METHOD FOR IDENTIFYING ALLERGENIC PROTEINS AND PEPTIDES

FIELD OF THE INVENTION:

[0001] The present invention relates to a method for identifying allergenic proteins and peptides. More specifically, the present invention provides a method for identifying allergenic milk proteins and/or peptides comprising the steps of: providing at least one expression library comprising DNA or cDNA derived from the mammary gland tissue of a lactating mammal, preferably a lactating cow, expressing at least one protein or peptide encoded by said expression library, determining the binding capacity of said at least one protein or peptide to IgE of at least one serum of an individual who is sensitive to mammalian milk, preferably cow's milk, contacting the at least one protein or peptide exhibiting an IgE binding capacity as determined in step c) with basophil cells, eosinophil cells or mast cells and identifying the at least one protein or peptide as being allergenic when said basophil cells, eosinophil cells or mast cells release upon contact with at least one protein or peptide of step d) at least one mediator.

In this specification, a number of documents including patent applications and manufacturer's manuals is cited. The disclosure of these documents, while not considered relevant for the patentability of this invention, is herewith incorporated by reference in its entirety. More specifically, all referenced documents are incorporated by reference to the same extent as if each individual document was specifically and individually indicated to be incorporated by reference. None of these documents is acknowledged to be prior art to the present invention.

BACKGROUND OF THE INVENTION

[0002] Approximately 2-8% of infants younger than 3 years and about 2% of the adult population are affected by food hypersensitivities. About 80% of allergic reactions in children are the result of hypersensitivity to cow's milk (CM), hen's egg and legumes (e.g. peanuts and soybeans). In the adult population, however, allergy to fruits, peanuts and tree nuts represent the hypersensitivities with the highest prevalence.

[0003] Cow's milk is among the first foods introduced into an infant's diet and thus one of the most common causes of food allergy in young children. About 2.5% of neonates show adverse reactions to cow's milk in the first year of their life.

[0004] Symptoms of cow's milk allergy (CMA) are either of immediate or delayed type and they range from mild to severe reactions that involve the skin, the respiratory tract, gastrointestinal tract and in the worst case appear as life-threatening systemic reactions (anaphylaxis). In contrast to the cell-mediated delayed type reactions, immediate reactions are caused by the production of immunoglobulin E (IgE) antibodies in response to otherwise harmless antigens (Type I hypersensitivity). The interaction of IgE antibodies with the allergenic molecule leads to specific activation of effector cells (mast cells, basophil granulocytes) and to a subsequent release of inflammatory mediators like histamine, prostaglandine, and leukotriene which are responsible for the immediate-type allergic reactions.

[0005] Cow's milk contains more than 25 proteins and some of them are known to be allergenic. By the action of chymosin (rennin) or by acidification of milk to pH 4.6 two fractions are obtained: Twenty percent of the proteins are found in the whey fraction and 80% of proteins comprise the casein fraction. Allergenic molecules are contained in both fractions and considered either major or minor allergens depending on the incidence of documented allergic responses in the CMA population.

[0006] Major allergens present in cow's milk are alpha-lactalbumin, beta-lactoglobulin, alphaS1-casein, beta-casein and kappa-casein. Minor allergens present in cow's milk are alphaS2-casein, lactoferrin, bovine serum albumin and immunoglobulin.

[0007] Alpha-lactalbumin (Bos d 4), beta-lactoglobulin (Bos d 5), immunoglobulins (Bos d 7), BSA and lactoferrin are the well-known IgE-reactive components in whey. AlphaS1-casein, alphaS2-casein, beta-casein and kappa-casein are the potent allergens in the casein fraction (Bos d 8) (Wal, 2004).

[0008] In the whey fraction beta-lactoglobulin (BLG) and alpha-lactalbumin (ALA) are regarded as major allergens. Beta-lactoglobulin is a globular and very stable protein which belongs to the lipocalins, a protein superfamily, that bind hydrophobic ligands. Other allergens, like the major dog allergens Can f 1 and Can f 2 and allergens of other furred animals (horse, cat, and guinea pig) also belong to this protein superfamily. Beta-lactoglobulin naturally occurs in a dimeric form with a molecular weight of 36 kDa. There are two major isoforms of beta-lactoglobulin, the genetic variants A (BLGA) and B (BLGB), which differ in amino acids 64 and 118 (aspartic acid and valine in BLGA, glycine and alanine in BLGB). Stability and the fact that beta-lactoglobulin belongs to the family of lipocalins may explain the high allergenic potential of this molecule.

[0009] Alpha-lactalbumin is a 14 kDa acidic Ca^{2+} binding monomer stabilized by four disulfide bridges. It is a regulatory component in the galactosyltransferase system that synthesizes lactose. Sequence analysis showed high amino acid sequence homology to human alpha-lactalbumin (hALA) and lysozyme from hen's egg; a major allergen of hen's egg. The allergenicity of alpha-lactalbumin may be explained by its stability.

[00010] In suspension the proteins of the casein fraction form ordered aggregates (micelles) with a constant proportion of the individual molecules: alphaS1- and alphaS2-casein 37% each, beta- and kappa-casein 13% each. The four casein molecules have little primary structure homology, have different functional properties, but are all phosphorylated proteins with rheomorph, highly hydrated tertiary structures that can be easily degraded by some proteases. This sensitivity to proteolytic digestion is a rather unusual characteristic for an important allergen. Cow's milk caseins share amino acid sequence homologies of up to 90% with caseins of other mammals, like goat and sheep. This sequence homology might be the reason for the frequently observed cross-reactivity between cow's milk and milk from other animals.

[00011] The present invention addresses the need to provide methods which allow to identify allergenic proteins and peptides present in a biological source. The present invention thereby also addresses the need to provide methods which allow to identify non-allergenic proteins and peptides present in a biological source, specifically in milk from a mammal and in particular in cow milk and nutritional formulations containing such milk. Moreover, the invention provides means and methods for diagnosing and treating an allergy in an individual.

SUMMARY OF THE INVENTION

[00012] An aspect of the present invention relates to a method for identifying allergenic milk proteins and peptides, which comprises the steps of providing at least one expression library comprising DNA or cDNA derived from the mammary gland tissue of a lactating mammal, preferably a lactating cow, expressing at least one protein or peptide encoded by said expression library, determining the binding capacity of said at least one protein or peptide to IgE of at least one serum of an individual who is sensitive to mammalian milk, preferably cow's milk, contacting the said at least one protein or peptide exhibiting an IgE binding capacity with basophil cells, eosinophil cells or mast cells, and identifying the said at least one protein or peptide as being allergenic when said basophil cells, eosinophil cells or mast cells release at least one mediator upon contact with said at least one protein or peptide. The invention in another aspect provides a method for identifying at least one allergenic milk protein or peptide, which comprises the steps of providing at least one expression library

comprising DNA or cDNA derived from the mammary gland tissue of a lactating mammal, preferably a lactating cow, expressing at least one protein or peptide encoded by said expression library, determining the binding capacity of said at least one protein or peptide to IgE of at least one serum of an individual who is sensitive to mammalian milk, preferably cow's milk, contacting the said at least one protein or peptide exhibiting an IgE binding capacity with basophil cells, eosinophil cells or mast cells, and identifying the at least one protein or peptide as being allergenic when said basophil cells, eosinophil cells or mast cells release at least one mediator upon contact with said at least one protein or peptide. The invention thus relates to the method as described herein above, wherein the allergenic proteins and peptides are milk allergenic proteins and peptides, the at least one expression library comprises DNA or cDNA derived from the mammary gland tissue of a lactating mammal, preferably a lactating cow, and the individual who is sensitive to the allergenic source is an individual who is sensitive to mammalian milk, preferably cow milk.

[00013] According to a further aspect of the invention the methods of the invention described above can further comprise a step of determining the amino acid sequence of the at least one protein or peptide identified by the methods.

[00014] The present invention also provides a method for identifying allergenic proteins and/or peptides encoded by a DNA or cDNA expression library comprising the steps of providing at least one expression library comprising DNA or cDNA derived from at least one allergen source, expressing at least one protein or peptide encoded by said expression library, determining the binding capacity of said at least one protein or peptide to IgE of at least one serum of an individual who is sensitive to the at least one allergen source, in particular mammalian milk, preferably cow milk and nutritional formulations containing mammalian milk, preferably cow milk, contacting the said at least one protein or peptide exhibiting an IgE binding capacity with basophil cells, eosinophil cells or mast cells, and identifying the at least one protein or peptide as being allergenic when said basophil cells, eosinophil cells or mast cells release at least one mediator upon contact with said at least one protein or peptide. Thus, the invention provides a method for identifying at least one allergenic protein or peptide encoded by a DNA or cDNA expression library comprising the steps of providing at least one expression library comprising DNA or cDNA derived from at least one allergen source, expressing at least one protein or peptide encoded by said expression library, determining the binding capacity of said at least one protein or peptide to IgE of at least one serum of an individual who is sensitive to the at least one allergen source, contacting the said at least one protein or peptide exhibiting an IgE binding capacity with basophil cells eosinophil cells or mast cells, and identifying the at least one protein or peptide

as being allergenic when said basophil cells, eosinophil cells or mast cells release at least one mediator upon contact with said at least one protein or peptide.

[00015] A further aspect of the present invention relates to a method for detecting allergenic proteins and peptides in a cow milk comprising sample comprising the step of determining the presence of at least one protein or peptide from Figures 1A, 1B and 1C (SEQ ID Nos. 22-84) and Table 1A, 1B and Table 2 (SEQ ID Nos. 1-21).

[00016] According to this aspect of the invention the mammalian milk, preferably cow milk can be a hydrolysed mammalian milk, preferably hydrolysed cow milk. Furthermore, said presence of at least one protein or peptide can be determined by mass spectrometry. Preferably, the proteins and peptides present in the sample are isolated prior to mass spectrometry. Proteins and peptides can be isolated by an electrophoretic method or by high performance liquid chromatography. Preferably, the electrophoretic method can be two-dimensional electrophoresis.

[00017] Another aspect of the invention relates to a protein or peptide identified by the method of the invention. A further aspect of the invention relates to or a protein or peptide selected from Figures 1A, 1B, 1C (SEQ ID Nos 22-84), Tables 1A, 1B, or Table 2 (SEQ ID Nos. 1-21).

