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SUMMARY

In one aspect, the present disclosure refers to a CGRP peptide immunogen construct having about 30 or more amino acids, represented by the formulae: $(Th)_m$–$(A)_n$–(CGRP functional B epitope peptide)–X or (CGRP functional B epitope peptide)–$(A)_n$–$(Th)_m$–X or $(Th)_m$–$(A)_n$–(CGRP functional B epitope peptide)–$(A)_n$–$(Th)_m$–X wherein Th is a heterologous T helper epitope; A is a heterologous spacer; (CGRP functional B epitope peptide) is a B cell epitope peptide having the amino acid sequence selected from the group consisting of~~from 7 to about 30 amino acid residues derived from CGRP receptor binding or activation region with~~ SEQ ID NOs: 4-13 and 15-24~~) of CGRP (SEQ ID NOs: 1-3)~~; X is an α-COOH or α-$CONH_2$ of an amino acid; m is from 1 to about 4; and n is from 0 to about 10.

In another aspect, the present disclosure refers to an CGRP peptide immunogen construct comprising:

a. a B cell epitope comprising the amino acid sequence selected from the group consisting of SEQ ID NOs: 4-13 and 15-24~~from about 7 to about 30 amino acid residues from the CGRP sequence of SEQ ID NOs: 1 to 3~~;

b. a T helper epitope comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 74-115, and any combination thereof; and

employed for immunogenicity enhancement. This is in sharp contrast to standard methods that use a conventional carrier protein, such as KLH, toxoid, or other biological carriers used for such peptide antigenicity enhancement

In another aspect, the present disclosure refers to a pharmaceutical composition comprising:

a. a peptide immunogen construct as disclosed herein; and

b. a pharmaceutically acceptable delivery vehicle and/or adjuvant.

The present disclosure is also directed to pharmaceutical compositions and formulations for the prevention and/or treatment of migraine. In some examples, pharmaceutical compositions comprising a stabilized immunostimulatory complex, which is formed by mixing a CpG oligomer with a peptide composition containing a mixture of CGRP peptide immunogen constructs through electrostatic association, to further enhance the CGRP peptide immunogenicity towards the desired cross-reactivity with the full-length CGRP (e.g., SEQ ID NOs:1-3).

In other examples, pharmaceutical compositions comprising a peptide composition of a mixture of CGRP peptide immunogen constructs in contact with mineral salts including Alum gel (ALHYDROGEL) or Aluminum phosphate (ADJU-PHOS) to form a suspension formulation, or with MONTANIDE™ ISA 51 or 720 as adjuvant to form water-in-oil emulsions, that can be used for the prevention and/or treatment of migraine.

~~In one aspect, the present disclosure refers to a use of a pharmaceutical composition as disclosed herein in the generation of antibodies against CGRP in an animal.~~

Furthermore, the present disclosure also describes a method for the low cost manufacture and quality control of CGRP peptide immunogen constructs and formulations thereof, capable of preventing and/or treating migraine in animals.

In another aspect, the present disclosure refers to an isolated antibody or ~~epitope~~antigen-binding fragment thereof that specifically binds to CGRP peptide immunogen as disclosed herein~~the CGRP receptor binding or activation region of SEQ ID NOs: 4-13 and 15-24~~.

In another aspect, the present disclosure refers to a composition comprising the isolated antibody or antigen-binding fragment thereof as disclosed herein.

The present disclosure is also directed to antibodies directed against the disclosed CGRP peptide immunogen constructs. In particular, the CGRP peptide immunogen constructs of the present disclosure are able to stimulate the generation of highly specific functional antibodies that are cross-reactive with the full-length CGRP molecule. The disclosed antibodies bind with high specificity to CGRP without much, if any, directed to the heterologous Th epitopes employed for immunogenicity enhancement, which is in sharp contrast to antibodies produced using conventional proteins or other biological carriers used for such peptide immunogenicity

CLAIMS

1. A CGRP peptide immunogen construct having about 30 or more amino acids, represented by the formulae:

$(Th)_m–(A)_n–($ CGRP functional B epitope peptide$)–X$

or

(CGRP functional B epitope peptide$)–(A)_n–(Th)_m–X$

or

$(Th)_m–(A)_n–($ CGRP functional B epitope peptide$)–(A)_n–(Th)_m–X$

wherein

Th is a heterologous T helper epitope;

A is a heterologous spacer;

(CGRP functional B epitope peptide) is a B cell epitope peptide having the amino acid sequence selected from the group consisting of ~~from 7 to about 30 amino acid residues derived from CGRP receptor binding or activation region with~~ SEQ ID NOs: 4-13 and 15-24~~) of CGRP (SEQ ID NOs: 1-3)~~;

X is an α-COOH or α-$CONH_2$ of an amino acid;

m is from 1 to about 4; and

n is from 0 to about 10.

