PEPTIDE

FIELD OF THE INVENTION

The present invention relates to a peptide. In particular, it relates to peptides at least the core sequence of which is derivable from factor VIII (FVIII). The peptides can be used to reduce or prevent factor VIII inhibitor antibody formation, for example in haemophilia A treatment and acquired haemophilia.

BACKGROUND TO THE INVENTION

HAEMOPHILIA

Haemophilia belongs to a group of inheritable blood disorders that includes haemophilia A, haemophilia B (Christmas disease) and Von Willebrand's disease.

In haemophilia, the blood's ability to clot is severely reduced because an essential clotting factor is partly or completely missing, resulting in increased bleeding time. Haemophilia A is a deficiency of the clotting factor VIII, whereas Haemophilia B is a deficiency of clotting factor IX. In both diseases, the faulty gene is found on the X chromosome, so the conditions are X-linked. Haemophilia A is five times more common than haemophilia B.

Haemophilia is a lifelong inherited genetic condition, which affects females as carriers and males who inherit the condition. About a third of new diagnoses are where there is no previous family history. It appears world-wide and occurs in all racial groups. About 6,000 people are affected with haemophilia in the UK.

Haemophiliacs bleed for a prolonged period following injury. External injuries such as cuts and grazes do not usually pose serious problems: it is often possible to stop bleeding by applying a degree of pressure and covering the affected area (e.g with a plaster).

The main problem is internal bleeding into joints, muscles and soft tissues, which can occur spontaneously. Internal bleeding, such are haemorrhages into the brain, is very difficult to manage and can be fatal. Repeated bleeding in the joints causes acute pain and can cause arthritis and/or long-term joint damage leading to disability.

Treatment for haemophilia is usually by replacement of the missing clotting factor. In mild or moderate haemophilia injections may be given at the time a bleed occurs (on-demand therapy). However, in severe haemophilia regular prophylactic injections are given to help the blood to clot and minimise the likelihood of long term joint damage.

A potentially serious complication of coagulation factor replacement therapy for haemophilia A is the development of antibodies that neutralise the procoagulant function of factor VIII. Factor VIII inhibitors occur in approximately 25% of those with severe haemophilia A. Since patients with congenital haemophilia A can be genetically deficient in FVIII, the synthesis of inhibitors is an alloimmune response to the foreign protein administered to prevent or treat bleeding episodes.

CD4+ T cells play a central role in the immune response to FVIII. After being taken up by antigen-presenting cells (APCs), FVIII undergoes proteolytic degradation into peptide fragments (Reding *et al* (2006) Haemophilia 12(supp 6) 30-36). These peptides are then presented on the surface of the APC in association with MHC class II molecules. This complex is then recognised by the T cell receptor of a CD4+ cell specific for FVIII. In the presence of the appropriate costimulatory signals, this recognition ultimately causes the CD4+ cell to direct the synthesis of antibodies by B cells.

The incidence of inhibitor formation initially increases with the number of factor VIII treatments, but appears to plateau after 50-100 exposure days. Inhibitor formation is much more common in severe haemophilia than in moderate or mild disease and some molecular defects, most clearly large deletions and nonsense mutations in the factor VIII light chain, appear to predispose to inhibitor formation. Parameters such as the concentration, type (purified or recombinant) of replacement factor, and treatment history may also affect the likelihood of antibody production.

The management of haemophilia patients with inhibitors is an ongoing challenge. Immune tolerance induction (ITI) using a desensitization technique is successful in some patients with alloantibodies against factor VIII. This therapeutic approach requires ongoing exposure to factor replacement therapy, so is a long-term strategy.

Although ITI can be successful, a significant proportion (about 30%) of patients fail to respond to ITI. Patients with high inhibitor titres are much less likely to respond to treatment. Another significant contributing factor is age at the start of commencing ITI, with greatly decreased success rates when the patient is older than 20 (Hay *et al* (2005) Seminars in Thrombosis and Hemostasis 32:15-21)

When ITI therapy is unsuccessful, the inhibitor generally persists for life, and because such patients are usually high-responders, it is necessary to treat episodes of bleeding with FVIII bypassing products, such as activated prothombin complex concentrates (FEIBA™), and recombinant-activated FVII. However, the use of such agents is associated with adverse events such as disseminated intravascular coagulation, acute myocardial infarction, pulmonary embolus and thromboses (Acharya and DiMichele (2006) Best Practice & Research Clinical Haematology 19:51-66).

Immunosuppressive therapy is sometimes used for patients who fail to response to ITI. Treatment includes administration of immunosuppressive drugs such as cyclophosphamide, prednisone, azathioprine and cyclosporine which non-specifically target the immune system. These treatments can have side-effects associated with general immunosuppression.

There is renewed interest on selective B cell depletion using Rituximab™, a humanised monoclonal antibody to B cell CD20 antigen. However, infusion reactions, serum sickness and opportunistic infections have occurred in some children treated with this drug (DiMichele (2007) J Thromb Haemost 5:143-50).

ACQUIRED HAEMOPHILIA

Acquired haemophilia is a rare autoimmune condition which affects between 1 and 4 people in every million. In this condition, subjects who are not born with haemophilia

develop antibodies against one of the clotting factors such as factor VIII. It is thought that pregnancy and autoimmune diseases such as rheumatoid arthritis and cancer may increase the risk of developing acquired haemophilia. Although there are differences in the underlying immune mechanisms leading to their production, the clinical manifestations of FVIII inhibitors produced in response to coagulation factor replacement therapy and those produced in acquired haemophilia are similar.

Acquired haemophiliac patients have a mortality rate that approaches 25%, partly because of the association of acquired inhibitors with severe bleeding complications. The therapy of acquired autoantibody inhibitors is based primarily on the need to control or prevent acute hemorrhagic complications, which frequently are life and limb threatening and secondarily to eradicate the autoantibody to restore normal coagulation.

Some bleeds associated with low titre autoantibody inhibitors (< 5 Bethesda Units) may be treated effectively with FVIII concentrates administered at high doses. Porcine FVIII concentrate was formerly considered a critical first-line therapy for acquired hemophilia-related bleeding since it was the only replacement therapy that provided an opportunity to actually measure post-infusion FVIII coagulation activity levels in the laboratory. The product was removed from the marketplace in 2004 because of contamination of the porcine plasma pools by porcine parvovirus. Now, "bypassing" agents are most commonly used, but potential risks of thrombogenicity exist and there is only about 80% efficacy for each product. Plasma exchange via plasmapheresis and extracorporeal immunoadsorption may be necessary to temporarily reduce the inhibitor titer enough for bypassing agents or FVIII replacement to provide adequate hemostasis.

Eradication of autoantibody inhibitors depends on immunosuppressive measures, such as: (1) administration of corticosteroids with 30%–50% efficacy in 3–6 weeks; (2) use of cytotoxic and myelosuppressive chemotherapeutic agents, e.g., cyclophosphamide, cyclosporine, 2-chlorodeoxyadenosine; (3) immunomodulation with intravenous immunoglobulin; and (4) selective B-lymphocyte depletion with rituximab. Rituximab™ responders may require concurrent use of steroids and relapses may respond to retreatment.

Thus, all currently available methods for reducing alloantibody production associated with haemophilia A treatment, and autoantibody production in acquired haemophilia,

have shortcomings. There is therefore a need for improved methods to address the issue of anti-FVIII antibodies in haemophilia A and acquired haemophilia.

The present inventors have found that it is possible to prevent FVIII inhibitor antibody formation by pre-tolerising the patient with FVIII-derived peptides.

SUMMARY OF ASPECTS OF THE INVENTION

The first aspect of the present invention, therefore, relates to a peptide, at least part of the sequence of which is derivable from FVIII, which is capable of inducing or restoring tolerance to FVIII.

In a first embodiment, the present invention provides a peptide comprising one of the following FVIII-derived sequences:

GTLMVFFGNVDSSGI
TQTLHKFILLFAVFD
SLYISQFIIMYSLDG
PPIIARYIRLHPTHY
PPLLTRYLRIHPQSW
MHTVNGYVNRSLPGL
LGQFLLFCHISSHQH
DTLLIIFKNQASRPY
PRCLTRYYSSFVNME
TENIQRFLPNPAGVQ
DNIMVTFRNQASRPY
RYLRIHPQSWVHQIA

with one or more of the following modifications:

(i) removal of one or more hydophobic amino acid(s);

(ii) replacement of one or more hydrophobic amino acid(s) with charged hydrophilic amino acid(s); and

(iii) insertion of a charged amino acid at one or both terminus(i)

which modified peptide is capable of binding to an MHC class II molecule without further antigen processing and being recognised by a factor VIII specific T cell.

The "parent" (unmodified) peptide may be PRCLTRYYSSFVNME or DNIMVTFRNQASRPY

In a second embodiment the present invention provides a peptide comprising the sequence

$X(aa)_n$-core sequence -$(aa)_m$

wherein X is a charged hydrophilic residue;

aa is an amino acid;

n is an integer between 0 and 5;

m is an integer between 0 and 5; and

the "core sequence" is selected from the following group of FVIII-derived peptides: LYISQFIIM

FIIMYSLDG

IARYIRLHP

LIIFKNQAS

LTRYYSSFV

MVTFRNQAS

LRIHPQSWV

which peptide is capable of binding to an MHC class II molecule without further antigen processing and being recognised by a factor VIII specific T cell.

For example, the peptide may comprise the sequence

XDNIMVTFRNQAS.

In a third embodiment, the present invention provides a peptide comprising the sequence:

$Y(aa)_n$-core sequence-$(aa)_mZ$

wherein Y and Z are charged amino acids having reverse polarity;

aa is an amino acid;

n is an integer between 0 and 5;

m is an integer between 0 and 5; and

the "core sequence" is selected from the following group of FVIII-derived peptides:

LYISQFIIM

FIIMYSLDG

IARYIRLHP

LIIFKNQAS

LTRYYSSFV

MVTFRNQAS

LRIHPQSWV

which peptide is capable of binding to an MHC class II molecule without further antigen processing and being recognised by a factor VIII specific T cell.

For example, the peptide may comprise the sequence:

YDNIMVTFRNQASZ

In the third embodiment, for example, Y may be a positively charged amino acid and Z may be a negatively charged amino acid. Alternatively, Y may be a negatively charged amino acid and Z may be a positively charged amino acid.

A charged, hydrophilic amino acid may, for example be D, E, K, H or R. A positively charged amino acid may, for example be K, H or R. A negatively charged amino acid may, for example be D or E.

The peptide of the first aspect of the invention may, for example, comprise or consist of the sequence EDNIMVTFRNQASR.

