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(54) Title: USE OF IGBPMA FOR BINDING IGA

(57) Abstract: The present invention relates to use of an IGBPMA polypeptide in methods for binding, detecting, quantitating, re-moving and purifying IgA. The invention also relates to the use of IGBPMA for diagnostic and therapeutic purposes.



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USE OF IGBPMA FOR BINDING IGA

Field of the Invention

The invention relates to the use of an IGBPMA polypeptide as a reagent for binding IgA.

- 5 In particular, an IGBPMA polypeptide may be used for binding, detecting, quantitating, removing and purifying IgA. The invention also relates to the use of IGBPMA for diagnostic and therapeutic purposes.

Background to the Invention

- 10 IgA binding activity, for example for purification or removal of IgA can be provided by use of the IgA binding lectin, Jacalin. Jacalin was identified in jackfruit seeds in the 1970s and is a glycol-protein that binds specifically to the α -D-galactose side chain of IgA. Since Jacalin is a glycosylated lectin, it is purified from jackfruit for laboratory use. Purified Jacalin can be purchased from numerous bio-research companies, see for example www.vectorlabs.com,
15 www.piercenet.com (Thermo Scientific).

There is a need for provision of additional reagents for binding of IgA, both for general binding and for therapeutic applications.

IGBPMA (Immunoglobulin Binding Protein MA) is a protein previously described as having IgG and IgE binding activities, and which is expressed in tick salivary gland extracts.

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Summary of the Invention

- The present invention utilises IGBPMA polypeptides for binding of IgA. The inventors have surprisingly shown that IGBPMA polypeptides have IgA binding activity, whereas these polypeptides have only previously been described as having IgG or IgE binding activity. In
25 addition, the unexpected IgA binding activity of IGBPMA was found to be much stronger than its binding activity for IgG or IgE.

- The inventors' findings provide an additional reagent that can be used to bind IgA. This reagent can be produced by conventional recombinant techniques such as in microbiological culture and is thus suitable for large scale protein production. The reagent may be used for
30 biomedical and biochemical applications which require IgA binding, detection or removal. The inventors' findings also allow the binding activity of IGBPMA for IgA to be used for diagnostic

or therapeutic purposes in IgA-related diseases. The interaction between IGBPMA and IgA may also be assayed in order to identify compounds which exert a modulatory effect thereon.

The invention thus provides a method for binding IgA in a sample, comprising contacting said sample with an IGBPMA polypeptide under conditions suitable for binding of IgA to said polypeptide. The invention further provides a method for removing or purifying IgA from said sample, comprising contacting said sample with an IGBPMA polypeptide attached to a solid support under conditions suitable for binding of IgA to said polypeptide. The invention additionally provides a kit comprising an IGBPMA polypeptide, and reagents suitable for determining the presence of IgA in a sample. The invention also provides a method for identifying whether or not a subject is at risk of developing, or has, an IgA-related disease, said method comprising:

- (a) measuring the level of IgA in a sample derived from said subject by contacting said sample with an IGBPMA polypeptide and detecting binding of said polypeptide to IgA;
- (b) comparing the level of IgA in said sample to a normal level of IgA and thereby determining whether or not a subject is at risk of developing, or has, an IgA-mediated disease;

wherein an increased level of IgA in the sample compared to the normal level identifies the subject as being at risk of developing, or having, an IgA-mediated disease.

The invention additionally provides a method for preventing or treating an IgA-related disease in a subject, comprising administering an effective amount of an IGBPMA polypeptide or of a nucleic acid encoding said polypeptide. The invention further provides a method for identifying a compound that modulates binding of an IgA ligand to IgA, said method comprising contacting an IgA-ligand complex with an IGBPMA polypeptide in the presence of the compound, and measuring binding of said IgA ligand to IgA.

Brief description of the Figures

Figure 1 shows measurement of binding of recombinant IGBPMA (rIGBPMA) to immobilised BSA, IgG, IgA and IgM by surface plasmon resonance (BIAcore). X – axis : Time (seconds), y-axis : Response (Resonance units). Trace labelled as number 1 = rIGBPMA immobilised to BSA. Trace labelled as number 2 = rIGBPMA immobilised to IgG. Trace

labelled as number 3 = rIGBPMA immobilised to IgA. Trace labelled as number 4 = rIGBPMA immobilised to IgM.

Figure 2 shows the effect of preincubation of rIGBPMA with soluble BSA (panel A), IgG (panel B), IgA (panel C) or IgM (panel D) on binding of rIGBPMA to immobilised BSA, IgG, IgA and IgM. X – axis : Time (seconds), y-axis : Response (Resonance units). Trace labelled as number 1 = rIGBPMA immobilised to BSA. Trace labelled as number 2 = rIGBPMA immobilised to IgG. Trace labelled as number 3 = rIGBPMA immobilised to IgA. Trace labelled as number 4 = rIGBPMA immobilised to IgM.

Figure 3 shows measurement of secondary binding activities of IGBPMA by detection of binding of free BSA, IgA, IgG or IgM to immobilised rIGBPMA. X – axis : Time (seconds), y-axis : Response (Resonance units). Trace labelled as number 1 = rIGBPMA immobilised to BSA. Trace labelled as number 2 = rIGBPMA immobilised to IgG. Trace labelled as number 3 = rIGBPMA immobilised to IgA. Trace labelled as number 4 = rIGBPMA immobilised to IgM. Point number 5 = injection of rIGBPMA; point number 6 = injection of BSA; point number 7 = injection of IgA; point number 8: injection of IgA; point number 9 = injection of IgM.

Figure 4 shows a comparison of Jacalin and rIGBPMA binding to IgA. X – axis : Time (seconds), y-axis represents the difference in response (measured in resonance units, RU) between test flow cells (Fc -2,-3,-4) bearing immobilised IgA, IgG and IgE respectively and control flow cells (Fc-1) which were blank. The following steps of additions of Jacalin or rIGBPMA were performed at the indicated points. (1) 30 µl of Jacalin at 15 µg/ml for testing the Jacalin-IgA binding. (2) 30 µl of Jacalin at 100 µg/ml to saturate the Jacalin-IgA binding sites. (3) 30 µl of Jacalin at 15 µg/ml for obtaining the Jacalin-IgA binding capacity after Jacalin saturation. (4) 30 µl of rIGBPMA at 60 µg/ml for testing the rIGBPMA-IgA binding in Jacalin saturated surface. (5) 30 µl of rIGBPMA at 15 µg/ml to further test the rIGBPMA-IgA binding. (6) 30 µl of Jacalin at 15 µg/ml to obtain Jacalin-IgA binding after rIGBPMA-IgA binding and to compare with that of step 3. Trace labelled with points numbered 1 to 6 = IgA binding. Trace labelled 7 = IgG binding. Trace labelled 8 = IgE binding.

Figure 5, panels a-d show measurement of binding of Jacalin and rIGBPMA to IgA. X – axis : Time (seconds), y-axis represents the difference in response (measured in resonance units, RU) between test flow cells (Fc -2,-3,-4) bearing immobilised IgA, IgG and IgE respectively and control flow cells (Fc-1) which were blank. Trace and step numbering as for Figure 4.

Description of the sequences

SEQ ID NO: 1 is a nucleic acid sequence encoding mature IGBPMA.

SEQ ID NO: 2 is the amino acid sequence of mature IGBPMA.

5 SEQ ID NO: 3 is a nucleic acid sequence encoding full-length IGBPMA.

SEQ ID NO: 4 is the amino acid sequence of full-length IGBPMA.

SEQ ID NO: 5 is a nucleic acid sequence encoding a Jacalin polypeptide.

SEQ ID NO: 6 is a further nucleic acid sequence encoding a Jacalin polypeptide.

SEQ ID NO: 7 is a further nucleic acid sequence encoding a Jacalin polypeptide.

10 SEQ ID NO: 8 is a further nucleic acid sequence encoding a Jacalin polypeptide.

SEQ ID NO: 9 is the amino acid sequence of a Jacalin polypeptide.

SEQ ID NO: 10 is the amino acid sequence of a further Jacalin polypeptide.

SEQ ID NO: 11 is the amino acid sequence of a further Jacalin polypeptide.

SEQ ID NO: 12 is the amino acid sequence of a further Jacalin polypeptide.

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Detailed Description of the Invention

It is to be understood that different applications of the disclosed methods may be tailored to the specific needs in the art. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments of the invention only, and is not intended to be limiting. In addition as used in this specification and the appended claims, the singular forms “a”, “an”, and “the” include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to “a compound” includes “compounds”, reference to “a polypeptide” includes two or more such polypeptides, and the like. All publications, patents and patent applications cited herein, whether supra or infra, are hereby incorporated by reference in their entirety.

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Method for binding IgA

The invention provides a method for binding IgA in a sample. The binding of IgA is effected by contacting the sample with an IGBPMA polypeptide under conditions suitable for binding of IgA to said polypeptide.

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IGBPMA polypeptide

The method of the invention uses an IGBPMA polypeptide. An IGBPMA polypeptide is any polypeptide obtainable from an ectoparasite such as a tick or arthropod parasite and which has IgA binding activity. The ability of a polypeptide obtainable from an ectoparasite to bind IgA may be routinely determined by a person skilled in the art. Preferably, the IgA binding activity is similar to that of the polypeptide of SEQ ID NO:2, as described below.

IGBPMA polypeptides are obtainable in particular from the blood-feeding insect and acarine parasites, e. g. biting flies, cattle ticks and mites. As representative of such arthropod parasites may be mentioned for example, ticks of the species *Boophilus*, *Amblyomma*, *Argas*, *Rhipicephalus*, *Hyalomma*, *Ornithodoros*, *Dermacentor*, *Ixodes*; flies, particularly the myiasis, sucking and biting flies, such as *Oestrus ovis*, *Gasterophilus* spp, *Chrysomya* spp, *Calliphora* spp, *Hypoderma* spp, *Dermatobia* spp, *Cochliomyia* spp, *Stomoxys calcitrans*, *Hydrotaea irritans*, *Simulium* spp, *Lyperosia irritans*. *Haematobia* spp, *Tabanus* spp, *Phlebotomus* spp and *Glossina* spp, lice eg. *Haematopinus eurysternus*, *Linognathus vituli*, *Solenopotes capillatus*, *Linoanathus ovillus*, and *Menacanthus* spp; mites such as *Notoedres* spp, *Demodex* spp, *Sarcoptes* spp, *Chorioptes* spp, *Psoreraates* spp, *Dermanyssus* spp, *Ornithonyssus* spp, *Otodectes* spp and *Notoedres* spp; fleas eg. *Ctenocephalides canis* and *C. felis*; keds eg. *Melophagus ovinus* and bugs such as *Cimex* spp.

An IGBPMA coding sequence of the tick *Rhipicephalus appendiculatus* is shown in SEQ ID NO: 1 and encodes the mature IGBPMA protein shown in SEQ ID NO: 2. A further IGBPMA coding sequence is shown in SEQ ID NO:3 and encodes the full-length IGBPMA protein shown in SEQ ID NO: 4. These sequences are also disclosed in the Genbank database, accession number AF001868. A variant nucleic acid sequence comprising a silent mutation (T-C) at nt-648 of AF001868 is disclosed in WO 2007/051975.

The IGBPMA polypeptide preferably comprises the amino acid sequence of SEQ ID NO: 2 or a variant thereof. The IGBPMA polypeptide more preferably consists of the amino acid sequence of SEQ ID NO: 2. The IGBPMA polypeptide may comprises the amino acid sequence of SEQ ID NO: 4 or a variant thereof.

A variant of SEQ ID NO: 2 or of SEQ ID NO: 4 may comprise truncations, mutants or homologues thereof. A variant of SEQ ID NO: 2 or SEQ ID NO: 4 also includes any transcript variants thereof. A variant of SEQ ID NO: 2 or SEQ ID NO: 4 must bind IgA.

Any homologues mentioned herein are typically at least 40% homologous to the relevant region of SEQ ID NO: 2 or SEQ ID NO: 4. Homology can be measured using known methods.

