IMMUNOGENIC PEPTIDES WITH IMPROVED OXIDOREDUCTASE MOTIFS

BACKGROUND OF THE INVENTION

Several strategies have been described to prevent the generation of an unwanted immune response against an antigen. WO2008/017517 describes a new strategy using peptides comprising an MHC class II antigen of a given antigenic protein and an oxidoreductase motif. These peptides convert CD4+ T cells into a cell type with cytolytic properties called cytolytic CD4+ T cells. These cells are capable to kill via triggering apoptosis those antigen presenting cells (APC), which present the antigen from which the peptide is derived. WO2008/017517 demonstrates this concept for allergies and auto-immune diseases such as type I diabetes. Herein insulin can act as an auto-antigen.

WO2009101207 and Carlier et al. (2012) *Plos one* **7,10** e45366 further describe the antigen specific cytolytic cells in more detail.

WO2009101206 describes the use of peptides with an oxidoreductase motif and an MCH class II epitope of a soluble allo-antigen to prevent an immune response against such antigen when used in replacement therapies (e.g. unwanted immune response against injected insulin in diabetes patents).

WO2016059236 discloses further modified peptides wherein an additional Histidine is present in the proximity of the oxidoreductase motif.

In the design of a peptide against type I diabetes, many factors can be taken into account, such as the type of the auto-antigen (insulin, GAD 65, …), a specific domain and epitope of the auto-antigen, the oxidoreductase motif, the length and amino-acid acid sequence between the oxidoreductase motif and the epitope sequence.

In addition to the peptides comprising an MHC class II epitope of an allergen or antigen, WO2012069568A2 further disclosed the possibility of using NKT cell epitopes, binding the CD1d receptor and resulting in activation of cytolytic antigen-specific NKT cells, which have been shown to eliminate, in an antigen-specific manner, APC presenting said specific antigen.

Both strategies are building upon the use of oxidoreductase motifs of the [CST]X2C or CX2[CST] type. In order to improve the efficacy of a treatment using such immunogenic peptides, the search for more active peptides and/or more potent oxidoreductase motifs continues.

SUMMARY

The present disclosure provides novel immunogenic peptides comprising a T-cell epitope of an antigen and a oxidoreductase motif.

The inventors have tested the effect of adding different combinations of basic (charged) amino acids, either before, within, or after the traditionally used CXX[CST] or [CST]XXC oxidoreductase motifs.

By doing this, they found that in many cases, the oxidoreductase activity is changed when using specific combinations as claimed. This implies that the choice of a certain basic amino acid in the motif is not arbitrary but leads to an improved effect of the motif. More in particular, the inventors have shown that the use of basic amino acids K (lysine) or R (arginine) outperform the use of H (histidine). In some specific positions, also the K and R residues outperform each other and combinations of multiple basic amino acid residues in the motif seem to further increase these effects. These effects are displayed in the Figures and explained in the Examples section. Also the effect on the cellular level in model systems has been tested and confirms the improved activity of the immunogenic peptides of the disclosure.

The present disclosure relates to the following examples:

Example 1: An immunogenic peptide, said immunogenic peptide comprising:
a) an oxidoreductase motif,
b) a T-cell epitope of an antigenic protein, and
c) a linker between a) and b) of between 0 and 7 amino acids
wherein:
(i) said oxidoreductase motif is selected from the group comprising:
$(Z_2)^+(B^1)_n$[CST]XXC or $(Z_2)^+(B^1)_n$CXX[CST];
wherein $(Z_2)^+$ is a basic amino acid which is not H;
wherein X is any amino acid;
wherein (B^1) is any amino acid and wherein n is an integer from 0 to 3; or
(ii) said oxidoreductase motif is selected from the group comprising: [CST]X$(Z_2)^+$C or C$X(Z_2)^+$[CST];
wherein $(Z_2)^+$ is a basic amino acid which is not H;
wherein X is any amino acid;
wherein (B^1) is any amino acid and wherein n is an integer from 0 to 3; or
(iii) said oxidoreductase motif is selected from the group comprising: [CST]$(Z_1)^+$XC; or C$(Z_1)^+$X[CST],
wherein $(Z_1)^+$ is a basic amino acid which is not H or R, preferably K;
wherein X is any amino acid; or
(iv) said oxidoreductase motif is selected from the group comprising: [CST]XXC$(B^2)_m(Z_3)^+$, or CXX[CST]$(B^2)_m(Z_3)^+$,
wherein $(Z_3)^+$ is a basic amino acid,
preferably not H when the T-cell epitope is an NKT-cell epitope, more preferably R or K,
preferably a H, K or R or a non-natural basic amino acid such a L-ornithine when the T-cell epitope is an MHC class II epitope, more preferably R or K,
wherein X is any amino acid;

wherein (B^2) is any amino acid and wherein m is an integer from 0 to 3, with the proviso that when $(Z_3)^+$ is R, m is 0 and when $(Z_3)^+$ is H, m is 0 or 1.

Example 2: The immunogenic peptide according to example 1, wherein $(Z_1)^+$ is selected from the group comprising: K or a non-natural basic amino acid as defined herein and/or wherein either one of $(Z_2)^+$ and/or $(Z_3)^+$ is selected from the group comprising: K, R or a non-natural basic amino acid as defined herein.

Example 3: The immunogenic peptide according to example 1 or 2, wherein X is any amino acid except for C, S, or T.

Example 4: The immunogenic peptide according to any one of examples 1 to 3, wherein X is any amino acid except for basic amino acids.

Example 5: The immunogenic peptide according to any one of examples 1 to 4, wherein either one of $(Z_1)^+$, $(Z_2)^+$ and/or $(Z_3)^+$ is K or L-ornithine.

