Review Article

Recombinant Factor VIII for Haemophilia
An Overview of Production Technologies

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Normal blood coagulation depends on multiple coagulation factors mostly proteins and they circulate in the blood in different concentration. Haemophila A (classical haemophila) is a X linked recessive disorder caused by a deficiency of factor VIII. High frequency of haemophilia compared to other autosomal disorders has resulted a high demand of purified factor VIII for the treatment of haemophiliacs. In 1980s, production technology for the recombinant factor VIII came into the market and recent times many pharmaceutical companies are producing factor VIII for the use of the patients. Here we are over viewing various production technologies for factor VIII along with its purification and formulation strategies.

Introduction

Blood loss from the body must be stopped in case of injury in which blood vessels are cut or ruptured. This is accomplished by the solidification of blood known as coagulation or clotting. The stimulus generated due to the cut is propagated in a cascade; catalytically amplified at each step of the cascade in such a way that finally a rapid conversion of protein fibrinogen into its insoluble form (fibrin) takes place. Congregation and dumping of blood platelets at the site of vascular injury, thus effectively stop the blood leakage. Localised constriction of the blood vessels, minimise further flow of blood through the area. The blood coagulation process is dependent upon a large number of clotting factors, which act in sequential manner. At least 12 distinct factors participate in the coagulation process, along with several macromolecular cofactors[1] (Table 1 shows the names of different blood clotting factors). Except factor IV, all are proteins and most are proteolytic enzymes, present as zymogen and become sequentially activated. Blood clotting occurs via two distinct pathways. The final steps of these two clotting cascades are identical but the initial steps occur through two distinct pathways; extrinsic and intrinsic pathway. Clotting occurs much more rapidly when initiated via the extrinsic pathway. Both pathways are initiated when specific clotting proteins make contact with specific surface molecules exposed only upon damage to blood vessel.

Table 1 Name of the various blood clotting factors

Factor I	→	Fibrinogen
Factor II	→	Prothrombin
Factor III	→	Tissue thromboplastin
Factor IV	→	Calcium ion
Factor V	→	Labile factor
Factor VII	→	Stable factor
Factor VIII	→	Antihaemophilic factor
Factor IX	→	Christmas factor or plasma thromboplastin component (PTC)
Factor X	→	Stuart-power factor
Factor XI	→	Plasma Thromboplastin Antecedent (PTA)
Factor XII	→	Hageman factor
Factor XIII	→	Fibrin stabilising factor

Factor VIII and haemophilia

Haemophilia A (classical haemophilia), often called simply haemophilia is an X-linked recessive disorder which is caused by a deficiency of factor VIII. von Willebrand disease is a related disorder, also caused by a defect in the factor VIII complex. Intact factor VIII complex, usually purified from the blood, consists of two distinct gene products: factor VIII and multiple copies of von Willebrand factor (vWF). This complex display a molecular mass ranging from 1 - 2 million dalton, of which up to 15% is carbohydrate. The fully intact factor VIII complex is required to enhance the rate of activation of factor IX of the intrinsic system. Persons suffering from haemophilia A exhibit markedly reduced levels of (or complete absence of) factor VIII complex in their blood. Persons suffering from rare von Willebrand disease lack both components of mature factor VIII complex. The severity of the resultant disease is somewhat dependent upon the clotting disorders. Genetic defect characterized by (a) lack of expression or (b) altered amino acid sequence of any clotting factor can have serious clinical consequences. In order to promote effective clotting, both intrinsic and extrinsic coagulation pathways must be functional and the inhibition of one of these pathways will result in severely retired coagulation ability. The result is usually occurrence of spontaneous brushing and prolonged haemorrhage, which can be fatal. With the exception of tissue factor and Ca^{++} defects, all other clotting factors have been characterised, up to 90% of these, however, related to a deficiency of factor VIII while much of the remainder is due to the deficiency

in factor IX. Such clotting disorders are generally treated by ongoing administration of whole blood or more usually concentrates of relevant coagulation factors purified from whole blood. This entails accidental admission of blood borne disease, particularly hepatitis and AIDS. In turn this has hastened the development of blood coagulation factors produced by genetic engineering, several of which are now approved for general medical use. Persons completely devoid of factor VIII (or expression levels below 1% of normal level) will experience frequent, severe and often spontaneous bouts of bleeding. Persons expressing 5% or above of the normal complex level, experience less severe clinical symptoms[2]. Treatment normally entails administration of factor VIII complex purified from donated blood. More recently recombinant forms of the product have also become available.

