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ORIGINAL ARTICLE

Genetic analysis of patients with deep vein thrombosis during pregnancy and postpartum

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Abstract Deep vein thrombosis (DVT) is a serious pregnancy-related complication. Recent studies indicate that the genetic background for DVT differs with ethnicity. In our study, we enrolled 18 consecutive Japanese patients who had developed DVT during pregnancy and postpartum. We performed a genetic analysis of three candidate genes for DVT, protein S, protein C and antithrombin, in these patients. We found that four patients had missense mutations in the protein S gene, including the K196E mutation in two patients, the L446P mutation in one patient, and the D79Y and T630I mutations in one patient, as well as one patient with the C147Y mutation in the protein C gene. All five patients with genetic mutations had DVT in their first two trimesters. Nine of the patients without genetic mutations developed DVT in the first two trimesters, and four in the postpartum period. Thus, genetic mutations in the protein S gene were predominant in pregnant Japanese DVT women, and DVT in pregnant women with genetic mutations occurred more frequently at the early stage of pregnancy than postpartum. Considering the rapid decrease in protein S activity during pregnancy, we may need to assess thrombophilia in women before pregnancy.

Keywords Deep vein thrombosis · Protein S · Thrombophilia · Pregnancy

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1 Introduction

Venous thromboembolism is the leading cause of maternal deaths in Western countries [1]. The incidence of pregnancy-related venous thromboembolism was 13 per 10,000 deliveries [2]. A 30-year population-based study reported that the unadjusted incidence of deep vein thrombosis (DVT) was 151.8 per 100,000 woman-years [3]. Most studies have found that the risk for thrombosis were 3–12 times higher in postpartum than during pregnancy [3, 4]. One study, however, reported twice as many events antenatally as postpartum [5]. Most of these studies involved patients in Western countries. A study in Japan showed that pulmonary thromboembolism occurred in 0.02% of total births, and the mortality rate was 2.5 per 100,000 deliveries [6]. Women with pregnancy-related thrombosis tend to have inherited thrombophilia, thus the prevention of DVT during pregnancy and postpartum is important for pregnant women. Therefore, the identification of inherited or acquired thrombophilia in pregnant women is urgently needed for the prevention of pregnancy-related thrombosis.

In Caucasian populations, two thrombotic mutations, the factor V Leiden mutation and the prothrombin G20210A mutation, for venous thromboembolism are widely distributed, with 30–60% of women with pregnancy-related thrombosis having these mutations [7, 8]. Both mutations are well-established risk factors for venous thromboembolism during pregnancy and postpartum in Caucasian populations. Several prophylactic therapies for pregnant women, such as heparin administration in the perinatal period, are recommended based on the type of thrombophilia and history of thrombosis [9]. However, these two genetic mutations are not found in the Japanese population [10, 11]. Thus, Caucasians and Japanese have clear genetic differences for thrombosis [12].

Deficiencies in protein S, protein C, and antithrombin are well-known risk factors for DVT [13]. The frequency of protein C deficiency and antithrombin deficiency in the general Japanese population was estimated to be 0.13 and 0.15%, respectively, and was comparable to the Caucasian population [12, 14–16]. The frequency of protein S deficiency in Japanese, however, seemed to be higher than that in Caucasians [17, 18], although the assays for plasma protein S levels differed among the studies. Actually, the frequency of protein S deficiency in 2,690 individuals randomly selected from the general Japanese population was estimated to be 1.12%, higher than reported in Caucasian populations (0.03–0.13%) [17, 18]. In a study of Japanese patients with venous thromboembolism, the frequency of inherited protein S deficiency was higher than that in Caucasian patients [19, 20]. It was recently reported that 17% of Japanese patients with venous thromboembolism had genetic mutations in the protein S gene [20]; this was much higher than in selected Caucasian patients with thromboembolism (1.4–8.6%) [13]. Furthermore, we and others reported the significant association with a missense mutation, K196E, in the protein S gene and venous thromboembolism in Japanese populations [19, 21, 22]. The carriers of this mutation showed low protein S activity [19, 23]. The prevalence of this mutant allele in the general Japanese population was about 0.009, suggesting that a substantial proportion of the Japanese population carried the protein S E-allele and was at risk of developing DVT [12, 19, 21, 22, 24]. This mutation seems to be ethnically specific, because it has not so far been identified in Caucasians.

It is well recognized that plasma levels of protein S activity and antigen are significantly reduced during pregnancy [25] and in oral contraceptive users [26]. The activities of protein S, protein C, and antithrombin can be affected at the acute stage of thrombotic events or after antithrombotic therapies. Therefore, the plasma assay may have an intrinsic limitation for the diagnosis of thrombophilia, and alternative ways to diagnose thrombophilia are expected. Genetic analysis might fulfill this requirement if it is applicable.

In this study, we performed DNA analysis for the genes of protein S, protein C, and antithrombin in patients with DVT during pregnancy and postpartum. We measured their plasma activities of protein S, protein C, and antithrombin. Based on these analyses, we described the clinical characteristics of the DVT events in patients with genetic mutation.

2 Materials and methods

2.1 Study patients

In this study, 18 consecutive patients with DVT during pregnancy and postpartum were enrolled from two tertiary perinatal centers: the National Cerebral and Cardiovascular Center and the Osaka Medical Center and Research Institute for Maternal and Child Health. Both centers are located in the Osaka Prefecture, which has the third-largest population in Japan. Postpartum was defined as the first 3 months after delivery. DVT was diagnosed by ultrasonography, venography, or magnetic resonance imaging angiography. We enrolled only patients with symptomatic DVT. Each patient's age, body mass index, gestational weeks of DVT onset, complications of pregnancy, delivery mode, and other information were reviewed.

The protocol of this study was approved by the Ethics Review Committee of the National Cerebral and Cardiovascular Center and by that of the Osaka Medical Center and Research Institute for Maternal and Child Health. Only those who had given written informed consent for genetic analyses were included.

2.2 Activity measurements of protein S, protein C, antithrombin, and antiphospholipid syndrome screening

The plasma samples were obtained after at least 3 months' postpartum and at least 3 months without the use of warfarin. Samples were subjected to a thrombophilia screening, including prothrombin time, activated partial prothrombin time, and activities of protein S, protein C, and antithrombin. Protein S activity was measured as cofactor activity for activated protein C on the basis of the activated partial thromboplastin time assay using Staclot protein S (Diagnostica Stago, Asnieres, France) [18]. Protein C amidolytic activity was measured using S-2366 as a chromogenic substrate and Protac derived from *Agkistrodon contortrix* venom as the activator [16]. Antithrombin activity was measured as a heparin cofactor activity using chromogenic substrate S-2238 (Chromogenix AB, Stockholm, Sweden) [16, 27]. Samples were also subjected to an antiphospholipid syndrome screening of

lupus anticoagulant, anticardiolipin antibody, and anti-β2-glycoprotein-I antibody [28].

2.3 DNA sequencing of protein S, protein C, and antithrombin genes

We sequenced the entire coding region of protein S, protein C, and antithrombin genes in 18 patients with DVT. The method of direct sequencing using the 96-capillary 3730xl DNA Analyzer (Applied Biosystems Japan, Tokyo, Japan) has been described previously [20, 29]. We have adopted the numbering standards of the Nomenclature Working Group, wherein the A of the ATG of the initiator Met codon is denoted as nucleotide +1, and the initial Met residue is denoted as amino acid +1 [30].

