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New Insights Into the Treatment of Glanzmann Thrombasthenia

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ABSTRACT

Glanzmann thrombasthenia (GT) is a rare inherited autosomal recessive bleeding disorder of platelet function caused by a quantitative or qualitative defect of platelet membrane glycoprotein IIb/IIIa (integrin αIIbβ3), a fibrinogen receptor required for platelet aggregation. Bleeds in GT are variable and may be severe and unpredictable. Bleeding not responsive to local and adjunctive measures, as well as surgical procedures, is treated with platelets, recombinant activated factor VII (rFVIIa), or antifibrinolytics, alone or in combination. Although platelets are the standard treatment for GT, their use is associated with the risk of blood-borne infection transmission and may also cause the development of platelet antibodies (to human leukocyte antigens and/or αIIbβ3), potentially resulting in platelet refractoriness. Currently, where rFVIIa is approved for use in GT, this is mostly for patients with platelet antibodies and/or a history of platelet refractoriness. However, data from the prospective Glanzmann's Thrombasthenia Registry (829 bleeds and 206 procedures in 218 GT patients) show that rFVIIa was frequently used in nonsurgical and surgical bleeds, with high efficacy rates, irrespective of platelet antibodies/refractoriness status. The mechanisms underpinning rFVIIa effectiveness in GT have been studied. At therapeutic concentrations, rFVIIa binds to activated platelets and directly activates FX to FXa, resulting in a burst of thrombin generation. Thrombin converts fibrinogen to fibrin and also enhances GT platelet adhesion and aggregation mediated by the newly converted (polymeric) fibrin, leading to primary hemostasis at the wound site. In addition, thrombin improves the final clot structure and activates thrombin-activatable fibrinolysis inhibitor to decrease clot lysis.

Contents

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Glanzmann thrombasthenia (GT) is a rare inherited bleeding disorder of platelet function. For bleeding not responsive to local management and antifibrinolytics, treatment with systemic hemostatic agents is required. Platelet transfusion is the time-honored standard treatment for serious bleeding but is not without its shortcomings. Recombinant activated factor VII (rFVIIa) is emerging as an important therapeutic agent. This review provides an update on the general understanding of GT and its management, with emphasis on current thinking on the mechanisms of action of rFVIIa in GT. We will briefly touch on hemopoietic stem cell transplantation (HSCT) as a curative treatment in carefully selected patients with poor quality of life suffering from recurrent persistent bleeding. Finally, the prospect of gene therapy and the promising progress made in basic and animal research will be discussed.

GT: General Overview

Eduard Glanzmann, a Swiss pediatrician, first described GT in 1918 [1]. GT is characterized by absent or decreased platelet aggregation to physiologic agonists including adenosine diphosphate (ADP), epinephrine, collagen, and thrombin [2–4]; prolonged bleeding time or PFA-100 closure times [5,6]; and abnormal clot retraction [2,7] (Table 1). Platelet number and morphology are usually normal [2], as is platelet agglutination to ristocetin (Table 1) [8], although a few families with macrothrombocytopenia have been reported [2,9]. These laboratory findings are unique to GT. Although platelets in GT patients can undergo shape change upon stimulation, adhere to exposed subendothelial tissue, and initiate secretion from storage granules [4], they cannot form platelet aggregates and thrombi at the site of vascular injury [10].

Platelet dysfunction in GT is caused by a quantitative or qualitative defect of the platelet membrane glycoprotein (GP) IIb/IIIa (integrin αIIbβ3) complex [3,4,11]. αIIbβ3 is a heterodimeric molecular complex acting as a fibrinogen receptor [12,13], which is important for mediating platelet aggregation induced by physiologic agonists and for fibrin clot retraction. When platelets are activated, αIIbβ3 responds to inside-out signaling and transforms from its bent resting state to a straight active configuration required for fibrinogen binding [14–16]. Other αIIbβ3 ligands include the adhesive proteins von Willebrand factor, fibronectin, vitronectin, and CD40L [17–19]. Platelets with deficient or defective αIIbβ3 cannot bind to these adhesive proteins when stimulated (Figure), which accounts for the characteristic GT laboratory findings. GT is a rare autosomal recessive disorder with an incidence of approximately 1 per million. However, in areas where marriage between close family relatives is common, the incidence can be as high as 1:200000 [20]. The genetic defect resides on the *ITGA2B* or *ITGB3* gene encoding αIIb and β3, both located on chromosome 17 (12q21) [3,4,9,21,22]. Mutations affecting either gene can result in GT. The multitude of mutations reported and how they result in quantitative or qualitative αIIbβ3 defects in GT have recently been extensively reviewed by Nurden and colleagues [4,9]. Details are also available at http://sinaicentral.mssm.edu/intranet/research/glanzmann/menu. Currently (as of October 25, 2015), there are 255 mutation records on the *ITGA2B* gene and 164 mutation records on the *ITGB3* gene. Although the majority are missense mutations, nonsense mutations (causing premature termination), small deletions and insertions (with in- or out-of-frame shifts, some causing altered splicing or premature termination and loss of transcript), as well as intronic splice site alterations are also reported. Integrin synthesis occurs in the megakaryocytes with αIIbβ3 complex formation in the endoplasmic reticulum. Any noncomplexed or incorrectly folded gene products will fail to undergo processing in the Golgi apparatus and are rapidly degraded intracellularly [4,9,23,24]. In GT, homozygosity of the same mutation is often a result of consanguineous marriage, although many other patients may be compound heterozygous for different mutations from each parent. Bleeding symptoms are confined to GT patients who are homozygous or compound heterozygous for αIIbβ3 mutations, whereas simple heterozygotes are asymptomatic [11], so family bleeding history may be absent. Integrin subunit αIIb expression is exclusively for αIIbβ3 in platelets. However, subunit β3 is involved in other integrins outside platelets. The impact of β3 loss (that results in GT) on other tissues is currently unknown.

