%)	19/19 (100%)	12 (63%)	14.8
Hino et al. (Japan)	2003	30	21 (70%)	18/21 (86%)	10 (56%)	15
Hashino et al. (Japan)	2003	22	14 (64%)	13/14 (93%)	5 (39%)	15
Ando et al. (Japan)	2003	61	50 (82%)	27/29 (93%)	16 (59%)	11
Michel et al. (USA)	2004	76	16 (21%)	14/16 (93%)	0/14 (0%)	11.5
Veneri et al. (Italy)	2004	21	19 (91%)	19/21 (91%)	14 (74%)	18.1
Takahashi et al. (Japan)	2004	20	15 (75%)	13/15 (87%)	7 (54%)	4
Fujimura et al. (Japan)	2004	435	300 (69%)	161/207 (78%)	79/122 (65%)	12<
Ando et al. (Japan)	2004	20	17 (85%)	15/17 (88%)	10 (67%)	24
Sato et al. (Japan)	2004	53	39 (74%)	27/32 (84%)	15 (56%)	6<
Kurtoglu et al. (Turkey)	2004	38	26 (68%)	/	/	/
Inaba et al. (Japan)	2005	35	25 (71%)	25	11 (44%)	6
Stasi R et al. (Italy)	2005	137	64 (47%)	52	17 (33%)	12<
Total		1135	722 (64%)	384/463 (83%)	224 (49%)	12.3<

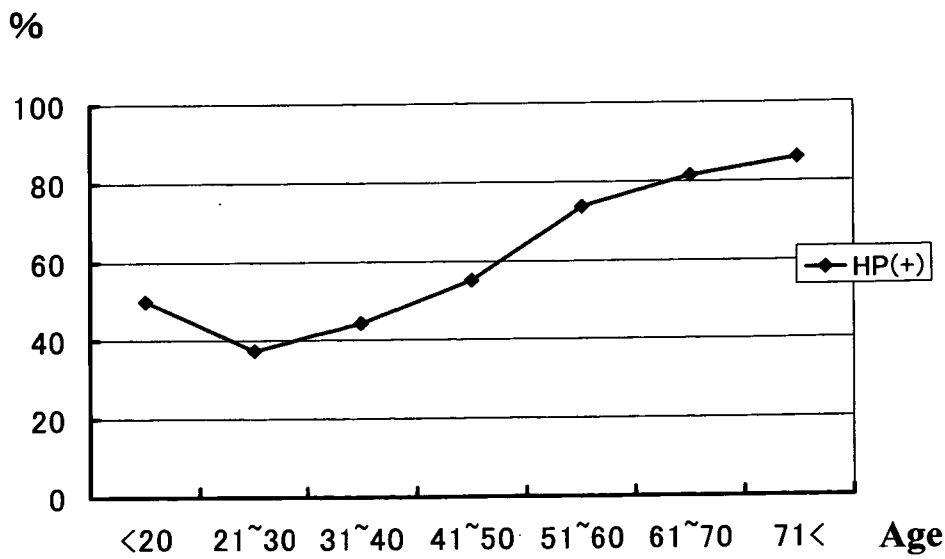

Fig. 1 *Helicobacter pylori* positive rate in each age group of ITP cases in Japan

Table 2 The Platelet response at 12 months after eradication in eradication success group and non-success group (Retrospective analysis in Japan2003)
The platelet count before eradication did not influence the platelet response by eradication significantly in both groups. In eradication success group CR, PR and NR rate were 23%, 42% and 35%, respectively. On the other hands, platelet response was found in 27%, even though in eradication non-success group. It was suspected that the inadequate timing of C^{14}-Urea breath test after eradication or the misdiagnose of *Helicobacter pylori* infection.

Platelet count Before eradication(X10^4)	CR	PR	NR	Total
Eradication				
Success group(n=122)				
<1	0	2(67)	1(33)	3
1～3	8(20)	23(58)	9(23)	40
3～5	8(21)	16(42)	14(37)	38
5～10	12(29)	10(24)	19(46)	41
Total	28(23)	51(42)	43(35)	122
Eradication				
Non-success group (n=33)				
<1	0	0	2(100)	2
1～3	1(11)	1(11)	7(78)	9
3～5	1(7)	3(21)	10(71)	14
5～10	3(38)	0	5(63)	8
Total	5(15)	4(12)	24(73)	33

の血小板増加を維持したことになる。この頻度は除菌不成功群に比し有意に高く，ピロリ菌の除去が血小板増加に密接に関わっていることが明らかとなった。

今回の調査で除菌不成功群においても 33 例中 9 例は 12 ヶ月後も血小板数が増加（27%）していることが判った。9 例中 5 例は CR（15%），4 例は PR である（12%）。(Table 2) この除菌不成功群における血小板増加が認められた理由は，①除菌効果の判定が誤り，②除菌判定時期が問題，等が考えられ除菌効果の判定方法，判定時期が重要であることが伺える。

以上のような HP 陽性 ITP に対するピロリ菌の除去が血小板増加反応に直接的に関係していることは以下の報告からも支持される。すなわち

① SLE に伴う血小板減少においては HP 菌陽性であっても，除菌による血小板増加反応は認められず，除菌による血小板増加は ITP 特有のものである[9]。

また② HP 菌陰性 ITP 症例に無作為的に除菌を試みた報告では血小板増加反応を示した症例は認められず，除菌療法による非特異的血小板増加作用ではない[14]。③さらに HP 菌陽性 ITP 症例を無作為的に除菌療法を行う群（13 例）と行わない群（12 例）の 2 群に分け血小板増加反応を検討した報告では除菌群のみに血小板増加が認められる[23]，等である。今回の共同研究を含め多くの報告において除菌成功群では不成功群に比し有意に血小板増加効果が認められたことから（$p<0.0001$），この様な ITP 症例を HP 関連 ITP と呼称する根拠としている。

2) 除菌による血小板増加反応の特徴

本邦での研究結果を含め多くの報告は性差，平均年齢，除菌前の血小板数，除菌直前の ITP 治療の有無，治療の内容など所謂 ITP の臨床病態や過去の治療経過は除菌による血小板増加効果に影響を与えていない。すなわちステロイド療法や摘脾に対して不応性の症例に対しても有効性が認められる点は大きな利点である[9,11,16]。Stasi らは除菌前の血小板減少が軽微である症例（4.8 万前後）群に血小板増加効果が有意に認められ予後を予測する上で参考になると報告しているが，本邦の成績では同様の傾向はあるものの有意差は認められていない[24]。

