

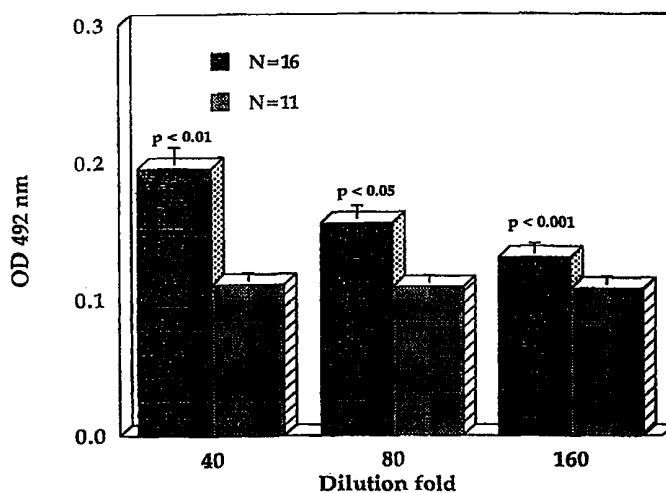


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(54) Title: AUTOIMMUNE INNER EAR DISEASE ANTIGEN AND DIAGNOSTIC ASSAY

Summary of ELISA results demonstrating the reactivity of sera from Meniere's disease patients with GST-Raf-1 (SEQ.ID.NO.:4).



(57) Abstract

A 28 kD antigen present in the membranous structure of the inner ear and an immunoassay method for diagnosing Meniere's disease using the 28 kD antigen as a target substance for detecting target binding substance in biological fluid from an animal or human having symptoms of a disease of the inner ear.

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10 AUTOIMMUNE INNER EAR DISEASE ANTIGEN AND
 DIAGNOSTIC ASSAY

 FIELD OF THE INVENTION

 This invention relates to the field of immunology
15 and more specifically relates to an antigen of the membranous
 structures of the inner ear and to an immunoassay method for
 detecting an autoimmune disease of the membranous structures
 of the inner ear.

20 BACKGROUND OF THE INVENTION

 Hearing problems can result from a variety of
 disorders, diseases or traumas of the inner ear. Symptoms of
 inner ear problems include, but are not limited to, hearing
 loss, dizziness, vertigo and tinnitus. Several inner ear diseases
25 have recently been classified as autoimmune diseases. These
 include, but are not limited to, Meniere's disease, progressive
 bilateral sensorineural hearing loss (PSHL), otosclerosis and
 sudden hearing loss.

 Meniere's disease, although idiopathic by
30 definition, has been ascribed to a variety of causes, among
 which are autoimmune factors. There is evidence to suggest
 that antibodies generated against inner ear proteins cause inner
 ear inflammation and swelling that can result in a complete
 loss of hearing (Dereby, M. *Otolaryngology-Head & Neck*
35 *Surgery* 114:360-365, 1996). Symptoms typically associated

with Meniere's disease include ringing in the ears, dizziness, a sense of fullness or pressure in the ears, and progressive deafness. These symptoms may be produced by a sudden influx of fluid into the endolymphatic sac, resulting in a rupture of Reisser's membrane in the cochlea. Immunological derangement of the endolymphatic sac or other membranous structures of the inner ear could initiate a cascade of reactions leading to endolymphatic hydrops and presenting as Meniere's disease (Soliman, A. *American Journal of Otology* 17:70-80, 1996). There are at least four million Meniere's disease patients in the United States, and many more patients report symptoms associated with Meniere's disease but cannot be positively diagnosed.

Researchers have attempted to isolate the antigen or antigens responsible for autoimmune inner ear diseases. A protein is considered to be a potential antigen if it is reactive with antibodies produced by patients exhibiting autoimmune inner ear diseases.

For example, antibodies in the sera of patients having inner ear disease have been found to react with protein bands of 58 kD and of 30 kD on Western blots of guinea pig inner ear extracts (Cao, M. et al., *Laryngoscope* 106:207-212, 1996). The 58 kD band was shown to be nonspecific to the inner ear when antibodies reacted with a 58 kD band on Western blots of guinea pig brain, lung and liver. In contrast, the 30 kD band was specific to the inner ear. Antibodies from patients reacted with a 30 kD band on Western blots of extracts from Corti's organ, the spiral ganglion and the acoustic nerve fiber, but not with extracts from the spinal ligament and the stria vascularis.