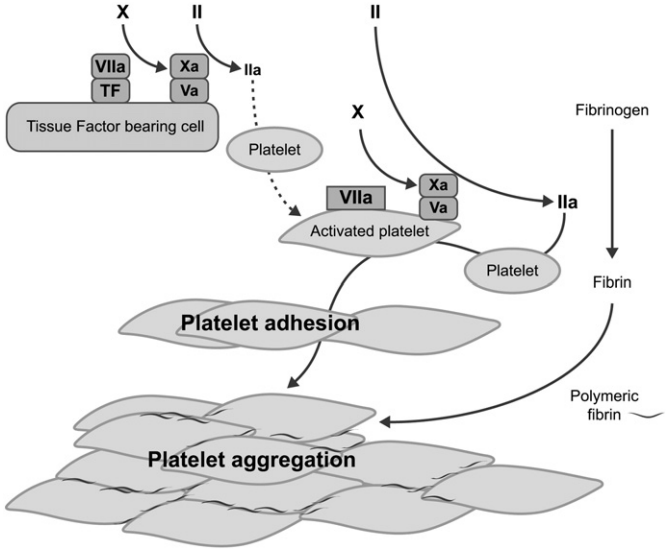

Figure. Adhesion and aggregation in GT platelets deficient in GPIIb-IIIa (integrin αIIbβ3): role of factor VIIa. FVIIa-Tissue Factor (TF) complex on TF-bearing cells at the site of vascular injury activates FX to FXa [25]. FXa-FVa on the TF-bearing cells initiates generation of a small amount of thrombin (FIIa) that is insufficient to provide fibrin formation but sufficient to activate GT platelets, causing degranulation and release of FV. FVIIa binds weakly to the negatively charged phospholipid surface [26] of the activated platelets, with the binding enhanced by the GPIb/IX/V complex [27]. FVIIa at high concentration (attained by high-dose rFVIIa therapy) can directly activate FX to FXa to mediate generation of a high concentration of thrombin (thrombin burst) [26]. The augmented thrombin generation results in an increased number of activated platelets deposited (adhesion) to the wound site and an increased available platelet procoagulant surface to facilitate more thrombin generation and more platelet activation [28,29]. The augmented thrombin generated also converts fibrinogen to fibrin. GT platelets lack the fibrinogen receptor (integrin αIIbβ3); therefore, these platelets cannot use fibrinogen for aggregation. However, binding of fibrin/polymeric fibrin to an unidentified platelet surface receptor can mediate aggregation of GT platelets at the wound site (albeit less potently than fibrinogen-mediated aggregation of normal platelets) [28,29] following the initial platelet adhesion, resulting in the formation of a primary hemostatic plug (adopted from Poon [30] with permission from the publisher).

GT can be classified according to platelet membrane αIIbβ3 protein levels measured using flow cytometry using monoclonal antibodies CD41 (for αIIb) and CD61 (for β3) [31,32]. As shown in Table 1,

Table 1
Diagnosis and classification of GT

Type	Proportion of GT patients	αIIbβ3 expression (platelet membrane)	αIIbβ3 (%) (flow cytometry: CD41: αIIb CD61: β3)	Platelet aggregation (ADP, EP, collagen, thrombin)	Platelet agglutination (ristocetin)	Bleeding time/ closure times (PFA-100)	Clot retraction	α-Granule pool fibrinogen
Type I	~75%	Absent or trace expression	0-5	Nil	Normal	Prolonged	Nil	Nil
Type II	~15%	Substantially reduced	5-20	Nil	Normal	Prolonged	Residual	Subnormal
Variant	~10%	Abnormal αIIbβ3 which cannot bind fibrinogen	>20	Nil/abnormal	Normal	Prolonged	Variable	Variable

Platelet count and morphology are usually normal, although a few families have been reported to have macrothrombocytopenia (see text). Abbreviations: ADP, adenosine 5'-diphosphate; EP, epinephrine.

approximately 75% of GT patients are type I (<5% αIIbβ3, absent platelet α-granule pool fibrinogen, and absent clot retraction), 15% are type II (5%-20% αIIbβ3 with diminished platelet fibrinogen and normal or diminished clot retraction), whereas 10% are variant GT (>20% αIIbβ3, defective receptor function with variable platelet fibrinogen content and clot retraction) [11,33,34].

