(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
15 February 2007 (15.02.2007)

PCT

(10) International Publication Number
WO 2007/019376 A2

(51) International Patent Classification:
A61K 51/00 (2006.01)

(21) International Application Number:
PCT/US2006/030583

(22) International Filing Date: 3 August 2006 (03.08.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/705,282 3 August 2005 (03.08.2005) US

(71) Applicants *(for all designated States except US)*: **RQ BIOSCIENCE, INC.** [US/US]; 297 Turnpike Road, Apartment 101, Westborough, MA 01581 (US). **NEW ENGLAND MEDICAL CENTER HOSPITALS, INC.** [US/US]; 750 Washington Street, Boston, MA 02111 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **QIU, Robert, Sidong** [US/US]; 297 Turnpike Road, Apartment 101, Westborough, MA 01581 (US). **QIU, Jiazhou** [US/US]; 297 Turnpike Road, Westborough, MA 01581 (US). **PLAUT, Andrew, G.** [US/US]; 22 Peacock Farm Road, Lexington, MA 02421-6340 (US).

(74) Agent: **ELBING, Karen, L.**; CLARK & ELBING LLP, 101 Federal Street, Boston, MA 02110 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2007/019376 A2

(54) **Title:** METHODS AND COMPOSITIONS FOR DIAGNOSIS OF IGA-AND IGM-MEDIATED KIDNEY DISEASES

(57) **Abstract:** The present invention features noninvasive methods for diagnosing IgA or IgM kidney disorders, such as IgA nephropathy, Henoch-Schδnlein purpura, and IgM nephropathy, in a mammal. The invention also features compositions and kits useful in diagnosing these disorders.

METHODS AND COMPOSITIONS FOR DIAGNOSIS OF IgA- AND IgM-MEDIATED KIDNEY DISEASES

Background of the Invention

The invention relates to the field of diagnostic methods for kidney diseases and compositions and kits useful in the diagnosis of such diseases.

IgA nephropathy (IgAN, Berger's disease) is characterized by the deposition of IgA1 in the mesangium of the renal glomerulus and is the most common glomerulonephritis worldwide. The IgA deposits arise spontaneously, usually in the second or third decade of life and are thought to be immune complexes. The antigen(s) are unknown; IgA itself may be the antigen. The prevalence of the disease is high in the U.S. and Europe, but highest in Asia. The incidence in Japan may be 40-50% of all renal biopsies. Persistent or intermittently detected microscopic hematuria and proteinuria for many years is the clinical feature of this disease, and more than 50% of patients also develop hypertension. It is not a benign illness as once believed, with about 15-40% of patients eventually developing renal failure. Indeed, IgA nephropathy is the main cause of end-stage renal disease in patients with primary glomerular disease that eventually come to renal transplantation.

Henoch-Schönlein purpura (HSP) is another IgA1-mediated post-infectious vasculitis, most often in children ages 2-11 yrs, with kidney damage very similar to IgAN. Prevalance is 22/100,000 under age 14, and 70/100,000 in the group age 4 to 6 years old. HSP is so similar to IgAN that the notion that IgAN is a renal-limited form of HSP has recently gained acceptance (Smith and Feehally, Springer Semin. Immunopathol. 24:477–493, 2003).

IgM nephropathy (IgMN) causes nephrotic syndrome and is characterized by IgM mesangial deposits. It is speculated that these deposits are derived from circulating IgM aggregates or immune complexes, similar to IgAN. IgMN is

often seen in children, and the clinical feature of IgMN is very similar to minimal change disease (MCD), in which nephrotic syndrome is the main clinical manifestation, but IgMN results in a significantly higher incidence of hypertension then MCD.

Prior to the present invention, diagnosis of these diseases were by renal biopsy, which removes a sample of the kidney tissue for pathological examination. A renal pathologist does immunofluorescent staining of kidney tissues. This requires sequential application of anti-human IgA antibody, followed by development with a fluorochrome-conjugated second antibody to localize the IgA-based antibody-antigen complexes for the diagnosis of IgAN and HSP. In the diagnosis of IgMN, the same procedures can be applied, but the antibody used specifically binds human IgM. If the test detects high levels of glomerular IgA, more so than co-existing IgG, and with complement components variably present, the diagnosis of IgA nephropathy is made. This is the same for IgMN, in which the deposition of IgM in the glomerulus defines the illness.

Biopsy is usually done with the patient lying in the prone position, the kidney having been localized using ultrasound or CAT scan. Under local anesthesia, a small incision is made in the skin. Using an appropriate breath-holding protocol, a biopsy needle is used to take a sample the size of 1-1.5cm x 2mm, and the needle is removed. The patient remains in the hospital, lying supine for 12 to 24 hours, with monitoring to detect complications which may include bleeding, pain, arteriovenous fistula, urinary tract infection, and in rare cases, death.

Bleeding is the most common complication of renal biopsy. Although prior to biopsy patients are tested for the ability to coagulate, most patients experience microscopic hematuria for a short time. Rarely, bleeding is severe enough to require a blood transfusion or surgery. It has been estimated that surgery is required to control the bleeding in 0.1 to 0.4 percent of percutaneous

renal biopsies, and removal of the kidney to control hemorrhage is required in approximately 0.06 percent (6 per 10,000) of completed needle biopsies.

Pain is a common problem and is transient. Pain lasting more than 12 hours occurs in approximately 4 percent of biopsies. Severe or prolonged pain can occur if a blood clot obstructs one of the ureters or in the event of a large subcapsular hematoma.

Arteriovenous fistula between two blood vessels can result from damage to the walls of an adjacent artery and vein caused by the biopsy needle. Such fistulas are rare and usually close spontaneously in one to two years.

Death occurs in approximately 0.1% of renal biopsy cases.

Renal biopsy is not appropriate for all patients. Contraindications include an uncorrectable bleeding condition, small kidneys, severe hypertension that cannot be medically controlled, multiple bilateral renal cysts or a renal tumor, hydronephrosis (a condition in which the flow of urine is obstructed leading to kidney damage), active infection of the tissues in or surrounding the kidney, inability of the patient to cooperate, and a solitary native kidney. Alternatives to percutaneous biopsy are the open surgery biopsy and transjugular renal biopsy

Thus there is a need for safer, more reliable, and less invasive methods for diagnosing IgA nephropathy, HSP, and IgM nephropathy.

Summary of the Invention

The present invention features a method for diagnosing an IgA or IgM kidney disease in a mammal (e.g., a human) which includes administering (e.g., intravenously) to the mammal a compound which specifically binds IgA or IgM and detecting the compound in the kidney of the mammal, where an increase in the amount of the compound in the kidney of the mammal relative to the amount in the kidney of a mammal without the kidney disease is diagnostic of the IgA or IgM kidney disease in the mammal.

The compound may include a peptide (e.g., human FcαR1 (SEQ ID NO:2), human Fcα/μ receptor (SEQ ID NO:6), polymeric Ig receptor (SEQ ID NO:7), Sir22 (SEQ ID NO:3), streptococcal IgA-binding peptide (Sap; SEQ ID NO:4), a modified Z-domain protein (SEQ ID NO:12), and YDWIPSSAW (SEQ ID NO:9), or an IgA- or IgM-binding fragment of these peptides). The peptide may include a modification of an N-terminal cap, a C-terminal cap, a D-amino acid, an amino acid surrogate, a peptidomimetic sequence, or a spacer. The compound may include an antibody or an IgA- or IgM-binding fragment of an antibody (e.g., anti-human IgA or anti-human IgM). The compound may also include a qdot. The compound may be linked to a a radioactive label (e.g., ^{99m}Tc, ^{111}In, ^{66}Ga, ^{67}Ga, ^{68}Ga, ^{86}Y, ^{90}Y, ^{201}Tl, ^{55}Co, ^{60}Cu, ^{61}Cu, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{82}Rb, $^{185/187}$Re, and $^{186/188}$Re). The linkage may be by a bifunctional chelating agent, which may include N_3S, N_2S_2, PnAO, HYNIC, $[M(CO)_3(H_2O)_3]^+$, PADA, DTPA, DOTA, histidine, a tripeptide (e.g., Lys-Gly-Cys, Cys-Gly-Cys, and Gly-Gly-Cys), and a tetrapeptide (e.g., Gly-Ala-Gly-Gly or Cys-Gly-Cys-Gly). The compound may be linked to a paramagnetic substance (e.g., gadolinium). The detection may be carried out by an imaging technique (e.g., SPECT, PET, planar scan and MRI).

In a further embodiment, the compound includes a galactose and a first member of a binding pair (e.g., streptavidin). The administration of the compound may further include administration of galactose-Ficoll. The detection may then be performed by administering to the mammal a radioactively labeled (e.g., ^{99m}Tc, ^{111}In, ^{66}Ga, ^{67}Ga, ^{68}Ga, ^{86}Y, ^{90}Y, ^{201}Tl, ^{55}Co, ^{60}Cu, ^{61}Cu, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{82}Rb, $^{185/187}$Re, or $^{186/188}$Re labeled) compound (e.g., human serum albumin) conjugated to a second member of a binding pair (e.g., biotin) and a galactose followed by detecting the radioactively labeled compound in the kidney of the mammal.

A second aspect of the invention is a composition including an IgA- or IgM-binding compound, a bifunctional chelating agent, and a detectable label in

a pharmaceutically acceptable carrier, where the IgA- or IgM-binding compound is linked to the detectable label through the bifunctional chelating agent. The compound may include a peptide (e.g., human FcαR1 (SEQ ID NO:2), human Fcα/μ receptor (SEQ ID NO:6), polymeric Ig receptor (SEQ ID NO:7), Sir22 (SEQ ID NO:3), streptococcal IgA-binding peptide (Sap; SEQ ID NO:4), a modified Z-domain protein (SEQ ID NO:12), and YDWIPSSAW (SEQ ID NO:9), or an IgA- or IgM-binding fragment of one of these peptides). The peptide may include a modification of an N-terminal cap, a C-terminal cap, a D-amino acid, an amino acid surrogate, a peptidomimetic sequence, or a spacer. The compound may also include an antibody (e.g., anti-human IgA or anti-human IgM), or an IgA- or IgM-binding fragment of the antibody. The bifunctional chelating agent may be N_3S, N_2S_2, PnAO, HYNIC, $[M(CO)_3(H_2O)_3]^+$, PADA, DTPA, DOTA, histidine, a tripeptide (e.g., Lys-Gly-Cys, Cys-Gly-Cys, or Gly-Gly-Cys.), or a tetrapeptide (e.g., Gly-Ala-Gly-Gly or Cys-Gly-Cys-Gly).

In a third aspect, the invention provides a kit including an IgA- or IgM-binding compound, a bifunctional chelating agent; a detectable label in a pharmaceutically acceptable carrier; and instructions for use for detecting an IgA or IgM kidney disease in a mammal. The compound may include a galactose and a first member of a binding pair (e.g., streptavidin); and the detectable label may include a radioactively labeled peptide (e.g., human serum albumin), the radioactively labeled(e.g., ^{99m}Tc, ^{111}In, ^{66}Ga, ^{67}Ga, ^{68}Ga, ^{86}Y, ^{90}Y, ^{201}Tl, ^{55}Co, ^{60}Cu, ^{61}Cu, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{82}Rb, $^{185/187}Re$, or $^{186/188}Re$ labeled) peptide including a galactose and a second member of a binding pair (e.g., biotin).

By "IgA or IgM kidney disease" is meant a disorder of the kidney mediated by IgA or IgM deposition in the kidney. These disorders include, as examples, IgA nephropathy, Henoch-Schönlein purpura, and IgM nephropathy.

By "specifically binds" is meant a compound which recognizes and binds, a compound, for example, IgA or IgM, but which does not substantially

recognize and bind other molecules in a sample, for example, a biological sample, which naturally includes that compound. In one example, an antibody that specifically binds to IgA1 (SEQ ID NO:1) recognizes a 13 amino acid region present in IgA1 and absent in IgA2. Desirably, the compound binds the compound of interest, for example, IgA, at least 5-fold, 10-fold, 25-fold, 50-fold, 100-fold, or 1000-fold more strongly than it binds other components of the sample.

By "N-terminal cap" is meant any chemical modification to the amino-terminal end of a peptide or protein. In the present invention, the addition of an N-terminal cap to a peptide or protein is intended to decrease the rate of in vivo degradation of the peptide or protein as compared to the uncapped protein. Examples of N-terminal caps include acetylation and peptide cyclization.

By "C-terminal cap" is meant any chemical modification to the carboxy-terminal end of a peptide or protein. In the present invention, the addition of a C-terminal cap to a peptide or protein is intended to decrease the rate of in vivo degradation of the peptide or protein as compared to the uncapped protein. Examples of C-terminal caps include amidating or reducing the C-terminus, and peptide cyclization.

By "peptidomimetic" is meant a molecule that mimics characteristics of peptides, including the ability to recognize biomolecules. In the present invention, peptidomimetics are inserted into peptides or proteins to prevent in vivo degradation by endopeptidases and exopeptidases.

By "spacer" is meant a small molecule that is inserted in place of an amino acid in a peptide sequence either internally or at either the N- or C-termini. An examples of a spacer includes aminohexanoic acid. Like peptidomimetics, spacers are used in the present invention to prevent peptide degradation.

By "amino acid surrogate" is meant any chemical compound that can be placed into a peptide or protein in place of an amino acid. Examples of amino

acid surrogates include spacers, peptidomimetics, imino acids, and amino acids with methylated amide and/or methylated side chain nitrogens.

By "fragment" is meant a chain of at least 4, 5, 6, 8, 10, 15, 20, or 25 amino acids or nucleotides which comprises any portion of a larger peptide or polynucleotide.

By "peptide" is meant any chain of amino acids, or analogs thereof, regardless of length or post-translational modification (for example, glycosylation or phosphorylation).

By "qdot" is meant a fluorescent semiconductor nanocrystal. Example of materials from which qdots are made include CdS, CdSe, CdTe, CdHgTe/ZnS, InP, InAs, and PbSe.

Other features and advantages of the invention will be apparent from the following Detailed Description, the drawings, and the claims.

Brief Description of the Drawings

Figure 1 is a diagram of dimeric IgA1 (SEQ ID NO:1), its hinge region (SEQ ID NO:14), and the O-glycan sites (Thr225, Thr228, Ser230, Ser232, Thr236).

Figure 2 is a diagram of the structure and characteristics of the Fc-receptor for IgA (FcαR1/CD89; SEQ ID NO:2). The extracellular domains 1 and 3 (EC-1 and EC-2) as well as the associated pair of γ-chains with their signaling (ITAM) motifs are depicted. Furthermore the characteristics of CD89, its cellular distribution, and its known functions are summarized. (From: Westerhuis, *Pathogenetic Aspects of IgA-Nephropathy* 2001 PrintPartners Ipskamp, Enschede)

Figure 3 is a schematic representation of the streptococcal Sir22 (M22; SEQ ID NO:3) protein and sequence of the Sap peptide (SEQ ID NO:4) derived from this protein. The Sap peptide includes the 29-residue IgA-binding region (horizontal arrows labeled "IgA") and the 10 residues on either side of this

region. In addition, Sap includes a C-terminal cysteine residue not present in Sir22 that promotes its dimerization. The regions in Sir22 known to include binding sites for C4BP (the human complement regulator), IgA, and IgG are indicated. The C4BP binding region is a hypervariable domain. (The horizontal arrows under designated "C4BP" and "IgG" indicate corresponding regions for binding C4BP and IgG. There are some sequence overlaps for each of these three binding regions). The position of the conserved C repeat region is also indicated. The C-terminal end of Sir22 is covalently bound to peptidoglycan in the bacterial cell wall.

Figure 4 is a schematic representation of an intact Ig together with Fab and Fv fragments and single V (colored ovals; dots represent antigen-binding sites) and C domains (uncolored). Engineered recombinant antibodies are shown as scFv monomers, dimers (diabodies), trimers (triabodies), and tetramers (tetrabodies), with linkers represented by a black line. Minibodies are shown as two scFv modules joined by two C domains. Also shown are Fab dimers (conjugates by adhesive polypeptide or protein domains) and Fab trimers (chemically conjugated). Colors denote different specificities for the bispecific scFv dimers (diabodies) and Fab dimers and trimers.

Figure 5 is a diagram of structures of bifunctional chelating agents (BFCAs) for ^{99m}Tc. Triamidethiols (N_3S), diamidedithiols (N_2S_2), propyleneamineoxime (PnAO), and hydrazinonicotinic acid (HYNIC) are shown.

Figure 6 is a diagram of structures of picolylamine-N,N-diacetic acid (PADA) and its complex with Tc. PADA and its complex after reaction with the aquaion $[Tc(CO)_3(H_2O)_3]^+$ are shown.

Figure 7 is a diagram of structures of BFCAs for ^{111}In. Diethyl-enetriaminepentaacidic acid (DTPA) and tetraazacyclo-dodecanetetraacidic acid (DOTA) are shown.

Figure 8 is a list of amino acid and nucleic acid sequences including IgA1 C-region (SEQ ID NO:1), CD89 (SEQ ID NO:2), Sir22 (SEQ ID NO:3), Sap (SEQ ID NO:4), soluble CD89 (SEQ ID NO:5), Human Fcα/μR (SEQ ID NO:6), pIgR (SEQ ID NO:7), CD71 (SEQ ID NO:8), IgM binding peptide (SEQ ID NO:9), Staph Protein A (SEQ ID NO:10), Z-domain (SEQ ID NO:11), modified Z-domain (SEQ ID NO:12), B-domain (SEQ ID NO:13), the IgA1 hinge region (SEQ ID NO:14), IgM mu chain amino acid sequence (SEQ ID NO:15), IgM mu chain nucleic acid sequence (SEQ ID NO:16), and the IgM hinge sequence (SEQ ID NO:17).

Detailed Description

The present invention uses radiologic scans to identify patients who have glomerular-based renal diseases, such as IgA nephropathy (IgAN), Henoch-Schönlein purpura (HSP), and IgM nephropathy (IgMN). IgAN is characterized by a time-dependent (years) deposition of IgA1 immunoglobulins (SEQ ID NO:1) into the glomeruli of the kidney cortex. HSP is closely related to IgAN, with the same pattern of IgA1 deposition and kidney injury. IgMN is characterized by IgM deposition in the mesangium of glomeruli. To identify those patients with renal disease who have IgA1 or IgM deposition, it has been necessary to carry out a closed needle biopsy of the kidney, a somewhat painful procedure of moderate risk that yields a tissue core for pathological examination. With the present invention IgA1 or IgM deposition can be diagnosed using radiologic imaging methods in which the IgA or IgM deposits in the kidney cortex are detected using an injected, labeled IgA-binding or IgM-binding molecule.