Example 6: The immunogenic peptide according to any one of examples 1 to 5, wherein said T cell epitope of an antigenic protein is an NKT cell epitope or an MHC class II T cell epitope.

Example 7: The immunogenic peptide according to any one of examples 1 to 6, wherein said epitope has a length of between 7 and 30 amino acids, preferably between 7 and 25 amino acids, more preferably between 7 and 20 amino acids.

Example 8: The immunogenic peptide according to any one of examples 1 to 7, having a length of between 11 and 50 amino acids, preferably between 11 and 40 amino acids, more preferably between 11 and 30 amino acids.

Example 9: The immunogenic peptide according to any one of examples 1 to 8, wherein said antigenic protein is an auto-antigen, a soluble allofactor, an alloantigen shed by the graft, an antigen of an intracellular pathogen, an antigen of a viral vector used for gene therapy or gene vaccination, a tumor-associated antigen or an allergen.

Example 10: The immunogenic peptide according to any one of examples 1 to 9, wherein at least one X in the motif is P or Y.

Example 11: The immunogenic peptide according to any one of examples 1 to 10, wherein the linker is of between 0 and 4 amino acids.

Example 12: The immunogenic peptide according to any one of examples 1 to 11, wherein said oxidoreductase motif does not naturally occur within a region of 11 amino acids N-terminally or C-terminally of the T-cell epitope in said antigenic protein.

Example 14: The immunogenic peptide according to any one of examples 1 to 13, wherein the T-cell epitope does not naturally comprise said oxidoreductase motif.

Example 15: The immunogenic peptide according to any one of examples 1 to 14, for use in medicine, more particularly for use in treating and/or prevention of an autoimmune disease, an infection with an intracellular pathogen, a tumor, an allograft rejection, or an immune response to a soluble allofactor, to an allergen exposure or to a viral vector used for gene therapy or gene vaccination.

Example 16: A method for preparing an immunogenic peptide according to any one of examples 1 to 13, comprising the steps of:

(a) providing a peptide sequence consisting of a T-cell epitope of said antigenic protein, and
(b) linking to said peptide sequence the oxidoreductase motif, such that said motif and said epitope are either adjacent to each other or separated by a linker of at most 7 amino acids.

Example 17: A method for obtaining a population of antigen-specific cytolytic CD4+ T cells, against APC presenting said antigen, the method comprising the steps of:

- providing peripheral blood cells;
- contacting said cells with an immunogenic peptide according to any one of examples 1 to 14, more particularly comprising:
  a) an oxidoreductase motif,
  b) a T-cell epitope of an antigenic protein, and
  c) a linker between a) and b) of between 0 and 7 amino acids
  wherein:

(i) said oxidoreductase motif is selected from the group comprising:
$(Z_2)^+(B^1)_n$[CST]XXC or $(Z_2)^+(B^1)_n$CXX[CST];
wherein $(Z_2)^+$ is a basic amino acid which is not H;
wherein X is any amino acid;
wherein (B^1) is any amino acid and wherein n is an integer from 0 to 3; or
(ii) said oxidoreductase motif is selected from the group comprising: [CST]X$(Z_2)^+$C or CX$(Z_2)^+$[CST];

wherein $(Z_2)^+$ is a basic amino acid which is not H;
wherein X is any amino acid;
wherein (B^1) is any amino acid and wherein n is an integer from 0 to 3; or
(iii) said oxidoreductase motif is selected from the group comprising: $[CST](Z_1)^+XC$; or $C(Z_1)^+X[CST]$,
wherein $(Z_1)^+$ is a basic amino acid which is not H or R, preferably K;
wherein X is any amino acid; or
(iv) said oxidoreductase motif is selected from the group comprising: $[CST]XXC(B^2)_m(Z_3)^+$, or $CXX[CST](B^2)_m(Z_3)^+$,
wherein $(Z_3)^+$ is a basic amino acid,
preferably not H when the T-cell epitope is an NKT-cell epitope, more preferably R or K,
preferably a H, K or R or a non-natural basic amino acid such a L-ornithine when the T-cell epitope is an MHC class II epitope, more preferably R or K,
wherein X is any amino acid;
wherein (B^2) is any amino acid and wherein m is an integer from 0 to 3, with the proviso that when $(Z_3)^+$ is R, m is 0 and when $(Z_3)^+$ is H, m is 0 or 1; and
- expanding said cells in the presence of IL-2.

Example 18: A method for obtaining a population of antigen-specific NKT cells, the method comprising the steps of:
- providing peripheral blood cells;
- contacting said cells with an immunogenic peptide according to any one of examples 1 to 14, more particularly comprising:
  a) an oxidoreductase motif,
  b) a T-cell epitope of an antigenic protein, and
  c) a linker between a) and b) of between 0 and 7 amino acids
  wherein:

(i) said oxidoreductase motif is selected from the group comprising:
$(Z_2)^+(B^1)_n[CST]XXC$ or $(Z_2)^+(B^1)_nCXX[CST]$;
wherein $(Z_2)^+$ is a basic amino acid which is not H;
wherein X is any amino acid;
wherein (B^1) is any amino acid and wherein n is an integer from 0 to 3; or
(ii) said oxidoreductase motif is selected from the group comprising: $[CST]X(Z_2)^+C$ or $CX(Z_2)^+[CST]$;
wherein $(Z_2)^+$ is a basic amino acid which is not H;
wherein X is any amino acid;
wherein (B^1) is any amino acid and wherein n is an integer from 0 to 3; or

(iii) said oxidoreductase motif is selected from the group comprising: [CST]$(Z_1)^+$XC; or C$(Z_1)^+$X[CST],

wherein $(Z_1)^+$ is a basic amino acid which is not H or R, preferably K;

wherein X is any amino acid; or

(iv) said oxidoreductase motif is selected from the group comprising: [CST]XXC$(B^2)_m(Z_3)^+$, or CXX[CST]$(B^2)_m(Z_3)^+$,

wherein $(Z_3)^+$ is a basic amino acid,

preferably not H when the T-cell epitope is an NKT-cell epitope, more preferably R or K,

preferably a H, K or R or a non-natural basic amino acid such a L-ornithine when the T-cell epitope is an MHC class II epitope, more preferably R or K,

wherein X is any amino acid;

wherein (B^2) is any amino acid and wherein m is an integer from 0 to 3, with the proviso that when $(Z_3)^+$ is R, m is 0 and when $(Z_3)^+$ is H, m is 0 or 1; and

- expanding said cells in the presence of IL-2.