しかし極度に血小板数が少ない 1 万以下の症例では血小板増加反応が認められても軽度である傾向がある。但し出血傾向などは消失し臨床的に有用であると考えられている。

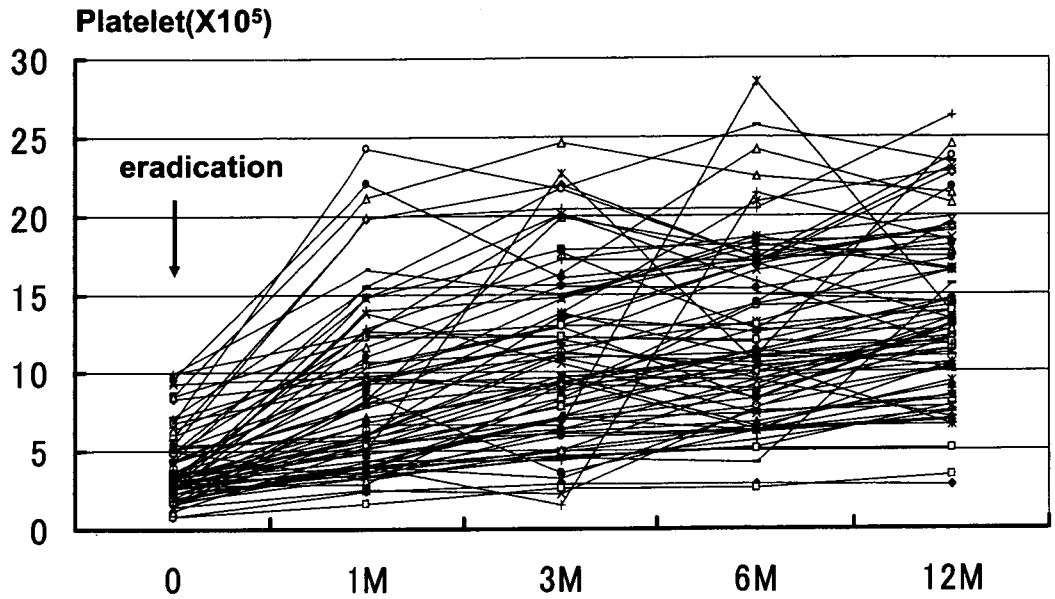

Fig. 2 Platelet response curve for 12 months in eradication success group (79 ITP cases)

血小板増加反応は除菌後1ヶ月頃には認められ，再発例がなく1年以上血小板増加が持続することも特徴である（Fig. 2）。多くの報告でも血小板増加反応は長期に持続しており再発がないか少ない。一般的に除菌後のピロリ菌再感染については明らかとなっていないが，最近Tsutsumiらによって再感染，再発例が報告され，再除菌により増加反応を示したと記載されている[26]。

ITPとしての罹病期間に関しては罹病期間が短い群（6.52±4.67年）が長い群（9.85±7.77年）に比し有意に除菌成功後の血小板増加を来たした（P<0.0001）。Tasaiらも罹病期間1年前後の症例が3年未満の症例に比し血小板増加反応が良好との報告を行っている[24]。

以上の臨床研究からITPの中にはHP菌が発症にかかわっている症例が存在し除菌療法が血小板増加に有用であることが確認できた。

ヘリコバクタピロリ菌感染による血小板減少機序

明確なエビデンスはないが局所のHP菌に対する免疫反応系が遠隔の細胞に障害を及ぼしていると考えられている。

HP菌体成分のうち細胞外毒素である95Kdの空胞化因子VacA（6面体陰イオン選択性，電位依存性チャンネルを形成するとともにミトコンドリア膜を標的としアポトーシスを引き起こす）やCag－PAI（cag pathogenicity island）がコードする蛋白の一つであるCagA（cytotoxin-associated antigen A）は直接細胞障害や炎症反応，細胞増殖反応に関わるとともに強い抗原性を有しており，菌体表層のLewis抗原様側鎖と共に，これら抗原に対する免疫反応が検討されている[2]。

しかし一般のHP菌陽性症例においては，HP菌に対する特異的な全身性の免疫反応は末梢血リンパ球の検索からは認められていない。また非臓器特異的，特異的自己抗体（抗核抗体，抗平滑筋抗体，抗マイクロソーム抗体など）の出現頻度はコントロールと差がないと報告されている[27,28]。しかし最近小児においてはHP菌陽性群においては臨床症状とは関係なく陰性群に比し，胃壁細胞抗体，マイクロゾーム抗体，の出現頻度が有意に高く，小児におけるHP菌感染は自己免疫的胃炎や他の自己免疫疾患の誘引となると報告され[29]，局所のHP感染から全身的免疫反応への伸展については今後さらなる議論が必要である。

ITPに関しては除菌により血小板が増加した症例ではTh1/Th2の比が除菌後上昇する報告は，HP菌感染が全身的な免疫反応の不均衡をひき起していることを裏付ける報告として興味深い[30]。また最近HP陽性ITP症例においては末梢血に特異的なT cellのクローナルな増殖が認められ除菌効果に伴ってクローンが消失すると報告され，HP菌に対する全身的な免疫反応が血小板減少と関連している事が示されている[31]。

1) Lewis抗体の関与

HP感染症例には高いLewis抗体価を示す例がある。この抗Lewis抗体が認識するエピトープを有する組織にこの抗体が結合し自己免疫疾患を起こす（molecular mimicry）。例えば胃壁細胞のH^+-K^+-ATPaseの一部はLewis抗原エピトープを有し抗Lewis抗体が壁細胞障害を生じ胃炎を引き起こす説もある[32,33]。

この抗 Lewis 抗体が血小板に非特異的に吸着され血小板減少を引き起こすと考えられる[34]。

2) 分子相同性

従来より微生物の産生物質，たとえばリポポリサッカライドや DNA などはアジュバントとして働き無関係の抗原に対して免疫反応を起こし，自己抗原に対しても異種抗原と同じように T-cell の反応を引き起こし易いと言われている[35]。また一方，抗原分子相同性により（molecular mimicry）微生物由来の抗原ペプチドと自己抗原ペプチドの間に交差性があれば自己抗体として認識され自己免疫疾患が発症すると推測される。

HP 菌陽性 ITP 血小板から誘出した抗体は HP 菌の Cag A 抗原と反応することが免疫ブロッティング法で明らかにされた[15]。さらに除菌により血小板数が回復すると Cag A 抗原と反応する抗体は誘出液中には認められなくなるが，除菌による血小板数の増加が認められない症例においては抗体の消失は認められない。また興味あることに HP 菌陰性 ITP 症例の血小板誘出液は Cag A を認識することから，ITP の血小板に結合している血小板由来のペプチドに対する自己血小板抗体は抗原相同性のある CagA と反応したものと理解される。

CagA に対する抗原相同性については同様な方法で検討したフランスのグループは否定的な検討結果を報告している[13]。これには本邦と欧米との間で HP 菌株に差がある可能性もあり CagA 抗原との分子相同性については今後多くの地域で検討すべきである。

この他 HP 陽性 ITP 症例の血小板には anti-Cag A 抗体と反応する 55kDa 抗原が存在するのに対し正常血小板にはこの蛋白は存在せず，55kDa 蛋白と反応し血小板寿命を短縮するとの報告もある[36]。

また Cag A IgG 抗体価が高い症例には除菌による血小板増加反応を示す症例が有意に多く，除菌効果の予測に応用可能であるとの報告は，Cag A 抗原が ITP 発症の免疫反応に関わっている事を別の角度から示唆したものである[23]。

いずれにしても Cag A 抗原とこれに対する抗体が血小板減少の発症に関連している報告が集積されつつあるのが現状である。

3) vWF，抗 HP 菌抗体による血小板活性化反応による[37]

