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(54) **METHOD FOR IDENTIFYING ALLERGENIC PROTEINS AND PEPTIDES**

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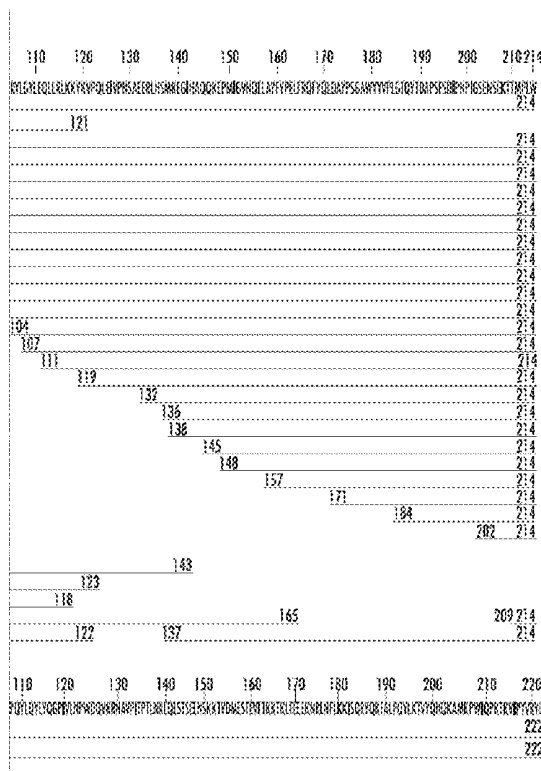
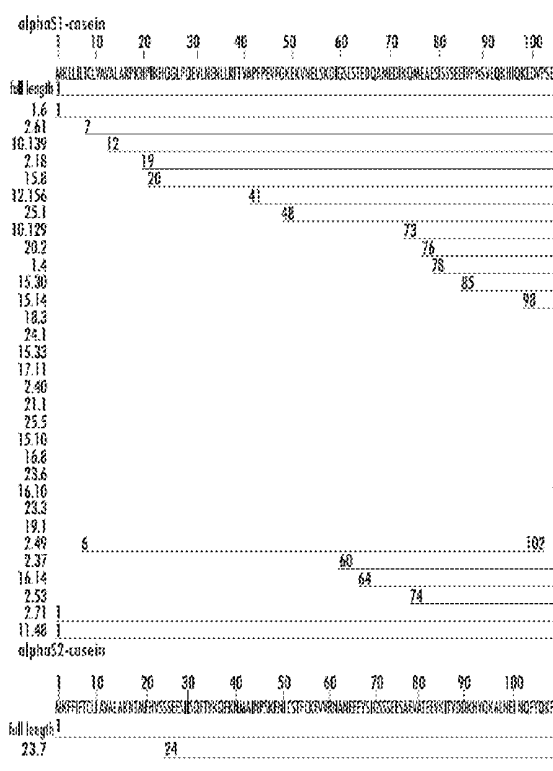
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(57) **ABSTRACT**

A method for identifying allergenic proteins and peptides. More specifically, a method for identifying allergenic milk proteins and/or peptides including the steps of: providing at least one expression library comprising DNA or cDNA derived from the mammary gland tissue of 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 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.



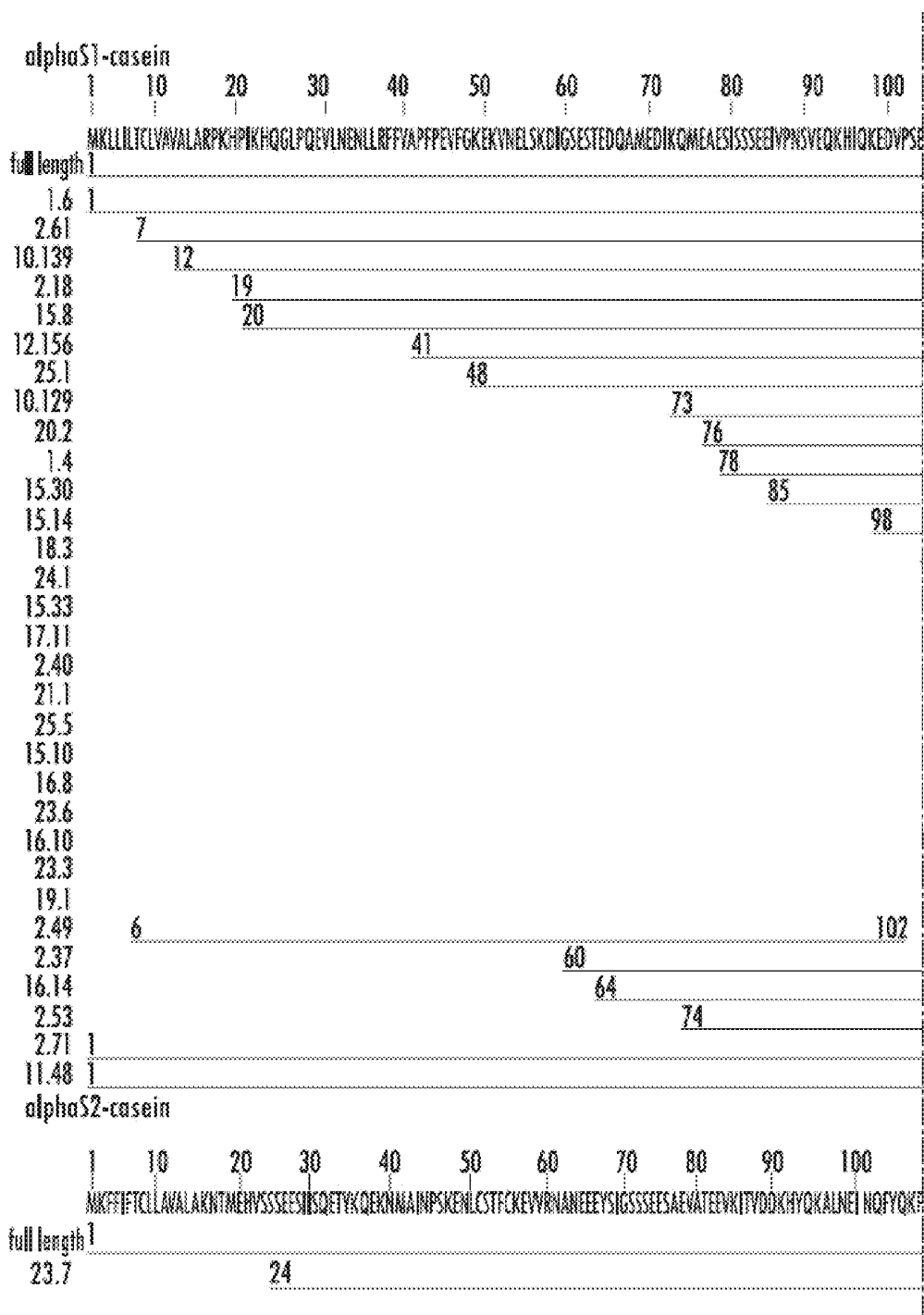
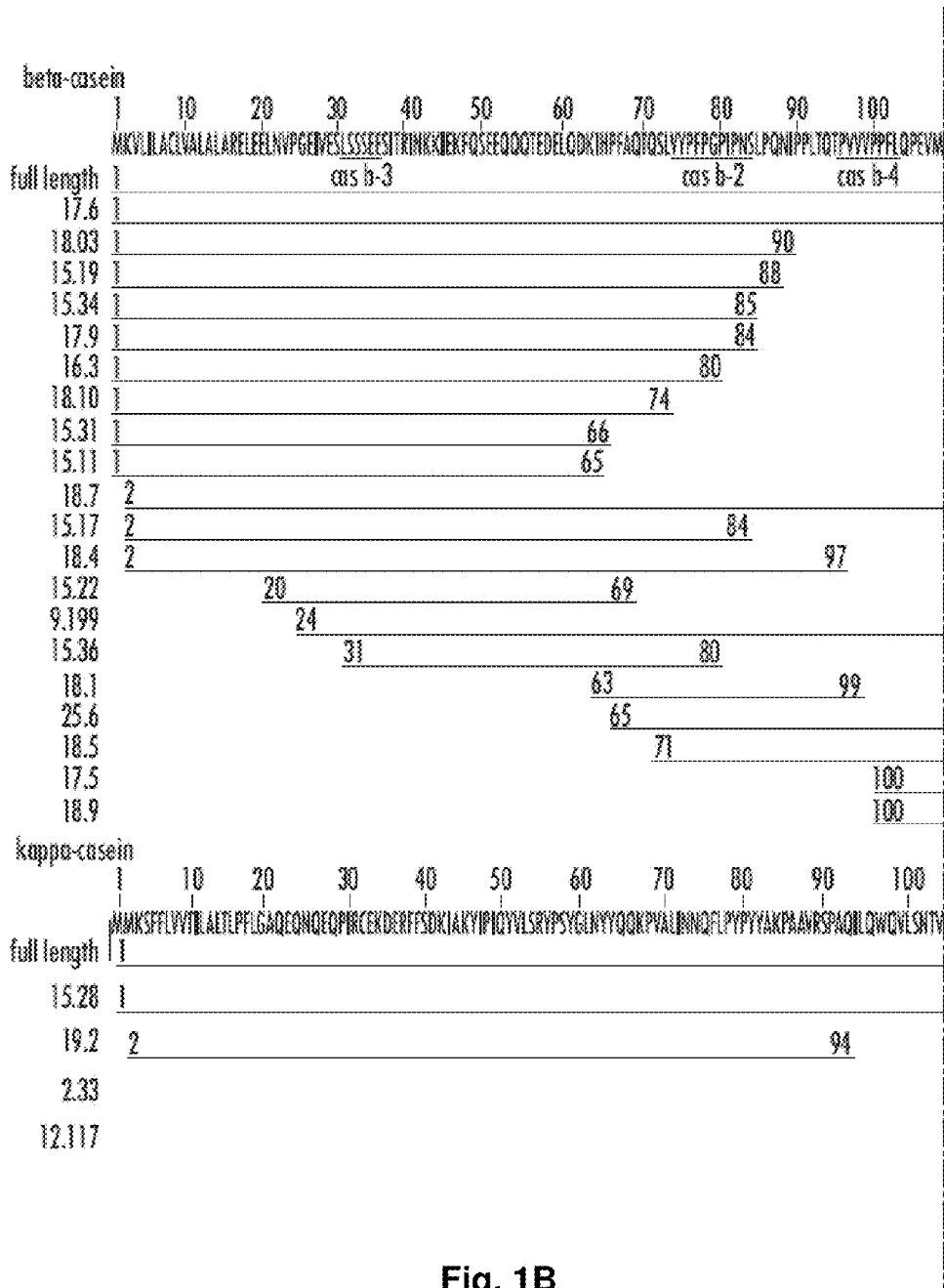


Fig. 1A



Matchline to Figure 1B continued

Fig. 1B

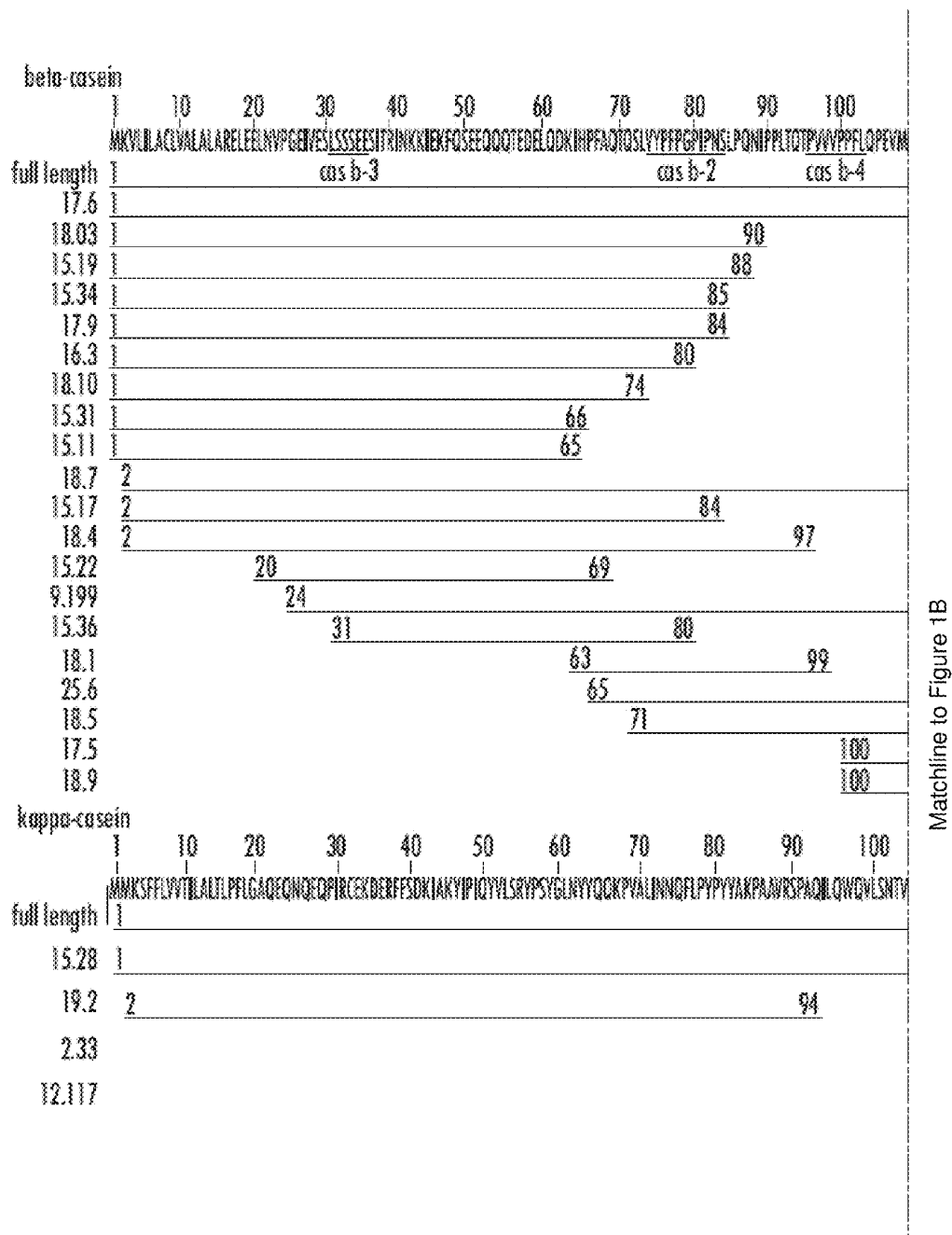


Fig. 1B
continued

alpha-lactalbumin

1 10 20 30 40 50 60 70 80 90
 MSFVSLLLVGLFHATQAEQLTKCEVFRFLKDLKGYGGVSLPEWVCTTFHTSGYDTQALVQNDSTVEYGLFQINNKIWKCKDDQNPSSNICNISCDFLD

bovine serum albumin

1 10 20 30 40 50 60 70 80 90
 MKWYTFISLLLFSSAYSRGVFRDTHKSEIAHRFKDLGEEHFKGLVLIAFSQYLQQCPFDEHVKLVNELEFAKTCVADESHAGCEKSLHTLFGDE
 210 220 230 240 250 260 270 280 290
 KIEIMREKVLTSARQRLRCASIQKFGERALKAWSVARLSQNFPAEFYEVTKLVDTLTKVHKECCHGDILLECADDRADLANVICNDQDTSSKL
 410 420 430 440 450 460 470 480 490
 EPQNLKQNCQQFEKLGEGYGFQNALIVRYTRKVPQVSTPTLVEVSRSLGKVGTRCCTKPESERMPC TEDYLSLILNRLCVLHEKTPYSEKVTKC

lactoferrin

1 10 20 30 40 50 60 70 80 90
 MKLFPALLSLGALGLCLAAPRKNVRWCTISQPEWFKCRRWQWRMKNKLGAPSTCVRRFALECRRAIAEKKADAVTLGGMVFAGRDPYKLR
 210 220 230 240 250 260 270 280 290
 ACSREPYFGYSGAFKCLDQDAGQVAVFKETTVFENLPEKADRDQYELLCLNNSRAPHYDAFKECHLAQVPSHAVVARSSVDGKEDLWKLKSKAQ
 410 420 430 440 450 460 470 480 490
 VLVLKGEADALNLDGGYITAGKCGLVPLAENRKSXKHSIDCVLRPTGEGYLAVAVYKKA NEGLTWNLSKDKRSCHTAYDRTAGWNPMLIVNQ
 610 620 630 640 650 660 670 680
 TEAQSCHLAVAPNHAVVSRSDRAAHVKQVLLHQQALFGKNGKNCDFKCLFKSETKNLLFNDNTECLAKLGGRPYEEYLGTEYV

