Review

Blood coagulation factor VIII: An overview

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Factor VIII (FVIII) functions as a co-factor in the blood coagulation cascade for the proteolytic activation of factor X by factor IXa. Deficiency of FVIII causes hemophilia A, the most commonly inherited bleeding disorder. This review highlights current knowledge on selected aspects of FVIII in which both the scientist and the clinician should be interested.

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1. Introduction

Factor VIII (FVIII) is a glycoprotein cofactor that serves as a critical component in the intrinsic blood coagulation pathway (Kaufman 1992). Insufficient expression of FVIII or expression of nonfunctional FVIII results in hemophilia A, one of the most common severe hereditary bleeding disorders. This article gives a general overview on current knowledge of blood coagulation FVIII: especially, its lifespan; mutations in the gene which cause hemophilia A; replacement therapy for the treatment of hemophilia A; and the development of inhibitors in patients with hemophilia A.

2. Lifespan

2.1 *FVIII gene*

The gene of FVIII is located at the tip of the long arm of X chromosome (Gitschier *et al* 1984). It spans over 180 kb. It comprises 26 exons, which encode a polypeptide chain of 2351 amino acids (Vehar *et al* 1984). This includes a single peptide of 19 amino acids and a mature protein of 2332 amino acids. FVIII is a large multidomain glycoprotein with domain structure A1-A2-B-A3-C1-C2 (Vehar *et al* 1984; Lenting *et al* 1998) (figure 1). The heavy chain is composed of domains A1-A2-B, while the light chain is composed of domains A3-C1-C2. The A domains are bordered by short spacers that contain clusters of aspartic acid and glutamic acid residues, called acidic regions. The large B domain is encoded by a single large exon and has no detectable homology to any other known genes. It is extensively glycosylated on asparagine, serine, and threonine residues. The C domains occur twice in the carboxy terminus of the FVIII light chain and exhibit homology to proteins that bind glycoconjugates and negatively charged phospholipids.

FVIII, a divalent metal ion-dependent heterodimer, contains a single copper atom but, the role of this metal in the structure and function of the cofactor is unclear. Furthermore, association of FVIII heavy chain (A1-A2-B domains) and light chain (A3-C1-C2 domains) is metal ion-dependent with residues in the A1 and A3 domains, which contain the interactive sites. Both Ca(II) and Mn(II) support this interaction, which is of interest since Ca(II) sites are typically formed by carboxylate moieties while Mn(II) can bind carboxylates as well as co-ordinated histidine residues (Martin 1986). The divalent metal ion binding site(s) required for subunit association has (have) not been identified.

2.2 *Secretion and activation*

The liver is the major site of FVIII synthesis (Wion *et al* 1985). The significant contribution of extrahepatic bio-

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Abbreviations used: FVIII, Factor VIII; ER, endoplasmic reticulum; ITI, immune tolerance induction; vWF, von Willebrant factor.

synthesis of FVIII in health and disease states is still not known.

The absence of a naturally derived cell line that expresses FVIII prevents the study of FVIII biosynthesis in its natural host cell. The expression of FVIII in mammalian cells transfected with the FVIII gene has allowed analysis of the biosynthesis and processing of this complex glycoprotein.

The initial stage of secretion involves the translocation of the mature 2332 amino acids polypeptide into the lumen of the endoplasmic reticulum (ER), where glycosylation occurs. Within the ER, FVIII appears to interact with a number of chaperone proteins, including calreticulin, calnexin and the IgG-binding protein (Marquette *et al* 1995; Swaroop *et al* 1997; Pipe *et al* 1998). Due to the interaction with these chaperone proteins, a significant proportion of the FVIII molecules is retained within the ER, thereby limiting the transport of FVIII to the Golgi apparatus. The mechanism responsible for the transport from the ER to the Golgi apparatus is not elucidated yet.