For example the UWGCG Package provides the BESTFIT program which can be used to calculate homology (for example used on its default settings) (Devereux *et al* (1984) Nucleic Acids Research 12, 387-395). The PILEUP and BLAST algorithms can be used to calculate homology or line up sequences (typically on their default settings), for example as described in

5 Altschul S. F. (1993) J Mol Evol 36:290-300; Altschul, S, F *et al* (1990) J Mol Biol 215:403-10. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/>). This algorithm involves first identifying high scoring sequence pair (HSPs) by identifying short words of length W in the query sequence that either match or satisfy some positive-valued threshold score T when aligned

10 with a word of the same length in a database sequence. T is referred to as the neighbourhood word score threshold (Altschul *et al*, supra). These initial neighbourhood word hits act as seeds for initiating searches to find HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Extensions for the word hits in each direction are halted when: the cumulative alignment score

15 falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment. The BLAST program uses as defaults a word length (W) of 11, the BLOSUM62 scoring matrix (see Henikoff and Henikoff (1992) Proc. Natl. Acad. Sci. USA 89: 10915-10919) alignments (B) of 50, expectation (E) of 10, M=5, N=4, and a

20 comparison of both strands.

The BLAST algorithm performs a statistical analysis of the similarity between two sequences; see e.g., Karlin and Altschul (1993) Proc. Natl. Acad. Sci. USA 90: 5873-5787. One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)),

25 which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a sequence is considered similar to another sequence if the smallest sum probability in comparison of the first sequence to the second sequence is less than about 1, preferably less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

30 In preferred embodiments, a variant sequence may be at least 55%, 65%, 70%, 75%, 80%, 85%, 90% and more preferably at least 95%, 97% or 99% homologous to a particular region of SEQ ID NO: 2 or SEQ ID NO: 4 over at least 20, preferably at least 30, for instance at

least 40, 60, 100, 200, 300 or more contiguous amino acids, or even over the entire sequence of the variant. Alternatively, the variant sequence may be at least 55%, 65%, 70%, 75%, 80%, 85%, 90% and more preferably at least 95%, 97% or 99% homologous to full-length SEQ ID NO: 2 or SEQ ID NO:4. Typically the variant sequence differs from the relevant region of SEQ ID NO: 2 or SEQ ID NO: 4 by at least, or less than, 2, 5, 10, 20, 40, 50 or 60 mutations (each of which can be substitutions, insertions or deletions). A variant IGBPMA polypeptide of the invention may have a percentage identity with a particular region of SEQ ID NO: 2 or SEQ ID NO: 4 which is the same as any of the specific percentage homology values (i.e. it may have at least 40%, 55%, 80% or 90% and more preferably at least 95%, 97% or 99% identity) across any of the lengths of sequence mentioned above.

Variants of SEQ ID NO: 2 or SEQ ID NO: 4 also include truncations. Any truncation may be used so long as the variant is still able to bind IgA. Truncations will typically be made to remove sequences that are non-essential for IgA binding and/or do not affect conformation of the folded protein, in particular folding of the IgA binding domain. Truncations may also be selected to improve solubility of the IGBPMA polypeptide. Appropriate truncations can routinely be identified by systematic truncation of sequences of varying length from the N- or C-terminus. Truncations may remove all other sequences except for the IgA binding domain.

Variants of SEQ ID NO: 2 or SEQ ID NO: 4 further include mutants which have one or more, for example, 2, 3, 4, 5 to 10, 10 to 20, 20 to 40 or more, amino acid insertions, substitutions or deletions with respect to a particular region of SEQ ID NO: 2. Deletions and insertions are made preferably outside of the IgA binding domain as described below. Insertions are typically made at the N- or C-terminal ends of a sequence derived from SEQ ID NO: 2 or SEQ ID NO: 4, for example for the purposes of recombinant expression as detailed below. A sequence may be inserted to facilitate purification of the IGBPMA polypeptide, such as an affinity tag. Another common N-terminal insertion is a signal peptide to assist secretion in a cell system where the variant sequence derived from SEQ ID NO: 2 or SEQ ID NO: 4 does not contain one.

Substitutions are also typically made in regions that are non-essential for IgA binding and/or do not affect conformation of the folded protein. Such substitutions may be made to improve solubility or other characteristics of the IGBPMA polypeptide. Although not generally preferred, substitutions may also be made in the IgA binding domain, i.e. at residues which affect binding to or contact with IgA, so long as such substitutions do not significantly affect binding

activity for IgA. These substitutions may be made to improve binding to IgA or to map the binding site of a compound which modulates binding of an IGBPMA polypeptide to IgA.

Substitutions preferably introduce one or more conservative changes, which replace amino acids with other amino acids of similar chemical structure, similar chemical properties or similar side-chain volume. The amino acids introduced may have similar polarity, hydrophilicity, hydrophobicity, basicity, acidity, neutrality or charge to the amino acids they replace. Alternatively, the conservative change may introduce another amino acid that is aromatic or aliphatic in the place of a pre-existing aromatic or aliphatic amino acid. Conservative amino acid changes are well known in the art and may be selected in accordance with the properties of the 20 main amino acids as defined in Table A. Where amino acids have similar polarity, this can also be determined by reference to the hydropathy scale for amino acid side chains in Table B.

Table A – Chemical properties of amino acids

Ala	aliphatic, hydrophobic, neutral	Met	hydrophobic, neutral
Cys	polar, hydrophobic, neutral	Asn	polar, hydrophilic, neutral
Asp	polar, hydrophilic, charged (-)	Pro	hydrophobic, neutral
Glu	polar, hydrophilic, charged (-)	Gln	polar, hydrophilic, neutral
Phe	aromatic, hydrophobic, neutral	Arg	polar, hydrophilic, charged (+)
Gly	aliphatic, neutral	Ser	polar, hydrophilic, neutral
His	aromatic, polar, hydrophilic, charged (+)	Thr	polar, hydrophilic, neutral
Ile	aliphatic, hydrophobic, neutral	Val	aliphatic, hydrophobic, neutral
Lys	polar, hydrophilic, charged(+)	Trp	aromatic, hydrophobic, neutral
Leu	aliphatic, hydrophobic, neutral	Tyr	aromatic, polar, hydrophobic

Table B. Hydropathy scale

Side Chain	Hydropathy
Ile	4.5
Val	4.2

	Leu	3.8
	Phe	2.8
	Cys	2.5
	Met	1.9
5	Ala	1.8
	Gly	-0.4
	Thr	-0.7
	Ser	-0.8
	Trp	-0.9
10	Tyr	-1.3
	Pro	-1.6
	His	-3.2
	Glu	-3.5
	Gln	-3.5
15	Asp	-3.5
	Asn	-3.5
	Lys	-3.9
	Arg	-4.5

20 A variant of SEQ ID NO:2 or SEQ ID NO: 4 preferably has a binding activity for IgA which is the same as or improved with respect to the native polypeptide of SEQ ID NO:2 or SEQ ID NO: 4. However, variants which have reduced IgA binding activity compared to SEQ ID NO:2 or SEQ ID NO: 4 may also be used, provided that they retain sufficient binding activity for the relevant application. A variant of SEQ ID NO:2 or SEQ ID NO: 4 may thus have an IgA
25 binding activity that is at least 40%, 50%, 60%, 65%, 70%, 75%, and more preferably at least 80%, 85%, 90%, 95%, or 99% of the IgA binding activity of SEQ ID NO: 2 or SEQ ID NO: 4 respectively. The binding activity of the variant for IgA is preferably measured under the same conditions as the binding activity of SEQ ID NO:2 and SEQ ID NO: 4 for IgA, which is described below.

30 *Sample*

The method is preferably carried out *in vitro* or *ex vivo*. The method may be carried out on any sample. The sample may be of any origin, but is typically obtained from a human or animal body.

The sample is preferably a fluid sample. The sample may comprise a body fluid. The
35 sample is preferably obtained from a mucosal surface. The sample may be saliva, colostrum, tears, sweat, gut fluid or other mucous secretions. The sample may be a mucous secretion from the kidney, genitourinary tract, gastrointestinal tract, prostate or respiratory epithelium. The sample may be mucus, blood, plasma or serum. The sample may be an IgA deposit, for example

obtained from the kidney. The sample can be a cell or tissue sample. The sample may be from an immunised host animal expressing IgA. The sample may be a culture medium or a cell lysate from a cell culture expressing IgA. The cell culture may be prokaryotic or eukaryotic. The sample is typically processed prior to contacting with an IGBPMA polypeptide.

5 The sample typically contains IgA or is suspected of containing IgA. The IgA may be from any source. The IgA may be from any species, such as any mammalian or avian species. The IgA may be polymeric such as dimeric, or monomeric IgA. The IgA may be of any IgA isotype, such as IgA1 or IgA2. Preferably, the IgA is human IgA. The IgA may be recombinant IgA, humanised IgA, chimeric IgA or an IgA antibody fragment. The IgA may be glycosylated
10 IgA, for example galactosylated IgA, such as galactosylated IgA1. The IgA may be aberrantly glycosylated IgA, for example galactose-deficient IgA or IgA1. The IgA or IgA1 may have no galactose present. The IgA may be an autoantibody. The IgA may be a glycan-specific antibody.

 The sample may be previously determined as having IgA present. Methods for detecting and quantitating IgA are known in the art. Detection of IgA may also be carried out in the
15 context of a method of the invention, i.e. by detecting the interaction between IGBPMA and IgA, as described below. The method may also be used on a sample in order to confirm there is no IgA present or for the purposes of removing any IgA that might be present, even if there is no previous indication of the presence of IgA in the sample.

 The sample is contacted with an IGBPMA polypeptide. The method can be carried out
20 using any IGBPMA polypeptide in any form. Suitable IGBPMA polypeptides are discussed in more detail below. The IGBPMA polypeptide can be in solution. The solution may comprise a purified or substantially purified recombinant IGBPMA polypeptide in a suitable buffer. Such buffers are known in the art. Alternatively, the solution may be a culture medium or a cell lysate from a cell culture expressing an IGBPMA polypeptide.

25 Preferably, the IGBPMA polypeptide is immobilised on a solid support, such as a platform or surface. The immobilisation may be reversible or irreversible. Suitable platforms or surfaces are known in the art. Examples include plates, beads, microbeads, membranes, chips, columns, crystals and ceramics. The IGBPMA polypeptide may be immobilised on a sensor chip, such as a sensor chip which permits detection of interactions of the immobilised IGBPMA
30 with a ligand. The sensor chip may be suitable for detection of binding interactions by surface plasmon resonance.

Examples of solid support materials include glass, plastics, synthetic polymers, nitrocellulose, nylon, ceramics, metals, resins, gels and membranes. A suitable platform is a standard 96 or 384 well plate. The IGBPMA may be anchored to a lipid-containing membrane. The membrane may be natural or artificial. Suitable membranes are known in the art.

5 Suitable binding conditions for binding of an IGBPMA polypeptide to IgA include physiological pH and salt concentrations, such as those present in whole blood or serum. A preferred binding buffer is 50mM Tris, 100mM NaCl, 20mM CaCl₂, 20mM MgCl₂, pH 7.2. This preferred binding buffer may be used to compare the binding activity of a variant of SEQ ID NO:2 or SEQ ID NO: 4 with that of SEQ ID NO:2 or SEQ ID NO: 4 itself, as described above.

10 The skilled person would be able to select other suitable binding conditions and buffers on the basis of their common general knowledge.

The method of the invention may be carried out for any purpose where binding of IgA is required. In particular, the method may be carried out for the purpose of detecting, sequestering, neutralising, removing or purifying IgA present in the sample.

15 The method for binding IgA in a sample may further comprise contacting of the sample with a further IgA-binding agent. Preferably the further IgA-binding agent is a Jacalin polypeptide. As discussed below, analysis of binding of IGBPMA and Jacalin to IgA indicates that these IgA-binding agents bind to different sites on IgA. Accordingly, sequential binding with an IGBPMA polypeptide and a Jacalin polypeptide may be used to provide a greater level
20 of IgA binding in a sample. The method for binding IgA in a sample may comprise first contacting the sample with an IGBPMA polypeptide to bind IgA, and then subsequently contacting the sample with a further IgA-binding agent to further bind IgA. Preferably, the further IgA-binding agent is a Jacalin polypeptide. Alternatively, the method may comprise first contacting the sample with an IgA-binding agent other than IGBPMA, and then subsequently
25 contacting the sample with an IGBPMA polypeptide to further bind IgA. Preferably, the IgA-binding agent other than IgA is a Jacalin polypeptide. The method may comprise adding IGBPMA and a further IgA-binding agent to the sample simultaneously or concurrently to bind IgA.