HP 菌のある株は vWF，抗 HP 菌抗体の存在下で血小板凝集反応を引き起こすことが報告された。この凝集反応は HP 菌が抗 HP 菌抗体，vWF を結合し，血小板膜 GPIb/V/IX 複合体およびその近傍に位置するとされる FcγRIIA を介してシグナルが GPIIb/IIIa 複合体へ伝達され血小板凝集反応が生じると考えられている。血小板活性化が局所の炎症反応，潰瘍形成に，また心血管障害に，さらには血小板活性化による慢性的血小板消費による血小板減少が引き起こされるとする報告もある。

ピロリ菌感染によって ITP を生じる症例の特徴

ピロリ菌感染者は非常に多いにもかかわらず ITP を発症する症例はそのごく一部で，多くは ITP を発症しない。これについては免疫反応の個体差の観点から HLA 系を検索した報告がある。それによると HP 菌陽性 ITP では HLA-DRB1*11，14，と HLA-DQB1*03 が HP 菌陰性 ITP に比し有意に高く，HLA-DRB1*03 が有意に低い結果が得られている。このような HLA 系を有するヒトが HP 菌感染により HP 関連 ITP を発症する可能性が示唆される[38,39]。しかし HLA 系頻度は人種によって差がありこれを普遍化することはできないが，今後各々の人種間で検討し結論を出す必要がある。

除菌療法の副作用

除菌の副作用に関して詳細な報告はない。本邦の集計では 222 例中 39 例（17.6%）に何らかの副作用が認められた[16]。多くは消化器症状で 25 例（64%）に認められ，その内訳は軟便，下痢胃部不快感などであった。この他蕁麻疹などの皮疹 9 例（23%）が認められている。重篤な症例は血小板減少が増悪した 1 例のみで安全におこなえる治療と思われる。ただし血小板数が 1 万以下の症例に関しては消化管出血，その他出血傾向の増悪など慎重な判断が必要である。

ピロリ関連 ITP に対する除菌療法の位置付け

従来より ITP は原因不明の自己免疫疾患として捉えられている[40~42]。しかしここに示したように HP 菌関連 ITP が存在することも理解可能で，免疫学的原因がより明確に推測される ITP の一つと捉えることができる。これらに対して従来とは全く異なった治療法で 60%以上に良好な治療成績が得られたことは医療経済を含め画期的なことである。特に従来より第一選択薬とされているステロイド治療を回避することが出来る症例が出てくることから副作用，合併症対策への治療費，治療期間が削減されより良い QOL が期待できると考えられる。

そこで HP 菌陽性 ITP 症例においてはまず HP 菌の関与を除き，除菌により血小板増加反応を示した症例は HP 関連 ITP として ITP の中で位置付けてはどうかと考えている（Fig. 3）[43]。

ITP 治療ガイドライン

以上を踏まえて「血液凝固異常症に関する研究」班で ITP 治療ガイドライン作成した（Fig. 4）。その概略とし

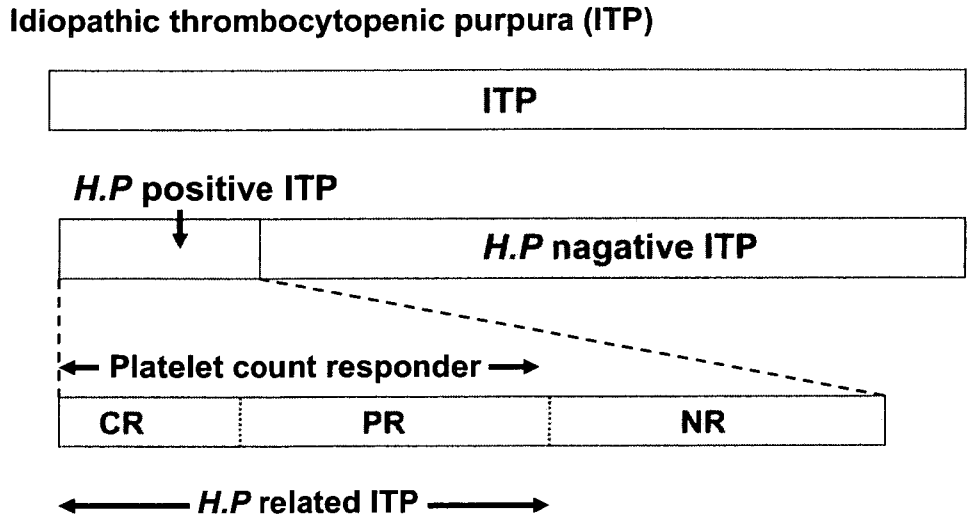

Fig. 3　Clinical situation of *Helicobacter pylori* related ITP (Hypothesis)

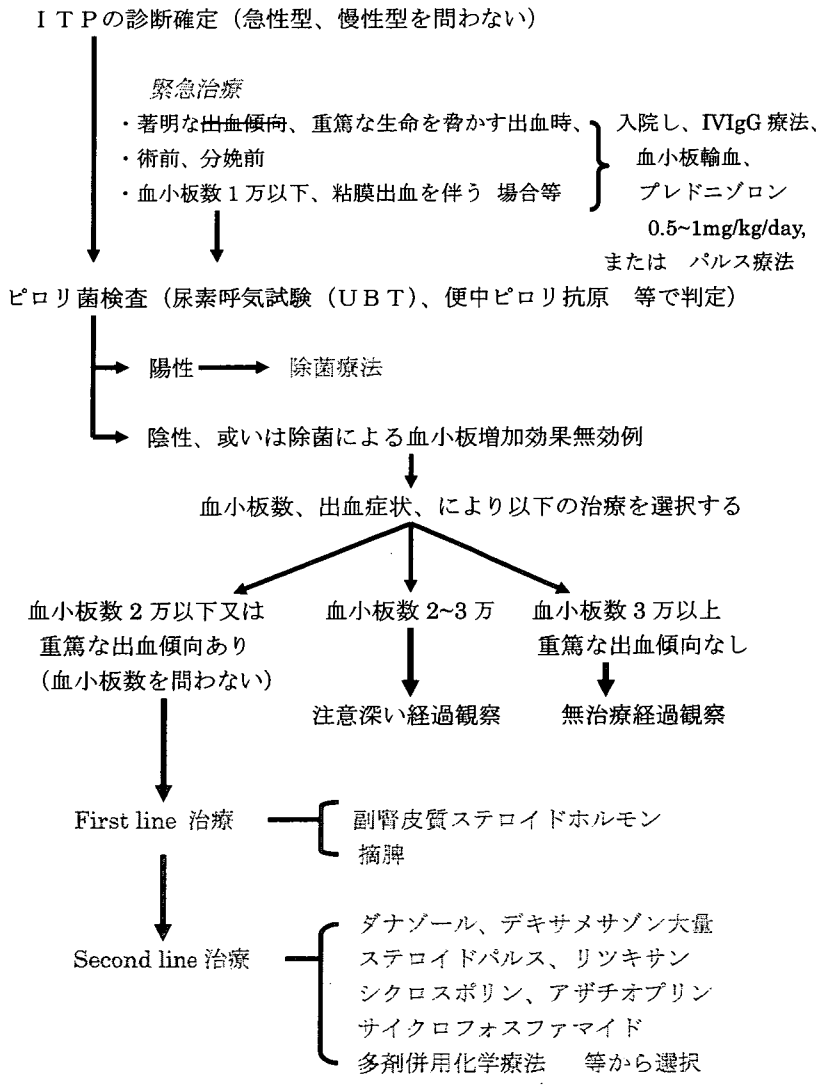

Fig. 4　Guideline for adult ITP treatment (2004)

