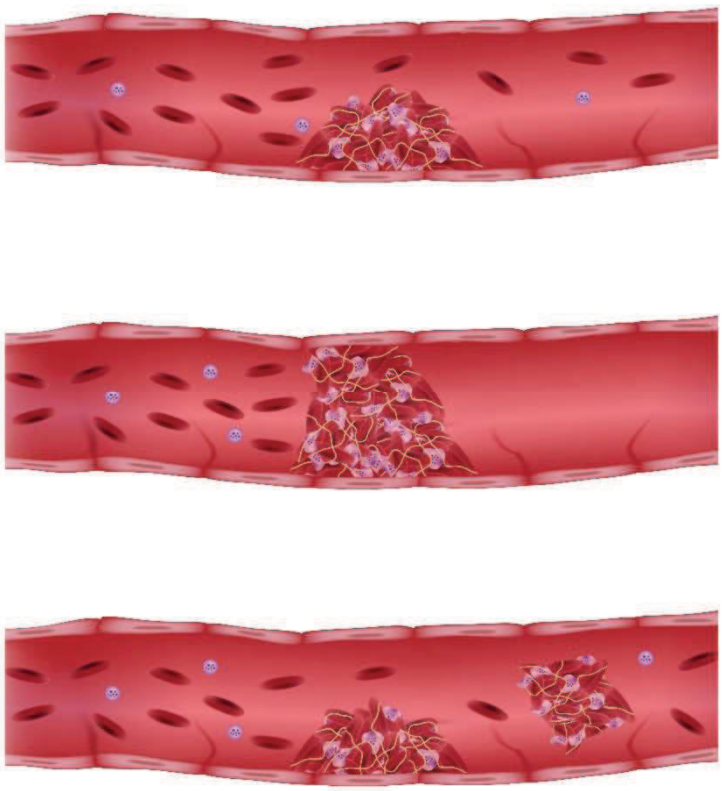
Products for Research on Factor VIII



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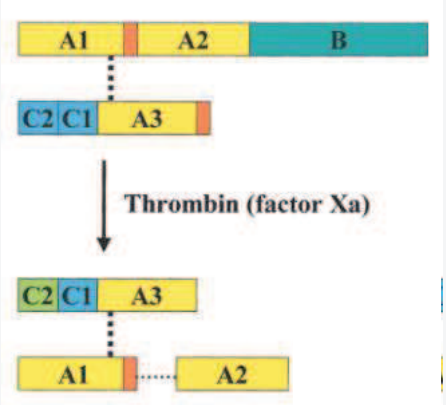
Content

Factor VIII Description

Description

Factor VIII (FVIII) was first described in 1937; in 1979 it was characterized at the protein level.

FVIII consists of six domains: A1-A2-B-A3-C1-C2. The C domains mediate phospholipid and membrane binding. Activation to FVIIIa results in the cleavage and release of the B domain. Thus, FVIIIa is divided into a heavy chain, consisting of the A1-A2 domains, and a light chain, consisting of the A3-C1-C2 domains. In the presence of calcium both form a complex in a non-covalent manner.



Physiology

FVIII is synthesized and released into the bloodstream by the vascular, glomerular, and tubular endothelium, and the sinusoidal cells of the liver.
It circulates in the bloodstream in a stable inactive form, bound to von Willebrand factor. Binding is released when the complex encounters injuries to the blood vessel: FVIII is then activated (by Thrombin) to FVIIIa and separates from the von Willebrand factor.
In the presence of Ca^{2+} and phospholipids, FVIIIa forms a complex with factor IX that converts factor X to the activated form Xa. Xa generates thrombin from prothrombin. When FVIIIa is no longer protected by vWF, it is prone to proteolytic inactivation (most prominently by activated protein C) and quickly cleared from the circulation.

Pathophysiology

FVIII is essentially involved in blood clotting. Defects in the FVIII gene result in the recessive X-linked bleeding disorder “Hemophilia A”. To restore hemostasis, Hemophilia A patients are given

(i) FVIII concentrates prepared from donated blood or
(ii) (ii) recombinant FVIII. A major side effect of such treatment is the development of antibodies against FVIII which inhibit its activity; accordingly, such antibodies have been termed “inhibitors”.

On the other hand, individuals with high levels of factor VIII are at increased risk for deep vein thrombosis and pulmonary embolism.