Matchline to Figure 1C continued

Fig. 1C

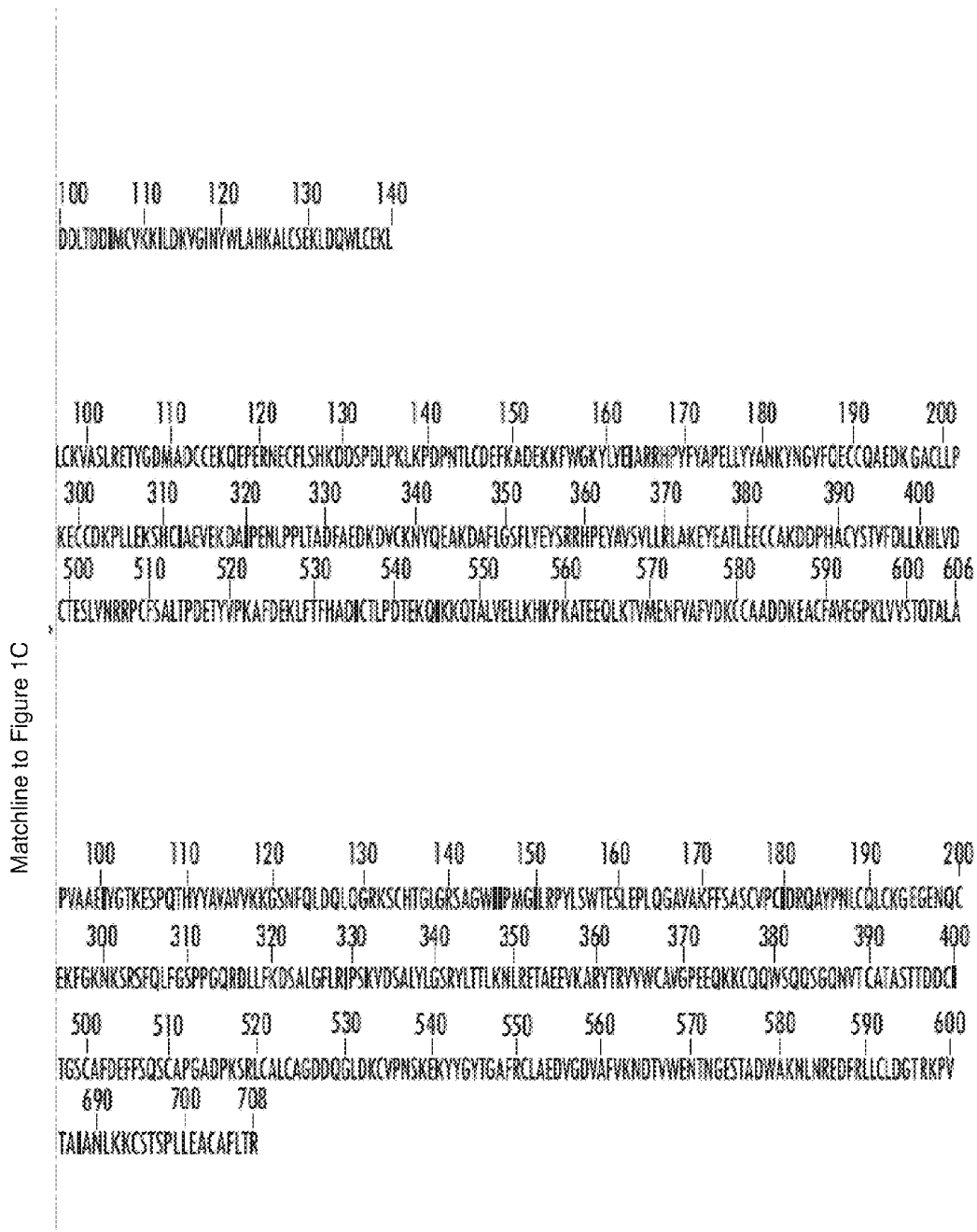


Fig. 1C
continued

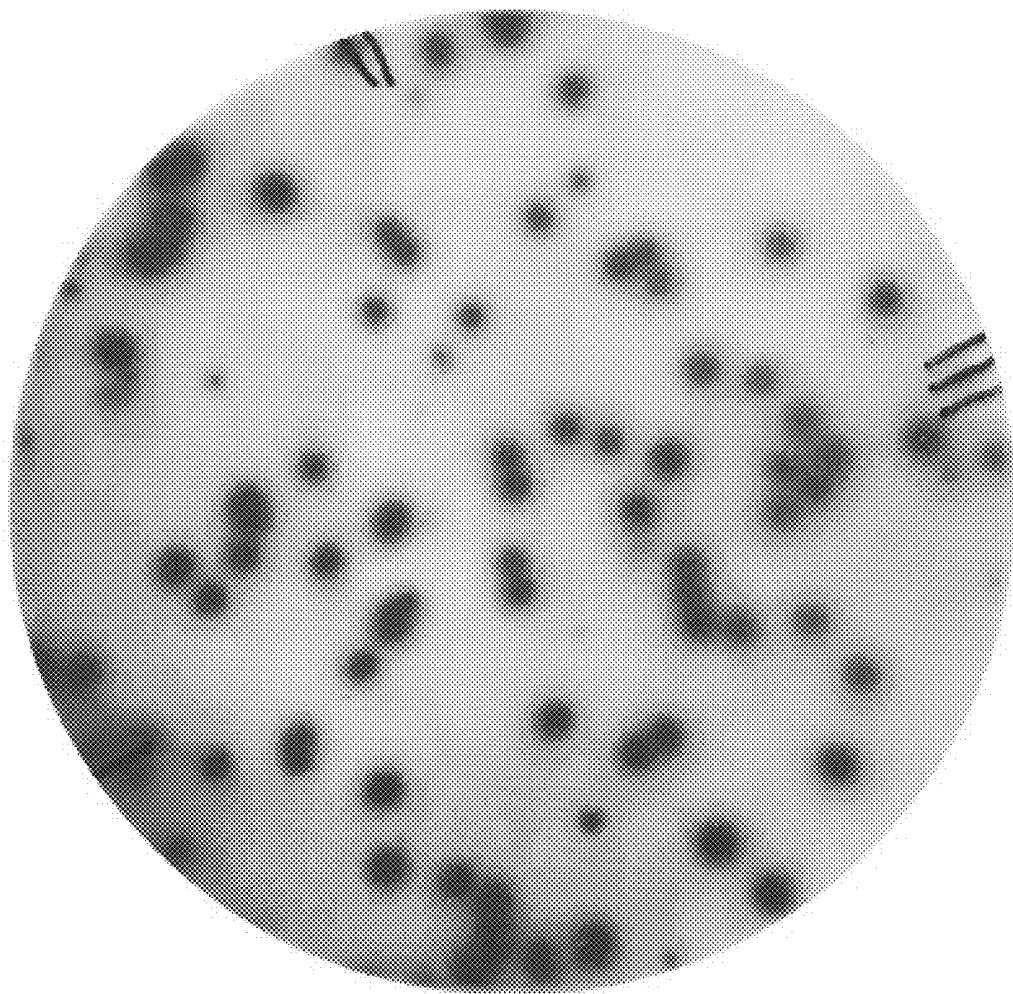


Fig. 2

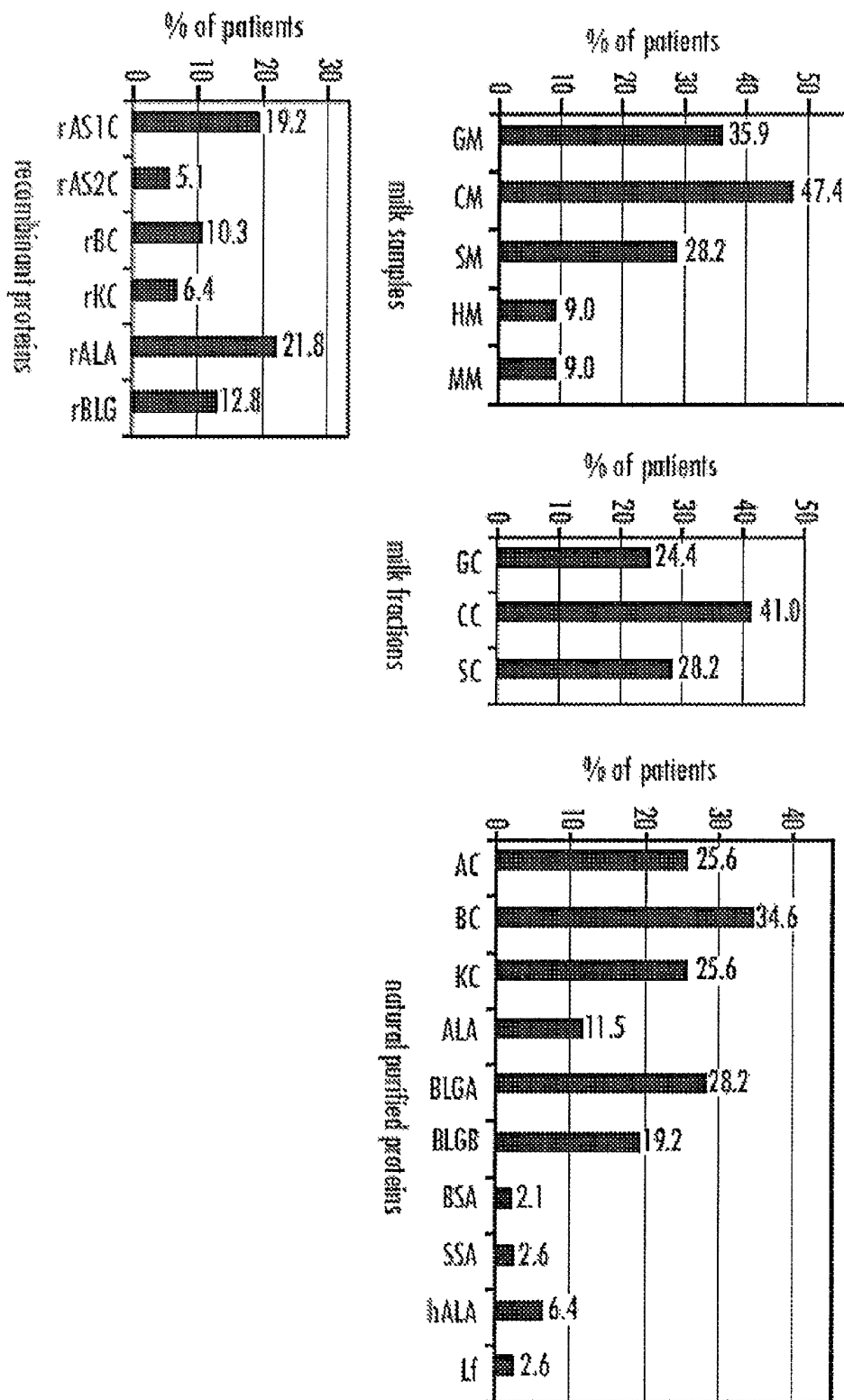


Fig. 3

Milk component	Name	MW (kDa)
purified proteins		
α -casein	Bos d 8 alpha	25.5 (α S1) 26 (α S2)
β -casein	Bos d 8 beta	24.0
κ -casein	Bos d 8 kappa	19.0
α -lactalbumin	Bos d 4	14.2
β -lactoglobulin, variant A	Bos d 5 A	18.3
β -lactoglobulin, variant B	Bos d 5 B	18.3
lactoferrin	Bos d lactoferrin	80
bovine serum albumin	Bos d 6	66.3
sheep serum albumin	Ovi a SSA	24.3
human α -lactalbumin	Hom s lactalbumin	16.2
milk fractions		
cow casein	Bos d 8	19 - 26
sheep casein	Ovi a casein	24.3
goat casein	Cap h casein	24.3
recombinant proteins		
τ α S1-casein	Bos d 8 alphaS1	23.9
τ α S2-casein	Bos d 8 alphaS2	25.3
τ β -casein	Bos d 8 beta	24.5
τ κ -casein	Bos d 8 kappa	19.9
τ α -lactalbumin	Bos d 4	19.2
τ β -lactoglobulin	Bos d 5 B	15.1
recombinant fragments		
BSA fragment 1, AA 1-190		23.9
BSA fragment 2, AA 200-389		22.6
BSA fragment 3, AA 390-590		23.4
α S1-casein peptides		
Cas1, AA 1-31		3.8
Cas2, AA 32-64		3.8
Cas3, AA 65-100		4.3
Cas4, AA 101-133		3.9
Cas5, AA 134-167		4.2
Cas6, AA 168-199		3.6
α -lactalbumin peptides		
Lac1, AA 1-19		2.3
Lac2, AA 15-34		2.2
Lac3, AA 30-49		2.2
Lac4, AA 45-64		2.4
Lac5, AA 60-79		2.3
Lac6, AA 75-94		2.3
Lac7, AA 90-109		2.3
Lac8, AA 105-123		2.2
whole milk samples		
cow's milk	Bos d	
sheep's milk	Ovi a	
goat's milk	Cap h	
mare's milk	Equ c	
human milk	Hom s	

Matchline to Figure 6b

Fig. 4a

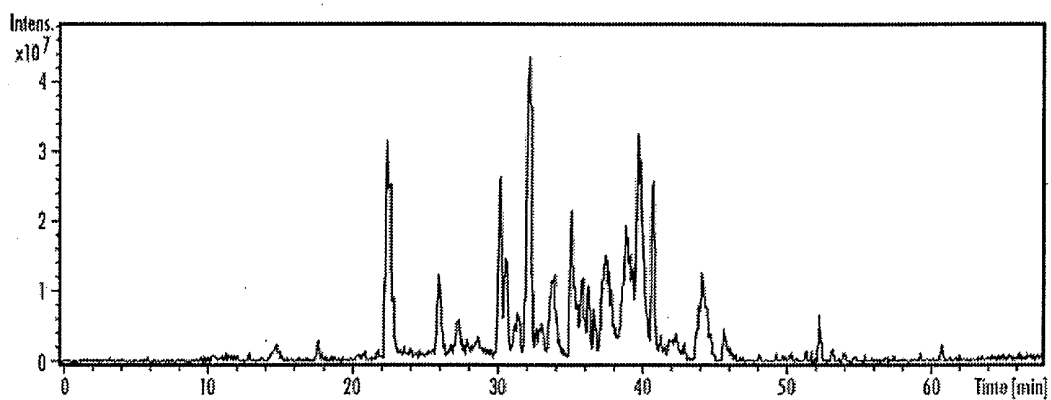


Fig. 5

Mascot Search Results

Protein View

Match to: **CASB_BOVIN** Score: 144

Beta-casein precursor - Bos taurus (Bovine)

Found in search of D:\Data\milk 0.3171.07.mgf

Nominal mass (Mr): **25149** ; Calculated pI value: **5.26**

NCBI BLAST search of CASB_BOVIN against nr

Unformatted sequence string for pasting into other applications

Taxonomy: Bos taurus

Fixed modifications: Carboxymethyl (C) ↕

Variable modifications: Oxidation (M) ↕

No enzyme cleavage specificity

Sequence Coverage: **11%**

Matched peptides shown in **Bold**

1 MKVLILACLV ALALARELEE LNVPGEIVES LSSSEESITR INKKIEKFQS
51 EEQQQTEDEL QDKIHPPAQT QSL **VYFPGP** IPNSLPQNIP PLTQT **PVVVP**
101 P FLQPEVMGV SKVKEAMAPK HKEMPPPKYP VEPFTESQSL TLTDVENLHL
151 PLPLLOSWM H **QPHQPLPPTV** MFPPQSVLSL SQSKVLPVPQ KAVPYPQRDM
201 PIQAFLLYQE PVLGPVRGPF PIIV

Fig. 6

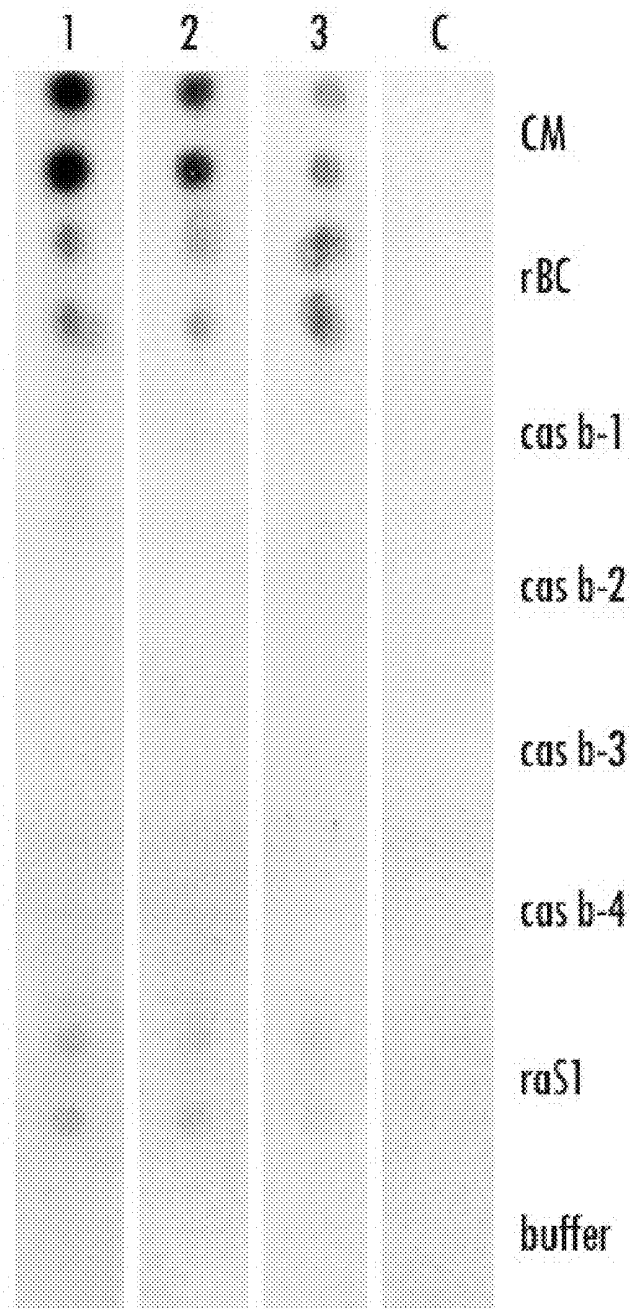


Fig. 7

METHOD FOR IDENTIFYING ALLERGENIC PROTEINS AND PEPTIDES

RELATED APPLICATION

[0001] This application claims priority from and benefit of U.S. Provisional Patent Application Ser. No. 61/196,416, entitled Method For Identifying Allergenic Proteins And Peptides, filed Oct. 17, 2008, the disclosure of which is hereby incorporated by reference herein in its entirety.

