Congenital Bleeding Disorders

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Both clinical and basic problems related to the congenital bleeding disorders continue to confront hematologists. On the forefront are efforts to bring genetic correction of the more common bleeding disorders such as hemophilia A to the clinic in a safe and accessible manner. A second issue, particularly for patients with hemophilia, is the development of inhibitors—questions of how they arise and how to prevent and treat these problems that confound otherwise very successful replacement therapy and allow patients to maintain normal lifestyles. A third issue is the continuing question of diagnosis and management of von Willebrand disease, the most common congenital bleeding disorder, especially in individuals who have borderline laboratory values, but have a history of clinical bleeding.

In Section I, Dr. Christopher Walsh discusses general principles of effective gene transfer for the hemophilias, specific information about viral vectors and non-viral gene transfer, and alternative target tissues for factor VIII and factor IX production. He highlights information about the immune response to gene transfer and reviews data from the hemophilia gene transfer trials to date. The future prospects for newer methods of therapy such as RNA repair and the use of gene-modified circulating endothelial progenitors are presented as possible alternatives to the more traditional gene therapy approaches.

In Section II, Dr. Nigel Key focuses on inhibitor development in patients with hemophilia A. He reviews the progress in our understanding of the risk factors and presents newer information about the immunobiology of inhibitor development. He discusses the natural history of these inhibitors and the screening, laboratory diagnosis, and treatment, including the use of different modalities for the treatment of acute bleeding episodes. Dr. Key also presents information about the eradication of inhibitors by immune tolerance induction and reviews recent information from the international registries regarding the status and success of immune tolerance induction.

In Section III, Dr. Margaret Rick discusses the diagnosis, classification, and management of von Willebrand disease. Attention is given to the difficulty of diagnosis in patients with mild bleeding histories and borderline laboratory test results for von Willebrand factor. She presents the value of different laboratory assays for both diagnosis and classification, and she relates the classification of von Willebrand disease to the choice of treatment and to the known genetic mutations. Practical issues of diagnosis and treatment, including clinical cases, will be presented.

I. Gene Transfer for the Hemophilias

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Genetic correction of the hemophilias is a model system for developing a basic understanding of how gene therapy will be achieved. The goals for hemophilia gene transfer require the long-term therapeutic production of the coagulant protein without stimulation of an immune response to the transgene product or the vector. Several groups have demonstrated sustained expression of clotting factors at therapeutic levels in animal models of hemophilia leading to Phase I clinical trials. To date, 5 different trials, 3 for hemophilia A and 2 for hemophilia B, have enrolled approximately 40 patients with severe hemophilia. Here we will focus on the current gene transfer strategies for treating the hemophilias.

Current treatment for hemophilia-related bleeding episodes uses intravenous infusion of purified and recombinant factor proteins, which is effective but transient because of the short half-life of the proteins. This treatment is expensive, restricts the prophylactic use of factors that can lead to crippling joint disease, and may transmit infectious agents. Effective hemophilia gene transfer requires that a sustained, long-term (for years) production of coagulation factor at therapeutic levels be generated. Thus, the method of gene delivery must

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be safe, and the risk of immune response to potential neoantigens must be minimal. Given recent scientific and technical developments, genetic correction of hemophilic patients is now viewed as an achievable goal.

The factor VIII and IX genes (*F8* and *F9* respectively) and protein products (FVIII and FIX) have been extensively studied.[1] Many tissues and cell types (skeletal muscle, liver, spleen, and skin) are capable of producing and expressing fully functional FIX protein. Although there is debate as to the minimally hemostatic factor level, ≥ 5% of the normal factor level is sufficient to convert a severely affected patient with frequent spontaneous bleeding episodes (patient with < 1% factor level) to a moderate or mildly affected level. Coagulation assays are standardized, and animal models (knockout mice and hemophilic canines) that mimic the human phenotype are available for testing. A variety of gene transfer approaches are currently being tested both in the laboratory and in the clinic. Results of 2 clinical trials, using nonviral and viral-based gene transfer approaches, show that despite low factor levels, patients required less factor infusion and reported fewer bleeding episodes. Although neither trial included a placebo arm, these results support clinical observations that even relatively low levels of factor dramatically reduce spontaneous bleeding. Despite the current excitement, there is a need for improved vectors. Here we will review the recent advances over the past 2 years in this field, which mirror the advances in the field of gene transfer in general.

Viral Vectors

Depending on the biology of the particular viral vector used, vectors exhibit either long-term gene expression (months to years) as a result of integration into the host cell genome or transient expression (weeks to months) due to the lack of stable persistence of the transferred gene within the target cell. Vector transgene expression is regulated within target cells depending on the transcriptional elements used regardless of whether the transgene is in an integrated or non-integrated state.

Adenovirus vectors (rAd)

Hemostatic levels of factors VIII and IX were reached with first- and second-generation adenovirus vectors. Unfortunately, the exuberant cell-mediated immune response engendered by this vector leads to inflammatory responses directed at transduced cells with the attendant loss of protein expression. Newer gutless adenovirus with a minimum of endogenous adenoviral genes may limit the immune response, but as a consequence gene expression is reduced, requiring more vector to achieve the desired outcome.

Adeno-associated virus (AAV)

rAAV has recently come to the fore because it is relatively easy to prepare, can infect both dividing and nondividing cells, and does not stimulate a cytotoxic lymphocyte response to infected cells. It does engender a humoral immune response to its protein capsid coat. Using rAAV2, therapeutic levels of FIX and FVIII were demonstrated in knockout mice and hemophilic canines.[2] Eight serotypes of AAV have been isolated and cloned (AAV1-8). Of these, AAV2 was the first cloned and most extensively studied. Surprisingly, other serotypes yield FIX expression at levels 2 logs greater than AAV2 following skeletal muscle injection into mice[3] and produce sustained supratherapeutic factor levels leading to complete loss of the bleeding diathesis. Such levels are achieved as a result of increasingly effective skeletal muscle gene transfer. Here a linear relationship exists between input vector and factor expression. A unique side benefit is the lack of an immune inhibitor response, presumably because of continuous factor production as the major determinant for inducing tolerance. This result is reminiscent of immune tolerance strategies currently used in the clinic with protocols using repeated, frequent infusion of factor.

AAV1 produces robust transgene expression in muscle, but the exact mechanism is unclear. Data using AAV1 suggested that the number of transduced muscle fibers infected increases significantly with AAV1 compared with AAV2. The reasonable working hypothesis explaining the serotype transduction differences lies in the level of virus specificity for binding cell receptors. At present, the receptor for AAV1 has not been identified but appears distinct from AAV2's.

AAV serotypes 7 and 8 recently isolated from primates infected with high-dose adenovirus[4] are not neutralized by heterologous antisera raised to the other serotypes. rAAV7 and 8 vector particles carrying the alpha-1-antitrypsin complementary DNA (cDNA) were compared for transducing effectiveness in mice. AAV7 was equivalent to AAV1 in efficient expression in skeletal muscle, whereas AAV8 expressed protein at a 10- to 100-fold greater rate in liver-directed expression compared with all other serotypes. These data confirm that relatively small differences in the capsid structure produce striking differences in transgene expression in a wide variety of tissues.