Example 19: A method for obtaining a population of antigen-specific cytolytic CD4+ T cells, against APC presenting said antigen, the method comprising the steps of:

- providing an immunogenic peptide according to any one of examples 1 to 14, more particularly comprising:
  a) an oxidoreductase motif,
  b) a T-cell epitope of an antigenic protein, and
  c) a linker between a) and b) of between 0 and 7 amino acids
  wherein:

(i) said oxidoreductase motif is selected from the group comprising:

$(Z_2)^+(B^1)_n$[CST]XXC or $(Z_2)^+(B^1)_n$CXX[CST];

wherein $(Z_2)^+$ is a basic amino acid which is not H;

wherein X is any amino acid;

wherein (B^1) is any amino acid and wherein n is an integer from 0 to 3; or

(ii) said oxidoreductase motif is selected from the group comprising: [CST]X$(Z_2)^+$C or C$X(Z_2)^+$[CST];

wherein $(Z_2)^+$ is a basic amino acid which is not H;

wherein X is any amino acid;

wherein (B^1) is any amino acid and wherein n is an integer from 0 to 3; or

(iii) said oxidoreductase motif is selected from the group comprising: [CST]$(Z_1)^+$XC; or C$(Z_1)^+$X[CST],

wherein $(Z_1)^+$ is a basic amino acid which is not H or R, preferably K;

wherein X is any amino acid; or

(iv) said oxidoreductase motif is selected from the group comprising: [CST]XXC$(B^2)_m(Z_3)^+$, or CXX[CST]$(B^2)_m(Z_3)^+$,

wherein $(Z_3)^+$ is a basic amino acid,

preferably not H when the T-cell epitope is an NKT-cell epitope, more preferably R or K,

preferably a H, K or R or a non-natural basic amino acid such a L-ornithine when the T-cell epitope is an MHC class II epitope, more preferably R or K,

wherein X is any amino acid;

wherein (B^2) is any amino acid and wherein m is an integer from 0 to 3, with the proviso that when $(Z_3)^+$ is R, m is 0 and when $(Z_3)^+$ is H, m is 0 or 1;

- administering said peptide to a subject; and
- obtaining said population of antigen-specific cytolytic CD4+ T cells from said subject.

Example 20: A method for obtaining a population of antigen-specific NKT cells, the method comprising the steps of:

- providing an immunogenic peptide according to any one of examples 1 to 14, more particularly comprising::
  a) an oxidoreductase motif,
  b) a T-cell epitope of an antigenic protein, and
  c) a linker between a) and b) of between 0 and 7 amino acids
  wherein:

(i) said oxidoreductase motif is selected from the group comprising:

$(Z_2)^+(B^1)_n$[CST]XXC or $(Z_2)^+(B^1)_n$CXX[CST];

wherein $(Z_2)^+$ is a basic amino acid which is not H;

wherein X is any amino acid;

wherein (B^1) is any amino acid and wherein n is an integer from 0 to 3; or

(ii) said oxidoreductase motif is selected from the group comprising: [CST]X$(Z_2)^+$C or CX$(Z_2)^+$[CST];

wherein $(Z_2)^+$ is a basic amino acid which is not H;

wherein X is any amino acid;

wherein (B^1) is any amino acid and wherein n is an integer from 0 to 3; or

(iii) said oxidoreductase motif is selected from the group comprising: [CST]$(Z_1)^+$XC; or C$(Z_1)^+$X[CST],

wherein $(Z_1)^+$ is a basic amino acid which is not H or R, preferably K;

wherein X is any amino acid; or

(iv) said oxidoreductase motif is selected from the group comprising: [CST]XXC$(B^2)_m(Z_3)^+$, or CXX[CST]$(B^2)_m(Z_3)^+$,

wherein $(Z_3)^+$ is a basic amino acid,

preferably not H when the T-cell epitope is an NKT-cell epitope, more preferably R or K,

preferably a H, K or R or a non-natural basic amino acid such a L-ornithine when the T-cell epitope is an MHC class II epitope, more preferably R or K,
wherein X is any amino acid;
wherein (B^2) is any amino acid and wherein m is an integer from 0 to 3, with the proviso that when $(Z_3)^+$ is R, m is 0 and when $(Z_3)^+$ is H, m is 0 or 1;

- administering said peptide to a subject; and
- obtaining said population of antigen-specific NKT cells from said subject.

Example 21: The population of antigen-specific cytolytic CD4+ T cells or NKT cells obtainable by the method of examples 17 to 20 for use in the treatment and/or prevention of an autoimmune disease, an infection with an intracellular pathogen, a tumor, an allograft rejection, or an immune response to a soluble allofactors, to an allergen exposure or to a viral vector used for gene therapy or gene vaccination.

Example 22: A method of treating and/or preventing an autoimmune disease, an infection with an intracellular pathogen, a tumor, an allograft rejection, or an immune response to a soluble allofactors, to an allergen exposure or to a viral vector used for gene therapy or gene vaccination in an individual, comprising the steps of administering the immunogenic peptide according to any one of examples 1 to 13 or the cell population according to any one of examples 17 to 20 to said individual.