[00018] A further aspect of the invention relates to at least one protein or peptide of the invention for use in the diagnosis of an allergy or a predisposition for an allergy in an individual. It is preferred that the allergy is a milk allergy.

[00019] A different aspect of the invention refers to a method of diagnosing an allergy or a predisposition for an allergy in an individual comprising administering at least one protein or peptide as mentioned herein to an individual suspected of being allergic or becoming allergic and assessing whether the individual developes an allergic reaction against the protein or peptide.

[00020] According to this aspect of the invention it is preferred that the allergy or the predisposition for an allergy is a milk allergy or a predisposition for a milk allergy. The method of this aspect preferably comprises additionally performing a skin test and/or a blood test. The skin test is preferably selected from (i) a skin prick test, (ii) an intradermal test, (iii) a skin patch test, or (iv) any combination of tests of (i) to (iii), wherein a positive result of the tests (i) to (iv) is indicative of an allergy or a predisposition for an allergy in an individual. The blood test preferably comprises the steps of (i) contacting at least one protein or peptide of the invention with a blood sample, serum sample or plasma sample from said individual, and (ii) determining if said at least one protein or peptide binds to an IgE antibody in said blood sample, serum sample or plasma sample, wherein binding of said at least one protein or peptide to an IgE antibody is indicative of an allergy or a predisposition of an allergy in said

individual; and/or (i') contacting at least one protein or peptide of the invention with basophil cells, eosinophil cells or mast cells of said individual, and (ii') determining if said basophil cells, eosinophil cells or mast cells release upon contact with the at least one protein or peptide at least one mediator, or degranulate upon contact with the at least one protein or peptide, wherein release of said at least one mediator upon contact with said at least one protein or peptide or degranulation upon contact with said at least one protein or peptide is indicative of an allergy or a predisposition for an allergy in said individual.

[00021] Another aspect of the invention refers to at least one protein or peptide of the invention for use in an allergen-immunotherapy of an allergy in an individual. It is preferred that the allergy is a milk allergy.

[00022] In a further aspect the invention relates to a method of allergen-immunotherapy for an allergy in an individual comprising administering at least one protein or peptide of the invention. It is preferred that the allergy is a milk allergy.

[00023] In another aspect the invention relates to a method for determining an allergy or a predisposition for an allergy in an individual, the method comprising a) contacting at least one protein or peptide of the invention with a blood sample, serum sample or plasma sample, wherein said blood sample, serum sample or plasma sample is or has been isolated from said individual, and b) determining if said at least one protein or peptide binds to an IgE antibody in said blood sample, serum sample or plasma sample, wherein binding of said at east one protein or peptide to an IgE antibody is indicative for an allergy or a predisposition for an allergy in said individual; and/or a') contacting at least one protein or peptide of the invention with basophil cells, eosinophil cells or mast cells wherein said basophil cells, eosinophil cells or mast cells are or have been isolated from said individual, and b') determining if said basophil cells, eosinophil cells or mast cells (i) release upon contact with the at least one protein or peptide at least one mediator, or (ii) degranulate upon contact with the at least one protein or peptide, wherein release of said at least one mediator upon contact with said at least one protein or peptide or degranulation upon contact with said at least one protein or peptide is indicative for an allergy or a predisposition for an allergy in said individual.

[00024] According to this aspect of the invention it is preferred that the allergy or the predisposition for an allergy is a milk allergy or the predisposition for a milk allergy. Preferably the method according to this aspect further comprises discriminating individuals with a severe allergy from individuals which are sensitized but asymptomatic, and/or discriminating individuals who grow out of an allergy from individuals who do not grow out of an allergy.

[00025] In a further aspect the invention refers to a method for identifying a IgE-reactive non-allergenic milk protein or peptide encoded by a DNA or cDNA of at least one expression library comprising the steps of: a) providing at least one expression library comprising DNA or

cDNA derived from the mammary gland tissue of a lactating mammal, preferably a lactating cow, b) expressing at least one protein or peptide encoded by said expression library, c) determining the binding capacity of said at least one protein or peptide to IgE of at least one serum of an individual who is sensitive to mammalian milk, preferably cow's milk, d) contacting the at least one protein or peptide exhibiting an IgE binding capacity as determined in step c) with basophil cells, eosinophil cells or mast cells, e) determining if said basophil cells, eosinophil cells or mast cells (i) release upon contact with the at least one protein or peptide at least one mediator, or (ii) degranulate upon contact with the at least one protein or peptide, and f) identifying the at least one protein or peptide as being non-allergenic when said basophil cells, eosinophil cells or mast cells (i') do not release upon contact with the at least one protein or peptide at least one mediator, or (ii') do not degranulate upon contact with the at least one protein or peptide.

[00026] According to this aspect of the invention, the at least one protein or peptide identified by the method or specifically disclosed herein is for use in treating an allergy. Also provided in accordance with the present invention is a method of treating an allergy in an individual comprising administering at least one protein or peptide identified by the method or specifically disclosed herein.

[00027] It is preferred in all applicable embodiments of the invention that said individual or patient is a human. The eosinophil cells, mast cells or basophil cells are as a rule mammalian and may be of human origin. In general, the term "lactating mammal" includes mammals of the species goat, sheep, horse, buffalo, camel but is not limited thereto. Cow is a particularly preferred species.

BRIEF DESCRIPTION OF THE DRAWINGS

[00028] Figure 1A shows the deduced amino acid sequences of cDNAs encoding IgE-reactive full-length alphaS1- and alphaS2-casein and IgE-reactive alphaS1- and alphaS2-casein fragments. The amino acid sequence of full-length alphaS1- and alpha-S2-casein are shown at the top. The sequence of IgE-reactive alphaS1- and alphaS2-casein fragments (clone numbers right margin) are displayed as lines. Numbers indicate the first and last amino acid of each clone.

[00029] Figure 1B shows the deduced amino acid sequences of cDNAs encoding IgE-reactive full-length beta-, kappa-casein and beta-lactoglobulin and IgE-reactive fragments of these proteins.

[00030] The amino acid sequence of full-length beta-, kappa-casein and beta-lactoglobulin are shown at the top. The sequence of IgE-reactive fragments (clone numbers right margin)

are displayed as lines. The numbers indicate the first and last amino acid of each clone. Underlined sequences in the beta-casein sequence (top) correspond to non-allergenic peptides identified by mass spectrometric analysis of an extensively hydrolysed hypoallergenic milk formula.

[00031] Figure 1C shows amino acid sequences of alpha-lactalbumin, bovine serum albumin and lactoferrin.

[00032] Figure 2 shows an IgE screening of an expression cDNA library from bovine mammary glands. E.coli cells Y1090 infected with phages carrying the cDNA library were induced to synthesize recombinant proteins. These proteins were transferred to a nitrocellulose filter and exposed to serum IgE from milk allergic patients followed by incubation with 125I-labeled anti-human IgE antibodies. In this autoradiogram of the filter the IgE-reactive clones appear as black dots.

[00033] Figure 3 shows the allergenic activity of the milk samples and components. Biological activity was determined in human RBL assays. The percentages of patients' sera (n = 78) giving a positive reaction in human RBL assays are shown as black bars (y-axis). Analyzed were the following milk components: milk samples from different species (GM, goat's milk; CM, cow's milk; SM, sheep's milk; HM, human milk; MM, mare's milk), the casein fraction of different species (GC, goat casein; CC, cow casein; SC, sheep casein), natural purified proteins (ACalpha-casein; BC, beta-casein; KC, kappa-casein; ALA, alpha-lactalbumin, BLGB, beta-lactoglobulin variant B; BLGA, beta-lactoglobulin variant A; BSA, bovine serum albumin; SSA, sheep serum albumin; hALA, human alpha-lactalbumin; Lf, lactoferrin), recombinant proteins (rAS1C, recombinant alphaS1-casein; rAS2C, recombinant aS2-casein; rBC, recombinant beta-casein; rKC, recombinant kappa-casein; rALA, recombinant alpha-lactalbumin; rBLG, recombinant beta-lactoglobulin), recombinant fragments of BSA (F1, recombinant fragment 1 of BSA; F2, recombinant fragment 2 of BSA; F3, recombinant fragment 3 of BSA) and synthetic peptides of aS1-casein (Cas1 – Cas6).

[00034] Figure 4 shows a list of allergen sequences found in milk.

[00035] Figure 5 shows the separation of a hydrolyzed milk formula using nano liquid chromatography followed by electrospray ionization MS. Presentation of total ion chromatogram of 1µg hydrolyzed milk sample. Retention times are shown on the x-axis and signal intensities in cps (counts per second) are displayed on the y-axis.

[00036] Figure 6 shows a Mascot search and the identification of beta-casein-derived peptides in hydrolyzed cow's milk.

[00037] Figure 7 shows beta-casein-derived peptides identified in hydrolyzed cow's milk as being not IgE-reactive.

DETAILED DESCRIPTION

[00038] It is an object of the present invention to provide a method for identifying allergenic milk proteins and/or peptides comprising the steps of providing at least one expression library comprising DNA or cDNA derived from the mammary gland tissue of a lactating mammal, preferably a lactating cow, expressing at least one protein or peptide encoded by said expression library, determining the binding capacity of said at least one protein or peptide to IgE of at least one serum of an individual who is sensitive to mammalian milk, preferably cow's milk, contacting the at least one protein or peptide exhibiting an IgE binding capacity with basophil cells, eosinophil cells or mast cells, and identifying the at least one protein or peptide as being allergenic when said basophil cells, eosinophil cells or mast cells release at least one mediator upon contact with the at least one protein or peptide.

The present invention also provides a method which comprises the following steps: providing at least one expression library comprising DNA or cDNA derived from at least one allergen source, expressing at least one protein or peptide encoded by said expression library, determining the binding capacity of said at least one protein or peptide to IgE of at least one serum of an individual who is sensitive to the at least one allergen source, contacting the at least one protein or peptide exhibiting an IgE binding capacity with basophil cells, eosinophil cells or mast cells, and identifying the at least one protein or peptide as being allergenic when said basophil cells, eosinophil cells or mast cells release at least one mediator upon contact with the at least one protein or peptide.

