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10 **SUMMARY**

The present disclosure is directed to individual peptide immunogen constructs targeting the extracellular membrane proximal domain of membrane-bound IgE (IgE EMPD) for the treatment and/or prevention of IgE mediated allergic diseases. The present disclosure is also directed to compositions containing the peptide immunogen constructs, methods of making and using the peptide immunogen constructs, and antibodies produced by the peptide immunogen constructs.

Thus, in one aspect, the present disclosure refers to an IgE EMPD peptide immunogen construct represented by the formulae: $(Th)_m-(A)_n-(\text{IgE EMPD fragment})-X$ or $(\text{IgE EMPD fragment})-(A)_n-(Th)_m-X$ or $(Th)_m-(A)_n-(\text{IgE EMPD fragment})-(A)_n-(Th)_m-X$ wherein Th is a heterologous T helper epitope; A is a heterologous spacer; (IgE EMPD fragment) is a B cell epitope having about 20 to about 40 amino acid residues from the central intramolecular loop of IgE EMPD; X is an α -COOH or α -CONH₂ 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 IgE EMPD peptide immunogen construct comprising: a B cell epitope comprising about 20 to about 40 amino acid residues from the IgE EMPD sequence of SEQ ID NO: 1 or SEQ ID NO: 2; a T helper epitope comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 59-87; and an optional heterologous spacer selected from the group consisting of an amino acid, Lys-, Gly-, Lys-Lys-Lys-, (α , ϵ -N)Lys, and ϵ -N-Lys-Lys-Lys-Lys (SEQ ID NO: 129), wherein the B cell epitope is covalently linked to the T helper epitope directly or through the optional heterologous spacer.

In yet another aspect, the present disclosure refers to a composition comprising a peptide immunogen construction as disclosed herein.

In yet another aspect, the present disclosure refers to a pharmaceutical composition comprising: a. a peptide immunogen construct as disclosed herein; and b. and a pharmaceutically acceptable delivery vehicle and/or adjuvant.

In yet another aspect, the present disclosure refers to an isolated antibody or epitope-binding

fragment thereof that specifically binds to the B cell epitope of the IgE EMPD peptide immunogen construct as disclosed herein.

In yet another aspect, the present disclosure refers to an isolated antibody or epitope-binding fragment thereof that specifically binds to the B cell epitope of the IgE EMPD peptide immunogen construct as disclosed herein.

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

The disclosed peptide immunogen constructs contain about 20 or more amino acids. The peptide immunogen constructs contain a B cell epitope from the 67 amino acid sequence of the full-length IgE EMPD (SEQ ID NO: 1). The B cell epitope can be linked to a heterologous T helper cell (Th) epitope derived from pathogen proteins through an optional heterologous spacer. The disclosed peptide immunogen constructs stimulate the generation of highly specific antibodies directed against IgE EMPD and can bind to recombinant IgE EMPD-containing protein, IgE EMPD on mIgE bearing B cells, and/or a recombinant, soluble IgE EMPD protein containing the Fc portion of human IgG1 and the IgE EMPD of human membrane-bound IgE (referred to as “ γ 1-em67”). The disclosed peptide immunogen constructs can be used as an allergen-independent, cost-effective, universal immunotherapy for global patients suffering from IgE mediated allergic diseases.

The B cell epitope portion of the peptide immunogen constructs have amino acid sequences from the full-length IgE EMPD sequence (SEQ ID NO: 1). In some examples, the B cell epitope has a sequence containing the internal intra-molecular loop formed by endogenous cysteines (C18-C39), according to numbering of the full-length IgE EMPD sequence (SEQ ID NO: 1). In certain specific examples, the B cell epitope has an amino acid sequence of IgE EMPD-1-39 (SEQ ID NO: 5), IgE EMPD-7-40 (SEQ ID NO: 6), IgE EMPD-19-38 (SEQ ID NO: 8), or IgE EMPD-1-40 (SEQ ID NO: 9).

The peptide immunogen constructs of the present disclosure can contain a heterologous

Th epitope amino acid sequence derived from a pathogenic protein (e.g., SEQ ID NOs: 59 to 87). In certain examples, the heterologous Th epitope is derived from natural pathogens, such as Diphtheria Toxin (SEQ ID NO: 63), Plasmodium Falciparum (SEQ ID NO: 64), Cholera Toxin (SEQ ID NO: 66). In other examples, the heterologous Th epitope is an idealized artificial Th epitope derived from Measles Virus Fusion protein (MVF 1 to 5) or Hepatitis B Surface Antigen (HBsAg 1 to 3) in the form of either single sequence or combinatorial sequences (e.g., SEQ ID NOs: 70, 69, and 71).

