[0007] Certain approaches have been explored to address the challenges associated with treating hemophilia by administration of FVIII protein. For example, WO 03/087161 discloses modified FVIII proteins, in which the immune characteristics of the FVIII protein are modified by reducing or removing the number of potential T-cell epitopes present on the protein. A number of regions that include T-cell epitopes along the FVIII protein were identified, including, e.g., $FVIII^{2030-2044}$. According to the disclosure, removal of such regions could be used to provide functional FVIII protein that did not induce production of anti-FVIII antibodies. WO 09/071886 also discloses specific regions of FVIII protein that were predicted to give rise to HLA-DR2 binding peptides that are involved in a patient's immune response, such as, e.g., $FVIII^{475-495}$, $FVIII^{542-562}$, $FVIII^{1785-1805}$, and $FVIII^{2158-2178}$. The peptides were identified for possible use in inducing immune tolerance in a patient.

[0008] While there have been advances in identifying regions of FVIII protein involved in the immune response, there is still a need to identify other regions of FVIII protein that can be used for developing other therapeutic peptides and methodologies that can, for example, be used to treat patients having hemophilia A.

BRIEF SUMMARY OF THE INVENTION

[0009] The present ~~invention~~disclosure is based on the identification of regions of FVIII protein related to the immune response against FVIII molecules. More specifically, a FVIII peptide including the region of FVIII protein can be used to induce tolerance to human FVIII in patients with, e.g., hemophilia A. Furthermore, the FVIII peptides can be used for immunodiagnostic purposes to monitor patients with hemophilia A during replacement therapy and during immune tolerance induction therapy.

[0009a] In one aspect, there is provided the use of a therapeutically effective amount of a peptide having an amino acid sequence consisting of:

$$(R^1)_x\text{-}P\text{-}(R^2)_y,$$

wherein:

P is an amino acid sequence having at least 90% identity to a sequence of SEQ ID NO:344;

R^1 is an amino acid sequence consisting of from 1 to 80 amino acids;

R^2 is an amino acid sequence consisting of from 1 to 80 amino acids; and

each of x and y are independently zero or one

in the manufacture of a medicament for inducing an immune tolerance to FVIII in a subject in need thereof, wherein said subject i) is being treated with FVIII therapy or ii) is being treated with an immune tolerance induction therapy.

[0009b] In another aspect, there is provided a peptide consisting of the amino acid sequence:

$$(R^1)_x\text{-}P\text{-}(R^2)_y,$$

wherein:

P is an amino acid sequence having at least 90% identity to a sequence of SEQ ID NO:344;

R^1 is an amino acid sequence consisting of from 1 to 80 amino acids;

R^2 is an amino acid sequence consisting of from 1 to 80 amino acids; and

each of x and y are independently zero or one.

[0009c] In another aspect, there is provided a composition comprising a peptide as defined above.

[0009d] In another aspect, there is provided a method of making a FVIII peptide, the method comprising the steps of:

a) providing a culture of cells comprising a polynucleotide that encodes a FVIII peptide as defined above; and

b) expressing the peptide in the culture of cells.

[0009e] In another aspect, there is provided a fusion protein comprising:

a Factor VIII peptide as defined above; and a reporter peptide.

[0010] In one ~~aspect~~embodiment, the present invention provides a method of inducing an immune tolerance to FVIII in a subject in need thereof, the method comprising a step of: administering to the subject a therapeutically effective amount of a peptide having an amino acid sequence consisting of: $(R^1)_x$-P-$(R^2)_y$, wherein: P is an amino acid sequence having at least 85% identity to at least nine consecutive amino acids of a sequence selected from SEQ ID NOS:10, 68, 344, and 740; R^1 is an amino acid sequence consisting of from 1 to 80 amino acids; R^2 is an amino acid sequence consisting of from 1 to 80 amino acids; and each of x and y are independently zero or one.

[0025] In one embodiment of the methods provided above, x is one and y is zero.