About one in 10,000 males are born with a defect in the factor VIII complex and there are approximately 25,000 haemophiliac subjects currently resident in the USA. Haemophilia A affects 10-20 per 1000,000 male. High frequency of haemophilia compared to other autosomal disorders results from the position of this section of the gene on the X chromosome. Haemophilia as a disease has been described as early as the 10th century when the Moorish physician Khalaf Abu Abbas Abu-al-Kasim referred to a haemophilia disorder in a medical handbook. It became well known as “Royal Disease” in 18th century when Queen Victoria of England spread the disease to the ruling families of Europe and Russia. Transmission of haemophilia is a recessive X linked trait. Female can transmit haemophilia to their sons, while a male haemophiliac will be normal. In contrast daughter of male haemophiliacs may transmit the disease to half of their sons.

Biosynthesis and secretion of factor VIII

The structure of factor VIII was elucidated way back in 1984[3]. The gene for factor VIII is located at the tip of the long arm of the X chromosome, spans over 186 kb and as such one of the largest known genes it consists of 26 exons ranging in size from 69 bp to 3106 bp and introns as large as 32.4kb. The gene comprises nearly 1% of the X-chromosome[4]. Several lines of evidence indicate that liver hepatocyte are the major factor VIII producing cells[(5-7)]. Firstly, the factor VIII mRNA is present in the hepatocytes but not in sinusoidal cells. Secondly, the promoter region of the factor VIII gene comprises responsive elements that are characteristic for hepatocyte specific expression and finally in immunoultrastructural studies, factor VIII protein has been detected in the rough endoplasmic reticulum (ER) and the golgi apparatus of hepatocyte.

Studies on factor VIII biosynthesis and secretion have been limited by the lack of human cell lines that properly express significant amount of factor VIII. Analysis of the process has been therefore restricted to autologous gene expression. Low level of expression is associated with low level of steady state m-RNA and inefficient secretion. The initial stage of secretion involves the translocation of the mature 2332 amino acid polypeptide into the lumen of ER where N-linked glycosylation occurs. Within the ER, factor VIII appears to interact with a number of chaperon proteins including calreticulin, calnexin and IG binding proteins (BiP). Due to the interaction with these chaperon proteins, a significant proportion of factor VIII molecule is retained within the ER thereby limiting the transport of factor VIII to the Golgi apparatus. The mechanism of transport from the ER to Golgi apparatus is not yet elucidated. However, recent studies indicate that this step involves an intracellular membrane lectin: endoplasmic reticule Golgi intermediate compartment - 53 (ERGIC-53). Within the Golgi apparatus, factor VIII is subjected to further processing including modification of the N-linked oligosaccharides to complex type structures, O-linked glycosylation and sulfation of specific tyrosine residues. In addition, factor VIII also undergoes intracellular proteolysis. It has been noted that one of the main reasons for the poor secretion of factor VIII is located in codon 2307. The replacement of Arg 2307 by Glu or Leu leads to the poor secretion of factor VIII[8].

Structure of factor VIII

In plasma, factor VIII protein exists as a metal ion complex of 200 kDa heavy chain and 80 kDa light chain doublet and interacts with vWF secreted from vascular endothelial cells. The presence of vWF is required to stabilise factor VIII in the plasma. The triggering of the coagulation cascade and subsequent generation of serine protease, cleaves factor VIII by a process known as multiple proteolytic cleavage[9]. The mature factor VIII protein contains 2332 amino acids arranged in six domains, namely A1 (residues 1-336), A2 (372-710), B (741-1648), A3 (1896-2019), C1 (2020-2172), C2 (2173-2332). The A-domains are surrounded by acidic regions, (a1, a2, a3) occur twice in the heavy chain and once in the light chain, have homology to ceruloplasmin, a copper binding plasma protein. The light chain also contains a single B-domain, and two C-domains along with the A-domain. There are seven disulphide bridges in the mature molecule along with some free cysteine residues. Factor VIII contains 25 consensus sequences that allow N-linked glycosylation, 19 of which are located in the B-domain. The entire molecule is highly glycosylated. The B-domain is proteolytically released upon activation by thrombin or by factor Xa and is not required for the procoagulant activity *in vitro* or *in vivo*[9].