When one biopsies the kidney in order to make the diagnosis of kidney disease, the key criteria for diagnosis of IgAN, HSP, and IgMN is the presence of dominant IgA1 or IgM deposits in the kidney glomeruli, the only site of IgA or IgM deposition. As normal persons and those with non-IgAN, non-IgM

kidney diseases do not have significant amounts of IgA1 or IgM in their renal glomeruli, the present invention represents a macro-scale, non-invasive detection method for IgA or IgM in the kidney cortex. Another advantage of the present invention is that scanning provides information about the entire kidney cortices in both kidneys, thus reducing sampling error inherent in needle biopsy methods (Sund et al., Nephrol. Dial. Transplant. 14:2445-2454, 1999).

The invention features several IgA-specific peptides that target IgA1, available radioisotopes by which they may be labeled, and linkers used for this labeling. In one embodiment, radionuclide-labeled IgA-binding peptides or proteins are used as diagnostic radiopharmaceuticals to detect IgA deposits in the kidney. The injected radiolabeled IgA-binding peptides bind to IgA throughout the body. Because there is little IgA deposited in the glomeruli of a healthy kidney, IgA deposits in the glomeruli of patients with IgAN result in a high concentration of the emission of radiation from the cortex of the kidney, where the glomeruli are located. This radiation can be detected by various nuclear imaging techniques and therefore may be used to diagnose IgAN. For diagnosis of the kidney disease, antibodies of human origin, or antibodies that are humanized, or animal monoclonal antibodies with specificity for human IgA1 are well-suited as IgA binding compounds, and can also be used to detect IgA1 deposits in the glomeruli.

Additional types of nephropathies including IgM nephropathy can be diagnosed similarly. Replacing an IgA-binding protein or antibody with an anti-human IgM antibody or its fragment, for example, IgM can be detected in IgM nephropathy.

IgA Structure

Human immunoglobulin A (IgA) synthesis exceeds the combined total of all the other immunoglobulin classes (Rifai et al., J. Exp. Med. 191:2171–2181, 2000). It is estimated that 66 mg of IgA/kg of body weight is produced every day,

compared with 34 mg of IgG and 7.9 mg of IgM. There are two isotypes of IgA, IgA1 (SEQ ID NO:1) and IgA2. On mucosal surfaces (e.g., gut, respiratory tract, genital tract) both IgA1 and IgA2 are present, synthesized by local B cells. In the blood, however, IgA1 predominates, produced by B cells in the bone marrow, lymph nodes, and spleen (Donadio and Grande, N. Engl. J. Med. 347:738-748, 2002).

The main difference between the IgA1 (SEQ ID NO:1) and IgA2 subclasses is a 13 amino acid deletion in the IgA2 hinge region. This segment in IgA1 contains several serine and threonine amino acid residues that are O-glycosylated. The absence of this region in IgA2 explains the lack of O-linked oligosaccharides on IgA2 (Figure 1).

IgM Structure

IgM (SEQ ID NO:15) can be found as a monomer on the surface of the B lymphocyte, but in circulation it exists mainly as a pentamer after being secreted from plasma cells. The IgM pentamer has a molecular mass of ~850-1,000 kDa while each monomer is ~180 kDa. IgM represents ~10% of total serum Ig and is the first isotype of antibody synthesized during a primary humoral response.

The normal plasma IgM level in adults is about 1 mg/ml with a half-life about 5 days. This compares with IgG level of 12 mg/ml with 25 days of half-life and IgA of 1.8 mg/ml with half-life of 6 days.

Carbohydrates constitute about 12% of the IgM protein by weight. The mu heavy chain of IgM consists of 4 CH domains. (Only mu and epsilon (IgE) heavy chains each have four constant heavy region domains---CH1, CH2, CH3 & CH4, while gamma (IgG), alpha (IgA), and delta (IgD) each have 3 constant heavy region domains.)

Due to the presence of 10 identical antigen-binding sites on a pentamer, IgM is an excellent agglutinating antibody. In addition, IgM is also efficient at

activating complement. The IgM monomers are joined together as a pentamer through interchain disulfide bonds, and also by J chain, a 15 kDa peptide that attaches to two monomers of IgM and places the pentamer into a closed, apparently circular, conformation. (J chain also serves to link monomers of IgA together, forming dimers.)

Diagnostic Compositions

The components of the diagnostic radiopharmaceutical for use herein include (1) a compound, peptide, or protein that specifically binds human IgA or IgM and (2) a compound (e.g., a radioisotope) capable of detection by a radiologic imaging technique (e.g., SPECT) chelated to the compound, peptide, or protein in a pharmaceutically acceptable carrier. Preferable agents include a bifunctional chelating agent (BFCA), which is used to chelate (1) and (2).

While any IgA-binding or IgM-binding compound, protein, or peptide may be used as a biomolecule for diagnosis, preferred candidates meet several or all of the following criteria: high specificity and affinity for human IgA or IgM; for IgA-mediated disorders, ability to differentiate IgA1 from IgA2; small molecules, as these are less immunogenic; a protein of human origin; easily expressed or chemically synthesized; easy to produce and modify; and appropriately glycosylated, containing galactose for asialoglycloprotein receptor (ASGPR) clearance thereby minimizing renal tubule retention.

IgA- and IgM-binding Compounds/Peptides

IgA- and IgM-binding proteins or peptides can include native human IgA-binding receptors on cells; receptors that recognize both IgA and IgM; bacterial IgA-binding proteins and their derived peptides; anti-human IgA or IgM antibodies and their smaller derivatives (e.g., Fab); and IgA- and IgM-specific peptides discovered using methods such as phage display.

IgA-specific Receptors

The natural functions of IgA-specific receptors include recruitment of inflammatory cells and mediators to inflammatory sites (CD89; SEQ ID NO:2), and the distribution of IgA, e.g., entry of IgA into secretions (pIgR; SEQ ID NO:7).

Human FcαR1 (CD89; SEQ ID NO:2; see Figure 2) is a membrane glycoprotein that contains two extracellular Ig-like domains (206 aa), a membrane-spanning region (19 aa), and a cytoplasmic tail (31 aa). At the transmembrane region, a positively charged arginine residue is necessary for association of CD89 with the FcR γ-chain. Like other Fc receptors lacking an intracellular signaling motif, FcαR's signaling into the cell is initiated via its association with FcR γ-chain. The FcR γ-chain is a homodimer-signaling unit with a size of 10 kDa. Binding to CD89 leads to phosphorylation of the intracellular, immunoreceptor tyrosine-based activation motif (ITAM) on the γ-chain, activating the signaling pathways downstream into the cell. FcαR1 binds both the monomeric and dimeric forms of IgA1 and IgA2. Transfection studies in leukocytes show that the FcαR1 does not bind IgG. FcαR1 is expressed only by myeloid cells, including neutrophils, monocytes, macrophages, and eosinophils. It was proposed that FcαR1 plays a role in the removal of IgA-antigen complexes from the circulation (Mattu et al., J. Bio. Chem. 273:2260–2272, 1998; Leung et al., J. Am. Soc. Nephrol. 11:241-249, 2000; Westerhuis, *Pathogenetic Aspects of IgA-Nephropathy 2001*, Chapter 1, Section IV: IgA receptors and IgAN, 2001, PrintParters, Ipskamp, Enschede).

A 206 amino acid soluble portion of recombinant CD89 (SEQ ID NO:5) has been successfully expressed in several research labs, and such a soluble receptor has the potential to be used as an IgA-detecting peptide for diagnosis of IgAN. Despite some controversial reports that soluble CD89 might exacerbate IgAN (Pierre Launay et al., J. Exp. Med. 191:1999-2009), this fragment is preferred for binding to IgA due to its high specificity and affinity. This protein

is glycosylated, which likely accelerates its clearance via the asialoglycoprotein receptor (ASGPR). This minimizes background noise of unbound ligand in circulation and likely minimizes the clearance of radioactive nuclides through the renal degradation system, which also reduces background noise. More preferably, a smaller fragment of this peptide that retains IgA-binding activity is used.

Receptors Specifically Binding Both IgA and IgM

The human Fcα/μ receptor (Fcα/μ R; SEQ ID NO:6) binds both IgA and IgM with intermediate to high affinity. Fcα/μ R is constitutively expressed on the majority of B-lymphocytes and macrophages (Sakamoto et al., Eur. J. Immunol. 31:1310-1316, 2001; Shibuya et al., Nat. Immunol. 1:441-446, 2000).

The polymeric Ig receptor (pIgR; SEQ ID NO:7) is an integral membrane component localized on the basolateral surface of secretory epithelial cells. It mediates the trans-epithelial transport of polymeric Ig, mainly dimeric IgA and pentameric IgM. pIgR is on most human secretory epithelia, including intestine, bronchus, salivary glands, renal tubule, and uterus, and it binds to J chains on polymeric immunoglobulins. Binding of IgA results in the protein being transferred from the lamina propria of the mucosa through the epithelial cell to the gut (or other IgA secretory sites) to reach the cell-free fluids bathing the mucus membranes. Secretory IgA (sIgA) is responsible for neutralization of microbes and toxins and prevents unwanted antigens from passing through the mucosal barrier (Mattu et al., J. Bio. Chem. 273:2260–2272, 1998; Leung et al., J. Am. Soc. Nephrol. 11:241-249, 2000).

Recently, other IgA receptors have been proposed, including the transferrin receptor (CD71; SEQ ID NO:8) expressed on mesangial cells (Haddad et al., J. Am. Soc. Nephrol. 14:327–337, 2003). Although the role of this protein in IgA1 deposition diseases is unknown, it may also be used in the present invention.

Bacterial IgA-binding Peptides

Bacterial surface proteins that bind human IgA-Fc have been described in both group A streptococci (*Streptococcus pyogenes*) and group B streptococci (*Streptococcus agalactiae*; Sandin et al., J. Immunol. 169:1357-1364, 2002; Pleass et al., J. Biol. Chem. 276:8197-8204, 2001; Johnsson et al., J. Biol. Chem. 274:14521-14524, 1999; Stenbere et al., J. Biol. Chem. 269:13458–13464, 1994). The IgA-binding proteins of *S. pyogenes* are members of the M protein family, a heterogeneous family of dimeric proteins that are important virulence factors. All M proteins bind one or more human plasma proteins, and about 50% of all *S. pyogenes* strains express an M protein that binds IgA-Fc. M proteins have structural features that foster IgA binding, for example, in several instances a heptad repeat pattern forms a coiled-coil dimer, which binds to specific sites on IgA. Protein Sir22 (also named protein M22, as it is among the M proteins; SEQ ID NO:3), which has been studied in detail by Sandin et al. (J. Immunol. 2002, 169:1357-1364), has such a structure with a stretch of 29 amino acids sufficient for IgA binding. A 50-residue synthetic peptide (SEQ ID NO:4) has been designed that includes the 29-residue IgA-binding region and which specifically binds to human IgA. This 50-mer, designated Streptococcal IgA-binding peptide or Sap (SEQ ID NO:4), has the properties of an isolated IgA-binding domain, and its binding site on IgA is known to be in the Fc region, the same site that binds human CD89. Sap is a homologue of amino acids 35–83 of Sir22 and was designed to include a C-terminal cysteine residue not present in Sir22. The cysteine was introduced to bring about dimerization of the Sap peptide, a process that can be enhanced by incubation with $CuCl_2$. The IgA-binding tests conducted by Lindahl et al. indicate that Sap dimerization is essential for IgA binding. Sap peptide immobilized on a solid support has been shown to deplete all isotypes, monomers and polymers of IgA from human serum, and eluates from Sap chromatographic columns contain only IgA. Thus, dimerized Sap represents a

preferred IgA-binding peptide for IgAN diagnosis, having both IgA-binding specificity and apparent high affinity. Additionally, Sap can be synthesized by solid phase peptide synthesis (SPPS) methods, and a bifunctional chelating agent (BFCA; see below for details) can be added to either the N- or C-terminus while the residue is still in the solid phase on the resin, using methods standard in the art. Sap analogs are small (about 5.5 kDa for a monomer and 11 kDa when dimerized), thus minimizing antigenicity.

In *Staphylococcus aureus* protein A (SPA; SEQ ID NO:10), one can find five Ig binding domains. SPA binds strongly to the Fc region of immunoglobulins. Z-domain (SEQ ID NO:11) is a 58-aa residue recombinant protein derived from one of these five homologous domains (the B domain; SEQ ID NO:13) in SPA. Z-domain was originally developed by changing a few amino acids of the B domain to enhance the stability of the latter as a gene-fusion partner for affinity purification of recombinant proteins by using IgG-containing resins. Using phage display technology, researchers at Karolinska Institute in Sweden modified the IgG-binding Z-domain into an IgA-binding peptide designated as $Affibody_{IgA1}$ or modified Z-domain (SEQ ID NO:12). (Graille et al., Proc. Natl. Acad. Sci. U.S.A. 97:5399-5404, 2000; Wahlberg et al., Proc. Natl. Acad. Sci. U.S.A. 100:3185-3190, 2003; Rönnmark et al., Eur. J. Biochem. 269: 2647-2655, 2002; Braisted and Wells, Proc. Natl. Acad. Sci. U.S.A. 93:5688-5692, 1996). This peptide binds both human IgA1 and IgA2 with high specificity and affinity. The original IgG binding affinity was completely lost with these modifications. The binding site on IgA is believed to be in the Fc region. Given its binding to human IgA, this peptide is also preferred for IgAN diagnosis.

Anti-human IgA or IgM Antibodies or Their Antigen-binding Fragments

Polyclonal or monoclonal anti-human IgA or IgM antibodies may also be used for the diagnosis of IgA or IgM kidney diseases. These antibodies may be

derived from many sources including immunized animals or they may be prepared as animal/human chimeric proteins or humanized chimeric proteins, or human origin protein; all are strategies known in the art for making protein infusion therapies and/or diagnostic reagents. Both whole molecules and Ag-binding fragments of these antibodies can be used in the diagnostic methods and compositions of the invention. However, a preferred reagent is a monoclonal antibody of human origin, and, in the case of IgAN or HSP, preferably one that differentiates IgA1 from IgA2. Also, it is preferred that subunits of the antibody protein, which are preferred over the full-length antibody, maintain IgA- or IgM-recognition specificity (Reff et al., Canc. Control 9:152-166, 2002; Gorman and Clark, Semin. Immunol. 2:457-66, 1990; *Antibodies as Medicines* 2000 by Biotech Analytics).

In a preferred embodiment, small antibody fragments of anti-human IgA1 or anti-human IgM (e.g., human-originated fragments, humanized chimeric fragments, chimeric fragments, and animal origin fragments) are used. The fragments of the antibody that retain antigen-binding activity may be monovalent Fab, Fv, or scFv; or bivalent F(ab)'2 or diabodies. (See the schematic diagrams (Hudson and Souriau, Nat. Med. 9:129-134, 2003) in the Figure 4 below). Preferably, the Fc region is deleted from the molecule.

In one particular example, the anti-human IgA1 antibody is directed at epitopes in the hinge region because, as described above, this region is unique to IgA1. Such human origin anti-human IgA1 hinge region Fab may be readily made by phage display technology through a large phage antibody library developed by commercial providers such as Cambridge Antibody Technology (Cambridge, UK). This technology has advantages over methods for raising conventional monoclonal antibodies in being rapid, and in allowing access to the gene for ease of modification.

In another example, anti-IgM monoclonal antibodies, raised using methods standard in the art (e.g., those described herein), are used to diagnose

IgMN. These antibodies can be raised using the unique mu chain hinge sequences that bridge CH1 and CH2 domains of the constant region as a target antigen (PLPVIAELPPKVSVFVPPRDGFFGNP; SEQ ID NO:17). In addition, IgM-specific antibodies can be raised against isolated, intact Fc regions of the IgM protein, such Fc produced by trypsin cleavage at high temperature (Plaut and Tomasi, Proc. Natl. Acad. Sci. USA, 65:318-322, 1970).

IgM-binding Compounds

With the use of phage display technology, a peptide, YDWIPSSAW (SEQ ID NO:9), has been identified that binds with high affinity to murine IgM. This peptide also inhibits human rheumatoid factor (RF, mainly IgM class of anti-IgG autoimmune antibody) induced agglutination of IgG-coated latex beads, and shows no binding abilities to other immunoglobulins (Pati M. Glee et al. J Immunol, 1999, 163: 826-833).

Identifying Novel IgA- or IgM-binding Compounds

The methods and compositions of the invention may also employ novel IgA-binding or IgM-binding compounds identified using techniques such as phage display. Phage display is a combinatorial screening technique, allowing the discovery and characterization of proteins that interact with a desired target by using multiple genes from a gene bank (George P. Smith, Science 228:1335, 1985). These genes represent a required diversity generated by DNA recombinant technology; therefore, each phage displays a unique random peptide. These genes are inserted into phages by replacing preexisting genes, thus creating a phage library. Premade libraries are readily available (e.g., Novagen T7Select) with accompanying kits. Novagen's T7 system is a lytic phage display that is preferred for cDNA libraries (J. Imm. Meth. 231:39; Nature Biotech. 19:1193), but other phages may also be used, such as non-lytic M13 bacterial filamentous phage, which is based on N-terminal fusion to surface coat

proteins pIII and pVIII. The modified phages will then express the protein coded by the inserted DNA on its surface coat. In the case of M13, pIII display has a lower valency (1-5 per virion) and thus can be used to find high affinity compounds whereas pVIII has high valency (~200 per virion) and can be used to find very low affinity binding compounds. Phage display services are commercially available through companies including Dyax Corp. of Cambridge, Mass., which offers four phage libraries: human antibody, proteins, structured peptides, and linear peptides. The phages from an entire phage library are incubated with the targeted proteins, (e.g., the alpha chain of IgA or the mu chain of IgM). Phages that display peptides with high affinity and specificity for IgA or IgM are then selected. The polynucleotide sequences coding for these peptides are identified and sequenced, and additional peptides can be produced for further analysis. The highest affinity and specificity protein, along with other qualities such as immunogencity, are then chosen as the final candidate to be used in the methods or compositions of the invention. Phage display gene banks or library may include millions of related genes, and thus may be employed to identify IgA- or IgM-binding compounds useful for the diagnostic methods and compositions of the invention.

Polypeptide Expression

In general, polypeptides for use in the invention may be produced by any standard technique; for example, by transformation of a suitable host cell with all or part of a polypeptide-encoding polynucleotide molecule or fragment thereof in a suitable expression vehicle.

Those skilled in the field of molecular biology will understand that any of a wide variety of expression systems may be used to provide the recombinant polypeptide. The precise host cell used is not critical to the invention. A polypeptide for use in the invention may be produced in a prokaryotic host (e.g., *E. coli*) or in a eukaryotic host (e.g., *Saccharomyces cerevisiae*, insect cells, e.g.,

Sf21 cells, or mammalian cells, e.g., NIH 3T3, HeLa, or preferably COS cells). Such cells are available from a wide range of sources (e.g., the American Type Culture Collection, Rockland, Md.; also, see, e.g., *Current Protocols in Molecular Biology*, Eds. Ausubel et al., John Wiley and Sons). The method of transformation or transfection and the choice of expression vehicle will depend on the host system selected. Transformation and transfection methods are described, e.g., in Ausubel et al. (supra); expression vehicles may be chosen from those provided, e.g., in *Cloning Vectors: A Laboratory Manual* (Pouwels, P. H. et al., 1985, Supp. 1987).