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Factor VIII Description – Scientific Evidence

The evolving understanding of factor VIII binding sites and implications for the treatment of hemophilia A.

Gilbert GE, Blood Rev. 2019 Jan;33:1-5.

Summary:
This publication provides a current review of Factor VIII biology with a special emphasis on platelet interaction sites. The authors assume that understanding of these binding sites is a starting point to overcome limitations of current assays. They conclude that "Refined models of FVIII binding sites have the potential to improve FVIII assays, possibly improving bleeding risk stratification for patients with mild and moderate hemophilia A. They may also support earlier and more accurate detection of inhibitors, before they are clinically evident."

Factor VIII Activity and Inhibitor Assays in the Diagnosis and Treatment of Hemophilia A.

Castellone DD and Adcock DM, Semin Thromb Hemost. 2017 Apr;43(3):320-330.

Summary:
An informative review of the characteristics and pitfalls of Factor VIII Activity and Inhibitor Assays in the Diagnosis and Treatment of Hemophilia A.

The factor VIII protein and its function.

Mazurkiewicz-Pisarek A et al., Acta Biochim Pol. 2016;63(1):11-16.

Summary:
A 2016 review on Factor VIII biology for informative light reading.

Mild hemophilia A patient with novel Pro1809Leu mutation develops an anti-C2 antibody inhibiting allogeneic but not autologous factor VIII activity.

Yada K et al., J Thromb Haemost. 2015 Oct;13(10):1843-1853.

Summary:
Yada and colleagues analyzed a patient with mild hemophilia caused by heP1809L mutation in the A3 domain. They utilized the monoclonal antibodies ESH4 and EH8 to dissect the binding characteristics of this mutant FVIII. They took their findings to conclude that "the P1809L mutation in A3 induced the conformational change in the FVIII molecule that hampered antigenic determinant(s) located in the C2 domain and might result in the inhibitor development."

Platelet binding sites for factor VIII in relation to fibrin and phosphatidylserine.

Gilbert GE et al., Blood. 2015 Sep 3;126(10):1237-1244.

Summary:
Activated platelets expose binding sites for Factor VIII. Gilbert and colleagues analyzed the nature of these. They suspected that not phosphatidyl serine (PS) but rather soluble fibrin (bound to the αIIbβ3 integrin of platelets) might be the major binding mechanism. The used mutant factor VIII and the antibodies ESH8 and ESH4 (against the Factor VIII C2 domain) for their studies which allowed them to conclude "platelet-bound SF [soluble fibrin] is a component of functional fVIII binding sites."

Factor VIII Description – Scientific Evidence

Native plasma-derived FVIII/VWF complex has lower sensitivity to FVIII inhibitors than the combination of isolated FVIII and VWF proteins. Impact on Bethesda assay titration of FVIII inhibitors.

Bravo MI et al., Haemophilia. 2014 Nov;20(6):905-911.

Summary:
The original Bethesda method was developed to standardize measurement of inhibitors in a factor VIII neutralization assay. Bravo and colleagues used the monoclonal antibody ESH8 as model inhibitory antibody to compare variations of the Bethesda assay in which either Normal plasma, plasma derived FVIII/VWF complex or isolated FVIII (recombinant FVIII, B-domain deleted vs. plasma-derived FVIII) were used. Thrombin Generation Assays were performed with FVIII-deficient plasma spiked with the FVIII-VWF mixtures with and without the ESH-8 antibody. The authors took their findings to conclude that "VWF protection against FVIII inhibitor activity might be higher with native pd [plasma-derived] FVIII/VWF complex than with the corresponding compound formed from the isolated proteins. Bethesda assay titration using different FVIII concentrates would be advisable to guide the treatment of inhibitor patients."

Replacing the factor VIII C1 domain with a second C2 domain reduces factor VIII stability and affinity for factor IXa.

Wakabayashi H and Fay PJ, J Biol Chem. 2013 Oct 25;288(43):31289-31297.

Summary:
Wakabayashi and Fay studied the role of the Factor VIII C1 domain in Factor VIII function. The used genetically engineered recombinant FVIII variants as well as the monoclonal antibodies against distinct FVIII domains; among them ESH4 and ESH8. They took their data to suggest that "the C1 domain resides in close proximity to FIXa in the FXase complex and contributes a critical role to FVIII structure and function."