TECHNICAL FIELD

[0002] The present disclosure relates to a method for identifying allergenic proteins and peptides. More specifically, the present disclosure 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 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 cow's milk, contacting the at least one protein or peptide exhibiting an IgE binding capacity as determined 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.

BACKGROUND OF THE DISCLOSURE

[0003] 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.

[0004] 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.

[0005] 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.

[0006] 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.

[0007] 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.

[0008] 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).

[0009] 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.

[0010] 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.

[0011] 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.

[0012] The present disclosure addresses the need to provide methods which allow to identify allergenic proteins and peptides present in a biological source. The present disclosure thereby also addresses the need to provide methods which allow one to identify non-allergenic proteins and peptides present in a biological source, in particular in cow milk and nutritional formulations containing cow milk. Moreover, the disclosure provides methods for diagnosing and treating an allergy in an individual.

SUMMARY OF THE DISCLOSURE

[0013] An embodiment of the present disclosure relates to a method for identifying allergenic milk proteins and pep-

tides, which comprises the steps of providing at least one expression library comprising DNA or cDNA derived from the mammary gland tissue of 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 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. Thus, the disclosure in another embodiment 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 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 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. In even other words the disclosure relates to the method as described in paragraph [0012], 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 cow, and the individual who is sensitive to the allergenic source is an individual who is sensitive to cow milk.

[0014] According to a further embodiment the disclosed methods can further comprise a step of determining the amino acid sequence of the at least one protein or peptide identified by the methods.

[0015] The present disclosure 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 cow milk and nutritional formulations containing 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 disclosure 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.

[0016] A further embodiment 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 FIGS. 1A, 1B and 1C (SEQ ID Nos. 22-84) and Table 1A, 1B and Table 2 (SEQ ID Nos. 1-21).

[0017] According to this embodiment of the disclosure, the cow milk can be a 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.

[0018] Another embodiment of the disclosure relates to a protein or peptide identified by the method. A further embodiment relates to a protein or peptide selected from FIGS. 1A, 1B, 1C (SEQ ID Nos 22-84), Tables 1A, 1B, or Table 2 (SEQ ID Nos. 1-21).

[0019] A further embodiment relates to at least one protein or peptide 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.

[0020] A different embodiment of the disclosure 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 developed an allergic reaction against the protein or peptide.

[0021] According to this embodiment, 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 embodiment preferably comprises additionally 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 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 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.

[0022] Another embodiment of the disclosure refers to at least one protein or peptide for use in an allergen-immunotherapy of an allergy in an individual. It is preferred that the allergy is a milk allergy.

[0023] In a further embodiment the disclosure relates to a method of allergen-immunotherapy for an allergy in an individual comprising administering at least one protein or peptide of the disclosure. It is preferred that the allergy is a milk allergy.

[0024] In another embodiment the disclosure 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 disclosure 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 the disclosure 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.

[0025] According to this embodiment, 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 embodiment 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.

[0026] In a further embodiment the disclosure 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 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) 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 the at least one mediator upon contact with the at least one protein or peptide or degranulation upon contact with the at least one protein or peptide is indicative for an allergy or a predisposition for an allergy in said individual.

[0027] According to this embodiment, 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 disclosure 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.

[0028] It is preferred in all applicable embodiments of the disclosure 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.

BRIEF DESCRIPTION OF THE DRAWINGS

[0029] FIG. 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 alphaS2-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.

[0030] FIG. 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.

[0031] 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.

[0032] FIG. 1C shows amino acid sequences of alpha-lactalbumin, bovine serum albumin and lactoferrin.

[0033] FIG. 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.

[0034] FIG. 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 alphaS2-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 alphaS1-casein (Cast1-Cas6).

[0035] FIG. 4 shows a list of allergen sequences found in milk.

[0036] FIG. 5 shows the separation of a hydrolyzed milk formula using nano liquid chromatography followed by electrospray ionization MS. Presentation of total ion chromatogram of 1 μ g 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.

[0037] FIG. 6 shows a Mascot search and the identification of beta-casein-derived peptides in hydrolyzed cow's milk.

[0038] FIG. 7 shows beta-casein-derived peptides identified in hydrolyzed cow's milk as being not IgE-reactive.

DETAILED DESCRIPTION

[0039] One aspect of the present disclosure is 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 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 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.

[0040] The present disclosure 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.

[0041] The method according to the present disclosure 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 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 disclosure. 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.

[0042] 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 disclosure 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 disclosure include β -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).

[0043] 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 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.

[0044] 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.

[0045] 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, the present disclosure may use the term protein and peptide for the same type of molecule.

[0046] Allergens (substances which are capable to provoke an allergic reaction in an individual) are synthesized by various organisms, including plants and animals. According to a preferred embodiment of the present disclosure 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 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.

[0047] 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.

[0048] 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.

[0049] 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 cow milk or nutritional formulations containing 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 disclosure, 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.

[0050] 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.

[0051] 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 isopropylthio- β -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, HTLV1, HIV1, 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.

[0052] 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 inoculate 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.

[0053] 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.

[0054] 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.

[0055] 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.

[0056] The solid support to be used according to the present disclosure 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.

[0057] 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)).

[0058] 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).

[0059] According to a preferred embodiment of the present disclosure, the method further comprises a step of determining the amino acid sequence of the at least one protein or peptide identified in step e).

[0060] The amino acid sequence of the proteins or peptides encoded and expressed by the DNA or cDNA library may 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.

[0061] 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; U.S. Pat. No. 6,799,121). The amino acid sequence of the proteins and peptides may be compared to amino acid sequences of known proteins.

[0062] 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).

[0063] As mentioned, another embodiment of the present disclosure 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 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 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.

[0064] In another embodiment of the disclosure 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 disclosure 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 embodiments also apply to this embodiment of the disclosure.

[0065] A further embodiment of the present disclosure 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 FIGS. 1A, 1B and 1C (SEQ ID Nos. 22-84) and Table 1A, 1B and Table 2 (SEQ ID Nos. 1-21). The term "cow milk comprising sample" in accordance with the present application refers to a food sample or a nutrition sample containing cow milk. Non-limiting examples are cow milk, curd, cream, butter, yoghurt and food containing any of these. Within said cow milk comprising sample said at least one of the proteins and peptides is present in an amount which allows for determining the allergenicity of a cow milk comprising sample. Usually amount in the range of micrograms or nanograms of a protein or peptide is sufficient to determine allergenicity.

[0066] The proteins and peptides identified in FIGS. 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.

[0067] The method of the present disclosure is particularly suited to determine the allergenicity of a hydrolysed cow milk.

[0068] 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 FIGS. 1A, 1B and 1C (SEQ ID Nos. 22-84) and Table 1A, 1B and Table 2 (SEQ ID Nos. 1-21).

[0069] The at least one protein and/or peptide is preferably determined by mass spectrometry. In order to identify the cow milk components present in a sample comprising cow milk mass spectrometry may be applied. Mass spectrometry allows to identify the milk components as identified in FIGS. 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 FIGS. 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.

[0070] 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.

[0071] 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 disclosure 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 November 11, p. 28323-28328) the findings of the present disclosure 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 (discontinuous) 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, dispensable. 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 from 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 disclosure can reliably assess allergenicity of a protein or peptide of the disclosure for a particular individual, i.e. specifically distinguish between non-allergenic IgE. It was in particular surprising that the methods of the disclosure 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.

[0072] 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.

[0073] The allergenic proteins or peptides specifically disclosed herein or identified by the described methods of the disclosure may be used to diagnose an allergy or a predisposition to an allergy in an individual, preferably a milk allergy or predisposition for a milk allergy. Accordingly, in one embodiment the disclosure 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 identified by the methods described herein to an individual suspected of being allergic or becoming allergic and assessing whether the individual developed an allergic reaction against the protein or peptide. Means and ways of administration are described herein below.

[0074] 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 identified 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 disclosure 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 disclosure 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.

[0075] In addition and according to another embodiment of the disclosure, the allergenic proteins or peptides specifically disclosed herein or identified by the described methods of the disclosure may be used in an allergen-immunotherapy of an allergy in an individual. Accordingly, an embodiment of the disclosure 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 identified by the methods described herein. It is preferred that the allergy is a milk allergy.

[0076] Allergen-immunotherapy (also termed hyposensitization therapy, immunologic desensitization or allergen-specific immunotherapy) in accordance with the present disclosure 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.

[0077] It is preferred that the at least one protein or peptide of the disclosure 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 disclosure, the term "pharmaceutical composition" relates to a composition for administration to a patient, preferably a human patient. The pharmaceutical composition of the disclosure comprises the compounds recited above. It may, optionally, comprise further molecules capable of altering the characteristics of the compounds of the disclosure 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 disclosure 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 disclosure 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 iontophoresis, 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 disclosure can be administered as sole active agent or can be administered in combination with other agents.

[0078] In another embodiment the disclosure 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 meth-

ods 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 disclosure the allergy or predisposition is a milk allergy or a predisposition to a milk allergy. In a further preferred embodiment of the disclosure 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 of 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.

[0079] 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

[0080] In another embodiment the disclosure 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: providing at least one expression library comprising DNA or cDNA derived from the mammary gland tissue of 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 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 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 the at least one mediator upon contact with the at least one protein or peptide or degranulation upon contact with the at least one protein or peptide is indicative for an allergy or a predisposition for an allergy in said individual. In this regard, it is noted that a non-allergenic milk protein or peptide is in general 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 disclosure. Non-allergenic milk proteins or peptides are defined by their IgE-reactivity but a lack of allergenic activity on these cells.

[0081] The IgE-reactive non-allergenic milk proteins or peptides identified by the method according to the disclosure

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 disclosure 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.

[0082] 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

[0083] 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 FIG. 1A-C (SEQ ID Nos. 22-84). The obtained IgE-reactive allergen fragments allow drawing conclusions about the location of IgE epitopes.

Experimental Protocol

[0084] 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, Calif.). *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 -labeled anti-IgE antibody (IBL, Hamburg, Germany). Membranes were exposed to X-ray films (Kodak, Rochester, N.Y.). 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 FIG. 2).

[0085] 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 ana-

lyzed 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

[0086] 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 Fc ϵ RI 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 (FIG. 3).

[0087] The most potent cell-activating component was cow's milk with a stimulation rate of 47.4% (FIG. 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).

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 purified 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

TABLE 2-continued

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)
recombinant BSA fragments	BSA_F1	4.3	2.1
	BSA_F2	4.3	8.5
	BSA_F3	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

[0088] 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.

[0089] 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.

[0090] 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.

[0091] 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. FIG. 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

[0092] 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.

[0093] 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)

[0094] Humanized Rat Basophil Leukaemia (RBL) cells (clone RBL-703/21) were incubated in microtiter plates overnight at 37° C. (7% CO₂, 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% D₂O and 0.1% BSA/HAS for 1 hour at 37° C. (7% CO₂, 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% CO₂, 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: [(F_{1s}-F_{1sp}): (F_{1z}-F_{1sp})]×100 (where F_{1s} is the fluorescence of the sample; F_{1sp} is the fluorescence of the spontaneous release and F_{1z} 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

[0095] 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 FIG. 5 and the extracted mass spectra demonstrated that all proteins had been hydrolyzed to small peptides of a maximum length of 14 amino acids.

[0096] In particular, FIG. 5 shows the Separation of a hydrolyzed milk formula using nano liquid chromatography followed by electrospray ionization MS. Presentation of total ion chromatogram of 1 µg 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.

[0097] 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, FIG. 6). In FIG. 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: HQPHQLPPT (calculated mass of 1.25 kDa, corresponding to aa 160-170 of beta-casein), VYFPFGPIPN (calculated mass of 1.19 kDa, corresponding to aa 74-84 of beta-casein), SSSSE (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 FIG. 7 IgE reactivity testing of these peptides is shown (Table 1: cas b-1, 2, 3 and 4; FIG. 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 (rASIC) 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 ¹²⁵I-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 (FIG. 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.

TABLE 1

Pep- tide	Sequence	Length (aa)	pI	MW (kDa)
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.				
Cas1	RPKHPKIQGLPQEVLNENLLRFFVA PFPEVC	32	8.22	3.75
Cas2	FGKEKVNELSKDIGSESTEDQAMEDI KQMEAES	33	4.18	3.70
Cas3	ISSSEIIVPNSVEQKHIQKEDVPSE YLGYEQLLRC	36	4.80	4.21
Cas4	CLKKYKVPQLEIVPNSAEERLHSMK EGIHIAQOKE	34	8.14	3.96

TABLE 1-continued

Pep-tide	Sequence	Length (aa)	pI	MW (kDa)
A) Synthetic peptides of α S1-casein and BSA fragments. The name of the peptides, their amino acid sequence, length, pI and molecular weight in kDa are listed.				
Cas5	CPMIGVNQELAYFYPELFRQFYQLD AYPSGAWYYV	35	4.14	4.24
Cas6	PLGTQYTDAPSFSDIPNPIGSENSE KTTMPLWC	33	3.92	3.60
BSA F1	MRGVFRRDRTHKSEIAHRFKDLGEEH FKGLVLIAPFQYLVQCPDFEHVKLNV ELTEFAKTCVADESHAGCEKSLHTLF GDELCKVASLRETYGDMADCCCKQ EPERNECFLSHKDDSPDLKPKLPDPN TLCDEPKADEKKFWGKLYEYIARRH PYFYAPELLEYANKYNGVFQECQQA EDKGACLLPKIETMREKVLTSHHH HHH	190	5.95	23.9
BSA F2	MARQRLRCASIQKFGERALKAWSV ARLSQKEPKAEFVEVTKLVDTDLTKV HKECCHGDLLECADDRADLAKYICD NQDTISSKLKCECCDKPLLEKSHCIAE VEKDAIPENLPLPLTADFAEDKDVCK NYQEAQDAFLGSFLYEYSRRHPEYA VSVLLRLAKEYEATLECCAKDDPH ACYSTVFDKLLKHLVDEHHHHH	189	5.94	22.6
BSA F3	MPQNLIKQNCDOFEKLGEGYGFQNAL IVRYTRKVPQVSTPTLVEVSRSLGKV GTRCCTKPESEMPCTEDYLSLWNR LCVLHEKTPVSEKVTCCCTESLVNR RPCFSALTPDETYVPKAFDEKLFTFH ADICTLPDTEKQIKKQATLVLELLKHK PKATEEQLKTVMENFVAFVKCCA ADDKEACFAVEGPKLVVSTQTALAH HHHHH	200	7.54	23.4
B) Synthetic peptides of β -casein. The name of the peptides, their amino acid sequence, length, pI and molecular weight in kDa are listed.				
cas b-1	HQP HQPLPPTV	11	6.92	1.25
cas b-2	VYPFP GPIIPNS	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:

[0098] 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 μ g 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 ¹²⁵I-labelled anti-human IgE antibodies (IBL, Germany).