The activation of FVIII coincides with proteolysis of both the heavy and light chain (Fulcher *et al* 1983; Eaton *et al* 1986). Cleavage within the heavy chain after arginine residue 740 generates a 90 kDa polypeptide, which is subsequently cleaved after arginine residue 372 to generate polypeptides of 50 and 43 kDa (Eaton *et al* 1986). Concomitantly, the 80 kDa light chain is cleaved after arginine residue 1689 to generate a 73 kDa polypeptide. The appearance of the 50, 43 and 73 kDa polypeptides correlates with peak thrombin activation (Fulcher *et al* 1983; Eaton *et al* 1986). Cleavage at 1689 releases FVIII from von Willebrant factor (vWF), relieving the vWF inhibition of FVIII phospholipid interaction (Pitmann and Kaufman 1988) and permitting the interaction of FVIII with platelets.

It will be of interest to know why FVIII becomes a potent cofactor of factor IXa once it is cleaved within the heavy and light chain and what is the structural basis for such a dramatic increase in biological activity.

2.3 *Inactivation and clearance from circulation*

Proteolysis of activated FVIII by FXa, or by activated protein C, or by thrombin results in the inactivation of FVIII (Foster and Zimmerman 1989). At present, little is known about the mechanism by which FVIII is cleared from the circulation (Lenting *et al* 1998).

3. Mutation

A variety of gene defects viz. point mutations, deletions, duplications, insertions in FVIII gene have been identified which cause hemophilia A (Tuddenham *et al* 1991;

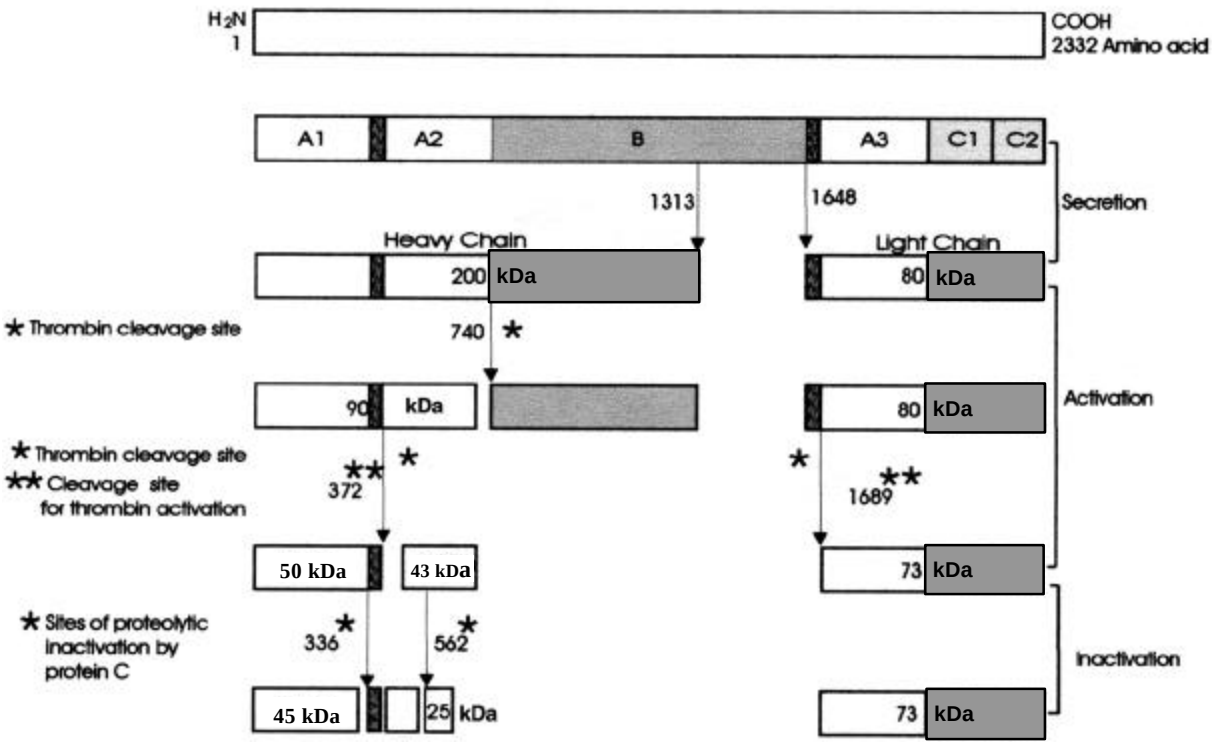

Figure 1. Structural domains of FVIII protein and its processing.