Lentivirus

Lentiviral vectors have the potential to play an important role in hemophilia gene therapy. Lentivirus vectors derived from either human immundeficiency virus (HIV)-1, equine infectious anemia virus (EIAV) and the feline leukemia virus (FeLV) are integrating vec-

tors that have been modified to infect a broad range of cell types. The key feature of lentiviral biology is that target cells are not required to undergo cell cycling for transfer of the proviral complex to the nucleus. This feature allows for lentiviral gene transfer into quiescent, non-cycling cells. One study used HIV-based lentiviral vectors containing human FVIII (hFVIII) or human FIX (hFIX) cDNA for portal vein injection into C57Bl/6 mice. Increasing doses of hFIX-expressing lentivirus resulted in a dose-dependent, sustained increase in plasma hFIX levels up to approximately 50–60 ng/mL (normal 5000 ng/mL). Partial hepatectomy resulted in a 4- to 6-fold increase in plasma hFIX, up to 350 ng/mL, compared with the nonhepatectomized animals, suggesting that cell division enhanced vector transduction. In studies with mice using lentiviral vectors for FVIII gene transfer, the expression of plasma hFVIII reached 30 ng/mL (15% of normal) but was transient as the plasma levels fell concomitant with the formation of anti-hFVIII antibodies.[5-7]

Nonviral gene transfer

Because of the potential safety issues associated with viral vectors, an alternate approach is to use naked plasmid DNA that carries the gene of interest along with genetic elements that promote integration of the transgene within the genome. Early attempts at using such a system have provided encouraging results.[8]

Alternative Target Organs/Tissues for Factor VIII and IX Production

The liver is the principal organ synthesizing the coagulation factors. However, other organs can synthesize FIX and FVIII. FIX can be expressed in skeletal muscle, fibroblasts, keritinocytes, intestinal mucosa, cells lining the amniotic cavity, and marrow stroma. FVIII transgene–expressing endothelial cells in the circulation are capable of secreting high levels of FVIII for a sustained period in animal models.[9] Circulating endothelial cells obtained from peripheral blood are expanded ex vivo and then genetically modified to express the gene of interest. The coexpression of FVIII and von Willebrand factor (vWF) in endothelial cells may explain the high factor levels observed in vivo. The half-life of fully differentiated endothelial cells remains to be determined. Ex vivo gene transfer of gene-modified hematopoietic progenitor cells and marrow stroma is also capable of FVIII secretion in vivo,[10] but factor levels are substantially lower than other target organs such as liver.

Immune System Response to Gene Transfer of Coagulation Factors

The antibody responses to exogenous factor replacement, termed *inhibitors*, affect nearly 20% of FVIII patients and 3% of FIX patients. Inhibitory antibodies that bind to the particular regions of the factor molecule inactivate the factor by changing its protein conformation.[11] In general, the immune response seems to result from the type of genetic mutation. For example, a large deletion in the factor VIII gene and complete loss of protein typically lead to a greater incidence of inhibitor formation. Bleeding episodes in patients with inhibitors are difficult to manage with current therapy that relies on activated bypass factors and recombinant factor VIIa. Will a constant source of factor engender high-titer inhibitory antibodies, negate any positive benefit, and worsen bleeding? Because the answer to this question is unknown, current gene transfer clinical trials exclude patients with inhibitors.

Activation of $CD4^+$ subsets in humans and Th-1 and Th-2 lymphocytes in mice suggests that major histocompatibility complex (MHC) class I and II mechanisms are both involved in inhibitor formation. Involvement of both central (marrow, thymus) and peripheral (lymph nodes, Peyer's patch) mechanisms inducing tolerance to factor VIII has been described but is poorly understood. In addition to the immune response to the transgene factor proteins, immune responses to viral vector capsid of adenovirus and AAV prevents readministration of vectors.

Hemophilia gene transfer clinical trials

Within the past 2 years, 5 gene transfer trials have been approved in the United States (3 for hemophilia A, 2 for hemophilia B). All 5 trials were sponsored by biotech firms that developed vectors specifically for FVIII and FIX. The first hemophilia gene transfer trial (Trial 1, **Table 1**) was a Phase I dose escalation study enrolling 13 subjects with severe hemophilia A.[12] Subjects received an amphotropic retroviral vector carrying a B-domain deleted hFVIII gene by intravenous infusion. Infusions of vector ranging from 3×10^7 to 9×10^8 viral particles **(**vp**)**/kg were administered to patients with documented HIV and hepatitis C virus (HCV) infections and were well tolerated. FVIII was measured, and no subject had sustained factor VIII levels > 1% of normal levels. Although there was, on occasion, reduced self-infusion of factor, overall no significant decrease in bleeding frequency was observed, and there was no objective correlation between vector dose and FVIII activity response. This clinical outcome is consistent with the limited capability of retroviral integration into nondividing liver cells and the lack of

Table 1. Summary of hemophilia gene transfer trials.

Sponsor	Trial No.	*n*	Vector/Route	Factor Level†	Side Effects
Chiron	1—Phase I	13	Retrovirus/IV	0–1%	None
TKT	2—Phase I	6	Plasmid/omentum	0–4%	None
Avigen	3—Phase I	9	AAV2/IM	0–1%	None
Avigen	4—Phase I	6	AAV2/intrahepatic	3–12%	Elevated transaminase‡
Genstar	5—Phase I	3	Adenovirus/IV	0–1%	Elevated transaminase,‡ thrombocytopenia

Abbreviations: AAV2, adeno-associated virus, type 2; IM, intramuscular; IV, intravenous.
†Range of factor levels indicated; all patients were at < 1% before study.
‡Transient elevated transaminase levels/dose-dependent.

a liver-specific promoter in the retroviral vector used.

A trial (Trial 2) has used a nonviral approach and a FVIII plasmid that was electroporated into autologous skin fibroblasts. Cells were expanded in vitro and 100 or 400 million cells were subsequently injected into the greater omentum.[13] Increases of FVIII above pretreatment levels were measured in 4 of 6 patients with either a concomitant reduction in the use of recombinant FVIII or a decreased number of spontaneous bleeding episodes. However, FVIII decreased to pretreatment levels in all patients after 12 months. The decline in factor expression may have been due to gene silencing, immunological clearance, or senescence of the fibroblasts after reimplantation.

Trial 3 using an AAV2 vector carrying hFIX cDNA injected intramuscularly was carried out in 9 patients in a dose escalation study.[14,15] One patient receiving the lowest dose (2×10^{11} vp/kg) was reported to maintain factor levels at 1% to 2% and had a 50% reduction in both factor usage and bleeding episodes for up to 40 months postinjection. No evidence of inhibitor development was reported despite preclinical data in dogs showing a transient inhibitor response. Dissemination of virus was transiently detectable in all body fluids, excluding semen. At higher doses of virus, no significant plasma factor levels were reported. However, as reported in Trial 1, investigators found a patient self-reported reduction in the frequency of factor usage signifying treatment effectiveness. An increase in the number of injection sites from 10 to 90 produced no significant increase in factor level. Molecular analysis of virus dissemination was detectable in all body fluids (saliva, blood, and urine) but not in germ cells. All patients had low (1:100–1000) preinjection anti-AAV2 neutralizing titers that increased after vector administration. High-titer neutralizing antibodies to AAV developed in all patients at levels sufficient to preclude readministration of vector. Muscle biopsy confirmed previous observations of AAV2 tropism in animals, showing that only slow-twitch muscle fibers expressed factor IX. No placebo control group was incorporated into this study.