[00039] The method according to the present invention is particularly suited to identify proteins and peptides exhibiting allergenic properties. In order to identify allergenic properties of proteins or peptides only amounts in the range of micrograms or nanograms are required. These proteins and peptides are encoded by a DNA or cDNA library which is obtained from a source which is known to contain or synthesize proteins and/or peptides provoking an allergenic reaction in an individual. The allergen source may comprise different types of tissues and cells which may be responsible for the biosynthesis of the allergens. If these cells and tissues are known it is possible to specifically create DNA or cDNA libraries from said cells and tissues, which can be used in the method according to the present invention. For example, milk allergens from a mammal, in particular from a cow, can be identified by isolating DNA or cDNA from the mammary gland tissue of lactating mammals.

[00040] The expressed proteins and peptides of the DNA and cDNA expression library are contacted with IgE of at least one serum of at least one individual who is sensitive to the at least one allergen source. In this first step those proteins and peptides are identified which

are capable to bind to IgE, which is one prerequisite for allergic reactions. The term "IgE-reactive" in accordance with the present invention means the capacity of an amino acid sequence to bind IgE. In a further step the peptides and proteins binding to IgE are further contacted with basophil cells, eosinophil cells or mast cells, which have been previously loaded with IgE from at least one individual which is known to be allergic against at least one allergen of the allergen source. Basophil cells, eosinophil cells or mast cells carrying allergen specific IgE molecules release upon contact with a respective allergen mediators such as histamine and/or other allergic mediators released by basophil cells, eosinophil cells or mast cells, which indicates that the peptides and proteins capable to bind to IgE are also capable to induce degranulation of basophil cells, eosinophil cells or mast cells after being contacted with these cells. Suitable allergenic mediators preferably are histamine, heparin, prostaglandine, leukotriene, chemokines, cytokines. Other allergenic mediators that may be useful in the context of the present invention include ß-hexosaminidase, eosinophil peroxidase, ribonuclease (RNase), deoxyribonuclease, lipase, plasminogen and major basic protein. The person skilled in the art is well aware of mediators released by these cells which can be identified by common textbook knowledge (see for example Janeway *et al.* 2002: Immunology, Spektrum Akademischer Verlag; Auflage: 5. Auflage; Paul *et al.* 1989: Fundamental Immunology, Raven Press Ltd.; Second edition).

[00041] Those members of the DNA or cDNA library which bind to IgE of an allergic individual and are capable to induce degranulation of basophil cells, eosinophil cells or mast cells can be isolated and their DNA or cDNA insert can be sequenced by methods known in the art. Alternatively, the proteins and peptides expressed by the respective library members can be isolated by methods known in the art and analysed by mass spectrometry or amino acid sequencing, for instance.

[00042] The members of the DNA or cDNA library may be derived from any source carrying biological material which is known or unknown to provoke allergenic reactions when contacted with an individual. Hence, the term "allergen source" as used herein refers to any kind of biological material capable to synthesize allergens.

[00043] The term "peptide" or "protein" is intended to mean a sequence of amino acids held together by peptide bonds. "Peptide" as used herein means that the amino acid containing molecule contains essentially up to 250 amino acids, such as up to 200 amino acids, such as up to 150 amino acids, such as up to 100 amino acids, such as up to 50 amino acids, such as up to 45 amino acids, especially such as up to 40 amino acids, such as up to 30 amino acids, such as up to 20 amino acids, and preferably more than 2 amino acid residues. "Protein", as used herein, means that the amino acid containing molecule contains

essentially more than 250 amino acids. In the upper range (i.e. around 250 amino acids), the present invention may use the term protein and peptide for the same type of molecule.

[00044] Allergens (substances which are capable to provoke an allergenic reaction in an individual) are synthesized by various organisms, including plants and animals. According to a preferred embodiment of the present invention the at least one allergen source is an animal, more particularly a mammal. Animals are known to be a source of allergens. These allergens are usually present in animals (e.g. cat and dog) dander or skin flakes, as well as their saliva and urine. Mammals, for instance, secrete allergens also in milk. Consequently mammals like cow, horse, goat, sheep, camel and buffalo may comprise a high amount of allergens. The cDNA and DNA used to identify such allergens may be isolated from lactating mammals, whereby the DNA or cDNA is preferably obtained from the mammary gland tissue.

[00045] Another source of allergens are mites like house dust mites, fish, egg etc. These animals are known to produce substances which cause allergic reaction in individuals.

[00046] A further allergen source are plants. In particular weeds and nuts are known to produce allergens. Of course also trees, like birch, are a source of allergens.

[00047] The DNA (such as genomic DNA and other types of DNA with the exception of cDNA) or cDNA can be obtained from the allergen source such as mammalian milk, preferably cow milk or nutritional formulations containing mammalian milk, preferably cow milk by methods known in the art. These nucleic acid molecules are preferably obtained from cells and tissues which are known or suspected to produce allergens. However, in order to reduce the amount of clones to be screened to identify allergens it is preferred to insert cDNA molecules (cDNA molecules are derived from mRNA) into expression libraries, because these molecules reflect the pool of proteins and peptides expressed in the respective cells and tissue. In accordance with the present invention, any method may be used to prepare a cDNA from a cell that expresses (potentially) the allergen. Such methods are well-known to a person of skill in the art (see, for example, Sambrook et al., "Molecular Cloning: A Laboratory Manual," 2nd ED. (1989) and Ausubel, F. M. et al., "Current Protocols in Molecular Biology," (Current Protocol, 1994)). There are also numerous commercially available kits for obtaining double-stranded cDNA, for example, the Superscript II or Superscript III kit (Invitrogen, USA, catalog # 18580008), the Great Lengths cDNA Synthesis Kit (Clontech, USA, catalog # K-1048-1), the cDNA Synthesis Kit (Stratagene, USA, catalog #200301), and the like.

[00048] The cDNA and DNA molecules may be ligated to linker DNA sequences containing suitable restriction enzyme recognition sites. Such linker DNAs are also known in the art and commercially available, for example, from Promega Corporation, USA and from New England Biolabs, USA. The cDNA and DNA molecules may be further subjected to

restriction enzyme digestion, size fractionation on columns or gels, or any other suitable method known to a person of ordinary skill in the art.

[00049] The cDNA and DNA library is then inserted into expression vectors which may comprise a nucleotide sequence encoding a tag, sequences that direct DNA replication in bacterial cells, sequences that direct DNA transcription and mRNA translation in eukaryotic cells and the like. Suitable expression vectors which comprise the described regulatory elements are known in the art. The cDNA or DNA molecule as described herein above may be designed for direct introduction or for introduction via liposomes, phage vectors or viral vectors (e.g. adenoviral, retroviral) into a cell. A typical mammalian expression vector contains the promoter element, which mediates the initiation of transcription of mRNA, the protein coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. Moreover, elements such as origin of replication, drug resistance gene, regulators (as part of an inducible promoter) may also be included. The *lac* promoter is a typical inducible promoter, useful for prokaryotic cells, which can be induced using the lactose analogue *isopropylthiol-b-D-galactoside*. ("IPTG"). For recombinant expression and secretion, the cDNA or DNA molecule of interest may be ligated (described in Ghahroudi et al, 1997, FEBS Letters 414:521-526). Additional elements might include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription can be achieved with the early and late promoters from SV40, the long terminal repeats (LTRs) from retroviruses, e.g., RSV, HTLVI, HIVI, and the early promoter of the cytomegalovirus (CMV). However, cellular elements can also be used (e.g., the human actin promoter). Alternatively, the recombinant protein or peptide can be expressed in stable cell lines that contain the gene construct integrated into a chromosome. The co-transfection with a selectable marker such as dhfr, gpt, neomycin, hygromycin allows the identification and isolation of the transfected cells. The expression vectors will preferably include at least one selectable marker. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as *E. coli*, *Streptomyces* and *Salmonella typhimurium* cells; fungal cells, such as yeast cells; insect cells such as Drosophila S2 and Spodoptera Sf9 cells; animal cells such as CHO, COS, 293 and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art. The insertion of the cDNA and DNA library (cDNA or DNA pool of a specific cell or tissue) molecules into expression vectors results in expression libraries.

[00050] The expression library used to express the cDNA and DNA molecules of the allergen source is preferably a phage display library or a bacterial expression library. The members ("constructs") of these libraries are then inserted into cells capable to express the proteins and peptides encoded by the cDNA and DNA. Preferred cells are selected on the

type of vectors used to create the cDNA and DNA libraries. If the vectors are designed for bacterial expression, the cells are bacterial cells. The cDNA and DNA libraries are introduced into the cells using methods (e.g. transformation), well-known to a person of ordinary skill in the art and described, e.g., in Sambrook et al., Molecular Cloning: a Laboratory Manual, 2nd Ed., Cold Spring Harbor Press (Cold Spring Harbor, N.Y., 1989). The next steps of culturing bacterial cells to select for transformants and to produce individual bacterial colonies (clones) are well known in the art. Following selection of transformants on agar plates, the cultured bacterial colonies may be picked individually and used to innoculate liquid culture media arranged in arrays in a grid pattern to form gridded bacterial stocks, for example, in 96-well microtiter plates. This arrangement allows representative growth of each bacterial clone in an independent well and facilitates subsequent sub-selection of positive scoring pools of clones.

[00051] It is particularly preferred to use vectors for the expression library which are able to secrete the produced proteins and peptides to the exterior of the cell. The secreted proteins and peptides may further comprise a membrane anchoring domain which allows to immobilise the expressed molecules on the surface of the cells. Methods and means to produce such libraries are known to the person skilled in the art.

[00052] In order to determine the binding of allergen specific IgE molecules to the peptides and/or proteins encoded by the cDNA and DNA libraries the at least one protein or peptide is immobilized on a solid support. The solid support is afterwards contacted with IgE molecules derived from individuals suffering from an allergy and the binding of said IgE molecules on said solid support is determined.

[00053] The binding of IgE to proteins and peptides immobilized on a solid support can be achieved by using antibodies or fragments thereof capable to bind to IgE. Such antibodies are well known in the art.

[00054] The solid support to be used according to the present invention may be a membrane, preferably a nitrocellulose membrane. In a particularly preferred embodiment these membranes are contacted with the bacterial colonies carrying the cDNA or DNA library present on an agar plate in order to form a replica of these colonies. This replica may be used to detect binding of allergen specific IgE to specific colonies.