Antibodies against a 30 kD cochlear protein have been reported in the serum of some patients with Meniere's disease (Joliat, T. et al., *Ann. Otol. Rhinol. Laryngol.* 101:1001-1006, 1994 and Cao, M. et al., *Laryngoscope* 106:207-212, 1996). This 30 kD protein has been identified as

the major peripheral myelin protein "P0" and is believed to be associated with acoustic nerve and spiral ganglion (Cao, M. et al., *Laryngoscope* 106:207-212 (1996). Antibodies reactive with the 30 kD protein are not specific for Meniere's disease as these antibodies have been found in patients having other autoimmune diseases such as progressive bilateral sensorineural hearing loss (PSNHL), otosclerosis and sudden deafness and in control subjects. (Cao, M. et al., IMMUNOBIOLOGY IN OTOTOLOGY, RHINOLOGY AND LARYNGOLOGY (eds. Mogi, G., Veldman, J., and Kawauchi, H.) Kugler Publ. Amsterdam/New York, (1994) pp. 263-268).

Antibodies against a 68 kD protein in extracts from bovine inner ear have been reported in the serum of PSNHL patients (Harris J. and Sharp P. *Laryngoscope* 100:516-524, 1990). This 68 kD protein has been identified as a 70 kD heat shock protein that has been implicated in other autoimmune diseases such as Lyme's disease and ulcerative colitis (Billings P. et al. *Ann. Otol. Rhinol. Laryngol.* 104:181-188, 1995).

An early diagnosis of autoimmune inner ear disease is critical. Prompt treatment of the disease at an early stage of the illness may preserve any remaining inner ear function. Moreover, the ability to distinguish antigenic epitopes of the inner ear relevant to the pathogenesis of specific autoimmune inner ear diseases will enable clinical investigation and research on autoimmune inner ear disease, and will further enable the clinical diagnosis of autoimmune inner ear diseases and immunologic therapy.

As the availability of human inner ear tissue is extremely limited, there is an on-going need for the identification of disease-specific antigens and for the development of simple, sensitive and reproducible assays for the detection and differential diagnosis of autoimmune inner ear diseases.

SUMMARY OF THE INVENTION

An isolated 28 kD antigen present in the membranous structure of the inner ear and an immunoassay method for diagnosing Meniere's disease using the 28 kD antigen as a target substance for detecting target binding substance in biological fluid from an animal or human having symptoms of a disease of the inner ear are provided.

It is an object of the present invention to provide an antigen identified in the membranous structure of the inner ear that reacts specifically with antibodies from the sera of patients having an autoimmune disease of the membranous structures of the inner ear.

It is a further object of the present invention to provide a simple, rapid, sensitive and reproducible method for detecting antibodies to antigens from the membranous structures of the inner ear.

It is a further object of the present invention to provide an isolated antigen from the membranous structures of the inner ear that reacts specifically with antibodies in the sera of patients having Meniere's disease.

It is a further object of the present invention to provide a sensitive blood test for the detection of Meniere's disease in the early stages of the disease.

It is a further object of the present invention to provide an immunoassay that can distinguish Meniere's disease from other autoimmune ear diseases.

It is a further object of the present invention to provide an immunoassay that can monitor the progression of Meniere's disease or the effects of treatment for Meniere's disease.

It is a further object of the present invention to provide an antigen to be used for the immunotherapeutic treatment of Meniere's disease.

These and other objects of the present invention will become apparent after reading the following detailed

description of the disclosed embodiments and the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

5 Figure 1 is a Western blot demonstrating that GST-Raf-1 (SEQ.ID.NO.:4) is present in the membranous portion of the inner ear, but is not present in the neural portion of the inner ear, in facial nerve or in brain tissue.

10 Figure 2 is a Western blot demonstrating the reactivity of sera from Meniere's disease patients with the 28 kD protein SEQ.ID.NO.:1, (left panel) and with GST-Raf-1, SEQ.ID.NO.:4, (right panel).