A dose-escalation study based on AAV2 vectors (Trial 4) carrying hFIX cassettes delivered via the hepatic artery has begun. Six subjects have received rAAV2 ranging in doses from 8×10^{10} to 2×10^{12} vector genomes (vg)/kg via hepatic artery delivery. All subjects had severe hemophilia B (FIX < 1%), and all had positive HCV serology without evidence of fibrosis on biopsy. In 4 subjects receiving low and intermediate virus doses, no vector toxicity was observed, but patients failed to achieve factor IX levels > 2%. The trial was temporarily halted because of detection of the transgene in seminal fluid, and the trial resumed based on data that germ cells were not infected with the virus. Two patients in the high-dose cohort received 2×10^{12} vg/kg. Patients had circulating factor IX levels from 3% to 12% of normal hFIX 2 to 3 weeks after injection; however, 6 weeks after vector infusion the FIX levels dropped to baseline (< 1.0% of normal). The loss of FIX coincided with a 9-fold elevation of the liver transaminases. Whether these results represent toxicity of the vector alone or result from underlying liver disease remains to be determined.

Trial 5 used a "gutless" adenoviral vector carrying the hFVIII cDNA. It was hypothesized that deletion of the endogenous adenovirus coding region retained in earlier vectors would lessen or eliminate the well-described cytotoxic immune response against the virus. Nonhuman primates who received high doses intravenously developed elevated serum transaminases and thrombocytopenia. Intravenous administration of gutless adenoviral vector in 3 severe hemophilia A patients was performed. At low dose, no observed factor VIII level was achieved, and at higher doses one patient developed marked thrombocytopenia and elevated transaminases without a significant elevation in FVIII.

What do these clinical results tell us? Although the goal of long-term therapeutic factor expression has not been achieved, the data are encouraging. Detectable

factor levels were observed. Given the potential immune response to the vector and the transgene protein, it was reassuring that the development of FIX or FVIII inhibitors was not detected in any of the 5 trials. Hepatic toxicity (Grade II-III) was reported in 2 of the trials where the liver was the target organ. Factor levels predicted from animal models were not observed in subjects except for rAAV2 infusions into liver circulation. rAAV2 toxicity observed in human subjects was not observed in mouse, dog, or nonhuman primate animal models at equivalent vector doses, whereas preclinical testing in animals with gutless adenoviral vector predicted the observed toxicity in humans. Thus, although testing of new factor proteins in hemophilic animals is traditionally used because of pharmacokinetic profiles similar to those seen in humans, such extrapolation using gene transfer vectors is not clear-cut, and animal studies may not be predictive of the clinical outcome.[16-18] These outcomes reflect species differences in terms of the rate of cell infectivity, gene expression, protein modification, processing, and immune response. Testing in different animal models serves to confirm the validity of each new approach.

Future Prospects

RNA repair

A novel approach for genetic correction is the use of pre-messenger RNA (pre-mRNA) repair. RNA transsplicing uses endogenous splicing mechanisms to correct a portion of the defective RNA. A pre-mRNA containing a portion of correct gene sequence is designed to base pair with the pre-mRNA transcribed from the defective gene. The designed pre-mRNA also contains all the requisite splicing signals that allow 2 independent mRNAs to splice together, resulting in a correct copy of mRNA that is translated into a normal protein.[19] The advantage of this system is that large genes that are unable to be packaged into viral vectors, or genes that contain large regulatory elements, could be corrected by using the smaller spliced sequences. Such a system was developed for FVIII correction. The *F*8 exon 16 knockout mice were used to test transsplicing in vivo. Here, a designed pre-mRNA encoded for exons 16 to 26, the region not transcribed in the knockout mice; 2% to 6% factor VIII levels were generated, sufficient amounts to prevent a bleeding challenge.[20] After direct injection of a plasmid encoding a pre-mRNA, factor VIII was detected over 3 to 5 days; by comparison, injection of AAV containing transsplicing constructs yields ~2% to 4% FVIII for 3 to 4 months. As transsplicing efficiency improves, RNA repair may be useful for the treatment of autosomal dominant disorders or cDNA too large to be packaged by current viral vectors.

Gene-modified circulating endothelial progenitor

The use of blood outgrowth endothelial cells (BOEC) as a source of cells synthesizing factor VIII has been described.[9] These circulating endothelial progenitor cells are isolated from peripheral blood, expanded in culture, and modified genetically to carry the normal h*F8* gene. A significant advantage is the synthesis of vWF in these BOEC clones that are expanded; vWF serves as the carrier protein necessary for factor VIII stability in plasma. Major questions regarding the use of BOEC include their half-life, their distribution in vivo, their rate of expansion in vivo, and the potential for uncontrolled growth following transplantation.

Gene-modified stem cell therapy

Recent reports on the plasticity of stem cells derived from adult tissue such as liver, brain, muscle, skin, and fat cells have generated enormous interest in using stem cells for the genetic correction of hemophilia. Cells that co-purify with mesenchymal stem cells termed multipotent adult progenitor cells or MAPCs derived from marrow stroma can be induced to differentiate into cell types with neuroectoderm, endoderm, and mesoderm characteristics.[21] When MAPCs are injected into irradiated animals, they differentiate into hematopoietic lineages as well as epithelium of the liver, gut, and lung. MAPCs could potentially be genetically modified to synthesize coagulation factors before retransplantation. Advantages include ex vivo expansion and gene modification with selected clones that produce high levels of factor. Using autologous stem cells derived from each patient would avoid transplantation rejection and the need for immunosuppression. Current disadvantages include the long lead time (months) required to generate the number of cells for transplantation and the ability to control the differentiated fates of the transplanted multipotential cells.

Summary

Data suggest that genetic correction of the hemophilias is feasible. The subjective reporting by patients of decreased bleeding episodes at nominal levels of factor strongly hint that reasonable factor levels might be reached and that a major breakthrough in the treatment of hemophilia is ahead. Gene transfer for hemophilia requires a combination of vector delivery systems, animal models, and clinical studies designed to answer specific questions. These studies will both improve the treatment of hemophilia patients and instruct others in the field of gene transfer. It is hoped that this work will

represent a milestone in the use of genetics for treatment of human ailments.

II. Inhibitors in Congenital Coagulation Disorders

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Inhibitory antibodies that neutralize the procoagulant function of therapeutically administered clotting factors have been described as a complication of a number of congenital factor deficiency states, including deficiencies of factors VIII and IX (hemophilia A and B respectively), factor XI, and von Willebrand factor. Rarely, inhibitors have also been described in congenital deficiencies of factors V, VII, X, and XIII. Although the presence of these inhibitors generally does not change the site, frequency, or initial severity of bleeding, it is a feared complication because of the resultant difficulty in achieving hemostasis in the event of bleeding.