3 Results

3.1 DVT history of enrolled patients

We enrolled 18 Japanese symptomatic DVT patients in this study, and only one patient had previous DVT event. All patients were negative for the antiphospholipid syndrome. Thirteen patients were primiparous and five were multiparous. One patient without genetic mutation had a history of miscarriage. One patient without genetic mutation had a history of first trimester artificial abortion that was also complicated with DVT at the time. As an additional risk factor, two out of 13 DVT patients without genetic mutation showed hyperemesis, but all five patients with genetic mutation did not show hyperemesis. Other risk factors such as bed rest, preeclampsia, multiple pregnancy, and preterm labor were not observed in all 18 patients. One patient without genetic mutation had the travelers' thrombosis in the first trimester. One patient without genetic mutation showed paradoxical embolism after DVT postpartum.

3.2 Identification of genetic mutation in DVT patients

We sequenced the coding regions of the protein S, protein C, and antithrombin genes in the 18 DVT patients and identified missense mutations in the protein S gene in four cases, and in the protein C gene in one case, but not in the antithrombin gene (Table 1). Two patients, cases 1 and 2, had the K196E mutation in the protein S gene; this is the most popular thrombophilic mutation in the Japanese population [19, 21, 24]. These two patients had protein S anticoagulant activity above 50% (Table 1). Case 3 had a missense mutation, L446P, in the protein S gene. Case 4 had two missense mutations, D79Y and T630I, in the protein S gene with very low anticoagulant activity of 4%, with family history of DVT in her father. The protein S anticoagulant activities during pregnancy in cases 2, 3, and 4 were decreased to 25, <20, and <1%, respectively. Case 5 had the C147Y mutation in the protein C gene with 45% amidolytic activity. Her protein C activity did not change during pregnancy (Table 1). None of the 18 patients with DVT had nonsynonymous mutations in the antithrombin gene. All patients were not obese with body mass index between 18 and 24. Case 1, 2, and 3 had term vaginal delivery; however, case 4 and 5 had cesarean section due to other obstetric indication.

3.3 Onset of DVT in patients with genetic mutation

Table 2 shows the onset of the DVT events in patients with or without genetic mutation. DVT was found in all five patients with genetic mutations in their first and second trimesters, but not in postpartum. In 13 patients without genetic mutations, DVT events occurred in postpartum for four patients and in the first and second trimesters for nine patients. Two out of four patients without genetic mutation underwent cesarean section. Thus, DVT in pregnant patients with genetic mutation tended to occur in the first and second trimesters and not postpartum.

4 Discussion

Although the relationship between DVT and genetic mutations in protein S, protein C, and antithrombin genes is well established, the clinical courses of DVT patients with genetic mutation among Japanese women during pregnancy and postpartum have not been well characterized. Recent genetic analysis of inherited thrombophilia revealed ethnic differences in DVT between Caucasians and Asians [19, 21], suggesting that the study of venous thromboembolism within individual ethnic populations is highly valuable [12]. It has been established that Caucasians have factor V Leiden mutation and prothrombin G20210A mutation as genetic risk factors for DVT, whereas Japanese do not carry them [10, 11]. However, Japanese have the K196E mutation in the protein S gene as a genetic risk for DVT [19, 21, 22]. The study of DVT in a Japanese population without factor V Leiden mutation or prothrombin G20210A mutation may reveal different clinical characteristics and give rise to hitherto unrecognized issues. In particular, sub-group analyses, such as DVT during pregnancy and postpartum, would be valuable. In the present study, we enrolled 18 pregnant Japanese women with DVT and found that five out of 18 patients (28% patients) had genetic mutations in the protein S or protein C gene. None carried mutations in the antithrombin gene.

The question of when DVT events occur in pregnant women with genetic mutations has been debated. Studies of

Table 1 Nonsynonymous mutations identified in protein S and protein C genes in patients ($n = 18$) with DVT during pregnancy and postpartum

Patient	cDNA[a]	Region	Amino acid change	Protein S[b] or protein C[c] activity (%)	Protein S[b] or protein C[c] activity, during pregnancy (%)	Age	Gravida	Parity	Body mass index	Family history	Other complications of pregnancy	Onset of DVT (weeks of gestation)	Delivery mode	Recurrence of DVT	Complication of PTE (weeks of gestation)
Protein S gene															
Case 1	c.586	Exon 6	K196E	57[b]	n.d.	30	1	1	18.6	None	None	27	TVD	None	27
Case 2	c.586	Exon 6	K196E	68[b]	25[b]	27	0	0	20.3	None	None	10	TVD	None	None
Case 3	c.1337	Exon 12	L446P	13[b,d]	<20[b]	30	0	0	18.8	None	None	27	TVD	None	None
Case 4	c.235	Exon 3	D79Y	4[b]	<1[b]	35	0	0	22.5	Father	None	6	C/S	None	None
	c.1889	Exon 15	T630I												
Protein C gene															
Case 5	c.440	Exon 6	C147Y	45[c]	57[c]	28	0	0	24.2	None	None	20	C/S	None	None

TVD term vaginal delivery, *C/S* cesarean section, *PTE* pulmonary thromboembolism

[a] Position from A of initial ATG in cDNA

[b] Protein S anticoagulant activity

[c] Protein C amidolytic activity

[d] Protein S activity was obtained under warfarin treatment

pregnant Caucasian women have reported a 3- to 12-times higher risk of thrombosis postpartum than during pregnancy [3, 4]. On the other hand, a large retrospective study found that events were twice as likely during pregnancy as postpartum [5]. In our new study, we found that Japanese patients with genetic mutations manifested DVT events in their first two trimesters (Table 2). In particular, pregnant Japanese patients with genetic mutation had no DVT events postpartum. Although this trend went against previous findings [3, 4], it was consistent with the results that there were twice as many DVT events during pregnancy as postpartum [5]. DVT onset at the early stage of pregnancy in patients with genetic mutation might be reasonable, since genetic mutation accelerates DVT onset, and patients with mutation might have DVT events in their early stage of pregnancy.

In the present study, we enrolled 18 pregnant Japanese women with DVT and found that four out of 18 patients (22% patients) had genetic mutations in the protein S gene. A previous study on thrombophilia activity screening in Japanese patients with DVT reported a high prevalence of protein S deficiency [31], and this was later confirmed by genetic analysis [19]. Taken together with these previous findings, our study reinforced the theory that protein S deficiency is an important risk factor for DVT in Japanese. This observation was in stark contrast to the case in Caucasians, in whom factor V Leiden and prothrombin G20210A mutations are involved in almost 50% of all DVT cases in pregnant women [8]. It is well known that the level of protein S activity was decreased immediately after pregnancy [25]. Therefore, predisposed thrombophilia should be considered in the care of patients with pregnancy-related complications, and antithrombotic prophylactic therapy might be applicable for those patients. Also, it might be good for women of child-bearing years to know their own thrombophilic nature.

A previous study reported on DNA sequence analyses of the protein S, protein C, and antithrombin genes in 173 Japanese DVT patients [20]. In this study, 55 patients (accounting for 32% of total patients) had nonsynonymous mutations in one of three genes. Among the three genes, mutations in the protein S gene were predominant, being found in 29 patients (17% of the total). Among various nonsynonymous mutations in the protein S gene, the K196E mutation was most prevalent. It was found in one out of 55–70 Japanese individuals, from analyses of general Japanese populations [19, 21, 22, 24]. In our study, we sequenced three genes in 18 patients with pregnancy-related thrombosis and identified missense mutations in five patients (accounting for 28% of the patients). Among five patients, four (22% of the total) had missense mutations in the protein S gene, which reconfirmed the predominance of inherited protein S deficiency in Japanese patients with

Table 2 Onset of DVT according to trimester of pregnancy and postpartum, and according to delivery mode

	Onset of DVT				Delivery mode			Complication of PTE
	First trimester	Second trimester	Third trimester	Postpartum period	Artificial abortion	Term vaginal delivery	Term cesarean section	
Patients with genetic mutation								
Protein S ($n = 4$)	2	2	0	0	0	3	1	1
Protein C ($n = 1$)	0	1	0	0	0	0	1	0
Total ($n = 5$)	2	3	0	0	0	3	2	1[a]
Patients without genetic mutation								
Total ($n = 13$)	4	5	0	4[c]	1[d]	8	4	1[b]

PTE pulmonary thromboembolism

[a] PTE events with genetic mutation occurred during the second trimester

[b] PTE events in the patients without genetic mutation occurred postpartum after cesarean section

[c] Two out of 4 patients without genetic mutation underwent cesarean section

[d] First trimester

DVT. Two of these patients had K196E mutation. Thus, K196E mutation in the protein S gene would be a genetic risk for not only DVT in general, but also for pregnancy-related DVT.