て

1. ITP 治療の緊急性を要する症例は別として，まずヘリコバクターピロリ菌感染が関係している ITP か否かを検査し，陽性であればインフォームドコンセントの基にまず除菌療法を行う。
2. 除菌療法に反応しない症例に関しては従来の標準的治療を行う，を大枠としている。さらに今回提案したプロトコールには治療目標を 3 段階に定め，各治療ごとの評価を可能とするとともに，各治療の対象を具体的に示した点，個々の症例の病状を勘案して薬物量や治療法を選択出来る，などが盛り込まれている。紙面の都合上詳しくは難治性疾患克服事業報告書を参照していただきたい[44]。

なお HP 陽性 ITP に対する除菌療法については現在保険収載を目標に関係方面へ働きかけを行っている。

レトロスペクティブ研究のまとめ

現時点で本邦における HP 関連 ITP は以下のようにまとめられる。

1. ITP におけるピロリ菌陽性率は加齢と共に高くなり 50 歳以上では 70%以上で本邦の一般的傾向と違いはない。HP 陽性率に性差はない。
2. ピロリ菌陽性 ITP 群は陰性群に比し，平均年齢が有意に高い。
3. 除菌成功群に血小板増加例が有意に多い（63%）。
4. 除菌前の ITP 治療が除菌後の血小板増加に影響しない。
5. 除菌による血小板増加例は ITP 罹病期間が短い。
6. 血小板増加反応を示した症例の再発例は認めていない。
   血小板増加反応は除菌後約 1 ヶ月で認められ，増加効果は長期に持続する。
7. 副作用は 17.6%に認め消化器症状が主で，重篤な症例は出血傾向が増悪した 1 例である。
8. 以上のエビデンスから HP 陽性 ITP に対しては除菌療法を組み込んだ治療ガイドラインを作成した。

ま と め

所謂特発性血小板減少性紫斑病と診断していた症例に対しピロリ除菌が血小板増加効果をもたらすことは本症にとってエポックメイキングな事である。しかしながらこのような現象が一部の国に限られていること（日本，イタリア，台湾など）についての更なる解析が必要である。そこが明確にされない現状では除菌療法及びその位置付けが国際的に認知されにくい現実となっている。しかしながら一方では世界各国でこのようなエビデンスに目が向けられており，所謂 HP 関連 ITP が HIV 関連 ITP と同様な位置づけが来る日を期待している。いずれにしてもこれからも症例の集積と，臨床的解析をさらに推し進め，海外の状況にも目を向けながらコンセンサスを得たこの疾患の位置づけを示す必要がある[45,46]。

謝　辞

本邦でのレトロスペクティブ研究は厚生労働省　難治性疾患克服事業　血液凝固異常症に関する調査研究班　班長　池田康夫教授（慶応義塾大学）のもとで，大阪大学　倉田義之先生，慶応義塾大学　桑名正隆先生，並びに北海道大学，旭川赤十字病院，昭和大学藤が丘病院，東京都立駒込病院，東京女子医科大学，東京医科大学，関西医科大学，広島大学，広島赤十字原爆病院の各血液内科の先生方の協力を得て行われたもので，改めて深謝いたします。

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Quantitative RT-PCR analysis of sphingolipid metabolic enzymes in acute leukemia and myelodysplastic syndromes

Leukemia (2006) **20**, 2042–2046. doi:10.1038/sj.leu.2404386; published online 7 September 2006

Sphingolipids and their metabolites recently appeared as a potent class of regulators of cell proliferation, survival as well as apoptosis.[1] The ceramide/S1P rheostat has been proposed as the model to determine the cell's fate.[2] This rheostat is being increasingly recognized as a critical element in tumor cell proliferation and chemotherapy. Sphingomyelinase is responsible for the first step of the sphingomyelin-catabolic pathway and produces ceramide, which figures mostly as the proapoptic

factor in response to various reagents including anti-cancer drug or radiation. On the contrary, sphingosine kinase (SPHK) is the enzyme that produces sphingosine 1–phosphate (S1P) from sphingosine. S1P binds to five G-protein coupled receptors called S1P receptors. S1P promotes cell survival or motion as the first or second cellular messenger in response to various agonists. Therefore, enzymes in this pathway provide potential targets for new anti-cancer drugs.

Interestingly, overexpression of SPHK1 is thought to be oncogenic, and renders transfected cells chemoresistant.[2] SPHK1 mRNA was significantly higher in various cancer tissues than in their normal counterparts.[3] In prostate cancer cell lines, we reported the inverse relationship between SPHK1 level and anti-cancer drug sensitivity.[4] The quantity of each cellular sphingolipid metabolite was thought to be determined by the complex balance between each metabolic enzyme activity and substrate. However, no analysis of gene expression of sphingolipid metabolizing enzymes including SPHK1 of acute leukemia or related diseases has been reported.

In the present study, we performed quantitative RT-PCR assay to measure the mRNA levels of nine major enzymes involved in the sphingolipid metabolic pathway including sphingosine kinase 1 (SPHK1), sphingosine kinase 2 (SPHK2), acid sphingomyelinase (ASMase), neutral sphingomyelinase 2 (NSMase2), acid ceramidase (ACDase), sphingosine 1–phosphate lyase (SPL), sphingosine 1–phosphate phosphatase 1 (SPP1), glucosyl ceramide synthase (GlcCer Syn), sphingomyelin synthase 1 (SM Syn). Multidrug-resistant gene (MDR) and BCL2 were also measured to examine the chemoresistance gene expression in the current samples.

Quantitative PCR was performed with Power SYBR Green master mix (Applied Biosystems, Foster City, CA, USA) in duplicate using primer sets described in Table 1, and ABI PRISM 7000 sequence detection systems (Applied Biosystems) were used for the measurement. ABL gene expression was measured as the internal control with Taqman probe as shown in Table 1 according to the recommendation by Beillard *et al.*[5] The specificity of PCR product was confirmed in the preliminary experiments using cell lines. Standard curve was created using cDNA fragment of each enzyme produced by the PCR method and then inserted into the cloning vector. The relative gene expression level was calculated as the ratio of each gene expression/ABL gene expression.

After obtaining informed consent, bone marrow cells were collected from 19 patients with acute leukemia and 60 patients with myelodysplastic syndromes (MDS) (28 RA (refractory anemia), 21 RAEB (refractory anemia with excessive blast) and 11 RAEB-t (RAEB in transformation) according to FAB classification) mostly at their initial diagnosis or before any treatment. For the normal control, bone marrow samples for the disease staging were used from 11 patients with non-Hodgkin's lymphoma without bone marrow invasion. Mononuclear cells were collected and RNA was extracted. The first strand cDNA was prepared using the Super Script First-Strand System (Invitrogen). CML-BC and Ph_1 + ALL samples were omitted because the ABL gene was used as the internal control of RT-PCR assay. Patient characteristics are provided as the Supplementary Data.