This review will primarily focus on hemophilia A, where progress has been made in recent years in our understanding of risk factors, immunobiology, natural history, and therapy of FVIII inhibitors.

Screening and Laboratory Diagnosis

FVIII inhibitors should be quantified using a Bethesda assay modified according to the Nijmegen method, in which false positive results are avoided by the addition of a buffer to prevent pH shift during the 2-hour incubation.[1] High responder antibodies are defined as those with a peak activity > 5 BU/mL that are associated with anamnesis. In many, but not all high responder patients, the inhibitor titer will regress to low (1–2 BU) or even undetectable levels following several months of abstinence from FVIII. However, the titer will typically begin to rise within 3–7 days of reexposure to FVIII in an anamnestic response.

Occasionally, nonneutralizing FVIII inhibitors are encountered which do not produce any significant interference with the procoagulant functions of FVIII, but do accelerate in vivo clearance. When suspected, a factor recovery and half-life study, conducted over 24–48 hours, may demonstrate reduced (< 66% expected) recovery, and an abnormally short half-life (< 6 hours).

Epidemiology and Risk Factors for Inhibitor Development

Prospective studies in previously untreated severe hemophilia A patients have shown that inhibitors develop in 22%–31%, with a median of 9–11 FVIII exposure days before inhibitor appearance.[2] Up to one third of these inhibitors are transient and of relatively little clinical significance, while about 80% of the remainder are of the high responder type. It is generally agreed that the incidence of inhibitors is no greater with first or second generation rFVIII products compared to intermediate purity products, although the jury is still out with respect to the third generation rFVIII, Advate™. While the inhibitor formation rate is higher than would be expected by chance in brother pairs, the concordance rate is less than 100% in monozygotic twins.[3] Certain races, especially those of African origin, have a higher risk of inhibitor development. These observations suggest a genetic predisposition, but with incomplete penetrance. The association of inhibitors with HLA class II determinants is weak at most.[4] However, more disruptive mutations in the FVIII gene, including the intron 22 inversions, large gene deletions, and stop codons, are associated with about a 35% risk of inhibitor formation, compared to only about 5% in those with missense mutations and small deletions.[5] As expected, the more profound mutations tend to be associated with an absence of circulating factor VIII antigen (CRM negative). On the face of it, the absence of tolerizing FVIII antigen conveniently explains the immune response to a "foreign" protein. However, while the risk of inhibitor formation in patients with mild or moderate hemophilia is much lower (3%–13%), certain missense CRM^+ FVIII gene mutations that cause mild or moderate hemophilia A are also a risk factor for inhibitor development.[6] Mutations that result in a stable abnormal conformation in the FVIII molecule (for example, due to the introduction of a Cys residue that may lead to the formation of aberrant disulfide bonds) are at particularly high risk of inhibitor development. Interestingly, inhibitors in these patients with less severe variants of hemophilia tend to develop at times of intense factor exposure, usually in the setting of a major injury or surgery, indicating that the risk of antibody formation may in part be determined by the context in which the potential immunogen is presented to the immune system.[7] Intriguingly, recent reports suggest a possible heightened risk of inhibitor formation in previously treated, seemingly lower risk patients—many of whom had less severe variants of hemophilia—when FVIII is administered by continuous infusion.[8] Whether this is a function of continuous high-level exposure, or structural/conformational modification of the reconstituted FVIII molecule in the syringe pump/tubing is unclear. The latter possibility is suggested by the

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2 outbreaks of inhibitors that occurred in Northern Europe in the 1990s; in one of these, partial autolysis of FVIII that produced a 40 kD fragment during fractionation was shown to be responsible for the development of anti-C2 domain inhibitors.[9]

Finally, recent reports have suggested that the incidence of inhibitors is reduced in children who avoided exposure to FVIII early in life, with very few reported inhibitors in patients receiving their first FVIII exposure at age > 1.5 years.[10,11] However, these studies were not controlled for FVIII genotype, and are in apparent contradiction to reports of the first appearance of FVIII inhibitors in adult patients after their first intensive FVIII exposure (usually during a surgical procedure).[12]

In summary, it seems likely that a complex interplay of several variables—many of which are incompletely understood—determines the risk of inhibitor formation.

Immunobiology of FVIII Inhibitors

FVIII inhibitors are polyclonal high-affinity immunoglobulin G (IgG) molecules, whose synthesis depends on the activation of CD4+ T cells specific for FVIII. Epitopes recognized by FVIII inhibitory antibodies tend to be concentrated in the A2, A3, and C2 domains of FVIII. These antibodies neutralize FVIII procoagulant function by binding to certain critical locations, such as the FIXa, vWF, or phospholipid binding sites. It has also been proposed that some IgG inhibitory antibodies neutralize FVIII as a result of their intrinsic proteolytic activity,[13] although this requires further confirmation. FVIII inhibitor synthesis is initiated when FVIII is endocytosed and proteolytically degraded within an antigen presenting cell (APC), and short component peptides on cell surface MHC class II molecules are presented to $CD4^+$ cells. Recognition of this peptide/MHC II complex by the cognate T cell(s) induces activation and clonal expansion. Costimulatory ligand pairs on the APC and T cell are required for full activation. Cytokines secreted by the expanded T-cell clone then promote B cell synthesis of inhibitory antibody to FVIII. Elimination of FVIII-specific T-cell clones, or prevention of T-cell activation would constitute "antigen-specific" immune tolerance to FVIII. Hypothetically, this could be achieved by exposure to a small number of "immuno-dominant" universal $CD4^+$ epitope peptide sequences (i.e., those that are recognized by the vast majority of patients) administered in the appropriate dose and route. These antigens may be capable of inducing peripheral T-cell tolerance through clonal anergy or deletion, or induction of immunoregulatory T-cell subsets that downregulate the unwanted immune response.[14] Blocking of costimulatory pathways following FVIII exposure may also be a valid therapeutic strategy. This approach was initiated in a clinical trial using a humanized monoclonal antibody to CD40 ligand prior to its withdrawal due to thrombotic complications.[15] Another hypothetical approach to prevent or treat existing FVIII inhibitors is the use of anti-idiotype antibodies. Theoretically, one could passively immunize patients with pooled immunoglobulin enriched for anti-idiotypic antibodies to FVIII, or alternatively, induce active immunization by administration of appropriate idiotypes.[16]

Eradication of FVIII Inhibitors: Immune Tolerance Induction

Immune tolerance induction (ITI) is a process by which a subject is made tolerant to FVIII (or FIX) by repeated daily exposure to FVIII (FIX) over several months to years. Since the first description of successful ITI by Brackmann in 1977, a number of protocols that vary in the dose schedule of FVIII, and in the presence or absence of immunosuppressive therapy, have been proposed. Most protocols (with the exception of the Malmo protocol) now rely on the use of FVIII alone, due to a growing reluctance to risk the toxicities of immunosuppressive agents such as cyclophosphamide or steroids. The value of immunosuppression in ITI for patients with congenital hemophilia has never been adequately evaluated, although it is well established in acquired hemophilia. However, the successful use of rituximab in the elimination of refractory FVIII inhibitors may be worthy of further study.[17]