Bowen 2002). Point mutations are the most prevalent type of defect, probably underlying the disease in 90–95% of patients. It occurs in the hemophilias and is comprised of missense mutations (these change a codon so that a different amino acid is encoded), nonsense point mutations (these change an amino acid codon into a translation stop codon), and mRNA splice-site point mutations (these corrupt a true mRNA splice site, or create a novel one) (Bowen 2002). Deletions are second most common gene defects (5–10% of patients) but duplications are extremely rare. Inversion gene defect is involved in intron 22 at FVIII gene which causes severe hemophilia A and is found in 40%–50% of patients with severe disease (Lakich *et al* 1993; Naylor *et al* 1993). The FVIII gene is completely disrupted: introns 1 to 22 are moved away from their normal context and their orientation is inverted. The intron 22 inversion is principally an error of DNA replication during spermatogenesis in males and not during oogenesis in females (Antonarakis *et al* 1995). In hemophilia A families, in which the intron 22 inversion is the causative gene defect, the defect can often be shown to have originated in an unaffected male relative and in sporadic cases, this is often the patient's grandfather on his mother's side of the family (Antonarakis *et al* 1995). The distal inversion is more common than the proximal inversion (Antonarakis *et al* 1995).

The binding of FVIII to vWF is essential for the survival of FVIII in the circulation. Two peptide regions of FVIII are implicated to be involved in binding vWF: one at the aminoterminal end of intact FVIII light chain (Viot *et al* 1995; Saenko and Scandella 1997) and one of the region is at the carboxyterminal end (Saenko *et al* 1994; Saenko and Scandella 1997). Mutations affect binding of FVIII to vWF. Within the A3 domain, sulphation of tyrosine 1680 is crucial for FVIII interaction with vWF (Leyte *et al* 1991). Missense mutations, resulting in the replacement of this tyrosine residue, result in decreased FVIII binding to vWF (Leyte *et al* 1991). Similarly, retention of the tyrosine residue, but prevention of sulphation, also decreases FVIII binding to vWF (Pittman *et al* 1992). Clearly, the loss of sulphation is crucial, whether or not this is accompanied by structural/folding changes brought about by a mutant amino acid substitution. Recently, mutations have also been identified within the C1 domain that decreases FVIII binding to vWF (Gilles *et al* 1999; Jacquemin *et al* 2000). Missense mutations (arginine 593 to cysteine and asparagine 618 to serine) also cause intracellular accumulation of FVIII (Roelse *et al* 2000).

The incidence of hemophilia is nearly 1 in 5000 males (Lozier and Kessler 2000). India has a population of over 1000 million and it is estimated that there are at least 50,000 severe hemophilia patients, and only 15% of estimated number have been diagnosed to have hemophilia (Srivastava *et al* 1998).

Hemophilia is classified as mild, moderate or severe, based on the factor level in plasma and the severity of clinical disease. Approximately 60% of patients with mutant gene for FVIII have severe hemophilia (the level of FVIII activity is less than 1·0% of normal) whereas the remainder have moderate or mild hemophilia (FVIII activity, 1·0 to 5·0% of normal or more than 5·0% of normal respectively) (Lozier and Kessler 2000). In severe hemophilia, spontaneous bleeding into joints, soft tissues and vital organs is frequent, whereas in mild hemophilia, bleeding usually occurs only after trauma or surgery (Richard 1997).

The FVIII gene of more than 2500 hemophilia A patients has been examined for molecular defects using a variety of methods, and a database of mutations in the gene has been published (Tuddenham *et al* 1991; Antonarakis *et al* 1995) and is now constantly updated on the website http://europium.csc.mrc.ac.uk (Wacey *et al* 1996).

4. Replacement therapy

Hemorrhagic episodes in patients with hemophilia A can be managed by replacing FVIII. Several plasma products are available for use in raising FVIII to hemostatic levels. Fresh frozen plasma and cryoprecipitate both contain FVIII and are being used for treatment. A disadvantage of plasma is that large volumes must be infused to achieve and maintain even minimum levels of FVIII.

