

TREATMENT AND PREVENTION OF HOUSE DUST MITE ALLERGIES

TECHNICAL FIELD

[0001] The present invention relates to the field of immunotherapy of patients suffering from an allergy, in particular of house dust mite allergy.

BACKGROUND ART

[0002] More than 25% of the population in industrialised countries suffer from IgE-mediated allergies. Allergic patients are characterized by the increased production of IgE antibodies against per se harmless antigens (i.e., allergens). The immediate symptoms of Type I allergy (allergic rhinoconjunctivitis, asthma, dermatitis, anaphylactic shock) are caused by allergen-induced cross-linking of mast cell-bound IgE antibodies and the release of biologically active mediators (e.g., histamine, leukotrienes).

[0003] WO 2012/168487 describes the use of a surface polypeptide of a virus of the hepadnaviridae family (e.g. Hepatitis B virus) as a carrier protein for allergen fragments.

[0004] In Banerjee S et al. (J Immunol 192(2014):4867-4875) proteins comprising two or four identical allergen fragments of Der p 23 fused to the N- and C-terminus of PreS are disclosed. According to Banerjee S et al. the fusion protein comprising two identical Der p 23 fragments on the C-terminus of PreS and other two identical Der p 23 fragments on the N-terminus of PreS induced the formation of Der p 23 specific antibodies showing a significant IgE inhibition compared to fusion proteins comprising the same Der p 23 fragments on both termini of PreS.

[0005] Curin M et al. (Allergy 73(2018):1653-1661) discloses fragments of Der p 5 and Der p 21 which can be used for the further development of vaccines.

[0006] In EP 2 727 934 allergen fragments of several Der p allergens lacking IgE reactivity and exhibiting T cell reactivity were tested.

- [0007] Huey-Jy H et al. (J Immunol 196(2016): Suppl 1 192, 5) describes polypeptides comprising inter alia fragments of Der p 1, Der p 2, Der p 7 and Der p 8.
- [0008] Bussi eres L et al. (Int Arch Allergy Immunol 153(2010):141-151) as well as EP 1 908 776 describe fusion proteins comprising mature Der p 1 and Der p 2.
- [0009] WO 2009/118642 discloses fusion polypeptides which comprise fragments of Der p 1 and Der p 2 having a length of at least 50 amino acid residues.
- [0010] Chen A et al. (Mol Immunol 45(2008):2486-2498), Cas-set A et al. (Int Arch Allergy Immunol 159(253-262), WO 2015/070925 and Chen K-W et al. (Allergy 67(2012):609-621) disclose fragments of Der p allergens. Some of these fragments may be bound to carrier proteins.
- [0011] House-dust mites (HDMs) represent one of the most important allergen sources worldwide. Almost 10% of the population and more than 50% of allergic patients are sensitized to mite allergens. The HDM *Dermatophagoides pteronyssinus* (Der p) and *Dermatophagoides farina* (Der f) are prevalent worldwide. The allergens of Der p and Der f comprise more than 30 proteins or glycoproteins of which most have been characterized so far. Group 1, 2 and 23 allergens (Der p 1, Der p 2 and Der p 23 as well as Der f 1 and Der f 2 and Der f 23) represent very important allergens from HDM, to which more than 80% of HDM allergic patients are sensitized. However, it has been shown recently that other HDM allergens (e.g., Der p 5, Der p 7 and Der p 21 as well as Der f 5, Der f 7 and Der f 21) represent important HDM allergens and cause sensitization and allergic symptoms in 15 to >40% of HDM allergic patients (Posa et al., J Allergy Clin Immunol. 2017 Feb;139(2):541-549.e8). In this context it was found that current allergen extract-based HDM vaccines fail to induce sufficient protective IgG antibodies against Der p 5, Der p 7, Der p 21 and Der p 23 and leave many HDM allergic patients untreated (Selection of house dust mite-allergic patients by molecular diagnosis may enhance success of specific immunotherapy. Chen KW, Ziegelmayer P, Ziegelmayer R, Lemell P, Horak F, Bunu CP, Valenta R,

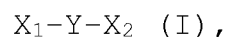
Vrtala S. J Allergy Clin Immunol. 2019 Mar;143(3):1248-1252.e12. doi: 10.1016/j.jaci.2018.10.048. Epub 2018 Nov 14) Therefore, it was recognized as an important task to discover and develop a vaccine for allergy immunotherapy of house dust mite allergy, which provides complete protection of HDM allergic patients by inducing blocking antibodies against Der p 1, Der p 2, Der p 5, Der p 7, Der p 21 and Der p 23 as well as the corresponding Der f allergens.

SUMMARY OF THE INVENTION

[0012] Currently approved immunotherapy products for house dust mite allergies are based on extracts from HDM bodies and fecal particles. These products contain variable concentrations of Der p 1, Der p 2, Der f 1 and Der f 2. They do not contain all relevant HDM allergens and especially concentrations of Der p 23 and Der f 23 are very low. Therefore they provide only an incomplete solution for the treatment or prevention of patients with house dust mite allergies (Casset et al., Int Arch Allergy Immunol. 2013; 161(3): 287-288). For instance, sublingual tablets marketed by ALK Abelló achieve only a mean reduction of allergy symptoms by 18% compared to placebo (Demoly et al., J. Allergy Clin. Immunol. 2016; 137: 444-451). Due to the relatively high number of different house dust mite allergens a potential immunotherapy would require the administration of many different proteins and polypeptides in order to cover at least the most important house dust mite allergens.

[0013] Hence, it is an object of the present invention to provide a vaccine and respective components comprised therein which can be used in the treatment and/or prevention of house dust mite allergies caused by all six major allergens.

[0014] This object is solved by one or more fusion protein(s) having formula (I)



wherein X_1 and X_2 comprise each four to eight allergen fragments or variants thereof fused to each other, wherein said allergen fragments are derived from at least two allergens of the genus *Dermatophagoides*, and wherein Y is a carrier protein.

[0015] Another aspect of the present invention relates to a pharmaceutical preparation comprising at least one fusion protein as described above.

[0016] A further aspect of the present invention relates to a fusion protein or a pharmaceutical preparation of the present invention for the use in the treatment or the prevention of an allergy caused by an allergen of a house dust mite.

[0017] Another aspect of the present invention relates to a nucleic acid molecule encoding a fusion protein according to the present invention.

[0018] A further aspect of the present invention relates to a vector comprising a nucleic acid molecule according to the present invention.

[0019] Yet another aspect of the present invention relates to a host cell comprising a nucleic acid molecule or a vector according to the present invention.

BRIEF DESCRIPTION OF THE FIGURES

[0020] Fig. 1 shows a schematic representation of constructs Der p 1-2 C3 and Der p 5 7 21 23_P6 (large). PreS stands for the surface antigen PreS of the hepatitis B virus. "P" indicates the peptides derived from house dust mite allergen Der p 1, Der p 2, Der p 5, Der p 7, Der p 21 and Der p 23 as mentioned in Table I.

[0021] Fig. 2 shows the purified PreS fusion proteins analyzed by SDS-PAGE.

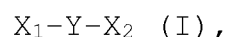
[0022] Fig. 3 shows the results of an IgE reactivity assay of Der p 1-2 C3 and Der p 5 7 21 23_P6 (large) as determined by immunoblots.

[0023] Fig. 4 shows the results of a basophil activation assay demonstrating that Der p 1-2 C3 lacks allergenic activity.

- [0024] Fig. 5 shows the results of a basophil activation assay demonstrating that Der p 5 7 21 23_P6 lacks allergenic activity.
- [0025] Fig. 6 shows the inhibition of patients' IgE binding to Der p 1 and Der p 2 with anti-sera specific for Der p 1-2 C3.
- [0026] Fig. 7 shows the inhibition of patients' IgE binding to Der p 5, Der p 7, Der p 21 and Der p 23 with anti-sera specific for Der p 5 7 21 23_P6.
- [0027] Figs. 8A to 8F show serum levels of IgGs specifically binding to house dust mite allergens Der p 1 (Fig 8A), Der p 2 (Fig 8B), Der p 5 (Fig 8C), Der p 7 (Fig 8D), Der p 21 (Fig 8E) and Der p 23 (Fig 8F) determined by ELISA from rabbit sera immunized with 20µg BM35 ("BM35 20"), 40µg BM35 ("BM35 40"), 80µg BM35 ("BM35 80"), Tyro-SIT ("Bencard"), Alutard SQ 503 ("ALK D.pter"), Alutard SQ 510 ("ALK Dp/Df"), ACAROID ("Allergoph."), PURETHAL Milbenmischung ("HAL"), CLUSTOID Milben Injektionssuspension ("Roxal") and nDer p1, rDer p 2, rDer p 5, rDer p 7, rDer p 21 and rDer p 23. The samples were retrieved at days 38 and 66 after the first injection.
- [0028] Figs. 9A to 9F show serum titers of IgGs specifically binding to house dust mite allergens Der p 1 (Fig 9A), Der p 2 (Fig 9B), Der p 5 (Fig 9C), Der p 7 (Fig 9D), Der p 21 (Fig 9E) and Der p 23 (Fig 9F) determined by ELISA from rabbit sera immunized with 20µg BM35 ("BM35 20"), 40µg BM35 ("BM35 40") and 80µg BM35 ("BM35 80") of sera retrieved before the first injection of said immunogens and at days 38 and 66 after the first injection. As controls serum titers of IgG of the respective allergens were determined in serum samples of rabbits vaccinated with allergens nDer p 1, rDer p 2, rDer p 5, rDer p 7, rDer p 21 and rDer p 23.

DESCRIPTION OF EMBODIMENTS

- [0029] The present invention relates to one or more fusion protein(s) having formula (I)



wherein X_1 and X_2 comprise each four to eight allergen fragments or variants thereof fused to each other, wherein said allergen fragments are derived from at least two allergens of the genus *Dermatophagoides*, and wherein Y is a carrier protein.

[0030] It turned surprisingly out that a fusion protein having an "architecture" as defined by formula (I) induces the *in vivo* formation of antibodies directed to the at least two allergens of the genus *Dermatophagoides*. In particular the presence of four to eight allergen fragments at the C- and N-terminus of a carrier protein has advantageous effect in regard to the induction of antibodies inhibiting the interaction of allergen specific IgEs to their respective allergen. The formation of such antibodies allows to reduce or even to prevent allergic reactions resulting in the treatment of allergies caused by allergens of the genus *Dermatophagoides*.

[0031] "Fusion protein", as used herein, refers to a protein or polypeptide created by attaching two or more polypeptides and/or peptides to each other. Fusion proteins can be produced by recombinant DNA technology or through chemical covalent conjugation.

[0032] "Allergen fragment", as used herein, refers to a peptide or polypeptide stretch derived from an allergen by fragmentation.

[0033] The four to eight, preferably the four to six allergen fragments, which are fused to each other, are derived from at least two, preferably at least three, more preferably at least four, in particular from one, two, three or four, allergens of one or more house dust mites of the genus *Dermatophagoides*. These allergen fragments consist of 8 to 100, preferably 8 to 80, more preferably 8 to 60, more preferably 10 to 60, more preferably 10 to 50, more preferably 15 to 50, more preferably 20 to 50, more preferably 25 to 50, consecutive amino acid residues of said allergen.

[0034] The fusion protein of the present invention may comprise more than one fragment derived from the same aller-

gen. In such a case the fragments may be derived from different (i.e. non-adjacent) regions or from adjacent regions of the same allergen. In the latter case the order of the fragments may be different than in the allergen from which the fragments are derived from.