[00055] Basophil cells, eosinophil cells or mast cells which have been loaded with allergen specific IgE from individuals suffering from an allergy can be used to determine the allergenic potential of the proteins or peptides encoded by the DNA or cDNA library. The basophil cells, eosinophil cells or mast cells may be of any mammal origin, whereby it is preferred to use humanized rat basophil leukaemia (RBL) cells (e.g. clone RBL-703/21). Cells can be humanized by introducing and expressing DNA that encodes all or a portion of a human Fc-receptor, preferably DNA that encodes all or a portion of a human IgE-receptor I. Methods for

the production of humanized cells are known in the art. Preferably the method as described in Hoffmann et al. (loc. lit.) is used for the production of humanized cell cultures. Methods for the production of stripped basophils are described in or Kleine Budde et al. (Int Arch Allergy Immunol, 126(4)).

[00056] After the identification of the proteins/peptides which are able to bind to allergen specific IgE the amino acid sequence of the at least one protein or peptide exhibiting an IgE binding capacity as determined in step c) is preferably determined and the at least one protein or peptide which is able to bind to allergen specific IgE is chemically synthesized or recombinantly produced prior to step d) and may be used in step d).

[00057] According to a preferred embodiment of the present invention the method according to the present invention further comprises a step of determining the amino acid sequence of the at least one protein or peptide identified in step e).

[00058] The amino acid sequence of the proteins or peptides encoded and expressed by the DNA or cDNA library may, e.g., be determined by mass spectrometry or by Edman degradation. If such a method is used, the proteins and/or peptides expressed by the DNA or cDNA library have to be isolated prior to amino acid sequencing. The isolation may be achieved by methods known in the art. In order to facilitate the isolation of the proteins and peptides they may be fused to a tag (e.g. histidine tag). Alternatively, it is also possible to isolate the respective DNA or cDNA clone and to sequence the DNA or cDNA insert.

[00059] Suitable methods for determining an amino acid sequence of the proteins and peptides include, but are not limited to, Edman degradation, (tandem) mass spectrometry and the like (see e.g. Edman, P. Mol. Biol. Biochem. Biophys., (1970), 8: 211-255; US 6,799,121). The amino acid sequence of the proteins and peptides may be compared to amino acid sequences of known proteins.

[00060] The term "mass spectrometry" as used herein includes various methods such as tandem mass spectrometry, matrix assisted laser desorption ionization (MALDI) time-of-flight (TOF) mass spectrometry, MALDI-TOF-TOF mass spectrometry, MALDI Quadrupole-time-of-flight (Q-TOF) mass spectrometry, electrospray ionization (ESI) -TOF mass spectrometry, ESI-Q-TOF, ESI-TOF-TOF, ESI-ion trap mass spectrometry, ESI Triple quadrupole mass spectrometry, ESI Fourier Transform mass spectrometry (FTMS), MALDI-FTMS, MALDI-Ion Trap-TOF, and ESI-Ion Trap TOF. These mass spectrometry methods are well known in the art (see e.g. Gary Siuzdak, "Mass Spectrometry for Biotechnology", Academic Press, NY, (1996)). At its most basic level, mass spectrometry involves ionizing a molecule and then measuring the mass of the resulting ion. Since molecules ionize in a way that is well known, the molecular weight of the molecule can generally be accurately determined from the mass of the ion. Tandem mass spectrometry, for instance, may be used to identify proteins because

it can provide information in addition to parent ion molecular weight. Tandem mass spectrometry involves first obtaining a mass spectrum of the ion of interest, then fragmenting that ion and obtaining a mass spectrum of the fragments. Tandem mass spectrometry thus provides both molecular weight information and a fragmentation pattern that can be used in combination along with the molecular weight information to identify the exact sequence of a peptide or protein (see e.g. Hunt et al. (1986) PNAS USA 83:6233-6237; Shevchenko et al. (1996) PNAS USA 93:14440-14445; Figeys et al. (1996) Anal. Chem. 68:1822-1828 and Wilm et al. (1996) Nature 379:466-469.

[00061] As mentioned, another aspect of the present invention relates to a method for identifying allergenic milk proteins and/or peptides comprising the steps of providing at least one expression library comprising DNA or cDNA derived from the mammary gland tissue of a lactating mammal, preferably a lactating cow, expressing at least one protein or peptide encoded by said expression library, determining the binding capacity of said at least one protein or peptide to IgE of at least one serum of an individual who is sensitive to mammalian milk, preferably cow's milk, contacting the at least one protein or peptide exhibiting an IgE binding capacity with basophil cells, eosinophil cells or mast cells, and identifying the at least one protein or peptide as being allergenic when said basophil cells, eosinophil cells or mast cells release at least one mediator upon contact with the at least one protein or peptide.

In another aspect of the invention the method further comprises a step of determining the amino acid sequence of the at least one protein or peptide identified by the method previously discussed. Thus, the method of the invention for identifying allergic proteins and/or peptides described above is preferably employed for the identification of allergic milk proteins and/or peptides. Accordingly, all of the above definitions and preferred aspect also apply to this aspect of the invention.

[00062] A further aspect of the present invention relates to a method for determining the allergenicity of a cow milk comprising sample comprising the step of determining the presence of at least one of the proteins and peptides of Figures 1A, 1B and 1C (SEQ ID Nos. 22-84) and Table 1A, 1B and Table 2 (SEQ ID Nos. 1-21). The term "mammalian milk, preferably cow milk comprising sample" in accordance with the present application refers to a food sample or a nutrition sample containing mammalian milk, preferably cow milk. Non-limiting examples are (cow) milk, curd, cream, butter, yoghourt and food containing any of these. Within said milk, preferably cow milk comprising sample said at least one of the proteins or peptides is present in an amount which allows for determining the allergenicity of a milk, preferably cow milk comprising sample. Usually amount in the range of micrograms or nanograms of a protein or peptide is sufficient to determine allergenicity.

[00063] The proteins and peptides identified in Figures 1A, 1B and 1C (SEQ ID Nos. 22-84) and Table 1A, 1B and Table 2 (SEQ ID Nos. 1-21) can be used to determine whether a sample comprising cow milk comprises allergenic molecules. If only one of said proteins or peptides is present in a cow milk comprising sample, the sample is considered as being allergenic.

[00064] The method of the present invention is particularly suited to determine the allergenity of a hydrolysed mammalian milk, preferably cow milk.

[00065] The at least one protein and peptide is preferably determined by an immunoassay involving antibodies or fragments thereof binding to the proteins and peptides identified in Figures 1A, 1B and 1C (SEQ ID Nos. 22-84) and Table 1A,1B and Table 2 (SEQ ID Nos. 1-21).

[00066] The at least one protein and/or peptide is preferably determined by mass spectrometry. In order to identify the mammalian milk, preferably cow milk components present in a sample comprising mammalian milk, preferably cow milk, mass spectrometry may be applied. Mass spectrometry allows to identify the milk components as identified in Figures 1A, 1B and 1C (SEQ ID Nos. 22-84) and Table 1A, 1B and Table 2 (SEQ ID Nos. 1-21). A comparison between the fragments present in the sample with the proteins and peptides of Figures 1A, 1B and 1C (SEQ ID Nos. 22-84) and Table 1A, 1B and Table 2 (SEQ ID Nos. 1-21) allows to determine whether said sample comprises allergenic substances derived from cows milk.

[00067] In order to achieve better results the proteins and peptides present in the sample are preferably isolated prior to mass spectrometry. This isolation may be done by an electrophoretic method, preferably two-dimensional electrophoresis, or high performance liquid chromatography.

Screening of product/proteins/peptides in the IgE binding assay (step c)) and basophil (humanized mast cells) degranulation indeed provides data on potential allergenicity, and these methods are used in clinical practice. However, the cDNA expression library data will add a complete list of potential allergenic peptides/peptide sequences for particular allergens (e.g. cow's milk). The present invention combines these methods (improved detection of potential allergenic structures), enabling using mass spectrometry to verify the presence of these potential allergenic structures in product matrices. This combination of a sequence database of allergenic milk proteins and peptides established in combination with IgE reactivity and degranulation (bioactivity) should serve as a basis for the development of a more reliable, sensitive and reproducible method for the assessment and prediction of allergenicity of products containing milk. Although, a similar method has been used to for the identification of grass pollen allergens (Ball et al. 1994, Journal of Biological Chemistry, Vol.

269; Issue of Nov 11, p. 28323-28328) the findings of the present invention are nevertheless surprising, as the skilled person would not have considered that the method according to the teachings of Ball et al. 1994 or a similar method could successfully be applied to milk allergens. To explain further, IgE-binding of grass pollen allergens as well as Ig-E binding to other respiratory allergens depends critically on amino acid residues which are spread over the allergen. Thus, grass pollen allergens and respiratory allergens in general assemble into conformational (discontinous) IgE epitopes. Vrtatla et al. (J. Clin. Invest, Vol 99, No 7, p. 1673-1681) show that loss of this conformation, e.g. in recombinant fragments derived from conformational allergens, leads to drastically reduced capacity of IgE-binding and histamine release from patients' basophils. In contrast to grass pollen allergens and respiratory allergens, milk allergens and food allergens in general have epitopes which are not conformational but unfolded (Järvinen et al., Int Arch Allergy Immunol, Vol 126, p. 111-118 and Järvinen et al., Allergy, Vol 62, p. 758-765). The prior art thus only recognized IgE-binding of unfolded allergens derived e.g. from milk and egg. The release of a mediator from basophil cells, mast cells, or eosinophil cells has not been shown or suggested to the knowledge of the inventors. Considering the teaching of Vrtatla et al (loc. lit.) the skilled person would assume that fragments of allergens and recombinantly produced allergens of milk which do not have IgE-reactivity would also not have the capacity to induce histamine release from patients' basophils. Similarly, considering the teaching of Vrtatla et al (loc. lit.) the skilled person would assume that fragments of allergens and recombinantly produced allergens of milk which do have IgE-reactivity would also have the capacity to induce histamine release from patients' basophils. The step of further testing histamine release of IgE reactive milk proteins and peptides therefore appeared, according to the prior art, dispensible. In contrast, the inventors have surprisingly found that only a method including the steps of IgE reactivity and release of a mediator from basophil cells, mast cells or eosinophil cells can reliably identify allergenic milk proteins and peptides. It is further noted that only the combined testing of IgE reactivity and release of a mediator allows for the reliable assessment of allergenicity of a protein or peptide derived form milk. Depending on the individual to be tested proteins and peptides can either induce the release of a mediator or not. Thus, non-allergenic proteins and peptides which only display IgE-reactivity without mediating release of a mediator from basophil cells, mast cells or eosinophil cells can be distinguished from allergenic proteins or peptides (allergens). Therefore, only the method according to the invention can reliably assess allergenicity of a protein or peptide of the invention for a particular individual, i.e. specifically distinguish from non-allergenic IgE-binding. It was in particular surprising that the methods of the invention led to the identification of the

peptides indicated in Tables 1A, 1B and Table 2 (SEQ ID Nos. 1-22), since these peptides are all unfolded peptides and hence do not have conformational epitopes.