Figure 1 shows the message levels of sphingolipid metabolic enzymes as well as BCL2 and MDR. We mainly focused on the statistical difference between AL and normal control by using one-way factorial analysis of variance and multiple comparison test (Bonferroni/Dunn's method). Statistical analysis was performed using Microsoft Excel software and Stat view version 5 (SAS Institute Inc., Cary, NC, USA). We used RNA from total mononuclear cells of bone marrow aspirates instead of purified hematopoietic stem cells or blast cells. Therefore, the heterogeneity of bone marrow component might have affected our results.

Among enzymes examined, AL also showed noticeable increases of SPHK1 message as compared to normal population. Some AL showed more than 2 log order higher SPHK1 gene expression as compared to the normal control. SPHK2 did not show significant differences between groups analyzed. It is also of note that SPHK1 expression of RAEB-t is also significantly higher than normal, although we could not see significant differences in SPHK1 gene expression between RAEB and normal control. There was no correlation between the SPHK1 gene expression level and abnormal karyotypes with poor prognosis such as seven monosomy or complex abnormality (data not shown), suggesting that SPHK1 gene expression is independent from karyotype abnormality. Although we did not make a sequential analysis of the same MDS patient, SPHK1 might be a candidate of the surrogate marker of AL and MDS, because its expression gradually increased during the progression of MDS and high in AL.

Okazaki's group[6] reported the correlation between chemoresistance and the increase of glucosylceramide synthase and spingomyelin synthase in leukemia cell lines and a small clinical sample. The increase of these enzyme activities might decrease the cellular ceramide level. However, the significance of these findings has not been repeated by others. In our analysis, the message levels of these genes did not show any significant difference between AL and normal control. ASMase, ACDase and SPL were not different between AL and normal control.

Interestingly, NSMase2 was decreased in AL, RAEB and RA samples as compared to normal control. Among SMases, NSMase2 has been cloned recently and was reported to play as a growth suppressor linking confluence to the G0/G1 cell cycle checkpoint.[7] The decrease of NSMase2 in AL is an unexpected and novel finding. Considering the sphingolipid rheostat model, either the increase of SPHK1 or the decrease of NSMase2 gene expression results in the decrease of ceramide/S1P ratio in leukemia blast cells, which might stimulate cell proliferation or survival. Although the regulatory mechanism of ASMase gene expression was reported in cell lines, the transcriptional regulation of NSMase2 has not been clarified yet, and is an interesting topic for future analysis. Statistical significance was also observed in SPP1 only between AL and normal control but not between MDS and normal control. As for SPL and SPP1, which convert S1P to phosphoethanolamine and palmitaldehyde, or sphingosine, respectively, we only observed the increase in SPP1 message but not SPL. The increase of SPP1 expression is also thought to modulate the ceramide/S1P rheostat, however, the significance of this finding remains to be determined.

As to other well-known chemoresistance genes, MDR showed no significant difference between AL and normal control. On the contrary, statistical significance was observed in BCL2 between AL and normal, but not between MDS and normal control. We analyzed the relationship between SPHK1 and MDR or SPHK1 and BCL2 gene expression. In AL, there was no relationship between SPHK1 gene expression level and two well-recognized genes of chemoresistance, MDR and BCL2, of the same sample (precise data not shown). This could suggest that SPHK1 is not always located downstream of BCL2 in AL.

Furthermore, it was also revealed that there are no correlationships between SPHK1 and NSMase2, between SPHK1 and SPP1 gene expression, or between SPHK1 and SPHK2 gene expression.

Table 1 Primer sets of quantitative RT-PCR

Hugo gene nomenclature	*chromosome*	*Alternate name*	*GenBank accession no.*	*Amplicon location relative to transcription start (bp)*	*% GC*	*Forward primer sequence*	*Reverse primer sequence*	*Annealing Temperature*
SPHK1	17	Sphingosine kinase 1	NM_021972	+821/+1060	58.6	TCCTGGCACTGCTGCACTC	TAACCATCAATTCCCCATCCAC	61.0
SPHK2	19	Sphingosine kinase 2	NM_020126	+26/+186	70.9	AGCAGCAGGACCAGAGGCCA	GGTGAGGGCAAAGCGTGGG	67.0
SPL	10	Sphingosine-1-phosphate lyase 1	NM_003901	+794/+934	51.5	TGGAGGTGGATGTGCGGGCAA	CCCAGACAAGCGTCGACATGAAG	62.0
SPP1	4	Sphingosine-1-phosphate phosphatase 1	NM_030791	+637/+772	44.9	ACCGCCATCCCCATTTCT	AGGAATCCAGCAATAATATCCAG	59.0
ASMase	11	Acid sphingomyelinase	NM_000543	+1073/+1220	56.0	AAGCCCTGCGCACCCTCAGAA	CCTGAAGCTCCCCCACCAGCC	64.0
NSMase2	2	Neutral sphingomyelinase II	NM_018667	+1535/+1645	60.0	ACTTTGATAACTGCTCCTCTGAC	TTCGTGTCCAGCAGAGTACC	63.0
ACDase	8	Acid ceramidase	NM_177924	+875/+968	47.8	GATATTGGCCCCAGCCTACTTT	ACCCTGCTTAGCATCGAGTTCA	60.0
GlcCer synthase	9	Glucosylceramide synthase	NM_003358	+144/+341	37.5	CAAGCTCCCAGGTGTCTCTCTTC	GATTAATGCCAACTTTTTTACCACCTA	64.0
SM synthase1	10	Sphingomyelin synthase 1	NM_147156	+757/+894	48.4	GAAGCCCAACTGCGAAGAATAA	AGAGTCGCCGAGG GGAATAC	60.0
MDR	7	Multidrug resistance 1	NM_000927	+1141/+1349	48.7	AGTGGGCACAAACCAGATAA	CTGTCCATCAACACTGACCA	63.0
BCL2	18	BCL2 (B-cell lymphoma type 2)	NM_000633	+337/+583	61.7	GCCGAGATGTCCAGCCAG	AGTTCCACAAAGGCATCCCA	62.0
ABL	22	PCR primer	Sense			CCCAACCTTTTCGTTGCACTGT		
			Antisense			CGGCTCTCGGAGGAGACGTAGA		
		Taqman probes	Sense			ACTAAAGGTGAAAAGCTCCGGGTC-FITC		
			Antisense			LCRed640-TAGGCTATAATCACAATGGGGAATGG		