There are limitations to the present study. This was a small-scale retrospective study with 18 patients. We performed genetic analysis in those patients and identified five patients with genetic mutation. To understand the DVT risk in pregnant Japanese patients with inherited or acquired thrombophilia, we will have to recruit patients consecutively and perform thrombophilic screening, including genetic analysis, in the future evaluation.

In conclusion, we identified inherited thrombophilia in pregnant Japanese women with DVT and found protein S deficiency to be a predominant cause of thrombophilia. By DNA sequence analysis, we found two patients with a K196E mutation in the protein S gene that is prevalent in the Japanese population. Since pregnant women showed reduced protein S levels, a diagnosis of protein S deficiency based on its activity has an intrinsic limitation. Since the onset of DVT tends to occur at an early stage during pregnancy, the genetic analysis might be an alternative diagnostic tool.

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More on: racial differences in venous thromboembolism

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See also Zakai NA, McClure LA. Racial differences in venous thromboembolism. *J Thromb Haemost* 2011; **9**: 1877–82.

We read with great interest the review article by Zakai and McClure [1] regarding racial differences in venous thromboembolism (VTE). While racial differences in the incidence of VTE have been reported, the race-specific genetic risk factors for VTE have remained obscure until recently. Factor V Leiden mutation and prothrombin G20210A mutation are commonly found in populations of European origin as modest genetic risk factors for VTE [1], and Asians have neither mutation. Although the incidence of VTE in African-Americans is more than 5-fold greater than that in Asian-ancestry populations, and European and Hispanic populations are at intermediate risk of VTE, Asian populations may have race-specific mutations for VTE. Recently, we identified a protein S K196E mutation as a genetic risk factor for VTE in a Japanese population [2–4]. As Zakai and McClure have described a high prevalence of protein C and protein S deficiency in Japanese, Taiwanese and Thai populations with VTE, the protein S K196E mutation can account for the high prevalence of protein S deficiency in Japanese subjects. An *in vitro* study showed that the recombinant protein S with the K196E mutation lost activated protein C-dependent anticoagulant activity [5].

So far, three independent case–control studies, all performed in Japan, have reached the same conclusion – that the protein S K196E mutation is a risk factor for VTE, with odds ratios between 3.74 and 8.56 [2–4]. The prevalence of this mutation has been examined in four cohorts, and 77 heterozygous carriers have been identified in 4319 Japanese subjects [2–4,6], indicating a mutant allele frequency of 0.0089. None were homozygotes. The total population of Japan is now 127 million. Therefore, approximately 10 000 Japanese may be homozygotes. Three VTE patients homozygous for protein S K196E mutation have been identified with a prevalence of one homozygote out of approximately 85 VTE patients [2,3]. One homozygote showed 78% total protein S antigen, 94% free protein S antigen, and 35% protein S anticoagulant activity [2]. Another homozygote showed 39% protein S anticoagulant activity but no data on antigen levels. The third homozygote

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did not have any antigen or activity data [3]. The protein S K196E mutation can be found in VTE patients with congenital protein C deficiency, facilitating the development of VTE [4,7], and is frequently present in VTE patients with pregnancy [8]. The genotype-phenotype study of the Japanese general population showed that the individuals heterozygous for the mutant E-allele had 16% lower protein S anticoagulant activity than wild-type subjects [9].

The protein S K196E mutation seems to be race specific, because so far this mutation has not been identified in a Caucasian population. We believe that the protein S K196E mutation should be present in other east Asian populations, including the Chinese and Koreans; however, so far there are no reports on those populations. Some genetic mutations specific to eastern Asian populations, in particular the plasminogen A610 T mutation with an allele frequency of 0.020 in Japanese subjects and the ADAMTS13 P475S mutation with an allele frequency of 0.050 in Japanese subjects, have been reported in other east Asian populations [10]. These mutations are low-frequency variations, with allele frequencies between 0.05% and 0.5% [11]. Factor V Leiden mutation and prothrombin G20210A mutation in Caucasian populations are also regarded as low-frequency variations. Thus, as previously described [11], the low-frequency genetic variations would be important for specific phenotypes such as VTE, and the protein S K196E mutation is a race-specific genetic risk factor for VTE. Recent genomewide genetic analysis in Asian populations revealed the southern migration route to eastern Asia [12]. The low-frequency genetic variation should have occurred recently and should be fixed within a specific population. This accumulating body of evidence strongly suggests that genetic studies should be carried out in each ethnic population and that studies of common variations as well as low-frequency variations would be valuable.

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Disclosure of Conflict of Interests

The authors state that they have no conflict of interest.

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This proof-of-principle study illustrates that NGS enables rapid genetic diagnosis of a PFD in a single test. In this example, we were able to restrict SNV mapping to a subgroup of 57 genes implicated in secretion, so that it was feasible to use Sanger sequencing to determine whether each potential SNV was a true-positive or a false-positive call. Although successful in HPS, restricting analysis to a subgroup of genes is likely to reduce the diagnostic yield of NGS for other PFDs where it is less easy to select a candidate gene list. Strategies such as whole-exome sequencing may circumvent this difficulty by increasing the overall sensitivity of NGS for pathogenic SNVs. However, increasing the number of mapped genes is also expected to yield significantly larger numbers of irrelevant SNVs and false-positive calls. Alternative NGS strategies require further evaluation in other PFDs to determine the optimum diagnostic and cost-effective approach.

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Disclosure of Conflict of Interest

The authors state that they have no conflict of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. The 216 candidate PFD genes.
Data S2. The accessible internet-based resources used in this work.

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RGS2 deficiency in mice does not affect platelet thrombus formation at sites of vascular injury

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RGS2 is a member of the regulator of G-protein signaling (RGS) protein family that plays a key role in the regulation of G-protein-coupled receptors. RGS2 inhibits Gq-mediated and Gi-mediated signaling by activating the intrinsic GTPase of the Gα subunit [1,2]. RGS2 also interacts with Gs and adenylyl cyclase, and suppresses Gs signaling independently of GTPase-activating protein activity [2,3]. In platelets, the activation of the Gq-coupled or Gi-coupled receptors by thrombin, thromboxane A_2 and ADP stimulates platelet aggregation, whereas the activation of the Gs-coupled prostacyclin receptor inhibits aggregation [4,5]. Recently, active roles of RGS proteins in regulating platelet G-protein-coupled receptors have been

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reported [6,7]. Mice carrying a G186S mutation in the α subunit of Gi2 that impairs RGS–Gi2 interactions showed increased platelet aggregation in vitro and enhanced platelet thrombus formation after vascular injury *in vivo* [6]. These results indicate that platelet RGS proteins are essential for regulating thrombogenesis at the sites of vascular injury. A G23D mutation in the human *RGS2* gene was reported to increase the proportion of RGS2 isoforms that strongly interact with adenylyl cyclase. Platelets from carriers of this mutation showed decreased Gs activity after stimulation of the prostacyclin receptor, suggesting that RGS2 is a negative regulator of platelet Gs signaling [7]. In this study, we analyzed platelet thrombus formation in RGS2-deficient mice to fully characterize the physiologic importance of RGS2 in platelets.