In a second aspect, the present invention provides a composition, such as a pharmaceutical composition comprising one or more peptide(s) of the first aspect of the invention. The composition may comprise a plurality of peptides wholly or partly derivable from FVIII which are capable of inducing or restoring tolerance to FVIII.

The composition may be in the form of a kit, in which the plurality of peptides are provided separately for separate, subsequent, sequential or simultaneous administration.

The peptide or a composition of the invention may be for use in suppressing, reducing, or preventing the development of factor VIII inhibitor antibodies.

The present invention also provides the use of such a peptide or composition in the manufacture of a medicament to suppress, reduce or prevent the development of factor VIII inhibitor antibodies.

The present invention also provides a method for suppressing, preventing or reducing the development of Factor VIII inhibitor antibodies in a subject, which comprises the step of administration of such a peptide or composition to the subject.

The subject may be deficient in FVIII. In particular the subject may have haemophilia A, and may be, or be about to, undergo factor VIII replacement therapy.

Alternatively the subject may have, or be at risk from contracting, acquired haemophilia.

Factor VIII inhibitors are found more frequently in individuals expressing HLA-DR2. The subject treated by the method of the invention may therefore be HLA-DR2 positive.

DESCRIPTION OF THE FIGURES

Figure 1: Recall responses for lymph node cells (LNC) from FVIII+DR2+ mice primed with rhFVIII/CFA

a) LNC proliferation to FVIII peptides 1-6

b) LNC proliferation to FVIII peptides 7-12

c) LNC proliferation to FVIII peptides 1, 3 and 11

Figure 2: Representative examples of FVIII+DR2+ T cell hybridoma clones specific for FVIII-derived peptides

Figure 3: Recall responses for LNC from FVIII-DR2+ mice primed with rhFVIII/CFA

Figure 4: Representative examples of FVIII-DR2+ T cell hydridoma clones specific for FVIII-derived peptides

Figure 5: FVIII-/- clones specific for a) DNIMV and b) PRCLT

Figure 6: Recall responses for LNC to FVIII for FVIII+DR2+ mice treated 3x i.p. with peptide prior to priming with rhFVIII/CFA.

Figure 7: Determination of the range of peptide epitopes capable of functions as apitopes using FVIII-DR2+ T cell hydridoma clones specific for FVIII-derived overlapping peptides. The original peptide is termed 0. One amino acid shift towards the N-terminal is -1, two amino acid shits towards the N-terminal is -2 etc. One shift towards the C-terminal is +1 etc.

Figure 8: Lymph node cell IFN-gamma production in response to FVIII for FVIII-DR2+ mice treated with FVIII-derived peptides PRCLT, DNIMV or a mixture of both of these.

Figure 9: Responses from naive or tolerised mice stimulated with either EDNIMVTFRNQASR (EDNIMV) or a control peptide (DNIMV).

DETAILED DESCRIPTION

PEPTIDE

The present invention relates to a peptide.

The term "peptide" is used in the normal sense to mean a series of residues, typically L-amino acids, connected one to the other typically by peptide bonds between the α-amino and carboxyl groups of adjacent amino acids. The term includes modified peptides and synthetic peptide analogues.

The peptide of the present invention may be made using chemical methods (Peptide Chemistry, A practical Textbook. Mikos Bodansky, Springer-Verlag, Berlin.). For example, peptides can be synthesized by solid phase techniques (Roberge JY *et al* (1995) Science 269: 202-204), cleaved from the resin, and purified by preparative high performance liquid chromatography (e.g., Creighton (1983) Proteins Structures And Molecular Principles, WH Freeman and Co, New York NY). Automated synthesis may

be achieved, for example, using the ABI 43 1 A Peptide Synthesizer (Perkin Elmer) in accordance with the instructions provided by the manufacturer.

The peptide may alternatively be made by recombinant means, or by cleavage of ta peptide from factor VIII followed by modification of one or both ends. The composition of a peptide may be confirmed by amino acid analysis or sequencing (e.g., the Edman degradation procedure).

For practical purposes, there are various other characteristics which the peptide may show. For example, it is important that the peptide is sufficiently stable *in vivo* to be therapeutically useful. The half-life of the peptide *in vivo* may be at least 10 minutes, 30 minutes, 4 hours, or 24 hours.

The peptide may also demonstrate good bioavailability *in vivo*. The peptide may maintain a conformation *in vivo* which enables it to bind to an MHC molecule at the cell surface without due hindrance.

CORE RESIDUES

In an adaptive immune response, T lymphocytes are capable of recognising internal epitopes of a protein antigen. Antigen presenting cells (APC) take up protein antigens and degrade them into short peptide fragments. A peptide may bind to a major histocompatability complex (MHC) class I or II molecule inside the cell and be carried to the cell surface. When presented at the cell surface in conjunction with an MHC molecule, the peptide may be recognised by a T cell (via the T cell receptor (TCR)), in which case the peptide is a T cell epitope.

An epitope is thus a peptide derivable from an antigen which is capable of binding to the peptide-binding groove of a MHC class I or II molecule and be recognised by a T cell.

The minimal epitope is the shortest fragment derivable from an epitope, which is capable of binding to the peptide-binding groove of a MHC class I or II molecule and being recognised by a T cell. For a given immunogenic region, it is typically possible

to generate a "nested set" of overlapping peptides which act as epitopes, all of which contain the minimal epitope but differ in their flanking regions.

By the same token, it is possible to identify the minimal epitope for a particular MHC molecule:T cell combination by measuring the response to truncated peptides. For example if a response is obtained to the peptide comprising residues 1-15 in the overlapping library, sets which are truncated at both ends (i.e. 1-14, 1-13, 1-12 etc. and 2-15, 3-15, 4-15 etc.) can be used to identify the minimal epitope.

The present invention provides peptides comprising a "core residue" sequence of FVIII which selected from the following list:

LYISQFIIM
FIIMYSLDG
IARYIRLHP
LIIFKNQAS
LTRYYSSFV
MVTFRNQAS
LRIHPQSWV

These core residue sequences were predicted using HLA-DR2 binding algorithms to represent or comprise the minimal epitope for each region, as shown in the Examples.

APITOPES

The present inventors have previously determined that there is a link between the capacity of a peptide to bind to an MHC class I or II molecule and be presented to a T cell without further antigen processing, and the peptide's capacity to induce tolerance *in vivo* (WO 02/16410). If a peptide is too long to bind the peptide binding groove of an MHC molecule without further processing (e.g. trimming), or binds in an inappropriate conformation then it will not be tolerogenic *in vivo*. If, on the other hand, the peptide is of an appropriate size and conformation to bind directly to the MHC peptide binding groove and be presented to a T cell, then this peptide can be predicted to be useful for tolerance induction.

It is thus possible to investigate the tolerogenic capacity of a peptide by investigating whether it can bind to an MHC class I or II molecule and be presented to a T cell without further antigen processing *in vitro*.

The peptides of the present invention are apitopes (Antigen Processing-Indepent epiTOPES) in that they are capable of binding to an MHC class II molecule and stimulating a response from factor VIII specific T cells without further antigen processing. Such apitopes can be predicted to cause tolerance to FVIII, following the rule-based method described in WO 02/16410.

A peptide of the present invention may be any length that is capable of binding to an MHC class I or II molecule without further processing. Typically, the peptide of the present invention is capable of binding MHC class II.

Peptides that bind to MHC class I molecules are typically 7 to 13, more usually 8 to 10 amino acids in length. The binding of the peptide is stabilised at its two ends by contacts between atoms in the main chain of the peptide and invariant sites in the peptide-binding groove of all MHC class I molecules. There are invariant sites at both ends of the groove which bind the amino and carboxy termini of the peptide. Variations is peptide length are accomodated by a kinking in the peptide backbone, often at proline or glycine residues that allow the required flexibility.

Peptides which bind to MHC class II molecules are typically between 8 and 20 amino acids in length, more usually between 10 and 17 amino acids in length, and can be longer (for example up to 40 amino acids). These peptides lie in an extended conformation along the MHC II peptide-binding groove which (unlike the MHC class I peptide-binding groove) is open at both ends. The peptide is held in place mainly by main-chain atom contacts with conserved residues that line the peptide-binding groove.

PEPTIDE SEQUENCES

The first embodiment of the invention relates to a peptide comprising one of the following FVIII-derived sequences:

GTLMVFFGNVDSSGI

TQTLHKFILLFAVFD
SLYISQFIIMYSLDG
PPIIARYIRLHPTHY
PPLLTRYLRIHPQSW
MHTVNGYVNRSLPGL
LGQFLLFCHISSHQH
DTLLIIFKNQASRPY
PRCLTRYYSSFVNME
TENIQRFLPNPAGVQ
DNIMVTFRNQASRPY
RYLRIHPQSWVHQIA

with one or more of the following modifications:

(i) removal of one or more hydophobic amino acid(s);

(ii) replacement of one or more hydrophobic amino acid(s) with charged hydrophilic amino acid(s); and

(iii) insertion of a charged amino acid at one or both terminus(i)

which modified peptide is capable of binding to an MHC class II molecule without further antigen processing and being recognised by a factor VIII specific T cell.

A list of standard amino acids, together with their side chain polarity, charge and hydropathy index is given in Table 1.

Table 1

Amino Acid	3-Letter	1-Letter	Side chain polarity	Side chain charge (pH 7)	Hydropathy index
Alanine	Ala	A	nonpolar	neutral	1.8
Arginine	Arg	R	polar	positive	-4.5
Asparagine	Asn	N	polar	neutral	-3.5
Aspartic acid	Asp	D	polar	negative	-3.5
Cysteine	Cys	C	nonpolar	neutral	2.5
Glutamic acid	Glu	E	polar	negative	-3.5
Glutamine	Gln	Q	polar	neutral	-3.5
Glycine	Gly	G	nonpolar	neutral	-0.4
Histidine	His	H	polar	positive	-3.2
Isoleucine	Ile	I	nonpolar	neutral	4.5
Leucine	Leu	L	nonpolar	neutral	3.8
Lysine	Lys	K	polar	positive	-3.9

Methionine	Met	M	nonpolar	neutral	1.9
Phenylalanine	Phe	F	nonpolar	neutral	2.8
Proline	Pro	P	nonpolar	neutral	-1.6
Serine	Ser	S	polar	neutral	-0.8
Threonine	Thr	T	polar	neutral	-0.7
Tryptophan	Trp	W	nonpolar	neutral	-0.9
Tyrosine	Tyr	Y	polar	neutral	-1.3
Valine	Val	V	nonpolar	neutral	4.2

Hydrophobic amino acids include: G, C, M, A, P, I, L, V and the aromatic amino acids F and W. Hydrophobic amino acids may be removed from the ends of the sequence or within the sequence.