Classification of recombinant factor VIII

There are two types of recombinant factor VIII products: the first generation products - contain albumin as stabilizer in the final formulation e.g. Kogenate/Helixate, Recombinate and the second generation products - contain sucrose as stabilizer in the final formulation. e.g. ReFacto; KOGENATE/ Kogenate FS. They are further classified as natural full-length recombinant factor VIII molecule, contains complete factor VIII molecule as it occurs naturally in the body. B-domain deleted recombinant factor VIII molecule ReFacto.

B-Domain deleted recombinant factor VIII

The molecule of B-domain deleted recombinant factor VIII does not comprise of natural full-length factor VIII. The molecule is more stable and yield is higher. Thus removal of B-domain has no adverse effect on protein activity, omitting this region have the benefit of increasing expression and decreasing the overall size of the cDNA. There are three types of single chain cDNA encoding B-domain deleted derivatives, taking care that Arg 740 and Glu 1649 are important for processing.

Type I constructs: Arg 747, Arg 752 or Arg 776 in the N-terminal region of factor VIII B domain were fused to the Glu 1649 of the 80 KDa subunit.

Type II constructs: rVIII-S Q, Ser 743 was fused to Glu 1638 creating a link of 14 amino acids between 90 KDa and 80 KDa subunits.

Type III constructs: B-domain was completely removed or replaced with 1-4 Arg residues[10].

Licensed factor VIII preparations

Earlier whole blood transfusions were used to provide the deficient clotting factor VIII. This approach was only successful in the treatment of minor bleeding episodes. Then came the concentrated factor VIII products, in early 1950's, after the discovery of cryoprecipitate. The main problem with these type of products was the risk of viral contamination and transmission of various diseases like viral hepatitis, AIDS etc. Moreover these preparations were quite expensive too. In 1984, factor VIII gene was cloned and research began in the production of recombinant factor VIII (rFVIII) products. Recombinant factor VIII has biological activity comparable to that of plasma derived factor VIII and is safe and efficacious too[11]. Then in 1992, Baxter in conjuction with Genetic Institute introduced the world's first genetically engineered factor VIII under the name of 'Recobinate and Refacto'. Then in Feb 1993, Genetech produced second recombinant factor VIII product named 'Kogenate'. Till now there are five products licensed in the market under different brand names[12]. Table 2 shows different licensed factor VIII preparations available in the market.

Principles for the production and development of factor VIII by recombinant techniques

Factor VIII is one of the largest genes cloned to date and it is the largest cloned protein currently being used. But the production yield is low. Three reasons can be attributed that limit factor VIII expression 1) lack of proper transport of premature translation product from the endoplasmic reticulum to golgi apparatus, 2) High levels of von-Willebrand factor are required for stable production of factor VIII[13] and 3) chaperonin identified as immunoglobulin binding protein (BiP) within the ER (3). In addition to these, the 110 amino acid regions within the A1 domain inhibit factor VIII secretion from the mammalian cell lines[14]. Therefore manufacturing of such a complicated product must follow standard development procedure like, a) optimisation of the expression system b) preparation of cell banks. c) Culture medium designing and optimisation d) technical culture system e) purification process and f) formulation.