There are several existing ITI registries that have begun to define favorable or adverse clinical characteristics that determine responsiveness to ITI. These registries include the International Immune Tolerance Registry (IITR), the North American (NAIT), and German and Spanish Registries. There is broad agreement that the overall likelihood of success with ITI is in the range of 70% ± 10%. Certain favorable clinical features have been identified, including a low inhibitor titer (both historically and immediately prior to initiation of ITI), and possibly a shorter interval from initial identification of inhibitor until commencement of ITI. It remains controversial whether the daily dose of FVIII is predictive of overall success rate; whereas the IITR found that a daily dose ≥ 200 U/kg/day was superior to lower doses, particularly for patients with inhibitor titers > 10 BU/mL at the time of ITI initiation, such an association could not be corroborated by the NAIT. The NAIT data did however demonstrate that daily dose was inversely related to duration of therapy in cases of successful ITI.[18,19] Because of the enormous clinical and financial implications, an International Immune Tolerance Study was initiated in 2002 to compare the effi-

cacy, morbidity, and cost effectiveness of low versus high dose ITI. Eligible patients include those with severe hemophilia A with a FVIII inhibitor ≤ 12 months from diagnosis, and a historical peak inhibitor titer > 5 but < 200 BU/mL. One hundred fifty patients aged ≤ 7 years will be randomized to FVIII doses of 50 IU/kg three times weekly or 200 IU/kg/day. Further information is available at the study Web site, www.itistudy.com.

Although commonly initiated after ITI, it is unknown whether long-term prophylaxis prevents immunological relapse.[20] It is also unknown whether product type has any bearing on the likelihood of success, although preliminary data from the NAIT suggest a benefit of monoclonal over rFVIII.[21] It has also been observed that switching to pdFVIII may be successful in some patients failing ITI.[22]

Hemostatic Agents Used to Control Bleeding in Patients with FVIII Inhibitors

The management of bleeding in patients with FVIII inhibitors should take into consideration the current inhibitor titer, the potential for anamnestic response to FVIII-containing products, and the historical responsiveness of the patient to bypassing therapies. Potential risks with the use of bypassing agents, such as thrombosis, should also be considered. In the event of life- or limb-threatening hemorrhage, raising the plasma factor VIII level is always preferable if it can be achieved with some combination of antibody removal (plasmapheresis or protein A sepharose column therapy[23]), and/or high dose FVIII. This option has become more restricted recently due to concerns about potential contamination of porcine FVIII (Hyate C™) by porcine parvovirus. However, a Phase I study of recombinant B domain-deleted porcine FVIII is soon to be initiated. Furthermore, recombinant human/porcine FVIII hybrids, in which human FVIII sequences in the A2, activation peptide-A3 and C2 domains are replaced by less antigenic porcine sequences, could offer a therapeutic alternative for patients with inhibitors.[24]

Bypassing agents induce hemostasis in the absence of FVIII/FIX. As yet, no study has directly compared the hemostatic efficacy of activated prothrombin complex concentrates (APCCs) with recombinant factor VIIa (rFVIIa), although this trial is now under way in Scandinavia (the FENOC study). Although APCCs have been in use for about 30 years, no consensus exists regarding their hemostatic mechanism(s). It may be that the relatively high concentrations of activated factors VII and IX account for the hemostatic effect of Autoplex-T,[25] whereas a combination of factor Xa and prothrombin—a complex referred to as partial prothrombinase (PPT)—probably accounts for the FVIII bypassing activity of FEIBA. Improvement of the APTT in hemophilic plasma and correction of the cuticle bleeding time in a rabbit model of hemophilia was shown with both plasma-derived and recombinant mixtures of factors II and Xa in the appropriate molar ratio.[26] Potentially, the use of rPPT as a therapeutic agent would circumvent concerns about viral transmission associated with APCCs, and avoid the unwanted anamnestic responses that are due to the small amount of FVIII in the products, which occur in up to 30% of patients.

The mechanism of action, pharmacokinetics, and safety of rFVIIa have been reviewed previously in this forum and elsewhere.[27,28] However, with accumulating experience, questions about dosing and possible monitoring strategies have come to the fore. The dominant mechanism of action of high-dose FVIIa may be to generate thrombin on the platelet surface, independently of tissue factor.[29] The magnitude of this "thrombin burst" may correlate with hemostatic efficacy. Higher doses of rFVIIa than the currently approved 90–120 μg/kg may be superior in some patients who fail to respond to standard doses.[30] In a recent analysis of Phase IV data for rFVIIa that included 556 bleeding episodes in 39 patients, mostly in the setting of home therapy, the 97% response rate to high dose (200–346 μg/kg) rFVIIa was significantly better than the 84% response rate that was reported with doses < 200 μg/kg ($P < .001$).[31]

Bypassing therapies (such as rFVIIa) are generally administered according to empirical dosing guidelines, without any laboratory monitoring. A drawback of the usual platelet-poor plasma assays (including PT, FVII:C and FVIIa levels) is that artificial phospholipid vesicles may "de-emphasize" the relatively weak platelet-binding properties of FVIIa. Furthermore, it may be that differences in platelet procoagulant activity—that is, the ability to support FVIIa-mediated activation of FX—may account for the discrepant individual responsiveness to rFVIIa.[32] As might be expected, these plasma-based assays have failed to correlate with clinical outcomes with rFVIIa. Therefore, whole blood or platelet-rich plasma assays should theoretically provide a better system in which to study the effects of rFVIIa. Candidate monitoring strategies currently under investigation include rotational thromboelastography (rotTEG) which measures changes in whole blood clot elasticity,[33] the platelet contractile force,[34] and a modified whole blood activated clotting time, the ACT-LR assay.[35] As yet, however, none of these assays has been shown to predict individual responsiveness to rFVIIa, or to correlate with clinical outcomes in a sufficiently large patient sample.

Theoretically, the short half-life of rFVIIa (~2.5 hours) precludes the likelihood of successful prophy-

laxis in inhibitor patients. Although there has been some interest in the use of FEIBA for prophylaxis,[36] a recent study suggests that it may fail to arrest established arthropathy.[37]

Inhibitors in Congenital Factor IX Deficiency (Hemophilia B)

Inhibitors are relatively uncommon in hemophilia B, occurring in 2%-3% of severely affected individuals. As with FVIII, the incidence of inhibitors does not appear to be any higher with rFIX compared to pdFIX.[38] While over 2,000 mutations in the FIX gene have been described in hemophilia B, most are low-risk missense mutations. However, patients with gene deletions or rearrangements are at high risk of inhibitor formation (~50%) as are those with frameshift, premature stop, or splice-site site mutations (~20%). Patients with large deletions are at particular risk of anaphylactic reactions to FIX products, sometimes associated with demonstrable IgE antibodies to FIX.