Currently, there are two types of lyophilized FVIII concentrates (viz. plasma and recombinant) available commercially (table 1) (McEvoy 2001). Purified human plasma FVIII is isolated from pooled plasma of thousands of donors. With the advent of new sterilization techniques, donor screening and protein purification methodologies, the incidence of transmission of hepatitis B, hepatitis C or human immunodeficiency virus, is negligible (Fricke and Lamb 1993). However, these measures do not prevent the transmission of the highly thermoresistant parvovirus B19 (Azzi *et al* 1999). Few clinical complications due to parvovirus have been reported in patients with hemophilia (Yee *et al* 1995; Matsui *et al* 1999). Plasma-derived products are potentially capable of transmitting unknown viruses and therefore further study is needed to assess the relative safety of the various commercially available preparation of antihemophilic factor (human) in regard to transmission.

In addition to human plasma FVIII, porcine plasma FVIII (obtained from pooled porcine plasma) is also commercially available for human use. It is used in the management of bleeding in patients with acquired hemophilia who have spontaneously acquired inhibitors to antihemophilic factor (auto antibodies) (McEvoy 2001). But it should not be used as first line of replacement therapy. Now

porcine plasma is screened for the risk factor associated with porcine parvovirus using polymerase chain reaction test.

Recombinant FVIII is produced using mammalian cells: i.e. baby hamster kidney cells or chinese hamster ovary cells. It is a highly purified glycoprotein and provides a temporary replacement to prevent or control of bleeding episodes or to perform emergency or elective surgery in patients with hemophilia A.

Recently, Azzi *et al* (2001) reported that TT virus (Nishizawa *et al* 1997) occurs in first generation of recombinant FVIII product with human serum albumin as the source. However, the results differ from other studies (Kreil *et al* 2002). In view of safety, a new recombinant FVIII, formulated in sucrose instead of human serum albumin, has been licensed in the US and Europe (Abshire *et al* 2000). Another one of the new product lacks the large B domain, is under clinical trials (Osterberg *et al* 1997; Lusher *et al* 2000).

Gene therapy for hemophilia A is an attractive therapy (Kaufman 1999). There are few experimental reports of successful expression of FVIII (Hoeben *et al* 1992, 1993; Lynch *et al* 1993) using retroviral vectors and transplantation in nude mice (Hoeben *et al* 1993). Recently, Roth *et al* (2001) developed somatic cell gene therapy system in patients with severe hemophilia A. This system have some potential advantages over gene therapy based on viral vectors. Patients received a homogeneous clonal population of cells containing a single genetic modification, thereby minimizing the risk of insertional mutagenesis that might occur with viral vectors. Despite these advantages, the system has noteworthy disadvantages. The implantation procedure is moderately invasive, and the FVIII producing autologous fibroblasts must be prepared individually for each patient.

5. Inhibitors

Management of bleeding episodes in patients with hemophilia A consists of administering FVIII concentrates derived from plasma or recombinant FVIII. The development of antibodies against these FVIII is a major clinical complication as these inhibitory antibodies bind to infused FVIII, thereby reducing its half life and neutralizing its coagulant activity (Mannucci and Tuddenham 2001). Inhibitors are estimated to occur in 20%–35% of patients with severe hemophilia A (Lusher *et al* 1993) and usually

Table 1. Currently available factor VIII concentrate products.