Example 4

Epitope Mapping of Bovine Alpha-Lactalbumin (ALA) to Identify IgE Reactive Ala-Derived Peptides

Methods

[0099] 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, Calif.) and purified as described (Focke et al., *Faseb J* 15:2042-2044).

[0100] 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 25 μ l 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.

[0101] Results

Identification of ALA Epitopes which are Reactive with IgE Antibodies

[0102] 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	EQLTKCEVFPRELKDLKGYG	19	6.34	2.26
Lac2	LKGYGGVSLPEWVCTTFHTS	20	6.74	2.18
Lac3	TFHTSGYDTQA1YQNNDSSTE	20	4.31	2.23
Lac4	NDSTEYGLFPQINNK1WCKDD	20	4.42	2.40
Lac5	WCKDDQNPSSNICNISCDK	20	5.30	2.31
Lac6	ISCDKFLDDDLTDDIMCVKK	20	4.11	2.32

TABLE 2-continued

Synthetic ALA-derived peptides ^a				
Peptide Sequence	Length (aa)	Length		
		pI	MW (kDa)	
Lac7	MCVKKILDKVGINYWLAHKA	20	9.52	2.33
Lac8	LAHKALCSEKLDQWLCEKL	19	6.74	2.23

^aAbbreviations 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 I E 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

[0103] The disclosure furthermore comprises the following items:

- [0104] 1. Method for identifying allergenic proteins and peptides encoded by a DNA or cDNA expression library comprising the steps of:
- [0105] a) providing at least one expression library comprising DNA or cDNA derived from at least one allergen source,
- [0106] b) expressing at least one protein or peptide encoded by said expression library,
- [0107] 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,
- [0108] 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
- [0109] 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.
- [0110] 2. Method according to item 1, wherein the at least one allergen source is an animal.
- [0111] 3. Method according to item 2, wherein the animal is a mammal.
- [0112] 4. Method according to item 3, wherein the mammal is a cow.

- [0113] 5. Method according to item 3, wherein the mammal is a cat.
- [0114] 6. Method according to item 3, wherein the mammal is a dog.
- [0115] 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.
- [0116] 8. Method according to item 2, wherein the animal is a mite.
- [0117] 9. Method according to item 1, wherein the animal is a fish.
- [0118] 10. Method according to item 2, wherein the allergen source is an egg.
- [0119] 11. Method according to item 1, wherein the at least one allergen source is a plant.
- [0120] 12. Method according to item 11, wherein the plant is a weed.
- [0121] 13. Method according to item 11, wherein the plant is a nut.
- [0122] 14. Method according to item 11, wherein the plant is a tree.
- [0123] 15. Method according to item 1, wherein the expression library is a phage display library or a bacterial expression library.
- [0124] 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.
- [0125] 17. Method according to item 1, wherein the basophil cells are humanized rat basophil leukaemia (RBL) cells (clone RBL-703/21).
- [0126] 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).
- [0127] 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).
- [0128] 20. Method according to item 19, wherein the amino acid sequence is determined by mass spectrometry.
- [0129] 21. Method according to item 19, wherein the amino acid sequence is determined by Edman degradation.
- [0130] All cited patents and publications referred to in this application are incorporated herein by reference.
- [0131] The invention thus being described, it will be apparent that it may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the present invention and all such modifications as would be obvious to one skilled in the art are intended to be included in the scope of the following claims.

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Lys Glu

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 <211> LENGTH: 33
 <212> TYPE: PRT
 <213> ORGANISM: Bos taurus

<400> SEQUENCE: 6

Pro Leu Gly Thr Gln Tyr Thr Asp Ala Pro Ser Phe Ser Asp Ile Pro
 1 5 10 15

Asn Pro Ile Gly Ser Glu Asn Ser Glu Lys Thr Thr Met Pro Leu Trp
 20 25 30

Cys

<210> SEQ ID NO 7
 <211> LENGTH: 205
 <212> TYPE: PRT
 <213> ORGANISM: Bos taurus

<400> SEQUENCE: 7

Met Arg Gly Val Phe Arg Arg Asp Thr His Lys Ser Glu Ile Ala His
 1 5 10 15

Arg Phe Lys Asp Leu Gly Glu Glu His Phe Lys Gly Leu Val Leu Ile
 20 25 30

Ala Phe Ser Gln Tyr Leu Gln Gln Cys Pro Phe Asp Glu His Val Lys
 35 40 45

Leu Val Asn Glu Leu Thr Glu Phe Ala Lys Thr Cys Val Ala Asp Glu
 50 55 60

Ser His Ala Gly Cys Glu Lys Ser Leu His Thr Leu Phe Gly Asp Glu
 65 70 75 80

Leu Cys Lys Val Ala Ser Leu Arg Glu Thr Tyr Gly Asp Met Ala Asp
 85 90 95

Cys Cys Glu Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Ser His
 100 105 110

Lys Asp Asp Ser Pro Asp Leu Pro Lys Leu Lys Pro Asp Pro Asn Thr
 115 120 125

Leu Cys Asp Glu Phe Lys Ala Asp Glu Lys Lys Phe Trp Gly Lys Tyr
 130 135 140

Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu
 145 150 155 160

Leu Tyr Tyr Ala Asn Lys Tyr Asn Gly Val Phe Gln Glu Cys Cys Gln
 165 170 175

Ala Glu Asp Lys Gly Ala Cys Leu Leu Pro Lys Ile Glu Thr Met Arg
 180 185 190

Glu Lys Val Leu Thr Ser Ser His His His His His His
 195 200 205

<210> SEQ ID NO 8
 <211> LENGTH: 197
 <212> TYPE: PRT
 <213> ORGANISM: Bos taurus

<400> SEQUENCE: 8

Met Ala Arg Gln Arg Leu Arg Cys Ala Ser Ile Gln Lys Phe Gly Glu
 1 5 10 15

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Arg Ala Leu Lys Ala Trp Ser Val Ala Arg Leu Ser Gln Lys Phe Pro
    20                25                30
Lys Ala Glu Phe Val Glu Val Thr Lys Leu Val Thr Asp Leu Thr Lys
    35                40                45
Val His Lys Glu Cys Cys His Gly Asp Leu Leu Glu Cys Ala Asp Asp
    50                55                60
Arg Ala Asp Leu Ala Lys Tyr Ile Cys Asp Asn Gln Asp Thr Ile Ser
    65                70                75                80
Ser Lys Leu Lys Glu Cys Cys Asp Lys Pro Leu Leu Glu Lys Ser His
    85                90                95
Cys Ile Ala Glu Val Glu Lys Asp Ala Ile Pro Glu Asn Leu Pro Pro
    100                105                110
Leu Thr Ala Asp Phe Ala Glu Asp Lys Asp Val Cys Lys Asn Tyr Gln
    115                120                125
Glu Ala Lys Asp Ala Phe Leu Gly Ser Phe Leu Tyr Glu Tyr Ser Arg
    130                135                140
Arg His Pro Glu Tyr Ala Val Ser Val Leu Leu Arg Leu Ala Lys Glu
    145                150                155                160
Tyr Glu Ala Thr Leu Glu Glu Cys Cys Ala Lys Asp Asp Pro His Ala
    165                170                175
Cys Tyr Ser Thr Val Phe Asp Lys Leu Lys His Leu Val Asp Glu His
    180                185                190
His His His His His
    195

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<210> SEQ ID NO 9
<211> LENGTH: 208
<212> TYPE: PRT
<213> ORGANISM: Bos taurus

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<400> SEQUENCE: 9

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Met Pro Gln Asn Leu Ile Lys Gln Asn Cys Asp Gln Phe Glu Lys Leu
  1      5      10      15
Gly Glu Tyr Gly Phe Gln Asn Ala Leu Ile Val Arg Tyr Thr Arg Lys
  20      25      30
Val Pro Gln Val Ser Thr Pro Thr Leu Val Glu Val Ser Arg Ser Leu
  35      40      45
Gly Lys Val Gly Thr Arg Cys Cys Thr Lys Pro Glu Ser Glu Arg Met
  50      55      60
Pro Cys Thr Glu Asp Tyr Leu Ser Leu Ile Leu Asn Arg Leu Cys Val
  65      70      75      80
Leu His Glu Lys Thr Pro Val Ser Glu Lys Val Thr Lys Cys Cys Thr
  85      90      95
Glu Ser Leu Val Asn Arg Arg Pro Cys Phe Ser Ala Leu Thr Pro Asp
  100     105     110
Glu Thr Tyr Val Pro Lys Ala Phe Asp Glu Lys Leu Phe Thr Phe His
  115     120     125
Ala Asp Ile Cys Thr Leu Pro Asp Thr Glu Lys Gln Ile Lys Lys Gln
  130     135     140
Thr Ala Leu Val Glu Leu Leu Lys His Lys Pro Lys Ala Thr Glu Glu
  145     150     155     160
Gln Leu Lys Thr Val Met Glu Asn Phe Val Ala Phe Val Asp Lys Cys
  165     170     175

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Cys Ala Ala Asp Asp Lys Glu Ala Cys Phe Ala Val Glu Gly Pro Lys
 180 185 190

Leu Val Val Ser Thr Gln Thr Ala Leu Ala His His His His His His
 195 200 205

<210> SEQ ID NO 10
 <211> LENGTH: 11
 <212> TYPE: PRT
 <213> ORGANISM: Bos taurus

<400> SEQUENCE: 10

His Gln Pro His Gln Pro Leu Pro Pro Thr Val
 1 5 10

<210> SEQ ID NO 11
 <211> LENGTH: 11
 <212> TYPE: PRT
 <213> ORGANISM: Bos taurus

<400> SEQUENCE: 11

Val Tyr Pro Phe Pro Gly Pro Ile Pro Asn Ser
 1 5 10

<210> SEQ ID NO 12
 <211> LENGTH: 6
 <212> TYPE: PRT
 <213> ORGANISM: Bos taurus

<400> SEQUENCE: 12

Leu Ser Ser Ser Glu Glu
 1 5

<210> SEQ ID NO 13
 <211> LENGTH: 8
 <212> TYPE: PRT
 <213> ORGANISM: Bos taurus

<400> SEQUENCE: 13

Pro Val Val Val Pro Pro Phe Leu
 1 5

<210> SEQ ID NO 14
 <211> LENGTH: 19
 <212> TYPE: PRT
 <213> ORGANISM: Bos taurus

<400> SEQUENCE: 14

Glu Gln Leu Thr Lys Cys Glu Val Phe Arg Glu Leu Lys Asp Leu Lys
 1 5 10 15

Gly Tyr Gly

<210> SEQ ID NO 15
 <211> LENGTH: 20
 <212> TYPE: PRT
 <213> ORGANISM: Bos taurus

<400> SEQUENCE: 15

Leu Lys Gly Tyr Gly Gly Val Ser Leu Pro Glu Trp Val Cys Thr Thr
 1 5 10 15

Phe His Thr Ser

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20

<210> SEQ ID NO 16
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Bos taurus

<400> SEQUENCE: 16

Thr Phe His Thr Ser Gly Tyr Asp Thr Gln Ala Ile Val Gln Asn Asn
1 5 10 15

Asp Ser Thr Glu
20

<210> SEQ ID NO 17
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Bos taurus

<400> SEQUENCE: 17

Asn Asp Ser Thr Glu Tyr Gly Leu Phe Gln Ile Asn Asn Lys Ile Trp
1 5 10 15

Cys Lys Asp Asp
20

<210> SEQ ID NO 18
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Bos taurus

<400> SEQUENCE: 18

Trp Cys Lys Asp Asp Gln Asn Pro His Ser Ser Asn Ile Cys Asn Ile
1 5 10 15

Ser Cys Asp Lys
20

<210> SEQ ID NO 19
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Bos taurus

<400> SEQUENCE: 19

Ile Ser Cys Asp Lys Phe Leu Asp Asp Asp Leu Thr Asp Asp Ile Met
1 5 10 15

Cys Val Lys Lys
20

<210> SEQ ID NO 20
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Bos taurus

<400> SEQUENCE: 20

Met Cys Val Lys Lys Ile Leu Asp Lys Val Gly Ile Asn Tyr Trp Leu
1 5 10 15

Ala His Lys Ala
20

<210> SEQ ID NO 21
<211> LENGTH: 19
<212> TYPE: PRT

-continued

<213> ORGANISM: Bos taurus

<400> SEQUENCE: 21

Leu Ala His Lys Ala Leu Cys Ser Glu Lys Leu Asp Gln Trp Leu Cys
 1 5 10 15

Glu Lys Leu

<210> SEQ ID NO 22

<211> LENGTH: 214

<212> TYPE: PRT

<213> ORGANISM: Bos taurus

<400> SEQUENCE: 22

Met Lys Leu Leu Ile Leu Thr Cys Leu Val Ala Val Ala Leu Ala Arg
 1 5 10 15

Pro Lys His Pro Ile Lys His Gln Gly Leu Pro Gln Glu Val Leu Asn
 20 25 30

Glu Asn Leu Leu Arg Phe Phe Val Ala Pro Phe Pro Glu Val Phe Gly
 35 40 45

Lys Glu Lys Val Asn Glu Leu Ser Lys Asp Ile Gly Ser Glu Ser Thr
 50 55 60

Glu Asp Gln Ala Met Glu Asp Ile Lys Gln Met Glu Ala Glu Ser Ile
 65 70 75 80

Ser Ser Ser Glu Glu Ile Val Pro Asn Ser Val Glu Gln Lys His Ile
 85 90 95

Gln Lys Glu Asp Val Pro Ser Glu Arg Tyr Leu Gly Tyr Leu Glu Gln
 100 105 110

Leu Leu Arg Leu Lys Lys Tyr Lys Val Pro Gln Leu Glu Ile Val Pro
 115 120 125

Asn Ser Ala Glu Glu Arg Leu His Ser Met Lys Glu Gly Ile His Ala
 130 135 140

Gln Gln Lys Glu Pro Met Ile Gly Val Asn Gln Glu Leu Ala Tyr Phe
 145 150 155 160

Tyr Pro Glu Leu Phe Arg Gln Phe Tyr Gln Leu Asp Ala Tyr Pro Ser
 165 170 175

Gly Ala Trp Tyr Tyr Val Pro Leu Gly Thr Gln Tyr Thr Asp Ala Pro
 180 185 190

Ser Phe Ser Asp Ile Pro Asn Pro Ile Gly Ser Glu Asn Ser Glu Lys
 195 200 205

Thr Thr Met Pro Leu Trp
 210

<210> SEQ ID NO 23

<211> LENGTH: 121

<212> TYPE: PRT

<213> ORGANISM: Bos taurus

<400> SEQUENCE: 23

Met Lys Leu Leu Ile Leu Thr Cys Leu Val Ala Val Ala Leu Ala Arg
 1 5 10 15

Pro Lys His Pro Ile Lys His Gln Gly Leu Pro Gln Glu Val Leu Asn
 20 25 30

Glu Asn Leu Leu Arg Phe Phe Val Ala Pro Phe Pro Glu Val Phe Gly
 35 40 45

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Lys Glu Lys Val Asn Glu Leu Ser Lys Asp Ile Gly Ser Glu Ser Thr
50 55 60

Glu Asp Gln Ala Met Glu Asp Ile Lys Gln Met Glu Ala Glu Ser Ile
65 70 75 80

Ser Ser Ser Glu Glu Ile Val Pro Asn Ser Val Glu Gln Lys His Ile
85 90 95

Gln Lys Glu Asp Val Pro Ser Glu Arg Tyr Leu Gly Tyr Leu Glu Gln
100 105 110

Leu Leu Arg Leu Lys Lys Tyr Lys Val
115 120

<210> SEQ ID NO 24
 <211> LENGTH: 208
 <212> TYPE: PRT
 <213> ORGANISM: Bos taurus