RGS2-deficient ($Rgs2^{-/-}$) mice on the C57BL/6J genetic background [8,9] were provided by M. E. Medelsohn (Tufts University School of Medicine). Blood samples were collected from male mice aged 8–12 weeks. All animal procedures were approved by the Animal Care and Use Committees of the National Cerebral and Cardiovascular Center. We confirmed the presence of RGS2 transcripts in platelets of wild-type C57BL/6J ($Rgs2^{+/+}$) mice by RT-PCR (data not shown), as previously reported in rat platelets [10]. No RGS2 transcripts were detected in platelets of $Rgs2^{-/-}$ mice. Platelet counts were (810 ± 77) × 10^9 L^{-1} (mean ± standard deviation [SD], n = 20) for $Rgs2^{+/+}$ mice and (781 ± 84) × 10^9 L^{-1} (n = 20) for $Rgs2^{-/-}$ mice, with no significant difference ($P > 0.05$ by Student's t-test) between the genotypes.

We studied platelet Gs activity by using whole-blood aggregation-inhibition analysis. Platelet aggregation in whole blood was measured by a screen filtration pressure method with a WBA-Neo aggregometer (ISK, Tokyo, Japan) [11,12]. Four different concentrations (100, 200, 400 and 800 μg L^{-1}) of the Gs agonist prostaglandin E_1 (PGE_1) were added to whole blood 1 min before aggregation was initiated with 4 mg L^{-1} collagen. The 50% inhibitory concentrations (IC_{50}) of PGE_1 calculated from the dose–response curve were 308 ± 34 μg L^{-1} (mean ± SD, n = 8) for $Rgs2^{+/+}$ mice and 330 ± 75 μg L^{-1} (n = 8) for $Rgs2^{-/-}$ mice, with no significant difference between the genotypes. We also examined the inhibitory responses to PGE_1 on collagen-induced platelet aggregation in platelet-rich plasma, as previously reported in humans [7]. Aggregation was monitored by light transmission with an MCM Hema Tracer 704 aggregometer (Tokyo Koden, Tokyo, Japan), as described previously [13]. Again, the responses were comparable in $Rgs2^{+/+}$ mice (IC_{50} = 6.46 ± 2.77 μg L^{-1}, mean ± SD, n = 4) and $Rgs2^{-/-}$ mice (IC_{50} = 8.72 ± 2.69 μg L^{-1}, n = 4), suggesting normal platelet Gs activity in $Rgs2^{-/-}$ mice.

To clarify the consequences of RGS2 deficiency in platelet thrombus formation under physiologic blood flow, we performed a whole-blood perfusion assay on a collagen-coated surface, as described previously [13,14]. Under both low (200 s^{-1}) and high (1500 s^{-1}) shear rate conditions, we did not detect any difference in either rate or extent of thrombus accumulation between $Rgs2^{+/+}$ and $Rgs2^{-/-}$ mice (Fig. 1A).

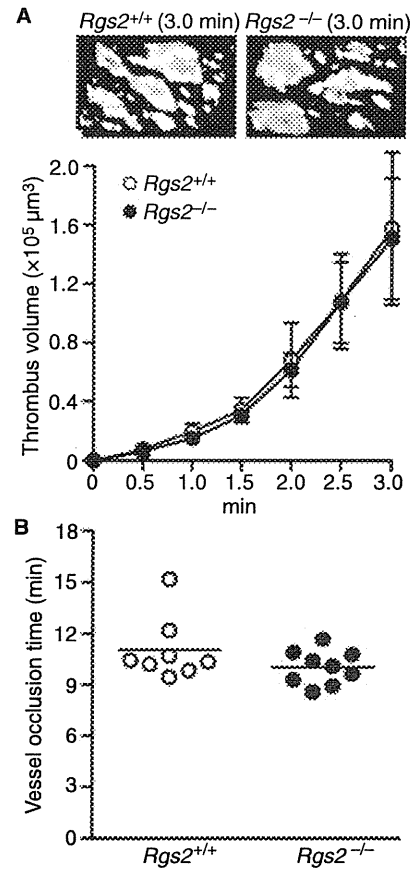

Fig. 1. Platelet thrombogenesis in RGS2-deficient mice. (A) In vitro thrombogenesis on a collagen surface under flow. Whole blood from $Rgs2^{+/+}$ or $Rgs2^{-/-}$ mice containing mepacrine-labeled platelets was perfused at a wall shear rate of 1500 s^{-1}. The cumulative thrombus volume, analyzed with a multidimensional imaging system, was measured every 0.5 min up to 3 min. Data are the mean ± standard error of the mean of 12 mice for each genotype. No significant differences were observed between $Rgs2^{+/+}$ and $Rgs2^{-/-}$ mice by two-way repeated measures analysis of variance. The typical images of thrombi at 3 min after perfusion are shown in the upper panels. The cumulative thrombus volume at a shear rate of 200 s^{-1} was also similar between the genotypes (n = 11). (B) *In vivo* thrombogenesis in ferric chloride-injured mesenteric arterioles. Endogenous platelets of live mice were fluorescently labeled with a DyLight488-conjugated rat IgG against mouse glycoprotein Ibβ, and observed in mesenteric arterioles after injury with 10% ferric chloride. The vessel occlusion time is shown. Student's t-test showed no significant differences between the genotypes. Time to first thrombus formation also did not differ between genotypes. The symbols represent data from a single mouse. The bars represent the mean values of groups.

These results suggest that RGS2 does not substantially contribute to the regulation of platelet thrombogenesis under flow. Similar results were obtained *in vivo* in thrombosis experiments using intravital microscopy. We applied 10% ferric chloride topically to the mesenteric arterioles of mice aged 3–5 weeks. Platelet thrombus formation in the arterioles was monitored with a fluorescence microscope and a CCD camera system, as described previously [14]. The diameters and shear rates of the studied arterioles were 123.2 ± 17.7 μm and 1291 ± 163 s^{-1} (mean ± SD, n = 8) for $Rgs2^{+/+}$ mice, and 118.3 ± 13.0 μm and 1299 ± 222 s^{-1} (n = 9) for $Rgs2^{-/-}$

mice, with no significant difference between genotypes. Both the time to first thrombus formation (> 30 μm) and the vessel occlusion time following injury were comparable between $Rgs2^{+/+}$ and $Rgs2^{-/-}$ mice, indicating that RGS2 does not play a significant role in the regulation of thrombogenesis at the site of arteriolar injury (Fig. 1B).

These results suggest that RGS2 deficiency has no impact on hemostasis *in vivo*. Platelets are thought to express at least 10 different RGS proteins; therefore, other RGS proteins are potentially important.

Addendum

F. Banno: designed research, performed experiments, analyzed and interpreted data, wrote the paper, and approved the final version to be published; T. Nojiri: performed experiments, analyzed data, and approved the final version to be published; S. Matsumoto: performed experiments, analyzed data, and approved the final version to be published; K. Kamide: interpreted data and approved the final version to be published; T. Miyata: designed research, interpreted data, wrote the paper, and approved the final version to be published.