Charged hydrophilic amino acids include: K, R, D, H and E. Hydrophobic amino acids may be exchanged with charged hydrophilic amino acids the ends of the sequence or within the sequence.

One or more charged amino acid(s) may be inserted at the N-terminus of the sequence. Advantageously, a positively charged amino acid is inserted or substituted at one terminus and a negatively charged amino acid is inserted/substituted at the other terminus in order to create a charge dipole.

Modification of the parent sequence should not significantly impair binding of the peptide to the peptide binding groove of an MHC molecule, its capacity to be recognised by a T cell, or its capacity to act as an apitope (bind to an MHC molcule and be presented to a T cell without further antigen processing). This may be readily tested using known antigen presentation assays and T-cell hybridomas.

The second embodiment of the invention relates to a peptide comprising the sequence

$X(aa)_n$-core sequence -$(aa)_m$

wherein X is a charged hydrophilic residue;

aa is an amino acid;

n is an integer between 0 and 5;

m is an integer between 0 and 5; and

the "core sequence" is selected from the following group of FVIII-derived peptides: LYISQFIIM

FIIMYSLDG

IARYIRLHP

LIIFKNQAS

LTRYYSSFV

MVTFRNQAS

LRIHPQSWV

which peptide is capable of binding to an MHC class II molecule without further antigen processing and being recognised by a factor VIII specific T cell.

The sequence $(aa)_n$ or $(aa)_m$ can be any sequence of between 4 and 5 amino acids. For example, the peptide may have the sequence:

XDNIMVTFRNQAS

in which case, n=3 and m=0.

The third embodiment of the present invention relates to a peptide comprising the sequence:

$Y(aa)_n$-core sequence-$(aa)_mZ$

wherein Y and Z are charged amino acids having reverse polarity;

aa is an amino acid;

n is an integer between 0 and 5;

m is an integer between 0 and 5; and

the "core sequence" is selected from the following group of FVIII-derived peptides:

LYISQFIIM

FIIMYSLDG

IARYIRLHP

LIIFKNQAS

LTRYYSSFV

MVTFRNQAS

LRIHPQSWV

which peptide is capable of binding to an MHC class II molecule without further antigen processing and being recognised by a factor VIII specific T cell.

For example, for the peptide EDNIMVTFRNQASR,

Y=E;
$(aa)_n$ = DNI
m=0; and
Z=R

The peptide may comprise the core residue sequence, together with additional flanking sequences at the N and/or C terminal end ($(aa)_n$ and $(aa)_m$ respectively), provided that the resulting peptide is capable of binding to an MHC class II molecule without further antigen processing.

The flanking N and/or C terminal sequences may be derivable from the sequences flanking the core residue sequences in human FVIII.

APIPS

Various antigen processing independent presentation systems (APIPS) are known, including:

a) fixed APC (with or without antibodies to CD28);
b) Lipid membranes containing Class I or II MHC molecules (with or without antibodies to CD28); and
c) purified natural or recombinant MHC in plate-bound form (with or without antibodies to CD28).

All of these systems are capable of presenting antigen in conjunction with an MHC molecule, but are incapable of processing antigen. In all these systems the processing function is either absent or disabled. This makes it possible to investigate whether a peptide can bind to an MHC class I or II molecule and be presented to a T cell without further antigen processing.

The use of fixed APC to investigate T cell responses is well known in the art, for example in studies to investigate the minimal epitope within a polypeptide, by measuring the response to truncated peptides (Fairchild *et al* (1996) Int. Immunol.

8:1035-1043). APC may be fixed using, for example formaldehyde (usually paraformaldehyde) or glutaraldehyde.

Lipid membranes (which may be planar membranes or liposomes) may be prepared using artificial lipids or may be plasma membrane/microsomal fractions from APC.

In use, the APIPS may be applied to the wells of a tissue culture plate. Peptide antigens are then added and binding of the peptide to the MHC portion of the APIPS is detected by addition of selected T cell lines or clones. Activation of the T cell line or clone may be measured by any of the methods known in the art, for example via ^{3}H-thymidine incorporation or cytokine secretion.

FACTOR VIII

The core sequence of peptide of the invention is derivable from factor VIII. One or both flanking sequences $((aa)_n$ and $(aa)_m)$ may also be derivable from factor VIII.

Factor VIII participates in the intrinsic pathway of blood coagulation; factor VIII is a cofactor for factor IXa which, in the presence of Ca+2 and phospholipids, converts factor X to the activated form Xa.

The factor VIII gene produces two alternatively spliced transcripts. Transcript variant 1 encodes a large glycoprotein, isoform a, which circulates in plasma and associates with von Willebrand factor in a noncovalent complex. This protein undergoes multiple cleavage events. Transcript variant 2 encodes a putative small protein, isoform b, which consists primarily of the phospholipid binding domain of factor VIIIc. This binding domain is essential for coagulant activity.

The complete 186,000 base-pair sequence of the human factor VIII gene was elucidated in the mid 1980s (Gitschier et al (1984) Nature 312 326-330). At the same time, DNA clones encoding the complete 2351 amino acid sequence were used to produce biologically active factor VIII in cultured mammalian cells (Wood *et al* (1984) Nature 312:330-337). The complete 2,351 amino acid sequence for human factor VIII is given in SEQ ID No. 1.

The core residues of peptide of the present invention are derivable from factor VIII. Optionally the flanking sequence(s) may also be derivable from factor VIII if they are the same as the sequences flanking the core sequence in the native FVIII polypeptide. This sequence may be obtainable or obtained from cleavage of the factor VIII sequence.

SOLUBILITY

The peptide of the first embodiment of the invention is a modified form of one of the following peptides:

GTLMVFFGNVDSSGI
TQTLHKFILLFAVFD
SLYISQFIIMYSLDG
PPIIARYIRLHPTHY
PPLLTRYLRIHPQSW
MHTVNGYVNRSLPGL
LGQFLLFCHISSHQH
DTLLIIFKNQASRPY
PRCLTRYYSSFVNME
TENIQRFLPNPAGVQ
DNIMVTFRNQASRPY
RYLRIHPQSWVHQIA

These peptides have already been shown to act as apitopes and be tolerogenic *in vivo* (See examples and Intenational patent application No PCT/GB2008/003996).

It has since come to light that solubility is an important consideration in peptide-mediated tolerance induction. Solubility may be improved by one or more of the following:

(i) removal of one or more hydrophobic residues;

(ii) addition/substitution to add one or more charged hydrophilic residues

(iii) placing positively and negatively charged amino acids at either end to create a charge dipole.

The modified peptide may be more soluble that the parent (unmodified) peptide. The modified peptide may have 2, 3, 4, or 5-fold greater solubility than the parent peptide. The peptide may be soluble at concentrations of up to 0.5 mg/ml, 1 mg/ml, or 5 mg/ml.

TOLERANCE

T cell epitopes play a central role in the adaptive immune response to any antigen, whether self or foreign. The central role played by T cell epitopes in hypersensitivity diseases (which include allergy, autoimmune diseases and transplant rejection) has been demonstrated through the use of experimental models. It is possible to induce inflammatory or allergic diseases by injection of synthetic peptides (based on the structure of T cell epitopes) in combination with adjuvant.

By contrast, it has been shown to be possible to induce immunological tolerance towards particular antigens by administration of peptide epitopes in soluble form. Administration of soluble peptide antigens has been demonstrated as an effective means of inhibiting disease in experimental autoimmune encephalomyelitis (EAE – a model for multiple sclerosis (MS)) (Metzler and Wraith (1993) Int. Immunol. 5:1159-1165; Liu and Wraith (1995) Int. Immunol. 7:1255-1263; Anderton and Wraith (1998) Eur. J. Immunol. 28:1251-1261); and experimental models of arthritis, diabetes, and uveoretinitis (reviewed in Anderton and Wraith (1998) as above). This has also been demonstrated as a means of treating an ongoing disease in EAE (Anderton and Wraith (1998) as above).

Tolerance is the failure to respond to an antigen. Tolerance to self antigens is an essential feature of the immune system, when this is lost, autoimmune disease can result. The adaptive immune system must maintain the capacity to respond to an enormous variety of infectious agents while avoiding autoimmune attack of the self antigens contained within its own tissues. This is controlled to a large extent by the sensitivity of immature T lymphocytes to apoptotic cell death in the thymus (central tolerance). However, not all self antigens are detected in the thymus, so death of self-reactive thymocytes remains incomplete. There are thus also mechanisms by which tolerance may be acquired by mature self-reactive T lymphocytes in the peripheral

tissues (peripheral tolerance). A review of the mechanisms of central and peripheral tolerance is given in Anderton *et al* (1999) (*Immunological Reviews* **169**:123-137).

In haemophilia A, patients have a defect in the factor VIII gene. This means that factor VIII is not recognised as a "self" antigen by the immune system. When factor VIII is administered during coagulation factor replacement therapy, therefore, an alloimmune response is generated to the foreign protein, leading to the production of FVIII inhibitor antibodies.

The peptides of the present invention are capable of inducing tolerance to factor VIII such that when FVIII is administered therapeutically, it does not induce an immune response and FVIII inhibitors do not develop.

Acquired haemophilia is an autoimmune disease in which tolerance to factor VIII breaks down. In this case, peptides of the present invention may be administered to reinstate tolerance to this self protein and curtail the pathogenic immune response.

Tolerance may result from or be characterised by the induction of anergy in at least a portion of CD4+ T cells. In order to activate a T cell, a peptide must associate with a "professional" APC capable of delivering two signals to T cells. The first signal (signal 1) is delivered by the MHC-peptide complex on the cell surface of the APC and is received by the T cell via the TCR. The second signal (signal 2) is delivered by costimulatory molecules on the surface of the APC, such as CD80 and CD86, and received by CD28 on the surface of the T cell. It is thought that when a T cell receives signal 1 in the absence of signal 2, it is not activated and, in fact, becomes anergic. Anergic T cells are refractory to subsequent antigenic challenge, and may be capable of suppressing other immune responses. Anergic T cells are thought to be involved in mediating T cell tolerance.

Without wishing to be bound by theory, the present inventors predict that peptides which require processing before they can be presented in conjunction with MHC molecules do not induce tolerance because they have to be handled by mature antigen presenting cells. Mature antigen presenting cells (such as macrophages, B cells and dendritic cells) are capable of antigen processing, but also of delivering both signals 1

and 2 to a T cell, leading to T cell activation. Apitopes, on the other hand, will be able to bind class II MHC on immature APC. Thus they will be presented to T cells without costimulation, leading to T cell anergy and tolerance.