Bleeding phenotype in GT is heterogeneous (from mild to severe), and the relationship to GT genetic mutations and GT type has not been elucidated. Linkage between the bleeding phenotype and the platelet α2 C807T polymorphism [35] has been reported in a small group of GT patients, suggesting that a milder bleeding phenotype occurs in patients with α2 807-T/T (compared with T/C or C/C) [35]. Typically, patients with GT develop bleeding symptoms early in childhood, although some symptomatic children may improve when they reach adulthood. Of the patients enrolled in the Glanzmann's Thrombasthenia Registry (GTR) [36,37], age of first bleeding symptom was available in 187 (enrolled at age 0.05-50 years). First bleed symptoms occurred at a median age of 1 year (mean, 5.6 years), with 53% by the age of 1 year and 85% by the age of 14 years (unpublished data). GTR is a prospective observational, international registry that enrolled 218 patients with GT from 45 sites in 15 countries worldwide and collected data on effectiveness and safety of rFVIIa and other treatment modalities on 829 bleeding (184 patients) and 204 surgical episodes (97 patients) [36,37]. The bleeding manifestations of 216 of these GT patients are listed in Table 2, which also provides comparative data reported from previous GT patient series [11,20,38]. Bleeding episodes are typically mucocutaneous and include easy bruising, purpura, epistaxis, gingival and other mouth bleeding, menorrhagia, and gastrointestinal bleeding [11,20]. Less frequent bleeding episodes include hemarthrosis and central nervous system bleeding. Bleeding complications are frequent after trauma and surgical procedures including dental extraction, as well as during/after child birth. Sociocultural factors and prevailing moral values may prevent some women from discussing menorrhagia even in clinical settings [39], which may account for the discrepancy in menorrhagia rates in different series.

Bleeding severity in GT is variable and unpredictable and appears to bear little relationship to GT classified according to platelet membrane αIIbβ3 protein levels (ie, types I, II, and variant) [11] or to genetic mutation, as the severity of bleeding may differ even among patients with the same genetic mutation [35], suggesting involvement of other genetic/nongenetic factors in determining the bleeding phenotype in these patients. Overall, GT is considered a severe bleeding disorder, and serious bleeding may be fatal, although overall the mortality rate in GT is relatively low. In a series of 64 French GT patients reported by George et al [11], 54 (84%) had a history of red cell transfusion. Epistaxis is common, particularly in children, and can be so severe as to require transfusion [11,40]. Menorrhagia can be a critical bleeding problem, with a particularly high risk of severe and prolonged bleeding requiring transfusion at menarche.

Hemostatic Management

Management options for the treatment of GT bleeding include local and adjunctive measures for mild bleeding. However, when local

Table 2
Number and percentage of GT patients who have had at least 1 clinical manifestation*

	GTR* Total N = 216 (M/F = 92/124) n (%)	George et al, 1990 [11]¶ Total N = 177 (M/F = 75/102) n (%)	Toogeh et al, 2004 [20]† Total N = 382 (M/F = 204/178) n (%)	Borhany et al, 2012 [38]‡ Total N = 43 (M/F = 20/23) n (%)
Epistaxis	159 (79.2)	129 (72.9)	190 (49.7)	29 (67.4)
Gingival bleeding	135 (61.9)	97 (54.6)	87 (22.8)	19 (44.1)
Dental bleeding§	82 (37.6)	–	–	–
Menorrhagia (female)	67/91** (73.6)	54/55 (98.2)	17/132¶¶ (12.9)	5/5¶¶ (100)
Bleeding at childbirth	7/91 (7.7)	–	–	–
Ruptured ovarian follicle/cyst	2/91 (2.2)	–	–	–
Easy bruising/purpura/petechiae	94 (43.1)	152 (85.9)	58 (15.1)	25 (58.1)
Subcutaneous hematoma	82 (37.6)	–	–	5 (11.6)
Muscle hematoma	28 (12.8)	–	–	–
Hematoma; unspecific	–	–	18 (4.7)	4 (9.3)
Gastrointestinal bleeding	50 (22.9)	22 (12.4)	18 (4.7)	9 (21.0)
Hemorrhoidal bleeding	12 (5.5)	–	–	–
Hemarthrosis	14 (6.4)	5 (2.8)	1 (0.3)	–
Hematuria	15 (6.9)	10 (5.6)	–	6 (14.0)
Circumcision bleeding	11 (5.0)	–	14/204 (6.8)	12/20 (60)
Cephalohematoma	1 (0.5)	–	–	–
Bleeding after minor trauma	10 (4.6)	–	–	–
Bleeding from vaccination	3 (1.4)	–	–	–
Umbilical cord bleeding	–	–	1 (0.3)	–
Hemoperitoneum	6 (2.8)	–	–	–
Hemopericardium	1 (0.5)	–	–	–
Intrahepatic hematoma	–	1 (0.6)	–	–
Central nervous system bleeding	4 (1.8)	3 (1.7)	1 (0.3)	–
Ear bleeding	–	–	–	4 (9.3)
Subconjunctival bleeding	2 (0.9)	–	–	–