Example 23: A method of treating or preventing an autoimmune disease, an infection with an intracellular pathogen, a tumor, an allograft rejection, or an immune response to a soluble allofactors, to an allergen exposure or to a viral vector used for gene therapy or gene vaccination in an individual, comprising the steps of:

- providing peripheral blood cells of said individual,
- contacting said cells with an antigenic peptide according to any of examples 1 to 13,
- expanding said cells, and
- administering said expanded cells to said individual.

In one aspect, the present disclosure refers to an immunogenic peptide, said immunogenic peptide comprising:
a) an oxidoreductase motif,
b) a T-cell epitope of an antigenic protein, and
c) a linker between a) and b) of between 0 and 7 amino acids
wherein:
(i) said oxidoreductase motif is selected from the group comprising:
$(Z_2)^+(B^1)_n$[CST]XXC or $(Z_2)^+(B^1)_n$CXX[CST];
wherein $(Z_2)^+$ is a basic amino acid which is not H;

wherein X is any amino acid;

wherein (B^1) is any amino acid and wherein n is an integer from 0 to 3; or

(ii) said oxidoreductase motif is selected from the group comprising: $[CST]X(Z_2)^+C$ or $CX(Z_2)^+[CST]$;

wherein $(Z_2)^+$ is a basic amino acid which is not H;

wherein X is any amino acid; or

(iii) said oxidoreductase motif is selected from the group comprising: $[CST](Z_1)^+XC$; or $C(Z_1)^+X[CST]$,

wherein $(Z_1)^+$ is a basic amino acid which is not H or R;

wherein X is any amino acid; or

(iv) said oxidoreductase motif is selected from the group comprising: $[CST]XXC(B^2)_m(Z_3)^+$, or $CXX[CST](B^2)_m(Z_3)^+$,

wherein $(Z_3)^+$ is K, R, or a non-natural basic amino acid such a L-ornithine,

wherein X is any amino acid;

wherein (B^2) is any amino acid and wherein m is an integer from 0 to 3, with the proviso that when $(Z_3)^+$ is R, m is 0.

In a preferred example of said oxidoreductase motifs as described herein, when present, B^1 and/or B^2 are each independently selected from: H, K, or R, preferably H.

Particularly preferred examples of the disclosure comprise one of the following oxidoreductase motifs:

KCXXC, wherein X is any amino acid, preferably RCPYC, RCGHC, or RCHGC;

KCXXC, wherein X is any amino acid, preferably KCPYC, KCGHC, or KCHGC;

KHCXXC, wherein X is any amino acid, preferably RHCPYC, RHCGHC, or RHCHGC;

KRCXXC, wherein X is any amino acid, preferably KHCPYC, KHCGHC, or KHCHGC;
CXRC, wherein X is any amino acid, preferably CGRC, CPRC, CHRC;
CXKC, wherein X is any amino acid, preferably CGKC, CPKC, CHKC;
CXXCK, wherein X is any amino acid, preferably CHGCK, CPYCK, CGHCK;
CXXCHK, wherein X is any amino acid, preferably CHGCHK, CPYCHK, CGHCHK;
CXXCR, wherein X is any amino acid, preferably CHGCR, CPYCR, CGHCR;
CXXCHR, wherein X is any amino acid, preferably CHGCHR, CPYCHR, CGHCHR;
KCKXC, RCKXC, KCRXC, or RCRXC, wherein X is any amino acid
KCXKC, RCXKC, KCXRC, or RCXRC, wherein X is any amino acid
KCKXCK, RCKXCK, KCRXCK, KCKXCR, RCRXCK, RCKXCR, KCRXCR, or RCRXCR, wherein X is any amino acid
KCKXCHK, RCKXCHK, KCRXCHK, KCKXCHR, RCRXCHK, RCKXCHR, KCRXCHR or RCRXCHR, wherein X is any amino acid
KCXXCK, KCXXCR, RCXXCK or RCXXCR, wherein X is any amino acid
KCXXCHK, KCXXCHR, RCXXCHK, RCXXCHR, wherein X is any amino acid
KCXKCK, RCXKCK, KCXRCK, KCXKCR, RCXRCK, RCXKCR, KCXRCR, or RCXRCR, wherein X is any amino acid
KCXKCHK, RCXKCHK, KCXRCHK, KCXKCHR, RCXRCHK, RCXKCHR, KCXRCHR, or RCXRCHR, wherein X is any amino acid.

In anyone of the above examples, the antigen from which the epitope is designed can be selected from the group comprising: pro-insulin, insulin, C-peptide, MOG and tetanus toxin.

In a preferred example of the disclosure, the linker comprises at least 1 amino acid, at least 2 amino acids, at least 3 amino acids, or at least 4 amino acids. Preferably, said linker comprises between 1 and 7 amino acids, such as between 2 and 7 amino acids, between 3 and 7 amino acids, or between 4 and 7 amino acids.

In a preferred example of the disclosure, especially in those aspects where the oxidoreductase motif is [CST]$(Z_1)^+$XC or C$(Z_1)^+$X[CST], said oxidoreductase motif is not CRLC and/or the immunogenic peptide is not CRLC-KVAPVIKAR-MM.

In another preferred example of the disclosure, the T-cell epitope does not comprise a basic amino acid at its N-terminal end, i.e. immediately adjacent to the linker or oxidoreductase motif, more particularly in case the linker is absent or only comprises 1 or 2 amino acids. More preferably, in all examples wherein the oxidoreductase motif is [CST]XXC$(B^2)_m(Z_2)^+$ or CXX[CST]$(B^2)_m(Z_2)^+$, the T-cell epitope does not comprise a basic

amino acid at its N-terminal end, i.e. immediately adjacent to the linker or oxidoreductase motif, more particularly in case the linker is absent or only comprises 1 or 2 amino acids.