[00068] An improved method to detect potential allergens by using the cDNA expression library followed up a phage peptide expression system and functional screening using IgE binding and IgE mediated basophil cell degranulation, eosinophil cell degranulation or mast cell degranulation. This approach enables monitoring/screening and forecasting the presence of potential food allergens in raw materials, ingredients and finished product for the purpose of developing and manufacturing nutritional formulations.

[00069] The allergenic proteins or peptides specifically disclosed herein or identified by the described methods of the invention may be used to diagnose an allergy or an predisposition to an allergy in a individual, preferably a milk allergy or predisposition for a milk allergy. Accordingly, in one embodiment the invention relates to a method for the diagnosis of an allergy or a predisposition to an allergy in an individual comprising administering at least one allergenic protein or peptide specifically disclosed herein or indentified by the methods described herein to an individual suspected of being allergic or becoming allergic and assessing whether the individual developes an allergic reaction against the protein or peptide. Means and ways of administration are described herein below.

[00070] The diagnosis of an allergy involves in general having a skin or blood test to find out what substance, or allergen, may trigger an allergic response in an individual. Skin tests are usually preferred because they are rapid, reliable, and generally less expensive than blood tests, but either type of test may be used. For a skin test a suitable amount of at least one allergenic protein or peptide specifically disclosed herein or indentified by the methods described herein is placed on or below the skin to determine if a allergic reaction develops. There are three types of skin tests preferred: (1) The skin prick test is done by placing a drop of a solution containing said at least one allergenic protein or peptide (allergen solution) on the skin, and a series of scratches or needle pricks allows the solution to enter the skin. If the skin develops a red, raised itchy area (called a wheal), it usually means that the person is allergic to that allergen. This is called a positive reaction. (2). During the intradermal test, a small amount of the allergen solution is injected into the skin. An intradermal allergy test may be done when a substance does not cause a reaction in the skin prick test but is still suspected as an allergen for that person. (3) For a skin patch test, the allergen solution is placed on a pad that is taped to the skin for about 24 to 72 hours. This test is used to detect a skin allergy called contact dermatitis. In a blood test an allergy in an individual may be determined by the steps of (i) contacting at least one protein or peptide identified by the methods of the invention or specifically disclosed herein with a blood sample, serum sample or plasma sample from said individual, and (ii) determining if said at least one protein or

peptide binds to an IgE antibody in said blood sample, serum sample or plasma sample, wherein binding of said at least one protein or peptide to an IgE antibody is indicative of an allergy or a predisposition of an allergy in said individual; and/or (i') contacting at least one protein or peptide identified by the methods of the invention or specifically disclosed herein with basophil cells, eosinophil cells or mast cells of said individual, and (ii') determining if said basophil cells, eosinophil cells or mast cells release upon contact with the at least one protein or peptide at least one mediator, or degranulate upon contact with the at least one protein or peptide, wherein release of said at least one mediator upon contact with said at least one protein or peptide or degranulation upon contact with said at least one protein or peptide is indicative of an allergy or a predisposition for an allergy in said individual.

[00071] In addition and according to another embodiment of the invention, the allergenic proteins or peptides specifically disclosed herein or identified by the described methods of the invention may be used in an allergen-immunotherapy of an allergy in an individual. Accordingly, an embodiment of the invention relates to a method for allergen-immunotherapy of an allergy in an individual comprising administering at least one allergenic protein or peptide specifically disclosed herein or indentified by the methods described herein. It is preferred that the allergy is a milk allergy.

[00072] Allergen-immunotherapy (also termed hyposensitization therapy, immunologic desensitization or allergen-specific immunotherapy) in accordance with the present invention is a form of immunotherapy for allergic disorders in which the patient is vaccinated with increasingly larger doses of an allergen with the aim of inducing immunologic tolerance. Allergen-immunotherapy is the only treatment strategy which treats the underlying cause of the allergic disorder. It is a highly cost effective treatment strategy which results in an improved quality of life and a reduction in allergic and allergen related symptoms. Immunotherapy has been shown to produce long term remission of allergic symptoms, reduce severity of associated allergic reactions as well as reduce the chances of developing new sensitizations to allergens. This is achieved via immunotherapy modulating the immune system response to allergens. Allergen immunotherapy can either reduce the need for medication, severity of symptoms or eliminate hypersensitivity altogether. Moreover, allergen-specific immunotherapy is the only treatment option that is known to modify the allergy disease process (with a possible chance of curing the disease), whereas other therapies merely suppress the symptoms.

[00073] It is preferred that the at least one protein or peptide of the invention for use in any of the herein described methods or uses for diagnosing or methods and uses of allergen immuno-therapy is present in a pharmaceutical composition. In accordance with the present invention, the term "pharmaceutical composition" relates to a composition for administration to

a patient, preferably a human patient. The pharmaceutical composition of the invention comprises the compounds recited above. It may, optionally, comprise further molecules capable of altering the characteristics of the compounds of the invention thereby, for example, stabilizing, modulating and/or activating their function. The composition may be in solid, liquid or gaseous form and may be, inter alia, in the form of (a) powder(s), (a) tablet(s), (a) solution(s) or (an) aerosol(s). The pharmaceutical composition of the present invention may, optionally and additionally, comprise a pharmaceutically acceptable carrier. Examples of suitable pharmaceutical carriers are well known in the art and include phosphate buffered saline solutions, water, emulsions, such as oil/water emulsions, various types of wetting agents, sterile solutions, organic solvents including DMSO etc. Compositions comprising such carriers can be formulated by well known conventional methods. These pharmaceutical compositions can be administered to the subject at a suitable dose. The dosage regimen will be determined by the attending physician and clinical factors. As is well known in the medical arts, dosages for any one patient depends upon many factors, including the patient's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. The therapeutically effective amount for a given situation will readily be determined by routine experimentation and is within the skills and judgement of the ordinary clinician or physician. The pharmaceutical composition for use in accordance with the present invention can be formulated in conventional manner according to methods found in the art, using one or more physiological carriers or excipient, see, for example Ansel et al., "Pharmaceutical Dosage Forms and Drug Delivery Systems", 7th edition, Lippincott Williams & Wilkins Publishers, 1999. The pharmaceutical composition may, accordingly, be administered orally, parenterally, such as subcutaneously, intravenously, intramuscularly, intraperitoneally, intrathecally, transdermally, transmucosally, subdurally, locally or topically via iontopheresis, sublingually, by inhalation spray, aerosol or rectally and the like in dosage unit formulations optionally comprising conventional pharmaceutically acceptable excipients. The pharmaceutical composition of the invention can be administered as sole active agent or can be administered in combination with other agents.

[00074] In another embodiment the invention relates to a method for determining an allergy or a predisposition for an allergy in an individual comprising contacting at least one protein or peptide disclosed herein or identified by the methods described herein with basophil cells, eosinophil cells or mast cells isolated from said individual, and identifying the at least one protein or peptide as being allergenic in said individual when said basophil cells, eosinophil cells or mast cells release upon contact with the at least one protein or peptide at least one mediator, or degranulate upon contact with the at least one protein or peptide. In a

preferred embodiment of the invention the allergy or predisposition is a milk allergy or a predisposition to a milk allergy. In a further preferred embodiment of the invention these methods for diagnosing an allergy or a predisposition to an allergy further comprises discriminating individuals with a severe allergy from individuals which are sensitized but asymptomatic, and/or discriminating individuals who grow out of an allergy from individuals who do not grow out an allergy. In this regards, the term "to grow out of an allergy" means that the allergy is not persistent but will decrease or preferably disappear when then individual having the allergy is becoming older. In this regard is preferred that the subject grows out of allergy during childhood and is not allergic as adult subject.

[00075] The at least one protein or peptide of the method for determining an allergy or a predisposition for an allergy in an individual may be immobilized on a solid support (e.g. nitrocellulose). The solid support is afterwards contacted with basophil cells, eosinophil cells or mast cells isolated from said individual and the release of a mediator or degranulation is determined. Alternatively, the cells may be immobilized on a solid support and contacted with the at least one protein or peptide. Moreover, the at least one protein or peptide may be a protein or a peptide isolated from its natural source or a recombinant protein or peptide produced by the methods described herein above

[00076] In another embodiment the invention relates to a method for identifying a IgE-reactive non-allergenic milk protein or peptide encoded by a DNA or cDNA of at least one expression library comprising the steps of: a) providing at least one expression library comprising DNA or cDNA derived from the mammary gland tissue of a lactating mammal, preferably a lactating cow, b) expressing at least one protein or peptide encoded by said expression library, c) determining the binding capacity of said at least one protein or peptide to IgE of at least one serum of an individual who is sensitive to mammalian milk, preferably cow's milk, d) contacting the at least one protein or peptide exhibiting an IgE binding capacity as determined in step c) with basophil cells, eosinophil cells or mast cells, e) determining if said basophil cells, eosinophil cells or mast cells (i) release upon contact with the at least one protein or peptide at least one mediator, or (ii) degranulate upon contact with the at least one protein or peptide, and f) identifying the at least one protein or peptide as being non-allergenic when said basophil cells, eosinophil cells or mast cells (i') do not release upon contact with the at least one protein or peptide at least one mediator, or (ii') do not degranulate upon contact with the at least one protein or peptide. In this regard, it is noted that a non-allergenic milk protein or peptide may in general be IgE-reactive but does not trigger the release of a mediator from basophil cells, eosinophil cells or mast cells or does not trigger degranulation of basophil cells, eosinophil cells or mast cells upon contact with said cells. Consequently, whether a milk protein or peptide is allergenic or non-allergenic can be determined by the

methods of the invention. Non-allergenic milk proteins or peptides are defined by their IgE-reactivity but a lack of allergenic activity on these cells.