Product	Manufacturer	Prepared	Purified	Viral inactivation methods
Alphanate	Alpha Therapeutic	Pooled human venous plasma	Heparine agarose chromatography	Solvent, detergent and heat treatment
Hemofil-M	Baxter	Pooled human venous plasma	Immunoaffinity chromatography using murine monoclonal antibody	Solvent and detergent.
Monarc-M	American Red Cross	Pooled human venous plasma	Immunoaffinity chromatography using murine monoclonal antibody	Solvent and detergent.
Monoclate-P	Aventis	Pooled human venous plasma	Affinity chromotography using murine monoclonal antibody	Pasteurization.
Humate-P*	Aventis	Pooled human venous plasma	–	Pasteurization
Koate-DVI		Pooled human venous plasma	–	Solvent and detergent
Hyate: c	Speywood	Porcine plasma	Polyelectrolyte fractionization	–
Helixate	Aventis, Behring	Recombinant, baby hamster kidney cells	Ion exchange chromatography, gel filtration, monoclonal antibody immunoaffinity chromatography	–
Kogenate FS	Bayer	Recombinant, baby hamster kidney cells	Ion exchange chromatography, gel filtration, monoclonal antibody, immunoaffinity chromatography	–
Helixate FS	Aventis, Behring	Recombinant, baby hamster kidney cells	Ion exchange chromatography, gel filtration, monoclonal antibody immunuaffinity chromatography	Solvent and detergent
Biociiate	Aventis, Behring	Recombinant, Chinese hamster ovary cells	Column chromatography and monoclonal antibody immunoaffinity chromatography	–
Recombinate	Hyland	Recombinant, Chinese hamster ovary cells	Column chromatography and monoclonal antibody immunoaffinity chromatography	–

they occur during the early phase of treatment when the patients are young. These inhibitors react with the A2, A3 or C2 domains and only vary occasionally with the A1 and B domains (Scandella 2000).

The mechanism of FVIII inhibitor development remains unresolved and complicated because no single factor can be counted as responsible. It appears that severely affected hemophiliacs with gene inversions, deletions or stop codon mutations in the FVIII gene, generate inhibitors more often than those with missense mutations (Schwaab *et al* 1995). Recent studies in the Netherlands, Belgium and Germany indicate that the manufacturing process may influence inhibitor development (Rosendaial *et al* 1993, 1997; Peerlinck *et al* 1993, 1997; Mauser-Bunschoten *et al* 1994).

Eradication of inhibitor is highly desirable and widely accepted goal of hemophilia care. The eradication of inhibitor is accomplished through immune tolerance induction (ITI) which typically involves the daily infusion of large doses of FVIII over many months to years as well as the use of immunosupressive agents. The efficacy rate of ITI ranges from 63% to 83% (Mariani *et al* 1994; Ghirardini *et al* 1996; Dimichele 1998). A major disadvantage of ITI is its high initial cost.

6. Conclusion

It is clear from this brief overview that considerable advances have been made in recent years in understanding some important aspects of FVIII. These findings have answered many questions, but at the same time have raised many more questions as well. Finding the answers to new questions will help to further improve our current strategies for the treatment of hemophilia A.

References

Abshire T C, Brackmann H H, Scharrer I, Hoots K, Gazengel C, Powell J S, Goring E, Kellermann E and Voshurgh E 2000 Sucrose formulated recombinant human antihemophilic factor VIII is safe and efficacious for treatment of hemophilia A in home therapy; *Thromb. Haemost.* **38** 811–816

Antonarakis S E, Kazazian H H and Tuddenham E G 1995 Molecular etiology of factor VIII deficiency in hemophilia A; *Hum. Mutat.* **5** 1–22

Antonarakis S E, Rossiter J P, Young M, Horst J, Demoerloose P, Sammer S S, Ketterling R P and Kazazian H H Jr, Negrier C, Vinciguerra C 1995 Factor VIII gene inversions in severe hemophilia A: results of an international consortium study; *Blood* **86** 2206–2212

Azzi A, De Santis R, Morfini M, Zakrzewska K, Musso R, Santagostina E and Castaman G 2001 TT virus contaminates first generation recombinant factor VIII concentrates; *Blood* **98** 2571–2573

Azzi A, Morfini M and Mannucci P M 1999 The transfusion associated transmission of parvovirus B19; *Transfus. Med. Rev.* **13** 194–204

Bowen D J 2002 Haemophilia A and Haemophilia B: molecular insights; *Mol. Pathal.* **55** 127–144

Dimichele D M 1998 Immune tolerance: a synopsis of the international experience; *Haemophilia* **4** 568–573

Eaton D, Rodriguez H and Vehar G A 1986 Proteolytic processing of human factor VIII. Correlation of specific cleavages by thrombin, factor Xa, and activated protein C with activation and inactivation of factor VIII coagulant activity; *Biochemistry* **25** 505–512