<400> SEQUENCE: 24

Thr Cys Leu Val Ala Val Ala Leu Ala Arg Pro Lys His Pro Ile Lys
1 5 10 15

His Gln Gly Leu Pro Gln Glu Val Leu Asn Glu Asn Leu Leu Arg Phe
20 25 30

Phe Val Ala Pro Phe Pro Glu Val Phe Gly Lys Glu Lys Val Asn Glu
35 40 45

Leu Ser Lys Asp Ile Gly Ser Glu Ser Thr Glu Asp Gln Ala Met Glu
50 55 60

Asp Ile Lys Gln Met Glu Ala Glu Ser Ile Ser Ser Ser Glu Glu Ile
65 70 75 80

Val Pro Asn Ser Val Glu Gln Lys His Ile Gln Lys Glu Asp Val Pro
85 90 95

Ser Glu Arg Tyr Leu Gly Tyr Leu Glu Gln Leu Leu Arg Leu Lys Lys
100 105 110

Tyr Lys Val Pro Gln Leu Glu Ile Val Pro Asn Ser Ala Glu Glu Arg
115 120 125

Leu His Ser Met Lys Glu Gly Ile His Ala Gln Gln Lys Glu Pro Met
130 135 140

Ile Gly Val Asn Gln Glu Leu Ala Tyr Phe Tyr Pro Glu Leu Phe Arg
145 150 155 160

Gln Phe Tyr Gln Leu Asp Ala Tyr Pro Ser Gly Ala Trp Tyr Tyr Val
165 170 175

Pro Leu Gly Thr Gln Tyr Thr Asp Ala Pro Ser Phe Ser Asp Ile Pro
180 185 190

Asn Pro Ile Gly Ser Glu Asn Ser Glu Lys Thr Thr Met Pro Leu Trp
195 200 205

<210> SEQ ID NO 25
 <211> LENGTH: 203
 <212> TYPE: PRT
 <213> ORGANISM: Bos taurus

<400> SEQUENCE: 25

Val Ala Leu Ala Arg Pro Lys His Pro Ile Lys His Gln Gly Leu Pro
1 5 10 15

Gln Glu Val Leu Asn Glu Asn Leu Leu Arg Phe Phe Val Ala Pro Phe
20 25 30

-continued

Met Pro Leu Trp
195

<210> SEQ ID NO 27
<211> LENGTH: 195
<212> TYPE: PRT
<213> ORGANISM: Bos taurus

<400> SEQUENCE: 27

Pro Ile Lys His Gln Gly Leu Pro Gln Glu Val Leu Asn Glu Asn Leu
1 5 10 15
Leu Arg Phe Phe Val Ala Pro Phe Pro Glu Val Phe Gly Lys Glu Lys
20 25 30
Val Asn Glu Leu Ser Lys Asp Ile Gly Ser Glu Ser Thr Glu Asp Gln
35 40 45
Ala Met Glu Asp Ile Lys Gln Met Glu Ala Glu Ser Ile Ser Ser Ser
50 55 60
Glu Glu Ile Val Pro Asn Ser Val Glu Gln Lys His Ile Gln Lys Glu
65 70 75 80
Asp Val Pro Ser Glu Arg Tyr Leu Gly Tyr Leu Glu Gln Leu Leu Arg
85 90 95
Leu Lys Lys Tyr Lys Val Pro Gln Leu Glu Ile Val Pro Asn Ser Ala
100 105 110
Glu Glu Arg Leu His Ser Met Lys Glu Gly Ile His Ala Gln Gln Lys
115 120 125
Glu Pro Met Ile Gly Val Asn Gln Glu Leu Ala Tyr Phe Tyr Pro Glu
130 135 140
Leu Phe Arg Gln Phe Tyr Gln Leu Asp Ala Tyr Pro Ser Gly Ala Trp
145 150 155 160
Tyr Tyr Val Pro Leu Gly Thr Gln Tyr Thr Asp Ala Pro Ser Phe Ser
165 170 175
Asp Ile Pro Asn Pro Ile Gly Ser Glu Asn Ser Glu Lys Thr Thr Met
180 185 190
Pro Leu Trp
195

<210> SEQ ID NO 28
<211> LENGTH: 174
<212> TYPE: PRT
<213> ORGANISM: Bos taurus

<400> SEQUENCE: 28

Ala Pro Phe Pro Glu Val Phe Gly Lys Glu Lys Val Asn Glu Leu Ser
1 5 10 15
Lys Asp Ile Gly Ser Glu Ser Thr Glu Asp Gln Ala Met Glu Asp Ile
20 25 30
Lys Gln Met Glu Ala Glu Ser Ile Ser Ser Ser Glu Glu Ile Val Pro
35 40 45
Asn Ser Val Glu Gln Lys His Ile Gln Lys Glu Asp Val Pro Ser Glu
50 55 60
Arg Tyr Leu Gly Tyr Leu Glu Gln Leu Leu Arg Leu Lys Lys Tyr Lys
65 70 75 80
Val Pro Gln Leu Glu Ile Val Pro Asn Ser Ala Glu Glu Arg Leu His
85 90 95

-continued

Ser Met Lys Glu Gly Ile His Ala Gln Gln Lys Glu Pro Met Ile Gly
 100 105 110

Val Asn Gln Glu Leu Ala Tyr Phe Tyr Pro Glu Leu Phe Arg Gln Phe
 115 120 125

Tyr Gln Leu Asp Ala Tyr Pro Ser Gly Ala Trp Tyr Tyr Val Pro Leu
 130 135 140

Gly Thr Gln Tyr Thr Asp Ala Pro Ser Phe Ser Asp Ile Pro Asn Pro
 145 150 155 160

Ile Gly Ser Glu Asn Ser Glu Lys Thr Thr Met Pro Leu Trp
 165 170

<210> SEQ ID NO 29
 <211> LENGTH: 167
 <212> TYPE: PRT
 <213> ORGANISM: Bos taurus

<400> SEQUENCE: 29

Gly Lys Glu Lys Val Asn Glu Leu Ser Lys Asp Ile Gly Ser Glu Ser
 1 5 10 15

Thr Glu Asp Gln Ala Met Glu Asp Ile Lys Gln Met Glu Ala Glu Ser
 20 25 30

Ile Ser Ser Ser Glu Glu Ile Val Pro Asn Ser Val Glu Gln Lys His
 35 40 45

Ile Gln Lys Glu Asp Val Pro Ser Glu Arg Tyr Leu Gly Tyr Leu Glu
 50 55 60

Gln Leu Leu Arg Leu Lys Lys Tyr Lys Val Pro Gln Leu Glu Ile Val
 65 70 75 80

Pro Asn Ser Ala Glu Glu Arg Leu His Ser Met Lys Glu Gly Ile His
 85 90 95

Ala Gln Gln Lys Glu Pro Met Ile Gly Val Asn Gln Glu Leu Ala Tyr
 100 105 110

Phe Tyr Pro Glu Leu Phe Arg Gln Phe Tyr Gln Leu Asp Ala Tyr Pro
 115 120 125

Ser Gly Ala Trp Tyr Tyr Val Pro Leu Gly Thr Gln Tyr Thr Asp Ala
 130 135 140

Pro Ser Phe Ser Asp Ile Pro Asn Pro Ile Gly Ser Glu Asn Ser Glu
 145 150 155 160

Lys Thr Thr Met Pro Leu Trp
 165

<210> SEQ ID NO 30
 <211> LENGTH: 142
 <212> TYPE: PRT
 <213> ORGANISM: Bos taurus

<400> SEQUENCE: 30

Lys Gln Met Glu Ala Glu Ser Ile Ser Ser Ser Glu Glu Ile Val Pro
 1 5 10 15

Asn Ser Val Glu Gln Lys His Ile Gln Lys Glu Asp Val Pro Ser Glu
 20 25 30

Arg Tyr Leu Gly Tyr Leu Glu Gln Leu Leu Arg Leu Lys Lys Tyr Lys
 35 40 45

Val Pro Gln Leu Glu Ile Val Pro Asn Ser Ala Glu Glu Arg Leu His
 50 55 60

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Ser Met Lys Glu Gly Ile His Ala Gln Gln Lys Glu Pro Met Ile Gly
65 70 75 80

Val Asn Gln Glu Leu Ala Tyr Phe Tyr Pro Glu Leu Phe Arg Gln Phe
85 90 95

Tyr Gln Leu Asp Ala Tyr Pro Ser Gly Ala Trp Tyr Tyr Val Pro Leu
100 105 110

Gly Thr Gln Tyr Thr Asp Ala Pro Ser Phe Ser Asp Ile Pro Asn Pro
115 120 125

Ile Gly Ser Glu Asn Ser Glu Lys Thr Thr Met Pro Leu Trp
130 135 140

<210> SEQ ID NO 31
 <211> LENGTH: 139
 <212> TYPE: PRT
 <213> ORGANISM: Bos taurus

<400> SEQUENCE: 31

Glu Ala Glu Ser Ile Ser Ser Ser Glu Glu Ile Val Pro Asn Ser Val
1 5 10 15

Glu Gln Lys His Ile Gln Lys Glu Asp Val Pro Ser Glu Arg Tyr Leu
20 25 30

Gly Tyr Leu Glu Gln Leu Leu Arg Leu Lys Lys Tyr Lys Val Pro Gln
35 40 45

Leu Glu Ile Val Pro Asn Ser Ala Glu Glu Arg Leu His Ser Met Lys
50 55 60

Glu Gly Ile His Ala Gln Gln Lys Glu Pro Met Ile Gly Val Asn Gln
65 70 75 80

Glu Leu Ala Tyr Phe Tyr Pro Glu Leu Phe Arg Gln Phe Tyr Gln Leu
85 90 95

Asp Ala Tyr Pro Ser Gly Ala Trp Tyr Tyr Val Pro Leu Gly Thr Gln
100 105 110

Tyr Thr Asp Ala Pro Ser Phe Ser Asp Ile Pro Asn Pro Ile Gly Ser
115 120 125

Glu Asn Ser Glu Lys Thr Thr Met Pro Leu Trp
130 135

<210> SEQ ID NO 32
 <211> LENGTH: 137
 <212> TYPE: PRT
 <213> ORGANISM: Bos taurus

<400> SEQUENCE: 32

Glu Ser Ile Ser Ser Ser Glu Glu Ile Val Pro Asn Ser Val Glu Gln
1 5 10 15

Lys His Ile Gln Lys Glu Asp Val Pro Ser Glu Arg Tyr Leu Gly Tyr
20 25 30

Leu Glu Gln Leu Leu Arg Leu Lys Lys Tyr Lys Val Pro Gln Leu Glu
35 40 45

Ile Val Pro Asn Ser Ala Glu Glu Arg Leu His Ser Met Lys Glu Gly
50 55 60

Ile His Ala Gln Gln Lys Glu Pro Met Ile Gly Val Asn Gln Glu Leu
65 70 75 80

Ala Tyr Phe Tyr Pro Glu Leu Phe Arg Gln Phe Tyr Gln Leu Asp Ala
85 90 95

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Tyr Pro Ser Gly Ala Trp Tyr Tyr Val Pro Leu Gly Thr Gln Tyr Thr
 100 105 110

Asp Ala Pro Ser Phe Ser Asp Ile Pro Asn Pro Ile Gly Ser Glu Asn
 115 120 125

Ser Glu Lys Thr Thr Met Pro Leu Trp
 130 135

<210> SEQ ID NO 33
 <211> LENGTH: 130
 <212> TYPE: PRT
 <213> ORGANISM: Bos taurus

<400> SEQUENCE: 33

Glu Ile Val Pro Asn Ser Val Glu Gln Lys His Ile Gln Lys Glu Asp
 1 5 10 15

Val Pro Ser Glu Arg Tyr Leu Gly Tyr Leu Glu Gln Leu Leu Arg Leu
 20 25 30

Lys Lys Tyr Lys Val Pro Gln Leu Glu Ile Val Pro Asn Ser Ala Glu
 35 40 45

Glu Arg Leu His Ser Met Lys Glu Gly Ile His Ala Gln Gln Lys Glu
 50 55 60

Pro Met Ile Gly Val Asn Gln Glu Leu Ala Tyr Phe Tyr Pro Glu Leu
 65 70 75 80

Phe Arg Gln Phe Tyr Gln Leu Asp Ala Tyr Pro Ser Gly Ala Trp Tyr
 85 90 95

Tyr Val Pro Leu Gly Thr Gln Tyr Thr Asp Ala Pro Ser Phe Ser Asp
 100 105 110

Ile Pro Asn Pro Ile Gly Ser Glu Asn Ser Glu Lys Thr Thr Met Pro
 115 120 125

Leu Trp
 130

<210> SEQ ID NO 34
 <211> LENGTH: 117
 <212> TYPE: PRT
 <213> ORGANISM: Bos taurus

<400> SEQUENCE: 34

Lys Glu Asp Val Pro Ser Glu Arg Tyr Leu Gly Tyr Leu Glu Gln Leu
 1 5 10 15

Leu Arg Leu Lys Lys Tyr Lys Val Pro Gln Leu Glu Ile Val Pro Asn
 20 25 30

Ser Ala Glu Glu Arg Leu His Ser Met Lys Glu Gly Ile His Ala Gln
 35 40 45

Gln Lys Glu Pro Met Ile Gly Val Asn Gln Glu Leu Ala Tyr Phe Tyr
 50 55 60

Pro Glu Leu Phe Arg Gln Phe Tyr Gln Leu Asp Ala Tyr Pro Ser Gly
 65 70 75 80

Ala Trp Tyr Tyr Val Pro Leu Gly Thr Gln Tyr Thr Asp Ala Pro Ser
 85 90 95

Phe Ser Asp Ile Pro Asn Pro Ile Gly Ser Glu Asn Ser Glu Lys Thr
 100 105 110

Thr Met Pro Leu Trp
 115

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<210> SEQ ID NO 35
<211> LENGTH: 111
<212> TYPE: PRT
<213> ORGANISM: Bos taurus

<400> SEQUENCE: 35
Glu Arg Tyr Leu Gly Tyr Leu Glu Gln Leu Leu Arg Leu Lys Lys Tyr
1          5          10
Lys Val Pro Gln Leu Glu Ile Val Pro Asn Ser Ala Glu Glu Arg Leu
20         25         30
His Ser Met Lys Glu Gly Ile His Ala Gln Gln Lys Glu Pro Met Ile
35         40         45
Gly Val Asn Gln Glu Leu Ala Tyr Phe Tyr Pro Glu Leu Phe Arg Gln
50         55         60
Phe Tyr Gln Leu Asp Ala Tyr Pro Ser Gly Ala Trp Tyr Tyr Val Pro
65         70         75         80
Leu Gly Thr Gln Tyr Thr Asp Ala Pro Ser Phe Ser Asp Ile Pro Asn
85         90         95
Pro Ile Gly Ser Glu Asn Ser Glu Lys Thr Thr Met Pro Leu Trp
100        105        110

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<210> SEQ ID NO 36
<211> LENGTH: 108
<212> TYPE: PRT
<213> ORGANISM: Bos taurus

<400> SEQUENCE: 36
Leu Gly Tyr Leu Glu Gln Leu Leu Arg Leu Lys Lys Tyr Lys Val Pro
1          5          10        15
Gln Leu Glu Ile Val Pro Asn Ser Ala Glu Glu Arg Leu His Ser Met
20         25         30
Lys Glu Gly Ile His Ala Gln Gln Lys Glu Pro Met Ile Gly Val Asn
35         40         45
Gln Glu Leu Ala Tyr Phe Tyr Pro Glu Leu Phe Arg Gln Phe Tyr Gln
50         55         60
Leu Asp Ala Tyr Pro Ser Gly Ala Trp Tyr Tyr Val Pro Leu Gly Thr
65         70         75         80
Gln Tyr Thr Asp Ala Pro Ser Phe Ser Asp Ile Pro Asn Pro Ile Gly
85         90         95
Ser Glu Asn Ser Glu Lys Thr Thr Met Pro Leu Trp
100        105

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<210> SEQ ID NO 37
<211> LENGTH: 104
<212> TYPE: PRT
<213> ORGANISM: Bos taurus

<400> SEQUENCE: 37
Glu Gln Leu Leu Arg Leu Lys Lys Tyr Lys Val Pro Gln Leu Glu Ile
1          5          10        15
Val Pro Asn Ser Ala Glu Glu Arg Leu His Ser Met Lys Glu Gly Ile
20         25         30
His Ala Gln Gln Lys Glu Pro Met Ile Gly Val Asn Gln Glu Leu Ala
35         40         45

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Tyr Phe Tyr Pro Glu Leu Phe Arg Gln Phe Tyr Gln Leu Asp Ala Tyr
 50 55 60
 Pro Ser Gly Ala Trp Tyr Tyr Val Pro Leu Gly Thr Gln Tyr Thr Asp
 65 70 75 80
 Ala Pro Ser Phe Ser Asp Ile Pro Asn Pro Ile Gly Ser Glu Asn Ser
 85 90 95
 Glu Lys Thr Thr Met Pro Leu Trp
 100

<210> SEQ ID NO 38
 <211> LENGTH: 95
 <212> TYPE: PRT
 <213> ORGANISM: Bos taurus