In a further example of the disclosure, the T-cell epitope does not comprise a basic amino acid in position 1, 2 and/or 3 counted from its N-terminal end, i.e. immediately adjacent to the linker or oxidoreductase motif, more particularly in case the linker is absent or only comprises 1 or 2 amino acids.

In a further example of the disclosure, either one of X, (B^1), and/or (B^2) can be a basic amino acid. In another example, either one of X, (B^1), and/or (B^2) is any amino acid except for C, S, or T. In yet a further example, either one of X, (B^1), and/or (B^2) is any amino acid except for a basic amino acid.

The peptides of the present disclosure have the advantage that cytolytic CD4+ T cells which have been generated using these peptides have an increased IFN-gamma and sFasL production compared to prior art peptides. Also Granzyme B production in said CD4+ T cells is believed to be increased.

The increased expression levels of these markers are indications of a greater capacity of the peptides of the present disclosure to generate cytolytic CD4+ T cells compared to the prior art peptides.

In another aspect, the present disclosure refers to the immunogenic peptide as described herein, for use in medicine.

In another aspect, the present disclosure refers to the immunogenic peptide as described herein, for use in treating and/or prevention of an autoimmune disease, an infection with an intracellular pathogen, a tumor, an allograft rejection, or an immune response to a soluble allofactor to an allergen exposure or to a viral vector used for gene therapy or gene vaccination.

In another aspect, the present disclosure refers to a use of the immunogenic peptide as described herein, in the manufacture of a medicament for treating and/or prevention of an autoimmune disease, an infection with an intracellular pathogen, a tumor, an allograft rejection, or an immune response to a soluble allofactor to an allergen exposure or to a viral vector used for gene therapy or gene vaccination.

In another aspect, the present disclosure refers to a method for preparing an immunogenic peptide as described herein, comprising the steps of:

(a) providing a peptide sequence consisting of a T-cell epitope of said antigenic protein, and

(b) linking to said peptide sequence the oxidoreductase motif, such that said motif and said epitope are either adjacent to each other or separated by a linker of at most 7 amino acids.

In another aspect, the present disclosure refers to a method for obtaining a population of antigen-specific cytolytic CD4+ T cells, against APC presenting said antigen, the method comprising the steps of:

- providing peripheral blood cells;
- contacting said cells with an immunogenic peptide comprising:
  a) an oxidoreductase motif,
  b) a T-cell epitope of an antigenic protein, and
  c) a linker between a) and b) of between 0 and 7 amino acids,
  wherein:

(i) said oxidoreductase motif is selected from the group comprising:
$(Z_2)^+(B^1)_n$[CST]XXC or $(Z_2)^+(B^1)_n$CXX[CST];
wherein $(Z_2)^+$ is a basic amino acid which is not H;
wherein X is any amino acid; or
(ii) said oxidoreductase motif is selected from the group comprising: [CST]X$(Z_2)^+$C or CX$(Z_2)^+$[CST];
wherein $(Z_2)^+$ is a basic amino acid which is not H;
wherein X is any amino acid;
wherein (B^1) is any amino acid and wherein n is an integer from 0 to 3; or
(iii) said oxidoreductase motif is selected from the group comprising: [CST]$(Z_1)^+$XC; or C$(Z_1)^+$X[CST],
wherein $(Z_1)^+$ is a basic amino acid which is not H or R;
wherein X is any amino acid; or
(iv) said oxidoreductase motif is selected from the group comprising: [CST]XXC$(B^2)_m(Z_3)^+$, or CXX[CST]$(B^2)_m(Z_3)^+$,
wherein $(Z_3)^+$ is K, R, or a non-natural basic amino acid such a L-ornithine,
wherein X is any amino acid;
wherein (B^2) is any amino acid and wherein m is an integer from 0 to 3, with the proviso that when $(Z_3)^+$ is R, m is 0; and

- expanding said cells in the presence of IL-2.

In another aspect, the present disclosure refers to a method for obtaining a population of antigen-specific NKT cells, the method comprising the steps of:

- providing peripheral blood cells;
- contacting said cells with an immunogenic peptide comprising:
  a) an oxidoreductase motif,

b) a T-cell epitope of an antigenic protein, and

c) a linker between a) and b) of between 0 and 7 amino acids,

wherein:

(i) said oxidoreductase motif is selected from the group comprising:

$(Z_2)^+(B^1)_n$[CST]XXC or $(Z_2)^+(B^1)_n$CXX[CST];

wherein $(Z_2)^+$ is a basic amino acid which is not H;

wherein X is any amino acid;

wherein (B^1) is any amino acid and wherein n is an integer from 0 to 3; or

(ii) said oxidoreductase motif is selected from the group comprising: [CST]X$(Z_2)^+$C or CX$(Z_2)^+$[CST];

wherein $(Z_2)^+$ is a basic amino acid which is not H;

wherein X is any amino acid; or

(iii) said oxidoreductase motif is selected from the group comprising: [CST]$(Z_1)^+$XC; or C$(Z_1)^+$X[CST],

wherein $(Z_1)^+$ is a basic amino acid which is not H or R;

wherein X is any amino acid; or

(iv) said oxidoreductase motif is selected from the group comprising: [CST]XXC$(B^2)_m(Z_3)^+$, or CXX[CST]$(B^2)_m(Z_3)^+$,

wherein $(Z_3)^+$ is K, R, or a non-natural basic amino acid such a L-ornithine,

wherein X is any amino acid;

wherein (B^2) is any amino acid and wherein m is an integer from 0 to 3, with the proviso that when $(Z_3)^+$ is R, m is 0; and

- expanding said cells in the presence of IL-2.