Foster P A and Zimmerman T S 1989 Factor VIII structure and function; *Blood Rev.* **3** 180–191

Fricke W A and Lamb M A 1993 Viral safety of clotting factors; *Semin. Thromb. Hemost.* **19** 54–61

Fulcher C A, Roberts J R and Zimmerman T S 1983 Thrombin proteolysis of purified factor VIII. Corelation of activation with generation of a specific polypeptide; *Blood* **61** 807–811

Ghirardini A, Puopolo M, Chiarotti F and Mariani G 1996 The international registry of immune tolerance: 1994; Update; *Vox Sang.* **70** 42–46

Gilles J G, Lavend'homme R, Peerlinck K, Jacquemin M G, Hoxlaerts M, Joorieux S, Mazurier C, Vermylen J and Saint-Remy J M 1999 Some factor VIII (FVIII) inhibitors recognize a FVIII epitope(s) that is present only on FVIII – vWF complexes; *Thromb. Haemost.* **82** 40–45

Gitschier J, Wood W I, Goralka T M, Wion K L, Chen E Y, Eaton D H, Vehar G A, Capon D J and Lawn R M 1984 Characterization of the human factor VIII gene; *Nature* (*London*) **312** 326–330

Hoeben R C, Einerhand M P W, Briet E, Van Ormondt H, Valerio D and Van der Eb A J 1992 Towards gene therapy in hemophilia A: retrovirus-mediated transfer of a factor VIII gene into murine haemotopoietic progenitor cells; *Thromb. Haemost.* **67** 341–345

Hoeben R C, Fallaux F J, Van Tilburg N H, Cramer S J, Van Briet E and Van Der Eb A J 1993 Towards gene therapy of hemophilia A: long term persistence of Factor VIII secreting fibroblasts after transplantation into immunodeficient mice; *Hum. Gene Ther*. **4** 179–186

Jacquemin M, Lavend'homme R, Benhida A, Vanzieleghem B, d'Oiron R, Lavergne J M, Brackmann H H, Schwaab R, Vanden Driessche T, Chuah M K L, Hoylaert S M, Gilles J G G, Peerlink K, Vermylen J and Saaint-Remy J M R 2000 A novel cause of mild/moderate hemophilia A: Mutations scattered in the factor VIII C1 domain reduce factor VIII binding to von Willebrand factor; *Blood* **96** 958–965

Kaufman R J 1992 Biological regulation of factor VIII activity; *Annu. Rev. Med*. **43** 325–339

Kaufman R J 1999 Advances toward gene therapy for hemophilia at the milennium; *Hum. Gene Ther.* **10** 2091–2107

Kreil T R, Zimmermann K, Pable S, Schwarz H P, Dorner F, Azzi A and Morfini 2002 TT virus does not contaminate first generation recombinant factor VIII concentrate; *Blood* **100** 2271–2272

Lakich D, Kazazian H H Jr, Antonarakis S E and Gitschier J 1993 Inversions disrupting the factor VIII gene are a common cause of severe haemophilia A; *Nat. Genet.* **5** 236–241

Lenting P J, van Mourik J A and Mertens K 1998 The life cycle of coagulation factor VIII in view of its structure and function; *Blood* **92** 3983–3996

Leyte A, van Schijndel H B, Niehrs C, Hunter W B, Verbeet M P, Mertens K and van Mourik J A 1991 Sulphation of Tyr1680 of human blood coagulation factor VIII is essential for the interaction of factor VIII with von Willebrand factor; *J. Biol. Chem.* **266** 740–746

Lozier J N and Kessler C N 2000 Clinical aspects and therapy of hemophilia; in *Hematology: basic principles and practice* (eds) R Hoffman, E Benz Jr and S Shattil (New York: Churchill Livingstone) pp 1883–1904

Lusher J M, Arkin S, Abildgaard C F and Schwartz R S 1993 Recombinant factor VIII for the treatment of previously untreated patients with haemophilia A – Safety, efficacy, and development of inhibitors; *N. Engl. J. Med.* **328** 453–459