A putative inhibitory mechanism in the tenase complex responsible for loss of coagulation function in acquired haemophilia A patients with anti-C2 autoantibodies.

Matsumoto T et al., Thromb Haemost. 2012 Feb;107(2):288-301.

Summary:
Acquired haemophilia A (AHA) is caused by the development of two classes of factor VIII autoantibodies, characterized by their so-called type 1 or type 2 inhibitory behavior. AHA results in more serious haemorrhagic symptoms than congenital severe haemophilia A (HA). The authors performed studies in which -among others - the monoclonal antibodies ESH4 and ESH8 were used as model antibodies for acquired auto-antibodies. They conclude that their results "support the concept that global coagulation might be more suppressed in AHA than in severe HA due to the inhibition of FIXa-dependent FX activation by steric hindrance in the presence of FVIII-anti-C2 autoantibodies. Additionally, AHA-type 1 inhibitors prevented FVIIIa-phospholipid binding, essential for the tenase complex, whilst AHA-type 2 antibodies decreased FXa generation by inhibiting thrombin-catalyzed FVIII activation. These two distinct mechanisms might, in part, contribute to and exacerbate the serious haemorrhagic symptoms in AHA."

Factor VIII Description – Scientific Evidence

A new method measuring the interaction between von Willebrand factor and coagulation factor VIII.

Karlman M et al., Thromb Res. 2011 Jan;127(1):47-50.

Summary:
Karlman and colleagues developed an ELISA-type test to study the interaction between Factor VIII and von Willebrand Factor. In brief: Microtiter plates were coated with a monoclonal antibody (ESH-8), reacting with the C2 domain of FVIII. Thereafter the wells were treated with recombinant FVIII. After washing, diluted plasma samples were added and incubated for 1h. Then HRP-conjugated antibodies against VWF were added and used for quantification of bound VWF. They conclude that they "have established a simple and reliable method to detect decreased binding of FVIII to von Willebrand factor in plasma samples. The method can conveniently be used to study large populations, as well as finding minor binding defects in patients."

A membrane-interactive surface on the factor VIII C1 domain cooperates with the C2 domain for cofactor function.

Lü J et al., Blood. 2011 Mar 17;117(11):3181-3189.

Summary:
Lü and colleagues analyzed the interaction of Factor VIII with phosphatidylserine (PS)-containing membranes. An interaction that is mediated by the Factor VIII tandem, lectin-homology, C1 and C2 domains. Mutant FVIII molecules and monoclonal antibodies including clone ESH4 were instrumental for these studies. They conclude that their results "identify a membrane-binding face of the factor VIII C1 domain, indicate an influence of the C1 domain on factor VIII binding to factor X, and indicate that cooperation between the C1 and C2 domains is necessary for full activity of the factor Xase complex."

Kinetic parameters of monoclonal antibodies ESH2, ESH4, ESH5, and ESH8 on coagulation factor VIII and their influence on factor VIII activity.

Egler C et al., J Mol Recognit. 2009 Jul-Aug;22(4):301-306.

Summary:
Out of the ESH series of anti-Factor VIII (FVIII) antibodies, the antibodies ESH2, ESH4, ESH5 and ESH8 inhibit the pro-coagulant activity of factor VIII. Egler and colleagues systematically characterized and compared the inhibitory activity (binding kinetics) of these antibodies, as a model system for inhibitory antibodies which are raised against injected FVIII in about 25% of hemophiliacs as a serious side effect of substitution therapy. The antibodies differed in their equilibrium dissociation constants in accordance with their lowest dissociation constant ESH8, and ESH4 reduced FVIII activity of normal human plasma over 90 % in a one-stage clot inhibition assay. The authors conclude by stating that their study "can provide data to rationally test peptides/mimotopes to remove or neutralize inhibitors of FVIII activity."

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Factor VIII Description – Scientific Evidence

Role of the C2 domain of factor VIIIa in the assembly of factor-X activating complex on the platelet membrane.

Ahmad SS and Walsh PN, Biochemistry. 2005 Oct 25;44(42):13858-13865.

Summary:
Ahmad and Walsh studied the role of the Factor VIII C2 domain in the assembly of factor-X activating complex on the platelet membrane. For their studies they used natural and recombinant Factor VIII variants, synthetic peptides and the monoclonal antibodies ESH4 and ESH8. The state that “a major platelet-binding site resides within residues 2303-2332 in the C2 domain of FVIIIa, and an additional site within residues 2248-2285 increases the stoichiometry and affinity of FVIIIa binding to activated platelets only in the presence of FIXa and FX but does not directly mediate FVIIIa binding to the platelet surface”.