[0026] In one embodiment of the methods provided above, x is zero and y is one.

[0027] In one embodiment of the methods provided above, x and y are both zero.

[0028] In one embodiment of the methods provided above, the peptide consists of from 9 to 100 amino acids.

[0029] In one embodiment of the methods provided above, the peptide consists of from 9 to 50 amino acids.

[0030] In one embodiment of the methods provided above, the peptide consists of from 9 to 25 amino acids.

[0031] In one embodiment of the methods provided above, administration of the pharmaceutical composition prevents development of anti-FVIII antibodies in the subject.

[0032] In one embodiment of the methods provided above, administration of the pharmaceutical composition reduces an amount anti-FVIII antibodies present in the subject.

[0033] In one ~~aspect~~embodiment, the present invention provides a peptide consisting of the amino acid sequence: $(R^1)_x$-P-$(R^2)_y$, wherein: P is an amino acid sequence having at least 85% identity to a sequence of at least nine consecutive amino acids of a sequence selected from SEQ ID NOS:10, 68, 159, 250, 344, 477, 568, 659, and 740; R^1 is an amino acid sequence consisting of from 1 to 80 amino acids; R^2 is an amino acid sequence consisting of from 1 to 80 amino acids; and each of x and y are independently zero or one.

[0034] In one embodiment of the peptides provided above, P is an amino acid sequence having at least 90% identity to a sequence of at least nine consecutive amino acids of SEQ ID NO:10.

[0035] In one embodiment of the peptides provided above, P is an amino acid sequence identical to a sequence of at least nine consecutive amino acids of SEQ ID NO:10.

[0036] In one embodiment of the peptides provided above, P is an amino acid sequence having at least 90% identity to a sequence of at least nine consecutive amino acids of SEQ ID NO:68.

[0037] In one embodiment of the peptides provided above, P is an amino acid sequence having at least 95% identity to a sequence of at least nine consecutive amino acids of SEQ ID NO:68.

[0053] In one embodiment of the peptides provided above, the peptide consists of from 9 to 25 amino acids.

[0054] *In one ~~aspect~~embodiment, the present invention provides a composition comprising a peptide as* described herein.

[0055] In one embodiment of the compositions provided above, the composition is formulated for pharmaceutical administration.

[0056] In one embodiment of the compositions provided above, the composition further comprises a second polypeptide, the second polypeptide consisting of the amino acid sequence: $(R^1)_x$-P-$(R^2)_y$, wherein: P is an amino acid sequence having at least 85% identity to a sequence of at least nine consecutive amino acids of a sequence selected from SEQ ID NOS:10, 68, 159, 250, 477, 568, 659, and 740; R^1 is an amino acid sequence consisting of from 1 to 80 amino acids; R^2 is an amino acid sequence consisting of from 1 to 80 amino acids; and each of x and y are independently zero or one.

[0057] In one ~~aspect~~embodiment, the present invention provides a method of making a FVIII peptide, the method comprising the steps of: a) providing a culture of cells comprising a polynucleotide that encodes a FVIII peptide according to any one of claims 24 to 41; and b) expressing the peptide in the culture of cells.

[0058] In one ~~aspect~~embodiment, the present invention provides a method of identifying a FVIII peptide-specific T cell, the method comprising: a) combining a plurality of CD4+ T cells with a peptide complexed with a MHC class II multimer, wherein the peptide is a FVIII peptide according to any one of claims 24 to 41; and b) identifying at least one of the members of the plurality of CD4+ T cells that is specific for the peptide complexed with the MHC class II multimer.

[0059] *In one embodiment of the methods provided above, the MHC class II multimer is a* MHC class II tetramer.

[0060] In one embodiment of the methods provided above, the peptide or MHC class II multimer further comprises a detectable moiety.

[0061] In one embodiment of the methods provided above, the method further comprises isolating at least one CD4+ T cell that is specific for the peptide.

[0062] In one embodiment of the methods provided above, the CD4+ T cell is isolated using flow cytometry.