~~2. The CGRP peptide immunogen construct according to claim 1, wherein the CGRP receptor binding or activation region is selected from the group consisting of SEQ ID NOs: 4-13 and 15-24.~~

~~3.~~2. The CGRP peptide immunogen construct according to ~~any of claims 1 or 2~~claim 1, wherein the Th epitope is selected from the group consisting of SEQ ID NOs: 74-115.

~~4.~~3. The CGRP peptide immunogen construct according to claim 1, wherein the peptide immunogen construct is selected from the group consisting of SEQ ID NOs: 120-127 and 130-180.

~~5.~~4. An CGRP peptide immunogen construct comprising:

a. a B cell epitope comprising ~~from about 7 to about 30 amino acid residues from the CGRP sequence of SEQ ID NOs: 1 to 3~~ the amino acid sequence selected from the group consisting of SEQ ID NOs: 4-13 and 15-24;

b. a T helper epitope comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 74-115, and any combination thereof; and

c. an optional heterologous spacer selected from the group consisting of an amino acid, Lys-, Gly-, Lys-Lys-Lys-, (α, ε-N)Lys, ε-N-Lys-Lys-Lys-Lys (SEQ ID NO: 72), Lys-Lys-Lys-ε-N-Lys (SEQ ID NO: 73), and Pro-Pro-Xaa-Pro-Xaa-Pro (SEQ ID NO: 71), and any combination thereof,

wherein the B cell epitope is covalently linked to the T helper epitope directly or through the optional heterologous spacer.

~~6.~~ 5. The CGRP peptide immunogen construct of claim ~~5~~4, wherein the B cell epitope is selected from the group consisting of SEQ ID NOs: 4-13 and 15-24.

~~7.~~6. The CGRP peptide immunogen construct of claim ~~5~~4, wherein the T helper epitope is selected from the group consisting of SEQ ID NOs: 68, 71, 74-82, 87, 89-92, 95, and any combination thereof.

~~8.~~7. The CGRP peptide immunogen construct of claim ~~5~~4, wherein the optional heterologous spacer is (α, ε-N)Lys, ε-N-Lys-Lys-Lys-Lys (SEQ ID NO: 72), Lys-Lys-Lys-ε-N-Lys (SEQ ID NO: 73), or Pro-Pro-Xaa-Pro-Xaa-Pro (SEQ ID NO: 71), where Xaa is any amino acid.

~~9.~~8. The CGRP peptide immunogen construct of claim ~~5~~4, wherein the T helper epitope is covalently linked to the amino terminus of the B cell epitope.

~~10.~~9. The CGRP peptide immunogen construct of claim ~~5~~4, wherein the T helper epitope is covalently linked to the amino terminus of the B cell epitope through the optional heterologous spacer.

~~11.~~10. A composition comprising an CGRP peptide immunogen construct according to claim 1.

~~12.~~11. A pharmaceutical composition comprising:

a. a peptide immunogen construct according to claim 1; and

b. a pharmaceutically acceptable delivery vehicle and/or adjuvant.

~~13.~~12. The pharmaceutical composition of claim ~~12~~11, wherein

a. the CGRP functional B epitope peptide is selected from the group consisting of SEQ ID NOs: 4-13 and 15-24;

b. the Th epitope is selected from the group consisting of SEQ ID NOs: 74-115; and

c. the heterologous spacer is selected from the group consisting of an amino acid, Lys-, Gly-, Lys-Lys-Lys-, (α, ε-N)Lys, ε-N-Lys-Lys-Lys-Lys (SEQ ID NO: 72), Lys-Lys-Lys- ε-N-Lys (SEQ ID NO: 73), and Pro-Pro-Xaa-Pro-Xaa-Pro (SEQ ID NO: 71), and any combination thereof; and

wherein the CGRP peptide immunogen construct is mixed with an CpG

oligodeoxynucleotide (ODN) to form a stabilized immunostimulatory complex.

~~14.~~13. The pharmaceutical composition of claim ~~12~~11, wherein

a. the CGRP peptide immunogen construct is selected from the group consisting of SEQ ID NOs: 120-127 and 130-180; and

wherein the CGRP peptide immunogen construct is mixed with an CpG oligodeoxynucleotide (ODN) to form a stabilized immunostimulatory complex.

~~15. Use of a pharmaceutical composition according to claim 12 in the generation of antibodies against CGRP in an animal.~~

~~16.~~14. An isolated antibody or ~~epitope~~antigen-binding fragment thereof that specifically binds to the CGRP peptide immunogen of claim 1.~~CGRP receptor binding or activation region of SEQ ID NOs: 4-13 and 15-24~~.

~~17. The isolated antibody or epitope binding fragment thereof according to claim 16 bound to the CGRP peptide immunogen construct.~~

~~18.~~15. A composition comprising the isolated antibody or ~~epitope~~antigen-binding fragment thereof according to claim 14~~16~~.

~~19.~~16. Use of a pharmaceutical composition of claim ~~12~~ 11 in the manufacture of a medicament for preventing and/or treating migraine in an animal.