Abbreviations: M/F = male/female.
*Data are available on 216 of 218 GT patients enrolled in GTR; bleeding manifestations include those recorded at GTR registration (whether bleeding symptoms were reported or not), admissions (whether treated or not), and those recorded with a history of manifestations before GTR registration/admissions. Of the 829 bleeding episodes (184 patients) treated prospectively and recorded in GTR, 199 (24%) were posttraumatic bleeds [36]. Whether some of the bleeding manifestations before GTR registration/admission were trauma induced (in addition to those reported as bleeding from minor trauma) were not clearly reported. The GTR recorded treatments to prevent surgical bleeding [37]. However, history of bleeding following procedures before GTR registration/admission (apart from dental bleeding, which includes deciduous tooth removal and dental extraction§) and following circumcision were not reported. The final number is higher than the total number of patients because many patients have more than 1 clinical manifestation (median 4, mean 3.69, range 0-10).
§Dental bleeding also includes deciduous teeth eruption and removal, and dental extractions.
**Menorrhagia: calculation based on number of women aged 12 years or older; not included in calculation: "vaginal bleeding" occurring in one 11-year-old girl.
¶¶Calculation based on women of reproductive age.
Patient population: *various ethnic groups from 15 countries; ¶various ethnic groups: 64 French plus 113 from the literature; †Iranians; ‡Pakistani. Although these are distinct rather than cumulative databases, it could not be ascertained whether patients from one database were reported in another. However, the timing of the different databases would make instances of overlapping less likely to occur.

management fails or for more serious bleeding and surgical coverage, the use of systemic hemostatic agents such as platelets and rFVIIa is required. Currently, rFVIIa is approved in the European Union for GT patients with platelet antibodies (to human leukocyte antigen [HLA] and/or αIIbβ3) with past or present platelet refractoriness, whereas in the United States, it is approved for GT patients with refractoriness to platelet transfusions (with or without antibodies to platelets), and in Canada, it is approved for patients with clinical refractoriness and/or platelet antibodies or when platelets are not immediately available [41–43]. However, data from the international, observational GTR, established in 2004 [36,37], suggest that rFVIIa is frequently used off-label for bleeding and surgical procedures, irrespective of platelet antibodies and/or platelet refractoriness, and the proportion of treatments rated "effective" was similar to those treated with other modalities. Nonsurgical bleeds were resolved in 91% (111/122) episodes treated with rFVIIa alone, 82.7% (86/104) treated with rFVIIa + antifibrinolytics, 72.7% (48/66) treated with rFVIIa + platelets ± antifibrinolytics, 78.8% (245/311) treated with platelets ± antifibrinolytics, and 84.7% (183/216) treated with antifibrinolytics [36]. For surgical bleeds in patients without platelet antibodies or refractoriness, the efficacy rate was 100% for both minor and major procedures using rFVIIa alone (minor 24/24; major 4/4), rFVIIa + antifibrinolytics (minor 17/17; major 3/3), and platelets ± antifibrinolytics (minor 11/11; major 5/5) [37]. In patients with antibodies and refractoriness undergoing minor procedures, efficacy rates were 88.9% (16/18) for rFVIIa alone, 100% (19/19) for rFVIIa + antifibrinolytics, 66.7% (2/3) for platelets ± antifibrinolytics, and 100% (3/3) for rFVIIa + platelets ± antifibrinolytics [37]. Eleven major procedures were performed in patients with platelet antibodies and/or refractoriness. Of these, effectiveness was 100% (2/2) for rFVIIa alone, 50% (2/4) for rFVIIa + antifibrinolytics, 100% (2/2) for platelets ± antifibrinolytics, and 66.7% (2/3) for rFVIIa + platelets ± antifibrinolytics. Dosage schedules of rFVIIa for both nonsurgical and surgical bleeds were in line with those previously reported or recommended for GT patients (≥80 μg/kg every ≤2.5 hours for nonsurgical bleeds; 90-140 μg/kg every ≤2.5 hours for ≥2 doses for minor surgery, and more doses for major surgery until hemostasis is secured) [36,37].

The GTR data also reveal a low frequency of adverse events (AEs) associated with rFVIIa use for both nonsurgical and surgical bleeds. Thirty-five AEs were associated with nonsurgical bleeds, 11 of which occurred in patients receiving treatments that included rFVIIa; 3 AEs in 1 patient were possibly or probably related to rFVIIa (nausea, dyspnea, and headache) [36]. There were no thromboembolic events among patients treated with rFVIIa for nonsurgical bleeds. Among patients undergoing surgery, 1 of 4 reported AEs was judged to be related to rFVIIa (nonfatal thromboembolic event in an adult woman treated with rFVIIa + platelets + antifibrinolytics) [37].

These results from the GTR, with the largest data collection thus far, suggest that rFVIIa may be effective with no safety concerns in patients with GT both with and without platelet antibodies and/or platelet refractoriness. However, generalization of these results cannot be made without caution, as data from registries are inherently limited by voluntary participation of enrollment centers from different countries with no guarantee of exhaustiveness in reporting all treatment cases. In this respect, although rFVIIa has recently gained approval in Canada for use in GT patients with refractoriness and/or platelet-specific antibodies, it is of note that rFVIIa is also approved where platelets are not immediately available [41].

Platelet Transfusion as Current Standard Treatment of GT

To date, the standard treatment for serious bleeding and coverage of surgical procedures has been platelet transfusion. Platelets are naturally effective in nonimmune quantitative and qualitative platelet disorders. However, in addition to allergic and immune reactions (anaphylaxis and transfusion-related acute lung injury being the most serious), other potential risks associated with platelet transfusion include blood-borne pathogen transmission and immunization. Furthermore, platelet concentrates, particularly HLA-matched single-donor and leukocyte-reduced apheresed platelets, may not be readily available in some areas or for emergency use.