Table 2 Licensed factor VIII preparations

Trade name	License holder	Manufacturing company
Kogenate	Bayer	Bayer
Helixate	Aventis Behring	Bayer
Recombinate	Baxter	Baxter
ReFacto	Wyeth Ayerst	Genetic Institute
KOGENATE/kogenateFS	Bayer	Bayer

a) Optimisation of expression system

DNA clones encoding complete 2351 amino acids for human factor VIII have been isolated and are used to produce biologically active factor VIII in cultured mammalian cells[2]. Mammalian cell lines are used for the proper and active post-translational modification (correct folding and proper glycosylation) of the product. Their main disadvantages are more complex fermentation, low yield and increased production cost. But as the product is a very large molecule and highly glycosylated, mammalian cell line is the only suitable choice[15]. Characterization of the cell lines for factor VIII production are given in Table 3.

Table 3 Characterization of cell lines for factor VIII

Status	Safety
Approximate 150 copies of the human factor VIII gene per cell.	Free from microbial contamination
No change in the sequence of the protein- coding region.	No foreign body or retrovirus.
No change after 9 months fermentation under production condition or after 5 years of storage at $-196^{o}C$.	No tumorogenicity of the host cell DNA, no infectivity of the cells or cell particle.

The cell lines used are of two types 1) Baby hamster kidney BHK-21 and 2) Chinese hamster ovary (CHO). They were particularly chosen for the fact that these are well studied system and repeatedly being used for the production of other recombinant proteins and has greater replicative efficiency and comparatively better infection resistivity. Plasmid named pSAT.8cl (2) and PRXPy VIII-I were constructed for the transfer of factor VIII gene into the mammalian cell lines. Full-length factor VIII plasmid pSAT.8cl consists of the following genes for factor VIII and dihyrofolate reductase (DHFR) coding

regions: fragments of plasmid like pBR322, pUC13, SV40 early promoter, ampicillin resistance gene, adenovirus major late promoter and various linkers containing sites for multiple restriction endonucleases. The plasmid is introduced into a CHO or BHK-21 cell lines by calcium phosphate co-precipitation method. The plasmid is selected on methotrexate containing media plates and clones with high copy number are selected. The process has been scaled up using suitable culture medium containing adequate nutrients, oxygen supply, appropriate pH, and temperature. The final cell line obtained is called Master Cell Bank (MCB) and is preserved in liquid nitrogen.

b) Preparation of cell banks

Formation of MCB follows thorough characterization of the cells with regard to copy number, integration sites, as well as safety features such as lack of microbial/viral contaminants and viral transmission risks. In the next step, working cell banks are created (WCB) from the MCB. This is done by culturing thawed MCB cells as briefly as possible under defined conditions in culture flasks, spinners, or even small-scale fermenters. These WCB are preserved in liquid nitrogen and are used to inoculate the culture medium in large-scale fermenters.

c) Culture medium

The next step involves the development of serum free culture media that can support cell growth, product formation and ensure product stability. Product stability is particularly important for large factor VIII molecule. Medium should contain minimum animal and human derived components to minimize viral contamination. Culture medium should be of precisely defined composition; containing nutrients, vitamins, salts, recombinant insulin, albumin and other essential components required for the production of factor VIII.

d) Technical culture system

Normally suspension cultures in deep tank fermenters are used, where cells are cultivated under controlled condition. Because of the lack of cell wall, mammalian cells are extremely fragile and are to be handled very carefully in fermenter. All the products are manufactured in the large fermenters ranging from 100 to 2500 L. Mostly continuous perfusion cultures with cell retention type fermenters are used. Various control parameters that are taken care, for recombinant factor VIII production in these fermenters are oxygen concentration, pH, temperature, agitation, glucose, concentration, absence of mycoplasma virus, perfusion rate, cell density, cell viability and sterility condition[16].