In another aspect, the present disclosure refers to a method for obtaining a population of antigen-specific cytolytic CD4+ T cells, against APC presenting said antigen, the method comprising the steps of:

- providing an immunogenic peptide comprising:

a) an oxidoreductase motif,

b) a T-cell epitope of an antigenic protein, and

c) a linker between a) and b) of between 0 and 7 amino acids,

wherein:

(i) said oxidoreductase motif is selected from the group comprising:

$(Z_2)^+(B^1)_n$[CST]XXC or $(Z_2)^+(B^1)_n$CXX[CST];

wherein $(Z_2)^+$ is a basic amino acid which is not H;

wherein X is any amino acid;

wherein (B^1) is any amino acid and wherein n is an integer from 0 to 3; or

(ii) said oxidoreductase motif is selected from the group comprising: [CST]X$(Z_2)^+$C or CX$(Z_2)^+$[CST];

wherein $(Z_2)^+$ is a basic amino acid which is not H;

wherein X is any amino acid; or

(iii) said oxidoreductase motif is selected from the group comprising: $[CST](Z_1)^+XC$; or $C(Z_1)^+X[CST]$,

wherein $(Z_1)^+$ is a basic amino acid which is not H or R;

wherein X is any amino acid; or

(iv) said oxidoreductase motif is selected from the group comprising: $[CST]XXC(B^2)_m(Z_3)^+$, or $CXX[CST](B^2)_m(Z_3)^+$,

wherein $(Z_3)^+$ is K, R, or a non-natural basic amino acid such a L-ornithine,

wherein X is any amino acid;

wherein (B^2) is any amino acid and wherein m is an integer from 0 to 3, with the proviso that when $(Z_3)^+$ is R, m is 0;

- administering said peptide to a subject; and
- obtaining said population of antigen-specific cytolytic CD4+ T cells from said subject.

In another aspect, the present disclosure refers to a method for obtaining a population of antigen-specific NKT cells, the method comprising the steps of:

- providing an immunogenic peptide comprising:
  a) an oxidoreductase motif,
  b) a T-cell epitope of an antigenic protein, and
  c) a linker between a) and b) of between 0 and 7 amino acids,
  wherein:

(i) said oxidoreductase motif is selected from the group comprising:

$(Z_2)^+(B^1)_n[CST]XXC$ or $(Z_2)^+(B^1)_nCXX[CST]$;

wherein $(Z_2)^+$ is a basic amino acid which is not H;

wherein X is any amino acid;

wherein (B^1) is any amino acid and wherein n is an integer from 0 to 3; or

(ii) said oxidoreductase motif is selected from the group comprising: $[CST]X(Z_2)^+C$ or $CX(Z_2)^+[CST]$;

wherein $(Z_2)^+$ is a basic amino acid which is not H;

wherein X is any amino acid; or

(iii) said oxidoreductase motif is selected from the group comprising: $[CST](Z_1)^+XC$; or $C(Z_1)^+X[CST]$,

wherein $(Z_1)^+$ is a basic amino acid which is not H or R;

wherein X is any amino acid; or

(iv) said oxidoreductase motif is selected from the group comprising: $[CST]XXC(B^2)_m(Z_3)^+$, or $CXX[CST](B^2)_m(Z_3)^+$,

wherein $(Z_3)^+$ is K, R, or a non-natural basic amino acid such a L-ornithine,

wherein X is any amino acid;

wherein (B^2) is any amino acid and wherein m is an integer from 0 to 3, with the proviso that when $(Z_3)^+$ is R, m is 0*;*

- administering said peptide to a subject; and
- obtaining said population of antigen-specific NKT cells from said subject.

In another aspect, the present disclosure refers to the population of antigen-specific cytolytic CD4+ T cells or NKT cells obtainable by the method as described herein for use in the treatment and/or prevention of an autoimmune disease, an infection with an intracellular pathogen, a tumor, an allograft rejection, or an immune response to a soluble allofactors, to an allergen exposure or to a viral vector used for gene therapy or gene vaccination.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: represents the kinetics of the redox activities of immunogenic peptides comprising a CHGC motif and an insulin T cell epitope. The basic amino acid is inserted at the N-terminus of the oxidoreductase motif, and the initial velocities of the redox activities are followed for 10 minutes. Peptides with oxidoreductase motifs without any charged amino acid or with an H at their N-terminus are used as control peptides. See table 1 for details.

Figure 2: represents the kinetics of the redox activities of immunogenic peptides comprising a CPYC motif and a tetanus toxin T cell epitope. The basic amino acid is inserted at the N-terminus of the oxidoreductase motif, and the initial velocities of the redox activities are followed for 10 minutes. Peptides with oxidoreductase motifs without any charged amino acid or with an H at their N-terminus are used as control peptides. See table 2 for details.