One particular bacterial expression system for polypeptide production is the *E. coli* pET expression system (Novagen, Inc., Madison, Wis.). According to this expression system, DNA encoding a polypeptide is inserted into a pET vector in an orientation designed to allow expression. As the gene encoding such a polypeptide is under the control of the T7 regulatory signals, expression of the polypeptide is achieved by inducing the expression of T7 RNA polymerase in the host cell. This is typically achieved using host strains which express T7 RNA polymerase in response to IPTG induction. Once produced, recombinant polypeptide is then isolated according to standard methods known in the art, for example, those described herein.

Another bacterial expression system for polypeptide production is the pGEX expression system (Pharmacia). This system employs a GST gene fusion system which is designed for high-level expression of genes or gene fragments as fusion proteins with rapid purification and recovery of functional gene products. The polypeptide of interest is fused to the carboxy- terminus of the glutathione S-transferase protein from *Schistosoma japonicum* and is readily purified from bacterial lysates by affinity chromatography using Glutathione Sepharose 4B. Fusion proteins can be recovered under mild conditions by elution with glutathione. Cleavage of the glutathione S-transferase domain from the fusion protein is facilitated by the presence of recognition sites for

site-specific proteases upstream of this domain. For example, polypeptides expressed in pGEX-2T plasmids may be cleaved with thrombin; those expressed in pGEX-3X may be cleaved with factor Xa.

Once the recombinant polypeptide is expressed, it is isolated, e.g., using affinity chromatography. In one example, an antibody (e.g., produced as described herein) raised against a polypeptide for use in the invention may be attached to a column and used to isolate the recombinant polypeptide. Lysis and fractionation of polypeptide-harboring cells prior to affinity chromatography may be performed by standard methods (see, e.g., Ausubel et al., supra).

Once isolated, the recombinant polypeptide can, if desired, be further purified, e.g., by high performance liquid chromatography (see, e.g., Fisher, *Laboratory Techniques In Biochemistry And Molecular Biology*, eds., Work and Burdon, Elsevier, 1980).

Polypeptides for use in the invention, particularly short peptide fragments, can also be produced by chemical synthesis (e.g., by the methods described in *Solid Phase Peptide Synthesis*, 2nd ed., 1984 The Pierce Chemical Co., Rockford, Ill.).

These general techniques of polypeptide expression and purification can also be used to produce and isolate useful peptide fragments or analogs (described herein).

The short peptides such as 50-mer Streptococcal IgA-binding peptide (Sap) and 58-mer modified Z-domain can also be chemically synthesized.

Antibody Production

To generate antibodies, any standard technique may also be used. For example, a coding sequence for IgA1, IgM, or fragments thereof (e.g., the hinge region of IgA1, amino acids 217-241) may be chemically synthesized or expressed as a C-terminal fusion with glutathione S-transferase (GST) (Smith and Johnson, Gene 67:31-40, 1988). The fusion protein is purified on

glutathione-Sepharose beads, eluted with glutathione, cleaved with thrombin (at the engineered cleavage site), and purified to the degree necessary for immunization of mice or rabbits. Primary immunizations are carried out with Freund's complete adjuvant or a similar adjuvant and subsequent immunizations with Freund's incomplete adjuvant. Antibody titres are monitored by ELISA or Western blot and immunoprecipitation analyses using the thrombin-cleaved polypeptide fragment of the GST fusion protein. Immune sera are affinity purified using CNBr-Sepharose-coupled polypeptide. Antiserum specificity is determined using a panel of unrelated GST proteins.

As an alternate or adjunct immunogen to GST fusion proteins, peptides corresponding to relatively unique immunogenic regions of IgA or IgM may be generated and coupled to keyhole limpet hemocyanin (KLH) through an introduced C-terminal lysine. Antiserum to each of these peptides is similarly affinity purified on peptides conjugated to BSA, and specificity tested in ELISA and Western blots using peptide conjugates, and by Western blot and immunoprecipitation using the polypeptide expressed as a GST fusion protein.

Alternatively, monoclonal antibodies which specifically bind IgA or IgM are prepared according to standard hybridoma technology (see, e.g., Kohler et al., Nature 256:495, 1975; Kohler et al., Eur. J. Immunol. 6:511, 1976; Kohler et al., Eur. J. Immunol. 6:292, 1976; Hammerling et al., In *Monoclonal Antibodies and T Cell Hybridomas*, Elsevier, N.Y., 1981; Ausubel et al., supra). Once produced, monoclonal antibodies are also tested for specificity by Western blot or immunoprecipitation analysis (by the methods described in Ausubel et al., supra). Antibodies which specifically recognize IgA, especially the IgA1 isotype, or IgM are considered to be useful in the invention. Alternatively monoclonal antibodies may be prepared using IgA or IgM and a phage display library (Vaughan et al., Nat. Biotechnol. 14:309-314, 1996).

Preferably, antibodies are produced using fragments of IgA or IgM which lie outside generally conserved regions (e.g., the 13 amino acid region in IgA1,

absent in IgA2; see Figure 1) or unique sequence in IgM hinge region; see SEQ ID NO:14 and appear likely to be antigenic by criteria such as high frequency of charged residues. In one specific example, such fragments are generated by standard techniques of PCR and cloned into the pGEX expression vector (Ausubel et al., supra). Fusion proteins are expressed in *E. coli* and purified using a glutathione agarose affinity matrix as described in Ausubel et al. (supra). To attempt to minimize the potential problems of low affinity or specificity of antisera, two or three such fusions are generated for each polypeptide, and each fusion is injected into at least two rabbits. Antisera are raised by serial injections, preferably including at least three booster injections.

Antibodies generated against IgA1 may be employed to diagnose IgAN and HSP, and antibodies anti-IgM may be used to diagnose IgMN.

Peptide Modifications

It may be desirable to modify peptides used in the methods and compositions of the invention, as peptides that enter the circulation are rapidly degraded in plasma. Modifications are therefore desirable to prolong their half-life without reducing biological activity and binding specificity. For example, the tetradecapeptide somatostatin has only a 3 minute half-life in human plasma, far too short to be used as a radiopharmaceutical in cancer diagnosis. Thus, its analog, octreotide was developed to have a 90 minute half-life in the human circulation (Langer and Beck-Sickinger, Curr. Med. Chem. Anti-Canc. Agents 1:71-93, 2001). To resist degradation by exopeptidases, the peptides may be capped at the N- and/or C-terminus. This includes, for example, acetylation of the N-terminus, amidating or reducing the C-terminus, and N to C cyclization. These methods are standard in the art. To reduce susceptibility to endopeptidases the peptide may be modified by introducing D-amino acids, amino acid surrogates, or peptidomimetic sequences and spacers. Other ways to improve stability include modification of peptide bonds, replacement of amino-

with imino- groups, methylation of amide nitrogens, and shortening of the natural amino acid sequence. Again, these methods are standard in the art.

Radiometals

As indicated above, the diagnostic reagent further includes a detectable label, such as a radioactive label like a radioactive metal. Various radioactive metals are used in scintigraphy and their use varies with scintigraphy type, diagnosis being attempted, and cost and availability of the isotope. A key determinant in the isotope chosen is the scinitigraphy type, and radioactive decay needed. SPECT and PET use different isotopes because SPECT uses gamma emitters, whereas PET uses positron emitters. Isotopes can be produced in four different ways, which affects their cost. Those produced in a generator come from the conversion of a long half-life radioactive isotope to one of shorter half-life. This makes them practical for use even in small hospitals, and their cost is lower. Other radioactive metals are more expensive as they are produced in nuclear reactors, cyclotrons, and linear accelerators, and this may limit availability for smaller hospitals, and those in more remote areas. As for diagnosis, the radiometal used is in part dictated by the time needed to reach and to bind to the target. In the case of IgA-binding peptides, a longer half-life in the range of 4-80 hours may be preferred.

Metastable technetium-99m (^{99m}Tc) is used in 85% of scans, which is about 7 million each year in the U.S. alone. The properties of ^{99m}Tc are virtually ideal for diagnostic imaging; the γ-radiation of 140 keV falls within the ideal range of today's gamma cameras, and a half-life of 6 hours is long enough to synthesize the labeled radiopharmaceuticals, perform quality control, inject it into the patient, and carry out imaging. But the half-life is short enough to enable administration of sufficiently high doses with minimal patient risk, and at the concentration levels used ($< 10^{-6}$ M), neither the resulting gamma radiation nor

the soft beta decay of ^{99}Tc is hazardous. ^{99m}Tc is readily available at low cost from its parent nuclide ^{99}Mo ($t_{1/2}$ 66 h), produced in a ^{99}Mo/^{99m}Tc generator.

Gamma-emitting Indium-111 (^{111}In) has been extensively used for labeling monoclonal antibodies and peptides. The first peptide radiopharmaceutical approved for clinical use by the FDA was the ^{111}In-labeled somatostatin analog OctreoScan® (Mallinckrodt, St. Loius, Mo). The chemistry and half-life of this isotope (67.9 h) makes it ideal for labeling immunoglobulins, where imaging may be performed several days after the initial injection. ^{111}In is produced in a cyclotron from Cadmium-111, and thus it is less available in comparison to ^{99m}Tc. Ion exchange and solvent extraction are the methods commonly used to separate Indium-111 from parent cadmium. However, for the purpose of radiolabeling an IgA-binding peptide, the longer half-life of ^{111}In compared to ^{99m}Tc provides the IgA-binding peptide more time to reach IgA deposits in the kidney and provides more time for clearance of circulating tagged IgA.

Galium-67 (^{67}Ga) is produced in a cyclotron from zinc-68 (^{68}Zn), and was the first nuclide produced for human use in 1953. The separation techniques may include solvent extraction and ion exchange, or both. The half-life of ^{67}Ga is over 78 hours, and its energy decay characteristics make it suitable for either gamma scintigraphy or PET imaging.

The radionuclides of copper offer a selection of diagnostic epitopes including ^{60}Cu, ^{61}Cu, ^{62}Cu, and ^{64}Cu. The positron-emitting ^{64}Cu is cyclotron produced. ^{64}Cu is preferred for labeling proteins, peptides, and agents with long blood clearance, which is likely an advantageous property of IgA-binding proteins. ^{64}Cu has a half-life of 12.7 hours.

Tables 1 and 2 below show the many gamma- and positron-emitting radiometals for diagnostic use, their decay characteristics, half-life, and methods of production (Anderson and Welch, Chem. Rev. 99:2219-2234, 1999). Any of these radiometals may be used in the methods and compositions described herein.

Table 1. Gamma- and Beta-Emitting Radionuclides

isotope	$t_{1/2}$ (h)	production methods	decay mode	E_γ (keV)	E_{β^-} (keV)
^{67}Cu	62.01	accelerator, ^{67}Zn(n,p)	β^- (100%)	91, 93, 185	577, 484, 395
^{67}Ga	78.26	cyclotron	EC (100%)	91, 93, 185, 296 388	
^{90}Y	64.06	^{90}Sr/^{90}Y generator	β^- (72%)		2288
^{111}In	67.9	cyclotron, ^{111}Cd(p,n)^{111}In	EC (100%)	245, 172	
^{99m}Tc	6.0	^{99}Mo/^{99m}Tc generator	IT (100%)	141	
^{201}Tl	72 h	cyclotron	EC (100%)	135, 167	
		^{203}Tl(p,3n)^{201}Pb(p,n)^{201}Tl	Hg X-rays		

Table 2. Positron-Emitting Radionuclides

isotope	$t_{1/2}$(h)	methods of production	decay mode	E_{β^+} (keV)
^{55}Co	17.5	cyclotron, ^{54}Fe(d,n)^{55}Co	β^+ (77%)	1513, 1037
			EC (23%)	
^{60}Cu	0.4	cyclotron, ^{60}Ni(p,n)^{60}Cu	β^+ (93%)	3920, 3000
			EC (7%)	2000
^{61}Cu	3.3	cyclotron, ^{61}Ni(p,n)^{61}Cu	β^+ (62%)	1220, 1150
			EC (38%)	940, 560
^{62}Cu	0.16	^{62}Zn/^{62}Cu generator	β^+ (98%)	2910
			EC (2%)	
^{64}Cu	12.7	cyclotron, ^{64}Ni(p,n)^{64}Cu	β^+ (19%)	656
			EC (41%)	
			β^- (40%)	
^{66}Ga	9.5	cyclotron, ^{63}Cu(α,nγ)^{66}Ga	β^+ (56%)	4150, 935
			EC (44%)	
^{68}Ga	1.1	^{68}Ge/^{68}Ga generator	β^+ (90%)	
			EC (10%)	1880, 770
^{82}Rb	0.022	^{82}Sr/^{82}Rb generator	β^+ (96%)	3150
			EC (4%)	
^{86}Y	14.7	cyclotron, ^{86}Sr(p,n)^{86}Y	β^+ (33%)	2335, 2019
			EC (66%)	1603, 1248
				1043

BFCAs

In radiopharmaceuticals, bifunctional chelating agents may be used to bridge the biological molecules and radiometals. There are many BFCAs available, any of which may be used in the invention. In one particular example, BFCAs that bind ^{99m}Tc have been most widely used because ^{99m}Tc is the metal used in 85% of the diagnostic scans currently in clinical use (Langer and Beck-Sickinger, Curr. Med. Chem. Anti-Canc. Agents 1:71-93, 2001). These BFCAs form a complex that has thermodynamic stability, kinetic inertness with respect to dissociation, and ability to stabilize the oxidation state of Tc. A variety of BFCAs have been developed for ^{99m}Tc including triamdiethiols N_3S, diamide-dithiols N_2S_2, propyleneamineoxime (PnAO), and hydrazinonicotinic acid (HYNIC). Some structures are shown in (Figure 5). A newer approach employs an organometallic species $[M(CO)_3(H_2O)_3]^+$ (M = ^{99}Tc, ^{99m}Tc, $^{185/187}$Re,

$^{186/188}$Re), which is a Tc/Re(I) precursor for labelling pharmacophores. The cationic species, available after short reaction times in high radiochemical purity, offer several advantages including maintenance of the oxidation state, stability in serum, and formation of stable complexes with biological ligands. Histidine and PADA (picolylamine-N,N-diacetic acid; Figure 6) have been identified as suitable BFCAs. Histidine has been used to radiolabel a neurotensin derivative, and PADA for the labelling of bombesin- and neuropeptide Y derivatives with ^{99m}Tc.

The BFCA of choice for Indium-111 is diethylene-triaminepentaacidic acid (DTPA), and a variety of peptides have been labeled with DTPA (Figure 7). The second most widely used chelator for ^{111}In is DOTA (tetraazacyclododecanetetraacidic acid) (Figure 7), which also can be used for complexing the therapeutic radionuclide Yttrium-90 (^{90}Y).

Tripeptides and tetrapeptides such as Lys-Gly-Cys, Cys-Gly-Cys, Gly-Gly-Cys, and Gly-Ala-Gly-Gly can also be used as BFCAs (Langer and Beck-Sickinger, Curr. Med. Chem. Anti-Canc. Agents 1:71-93, 2001). For example, ^{99m}Tc has been used to label vasoactive intestinal peptide (VIP), and its analog TP3654, which carries the chelating amino acid sequence GAGG on the C-terminus of VIP (Thakur et al., J. Nucl. Med., 41:107, 2000). In another report, ^{99m}Tc-labelling via N-terminal Ac-Cys-Gly-Cys-Gly (CGCG) chelation to α-melanocyte-stimulating hormone (MSH) resulted in promising candidates for peptide-based radio-diagnosis (Chen et al., Nucl. Med. Biol. 26:687-693, 1999).

Non Radioactive Labels

In addition to radiolabeled compounds, paramagnetic substances and quantum dot (qdot) technology can also be used in the methods and compositions of the invention. Coupling paramagnetic substances to an IgA-binding or IgM-binding compound allows imaging via MRI, and qdot allows fluorescent imaging.

Paramagnetic Substances

If an imaging technique (e.g., MRI) that does not require radioactivity is used in the present invention, a nonradioactive label may be substituted for the radiometal described above. For example, a paramagnetic substance (e.g., gadolinium) can be conjugated to an IgA-binding protein using methods standard in the art. When the compound is injected into a mammal and imaged by MRI, there is a difference in magnetic properties where the paramagnetic substances settle down. These differences can be used to identify IgA or IgM deposition in the glomeruli of the kidneys, and thus to diagnose an IgA or IgM kidney disease.

Quantum Dots

Quantum dots (qdots) are fluorescent semiconductor nanocrystals made from variety of materials, for example CdS, CdSe, CdTe, CdHgTe/ZnS, InP, InAs, and PbSe. For nanocrystals smaller than the so-called Bohr exciton radius (a few nanometers), energy levels are quantized, with values directly related to the crystal size (an effect called quantum confinement). Thus, they have some distinguishing characteristics from commonly used fluorophores. One advantage of the qdots is that their size and shape can be precisely controlled by the duration, temperature, and ligand molecules used in the synthesis. The qdots' absorption and emission properties can also be controlled, because they are composition- and size-dependent.

The core of qdots may be wrapped by polymer coating materials to make them soluble in aqueous solutions and to prevent the release of cytotoxic Cd^{2+} or Se^{2-} ions from the qdot core. To add function to qdots, the outermost layer can include conjugated compounds (e.g., antibodies and bioactive peptides) that impart specific functionalities (e.g., IgA or IgM binding). If reduction of accumulation in liver and bone marrow is desired, high molecular weight polyethylene glycol (PEG) molecules can be added to the coating.

In one embodiment, qdots may be used alone for imaging purposes. Qdots tagged with antibodies (e.g., anti-IgA or anti-IgM antibodies) or binding compounds (e.g., IgA- or IgM-binding compounds) may be used to target the qdots to specific molecules. Such qdots can be injected into a mammal, and imaged using fluorescent techniques as described by Gao et al. (Nat. Biotech. 22:969-976, 2004).

In another embodiment, qdots may be coupled to radiolabeled compounds (e.g., the radiometals described herein) or paramagnetic substances (e.g., gadolinium). These qdots can be injected into a mammal, and visualized using an appropriate imaging technique (e.g., MRI, SPECT, PET, or planar scan).

Formulation of Diagnostic Compositions

The compound(s) used in the compositions and methods the invention may be contained in any appropriate amount in any suitable carrier substance, and is generally present in an amount of 1-95% by weight of the total weight of the composition. The composition is preferably provided in a dosage form that is suitable for parenteral (e.g., intravenous) administration route. The diagnostic compositions may be formulated according to conventional pharmaceutical practice (see, e.g., Remington, *The Science and Practice of Pharmacy* (20th ed.), ed. A.R. Gennaro, Lippincott Williams & Wilkins, 2000 and *Encyclopedia of Pharmaceutical Technology*, eds. J. Swarbrick and J. C. Boylan, 1988-1999, Marcel Dekker, New York).

