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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 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 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

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.

The present disclosure is directed to portions of the Calcitonin Gene-Related Peptide (CGRP) that can be used as B cell epitopes. The present disclosure is also directed to peptide immunogen constructs containing B cell epitopes from CGRP, compositions containing the peptide immunogen constructs, methods of making and using the peptide immunogen constructs, and antibodies produced by the peptide immunogen constructs.

One example of the present disclosure is directed to portions of CGRP from different organisms as B cell epitopes in peptide immunogen constructs, as well as formulations thereof, that can be used for the prevention and/or treatment of migraine. The disclosed CGRP peptide immunogen constructs (SEQ ID NOs: 116-127 and 130-180) have 30 or more total amino acids and contain a functional B cell epitope peptide having about 7 to about 30 amino acids (SEQ ID NOs: 4-13, 15-19, and 20-24 of **Table 1**) derived from CGRP from human, marmoset, or rat/mouse (i.e., SEQ ID NOs: 1-3, respectively). The functional B cell epitope peptide can be linked through an optional heterologous spacer to a heterologous T helper cell (Th) epitope peptide derived from pathogen proteins (e.g., SEQ ID NOs: 74-115), to form the disclosed peptide immunogen construct.

The disclosed CGRP peptide immunogen constructs can contain a CGRP B cell epitope peptide having about 7 to about 30 amino acids. The B cell epitope peptide can be derived from the CGRP receptor binding region (e.g., SEQ ID NOs: 5-9 and 15-22, shown in **Table 1**) located at the C-terminal and central regions of the CGRP molecule. The B cell epitope peptide can also be derived from the CGRP receptor activation site around a cyclic C2-C7 loop (e.g. SEQ ID NOs: 4, 10-13 and 23-24, shown in **Table 1**) located at the N-terminal and central regions of the CGRP molecule. The designed CGRP B cell epitope peptide can be linked to a heterologous Th epitope derived from a pathogenic protein (e.g., SEQ ID NOs: 74-115 of **Table 2**) at either the N- or C-terminus of the CGRP peptide. The B cell and Th epitopes act together to stimulate the generation of highly specific antibodies cross-reactive with full-length CGRP (SEQ ID NO:1-3) of various species.

In certain examples, the heterologous Th epitopes employed to enhance the CGRP B cell epitope peptide are derived from natural pathogens EBV BPLF1 (SEQ ID NO: 112), EBV CP (SEQ ID NO: 109), Clostridium Tetani (SEQ ID NOs: 74, 77, 104, 106-108), Cholera Toxin (SEQ ID NO: 81), and Schistosoma mansoni (SEQ ID NO: 80), as well as those idealized artificial Th epitopes derived from Measles Virus Fusion protein (MVF 1 to 5) and Hepatitis B Surface Antigen (HBsAg 1 to 3) in the form of either single sequence or combinatorial sequences (e.g. SEQ ID NOs: 75, 82-99).

The disclosed CGRP peptide immunogen constructs, containing both designed B cell- and Th epitope peptides, act together to stimulate the generation of highly specific antibodies directed against CGRP functional sites, including the CGRP receptor binding region located at the C-terminal of the CGRP molecule or the cyclic C2-C7 loop involved in receptor activation, offer therapeutic immune responses to patients predisposed to, or suffering from, migraine.

In one aspect, the present disclosure refers to a composition comprising an CGRP peptide immunogen construct as disclosed herein.

Another example of the present disclosure is directed to peptide compositions containing a CGRP peptide immunogen construct. In some examples, the compositions contain one peptide immunogen construct. In other examples, peptide compositions comprising a mixture of CGRP peptide immunogen constructs. In certain examples, the mixture of CGRP peptide immunogen constructs have heterologous Th epitopes derived from different pathogens that can be used to allow coverage of as broad a genetic background in patients leading to a higher percentage in responder rate upon immunization for the prevention and/or treatment of migraine.