Of course, apitopes are also capable of binding to MHC molecules at the cell surface of mature APC. However, the immune system contains a greater abundance of immature than mature APC (it has been suggested that less than 10% of dendritic cells are activated, Summers et al. (2001) Am. J. Pathol. **159**: 285-295). The default position to an apitope will therefore be anergy/tolerance, rather than activation.

The induction of tolerance to FVIII can be monitored *in vivo* by looking for a reduction in the level of:

(i) FVIII inhibitory antibodies:

(ii) CD4+ T cells specific for FVIII

(iii) B cells capable of secreting FVIII inhibitory antibodies

by techniques known in the art.

It has been shown that, when tolerance is induced by peptide administration, the capacity of antigen-specific CD4+ T cells to proliferate is reduced. Also, the production of IL-2, IFN-γ and IL-4 production by these cells is down-regulated, but production of IL-10 is increased. Neutralisation of IL-10 in mice in a state of peptide-induced tolerance has been shown to restore completely susceptibility to disease. It has been proposed that a population of regulatory cells persist in the tolerant state which produce IL-10 and mediate immune regulation (Burkhart *et al* (1999) Int. Immunol. **11**:1625-1634).

The induction of tolerance can therefore also be monitored by various techniques including:

(a) the induction of anergy in CD4+ T cells (which can be detected by subsequent challenge with FVIII *in vitro*);

(b) changes in the CD4+ T cell population, including

(i) reduction in proliferation;

(ii) down-regulation in the production of IL-2, IFN-γ and IL-4; and

(iii) increase in the production of IL-10.

As used herein, the term "tolerogenic" means capable of inducing tolerance.

COMPOSITION

The present invention also relates to a composition, such as a pharmaceutical composition comprising one or more peptide(s) according to the invention.

The peptide may comprise a plurality of peptides, for example, two, three, four, five or six peptides.

The composition of the present invention may be for prophylactic or therapeutic use.

When administered for prophylactic use, the composition may reduce or prevent the generation of an immune response to FVIII. The level of immune response is less than would have been obtained in the patient had not been treated with the composition. The term "reduce" indicates that a partial reduction in immune response is observed, such as a 50%, 70%, 80% or 90% reduction in the response that would have been observed in the patient had not been treated with the composition (or in the response observed in an untreated patient over the same time-frame). The term "prevent" indicates that no appreciable immune response to FVIII is observed.

When administered for therapeutic use, the composition may suppress an already ongoing immune response to FVIII. The term "suppress" indicates a reduction in the level of an on-going immune response, compared to the level before peptide treatment, or the level which would have been observed at the same time point had the treatment not been given.

Treatment with the composition of the present invention may cause a reduction in levels of any or all of the following:

(i) FVIII inhibitory antibodies:

(ii) CD4+ T cells specific for FVIII

(iii) B cells secreting FVIII inhibitory antibodies.

Detection of all these factors can be carried out by techniques known in the art, such as ELISA, FACS etc.

Treatment with the composition of the present invention may also or alternatively cause anergy in CD4+ T cells specific for FVIII. Anergy can be detected by for example subsequent challenge with FVIII *in vitro*.

It is important to bear in mind that not all immune responses to FVIII are pathogenic. Non-inhibitory anti-FVIII antibodies may be found in haemophilia patients without inhibitors (Moreau *et al* (2000) Blood 95:3435-41) and approximately 15% of healthy blood donors (Algiman *et al* (1992) 89:3795-9).

FVIII inhibitors may be detected by the Nijmegen modification of the clotting Bethesda assay, in which the ability of the patient's plasma to inactivate FVIII in normal plasma is tested. A Bethesda unit is defined as the amount of antibody that neutralizes 50% of plasma FVIII activity, and titres of 0.6BU or greater suggest the presence of antibody.

Inhibitors are generally classified as low titre if the level is <5 BU and high titre if ≥ 5 BU.

The level of circulating FVIII inhibitory antibodies may be reduced to 90%, 75%, 50%, 20%, 10% 5% of the level of antibodies which would have been observed had the patient not received treatment.

The level of circulating FVIII inhibitory antibodies may be reduced to 5, 4, 3, 2, 1 or 0.5 BU.

The peptides and composition of the invention may increase the amount or proportion of therapeutically administered FVIII which is available to aid clotting in a patient. This is due to the reduction in FVIII inhibitors which may effectively remove a proportion of FVIII from exerting its therapeutic function. The peptide or composition

of the invention may increase the amount of available FVIII by, for example, 10%, 25%, 50% 75% or 100%.

The peptides and composition of the invention may thus reduce the amount of FVIII which needs to be administered to aid clotting in a patient.

FORMULATION

The composition may by prepared as an injectable, either as liquid solution or suspension; solid form suitable for solution in, or suspension in, liquid prior to injection may also be prepared. The preparation may also be emulsified, or the peptides encapsulated in liposomes. The active ingredients may be mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline (for example, phosphate-buffered saline), dextrose, glycerol, ethanol, or the like and combinations thereof.

In addition, if desired, the composition may contain minor amounts of auxiliary substances such as wetting or emulsifying agents and/or pH buffering agents. Buffering salts include phosphate, citrate, acetate. Hydochloric acid and/or sodium hydryoxide may be used for pH adjustment. For stabilisation, disaccharides may be used such as sucrose or trehalose.

If the composition comprises a plurality of peptides, the relative ratio of the peptides may be approximately equal. Alternatively the relative ratios of each peptide may be altered, for example, to focus the tolerogenic response on a particular sub-set of autoreactive T-cells or if it is found that one peptide works better than the others in particular HLA types.

After formulation, the composition may be incorporated into a sterile container which is then sealed and stored at a low temperature, for example 4°C, or it may be freeze-dried.

Conveniently the composition is prepared as a lyophilized (freeze dried) powder. Lyophilisation permits long-term storage in a stabilised form. Lyophilisation

procedures are well known in the art, see for example http://www.devicelink.com/ivdt/archive/97/01/006.html. Bulking agents are commonly used prior to freeze-drying, such as mannitol, dextran or glycine.

The composition may be administered in a convenient manner such as by the oral, intravenous (where water soluble), intramuscular, subcutaneous, sublingual, intranasal, intradermal or suppository routes or implanting (e.g. using slow release molecules).

The composition may advantageously be administered via intranasal, subcutaneous or intradermal routes.

The peptide and composition of the invention may be used to treat a human subject. The subject may have haemolphilia A, in particular severe haemophilia A. The subject may be genetically deficient in FVIII. The subject may have acquired haemophilia. The subject may have inhibitory anti-FVIII antibodies.

The subject may be undergoing or about to undergo coagulant replacement therapy with FVIII.

The subject may be undergoing or about to undergo gene therapy with the FVIII gene.

The subject may be an HLA-haplotype which is associated with a predisposition to develop inhibitory anti-FVIII alloantibodies or autoantibodies. The subject may express HLA-DR2. Methods for determining the HLA haplotype of an individual are known in the art.

Typically, a physician will determine the actual dosage which will be most suitable for an individual subject and it will vary with the age, weight and response of the particular patient.

In a preferred embodiment a "dose escalation" protocol may be followed, where a plurality of doses is given to the patient in ascending concentrations. Such an approach has been used, for example, for phospholipase A2 peptides in immunotherapeutic

applications against bee venom allergy (Müller *et al* (1998) J. Allergy Clin Immunol. 101:747-754 and Akdis et al (1998) J. Clin. Invest. 102:98-106).

KITS

Conveniently, if the composition comprises a plurality of peptides, they may be administered together, in the form of a mixed composition or cocktail. However, there may be circumstances in which it is preferable to provide the peptides separately in the form of a kit, for simultaneous, separate, sequential or combined administration.

The kit may also comprise mixing and/or administration means (for example a vapouriser for intranasal administration; or a syringe and needle for subcutaneous/intradermal dosing). The kit may also comprise instructions for use.

The pharmaceutical composition or kit of the invention may be used to treat and/or prevent a disease.

In particular, the composition/kit may be used to treat and/or prevent haemophilia A or acquired haemophilia.

HAEMOPHILIA A

Hemophilia A (classic hemophilia), is caused by the deficiency of Factor VIII.

Hemophilia A has an estimated incidence of 1 in 10,000 males, while hemophilia B is estimated to occur in one in 40,000 males. Approximately 1 woman in 5,000 is a carrier for hemophilia A, and 1 in 20,000 is a carrier of hemophilia B.

Hemophilia is typically divided into three classes: severe, moderate and mild, based on the level of clotting factor in the blood. In severe hemophilia, there is less than 1 percent of normal clotting factor. The degree of severity tends to be consistent from generation to generation.

Contrary to popular belief, minor cuts and wounds do not usually present a threat to hemophiliacs. Rather, the greatest danger comes from spontaneous bleeding that may

occur in joints and muscles. This is most prone to occur during years of rapid growth, typically between the ages of 5 and 15 years.

Repeated spontaneous bleeding in joints may cause arthritis, and adjacent muscles become weakened. Pressure on nerves caused by the accumulation of blood may result in pain, numbness, and temporary inability to move the affected area.

Haemophilia A is usually diagnosed with a blood test to determine the effectiveness of clotting and to investigate whether the levels of clotting factors are abnormal.

The development of purified clotting factors in the 1970s, isolated from donated blood, significantly improved the long-term outlook for hemophiliacs. Mild to moderate haemophiliacs can use treatment with FVIII on an *ad hoc* basis, whereas severe haemophiliacs may require regular, indefinite treatment.

Previously, patients were given factor VIII concentrates pooled from thousands of plasma donations. This lead to significant problems of contamination with viral pathogens, particularly the human immunodeficiency virus and the hepatitis viruses. Monoclonal antibody purification techniques, heat inactivation, and virucidal detergent treatments have rendered plasma-derived concentrates relatively safe.

Recombinant DNA technology has now provided a series of synthetic products,such as Recombinate™ and Kogenate™. Kogenate is made using baby hamster kidney cells expressing human factor VIII. The resulting factor is highly purified, eliminating any possibility of transmission of virus from plasma.

The peptide or composition of the present invention may be administered before and/or during factor VIII replacement therapy.

Hemophilia A is an ideal disease target for gene therapy since i) it is caused by a mutations in a single identified gene, ii) a slight increase in clotting factor levels *in vivo* can convert severe hemophilia into milder disease, and iii) current replacement therapies are considered suboptimal. Also, there is a wide range of safety if there is an "overshoot" of desired level of coagulation activity.

Unfortunately, to date the promise of gene therapy as a cure for haemophilia has not been realized, primarily because of difficulties in finding a gene delivery system which is sufficiently non-immunogenic to allow for long term expression of the clotting factor.

The peptides of the present invention would also be suitable for tolerising a subject prior to gene therapy with factor VIII and/or managing FVIII inhibitor formation in a patient following gene therapy.