30 Suitable binding conditions for binding of a Jacalin polypeptide to IgA are similar to those suitable for binding of an IGBPMA polypeptide to IgA. Such conditions include physiological pH and salt concentrations, such as those present in whole blood or serum. An example of a suitable binding buffer is 50mM Tris, 100mM NaCl, 20mM CaCl₂, 20mM MgCl₂,

pH 7.2. The skilled person would be able to select other suitable binding conditions and buffers on the basis of their common general knowledge.

Preferably, where the methods of the invention comprise binding of IgA with both an IgA polypeptide and a Jacalin polypeptide, the binding conditions will be the same for the step of binding of the IGBPMA polypeptide to IgA, and the step of binding of the Jacalin polypeptide to IgA. However, the methods of the invention may comprise altering the binding conditions between the step of binding of the IGBPMA polypeptide to IgA, and the step of binding of the Jacalin polypeptide to IgA, to optimise binding to IgA in both steps.

Jacalin polypeptide

The invention may comprise use of a Jacalin polypeptide to further bind IgA. A Jacalin polypeptide is any lectin polypeptide obtainable from Jackfruit and which has IgA binding activity. Typically, the Jacalin polypeptide has binding activity for galactosylated IgA. The ability of a lectin polypeptide obtainable from a Jackfruit species to bind IgA may be routinely determined by a person skilled in the art. Jacalin polypeptides are obtainable in particular from *Artocarpus heterophyllus* or *Artocarpus integrifolia*.

Jacalin-encoding nucleic acid sequences are shown in SEQ ID NOs 5 to 8. These sequences represent accession numbers L03795, L03798, L03796 and L03797 in the Genbank database. Amino acid sequences of Jacalin polypeptides are shown in SEQ ID NOs 9 to 12.

These sequences represent accession numbers AAA32677, AAA32680, AAA32678 and AAA32679 in the Genbank database.

A Jacalin polypeptide used in a method or kit of the invention preferably comprises the amino acid sequence of SEQ ID NO: 9, 10, 11 or 12, or a variant of any thereof. More preferably, the Jacalin polypeptide consists of the amino acid sequence of SEQ ID NO: 9, 10, 11 or 12. A Jacalin polypeptide may comprise or consist of an amino acid sequence encoded by the nucleic acid sequence of SEQ ID NO 5, 6, 7, or 8 or a variant of any thereof.

A variant of any of SEQ ID NOs 9 to 12 or of an amino acid sequence encoded by any of SEQ ID NOs 5 to 8 may comprise truncations, mutants or homologues thereof, or a transcript variant thereof. Any of the above variants must bind IgA.

Any homologues are typically at least 40% homologous to the relevant region of SEQ ID NO: 9, 10, 11 or 12 or to the relevant region of the amino acid sequence encoded by SEQ ID NO

5, 6, 7, or 8. Homology can be measured as discussed above in relation to variant IGBPMA polypeptides.

In preferred embodiments, a variant Jacalin sequence may be at least 55%, 65%, 70%, 75%, 80%, 85%, 90% and more preferably at least 95%, 97% or 99% homologous to a particular region of SEQ ID NO: 9, 10, 11, or 12 or to a particular region of the amino acid sequence encoded by SEQ ID NO 5, 6, 7, or 8. The above levels of homology are typically present over at least 20, preferably at least 30, for instance at least 40, 60, 100, 200 or more contiguous amino acids, or even over the entire sequence of the variant. Alternatively, the variant sequence may be at least 55%, 65%, 70%, 75%, 80%, 85%, 90% and more preferably at least 95%, 97% or 99% homologous to full-length SEQ ID NO: 9, 10, 11 or 12, or to the full-length amino acid sequence encoded by SEQ ID NO 5, 6, 7, or 8.

Typically the variant sequence differs from the relevant region of SEQ ID NO: 9, 10, 11, or 12, or to the relevant region of the amino acid sequence encoded by SEQ ID NO 5, 6, 7, or 8, by at least, or less than, 2, 5, 10, 20, 40, 50 or 60 mutations (each of which can be substitutions, insertions or deletions). A variant Jacalin polypeptide of the invention may have a percentage identity with a particular region of SEQ ID NO: 9, 10, 11, or 12, or with a particular region of the amino acid sequence encoded by SEQ ID NO 5, 6, 7, or 8, which is the same as any of the specific percentage homology values (i.e. it may have at least 40%, 55%, 80% or 90% and more preferably at least 95%, 97% or 99% identity) across any of the lengths of sequence mentioned above.

Variants of SEQ ID NOs 9, 10, 11, or 12, or of amino acid sequences encoded by SEQ ID NO 5, 6, 7, or 8 also include truncations. Any truncation may be used so long as the variant is still able to bind IgA. Truncations will typically be made to remove sequences that are non-essential for IgA binding and/or do not affect conformation of the folded protein, in particular folding of the IgA binding domain. Truncations may also be selected to improve solubility of the Jacalin polypeptide. Appropriate truncations can routinely be identified by systematic truncation of sequences of varying length from the N- or C-terminus. Truncations may remove all other sequences except for the IgA binding domain.

Variants of SEQ ID NOs 9, 10, 11, or 12, or of amino acid sequences encoded by SEQ ID NO 5, 6, 7, or 8 further include mutants which have one or more, for example, 2, 3, 4, 5 to 10, 10 to 20, 20 to 40 or more, amino acid insertions, substitutions or deletions with respect to a particular region thereof. Deletions and insertions are made preferably outside of the IgA

binding domain as described below. Insertions are typically made at the N- or C-terminal ends of a sequence derived from SEQ ID NOs 9, 10, 11, or 12, or of an amino acid sequence encoded by SEQ ID NO 5, 6, 7, or 8, for example for the purposes of recombinant expression. A sequence may be inserted to facilitate purification of the Jacalin polypeptide, such as an affinity tag.

- 5 Another common N-terminal insertion is a signal peptide to assist secretion in a cell system where the variant sequence derived from SEQ ID NOs 9, 10, 11, or 12, or derived from an amino acid sequence encoded by SEQ ID NO 5, 6, 7, or 8, does not contain one.

Substitutions are also typically made in regions that are non-essential for IgA binding and/or do not affect conformation of the folded protein. Such substitutions may be made to
10 improve solubility or other characteristics of the Jacalin polypeptide. Although not generally preferred, substitutions may also be made in the IgA binding domain, i.e. at residues which affect binding to or contact with IgA, so long as such substitutions do not significantly affect binding activity for IgA. These substitutions may be made to improve binding to IgA. Substitutions preferably introduce one or more conservative changes. The conservative changes discussed
15 above in relation to IGBPMA variants are equally applicable to Jacalin variants.

A variant of SEQ ID NOs 9, 10, 11, or 12, or of an amino acid sequence encoded by SEQ ID NO 5, 6, 7, or 8 preferably has a binding activity for IgA which is the same as or improved with respect to the native polypeptide. However, variants which have reduced IgA binding activity may also be used, provided that they retain sufficient binding activity for the relevant
20 application. A variant of SEQ ID NOs 9, 10, 11, or 12, or of an amino acid sequence encoded by SEQ ID NO 5, 6, 7, or 8, may thus have an IgA binding activity that is at least 40%, 50%, 60%, 65%, 70%, 75%, and more preferably at least 80%, 85%, 90%, 95%, or 99% of the IgA binding activity of the respective native polypeptide. The binding activity of the variant for IgA is preferably measured under the same conditions as the binding activity of the native Jacalin
25 polypeptide.

Detection of IgA

The binding of an IGBPMA polypeptide to IgA can allow for detection of the presence of IgA in a sample. This detection may also allow for quantitation of the level of IgA present in a sample, of the level of IgA removed from a sample, or of the level of IgA remaining in a sample
30 after binding.

Suitable means for detecting a binding interaction between two molecules are well known in the art. The detection of binding may be direct or indirect.

An IGBPMA polypeptide may include a detectable label, such as a fluorescent label. The label is typically attached to the IGBPMA polypeptide at a position such that it is suitable for monitoring binding of IgA to the IGBPMA polypeptide. The label may thus be attached to a site in the IgA binding domain of IGBPMA or at a position within IGBPMA whose conformation is affected by IgA binding. The signal from the label may be decreased, increased or changed in spectrum upon binding of IgA to the IGBPMA polypeptide as compared to the signal present in the absence of IgA. These changes in signal are linked to the binding of IgA to IGBPMA and thus can provide a direct measure of the level of IgA in the sample.

The presence of IgA in complex with an IGBPMA polypeptide may be determined. The IGBPMA polypeptide is preferably immobilised to a solid support for this purpose. The IgA present in complex on the solid support via its binding to the IGBPMA polypeptide can then be detected.

Detection of the IgA present in complex may be by any suitable means known for detection of IgA in the art. For example, the detection may be performed by an immunoassay utilising an anti-IgA antibody. The anti-IgA antibody can be conjugated to a detectable label, such as a fluorescent, radioactive, or enzymatic label. Detection of IgA may be carried out by ELISA for example using an anti-IgA antibody-HRP conjugate. The level of IgA may then be quantitated by adding a substrate for the conjugated HRP and monitoring the enzyme reaction colorimetrically. These assays are suitable for performing in a multi-well high-throughput format.

The amount of IgA removed from a sample may also be detected by elution of IgA bound to the IGBPMA polypeptide following binding. Similarly, any residual amount of IgA remaining in a sample after binding with an IGBPMA polypeptide may be determined. The eluted or residual IgA can be detected by any suitable means known for detection of IgA in the art.

The invention thus provides a method for detecting the presence or amount of IgA in a sample, comprising contacting said sample with an IGBPMA polypeptide under conditions suitable for binding of IgA to said polypeptide, and detecting IgA in said sample. The detection of IgA may be performed before, during, or after said contacting. The detection may be performed both before and after said contacting. The detection may comprise determining the presence of IgA. The detection may comprise quantitating the amount of IgA. The detection may comprise determining the presence of or quantitating the amount of glycosylated IgA, preferably the presence of or amount of galactosylated IgA. Such detection typically comprises a step of

determining the level of glycosylation or galactosylation present on IgA, such as the level of galactosylation present on IgA1. Methods for determining the glycosylation or galactosylation level of a given protein are known in the art. The detection may comprise determining the presence or amount of immune complexes comprising (i) poorly galactosylated or galactose-deficient IgA1 and/or (ii) glycan-specific IgA and/or glycan-specific IgG. These aspects are discussed further below in the context of diagnostic applications of the invention.

The method for detecting the presence or amount of IgA in a sample may further comprise detecting the presence or amount of IgG and/or IgE in said sample. Appropriate detection steps can be selected to independently detect each form of immunoglobulin.

Removal of IgA

The binding of an IGBPMA polypeptide to IgA can allow for the removal of IgA from a sample. This is useful where the presence of IgA is not desired in the sample, or where it is necessary to reduce levels of IgA below a threshold amount. The invention thus provides a method for removing IgA from a sample, comprising contacting said sample with an IGBPMA polypeptide under conditions suitable for binding of IgA to said polypeptide, and removing IgA from the sample. The method typically comprises collecting a sample in which IgA has been removed.

The removal of IgA may be effected by precipitating complexes of IgA and IGBPMA. Preferably, the IGBPMA polypeptide is attached to a solid support, such as on beads, a resin, or a column, and the sample is a fluid sample. IgA immobilised to the solid support via its interaction with IGBPMA can then be physically separated from the fluid sample.

The method may remove all IgA present in the sample, such that there is no IgA detectable in the sample after the step of removal. The method may also remove 40%, 50%, 60%, 65%, 70%, 75%, and more preferably at least 80%, 85%, 90%, 95%, or 99% of the IgA present in the sample. The contacting with an IGBPMA polypeptide may be performed in multiple iterations to remove further IgA from the sample. The amount of IgA to be removed from the sample may be selected according to the maximum level of IgA that can be tolerated for the desired application of the treated sample. The residual amount of IgA following carrying out the method may be less than 50%, 40%, 30%, 25%, 20%, more preferably less than 15%, 10%, 5%, 2%, or 1% of the starting amount if IgA present in the sample.