Parenteral Compositions

The diagnostic compositions may be administered parenterally by injection, infusion, or implantation (intravenous or the like) in dosage forms, formulations, or via suitable delivery devices or implants containing conventional, non-toxic pharmaceutically acceptable carriers and adjuvants. The

formulation and preparation of such compositions are well known to those skilled in the art of pharmaceutical formulation.

The composition may be in form of a solution, a suspension, an emulsion, an infusion device, or a delivery device for implantation, or it may be presented as a dry powder to be reconstituted with water or another suitable vehicle before use. Apart from the diagnostic compound(s), the composition may include suitable parenterally acceptable carriers and/or excipients. Furthermore, the composition may include suspending, solubilizing, stabilizing, pH-adjusting agents, tonicity adjusting agents, and/or dispersing agents.

As indicated above, the diagnostic compositions according to the invention may be in a form suitable for sterile injection. To prepare such a composition, the diagnostic compound(s) are dissolved or suspended in a parenterally acceptable liquid vehicle. Among acceptable vehicles and solvents that may be employed are water, water adjusted to a suitable pH by addition of an appropriate amount of hydrochloric acid, sodium hydroxide or a suitable buffer, 1,3-butanediol, Ringer's solution, dextrose solution, and isotonic sodium chloride solution. The aqueous formulation may also contain one or more preservatives (e.g., methyl, ethyl or n-propyl p-hydroxybenzoate). In cases where one of the compounds is only sparingly or slightly soluble in water, a dissolution enhancing or solubilizing agent can be added, or the solvent may include 10-60% w/w of propylene glycol or the like.

Dosages of Compounds

While the attending physician ultimately will decide the appropriate amount of the compound or compounds for diagnosis of an IgA or IgM kidney disease, typically 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 mCi of a radiolabeled compound will be administered intravenously in a pharmaceutically acceptable carrier. Dosages are determined based on several considerations, including the dosage required for imaging technique (e.g., SPECT) used and by the clearance and

absorption characteristics of the compound. Radioactive dosimetry can be used to ensure the maximum radiation allowed to each organ is not exceeded, based on published values.

Imaging Techniques

Any standard imaging technique can be used in the present invention, with scintigraphy being the preferred method. Those skilled in the art will know which types of scanner are used with specific radiographic agents.

Scintigraphy

Scintigraphy involves the use of radioactive isotopes to diagnose and treat various diseases. It has applications in neurology, cardiology, oncology, endocrinology, lymphatics, urinary function, gastroenterology, pulmonology, and other areas. Generally, the radioactive isotope is chemically bonded to a specific compound, and then injected intravenously. Different radioactive isotopes are used for different scintigraphy methods and three common methods are SPECT, PET, and planar scan (Anderson and Welch, Chem. Rev. 99:2219-2234, 1999; Langer and Beck-Sickinger, Curr. Med. Chem. Anti-Canc. Agents 1:71-93, 2001).

SPECT (Single Photon Emission Computer Tomography) is a nuclear imaging technique that uses gamma rays. After injection of an isotope, a rotating gamma camera collects images 360 degrees around a patient, selecting only photons of certain energy. The images from various levels of the body are processed and recombined to form a 3D image.

PET (Positron Emission Tomography) is a nuclear imaging technique that uses radioactive isotopes that decay by positron emission, resulting in the release of two photons in opposite directions. A 360-degree scanner detects these photons, but only paired photons are processed. The safety of PET scans is higher than body CT, with radiation absorbed from a PET scan averaging 5 mSv,

whereas the radiation absorbed from a CT scan of the body ranges from 6 – 16 mSv.

Planar scanners were among the first generation of scintillation detecting devices and are currently used in many diagnostic procedures. They are in common use and yield two-dimensional images.

MRI

In addition to scintigraphy methods, magnetic resonance imaging (MRI) can also be used in the methods of the invention. MRI uses strong magnetic fields to generate three dimensional tomographic images of patients' bodies and does not require radioactivity.

Radiological Optimization

To further optimize the diagnostic methods of the invention, renal tubule retention may be reduced and pretargeting strategies employed.

Renal Tubule Retention of Radioactive Chelates

Radiolabeled peptides, proteins, or their fragments smaller than approximately 60 kDa are filtered at the glomerulus and enter the renal tubule (Langer and Beck-Sickinger, Curr. Med. Chem. Anti-Canc. Agents 1:71-93, 2001; Thakur et al., J. Nucl. Med., 41:107, 2000). Under physiological conditions, the cells of the proximal tubule quantitatively reabsorb these peptides, which are then subject to lysosomal degradation. This reabsorption traps the radiometal chelate in the cell, resulting in high retention of the radiolabel in the renal medulla and reducing the scintigraphic sensitivity for detection of specific higher emissions from the medullary region of the kidney. However, in IgAN, IgA deposits are in the glomeruli located in the renal cortex, minimizing the problem of renal radiometal retention and the associated interference. Reduction of renal tubule retention in diagnosing an IgA or IgM

kidney disease may still be desirable, and can be accomplished by using more lipophilic molecules. These molecules have a high content of lipophilic amino acids in the peptide, or have the addition of a fatty acyl moiety on the molecule. In one example, such lipophilic modification can be seen in RC-160, a somatostatin analog with increased lipophilicty that has fewer tendencies towards renal excretion (P. Dasgupta and R. Mukherjee, Br J Pharmacol (2000) 129, 101−109). A second example is stearyl-Nle^{17}-VIP, a lipophilic analogue of 28-mer vasoactive intestinal peptide, VIP. (Gozes et al., J. Pharmacol. Exper. Therap. 273 (1995) 161-167). The lipophilic modification may promote metabolic degradation through the liver, rather than the kidney. Another way to reduce renal tubular accumulation of radiometals is to increase the size of the radiolabeled reagent to be large enough to avoid filtration through glomeruli (Langer and Beck-Sickinger, Curr. Med. Chem. Anti-Canc. Agents 1:71-93, 2001). A third method is to administer oral or injected basic amino acids (e.g., lysine) or their derivatives following administration of the diagnostic agent, thereby inhibiting renal tubular cell uptake of proteins and peptides and inducing a transient proteinuria (Behr et al., Eur. J. Nucl. Med. 25:201-212, 1998).

Pretargeting Strategies

Pretargeting delivery is commonly used in tumor radioimmunoscintigraphy (RIS) and radioimmunotherapy (RIT) (Anderson and Welch, Chem. Rev. 99:2219-2234, 1999; Langer and Beck-Sickinger, Curr. Med. Chem. Anti-Canc. Agents 1:71-93, 2001; Chang et al., Mol. Canc. Ther. 1:553-563, 2002; Rossi et al., Clin. Canc. Res. 9:3886s–3896s, 2003). Pretargeting reduces or clears unbound radiolabeled molecules in the circulation, at the time when bound concentration is highest. The most widely used system is the biotin-streptavidin (SA) pair. Generally, the non-radiolabeled mAb-SA is injected first. As the clearance of antibodies is slow in circulation, unbound antibodies need to be cleared before delivering radiolabeled biotin. Thus, after

1-2 days of the first injection, a galactosylated clearing agent that interacts with mAb-SA is injected. This agent quickly clears away any circulating mAb-SA, but it is unable to clear bound mAb-SA. After another 1-10 hours, the radiolabeled biotin-BFCA is injected. Therefore, the radioactive chelate is only delivered to bound locations instead of to the circulating antibodies. Other possible strategies include a similar approach with a binding pair other than biotin-streptavidin or a system that requires only two steps, for example, as described below.

Two Step Method

To reduce the intravascular IgA or IgM radioactivity background, a two step method may also be utilized. In this method, an anti-IgA1 antibody or an anti-IgM antibody is first prepared, from which an Fab domain is obtained by limited proteolysis. This Fab is then modified to include streptavidin (SA) and galactose (Gal). The streptavidin is added as a biotin ligand, and Gal is added to promote removal of circulating Fab-IgA1 or Fab-IgM complexes. As clearance of the SA-Gal-Fab by hepatic ASGPR (asialoglycoprotein receptors) is faster than binding to IgA or IgM, a suitable competitive inhibitor such as galactose-Ficoll, a synthesized polymer of galactose that effectively inhibits ASGPR function, is injected (Rifai et al., J. Exp. Med. 191:2171–2181, 2000). Simultaneously with or immediately following the galactose-Ficoll injection, the SA-Gal-Fab anti-IgA1 or SA-Gal-Fab anti-IgM, is injected into patients for pretargeting, binding to both circulating IgA1 and IgA1 deposited in the renal cortex, or circulating IgM and IgM deposited in the renal cortex. At 24-48 hours, when the effect of galactose-Ficoll ends, the circulating SA-Gal-Fab-IgA1 or SA-Gal-Fab-IgM complexes are cleared from the circulation via the hepatic ASGPR. However, the immobilized SA-Gal-Fab-IgA1 or SA-Gal-Fab-IgM complexes in the glomeruli persist, and are available for imaging.

Approximately one or two days after the first injection, with background vascular SA-Gal-Fab-IgA1 or SA-Gal-Fab-IgM complex substantially cleared, radiolabeled (^{99m}Tc or ^{111}In) biotin-HSA-Gal (biotinylated human serum albumin conjugated with galactose) is injected and binds to SA-Gal-Fab-IgA1 SA-Gal-Fab-IgM complexes in the kidney. After another 4-24 hours, the removal of unbound (free) radiolabeled Biotin-HSA-Gal from the circulation by hepatic ASGPR results in increased target to noise ratio in the renal cortex. In addition, albumin's size (66kDa) excludes the radioactive complex from being filtrated from glomeruli, thus reducing associated background radiation due to settling of radioactive chelates in the proximal tubule cells of the kidneys. Another advantage of this design is that streptavidin is a tetramer (MW: 4x13kDa=52kDa), binding 4 molecules of biotinylated ligands. This increases the imaging signal, with four moles of radioactive Biotin-HSA-Gal binding per mole streptavidin exposed in the renal cortex.

To carry out this method, anti-human IgA1 antibody or anti-human IgM antibody and its Fab fragment are produced to diagnose an IgA or IgM kidney disease. Mouse or chimeric monoclonal anti-human IgA1 hinge region or anti-human IgM antibodies are raised by conventional methods, for example, as described herein. The Fab fragment is then obtained by any standard technique, such as papain digestion, and then purified, for example, by gel-filtration chromatography followed by antigen-affinity chromatography.

To conjugate galactose molecules onto Fab, cyanomethyl-2,3,4,6-tetra-I-acetyl-1-thio-β-D-galactopyranoside (C-4141; Sigma) is dissolved in methanol at 0.1 M and mixed with 0.1 volume sodium methoxide (methanol sodium derivative; J.T. Baker Chemical Co., Phillipsburg, NJ). After 48 h at room temperature, this mixture is stored for weeks at 4°C. The conjugate is replaced in 0.25 M sodium borate buffer, pH 8.5, containing Fab at 1.0 mg/ml. After 2h at room temperature, the sample is dialyzed into

phosphate-buffered saline. The antigen-binding function of the antibody is not altered by the conjugation (Ong et al., Canc. Res. 51:1619-1626, 1991).

Streptavidin (SA; MW:~52 kDa) binds specifically with biotin (244 Da). It is derived from the bacterium *Streptomyces avidinii* and bears similarity to chicken egg-white avidin both in three-dimensional structure and its ability to bind biotin with extremely high affinity ($Kd=10^{-15}$ M). It is a tetrameric protein (4x13 kDa) capable of binding up to 4 biotin molecules. Unlike avidin, streptavidin is non-glycosylated and is essentially neutral in charge, whereas avidin (pI~10.5) is basic at neutral pH. Because of this, streptavidin has considerably less non-specific binding resulting in less background and has replaced avidin as the preferred reagent for applications where protein interactions may cause undesired background.

To conjugate SA to Gal-Fab, the Gal-Fab is chemically conjugated to SA (Genzyme, Cambridge, Mass) using succinimidyl 4-(*N*-maleimido-methyl) cyclohexane-1-carboxylate (SMCC). Excess SMCC is offered to SA in a 3:1 molar ratio in sodium borate at pH 8 containing 5% DMSO. After 30 min, the SMCC-SA is desalted by Sephadex G-25 (Amersham Pharmacia) gel filtration. The Gal-Fab is reduced with DTT, desalted by gel filtration and mixed in 1:1 molar ratio with SMCC-SA at 5 mg/ml total protein concentration in PBS. The reaction is monitored by size exclusion HPLC, and after sufficient conjugate has formed, *ca.* 50 min, the reaction is quenched by the addition of sodium tetrathionate to 5 mM to reoxidize unreacted thiols. The conjugate is separated from free SA by Q Sepharose chromatography. The conjugate is also separated from unconjugated Gal-Fab by affinity chromatography using immobilized iminobiotin (Pierce) equilibrated in 0.05 M sodium carbonate, 0.5 M sodium chloride, pH 11 and eluting with 0.05 M sodium acetate/0.5 M sodium chloride, pH 4. About 80% of the conjugate consists of SA/antibody in a 1:1 ratio, with the remainder primarily in a 2:1 ratio or higher (Axworthy et al., Proc. Natl. Acad. Sci. U.S.A. 97:1802–1807, 2000).

HSA (human serum albumin) is a single polypeptide chain protein containing 584 amino acids (66 kDa), and is the most abundant protein in the blood, accounting for about 60% of protein in the plasma. HSA is exclusively produced in liver, and unlike most other plasma proteins, it contains no carbohydrates. HSA has a half-life of about 19 days. The main functions of HSA include maintenance of colloidal osmotic pressure in the blood vessels, contribution to maintenance of acid/base metabolism, and transport of intrinsic substances and medications. Highly purified, virus free, recombinant human albumin in pharmaceutical excipient quality can be obtained commercially (e.g., GTC Biotherapeutics).

To prepare biotin-HSA-Gal, HSA is combined with 3-fold excess *N*-hydroxysuccinimide-LC-biotin (Pierce) in 0.5 M sodium borate (pH 8.5), 5% DMSO. After *ca.* 4 hr the solution is added to a 200-fold excess of freshly prepared neat 2-imino-2-methoxyethyl 1-thio-β-D-galactopyranoside. The mixture is stirred for 8 hr at room temperature and purified by diafiltration into PBS. The final material contains 1.6 moles of biotin per mole of HSA as determined by displacement of 2-(4′-hydoxyphenylazo)-benzoic acid (HABA) from SA and 30–40 moles of thiogalactose per mole of HSA as determined by a colorimetric anthrone assay (Axworthy et al., Proc. Natl. Acad. Sci. U.S.A. 97:1802–1807, 2000).

To conjugate bifunctional chelating agent (BFCA) DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) onto biotin-HSA-galactose, the COOH group of DOTA is activated by a carbodiimide reagent. Briefly, DOTA is dissolved in anhydrous DMSO at 80°C, and the solution is cooled under argon. A solution of *N*-hydroxy-2,5-pyrrolidinedione in DMSO is added dropwise to a stirred solution of DOTA, followed by the dropwise addition of *N,N'*-dicyclohexylcarbodiimide in DMSO. The molar ratio betweenDOTA:*N*-hydroxy-2,5-pyrrolidinedione:*N,N'*-dicyclohexylcarbodiimide is 1:1.4:0.8. The reaction mixture is stirred overnight and then filtered to

separate a by-product, dicyclohexylurea. The conjugation between DOTA and biotin-HSA-Gal is carried out at a molar ratio of 50:1 by adding an adequate volume of the DOTA-activated ester solution to the biotin-HSA-Gal dissolved in 0.1 M phosphate buffer (pH 8.0). After the overnight reaction, the conjugate is purified by a reverse-phase column in a FPLC system coupled with a UV detector and a radiodetector. A linear gradient method is applied using an aqueous 0.1% trifluoroacetic acid solution (solvent A) and methanol (solvent B). The eluents are delivered at a flow of 4 ml/min, starting from 0% of solvent A to 100% of solvent B in 37 ml. Two peaks corresponding to the Biotin-HSA-Gal conjugates with DOTA are observed in the UV profile. The retention volume for biotin-HSA-gal conjugates with DOTA differ from that of unconjugated biotin-HSA-gal. An integrated fraction collector is used to recover each compound and further analysis by MALDI-TOF (matrix-assisted laser desorption/ionization combined with time-of-flight) mass spectrometry (Bussolati et al., Canc. Res. 61:4393-4397, 2001) can be performed if desired.

To conjugate with the radiolabel, indium-111-chloride ($^{111}InCl_3$) with no carrier added is obtained from, for example, Mallinckrodt, Inc. (St. Louis, MO). DOTA-biotin-HSA-galactose conjugate is dissolved in 0.2 M ammonium acetate buffer (metal free; pH 7), and then incubated for 30 min at 100 C with $^{111}InCl_3$ at a ratio of about 20 MBq ^{111}In/1 nmol conjugate. The purity of radiolabeled conjugate is assessed by instant thin layer chromatography (ITLC). The ITLC system uses an ITLC-SG (silica gel) support (Gelman Sciences, Ann Arbor, MI) and 4 mM EDTA (pH 4.0) as a mobile phase. The peptide-bound activity remains at the origin, and the previously uncomplexed radiometal moves at the solvent front as an EDTA complex. The radiolabeling efficiency is typically greater than 97% and is critically dependent on the chemical purity (metal cations) of the $^{111}InCl_3$ (Smith-Jones et al., Endocrinology 140:5136-5148, 1999).

Galactose-Ficoll can be readily synthesized as follows. Cyanomethyl 1-thio-β-D-galactopyranoside is dissolved in methanol at an approximate ratio of 1.0 mmol per 10 ml, with the addition of 0.5 ml of 0.5 M sodium methoxide in methanol. After 48 hours of stirring at room temperature in a tightly capped glass vial, the methanol will be evaporated with a stream of nitrogen. To the dried residue is added 1 gram of Ficoll 70 or Ficoll 400 from Pharmacia or other manufacturers in 100 ml BBS (0.16 M sodium chloride-0.2 M sodium borate, pH 8.0). This is stirred at room temperature for 24 hours, then the reaction is stopped by adding 10 ml of 1.0 N acetic acid. The reaction mixture will be dialyzed exhaustively against distilled water (Plotz and Rifai, *Biochemistry* 1982, 21, 301-308; Lee at el. *Biochemistry* 1976, 15, 3956-3963).

Administration of the compounds of the invention may be intravenous. In one example, 2.0 mCi of ^{111}In is injected into patients intravenously over a period of 30-60 seconds. Images are taken 24-48 hours post injection as described below; however, the time between administration and imaging may be altered as desired or based on clearance and retention characteristics of the particular compounds used in the diagnostic method.