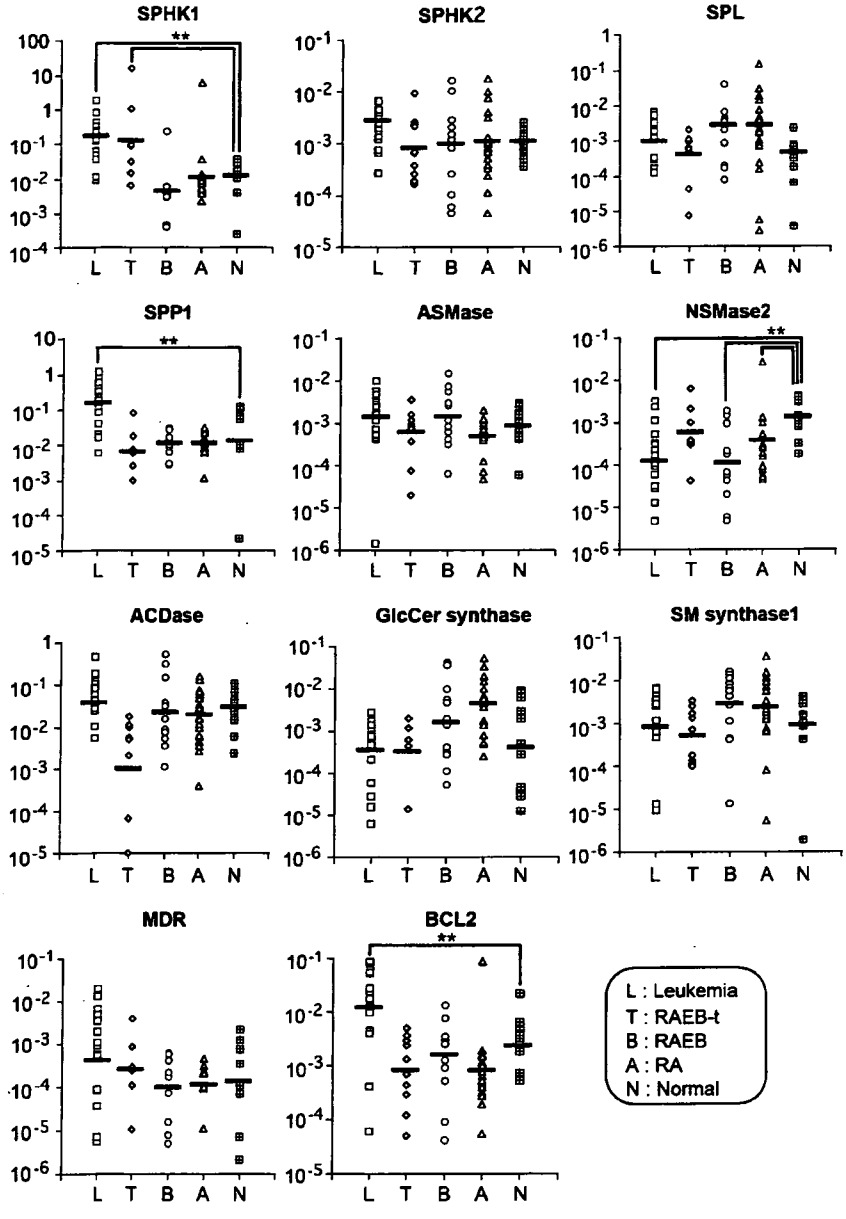

Figure 1 Relative message expression levels of sphingolipid metabolic enzymes in AL, MDS and normal control. Quantitative RT-PCR was performed with bone marrow RNA. The relative expression level was calculated with the enzyme gene expression/ABL gene expression level and was shown in the log scale. The classification of MDS was according to the FAB classification. Horizontal short bar denotes the mean value of the group. Statistical significances were analyzed by using one-way factorial analysis of variance and multiple comparison test (Bonferroni/Dunn's method). *Means $P<0.01$.

Apoptosis induction can be suitable for the treatment of diseases such as malignant tumors. The trials of novel inhibitors of SPHK1 are based on the hypothesis that the modification of ceramide/S1P rheostat induces apoptosis of malignant tumor cells and enhances chemosensitivity. Actually, a synthetic compound with SPHK inhibitor activity can induce apoptosis in tumor cells even with multidrug resistance.[3] As we[4] recently reported that SPHK1 activity is a chemotherapy sensor in prostate cancer cells, and overexpression of SPHK1 has been reported in solid tumors,[3] it is of interest to know whether this observation can be applied to hematological malignancies, especially acute leukemia and MDS. Such data are important because the relapse after intensive chemotherapy is still an unsolved problem in leukemia treatment, and new remedies are urgently required. Our data showed AL or RAEB-t cases with much higher SPHK1 gene expression compared to normal. Therefore, it is suggested that the development of new SPHK1 inhibitor is also beneficial for AL patients whose SPHK1 gene expression (and probably SPHK enzyme activity) is enhanced.

Bonhoure *et al.*[8] reported that sustained SPHK1 overexpresion can render HL60 cells chemoresistant by decreasing the cellular ceramide level and that a novel SPHK1 inhibitor, F-12509a, could recover chemosensitivity. Considering the molecular target of chemotherapy, enzyme inhibitors are more practical than agents for enzyme activation (NSMase2 in our case). Inhibitor of SPHK1 is almost at the stage of clinical investigation. In the present study, we could analyze only one time point of patients (mostly at their first diagnosis before chemotherapy) and could not measure enzyme activities due to the paucity of samples. The measurement of SPHK1 gene expression and/or SPHK enzyme activity of each patient might be necessary to assess the efficacy of enzyme inhibitors in future clinical settings. Sequential analysis of the same patients will also add further information.

The localization and activation of enzymes are also important factors to determine the final cellular ceramide/S1P rheostat. SPHK1 was reportedly activated by phosphorylation by agonists and translocated to membranes.[2] Therefore, further analysis of enzyme activation is necessary to conclude firmly that sphingolipid metabolizing enzymes such as SPHK1 are a novel and promising molecular target for acute leukemia chemotherapy.

Taken together, this is, to our knowledge, the first report of a gene expression profile of major sphingolipid metabolizing enzymes of AL and MDS using quantitative RT-PCR, It documents the increase of SPHK1 gene expression in AL and RAEB-t and the decrease of NSMase2 gene expression in AL and RAEB, suggesting that sphingolipid metabolizing enzymes such as SPHK1 could be a novel target for the chemotherapy of AL.

Acknowledgements

We express sincere thanks to Professor S Nakamura and other members of the Department of Laboratory Medicine, Nagoya University School of Medicine for their assistance. This work was partially supported by the Health and Labor Sciences Research Grants for the Research on Measures for Intractable Diseases from the Ministry of Health, Labor and Welfare.

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Supplementary Information accompanies the paper on the Leukemia website (http://www.nature.com/leu)

Journal of Thrombosis and Haemostasis, 4: 1738–1746

ORIGINAL ARTICLE

A classification of the fibrin network structures formed from the hereditary dysfibrinogens

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To cite this article: Sugo T, Endo H, Matsuda M, Ohmori T, Madoiwa S, Mimuro J, Sakata Y. A classification of the fibrin network structures formed from the hereditary dysfibrinogens. *J Thromb Haemost* 2006; 4: 1738–46.