[0035] The fusion protein of the present invention may also comprise one or more allergen fragments having the same or substantially the same amino acid sequence. "Substantially the same", as used herein, means that two or more sequences derived from the same allergen show at least 80%, preferably at least 85%, more preferably at least 90%, more preferably at least 95%, sequence identity.

[0036] The degree of identity of a first amino acid sequence to a second amino acid sequence can be determined by a direct comparison between both amino acid sequences using certain algorithms. Such algorithms are, for instance, incorporated in various computer programs or websites (e.g. <https://www.ncbi.nlm.nih.gov/>) (e.g. "BLAST 2 SEQUENCES (blastp)" (Tatusova et al. (1999) FEMS Microbiol. Lett. 174:247-25; Corpet F, Nucl. Acids Res. (1988) 16:10881-10890) with the following parameters: Matrix BLOSUM62; Open gap 11 and extension gap 1 penalties; gap x_dropoff50; expect 10.0 word size 3; Filter: none.

[0037] The fusion protein of the present invention may also comprise variants of allergen fragments. Particular preferred variants of allergen fragments comprise at least one, preferably at least two, more preferably at least three, amino acid exchanges compared to the allergen fragment. Particularly preferred is the exchange of at least one, preferably of at least two, more preferably of at least three, in particular of all, cysteine residues naturally occurring in the allergen fragment with other amino acid residues, preferably with serine, threonine, glycine, alanine, or leucine residues. Thus, a variant of the allergen fragment of the present invention preferably does not contain any cysteine residues.

[0038] The allergen fragments within the fusion protein of the present invention may be fused directly to each other or may be separated by a single amino acid residue or a

linker peptide consisting of two to 30, preferably two to 20, more preferably two to ten, more preferably two to five, amino acid residues. Also X_1/X_2 and the carrier protein Y may be separated by a single amino acid residue or a linker peptide as defined above.

[0039] The fusion protein of the present invention can be recombinantly produced in any expression system known in the art. Particularly preferred expression systems include bacteria (e.g. *E. coli*), yeast cells (e.g. *Pichia pastoris*) or insect cells like S2 cells from *Drosophila melanogaster*, Sf9 or Sf21 cells from *Spodoptera frugiperda*, or TNi cells from *Trichoplusia ni*.

[0040] The allergen fragments of the fusion protein of the present invention may be derived from any house dust mite. However, it is particularly preferred to use allergens from house dust mites which cause allergic reactions in most people. Therefore, it is preferred that the allergen fragments are derived from allergens of *Dermatophagoides pteronyssinus* and/or *Dermatophagoides farinae*.

[0041] According to another preferred embodiment of the present invention the at least two allergens are of *Dermatophagoides pteronyssinus* and selected from the group consisting of Der p 1, Der p 2, Der p 5, Der p 7, Der p 21 and Der p 23.

[0042] According to another preferred embodiment of the present invention the at least two allergens are of *Dermatophagoides farinae* and selected from the group consisting of Der f 1, Der f 2, Der f 5, Der f 7, Der f 21 and Der f 23.

[0043] Allergic people react differently to allergens derived from the same source. People suffering from house dust mite allergies caused by *Dermatophagoides pteronyssinus* and/or *Dermatophagoides farinae* react to allergens Der p 1 and Der p 2 as well as to Der p 5, Der p 7, Der p 21 and Der p 23 and to allergens Der f 1 and Der f 2 as well as to Der f 5, Der f 7, Der f 21 and Der f 23, respectively. Therefore, it is particularly preferred to provide a fusion protein comprising allergen fragments of one or more of these allergens.

[0044] According to a further preferred embodiment of the present invention the allergen fragments of the at least two allergens consist of 25 to 50 amino acid residues, preferably 28 to 48 amino acid residues, more preferably 30 to 45 amino acid residues.

[0045] According to a preferred embodiment of the present invention at least one, preferably at least two, more preferably at least three, in particular all, of the cysteine residues of the allergen fragments are substituted with serine, threonine, glycine, alanine, or leucine.

[0046] Some or all cysteine residues of the allergen fragments used in the fusion protein of the present invention may be substituted with other amino acid residues. The substitution of one or more cysteine residues may reduce the number of disulphide bonds potentially formed during the recombinant expression of the fusion proteins of the present invention or during its processing (e.g. purification of the fusion protein from inclusion bodies, manufacturing of a vaccine formulation). Furthermore, the substitution of cysteine residues within the allergen fragments results in the reduction or complete removal of free sulphur groups in the fusion protein preventing that such groups are able to react with other compounds.

[0047] According to a further preferred embodiment of the present invention the allergen fragment of Der p 1 consists of an amino acid sequence being at least 90%, preferably at least 92%, more preferably at least 95%, more preferably at least 97%, in particular 100%, identical to an amino acid sequence selected from the group consisting of TNACSSINGNAPAEIDLRQMRTVTPIRMQGGCGSCWAFSGVA (SEQ ID No. 1), ATESAYLAYRNQSLDLAEQELVDCASQHGCHGDTIPRGIEYIQ (SEQ ID No. 2), HNGVVQESYYRYVAREQSCRRPNAQRFGISN (SEQ ID No. 3), VRNSWDTNWGDNGYGYFAANIDLMMIEEYPYVVIL (SEQ ID No. 4), TNASSINGNAPAEIDLRQMRTVTPIRMQGGSGSSWAFSGVA (SEQ ID No. 5), ATESAYLAYRNQSLDLAEQELVDSASQHGSHGDTIPRGIEYIQ (SEQ ID No. 6) and HNGVVQESYYRYVAREQSSRRPNAQRFGISN (SEQ ID No. 7).

[0048] According to another preferred embodiment of the present invention the allergen fragment of Der p 2 consists of an amino acid sequence being at least 90%, preferably

at least 92%, more preferably at least 95%, more preferably at least 97%, in particular 100%, identical to an amino acid sequence selected from the group consisting of CHGSEPCIIHRGKPFQLEAVFEANQNSKTAK (SEQ ID No. 8), EVDVPGIDPNACHYMKCPLVKGGQYDIKYTWIVPKIAPKSEN (SEQ ID No. 9), HGSEPSIIHRGKPFQLEAVFEANQNSKTAK (SEQ ID No. 10) and EVDVPGIDPNASHYMKSPLVKGGQYDIKYTWIVPKIAPKSEN (SEQ ID No. 11).

[0049] According to a preferred embodiment of the present invention the allergen fragment of Der p 5 consists of an amino acid sequence being at least 90%, preferably at least 92%, more preferably at least 95%, more preferably at least 97%, in particular 100%, identical to an amino acid sequence selected from the group consisting of DYQNEFDLLMERIHEQIKKGELALFYLQ (SEQ ID No. 12) and EQYNLE-MAKKSGDILERDLKKEEARVKKIEV (SEQ ID No. 13).

[0050] According to another preferred embodiment of the present invention the fragments of Der p 7 consists of amino acid sequence being at least 90%, preferably at least 92%, more preferably at least 95%, more preferably at least 97%, in particular 100%, identical to amino acid sequence DPIHYDKITEEINKAVDEAVAAIEKSETFD (SEQ ID No. 14).

[0051] According to a further preferred embodiment of the present invention the fragments of Der p 21 consists of amino acid sequence being at least 90%, preferably at least 92%, more preferably at least 95%, more preferably at least 97%, in particular 100%, identical to amino acid sequence YNYEFALESIKLLIKKLDELAKKVKAVNPDEYY (SEQ ID No. 15).

[0052] According to a preferred embodiment of the present invention the fragments of Der p 23 consists of an amino acid sequence being at least 90%, preferably at least 92%, more preferably at least 95%, more preferably at least 97%, in particular 100%, identical to an amino acid sequence selected from the group consisting of GY-FADPKDPHKFYICSNWEAVHKDCPGNTRWNEDEETCT (SEQ ID No. 16) and GYFADPKDPHKFYISSNWEAVHKDSPGNTRWNEDEETST (SEQ ID No. 17).

[0053] According to a further preferred embodiment of the present invention the allergen fragment of Der f 1 con-

sists of an amino acid sequence being at least 90%, preferably at least 92%, more preferably at least 95%, more preferably at least 97%, in particular 100%, identical to an amino acid sequence selected from the group consisting of TSACRINSVNVPSSELDLRSLRTVTPIRMQGGCGSCWAFSGVA (SEQ ID No. 38), ATESAYLAYRNTSLDLSEQELVDCASQHGCHGDTIPRGIEYIQ (SEQ ID No. 39), QNGVVEERSYPYVAREQQCRRPNSQHYGISN (SEQ ID No. 40) and VRNSWDTTWGDSGYGYFQAGNNLMMIEQYPYVVIM (SEQ ID No. 41).

[0054] According to another preferred embodiment of the present invention the allergen fragment of Der f 2 consists of an amino acid sequence being at least 90%, preferably at least 92%, more preferably at least 95%, more preferably at least 97%, in particular 100%, identical to an amino acid sequence selected from the group consisting of HGSDPCIIHRGKPFNLEAIFDANQNTKTAK (SEQ ID No. 42) and EVDVPGIDTNACHYIKCPLVKGQQYDAKYTWNVPKIAPKSEN (SEQ ID No. 43).

[0055] According to a preferred embodiment of the present invention the allergen fragment of Der f 5 consists of an amino acid sequence being at least 90%, preferably at least 92%, more preferably at least 95%, more preferably at least 97%, in particular 100%, identical to an amino acid sequence selected from the group consisting of DYQNEFDLLMQRIHEQMRKGEEALLHLQ (SEQ ID No. 44) and ERYN-VEIALKSNEILERDLKKEEQRVKKIEV (SEQ ID No. 45).

[0056] According to another preferred embodiment of the present invention the fragments of Der f 7 consists of amino acid sequence being at least 90%, preferably at least 92%, more preferably at least 95%, more preferably at least 97%, in particular 100%, identical to amino acid sequence DPIHYDKITEEINKAIDDAIAAIEQSETID (SEQ ID No. 46).

[0057] According to a further preferred embodiment of the present invention the fragments of Der f 21 consists of amino acid sequence being at least 90%, preferably at least 92%, more preferably at least 95%, more preferably at least 97%, in particular 100%, identical to amino acid sequence YNFETAVSTIEILVKDLAELAKVKVAVKSDD (SEQ ID No. 47).

[0058] According to a preferred embodiment of the present invention the fragments of Der f 23 consists of an amino

acid sequence being at least 90%, preferably at least 92%, more preferably at least 95%, more preferably at least 97%, in particular 100%, identical to an amino acid sequence selected from the group consisting of GY-FADPKDPCKFYICSNWEAIIHKSCPGNTRWNEKELTCT (SEQ ID No. 48).

[0059] According to another preferred embodiment of the present invention X_1 comprises four allergen fragments of Der p 1 and two fragments of Der p 2 or three fragments of Der p 21 and three fragments of Der p23.

[0060] It turned surprisingly out that X_1 comprising or consisting of these allergen fragments in any order being fused to the N-terminal end of the carrier protein results in the formation of antibodies being able to inhibit the binding of the respective naturally occurring allergen to allergen specific IgE. This allows to use such a fusion protein as a vaccine to treat or prevent allergic reactions caused by the respective allergens.

[0061] According to a further preferred embodiment of the present invention X_2 comprises two to four allergen fragments of Der p 1 and two fragments of Der p 2 or four fragments of Der p 5, two fragments of Der p 7.

[0062] X_2 fused to the C-terminal end of the carrier protein may comprise or consist of the aforementioned allergen fragments in any order. It turned out that such allergen fragments on the C-terminal end of the carrier protein result in a fusion protein inducing the formation of antibodies being able to inhibit the binding of the respective naturally occurring allergen to allergen specific IgE. This allows to use such a fusion protein as a vaccine to treat or prevent allergic reactions caused by the respective allergens.