ITI is associated with less favorable outcomes in hemophilia B, with success rates in the 30%-40% range. Intensive factor replacement during ITI has been described to induce nephrotic syndrome as a late complication in several cases. Interestingly, animal models of gene therapy in hemophilia B suggest that hepatic, but not intramuscular, gene transfer may lead to tolerization even in the presence of a large FIX gene deletion.[39]

Inhibitors in Congenital Factor XI Deficiency

A recent study determined that the prevalence of acquired inhibitors in an unrelated Israeli population with FXI deficiency was about 6% (7 of 118), with titers ranging from 3 to 25 BU/mL. Affected patients had in common a FXI:C < 1 U/dL, and a history of exposure to plasma (sometimes on as few as 1 occasion). All were homozygous for the Glu117Stop (type II) mutation, giving a prevalence of 33% among patients with this genotype, compared to 0% with other genotypes.[40] Therapeutic options to control bleeding in patients with FXI inhibitors include rFVIIa,[41] antifibrinolytic agents, and local hemostatics, such as fibrin glue.

III. Von Willebrand Disease

*Margaret E. Rick, MD**

When Erik von Willebrand described the disease bearing his name in 1926, he recognized the disease as a unique bleeding disorder inherited in an autosomal dominant pattern in patients with normal platelet counts.[1] We now know that bleeding occurs in this disease because of abnormalities in platelet adhesion and aggregation and decreased factor VIII levels, all due to a decrease in quantity or a dysfunction of von Willebrand factor (VWF).

Von Willebrand disease (VWD) is the most common inherited bleeding disorder. Although a laboratory-defined decrease in the quantity of VWF occurs in up to 1% of the population,[2,3] probably less than 10% of this group has bleeding symptoms due specifically to their decreased VWF. This is due in part to the poor correlation between VWF levels and bleeding and in part to the high frequency of bleeding symptoms reported by normal subjects. A recent study analyzing the relationship of VWD and the frequency of bleeding symptoms in the general population suggests that reasons other than a mild decrease in VWF may account for the bleeding symptoms in some of the patients.[4]

Pathogenesis

Structure

VWF is an unusual extremely large multimeric glycoprotein composed of repeating units that are polymerized from dimer subunits by disulfide bonds. It is synthesized in endothelial cells and megakaryocytes and, after cleavage of a large propeptide, is released as a series of multimers, including ultralarge forms that are rapidly cleaved to a slightly smaller size.[5,6] Since the dimers are approximately 500,000 daltons and the multimers may contain 20 or more dimers, the family of circulating VWF multimers may be greater than 10 to 20 million daltons. The multimers are normally present in a coiled configuration and are uncoiled to a more linear composition under shear stress[7]; this exposes functional domains within each monomer that are important in several binding functions and for normal physiologic cleavage of the protein (**Figure 1**).

Function

VWF binds to the platelet receptor glycoprotein Ib (GPIb) and to subendothelial structures such as collagen, serving as a bridge between platelets and subendothelium in damaged vessels. It also bridges between adjacent platelets in vessels with high shear such as arterioles, leading to small platelet aggregates. During normal hemostasis following injury, VWF in the subendothelium and plasma binds to platelets as fibrin is being formed at the site of injury, localizing platelets and promoting aggregation.[8] The binding is dependent on the size of the multimers. If there is a

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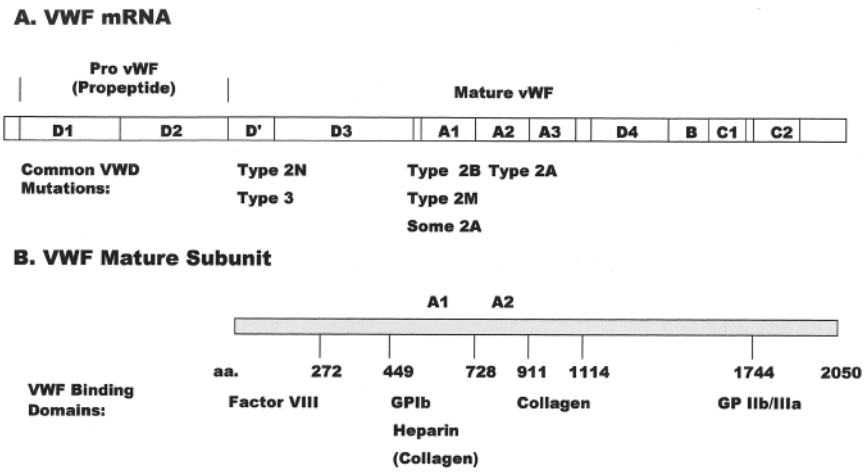

Figure 1. von Willebrand factor.

(A) mRNA showing the propeptide, designated gene regions (A-D), and below, locations where clusters of mutations occur in different types of VWD.

(B) Mature VWF peptide subunit (monomer) showing amino acid numbers (aa) and functional binding domains for ligands.

Abbreviations: mRNA, messenger RNA; VWD, von Willebrand disease; VWF, von Willebrand factor.

decrease in the more functional large VWF multimers, the patient may have a bleeding diathesis in spite of a normal concentration of VWF. In contrast, the ultralarge VWF forms that are initially released after synthesis are "sticky" and capable of binding to platelets in the circulation spontaneously without apparent further stimulation; this may lead to unwanted thrombus formation as seen in thrombotic thrombocytopenic purpura.[9] VWF also binds circulating factor VIII in a manner not dependent on the size of the multimers, "protecting" it in the complex and prolonging the factor VIII half-life from about 2 hours (in the absence of VWF) to 8 to 12 hours in the circulation.[10]

Diagnosis

Clinical presentation

Bleeding symptoms related to abnormal VWF function usually involve mucous membranes and mimic the bleeding seen with platelet dysfunction: bruising, epistaxis, oral bleeding, menorrhagia, and gastrointestinal bleeding. Patients may present at any age because of the wide range in severity of symptoms; those with marked decreases or qualitatively abnormal VWF function usually present earlier in life with bleeding at the time of mucous membrane–related procedures (tooth extractions, tonsillectomy) or at menarche. Serious gastrointestinal bleeding can occur, especially when it is associated with angiodysplasia.[11] Those rare patients who are homozygous or doubly heterozygous (type 3 VWD, see Classification below) have low factor VIII levels (2%-10%) as well as very low VWF levels and have additional symptoms including hemarthroses and soft tissue bleeding. A lack of correlation between VWF levels and symptoms has long been recognized; some of this poor correlation and the variability in symptoms for a given level of VWF may be due to the normal variation in intrinsic platelet function. As an example, variation in normal platelet function can result from the wide range of levels of collagen receptors, which vary 10-fold in normal subjects.[12]

Because of the physiologic variability in VWF levels (see below), the frequency of bleeding symptoms in the general population, and the overlap of laboratory reference ranges between normal subjects and patients with VWD, individuals who are suspected of having VWD based on clinical symptoms and who have just slightly low laboratory results should be tested on several occasions separated by weeks before a diagnosis of VWD is made. Family studies should be done if possible (see Laboratory Assays, below), and careful consideration should be given to the personal and family history of bleeding. As mentioned, bleeding symptoms are reported quite commonly in the normal population, making an isolated mild decrease in the VWF level questionable as the major criterion for a diagnosis of VWD. This question will likely be debated over the next few years.[4]

Physiologic variability

There is variation in the level of VWF associated with different blood groups, with type O subjects having VWF levels that are approximately 25% lower than those of other blood groups.[13] Common causes for increases in the level of VWF include pregnancy, adrenergic stimulation, estrogen replacement therapy, and inflammatory processes.[14]

Laboratory assays

Tests for VWD have traditionally included (1) VWF antigen level, (2) VWF activity (ristocetin cofactor, collagen binding), (3) factor VIII activity (abnormal in only moderate/severe disease), and (4) bleeding time. Bleeding time tests are not sensitive, however, and are

not done as often as they once were. Additional tests that aid in classifying the type of VWD once a diagnosis is established include a VWF multimer study and ristocetin-induced platelet aggregation (RIPA).