ACQUIRED HAEMOPHILIA

Acquired haemophilia is characterised by the presence of autoantibody inhibitors against FVIII in individuals with previously normal coagulation. It is a rare condition, with an estimated incidence of 1–3 per million population per year. The mortality rate associated with acquired autoantibody inhibitors approaches 25% versus the substantially lower risk of death in those with alloantibodies.

Compared to alloantibody inhibitor patients, acquired hemophilia is characterized by: (1) a more severe bleeding pattern; (2) higher incidence in older population; (3) occurrence in conjunction with identifiable underlying autoimmune diseases, lymphoproliferative or solid tumor malignancies, pregnancy, and use of certain antibiotics such as penicillin and sulfonamides in approximately 50% of cases; and (4) in vitro inhibitor activity that follow a type II pharmacokinetic pattern with incomplete neutralization of the targeted clotting factor activity by the autoantibody, typically resulting in residual factor VIII levels ranging between 2%–18% in patient plasma.

The peptide or composition of the present invention may be administered to a patient with acquired haemophilia, or to a patient believed to be at risk of developing acquired haemophilia due to, for example:

i) imminent treatment with, for example penicillin or a sulfornamide

ii) progession of a tumour or other malignancy

iii) imminent or early pregnancy.

The invention will now be further described by way of Examples, which are meant to serve to assist one of ordinary skill in the art in carrying out the invention and are not intended in any way to limit the scope of the invention.

EXAMPLES

Example 1: Selection of HLA-DR2 Factor VIII peptides

A series of FDVIII 15mer peptides were compared using three HLA-DR binding algorithms:
SYFPEITHI (http://www.syfpeithi.de/home.htm)
ProPred (http://www.imtech.res.in/raghava/propred/) and
and IEDB (http://www.immuneepitope.org/home.do).

Peptides were selected which were predicted to be HLA-DR2-binding by more than one of the programmes and flanking sequences were designed for the predicted core residues (table 2).

TABLE 2

Peptide No	FVIII First AA	Sequence in single amino acid code	Also referred to herein as:
1	2140	GTLMVFFGNVDSSGI	GTLMV
2	0208	TQTLHKFILLFAVFD	TQTLH
3	2114	SLYISQFIIMYSLDG	SLYIS
4	2161	PPIIARYIRLHPTHY	PPIIA
5	2318	PPLLTRYLRIHPQSW	PPLLT
6	250	MHTVNGYVNRSLPGL	MHTVN
7	322	LGQFLLFCHISSHQH	LGQFL
8	478	DTLLIIFKNQASRPY	DTLLI
9	545	PRCLTRYYSSFVNME	PRCLT
10	607	TENIQRFLPNPAGVQ	TENIQ
11	1788	DNIMVTFRNQASRPY	DNIMV
12	2322	RYLRIHPQSWVHQIA	RYLRI

Example 2: Investigating the response of HLA-DR2 restricted cells from factor VIII immunised mice to peptides

HLA-DR2 transgenic mice were immunised with human factor VIII in adjuvant. Draining lymph node cells were collected and restimulated *in vitro* with different concentrations of the 12 peptides from table 2. The results are shown in Figure 1.

HLA-DR2 restricted cells from factor VIII immunised mice clearly respond strongly to peptide DNIMV (1st amino acid 1788). There are also responses to peptides PRCLT (545) and PPIIA (2161).

Example 3: Investigating the response of T cells from HLA-DR2 mice to peptides

HLA-DR2 mice were first immunised with factor VIII in adjuvant. Spleen cells from immune mice were restimulated *in vitro* with factor VIII and the resulting lymphoblasts were fused with the BW5147 thymoma using polyethylene glycol.

T-cell hybridomas were selected in HAT medium and the hybridomas cloned and tested for their response to factor VIII. The hybridomas were then screened for their response to the 12 predicted peptides. Of the 27 hybridomas screened, 11 responded to DNIMV, 3 to PRCLT and 3 to PPIIA, although the response to PPIIA was weaker and less specific. The response of two hybridomas specific for DNIMV and PRCLT is shown in Figure 2.

Example 4 - Investigating the response of lymph node cells from FVIII-DR2+ mice to peptides

HLA-DR2 transgenic mice were crossed with factor VIII deficient mice to create a model of haemophilia expressing the human HLA class II MHC molecule.

These FVIII-DR2+ animals were immunised with factor VIII in adjuvant. Draining lymph nodes were isolated and tested for their response to the peptide panel. As shown in Figure 3, these cells responded well to PRCLT and DNIMV. There was a weak response to GTLMV and significant response to RYLRI.

Example 5 - Investigating the response of T cells from HLA-DR2 mice to peptides

Factor VIII deficient mice expressing HLA-DR2 were immunised with factor VIII in adjuvant. Spleen cells from the immunised mice were restimulated *in vitro* with factor VIII and the resulting lymphoblasts were fused with BW5147, as described above. T-cell hybridomas were screened for their response to the 12 predicted peptides. Yet again, the majority of hybridomas responded to peptides DNIMV and PRCLT. Of 19 hybridomas specific for factor VIII, 10 responded to DNIMV, 6 to PRCLT, 1 to PPIIA, 1 to SLYIS and 1 to DTLLI. Examples of responses by these hybridomas are shown in Figure 4.

Based on these experiments it is clear that two peptides DNIMV (first amino acid number 1788) and PRCLT (first amino acid 545) constitute the immunodominant T-cell epitopes in the HLA-DR2 restricted T-cell response to human factor VIII.

Example 6 - DNIMV and PRCLT behave as apitopes

In order to be an apitope, a peptide must be capable of binding to an MHC class I or II molecule without further antigen processing (i.e. trimming) and be presented to a T cell. In the present case, the capacity of peptides to be presented by fixed APC was investigated.

Mgar cells were either fresh or fixed with 1% paraformaldehyde. Clones were tested for antigenic specificity by culturing 100µl of hybridoma cells with $5x10^4$ Mgar cells in the presence and absence of 20µg/ml rhFVIII or peptide epitopes overnight. Supernatants were then collected and assessed for IL-2 production by ELISA. The fact that rhFVIII must be presented by live Mgar cells demonstrates that the intact protein requires antigen processing to be presented. Peptides DNIMV and PRCLT, on the other hand, are presented by both live and fixed Mgar cells indicating that these peptides function as apitopes (Figure 5).

Example 7 - Determination of the range of peptide epitopes capable of functioning as apitopes

The range of peptide epitopes capable of functioning as apitopes in the sequences surrounding DNIMV, PRCLT and the other peptides was identified by preparing panels of overlapping peptides (shown on pages 36-37) and screening these using the T-cell hybridomas using the same method as Example 5 (Figure 7).

Example 8 - DNIMV and PRCLT induce tolerance to whole factor VIII protein

HLA-DR2 transgenic mice were treated with either of the two soluble peptides, or PBS as a control, prior to immunisation with factor VIII in adjuvant. Draining lymph nodes were isolated and the cells restimulated *in vitro* with factor VIII protein in order to assess the immune status of the mice. As shown in Figure 6, treatment of mice with either DNIMV or PRCLT led to a substantial suppression of the immune response to factor VIII.

Example 9 - Investigation of whether DNIMV and PRCLT able to induce tolerance in the factor VIII knockout mouse

It was known from Example 8 that these two peptides are able to prevent the immune response to factor VIII in mice expressing endogenous factor VIII. The experiment was repeated with FVIII-DR2+ animals to determine whether these peptides also prevent the immune response to factor VIII in factor VIII deficient mice.

Example 10 – Investigation of whether DNIMV and PRCLT in combination are able to induce tolerance in the factor VIII knockout mouse

The two peptides which were shown to individually reduce the immune response to factor VIII in factor VIII deficient mice in Example 9 were combined. As shown in Figure 8, treatment of mice with both DNIMV and PRCLT led to a substantial suppression of the immune response to factor VIII, as shown by the decrease in IFN-gamma production. IFN-gamma is the major class switch lymphokine required for neutralising antibodies in the mouse. The effect demonstrated was greater than that observed using either peptide alone.

Example 11: The induction of tolerance using a modified peptide

The peptide DNIMV is partly, but not completely soluble. In order to improve the solubility of the peptide, a modified version was designed with the following sequence: EDNIMVTFRNQASR.

This is extended at the N-terminus to add a charged hydrophilic residue. It is also a truncated at the C-terminus to remove the proline and tyrosine residues. Furthermore by placing positively and negatively charged amino acids at either end of the peptide a charge dipole is created reported to increase solubility.

The modified peptide is more soluble than DNIMV and sufficiently soluble to allow intranasal peptide delivery. In order to assess the induction of tolerance using this peptide epitope, FVIII deficient mice were taken and half treated with the modified EDNIMV peptide (referred to as 'tolerised' in Figure 9). The mice were then immunised with DNIMV in CFA and 10 days later draining lymph nodes were collected and stimulated *in vitro* with either DNIMV or EDNIMV. Figure 9 shows the results for responses from either naïve or tolerised mice stimulated with either DNIMV or EDNIMV *in vitro*.

The results demonstrate that EDNIMV is able to recall an immune response from mice immunised with DNIMV. Furthermore, they demonstrate very clearly that mice tolerized with EDNIMV fail to mount an immune response to DNIMV in vivo as revealed by the lack of response to either DNIMV or EDNIMV *in vitro* after priming with DNIMV in CFA.

Example 12: EDNIMV induces tolerance to whole factor VIII protein

The experiment described in Example 8 is repeated for the modified peptide EDNIMV to demonstrate that EDNIMV is able to suppress the immune response to the whole factor VIII protein.