[0063] According to another preferred embodiment of the present invention the fusion protein comprises at least one polypeptide having amino acid sequence selected from the group consisting of SEQ ID No. 18, SEQ ID No. 19, SEQ ID No. 20, SEQ ID No. 21, SEQ ID No. 22, SEQ ID No. 23, SEQ ID No. 24 and/or SEQ ID No. 25.

[0064] The polypeptides consisting of amino acid sequences SEQ ID No. 18 and 19 comprise fragments of the allergens Der p 1 and Der p 2:

[0065] SEQ ID No. 18:

VRNSWDTNWGDNGYGYFAANIDLMMIEEYPYVVILHNGVVQESYYRYVAREQSSRRPN
AQRFGISNHGSEPSIIHRGKPFQLEAVFEANQNSKTAKHGSEPSIIHRGKPFQLEAVF
EANQNSKTAKVRNSWDTNWGDNGYGYFAANIDLMMIEEYPYVVILHNGVVQESYYRYV
AREQSSRRPNAQRFGISN

[0066] SEQ ID No. 19:

EVDVPGIDPNASHYMKSPVLVGQQYDIKYTWIVPKIAPKSENEVDVPGIDPNASHYM
KSPLVGKQQYDIKYTWIVPKIAPKSENATESAYLAYRNQSLDLAEQELVDSASQHGS
HGDITPRGIEYIQTNASSINGNAPAEIDLRQMRTVTPIRMQGGSGSSWAFSGVAATE
SAYLAYRNQSLDLAEQELVDSASQHGS HGDITPRGIEYIQTNASSINGNAPAEIDLR
QMRTVTPIRMQGGSGSSWAFSG

[0067] The polypeptide consisting of SEQ ID No. 18 may be X_1 or X_2 in formula (I), whereby in the most preferred embodiment of the present invention said polypeptide is X_1 in formula (I).

[0068] The polypeptide consisting of SEQ ID No. 19 may be X_1 or X_2 in formula (I), whereby in the most preferred embodiment of the present invention said polypeptide is X_2 in formula (I).

[0069] The polypeptides consisting of amino acid sequences SEQ ID No. 20 and 21 comprise fragments of the allergens Der p 5, Der p 7, Der p 21 and Der p 23:

[0070] SEQ ID No. 20:

YNYEFALESIKLLIKKLDELAKKVKAVNPDEYYNYEFALESIKLLIKKLDELAKKV
KAVNPDEYYNYEFALESIKLLIKKLDELAKKVKAVNPDEYYGYFADPKDPHKFYIS
SNWEAVHKDSPGNTRWNEDEETSTGYFADPKDPHKFYISSNWEAVHKDSPGNTRWNE
DEETSTGYFADPKDPHKFYISSNWEAVHKDSPGNTRWNEDEETST

[0071] SEQ ID No. 21:

DYQNEFDLLMERIHEQIKKGELALFYLDYQNEFDLLMERIHEQIKKGELALFYL
QDPIHYDKITEEINKAVDEAVAAIEKSETFDDPIHYDKITEEINKAVDEAVAAIEKS
ETFDEQYNLEMAKKSGDILERDLKKEEARVKKIEVEQYNLEMAKKSGDILERDLKKE
EARVKKIEV

[0072] The polypeptide consisting of SEQ ID No. 20 may be X₁ or X₂ in formula (I), whereby in the most preferred embodiment of the present invention said polypeptide is X₁ in formula (I).

[0073] The polypeptide consisting of SEQ ID No. 21 may be X₁ or X₂ in formula (I), whereby in the most preferred embodiment of the present invention said polypeptide is X₂ in formula (I).

[0074] The polypeptides consisting of amino acid sequences SEQ ID No. 22 and 23 comprise fragments of the allergens Der f 1 and Der f 2:

[0075] SEQ ID No. 22:

VRNSWDTTWGDSGYGYFQAGNNLMMIEQYPYVIMQNGVVEERSYPYVAREQQCRRP
NSQHYGISNHGSDPCIIHRGKPFNLEAIFDANQNTKTAKHGSDPCIIHRGKPFNLEA
IFDANQNTKTAKVRNSWDTTWGDSGYGYFQAGNNLMMIEQYPYVIMQNGVVEERSY
PYVAREQQCRRPNSQHYGISN

[0076] SEQ ID No. 23:

EVDVPGIDTNACHYIKCPLVKGQQYDAKYTWNVPKIAPKSENEVDVPGIDTNACHYI
KCPLVKGQQYDAKYTWNVPKIAPKSENATESAYLAYRNTSLDLSEQELVDCASQHGCHG
GDTIPRGIEYIQTSACRINSVNVPSSELDLRSLRTVTPIRMQGGCGSCWAFSGVAAT
ESAYLAYRNTSLDLSEQELVDCASQHGCHGDTIPRGIEYIQTSACRINSVNVPSSELD
LRSLRTVTPIRMQGGCGSCWAFSGVA

[0077] The polypeptide consisting of SEQ ID No. 22 may be X₁ or X₂ in formula (I), whereby in the most preferred embodiment of the present invention said polypeptide is X₁ in formula (I).

[0078] The polypeptide consisting of SEQ ID No. 23 may be X₁ or X₂ in formula (I), whereby in the most preferred embod-

iment of the present invention said polypeptide is X₂ in formula (I).

[0079] The polypeptides consisting of amino acid sequences SEQ ID No. 24 and 25 comprise fragments of the allergens Der p 5, Der p 7, Der p 21 and Der p 23:

[0080] SEQ ID No. 24:

YNFETAVSTIEILVKDLAELAKKVKAVKSDDYNFETAVSTIEILVKDLAELAKKVKAVKSDDYNFETAVSTIEILVKDLAELAKKVKAVKSDDGYFADPKDPCKFYICSNWEAIHKSCPGNTRWNEKELTCTGYFADPKDPCKFYICSNWEAIHKSCPGNTRWNEKELTCTGYFADPKDPCKFYICSNWEAIHKSCPGNTRWNEKELTCT

[0081] SEQ ID No. 25:

DYQNEFDLLMQRIHEQMRKGEEALLHLQDYQNEFDLLMQRIHEQMRKGEEALLHLQDPIHYDKITEEINKAIDDAIAAIEQSETIDDPIHYDKITEEINKAIDDAIAAIEQSETIDERYNVEIALKSNEILERDLKKEEQRVKKIEVERYNVEIALKSNEILERDLKKEEQRVKKIEV

[0082] The polypeptide consisting of SEQ ID No. 24 may be X₁ or X₂ in formula (I), whereby in the most preferred embodiment of the present invention said polypeptide is X₁ in formula (I).

[0083] The polypeptide consisting of SEQ ID No. 25 may be X₁ or X₂ in formula (I), whereby in the most preferred embodiment of the present invention said polypeptide is X₂ in formula (I).

[0084] According to a further preferred embodiment of the present invention the carrier protein is a surface polypeptide of a virus of a hepadnaviridae family or a fragment of the surface polypeptide.

[0085] According to a preferred embodiment of the present invention the virus of the hepadnaviridae family is Hepatitis B virus.

[0086] According to another preferred embodiment of the present invention the surface polypeptide of the virus of the hepadnaviridae family is PreS.

[0087] According to a further preferred embodiment of the present invention the fragment of the surface polypeptide is Hepatitis B PreS1 or Hepatitis B PreS2.

[0088] According to a preferred embodiment of the present invention the carrier protein comprises an amino acid sequence which is at least 90%, preferably at least 92%, more preferably at least 95%, more preferably at least 97%, in particular 100%, identical to SEQ ID No. 26.

[0089] The carrier protein used in the present invention may comprise or consist of the amino acid sequence SEQ ID No. 26 (GenBank Acc. No. AAT28678.1):

GGWSSKPRKGMGTNLSVPNPLGFFPDHQLDPAFGANSNNPDWDFNPIKDHWPAANQV
GVGAFGPGLTPPHGGILGWSPQAQGILTTVSTIPPPASTNRQSGRQPTPISPPLRDS
HPQAMQWNSTAFHQALQDPRVRGLYFPAGGSSSGTVNPAPNIASHISSISARTGDPV
TN

[0090] According to another preferred embodiment of the present invention the fusion protein comprises or consists of an amino acid sequence which is at least 90%, preferably at least 92%, more preferably at least 95%, more preferably at least 97%, in particular 100%, identical to SEQ ID No. 27, SEQ ID No. 28, SEQ ID No. 29, SEQ ID No. 30, SEQ ID No. 31, SEQ ID No. 32 or SEQ ID No. 33.

[0091] The fusion proteins of the present invention may comprise PreS as carrier protein Y and fragments of various allergens forming two polypeptides X₁ and X₂, respectively, being located adjacent to the carrier protein (see formula (I)). Particularly preferred fusion proteins comprise fragments of the allergens Der p 1, Der p 2, Der p 5, Der p 7, Der p 21 and Der p 23. Such fusion proteins may comprise or consist of the following amino acid sequences.

[0092] SEQ ID No. 27 ("Der p 1-2 C3")

VRNSWDTNWGDNGYGYFAANIDLMMIEEYPYVVILHNGVVQESYYRYVAREQSSRRP
NAQRFGISNHGSEPSIIHRGKPFQLEAVFEANQNSKTAKHGSEPSIIHRGKPFQLEA
VFEANQNSKTAKVRNSWDTNWGDNGYGYFAANIDLMMIEEYPYVVILHNGVVQESYY
RYVAREQSSRRPNAQRFGISNGWSSKPRKGMGTNLSVPNPLGFFPDHQLDPAFGAN

SNNPDWDFNPIKDHHPAANQVGVGAFGPGLTPPHGGILGWSPQAQGILTTVSTIPPP
ASTNRQSGRQPTPI SPPLRDSHPQAMQWNSTAFHQALQDPRVRGLYFPAGGSSSGTV
NPAPNIA SHISSISARTGDPVTNEVDVPGIDPNASHYMKSPLVKGQQYDIKYTWIVP
KIAPKSENEVDVPGIDPNASHYMKSPLVKGQQYDIKYTWIVPKIAPKSENATESAYL
AYRNQSLDLAEQELVDSASQHGSHGDTIPRGIEYIQTNASSINGNAPAEIDLRQMRT
VTPIRMQGGSGSSWAFSGVAATESAYLAYRNQSLDLAEQELVDSASQHGSHGDTIPR
GIEYIQTNASSINGNAPAEIDLRQMRTVTPIRMQGGSGSSWAFSG

[0093] SEQ ID No. 28 ("Der p 572123_P6 (large)")

YNYEFALESIKLLIKKLDELAKKVKAVNPDEYYYNYEFALESIKLLIKKLDELAKKVK
AVNPDEYYYNYEFALESIKLLIKKLDELAKKVKAVNPDEYYGYFADPKDPHKFYISSN
WEAVHKDSPGNTRWNEDEETSTGYFADPKDPHKFYISSNWEAVHKDSPGNTRWNEDEE
TSTGYFADPKDPHKFYISSNWEAVHKDSPGNTRWNEDEETSTGGWSSKPRKGMGTNLS
VPNPLGFFPDHQLDPAFGANSNNPDWDFNPIKDHHPAANQVGVGAFGPGLTPPHGGIL
GWSPQAQGILTTVSTIPPPASTNRQSGRQPTPI SPPLRDSHPQAMQWNSTAFHQALQD
PRVRGLYFPAGGSSSGTVNPAPNIA SHISSISARTGDPVTNDYQNEFDLLMERIHEQ
IKKGELALFYQLQDYQNEFDLLMERIHEQIKKGELALFYQLQDPIHYDKITEEINKAVD
EAVAAIEKSETFDDPIHYDKITEEINKAVDEAVAAIEKSETFDEQYNLEMAKKSGDIL
ERDLKKEEARVKKIEVEQYNLEMAKKSGDILERDLKKEEARVKKIEV