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Disclosure of Conflict of Interests

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話題

加齢とプロテインS*

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(((KEYWORDS)))　プロテインS，加齢，地域一般住民，吹田研究
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1．はじめに

プロテインSの血中抗原量や活性は，遺伝因子や環境因子により変動する．遺伝因子としてプロテインSの抗原量や活性に影響を与えるミスセンス変異やプロモータ領域の変異が挙げられる．環境因子として，年齢，性，ホルモン，妊娠，肝疾患，炎症が挙げられる．一方，プロテインSの抗凝固活性はC4BP結合型には見られず，C4BPに結合しない遊離型プロテインS(free protein S；fPS)が抗凝固活性を示す．プロテインS抗原量の測定は，総プロテインS(total protein S；tPS)抗原量およびC4BPに結合していないfPS抗原量が測定可能であり，fPS量がよりプロテインS抗凝固活性を反映すると考えられる．

本稿では，プロテインS抗原量および抗凝固活性の加齢による変動を中心に，これまでヨーロッパおよび本邦で行われた一般住民を対象にした研究の成果を紹介したい．

2．オランダで行われた小規模なプロテインS抗原量の研究[1)]

男性225名，女性168名(計393名)の健常人を対象に，tPS量，fPS量，プロテインC抗原量，X因子抗原量の測定が行われた．X因子はプロテインSやプロテインCと同様に，ビタミンK依存性の凝固因子なので，これらに関連して測定された．対象者の年齢は18～60歳以上であった．経口避妊薬を服用する女性は除外した．両プロテインS抗原量ともに，男性が女性より高い値を示したが，特に，50歳までのtPS量とfPS量はともに女性で有意な低値を示した．閉経前の女性はtPS量の低値を示したが，閉経後のtPS量は男性と差は見いだせなかった．fPS量は男性が有意に高く，閉経前の女性が最も低く，閉経後の女性はこれら2群の中間を示した．本研究は対象者総数が393名であり，これをさらに性別と10歳代に分けているので，一つのグループは40名程度と小規模であるものの，tPS量とfPS量の性差と，女性での閉経前後の変化を示した．

3．小児と100歳老齢者のプロテインS抗原量の研究[2,3)]

1～16歳までの246名の小児を対象に33種の凝血学的パラメーターを測定した研究が報告されている．この研究では，tPS量とfPS量も測定されている．両プロテインS抗原量は生後6か月で，すでに成人と同程度であった．しかし，生後1日目に測定すると，プロテインS抗原量は極めて低い値(0.3 U/ml程度)を示した[2)]．一方，100歳以上の高齢者(合計25名，男性9名，女性16名)のtPS量を測定した報告によると，100歳以上の老齢者のtPS量は18～50歳のグループや51～69歳のグループのtPS量と差が見られなかった．

*：Aging and protein S

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表　西スコットランドで測定された総プロテインS抗原量(tPS)と遊離プロテインS抗原量(fPS)

	人数	25歳未満	25～45歳未満	45歳以上	全体
tPS(%)					
男性	2,106	106(72～168)	109(72～162)	110(73～171)	108(72～164)
女性					
ホルモン°あり	487	84(56～136)	89(56～148)	95(57～157)	87(57～142)
ホルモンなし	1,195	96(65～141)	96(67～152)	99(71～146)	96(68～148)
fPS(%)					
男性	2,106	119(71～177)	115(68～177)	113(67～175)	115(68～176)
女性	1,682	96(51～155)	98(55～156)	100(60～152)	98(54～155)

tPS値(%)およびfPS(%)値は中央値，括弧内は2.5～97.5パーセンタイル値を示す．
女性のtPS量は経口避妊薬服用・ホルモン補充療法(°)で低値を示したので，その有無で分類した．

4．西スコットランドでのプロテインS抗原量の大規模な研究[4]

西スコットランドに在住の心臓，腎臓，肝臓に疾患をもたない3,788名(男性2,106名，女性1,682名，17～68歳)の血中プロテインS抗原量(tPS，fPS)が報告されている．男性の平均年齢は32歳，女性は35歳であった．女性の87%(1,467名)は閉経前であり，31%は経口避妊薬を服用していた．197名の女性は閉経しており，その15%はホルモン補充療法を受けていた．

表に西スコットランドで測定されたtPS量およびfPS量の年齢別の中央値と2.5～97.5%値を男女に別けて示した．両プロテインS抗原量は，ともに男性は女性より高値を示した．即ち，男性のtPS量は女性より平均13.6%高かった($p<0.001$)．男性ではtPS量は年齢により変化しなかったが，fPS量は加齢により少し減少した($p=0.001$)．女性では，tPS量は加齢により増加したが($p<0.001$)，fPS量は変わらなかった．閉経はtPS量に影響しなかった．経口避妊薬により約8%のtPS量の減少を認めた($p<0.001$)．fPS量は加齢により上昇した($p<0.02$)が，調整を行うとその有意差は消失し，最も大きな因子は閉経であった．経口避妊薬はfPS量に影響しなかった．閉経後の女性へのホルモン補充療法はtPS量，fPS量ともに影響を与えなかった．

表には，tPS量およびfPS量の年齢別の中央値と2.5～97.5%値を男女別に示している．全対象者の下方2.5%に当たるtPS量は56～73%であり，経口避妊薬を服用する若い女性が最も低く，男性が最も高かった．全対象者の下方2.5%に当たるfPS量でも，若い女性が51%と最も低く，男性が60%と最も高かった．

5．吹田研究で行ったプロテインS活性の大規模な研究[5]

吹田研究は，大阪府吹田市の住民を対象として1989年に始まったコホート研究である．住民台帳から30～70歳代まで10歳代ごとに男女別に無作為に抽出し，郵送で健診案内を郵送し参加を呼びかけた．15,200名を抽出した中で，これまでに8,360名が健診受診している．日本の多くのコホートが農漁村の在住者を対象とするのに対し，吹田研究は都市部の住民を対象とする点に特徴がある．筆者らは健診受診された2,690名(男性1,252名，女性1,438名，33～89歳)のプロテインS活性をStaclot protein S(Diagnostica Stago社)を用いて測定した．本研究の特徴は，計画健診により無作為に抽出された30歳代から80歳代までの広い年齢層の対象者のプロテインS活性(抗原量ではなく)を測定した点にあり，国民の3分の2を占める都市部住民の年齢と性別によるプロテインSの抗凝固活性の変化を把握できる．

プロテインS抗凝固活性は，男性が女性より高値を示した(92.6±21.4%，82.9±17.8%，平均値±標準誤差，$p<0.0001$)．特に男性では年齢により活性が低下したが(correlation coefficient，$r=-0.366$)，女性では変化が見られなかった．

プロテインS活性を性別，10歳年令別に区別した値を図に示す．これらの結果から，①男性のプロテインS活性は40歳代の102%をピークに，それ以降は徐々に低下し80歳代では78%にまで低下する，②一方，女性では30歳代・40歳代は79%と低値を示すものの，それ以降は下ることなく60齢代では逆に85%にまで上昇する，③男女