Methods

(i) Recall responses for DR2+ mice primed with rhFVIII

HLA-DR2+ murine MHC class II null mice were immunised with 40μg rhFVIII emulsified in Complete Freunds Adjuvant supplemented with 400μg heat-killed *M.tuberculosis H37Ra*, subcutaneously at the base of the tail. 10 days later the mice were sacrificed and the draining lymph nodes removed. Single cell suspensions were prepared and lymphocytes incubated at 4-5x10^5 cells per well in 96-well flat bottomed plates for 72 hours with the indicated concentrations of peptide or control antigens before pulsing with 0.5μCi/well tritiated thymidine for a further 16 hours. Plates were then frozen before cells were harvested onto glass filter mats and radioactive incorporation measured using a liquid scintillation β-counter

(ii) FVIII peptide specificity of T cell hybridomas generated from DR2+ mice

HLA-DR2+ murine MHC class II null mice were immunised as above. On day 10 draining lymph nodes were removed and lymphocytes cultured at 2.5x10^6 cells/ml, 1ml/well in 24 well plates in the presence of 20μg/ml rhFVIII for 3 days. Following this stimulation, lymphocytes were recovered, washed and fused with TCR$\alpha^-\beta^-$ BW fusion partner cells at a ratio of 4 BW cells to 1 lymphocyte, using polyethylene glycol as described by Nelson et al (1980) *PNAS* 77(5):2866. Fused cells were carefully washed and then plated out in flat bottomed 96 well plates for 2 days before the addition of HAT medium to select for T cell hybridomas. Cells were monitored for growth and approximately 10 days after fusions were performed, individual clones were selected and transferred to 24 well plates in HAT medium. Clones were maintained in HAT medium for at least 2 weeks before being weaned into HT medium and then complete medium. Clones were tested for antigenic specificity by culturing 100μl of hybridoma cells with 5x10^4 Mgar cells in the presence and absence of 20μg/ml rhFVIII overnight. Supernatants were then collected and assessed for IL-2 production by ELISA, with clones producing IL-2 in response to rhFVIII being considered positive for FVIII-specificity. To investigate the repertoire of predicted FVIII peptides FVIII-specific clones were again tested for IL-2 production, following overnight incubation with 20μg/ml of each of the 12 peptides.

(iii) Recall responses for FVIII-/- mice primed with rhFVIII

The same method was followed as for (i), except the mice were FVIII-deficient, HLA-DR2+ and murine MHC class II null.

(iv) FVIII peptide specificity of T cell hybridomas generated from FVIII-/- mice

The same method was followed as for (ii), except the mice were FVIII-deficient and HLA-DR2+.

(v) Tolerisation of FVIII-specific responses in DR2+ mice by pre-treatment with immunodominant FVIII peptides

HLA-DR2+ murine MHC class II null mice were treated 3 times with 100μg of DNIMV, PRCLT or PPIIA dissolved in PBS, or the equivalent volume of PBS alone. Peptides were administered intraperitoneally, with 3-4 days between each dose. Following the final administration, mice were primed with rhFVIII emulsified in complete Freunds adjuvant as for (i). 10 days later, draining lymph nodes were recovered and lymphocytes subsequently cultured in vitro with rhFVIII, or each of the tolerising peptides as well as control antigens, for 72 hours before the addition of tritiated thymidine as for (i).

(vi) Tolerisation of FVIII-specific responses in DR2+ mice by pre-treatment with a combination immunodominant FVIII peptides

HLA-DR2+ murine MHC class II null mice were treated 3 times with DNIMV, PRCLT or a combination of both DNIMV and PRCLT dissolved in PBS, or the equivalent volume of PBS alone. Peptides were administered intraperitoneally, over 8 days. Following the final administration, mice were primed with rhFVIII emulsified in complete Freunds adjuvant as for (i). 10 days later, draining lymph nodes were recovered and lymphocytes subsequently re-stimulated *in vitro* with rhFVIII. The supernatants were then collected and IFN-gamma was measured.

All publications mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described methods and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in

connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular immunology or related fields are intended to be within the scope of the following claims.

SEQ ID No.1

1 mqielstcff iclirfcfsa trryylgave lswdymqsdl gelpvdarfp prvpksfpfn
61 tsvvykktlf veftdhlfni akprppwmgl lgptiqaevy dtvvitlknm ashpvslhav
121 gvsywkaseg aeyddqtsqr ekeddkvfpg gshtyvwqvl kengpmasdp lcltysylsh
181 vdlvkdlnsg ligallvcre gslakektqt lhkfillfav fdegkswhse tknslmqdrd
241 aasarawpkm htvngyvnrs lpgligchrk svywhvigmg ttpevhsifl eghtflvrnh
301 rqasleispi tfltaqtlim dlgqfllfch isshqhdgme ayvkvdscpe epqlrmknne
361 eaedydddlt dsemdvvrfd ddnspsfiqi rsvakkhpkt wvhyiaaeee dwdyapivla
421 pddrsyksqy lnngpqrigr kykkvrfmay tdetfktrea iqhesgilgp llygevgdtl
481 liifknqasr pyniyphgit dvrplysrrl pkgvkhlkdf pilpgeifky kwtvtvedgp
541 tksdprcltr yyssfvnmer dlasgligpl licykesvdq rgnqimsdkr nvilfsvfde
601 nrswylteni qrflpnpagv qledpefqas nimhsingyv fdslqlsvcl hevaywyils
661 igaqtdflsv ffsgytfkhk mvyedtltlf pfsgetvfms menpglwilg chnsdfrnrg
721 mtallkvssc dkntgdyyed syedisayll sknnaieprs fsqnsrhpst rqkqfnatti
781 pendiektdp wfahrtpmpk iqnvsssdll mllrqsptph glslsdlqea kyetfsddps
841 pgaidsnnsl semthfrpql hhsgdmvftp esglqlrlne klgttaatel kkldfkvsst
901 snnlistips dnlaagtdnt sslgppsmpv hydsqldttl fgkkssplte sggplslsee
961 nndskllesg lmnsqesswg knvsstesgr lfkgkrahgp alltkdnalf kvsisllktn
1021 ktsnnsatnr kthidgpsll lenspsvwqn ilesdtefkk vtplihdrml mdknatalrl
1081 nhmsnkttss knmemvqqkk egpippdaqn pdmsffkmlf lpesarwiqr thgknslnsg
1141 qgpspkqlvs lgpeksvegq nflsekrkvv vgkgeftkdv glkemvfpss rnlfltnldn
1201 lhennthnqe kkiqeeiekk etliqenvvl pqihtvtgtk nfmknlflls trqnvegsyd
1261 gayapvlqdf rslndstnrt kkhtahfskk geeenlegig nqtkqiveky acttrispnt
1321 sqqnfvtqrs kralkqfrlp leetelekri ivddtstqws knmkhltpst ltqidyneke
1381 kgaitqspls dcltrshsip qanrsplpia kvssfpsirp iyltrvlfqd nsshlpaasy
1441 rkkdsgvqes shflqgakkn nlslailtle mtgdqrevgs lgtsatnsvt ykkventvlp
1501 kpdlpktsgk vellpkvhiy qkdlfptets ngspghldlv egsllqgteg aikwneanrp
1561 gkvpflrvat essaktpskl ldplawdnhy gtqipkeewk sqekspekta fkkkdtilsl
1621 nacesnhaia ainegqnkpe ievtwakqgr terlcsqnpp vlkrhqreit rttlqsdqee
1681 idyddtisve mkkedfdiyd edenqsprsf qkktrhyfia averlwdygm sssphvlmr
1741 aqsgsvpqfk kvvfqeftdg sftqplyrge lnehlgllgp yiraevedni mvtfrnqasr
1801 pysfysslis yeedqrqgae prknfvkpne tktyfwkvqh hmaptkdefd ckawayfsdv
1861 dlekdvhsgl igpllvchtn tlnpahgrqv tvqefalfft ifdetkswyf tenmerncra
1921 pcniqmedpt fkenyrfhai ngyimdtlpg lvmaqdqrir wyllsmgsne nihsihfsgh
1981 vftvrkkeey kmalylypg vfetvemlps kagiwrvecl igehlhagms tlflvysnkc
2041 qtplgmasgh irdfqitasg qygqwapkla rlhysgsina wstkepfswi kvdllapmii
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2161 ppiiaryirl hpthysirst lrmewmgcdl nscsmplgme skaisdaqit assyftnmfa
2221 twspskarlh lqgrsnawrp qvnnpkewlq vdfqktmkvt gvttqgvksl ltsmyvkefl
2281 isssqdghqw tlffqngkvk vfqgnqdsft pvvnsldppl ltrylrihpq swvhqialrm
2341 evlgceaqdl y

Overlapping peptide panels prepared in Example 7

Overlapping set for DTLLIIFKNQASRPY

1.	473-488	YGEVGDTLLIIFKNQ
2.	474-489	GEVGDTLLIIFKNQA
3.	475-490	EVGDTLLIIFKNQAS
4.	476-491	VGDTLLIIFKNQASR
5.	477-492	GDTLLIIFKNQASRP
6.	478-493	DTLLIIFKNQASRPY
7.	479-494	TLLIIFKNQASRPYN
8.	480-495	LLIIFKNQASRPYNI
9.	481-496	LIIFKNQASRPYNIY
10.	482-497	IIFKNQASRPYNIYP
11.	483-498	IFKNQASRPYNIYPH

Overlapping set for PRCLTRYYSSFVNME

1.	540-554	PTKSDPRCLTRYYSS
2.	541-555	TKSDPRCLTRYYSSF
3.	542-556	KSDPRCLTRYYSSFV
4.	543-557	SDPRCLTRYYSSFVN
5.	544-558	DPRCLTRYYSSFVNM
6.	545-559	PRCLTRYYSSFVNME
7.	546-560	RCLTRYYSSFVNMER
8.	547-561	CLTRYYSSFVNMERD
9.	548-562	LTRYYSSFVNMERDL
10.	549-563	TRYYSSFVNMERDLA
11.	550-564	RYYSSFVNMERDLAS

Overlapping set for DNIMVTFRNQASRPY

1.	1783-1797	RAEVEDNIMVTFRNQ
2.	1784-1798	AEVEDNIMVTFRNQA
3.	1785-1799	EVEDNIMVTFRNQAS
4.	1786-1800	VEDNIMVTFRNQASR
5.	1787-1801	EDNIMVTFRNQASRP
6.	1788-1802	DNIMVTFRNQASRPY
7.	1789-1803	NIMVTFRNQASRPYS
8.	1790-1804	IMVTFRNQASRPYSF
9.	1791-1805	MVTFRNQASRPYSFY
10.	1792-1806	VTFRNQASRPYSFYS
11.	1793-1807	TFRNQASRPYSFYSS

Overlapping set for SLYISQFIIMYSLDG

1.	2109-2123	RQKFSSLYISQFIIM
2.	2110-2124	QKFSSLYISQFIIMY

3. 2111-2125	KFSSLYISQFIIMYS
4. 2112-2126	FSSLYISQFIIMYSL
5. 2113-2127	SSLYISQFIIMYSLD
6. 2114-2128	SLYISQFIIMYSLDG
7. 2115-2129	LYISQFIIMYSLDGK
8. 2116-2130	YISQFIIMYSLDGKK
9. 2117-2131	ISQFIIMYSLDGKKW
10. 2118-2132	SQFIIMYSLDGKKWQ
11. 2119-2133	QFIIMYSLDGKKWQT