Blood-Borne Pathogen Transmission

The residual risk of blood-borne virus infection is now very low. Canadian data suggest that the risk per donor exposure was 1:7.8 million for HIV, 1:2.3 million for hepatitis C virus, 1:153 000 for hepatitis B virus, 1:4.3 million for human T-cell lymphotropic virus, and <1:1 million for West Nile virus [44,45]. In contrast, bacterial contamination of platelet concentrate remains the most prevalent risk for transfusion-associated infection because of the storage of platelets at 20°C-24°C, a condition that facilitates bacterial proliferation [46]. Process improvements and monitoring using bacterial culture systems reduced the risk (as of 2009) of transfusion-associated sepsis at around 1:12 000 for pooled whole-blood–derived platelets and 1:60 000 for single-donor (apheresis) platelets; these risks were much higher when improvements were not implemented, being 1:3000 for pooled whole-blood–derived platelets and 1:15 000 for single-donor platelets [47]. A recent review by Katus et al (2014) [48] estimated the residual risk of the transfusion of bacterially contaminated platelets to be 127 to 1885 per million units. Importantly, transfusion-associated sepsis is frequently not recognized by physicians, such that the actual prevalence may be higher, and the correlation between bacterial load and clinical outcome remains ill defined [49,50]. The 2011 report reviewing data from 12 Canadian Blood Agency production sites [51] suggested that although bacterial testing of platelet components and implementation of improved protocols were incrementally effective in reducing the risk of transfusion of bacterially contaminated platelet concentrates, false-negative bacterial culture screening results, implicated in adverse transfusion reaction, continued to occur [51]. Nonetheless, continuous efforts by the transfusion medicine community to reduce bacterial contamination have been effective. The last documented confirmed transfusion-transmitted bacterial infection in the United Kingdom was in 2009, predating universal bacterial culture of platelets. However, there were 2 "near misses" in 2014, where 2 separate units of apheresis platelets were observed by hospital blood banks to have clumps and were not transfused; culture of both units later identified growth of *Staphylococcus aureus* [52]. In the United States, there were only 2 Food and Drug Administration reports of transfusion-related fatalities due to bacterial contamination of apheresis platelets in 2012. There have been only 5 probable or likely septic transfusion reactions between 2007 and 2014 (out of approximately 646 000 platelet doses transfused) reported by the Canadian Blood Services (personal communication, Dr Mindy Goldman, Canadian Blood Services, Canada). In addition to the risk of known blood-borne pathogens, emerging agents, potential or actual, and their potential for severe clinical outcomes (most significantly variant Creutzfeldt-Jakob disease or variant Creutzfeldt-Jakob disease prions, dengue fever virus, *Babesia* sp, xenotropic murine leukemia-related virus, and protozoa) were recently reviewed by the blood banking/transfusion and bleeding disorders community [53–55].

Platelet Immunization

About 50% of nonimmune thrombocytopenic patients receiving platelet transfusion will develop antibodies against the HLAs [56–58], although the incidence is lower (15%-17%) if leukoreduction of the platelet component is practiced [56–58]. GT patients may also develop antibodies to the platelet membrane surface αIIbβ3 antigens. Such antibodies could conceivably result in inhibition of αIIbβ3 function or in the accelerated clearance of the αIIbβ3-bearing platelets. In our previous survey of 59 GT patients treated with rFVIIa, 29 patients (49%) had platelet antibodies (21 against αIIbβ3 and 13 against HLA, with 5 patients having antibodies against both) [59]. In the GTR, of 218 GT

patients treated with various hemostatic agents, 65 (30%) had platelet antibodies (47 against αIIbβ3 and 21 to HLA; unpublished data). In 1 study, 4 of 16 (25%) GT patients followed over a 30-year period developed antiplatelet antibodies: 2 against HLA, 1 against αIIbβ3, and 1 against both [60]. Recent studies suggest that anti-αIIbβ3 antibodies are more likely to develop in patients with homozygous or compound heterozygous mutations that result in premature protein termination [61]. Thus, anti-αIIbβ3 antibodies developed in as many as 13 of 16 (81%) patients with the French Manouche Gypsy mutation (with absence of the αIIbβ3 complex) but only in 2 of 8 (25%) patients with less drastic mutations [61]. Although these findings await confirmation in a larger study, which should take into account both the GT genotype and immune-response–modifying gene changes, this was the first study to suggest a possible relationship between αIIbβ3 gene mutation and the propensity to develop anti-αIIbβ3. Hence, the genetic analysis of GT patients appears to be important; for patients with known mutations that result in truncated or absent αIIbβ3 complex, it may be prudent to suggest that platelet transfusion should be avoided to prevent αIIbβ3 immunization [61]. In the absence of molecular data, some clinicians were concerned by the risk of immunization with platelet transfusion and preferred the use of rFVIIa [59].