15 Figure 3 is a summary of ELISA results demonstrating the reactivity of sera from Meniere's disease patients with GST-Raf-1 (SEQ.ID.NO.:4).

DETAILED DESCRIPTION OF THE INVENTION

20 As used herein, the term "28 kD antigen" refers to a protein extract or peptide from the membranous structures of the inner ear of a mammal, a protein or peptide having the amino acid sequence N'-IVQQFGFQRRASDDGKLTQ-C' (SEQ.ID.NO.:1) and antigenic variants thereof, a protein or peptide having the amino acid sequence N'-IVQQFGYQRRASDDGKLTD-C' (SEQ.ID.NO.:2) and antigenic variants thereof and a recombinant GST-Raf-1 protein or peptide having the amino acid sequence N'-MEHIQGAWKTISNGFGFKDAVFDGSSCISPTIVQ QFGYQRRASDDGKLTDPSKTSNTIRVFLPNKQRTVVNVR NGMSLHDCLMKALKVRGLQPECCAVFRLLEHKGKKAR LDWNTDAASLIGEELQVDFLDHVPLTTHNFARKTFLK-C' (SEQ.ID.NO.:4) and antigenic variants thereof.

35 As used herein, the term "target substance" refers to the 28 kD antigen or a nucleic acid molecule having a sequence encoding the 28 kD antigen of the membranous structures of the inner ear of a mammal.

As used herein, the term "immune sample" refers to samples having antibodies that interact specifically with the 28 kD antigen.

5 As used herein, the term "target-binding substance" refers to immune samples and to biological molecules, such as antibodies, which interact specifically with the 28 kD antigen. The term "target-binding substance" further include nucleic acid probes that hybridize under stringent hybridization conditions to a nucleic acid molecule
10 having a sequence encoding the 28 kD antigen of the membranous structures of the inner ear of a mammal.

As used herein, the term "preimmune sample" refers to samples not having antibodies that interact specifically with the 28 kD antigen.

15 As used herein, the term "membranous structures" refers to the basilar membrane, organ of Corti, stria vascularis, spiral ligament and vestibular epithelium of the inner ear.

20 As used herein, the term "neural structures" refers to the spiral ganglion, cochlear nerve in the modiolus and vestibular nerve in the temporal bone of the inner ear.

As used herein, the term "GST-Raf-1" refers to recombinant purified GST-Raf-1 protein (SEQ.ID.NO.:4) of human Raf-1 protein (SEQ.ID.NO.:6). Anti-Raf-1 is obtained
25 from Transduction Laboratories (Lexington KY).

The term "antigenic variant" refers to a protein or peptide having an amino acid sequence different from the protein or peptide to which it is compared, but having similar immunologic characteristics such as the ability to bind to one
30 or more antibodies that bind to the protein or peptide to which it is compared.

An antigen and a diagnostic assay method for detecting antibodies to the antigen in a biological sample are described herein. The antigen is the 28 kD antigen, as defined
35 above, and the diagnostic assay method is specific for detecting

antibodies to the 28 kD antigen. The 28 kD antigen is present in the membranous fraction of the inner ear, but is not present in the neural fraction of the inner ear, facial nerve or brain tissue (Suzuki et al., *ORL*, 59:10-17, 1997). The 28 kD antigen shows greater than 75% homology with residues 41 to 60 of Raf-1 protein from various species including, but not limited to, the human sequence (SEQ.ID.NO.:2) and the chicken sequence (SEQ.ID.NO.:3).

Preferably, the 28 kD antigen has the sequence of SEQ.ID.NO.:1, SEQ.ID.NO.:2, SEQ.ID.NO.:4 or antigenic variants thereof and reacts with a biological fluid from an individual having an autoimmune disease of the membranous structures of the inner ear. More preferably, the 28 kD antigen has the amino acid sequence of SEQ.ID.NO.:1 or SEQ.ID.NO.:2 or antigenic variants thereof and reacts with a biological fluid from an individual having Meniere's disease. Most preferably, the 28 kD antigen has the amino acid sequence of SEQ.ID.NO.:1 and antigenic variants thereof and reacts with a biological fluid from an individual having Meniere's disease.