<400> SEQUENCE: 38

Lys Val Pro Gln Leu Glu Ile Val Pro Asn Ser Ala Glu Glu Arg Leu
 1 5 10 15
 His Ser Met Lys Glu Gly Ile His Ala Gln Gln Lys Glu Pro Met Ile
 20 25 30
 Gly Val Asn Gln Glu Leu Ala Tyr Phe Tyr Pro Glu Leu Phe Arg Gln
 35 40 45
 Phe Tyr Gln Leu Asp Ala Tyr Pro Ser Gly Ala Trp Tyr Tyr Val Pro
 50 55 60
 Leu Gly Thr Gln Tyr Thr Asp Ala Pro Ser Phe Ser Asp Ile Pro Asn
 65 70 75 80
 Pro Ile Gly Ser Glu Asn Ser Glu Lys Thr Thr Met Pro Leu Trp
 85 90 95

<210> SEQ ID NO 39
 <211> LENGTH: 83
 <212> TYPE: PRT
 <213> ORGANISM: Bos taurus

<400> SEQUENCE: 39

Glu Glu Arg Leu His Ser Met Lys Glu Gly Ile His Ala Gln Gln Lys
 1 5 10 15
 Glu Pro Met Ile Gly Val Asn Gln Glu Leu Ala Tyr Phe Tyr Pro Glu
 20 25 30
 Leu Phe Arg Gln Phe Tyr Gln Leu Asp Ala Tyr Pro Ser Gly Ala Trp
 35 40 45
 Tyr Tyr Val Pro Leu Gly Thr Gln Tyr Thr Asp Ala Pro Ser Phe Ser
 50 55 60
 Asp Ile Pro Asn Pro Ile Gly Ser Glu Asn Ser Glu Lys Thr Thr Met
 65 70 75 80
 Pro Leu Trp

<210> SEQ ID NO 40
 <211> LENGTH: 79
 <212> TYPE: PRT
 <213> ORGANISM: Bos taurus

<400> SEQUENCE: 40

His Ser Met Lys Glu Gly Ile His Ala Gln Gln Lys Glu Pro Met Ile
 1 5 10 15
 Gly Val Asn Gln Glu Leu Ala Tyr Phe Tyr Pro Glu Leu Phe Arg Gln
 20 25 30

-continued

 Pro Leu Trp
 65

<210> SEQ ID NO 44
 <211> LENGTH: 58
 <212> TYPE: PRT
 <213> ORGANISM: Bos taurus

<400> SEQUENCE: 44

Leu Ala Tyr Phe Tyr Pro Glu Leu Phe Arg Gln Phe Tyr Gln Leu Asp
 1 5 10 15
 Ala Tyr Pro Ser Gly Ala Trp Tyr Tyr Val Pro Leu Gly Thr Gln Tyr
 20 25 30
 Thr Asp Ala Pro Ser Phe Ser Asp Ile Pro Asn Pro Ile Gly Ser Glu
 35 40 45
 Asn Ser Glu Lys Thr Thr Met Pro Leu Trp
 50 55

<210> SEQ ID NO 45
 <211> LENGTH: 44
 <212> TYPE: PRT
 <213> ORGANISM: Bos taurus

<400> SEQUENCE: 45

Leu Asp Ala Tyr Pro Ser Gly Ala Trp Tyr Tyr Val Pro Leu Gly Thr
 1 5 10 15
 Gln Tyr Thr Asp Ala Pro Ser Phe Ser Asp Ile Pro Asn Pro Ile Gly
 20 25 30
 Ser Glu Asn Ser Glu Lys Thr Thr Met Pro Leu Trp
 35 40

<210> SEQ ID NO 46
 <211> LENGTH: 31
 <212> TYPE: PRT
 <213> ORGANISM: Bos taurus

<400> SEQUENCE: 46

Leu Gly Thr Gln Tyr Thr Asp Ala Pro Ser Phe Ser Asp Ile Pro Asn
 1 5 10 15
 Pro Ile Gly Ser Glu Asn Ser Glu Lys Thr Thr Met Pro Leu Trp
 20 25 30

<210> SEQ ID NO 47
 <211> LENGTH: 13
 <212> TYPE: PRT
 <213> ORGANISM: Bos taurus

<400> SEQUENCE: 47

Gly Ser Glu Asn Ser Glu Lys Thr Thr Met Pro Leu Trp
 1 5 10

<210> SEQ ID NO 48
 <211> LENGTH: 97
 <212> TYPE: PRT
 <213> ORGANISM: Bos taurus

<400> SEQUENCE: 48

Leu Thr Cys Leu Val Ala Val Ala Leu Ala Arg Pro Lys His Pro Ile
 1 5 10 15

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Lys His Gln Gly Leu Pro Gln Glu Val Leu Asn Glu Asn Leu Leu Arg
 20 25 30
 Phe Phe Val Ala Pro Phe Pro Glu Val Phe Gly Lys Glu Lys Val Asn
 35 40 45
 Glu Leu Ser Lys Asp Ile Gly Ser Glu Ser Thr Glu Asp Gln Ala Met
 50 55 60
 Glu Asp Ile Lys Gln Met Glu Ala Glu Ser Ile Ser Ser Ser Glu Glu
 65 70 75 80
 Ile Val Pro Asn Ser Val Glu Gln Lys His Ile Gln Lys Glu Asp Val
 85 90 95

Pro

<210> SEQ ID NO 49
 <211> LENGTH: 84
 <212> TYPE: PRT
 <213> ORGANISM: Bos taurus

<400> SEQUENCE: 49

Gly Ser Glu Ser Thr Glu Asp Gln Ala Met Glu Asp Ile Lys Gln Met
 1 5 10 15
 Glu Ala Glu Ser Ile Ser Ser Ser Glu Ile Val Pro Asn Ser Val
 20 25 30
 Glu Gln Lys His Ile Gln Lys Glu Asp Val Pro Ser Glu Arg Tyr Leu
 35 40 45
 Gly Tyr Leu Glu Gln Leu Leu Arg Leu Lys Lys Tyr Lys Val Pro Gln
 50 55 60
 Leu Glu Ile Val Pro Asn Ser Ala Glu Glu Arg Leu His Ser Met Lys
 65 70 75 80

Glu Gly Ile His

<210> SEQ ID NO 50
 <211> LENGTH: 60
 <212> TYPE: PRT
 <213> ORGANISM: Bos taurus

<400> SEQUENCE: 50

Thr Glu Asp Gln Ala Met Glu Asp Ile Lys Gln Met Glu Ala Glu Ser
 1 5 10 15
 Ile Ser Ser Ser Glu Glu Ile Val Pro Asn Ser Val Glu Gln Lys His
 20 25 30
 Ile Gln Lys Glu Asp Val Pro Ser Glu Arg Tyr Leu Gly Tyr Leu Glu
 35 40 45
 Gln Leu Leu Arg Leu Lys Lys Tyr Lys Val Pro Gln
 50 55 60

<210> SEQ ID NO 51
 <211> LENGTH: 45
 <212> TYPE: PRT
 <213> ORGANISM: Bos taurus

<400> SEQUENCE: 51

Gln Met Glu Ala Glu Ser Ile Ser Ser Ser Glu Glu Ile Val Pro Asn
 1 5 10 15
 Ser Val Glu Gln Lys His Ile Gln Lys Glu Asp Val Pro Ser Glu Arg
 20 25 30

-continued

His Ala Gln Gln Lys Glu Pro Met Ile Gly Val Asn Gln Glu Leu Ala
130 135 140

Tyr Phe Tyr Pro Glu Leu Phe Arg Gln Phe Tyr Gln Leu Asp Ala Tyr
145 150 155 160

Pro Ser Gly Ala Trp Tyr Tyr Val Pro Leu Gly Thr Gln Tyr Thr Asp
165 170 175

Ala Pro Ser Phe Ser Asp Ile Pro Asn Pro Ile Gly Ser Glu Asn Ser
180 185 190

Glu Lys Thr Thr Met Pro Leu Trp
195 200

<210> SEQ ID NO 54
 <211> LENGTH: 222
 <212> TYPE: PRT
 <213> ORGANISM: Bos taurus

<400> SEQUENCE: 54

Met Lys Phe Phe Ile Phe Thr Cys Leu Leu Ala Val Ala Leu Ala Lys
1 5 10 15

Asn Thr Met Glu His Val Ser Ser Ser Glu Glu Ser Ile Ile Ser Gln
20 25 30

Glu Thr Tyr Lys Gln Glu Lys Asn Met Ala Ile Asn Pro Ser Lys Glu
35 40 45

Asn Leu Cys Ser Thr Phe Cys Lys Glu Val Val Arg Asn Ala Asn Glu
50 55 60

Glu Glu Tyr Ser Ile Gly Ser Ser Ser Glu Glu Ser Ala Glu Val Ala
65 70 75 80

Thr Glu Glu Val Lys Ile Thr Val Asp Asp Lys His Tyr Gln Lys Ala
85 90 95

Leu Asn Glu Ile Asn Gln Phe Tyr Gln Lys Phe Pro Gln Tyr Leu Gln
100 105 110

Tyr Leu Tyr Gln Gly Pro Ile Val Leu Asn Pro Trp Asp Gln Val Lys
115 120 125

Arg Asn Ala Val Pro Ile Thr Pro Thr Leu Asn Arg Glu Gln Leu Ser
130 135 140

Thr Ser Glu Glu Asn Ser Lys Lys Thr Val Asp Met Glu Ser Thr Glu
145 150 155 160

Val Phe Thr Lys Lys Thr Lys Leu Thr Glu Glu Glu Lys Asn Arg Leu
165 170 175

Asn Phe Leu Lys Lys Ile Ser Gln Arg Tyr Gln Lys Phe Ala Leu Pro
180 185 190

Gln Tyr Leu Lys Thr Val Tyr Gln His Gln Lys Ala Met Lys Pro Trp
195 200 205

Ile Gln Pro Lys Thr Lys Val Ile Pro Tyr Val Arg Tyr Leu
210 215 220

<210> SEQ ID NO 55
 <211> LENGTH: 199
 <212> TYPE: PRT
 <213> ORGANISM: Bos taurus

<400> SEQUENCE: 55

Ser Ser Glu Glu Ser Ile Ile Ser Gln Glu Thr Tyr Lys Gln Glu Lys
1 5 10 15

-continued

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Asn Met Ala Ile Asn Pro Ser Lys Glu Asn Leu Cys Ser Thr Phe Cys
      20                               25                               30
Lys Glu Val Val Arg Asn Ala Asn Glu Glu Glu Tyr Ser Ile Gly Ser
      35                               40                               45
Ser Ser Glu Glu Ser Ala Glu Val Ala Thr Glu Glu Val Lys Ile Thr
      50                               55                               60
Val Asp Asp Lys His Tyr Gln Lys Ala Leu Asn Glu Ile Asn Gln Phe
      65                               70                               75                               80
Tyr Gln Lys Phe Pro Gln Tyr Leu Gln Tyr Leu Tyr Gln Gly Pro Ile
      85                               90                               95
Val Leu Asn Pro Trp Asp Gln Val Lys Arg Asn Ala Val Pro Ile Thr
      100                              105                              110
Pro Thr Leu Asn Arg Glu Gln Leu Ser Thr Ser Glu Glu Asn Ser Lys
      115                              120                              125
Lys Thr Val Asp Met Glu Ser Thr Glu Val Phe Thr Lys Lys Thr Lys
      130                              135                              140
Leu Thr Glu Glu Glu Lys Asn Arg Leu Asn Phe Leu Lys Lys Ile Ser
      145                              150                              155                              160
Gln Arg Tyr Gln Lys Phe Ala Leu Pro Gln Tyr Leu Lys Thr Val Tyr
      165                              170                              175
Gln His Gln Lys Ala Met Lys Pro Trp Ile Gln Pro Lys Thr Lys Val
      180                              185                              190
Ile Pro Tyr Val Arg Tyr Leu
      195

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<210> SEQ ID NO 56

<211> LENGTH: 224

<212> TYPE: PRT

<213> ORGANISM: Bos taurus

<400> SEQUENCE: 56

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Met Lys Val Leu Ile Leu Ala Cys Leu Val Ala Leu Ala Leu Ala Arg
  1      5      10      15
Glu Leu Glu Glu Leu Asn Val Pro Gly Glu Ile Val Glu Ser Leu Ser
  20      25      30
Ser Ser Glu Glu Ser Ile Thr Arg Ile Asn Lys Lys Ile Glu Lys Phe
  35      40      45
Gln Ser Glu Glu Gln Gln Thr Glu Asp Glu Leu Gln Asp Lys Ile
  50      55      60
His Pro Phe Ala Gln Thr Gln Ser Leu Val Tyr Pro Phe Pro Gly Pro
  65      70      75      80
Ile Pro Asn Ser Leu Pro Gln Asn Ile Pro Pro Leu Thr Gln Thr Pro
  85      90      95
Val Val Val Pro Pro Phe Leu Gln Pro Glu Val Met Gly Val Ser Lys
  100     105     110
Val Lys Glu Ala Met Ala Pro Lys His Lys Glu Met Pro Phe Pro Lys
  115     120     125
Tyr Pro Val Glu Pro Phe Thr Glu Ser Gln Ser Leu Thr Leu Thr Asp
  130     135     140
Val Glu Asn Leu His Leu Pro Leu Pro Leu Leu Gln Ser Trp Met His
  145     150     155     160
Gln Pro His Gln Pro Leu Pro Pro Thr Val Met Phe Pro Pro Gln Ser
  165     170     175

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Val Leu Ser Leu Ser Gln Ser Lys Val Leu Pro Val Pro Gln Lys Ala
 180 185 190

Val Pro Tyr Pro Gln Arg Asp Met Pro Ile Gln Ala Phe Leu Leu Tyr
 195 200 205

Gln Glu Pro Val Leu Gly Pro Val Arg Gly Pro Phe Pro Ile Ile Val
 210 215 220

<210> SEQ ID NO 57
 <211> LENGTH: 181
 <212> TYPE: PRT
 <213> ORGANISM: Bos taurus

<400> SEQUENCE: 57

Met Lys Val Leu Ile Leu Ala Cys Leu Val Ala Leu Ala Leu Ala Arg
 1 5 10 15

Glu Leu Glu Glu Leu Asn Val Pro Gly Glu Ile Val Glu Ser Leu Ser
 20 25 30

Ser Ser Glu Glu Ser Ile Thr Arg Ile Asn Lys Lys Ile Glu Lys Phe
 35 40 45

Gln Ser Glu Glu Gln Gln Gln Thr Glu Asp Glu Leu Gln Asp Lys Ile
 50 55 60

His Pro Phe Ala Gln Thr Gln Ser Leu Val Tyr Pro Phe Pro Gly Pro
 65 70 75 80

Ile Pro Asn Ser Leu Pro Gln Asn Ile Pro Pro Leu Thr Gln Thr Pro
 85 90 95

Val Val Val Pro Pro Phe Leu Gln Pro Glu Val Met Gly Val Ser Lys
 100 105 110

Val Lys Glu Ala Met Ala Pro Lys His Lys Glu Met Pro Phe Pro Lys
 115 120 125

Tyr Pro Val Glu Pro Phe Thr Glu Ser Gln Ser Leu Thr Leu Thr Asp
 130 135 140

Val Glu Asn Leu His Leu Pro Leu Pro Leu Leu Gln Ser Trp Met His
 145 150 155 160

Gln Pro His Gln Pro Leu Pro Pro Thr Val Met Phe Pro Pro Gln Ser
 165 170 175

Val Leu Ser Leu Ser
 180

<210> SEQ ID NO 58
 <211> LENGTH: 90
 <212> TYPE: PRT
 <213> ORGANISM: Bos taurus

<400> SEQUENCE: 58

Met Lys Val Leu Ile Leu Ala Cys Leu Val Ala Leu Ala Leu Ala Arg
 1 5 10 15

Glu Leu Glu Glu Leu Asn Val Pro Gly Glu Ile Val Glu Ser Leu Ser
 20 25 30

Ser Ser Glu Glu Ser Ile Thr Arg Ile Asn Lys Lys Ile Glu Lys Phe
 35 40 45

Gln Ser Glu Glu Gln Gln Gln Thr Glu Asp Glu Leu Gln Asp Lys Ile
 50 55 60

His Pro Phe Ala Gln Thr Gln Ser Leu Val Tyr Pro Phe Pro Gly Pro
 65 70 75 80

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Ile Pro Asn Ser Leu Pro Gln Asn Ile Pro
85 90