The method for removal of IgA may be applied to remove IgA from serum products, or for removal of IgA in dialysis or transfusion of whole blood. The method may be used to remove

IgA from blood from normal donors in order for the thus processed blood to be transfused to IgA deficient patients. Such a method is carried out ex vivo or in vitro. The method for removal of IgA may be used for kidney dialysis. Such a method is carried out ex vivo. The method for removal of IgA may also be applied for removal of IgA from a preparation containing other immunoglobulins, such as IgG, IgM and IgE. The use of an IGBPMA polypeptide for selective binding of IgA is discussed below.

The method for removing IgA from a sample may further comprise removing IgG and/or IgE in said sample. The amount of IgG and/or IgE removed from the sample, or the residual amount of IgG and/or IgE following carrying out the method, may be any of the percentage values given above in connection with IgA removal. Appropriate binding conditions can be selected to bind and then remove IgA and additional immunoglobulins, or to bind and remove only IgA, as discussed below.

The method for removing IgA from a sample may further comprise contacting of the sample with a further IgA-binding agent. Preferably the further IgA-binding agent is a Jacalin polypeptide. The method for removing IgA from a sample may comprise first contacting the sample with an IGBPMA polypeptide to remove IgA, and then subsequently contacting the sample with a further IgA-binding agent to further remove IgA. Preferably, the further IgA-binding agent is a Jacalin polypeptide. Alternatively, the method may comprise first contacting the sample with an IgA-binding agent other than IGBPMA, and then subsequently contacting the sample with an IGBPMA polypeptide to further remove IgA. Preferably, the IgA-binding agent other than IgA is a Jacalin polypeptide. The method may comprise adding IGBPMA and a further IgA-binding agent to the sample simultaneously or concurrently to remove IgA.

In the above methods, the further IgA-binding agent, which is preferably a Jacalin polypeptide, is preferably attached to a solid support, such as on beads, a resin, or a column. In preferred methods, both the IGBPMA polypeptide and the further IgA-binding agent, which is preferably a Jacalin polypeptide, are attached to the same solid support. The methods may thus comprise contacting the sample with a solid support, such as beads, a resin, or a column, which comprises an IGBPMA polypeptide and a further IgA binding agent, preferably a Jacalin polypeptide. The IGBPMA polypeptide and further IgA binding agent, which is preferably a Jacalin polypeptide, may be immobilised to the same beads or a mixture of beads may be provided, some having an IGBPMA polypeptide attached and others the further IgA binding agent, preferably a Jacalin polypeptide, attached.

Purification of IgA

The binding of an IGBPMA polypeptide to IgA also allows for purification of IgA. This is useful in particular for the isolation of IgA from serum products and for the provision of recombinant, polyclonal or monoclonal IgA antibody preparations. The invention thus provides a method for purifying IgA from a sample, comprising contacting said sample with an IGBPMA polypeptide under conditions suitable for binding of IgA to said polypeptide, and purifying IgA from said sample. The method typically comprises collecting a sample in which IgA has been enriched.

Preferably, the IGBPMA polypeptide is attached to a solid support, such as on beads, a resin, or a column, and the sample is a fluid sample. IgA immobilised to the solid support via its interaction with IGBPMA can then be separated from the fluid sample.

The method then includes a step of eluting the immobilised IgA from the solid support. Suitable elution conditions for disrupting the interaction between IGBPMA and IgA can be selected by the skilled person. The elution conditions may comprise use of a weak acid or alkali solution, such as 10 mM HCl or 10 mM NaOH. The eluted IgA can then be formulated into a suitable buffer, for example for further storage.

The method may purify all IgA present in the sample, such that there is no IgA detectable in the sample after purification. The method may purify 40%, 50%, 60%, 65%, 70%, 75%, and more preferably at least 80%, 85%, 90%, 95%, or 99% of the IgA present in the sample. The contacting with an IGBPMA polypeptide may be performed in multiple iterations to purify further IgA from the sample. The method for purifying IgA may further comprise contacting with the sample with a further IgA binding agent. Preferably the further IgA-binding agent is Jacalin. The method may comprise first purifying IgA with IGBPMA and then subsequently further purifying IgA with the further IgA-binding agent. Alternatively, the method may comprise first purifying IgA with an IgA binding agent other than IGBPMA and then subsequently further purifying IgA with IGBPMA. The method may comprise adding IGBPMA and a further IgA-binding agent to the sample simultaneously or concurrently to purify IgA.

Similarly to the above discussion of methods for removing IgA, methods for purifying IgA which comprise binding of IgA with a further IgA-binding agent preferably comprise attachment of the further IgA-binding agent to a solid support. The solid support may comprise beads, a resin, or a column. Both the IGBPMA polypeptide and the further IgA-binding agent, which is preferably a Jacalin polypeptide, may be attached to the same solid support. The

methods for purifying IgA may thus comprise contacting the sample with a solid support, such as beads, a resin, or a column, which comprises an IGBPMA polypeptide and a further IgA binding agent, preferably a Jacalin polypeptide. The IGBPMA polypeptide and further IgA binding agent, which is preferably a Jacalin polypeptide, may be immobilised to the same beads or a mixture of beads may be provided, some having an IGBPMA polypeptide attached and others having the further IgA binding agent, preferably a Jacalin polypeptide, attached.

The method may selectively purify only IgA and not other immunoglobulins from a sample containing multiple immunoglobulins. For example, the method may purify only IgA from a sample containing IgA and IgM. The method may purify only IgA from a sample containing IgA, IgG and optionally also IgM. The method may purify only IgA from a sample containing IgA, IgE and optionally also IgG and/or IgM. Selective purification may be useful to remove contaminating IgA from preparations of other immunoglobulins.

In order to provide for selective binding of IgA, the skilled person may select appropriate conditions which are suitable for selective binding of IgA to an IGBPMA polypeptide. The IGBPMA polypeptide of SEQ ID NO:2 has greatly increased binding activity for IgA compared for example to its previously reported binding activity for IgG. Accordingly, conditions may be selected under which the weaker binding activity for IgG is disrupted, but the stronger binding activity for IgA is maintained. For example, a salt gradient may be used during elution of immunoglobulins bound to IGBPMA, whereby immunoglobulins that bind IGBPMA more weakly, such as IgG, are eluted using a salt concentration at which IgA remains bound to IGBPMA. Similar considerations apply for selective binding of IgA but not IgM, or IgA but not IgE.

In other applications, it may be of interest to purify IgA and also other immunoglobulins. The method for purifying IgA from a sample may thus further comprise purifying IgG and/or IgE from said sample. Suitable conditions can be selected under which IgA and the other immunoglobulins can all bind to one or more IGBPMA polypeptides. The amount of IgG and/or IgE purified from the sample may be any of the percentage values given above in connection with IgA purification efficiency.

Neutralising and sequestering IgA

The invention also provides a method for neutralising or sequestering IgA in a sample, comprising contacting said sample with an IGBPMA polypeptide under conditions suitable for binding of IgA to said polypeptide. This method allows for a sample containing IgA

to be used safely in applications where IgA activity is not desirable without removing IgA from the sample. The method may comprise masking a region of IgA which mediates an undesired IgA activity by contacting IgA with IGBPMA to form a neutralised IgA-IGBPMA complex. . The method for neutralising or sequestering IgA may further comprise contacting with the sample with a further IgA binding agent. Preferably the further IgA-binding agent is Jacalin. As discussed below, IGBPMA and Jacalin bind to IgA at different sites and so can be used in combination to mask different regions of IgA, thus further neutralising or sequestering IgA.

Kit for binding IgA

In a related aspect to the above methods, the invention provides a kit comprising an IGBPMA polypeptide and reagents suitable for detection of IgA. The invention also provides a kit comprising an IGBPMA polypeptide and a further IgA-binding agent, and optionally reagents suitable for detection of IgA. In either of the above kits, the IGBPMA polypeptide is preferably a polypeptide comprising SEQ ID NO: 2 or a variant thereof. The IGBPMA polypeptide may be preferably bound to a solid support, such as attached to beads, a resin or a column.

Where a kit of the invention comprises a further IgA binding agent, the further IgA binding agent is preferably a Jacalin polypeptide. The further IgA binding agent, which is preferably a Jacalin polypeptide, is preferably bound to a solid support, such as attached to beads, a resin or a column. A kit of the invention may comprise two separate solid supports, one having the IGBPMA polypeptide attached, and one having a further IgA binding agent, preferably a Jacalin polypeptide, attached. The two separate solid supports may be independently selected from beads, a resin or a column.

Alternatively, a kit of the invention may comprise a single solid support having both an IGBPMA polypeptide and a further IgA-binding agent, preferably a Jacalin polypeptide, attached. The single solid support may comprise beads, a resin or a column. The single solid support may comprise a mixture of beads, some having an IGBPMA polypeptide attached and others having the further IgA binding agent, preferably a Jacalin polypeptide, attached.

Alternatively, the IGBPMA polypeptide and further IgA binding agent, which is preferably a Jacalin polypeptide, may be immobilised to the same beads.

Where a kit of the invention comprises reagents suitable for detection of IgA, these may include for example an anti-IgA antibody, preferably conjugated to a detectable label, such as a fluorescent, radioactive, or enzymatic label.. Any kit of the invention may further comprise

reagents suitable for detection of IgG and/or IgE. Any kit of the invention may include suitable buffers and other factors which are required for binding and/or detection of IgA as described above.

5 **Diagnostic method**

As discussed above, the inventors have surprisingly demonstrated that an IGBPMA polypeptide has IgA binding activity. Thus, an IGBPMA polypeptide may be used for detection of IgA in the context of an IgA-related disease.

10 The invention therefore provides a method for identifying whether or not a subject is at risk of developing, or has, an IgA-related disease, said method comprising:

- (a) measuring the level of IgA in a sample derived from said subject by contacting said sample with an IGBPMA polypeptide and detecting binding of said polypeptide to IgA;
 - (b) comparing the level of IgA in said sample to a normal level of IgA and thereby determining whether or not a subject is at risk of developing, or has, an IgA-mediated
- 15 disease;

wherein an increased level of IgA in the sample compared to the normal level identifies the subject as being at risk of developing, or having, an IgA-mediated disease.

The level of IgA present in the sample may be measured by any method of binding and detecting IgA described herein. The level of IgA is then compared with a normal level of IgA..

20 The method of binding and detecting IgA may comprise measuring the level of glycosylated, such as galactosylated, IgA or IgA1 present in the sample. The level of glycosylated, such as galactosylated, IgA or IgA1 is then compared or a normal level of IgA or IgA1 glycosylation or galactosylation

A person skilled in the art will be able to determine a normal level of IgA. It will

25 typically be the average level of IgA observed in a representative sample of a healthy population. Specifically, the population does not have any IgA-related disease or any other disease or condition that is likely to result in altered IgA expression. This will allow for a statistically significant diagnosis to be performed on the basis of comparison with the normal level i.e. one which takes into account natural variation in IgA levels that are observed in the sample

30 population. Similarly, a person skilled in the art will be able to determine a normal level of IgA or IgA1 glycosylation or galactosylation.

The level of IgA or of glycosylated/galactosylated IgA or IgA1 in a sample from a subject can also be used to monitor the progression of an IgA-related disease in a subject or the suitability of a treatment. The IgA level or the level of glycosylation or galactosylation present on IgA or IgA1 may be measured at suitable time intervals after diagnosis.

5 The IgA-related disease may be an IgA-mediated disease, for example a disease associated with inappropriate IgA activation. The method may comprise detecting the presence of a disease associated with IgA autoantibodies. Particular IgA-mediated diseases of interest include celiac diseases, Linear IgA Dermatitis and IgA Nephropathy.