Overlapping set for PPIIARYIRLHPTHY

1. 2156-2170	HNIFNPPIIARYIRL
2. 2157-2171	NIFNPPIIARYIRLH
3. 2158-2172	IFNPPIIARYIRLHP
4. 2159-2173	FNPPIIARYIRLHPT
5. 2160-2174	NPPIIARYIRLHPTH
6. 2161-2175	PPIIARYIRLHPTHY
7. 2162-2176	PIIARYIRLHPTHYS
8. 2163-2177	IIARYIRLHPTHYSI
9. 2164-2178	IARYIRLHPTHYSIR
10. 2165-2179	ARYIRLHPTHYSIRS
11. 2166-2180	RYIRLHPTHYSIRST

Overlapping set for RYLRIHPQSWVHQIA

1. 2317-2331	PPLLTRYLRIHPQSW
2. 2318-2332	PLLTRYLRIHPQSWV
3. 2319-2333	LLTRYLRIHPQSWVH
4. 2320-2334	LTRYLRIHPQSWVHQ
5. 2321-2335	TRYLRIHPQSWVHQI
6. 2322-2336	RYLRIHPQSWVHQIA
7. 2323-2337	YLRIHPQSWVHQIAL
8. 2324-2338	LRIHPQSWVHQIALR
9. 2325-2339	RIHPQSWVHQIALRM
10. 2326-2340	IHPQSWVHQIALRME
11. 2327-2341	HPQSWVHQIALRMEV

CLAIMS

1. A peptide comprising one of the following FVIII-derived sequences:

GTLMVFFGNVDSSGI
TQTLHKFILLFAVFD
SLYISQFIIMYSLDG
PPIIARYIRLHPTHY
PPLLTRYLRIHPQSW
MHTVNGYVNRSLPGL
LGQFLLFCHISSHQH
DTLLIIFKNQASRPY
PRCLTRYYSSFVNME
TENIQRFLPNPAGVQ
DNIMVTFRNQASRPY
RYLRIHPQSWVHQIA

with one or more of the following modifications:

(i) removal of one or more hydophobic amino acid(s);

(ii) replacement of one or more hydrophobic amino acid(s) with charged hydrophilic amino acid(s); and

(iii) insertion of a charged amino acid at one or both terminus(i)

which modified peptide is capable of binding to an MHC class II molecule without further antigen processing and being recognised by a factor VIII specific T cell.

2. A peptide comprising the sequence

$X(aa)_n$-core sequence -$(aa)_m$

wherein X is a charged hydrophilic residue;

aa is an amino acid;

n is an integer between 0 and 5;

m is an integer between 0 and 5; and

the "core sequence" is selected from the following group of FVIII-derived peptides: LYISQFIIM

FIIMYSLDG

IARYIRLHP

LIIFKNQAS

LTRYYSSFV

MVTFRNQAS

LRIHPQSWV

which peptide is capable of binding to an MHC class II molecule without further antigen processing and being recognised by a factor VIII specific T cell.

3. A peptide according to claim 1, which comprises the sequence XDNIMVTFRNQAS.

4. A peptide comprising the sequence:

$Y(aa)_n$-core sequence-$(aa)_mZ$

wherein Y and Z are charged amino acids having reverse polarity;

aa is an amino acid;

n is an integer between 0 and 5;

m is an integer between 0 and 5; and

the "core sequence" is selected from the following group of FVIII-derived peptides:

LYISQFIIM

FIIMYSLDG

IARYIRLHP

LIIFKNQAS

LTRYYSSFV

MVTFRNQAS

LRIHPQSWV

which peptide is capable of binding to an MHC class II molecule without further antigen processing and being recognised by a factor VIII specific T cell.

5. A pepide according to claim 4, which comprises the sequence: YDNIMVTFRNQASZ

6. A peptide according to claim 4 or 5, wherein Y is a positively charged amino acid and Z is a negatively charged amino acid.

7. A peptide according to claim 4, 5 or 6, wherein Z is K or R.

8. A peptide according to any of claims 2 to 7, wherein X or Y is D or E.

9. A peptide according to any preceding claim, which comprises the sequence EDNIMVTFRNQASR.

10. A peptide according to any preceding claim which consists of the sequence EDNIMVTFRNQASR.

11. A composition comprising a plurality of peptides, including one or more peptide(s) according to any preceding claim.

12. A peptide according to any of claims 1 to 10, or a composition according to claim 11, for use in suppressing or preventing the production of factor VIII inhibitor antibodies *in vivo*.

13. The use of a peptide according to any of claims 1 to 10, or a composition according to claim 11, in the manufacture of a medicament to suppress or prevent the production of factor VIII inhibitor antibodies *in vivo*.

14. A method for suppressing or preventing the production of factor VIII inhibitor antibodies in a subject, which comprises the step of administration of a peptide according to any of claims 1 to 10, or a composition according to claim 11, to the subject.

15. A method for treating haemophilia in a subject which comprises the step of administration of a peptide according to any of claims 1 to 10, or a composition according to claim 11, to the subject.

16. A method according to claim 14 or 15, wherein the subject has haemophilia A, and is undergoing, or is about to undergo, factor VIII replacement therapy.

17. A method according to claim 14 or 15, wherein the subject has, or is at risk from contracting, acquired haemophilia.

18. A method according to any of claims 14 to 17, wherein the subject is HLA-DR2.

SEQUENCE LISTING

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Trp Asp Tyr Met Gln Ser Asp Leu Gly Glu Leu Pro Val Asp Ala Arg
35 40 45

Phe Pro Pro Arg Val Pro Lys Ser Phe Pro Phe Asn Thr Ser Val Val
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Tyr Lys Lys Thr Leu Phe Val Glu Phe Thr Asp His Leu Phe Asn Ile
65 70 75 80

Ala Lys Pro Arg Pro Pro Trp Met Gly Leu Leu Gly Pro Thr Ile Gln
85 90 95

Ala Glu Val Tyr Asp Thr Val Val Ile Thr Leu Lys Asn Met Ala Ser
100 105 110

His Pro Val Ser Leu His Ala Val Gly Val Ser Tyr Trp Lys Ala Ser
115 120 125

Glu Gly Ala Glu Tyr Asp Asp Gln Thr Ser Gln Arg Glu Lys Glu Asp
130 135 140

Asp Lys Val Phe Pro Gly Gly Ser His Thr Tyr Val Trp Gln Val Leu
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Tyr Leu Ser His Val Asp Leu Val Lys Asp Leu Asn Ser Gly Leu Ile
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Gly Ala Leu Leu Val Cys Arg Glu Gly Ser Leu Ala Lys Glu Lys Thr
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Gln Thr Leu His Lys Phe Ile Leu Leu Phe Ala Val Phe Asp Glu Gly
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Lys Ser Trp His Ser Glu Thr Lys Asn Ser Leu Met Gln Asp Arg Asp
225 230 235 240

Ala Ala Ser Ala Arg Ala Trp Pro Lys Met His Thr Val Asn Gly Tyr
245 250 255

Val Asn Arg Ser Leu Pro Gly Leu Ile Gly Cys His Arg Lys Ser Val
260 265 270

Tyr Trp His Val Ile Gly Met Gly Thr Thr Pro Glu Val His Ser Ile
275 280 285

Phe Leu Glu Gly His Thr Phe Leu Val Arg Asn His Arg Gln Ala Ser
290 295 300

Leu Glu Ile Ser Pro Ile Thr Phe Leu Thr Ala Gln Thr Leu Leu Met
305 310 315 320

Asp Leu Gly Gln Phe Leu Leu Phe Cys His Ile Ser Ser His Gln His
325 330 335

Asp Gly Met Glu Ala Tyr Val Lys Val Asp Ser Cys Pro Glu Glu Pro
340 345 350

Gln Leu Arg Met Lys Asn Asn Glu Glu Ala Glu Asp Tyr Asp Asp Asp
355 360 365

Leu Thr Asp Ser Glu Met Asp Val Val Arg Phe Asp Asp Asp Asn Ser
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Pro Ser Phe Ile Gln Ile Arg Ser Val Ala Lys Lys His Pro Lys Thr
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Trp Val His Tyr Ile Ala Ala Glu Glu Glu Asp Trp Asp Tyr Ala Pro
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Ala Tyr Thr Asp Glu Thr Phe Lys Thr Arg Glu Ala Ile Gln His Glu
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Leu Ile Ile Phe Lys Asn Gln Ala Ser Arg Pro Tyr Asn Ile Tyr Pro
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His Gly Ile Thr Asp Val Arg Pro Leu Tyr Ser Arg Arg Leu Pro Lys
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Gly Val Lys His Leu Lys Asp Phe Pro Ile Leu Pro Gly Glu Ile Phe
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Lys Tyr Lys Trp Thr Val Thr Val Glu Asp Gly Pro Thr Lys Ser Asp
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580 585 590

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Pro Glu Phe Gln Ala Ser Asn Ile Met His Ser Ile Asn Gly Tyr Val
625 630 635 640

Phe Asp Ser Leu Gln Leu Ser Val Cys Leu His Glu Val Ala Tyr Trp
645 650 655

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690 695 700

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725 730 735

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740 745 750

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755 760 765

Ser Thr Arg Gln Lys Gln Phe Asn Ala Thr Thr Ile Pro Glu Asn Asp
770 775 780

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785 790 795 800

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Pro Thr Pro His Gly Leu Ser Leu Ser Asp Leu Gln Glu Ala Lys Tyr
820 825 830

Glu Thr Phe Ser Asp Asp Pro Ser Pro Gly Ala Ile Asp Ser Asn Asn
835 840 845

Ser Leu Ser Glu Met Thr His Phe Arg Pro Gln Leu His His Ser Gly
850 855 860

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900 905 910

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915 920 925

Pro Val His Tyr Asp Ser Gln Leu Asp Thr Thr Leu Phe Gly Lys Lys
930 935 940

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945 950 955 960

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<400> 17

Leu Ile Ile Phe Lys Asn Gln Ala Ser
1 5

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<211> 9
<212> PRT
<213> Homo sapiens

<400> 18

Leu Thr Arg Tyr Tyr Ser Ser Phe Val
1 5

<210> 19
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<212> PRT
<213> Homo sapiens

<400> 19

Met Val Thr Phe Arg Asn Gln Ala Ser
1 5

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<213> Homo sapiens

<400> 20

Leu Arg Ile His Pro Gln Ser Trp Val
1 5

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<222> (1)..(1)
<223> Xaa is a charged hydrophilic amino acid residue

<400> 21

Xaa Asp Asn Ile Met Val Thr Phe Arg Asn Gln Ala Ser
1 5 10

<210> 22
<211> 14
<212> PRT
<213> Homo sapiens

<220>
<221> MISC_FEATURE
<222> (1)..(1)
<223> Xaa is a charged amino acid with reverse polarity to Xaa at position 14