Lusher J M, Petrini P and Angiolillo A 2000 Antibody and inhabitor patterns in previously untreated patients (PUPs) treated exclusively with B-domain deleted factor VIII (BDDrFVIII); *Blood* **96** 266

Lynch C M, Israel D I, Kaufman R J and Miller D 1993 Sequences in the coding region of clotting factor VIII act as dominant inhibitors of RNA accumulation and protein production; *Hum. Gene Ther.* **4** 259–273

Mannucci P M and Tuddenham E G D 2001 Medical Progress: The haemophilias – from Royal genes to gene therapy; *N. Engl. J. Med.* **344** 1773–1779

Mariani G, Ghirardini A and Bellocco R 1994 Immune tolerance in hemophilia – Principal results from the international registry. Report of the factor VIII and IX subcommittee; *Thromb. Haemost.* **72** 155–158

Marquette K A, Pittman D D and Kaufman R J 1995 A 110 amino acid region within the A1-domain of coagulation factor VIII inhibits secretion from mammalian cells; *J. Biol. Chem.* **270** 10297–10303

Martin R B 1986 *Metal ions in biological system* (ed.) H Segel (New York: Marcel Dekker) pp 20–21

Matsui H, Sugimoto M, Tsuji S, Shima M, Giddings J and Yoshioka A 1999 Transient hypoplastic anemia caused by primary human parvovirus B19 infection in previously untreated patient with hemophilla transfused with a plasma derived monoclonal antibody purified factor VIII concentrate; *J. Pediatr. Hematol. Oncol.* **21** 74–76

Mauser-Bunschoten E P, Roosendaal F R, Nieuwenhuis H P, Rosendaal G, Briet E and van den Berg H M 1994 Clinical course of factor VIII inhibitors developed after exposure to a pasteurised Dutch concentrate compared to classic inhibitors in haemophilia A; *Thromb. Haemost.* **71** 703–706

McEvoy G K, 2001 *AHFS Drug Information* (Bethesda: American Society of Health System Pharmacists) pp 1412–1428

Naylor J, Brinke A, Hassock S, Green P M and Giannelli F 1993 Characteristic mRNA abnormality found in half the patients with severe haemophilia A is due to large DNA inversions; *Hum. Mol. Genet.* **2** 1973–1978

Nishizawa T, Okamoto N, Konishi K, Yoshizawa H, Miyakawa Y and Mayumi M 1997 A novel DNA virus (TTV) associated with elevated transaminase levels in posttransfusion hepatitis of unknown etiology; *Biochem. Biophys. Res. Commun.* **241** 92–97

Osterberg T, Fatouros A and Mikaelsson M 1997 Development of a freeze dried albumin free formulation of recombinant factor VIII SQ; *Pharm. Res.* **14** 892–898

Peerlinck K, Arnout J, di Giampattista M, Gillies J G, Laub R, Jacquemin M, Saint J M R and Vermylen J 1997 Factor VIII inhibitors in previously treated haemophilia A patients with a double virus inactivated plasma derived concentrate; *Thromb. Haemost.* **77** 80–86

Peerlinck K, Arnout J, Gilles J G, Saint-Remy J M and Vermylen J 1993 A higher than expected incidence of factor VIII inhibitor in multitransfused haemophilia A patients treated with a intermediate purity pasteurized factor VIII concentrate; *Thromb. Haemost.* **69** 115–118

Pipe S W, Morris J A, Shah J and Kaufman R J 1998 Differential interaction of coagulation factor VIII and factor V with protein chaperones calnexin and calreticulin; *J. Biol. Chem.* **273** 8537–8544

Pitmann D D and Kaufman R J 1988 Proteolytic requirements for thrombin activation of antihemophilic factor VIII; *Proc. Natl. Acad. Sci. USA* **85** 2429–2433

Pittman D D, Wang J H and Kaufman R J 1992 Identification and functional importance of tyrosine sulfate residues within recombinant factor VIII; *Biochemistry* **31** 3315–3325