Any of the several SPECT scanners commercially available may be used in this method (e.g., the GE MyoSIGHT, General Electric Company). One skilled in the art will know what settings (e.g., collimators, window size, and energies) can be used with specific radiometals or isotopes. In one example, a medium energy collimator is used for ^{111}In, with symmetric windows of 15-20% set at the 173 and 247 keV (the photopeaks of ^{111}In). In another example, a low energy collimator is used for ^{99m}Tc, with symmetric windows of 20% set at 140 keV. The number of images to be taken depends on the capabilities of the scanning system, and the desired image quality. A 64 x 64 matrix set of images may be taken, usually with the camera moving 360 degrees with 64 stops. If higher image quality is desired, a 128 x 128 or 256 x 256 matrix set of images may be taken. The scan may take 30-60 minutes during which time the patient

must remain absolutely still. Additional patient preparation (e.g., oral hydration, cathartics, and enemas), if desired, may be performed to obtain optimal images.

The images can be used to determine the amount of radioactivity in the kidney of the patient. By comparing this amount of radioactivity to the radioactivity in the kidney of a patient known not to have IgA nephropathy or HSP, an increase in radioactivity detected in the kidney is diagnostic of IgA nephropathy or HSP.

Similarly, in the case where anti-human IgM is used in place of anti-human IgA, an increase in the amount of radioactivity determined from the images relative to the radioactivity in the kidney of a patient known not to have IgM nephropathy is diagnostic of IgM nephropathy.

Other Embodiments

All publications, patent applications including U.S. provisional application No. 60/705,282, filed August 3, 2005, and patents mentioned in this specification are herein incorporated by reference.

Various modifications and variations of the described method and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific desired embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments

What is claimed is:

CLAIMS

1. A method for diagnosing an IgA or IgM kidney disease in a mammal, said method comprising:

(a) administering to said mammal a compound which specifically binds IgA or IgM; and

(b) detecting said compound in the kidney of said mammal,

wherein an increase in the amount of said compound in said kidney of said mammal relative to the amount in the kidney of a mammal without said kidney disease is diagnostic of said IgA or IgM kidney disease in said mammal.

2. The method of claim 1, wherein said mammal is a human.

3. The method of claim 1, wherein said administration is intravenous.

4. The method of claim 1, wherein said compound comprises a peptide.

5. The method of claim 4, wherein said peptide is selected from the group consisting of human FcαR1 (SEQ ID NO:2), human Fcα/μ receptor (SEQ ID NO:6), polymeric Ig receptor (SEQ ID NO:7), Sir22 (SEQ ID NO:3), streptococcal IgA-binding peptide (Sap; SEQ ID NO:4), a modified Z-domain protein (SEQ ID NO:12), and YDWIPSSAW (SEQ ID NO:9), or an IgA- or IgM-binding fragment thereof.

6. The method of claim 4, wherein said peptide comprises a modification selected from the group consisting of an N-terminal cap, a C-terminal cap, a D-amino acid, an amino acid surrogate, a peptidomimetic sequence, and a spacer.

7. The method of claim 1, wherein said compound comprises an antibody, or an IgA- or IgM-binding fragment thereof.

8. The method of claim 7, wherein said antibody is anti-human IgA, or an IgA-binding fragment thereof.

9. The method of claim 7, wherein said antibody is anti-human IgM, or an IgM-binding fragment thereof.

10. The method of claim 1, wherein said compound comprises a qdot.

11. The method of claim 1, wherein said compound is linked to a radioactive label.

12. The method of claim 11, wherein said label is selected from the group consisting of ^{99m}Tc, ^{111}In, ^{66}Ga, ^{67}Ga, ^{68}Ga, ^{86}Y, ^{90}Y, ^{201}Tl, ^{55}Co, ^{60}Cu, ^{61}Cu, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{82}Rb, $^{185/187}Re$, and $^{186/188}Re$.

13. The method of claim 11, wherein said compound is linked to said radioactive label by a bifunctional chelating agent.

14. The method of claim 13, wherein said bifunctional chelating agent is selected from the group consisting of N_3S, N_2S_2, PnAO, HYNIC, $[M(CO)_3(H_2O)_3]^+$, PADA, DTPA, DOTA, histidine, a tripeptide, and a tetrapeptide.

15. The method of claim 14, wherein said tripeptide is Lys-Gly-Cys, Cys-Gly-Cys, or Gly-Gly-Cys.

16. The method of claim 14, wherein said tetrapeptide is Gly-Ala-Gly-Gly or Cys-Gly-Cys-Gly.

17. The method of claim 1, wherein said compound is linked to a paramagnetic substance.

18. The method of claim 17, wherein said paramagnetic substance is gadolinium.

19. The method of claim 1, wherein said detection is carried out by an imaging technique.

20. The method of claim 19, wherein said imaging technique is selected from the group consisting of SPECT, PET, planar scan, and MRI.

21. The method of claim 1, wherein said compound further comprises a galactose and a first member of a binding pair.

22. The method of claim 21, wherein said first member of a binding pair is streptavidin.

23. The method of claim 21, wherein said administration further comprises administration of galactose-Ficoll.

24. The method of claim 21, wherein said detection is performed by administering to said mammal a radioactively labeled compound conjugated to both (a) a second member of a binding pair and (b) a galactose, followed by detecting said radioactively labeled compound in said kidney of said mammal.

25. The method of claim 24, wherein said radioactively labeled compound is labeled with an isotope selected from the group consisting of ^{99m}Tc, ^{111}In, ^{66}Ga, ^{67}Ga, ^{68}Ga, ^{86}Y, ^{90}Y, ^{201}Tl, ^{55}Co, ^{60}Cu, ^{61}Cu, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{82}Rb, $^{185/187}$Re, and $^{186/188}$Re.

26. The method of claim 24, wherein said radiolabeled compound is human serum albumin.

27. The method of claim 24, wherein said second member of a binding pair is biotin.

28. A composition comprising:

(a) an IgA- or IgM-binding compound;

(b) a bifunctional chelating agent; and

(c) a detectable label in a pharmaceutically acceptable carrier,

wherein said IgA- or IgM-binding compound is linked to said detectable label through said bifunctional chelating agent.

29. The composition of claim 28, wherein said compound comprises a peptide.

30. The composition of claim 29, wherein said peptide is selected from the group consisting of human FcαR1 (SEQ ID NO:2), human Fcα/μ receptor (SEQ ID NO:6), polymeric Ig receptor (SEQ ID NO:7), Sir22 (SEQ ID NO:3), streptococcal IgA-binding peptide (Sap; SEQ ID NO:4), a modified Z-domain protein (SEQ ID NO:12), and YDWIPSSAW (SEQ ID NO:9), or an IgA- or IgM-binding fragment thereof.

31. The composition of claim 29, wherein said peptide comprises a modification selected from the group consisting of an N-terminal cap, a C-terminal cap, a D-amino acid, an amino acid surrogate, a peptidomimetic sequence, and a spacer.

32. The composition of claim 28, wherein said compound comprises an antibody, or an IgA- or IgM- binding fragment thereof.

33. The composition of claim 32, wherein said antibody is anti-human IgA, or an IgA-binding fragment thereof.

34. The composition of claim 32, wherein said antibody is anti-human IgM, or an IgM-binding fragment thereof

35. The composition of claim 28, wherein said bifunctional chelating agent is selected from the group consisting of N_3S, N_2S_2, PnAO, HYNIC, $[M(CO)_3(H_2O)_3]^+$, PADA, DTPA, DOTA, histidine, a tripeptide, and a tetrapeptide.

36. The composition of claim 35, wherein said tripeptide is Lys-Gly-Cys, Cys-Gly-Cys, or Gly-Gly-Cys.

37. The composition of claim 35, wherein said tetrapeptide is Gly-Ala-Gly-Gly or Cys-Gly-Cys-Gly.

38. The composition of claim 28, wherein said detectable label is a radioactive label.

39. The composition of claim 38, wherein said radioactive label is selected from the group consisting of ^{99m}Tc, ^{111}In, ^{66}Ga, ^{67}Ga, ^{68}Ga, ^{86}Y, ^{90}Y, ^{201}Tl, ^{55}Co, ^{60}Cu, ^{61}Cu, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{82}Rb, $^{185/187}$Re, and $^{186/188}$Re.

40. A kit comprising:

(a) an IgA- or IgM-binding compound;

(b) a bifunctional chelating agent; and

(c) a detectable label in a pharmaceutically acceptable carrier,

(d) instructions for use for detecting an IgA or IgM kidney disease in a mammal.

41. The kit of claim 40, wherein said compound further comprises a galactose and a first member of a binding pair; and said detectable label comprises a radioactively labeled peptide, said radioactively label peptide comprising a galactose and a second member of a binding pair.

42. The kit of claim 41, wherein said radiolabel is selected from the group consisting of ^{99m}Tc, ^{111}In, ^{66}Ga, ^{67}Ga, ^{68}Ga, ^{86}Y, ^{90}Y, ^{201}Tl, ^{55}Co, ^{60}Cu, ^{61}Cu, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{82}Rb, $^{185/187}$Re, and $^{186/188}$Re.

43. The kit of claim 41, wherein said first member of a binding pair is streptavidin, and said second member of a binding pair is biotin.

44. The kit of claim 41, wherein said radioactively labeled peptide is human serum albumin.

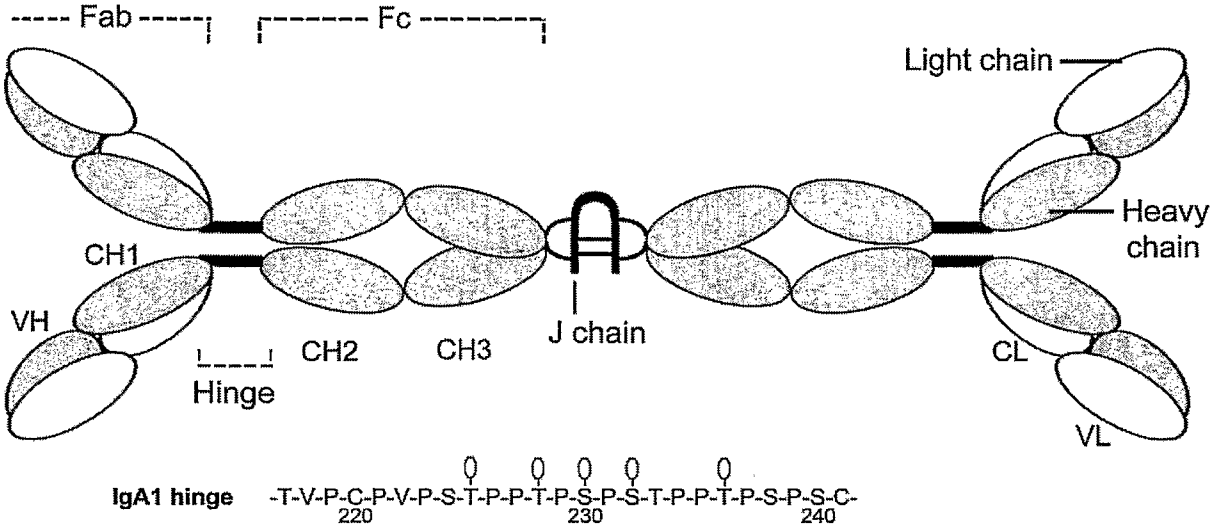

Fab
Fc
Light chain
Heavy chain
CH1
VH
CH2
CH3
J chain
CL
VL
Hinge
IgA1 hinge
-T-V-P-C-P-V-P-S-T-P-P-T-P-S-P-S-T-P-P-T-P-S-P-S-C-
220
230
240

Figure 1

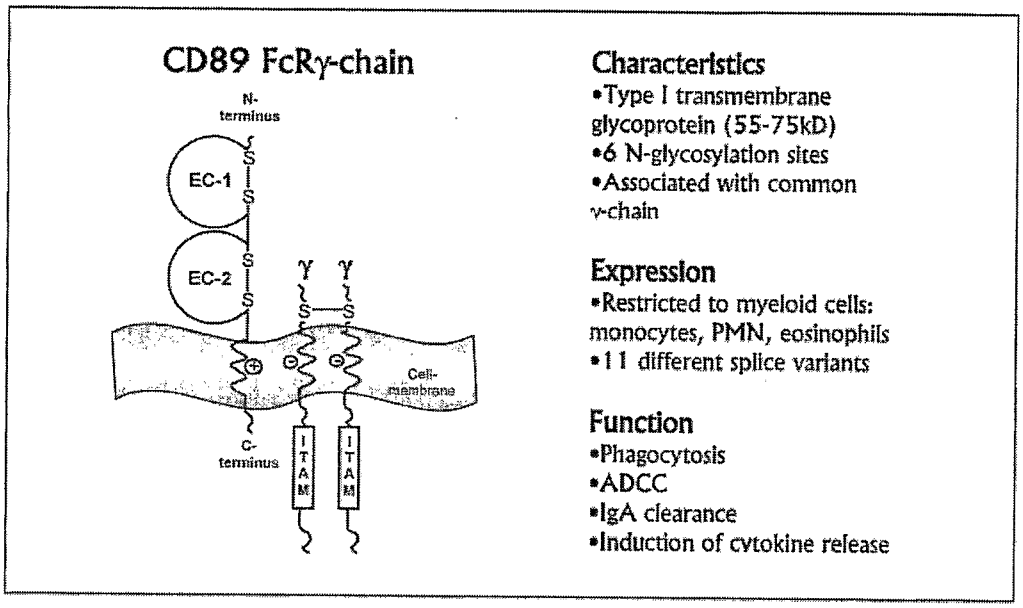

CD89 FcRγ-chain
N-terminus
EC-1
EC-2
γ γ
S
Cell-membrane
C-terminus
ITAM
ITAM
Characteristics
•Type I transmembrane glycoprotein (55-75kD)
•6 N-glycosylation sites
•Associated with common γ-chain
Expression
•Restricted to myeloid cells: monocytes, PMN, eosinophils
•11 different splice variants
Function
•Phagocytosis
•ADCC
•IgA clearance
•Induction of cytokine release

Figure 2

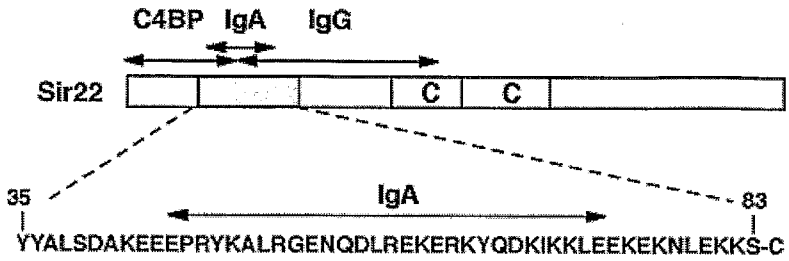

C4BP
IgA
IgG
Sir22
C
C
35
IgA
83
YYALSDAKEEEPRYKALRGENQDLREKERKYQDKIKKLEEKEKNLEKKS-C

Figure 3

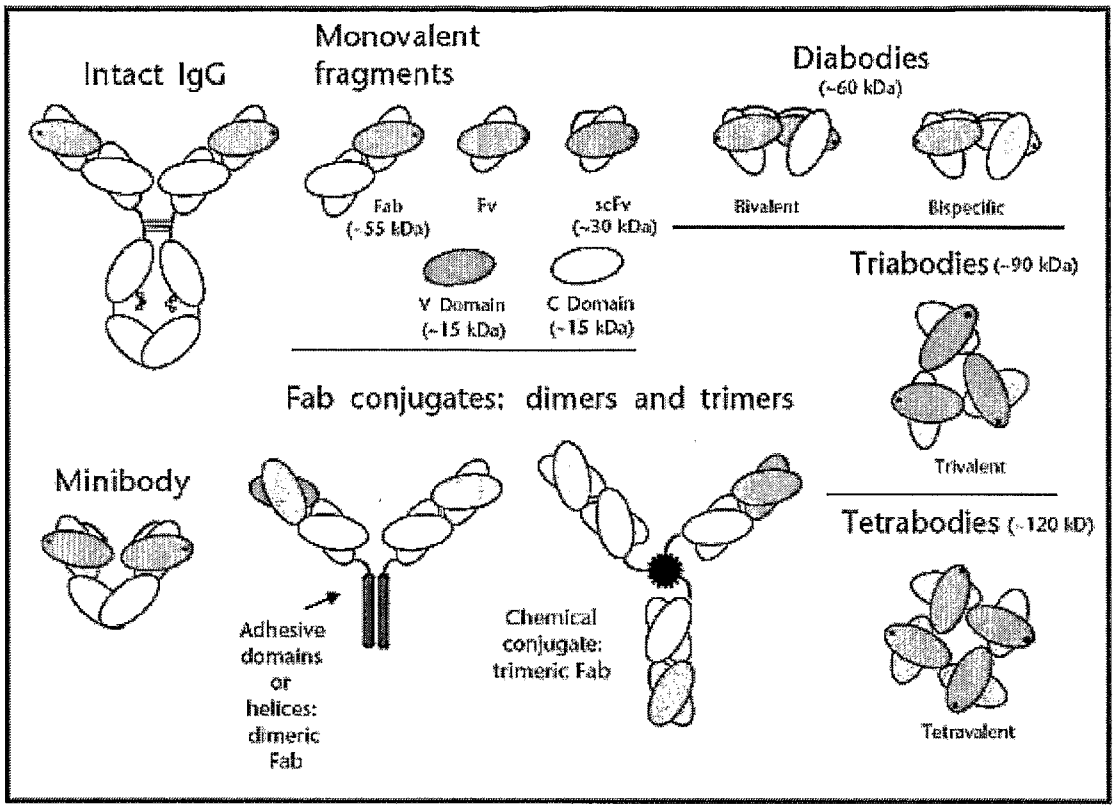
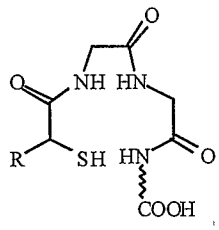
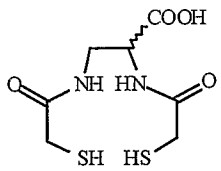

Intact IgG
Monovalent fragments
Fab (~55 kDa)
Fv
scFv (~30 kDa)
V Domain (~15 kDa)
C Domain (~15 kDa)
Diabodies (~60 kDa)
Bivalent
Bispecific
Triabodies (~90 kDa)
Trivalent
Tetrabodies (~120 kD)
Tetravalent
Fab conjugates: dimers and trimers
Minibody
Adhesive domains or helices: dimeric Fab
Chemical conjugate: trimeric Fab

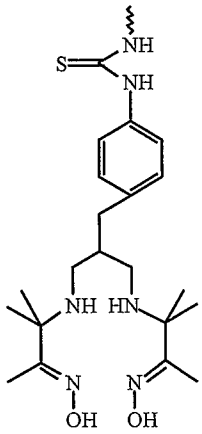
Figure 4



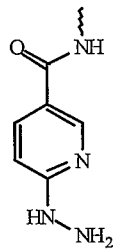
N_3S



N_2S_2



PnAO



HYNIC

Figure 5

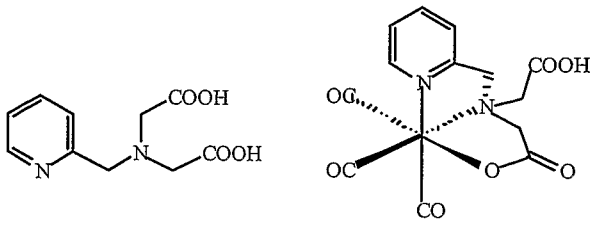
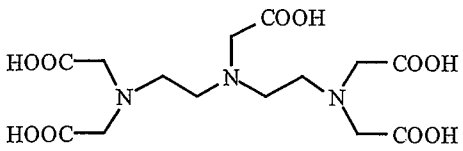
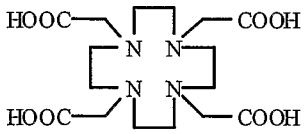