The method may comprising identifying whether or not a subject is at risk of developing, or has, a disease associated with IgA autoantibodies, by measuring the level of glycosylated/galactosylated IgA or IgA1 in a sample from a subject. The method may comprise measuring the level of IgA or IgA1 that does not have any galactose present. Preferably, the disease is IgA nephropathy. For example, the presence of poorly galactosylated IgA1 may be a trigger for generation of autoantibodies (Boyd JK et al, Kidney Intl 8 Feb (2012)). Additionally, the presence of immune complexes comprising poorly galactosylated IgA1 and glycan-specific IgA and/or IgG antibodies is indicative of a risk of developing, or the presence of, IgA nephropathy. The method may thus comprise measuring the level of immune complexes comprising poorly galactosylated or galactose-deficient IgA1 and glycan-specific IgA and/or IgG antibodies. The known IgA binding agent Jacalin is a galactose-binding lectin and so is unsuitable for measuring the level of poorly galactosylated IgA1.

The IgA-related disease may be a disease caused by IgA deficiency, such as an immunodeficiency. An example is selective IgA deficiency. The method may comprise identifying whether a subject has an IgA deficiency before carrying out a blood transfusion. Such a method may comprise identifying that a subject has an IgA deficiency, carrying out a method for removing IgA according to the invention on blood to be transfused to the subject, and then administering the blood from which IgA has been removed to the subject. The blood may be from a normal donor. Such a method may comprise identifying that a subject has an IgA deficiency and then administering blood from an IgA deficient donor to said subject.

In one embodiment, the invention relates to identifying whether or not the subject is at risk of developing the disease. The invention therefore relates to the diagnosis of susceptibility of a subject to the disease. This may allow for an early prophylactic or palliative treatment to prevent development of the disease. The invention may be used to confirm

susceptibility in subjects already suspected as being at risk or selected as being predisposed to developing the disease.

In another embodiment, the invention relates to identifying whether or not the subject has the disease. The invention therefore relates to the diagnosis of the disease. Typically, the subject has the disease or displays symptoms of the disease. The method may therefore be carried out on subjects who display preliminary symptoms of the disease.

The method of identifying whether or not a subject is at risk of developing, or has, an IgA-related disease of the invention is typically carried out *in vitro* or *ex vivo* on a sample derived from the subject. The sample is preferably a fluid sample. The sample typically comprises a body fluid. The sample may be saliva, colostrum, tears, sweat, gut fluid or other mucous secretions. The sample may be a mucous secretion from the kidney, genitourinary tract, gastrointestinal tract, prostate or respiratory epithelium. The sample may be mucus, blood, plasma or serum. The sample may be an IgA deposit, for example obtained from the kidney. The sample can be a cell or tissue sample. The sample is typically processed prior to its use in measuring the level of IgA.

Typically, the subject is human. However, it may be non-human. For instance, the subject can be a commercially farmed animal, such as a horse, cow, sheep or pig, or may be a pet such as a cat or a dog. Preferred non-human animals include, but are not limited to, primates, such as a marmoset or monkey. The subject can be a human or non-human animal undergoing treatment for an IgA-related disease.

Method of treatment and medical use

The invention also provides a method for preventing or treating an IgA-related disease in a subject, comprising administering an effective amount of an IGBPMA polypeptide or of a nucleic acid encoding said polypeptide. The invention further provides an agent which is an IGBPMA polypeptide or a nucleic acid encoding said polypeptide, for use in a method for preventing or treating an IgA-related disease. The invention additionally provides use of an IGBPMA polypeptide or a nucleic acid encoding said polypeptide in the manufacture of a medicament for preventing or treating an IgA-related disease.

The IGBPMA polypeptide preferably comprises SEQ ID NO:2 or SEQ ID NO: 4 or a variant of either thereof.

The nucleic acid may be any nucleic acid which encodes an IGBPMA polypeptide as described herein. The nucleic acid preferably comprises a nucleic acid sequence encoding SEQ ID NO: 2 or comprises the nucleic acid sequence of SEQ ID NO:1 or SEQ ID NO:3. The nucleic acid may comprise a variant of SEQ ID NO:1 or a variant of SEQ ID NO: 3 or a variant of a nucleic acid sequence encoding SEQ ID NO:2. The variant sequence may be at least 55%, 65%, 70%, 75%, 80%, 85%, 90% and more preferably at least 95%, 97% or 99% homologous to full-length SEQ ID NO: 1 or full-length SEQ ID NO: 3 or to a nucleic acid sequence encoding SEQ ID NO:2. The nucleic acid may be provided in the form of an expression cassette which includes control sequences operably linked to the inserted sequence, thus allowing for expression of an IGBPMA polypeptide *in vivo* in a targeted subject. These expression cassettes, in turn, are typically provided within vectors (e.g., plasmids or recombinant viral vectors) which are suitable for use as reagents for nucleic acid immunization.

Such an expression cassette may be administered directly to a host subject. Alternatively, a vector comprising an IGBPMA-encoding nucleic acid may be administered to a host subject.

In all these embodiments, the IGBPMA polypeptide or its encoding nucleic acid may be administered in order to prevent the onset of one or more symptoms of the disease. In this embodiment, the subject can be asymptomatic. The subject may have a predisposition to the disease as described above. A prophylactically effective amount of the antagonist is administered to such a subject. A prophylactically effective amount is an amount which prevents the onset of one or more symptoms of the disease.

Alternatively, the IGBPMA polypeptide or its encoding nucleic acid may be administered once the symptoms of the disease have appeared in a subject i.e. to cure existing symptoms of the disease. A therapeutically effective amount of the antagonist is administered to such a subject. A therapeutically effective amount is an amount which is effective to ameliorate one or more symptoms of the disease.

The subject may be any of those discussed above in relation to the diagnostic method. The subject is preferably identified as being at risk of, or having, the disease using the method of the invention described above.

The IgA-related disease may be an IgA-mediated disease, for example a disease associated with inappropriate IgA activation. Particular IgA-mediated diseases of interest include celiac diseases, Linear IgA Dermatitis and IgA Nephropathy.

The IGBPMA polypeptide or its encoding nucleic acid can be formulated into pharmaceutical compositions. These compositions may comprise, in addition to one of the above substances, a pharmaceutically acceptable excipient, carrier, buffer, stabiliser or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material may depend on the route of administration, e.g. oral, intravenous, cutaneous or subcutaneous, nasal, intramuscular, intraperitoneal routes.

Pharmaceutical compositions for oral administration may be in tablet, capsule, powder or liquid form. A tablet may include a solid carrier such as gelatin or an adjuvant. Liquid pharmaceutical compositions generally include a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included.

For intravenous, cutaneous or subcutaneous injection, or injection at the site of affliction, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilisers, buffers, antioxidants and/or other additives may be included, as required.

For delayed release, the IGBPMA polypeptide or its encoding nucleic acid may be included in a pharmaceutical composition which is formulated for slow release, such as in microcapsules formed from biocompatible polymers or in liposomal carrier systems according to methods known in the art.

The dose of the IGBPMA polypeptide or its encoding nucleic acid may be determined according to various parameters, especially according to the substance used; the age, weight and condition of the patient to be treated; the route of administration; and the required regimen. Again, a physician will be able to determine the required route of administration and dosage for any particular patient. A typical daily dose is from about 0.1 to 50 mg per kg of body weight, according to the activity of the specific IGBPMA polypeptide or encoding nucleic acid, the age, weight and conditions of the subject to be treated and the frequency and route of administration. Preferably, daily dosage levels are from 5 mg to 2 g. That dose may be provided as a single dose

or may be provided as multiple doses, for example taken at regular intervals, for example 2, 3 or 4 doses administered daily.

Screening methods

5 The identification of the interaction between IGBPMA and IgA permits screening for compounds which have a modulatory effect on this interaction.

The invention thus provides a method for identifying a compound that modulates binding of an IGBPMA polypeptide to IgA, said method comprising contacting an IGBPMA polypeptide with IgA in the presence of the compound, and detecting binding of said polypeptide to IgA.

10 The method typically comprises comparing the binding of said polypeptide to IgA as measured in the presence of said compound with a control value obtained for binding of said polypeptide to IgA in the absence of said compound, thereby determining whether said compound is a modulator of binding of an IGBPMA polypeptide to IgA. If for example the binding observed in the presence of the compound is reduced, this may indicate that the
15 compound competes for binding of IGBPMA with IgA or vice-versa.

Preferably, the compound is further identified as having IgA binding activity. Compounds that have IgA binding activity may be used in the methods of therapy and medical uses described herein. For example, the compound may bind IgA in a region which mediates the interaction of IgA with IGBMPA, thereby having a modulatory effect on binding of IgA to
20 IGBPMA. Thus, the method may comprise a further step of determining whether the compound binds to IgA

The invention further provides for a method which is for identification of ligands of IgA by binding of IGBPMA to IgA and determining ligands associated with the bound IgA. The method for identifying ligands of IgA typically comprises carrying out a method for removing or
25 purifying IgA from a sample according to the invention, and then identifying a ligand which is present in complex with IgA. The identification may be made by any means. For example, the identification may be made by an immunoassay or by mass spectrometry. The identification may be made while the ligand is in complex with IgA and IGBPMA, or following dissociation of this complex.

30 In a related aspect, the invention also provides a method for identifying a compound that modulates binding of an IgA ligand to IgA, said method comprising contacting an IgA-ligand

complex with an IGBPMA polypeptide in the presence of the compound, and measuring binding of said IgA ligand to IgA.

The method typically comprises comparing the binding of said IgA ligand to IgA as measured in the presence of said compound with a control value obtained for binding of said IgA ligand to IgA in the absence of said compound, thereby determining whether said compound is a modulator of binding of the IgA ligand to IgA. If for example the binding observed in the presence of the compound is reduced, this may indicate that the compound competes for binding of the IgA ligand with IgA or vice-versa.

The IgA ligand may have been previously identified by the method for identification of ligands of IgA of the invention, or may be a known IgA ligand. The measurement of binding of the IgA ligand to the IGBPMA polypeptide may be performed while the ligand is in complex with IgA and IGBPMA, or following dissociation of this complex. For example, the amount of IgA ligand bound to an IGBPMA-IgA complex can be determined following contacting with the compound. The amount of IgA ligand released following dissociation of this complex can be determined following contacting with the compound. The measurement of binding of the IgA ligand to IgA may be performed by any suitable means, such as by an immunoassay, or by use of an IgA ligand which is conjugated to a detectable label, such as a fluorescent label.

Any compound(s) can be used in the screening methods of the invention. The compound(s) may be any chemical compound(s) used in drug screening programmes. They may be natural or synthetic. Extracts of plants which contain several characterised or uncharacterised components may also be used. Typically, organic molecules will be screened, preferably small organic molecules which have a molecular weight of from 50 to 2500 Daltons.

Examples

Methods and Materials

Plasmid construct

Encoding sequence of IGBPMA (SEQ ID NO: 1) was cloned into pET23 plasmid (Novagen) as described in WO 2007/051975. The pET23-IGBPMA vector was used to transform E.coli C41 (DE3) cells (Lucigen). The encoded IGBPMA protein has a C-terminal 6 x His tag.

Protein expression

Ten µl of IGBPMA-C41 glycerol stock was added into 30 ml sterile tube with 10 ml LB medium containing 50 µg/ml Ampicillin. The culture was grown overnight at 37°C with shaking

at 180 rpm. The overnight culture was subcultured in 1 litre of LB-Amp for 6 h at 37°C with shaking of 180 rpm. The culture was chilled on ice and Isopropyl β -D-1-thiogalactopyranoside (IPTG) was added to final concentration of 1mM. The induced culture was shaken at 180 rpm overnight at 16°C. The cells were harvested by centrifugation at 4000 rpm for 10 min at 4°C, and resuspended in 20 ml of Tris-buffered saline (TBS, 50 mM Tris, 500 mM NaCl, pH 7.2).

Recombinant protein purification

The cell suspension was treated by sonication in an ice bath with three cycles of 8 min (6 second on, 4 second off) with 1 min interval between each cycle. After clarification at 15000 rpm in 4°C, the supernatant was passed through the Talon resin column (Clontech) at room temperature. The column was washed for at least 30 times the column volume using TBS, then 10 times with 5 mM imidazole in TBS, and finally eluted with 100 mM in TBS. A typical run can yield 10 mg of rIGBPMA.