Low molecular weight peptides restore the procoagulant activity of factor VIII in the presence of the potent inhibitor antibody ESH8.

Villard S et al., J Biol Chem. 2002 Jul 26;277(30):27232-27239.

Summary:
The antibody ESH8 inhibits Factor VIII activity. Villard and colleagues used ESH8 to identify peptides which mimic the Factor VIII antigenic epitope and which interfere with the inhibitory activity of ESH8; so-called “peptide decoys”. By using the phage display technology, they identified peptides which specifically bound to ESH8; these peptides were then analyzed in functional Factor VIII assays. The authors summarize that “the ability of the selected peptides to neutralize the inhibitory activity of ESH8 was demonstrated in functional tests as well as in vivo in a murine model of hemophilia A. This study demonstrates the potential of this approach to neutralize the activity of potent inhibitory Abs.

Intrinsic pathway of blood coagulation contributes to thrombogenicity of atherosclerotic plaque.

Ananyeva NM et al., Blood. 2002 Jun 15;99(12):4475-4485.

Summary:
It this publication evidence was presented for a role of the intrinsic pathway of coagulation in the thrombogenicity of atherosclerotic lesions after exposure of subendothelial smooth muscle cells and macrophages to blood flow. The antibody ESH8 was used for immuno-histochemical detection Factor VIII in atherosclerotic lesions.

Slowed release of thrombin-cleaved factor VIII from von Willebrand factor by a monoclonal and a human antibody is a novel mechanism for factor VIII inhibition.

Saenko EL et al., J Biol Chem. 1996 Nov 1;271(44):27424-27431.

Summary:
The anti-Factor VIII (FVIII) C2 domain monoclonal antibody ESH8 inhibits FVIII activity only when FVIII is bound to von Willebrand factor (vWf). The inhibitory mechanism is due to a slowed rate of FVIIIa release from vWF which allows time for inactivation of unstable FVIIIa prior to its participation in the formation of the Factor Xase complex. As similar mechanism was found for an inhibitory antibody from a hemophilia A patient.

Factor VIII Description – Scientific Evidence

In vivo production of human factor VII in mice after intrasplenic implantation of primary fibroblasts transfected by receptor-mediated, adenovirus-augmented gene delivery.

Zatloukal K et al., Proc Natl Acad Sci U S A. 1994 May 24;91(11):5148-5152.

Summary:
This is a seminal publication on the initial steps towards gene therapy for Factor VIII deficiency. The study was performed in mice. Upon adenovirus-augmented gene delivery high expression levels of the B-domain-deleted human factor VIII were found in primary mouse fibroblasts and myoblasts. In this study the anti-Factor VIII antibodies ESH4 and ESH8 were used for immuno-histochemistry and to set up an enzyme-linked immunosorbent assay (ELISA) for Factor VIII quantification.

Development, optimization and use of an enzyme linked immunosorbent assay (ELISA) to measure factor VIII antigen utilizing monoclonal antibodies.

Hornsey VS et al., Transfus Med. 1992 Sep;2(3):223-9.

Summary:
Hornsey and colleagues describe the development of an enzyme-Linked immunosorbent assay for Factor VIII by using the monoclonal antibodies ESH4 and ESH8.

The production and characterization of a panel of ten murine monoclonal antibodies to human procoagulant factor VIII.

Griffin BD et al., Thromb Haemost. 1986 Feb 28;55(1):40-46.

Summary:
This is the seminal publication on the production and characterization of the ESH antibody series against Factor VIII. Griffin and colleagues studied these monoclonal antibodies for their binding specificity by immuno-adsorption and for their impact on FVIII activity.