VWF antigen (VWF Ag) is a quantitative test that is usually carried out in an ELISA format using antibodies specific for VWF; if other methods are used (i.e., turbidometric methods), one needs to be aware that false-positive tests can occur because of rheumatoid factors. **VWF activity (ristocetin cofactor)** tests the functional ability of VWF to bind to platelets in the presence of ristocetin, an antibiotic that promotes the binding of VWF to platelets. Ristocetin is added at 1.2 mg/mL to a mixture of patient plasma (the VWF source) and washed normal platelets. The end point in the assay is clumping of adjacent platelets caused by the bridging action of the plasma VWF. Since the binding takes place between VWF and a platelet GPIb receptor molecule, a membrane containing this receptor can be used, and lyophilized platelet fragments or formalinized platelets have been employed in addition to freshly washed platelets in various ristocetin cofactor assays. Using dilutions of a standard plasma to establish a calibration curve, ristocetin assays are reasonably quantitative to about 6%, but there remains variability from lab to lab because of reagents and technique. A different functional assay, **VWF collagen binding activity,** is more easily quantitated than is the ristocetin cofactor assay. It measures the amount of VWF from plasma that is bound to microtiter plate wells previously coated with collagen.[15] A more global test of VWF-platelet interaction can be performed in a **platelet function analyzer**; the test has been used as a surrogate for the bleeding time. In this test, the patient's own platelets and VWF are employed, so the test is not specific for VWF abnormalities.[16] **Factor VIII activity** is usually performed in the traditional coagulation factor assay; this test will be abnormal only when the patient has a sufficiently low level of VWF to cause a low factor VIII, and it should not be used to exclude VWD.

Two further tests are used for classification of VWD subtypes: **VWF multimer distribution** by gel assays and **RIPA. VWF multimer** gels provide visualization of the size distribution of VWF multimers in plasma, particularly for assessing whether high-molecular-weight multimers are decreased or absent. Electrophoresis is performed on diluted plasma in agarose gels, the proteins are transferred to a membrane, and VWF is visualized using antibodies to VWF and an immunofluorescence end point.[17] Some type 2 VWD patients (see Classification, below) have decreases in the more functional large multimers. **RIPA** is used primarily to assess whether there is a "gain of function" mutation in the patient's VWF; this occurs in type 2B VWD. The patient's plasma (source of VWF) and platelets, instead of *normal* platelets or platelet membranes (standard in the ristocetin cofactor assay), are used in RIPA; different concentrations of ristocetin are added to aliquots of the patient's platelet-rich plasma, and platelet aggregation is assessed (present or absent). Concentrations of ristocetin below approximately 0.6 mg/mL do not cause aggregation in normal subjects but will cause aggregation in patients with type 2B VWD.

Genetic testing for diagnosis of the specific gene defect in type 2 and type 3 VWD patients is available in specialized laboratories and some research centers. The specific defect is usually identified by direct sequencing of the suspect area of the patient's gene (see **Figure 1**). Since the gene defect in the majority of type 1 patients is unknown at this time, genetic testing is not usually performed in this population.

Classification

VWD is categorized into 3 types (**Table 2**).[18] **Type 1** includes approximately 75% to 80% of patients and is a quantitative decrease in a structurally normal VWF. The majority of patients with type 1 do not have an identified causal mutation in the VWF gene, but in some patients mutations have been recognized that result in retention of VWF or inhibition of multimer assembly within the endothelial cell[19]; it is also possible that increased clearance from the circulation may account for decreased levels of VWF.[20] Laboratory assays show a concomitant decrease in VWF activity and antigen levels, a decreased RIPA, and a normal distribution of VWF multimers, though their intensity may be diminished because of the lower VWF concentration. Factor VIII is modestly decreased or in the low normal range. Patients usually have mild or moderate bleeding. **Type 2** includes 4 subtypes (A, B, M, N). **Type 2A** accounts for 10% to 15% of VWD. The majority of mutations in type 2A cause substitutions within a normal cleavage site in the A2 domain of VWF (**Figure 1**), and some make it more susceptible to proteolysis by the VWF cleaving protease (ADAMTS13). The mutations in type 2A VWD may either cause a defect in intracellular transport (2A, type 1) or render the molecule more susceptible to proteolysis (2A, type 2) (**Figure 1**). Laboratory testing typically shows a more marked decrease in VWF activity assays compared with antigen (because of the loss of the more functional high-molecular-weight multimers), a decreased RIPA, and an absence of the high-molecular-weight multimers on agarose gels. The factor VIII may be normal or decreased. Patients usually have moderate to severe bleeding symptoms and present before adulthood. **Type 2B** accounts for ap-

Table 2. Classification and treatment.[†]

Type	VWF Activity	Ag	RIPA	Multimer Pattern	Treatment	Comments
Type 1	↓	↓	↓	Uniform ↓	DDAVP 0.3 μg/kg IV in 50 mL saline over 20 minutes, or nasal spray 300 μg for weight >50 kg or 150 μg for <50 kg Replacement VWF concentrate at 20-30 IU/kg q12h	Perform therapeutic trial first. Most type 1 respond to DDAVP. Give 1-3 doses q12h; monitor for hyponatremia. Give VWF for 3-10 days for major bleeding. Monitor lab assays and clinical status.
Type 2						
2A	↓↓	↓	↓	↓ Large and Intermediate	DDAVP as in type 1	Many type 2A patients respond, but response may not be as marked as in type 1. Perform therapeutic trial prior to use.
					Replacement VWF concentrate	Administer as for type 1.
2B	↓↓	↓	↑	↓ Large	Possibly DDAVP (see comment) as in type 1	DDAVP may worsen thrombocytopenia; perform therapeutic trial and measure platelet count.
					Replacement VWF concentrate	Administer as for type 1
2M	↓	↓	↓	Normal	DDAVP as in type 1	Perform therapeutic trial
					Replacement VWF concentrate	Administer as for type 1
2N	NlNl	Nl	Normal	DDAVP as in type 1 (see comment)		$T^{1/2}$ of the increased level of factor VIII may be shortened due to lack of binding by abnormal VWF.
					Replacement VWF concentrate	Administer as for type 1
Type 3	↓↓↓	↓↓↓	↓↓↓	Undetectable	Replacement VWF concentrates Platelet transfusions if inadequate response to VWF replacement	Administer as for type 1; increase initial dose to 50 IU/kg

Abbreviations: DDAVP, desmopressin acetate; IV, intravenous; Nl, normal; RIPA, ristocetin-induced platelet aggregation; VWD, von Willebrand disease; VWF, von Willebrand factor.