Summary. *Objective:* The main objective was to study the relationships of the molecular defects in 38 dysfibrinogens with their fibrin networks. *Methods and results:* Scanning electron microscopic analyses revealed that all the fibrins formed under the same conditions had networks composed of either normal thickness fibers or thin fibers, accompanied by a variety of alterations in the network structure and characteristics. We classified these fibrin networks into five classes, designated normal, less-ordered, porous A, porous B and lace-like networks. The dysfibrinogens with defects in fibrinopeptide A release or the E:D binding sites formed normal or less-ordered networks, while those with defects in the D:D association formed porous A networks composed of many tapered terminating fibers, despite having fibers of normal width, and containing many pores or spaces. The porous B and lace-like networks were composed of highly branched thin fibers because of defects in the lateral association among protofibrils, and the major difference between them was the porosity of the porous B networks. All the porous B networks were easily damaged by mechanical stress, whereas the lace-like networks retained high resistance to such stress, indicating that the network strength was not dependent on the fiber width, but on the porosity that led to fragility of the network. *Conclusion:* Impairment of the D:D association is the major disturbing factor that leads to the formation of porous fibrin networks. The porosity may be introduced by severe impairment of the D:D association, as well as the lateral association, as has often been observed by extra glycosylation or defects in Ca^{2+} binding.

Keywords: dysfibrinogen, dysfibrinogenemia, fibrin, fibrinogen, network.

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Received 5 January 2006, accepted 5 May 2006

Introduction

Fibrinogen is a 340 kDa glycoprotein that is present in the blood of all vertebrates, where it participates in the final step of blood coagulation. Fibrinogen forms an insoluble gel on conversion to fibrin by the action of the proteolytic enzyme thrombin, which itself is activated by a cascade of enzymatic reactions triggered by injury or contact with a foreign surface. Fibrin formation involves a series of highly ordered molecular interactions, and the mechanisms underlying these interactions have been extensively studied over the past 40 years [1–2]. Recent crystallographic studies of particular domains and segments of fibrinogen and fibrin have revealed the details of these molecular interactions in fibrin assembly [3–8], and provided insights into the molecular abnormalities in hereditary dysfibrinogens. Dysfibrinogens can be roughly divided into two groups: (i) defective thrombin-catalyzed conversion of fibrinogen molecules to fibrin monomers and (ii) defective fibrin polymerization because of structural alterations in polymerization sites comprising the E:D binding site and end-to-end D:D abutment interface and lateral association sites in the carboxyl terminal α-chain (αC-domain). Over the past 40 years, many cases of hereditary dysfibrinogenemia have been reported and more than 300 variants have been identified at the molecular level, gene level or both [9–13] (see the fibrinogen database: http://www.geht.org/databaseang/fibrinogen). To date, there have been several reports of electron microscopic analyses of individual molecules of fibrinogen and fibrin monomers, fibrin fibers and networks [14–23]. For some dysfibrinogens, conflicting structures have been reported by different laboratories, even for fibrin molecules with identical amino acid substitutions. We have studied more than 40 kinds of dysfibrinogens, covering defects in most of the functional sites. Here, we focused on the relationships between these functional defects and the structural alterations of the fibrin networks, and considered how these relationships could explain the clinical manifestations of each specific type of dysfibrinogenemia.

Materials and methods

Normal fibrinogen and dysfibrinogens (three homozygous and 35 heterozygous) were prepared from individual patient plasma

samples, mostly by previously described ammonium sulfate precipitation methods [18], and stored at −80 °C at concentrations of 6.2–12 mg mL^{-1} until analysis. Three of the dysfibrinogens, namely Fbg Marburg, Giessen and Osaka VI, were purified using a calcium ion-dependent immunoaffinity column, and each eluate (A_{280} = 1.7–2.1) was treated with 5 mM $CaCl_2$ for 30 min, extensively dialyzed against 10 mM Tris–HCl, pH 7.4 containing 0.15 M NaCl (Tris-buffered saline, TBS) and stored at −80 °C as described previously [20]. The location names of the dysfibrinogens used in our experiments were: Asahikawa II-IV, Asahi, Bremen, Caracas II, Giessen, Hamasaka, Kamogawa, Kawaguchi, Kumamoto, Kurashiki, Kyoto II and III, Lima, Niigata, Marburg, Mitaka, Morioka I and II, Oita, Osaka II-VI, Pretoria, Saga, Tenri, Tochigi I and II, and Tokyo II-VIII. Each variant dysfibrinogen is expressed by its substitution, such as γR375G, and the location name is used for the variants with carboxyl terminal mutations or variants of AαR16C and γR275C to specify the respective dysfibrinogens. From the peptide mapping analyses, most of the heterozygous dysfibrinogens, except Fbg Tokyo V [23], were estimated to contain almost a 1:1 ratio of normal and variant fibrinogens. All the dysfibrinogens introduced to have an unpaired Cys did not contain the free SH-group, but the substances that paired by the disulfide bridge were not identified except four dysfibrinogens, Fbg Kawaguchi bridged between Aα16Cys intramolecularly [24], Osaka II with Cys [25] and Fbg Marburg and Giessen with serum albumin [26,27].

Conversion of fibrinogen to fibrin

The normal fibrinogen and dysfibrinogens were each diluted to 1.0 mg mL^{-1} with TBS as working solutions by adjusting the absorbance at 280 nm. For most of the experiments involving turbidity assays, the permeation study and the preparation of specimens for scanning electron microscopy (SEM), the fibrinogen concentration was 1 mg mL^{-1} and the final concentration of α-thrombin was 0.06 U mL^{-1}. Fibrin formation was initiated in a separate tube by the addition of α-thrombin (6 NIH U mL^{-1}) to a fibrinogen solution (100–500 μL), and the mixture was immediately transferred to a second container for the respective experiments and incubated at 25 °C for 17 h. For the compaction experiments, the working solution was diluted to 0.6 mg mL^{-1} with TBS and clotted by α-thrombin at 0.06 U mL^{-1} in a specified tube as described below.

Turbidity assay

Prior to fibrin formation, the absorbance at 350 nm of each fibrinogen solution in a disposable semi-micro cuvette (Plastibrand®; Brand GmbH, Wertheim, Germany) was measured as a reference, and designated A0. The turbidity assay and specimen preparation were performed with the same reaction mixture. After thrombin addition, 100 μL of the reaction mixture was transferred to a cuvette and incubated at 25 °C overnight. After the 17 h incubation, the turbidity of the fibrin was expressed by the increase in the absorbance at 350 nm relative to A0. The values from two separate experiments were averaged as the turbidity of the patient fibrin, except for γR375G fibrin, which was evaluated by one experiment. For some of the dysfibrinogens, we did not perform a turbidity assay because of the limited amount of sample available.