<220>
<221> MISC_FEATURE
<222> (14)..(14)
<223> Xaa is a charged amino acid with reverse polarity to Xaa at position 1

<400> 22

Xaa Asp Asn Ile Met Val Thr Phe Arg Asn Gln Ala Ser Xaa
1 5 10

<210> 23
<211> 14
<212> PRT
<213> Homo sapiens

<400> 23

Glu Asp Asn Ile Met Val Thr Phe Arg Asn Gln Ala Ser Arg
1 5 10

<210> 24

<211> 15
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<213> Homo sapiens

<400> 24

Tyr Gly Glu Val Gly Asp Thr Leu Leu Ile Ile Phe Lys Asn Gln
1 5 10 15

<210> 25
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<213> Homo sapiens

<400> 25

Gly Glu Val Gly Asp Thr Leu Leu Ile Ile Phe Lys Asn Gln Ala
1 5 10 15

<210> 26
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<213> Homo sapiens

<400> 26

Glu Val Gly Asp Thr Leu Leu Ile Ile Phe Lys Asn Gln Ala Ser
1 5 10 15

<210> 27
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<213> Homo sapiens

<400> 27

Val Gly Asp Thr Leu Leu Ile Ile Phe Lys Asn Gln Ala Ser Arg
1 5 10 15

<210> 28
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<213> Homo sapiens

<400> 28

Gly Asp Thr Leu Leu Ile Ile Phe Lys Asn Gln Ala Ser Arg Pro
1 5 10 15

<210> 29
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<400> 29

Thr Leu Leu Ile Ile Phe Lys Asn Gln Ala Ser Arg Pro Tyr Asn
1 5 10 15

<210> 30
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<400> 30

Leu Leu Ile Ile Phe Lys Asn Gln Ala Ser Arg Pro Tyr Asn Ile
1 5 10 15

<210> 31
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<213> Homo sapiens

<400> 31

Leu Ile Ile Phe Lys Asn Gln Ala Ser Arg Pro Tyr Asn Ile Tyr
1 5 10 15

<210> 32
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<213> Homo sapiens

<400> 32

Ile Ile Phe Lys Asn Gln Ala Ser Arg Pro Tyr Asn Ile Tyr Pro
1 5 10 15

<210> 33
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<212> PRT
<213> Homo sapiens

<400> 33

Ile Phe Lys Asn Gln Ala Ser Arg Pro Tyr Asn Ile Tyr Pro His
1 5 10 15

<210> 34
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<213> Homo sapiens

<400> 34

Pro Thr Lys Ser Asp Pro Arg Cys Leu Thr Arg Tyr Tyr Ser Ser
1 5 10 15

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<400> 35

Thr Lys Ser Asp Pro Arg Cys Leu Thr Arg Tyr Tyr Ser Ser Phe

1 5 10 15

<210> 36
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<213> Homo sapiens

<400> 36

Lys Ser Asp Pro Arg Cys Leu Thr Arg Tyr Tyr Ser Ser Phe Val
1 5 10 15

<210> 37
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<213> Homo sapiens

<400> 37

Ser Asp Pro Arg Cys Leu Thr Arg Tyr Tyr Ser Ser Phe Val Asn
1 5 10 15

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<213> Homo sapiens

<400> 38

Asp Pro Arg Cys Leu Thr Arg Tyr Tyr Ser Ser Phe Val Asn Met
1 5 10 15

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<400> 39

Arg Cys Leu Thr Arg Tyr Tyr Ser Ser Phe Val Asn Met Glu Arg
1 5 10 15

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<400> 40

Cys Leu Thr Arg Tyr Tyr Ser Ser Phe Val Asn Met Glu Arg Asp
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<400> 41

Leu Thr Arg Tyr Tyr Ser Ser Phe Val Asn Met Glu Arg Asp Leu
1 5 10 15

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<400> 42

Thr Arg Tyr Tyr Ser Ser Phe Val Asn Met Glu Arg Asp Leu Ala
1 5 10 15

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<400> 43

Arg Tyr Tyr Ser Ser Phe Val Asn Met Glu Arg Asp Leu Ala Ser
1 5 10 15

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<400> 44

Arg Ala Glu Val Glu Asp Asn Ile Met Val Thr Phe Arg Asn Gln
1 5 10 15

<210> 45
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<400> 45

Ala Glu Val Glu Asp Asn Ile Met Val Thr Phe Arg Asn Gln Ala
1 5 10 15

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<400> 46

Glu Val Glu Asp Asn Ile Met Val Thr Phe Arg Asn Gln Ala Ser
1 5 10 15

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<400> 47

Val Glu Asp Asn Ile Met Val Thr Phe Arg Asn Gln Ala Ser Arg
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<400> 48

Glu Asp Asn Ile Met Val Thr Phe Arg Asn Gln Ala Ser Arg Pro
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<400> 49

Asn Ile Met Val Thr Phe Arg Asn Gln Ala Ser Arg Pro Tyr Ser
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<400> 50

Ile Met Val Thr Phe Arg Asn Gln Ala Ser Arg Pro Tyr Ser Phe
1 5 10 15

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<400> 51

Met Val Thr Phe Arg Asn Gln Ala Ser Arg Pro Tyr Ser Phe Tyr
1 5 10 15

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<400> 52

Val Thr Phe Arg Asn Gln Ala Ser Arg Pro Tyr Ser Phe Tyr Ser
1 5 10 15

<210> 53
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<212> PRT
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<400> 53

Thr Phe Arg Asn Gln Ala Ser Arg Pro Tyr Ser Phe Tyr Ser Ser
1 5 10 15

<210> 54
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<213> Homo sapiens

<400> 54

Arg Gln Lys Phe Ser Ser Leu Tyr Ile Ser Gln Phe Ile Ile Met
1 5 10 15

<210> 55
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<213> Homo sapiens

<400> 55

Gln Lys Phe Ser Ser Leu Tyr Ile Ser Gln Phe Ile Ile Met Tyr
1 5 10 15

<210> 56
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<213> Homo sapiens

<400> 56

Lys Phe Ser Ser Leu Tyr Ile Ser Gln Phe Ile Ile Met Tyr Ser
1 5 10 15

<210> 57
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<213> Homo sapiens

<400> 57

Phe Ser Ser Leu Tyr Ile Ser Gln Phe Ile Ile Met Tyr Ser Leu
1 5 10 15

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<400> 58

Ser Ser Leu Tyr Ile Ser Gln Phe Ile Ile Met Tyr Ser Leu Asp
1 5 10 15

<210> 59
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<400> 59

Leu Tyr Ile Ser Gln Phe Ile Ile Met Tyr Ser Leu Asp Gly Lys
1 5 10 15

<210> 60
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<400> 60

Tyr Ile Ser Gln Phe Ile Ile Met Tyr Ser Leu Asp Gly Lys Lys
1 5 10 15

<210> 61
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<400> 61

Ile Ser Gln Phe Ile Ile Met Tyr Ser Leu Asp Gly Lys Lys Trp
1 5 10 15

<210> 62
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<400> 62

Ser Gln Phe Ile Ile Met Tyr Ser Leu Asp Gly Lys Lys Trp Gln
1 5 10 15

<210> 63
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<213> Homo sapiens

<400> 63

Gln Phe Ile Ile Met Tyr Ser Leu Asp Gly Lys Lys Trp Gln Thr
1 5 10 15

<210> 64
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<400> 64

His Asn Ile Phe Asn Pro Pro Ile Ile Ala Arg Tyr Ile Arg Leu
1 5 10 15

<210> 65
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Asn Ile Phe Asn Pro Pro Ile Ile Ala Arg Tyr Ile Arg Leu His
1 5 10 15

<210> 66
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<213> Homo sapiens

<400> 66

Ile Phe Asn Pro Pro Ile Ile Ala Arg Tyr Ile Arg Leu His Pro
1 5 10 15

<210> 67
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<213> Homo sapiens

<400> 67

Phe Asn Pro Pro Ile Ile Ala Arg Tyr Ile Arg Leu His Pro Thr
1 5 10 15

<210> 68
<211> 15
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<400> 68

Asn Pro Pro Ile Ile Ala Arg Tyr Ile Arg Leu His Pro Thr His
1 5 10 15

<210> 69
<211> 15
<212> PRT
<213> Homo sapiens

<400> 69

Pro Ile Ile Ala Arg Tyr Ile Arg Leu His Pro Thr His Tyr Ser
1 5 10 15

<210> 70
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<400> 70

Ile Ile Ala Arg Tyr Ile Arg Leu His Pro Thr His Tyr Ser Ile
1 5 10 15

<210> 71
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<400> 71

Ile Ala Arg Tyr Ile Arg Leu His Pro Thr His Tyr Ser Ile Arg
1 5 10 15

<210> 72
<211> 15
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<213> Homo sapiens

<400> 72

Ala Arg Tyr Ile Arg Leu His Pro Thr His Tyr Ser Ile Arg Ser
1 5 10 15

<210> 73
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<212> PRT
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<400> 73

Arg Tyr Ile Arg Leu His Pro Thr His Tyr Ser Ile Arg Ser Thr
1 5 10 15

<210> 74
<211> 15
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<400> 74

Pro Pro Leu Leu Thr Arg Tyr Leu Arg Ile His Pro Gln Ser Trp
1 5 10 15

<210> 75
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<400> 75

Pro Leu Leu Thr Arg Tyr Leu Arg Ile His Pro Gln Ser Trp Val
1 5 10 15

<210> 76
<211> 15
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<400> 76

Leu Leu Thr Arg Tyr Leu Arg Ile His Pro Gln Ser Trp Val His
1 5 10 15

<210> 77
<211> 15
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<400> 77

Leu Thr Arg Tyr Leu Arg Ile His Pro Gln Ser Trp Val His Gln
1 5 10 15

<210> 78
<211> 15
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<400> 78

Thr Arg Tyr Leu Arg Ile His Pro Gln Ser Trp Val His Gln Ile
1 5 10 15

<210> 79
<211> 15
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<213> Homo sapiens

<400> 79

Tyr Leu Arg Ile His Pro Gln Ser Trp Val His Gln Ile Ala Leu
1 5 10 15

<210> 80
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<400> 80

Leu Arg Ile His Pro Gln Ser Trp Val His Gln Ile Ala Leu Arg
1 5 10 15

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<400> 81

Arg Ile His Pro Gln Ser Trp Val His Gln Ile Ala Leu Arg Met
1 5 10 15

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Ile His Pro Gln Ser Trp Val His Gln Ile Ala Leu Arg Met Glu
1 5 10 15

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<400> 83

His Pro Gln Ser Trp Val His Gln Ile Ala Leu Arg Met Glu Val
1 5 10 15