[†]Antifibrinolytic agents such as epsilon aminocaproic acid (50 mg/kg 4 times daily for 3-5 days; maximum 20 g/d) are often used in conjunction with other therapy; they are especially useful for mucosal bleeding (e.g., in dental procedures).

proximately 5% of VWD. Type 2B mutations result in an abnormal structure of the binding site for platelet GPIb (A1 domain, **Figure 1**) and are responsible for a "gain of function" defect that allows spontaneous binding of the abnormal VWF to platelets in the circulation. Thrombocytopenia may also result, because of removal of platelet aggregates with bound VWF. Laboratory assays show a more marked decrease in VWF ristocetin cofactor activity than in antigen level (similar to type 2A), an *increased* reactivity to low concentrations of ristocetin in RIPA, and a decrease in the high-molecular-weight multimers of VWF. Factor VIII may be normal or low. Patients may have thrombocytopenia and usually have moderate to severe bleeding symptoms. An identical clinical and laboratory phenotype is seen with an abnormality in the platelet GPIb receptor that causes the receptor to bind normal VWF with greater affinity[21]; it is called pseudo- or platelet-type VWD and can be distinguished from type 2B VWD by mixing studies with patient platelets and normal plasma using RIPA. **Type 2M** is very uncommon and results from mutations affecting the A1 domain (in a different area from those mutations in type 2B). Laboratory tests show decreased VWF antigen and activity, decreased binding of VWF to platelets, and a normal multimer size distribution, though VWF bands with abnormal migration may be present and abnormally large multimers may be seen (Vicenza variant). **Type 2N** is an uncommon variant caused by mutations in the amino terminus of the mature VWF monomer that decrease binding for factor VIII and result in rapid clearance of factor VIII. Platelet-related VWF function is normal, so symptoms manifest as soft tissue and joint bleeding, as expected with decreased factor VIII. Because there is 50 to 100 times more VWF in the circulation than factor VIII, patients must be homozygous or doubly heterozygous in order to have decreased factor VIII. Laboratory studies show decreased factor VIII (2%-10%) and normal VWF function and antigen, RIPA, and multimer distribution. The diagnosis is established

by performing a binding assay for factor VIII that uses the patient's VWF as the binding partner. Patients have mild to moderate bleeding related to the decreased factor VIII. This variant may initially be confused with hemophilia A. The presence of affected females in the family is an important clue that this diagnosis should be considered. **Type 3** VWD is rare and is identified by a severe deficiency of VWF accompanied by a moderate deficiency of factor VIII. It is inherited in a homozygous or doubly heterozygous manner and is caused by a variety of mutations, including larger deletions. Laboratory tests show absent or very low VWF activity, antigen, and RIPA, and multimers are not visualized; factor VIII is usually about 5%. Patients have a severe bleeding disorder, manifesting symptoms in childhood.

Treatment

Besides a diagnosis of VWD, the patient's past response to bleeding challenges, current medications, and general medical condition should be considered to help in selecting appropriate treatment for bleeding (**Table 2**). Three major treatment modalities are used for patients with VWD: (1) DDAVP (desmopressin acetate), (2) replacement therapy with plasma-derived factor VIII/VWF concentrates, and (3) adjunctive therapies such as antifibrinolytic agents and topical therapies. The majority of patients with mild to moderate symptomatic type 1 and some patients with type 2 VWD can be treated with **DDAVP**, a medication that indirectly causes release of VWF and factor VIII from storage sites, increasing the levels of both factors 2- to 5-fold within 45 minutes following intravenous administration; the effect usually lasts about 6 hours.[22] Patients should initially undergo a therapeutic trial prior to using DDAVP for treatment to ensure that VWF levels increase as expected over baseline (2- to 5-fold). Type 2B patients are at risk for worsening thrombocytopenia after DDAVP, and they—as well as other type 2 and type 1 patients—should be evaluated with a therapeutic trial prior to using DDAVP for invasive procedures; platelet counts should be evaluated along with VWF levels in type 2B patients. DDAVP can also be given as a nasal spray, and levels peak approximately 2 hours after intranasal delivery. Intranasal dosing is particularly useful since the patient can have rapid access to medication at home at the start of a bleeding episode. It has been especially helpful in reducing excessive menstrual bleeding in women with VWD. Since tachyphylaxis and serious hyponatremia can occur after repeated doses, it is recommended that desmopressin be given at 8- to 12-hour intervals for 2 to 3 doses and then at intervals of 48 hours. If nonsteroidal anti-inflammatory agents are part of the patient's regimen, they should be used with caution in conjunction with DDAVP because of their potential to aggravate hyponatremia.[23] **VWF concentrates** should be used when bleeding is not controlled with desmopressin or if there is more serious bleeding; they are given prophylactically and following surgery or trauma for 2 to 14 days, as dictated by the clinical situation. Intermediate-purity plasma-derived factor VIII concentrates contain VWF (not recombinant or monoclonally purified factor VIII concentrates), and they are administered intravenously at approximately 12-hour intervals. One US product is labeled with VWF ristocetin cofactor units (Humate-P^{Rx}); other more highly purified plasma VWF products are available in Europe. Doses and duration of treatments are noted in **Table 2**. Since levels of VWF do not correlate well with bleeding, the patient may require adjustment in dosing in spite of laboratory testing that shows an "adequate" VWF level. Cryoprecipitate is generally not recommended because of the lack of viral inactivation for this product. **Adjunctive therapies** include antifibrinolytic agents such as epsilon aminocaproic acid (Amicar) and topical agents such as topical thrombin, Gelfoam, and fibrin sealant. Epsilon aminocaproic acid may be particularly helpful for dental procedures.

Since VWF levels increase 2- to 3-fold during the last 2 trimesters of pregnancy, treatment may not be required for delivery in type 1 VWD patients whose VWF levels have reached the normal range during the third trimester. VWF does drop rapidly within the 24 hours after delivery, however, and if bleeding occurs, DDAVP is recommended as the initial treatment for those patients known to be responsive. In more severely affected type 1 patients and in type 2 patients, DDAVP can be administered prophylactically to responsive patients after the onset of labor before delivery. If DDAVP is not effective for the bleeding, replacement therapy should be given. In patients with type 2B VWD whose platelets have decreased during pregnancy, platelets and VWD replacement therapy should be given instead of DDAVP.

Acquired VWD

Acquired VWD is very uncommon and may occur spontaneously or in association with diseases affecting the immune system (benign or malignant), cardiac valvular diseases, myeloproliferative disorders, solid tumors, and hypothyroidism. Mechanisms responsible for decreased VWF include antibodies, consumption or binding to cells, proteolysis, or decreased production of VWF.[24-26] Laboratory evaluation usually shows decreased VWF activity and antigen and often shows a decrease in the high-molecular-weight multimers; the VWF propeptide, which is not targeted by antibody and

not consumed or bound, is normal or elevated.[27] Antibodies have been found in less than a third of the cases and are difficult to demonstrate in the laboratory.[26] A trial of DDAVP is usually given as initial treatment, and if the response is not adequate, either replacement therapy or intravenous immunoglobulin (IVIG) is used.[25] The response to replacement therapy is usually satisfactory, despite the possible presence of antibodies to VWF.

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III. Von Willebrand Disease

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