Detection of IgA binding activity

Commercially available purified proteins (bovine serum albumin, Sigma; human IgG, MP Biomedicals; human IgM, MP Biomedicals; and human serum IgA, MP Biomedicals) were dissolved to 1 mg/ml in phosphate buffered saline (PBS; 20 mM phosphate, 130 mM NaCl, pH 7.2). The built-in surface preparation wizard of BIAcore 2000 (BIAcore AB) was used to immobilize the immunoglobulins onto a sensor chip CM5 (BIAcore AB chip coated with carboxymethyl dextran) at a target level of 5,000 resonance units (RU) using standard amine coupling chemistry (as described in the BIAcore 2000 Instrument Handbook and BIAApplication Handbook) at coupling pH 4.5 (10 mM acetate). Ligand protein was immobilized onto each of the 4 flow cells (fc) in the sensor chip by: fc1, BSA (4067.2 RU); fc2, human IgG (5415.3 RU); fc3, human IgA (5636.2 RU); and fc4, human IgM (4272.9 RU). The flow-cells were washed once with 30 μ l of 10 mM NaOH then 30 μ l of 10 mM HCl at a flow rate of 10 μ l/min before testing for binding activity. 30 μ l of purified rIGBPMA at 0.1 mg/ml (diluted in binding buffer, 50 mM Tris, 100 mM NaCl, 20 mM CaCl₂, 20 mM MgCl₂, pH 7.2) was injected into the flow cells at 10 μ l/min. The flow cells were washed with the binding buffer at 10 μ l/min.

Binding inhibition test

50 μ l of rIGBPMA at 0.1 mg/ml in the binding buffer was mixed and incubated with 50 μ l of BSA, or human IgG, or human IgA, or human IgM (1 mg/ml) at room temperature for 1h. The mixtures were centrifuged at 10000 rpm for 10 min. 30 μ l of the supernatant was injected

into the flow cells at 10 µl/min, to test if rIGBPMA pre-incubation with free ligands would inhibit its binding to immobilised ligands.

Secondary binding test

60 µl of 0.1 mg/ml rIGBPMA was injected into the flow cells to allow rIGBPMA to bind to the ligands on the chip (BSA, human-IgG, -IgA, -IgM). 30 µl of 0.1 mg/ml BSA, human IgA, human IgG, and human IgM were sequentially injected into the flow cells. This was to determine whether or not the ligand bound rIGBPMA was still able to bind to free ligands.

Example 1 – Investigation of binding of rIGBPMA to various immunoglobulins

Tick salivary gland derived Ig-binding protein MA (IGBPMA) was expressed with 6-his tags at the C-terminus in E.coli C41 (DE3) cells. Soluble recombinant protein (rIGBPMA) was extracted and purified by using the TALON metal affinity resin. The purified rIGBPMA was used to detect immunoglobulin (Ig) binding activities by using Surface Plasmon Resonance (SPR) technology (BIAcore 2000).

The results are shown in Figure 1. The rIGBPMA showed a strong binding activity to Human IgA, compared to Bovine Serum Albumin (BSA), Human IgG, and Human IgM. The binding activity appeared to be due to specific binding of rIGBPMA to Human IgA, rather than any non-specific interaction.

Fig.1 shows that rIGBPMA bound to human IgA and IgM but not BSA and human IgG. The IgA binding was about 8 times stronger than the IgM binding. The finding that IgG binding was not present may be explained by the protein expression protocol for IGBPMA. In previous studies (WO 2007/051975), IgG binding was observed when soluble rIGBPMA was produced by using the protein denaturation-refolding protocol. Alternative explanations of why IgG binding was not observed include the possibility that the IgG used was not presenting the IgG structural conformation recognised by IGBPMA.

Example 2 – Confirmation of specificity of binding of rIGBPMA to IgA

SPR analysis was used to investigate the effect of preincubation with free BSA, IgM, IgG or IgA on the ability of immobilised IGBPMA to bind these ligands. Results are shown in Figure 2. Fig. 2 shows rIGBPMA retained its human IgA binding activity (trace number 3) after incubation with BSA (Panel-A) and human IgG (Panel-B), but no longer had binding activity after incubation with IgA (Panel-C) and IgM (Panel-D). This inhibition test confirmed the direct

binding result shown in Fig.1, and further indicated that rIGBPMA could bind to free immunoglobulins as well as to immobilised molecules.

Example 3 – Investigation of secondary binding activities of rIGBPMA

5 SPR analysis was used to investigate whether ligand-bound rIGBPMA was able to bind to free ligands. When rIGBPMA was bound onto the immobilised ligands, injections of the free immunoglobulin ligand molecules into the flow cells should detect secondary binding activities. Results are shown in Figure 3.

Fig. 3 shows that the IgA bound rIGBPMA (trace number 4 after Point 5) was not able to
10 react to injections of either free BSA (Point 6), human IgA (Point 7), or human IgG (Point 8), but was able to further bind to free human IgM (Point 9). However, the injected free IgM was also bound by all flow cells, strongly suggesting that the secondary binding was derived from non-specific human IgM antibody interacting with the proteins on the chip surface. This result also indicated that the rIGBPMA-IgM binding activity shown in Figs. 1-2 included nonspecific IgM
15 antibody activities to rIGBPMA, although such nonspecific binding might not be solely responsible for the rIGBPMA-IgM signal.

The rIGBPMA-IgA binding, however, was genuinely mediated by rIGBPMA binding to the IgA molecule.

In conclusion, rIGBPMA displayed strong binding activity to human IgA. Its affinity to
20 human IgM, however, needs to be further clarified.

Example 4 - Recombinant IGBPMA (rIGBPMA) binds to human IgA differently to Jacalin

Purified rIGBPMA was produced using the recombinant protein purification protocol described for his-tagged IGBPMA above. Both rIGBPMA and Jacalin (Vetor Laboratories Inc.,
25 Burlingame, CA94010, USA, Jacalin L-1150, Lot W0721) was diluted in Tris-buffered saline (TBS, 50 mM Tris, 150 mM NaCl, 20 mM MgCl₂, 20 mM CaCl₂, pH=7.2) at 1 mg/ml before use.

Human IgA (AbD Native Human IgA, Serotec, 5111-5504, Batch No: 210612) was immobilised to Flow cell (Fc) 2 on a Biacore sensor chip CM5 surface by using the
30 immobilisation wizard of Biacore 3000 with a target level of 1000 RU. Fc1 was deactivated and used as blank control. Fc3 and Fc4 were used to immobilise comparable levels of Human IgG and Human IgE as more binding controls. After completion of the immobilisation wizard, the Fc

surface was washed by 10 µl of Glycine-HCl (100 mM Glycine, 150 NaCl, pH 2.6) followed by 10 µl of 50 mM NaOH, at flow rate of 20 µl/min. TBS was used as the running buffer in the binding test.

The binding test was performed using steps 1 to 6, details in Brief description of Figures above, all with flow rate of 20 µl/min. The data shown in Figure 4 represents the signal difference between testing Fc and the blank Fc1, i.e., Fc2-Fc1 (trace with numbered points 1 to 6), Fc3-Fc1 (trace numbered 7), and F4-Fc1 (trace numbered 8).

Clear Jacalin-IgA binding was observed at steps-I but Jacalin did not bind to IgG or IgE. Approximately 430 RU (peak) of IgA binding was obtained in Step I (Fig. 5a). Saturation was achieved in step 2 as a big disassociation occurred after injection of Jacalin was stopped (Fig. 4). This was confirmed by Step 3 in which an additional injection of 30 µl of 15 µg/ml Jacalin only gained approximately 80 RU of binding signal (Fig. 5b), significantly lower than that of Step 1 (Fig. 5a).

In Steps 4 and 5, clear rIGBPMA-IgA binding was detected on the Jacalin saturated surface (Fig. 5c). Approximately 350 RU of rIGBPMA-IgA binding was obtained at the peak of Step-V. Although the baseline of the IgA binding trace was not flat due to disassociation of Jacalin-IgA binding in the previous steps, the rIGBPMA-IgA binding was not the result of rIGBPMA replacement of Jacalin on the chip surface. This is because approximately 115 RU of Jacalin-IgA binding was obtained at the peak of Step 6 (Fig. 5d), larger than that of Step 3 of 80 RU (Fig. 5b).

If the rIGBPMA-IgA binding was a replacement of the Jacalin-IgA binding, the Jacalin-IgA binding signal of Step 6 should be considerably smaller than that of Step 3 because the baseline level was much higher at the beginning of Step 6, i.e., more IgA binding sites were occupied, than the beginning of Step 3 (Figs. 4, 5c). The most likely explanation of the observed binding pattern is therefore that Jacalin and rIGBPMA bind to IgA independently. As discussed above, Jacalin is a lectin which binds to a galactose side chain on IgA and can thus only bind galactosylated IgA. In contrast, IGBPMA binds to a different site on IgA and can thus have utility in binding a wider range of types of IgA.

CLAIMS

1. A method for binding IgA in a sample, comprising contacting said sample with an IGBPMA polypeptide under conditions suitable for binding of IgA to said polypeptide.
5
2. The method according to claim 1, wherein said IGBPMA polypeptide comprises the amino acid sequence of SEQ ID NO: 2 or a variant thereof.
3. The method according to claim 1 or 2, which further comprises detecting for IgA before,
10 during or after said contacting.
4. The method according to claim 3, which comprises quantitating the level of IgA before, during or after said contacting.
- 15 5. The method according to claim 3 or 4, which comprises detecting or quantitating IgA present in complex with said IGBPMA polypeptide during or after said contacting.
6. The method according to any one of the preceding claims, wherein said IGBPMA polypeptide is attached to a solid support.
20
7. The method according to any one of the preceding claims, which further comprises binding of IgE and/or IgG to said IGBPMA polypeptide.
8. A method for removing or purifying IgA from said sample, comprising contacting said
25 sample with an IGBPMA polypeptide attached to a solid support under conditions suitable for binding of IgA to said polypeptide.
9. The method according to claim 8, which comprises collecting a sample in which IgA has been removed.
30
10. The method according to claim 8, which comprises collecting a sample in which IgA has been enriched.

11. The method according to claim 8, which comprises eluting IgA from said solid support after said contacting.
12. The method according to claim 10 or 11, which further comprises identifying a ligand
5 which is present in complex with IgA.
13. The method according to any one of claims 8 to 11, which further comprises removing or purifying IgE and/or IgG from said sample.
- 10 14. The method according to any one of the preceding claims, which further comprises contacting said sample with a further IgA-binding agent under conditions suitable for binding of IgA to said agent, optionally wherein said further IgA-binding agent is a Jacalin polypeptide.
- 15 15. A kit comprising an IGBPMA polypeptide, and reagents suitable for determining the presence of IgA in a sample.
16. The kit according to claim 15, which comprises reagents suitable for determining the presence of IgE and/or IgG in a sample.
- 20 17. A kit comprising an IGBPMA polypeptide and a further IgA-binding agent.
18. The kit according to claim 17, wherein said further IgA-binding agent is a Jacalin polypeptide.
- 25 19. A method for identifying whether or not a subject is at risk of developing, or has, an IgA-related disease, said method comprising:
- (a) measuring the level of IgA in a sample derived from said subject by contacting said sample with an IGBPMA polypeptide and detecting binding of said polypeptide to IgA;
- 30 (b) comparing the level of IgA in said sample to a normal level of IgA and thereby determining whether or not a subject is at risk of developing, or has, an IgA-mediated disease;

wherein an increased level of IgA in the sample compared to the normal level identifies the subject as being at risk of developing, or having, an IgA-mediated disease.

20. A method for preventing or treating an IgA-related disease in a subject, comprising
5 administering an effective amount of an IGBPMA polypeptide or of a nucleic acid encoding said polypeptide.

21. A method for identifying a compound that modulates binding of an IgA ligand to IgA,
said method comprising contacting an IgA-ligand complex with an IGBPMA polypeptide in the
10 presence of the compound, and measuring binding of said IgA ligand to IgA.