e) Purification system

Purification process is the most critical one, in which the design must consider the following critical contaminations 1) cell derived (cell substrate DNA, cell substrate proteins) 2) media derived (bioactive proteins like insulin). Filtration separates host cells and cellular material from recombinant factor VIII. Then the product is purified using immunoaffinity chromatography. Factor VIII-von Willebrand factor complex is therefore pulled down by column chromatography using antibody raised against von Willebrand factor. This process also removes the impurities like bacteria, virus etc. 0.25-0.3 M calcium chloride is used to elute factor VIII from the column. von Willebrand factor remains bound to the monoclonal antibodies[17]. It has been noted that super porous gel support speed up the immunoaffinity chromatography with 1000-fold purification than normal agarose matrices[18]. Further purification of factor VIII is achieved by using ion exchange chromatography, which utilize the electrical charges of the molecule to remove unwanted proteins and host cell DNA. The product is further purified by size-exclusion chromatography using Sepharose CL-6B and TSK matrices[19]. All these chromatographic purification steps are strictly monitored for viral contamination and final purification of the product. Sometimes additional solvent detergent treatment using Triton X-100 are also done. In recent times solvents like tri-n-butyl phosphate and detergent like octoxynol are used in viral inactivation steps. These detergents dissolve the protective coating around lipid-enveloped viruses there by killing it[20].

f) Formulation

The large factor VIII molecule requires stabilizers like albumin and sucrose. But the use of albumin enhances the chances of viral contamination, therefore in the second-generation products; sucrose is used in the final formulation. The final products are filled into bottles and freeze dried. Before each lot of recombinant factor VIII is released, the final product is tested several times. The production cells that are used to produce this lot are also tested *in vitro* and *in vivo* to make sure that there is no virus.

Manufacturing of licensed recombinant factor VIII preparations

a) Manufacturing of Kogenate

The parent cell line used to manufacture kogenate is the Baby Hamster kidney cell line (BHK 21), which was established in 1962. Full-length factor VIII gene is assembled in the plasmid along with tandem promoter and dihydrofolate reductase (DHFR) gene as amplifier. The plasmid is cloned into BHK-21 cells. These transformed cells are selected on methotrexate to amplify the copy number of the factor VIII coding region. The clone with highest copy number is selected and further amplified; it is then adapted to suspension condition in spinner and fermenter culture and finally deposited as cell bank R3 for manufacturing. Cell safety characterization of the R3 cells before and after production revealed neither adventitious

agents nor tumorogenic activity in the cell line. Medium for Kogenate production consists of recombinant insulin and human albumin. These two proteins supplement in addition to some other synthetic compounds, which are required for stabilisation of the full-length recombinant factor VIII. The medium is sterilised by filtration. It is bovine serum free and does not contain von Willebrand Factor for stabilisation. Production of kogenate is high cell density, continuous perfusion culture type and carried out in fermenters of up to 500 L. The cells are cultivated in controlled conditions for six months. During the fermentation cycle, culture fluid containing recombinant factor VIII is continuously removed from the fermenter and is replaced with the fresh medium. High cell density maintained by separating the cells from the harvest fluid in an external cell retention system and recycling them back to fermenter. The advantages of the perfusion culture at high cell density are 1) high degree of culture control beyond basic oxygen, pH and temperature. In perfusion culture optimum conditions are maintained in fermenters all the time by perfusion of raw materials and 2) reduction of the fermentation volume. (Perfusion fermenter of 500 L is generally used for kogenate production.)

The purification system is generally carried out batch wise. Initial purification involves various filtration techniques followed by various chromatographic procedures. The first step in chromatography involves anion exchange column for removal of media additives, host cell DNA and other impurities. Subsequently 'weak virus inactivation step' is carried out by incubating the solution at 40^{o}C for 8 hour in buffer containing imidazole and polysorbate[21]. The product is further purified using immunoaffinity chromatography. Then gel filtration based on size exclusion is performed, followed again by the immunoaffinity chromatography purification and anion exchange chromatography. Finally the product is formulated by adding excipients such as histidine, calcium, glycine and human serum albumin (as stabilizer). It is then freeze dried at the three different fill sizes 250, 500 and 1000 IU of recombinant factor VIII.