<210> SEQ ID NO 59
<211> LENGTH: 88
<212> TYPE: PRT
<213> ORGANISM: Bos taurus

<400> SEQUENCE: 59

Met Lys Val Leu Ile Leu Ala Cys Leu Val Ala Leu Ala Leu Ala Arg
1 5 10 15
Glu Leu Glu Glu Leu Asn Val Pro Gly Glu Ile Val Glu Ser Leu Ser
20 25 30
Ser Ser Glu Glu Ser Ile Thr Arg Ile Asn Lys Lys Ile Glu Lys Phe
35 40 45
Gln Ser Glu Glu Gln Gln Gln Thr Glu Asp Glu Leu Gln Asp Lys Ile
50 55 60
His Pro Phe Ala Gln Thr Gln Ser Leu Val Tyr Pro Phe Pro Gly Pro
65 70 75 80
Ile Pro Asn Ser Leu Pro Gln Asn
85

<210> SEQ ID NO 60
<211> LENGTH: 85
<212> TYPE: PRT
<213> ORGANISM: Bos taurus

<400> SEQUENCE: 60

Met Lys Val Leu Ile Leu Ala Cys Leu Val Ala Leu Ala Leu Ala Arg
1 5 10 15
Glu Leu Glu Glu Leu Asn Val Pro Gly Glu Ile Val Glu Ser Leu Ser
20 25 30
Ser Ser Glu Glu Ser Ile Thr Arg Ile Asn Lys Lys Ile Glu Lys Phe
35 40 45
Gln Ser Glu Glu Gln Gln Gln Thr Glu Asp Glu Leu Gln Asp Lys Ile
50 55 60
His Pro Phe Ala Gln Thr Gln Ser Leu Val Tyr Pro Phe Pro Gly Pro
65 70 75 80
Ile Pro Asn Ser Leu
85

<210> SEQ ID NO 61
<211> LENGTH: 84
<212> TYPE: PRT
<213> ORGANISM: Bos taurus

<400> SEQUENCE: 61

Met Lys Val Leu Ile Leu Ala Cys Leu Val Ala Leu Ala Leu Ala Arg
1 5 10 15
Glu Leu Glu Glu Leu Asn Val Pro Gly Glu Ile Val Glu Ser Leu Ser
20 25 30
Ser Ser Glu Glu Ser Ile Thr Arg Ile Asn Lys Lys Ile Glu Lys Phe
35 40 45
Gln Ser Glu Glu Gln Gln Gln Thr Glu Asp Glu Leu Gln Asp Lys Ile
50 55 60

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His Pro Phe Ala Gln Thr Gln Ser Leu Val Tyr Pro Phe Pro Gly Pro
65 70 75 80

Ile Pro Asn Ser

<210> SEQ ID NO 62

<211> LENGTH: 80

<212> TYPE: PRT

<213> ORGANISM: Bos taurus

<400> SEQUENCE: 62

Met Lys Val Leu Ile Leu Ala Cys Leu Val Ala Leu Ala Leu Ala Arg
1 5 10 15

Glu Leu Glu Glu Leu Asn Val Pro Gly Glu Ile Val Glu Ser Leu Ser
20 25 30

Ser Ser Glu Glu Ser Ile Thr Arg Ile Asn Lys Lys Ile Glu Lys Phe
35 40 45

Gln Ser Glu Glu Gln Gln Thr Glu Asp Glu Leu Gln Asp Lys Ile
50 55 60

His Pro Phe Ala Gln Thr Gln Ser Leu Val Tyr Pro Phe Pro Gly Pro
65 70 75 80

<210> SEQ ID NO 63

<211> LENGTH: 74

<212> TYPE: PRT

<213> ORGANISM: Bos taurus

<400> SEQUENCE: 63

Met Lys Val Leu Ile Leu Ala Cys Leu Val Ala Leu Ala Leu Ala Arg
1 5 10 15

Glu Leu Glu Glu Leu Asn Val Pro Gly Glu Ile Val Glu Ser Leu Ser
20 25 30

Ser Ser Glu Glu Ser Ile Thr Arg Ile Asn Lys Lys Ile Glu Lys Phe
35 40 45

Gln Ser Glu Glu Gln Gln Thr Glu Asp Glu Leu Gln Asp Lys Ile
50 55 60

His Pro Phe Ala Gln Thr Gln Ser Leu Val
65 70

<210> SEQ ID NO 64

<211> LENGTH: 66

<212> TYPE: PRT

<213> ORGANISM: Bos taurus

<400> SEQUENCE: 64

Met Lys Val Leu Ile Leu Ala Cys Leu Val Ala Leu Ala Leu Ala Arg
1 5 10 15

Glu Leu Glu Glu Leu Asn Val Pro Gly Glu Ile Val Glu Ser Leu Ser
20 25 30

Ser Ser Glu Glu Ser Ile Thr Arg Ile Asn Lys Lys Ile Glu Lys Phe
35 40 45

Gln Ser Glu Glu Gln Gln Thr Glu Asp Glu Leu Gln Asp Lys Ile
50 55 60

His Pro
65

<210> SEQ ID NO 65

-continued

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<211> LENGTH: 65
<212> TYPE: PRT
<213> ORGANISM: Bos taurus

<400> SEQUENCE: 65

Met Lys Val Leu Ile Leu Ala Cys Leu Val Ala Leu Ala Leu Ala Arg
1           5           10           15
Glu Leu Glu Glu Leu Asn Val Pro Gly Glu Ile Val Glu Ser Leu Ser
20           25           30
Ser Ser Glu Glu Ser Ile Thr Arg Ile Asn Lys Lys Ile Glu Lys Phe
35           40           45
Gln Ser Glu Glu Gln Gln Gln Thr Glu Asp Glu Leu Gln Asp Lys Ile
50           55           60

His
65

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<210> SEQ ID NO 66
<211> LENGTH: 223
<212> TYPE: PRT
<213> ORGANISM: Bos taurus

<400> SEQUENCE: 66

Lys Val Leu Ile Leu Ala Cys Leu Val Ala Leu Ala Leu Ala Arg Glu
1           5           10           15
Leu Glu Glu Leu Asn Val Pro Gly Glu Ile Val Glu Ser Leu Ser Ser
20           25           30
Ser Glu Glu Ser Ile Thr Arg Ile Asn Lys Lys Ile Glu Lys Phe Gln
35           40           45
Ser Glu Glu Gln Gln Gln Thr Glu Asp Glu Leu Gln Asp Lys Ile His
50           55           60
Pro Phe Ala Gln Thr Gln Ser Leu Val Tyr Pro Phe Pro Gly Pro Ile
65           70           75           80
Pro Asn Ser Leu Pro Gln Asn Ile Pro Pro Leu Thr Gln Thr Pro Val
85           90           95
Val Val Pro Pro Phe Leu Gln Pro Glu Val Met Gly Val Ser Lys Val
100          105          110
Lys Glu Ala Met Ala Pro Lys His Lys Glu Met Pro Phe Pro Lys Tyr
115          120          125
Pro Val Glu Pro Phe Thr Glu Ser Gln Ser Leu Thr Leu Thr Asp Val
130          135          140
Glu Asn Leu His Leu Pro Leu Pro Leu Leu Gln Ser Trp Met His Gln
145          150          155          160
Pro His Gln Pro Leu Pro Pro Thr Val Met Phe Pro Pro Gln Ser Val
165          170          175
Leu Ser Leu Ser Gln Ser Lys Val Leu Pro Val Pro Gln Lys Ala Val
180          185          190
Pro Tyr Pro Gln Arg Asp Met Pro Ile Gln Ala Phe Leu Leu Tyr Gln
195          200          205
Glu Pro Val Leu Gly Pro Val Arg Gly Pro Phe Pro Ile Ile Val
210          215          220

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<210> SEQ ID NO 67
<211> LENGTH: 83
<212> TYPE: PRT
<213> ORGANISM: Bos taurus

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-continued

<400> SEQUENCE: 67

Lys Val Leu Ile Leu Ala Cys Leu Val Ala Leu Ala Leu Ala Arg Glu
 1 5 10 15
 Leu Glu Glu Leu Asn Val Pro Gly Glu Ile Val Glu Ser Leu Ser Ser
 20 25 30
 Ser Glu Glu Ser Ile Thr Arg Ile Asn Lys Lys Ile Glu Lys Phe Gln
 35 40 45
 Ser Glu Glu Gln Gln Gln Thr Glu Asp Glu Leu Gln Asp Lys Ile His
 50 55 60
 Pro Phe Ala Gln Thr Gln Ser Leu Val Tyr Pro Phe Pro Gly Pro Ile
 65 70 75 80
 Pro Asn Ser

<210> SEQ ID NO 68

<211> LENGTH: 96

<212> TYPE: PRT

<213> ORGANISM: Bos taurus

<400> SEQUENCE: 68

Lys Val Leu Ile Leu Ala Cys Leu Val Ala Leu Ala Leu Ala Arg Glu
 1 5 10 15
 Leu Glu Glu Leu Asn Val Pro Gly Glu Ile Val Glu Ser Leu Ser Ser
 20 25 30
 Ser Glu Glu Ser Ile Thr Arg Ile Asn Lys Lys Ile Glu Lys Phe Gln
 35 40 45
 Ser Glu Glu Gln Gln Gln Thr Glu Asp Glu Leu Gln Asp Lys Ile His
 50 55 60
 Pro Phe Ala Gln Thr Gln Ser Leu Val Tyr Pro Phe Pro Gly Pro Ile
 65 70 75 80
 Pro Asn Ser Leu Pro Gln Asn Ile Pro Pro Leu Thr Gln Thr Pro Val
 85 90 95

<210> SEQ ID NO 69

<211> LENGTH: 50

<212> TYPE: PRT

<213> ORGANISM: Bos taurus

<400> SEQUENCE: 69

Glu Leu Asn Val Pro Gly Glu Ile Val Glu Ser Leu Ser Ser Ser Glu
 1 5 10 15
 Glu Ser Ile Thr Arg Ile Asn Lys Lys Ile Glu Lys Phe Gln Ser Glu
 20 25 30
 Glu Gln Gln Gln Thr Glu Asp Glu Leu Gln Asp Lys Ile His Pro Phe
 35 40 45
 Ala Gln
 50

<210> SEQ ID NO 70

<211> LENGTH: 116

<212> TYPE: PRT

<213> ORGANISM: Bos taurus

<400> SEQUENCE: 70

Pro Gly Glu Ile Val Glu Ser Leu Ser Ser Ser Glu Glu Ser Ile Thr
 1 5 10 15

-continued

Arg Ile Asn Lys Lys Ile Glu Lys Phe Gln Ser Glu Glu Gln Gln Gln
 20 25 30

Thr Glu Asp Glu Leu Gln Asp Lys Ile His Pro Phe Ala Gln Thr Gln
 35 40 45

Ser Leu Val Tyr Pro Phe Pro Gly Pro Ile Pro Asn Ser Leu Pro Gln
 50 55 60

Asn Ile Pro Pro Leu Thr Gln Thr Pro Val Val Val Pro Pro Phe Leu
 65 70 75 80

Gln Pro Glu Val Met Gly Val Ser Lys Val Lys Glu Ala Met Ala Pro
 85 90 95

Lys His Lys Glu Met Pro Phe Pro Lys Tyr Pro Val Glu Pro Phe Thr
 100 105 110

Glu Ser Gln Ser
 115

<210> SEQ ID NO 71
 <211> LENGTH: 50
 <212> TYPE: PRT
 <213> ORGANISM: Bos taurus

<400> SEQUENCE: 71

Leu Ser Ser Ser Glu Glu Ser Ile Thr Arg Ile Asn Lys Lys Ile Glu
 1 5 10 15

Lys Phe Gln Ser Glu Glu Gln Gln Gln Thr Glu Asp Glu Leu Gln Asp
 20 25 30

Lys Ile His Pro Phe Ala Gln Thr Gln Ser Leu Val Tyr Pro Phe Pro
 35 40 45

Gly Pro
 50

<210> SEQ ID NO 72
 <211> LENGTH: 37
 <212> TYPE: PRT
 <213> ORGANISM: Bos taurus

<400> SEQUENCE: 72

Lys Ile His Pro Phe Ala Gln Thr Gln Ser Leu Val Tyr Pro Phe Pro
 1 5 10 15

Gly Pro Ile Pro Asn Ser Leu Pro Gln Asn Ile Pro Pro Leu Thr Gln
 20 25 30

Thr Pro Val Val Val
 35

<210> SEQ ID NO 73
 <211> LENGTH: 110
 <212> TYPE: PRT
 <213> ORGANISM: Bos taurus

<400> SEQUENCE: 73

His Pro Phe Ala Gln Thr Gln Ser Leu Val Tyr Pro Phe Pro Gly Pro
 1 5 10 15

Ile Pro Asn Ser Leu Pro Gln Asn Ile Pro Pro Leu Thr Gln Thr Pro
 20 25 30

Val Val Val Pro Pro Phe Leu Gln Pro Glu Val Met Gly Val Ser Lys
 35 40 45

-continued

Val Lys Glu Ala Met Ala Pro Lys His Lys Glu Met Pro Phe Pro Lys
50 55 60

Tyr Pro Val Glu Pro Phe Thr Glu Ser Gln Ser Leu Thr Leu Thr Asp
65 70 75 80

Val Glu Asn Leu His Leu Pro Leu Pro Leu Leu Gln Ser Trp Met His
85 90 95

Gln Pro His Gln Pro Leu Pro Pro Thr Val Met Phe Pro Pro
100 105 110

<210> SEQ ID NO 74
 <211> LENGTH: 99
 <212> TYPE: PRT
 <213> ORGANISM: Bos taurus

<400> SEQUENCE: 74

Gln Ser Leu Val Tyr Pro Phe Pro Gly Pro Ile Pro Asn Ser Leu Pro
1 5 10 15

Gln Asn Ile Pro Pro Leu Thr Gln Thr Pro Val Val Val Pro Pro Phe
20 25 30

Leu Gln Pro Glu Val Met Gly Val Ser Lys Val Lys Glu Ala Met Ala
35 40 45

Pro Lys His Lys Glu Met Pro Phe Pro Lys Tyr Pro Val Glu Pro Phe
50 55 60

Thr Glu Ser Gln Ser Leu Thr Leu Thr Asp Val Glu Asn Leu His Leu
65 70 75 80

Pro Leu Pro Leu Leu Gln Ser Trp Met His Gln Pro His Gln Pro Leu
85 90 95

Pro Pro Thr

<210> SEQ ID NO 75
 <211> LENGTH: 77
 <212> TYPE: PRT
 <213> ORGANISM: Bos taurus

<400> SEQUENCE: 75

Pro Pro Phe Leu Gln Pro Glu Val Met Gly Val Ser Lys Val Lys Glu
1 5 10 15

Ala Met Ala Pro Lys His Lys Glu Met Pro Phe Pro Lys Tyr Pro Val
20 25 30

Glu Pro Phe Thr Glu Ser Gln Ser Leu Thr Leu Thr Asp Val Glu Asn
35 40 45

Leu His Leu Pro Leu Pro Leu Leu Gln Ser Trp Met His Gln Pro His
50 55 60

Gln Pro Leu Pro Pro Thr Val Met Phe Pro Pro Gln Ser
65 70 75

<210> SEQ ID NO 76
 <211> LENGTH: 64
 <212> TYPE: PRT
 <213> ORGANISM: Bos taurus

<400> SEQUENCE: 76

Pro Pro Phe Leu Gln Pro Glu Val Met Gly Val Ser Lys Val Lys Glu
1 5 10 15

Ala Met Ala Pro Lys His Lys Glu Met Pro Phe Pro Lys Tyr Pro Val
20 25 30

-continued

Trp Gln Val Leu Ser Asn Thr Val Pro Ala Lys Ser Cys Gln Ala Gln
 100 105 110

Pro Thr Thr Met Ala Arg His Pro
 115 120

<210> SEQ ID NO 79
 <211> LENGTH: 93
 <212> TYPE: PRT
 <213> ORGANISM: Bos taurus

<400> SEQUENCE: 79

Met Lys Ser Phe Phe Leu Val Val Thr Ile Leu Ala Leu Thr Leu Pro
 1 5 10 15
 Phe Leu Gly Ala Gln Glu Gln Asn Gln Glu Gln Pro Ile Arg Cys Glu
 20 25 30
 Lys Asp Glu Arg Phe Phe Ser Asp Lys Ile Ala Lys Tyr Ile Pro Ile
 35 40 45
 Gln Tyr Val Leu Ser Arg Tyr Pro Ser Tyr Gly Leu Asn Tyr Tyr Gln
 50 55 60
 Gln Lys Pro Val Ala Leu Ile Asn Asn Gln Phe Leu Pro Tyr Pro Tyr
 65 70 75 80
 Tyr Ala Lys Pro Ala Ala Val Arg Ser Pro Ala Gln Ile
 85 90

<210> SEQ ID NO 80
 <211> LENGTH: 60
 <212> TYPE: PRT
 <213> ORGANISM: Bos taurus