[0094] SEQ ID No. 29 ("Der p 1-2 C1")

VRNSWDTNWGDNGYGYFAANIDLMMIEEYPYVVILHNGVVQESYYRYVAREQSSRRP
NAQRFGISNVRNSWDTNWGDNGYGYFAANIDLMMIEEYPYVVILHNGVVQESYYRYV
AREQSSRRPNAQRFGISNHGSEPSIIHRGKPFQLEAVFEANQNSKTAKHGSEPSIIH
RGKPFQLEAVFEANQNSKTAKGGWSSKPRKGMGTNLSVPNPLGFFPDHQLDPAFGAN
SNNPDWDFNPIKDHHPAANQVGVGAFGPGLTPPHGGILGWSPQAQGILTTVSTIPPP
ASTNRQSGRQPTPI SPPLRDSHPQAMQWNSTAFHQALQDPRVRGLYFPAGGSSSGTV
NPAPNIA SHISSISARTGDPVTNEVDVPGIDPNASHYMKSPLVKGQQYDIKYTWIVP
KIAPKSENEVDVPGIDPNASHYMKSPLVKGQQYDIKYTWIVPKIAPKSENATESAYL
AYRNQSLDLAEQELVDSASQHGSHGDTIPRGIEYIQTNASSINGNAPAEIDLRQMRT
VTPIRMQGGSGSSWAFSGVAATESAYLAYRNQSLDLAEQELVDSASQHGSHGDTIPR
GIEYIQTNASSINGNAPAEIDLRQMRTVTPIRMQGGSGSSWAFSG

[0095] SEQ ID No. 30 ("Der p 1-2 C2")

VRNSWDTNWGDNGYGYFAANIDLMMIEEYPYVVILHNGVVQESYYRYVAREQSSRRP
NAQRFGISNVRNSWDTNWGDNGYGYFAANIDLMMIEEYPYVVILHNGVVQESYYRYV

AREQSSRRPNAQRFGISNHGSEPSIIHRGKPFQLEAVFEANQNSKTAKHGSEPSIIH
RGKPFQLEAVFEANQNSKTAKGGWSSKPRKGMGTNLSVPNPLGFFPDHQLDPAFGAN
SNNPDWDFNPIKDHHPAANQVGVGAFGPGLTPPHGGILGWSPQAQGILTTVSTIPPP
ASTNRQSGRQPTPISPPLRDSHPQAMQWNSTAFHQALQDPRVRGLYFPAGGSSSGTV
NPAPNIASHISSISARTGDPVTNATESAYLAYRNQSLDLAEQELVDSASQHGSHGDT
IPRGIEYIQTNASSINGNAPAEIDLQMRVTPTIRMQGGSGSSWAFSGVAEVDVPGI
DPNASHYMKSPVLKGGQYDIKYTWIVPKIAPKSENEVDVPGIDPNASHYMKSPVLKGG
QYDIKYTWIVPKIAPKSENATESAYLAYRNQSLDLAEQELVDSASQHGSHGDTIPR
GIEYIQTNASSINGNAPAEIDLQMRVTPTIRMQGGSGSSWAFSG

[0096] SEQ ID No. 31 ("Der p 5.7.21.23_P4P5")

YNYEFALESIKLLIKKLDELAKKVKAVNPDEYYYNYEFALESIKLLIKKLDELAKKV
KAVNPDEYYYNYEFALESIKLLIKKLDELAKKVKAVNPDEYYGYFADPKDPHKFYIC
SNWEAVHKDCPGNTGYFADPKDPHKFYICSNWEAVHKDCPGNTGGWSSKPRKGMGTN
LSVPNPLGFFPDHQLDPAFGANSNNPDWDFNPIKDHHPAANQVGVGAFGPGLTPPHG
GILGWSPQAQGILTTVSTIPPPASTNRQSGRQPTPISPPLRDSHPQAMQWNSTAFHQ
ALQDPRVRGLYFPAGGSSSGTVNPAPNIASHISSISARTGDPVTNKFYICSNWEAVH
KDCPGNTRWNEDEETCTKFYICSNWEAVHKDCPGNTRWNEDEETCTDYQNEFDLLM
ERIHEQIKKGELALFYLDYQNEFDLLMERIHEQIKKGELALFYLDPIHYDKITE
EINKAVDEAVAAIEKSETFDDPIHYDKITEEINKAVDEAVAAIEKSETFDEQYNLEM
AKKSGDILERDLKKEEARVKKIEVEQYNLEMAKKSGDILERDLKKEEARVKKIEV

[0097] SEQ ID No. 32 ("Der p 5_7_21 C1")

YNYEFALESIKLLIKKLDELAKKVKAVNPDEYYYNYEFALESIKLLIKKLDELAKKV
KAVNPDEYYYNYEFALESIKLLIKKLDELAKKVKAVNPDEYYYNYEFALESIKLLIK
KLDELAKKVKAVNPDEYYDPIHYDKITEEINKAVDEAVAAIEKSETFDDPIHYDKIT
EEINKAVDEAVAAIEKSETFDGGWSSKPRKGMGTNLSVPNPLGFFPDHQLDPAFGAN
SNNPDWDFNPIKDHHPAANQVGVGAFGPGLTPPHGGILGWSPQAQGILTTVSTIPPP
ASTNRQSGRQPTPISPPLRDSHPQAMQWNSTAFHQALQDPRVRGLYFPAGGSSSGTV
NPAPNIASHISSISARTGDPVTNDYQNEFDLLMERIHEQIKKGELALFYLDYQNE
FDLLMERIHEQIKKGELALFYLDPIHYDKITEEINKAVDEAVAAIEKSETFDDPI
HYDKITEEINKAVDEAVAAIEKSETFDEQYNLEMAKKSGDILERDLKKEEARVKKIE
VEQYNLEMAKKSGDILERDLKKEEARVKKIEV

[0098] SEQ ID No. 33 ("Der p 5_7_21 C2")

YNYEFALESIKLLIKKLDELAKKVKAVNPDEYYYNYEFALESIKLLIKKLDELAKKV
KAVNPDEYYYNYEFALESIKLLIKKLDELAKKVKAVNPDEYYYNYEFALESIKLLIK
KLDELAKKVKAVNPDEYYDYQNEFDFLLMERIHEQIKKGELALFYLQDYQNEFDFLL
MERIHEQIKKGELALFYLQGGWSSKPRKGMGTNLSVPNPLGFFPDHQLDPAFGANSN
NPDWDFNPIKDHHPAANQVGVGAFGPGGLTPPHGGILGWSPQAQGILTTVSTIPPPAS
TNRQSGRQPTPISPPLRDSHPQAMQWNSTAFHQALQDPRVRGLYFPAGGSSSGTVNP
APNIAHSHISSISARTGDPVTNDPIHYDKITEEINKAVDEAVAAIEKSETFDDPIHYD
KITEEINKAVDEAVAAIEKSETFDDPIHYDKITEEINKAVDEAVAAIEKSETFDDPI
HYDKITEEINKAVDEAVAAIEKSETFDEQYNLEMAKKSGDILERDLKKEEARVKKIE
VEQYNLEMAKKSGDILERDLKKEEARVKKIEV

[0099] SEQ ID No. 36 ("Der f 1/2 C3")

VRNSWDTTWGDSGYGYFQAGNNLMMIEQYPYVIMQNGVVEERSYPYVAREQQSRRP
NSQHYGISNHGSDPSIIHRGKPFNLEAIFDANQNTKTAKHGS DPSIIHRGKPFNLEA
IFDANQNTKTAKVRNSWDTTWGDSGYGYFQAGNNLMMIEQYPYVIMQNGVVEERSY
PYVAREQQSRRPNSQHYGISNGGWSSKPRKGMGTNLSVPNPLGFFPDHQLDPAFGAN
SNNPDWDFNPIKDHHPAANQVGVGAFGPGGLTPPHGGILGWSPQAQGILTTVSTIPPP
ASTNRQSGRQPTPISPPLRDSHPQAMQWNSTAFHQALQDPRVRGLYFPAGGSSSGTV
NPAPNIAHSHISSISARTGDPVTNEVDVPGIDTNASHYIKSPLVKGQQYDAKYTWNVP
KIAPKSENEVDVPGIDTNASHYIKSPLVKGQQYDAKYTWNVPKIAPKSENATESAYL
AYRNTSLDLSEQELVDSASQHGSHGDTIPRGIEYIQTSASRINSVNVPSSELDLRSLR
TVTPIRMQGGSGSSWAFSGVAATESAYLAYRNTSLDLSEQELVDSASQHGSHGDTIP
RGIEYIQTSASRINSVNVPSSELDLRSLRTVTPIRMQGGSGSSWAFSG

[0100] SEQ ID No. 37 ("Der f 5 7 21 23_P6")

YNFETAVSTIEILVKDLAELAKKVKAVKSDDYNFETAVSTIEILVKDLAELAKKVK
VKSDDYNFETAVSTIEILVKDLAELAKKVKAVKSDDGYFADPKDPSKFYISSNWEAI
HKSSPGNTRWNEKELTSTGYFADPKDPSKFYISSNWEAIHKSSPGNTRWNEKELTST
GYFADPKDPSKFYISSNWEAIHKSSPGNTRWNEKELTSTGGWSSKPRKGMGTNLSVP
NPLGFFPDHQLDPAFGANSNNPDWDFNPIKDHHPAANQVGVGAFGPGGLTPPHGGILG
WSPQAQGILTTVSTIPPPASTNRQSGRQPTPISPPLRDSHPQAMQWNSTAFHQALQD
PRVRGLYFPAGGSSSGTVNPAPNIAHSHISSISARTGDPVTNDYQNEFDFLLMQRIHE
QMRKGEEALLHLQDYQNEFDFLLMQRIHEQMRKGEEALLHLQDPIHYDKITEEINKA
IDDAIAAIEQSETIDDPHYDKITEEINKAIDDAIAAIEQSETIDERYNVEIALKSN
EILERDLKKEEQRVKKIEVERYNVEIALKSNEILERDLKKEEQRVKKIEV

[0101]

- [0102] Another aspect of the present invention relates to a pharmaceutical preparation (i.e. vaccine formulation) comprising at least one fusion protein according to the present invention.
- [0103] According to a preferred embodiment of the present invention the preparation comprises a fusion protein comprising amino acid sequence SEQ ID No. 27 and a fusion protein comprising amino acid sequence SEQ ID No. 28.
- [0104] According to a further preferred embodiment of the present invention said preparation comprises 10 ng to 1 g, preferably 100 ng to 10 mg, especially 0.5 µg to 200 µg of the fusion protein of the present invention or a nucleic acid molecule encoding said fusion protein or a vector comprising said nucleic acid molecule.
- [0105] According to a particularly preferred embodiment of the present invention the fusion protein of the present invention is administered to an individual at least once in an amount of 0.01 pg/kg body weight to 5 mg/kg body weight, preferably 0.1 pg/kg body weight to 2 mg/kg body weight.
- [0106] According to further preferred embodiment of the present invention the fusion protein is administered to a patient in an amount of 5 to 100 µg, preferably 10 to 80 µg, more preferably 10 to 40 µg, either independent from the body weight (i.e. a dose may comprise 15, 20, 25, 30, or 80 µg) or per kg body weight.
- [0107] The amount of fusion protein that may be combined with excipients to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. The dose of the polypeptide construct may vary according to factors such as the disease state, age, sex and weight of the individual, and the ability to elicit the desired antibody response in the individual. The dosage regime may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation. The dose of the polypeptide construct may also be varied to provide optimum preventative dose

response depending upon the circumstances. For instance, the fusion protein of the present invention may be administered to an individual at intervals of several days, one or two weeks or even months depending always on the level of allergen specific IgG induction.