Figure 6



DTPA



DOTA

Figure 7

IgA1 C-region
SEQ ID NO:1

```
1   ASPTSPKVFP LSLCSTQPDG NVVIACLVQG FFPQEPLSVT WSESGQGVTA RNFPPSQDAS
61  GDLYTTSSQL TLPATQCLAG KSVTCHVKHY TNPSQDVTVP CPVPSTPPTP SPSTPPTPSP
121 SCCHPRLSLH RPALEDLLLG SEANLTCTLT GLRDASGVTF TWTPSSGKSA VQGPPERDLC
181 GCYSVSSVLP GCAEPWNHGK TFTCTAAYPE SKTPLTATLS KSGNTFRPEV HLLPPPSEEL
241 ALNELVTLTC LARGFSPKDV LVRWLQGSQE LPREKYLTWA SRQEPSQGTT TFAVTSILRV
301 AAEDWKKGDT FSCMVGHEAL PLAFTQKTID RLAGKPTHVN VSVVMAEVDG TCY
```

human FcαR1 CD89
SEQ ID NO:2

```
  1 dfpmpfisak sspvipldgs vkiqcqaire ayltqlmiik nstyreigrr lkfwnetdpe
 61 fvidhmdank agryqcqyri ghyrfrysdt lelvvtglyg kpflsadrgl vlmpgenisl
121 tcssahipfd rfslakegex slpqhqsgeh panfslgpvd lnvsgiyrcy gwynrspylw
181 sfpsnalelv vt
```

Sir22
SEQ ID NO:3

```
  1 MARKDTNKQY SLRKLKTGTA SVAVAVAVLG AGFANQTTVK AESSNNAESSN ISQESKLIN
 61 TLTDENEKLR EELQQYYALS DAKEEEPRYK ALRGENQDLR EKERKYQDKIK KLEEKEKNL
121 EKKSEDVERH YLKKLDQEHK EQQERQKNLE ELERQSQREI DKRYQEQLQKQ QQLETEKQI
181 SEASRKSLSR DLEASRAAKK KVEADLAALN AEHQKLKEEK QISDASRQGLS RDLEASREA
241 KKKVEADLAE ANSKLQALEK LNKELEEGKK LSEKEKAELQ ARLEAEAKALK EQLAKQAEE
301 LAKLKGNQTP NAKVAPQANR SRSAMTQQKR TLPSTGEAAN PFFTAAAATVM VSAGMLALK
361 RKEEN
```

Sap
SEQ ID NO:4

```
  1 YYALSDAKEEEPRYKALRGENQDLREKERKYQDKIKKLEEKEKNLEKKSC
```

soluble CD89
SEQ ID NO:5

```
  1 mdpkqttllc lvlclgqriq aqegdfpmpf isaksspvip ldgsvkiqcq aireayltql
 61 miiknstyre igrrlkfwne tdpefvidhm dankagryqc qyrighyrfr ysdtlelvvt
121 glygkpflsa drglvlmpge nisltcssah ipfdrfslak egelslpqhq sgehpanfsl
181 gpvdlnvsgi yrcygwynrs pylwsfpsna lelvvtdsih qdyttqnlir mavaglvlva
241 llailvenwh shtalnkeas advaepswsq qmcqpgltfa rtpsvck
```

Figure 8

Human Fcα/μR
SEQ ID NO:6

```
  1 mdgeatvkpg eqvplwthgw ppddpspsfa agssfalpqk rphprwlweg slpsrthlra
 61 mgtlrpsspl cwreessfaa pnslkgsrlv sgepggavti qchyapssvn rhqrkywcrl
121 gpprwicqti vstnqythhr yrdrvaltdf pqrglfvvrl sqlspddigc ylcgigsenn
181 mlflsmnlti sagpastlpt atpaageltm rsygtaspva nrwtpgttqt lgqgtawdtv
241 astpgtsktt asaegrrtpg atrpaapgtg swaegsvkap apipesppsk srsmsntteg
301 vwegtrssvt nraraskdrr emtttkadrp rediegvria ldaakkvlgt igppalvset
361 laweilpqat pvskqqsqgs igettpaagm wtlgtpaadv witsmeaasg egsaagdlda
421 atgdrgpqat lsqtpavgpw gppgkessvk ratcpglstl keikvsavlt qnphsllcvs
481 aaqeaervtl iqmthflevn pqadqlphve rkmlqddslp agasltaper npgp
```

pIgR
SEQ ID NO:7

```
  1 mllfvltcll avfpaistks pifgpeevns vegnsvsitc yypptsvnrh trkywcrqga
 61 rggcitliss egyvsskyag ranltnfpen gtfvvniaql sqddsgrykc glginsrgls
121 fdvslevsqg pgllndtkvy tvdlgrtvti ncpfktenaq krkslykqig lypvlvidss
181 gyvnpnytgr irldiqgtgq llfsvvinql rlsdagqylc qagddsnsnk knadlqvlkp
241 epelvyedlr gsvtfhcalg pevanvakfl crqssgencd vvvntlgkra pafegrilln
301 pqdkdgsfsv vitglrkeda grylcgahsd gqlqegspiq awqlfvnees tiprsptvvk
361 gvaggsvavl cpynrkesks ikywclwega qngrcpllvd segwvkaqye grlslleepg
421 ngtftvilnq ltsrdagfyw cltngdtlwr ttveikiieg epnlkvpgnv tavlgetlkv
481 pchfpckfss yekywckwnn tgcqalpsqd egpskafvnc densrlvslt lnlvtradeg
541 wywcgvkqgh fygetaavyv aveerkaags rdvslakada apdekvldsg freienkaiq
601 dprlfaeeka vadtrdqadg srasvdsgss eeqggssral vstlvplglv lavgavavgv
661 ararhrknvd rvsirsyrtd ismsdfensr efgandnmga ssitqetslg gkeefvatte
721 sttetkepkk akrsskeeae maykdfllqs stvaaeaqdg pqea
```

CD71
SEQ ID NO:8

```
MMDQARSAFS NLFGGEPLSY TRFSLARQVD GDNSHVEMKL AVDEEENADN  50
NTKANVTKPK RCSGSICYGT IAVIVFFLIG FMIGYLGYCK GVEPKTECER 100
LAGTESPVRE EPGEDFPAAR RLYWDDLKRK LSEKLDSTDF TSTIKLLNEN 150
SYVPREAGSQ KDENLALYVE NQFREFKLSK VWRDQHFVKI QVKDSAQNSV 200
IIVDKNGRLV YLVENPGGYV AYSKAATVTG KLVHANFGTK KDFEDLYTPV 250
NGSIVIVRAG KITFAEKVAN AESLNAIGVL IYMDQTKFPI VNAELSFFGH 300
AHLGTGDPYT PGFPSFNHTQ FPPSRSSGLP NIPVQTISRA AAEKLFGNME 350
GDCPSDWKTD STCRMVTSES KNVKLTVSNV LKEIKILNIF GVIKGFVEPD 400
HYVVVGAQRD AWGPGAAKSG VGTALLLKLA QMFSDMVLKD GFQPSRSIIF 450
ASWSAGDFGS VGATEWLEGY LSSLHLKAFT YINLDKAVLG TSNFKVSASP 500
LLYTLIEKTM QNVKHPVTGQ FLYQDSNWAS KVEKLTLDNA AFPFLAYSGI 550
PAVSFCFCED TDYPYLGTTM DTYKELIERI PELNKVARAA AEVAGQFVIK 600
LTHDVELNLD YERYNSQLLS FVRDLNQYRA DIKEMGLSLQ WLYSARGDFF 650
RATSRLTTDF GNAEKTDRFV MKKLNDRVMR VEYHFLSPYV SPKESPFRHV 700
FWGSGSHTLP ALLENLKLRK QNNGAFNETL FRNQLALATW TIQGAANALS 750
GDVWDIDNEF                                           760
```

Figure 8

IgM binding peptide
SEQ ID NO:9

YDWIPSSAW

SEQ ID NO:10
Staph Protein A

```
  1 meqritlkea wdqrngfiqs lkddpsqsan vlgeaqklnd sqapkadaqq nnfnkdqqsa
 61 fyeilnmpnl neaqrngfiq slkddpsqst nvlgeakkln esqapkadnn fnkeqqnafy
121 eilnmpnlne eqrngfiqsl kddpsqsanl lseakklnes qapkadnkfn keqqnafyei
181 lhlpnlneeq rngfiqslkd dpsqsanlla eakklndaqa pkadnkfnke qqnafyeilh
241 lpnlteeqrn gfiqslkddp gnsrgsvdlq itn
```

Z-domain
SEQ ID NO:11

```
  1 vdnkfnkeqq nafyeilhlp nlneeqrnaf iqslkddpsq sanllaeakk lndaqapk
```

Modified Z-domain
SEQ ID NO:12

```
  1 vdnkfnketi qasqeirllp nlngrqklaf ihsllddpsq sanllaeakk lndaqapk
```

B-domain
SEQ ID NO:13

```
  1 adnkfnkeqq nafyeilhlp nlneeqrngf iqslkddpsq sanllaeakk lndaqapk
```

IgA1 hinge region
SEQ ID NO:14

TVPCPVPSTPPTPSPSTPPTPSPSC

Figure 8

IgM mu chain amino acid sequence
SEQ ID NO:15

```
  1 mdwtwrflfv vaaatgvqsq vqlvqsgaev kkpgssvkvs ckasggtfss yaiswvrqap
 61 gqglewmggi ipifgtanya qkfqgrvtit adeststaym elsslrsedt avyycaktgi
121 lgpyssgwyp nsdyyyygmd vwgqgttvtv ssgsasaptl fplvscensp sdtssvavgc
181 laqdflpdsi tfswkyknns disstrgfps vlrggkyaat sqvllpskdv mqgtdehvvc
241 kvqhpngnke knvplpviae lppkvsvfvp prdgffgnpr sksklicqat gfsprqiqvs
301 wlregkqvgs gvttdqvqae akesgpttyk vtstltikes dwlsqsmftc rvdhrgltfq
361 qnassmcvpd qdtairvfai ppsfasiflt kstkltclvt dlttydsvti swtrqngeav
421 kthtnisesh pnatfsavge asiceddwns gerftctvth tdlpsplkqt isrpkgvalh
481 rpdvyllppa reqlnlresa titclvtgfs padvfvqwmq rgqplspeky vtsapmpepq
541 apgryfahsi ltvseeewnt getytcvvah ealpnrvter tvdkstegev sadeegfenl
601 watastfivl fllslfystt vtlfkvk
```

IgM mu chain nucleic acid sequence
SEQ ID NO:16

```
   1 gctctagaac tagtggatcc cccgggctgc aggaattctc taaagaagcc
cctgggagca
  61 cagctcatca ccatggactg gacctggagg ttcctctttg tggtggcagc
agctacaggt
 121 gtccagtccc aggtgcagct ggtgcagtct ggggctgagg tgaagaagcc
tgggtcctcg
 181 gtgaaggtct cctgcaaggc ttctggaggc accttcagca gctatgctat
cagctgggtg
 241 cgacaggccc ctggacaagg gcttgagtgg atgggaggga tcatccctat
ctttggtaca
 301 gcaaactacg cacagaagtt ccagggcaga gtcacgatta ccgcggacga
atccacgagc
 361 acagcctaca tggagctgag cagcctgaga tctgaggaca cggccgtgta
ttactgtgcg
 421 aaaaccggga tcctggggcc gtatagcagt ggctggtacc cgaactcgga
ctactactac
 481 tacggtatgg acgtctgggg ccaagggacc acggtcaccg tctcctcagg
gagtgcatcc
 541 gccccaaccc ttttcccct cgtctcctgt gagaattccc cgtcggatac
gagcagcgtg
 601 gccgttggct gcctcgcaca ggacttcctt cccgactcca tcactttctc
ctggaaatac
 661 aagaacaact ctgacatcag cagcacccgg ggcttcccat cagtcctgag
aggggcaag
 721 tacgcagcca cctcacaggt gctgctgcct tccaaggacg tcatgcaggg
cacagacgaa
 781 cacgtggtgt gcaaagtcca gcaccccaac ggcaacaaag aaaagaacgt
gcctcttcca
 841 gtgattgctg agctgcctcc caaagtgagc gtcttcgtcc caccccgcga
cggcttcttc
 901 ggcaaccccc gcagcaagtc caagctcatc tgccaggcca cgggtttcag
tccccggcag
 961 attcaggtgt cctggctgcg cgaggggaag caggtggggt ctggcgtcac
cacggaccag
1021 gtgcaggctg aggccaaaga gtctgggccc acgacctaca aggtgaccag
cacactgacc
1081 atcaaagaga gcgactggct cagccagagc atgttcacct gccgcgtgga
tcacaggggc
1141 ctgaccttcc agcagaatgc gtcctccatg tgtgtccccg atcaagacac
agccatccgg
```

Figure 8

1201 gtcttcgcca tccccccatc ctttgccagc atcttcctca ccaagtccac
caagttgacc
1261 tgcctggtca cagacctgac cacctatgac agcgtgacca tctcctggac
ccgccagaat
1321 ggcgaagctg tgaaaaccca caccaacatc tccgagagcc accccaatgc
cactttcagc
1381 gccgtgggtg aggccagcat ctgcgaggat gactggaatt ccggggagag
gttcacgtgc
1441 accgtgaccc acacagacct gccctcgcca ctgaagcaga ccatctcccg
gcccaagggg
1501 gtggccctgc acaggcccga tgtctacttg ctgccaccag cccgggagca
gctgaacctg
1561 cgggagtcgg ccaccatcac gtgcctggtg acgggcttct ctcccgcgga
cgtcttcgtg
1621 cagtggatgc agaggggggca gcccttgtcc ccggagaagt atgtgaccag
cgccccaatg
1681 cctgagcccc aggccccagg ccggtacttc gcccacagca tcctgaccgt
gtccgaagag
1741 gaatggaaca cgggggagac ctacacctgc gtggtggccc atgaggccct
gcccaacagg
1801 gtcaccgaga ggaccgtgga caagtccacc gaggggggagg tgagcgccga
cgaggagggc
1861 tttgagaacc tgtgggccac cgcctccacc ttcatcgtcc tcttcctcct
gagcctcttc
1921 tacagtacca ccgtcacctt gttcaaggtg aaatgatccc aacagaagaa
catcggagac
1981 cagagagagg aactcaaagg ggcgctgcct ccgggtctgg ggtcctggcc
tgcgtggcct
2041 gttggcacgt gtttctcttc ccgcccggcc tccagttgtg tgctctcaca
caggcttcct
2101 tctcgaccgg caggggctgg ctggcttgca ggccacgagg tgggctctac
cccacactgc
2161 tttgctgtgt atacgcttgt tgccctgaaa taaatatgca cattttatcc atg

IgM hinge sequence
SEQ ID NO:17

PLPVIAELPPKVSVFVPPRDGFFGNP

Figure 8

SEQUENCE LISTING

<110> RQ Bioscience, Inc.
New England Medical Center Hospitals, Inc.

<120> Methods and Compositions For Diagnosis of IgA- and IgM-Mediated Kidney Diseases

<130> 50400/002WO2

<150> US 60/705,282
<151> 2005-08-03

<160> 17

<170> PatentIn version 3.3

<210> 1
<211> 353
<212> PRT
<213> Homo sapiens

<400> 1

Ala Ser Pro Thr Ser Pro Lys Val Phe Pro Leu Ser Leu Cys Ser Thr
1 5 10 15

Gln Pro Asp Gly Asn Val Val Ile Ala Cys Leu Val Gln Gly Phe Phe
20 25 30

Pro Gln Glu Pro Leu Ser Val Thr Trp Ser Glu Ser Gly Gln Gly Val
35 40 45

Thr Ala Arg Asn Phe Pro Pro Ser Gln Asp Ala Ser Gly Asp Leu Tyr
50 55 60

Thr Thr Ser Ser Gln Leu Thr Leu Pro Ala Thr Gln Cys Leu Ala Gly
65 70 75 80

Lys Ser Val Thr Cys His Val Lys His Tyr Thr Asn Pro Ser Gln Asp
85 90 95

Val Thr Val Pro Cys Pro Val Pro Ser Thr Pro Pro Thr Pro Ser Pro
100 105 110

Ser Thr Pro Pro Thr Pro Ser Pro Ser Cys Cys His Pro Arg Leu Ser
115 120 125

Leu His Arg Pro Ala Leu Glu Asp Leu Leu Leu Gly Ser Glu Ala Asn
130 135 140

Leu Thr Cys Thr Leu Thr Gly Leu Arg Asp Ala Ser Gly Val Thr Phe
145 150 155 160

Thr Trp Thr Pro Ser Ser Gly Lys Ser Ala Val Gln Gly Pro Pro Glu
165 170 175

Arg Asp Leu Cys Gly Cys Tyr Ser Val Ser Ser Val Leu Pro Gly Cys
180 185 190

Ala Glu Pro Trp Asn His Gly Lys Thr Phe Thr Cys Thr Ala Ala Tyr
195 200 205

Pro Glu Ser Lys Thr Pro Leu Thr Ala Thr Leu Ser Lys Ser Gly Asn
210 215 220

Thr Phe Arg Pro Glu Val His Leu Leu Pro Pro Pro Ser Glu Glu Leu
225 230 235 240

Ala Leu Asn Glu Leu Val Thr Leu Thr Cys Leu Ala Arg Gly Phe Ser
245 250 255

Pro Lys Asp Val Leu Val Arg Trp Leu Gln Gly Ser Gln Glu Leu Pro
260 265 270

Arg Glu Lys Tyr Leu Thr Trp Ala Ser Arg Gln Glu Pro Ser Gln Gly
275 280 285

Thr Thr Thr Phe Ala Val Thr Ser Ile Leu Arg Val Ala Ala Glu Asp
290 295 300

Trp Lys Lys Gly Asp Thr Phe Ser Cys Met Val Gly His Glu Ala Leu
305 310 315 320

Pro Leu Ala Phe Thr Gln Lys Thr Ile Asp Arg Leu Ala Gly Lys Pro
325 330 335

Thr His Val Asn Val Ser Val Val Met Ala Glu Val Asp Gly Thr Cys
340 345 350

Tyr

<210> 2
<211> 192
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<222> (140)..(140)
<223> Xaa can be any naturally occurring amino acid