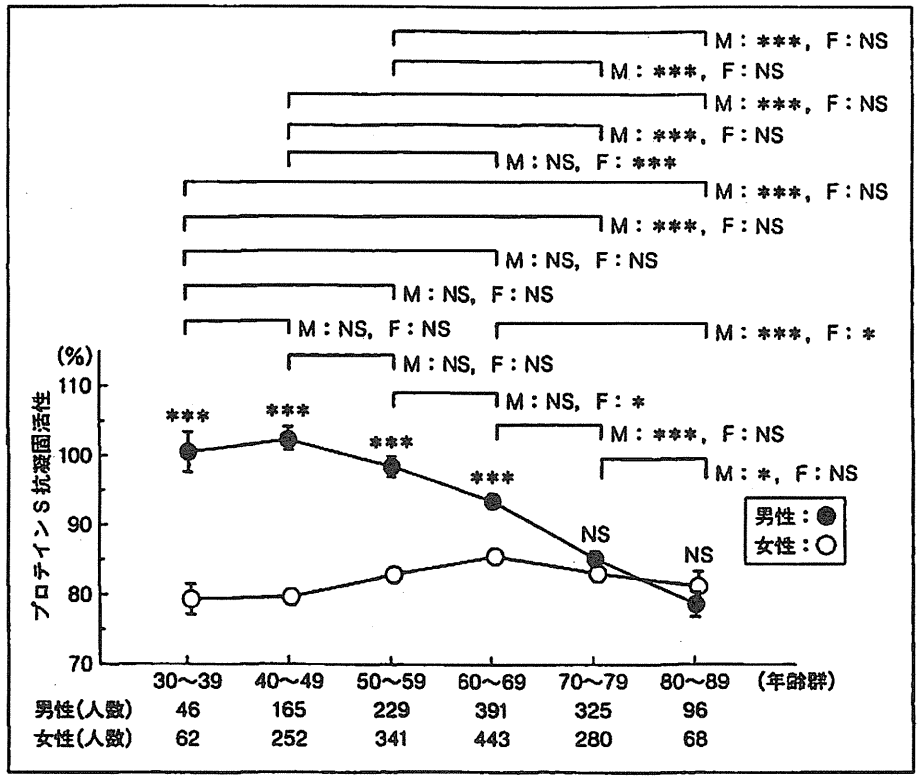

図　日本人2,698名のプロテインS抗凝固活性
男性1,252名，女性1,446名のプロテインS活性を10歳代ごとに平均値+/−標準誤差を示す．年齢別の男女群間の比較はMann-Whitney検定を用いた．男女別年齢群間の比較はSteel-Dwass検定を用いた．
***：$p<0.001$，**：$p<0.01$，*：$p<0.05$，NS：not significant.

で比較すると，30歳代～60歳代までは，男性のプロテインS活性は女性より明らかに高値を示す，④しかし，70歳代・80歳代では男女差がみられない，ことが明らかとなった．

男性のプロテインS活性が女性より高いという結果は，西スコットランドでのプロテインS抗原量の研究とよく一致する．しかし，男性のプロテインS活性は加齢により低下するという結果は，西スコットランドの抗原量の研究と一致しない．これは研究対象者の年齢の違いで説明される．すなわち，吹田研究は80歳代という高齢までの活性を測定しているので，加齢による活性の低下を観察したが，西スコットランドの研究では，25歳未満，25～45歳未満，45歳以上，という大まかな年齢の分け方をしているので，加齢による抗原量の低下を見いだせなかったと考えられる．

6．おわりに

プロテインSの抗原量と活性には性差がみられ，特に若い女性では低下が大きく，プロテインSの検査値を見るときに注意が必要である．男性は，70歳代・80歳代の高齢者でプロテインS活性の低下がみられた．こういった性と年齢によるプロテインS抗原量と活性の変化は，欠乏症の同定の際に，考慮すべきであろう．

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二次止血：凝固のメカニズム

宮田敏行*

abstract

凝固反応は組織因子と活性型Ⅶ（Ⅶa）の複合体形成で開始する．組織因子は血管中膜にある平滑筋細胞や外膜にある線維芽細胞に恒常的に発現しており，血管損傷により血液がこれらの細胞に接触すると，Ⅶa-組織因子複合体が形成される．この複合体はⅨやⅩを活性化させ少量のトロンビンを形成する．このトロンビンが凝固の補因子であるⅧとⅤを活性化することにより，凝固反応が増幅され，大量のトロンビンが生成される．トロンビンはフィブリンを形成するだけでなく，血小板上のトロンビン受容体であるprotease activated receptorを活性化させる．血小板は内皮下層マトリックスに存在するコラーゲンで活性化される．このように，循環系を維持するため，血管の損傷部位局所では，凝固反応と血小板の活性化が並行して進み，血栓・止血反応を行う．

Ⅰ はじめに

血液凝固は血小板凝集と相互に反応し止血・血栓に働く．組織因子に活性型Ⅶ（Ⅶa）が結合することで始まる凝固反応は，組織因子を発現した局所で進行し，トロンビンとフィブリンを生成する[1]．凝固反応で生成したトロンビンは，フィブリンの生成だけでなくトロンビン受容体であるprotease activated receptor（PAR）を介して血小板を活性化する能力を有し，血栓形成の主要な因子として働く（図1）[2]．循環血中の血小板は，コラーゲンを露出した損傷部位に集まり，コラーゲン依存性の血小板血栓を形成する[3]．本稿では，血栓形成における凝固反応の役割を解説する．

Ⅱ 凝固開始作動因子：組織因子

組織因子は膜タンパク質であり，いろいろな細胞上に発現し多様な機能を発揮する．組織因子は凝固反応の開始に加え，血管新生，癌の進行・転移，卵黄嚢血管系の維持に重要な役割を果たしている．組織因子は血管外膜の線維芽細胞，血管壁中膜の血管平滑筋細胞に恒常的に発現している（図2）．また，組織因子は多くの非血管系細胞に恒常的に発現し，単球と血管内皮細胞での発現は刺激により誘導される．顆粒球と血小板上に機能を示すほどの組織因子が存在するかについては議論のあるところである．血管内皮細胞は，流血中のⅦaが組織因子と結合することを遮断する機能を有し，損傷部位以外での凝固の開始を阻止している．組織因子は循環血中のマイクロパーティクル上にも存在し，この血中由来の組織因子が病理的な過程で血栓形成に関与する可能性が指摘されている（後述）．

止血に際しては，血管の中膜や外膜に存在する細胞に恒常的に発現している組織因子に血中のⅦaが結合し，凝固反応が開始される（図3）．Ⅶの0.5～1%がⅦaの形で血中を循環しており，このⅦaが凝固の

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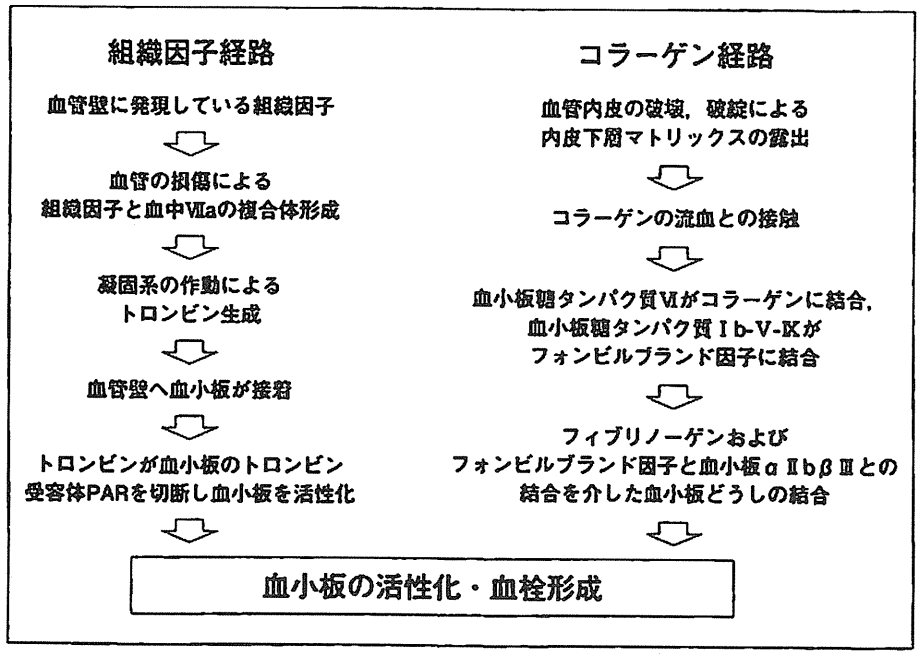