[0108] In a preferred embodiment of the present invention the fusion protein and the pharmaceutical preparation of the present invention are applied between 2 and 10, preferably between 2 and 7, even more preferably up to 5 times. In a further preferred embodiment of the present invention, single booster applications are given between 3 months and 5 years following the first dosing schedule. These booster application may be repeated between 2 and 10 times, preferably between 2 and 5 times and most preferably between 2 and 3 times. In a particularly preferred embodiment the time interval between the subsequent vaccinations is chosen to be between 2 weeks and 5 years, preferably between 3 weeks and up to 3 years, more preferably between 3 weeks and 1 year. The repeated administration of the fusion protein of the present invention may maximize the final effect of the treatment.

[0109] In a particularly preferred embodiment of the present invention the fusion protein and/or the pharmaceutical preparation of the present invention may be applied using 3 to 6, preferably 5, monthly injections followed by booster injections as mentioned above given every 1 to 6, preferably 3 to 4 months, for at least one, preferably at least two, more preferably from two to six, more preferably from three to five years.

[0110] According to another preferred embodiment of the present invention said preparation further comprises at least one adjuvant, pharmaceutical acceptable excipient and/or preservative.

[0111] The fusion protein and the pharmaceutical preparation of the present invention can be administrated subcutaneously, intramuscularly, intravenously, mucosally etc. Depending on the dosage form and administration route the polypeptide construct of the present invention may be combined with excipients, diluents, adjuvants and/or car-

riers. A preferred adjuvant is aluminum hydroxide. Suitable protocols for the production of vaccine formulations are known to the person skilled in the art and can be found e.g. in "Vaccine Protocols" (A. Robinson, M. P. Cranage, M. Hudson; Humana Press Inc., U. S.; 2nd edition 2003).

[0112] The fusion protein of the present invention may be formulated also with other adjuvants regularly used in vaccines. For instance, suitable adjuvants may be MF59, aluminum phosphate, calcium phosphate, cytokines (e.g. IL2, IL-12, GM-CSF), saponins (e.g. QS21), MDP derivatives, CpG oligonucleotides, LPS, MPL, polyphosphazenes, emulsions (e.g. Freund's, SAF), liposomes, virosomes, iscoms, cochleates, PLG microparticles, poloxamer particles, virus-like particles, heat-labile enterotoxin (LT), cholera toxin (CT), mutant toxins (e.g. LTK63 and LTR72), microparticles and/or polymerized liposomes. Suitable adjuvants are commercially available as, for example, AS01B (MPL and QS21 in a liposome formulation), AS02A, AS15, AS-2, AS-03 and derivatives thereof (GlaxoSmithKline, USA); CWS (cell-wall skeleton), TDM (trehalose-6,6'-dimycolate), LeIF (Leishmania elongation initiation factor), aluminum salts such as aluminum hydroxide gel (alum) or aluminum phosphate; salts of calcium, iron or zinc; an insoluble suspension of acylated tyrosine; acylated sugars; cationically or anionically derivatized polysaccharides; polyphosphazenes; biodegradable microspheres; monophosphoryl lipid A and quil A. Cytokines, such as GM-CSF or interleukin-2, -7 or -12 may also be used as adjuvants. Preferred adjuvants for use in eliciting a predominantly Th1-type response include, for example, a combination of monophosphoryl lipid A, preferably 3-O-deacylated monophosphoryl lipid A (3D-MPL), optionally with an aluminum salt. Aqueous formulations comprising monophosphoryl lipid A and a surfactant have been described in WO 98/43670.

[0113] Another preferred adjuvant is a saponin or saponin mimetic] or derivatives, preferably QS21 (Aquila Biopharmaceuticals Inc.), which may be used alone or in combina-

tion with other adjuvants. For example, an enhanced system involves the combination of a monophosphoryl lipid A and saponin derivative, such as the combination of QS21 and 3D-MPL. Other preferred formulations comprise an oil-in-water emulsion and tocopherol. A particularly potent adjuvant formulation is QS21, 3D-MPL and tocopherol in an oil-in-water emulsion. Additional saponin adjuvants for use in the present invention include QS7 (described in WO 96/33739 and WO 96/11711) and QS17 (described in US 5,057,540 and EP 0 362 279 B1).

[0114] The pharmaceutical preparation of the present invention comprises most preferably aluminum hydroxide as adjuvant.

[0115] Another aspect of the present invention relates to a nucleic acid molecule encoding a fusion protein according to the present invention. The nucleic acid molecule of the present invention can be an RNA or a DNA molecule. The nucleic acid molecule may be part of a vector (e.g. protein expression vector, integration vector, cloning vector) which can be transfected or introduced in any kind of biological cell. Preferred cells include bacterial cells such as *Escherichia coli*, yeast cells such as *Pichia pastoris* or *Saccharomyces cerevisiae*, plant cells, mammal cells and insect cells. Means and methods for obtaining such nucleic acid molecules, vectors and cells are well known to the person skilled in the art.

[0116] The nucleic acid molecule encoding a fusion protein according to the present invention can also be used directly for vaccinating subjects in need thereof. These nucleic acid molecules may be RNA and/or DNA molecules.

[0117] A further aspect of the present invention relates to a vector comprising a nucleic acid molecule according to the present invention.

[0118] According to a preferred embodiment of the present invention said vector is an expression or a cloning vector. The vector can be a bacterial, fungal, insect, viral or mammalian vector.

[0119] The vector of the present invention may preferably be employed for cloning and expression purposes in various

hosts like bacteria, yeasts, filamentous fungi, mammalian cells, insect cells, plant cells or any other prokaryotic or eukaryotic cells. Therefore, said vector comprises besides a nucleic acid encoding for a fusion protein according to the present invention host specific regulatory sequences.

[0120] Yet another aspect of the present invention relates to a host cell comprising a nucleic acid molecule or a vector according to the present invention.

[0121] A further aspect of the present invention relates to a fusion protein according to or pharmaceutical preparation according to the present invention for the use in the treatment or the prevention of an allergy caused by an allergen of a house dust mite, in particular caused by Der p 1, Der p 2, Der p 5, Der p 7, Der p 21 or Der p 23.

[0122] The terms "preventing" and "prevention", as used herein, refer to the prevention or inhibition of the recurrence, onset and development of an allergy or a symptom thereof in a subject resulting from the administration of the fusion protein or pharmaceutical preparation according to the present invention. In some embodiments "preventing" and "prevention" refers to the reduction of the risk to develop an allergy against specific allergens. The term "preventing" covers measures not only to prevent the occurrence of an allergy, but also to arrest its progress and reduce its consequences once established.

[0123] The terms "treatment" and "treating", as used herein, refer to the reduction or inhibition of the progression and duration of an allergy, the reduction or amelioration of the severity of the allergy and the amelioration of one or more symptoms thereof. "Treatment" encompasses also the improvement and/or reversal of the symptoms of an allergy or allergic reactions. A fusion protein which causes an improvement in any parameter associated with allergy may be identified as a therapeutic fusion protein or conjugate. The term "treatment" refers to both therapeutic treatment and prophylactic measures. For example, those who may benefit from treatment with compositions

and methods of the present invention include those already with an allergy as well as those in which the allergy is to be prevented.

[0124] The present invention is further illustrated by the following examples, however, without being restricted thereto.

EXAMPLES

[0125] Example 1: Design of allergen fragments to be used in the fusion proteins of the present invention

[0126] Peptides spanning the allergen sequence of interest were identified based on the prediction of surface exposure of amino acids as determined by ProtScale bioinformatics tool from the ExPASy server (<http://web.expasy.org/protscale/>). If peptides contained no cysteine residues in their sequence cysteines were added at their N- or C-terminus in order to allow them to couple to keyhole limpet hemocyanin (KLH). Peptides were synthesized using an Applied Biosystems peptide synthesizer Model 433A (Foster City, USA) and subsequently purified by preparative High-performance liquid chromatography (HPLC) (Dionex, Thermofischer Scientific, USA) (Focke et al., FASEB J. 2001; 15(11):2042-4). The size and identity of the peptides were confirmed by mass spectrometry (Bruker, Austria).

[0127] Table I lists allergen fragments derived from house dust mite allergens Der p 1 (TNAC SINGNAPAEIDLRQMRTVTPIRMQGGCGSCWAFSGVAATESAYLAYRNQSLD LAEQELVDCASQHGCHGDTIPRGIEYIQHNGVVQESYYRYVAREQSCRRPNAQRFGI SNYCQIYPPNVNKIREALAQTHSAIAVIIGIKDLDAFRHYDGRTIIQRDNGYQPNYH AVNIVGYSNAQGVDYWIVRNSWDTNWDNGYGYFAANIDLMMIEEYPYVVIL; SEQ ID No. 34), Der p 2 (DQVDVKDCANHEIKKVLVPGCHGSEPCIIHRGKPFQLEAVFEANQNSKTAKIEIKA SIEGLEVDVPGIDPNACHYMKCPLVKGQQYDIKYTWIVPKIAPKSENVVVTVKVMet GDNGVLACAIATHAKIRD; SEQ ID No. 35), Der p 5 (GenBank Acc. No.: X17699), Der p 7 (GenBank Acc. No.: U37044), Der p 21 (GenBank Acc. No.: DQ354124) and Der p 23 (GenBank Acc. No.: EU414751.1) which were tested in regard to IgE

reactivity, basophil activation, immunogenicity and blocking of allergen specific IgE molecules.