[00077] The IgE-reactive non-allergenic milk proteins or peptides identified by the method according to the invention may be used to saturate mast cell-bound IgE prior to allergen exposure and may be useful candidates for a safe immunotherapy of allergic diseases. Accordingly, the invention relates in another embodiment to a method for the treatment of an allergy in an individual comprising administering at least one IgE-reactive non-allergenic milk protein or peptide to said individual. It is preferred that the at least one IgE-reactive non-allergenic protein or peptide is formulated in a pharmaceutical composition as described herein above.

The examples illustrate the invention. The broad scope of this invention is best understood with reference to the following examples, which are not intended to limit the inventions to the specific embodiments.

EXAMPLES:

Example 1: *Identification of cDNA sequences coding for IgE-reactive milk components and fragments thereof.*

[00078] For identification of IgE-reactive proteins and IgE-reactive protein fragments contained in cow's milk an expression cDNA library using mammary gland tissue from a lactating cow was first constructed. This library was screened with the sera of cow's milk allergic patients. cDNAs coding for IgE-reactive full length alphaS1-, alphaS2-, beta-, kappa-casein and beta-lactoglobulin as well as IgE-reactive fragments thereof were identified. This is a rather surprising result as the mammalian proteins were produced in a bacterial system that does not add eukaryotic post translational modifications that may sometimes play a role in IgE binding as reported for major house dust mite allergens (Jacquet et al., 2002). The deduced amino acid sequences are shown in Figure 1 A-C (SEQ ID Nos. 22-84). The obtained IgE-reactive allergen fragments allow drawing conclusions about the location of IgE epitopes.

Experimental Protocol

[00079] Bovine mammary glands were obtained from a cow (race "Fleckvieh") and fresh tissue was immediately frozen and stored at -80°C until use. Total RNA from the tissue was isolated and cDNA obtained using a cDNA Synthesis System. The purified cDNA was inserted

into lambda phages using the Lambda gt11/EcoR I/CIAP-Treated/Gigapack III Cloning Kit (Stratagene, La Jolla, CA). *E. coli* Y1090 cells were infected with the phage library, plated onto LB Amp plates (145 mm diameter). Bacterial protein expression was induced by applying a nitrocellulose membrane (Whatman Schleicher & Schuell, Dassel, Germany). The adsorbed proteins were incubated with sera from CMA patients and bound IgE antibodies were detected with a ^{125}I-labled anti-IgE antibody (IBL, Hamburg, Germany). Membranes were exposed to X-ray films (Kodak, Rochester, NY). Positive clones whose proteins were capable of binding human IgE appeared as dark spots on the X-ray films (an example of an autoradiogram of such a membrane is shown in Figure 2).

[00080] Phage DNA of the positive clones was amplified using lambda gt11 forward primer (5' CGG GAT CCC GGT TTC CAT ATG GGG ATT GGT GGC GAC GAC TCC TGG AGC CCG TGA GTA TCG GCG GAA TTC 3') and lambda gt11 reverse primer (5' GAA TTC CAG CTG AGC GCC GGT CGC TAC CAT TAC CAG TTG GTC TGG TGT CAA CGG GAT CGC CG 3') and the amplified PCR products were sequenced. The obtained nucleotide sequences were analyzed by comparing them with a sequence database (http://www.ncbi.nlm.nih.gov/BLAST/).

Example 2: *Discrepancy of allergenic activity and IgE reactivity of milk components*

[00081] In order to evaluate whether a molecule has allergen potential, not only its IgE reactivity but also its biological activity needs to be demonstrated. A scientifically approved model to test biological reactivity of molecules is induction of histamine release from human basophil granulocytes (Purohit 2005). For this purpose a rat basophil leukaemia cell line expressing the α-chain of the human FcεRI receptor (Hoffmann et al., Int Arch Allergy Immunol, Vol 126(4), p. 277-285) was used. Different milk components (n=33) including whole milk extracts from different species, milk fractions, purified natural and recombinant cow's milk proteins, and recombinant protein fragments were tested. The percentages of 78 patients whose serum IgE antibodies trigger β-hexosaminidase release of the humanized RBL cells in response to the individual milk components were determined (Figure 3).

[00082] The most potent cell-activating component was cow's milk with a stimulation rate of 47.4% (Figure 3, panel "milk samples"). But also goat's and sheep's milk gave values of 35.9 and 28.2%, respectively, confirming cross-reactivity of milk proteins from different species. Of the single components, the purified proteins, the caseins (AC, BC, KC) as well as beta-lactoglobulin A (BLGA) showed the highest activities and triggered a release in case of 25 to 34.6% of the patients' sera. Interestingly, the recombinant form of alpha-lactalbumin (rALA) triggered a much higher release than its natural counterpart. Each of the synthetic

peptides had a rather low cell activating potential as stimulation rates of 2.6 to 7.7% were reached. The biological activity was compared to IgE reactivity (Table 2).

[00083] *Table 2:* IgE binding capacity and biological activity of milk components; discrepancy between IgE and allergic activity of milk components. Milk components show higher IgE reactivity than allergenic activity in human RBL assays.

		biological activity (% of patients)	**IgE binding (% of patients)**
milk samples	GM	35,9	69,2
	CM	47,4	85,9
	SM	28,2	82,1
	HM	9,0	17,9
	MM	9,0	29,5
casein fractions	GC	24,4	46,2
	CC	41,0	52,6
	SC	28,2	46,2
natural pruified proteins	AC	25,6	50,0
	BC	34,6	44,9
	KC	25,6	30,8
	ALA	11,5	65,4
	BLGA	28,2	50,0
	BLGB	19,2	51,3
	BSA	2,1	6,4
	SSA	2,6	1,3
	hALA	6,4	30,8
	Lf	2,6	5,1
recombinant proteins	rAS1C	19,2	48,7
	rAS2C	5,1	9,0
	rBC	10,3	55,1
	rKC	6,4	21,8
	rBLG	12,8	23,1
	rALA	21,8	25,6

		biological activity (% of patients)	IgE binding (% of patients)
recombinant BSA fragments	BSA_F 1	4,3	2,1
	BSA_F 2	4,3	8,5
	BSA_F 3	8,5	8,5
synthetic AS1C peptides	Cas 1	6,4	43,6
	Cas 2	3,8	15,4
	Cas 3	2,6	46,2
	Cas 4	3,8	35,9
	Cas 5	5,1	41,0
	Cas 6	7,7	37,2

[00084] The vast majority of the tested milk components like cow's and goats milk, purified natural beta-lactoglobulin variant A and B (BLGA and BLGB) and the recombinant alphaS1- and (rAS1C) alphaS2-casein (rAS2C) showed about two times higher percentages of IgE reactivity than biological activity. Such findings can be explained by the presence of IgE binding epitopes, which are able to bind IgE, but are not able to cross-link receptor, bound IgE and trigger the release of mediators. Only in case of five components (recombinant BSA fragment 3 (F3), recombinant alpha-lactalbumin (rALA), cow casein fraction (CC), natural purified kappa- (KC) and beta-casein (BC)) the percentages of IgE reactivity and the biological activity reached similar values. The extent of degranulation with the synthetic alphaS1-casein-derived peptides was rather low ranging from about a tenth (Cas 3, Cas 4, Cas 5) to one fourth (Cas 2, Cas 6) of the activity of the complete protein.

[00085] It was found that many of the IgE-reactive alphaS1-casein peptides could not induce degranulation in the basophil release assay and thus represent IgE-reactive haptens. Thus, these peptides (haptens) are non-allergenic. This result implies that exclusive IgE testing would lead to the false positive conclusions regarding allergenicity assessment. On the other hand IgE-reactive haptens may be useful as therapeutic agents to saturate mast cell-bound IgE prior to allergen exposure and may be useful candidates for a safe immunotherapy of allergic diseases. AlphaS1-casein-derived peptides that bound IgE and triggered basophil release have been further identified and a few patients' sera were identified that did not show

IgE reactivity to some peptides but induced basophil degranulation with these peptides. The latter result can be explained by the higher sensitivity of the basophil release assay.

[00086] In summary, comparison between measurements of IgE reactivity and biological activity reveals that the patients' sera show higher capacity in IgE binding than in the induction of basophil degranulation. The fact that allergenicity assays reflect the potential of a molecule to cause allergic symptoms implies that the measurement of IgE reactivity alone can not be used to identify with certainty allergenic components, but need to be complemented by a biological assay.

[00087] The combination of both assays, evaluation of IgE binding capacity and biological reactivity, allow to generate a data base containing allergenic proteins/fragments/peptides that can be detected by mass spectrometry. Figure 4 shows a list of sequences of allergenic milk peptides and proteins which represents a prototype of such a data base. Milk components tested for serum IgE reactivity as for allergenic activity are listed. Information regarding the allergen names, the molecular weights (MW) in kilo Dalton (kDa), the function and preparation of the allergens as well as references are listed.

Experimental protocol

[00088] Sera from 78 patients who were selected according to a positive case history, positive skin-prick reactions and determination of specific IgE to cow's milk extract using the CAP-FEIA System (Phadia, Uppsala, Sweden) were used. Sera were obtained from five adults and 73 children (30 female and 43 male) from Austria, Germany, Italy, Spain and France.