b) Manufacturing of Recombinate

The parental cell line is the widely used CHO cell line, which is thought to be mini-manufacturing plant for factor VIII. The CHO cells are ideal hosts for recombinate. The cells grow exceptionally well inside the bioreactor; are well studied and have safely been used since 1951. These cells have also been successfully used in the production of other recombinant proteins used as therapeutics, such as insulin for diabetes, human growth hormone for children with growth hormone deficiency etc. The full-length human factor VIII molecule was selected and cloned into CHO cells using a plasmid having DHFR gene. High productive clones are generated using methotrexate amplification. However, it was found that yield of rFVIII secreted into culture medium were low when bovine serum free culture medium was used. This could be overcome with the addition of external vWF to the medium. Therefore, human vWF gene was also inserted into the rFVIII secreting cells using a separate transfection vector containing the human vWF gene and gene for adenosine deaminase as marker. The marker allowed for the selection of vWF co-expressing cells in the presence of deoxycoformycin. The vWF gene is added as it acts as a natural stabilizer for the factor VIII protein in the body, protects the fragile structure of rFVIII molecule during processing and helps to produce a greater amount of intact factor VIII molecule.

The cell line was adapted to serum free conditions before the cell banks were established and stored in liquid nitrogen. Cell safety characterization of the production cell line before and after the typical production cycle should reveal no microbial contamination or viral safety problem. Recombinate cell cultivation for manufacturing is performed in a serum free medium containing the bovine additives insulin, albumin, and aprotinin (which is a protease inhibitor) to minimize the degradation of recombinant factor VIII in supernatant. The large-scale technical culture system is a modification of a 2500-L batch culture in which the cells are cultivated under controlled conditions with respect to oxygen, pH, temperature and agitation. The operational mode is called batch-refed process or fed batch process. Thawing the cells from the WCB starts a production cycle expand them through tissue culture, flask and spinner cultures up to 8-liter column before fermenters are inoculated. As the product and cell growth is simultaneous, product has to be harvested before the cells attain stationary growth phase. The removed fermentation volume is replaced with the fresh medium. The sequence of growth and harvest may be continued for up to 55 days. The purification process of Recombinate begins with depth filter to remove the cells and cell debris. Followed of three column chromatography steps Immunoaffinity chromatography using immobilized monoclonal antibodies is the main purification step. It is followed by two ion-exchange column chromatographies, with anion and cation exchanger in series for the removal of additional host cell impurities and media impurities. Finally, the Recombinate is formulated using Human Serum Albumin (HSA), polyethylene glycol-3350, histidine and calcium. It is freeze dried at fill sizes of 250, 500 and 1000 IU.

c) Manufacturing of ReFacto

ReFacto differs from the other recombinant factor VIII preparations, as it does not comprise the natural full-length factor VIII molecule, it lacks central part of B-domain. The key advantages are increased stability of the construct and simplified production. Recombinant factor VIII SQ is produced in CHO cells cultured in serum free medium containing HSA. CHO cells were transfected with the rFVIII SQ gene, containing DHFR gene sequence and amplified using methotrexate selection system. Later, cell line was adapted to suspension condition in order to use them for fermenter cultures, and the cell banks were prepared. In contrast to

the processes developed for the production of full-length rFVIII, the fermentation of Refacto producing cells is divided into two separate phases; cell growth phase and production phase. An initial cell multiplication is followed by the specific production phase under stationary growth condition. The phase change is triggered by the addition of butyrate, shifts in oxygen concentration and temperature. The large-scale technical culture system for Refacto is the continuous perfusion culture with cell retention system.

A typical manufacturing cycle starts with the expansion of WCB cells in a batch-refed mode in tissue culture flask, until the first fermenter of 50 L can be innoculated. This culture is then used to innoculate the final production fermenter of 500 L, which is also run in perfusion model. Once the final cell density is reached, the culture is switched from growth to product accumulation phase by adding butyrate and adjusting temperature and oxygen concentration. Fermentation is then continued in perfusion mode and product is continuously collected. The purification process for Refacto begins with the removal of the remaining cells and cell debris accumulated in the harvest by filtration. The purification process itself consists of a five-step chromatography process with the addition of specific virus inactivation step using Tri-n-butyl phosphate and Triton X-100. The following column chromatography sequence is performed for the purification steps. 1) Cation exchange chromatography followed by virus inactivation step, 2) immunoaffinity chromatography, 3) anion exchange chromatography, 4) hydrophobic chromatography using butyl-sepharose, and 5) gel filtration chromatography. Finally ReFacto is formulated using HSA and vWF free formulation containing sucrose, polysorbate 80, L - histidine, sodium chloride and calcium chloride. Product is freeze-dried and packaged into 250-500 and 1000 IU fill sizes.