Table I: Allergen fragments

<i>peptides</i>	<i>Position on al- lergen</i>	<i>SEQ ID No.</i>	<i>Amino acid sequence</i>
Der p 1			
Der p 1 P1	1-41	1	TNACSINGNAPAEIDLRQMRTVTPIRMQGGCGSCWAFSGVA
Der p 1 P1	1-41	5	TNA <u>SS</u> INGNAPAEIDLRQMRTVTPIRMQGG <u>SGS</u> WAFSGVA
Der p 1 P2	42-84	2	ATESAYLAYRNQSLDLAEQELVDCASQHGCHGDTIPRGIEYIC
Der p 1 P2	42-84	6	ATESAYLAYRNQSLDLAEQELVD <u>S</u> ASQHG <u>S</u> HGDTIPRGIEYIC
Der p 1 P3	85-115	3	HNGVVQESYYRYVAREQSCRRPNAQRFGISN
Der p 1 P3	85-115	7	HNGVVQESYYRYVAREQ <u>S</u> RRPNAQRFGISN
Der p 1 P4	99-135	49	REQSCRRPNAQRFGISNYCQIYPPNVNKIREALAQTH
Der p 1 P5	145-175	50	KDLDAFRHYDGRTIIQRDNGYQPNYHAVNIV
Der p 1 P6	155-187	51	GRTIIQRDNGYQPNYHAVNIVGYSNAQGVDYWI
Der p 1 P7	175-208	52	VGYSNAQGVDYWIVRNSWDTNWGDNGYGYFAANI
Der p 1 P8	188-222	4	VRNSWDTNWGDNGYGYFAANIDLMMIEEYPYVVIL
Der p 2			
Der p 2 P1	1-33	53	DQVDVKDCANHEIKKVLVPGCHGSEPCIIHRGK
Der p 2 P2	21-51	8	CHGSEPCIIHRGKPFQLEAVFEANQNSKTAK
Der p 2 P2a	22-51	10	HGSEP <u>S</u> IIHRGKPFQLEAVFEANQNSKTAK
Der p 2 P3	42-73	54	EANQNSKTAKIEIKASIEGLEVDVPGIDPNAC
Der p 2 P4	62-103	9	EVDVPGIDPNACHYMKCPLVKGQQYDIKYTWIVPKIAPKSEN
Der p 2 P4a	62-103	11	EVDVPGIDPNA <u>S</u> HYMK <u>S</u> PLVKGQQYDIKYTWIVPKIAPKSEN
Der p 2 P5	98-129	55	APKSENVVTVKVMGDNGVLACAIATHAKIRD
Der p 5			
Der p 5 P1	1-34	56	EDKKHDYQNEFDLLMERIHEQIKKGELALFYLQ
Der p 5 P2	24-59	57	KKGELALFYLQEQINHFEEKPTKEMKDKIVAEMDTI
Der p 5 P3	64-94	58	DGVRGVLDRMLQRKDLDFEQYNLEMAKKSG
Der p 5 P4	78-113	59	DLDFEQYNLEMAKKSGDILERDLKKEEARVKKIEV
Der p 5 P1-1	1-29	60	EDKKHDYQNEFDLLMERIHEQIKKGELA
Der p 5 P1-2	6-34	12	DYQNEFDLLMERIHEQIKKGELALFYLQ
Der p 5 P4-1	78-108	61	DLDFEQYNLEMAKKSGDILERDLKKEEARV
Der p 5 P4-2	83-113	13	EQYNLEMAKKSGDILERDLKKEEARVKKIEV
Der p 21			
Der p 21 P1	1-34	62	FIVGDKKEDEWRMAFDRLMEELETKIDQVEKGL
Der p 21 P2	35-71	63	LHLSEQYKELEKTKSKELKEQILRELTIGENFMKGAL
Der p 21 P3	67-95	64	MKGALKFFEMEAKRTDLNMFERYNYEFAL
Der p 21 P4	89-121	15	YNYEFALESIKLLIKKLDELAKKVKAVNPDEYY

Der p 7

Der p 7 P1	1-30	14	DPIHYDKITEEINKAVDEAVAAIEKSETFD
Der p 7 P2	20-50	65	VAAIEKSETFDPMKVPDHSKFERHIGIIDL
Der p 7 P3	50-80	66	LKGELDMRNIQVRGLKQMKRVGDANVKSE DG
Der p 7 P4	90-125	67	VHDDVVSMEYDLAYKLGD LHPNTHVISDIQDFVVEL
Der p 7 P5	123-148	68	VELSLEVSEEGNMTLTSFEVRQFANV
Der p 7 P6	149-176	69	VNHIGGLSILDPIFAVLSDVLTAIFQDT
Der p 7 P7	170-198	70	TAIFQDTVRAEMTKVLAPAFKKELERNQ

Der p 23

Der p 23 P1	1-32	71	MANDNDDDPTTTVHPTTTEQPDDKFECPSRFG
Der p 23 P2	15-48	72	PTTTEQPDDKFECPSRFGYFADPKDPHKFYICSN
Der p 23 P3	32-70	16	GYFADPKDPHKFYICSNWEAVHKDCPGNTRWNEDEETCT
Der p 23 P3a	32-70	73	GYFADPKDPHKF <u>A</u> ICSNW <u>AA</u> VHK <u>A</u> CPGNTRWN <u>AAAA</u> TCT
Der p 23 P3b	32-37	74	GYFADPKDPH <u>A</u> FYICSNWEAV <u>AA</u> DCPGNTRWNEDEETCT
Der p 23 P4	32-60	75	GYFADPKDPHKFYICSNWEAVHKDCPGNT
Der p 23 P4a	32-60	76	GYFADPKDPHKFYI <u>S</u> SNWEAVHKD <u>S</u> PGNT
Der p 23 P5	42-70	77	KFYICSNWEAVHKDCPGNTRWNEDEETCT
Der p 23 P5a	42-70	78	KFYI <u>S</u> SNWEAVHKD <u>S</u> PGNTRWNEDEET <u>S</u> T
Der p 23 P5b	47-70	79	SNWEAVHKDCPGNTRWNEDEETCT
Der p 23 P6	32-70	17	GYFADPKDPHKFYI <u>S</u> SNWEAVHKD <u>S</u> PGNTRWNEDEET <u>S</u> T
Der p 23 P7	32-64	80	GYFADPKDPHKFYICSNWEAVHKDCPGNTRWNE
Der p 23 P8	32-68	81	GYFADPKDPHKFYICSNWEAVHKDCPGNTRWNEDEET

Der f 1

Der f 1 P1		38	TSACRINSVNVPSELDLRSLRTVTPIRMQGGCGSCWAFSGVA
Der f 1 P2		39	ATESAYLAYRNTSLDLSEQELVDCASQHGHGDTIPRGIEYIC
Der f 1 P3		40	QNGVVEERSYPYVAREQQCRRPNSQHYGISN
Der f 1 P8		41	VRNSWDTTWGDSGYGYFQAGNNLMMIEQYPYVVIM

Der f 2

Der f 2 P2a		42	HGSDPCIIHRGKPFNLEAIFDANQNTKTAK
Der f 2 P4a		43	EVDVPGIDTNACHYIKCPLVKGQQYDAKYTWNVPKIAPKSEN

Der f 5

Der f 5 P1-2		44	DYQNEFDLLMQRIHEQMRKGEEALLHLQ
Der f 5 P4-2		45	ERYNVEIALKSNEILERDLKKEEQRVKKIEV

Der f 7

Der f 7 P1		46	DPIHYDKITEEINKAIDDAIAAAIEQSETID
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Der f 21

Der f 21 P4		47	YNFETAVSTIEILVKDLAELAKKVKAVKSDD
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Der f 23

Der f 23 P6		48	GYFADPKDPCKFYICSNWEAIIHKSCPGNTRWNEKELTCT
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[0128] IgE reactivity of peptides was determined by dot blot analysis. For this purpose 0.5µg aliquots of each peptide, of corresponding allergen and as a control human serum albumin (HSA) were dotted onto Whatman Protran nitrocellulose membrane (GE healthcare, Little Chalfont, UK). After blocking three times for 20min with gold buffer (50mM sodium phosphate [pH 7.4], 0.5% [v/v] Tween-20, 0.5% [w/v] BSA, and 0.05% [w/v] sodium azide), membranes were incubated with HDM-allergic patients' sera (1:10 in gold buffer) or with serum from a non-allergic person (1:10 in gold buffer) overnight at 4°C. Bound IgE antibodies were detected with 1:10 diluted I125-labeled anti-human IgE Abs (Demeditec Diagnostics, Kiel, Germany) and visualized by autoradiography (Kodak XOMAT film).

[0129] To determine the immunogenicity of the peptides rabbits were immunized three times (first booster injection after 4 weeks and a second booster injection after 7 weeks) with each of the KLH-conjugated peptides (200µg/injection) and, for control purposes, with respective allergen (200µg/injection) using once Freund's complete and twice Freund's incomplete adjuvant (Charles River, Chatillon sur Chalaronne, France) and/or Al(OH)₃ (Serva). Rabbit immune responses were analyzed by ELISA titrations. For the measurement of specific rabbit IgG antibodies, ELISA plates were coated overnight with respective allergen. After blocking, the plates were incubated overnight with serial dilutions of the corresponding rabbit anti-sera, or serum from a non-immunized rabbit (1:500, 1:2.000, 1:10.000 and 1:20.000). Bound rabbit IgG antibodies were detected with a 1:1.000 diluted horseradish peroxidase-labelled donkey anti-rabbit IgG antiserum (Amersham Biosciences, Little Chalfont, UK).

[0130] To determine the blocking capacity of peptide IgGs ELISA plates (Nunc, Roskilde, Denmark) coated overnight with 1µg/mL of respective allergen were pre-incubated for 24hours with each of the anti-peptide antisera, anti-allergen antiserum, or, for control purposes, with serum

from a non-immunized rabbit (all in a dilution of 1:50) and then washed. After overnight incubation with sera from HDM allergic patients (diluted 1:10), bound IgE antibodies were detected with horseradish peroxidase-labelled goat anti-human IgE antibodies (KPL, Gaithersburg, MD). The percentage reduction of IgE binding achieved by pre-incubation with rabbit antisera was calculated as follows: $100 - (\text{ODI}/\text{ODP}) \times 100$. ODI and ODP represent optical density values after pre-incubation with the rabbit immune serum or normal rabbit serum, respectively.

[0131] To test the allergenic activity of the peptides, rat basophil leukemia cells (RBL) expressing human high-affinity IgE receptor FcεRI (1×10^5 /well) were loaded overnight with sera from the HDM-allergic patients and, for control purposes, with the serum from one non-allergic individual at a dilution of 1: 10. Cells were washed three times with Tyrode's buffer (Sigma, Austria) and exposed to serial dilutions of allergen and peptides for 1h. Supernatants were analysed for β-hexosaminidase activity as described previously(. Experiments were carried out in triplicates, and results are presented as mean percentages of total β-hexosaminidase released after addition of 1%Triton X-100 +/- SE of the mean (SEM).

Table II

<i>peptides</i>	<i>IgE reactivity (dot blot)</i>	<i>basophil activation</i>	<i>immunogenicity</i>	<i>blocking of IgE</i>
Der p 1				
Der p 1 P1	-	-	yes	52% CFA
Der p 1 P2	-	-	yes	51% CFA
Der p 1 P3	-	-	yes	19% CFA
Der p 1 P4	-	-	yes	25% CFA
Der p 1 P5	-	-	yes	18% CFA
Der p 1 P6	-	-	yes	4% CFA
Der p 1 P7	-	-	yes	35% CFA
Der p 1 P8	-	-	yes	45% CFA
Der p 2				
Der p 2 P1	-	-	yes	41% CFA

Der p 2 P2	-	-	yes	70% CFA
Der p 2 P3	-	-	yes	78% CFA
Der p 2 P4	-	-	yes	73% CFA
Der p 2 P5	-	-	poor	3% CFA

Der p 5

Der p 5 P1	+	nd	yes	35% A1OH, 53% CFA
Der p 5 P2	-	-	yes	23% A1OH, 37% CFA
Der p 5 P3	+	-	yes	31% A1OH, 69% CFA
Der p 5 P4	+	nd	yes	42% A1OH, 83% CFA
Der p 5 P1-1	+	nd	nd	nd
Der p 5 P1-2	-	-	yes	45% A1OH, 69% CFA
Der p 5 P4-1	+	nd	nd	nd
Der p 5 P4-2	-	nd	yes	45% A1OH, 78% CFA

Der p 21

Der p 21 P1	+	-	yes	54% A1OH, 55% CFA
Der p 21 P2	+	-	yes	14% A1OH, 39% CFA
Der p 21 P3	+	-	poor	<10%
Der p 21 P4	-	-	yes	64% A1OH, 81% CFA

Der p 7

Der p 7 P1	-	-	yes	66%A1OH, 65% CFA
Der p 7 P2	-	-	yes	26% A1OH, 62% CFA
Der p 7 P3	-	-	yes	26% A1OH, 33% CFA
Der p 7 P4	-	-	yes	15% A1OH, 46% CFA
Der p 7 P5	-	-	poor	0% A1OH, 0% CFA
Der p 7 P6	-	-	poor	5% A1OH, 9% CFA
Der p 7 P7	-	-	yes	22% A1OH, 52% CFA

Der p 23

Der p 23 P1	-	-	poor	nd
Der p 23 P2	-	-	yes	19%
Der p 23 P3	+	nd	yes	nd
Der p 23 P3a	+	nd	poor	nd
Der p 23 P3b	+	nd	no	nd

Der p 23 P4	-	-	yes	33%
Der p 23 P4a	*	*	*	*
Der p 23 P5	+	-	yes	39%
Der p 23 P5a	*	*	*	*
Der p 23 P5b	*	*	*	*
Der p 23 P6	-	nd	yes	70%
Der p 23 P7	-	nd	yes	5%
Der p 23 P8	-	nd	yes	6%

nd: not done; bold and underlined letters: cysteine residues replaced with serine or alanine residues; + indicates IgE reactivity; - indicates no IgE reactivity or no basophil activation.