The development of platelet antibodies may be associated with refractoriness to future platelet transfusion. This occurs in approximately 50% of patients with anti-HLA antibodies [58,62,63] and is frequent in patients with anti-αIIbβ3 antibodies [59]. Transfused platelets in GT patients with anti-αIIbβ3 may not always be removed immediately [61,64], postulated to be dependent on antibody isotype. Flow cytometry is required to assess the presence of surviving donor platelets after transfusion [64], but this is not always available in every center at all times. Without readily available methods to predict which patients with a history of anti-HLA or anti-αIIbβ3 will be refractory to platelets, the avoidance of platelet transfusion is preferred in these immunized GT patients. However, for severely bleeding GT patients, when alternative therapies fail to secure hemostasis, transfusion with large doses of platelets, with or without antibody removal by plasmapheresis [65] or immunoabsorption [66], should still be attempted.

In contrast, for some patients with a history of platelet refractoriness, antibodies are not always detectable, possibly because the refractoriness is due to the presence of other clinical factors (eg, sepsis, splenomegaly), presumably because the antibodies have disappeared in the absence of new transfusions, or because the antibody titers may be below that able to be detected by the antibody assay used. In an earlier survey of 59 GT patients treated with rFVIIa, platelet antibodies were detected in 17 of 23 patients with a history of platelet refractoriness [59], whereas in the GTR, a history of platelet antibodies was reported in only 24 of 34 patients with a history of refractoriness (unpublished data). Anti-αIIbβ3 may also transfer across the placenta during pregnancy, potentially causing thrombocytopenia and bleeding in the fetus and/or the newborn infant [60,61,67–70]. Thus, it is prudent to avoid platelet transfusion in girls and women of reproductive age to prevent anti-αIIbβ3 immunization.

Recombinant Activated Human Factor VII

Pharmacokinetics and Clearance of High-Dose rFVIIa

The pharmacokinetics of rFVIIa have been studied in hemophilia and FVII deficiency. In severe hemophilia patients in the nonbleeding state who were given a single dose of rFVIIa at 90 μg/kg, the estimated mean initial and terminal half-life was 0.6 and ~2.6 hours, respectively [71]. A similar mean terminal half-life was also reported in children (2.6 vs 3.1 hours in adults; rFVIIa dose: 90-180 μg/kg) [72] and in patients with FVII deficiency (~3 hours, nonbleeding state) [73]. The plasma level of FVII increased proportionally with increasing dose when multiple doses were tested. Of note is the finding of Villar and colleagues [72] that the total body clearance normalized for body weight was significantly faster in children than in adults (FVII:C, 58 vs 39 mL kg^{-1} h^{-1} and FVIIa, 78 vs 53 mL kg^{-1} h^{-1}). A trend toward a larger volume of distribution at steady state in children than in adults was also observed [72]. These findings suggest that higher doses of rFVIIa may be needed in children to achieve the same plasma levels as adults; results from the GTR demonstrate that children required more rFVIIa (expressed in μg/kg) to stop bleeding than that reported for adults [74].

In the circulation, infused rFVIIa is principally cleared by its complex formation with antithrombin, accounting for 65% of an intravenous 90-μg/kg dose of rFVIIa in a hemophilia patient [71]. In mice, hepatocytes and Kupffer cells were also involved in the hepatic clearance and metabolism of both full-length rFVIIa and rFVIIa complexed with antithrombin and α2-macroglobulin [75,76].

How Does High-Dose rFVIIa Work in GT?

Physiologically, FVIIa exerts its hemostatic effect after being complexed with tissue factor (TF). In normal individuals, FVIIa-TF complex formation on TF-bearing cells at the site of vascular injury activates FX and FIX to result in an initial thrombin generation that activates a number of clotting factors (eg, FV, FVIII, FXI) as well as platelets. Activated platelets are recruited to the wound site where they aggregate, mediated in part by the binding of soluble fibrinogen to platelet surface αIIbβ3 [12,14–16], to form the primary hemostatic plug. Activated platelets at the wound site support further coagulation activation to result in thrombin generation sufficient for fibrin formation and hemostasis (the "thrombin burst") [25].

In GT, although this TF mechanism is important in generating the initial thrombin on TF-bearing cells required to initiate platelet activation at the site of tissue injury, the initial thrombin generated is not sufficient to result in platelet aggregate formation required to support thrombin burst at the vascular injury site (because of the lack of αIIbβ3 receptors for fibrinogen binding). GT platelets therefore have impaired thrombin generation capacity [77,78]. Experimental evidence suggests that high-dose rFVIIa supports hemostasis in GT via a TF-independent mechanism (Figure). High-dose rFVIIa can bind with a low affinity (Kd ~100 nmol/L) to the negatively charged phospholipid surface exposed on activated platelets [26], enhanced by the GPIb/IX/V complex on the platelet membrane surface [27]. At high concentrations, bound rFVIIa was able to activate FX to FXa directly, resulting in a burst of thrombin generation and enhancing hemostasis in GT by improving GT platelet adhesion and aggregation [28,29].