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Fig. 1

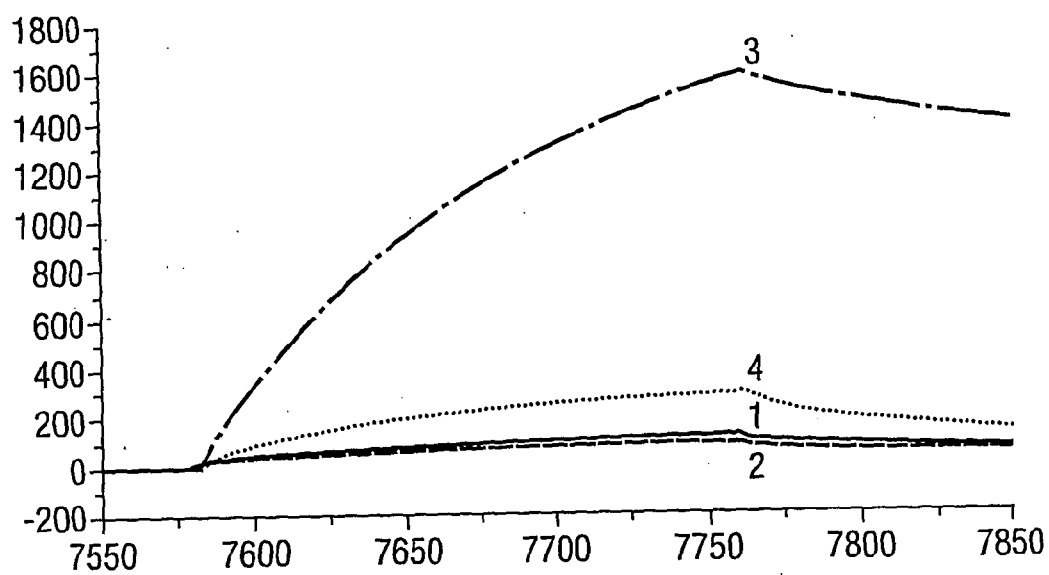


Fig. 2B

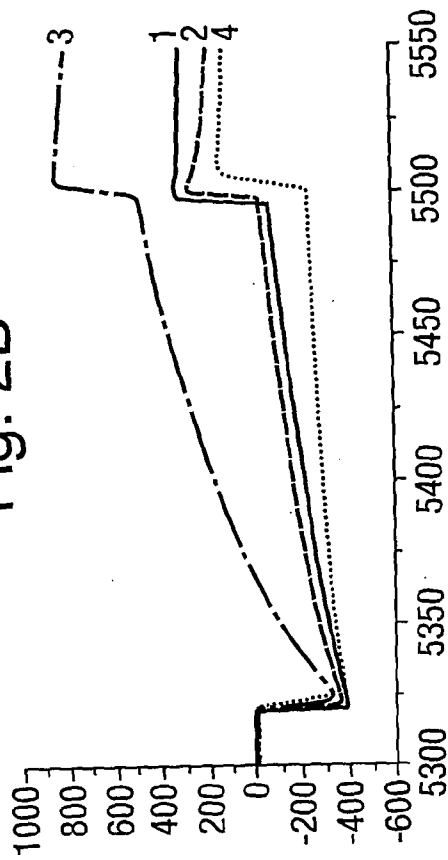


Fig. 2D

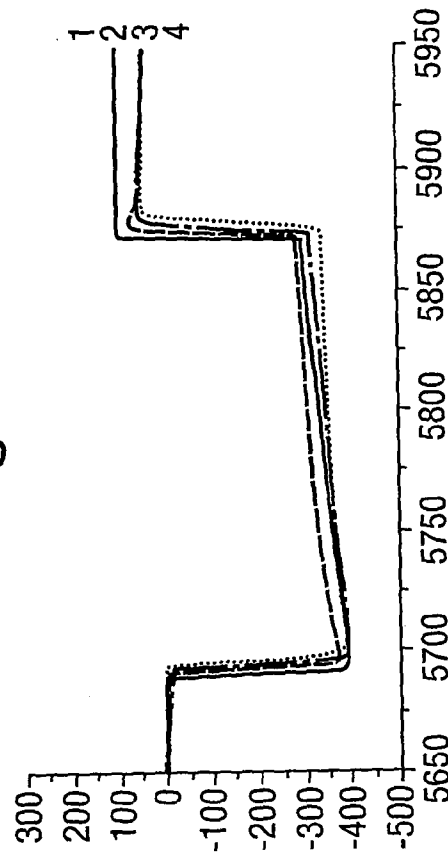


Fig. 2A

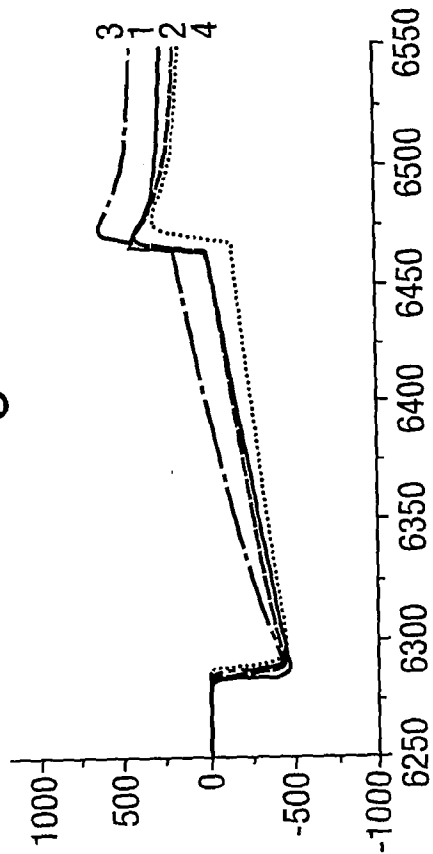
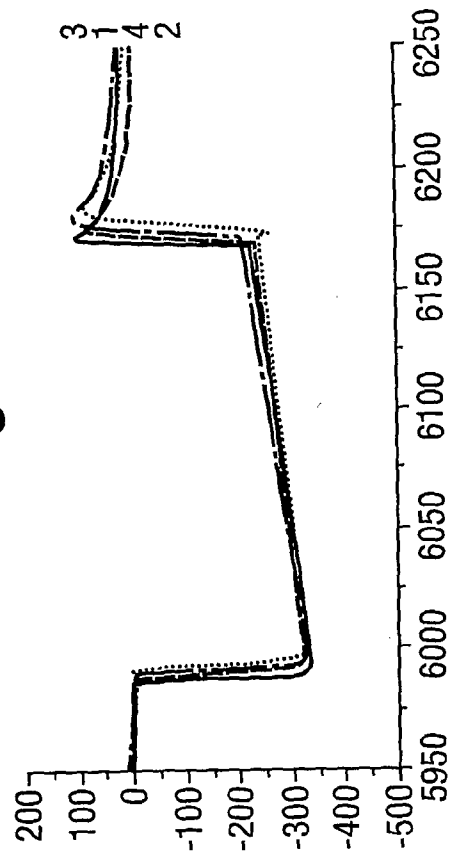


Fig. 2C



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Fig. 3

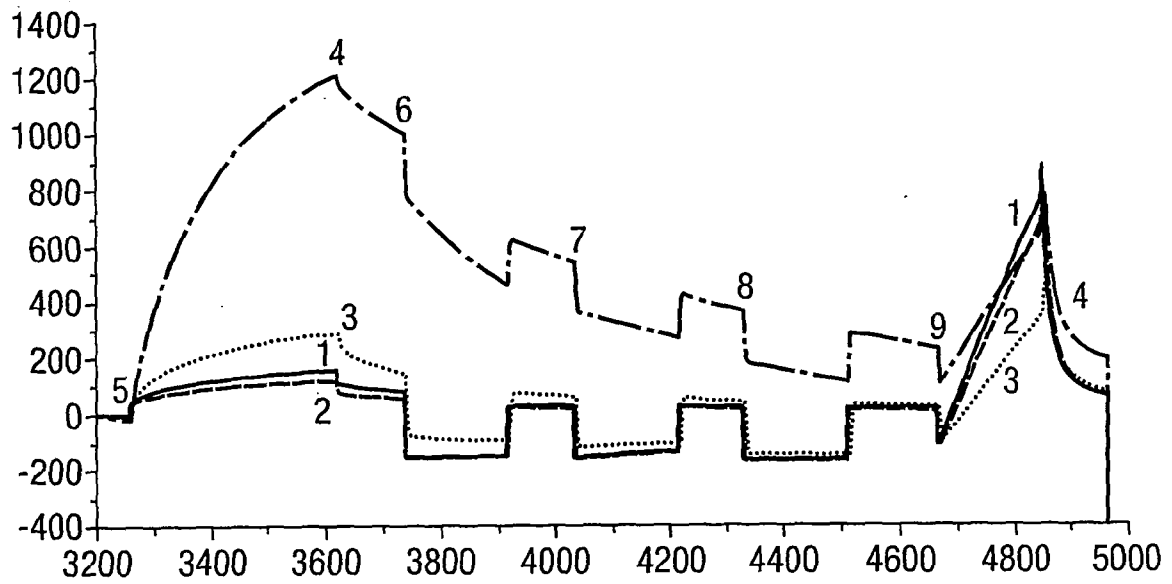
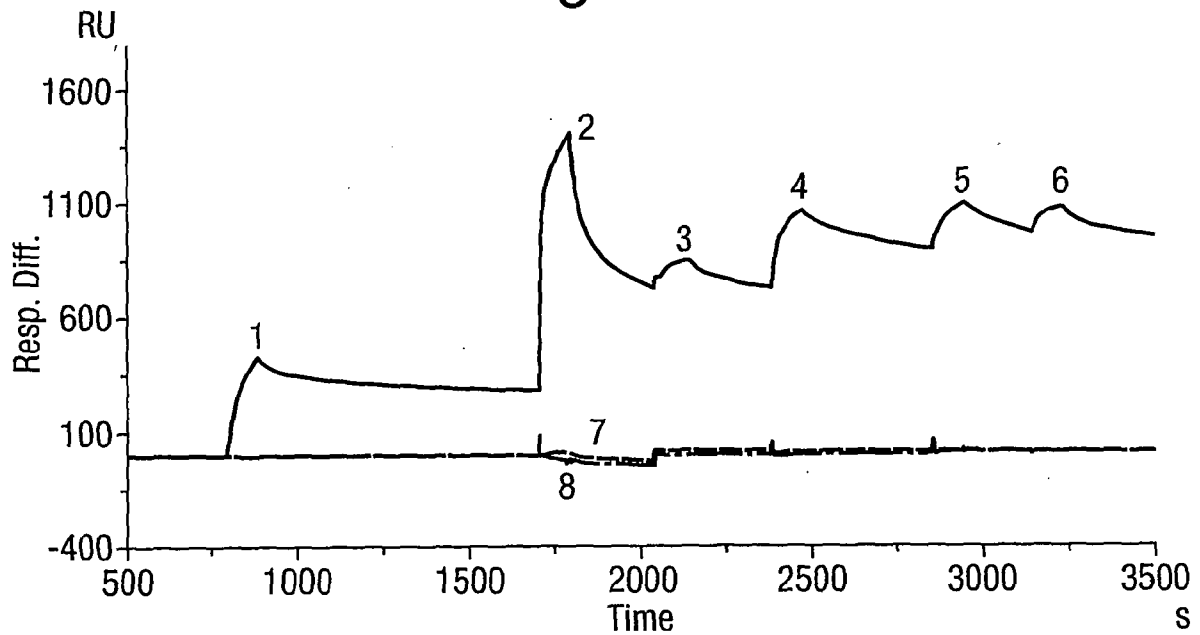