[0132] Example 2: Recombinant production of proteins

[0133] Genes (codon-optimized for *Escherichia coli* expression) coding for fusion proteins Der p 1-2 C3 and Der p 5 7 21 23_P6 (large) were synthesized (ATG: biosynthetics, Merzhausen, Germany and GenScript, Piscataway, USA) and inserted into the NdeI/XhoI sites of pET-27b (Novagen, Germany). Recombinant proteins were expressed in *E.coli* strain BL21-Gold (DE3). Der p 1-2 C3 (see Fig. 1; SEQ ID No. 27) expression was induced at OD600 0.4 by adding 0.5mM IPTG to bacterial cultures and incubating for 3h at 37°C. Der p 5 7 21 23_P6 (large) (see Fig. 1; SEQ ID No. 28) was induced at OD600 0.6 by adding 1mM IPTG and incubating cultures for 2.5h at 37°C.

[0134] Upon harvesting and adding protease inhibitors, inclusion body preparation was performed to remove soluble bacterial proteins. Pellets containing partially purified Der p 1-2 C3 proteins were dissolved in 6M Urea, 10mM Tris PH 8, 4% and isopropanol and purification was continued by anion exchange chromatography using a HiTrap DEAE Sepharose FF (GE healthcare) column. Elution of the protein from the column was achieved with linearly increasing NaCl concentration. Elution fractions of high purity were united and stepwise dialysis was performed to remove urea and salts and to enable proteins refolding without aggregation. Der p 1-2 C3 protein was incubated for a minimum of 4-5h at 4°C subsequently in the follow-

ing solutions: 1) 6M Urea, 154mM NaCl, 10mM Tris PH 8, 4% isopropanol, 2) 4M Urea, 103mM NaCl, 2mM Hepes, PH 8, 3) 2M Urea, 50.2mM NaCl, 2mM Hepes, PH 8, 4) 1M Urea, 25mM NaCl, 2mM Hepes, PH 8, 5) 0.5M Urea, 12.5mM NaCl, 2mM Hepes, PH 8, 6) 2mM Hepes, PH 8.

[0135] Cell lysate containing Der p 5 7 21 23_P6 (large) was dissolved in 6M Urea, 10mM Tris PH 7.5, 4% isopropanol were applied to HiTrap DEAE Sepharose FF (GE healthcare) column and elution of the protein from the column was achieved with linearly increasing NaCl concentration. Pure fractions were united and dialyzed as follows. Stepwise dialysis conditions Der p 5 7 21 23_P6 (large): incubate protein for minimum of 4-5h at 4°C subsequently in the following solutions: 1) 6M Urea, 100mM NaCl, 10mM Tris PH 7.5, 4% isopropanol, 2) 4M Urea, 100mM NaCl, 2mM Hepes, PH 7.5, 3) 2M Urea, 100mM NaCl, 2mM Hepes, PH 7.5, 4) 1M Urea, 100mM NaCl, 2mM Hepes, PH 7.5, 5) 0.5M Urea, 100mM NaCl, 2mM Hepes, PH 7.5, 6) 100mM NaCl, 2mM Hepes, PH 7.5, 7) 50mM NaCl, 2mM Hepes, PH 7.5, 8) 25mM NaCl, 2mM Hepes, PH 7.5, 9) 12.5mM NaCl, 2mM Hepes, PH 7.5, 10) 2mM Hepes, PH 7.5.

[0136] Purity of the proteins was checked by SDS-PAGE and Coomassie staining (see Fig. 2). Size exclusion chromatography was used to confirm that no oligomerization of the refolded proteins occurs.

[0137] Example 3: Determination of IgE reactivity

[0138] IgE reactivity of the constructs of example 2 was determined by immunoblot (Curin M, et al. Sci Rep. 22(2017):12135). Aliquots containing 0.5 µg of purified nDer p 1 (a natural isolate; Accession number: PDB: 3RVW_A; reference for methods: Hales, B.J. et al. Clin Exp Allergy. 30(2000): 934-943), rDer p 2 (a recombinantly produced Der p 2; sequence published in Chen KW et al, Allergy. 67(2012):609-21; reference for methods: Chen, K., et al. Mol Immunol. 45(2008): 2486-2498.), rDer p 5 (GeneBank: X17699; reference methods: Weghofer M, et al. Int Arch Allergy Immunol. 147(2008):101-9.), rDer p 7 (GeneBank: U37044; reference for methods: Resch Y, et al. Clin Exp Allergy. 41(2011):1468-77), rDer p 21 (GeneBank:

DQ354124; reference methods: Weghofer M, et al. Allergy. 63(2008):758-67.), rDer p 23(GeneBank: EU414751.1; reference methods: Weghofer M, et al. J Immunol. 190(2013):3059-67), Der p 1-2 C3 (see example 2), Der p 5 7 21 23_P6 (large) (see example 2) and for control purpose BSA were dotted onto nitrocellulose membranes (Schleicher & Schuell, Germany). Membranes were blocked with gold buffer (50 mM sodium phosphate [pH 7.4], 0.5% [v/v] Tween-20, 0.5% [w/v] BSA, and 0.05% [w/v] sodium azide), three times for 20 min and then incubated with house dust mite-allergic patients' sera (diluted 1:10 in gold buffer) and with a serum from a non-allergic person (1:10 in gold buffer) overnight at 4°C. Bound IgE was detected with 1:10 diluted 125I-labeled anti-human IgE Abs (Demeditec Diagnostics, Kiel, Germany) and visualized by autoradiography (Kodak XOMAT film) as described previously (Curin et al Sci Rep. 22(2017):12135).

[0139] IgE reactivity of the Der p 1-2 C3 was compared with that of Der p 1 and Der p 2 in 20 HDM-sensitized patients by dot-blot assay (Figure 3A). While allergic patients reacted with Der p 1 and Der p 2 no patient showed relevant IgE reactivity with Der p 1-2 C3. No reactivity was observed with serum from a non-allergic subject (NA), and the control protein BSA also showed no IgE reactivity (Figure 3A). In the next experiments IgE reactivity of Der p 5 7 21 23_P6 (large) was compared with IgE reactivity to Der p 5, Der p 7, Der p21 and Der p 23. 18 HDM-sensitized patients reacted with Der p 5, Der p 7, Der p 21 and Der p 23 in a patient dependent manner but neither of tested patients reacted with Der p 5 7 21 23_P6 (large) or with control protein BSA (Figure 3B).

[0140] Example 4: IgE competition assay

[0141] Information regarding the capacity of the peptides to induce blocking antibodies is important since blocking antibodies were shown to play a major role in immunotherapy of allergies.

[0142] In order to examine the ability of immunoglobulins (in particular IgGs) induced by the administration of Der p 1-2 C3 and Der p 5 7 21 23_P6 (large) to mammals to in-

hibit the binding of house dust mite allergic patients' IgE to house dust mite allergens ELISA competition experiments were performed.

[0143] IgE ELISA competition assays were done to analyse the inhibition of human IgE binding to nDer p 1, rDer p 2, rDer p 5, rDer p 7, rDer p 21, rDer p 23 by anti-Der p 1-2 C3 and anti-Der p 5 7 21 23_P6 (large)-specific rabbit IgG. Briefly, ELISA plates (Nunc, Denmark) coated overnight with 1 µg/mL of respective allergen were pre-incubated for 24 hours with anti-Der p 1-2 C3 antiserum, anti-Der p 5 7 21 23_P6 (large), respective anti-allergen antiserum for a comparison (anti-nDer p 1, anti-rDer p 2, anti-rDer p 5, anti-rDer p 7, anti-rDer p 21 or anti-rDer p 23 antiserum), or, for control purposes, with serum from a non-immunized rabbit (all diluted 1:3) and then washed. After overnight incubation with sera from HDM-allergic patients (diluted 1:10), bound IgE antibodies were detected with horseradish peroxidase-labelled goat anti-human IgE antibodies (KPL, Gaithersburg, MD). The percentage reduction of IgE binding achieved by pre-incubation with rabbit antisera was calculated as follows: $100 - (\text{ODI}/\text{ODP}) \times 100$ (see Figs. 6 and 7). ODI and ODP represent optical density values after pre-incubation with the rabbit immune serum or normal rabbit serum, respectively (see Curin M, et al. Sci Rep. 22(2017):12135).

[0144] The anti-Der p 1-2 C3 and anti-Der p 5 7 21 23_P6 (large)-specific rabbit IgG used in this example were obtained as follows. Rabbits (Charles River, France) were immunized three times (first booster injection after 4 weeks and a second booster injection after 7 weeks) with Der p 1-2 C3 or with Der p 5 7 21 23_P6 (large) (200 µg/injection). Al(OH)₃ (Serva) was used as adjuvant. 2 rabbits per protein were immunized. Rabbit immune responses were analyzed by ELISA titrations.

[0145] The inhibition of patients IgE binding to nDer p 1 achieved with anti-Der p 1-2 C3 antibodies was somewhat lower but close (53% and 58% mean inhibition) to inhibition with anti-nDer p 1 (78% mean inhibition). The inhibition of IgE binding to rDer p 2 achieved with rabbit

anti-Der p 1-2 C3 antibodies (89% and 85 % mean) was comparable with that obtained with rabbit anti-Der p 2 (mean 84%) (Figure 6). The inhibition of patients' IgE binding to Der p 5 achieved with anti-Der p 5 7 21 23_P6 (large) (mean inhibitions 76% and 85%) was comparable to inhibition with anti-Der p 5 (mean inhibition 88%) whereas inhibition to Der p 21 was somewhat lower by Der p 5 7 21 23_P6 (large) (mean inhibitions 58% and 58%) than with anti-Der p 21 (mean inhibition 87%). Inhibitions to Der p 7 and Der p 23 were higher by Der p 5 7 21 23_P6 (large) (74% both rabbits for Der p 7 and 47% and 42% for Der p 23) than with anti Der p 7 (58%) and anti-Der p 23 (7%) (Figure 7).

[0146] Example 5: Allergenic activity as determined by a basophil activation assay

[0147] To test the allergenic activity of the constructs of the present invention, in particular Der p 1-2 C3 and Der p 5 7 21 23_P6 (large), rat basophil leukemia cells (RBL) expressing human high-affinity IgE receptor FcεRI (1 x 10⁵/well) were loaded overnight with sera from the house dust mite-allergic patients at a dilution of 1:10. Cells were washed three times with Tyrode's buffer (Sigma, Austria) and exposed to serial dilutions of allergen (100 ng/mL, 10 ng/mL and 1 ng/mL of of nDer p 1, rDer p 2, rDer p 5, rDer p 7, rDer p 21, rDer p 23, Der p 1-2 C3 and Der p 5 7 21 23_P6 (large), respectively) for 1 h. Serum without allergen or allergen without serum were used as negative controls. Supernatants were analysed for β-hexosaminidase activity as described previously (Hartl et al Allergy 59(2004): 65-73). Experiments were carried out in triplicates, and results are presented as mean percentages of total β-hexosaminidase released after addition of 1%Triton X-100 +/- SE of the mean (SEM) (see Figs. 4 and 5).