Richard K A 1997 The diagnosis of hemophilia A and B and von Willebrand's disease; in *Hemophilia* (eds) C D Forbes, L M Aledort and R Madhok (London: Chapman and Hall Medical) pp 53–62

Roelse J C, De Laaf R T, Timmermans S M, Peters M, Van Mourik J A and Voorberg J 2000 Intracellular accumulation of factor VIII induced by missense mutations Arg 593 – Cys and Asn 618 – Ser explains cross-reacting material reduced haemophilia A; *Br. J. Haematol.* **108** 241–246

Rosendaal F R 1997 Factor VIII inhibitors on a SD treated and pasteurised concentrate associated with specific batches and batch characteristics; *Thromb. Haemost.* **78** 590–594

Rosendaial F R, Nieuwenhuis H K, van den Berg H M, Heijboer H, Mauser-Bunschoten E P, van der Meer J, Smit C, Strengers P F and Briet E 1993 A sudden increase in factor VIII inhibiter development in multitransfused hemophilia A patients in the Netherlands. Dutch Hemophilia study Group; *Blood* **81** 2180–2186

Roth D A, Tawa N E Jr, O'Brien J M, Treco D A and Selden R F 2001 Nonviral transfer of the gene encoding coagulation factor VIII in patients with severe Hemophilia A; *N. Engl. J. Med.* **344** 1735–1742

Saenko E L and Scandella D 1997 The acidic region of the factor VIII light chain and the C2 domain together from the high affinity binding site for von Willebrand factor; *J. Biol. Chem.* **272** 18007–18014

Saenko E L, Shima M, Rejalakshmi K J and Scandella D 1994 A role for the C2 domain of factor VIII in binding to von Willebrand factor; *J. Biol. Chem.* **269** 11601–11605

Scandella D 2000 Properties of anti factor VIII inhibitor antibodies in hemophilia A patients; *Semin. Thromb. Hemost.* **26** 137–142

Schwaab R, Brackmann H H, Meyer C, Seehafer J, Kirchgesser M, Hacck A, Olek K, Tuddenham E G D and Oldenburg J 1995 Haemophilia A: Mutation type determines risk of inhibitor formation; *J. Thromb. Haemost.* **74** 1402–1406

Srivastava A, Chuansumrit A, Chandy M, Duraiswamy G and Karagus C 1998 Management of hemophilia in the developing countries; *Hemophilia* **4** 474–480

Swaroop M, Moussalli M, Pipe S W and Kaufman R J 1997 Mutagenesis of a potential immunoglobulin-binding-protein-binding site enhances secretion of coagulation factor VIII; *J. Biol. Chem.* **272** 24121–24124

Tuddenham E G D, Cooper D N, Gritschier J, Higuchim, Hoyer L W, Yoshioka A, Peake I R, Schwaab R, Olekk and Kazazian H H 1991 Haemophilia A: database of nucleotide substitutions, deletions, insertions and rearrangements of the factor VIII gene; *Nucleic Acids Res.* **19** 4821–4833

Vehar G A, Keyt B, Eaton D, Rodriguez H, O'Brien D P, Rotblat F, Oppermann H, Keck R, Wood W I, Harkins R N, Tuddenham E G D, Lawn R M and Capon D J 1984 Structure of human Factor VIII; *Nature* (*London*) **312** 337–342

Viot A J, Koppelman S J, van den Berg M H, Bouma B N and Sixma J J 1995 The affinity and stoichiometry of binding of human factor VIII to von Willebrand factor; *Blood* **85** 3150–3157

Wacey A I, Kemball-Cook G, Kazazian H H, Antonarakis S E, Schivaab R, Lindey P and Tuddenhum E G 1996 The haemophilia A mutation search test and resource site, home page of the factor VIII mutation database: HAMSTeRS; *Nucleic Acids Res.* **24** 100–102

Wion K L, Kelly D, Summerfield J A, Tuddenham E G D and Lawn R M 1985 Distribution of factor VIII mRNA and antigen in human liver and other tissues; *Nature* (*London*) **317** 726–729

Yee T T, Lee C A and Pasi K J 1995 Life threatening human parvovirus B19 infection in immunocompetent haemophilia; *Lancet* **345** 794–795

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