d) Manufacturing of Kogenate FS

The newest recombinant factor VIII product available is the second-generation product, called kogenate. This product combines the advantages of the natural full-length FVIII molecule with an albumin free formulation and improved virus safety profile incorporating solvent detergent viral inactivation methods too. The cell line used is BHK-derived cell line; but the composition of culture medium and the continuous perfusion fermentation are identical between kogenate and kogenate FS. However, a faster purification process has been developed consisting of six-step column chromatography and viral inactivation. The filtered cell-free fermentation harvest is first purified using anion-exchange chromatography, followed by virus inactivation using solvent (tri-n-butyl phosphate) and detergent (octoxynol), after which immunoaffinity chromatography is performed using immobilized monoclonal antibodies. The eluant is then subjected to purification process improved by the addition of immobilized metal affinity chromatography (IMAC). A column containing immobilized copper ion binds rFVIII by forming chelates. An imidazole buffer that acts as a competing agent subsequently elutes the column. This step removes additional trace levels of residual host proteins. The final steps of purification involve gel filtration, cation exchange chromatography, and flow through anion-exchange chromatography. Finally, the product is filtered and formulated in an albumin-free formulation consisting of sucrose, glycine, histidine, and calcium as stabilizers and buffers. It is formulated in 250, 500, and 1000 IU fill sizes.

Problems in recombinant factor VIII therapy

Although haemophilia A patients have been successfully treated with either plasma-derived or recombinant factor VIII, the most critical remaining problem is the appearance of antifactor VIII antibodies that activate factor VIII interaction with factors IX and X and phospholipid. Inhibition of factor VIII binding to vWF by antibodies also inactivates factor VIII because vWF maintains the required association of the Factor VIII heavy and light chains in the circulation[22]. Inhibitor epitopes are found in the Factor VIII domain A2, A3, and C2 and in the acidic region between a1 and a2[23-26]. This leads to prevention of Factor VIII binding to factor IX by anti-A3 antibodies, factor X binding by anti-acidic region antibodies and phospholipid, and vWF binding by anti-C2 antibodies. New approaches to solve the associated problems include immunosuppressive therapy and existing antibody eradication. Immunologists to solve this problem are studying them extensively.

Conclusion

The field of haemophilia has always been one of the great challenges, in which developments in basic science and clinical research have been rapidly translated into clinical practice. In the past, whole plasma was used as replacement therapy for factor VIII; then came plasma concentrate. But both the products had risk of transmitting viral diseases, moreover these plasma derivatives were not able to meet the demands of the patients. With the advancement in genetic engineering it became possible to recreate, in a laboratory, a process that produces the factor VIII protein in the human body. This full-length recombinant factor VIII protein was manufactured under strict conditions to minimize contamination. But the expression of factor VIII in the mammalian cell was quite low. To solve this problem of low expression, the B-domain deleted recombinant factor VIII came into the market. This product was more stable and less difficult to produce. Today recombinant factor VIII preparations are broadly divided into two classes, first generation recombinant factor VIII products that have albumin as stabilizer in the final formulation and second-generation recombinant factor VIII product that do not have albumin as stabilizer. Advantage of second-generation products is that there is very little possibility of viral contamination due to the absence of albumin. But one thing is to be noted; second generation products still contain albumin in the

culture media, thus possibility of viral contamination is not totally removed. Due to these reasons, scientists now are looking for new possibilities for the treatment of haemophilia. 'Gene therapy' is one such approach, which is coming up with greater promises and expectation in biotechnology community. In this approach the break-through is expected for the treatment of patients by directly delivering the gene for factor VIII. If such treatment becomes effective it will be favourable as compared to conventional haemophilia therapy and will require less frequent injection of per yearly dose of factor VIII. On the other hand direct administration of factor Xa will by-pass the non-functional steps in the coagulation cascade.

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