<400> 2

Asp Phe Pro Met Pro Phe Ile Ser Ala Lys Ser Ser Pro Val Ile Pro
1 5 10 15

Leu Asp Gly Ser Val Lys Ile Gln Cys Gln Ala Ile Arg Glu Ala Tyr
20 25 30

Leu Thr Gln Leu Met Ile Ile Lys Asn Ser Thr Tyr Arg Glu Ile Gly
35 40 45

Arg Arg Leu Lys Phe Trp Asn Glu Thr Asp Pro Glu Phe Val Ile Asp
50 55 60

His Met Asp Ala Asn Lys Ala Gly Arg Tyr Gln Cys Gln Tyr Arg Ile
65 70 75 80

Gly His Tyr Arg Phe Arg Tyr Ser Asp Thr Leu Glu Leu Val Val Thr
85 90 95

Gly Leu Tyr Gly Lys Pro Phe Leu Ser Ala Asp Arg Gly Leu Val Leu
100 105 110

Met Pro Gly Glu Asn Ile Ser Leu Thr Cys Ser Ser Ala His Ile Pro
115 120 125

Phe Asp Arg Phe Ser Leu Ala Lys Glu Gly Glu Xaa Ser Leu Pro Gln
130 135 140

His Gln Ser Gly Glu His Pro Ala Asn Phe Ser Leu Gly Pro Val Asp
145 150 155 160

Leu Asn Val Ser Gly Ile Tyr Arg Cys Tyr Gly Trp Tyr Asn Arg Ser
165 170 175

Pro Tyr Leu Trp Ser Phe Pro Ser Asn Ala Leu Glu Leu Val Val Thr
180 185 190

<210> 3
<211> 365
<212> PRT
<213> Streptococcus pyogenes

<400> 3

Met Ala Arg Lys Asp Thr Asn Lys Gln Tyr Ser Leu Arg Lys Leu Lys
1 5 10 15

Thr Gly Thr Ala Ser Val Ala Val Ala Val Ala Val Leu Gly Ala Gly
20 25 30

Phe Ala Asn Gln Thr Thr Val Lys Ala Glu Ser Ser Asn Asn Ala Glu
35 40 45

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

Glu Asn Glu Lys Leu Arg Glu Glu Leu Gln Gln Tyr Tyr Ala Leu Ser
65 70 75 80

Asp Ala Lys Glu Glu Glu Pro Arg Tyr Lys Ala Leu Arg Gly Glu Asn
85 90 95

Gln Asp Leu Arg Glu Lys Glu Arg Lys Tyr Gln Asp Lys Ile Lys Lys
100 105 110

Leu Glu Glu Lys Glu Lys Asn Leu Glu Lys Lys Ser Glu Asp Val Glu
115 120 125

Arg His Tyr Leu Lys Lys Leu Asp Gln Glu His Lys Glu Gln Gln Glu
130 135 140

Arg Gln Lys Asn Leu Glu Glu Leu Glu Arg Gln Ser Gln Arg Glu Ile
145 150 155 160

```
Asp Lys Arg Tyr Gln Glu Gln Leu Gln Lys Gln Gln Gln Leu Glu Thr
                165                 170                 175

Glu Lys Gln Ile Ser Glu Ala Ser Arg Lys Ser Leu Ser Arg Asp Leu
            180                 185                 190

Glu Ala Ser Arg Ala Ala Lys Lys Lys Val Glu Ala Asp Leu Ala Ala
        195                 200                 205

Leu Asn Ala Glu His Gln Lys Leu Lys Glu Glu Lys Gln Ile Ser Asp
    210                 215                 220

Ala Ser Arg Gln Gly Leu Ser Arg Asp Leu Glu Ala Ser Arg Glu Ala
225                 230                 235                 240

Lys Lys Lys Val Glu Ala Asp Leu Ala Glu Ala Asn Ser Lys Leu Gln
                245                 250                 255

Ala Leu Glu Lys Leu Asn Lys Glu Leu Glu Glu Gly Lys Lys Leu Ser
            260                 265                 270

Glu Lys Glu Lys Ala Glu Leu Gln Ala Arg Leu Glu Ala Glu Ala Lys
        275                 280                 285

Ala Leu Lys Glu Gln Leu Ala Lys Gln Ala Glu Glu Leu Ala Lys Leu
    290                 295                 300

Lys Gly Asn Gln Thr Pro Asn Ala Lys Val Ala Pro Gln Ala Asn Arg
305                 310                 315                 320

Ser Arg Ser Ala Met Thr Gln Gln Lys Arg Thr Leu Pro Ser Thr Gly
                325                 330                 335

Glu Ala Ala Asn Pro Phe Phe Thr Ala Ala Ala Ala Thr Val Met Val
            340                 345                 350

Ser Ala Gly Met Leu Ala Leu Lys Arg Lys Glu Glu Asn
        355                 360                 365
```

<210> 4
<211> 50
<212> PRT
<213> Artificial sequence

<220>
<223> Sap

<400> 4

Tyr Tyr Ala Leu Ser Asp Ala Lys Glu Glu Glu Pro Arg Tyr Lys Ala
1 5 10 15

Leu Arg Gly Glu Asn Gln Asp Leu Arg Glu Lys Glu Arg Lys Tyr Gln
20 25 30

Asp Lys Ile Lys Lys Leu Glu Glu Lys Glu Lys Asn Leu Glu Lys Lys
35 40 45

Ser Cys
50

<210> 5
<211> 287
<212> PRT
<213> Homo sapiens

<400> 5

Met Asp Pro Lys Gln Thr Thr Leu Leu Cys Leu Val Leu Cys Leu Gly
1 5 10 15

Gln Arg Ile Gln Ala Gln Glu Gly Asp Phe Pro Met Pro Phe Ile Ser
20 25 30

Ala Lys Ser Ser Pro Val Ile Pro Leu Asp Gly Ser Val Lys Ile Gln
35 40 45

Cys Gln Ala Ile Arg Glu Ala Tyr Leu Thr Gln Leu Met Ile Ile Lys
50 55 60

Asn Ser Thr Tyr Arg Glu Ile Gly Arg Arg Leu Lys Phe Trp Asn Glu
65 70 75 80

Thr Asp Pro Glu Phe Val Ile Asp His Met Asp Ala Asn Lys Ala Gly
85 90 95

Arg Tyr Gln Cys Gln Tyr Arg Ile Gly His Tyr Arg Phe Arg Tyr Ser
100 105 110

```
Asp Thr Leu Glu Leu Val Val Thr Gly Leu Tyr Gly Lys Pro Phe Leu
        115                 120                 125

Ser Ala Asp Arg Gly Leu Val Leu Met Pro Gly Glu Asn Ile Ser Leu
    130                 135                 140

Thr Cys Ser Ser Ala His Ile Pro Phe Asp Arg Phe Ser Leu Ala Lys
145                 150                 155                 160

Glu Gly Glu Leu Ser Leu Pro Gln His Gln Ser Gly Glu His Pro Ala
                165                 170                 175

Asn Phe Ser Leu Gly Pro Val Asp Leu Asn Val Ser Gly Ile Tyr Arg
            180                 185                 190

Cys Tyr Gly Trp Tyr Asn Arg Ser Pro Tyr Leu Trp Ser Phe Pro Ser
        195                 200                 205

Asn Ala Leu Glu Leu Val Val Thr Asp Ser Ile His Gln Asp Tyr Thr
    210                 215                 220

Thr Gln Asn Leu Ile Arg Met Ala Val Ala Gly Leu Val Leu Val Ala
225                 230                 235                 240

Leu Leu Ala Ile Leu Val Glu Asn Trp His Ser His Thr Ala Leu Asn
                245                 250                 255

Lys Glu Ala Ser Ala Asp Val Ala Glu Pro Ser Trp Ser Gln Gln Met
            260                 265                 270

Cys Gln Pro Gly Leu Thr Phe Ala Arg Thr Pro Ser Val Cys Lys
        275                 280                 285
```

<210> 6
<211> 534
<212> PRT
<213> Homo sapiens

<400> 6

```
Met Asp Gly Glu Ala Thr Val Lys Pro Gly Glu Gln Val Pro Leu Trp
1               5                   10                  15
```

```
Thr His Gly Trp Pro Pro Asp Asp Pro Ser Pro Ser Phe Ala Ala Gly
            20                  25                  30

Ser Ser Phe Ala Leu Pro Gln Lys Arg Pro His Pro Arg Trp Leu Trp
        35                  40                  45

Glu Gly Ser Leu Pro Ser Arg Thr His Leu Arg Ala Met Gly Thr Leu
    50                  55                  60

Arg Pro Ser Ser Pro Leu Cys Trp Arg Glu Glu Ser Ser Phe Ala Ala
65                  70                  75                  80

Pro Asn Ser Leu Lys Gly Ser Arg Leu Val Ser Gly Glu Pro Gly Gly
                85                  90                  95

Ala Val Thr Ile Gln Cys His Tyr Ala Pro Ser Ser Val Asn Arg His
            100                 105                 110

Gln Arg Lys Tyr Trp Cys Arg Leu Gly Pro Pro Arg Trp Ile Cys Gln
        115                 120                 125

Thr Ile Val Ser Thr Asn Gln Tyr Thr His His Arg Tyr Arg Asp Arg
    130                 135                 140

Val Ala Leu Thr Asp Phe Pro Gln Arg Gly Leu Phe Val Val Arg Leu
145                 150                 155                 160

Ser Gln Leu Ser Pro Asp Asp Ile Gly Cys Tyr Leu Cys Gly Ile Gly
                165                 170                 175

Ser Glu Asn Asn Met Leu Phe Leu Ser Met Asn Leu Thr Ile Ser Ala
            180                 185                 190

Gly Pro Ala Ser Thr Leu Pro Thr Ala Thr Pro Ala Ala Gly Glu Leu
        195                 200                 205

Thr Met Arg Ser Tyr Gly Thr Ala Ser Pro Val Ala Asn Arg Trp Thr
    210                 215                 220

Pro Gly Thr Thr Gln Thr Leu Gly Gln Gly Thr Ala Trp Asp Thr Val
225                 230                 235                 240
```

```
Ala Ser Thr Pro Gly Thr Ser Lys Thr Thr Ala Ser Ala Glu Gly Arg
                245                 250                 255

Arg Thr Pro Gly Ala Thr Arg Pro Ala Ala Pro Gly Thr Gly Ser Trp
            260                 265                 270

Ala Glu Gly Ser Val Lys Ala Pro Ala Pro Ile Pro Glu Ser Pro Pro
        275                 280                 285

Ser Lys Ser Arg Ser Met Ser Asn Thr Thr Glu Gly Val Trp Glu Gly
    290                 295                 300

Thr Arg Ser Ser Val Thr Asn Arg Ala Arg Ala Ser Lys Asp Arg Arg
305                 310                 315                 320

Glu Met Thr Thr Thr Lys Ala Asp Arg Pro Arg Glu Asp Ile Glu Gly
                325                 330                 335

Val Arg Ile Ala Leu Asp Ala Ala Lys Lys Val Leu Gly Thr Ile Gly
            340                 345                 350

Pro Pro Ala Leu Val Ser Glu Thr Leu Ala Trp Glu Ile Leu Pro Gln
        355                 360                 365

Ala Thr Pro Val Ser Lys Gln Gln Ser Gln Gly Ser Ile Gly Glu Thr
    370                 375                 380

Thr Pro Ala Ala Gly Met Trp Thr Leu Gly Thr Pro Ala Ala Asp Val
385                 390                 395                 400

Trp Ile Thr Ser Met Glu Ala Ala Ser Gly Glu Gly Ser Ala Ala Gly
                405                 410                 415

Asp Leu Asp Ala Ala Thr Gly Asp Arg Gly Pro Gln Ala Thr Leu Ser
            420                 425                 430

Gln Thr Pro Ala Val Gly Pro Trp Gly Pro Pro Gly Lys Glu Ser Ser
        435                 440                 445

Val Lys Arg Ala Thr Cys Pro Gly Leu Ser Thr Leu Lys Glu Ile Lys
    450                 455                 460
```