[0148] It was found that Der p 1 and Der p 2 induced release of β-hexosaminidase from basophils loaded with serum IgE from HDM-allergic patients whereas the Der p 1-2 C3 did not activate the basophils (Fig 4). Next set of experiments showed that Der p 5, Der p 7, Der p 21 and

Der p 23 induced release of β -hexosaminidase from basophils loaded with serum IgE in a patient dependent manner whereas Der p 5 7 21 23_P6 (large) did not induce basophil release for the same patients' sera (Figure 5). These data indicate that the constructs Der p 1-2 C3 and Der p 5 7 21 23_P6 (large) do not exhibit allergenic activity.

[0149] Example 6: Formation of allergen-specific IgGs induced by BM35 compared to commercial HDM vaccines

[0150] Groups of two New Zealand white rabbits were immunized each subcutaneously with various doses of BM35 (a preparation comprising the fusion proteins comprising amino acid sequence SEQ ID No. 27 and 28 in a weight ratio of 1:1) and with commercial preparations according to the manufacturers indications (see Table III). Control rabbits were immunized with allergens nDer p1, rDer p 2, rDer p 5, rDer p 7, rDer p 21 and rDer p 23.

Table III

Product	Company	Administration scheme
Tyro-SIT	Bencard Allergie GmbH	Week 1: 0.1 ml Week 2: 0.3 ml Week 3: 0.5 ml Week 4: 0.1 ml Week 5: 0.3 ml Week 6: 0.5 ml Week 9: 0.5 ml Week 13: 0.5 ml Week 17: 0.5 ml
Alutard SQ 503 and 510	ALK-Abelló	Week 1: 0.2 ml Week 2: 0.4 ml Week 3: 0.8 ml Week 4: 0.2 ml Week 5: 0.4 ml Week 6: 0.8 ml Week 7: 0.2 ml Week 8: 0.4 ml Week 9: 0.8 ml

		Week 10: 0.2 ml Week 11: 0.4 ml Week 12: 0.6 ml Week 13: 0.8 ml Week 14: 1 ml Week 18: 1 ml
ACAROID	Allergopharma	Week 1: 0.1 ml Week 2: 0.2 ml Week 3: 0.4 ml Week 4: 0.6 ml Week 5: 0.1 ml Week 6: 0.2 ml Week 7: 0.4 ml Week 8: 0.6 ml Week 10: 0.6 ml Week 13: 0.6 ml Week 17: 0.6 ml
PURETHAL Milbenmischung	HAL Allergy	Week 1: 0.05 ml Week 2: 0.1 ml Week 3: 0.2 ml Week 4: 0.3 ml Week 5: 0.4 ml Week 6: 0.5 ml Week 8: 0.5 ml Week 10: 0.5 ml Week 12: 0.5 ml Week 15: 0.5 ml Week 19: 0.5 ml
CLUSTOID Milben In- jektionssuspension	Roxall Medizin GmbH	Week 1: 0.2 ml (after 15 min 0.5 ml) Week 4: 0.5 ml Week 8: 0.5 ml Week 12: 0.5 ml Week 16: 0.5 ml

[0151] Serum samples were taken from the rabbits on the day of first immunization and on days 38 and 66 after the first immunization in order to monitor the formation of

IgGs specifically binding to house dust mite allergens nDer p 1, rDer p 2, rDer p 5, rDer p 7, rDer p 21 and rDer p 23 in order to determine house dust mite allergen-specific antibody responses. Allergen-specific rabbit IgG responses were measured by diluting sera 1:500 and by using ELISA. Bound rabbit IgG was detected with 1:2000 diluted donkey anti-rabbit horseradish peroxidase-coupled IgG antibodies (NA 934; GE Healthcare UK Limited, Chalfont St Giles, United Kingdom). Color development was done with 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid). Sera obtained after immunization with wild type house dust mite allergens served as positive controls.

[0152] The results depicted in Figs. 8 and 9 clearly show that BM35 is able to induce the formation of a much higher titer of allergen specific IgGs compared to the commercial products. It is particularly surprising that BM35 induced the formation of antibodies directed to all 6 house dust mite allergens whereas the commercial products resulted in a much lower allergen specific IgG titer and only for single allergens. Thus, all commercial products showed a weaker induction of IgGs specifically binding to a very limited number of allergens compared to BM35.

CLAIMS

1. A fusion protein having formula (I)

$$X_1-Y-X_2 \text{ (I),}$$

in which X_1 and X_2 comprise each four to eight allergen fragments or variants thereof fused to each other, in which the allergen fragments are derived from at least two allergens of *Dermatophagoides pteronyssinus* selected from the group consisting of Der p 1, Der p 2, Der p 5, Der p 7, Der p 21 and Der p 23, and in which Y is a carrier protein, in which the fusion protein comprises at least one polypeptide having amino acid sequence selected from the group consisting of SEQ ID No. 18, SEQ ID No. 19, SEQ ID No. 20, SEQ ID No. 21, SEQ ID No. 22, SEQ ID No. 23, SEQ ID No. 24 and/or SEQ ID No. 25, in which:

(a) the allergen fragment of Der p 1 consists of an amino acid sequence selected from the group consisting of

TNACSINGNAPAEIDLRQMRTVTPIRMQGGCGSCWAFSGVA (SEQ ID No. 1),
ATESAYLAYRNQSLDLAEQELVDCASQHGCHGDTIPRGIEYIQ (SEQ ID No. 2),
HNGVVQESYYRYVAREQSCRRPNAQRFGISN (SEQ ID No. 3),
VRNSWDTNWGDNGYGYFAANIDLMMIEEYPYVVIL (SEQ ID No. 4),
TNASSINGNAPAEIDLRQMRTVTPIRMQGGSGSSWAFSGVA (SEQ ID No. 5),
ATESAYLAYRNQSLDLAEQELVDSASQHGSHGDTIPRGIEYIQ (SEQ ID No. 6)
and HNGVVQESYYRYVAREQSSRRPNAQRFGISN (SEQ ID No. 7), and/or

(b) the allergen fragment of Der p 2 consists of an amino acid sequence selected from the group consisting of CHGSEPCIIHRGKPFQLEAVFEANQNSKTAK (SEQ ID No. 8), EVDVPGIDPNACHYMKCPLVKGQQYDIKYTWIVPKIAPKSEN (SEQ ID No. 9), HGSEPSIIHRGKPFQLEAVFEANQNSKTAK (SEQ ID No. 10) and EVDVPGIDPNASHYMKSPKLVKGQQYDIKYTWIVPKIAPKSEN (SEQ ID No. 11), and/or

(c) the allergen fragment of Der p 5 consists of an amino acid sequence selected from the group consisting of DYQNEFDLFLMERIHEQIKKGELALFYLQ (SEQ ID No. 12) and EQYNLEMAKKSGDILERDLKKEEARVKKIEV (SEQ ID No. 13), and/or

(d) the allergen fragment of Der p 7 consists of amino acid sequence DPIHYDKITEEINKAVDEAVAAIEKSETFD (SEQ ID No. 14), and/or

(e) the allergen fragment of Der p 21 consists of amino acid sequence
YNYEFALESIKLLIKKLDELAKKVKAVNPDEYY (SEQ ID No. 15), and/or

(f) the allergen fragment of Der p 23 consists of an amino acid sequence selected
from the group consisting of

5 GYFADPKDPHKFYICSNWEAVHKDCPGNTRWNEDEETCT (SEQ ID No. 16) and
GYFADPKDPHKFYISSNWEAVHKDSPGNTRWNEDEETST (SEQ ID No. 17),
and/or

(g) the allergen fragment of Der f 1 consists of an amino acid sequence selected from
the group consisting of

10 TSACRINSVNVPSELRLSLRTVTPIRMQGGCGSCWAFSGVA (SEQ ID No. 38),
ATESAYLAYRNTSLDLSEQELVDCASQHGCHGDTIPRGIEYIQ (SEQ ID No. 39),
QNGVVEERSYPYVAREQQCRRPNSQHYGISN (SEQ ID No. 40) and
VRNSWDTTWGDSGYGYFQAGNNLMMIEQYPYVVIM (SEQ ID No. 41), and/or

15 (h) the allergen fragment of Der f 2 consists of an amino acid sequence selected from
the group consisting of HGSDPCIIHRGKPFNLEAIFDANQNTKTAK (SEQ ID No.
42) and EVDVPGIDTNACHYIKCLVKGGQYDAKYTWNVPKIAPKSEN (SEQ ID
No. 43), and/or

20 (i) the allergen fragment of Der f 5 consists of an amino acid sequence selected from
the group consisting of DYQNEFDLLMQRIHEQMRKGEEALLHLQ (SEQ ID No.
44) and ERYNVEIALKSNEILERDLKKEEQRVKKIEV (SEQ ID No. 45), and/or

(j) the allergen fragment of Der f 7 consists of an amino acid sequence
DPIHYDKITEEINKAIDDAIAAIEQSETID (SEQ ID No. 46), and/or

25 (k) the allergen fragment of Der f 21 consists of an amino acid sequence being at least
90% identical to amino acid sequence
YNFETAVSTIEILVKDLAELAKKVKAVKSDD (SEQ ID No. 47), and/or

(l) the allergen fragment of Der f 23 consists of an amino acid sequence
GYFADPKDPCKFYICSNWEAIIHKSCPGNTRWNEKELTCT (SEQ ID No. 48).

2. A fusion protein according to Claim 1, in which the allergen fragments of the at least
two allergens consist of 25 to 50 amino acid residues.

3. A fusion protein according to Claim 1, in which the allergen fragments of the at least two allergens consist of 28 to 48 amino acid residues.
4. A fusion protein according to Claim 1, in which the allergen fragments of the at least two allergens consist of 30 to 45 amino acid residues.
- 5 5. A fusion protein according to any preceding claim, in which at least one of the cysteine residues of the allergen fragments are substituted with serine, threonine, glycine, alanine, or leucine.
6. A fusion protein according to Claim 1, in which the carrier protein is a surface polypeptide of a virus of a hepadnaviridae family or a fragment of the surface polypeptide.
- 10 7. A fusion protein according to any preceding claim, in which the fusion protein comprises amino acid sequence SEQ ID No. 27 or amino acid sequence SEQ ID No. 28.
8. A nucleic acid molecule encoding a fusion protein according to any preceding claim.
9. A vector comprising a nucleic acid molecule according to Claim 8.
10. A host cell comprising a nucleic acid molecule according to Claim 8 or a vector
15 according to Claim 9.
11. A pharmaceutical preparation comprising at least one fusion protein according to any of Claims 1 to 7 or at least one nucleic acid molecule according to Claim 8.
12. A pharmaceutical preparation according to Claim 11, in which two fusion proteins or nucleic acid molecules comprised in the preparation have a weight ratio of 1:10 to 10:1.
- 20 13. A pharmaceutical preparation according to Claim 11 or 12, in which the preparation comprises a fusion protein comprising amino acid sequence SEQ ID No. 27 and/or a fusion protein comprising amino acid sequence SEQ ID No. 28 or a nucleic acid molecule encoding a fusion protein comprising amino acid sequence SEQ ID No. 27 and/or a fusion protein comprising amino acid sequence SEQ ID No. 28.
- 25 14. A fusion protein according to any of Claims 1 to 7, a nucleic acid molecule according to Claim 8 or a pharmaceutical preparation according to any of Claims 11 to 13 for use in the treatment or prevention of an allergy caused by an allergen of a house dust mite.