Enhanced thrombin generation from rFVIIa bound to activated platelets at high concentrations also improves fibrin clot structure not only in hemophilia plasma [79] but also in plasma from a patient with GT, improving hemostasis by decreasing clot permeability and tightening the fibrin network [80]. Similar improvement in fibrin structure was observed using confocal 3-dimensional microscopy of clots formed during thrombin generation after the use of GT platelet-rich plasma obtained after in vivo administration of therapeutic rFVIIa [78]. Two other recent observations of rFVIIa action in hemophilia and other bleeding disorders may also be pertinent in GT: (1) rFVIIa added to normal, FVII-deficient, and Bernard-Soulier syndrome platelet-rich plasma was shown to internalize to the platelet cytoplasm and redistribute to the open canalicular system and α-granules [81], thereby improving platelet aggregation and fibrin generation in perfusion studies. Whether this rFVIIa action occurs in GT has not yet been studied. (2) Infusion of rFVIIa in hemophilia patients with inhibitors also results in a transient increase in procoagulant platelet microparticles that promotes hemostasis [82]. Whether rFVIIa also releases platelet microparticles in GT remains to be explored.

Curative Management

HSCT as Current Curative Treatment

Although currently available treatment strategies for GT are very effective with a good safety profile, a small number of GT patients may continue to have persistent and recurrent life-threatening bleeding refractory to current hemostatic treatments, particularly patients with platelet antibodies and refractoriness. Management with HSCT is a

possibility for these patients and has been successfully performed, resulting in the correction of the bleeding disorder and improved quality of life. Between 1981 and 2015, 43 GT patients who underwent an allogeneic HSCT (age: median 9 years, range 1-53) have been registered with the Center for International Blood and Marrow Transplantation Research (CIBMTR) with various donor and graft sources and conditioning regimens. As of October 16, 2015, 35 (81%) transplanted patients remained alive, 3 (7%) were dead, and 5 (12%) were missing at a median 47 months (range, 3-120) of follow-up of survivors (preliminary data obtained from the Statistical Center of the CIBMTR; the analysis has not been reviewed or approved by the Advisory or Scientific Committees of the CIBMTR). A review of the literature found reports of at least 16 children (7 boys, 9 girls; age 13 months to 16 years, median 4.22 years) who had undergone 17 HSCTs, primarily for type I GT with a serious bleeding phenotype [83–91]. Of the 16 transplants that were successful, HLA-matched stem cell transplantation (SCT) sources included bone marrow from siblings (n = 10, 5 with heterozygous GT [84,85,88,90,91], 5 presumed normal [83,86,87]) and unrelated normal donors (n = 2) [83,86], peripheral blood from matched family members (n = 2) and unrelated cord bloods (n = 2) [89]. Successful conditioning regimens included full-intensity (myeloablative) conditioning in 13 [83–91] and reduced-intensity conditioning in 3 [86]. All but 1 of the successful transplantations resulted in complete engraftment. One child receiving peripheral blood stem cells from a matched family donor following reduced-intensity conditioning resulted in mixed chimerism with 30% of the donor cells, which was sufficient to correct the bleeding disorder [86]. The only HSCT failure was the first patient reported by Bellucci and colleagues [84]; the boy rejected the initially transplanted graft following reduced-intensity conditioning but was successfully retransplanted from the same sibling donor using a full-intensity conditioning regimen. It is uncertain whether reporting bias exists such that cases of unsuccessful transplantation have not been reported.

In addition to the 16 children transplanted primarily for GT, a 52-year-old man with acute myelogenous leukemia (FAB-M2) and type I GT as a comorbid condition was also reported to have successfully undergone transplantation of marrow stem cell from an unrelated donor following a full-intensity conditioning regimen [92].

Although HSCT merits careful consideration for the treatment of selected GT patients with persistent and life-threatening hemorrhage, the decision to transplant must be weighed against the significant risks associated with HSCT. Paradoxically, serious hemorrhage may be caused during the peritransplant period, especially when anti-αIIbβ3 development coincides with routine procedures including central venous catheter placement or conditioning-associated mucosal disruption [89], and during the thrombocytopenic phase [91]. For example, tunneled central venous catheter placement almost resulted in a fatal hemorrhage in 1 patient [89]. Graft-vs-host disease (acute and/or chronic) may also develop and require immunosuppressive treatment. Full-intensity conditioning may also carry considerable adverse effects such as infertility, growth retardation, and the risk of secondary malignancy [86]. Although reduced-intensity conditioning may reduce morbidity and mortality, the risk of graft rejection is higher than with full-intensity conditioning and HSCT [84,86,89].