図1
2つの経路による血小板活性化を通した血栓形成メカニズム

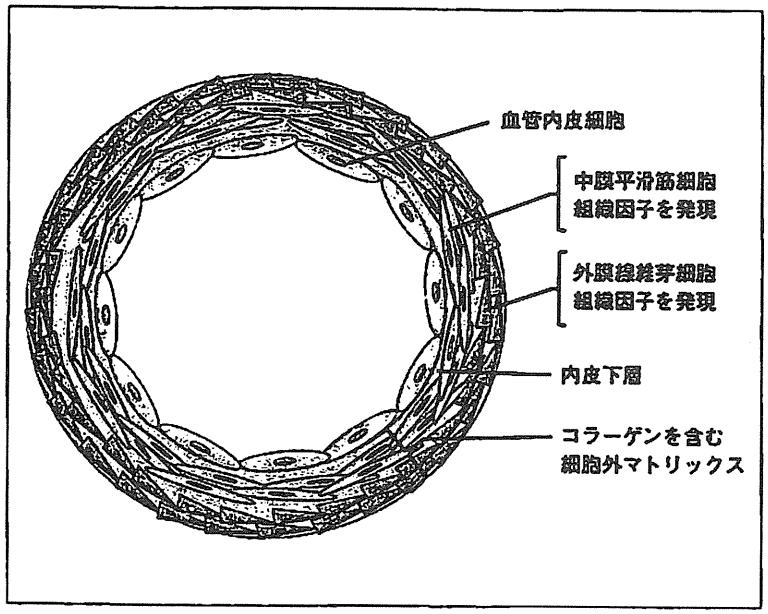

図2
血管の構築，凝固開始作動因子である組織因子と血小板活性化惹起因子であるコラーゲンの分布
血管内皮細胞は血管の内腔を被覆し，それを血管壁細胞（ペリサイト，血管平滑筋細胞）が細胞外マトリックス成分とともに包囲している．大血管系は3層構造をとる．すなわち，結合組織で支持された内皮細胞からなる内膜，コラーゲン，エラスチン，プロテオグリカンを含む細胞外マトリックスと平滑筋細胞を有する中膜，最も外側にある外膜は線維芽細胞や神経を支持するコラーゲン束を有する．内膜と中膜の間には内弾性板，中膜と外膜の間には外弾性板があり，それぞれを分離している．凝固反応を作動させる組織因子は血管中膜（平滑筋細胞）と外膜（線維芽細胞）に存在する．血小板活性化惹起因子であるコラーゲンは血管内皮下層のマトリックス中に存在する．通常，組織因子とコラーゲンは血液に接触しないが，血管の損傷によって血液と接触することにより，循環系を維持するために血栓形成を作動させる．コラーゲンは循環系維持の第一防御線であり，組織因子は第二防御線である．

開始反応を惹起する[4]．一方，アテローム血栓症では，アテローマのプラークの破裂により，細胞外マトリックス中にあるコラーゲンが血液に露出し，脂質をためたマクロファージ上に発現した組織因子が血液に露出する．これらの血栓誘発因子により，血栓の形成が始まると考えられる．

組織因子には活性型と不活性型があるとの報告がある[5]．不活性型組織因子の実体は明らかではないが，二量体や脂質膜の再構成に加えて，ジスルフィド結合の酸化還元状態の変化が報告されている．組織因子には2つのジスルフィド結合があり，1つは不安定なアロステリックジスルフィド結合で，還元型で遊離チオール基をもつものが不活性型．これが酸化されるとX因子活性化能を有するⅦa-組織因子複合体を形成できる立体構造になるという．この酸化還元反応はprotein disulfide isomeraseが触媒す

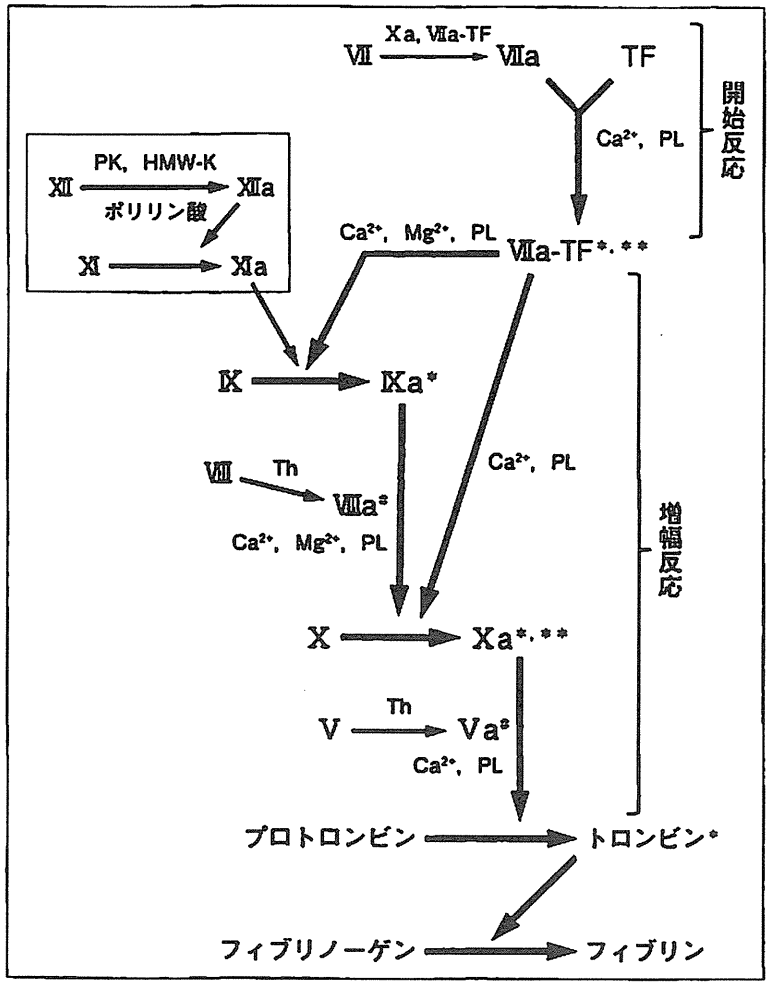

図3
凝固の開始反応と増幅反応を介したトロンビン生成

凝固は血管壁の組織因子と血液中のⅦa因子が複合体を形成することで開始される．Ⅶa-組織因子複合体はⅨおよびⅩを活性化し，少量のトロンビン（Th）が生成した後，このトロンビンが凝固反応の補因子であるⅧとⅤをⅧaとVaへと活性化することにより反応が増幅され，その結果大量のトロンビンが生成される．増幅反応はリン脂質（PL）膜上で効率よく進行する．トロンビンはフィブリノーゲンをフィブリンへと変換するだけでなく，血小板膜上のトロンビン受容体（PAR）を介して血小板を活性化させる．Ⅻ，プレカリクレイン（PK），高分子キニノーゲン（HMW-K）はガラス表面などの陰電荷の表面上でⅪを活性化させるが，生体内での血栓の形成に寄与するかは疑問視されている（四角枠で囲った）．最近，血小板から放出されるポリリン酸が陰電荷表面として働くことが示され，注目されている．凝固反応は血中の凝固制御因子により抑制される．制御因子としてプロテアーゼ阻害活性をもつアンチトロンビンと組織因子経路インヒビターが知られている．また，プロテインC抗凝固経路は，血管内皮細胞に発現するトロンボモジュリンと血管内皮細胞プロテインC受容体（EPCR）を介して，活性化プロテインCがⅧaとVaを分解し不活化することにより凝固を制御する．この際，プロテインSは不活化反応を促進する．

*：アンチトロンビンで活性が抑制される因子
**：組織因子経路インヒビターで活性が抑制される因子
#：プロテインS存在下で活性化プロテインCにより活性が抑制される因子

る．損傷部位にある活性化血小板や内皮細胞がこの酵素を放出し，組織因子のジスルフィド結合の形成を触媒することにより，組織因子の機能的に重要なジスルフィド結合の形成を促進すると考えられている．