[0063] In one ~~aspect~~embodiment, the present invention provides a fusion protein comprising a FVIII peptide as provided herein and a second peptide.

[0064] In one embodiment of the methods provided above, the second peptide is a reporter peptide.

[0065] In one embodiment of the methods provided above, the fusion protein is encoded by a nucleic acid.

[0066] In one embodiment of the methods provided above, the FVIII peptide is chemically linked to the second peptide.

[0067] In one ~~aspect~~embodiment, the FVIII peptides provided herein are used to induce immune tolerance towards human FVIII for the prevention of FVIII inhibitor development.

[0068] In one ~~aspect~~embodiment, the FVIII peptides provided herein are used to induce tolerance towards human FVIII for the treatment of patients with established FVIII inhibitors.

[0069] In one ~~aspect~~embodiment, the FVIII peptides provided herein are used to generate reagents suitable for direct staining of FVIII specific T cells (*e.g.*, MHC class II multimers or MHC class II tetramers) in immune monitoring of patients during replacement therapy or during immune tolerance induction therapy.

[0070] In one ~~aspect~~embodiment, the FVIII peptides provided herein are used to identify antigen specific T cells. In one embodiment, these reagents can be used to track FVIII specific T cells in *in vitro* and in *ex vivo* settings. In another embodiment, these reagents can be used to isolate and further characterize FVIII specific T cells. In one embodiment, fluorescent activated cell sorting (FACS) or single cell PCR can be used for these purposes.

[0071] In one ~~aspect~~embodiment, the FVIII peptides provided herein are used for immune monitoring of FVIII specific T cells during immune tolerance induction therapy.

[0072] In one ~~aspect~~embodiment, the FVIII peptides provided herein are used for immune monitoring of FVIII specific T cells during FVIII treatment.

[0073] In one ~~aspect~~embodiment, the FVIII peptides provided herein are used for immunodiagnostics of FVIII specific T cells during clinical development of new immune modulators for the prevention of FVIII inhibitors.

BRIEF DESCRIPTION OF THE DRAWINGS

[0074] Not applicable.

DETAILED DESCRIPTION OF THE INVENTION

I. Introduction

[0075] The present invention is related to Factor VIII (FVIII) peptides that can be used to induce tolerance to FVIII protein in, for example, patients with hemophilia A. Furthermore, the peptides can be used for immunodiagnostic purposes to monitor FVIII-specific T cells in patients with hemophilia A during replacement therapy and during immune tolerance induction therapy.

[0076] The present invention is based in-part on the discovery that several regions of FVIII, specifically $FVIII^{102-122}$, $FVIII^{246-266}$, and $FVIII^{1401-1424}$, are involved in the immune response mounted against FVIII protein during Factor VIII replacement therapy or connected with acquired hemophilia. The amino acid sequences of the regions identified are TVVITLKNMASHPVSLHAVGV (SEQ ID NO:740), AWPKMHTVNGYVNRSLPGLIG (SEQ ID NO:68), and QANRSPLPIAKVSSFPSIRPIYLT (SEQ ID NO:344), respectively. It is believed that the present invention provides for the first time identification of these FVIII protein regions and their relationship to the immune response to FVIII protein.

[0077] Peptides of the present invention include peptides having at least a portion of the regions $FVIII^{102-122}$, $FVIII^{246-266}$, and $FVIII^{1401-1424}$ that complexes with a MHC class II molecule to produce a T cell epitope capable of being recognized by T cells involved in a patient's immune response. In some embodiments, the peptides include at least nine contiguous amino acids that correspond to nine contiguous amino acids in $FVIII^{102-122}$, $FVIII^{246-266}$, or $FVIII^{1401-1424}$. As described further below, the peptides provided herein also include peptides longer than nine amino acids in length as well as variants of the $FVIII^{102-122}$, $FVIII^{246-266}$, and $FVIII^{1401-1424}$ sequences. Such an identification of the peptides of the present invention can have implications