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Factor VIII Products

1. Antigen, recombinant

Name	Source	Size	Art.No.
Coagulation Factor VIII Associated Protein 1 (F8A1), Mouse	recombinant	10 µg	6RPA148Mu01-10
		50 µg	6RPA148Mu01-50
		100 µg	6RPA148Mu01-100
Coagulation Factor VIII (F8), Canine	recombinant	10 µg	6RPB878Ca01-10
		50 µg	6RPB878Ca01-50
		100 µg	6RPB878Ca01-100
Coagulation Factor VIII (F8), Human	recombinant	10 µg	6RPB878Hu01-10
		50 µg	6RPB878Hu01-50
		100 µg	6RPB878Hu01-100
Coagulation Factor VIII (F8), Human	recombinant	10 µg	6RPB878Hu02-10
		50 µg	6RPB878Hu02-50
		100 µg	6RPB878Hu02-100
Coagulation Factor VIII (F8), Mouse	recombinant	10 µg	6RPB878Mu01-10
		50 µg	6RPB878Mu01-50
		100 µg	6RPB878Mu01-100
Coagulation Factor VIII (F8), Porcine	recombinant	10 µg	6RPB878Po01-10
		50 µg	6RPB878Po01-50
		100 µg	6RPB878Po01-100

2. Deficient Plasma

Name	Size	Art.No.
Factor VIII deficient human plasma	10 x 1 ml	938620

3. Antibodies (CE, IVD)

Name	Clone	Size	Art.No.
Factor VIII Related Antigen, RTU, polyclonal		2 ml	210147
		6 ml	210145
		25 ml	210146

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Factor VIII Products

4. Antibodies (Research-Use-Only)

Name	Clone	Size	Art.No.
Anti Coagulation Factor VIII Associated Protein 1 (F8A1), polyclonal, Mouse		50 µg 100 µg	6PAA148Mu01-50 6PAA148Mu01-100
Anti Coagulation Factor VIII (F8), polyclonal, Canine		50 µg 100 µg	6PAB878Ca01-50 6PAB878Ca01-100
Anti Coagulation Factor VIII (F8), polyclonal, Human		50 µg 100 µg	6PAB878Hu01-50 6PAB878Hu01-100
Anti coagulation Factor VIII (F8), polyclonal, Human		50 µg 100 µg	6PAB878Hu02-50 6PAB878Hu02-100
Anti coagulation Factor VIII (F8), polyclonal, Mouse		50 µg 100 µg	6PAB878Mu01-50 6PAB878Mu01-100
Anti coagulation Factor VIII (F8), polyclonal, Porcine		50 µg 100 µg	6PAB878Po01-50 6PAB878Po01-100
Anti Human Factor VIII	12H12	0,1 mg 1,0 mg	402063 402064
Anti Human Factor VIII	14E8	0,1 mg 1,0 mg	402065 402066
Anti Human Factor VIII	15D9	0,1 mg 1,0 mg	402067 402068
Anti Human Factor VIII	1C2	0,1 mg 1,0 mg	402069 402070
Anti Human Factor VIII	9C8	0,1 mg 1,0 mg	402071 402072
Murine MAb against human factor VIII, light chain	ESH-4	0,5 mg	938964
Murine MAb against human factor VIII, light chain	ESH-8	0,5 mg	938967
Murine MAb against human factor VIII, heavy chain	ESH-5	0,5 mg	938965
Rat anti mouse factor VIII	AMVIII-9035	0,1 mg 0,5 mg	400947 400948
Sheep anti human factor VIII, polyclonal		1,0 mg 5,0 mg	401194 400636
von Willebrand Factor /Factor VIII Related-Ag (Endothelial Marker), Concentrate	3E2D10	0,1 ml 0,5 ml	211274 210785
von Willebrand Factor /Factor VIII Related-Ag (Endothelial Marker), Concentrate	VWF635	0,1 ml 0,5 ml	211275 210786
von Willebrand Factor /Factor VIII Related-Ag (Endothelial Marker), Concentrate	3E2D10 + VWF635	0,1 ml 0,5 ml	211276 210836
von Willebrand Factor /Factor VIII Related-Ag (Endothelial Marker), Concentrate	IIIE2.34	0,1 ml 0,5 ml	211277 210837

Factor VIII Products

5. ELISA

Name	Size	Art.No.
Canine Coagulation Factor VIII (F8)	96 Tests	6SEB878Ca
Human Coagulation Factor VIII (F8)	96 Tests	6SEB878Hu
Mouse Coagulation Factor VIII (F8)	96 Tests	6SEB878Mu
Total Cyno monkey Factor VIII antigen assay	96 Tests	402192
	5 x 96 Tests	402193
Total human factor VIII antigen assay	96 Tests	402160
	5 x 96 Tests	402161
Total Rhesus monkey Factr VIII antigen assay	96 Tests	402190
	5 x 96 Tests	402191

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