[00089] Purified proteins and casein fractions were purchased from Sigma Aldrich (St. Louis, US). Recombinant proteins and recombinant BSA fragments were expressed in *E.coli* as described by (Vrtala et al., 1997). AlphaS1-casein peptides were synthesized on an Applied Biosystems peptide synthesizer Model 433A as described (Focke et al., 2001)

[00090] Humanized Rat Basophil Leukaemia (RBL) cells (clone RBL-703/21) were incubated in microtiter plates overnight at 37°C (7% CO_2, 95% humidity) with 50 µl human sera, diluted 1:10. Afterwards cells were washed and exposed to milk components, diluted to 0.3 µg/ml protein in Tyrodes buffer containing 50% D_2O and 0.1 % BSA/HAS for 1 hour at 37°C (7% CO_2, 95% humidity). Spontaneous release of the cells was also evaluated. Finally, the cell supernatants were incubated with 50 µl assay solution (0.1 M citric acid or sodium citrate, pH 4.5, 160 µM 4-methyl umbelliferyl-N-acetyl-β-D-glucosaminide) in new microtiter plates at 37 °C (7 % CO_2, 95 % humidity) for 1 h. The reactions were stopped by addition of 100 µl glycine buffer per well and the fluorescence measured at λ_{ex}: 360 and λ_{em}: 465 using a

fluorescence microplate reader. The specific hexosaminidase release of each sample was calculated using the formula: $[(Fl_S - Fl_{Sp}) : (Fl_Z - Fl_{Sp})] \times 100$ (where Fl_s is the fluorescence of the sample; Fl_{Sp} is the fluorescence of the spontaneous release and Fl_Z is the fluorescence of the total release).

Example 3: *Identification of proteins and/or peptides with and without allergenic activity in milk samples and in milk derived products by mass spectrometry*

[00091] A sequence database of allergenic milk proteins and peptides established by the combination of IgE reactivity and allergenic activity can serve as a basis for the development of a reliable, reproducible, analytical method for the assessment and prediction of allergenicity of milk samples and milk derived products. Mass spectrometry was used to detect potentially allergenic components in complex milk samples. As an example, mass spectrometry in combination with upstream high-performance liquid chromatography (HPLC) was applied for the analysis of a commercially available, extensively hydrolyzed hypoallergenic milk formula. Analysis of the chromatogram shown in Figure 5 and the extracted mass spectra demonstrated that all proteins had been hydrolyzed to small peptides of a maximum length of 14 amino acids.

[00092] In particular, Figure 5 shows the Separation of a hydrolyzed milk formula using nano liquid chromatography followed by electrospray ionization MS. Presentation of total ion chromatogram of 1µg hydrolyzed milk sample. Retention times are shown on the x-axis and signal intensities in cps (counts per second) are displayed on the y-axis.

[00093] A database search using the MASCOT Search algorithms resulted in the identification of several small peptides of which three were identified as milk-derived allergen, namely beta-casein (Bos d 8 beta, Figure 6). In Figure 6 a Mascot search and the identification of beta-casein-derived peptides in hydrolyzed cow's milk is depicted. At the top beta-casein, the only identified milk allergen in mass spectrometry, is mentioned with its molecular mass and calculated pI. Below the search parameters and the obtained sequence coverage of beta-casein are given. The bottom displays the beta-casein sequence with the matched peptides printed in bold letters. The beta-casein allergen was identified with peptide matches: HQPHQPLPPT (calculated mass of 1.25 kDa, corresponding to aa 160-170 of beta-casein), VYPFPGPIPN (calculated mass of 1.19 kDa, corresponding to aa 74-84 of beta-casein), SSSEE (calculated mass of 0.65 kDa, corresponding to aa 31-36 of beta-casein), and PVVVPP (calculated mass of 0.87 kDa, corresponding to aa 96-103 of beta-casein). In Figure 7 IgE reactivity testing of these peptides is shown (Table 1: cas b-1, 2, 3 and 4; Figure 7). Beta-casein-derived peptides identified in hydrolyzed cow's milk are not IgE-reactive. IgE

reactivity of nitrocellulose-dotted cow's milk (CM), recombinant beta-casein (rBC), synthetic beta-casein-derived peptides (cas b-1, cas b-2, cas b-3, cas b-4), and recombinant alphaS1-casein (rAS1C) were analysed by dot blot assay. Nitrocellulose-bound milk components were incubated with the sera from three cow's milk allergic patients (lanes 1, 2 and 3) and a non-allergic individual (lane C). Bound IgE antibodies were detected with ^{125}I-labeled anti-IgE antibodies. This figure and these results show that they represent non-allergenic peptides, i.e. they were too short to bind IgE antibodies and could be discriminated from allergenic and IgE-reactive peptides as defined in the data base of IgE-reactive and allergenic cow´s milk-derived peptides/proteins. Furthermore, they are different from the published major IgE binding regions of beta-casein (aa 1-16, aa 83-92, aa 135-144; Chatchatee 2001). None of the other IgE-reactive milk allergens and allergen fragments obtained by screening of the cDNA expression library (Figure 1) were detected in the hydrolyzed milk sample which therefore could be identified as a non-allergenic preparation. Hence it was demonstrated - using extensively hydrolyzed milk - that LC-MS analysis can be used for the identification of non-allergenic milk preparations/samples.

[00094] *Table 1.* A) Synthetic peptides of αS1-casein and BSA fragments. The name of the peptides, their amino acid sequence, length, pI and molecular weight in kDa are listed.

Peptide	**Sequence**	**Length (aa)**	**pI**	**MW (kDa)**
Cas1	RPKHPIKHQGLPQEVLNENLLRFFVAPF PEVC	32	8.22	3.75
Cas2	FGKEKVNELSKDIGSESTEDQAMEDIK QMEAES	33	4.18	3.70
Cas3	ISSSEEIVPNSVEQKHIQKEDVPSERYL GYEQLLRC	36	4.80	4.21
Cas4	CLKKYKVPQLEIVPNSAEERLHSMKEGI HAQQKE	34	8.14	3.96
Cas5	CPMIGVNQELAYFYPELFRQFYQLDAY PSGAWYYV	35	4.14	4.24
Cas6	PLGTQYTDAPSFSDIPNPIGSENSEKTT MPLWC	33	3.92	3.60
BSA F1	MRGVFRRDTHKSEIAHRFKDLGEEHFK GLVLIAFSQYLQQCPFDEHVKLVNELTE FAKTCVADESHAGCEKSLHTLFGDELC KVASLRETYGDMADCCEKQEPERNEC	190	5.95	23.9

Peptide	Sequence	Length (aa)	pI	MW (kDa)
	FLSHKDDSPDLPKLKPDPNTLCDEFKA DEKKFWGKYLYEIARRHPYFYAPELLY YANKYNGVFQECCQAEDKGACLLPKIE TMREKVLTSSHHHHHH			
BSA F2	MARQRLRCASIQKFGERALKAWSVAR LSQKFPKAEFVEVTKLVTDLTKVHKEC CHGDLLECADDRADLAKYICDNQDTIS SKLKECCDKPLLEKSHCIAEVEKDAIPE NLPPLTADFAEDKDVCKNYQEAKDAFL GSFLYEYSRRHPEYAVSVLLRLAKEYE ATLEECCAKDDPHACYSTVFDKLKHLV DEHHHHHH	189	5.94	22.6
BSA F3	MPQNLIKQNCDQFEKLGEYGFQNALIV RYTRKVPQVSTPTLVEVSRSLGKVGTR CCTKPESERMPCTEDYLSLILNRLCVLH EKTPVSEKVTKCCTESLVNRRPCFSAL TPDETYVPKAFDEKLFTFHADICTLPDT EKQIKKQTALVELLKHKPKATEEQLKTV MENFVAFVDKCCAADDKEACFAVEGP KLVVSTQTALAHHHHHH	200	7.54	23.4

[00095] B) Synthetic peptides of β-casein. The name of the peptides, their amino acid sequence, length, pI and molecular weight in kDa are listed.

Peptide	Sequence	Length (aa)	pI	MW (kDa)
cas b-1	HQPHQPLPPTV	11	6.92	1.25
cas b-2	VYPFPGPIPNS	11	5.49	1.19
cas b-3	LSSSEE	6	4.24	0.65
cas b-4	PVVVPPFL	8	5.96	0.87

Experimental protocol:

Hydrolyzed milk formula was analyzed by reversed-phase HPLC using a nano liquid chromatography system (Ultimate, LC Packings Dionex, The Netherlands) connected to the nanospray interface of a HCT-Ultra mass spectrometer (Bruker Daltonik, Germany). The obtained mass spectra were searched against the SwissProt protein databank using MASCOT (Matrix Science, London, United Kingdom) software search algorithms. For dot blot analysis 1 µg of protein was dotted onto nitrocellulose, and incubated with sera from milk allergic patients diluted 1:20 in PBST (PBS, 0.5% v/v Tween 20). Bound IgE antibodies were detected with 1:15 diluted ^{125}I-labelled anti-human IgE antibodies (IBL, Germany).

Example 4: *Epitope mapping of bovine alpha-lactalbumin (ALA) to identify IgE reactive ALA-derived peptides*

Methods

[00096] Synthesis of ALA-derived peptides: ALA-derived peptides (Lac1-Lac8) displayed in Table I were synthesized using the Fmoc (9 fluorenyl methoxy carbonyl)-strategy with HBTU [(2-/1H-Benzotriazol-1-yl)1,1,3,3, tetramethyluronium hexafluorophosphat]-activation (0.1 mmol small-scale cycles) on an Applied Biosystems peptide synthesizer Model 433A (Foster City, CA) and purified as described (Focke et al., *Faseb J* 15:2042-2044).

[00097] IgE reactivity testing to ALA-derived peptides:IgE reactivities of the ALA-derived peptides were determined by microarray analysis as described (Schulmeister et al., J Immunol. 182(11):7019-29). In brief, milk components were spotted onto a capillary-flow membrane on an ordinary microscope glass slide and incubated with 25µl of patients' sera. Bound IgE antibodies were detected with a fluorescence-conjugated anti-IgE antibody at a wavelength of 670 nm. The cut off level was set for each patient as the double value of the individual value gained with human serum albumin.

[00098] Results

Identification of ALA epitopes which are reactive with IgE antibodies

In order to identify IgE-reactive epitopes of ALA, we synthesized 8 peptides spanning the ALA sequence (Table 2). The 8 peptides had a length of 19-20 amino acids with 5 amino acid overlaps. The IgE reactivity of the 8 overlapping peptides was evaluated by microarray analysis using sera from the 36 patients showing IgE reactivity to rALA. We found that 30.6% of the patients reacted to Lac1, 33.3% to Lac2, 5.6% to Lac4, 2.8% to Lac5, Lac6, and Lac7, and 11.1% to Lac8 (Table 3). In total 19 of the 36 patients with IgE reactivity to rALA reacted at least with one ALA-derived synthetic peptide.