Val Ser Ala Val Leu Thr Gln Asn Pro His Ser Leu Leu Cys Val Ser
465 470 475 480

Ala Ala Gln Glu Ala Glu Arg Val Thr Leu Ile Gln Met Thr His Phe
485 490 495

Leu Glu Val Asn Pro Gln Ala Asp Gln Leu Pro His Val Glu Arg Lys
500 505 510

Met Leu Gln Asp Asp Ser Leu Pro Ala Gly Ala Ser Leu Thr Ala Pro
515 520 525

Glu Arg Asn Pro Gly Pro
530

<210> 7
<211> 764
<212> PRT
<213> Homo sapiens

<400> 7

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

Ser Thr Lys Ser Pro Ile Phe Gly Pro Glu Glu Val Asn Ser Val Glu
20 25 30

Gly Asn Ser Val Ser Ile Thr Cys Tyr Tyr Pro Pro Thr Ser Val Asn
35 40 45

Arg His Thr Arg Lys Tyr Trp Cys Arg Gln Gly Ala Arg Gly Gly Cys
50 55 60

Ile Thr Leu Ile Ser Ser Glu Gly Tyr Val Ser Ser Lys Tyr Ala Gly
65 70 75 80

Arg Ala Asn Leu Thr Asn Phe Pro Glu Asn Gly Thr Phe Val Val Asn
85 90 95

Ile Ala Gln Leu Ser Gln Asp Asp Ser Gly Arg Tyr Lys Cys Gly Leu
100 105 110

Gly Ile Asn Ser Arg Gly Leu Ser Phe Asp Val Ser Leu Glu Val Ser

115 120 125

Gln Gly Pro Gly Leu Leu Asn Asp Thr Lys Val Tyr Thr Val Asp Leu
130 135 140

Gly Arg Thr Val Thr Ile Asn Cys Pro Phe Lys Thr Glu Asn Ala Gln
145 150 155 160

Lys Arg Lys Ser Leu Tyr Lys Gln Ile Gly Leu Tyr Pro Val Leu Val
165 170 175

Ile Asp Ser Ser Gly Tyr Val Asn Pro Asn Tyr Thr Gly Arg Ile Arg
180 185 190

Leu Asp Ile Gln Gly Thr Gly Gln Leu Leu Phe Ser Val Val Ile Asn
195 200 205

Gln Leu Arg Leu Ser Asp Ala Gly Gln Tyr Leu Cys Gln Ala Gly Asp
210 215 220

Asp Ser Asn Ser Asn Lys Lys Asn Ala Asp Leu Gln Val Leu Lys Pro
225 230 235 240

Glu Pro Glu Leu Val Tyr Glu Asp Leu Arg Gly Ser Val Thr Phe His
245 250 255

Cys Ala Leu Gly Pro Glu Val Ala Asn Val Ala Lys Phe Leu Cys Arg
260 265 270

Gln Ser Ser Gly Glu Asn Cys Asp Val Val Val Asn Thr Leu Gly Lys
275 280 285

Arg Ala Pro Ala Phe Glu Gly Arg Ile Leu Leu Asn Pro Gln Asp Lys
290 295 300

Asp Gly Ser Phe Ser Val Val Ile Thr Gly Leu Arg Lys Glu Asp Ala
305 310 315 320

Gly Arg Tyr Leu Cys Gly Ala His Ser Asp Gly Gln Leu Gln Glu Gly
325 330 335

Ser Pro Ile Gln Ala Trp Gln Leu Phe Val Asn Glu Glu Ser Thr Ile

340 345 350

Pro Arg Ser Pro Thr Val Val Lys Gly Val Ala Gly Gly Ser Val Ala
355 360 365

Val Leu Cys Pro Tyr Asn Arg Lys Glu Ser Lys Ser Ile Lys Tyr Trp
370 375 380

Cys Leu Trp Glu Gly Ala Gln Asn Gly Arg Cys Pro Leu Leu Val Asp
385 390 395 400

Ser Glu Gly Trp Val Lys Ala Gln Tyr Glu Gly Arg Leu Ser Leu Leu
405 410 415

Glu Glu Pro Gly Asn Gly Thr Phe Thr Val Ile Leu Asn Gln Leu Thr
420 425 430

Ser Arg Asp Ala Gly Phe Tyr Trp Cys Leu Thr Asn Gly Asp Thr Leu
435 440 445

Trp Arg Thr Thr Val Glu Ile Lys Ile Ile Glu Gly Glu Pro Asn Leu
450 455 460

Lys Val Pro Gly Asn Val Thr Ala Val Leu Gly Glu Thr Leu Lys Val
465 470 475 480

Pro Cys His Phe Pro Cys Lys Phe Ser Ser Tyr Glu Lys Tyr Trp Cys
485 490 495

Lys Trp Asn Asn Thr Gly Cys Gln Ala Leu Pro Ser Gln Asp Glu Gly
500 505 510

Pro Ser Lys Ala Phe Val Asn Cys Asp Glu Asn Ser Arg Leu Val Ser
515 520 525

Leu Thr Leu Asn Leu Val Thr Arg Ala Asp Glu Gly Trp Tyr Trp Cys
530 535 540

Gly Val Lys Gln Gly His Phe Tyr Gly Glu Thr Ala Ala Val Tyr Val
545 550 555 560

Ala Val Glu Glu Arg Lys Ala Ala Gly Ser Arg Asp Val Ser Leu Ala

565 570 575

Lys Ala Asp Ala Ala Pro Asp Glu Lys Val Leu Asp Ser Gly Phe Arg
580 585 590

Glu Ile Glu Asn Lys Ala Ile Gln Asp Pro Arg Leu Phe Ala Glu Glu
595 600 605

Lys Ala Val Ala Asp Thr Arg Asp Gln Ala Asp Gly Ser Arg Ala Ser
610 615 620

Val Asp Ser Gly Ser Ser Glu Glu Gln Gly Gly Ser Ser Arg Ala Leu
625 630 635 640

Val Ser Thr Leu Val Pro Leu Gly Leu Val Leu Ala Val Gly Ala Val
645 650 655

Ala Val Gly Val Ala Arg Ala Arg His Arg Lys Asn Val Asp Arg Val
660 665 670

Ser Ile Arg Ser Tyr Arg Thr Asp Ile Ser Met Ser Asp Phe Glu Asn
675 680 685

Ser Arg Glu Phe Gly Ala Asn Asp Asn Met Gly Ala Ser Ser Ile Thr
690 695 700

Gln Glu Thr Ser Leu Gly Gly Lys Glu Glu Phe Val Ala Thr Thr Glu
705 710 715 720

Ser Thr Thr Glu Thr Lys Glu Pro Lys Lys Ala Lys Arg Ser Ser Lys
725 730 735

Glu Glu Ala Glu Met Ala Tyr Lys Asp Phe Leu Leu Gln Ser Ser Thr
740 745 750

Val Ala Ala Glu Ala Gln Asp Gly Pro Gln Glu Ala
755 760

<210> 8
<211> 760
<212> PRT
<213> Homo sapiens

<400> 8

Met Met Asp Gln Ala Arg Ser Ala Phe Ser Asn Leu Phe Gly Gly Glu
1 5 10 15

Pro Leu Ser Tyr Thr Arg Phe Ser Leu Ala Arg Gln Val Asp Gly Asp
20 25 30

Asn Ser His Val Glu Met Lys Leu Ala Val Asp Glu Glu Glu Asn Ala
35 40 45

Asp Asn Asn Thr Lys Ala Asn Val Thr Lys Pro Lys Arg Cys Ser Gly
50 55 60

Ser Ile Cys Tyr Gly Thr Ile Ala Val Ile Val Phe Phe Leu Ile Gly
65 70 75 80

Phe Met Ile Gly Tyr Leu Gly Tyr Cys Lys Gly Val Glu Pro Lys Thr
85 90 95

Glu Cys Glu Arg Leu Ala Gly Thr Glu Ser Pro Val Arg Glu Glu Pro
100 105 110

Gly Glu Asp Phe Pro Ala Ala Arg Arg Leu Tyr Trp Asp Asp Leu Lys
115 120 125

Arg Lys Leu Ser Glu Lys Leu Asp Ser Thr Asp Phe Thr Ser Thr Ile
130 135 140

Lys Leu Leu Asn Glu Asn Ser Tyr Val Pro Arg Glu Ala Gly Ser Gln
145 150 155 160

Lys Asp Glu Asn Leu Ala Leu Tyr Val Glu Asn Gln Phe Arg Glu Phe
165 170 175

Lys Leu Ser Lys Val Trp Arg Asp Gln His Phe Val Lys Ile Gln Val
180 185 190

Lys Asp Ser Ala Gln Asn Ser Val Ile Ile Val Asp Lys Asn Gly Arg
195 200 205

Leu Val Tyr Leu Val Glu Asn Pro Gly Gly Tyr Val Ala Tyr Ser Lys
210 215 220

Ala Ala Thr Val Thr Gly Lys Leu Val His Ala Asn Phe Gly Thr Lys
225 230 235 240

Lys Asp Phe Glu Asp Leu Tyr Thr Pro Val Asn Gly Ser Ile Val Ile
245 250 255

Val Arg Ala Gly Lys Ile Thr Phe Ala Glu Lys Val Ala Asn Ala Glu
260 265 270

Ser Leu Asn Ala Ile Gly Val Leu Ile Tyr Met Asp Gln Thr Lys Phe
275 280 285

Pro Ile Val Asn Ala Glu Leu Ser Phe Phe Gly His Ala His Leu Gly
290 295 300

Thr Gly Asp Pro Tyr Thr Pro Gly Phe Pro Ser Phe Asn His Thr Gln
305 310 315 320

Phe Pro Pro Ser Arg Ser Ser Gly Leu Pro Asn Ile Pro Val Gln Thr
325 330 335

Ile Ser Arg Ala Ala Ala Glu Lys Leu Phe Gly Asn Met Glu Gly Asp
340 345 350

Cys Pro Ser Asp Trp Lys Thr Asp Ser Thr Cys Arg Met Val Thr Ser
355 360 365

Glu Ser Lys Asn Val Lys Leu Thr Val Ser Asn Val Leu Lys Glu Ile
370 375 380

Lys Ile Leu Asn Ile Phe Gly Val Ile Lys Gly Phe Val Glu Pro Asp
385 390 395 400

His Tyr Val Val Val Gly Ala Gln Arg Asp Ala Trp Gly Pro Gly Ala
405 410 415

Ala Lys Ser Gly Val Gly Thr Ala Leu Leu Leu Lys Leu Ala Gln Met
420 425 430

Phe Ser Asp Met Val Leu Lys Asp Gly Phe Gln Pro Ser Arg Ser Ile
435 440 445

Ile Phe Ala Ser Trp Ser Ala Gly Asp Phe Gly Ser Val Gly Ala Thr
450 455 460

Glu Trp Leu Glu Gly Tyr Leu Ser Ser Leu His Leu Lys Ala Phe Thr
465 470 475 480

Tyr Ile Asn Leu Asp Lys Ala Val Leu Gly Thr Ser Asn Phe Lys Val
485 490 495

Ser Ala Ser Pro Leu Leu Tyr Thr Leu Ile Glu Lys Thr Met Gln Asn
500 505 510

Val Lys His Pro Val Thr Gly Gln Phe Leu Tyr Gln Asp Ser Asn Trp
515 520 525

Ala Ser Lys Val Glu Lys Leu Thr Leu Asp Asn Ala Ala Phe Pro Phe
530 535 540

Leu Ala Tyr Ser Gly Ile Pro Ala Val Ser Phe Cys Phe Cys Glu Asp
545 550 555 560

Thr Asp Tyr Pro Tyr Leu Gly Thr Thr Met Asp Thr Tyr Lys Glu Leu
565 570 575

Ile Glu Arg Ile Pro Glu Leu Asn Lys Val Ala Arg Ala Ala Ala Glu
580 585 590

Val Ala Gly Gln Phe Val Ile Lys Leu Thr His Asp Val Glu Leu Asn
595 600 605

Leu Asp Tyr Glu Arg Tyr Asn Ser Gln Leu Leu Ser Phe Val Arg Asp
610 615 620

Leu Asn Gln Tyr Arg Ala Asp Ile Lys Glu Met Gly Leu Ser Leu Gln
625 630 635 640

Trp Leu Tyr Ser Ala Arg Gly Asp Phe Phe Arg Ala Thr Ser Arg Leu
645 650 655

Thr Thr Asp Phe Gly Asn Ala Glu Lys Thr Asp Arg Phe Val Met Lys
660 665 670

Lys Leu Asn Asp Arg Val Met Arg Val Glu Tyr His Phe Leu Ser Pro
675 680 685

Tyr Val Ser Pro Lys Glu Ser Pro Phe Arg His Val Phe Trp Gly Ser
690 695 700

Gly Ser His Thr Leu Pro Ala Leu Leu Glu Asn Leu Lys Leu Arg Lys
705 710 715 720

Gln Asn Asn Gly Ala Phe Asn Glu Thr Leu Phe Arg Asn Gln Leu Ala
725 730 735

Leu Ala Thr Trp Thr Ile Gln Gly Ala Ala Asn Ala Leu Ser Gly Asp
740 745 750

Val Trp Asp Ile Asp Asn Glu Phe
755 760

<210> 9
<211> 9
<212> PRT
<213> Artificial sequence

<220>
<223> IgM binding peptide

<400> 9

Tyr Asp Trp Ile Pro Ser Ser Ala Trp
1 5

<210> 10
<211> 273
<212> PRT
<213> Staphylococcus aureus

<400> 10

Met Glu Gln Arg Ile Thr Leu Lys Glu Ala Trp Asp Gln Arg Asn Gly
1 5 10 15

Phe Ile Gln Ser Leu Lys Asp Asp Pro Ser Gln Ser Ala Asn Val Leu
20 25 30

Gly Glu Ala Gln Lys Leu Asn Asp Ser Gln Ala Pro Lys Ala Asp Ala

35 40 45

Gln Gln Asn Asn Phe Asn Lys Asp Gln Gln Ser Ala Phe Tyr Glu Ile
50 55 60

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

Ser Leu Lys Asp Asp Pro Ser Gln Ser Thr Asn Val Leu Gly Glu Ala
85 90 95

Lys Lys Leu Asn Glu Ser Gln Ala Pro Lys Ala Asp Asn Asn Phe Asn
100 105 110

Lys Glu Gln Gln Asn Ala Phe Tyr Glu Ile Leu Asn Met Pro Asn Leu
115 120 125

Asn Glu Glu Gln Arg Asn Gly Phe Ile Gln Ser Leu Lys Asp Asp Pro
130 135 140

Ser Gln Ser Ala Asn Leu Leu Ser Glu Ala Lys Lys Leu Asn Glu Ser
145 150 155 160

Gln Ala Pro Lys Ala Asp Asn Lys Phe Asn Lys Glu Gln Gln Asn Ala
165 170 175

Phe Tyr Glu Ile Leu His Leu Pro Asn Leu Asn Glu Glu Gln Arg Asn
180 185 190

Gly Phe Ile Gln Ser Leu Lys Asp Asp Pro Ser Gln Ser Ala Asn Leu
195 200 205

Leu Ala Glu Ala Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys Ala Asp
210 215 220

Asn Lys Phe Asn Lys Glu Gln Gln Asn Ala Phe Tyr Glu Ile Leu His
225 230 235 240

Leu Pro Asn Leu Thr Glu Glu Gln Arg Asn Gly Phe Ile Gln Ser Leu
245 250 255

Lys Asp Asp Pro Gly Asn Ser Arg Gly Ser Val Asp Leu Gln Ile Thr

260 265 270

Asn

<210> 11
<211> 58
<212> PRT
<213> Artificial Sequence

<220>
<223> Z-domain

<400> 11

Val Asp Asn Lys Phe Asn Lys Glu Gln Gln Asn Ala Phe Tyr Glu Ile
1 5 10 15

Leu His Leu Pro Asn Leu Asn Glu Glu Gln Arg Asn Ala Phe Ile Gln
20 25 30

Ser Leu Lys Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 12
<211> 58
<212> PRT
<213> Artificial Sequence

<220>
<223> Modified Z-domain

<400> 12

Val Asp Asn Lys Phe Asn Lys Glu Thr Ile Gln Ala Ser Gln Glu Ile
1 5 10 15

Arg Leu Leu Pro Asn Leu Asn Gly Arg Gln Lys Leu Ala Phe Ile His
20 25 30

Ser Leu Leu Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys

50 55

<210> 13
<211> 58
<212> PRT
<213> Staphylococcus aureus

<400> 13

Ala Asp Asn Lys Phe Asn Lys Glu Gln Gln Asn Ala Phe Tyr Glu Ile
1 5 10 15

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

Ser Leu Lys Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50 55

<210> 14
<211> 25
<212> PRT
<213> Homo sapiens

<400> 14

Thr Val Pro Cys Pro Val Pro Ser Thr Pro Pro Thr Pro Ser Pro Ser
1 5 10 15

Thr Pro Pro Thr Pro Ser Pro Ser Cys
20 25

<210> 15
<211> 627
<212> PRT
<213> Homo sapiens

<400> 15

Met Asp Trp Thr Trp Arg Phe Leu Phe Val Val Ala Ala Ala Thr Gly
1 5 10 15

Val Gln Ser Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys
20 25 30

Pro Gly Ser Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe
35 40 45

Ser Ser Tyr Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu
50 55 60

Glu Trp Met Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala
65 70 75 80

Gln Lys Phe Gln Gly Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser
85 90 95

Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val
100 105 110

Tyr Tyr Cys Ala Lys Thr Gly Ile Leu Gly Pro Tyr Ser Ser Gly Trp
115 120 125

Tyr Pro Asn Ser Asp Tyr Tyr Tyr Tyr Gly Met Asp Val Trp Gly Gln
130 135 140

Gly Thr Thr Val Thr Val Ser Ser Gly Ser Ala Ser Ala Pro Thr Leu
145 150 155 160

Phe Pro Leu Val Ser Cys Glu Asn Ser Pro Ser Asp Thr Ser Ser Val
165 170 175

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

Ser Trp Lys Tyr Lys Asn Asn Ser Asp Ile Ser Ser Thr Arg Gly Phe
195 200 205

Pro Ser Val Leu Arg Gly Gly Lys Tyr Ala Ala Thr Ser Gln Val Leu
210 215 220

Leu Pro Ser Lys Asp Val Met Gln Gly Thr Asp Glu His Val Val Cys
225 230 235 240

Lys Val Gln His Pro Asn Gly Asn Lys Glu Lys Asn Val Pro Leu Pro
245 250 255

Val Ile Ala Glu Leu Pro Pro Lys Val Ser Val Phe Val Pro Pro Arg
260 265 270

Asp Gly Phe Phe Gly Asn Pro Arg Ser Lys Ser Lys Leu Ile Cys Gln
275 280 285

Ala Thr Gly Phe Ser Pro Arg Gln Ile Gln Val Ser Trp Leu Arg Glu
290 295 300

Gly Lys Gln Val Gly Ser Gly Val Thr Thr Asp Gln Val Gln Ala Glu
305 310 315 320

Ala Lys Glu Ser Gly Pro Thr Thr Tyr Lys Val Thr Ser Thr Leu Thr
325 330 335

Ile Lys Glu Ser Asp Trp Leu Ser Gln Ser Met Phe Thr Cys Arg Val
340 345 350

Asp His Arg Gly Leu Thr Phe Gln Gln Asn Ala Ser Ser Met Cys Val
355 360 365

Pro Asp Gln Asp Thr Ala Ile Arg Val Phe Ala Ile Pro Pro Ser Phe
370 375 380

Ala Ser Ile Phe Leu Thr Lys Ser Thr Lys Leu Thr Cys Leu Val Thr
385 390 395 400

Asp Leu Thr Thr Tyr Asp Ser Val Thr Ile Ser Trp Thr Arg Gln Asn
405 410 415

Gly Glu Ala Val Lys Thr His Thr Asn Ile Ser Glu Ser His Pro Asn
420 425 430

Ala Thr Phe Ser Ala Val Gly Glu Ala Ser Ile Cys Glu Asp Asp Trp
435 440 445

Asn Ser Gly Glu Arg Phe Thr Cys Thr Val Thr His Thr Asp Leu Pro
450 455 460

Ser Pro Leu Lys Gln Thr Ile Ser Arg Pro Lys Gly Val Ala Leu His
465 470 475 480

Arg Pro Asp Val Tyr Leu Leu Pro Pro Ala Arg Glu Gln Leu Asn Leu
485 490 495

Arg Glu Ser Ala Thr Ile Thr Cys Leu Val Thr Gly Phe Ser Pro Ala
500 505 510

Asp Val Phe Val Gln Trp Met Gln Arg Gly Gln Pro Leu Ser Pro Glu
515 520 525

Lys Tyr Val Thr Ser Ala Pro Met Pro Glu Pro Gln Ala Pro Gly Arg
530 535 540

Tyr Phe Ala His Ser Ile Leu Thr Val Ser Glu Glu Glu Trp Asn Thr
545 550 555 560

Gly Glu Thr Tyr Thr Cys Val Val Ala His Glu Ala Leu Pro Asn Arg
565 570 575

Val Thr Glu Arg Thr Val Asp Lys Ser Thr Glu Gly Glu Val Ser Ala
580 585 590

Asp Glu Glu Gly Phe Glu Asn Leu Trp Ala Thr Ala Ser Thr Phe Ile
595 600 605

Val Leu Phe Leu Leu Ser Leu Phe Tyr Ser Thr Thr Val Thr Leu Phe
610 615 620

Lys Val Lys
625

<210> 16
<211> 2213
<212> DNA
<213> Homo sapiens

<400> 16
gctctagaac tagtggatcc cccgggctgc aggaattctc taaagaagcc cctgggagca 60

cagctcatca ccatggactg gacctggagg ttcctctttg tggtggcagc agctacaggt 120

gtccagtccc aggtgcagct ggtgcagtct ggggctgagg tgaagaagcc tgggtcctcg 180

gtgaaggtct cctgcaaggc ttctggaggc accttcagca gctatgctat cagctgggtg 240

cgacaggccc ctggacaagg gcttgagtgg atgggaggga tcatccctat ctttggtaca 300

gcaaactacg cacagaagtt ccagggcaga gtcacgatta ccgcggacga atccacgagc 360

acagcctaca tggagctgag cagcctgaga tctgaggaca cggccgtgta ttactgtgcg 420

aaaaccggga tcctggggcc gtatagcagt ggctggtacc cgaactcgga ctactactac 480

tacggtatgg acgtctgggg ccaagggacc acggtcaccg tctcctcagg gagtgcatcc 540

gccccaaccc ttttcccct cgtctcctgt gagaattccc cgtcggatac gagcagcgtg 600

gccgttggct gcctcgcaca ggacttcctt cccgactcca tcactttctc ctggaaatac 660

aagaacaact ctgacatcag cagcacccgg ggcttcccat cagtcctgag aggggcaag 720

tacgcagcca cctcacaggt gctgctgcct tccaaggacg tcatgcaggg cacagacgaa 780

cacgtggtgt gcaaagtcca gcaccccaac ggcaacaaag aaaagaacgt gcctcttcca 840

gtgattgctg agctgcctcc caaagtgagc gtcttcgtcc caccccgcga cggcttcttc 900

ggcaaccccc gcagcaagtc caagctcatc tgccaggcca cgggtttcag tccccggcag 960

attcaggtgt cctggctgcg cgaggggaag caggtggggt ctggcgtcac cacggaccag 1020

gtgcaggctg aggccaaaga gtctgggccc acgacctaca aggtgaccag cacactgacc 1080

atcaaagaga gcgactggct cagccagagc atgttcacct gccgcgtgga tcacaggggc 1140

ctgaccttcc agcagaatgc gtcctccatg tgtgtccccg atcaagacac agccatccgg 1200

gtcttcgcca tccccccatc ctttgccagc atcttcctca ccaagtccac caagttgacc 1260

tgcctggtca cagacctgac cacctatgac agcgtgacca tctcctggac ccgccagaat 1320

ggcgaagctg tgaaaaccca caccaacatc tccgagagcc accccaatgc cactttcagc 1380

gccgtgggtg aggccagcat ctgcgaggat gactggaatt ccggggagag gttcacgtgc 1440

accgtgaccc acacagacct gccctcgcca ctgaagcaga ccatctcccg gcccaagggg 1500

gtggccctgc acaggcccga tgtctacttg ctgccaccag cccgggagca gctgaacctg 1560

cgggagtcgg ccaccatcac gtgcctggtg acgggcttct ctcccgcgga cgtcttcgtg 1620

cagtggatgc agaggggggca gcccttgtcc ccggagaagt atgtgaccag cgccccaatg 1680

cctgagcccc aggccccagg ccggtacttc gcccacagca tcctgaccgt gtccgaagag 1740

gaatggaaca cgggggagac ctacacctgc gtggtggccc atgaggccct gcccaacagg 1800

gtcaccgaga ggaccgtgga caagtccacc gaggggggagg tgagcgccga cgaggagggc 1860

tttgagaacc tgtgggccac cgcctccacc ttcatcgtcc tcttcctcct gagcctcttc 1920

tacagtacca ccgtcacctt gttcaaggtg aaatgatccc aacagaagaa catcggagac 1980

cagagagagg aactcaaagg ggcgctgcct ccgggtctgg ggtcctggcc tgcgtggcct 2040

gttggcacgt gtttctcttc ccgcccggcc tccagttgtg tgctctcaca caggcttcct 2100

tctcgaccgg caggggctgg ctggcttgca ggccacgagg tgggctctac cccacactgc 2160

tttgctgtgt atacgcttgt tgccctgaaa taaatatgca cattttatcc atg 2213

<210> 17
<211> 26
<212> PRT
<213> Homo sapiens

<400> 17

Pro Leu Pro Val Ile Ala Glu Leu Pro Pro Lys Val Ser Val Phe Val
1 5 10 15

Pro Pro Arg Asp Gly Phe Phe Gly Asn Pro
20 25