Gene Therapy as Potential Future Curative Treatment

Although gene therapy remains experimental, the prospect that this technology may become a viable treatment option for patients has improved with the significant progress made in in vitro culture and in animal studies. Wilcox and colleagues [93] demonstrated that transduction of granulocyte-colony stimulating factor–mobilized peripheral blood stem cells with an oncoretrovirus vector encoding integrin β3 generated de novo synthesis of viable integrin αIIbβ3 complex on megakaryocytes from human GT patients with *ITGB3* mutation. Furthermore, in a murine GT model (*ITGB3* mutation), transplantation of bone marrow transduced with a lentivirus vector encoding β3 resulted in the correction of platelet function [94]. It was therefore hypothesized that transplanting autologous hematopoietic stem cells transduced with genes encoding normal integrin αIIb or β3 could result in de novo synthesis of a biologically normal integrin αIIbβ3 within megakaryocytes and allow trafficking of the entire receptor to the cell surface of the platelets produced [95]. A GT dog gene-therapy model (Great Pyrenees with *ITGA2B* mutation) was established by transplanting granulocyte-colony stimulating factor–mobilized autologous peripheral blood stem cells transduced with lentivirus vector encoding human αIIb [95]. For each of the 3 dogs, followed for 2, 4, and 5 years, the percentage of platelets that expressed αIIbβ3 stabilized to approximately 10% of the total platelet population [95]. Although this level of αIIbβ3 is considered pathologic in humans (in keeping with that observed in type II GT), it was sufficient to result in significantly decreased cutaneous bruising and shortening of the mucosal bleeding time [95]. Blood loss was reduced 20- to 135-fold as compared with the control dogs. Laboratory studies also demonstrated partial correction of aggregation of washed platelets to a mixture of activation agonists (ADP, epinephrine, and canine thrombin receptor–activating peptides: TRAP-1, -3, and -4), improved platelet adhesion on immobilized fibrinogen, and improved clot retraction [95].

Recently, Sullivan and colleagues [96] reprogrammed monocytes of 2 type I GT patients with *ITGA2B* mutations to induce pluripotent stem cells (iPSC). Insertion of GP1ba promoter-driven human αIIb cDNA into the AAS1 locus of the iPSCs resulted in high expression of αIIb mRNA and protein in the iPSC-derived megakaryocytes that responded to agonist stimulation, suggesting recovery of cell surface integrin αIIbβ3 expression and activation.

Although these studies represent a milestone toward gene therapy in human GT, further improvements are required for its clinical development. Efficient and safe vectors designed to enable transgene delivery and stable, high expression in humans are required. Furthermore, strategies need to be developed to overcome elimination of the vector or gene products by host immune response and also to prevent insertional mutagenesis of the transgene.

Conclusions

Since the time of its first description in 1918 [1], GT has become a well-understood congenital bleeding disorder of platelet membrane surface integrin αIIbβ3 with salient clinical, laboratory, biochemical, and genetic features. Because of the rarity of this disorder, understanding of its management has been hindered because few clinicians have extensive experience with GT, and randomized clinical trials are difficult to perform. Currently available hemostatic agents for GT patients with bleeding unresponsive to conservative treatment or undergoing surgical procedures include platelet transfusion and rFVIIa. Although platelet transfusion is a time-honored and effective treatment for GT patients, one of its disadvantages is that a proportion of patients who develop antibodies to HLA and/or αIIbβ3 are refractory to this treatment. In addition, as with other blood products, blood-borne pathogen transmission, in particular bacterial infections, is a concern, and much research is in progress to improve processes and to develop bacterial culture systems to lessen this risk. Recombinant FVIIa, as an alternative hemostatic agent, has been reported to be effective with a good safety profile. Much progress has been made over the past 10 years in understanding the mechanisms of action of rFVIIa in effecting hemostasis in GT. Curative treatment with HSCT is possible but only with considerable risk of HSCT-related adverse effects and should be considered exclusively for patients with persistent severe bleeding refractory to hemostatic treatment modalities following careful risk/benefit assessment. Gene therapy is a curative therapy for the future, and its progress is impressive and promising.

The GTR has prospectively collected data from 45 clinics from 15 countries on the treatment of 829 bleeding episodes in 184 GT patients, and 206 surgical procedures in 96 GT patients, with rFVIIa and/or

platelets and/or antifibrinolytics [36,37]. To date, the GTR represents the largest collection of data available on the treatment of bleeds in GT. It is hoped that these data will contribute toward the development of clinical "recommendations" for the management of GT patients using platelets or rFVIIa until a higher level of evidence may become available from randomized clinical trials (which are difficult to achieve because of the rarity of the disorder) or until a safe curative treatment is available.

Disclosures

MCP was chair of Novo Nordisk's expert panel on the GTR; has been an ad hoc speaker for Bayer, Novo Nordisk, and Pfizer; attended advisory board meetings of Biogen-IDEC, CSL-Behring, Novo Nordisk, Octapharma, and Pfizer; and received grant funding from CSL-Behring. GDM has been a speaker or a member of a speaker's bureau for Boehringer Ingelheim, Sanofi-Aventis, Bayer, Novo Nordisk, Pfizer, Biotest, and Grifols and has also acted as a consultant or ad hoc speaker/consultant for Boehringer Ingelheim, Eli Lilly, Sanofi-Aventis, Bayer, CSL Behring, Novo Nordisk, Pfizer, Biotest, and Grifols. Rd'O received fees or honoraria for attending advisory boards or speaking at symposia from Baxter, Novo Nordisk, Bayer, Pfizer, Sobi, and CSL Behring. RBZ has been a speaker for Sanofi-Aventis, LEO Pharma, GlaxoSmithKline, Bayer, Boehringer Ingelheim, Pfizer, Novo Nordisk, CSL Behring, MEDA, Novartis, Octapharma, Biotest, Wyeth, and AstraZeneca and received fees from Biotest, CSL Behring, GlaxoSmithKline, Novartis, Sanofi-Aventis, and Wyeth. He is a member of the advisory boards at Novo Nordisk, Pfizer, and Bayer. He received research funding from CSL Behring.

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