Figure 3: represents the kinetics of the redox activities of immunogenic peptides comprising a CPYC motif and a MOG T cell epitope. The basic amino acid is inserted at the N-terminus of the

CLAIMS

1. An immunogenic peptide, said immunogenic peptide comprising:
a) an oxidoreductase motif,
b) a T-cell epitope of an antigenic protein, and
c) a linker between a) and b) of between 0 and 7 amino acids
wherein:
(i) said oxidoreductase motif is selected from the group comprising:
$(Z_2)^+(B^1)_n$[CST]XXC or $(Z_2)^+(B^1)_n$CXX[CST];
wherein $(Z_2)^+$ is a basic amino acid which is not H;
wherein X is any amino acid;
wherein (B^1) is any amino acid and wherein n is an integer from 0 to 3; or
(ii) said oxidoreductase motif is selected from the group comprising: [CST]X$(Z_2)^+$C or CX$(Z_2)^+$[CST];
wherein $(Z_2)^+$ is a basic amino acid which is not H;
wherein X is any amino acid; or
(iii) said oxidoreductase motif is selected from the group comprising: [CST]$(Z_1)^+$XC; or C$(Z_1)^+$X[CST],
wherein $(Z_1)^+$ is a basic amino acid which is not H or R;
wherein X is any amino acid; or
(iv) said oxidoreductase motif is selected from the group comprising: [CST]XXC$(B^2)_m(Z_3)^+$, or CXX[CST]$(B^2)_m(Z_3)^+$,
wherein $(Z_3)^+$ is K, R, or a non-natural basic amino acid such a L-ornithine,
wherein X is any amino acid;
wherein (B^2) is any amino acid and wherein m is an integer from 0 to 3, with the proviso that when $(Z_3)^+$ is R, m is 0.

2. The immunogenic peptide according to claim 1, wherein $(Z_1)^+$ is selected from the group comprising: K or a non-natural basic amino acid and/or wherein either one of $(Z_2)^+$ and/or $(Z_3)^+$ each individually is selected from the group comprising: K, R or a non-natural basic amino acid.

3. The immunogenic peptide according to claim 1 or 2, wherein either one of $(Z_1)^+$, $(Z_2)^+$, and/or $(Z_3)^+$ is K or L-ornithine.

4. The immunogenic peptide according to any one of claims 1 to 3, wherein said T cell epitope of an antigenic protein is an NKT cell epitope or an MHC class II T cell epitope.

5. The immunogenic peptide according to any one of claims 1 to 4, wherein said epitope has a length of between 7 and 30 amino acids, preferably between 7 and 25 amino acids, more preferably between 7 and 20 amino acids.

6. The immunogenic peptide according to any one of claims 1 to 5, having a length of between 11 and 50 amino acids, preferably between 11 and 40 amino acids, more preferably between 11 and 30 amino acids.

7. The immunogenic peptide according to any one of claims 1 to 6, wherein said antigenic protein is an auto-antigen, a soluble allofactor, an alloantigen shed by the graft, an antigen of an intracellular pathogen, an antigen of a viral vector used for gene therapy or gene vaccination, a tumor-associated antigen or an allergen.

8. The immunogenic peptide according to any one of claims 1 to 7, wherein at least one X in the motif is P or Y.

9. The immunogenic peptide according to any one of claims 1 to 8, wherein the linker is of between 0 and 4 amino acids.

10. The immunogenic peptide according to any one of claims 1 to 9, wherein said oxidoreductase motif does not naturally occur within a region of 11 amino acids N-terminally or C-terminally of the T-cell epitope in said antigenic protein.

11. The immunogenic peptide according to any one of claims 1 to 10, wherein the T-cell epitope does not naturally comprise said oxidoreductase motif.

12. The immunogenic peptide according to any one of claims 1 to 11, for use in medicine.

13. The immunogenic peptide according to any one of claims 1 to 11, for use in treating and/or prevention of an autoimmune disease, an infection with an intracellular pathogen, a tumor, an allograft rejection, or an immune response to a soluble allofactor to an allergen exposure or to a viral vector used for gene therapy or gene vaccination.

14. A use of the immunogenic peptide according to any one of claims 1 to 11, in the manufacture of a medicament for treating and/or prevention of an autoimmune disease, an infection with an intracellular pathogen, a tumor, an allograft rejection, or an immune response to

a soluble allofactor to an allergen exposure or to a viral vector used for gene therapy or gene vaccination.

15. A method for preparing an immunogenic peptide according to any one of claims 1 to 11, comprising the steps of:

(a) providing a peptide sequence consisting of a T-cell epitope of said antigenic protein, and

(b) linking to said peptide sequence the oxidoreductase motif, such that said motif and said epitope are either adjacent to each other or separated by a linker of at most 7 amino acids.

16. A method for obtaining a population of antigen-specific cytolytic CD4+ T cells, against APC presenting said antigen, the method comprising the steps of:

- providing peripheral blood cells;
- contacting said cells with an immunogenic peptide comprising:

a) an oxidoreductase motif,

b) a T-cell epitope of an antigenic protein, and

c) a linker between a) and b) of between 0 and 7 amino acids,

wherein:

(i) said oxidoreductase motif is selected from the group comprising:

$(Z_2)^+(B^1)_n$[CST]XXC or $(Z_2)^+(B^1)_n$CXX[CST];

wherein $(Z_2)^+$ is a basic amino acid which is not H;

wherein X is any amino acid; or

(ii) said oxidoreductase motif is selected from the group comprising: [CST]X$(Z_2)^+$C or CX$(Z_2)^+$[CST];

wherein $(Z_2)^+$ is a basic amino acid which is not H;

wherein X is any amino acid;

wherein (B^1) is any amino acid and wherein n is an integer from 0 to 3; or

(iii) said oxidoreductase motif is selected from the group comprising: [CST]$(Z_1)^+$XC; or C$(Z_1)^+$X[CST],

wherein $(Z_1)^+$ is a basic amino acid which is not H or R;

wherein X is any amino acid; or

(iv) said oxidoreductase motif is selected from the group comprising: [CST]XXC$(B^2)_m(Z_3)^+$, or CXX[CST]$(B^2)_m(Z_3)^+$,

wherein $(Z_3)^+$ is K, R, or a non-natural basic amino acid such a L-ornithine,

wherein X is any amino acid;

wherein (B^2) is any amino acid and wherein m is an integer from 0 to 3, with the proviso that when $(Z_3)^+$ is R, m is 0; and

- expanding said cells in the presence of IL-2.