<400> SEQUENCE: 80

Pro Lys Lys Asn Gln Asp Lys Thr Glu Ile Pro Thr Ile Asn Thr Ile
 1 5 10 15
 Ala Ser Gly Glu Pro Thr Ser Thr Pro Thr Ile Glu Ala Val Glu Ser
 20 25 30
 Thr Val Ser Thr Leu Glu Ala Ser Pro Glu Val Ile Glu Ser Pro Pro
 35 40 45
 Glu Ile Asn Thr Val Gln Val Thr Ser Thr Ala Val
 50 55 60

<210> SEQ ID NO 81
 <211> LENGTH: 38
 <212> TYPE: PRT
 <213> ORGANISM: Bos taurus

<400> SEQUENCE: 81

Ser Thr Pro Thr Ile Glu Ala Val Glu Ser Thr Val Ser Thr Leu Glu
 1 5 10 15
 Ala Ser Pro Glu Val Ile Glu Ser Pro Pro Glu Ile Asn Thr Val Gln
 20 25 30
 Val Thr Ser Thr Ala Val
 35

<210> SEQ ID NO 82
 <211> LENGTH: 141
 <212> TYPE: PRT
 <213> ORGANISM: Bos taurus

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<400> SEQUENCE: 82

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Met Ser Phe Val Ser Leu Leu Leu Val Gly Ile Leu Phe His Ala Thr
1      5      10      15
Gln Ala Glu Gln Leu Thr Lys Cys Glu Val Phe Arg Glu Leu Lys Asp
20      25      30
Leu Lys Gly Tyr Gly Gly Val Ser Leu Pro Glu Trp Val Cys Thr Thr
35      40      45
Phe His Thr Ser Gly Tyr Asp Thr Gln Ala Ile Val Gln Asn Asn Asp
50      55      60
Ser Thr Glu Tyr Gly Leu Phe Gln Ile Asn Asn Lys Ile Trp Cys Lys
65      70      75      80
Asp Asp Gln Asn Pro His Ser Ser Asn Ile Cys Asn Ile Ser Cys Asp
85      90      95
Lys Phe Leu Asp Asp Asp Leu Thr Asp Asp Ile Met Cys Val Lys Lys
100     105     110
Ile Leu Asp Lys Val Gly Ile Asn Tyr Trp Leu Ala His Lys Ala Leu
115     120     125
Cys Ser Glu Lys Leu Asp Gln Trp Leu Cys Glu Lys Leu
130     135     140

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<210> SEQ ID NO 83

<211> LENGTH: 606

<212> TYPE: PRT

<213> ORGANISM: Bos taurus

<400> SEQUENCE: 83

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Met Lys Trp Val Thr Phe Ile Ser Leu Leu Leu Leu Phe Ser Ser Ala
1      5      10      15
Tyr Ser Arg Gly Val Phe Arg Arg Asp Thr His Lys Ser Glu Ile Ala
20      25      30
His Arg Phe Lys Asp Leu Gly Glu Glu His Phe Lys Gly Leu Val Leu
35      40      45
Ile Ala Phe Ser Gln Tyr Leu Gln Gln Cys Pro Phe Asp Glu His Val
50      55      60
Lys Leu Val Asn Glu Leu Glu Phe Ala Lys Thr Cys Val Ala Asp Glu
65      70      75      80
Ser His Ala Gly Cys Glu Lys Ser Leu His Thr Leu Phe Gly Asp Glu
85      90      95
Leu Cys Lys Val Ala Ser Leu Arg Glu Thr Tyr Gly Asp Met Ala Asp
100     105     110
Cys Cys Glu Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Ser His
115     120     125
Lys Asp Asp Ser Pro Asp Leu Pro Lys Leu Lys Pro Asp Pro Asn Thr
130     135     140
Leu Cys Asp Glu Phe Lys Ala Asp Glu Lys Lys Phe Trp Gly Lys Tyr
145     150     155     160
Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu
165     170     175
Leu Tyr Tyr Ala Asn Lys Tyr Asn Gly Val Phe Gln Glu Cys Cys Gln
180     185     190
Ala Glu Asp Lys Gly Ala Cys Leu Leu Pro Lys Ile Glu Thr Met Arg
195     200     205

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Glu	Lys	Val	Leu	Thr	Ser	Ser	Ala	Arg	Gln	Arg	Leu	Arg	Cys	Ala	Ser
210						215					220				
Ile	Gln	Lys	Phe	Gly	Glu	Arg	Ala	Leu	Lys	Ala	Trp	Ser	Val	Ala	Arg
225					230					235					240
Leu	Ser	Gln	Lys	Phe	Pro	Lys	Ala	Glu	Phe	Val	Glu	Val	Thr	Lys	Leu
				245					250					255	
Val	Thr	Asp	Leu	Thr	Lys	Val	His	Lys	Glu	Cys	Cys	His	Gly	Asp	Leu
			260					265					270		
Leu	Glu	Cys	Ala	Asp	Asp	Arg	Ala	Asp	Leu	Ala	Lys	Tyr	Ile	Cys	Asp
		275					280					285			
Asn	Gln	Asp	Thr	Ile	Ser	Ser	Lys	Leu	Lys	Glu	Cys	Cys	Asp	Lys	Pro
290						295					300				
Leu	Leu	Glu	Lys	Ser	His	Cys	Ile	Ala	Glu	Val	Glu	Lys	Asp	Ala	Ile
305					310					315					320
Pro	Glu	Asn	Leu	Pro	Pro	Leu	Thr	Ala	Asp	Phe	Ala	Glu	Asp	Lys	Asp
				325					330					335	
Val	Cys	Lys	Asn	Tyr	Gln	Glu	Ala	Lys	Asp	Ala	Phe	Leu	Gly	Ser	Phe
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Leu	Tyr	Glu	Tyr	Ser	Arg	Arg	His	Pro	Glu	Tyr	Ala	Val	Ser	Val	Leu
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Leu	Arg	Leu	Ala	Lys	Glu	Tyr	Glu	Ala	Thr	Leu	Glu	Glu	Cys	Cys	Ala
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Lys	Asp	Asp	Pro	His	Ala	Cys	Tyr	Ser	Thr	Val	Phe	Asp	Leu	Leu	Lys
385				390						395					400
His	Leu	Val	Asp	Glu	Pro	Gln	Asn	Leu	Ile	Lys	Gln	Asn	Cys	Asp	Gln
			405						410					415	
Phe	Glu	Lys	Leu	Gly	Glu	Tyr	Gly	Phe	Gln	Asn	Ala	Leu	Ile	Val	Arg
			420					425						430	
Tyr	Thr	Arg	Lys	Val	Pro	Gln	Val	Ser	Thr	Pro	Thr	Leu	Val	Glu	Val
		435					440						445		
Ser	Arg	Ser	Leu	Gly	Lys	Val	Gly	Thr	Arg	Cys	Cys	Thr	Lys	Pro	Glu
		450				455					460				
Ser	Glu	Arg	Met	Pro	Cys	Thr	Glu	Asp	Tyr	Leu	Ser	Leu	Ile	Leu	Asn
465					470					475					480
Arg	Leu	Cys	Val	Leu	His	Glu	Lys	Thr	Pro	Val	Ser	Glu	Lys	Val	Thr
			485						490					495	
Lys	Cys	Cys	Thr	Glu	Ser	Leu	Val	Asn	Arg	Arg	Pro	Cys	Phe	Ser	Ala
			500					505					510		
Leu	Thr	Pro	Asp	Glu	Thr	Tyr	Val	Pro	Lys	Ala	Phe	Asp	Glu	Lys	Leu
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Phe	Thr	Phe	His	Ala	Asp	Ile	Cys	Thr	Leu	Pro	Asp	Thr	Glu	Lys	Gln
	530					535					540				
Ile	Lys	Lys	Gln	Thr	Ala	Leu	Val	Glu	Leu	Leu	Lys	His	Lys	Pro	Lys
545					550					555					560
Ala	Thr	Glu	Glu	Gln	Leu	Lys	Thr	Val	Met	Glu	Asn	Phe	Val	Ala	Phe
				565					570					575	
Val	Asp	Lys	Cys	Cys	Ala	Ala	Asp	Asp	Lys	Glu	Ala	Cys	Phe	Ala	Val
			580				585						590		
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<212> TYPE: PRT
<213> ORGANISM: Bos taurus

<400> SEQUENCE: 84

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Pro Glu Trp Phe Lys Cys Arg Arg Trp Gln Trp Arg Met Lys Lys Leu
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Gly Ala Pro Ser Ile Thr Cys Val Arg Arg Ala Phe Ala Leu Glu Cys
 50          55          60

Ile Arg Ala Ile Ala Glu Lys Lys Ala Asp Ala Val Thr Leu Asp Gly
 65          70          75          80

Gly Met Val Phe Glu Ala Gly Arg Asp Pro Tyr Lys Leu Arg Pro Val
 85          90          95

Ala Ala Glu Ile Tyr Gly Thr Lys Glu Ser Pro Gln Thr His Tyr Tyr
100          105          110

Ala Val Ala Val Val Lys Lys Gly Ser Asn Phe Gln Leu Asp Gln Leu
115          120          125

Gln Gly Arg Lys Ser Cys His Thr Gly Leu Gly Arg Ser Ala Gly Trp
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Ile Ile Pro Met Gly Ile Leu Arg Pro Tyr Leu Ser Trp Thr Glu Ser
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Leu Glu Pro Leu Gln Gly Ala Val Ala Lys Phe Phe Ser Ala Ser Cys
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Val Pro Cys Ile Asp Arg Gln Ala Tyr Pro Asn Leu Cys Gln Leu Cys
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Lys Gly Glu Gly Glu Asn Gln Cys Ala Cys Ser Ser Arg Glu Pro Tyr
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Phe Gly Tyr Ser Gly Ala Phe Lys Cys Leu Gln Asp Gly Ala Gly Asp
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Val Ala Phe Val Lys Glu Thr Thr Val Phe Glu Asn Leu Pro Glu Lys
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Ala Asp Arg Asp Gln Tyr Glu Leu Leu Cys Leu Asn Asn Ser Arg Ala
245          250          255

Pro Val Asp Ala Phe Lys Glu Cys His Leu Ala Gln Val Pro Ser His
260          265          270

Ala Val Val Ala Arg Ser Ser Val Asp Gly Lys Glu Asp Leu Ile Trp
275          280          285

Lys Leu Leu Ser Lys Ala Gln Glu Lys Phe Gly Lys Asn Lys Ser Arg
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Ser Phe Gln Leu Phe Gly Ser Pro Pro Gly Gln Arg Asp Leu Leu Phe
305          310          315          320

Lys Asp Ser Ala Leu Gly Phe Leu Arg Ile Pro Ser Lys Val Asp Ser
325          330          335

Ala Leu Tyr Leu Gly Ser Arg Tyr Leu Thr Thr Leu Lys Asn Leu Arg
340          345          350

Glu Thr Ala Glu Glu Val Lys Ala Arg Tyr Thr Arg Val Val Trp Cys
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Ala	Val	Gly	Pro	Glu	Glu	Gln	Lys	Lys	Cys	Gln	Gln	Trp	Ser	Gln	Gln
370						375					380				
Ser	Gly	Gln	Asn	Val	Thr	Cys	Ala	Thr	Ala	Ser	Thr	Thr	Asp	Asp	Cys
385				390						395					400
Ile	Val	Leu	Val	Leu	Lys	Gly	Glu	Ala	Asp	Ala	Leu	Asn	Leu	Asp	Gly
				405					410					415	
Gly	Tyr	Ile	Tyr	Thr	Ala	Gly	Lys	Cys	Gly	Leu	Val	Pro	Val	Leu	Ala
			420					425					430		
Glu	Asn	Arg	Lys	Ser	Ser	Lys	His	Ser	Ser	Leu	Asp	Cys	Val	Leu	Arg
		435					440					445			
Pro	Thr	Glu	Gly	Tyr	Leu	Ala	Val	Ala	Val	Val	Lys	Lys	Ala	Asn	Glu
	450					455					460				
Gly	Leu	Thr	Trp	Asn	Ser	Leu	Lys	Asp	Lys	Lys	Ser	Cys	His	Thr	Ala
465					470					475					480
Val	Asp	Arg	Thr	Ala	Gly	Trp	Asn	Ile	Pro	Met	Gly	Leu	Ile	Val	Asn
				485					490					495	
Gln	Thr	Gly	Ser	Cys	Ala	Phe	Asp	Glu	Phe	Phe	Ser	Gln	Ser	Cys	Ala
			500					505					510		
Pro	Gly	Ala	Asp	Pro	Lys	Ser	Arg	Leu	Cys	Ala	Leu	Cys	Ala	Gly	Asp
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Asp	Gln	Gly	Leu	Asp	Lys	Cys	Val	Pro	Asn	Ser	Lys	Glu	Lys	Tyr	Tyr
	530					535					540				
Gly	Tyr	Thr	Gly	Ala	Phe	Arg	Cys	Leu	Ala	Glu	Asp	Val	Gly	Asp	Val
545					550					555					560
Ala	Phe	Val	Lys	Asn	Asp	Thr	Val	Trp	Glu	Asn	Thr	Asn	Gly	Glu	Ser
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Thr	Ala	Asp	Trp	Ala	Lys	Asn	Leu	Asn	Arg	Glu	Asp	Phe	Arg	Leu	Leu
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Cys	Leu	Asp	Gly	Thr	Arg	Lys	Pro	Val	Thr	Glu	Ala	Gln	Ser	Cys	His
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Leu	Ala	Val	Ala	Pro	Asn	His	Ala	Val	Val	Ser	Arg	Ser	Asp	Arg	Ala
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Ala	His	Val	Lys	Gln	Val	Leu	Leu	His	Gln	Gln	Ala	Leu	Phe	Gly	Lys
625					630					635					640
Asn	Gly	Lys	Asn	Cys	Pro	Asp	Lys	Phe	Cys	Leu	Phe	Lys	Ser	Glu	Thr
			645						650					655	
Lys	Asn	Leu	Leu	Phe	Asn	Asp	Asn	Thr	Glu	Cys	Leu	Ala	Lys	Leu	Gly
			660					665						670	
Gly	Arg	Pro	Thr	Tyr	Glu	Glu	Tyr	Leu	Gly	Thr	Glu	Tyr	Val	Thr	Ala
		675					680					685			
Ile	Ala	Asn	Leu	Lys	Lys	Cys	Ser	Thr	Ser	Pro	Leu	Leu	Glu	Ala	Cys
	690					695					700				
Ala	Phe	Leu	Thr	Arg											
705															

1. A method for identifying allergenic milk proteins and/or peptides, the method 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 at least one mediator upon contact with at least one protein or peptide of step d).

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, 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 selected from any of SEQ ID Nos. 1-84.

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

11. The at least one protein or peptide of claim 10 for use in the diagnosis of a milk allergy or a predisposition for a milk allergy in an individual.

12. 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 to an individual suspected of being allergic or becoming allergic and assessing whether the individual developed an allergic reaction against the protein or peptide.

13. The method of claim 12, wherein the method additionally comprises:

- a) a skin test; and/or
- b) a blood test.

14. The method of claim 13,

- 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 selected from any of SEQ ID Nos. 1-84 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 selected from any of SEQ ID Nos. 1-84 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.

15. At least one protein or peptide of claim 9 for use in an allergen-immunotherapy of an allergy in an individual.

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

17. 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 selected from any of SEQ ID Nos. 1-84 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 selected from any of SEQ ID Nos. 1-84 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.

18. The method of claim 17 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.

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

* * * * *

专利名称(译)	鉴定变应原蛋白和肽的方法		
公开(公告)号	US20100143262A1	公开(公告)日	2010-06-10
申请号	US12/581108	申请日	2009-10-16
[标]申请(专利权)人(译)	瓦伦塔鲁道尔夫 TOL RIC VAN HERZ UDO HOCHWALLNER主唱 FOCKE TEJKL MARGARTE 斯沃博达INES SCHULMEISTER ULRIKE		
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[标]发明人	VALENTA RUDOLPH TOL RIC VAN HERZ UDO HOCHWALLNER HEIDRUN FOCKE TEJKL MARGARTE SWOBODA INES SCHULMEISTER ULRIKE		
发明人	VALENTA, RUDOLPH TOL, RIC VAN HERZ, UDO HOCHWALLNER, HEIDRUN FOCKE-TEJKL, MARGARTE SWOBODA, INES SCHULMEISTER, ULRIKE		
IPC分类号	A61K49/00 C40B30/06 C07K7/08 C07K14/00 A61K38/08 A61K38/10 A61K38/16 C07K7/06 G01N33/53 A61P37/06		
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