Fig. 4



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Fig. 5a

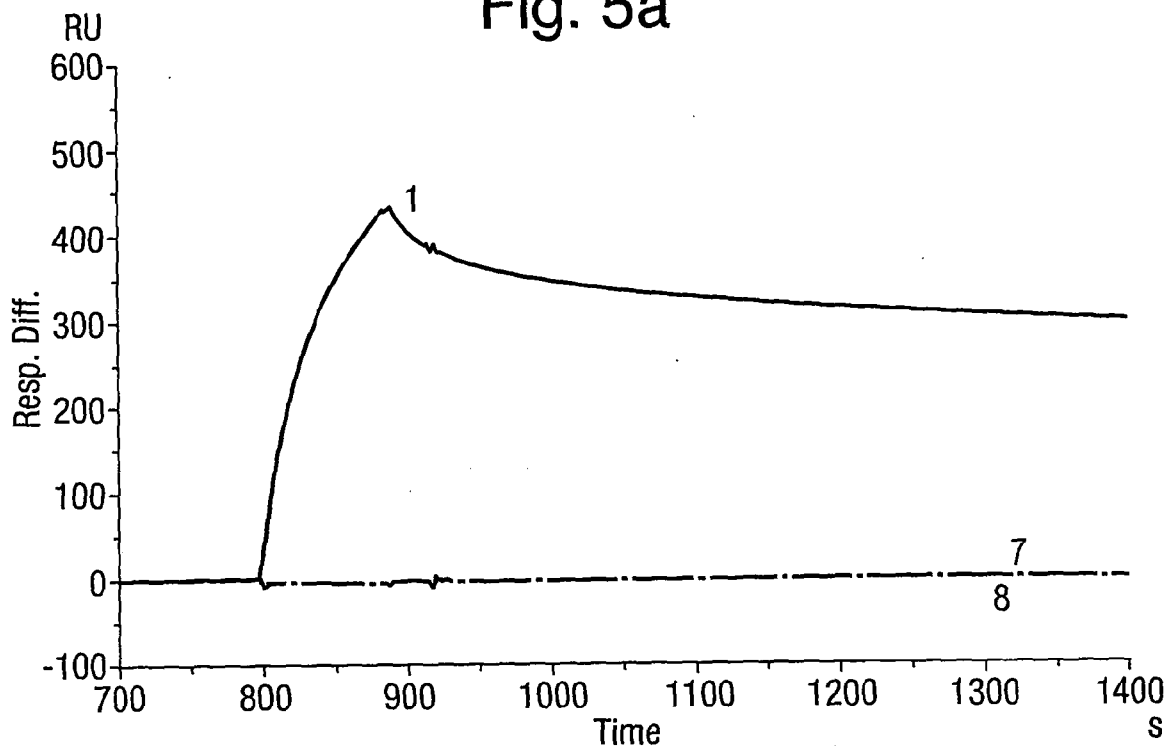
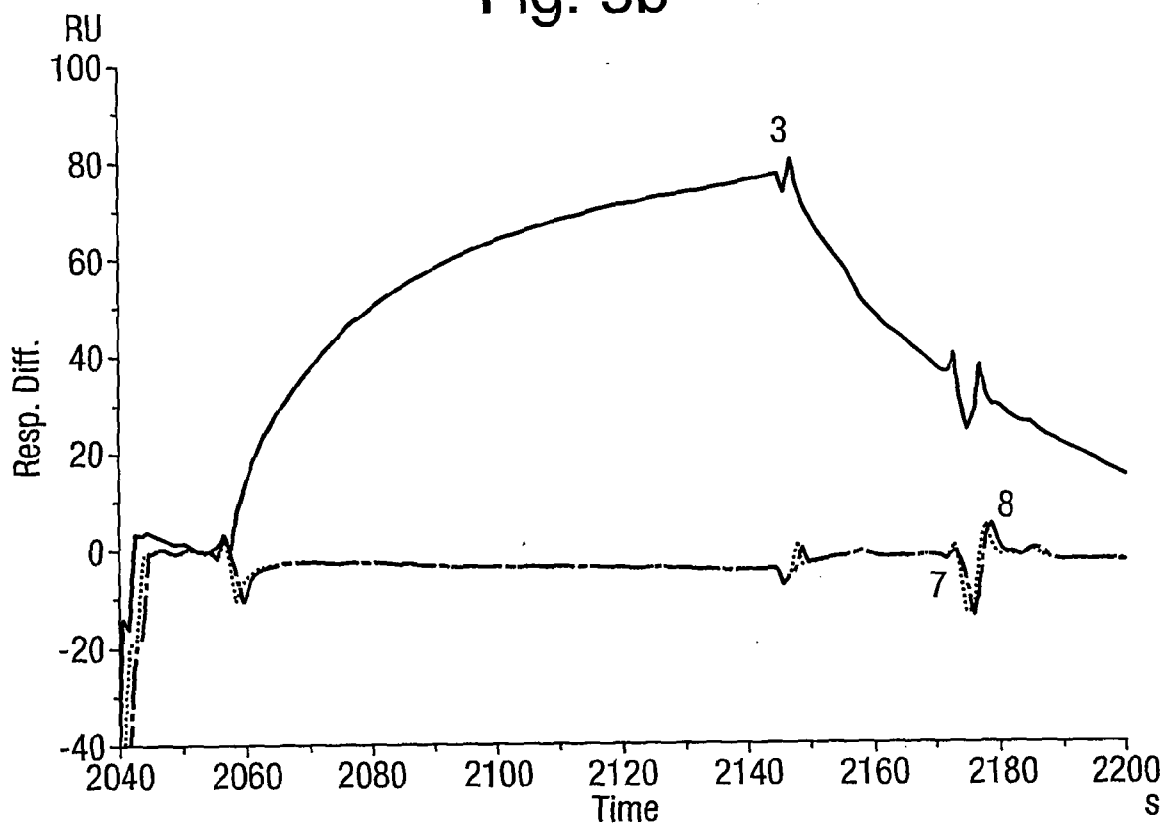


Fig. 5b



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Fig. 5c

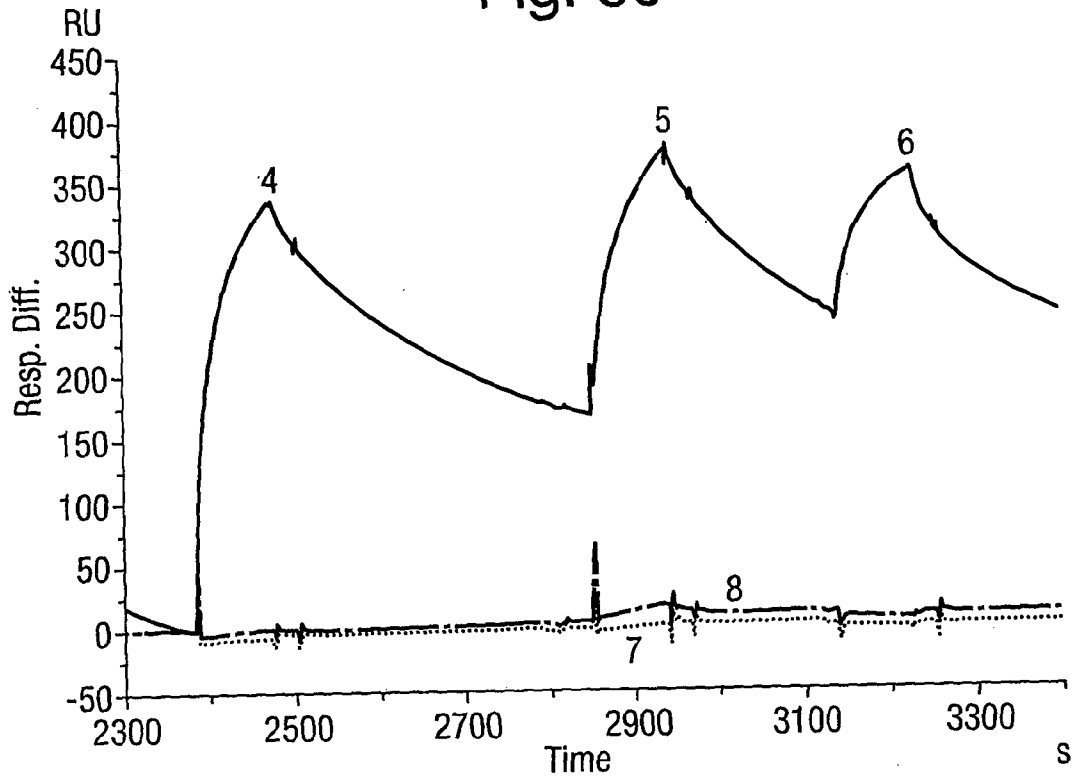
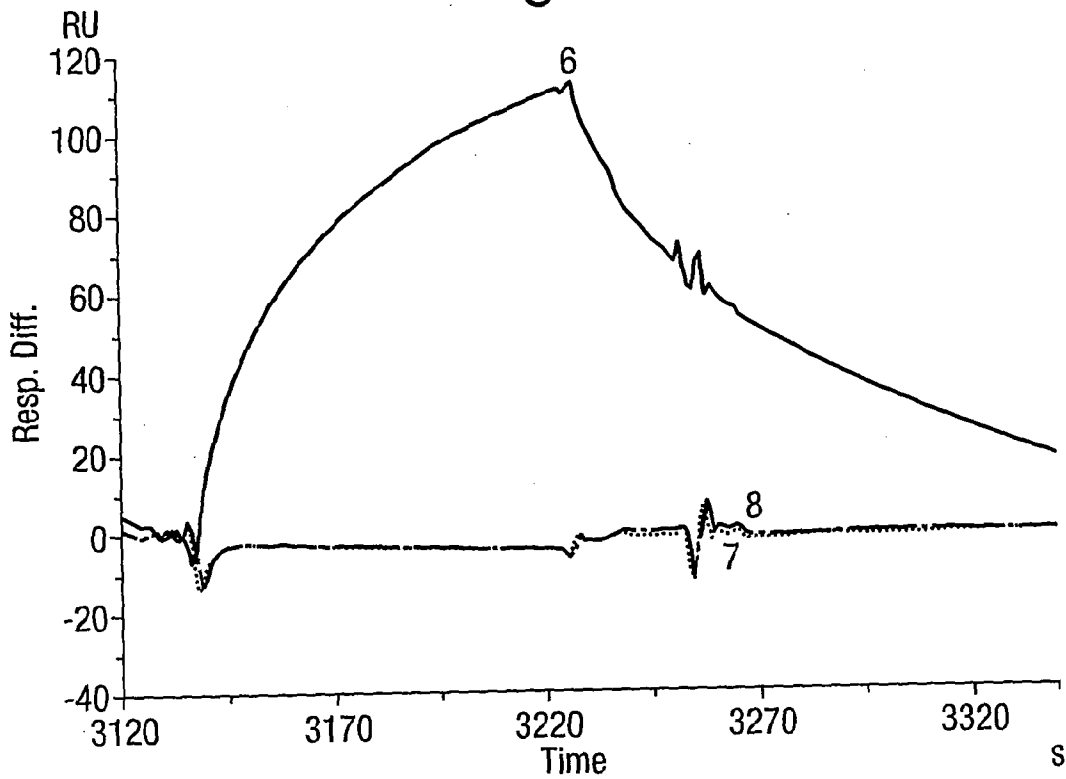


Fig. 5d



INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2013/050437

A. CLASSIFICATION OF SUBJECT MATTER
 INV. C07K14/435 A61K38/00 A61K38/17 G01N33/50
 ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2007/051975 A2 (NATURAL ENVIRONMENT RES COUNCI [GB]; WANG HUI [GB]; NUTTALL PATRICIA A) 10 May 2007 (2007-05-10) -----	1-21
A	WANG H ET AL: "Immunoglobulin G binding proteins in male Rhipicephalus appendiculatus ticks", 19951001; 19951000, vol. 17, no. 10, 1 October 1995 (1995-10-01), pages 517-524, XP009080575, ----- -/--	1-21



Further documents are listed in the continuation of Box C.



See patent family annex.

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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Date of the actual completion of the international search

4 June 2013

Date of mailing of the international search report

10/06/2013

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INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2013/050437

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WANG H ET AL: "IMMUNOGLOBULIN-G BINDING PROTEINS IN THE IXODID TICKS, RHIPICEPHALUS APPENDICULATUS, AMBLYOMMA VARIEGATUM AND IXODES HEXAGONUS", PARASITOLOGY, CAMBRIDGE UNIVERSITY PRESS, LONDON, GB, vol. 111, no. 2, 1 August 1995 (1995-08-01), pages 161-165, XP001011253, ISSN: 0031-1820 -----	1-21

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

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