SEM of fibrin clots

Specimens for SEM were prepared essentially according to a previously described method [20]. For comparisons of the fibrin clots from the 38 hereditary dysfibrinogens, the same conditions of clot formation were used as described above, except for Fbn Marburg, for which the thrombin concentration was 0.32 U mL^{-1} for complete gelation within 17 h. Aliquots of 35 and 55 μL of the reaction mixture were immediately transferred to 30- and 50-μL Plexiglas microdialysis cells, respectively, incubated at 25 °C for 17 h under humidified conditions, rinsed, dehydrated and subjected to critical-point-drying. After the critical-point-drying, the fibrin clots were removed from the cells, and the cylindrical clots were mounted on the specimen stage of an electron microscope to view the three faces, i.e. the top, back and side, and coated with gold-palladium as described previously [20]. Upon observation at 900-fold magnification, specimens with apparently compressed or broken fibrin were discarded, and those with a smooth surface were subjected to detailed observations at higher magnifications (4500–18 000-fold). Network images showing three-dimensionally homogenous fibrin at 4500-fold magnification were photographed as individual whole fibrin images, and abnormal fibrin images observed in partially compressed normal fibrin regions were omitted from the individual images. The fiber diameters and lengths between the branch points were estimated from 200–300 fibers in each 9000- or 18 000-fold magnified image, using with the Image Analysis Software MacSCOPE v2.5 (Mitani Co., Fukui, Japan). The fiber lengths and numbers of fiber ends in the 165 μm^2 area of each 4500-fold magnified image were counted. Some of the fiber crossing points may have been included as branch points, as it was difficult to distinguish between these two points, even at 18 000-fold magnification. For comparisons of the fiber widths in individual fibrin samples, the ratios of the numbers of fibers at 10- or 15-nm intervals relative to the total numbers of fibers were expressed as percentages.

Permeation studies

Permeability measurements were performed according to previously described methods with a slight modification [18]. The columns used in this experiment were created from 2-mL polystyrene tissue culture pipettes (3 mm in diameter), which had been precoated with fibrinogen at 1 mg mL^{-1} and air-dried before use. One end of each column was wrapped with nylon sheet to support the gel matrix and sealed with parafilm. Clotting was initiated in a separate tube with a mixture of fibrinogen and thrombin, and the mixture was immediately transferred to the column. The height of the gel matrix was

about 4.5 cm when 300 μL of the reaction mixture was used. After a 3.5-h incubation at 25 °C, 30 μL of TBS was layered on top of the gel and incubated for a further 13.5 h. Before the flow rate measurements, irregularities or defects in the fibrin matrix were examined by the passage of 0.01% basic fuchsin in TBS, and the column was then connected by plastic tubing to a reservoir containing TBS. The pressure gradient was 2-fold the clot height, and the pressure was kept constant during the experiment. The flow rate determined from the same column was reproducible in triplicate experiments unless the gel matrix was damaged during the experiment. Although the flow rates varied, with a maximal 1.5-fold difference among the different columns, we averaged the rates and calculated the permeation constant τ as described previously [18].

Compaction study

Compaction experiments were performed according to previously described methods with a minor modification [20]. Conical microfuge tubes were precoated with cooking oil and dried with a cotton swab. A fibrin matrix was formed in these tubes using 0.60 mg mL^{-1} fibrinogen and 0.06 NIH U mL^{-2} thrombin in TBS. After incubation at 25 °C for 17 h, the tubes were centrifuged three times in a swing rotor at 3000 × *g* for 1 min each, and the expelled buffer was withdrawn and measured. The volume of the compacted clots (estimated from the volume of expelled buffer) was expressed as a percentage of the original clot volume (750 μL) to give the percent compaction. The values from triplicate experiments were averaged in most cases, except for γR375G, Fbn Marburg and Giessen, for which the values were obtained from a single experiment. The percent compaction from six separate experiments using normal fibrin varied by twofold (2.0–4.8%), while in cases of higher compaction (> 7%), the maximal difference was < 1.1% in triplet experiments.

Results

After 17 h of incubation, all the abnormal fibrinogens clotted, but with a variety of turbidity values (A_{350} = 0.11–0.35). The clots with markedly lower turbidity (A_{350} < 0.22) consisted of thinner fibers, whereas the clots with slightly lower turbidity (A_{350} = 0.28–0.31) consisted of normal thickness fibers. The fibrin clots with low turbidity tended to be damaged by ordinary handling during the preparation of specimens for SEM. By careful manipulation of the samples, we could prepare less-damaged fibrin clots from most of the dysfibrinogens for comparisons of their fibrin network structures. As shown in Fig. 1 and Tables 1 and 2, the patient-derived fibrin networks were roughly classified into two categories, designated (i) networks composed of normal thickness fibers, and (ii) networks composed of thin fibers, and could be further classified into five classes according to the fiber characteristics and network architecture, namely normal, less-ordered, porous A, porous B and lace-like networks.

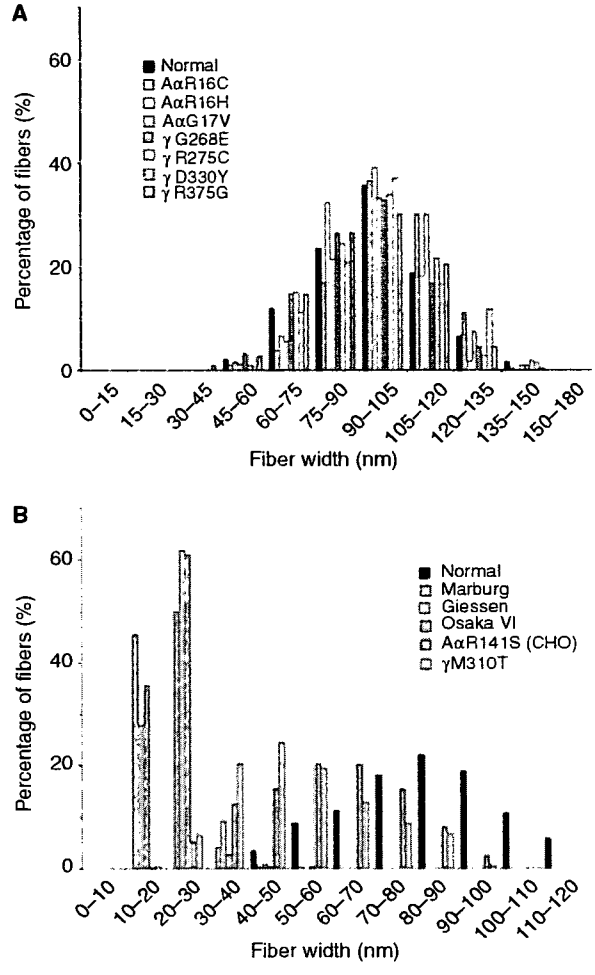

Fig. 1. Fiber distributions in the fibrins derived from patient fibrinogen samples. (A) Fibrins composed of normal thickness fibers. (B) Fibrins composed of thin fibers.

Fibrin network structures composed of normal thickness fibers

Normal fibrin networks were composed of uniformly packed fibers of 75–120 nm in width, and more than 10 μm in length with no visible fiber ends in the 165 μm^2 area of each 4500-fold magnified image. These networks also contained a few node structures, and more than three fibers protruded from each node. Most of the dysfibrinogens with an amino acid substitution in the amino-terminal region formed normal or less-ordered fibrin networks. The fibrins obtained from AαE11G, all three AαR16H, three out of eight AαR16C, AαG17V, AαP18L, AαR19G and γC139Y fibrinogens were indistinguishable from normal fibrin (Figs 2 and 3), and furthermore they all exhibited normal compaction and permeation activities as shown in Table 2. However, five of the AαR16C, γM268E, γD330Y, γR275S fibrinogens and the βN160S fibrinogen with extra glycosylation, βN160S(CHO), formed less-ordered networks in which the long normal thickness fibers were non-uniformly packed because of the presence of tangled nodes