Synergistic enhancement in CGRP immunogen constructs can be observed in the peptide compositions of this disclosure. The antibody response derived from the administration of such compositions containing CGPR peptide immunogen constructs was mostly (>90%) focused on the desired cross-reactivity against the CGRP functional site(s) or receptor binding region peptides (SEQ ID NOs: 4-13 and 15-24) without much, if any, directed to the heterologous Th epitopes

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-binding fragment thereof that specifically binds to the CGRP receptor binding or activation region of SEQ ID NOs: 4-13 and 15-24.

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

enhancement. Thus, the disclosed CGRP peptide immunogen constructs are capable of breaking the immune tolerance against self-CGRP, with a high responder rate, compared to other peptide or protein immunogens.

In some examples, the disclosed antibodies are directed against and specifically bind to the CGRP receptor binding sites on the C-terminal portion of the CGRP molecule (e.g., SEQ ID NOs: 5-9 and 15-22) when the peptide immunogen constructs are administered to a subject. The highly specific antibodies elicited by these CGRP peptide immunogen constructs can inhibit CGRP and CGRP receptor binding and the downstream activation event in the rise of cellular cAMP caused by the region around the cyclic C2-C7 loop of the CGRP, leading to effective prevention and/or treatment of migraine.

In other examples, the disclosed antibodies are directed against either the N-terminal

or central regions of CGRP around the cyclic C2-C7 loop responsible for the downstream cell activation event or against the C-terminal and central regions of the CGRP receptor binding site, when the peptide immunogen constructs of current invention are administered to a subject (e.g., SEQ ID NOs: 4, 10-13, and 23-24). The highly specific antibodies elicited by the CGRP peptide immunogen constructs can inhibit (1) CGRP and CGRP receptor binding and (2) the downstream activation event caused by the region around the cyclic C2-C7 loop of the CGRP, resulting in the suppression of the rise of cellular cAMP, thus leading to effective treatment of patients suffering from migraine.

In another aspect, the present disclosure refers to a use of a pharmaceutical composition as disclosed herein in the manufacture of a medicament for preventing and/or treating migraine in an animal.

Based on their unique characteristics and properties, the disclosed antibodies elicited by the CGRP peptide immunogen constructs are capable of providing a prophylactic immunotherapeutic approach to treating patients suffering from migraine.

In a further example, the present disclosure describes human monoclonal antibodies against CGRP induced by patients receiving compositions containing CGRP peptide immunogen constructs of this disclosure. An efficient method to make human monoclonal antibodies from B cells isolated from the blood of a human patient is described by Traggiai, E., et al, 2004, which is incorporated by reference.

The present disclosure is also directed to methods of making the disclosed CGRP peptide immunogen constructs, compositions, and antibodies. The disclosed methods describe the low cost manufacture and quality control of CGRP peptide immunogen constructs and compositions containing the constructs, which can be used in methods for treating patients suffering from migraine.

The present disclosure also includes methods for preventing and/or treating subjects predisposed to, or suffering from, migraine using the disclosed CGRP peptide immunogen constructs and/or antibodies directed against the CGRP peptide immunogen constructs. The methods for preventing and/or treating migraine in a subject include administering to the subject a composition containing a disclosed CGRP peptide immunogen construct. In certain examples, the compositions utilized in the methods contain a disclosed CGRP peptide immunogen construct in the form of a stable immunostimulatory complex with negatively charged oligonucleotides, such as CpG oligomers, through electrostatic association, which can be further supplemented with an adjuvant, for administration to patients suffering from migraine.

The disclosed methods also include dosing regimens, dosage forms, and routes for administering the CGRP peptide immunogen constructs to prevent and/or treat migraine in a subject.

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

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

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

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

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

12. A pharmaceutical composition comprising:
    a. a peptide immunogen construct according to claim 1; and
    b. a pharmaceutically acceptable delivery vehicle and/or adjuvant.

13. The pharmaceutical composition of claim 12, 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. The pharmaceutical composition of claim 12, 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. An isolated antibody or epitope-binding fragment thereof that specifically binds to the 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. A composition comprising the isolated antibody or epitope-binding fragment thereof according to claim 16.

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