Table 2. Synthetic ALA-derived peptides[a]

Peptide	Sequence	Length (aa)	pI	MW (kDa)
Lac1	EQLTKCEVFRELKDLKGYG	19	6.34	2.26
Lac2	LKGYGGVSLPEWVCTTFHTS	20	6.74	2.18
Lac3	TFHTSGYDTQAIVQNNDSTE	20	4.31	2.23
Lac4	NDSTEYGLFQINNKIWCKDD	20	4.42	2.40
Lac5	WCKDDQNPHSSNICNISCDK	20	5.30	2.31
Lac6	ISCDKFLDDDLTDDIMCVKK	20	4.11	2.32
Lac7	MCVKKILDKVGINYWLAHKA	20	9.52	2.33
Lac8	LAHKALCSEKLDQWLCEKL	19	6.74	2.23

[a]Abbreviations used in the table: Lac1-Lac8, ALA-derived peptides 1-8; aa, amino acids; pI, isoelectric point; MW, molecular weight; kDa, kilo Dalton.

Table 3. IgE binding capacity of alpha-lactalbumin-derived peptides tested with sera from the 36 patients with IgE reactivity to rALA

n=36		IgE binding (% of patients)
recombinant ALA	rALA	100.0
synthetic ALA peptides	Lac1	30.6
	Lac2	33.3
	Lac3	0
	Lac4	5.6
	Lac5	2.8
	Lac6	2.8
	Lac7	2.8
	Lac8	11.1

The invention furthermore comprises the following items:

1. Method for identifying allergenic proteins and peptides encoded by a DNA or cDNA expression library comprising the steps of:
    a) providing at least one expression library comprising DNA or cDNA derived from at least one allergen source,
    b) expressing at least one protein or peptide encoded by said expression library,

c) determining the binding capacity of said at least one protein or peptide to IgE of at least one serum of an individual who is sensitive to the at least one allergen source,

d) contacting the at least one protein or peptide exhibiting an IgE binding capacity as determined in step c) with basophil cells or mast cells and

e) identifying the at least one protein or peptide as being allergenic when said basophil cells release upon contact with at least one protein or peptide of step d) at least one mediator.

2. Method according to item 1, wherein the at least one allergen source is an animal.

3. Method according to item 2, wherein the animal is a mammal.

4. Method according to item 3, wherein the mammal is a cow.

5. Method according to item 3, wherein the mammal is a cat.

6. Method according to item 3, wherein the mammal is a dog.

7. Method according to item 2, wherein said mammal is a lactating mammal and the DNA or cDNA is obtained from the mammary gland tissue.

8. Method according to item 2, wherein the animal is a mite.

9. Method according to item 1, wherein the animal is a fish.

10. Method according to item 2, wherein the allergen source is an egg.

11. Method according to item 1, wherein the at least one allergen source is a plant.

12. Method according to item 11, wherein the plant is a weed.

13. Method according to item 11, wherein the plant is a nut.

14. Method according to item 11, wherein the plant is a tree.

15. Method according to item 1, wherein the expression library is a phage display library or a bacterial expression library.

16. Method according to item 1, wherein the IgE binding capacity is determined by immobilizing the at least one protein or peptide on a solid support, contacting the solid support with IgE and detecting binding of IgE on said solid support.

17. Method according to item 1, wherein the basophil cells are humanized rat basophil leukaemia (RBL) cells (clone RBL-703/21).

18. Method according to item 1, wherein the amino acid sequence of the at least one protein or peptide exhibiting an IgE binding capacity as determined in step c) is determined and the at least one protein or peptide chemically synthesized or recombinantly produced prior to step d).

19. Method according to item 1, wherein the method further comprises a step of determining the amino acid sequence of the at least one protein or peptide identified in step e).

20. Method according to item 19, wherein the amino acid sequence is determined by mass spectrometry.

21. Method according to item 19, wherein the amino acid sequence is determined by Edman degradation.

CLAIMS

1. A method for identifying allergenic milk proteins and/or peptides comprising the steps of:
   a) providing at least one expression library comprising DNA or cDNA derived from the mammary gland tissue of a lactating cow,
   b) expressing at least one protein or peptide encoded by said expression library,
   c) determining the binding capacity of said at least one protein or peptide to IgE of at least one serum of an individual who is sensitive to cow's milk,
   d) contacting the at least one protein or peptide exhibiting an IgE binding capacity as determined in step c) with basophil cells, eosinophil cells or mast cells and
   e) identifying the at least one protein or peptide as being allergenic when said basophil cells, eosinophil cells or mast cells release upon contact with at least one protein or peptide of step d) at least one mediator.

2. The method according to claim 1, wherein the method further comprises a step of determining the amino acid sequence of the at least one protein or peptide identified in step e).

3. A method for determining the allergenicity of a cow milk comprising sample comprising the step of determining the presence of at least one of the proteins and peptides of any of SEQ ID 1-84.

4. The method according to claim 3, wherein the cow milk is a hydrolysed cow milk.

5. The method according to claim 3 or 4, wherein the presence of the at least one protein and peptide is determined by mass spectrometry.

6. The method according to claim 5, wherein the proteins and peptides present in the sample are isolated prior to mass spectrometry.

7. The method according to claim 6, wherein the proteins and peptides are isolated by an electrophoretic method or by high performance liquid chromatography.

8. The method according to claim 7, wherein the electrophoretic method is two-dimensional electrophoresis.

9. A protein or peptide identified by the method of any of claim 1 or 2.

10. A protein or peptide selected from any of SEQ ID Nos. 1-84.

11. At least one protein or peptide of claims 9 or 10 for use in the diagnosis of an allergy or a predisposition for an allergy in an individual.

12. The at least one protein or peptide of claim 11 for use in the diagnosis of a milk allergy or a predisposition for a milk allergy in an individual.

13. A method of diagnosing an allergy or a predisposition for an allergy in an individual comprising administering at least one protein or peptide of claim 9 or 10 to an individual suspected of being allergic or becoming allergic and assessing whether the individual developed an allergic reaction against the protein or peptide.

14. The method of claim 13, wherein the allergy or the predisposition for an allergy is a milk allergy or a predisposition for a milk allergy.

15. The method of claim 13 or 14, wherein the method additionally comprises:
a) a skin test; and/or
b) a blood test.

16. The method of claim 15,
a) wherein the skin test is selected from
(i) a skin prick test,
(ii) an intra dermal test,
(iii) a skin patch test, or
(iv) any combination of tests of (i) to (iii),
wherein a positive result of the tests (i) to (iv) is indicative of an allergy or a predisposition for an allergy in an individual;
and/or
b) wherein the blood test comprises the steps of
(i) contacting at least one protein or peptide of claims 9 or 10 with a blood sample, serum sample or plasma sample from said individual, and

(ii) determining if said at least one protein or peptide binds to an IgE antibody in said blood sample, serum sample or plasma sample, wherein binding of said at least one protein or peptide to an IgE antibody is indicative of an allergy or a predisposition of an allergy in said individual;

and/or

(i') contacting at least one protein or peptide of claims 9 or 10 with basophil cells, eosinophil cells or mast cells of said individual, and

(ii') determining if said basophil cells, eosinophil cells or mast cells release upon contact with the at least one protein or peptide at least one mediator, or degranulate upon contact with the at least one protein or peptide, wherein release of said at least one mediator upon contact with said at least one protein or peptide or degranulation upon contact with said at least one protein or peptide is indicative of an allergy or a predisposition for an allergy in said individual.

17. At least one protein or peptide of claims 9 or 10 for use in an allergen-immunotherapy of an allergy in an individual.

18. A method of allergen-immunotherapy for an allergy in an individual comprising administering at least one protein or peptide of claim 9 or 10.

19. A method for determining an allergy or a predisposition for an allergy in an individual, the method comprising:

a) contacting at least one protein or peptide of claims 9 or 10 with a blood sample, serum sample or plasma sample, wherein said blood sample, serum sample or plasma sample is isolated from said individual, and

b) determining if said at least one protein or peptide binds to an IgE antibody in said blood sample, serum sample or plasma sample, wherein binding of said at least one protein or peptide to an IgE antibody is indicative for an allergy or a predisposition for an allergy in said individual;

and/or

a') contacting at least one protein or peptide of claims 9 or 10 with basophil cells, eosinophil cells or mast cells wherein said basophil cells, eosinophil cells or mast cells have been isolated from said individual, and

b') determining if said basophil cells, eosinophil cells or mast cells

(i) release upon contact with the at least one protein or peptide at least one mediator, or

(ii) degranulate upon contact with the at least one protein or peptide,

wherein release of said at least one mediator upon contact with said at least one protein or peptide or degranulation upon contact with said at least one protein or peptide is indicative for an allergy or a predisposition for an allergy in said individual.

20. The method of claim 19, wherein the allergy or the predisposition for an allergy is a milk allergy or the predisposition for a milk allergy.

21. The method of claims 19 or 20 further comprising

a) discriminating individuals with a severe allergy from individuals which are sensitized but asymptomatic, and/or

b) discriminating individuals who grow out of an allergy from individuals who do not grow out of an allergy.

22. A method for identifying a IgE-reactive non-allergenic milk protein or peptide encoded by a DNA or cDNA of at least one expression library comprising the steps of:

a) providing at least one expression library comprising DNA or cDNA derived from the mammary gland tissue of a lactating cow,

b) expressing at least one protein or peptide encoded by said expression library,

c) determining the binding capacity of said at least one protein or peptide to IgE of at least one serum of an individual who is sensitive to cow's milk,

d) contacting the at least one protein or peptide exhibiting an IgE binding capacity as determined in step c) with basophil cells, eosinophil cells or mast cells,

e) determining if said basophil cells, eosinophil cells or mast cells

(i) release upon contact with the at least one protein or peptide at least one mediator, or

(ii) degranulate upon contact with the at least one protein or peptide, and

f) identifying the at least one protein or peptide as being non-allergenic when said basophil cells, eosinophil cells or mast cells

(i') do not release upon contact with the at least one protein or peptide at least one mediator, or

(ii') do not degranulate upon contact with the at least one protein or peptide.

23. At least one protein or peptide identified by the method of claim 22 or claim 10 for use in treating an allergy.

24. A method of treating an allergy in an individual comprising administering at least one protein or peptide identified by the method of claim 22 or claim 10.