Ⅲ 凝固の主役を務めるトロンビン

組織因子を発現する局所で凝固反応が始まる．このⅦa-組織因子複合体はⅨとⅩを活性化し，活性型Ⅸ（Ⅸa）がⅩを活性型Ⅹ（Ⅹa）へ活性化させ，Ⅹaがプロトロンビンからトロンビンを生成させる（図3）．しかし，この反応は効率よく進まず，少量のトロンビンしか生成しない．このわずかな量のトロンビンがⅧとⅤを活性化させ，ⅧaとVaへと変換させる．これら活性型因子は，それぞれⅨaによるⅩの活性化（tenase活性とよぶ），およびⅩaによるプロトロンビンの活性化（プロトロンビナーゼ活性とよぶ）を大きく促進する能力をもつ．これにより，tenase活性とプロトロンビナーゼ活性が亢進し，その結果，大量のトロンビンが生成される．トロンビンは多機能プロテアーゼであり，フィブリノーゲンをフィブリンに変換するだけでなく，ⅧとⅤの活性化やPARを介した血小板活性化を行う．

血中には凝固を制御する因子が存在する（図3）．アンチトロンビンは，トロンビン，Ⅹa，Ⅸa，Ⅶa-組織因子複合体の活性を阻害することにより，凝固の増幅反応を制御する．組織因子経路インヒビター（tissue factor pathway inhibitor：TFPI）はⅦa-組織因子複合体およびⅩaを阻害することにより組織因子による開始反応を抑制する．TFPIは組織因子経路の活性化の初期には反応を抑制するが，大量の

トロンビンが生成されると反応を抑制することができない．プロテインCは血管内皮細胞プロテインC受容体（endothelial cell protein C receptor：EPCR）に結合し，トロンボモジュリンに結合したトロンビンで活性化される．生成した活性化プロテインCはプロテインS存在下でⅧaとⅤaを限定分解により不活性型へと変換し凝固を抑制する．

Tenase複合体とプロトロンビナーゼ複合体の活性には酸性リン脂質膜が必要である（図3）．これまで，活性化した血小板が凝固の場となる酸性リン脂質膜を提供すると考えられていた．しかし，トロンビンで活性化されない血小板をもつPar4欠損マウスでの研究によると，このマウスのフィブリン生成は正常であった[6]．このことは，生体内では活性化血小板以外の細胞が膜を提供する可能性を示唆している．

ガラス試験管を用いて採血すると，ガラス表面の陰電荷により凝固反応が惹起される．これを内因系凝固反応とよぶ．Ⅻ，高分子キニノーゲン，プレカリクレインの完全欠損症は凝固の接触経路の開始が欠損し，顕著な部分トロンボプラスチン時間の延長を示す．これらの患者はいずれも出血を示さないので，この反応は生体内の止血の開始には必要ではないと考えられている．しかし，マウスを用いた研究では，Ⅻ欠損やⅪ欠損は血栓形成が弱く，Ⅻを阻害すると出血なしに脳虚血傷害を保護することができた[7]．これらのマウスで得られた結果は，接触経路の開始を阻害することにより，血栓抑制ができる可能性を示唆している．最近，血小板から放出されるポリリン酸が陰電荷表面として働くことが示され，大変注目されている[8]．

Ⅳ 組織因子をもつマイクロパーティクル

組織因子は血液中のある種のマイクロパーティクルにも存在する．この粒子は，白血球，血小板，内皮細胞，平滑筋細胞，単球といった血液中の細胞に由来し，それらがもつタンパク質を保有する．血栓形成の過程で，血小板は血管壁に蓄積され活性化されて接着因子Pセレクチンを発現する．Pセレクチンはその受容体であるPセレクチン糖タンパク質リガンド1（P-selectin glycoprotein ligand-1：PSGL-1）をもつマイクロパーティクルを捕集することにより，組織因子を保有するマイクロパーティクルを捕らえ，組織因子を血小板血栓形成局所に集積させることができる[9]．

正常状態の血中には組織因子活性は検出されないが，組織因子を保有するマイクロパーティクルは存在する．たぶん組織因子保有マイクロパーティクルは不活性型組織因子を含み，パーティクルが血管損傷部位に集まったときにだけ活性化されるのだろう．病理的マイクロパーティクルは活性型組織因子を含み，血栓塞栓症の素因となる可能性がある．癌細胞もしくは炎症細胞に由来する組織因子保有マイクロパーティクルは血栓イベントを起こす可能性があり，現在，多くの研究が行われている．

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DICと外因系凝固反応，マイクロパーティクル

DIC, tissue factor dependent coagulation, and microparticle

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◎凝固の活性化は組織因子依存性にはじまり，ⅨやⅩの活性化に続いてトロンビンが形成される．このトロンビンの生成がフィブリン魂形成，凝固反応のさらなる増幅，血小板の活性化につながる．組織因子依存性の凝固を外因系凝固反応とよび，陰電荷の異物(ガラスなど)で惹起される内因性凝固反応と区別される．敗血症などの感染症に合併した播種性血管内凝固症候群(DIC)の発症では，炎症性サイトカインにより単球・マクロファージや血管内皮細胞に組織因子の発現誘導がみられ，これらの細胞上に発現した組織因子が凝固反応を作動させる．本稿ではDIC発症の基盤を理解することを目的として，組織因子で開始される外因系凝固反応と，最近注目されている組織因子含有マイクロパーティクルの血栓形成への関与を紹介する．

Key word：播種性血管内凝固症候群(DIC)，組織因子，マイクロパーティクル，血栓，止血

組織因子依存性の凝固反応機構

組織因子は1回膜貫通領域をもつ糖蛋白質である．組織因子は263アミノ酸残基からなり，219残基の細胞外領域，23残基の膜貫通領域，21残基の細胞内領域から構成される．細胞外領域はサイトカイン受容体スーパーファミリーと相同性を示し，単独およびⅦaとの複合体の立体構造も決定されている．組織因子は血液に接する細胞(血球系細胞，血小板，血管内皮細胞)には通常発現していない．組織因子は血管外膜の線維芽細胞，中膜の血液平滑筋細胞に恒常的に発現している．また，組織因子はこれら以外の種々の細胞にも発現しており，凝固反応の開始に加え，血管新生や癌の進行・転移などにも重要な役割を果たしている．

止血反応は血管の損傷部位から血液の流失を防ぐ生体防御機構であり，血管外膜に構成的に強く発現する組織因子が重要な役割を果たす．血液中には活性型Ⅶ(Ⅶa)がⅦの0.5～1%程度存在し，これが組織因子に結合することによりⅦa-組織因子複合体が形成され，Ⅸ活性化能とⅩ活性化能を発揮する(凝固開始反応とよぶ)(図1)．このⅦaはわずかな量なので，凝固の開始時に形成されるⅦa-組織因子複合体もわずかであり，こうして生成したXaからは少量のトロンビンしか生成しない．しかし，少量のトロンビンは凝固の補助因子であるⅧとⅤを活性化させる．活性型であるⅧaとⅤaが生成されると，Ⅹの活性化やプロトロンビンの活性化が効率よく進行する．さらに，トロンビンはⅪを活性化し凝固反応を促進する．また，Ⅶa-組織因子複合体はⅦの活性化も行い，これにより凝固がさらに進行することになる．これらの活性化反応が進んだ結果，大量のトロンビンが生成する(凝固増幅反応とよぶ)[1]．

トロンビンは多機能プロテアーゼである．フィブリノゲンのフィブリンへの変換やトランスグルタミナーゼであるⅫの活性化を行うだけでなく，血小板膜上のトロンビン受容体 protease activated receptor を活性化することにより血小板の活性化を行う(図1)．トロンビンにより活性化された血小板はADP放出を行い，血小板インテグリンの活性化が起こる．このように，トロンビンはきわめて多彩な機能，それも凝固の進展や血小板の活性化といった血栓形成につながる機能をもつ．こういった機能をもつトロンビンの形成は十