17. A method for obtaining a population of antigen-specific NKT cells, the method comprising the steps of:

- providing peripheral blood cells;
- contacting said cells with an immunogenic peptide comprising:

a) an oxidoreductase motif,

b) a T-cell epitope of an antigenic protein, and

c) a linker between a) and b) of between 0 and 7 amino acids,

wherein:

(i) said oxidoreductase motif is selected from the group comprising:

$(Z_2)^+(B^1)_n$[CST]XXC or $(Z_2)^+(B^1)_n$CXX[CST];

wherein $(Z_2)^+$ is a basic amino acid which is not H;

wherein X is any amino acid;

wherein (B^1) is any amino acid and wherein n is an integer from 0 to 3; or

(ii) said oxidoreductase motif is selected from the group comprising: [CST]X$(Z_2)^+$C or C$X(Z_2)^+$[CST];

wherein $(Z_2)^+$ is a basic amino acid which is not H;

wherein X is any amino acid; or

(iii) said oxidoreductase motif is selected from the group comprising: [CST]$(Z_1)^+$XC; or C$(Z_1)^+$X[CST],

wherein $(Z_1)^+$ is a basic amino acid which is not H or R;

wherein X is any amino acid; or

(iv) said oxidoreductase motif is selected from the group comprising: [CST]XXC$(B^2)_m(Z_3)^+$, or CXX[CST]$(B^2)_m(Z_3)^+$,

wherein $(Z_3)^+$ is K, R, or a non-natural basic amino acid such a L-ornithine,

wherein X is any amino acid;

wherein (B^2) is any amino acid and wherein m is an integer from 0 to 3, with the proviso that when $(Z_3)^+$ is R, m is 0; and

- expanding said cells in the presence of IL-2.

18. A method for obtaining a population of antigen-specific cytolytic CD4+ T cells, against APC presenting said antigen, the method comprising the steps of:

- providing an immunogenic peptide comprising:

a) an oxidoreductase motif,

b) a T-cell epitope of an antigenic protein, and

c) a linker between a) and b) of between 0 and 7 amino acids,

wherein:

(i) said oxidoreductase motif is selected from the group comprising:

$(Z_2)^+(B^1)_n$[CST]XXC or $(Z_2)^+(B^1)_n$CXX[CST];

wherein $(Z_2)^+$ is a basic amino acid which is not H;

wherein X is any amino acid;

wherein (B^1) is any amino acid and wherein n is an integer from 0 to 3; or

(ii) said oxidoreductase motif is selected from the group comprising: [CST]X$(Z_2)^+$C or C X$(Z_2)^+$[CST];

wherein $(Z_2)^+$ is a basic amino acid which is not H;

wherein X is any amino acid; or

(iii) said oxidoreductase motif is selected from the group comprising: [CST]$(Z_1)^+$XC; or C$(Z_1)^+$X[CST],

wherein $(Z_1)^+$ is a basic amino acid which is not H or R;

wherein X is any amino acid; or

(iv) said oxidoreductase motif is selected from the group comprising: [CST]XXC$(B^2)_m(Z_3)^+$, or CXX[CST]$(B^2)_m(Z_3)^+$,

wherein $(Z_3)^+$ is K, R, or a non-natural basic amino acid such a L-ornithine,

wherein X is any amino acid;

wherein (B^2) is any amino acid and wherein m is an integer from 0 to 3, with the proviso that when $(Z_3)^+$ is R, m is 0;

- administering said peptide to a subject; and
- obtaining said population of antigen-specific cytolytic CD4+ T cells from said subject.

19. A method for obtaining a population of antigen-specific NKT cells, the method comprising the steps of:

- providing an immunogenic peptide comprising:

a) an oxidoreductase motif,

b) a T-cell epitope of an antigenic protein, and

c) a linker between a) and b) of between 0 and 7 amino acids,

wherein:

(i) said oxidoreductase motif is selected from the group comprising:

$(Z_2)^+(B^1)_n$[CST]XXC or $(Z_2)^+(B^1)_n$CXX[CST];

wherein $(Z_2)^+$ is a basic amino acid which is not H;

wherein X is any amino acid;

wherein (B^1) is any amino acid and wherein n is an integer from 0 to 3; or

(ii) said oxidoreductase motif is selected from the group comprising: [CST]X$(Z_2)^+$C or C X$(Z_2)^+$[CST];

wherein $(Z_2)^+$ is a basic amino acid which is not H;

wherein X is any amino acid; or

(iii) said oxidoreductase motif is selected from the group comprising: [CST]$(Z_1)^+$XC; or C$(Z_1)^+$X[CST],

wherein $(Z_1)^+$ is a basic amino acid which is not H or R;

wherein X is any amino acid; or

(iv) said oxidoreductase motif is selected from the group comprising: [CST]XXC$(B^2)_m(Z_3)^+$, or CXX[CST]$(B^2)_m(Z_3)^+$,

wherein $(Z_3)^+$ is K, R, or a non-natural basic amino acid such a L-ornithine,

wherein X is any amino acid;

wherein (B^2) is any amino acid and wherein m is an integer from 0 to 3, with the proviso that when $(Z_3)^+$ is R, m is 0*;*

- administering said peptide to a subject; and
- obtaining said population of antigen-specific NKT cells from said subject.

20. The population of antigen-specific cytolytic CD4+ T cells or NKT cells obtainable by the method of any one of claims 16 to 19 for use in the treatment and/or prevention of an autoimmune disease, an infection with an intracellular pathogen, a tumor, an allograft rejection, or an immune response to a soluble allofactors, to an allergen exposure or to a viral vector used for gene therapy or gene vaccination.