In some examples, the peptide immunogen constructs contain a B cell epitope from IgE EMPD linked to a heterologous T helper cell (Th) epitope through an optional heterologous spacer. In certain examples, the peptide immunogen constructs contain a B cell antigenic site having more than about 20 amino acids from IgE EMPD-1-40 (SEQ ID NO: 9) linked to a heterologous Th epitope derived from a pathogenic protein (e.g., SEQ ID NOs: 59 to 87) through an optional heterologous spacer. In some examples, the optional heterologous spacer is a molecule or chemical structure capable of linking two amino acids and/or peptides together. In certain examples, the spacer is a naturally occurring amino acid, a non-naturally occurring amino acid, or a combination thereof. In specific examples, the peptide immunogen constructs have the amino acid sequence of SEQ ID NOs: 88-95, 98-124, and 130.

The present disclosure is also directed to compositions containing an IgE EMPD peptide immunogen construct. In some examples, the disclosed compositions contain more than one IgE EMPD peptide immunogen construct. In certain examples, the compositions contain a mixture of IgE EMPD G1-C39 peptide immunogen constructs (e.g., any combination of SEQ ID NOs: 88-95, 98-124, and 130) to cover a broad genetic background in patients. Compositions containing a mixture of peptide immunogen constructs can lead to a higher percentage in responder rate upon vaccine immunization for the treatment of IgE mediated allergic diseases compared to compositions containing only a single peptide immunogen construct.

The present disclosure is also directed to pharmaceutical compositions, including vaccine formulations, for the treatment and/or prevention of IgE mediated allergic diseases. In some examples, the pharmaceutical compositions contain the disclosed peptide immunogen constructs in the form of a stabilized immunostimulatory complex formed through electrostatic associations by mixing a CpG oligomer with a composition containing a peptide immunogen complex. Such stabilized immunostimulatory complexes are able to further enhance the immunogenicity of the peptide immunogen constructs. In some examples, the pharmaceutical compositions contain adjuvants such as mineral salts, including alum gel (ALHYDROGEL), aluminum phosphate (ADJUPHOS), or water-in-oil emulsions including MONTANIDE ISA 51 or 720.

The present disclosure is also directed to antibodies directed against the disclosed IgE EMPD peptide immunogen constructs. In particular, the peptide immunogen constructs of the present disclosure are able to stimulate the generation of highly specific antibodies that are cross-reactive with the IgE EMPD-1-52 amino acid sequence (SEQ ID NO: 2), the IgE EMPD 1-67 amino acid sequence (SEQ ID NO: 1), and fragments thereof (e.g., SEQ ID NOs: 5 and 6) when administered to a subject. The highly specific antibodies produced by the peptide immunogen constructs are cross reactive with recombinant IgE EMPD-containing protein, γ 1-em67, and/or IgE EMPD on membrane-bound IgE bearing B cells. The disclosed antibodies bind with high specificity to IgE EMPD without much, if any, directed to the heterologous Th epitopes employed for immunogenicity enhancement, which is in sharp contrast to the conventional protein or other biological carriers used for such peptide antigenicity enhancement.

The present disclosure also includes methods for treating and/or preventing IgE mediated allergic diseases using the disclosed peptide immunogen constructs and/or antibodies directed against the peptide immunogen constructs. In some examples, the methods for treating and/or preventing IgE mediated allergic diseases including administering to a host a composition containing a disclosed peptide immunogen construct. In certain examples, the compositions utilized in the methods contain a disclosed peptide immunogen construct in the form of a stable immunostimulatory complex with negatively charged oligonucleotides, such as CpG oligomers, through electrostatic association, which complexes are further supplemented, optionally, with mineral salts or oil as adjuvant, for administration to patients with IgE mediated allergic diseases. The disclosed methods also include dosing regimens, dosage forms, and routes for administering the peptide immunogen constructs to a host at risk for, or with, IgE mediated allergic diseases.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is an illustration that depicts the mechanism of the IgE-mediated allergic disease pathway. Naïve mature B cells begin by expressing membrane-bound IgM (mIgE). Upon encountering an allergen, these cells become activated with the help of cognate helper T (TH) cells that provide the B cells with necessary co-stimulatory signals and cytokines. Activated allergen-specific B cells, helped by a plethora of cytokines, such as IL-4 and IL-13, become IgE-committed B cells expressing mIgE through class-switching recombination. Those IgE-committed B cells terminally differentiate into IgE-secreting plasma cells. Most of the IgE-secreting plasma cells are short-lived and migrate to the site of inflammation then die off; however, a few long-lived cells migrate to their corresponding niches in the bone marrow. The allergen-specific IgE that is secreted from the plasma cells binds to the high-affinity IgE.Fc receptor, Fc ϵ RI, on the surface of blood basophils and tissue mast cells. Allergen-induced aggregation of IgE bound to Fc ϵ RI