The diagnostic assay for detecting the 28 kD antigen of the membranous structures of the inner ear can be, for example, an immunoassay. Such an immunoassay includes, but is not limited to, an ELISA, a Western blot assay, a competitive binding assay, a particle based immunoassay, a dual particle competitive immunoassay, and any of the other immunoassay methods known to those skilled in the art.

For example, in a conventional immunoassay, such as an ELISA, an inert solid-phase material, usually a plastic microtiter plate, is contacted with a solution containing the target substance (28 kD antigen) so that the target substance binds to, or coats, the solid phase material. The bound target substance is then contacted with an aqueous sample obtained from an individual having symptoms of inner ear disease, which may or which may not contain a target-binding

substance (anti-28 kD antibody). Unbound target-binding substance is removed, and the amount of reacted target-binding substance is quantitated using any of a number of detection devices known to those skilled in the art. For example, the bound target-binding substance may be detected with a second antibody to which has been attached a detectable label such as an enzyme, radioisotope or fluorescent molecule.

The target substance for use in the present invention includes, but is not limited to, protein extracted from the membranous structures of the inner ear, fractions of protein extracted from the membranous structures of the inner ear, and an isolated 28 kD antigen extracted and purified from the membranous structures of the inner ear. In addition, the target substance for use in the present invention also includes, but is not limited to, a protein or peptide having the amino acid sequence of SEQ.ID.NO.:1 or antigenic variants thereof, a protein or peptide having the amino acid sequence of SEQ.ID.NO.:2 or antigenic variants thereof, and a protein or peptide having the amino acid sequence of SEQ.ID.NO.:4 or antigenic variants thereof obtained using recombinant DNA technology or synthesized by methods known to those skilled in the art of peptide synthesis. It will be understood by those skilled in the art that the term "amino acid sequence of SEQ.ID.NO.:1, 2, or 4" includes antigenic variants thereof.

The concentration of target substance for use in the present invention can range between approximately 1 $\mu\text{g/ml}$ and 100 $\mu\text{g/ml}$. The more preferable range is between approximately 3 $\mu\text{g/ml}$ and 50 $\mu\text{g/ml}$. The most preferable range is between approximately 5 $\mu\text{g/ml}$ and 30 $\mu\text{g/ml}$. The target substance is dissolved in an aqueous solution and can be applied to an inert solid-phase support material by dipping, soaking, coating, spotting, spraying, blotting or other convenient means. Preferred methods include coating, spotting, spraying and blotting. More preferred methods include coating and blotting. For example, in an ELISA, a

preferred volume for coating is between about 10 μ l/well and 200 μ l/well. a more preferred volume for coating is between about 30 μ l/well and 150 μ l/well. A most preferred volume for coating is between about 50 μ l/well and 100 μ l/well.

5 Determination of the amount of target substance to be used for each method of application is well within the knowledge of one skilled in the art. For example, a standard target substance, target-binding substance assay combination can be used to determine the amount of target substance to be applied to the

10 inert solid-phase support material.

The solvent for use in the present invention can be any solvent that can solubilize the target-binding substance, and that is sufficiently miscible with water to be completely removed by subsequent thorough rinsing with an aqueous

15 solution. Such solvents include, but are not limited to phosphate buffered saline (PBS), tris(hydroxymethyl)amino methane (TRIS), N-2-hydroxyethylpeperazine-N'-2-ethanesulfonic acid (HEPES), citric acid-phosphate buffer and carbonate buffer. Such aqueous buffers and their appropriate

20 pHs are well known to those skilled in the art. Mixtures of solvents may also be used. Preferred solvents include 0.1 M carbonate buffer, pH 9.0, and citric acid-phosphate buffer, pH 5.0. These solvents may contain other chemicals including, but not limited to, SDS, Tween-20, bromphenol blue, glycerol and

25 dithiothreitol.

The solid phase, or inert solid-phase support material, for use in the present invention can be in the form of, but is not limited to, a membrane, a bead, a microtiter plate or any other solid-phase support form known to those skilled

30 in the art. Preferred forms include a membrane strip, a membrane well microtiter plate and a plastic well microtiter plate. More preferred forms include a membrane strip and a plastic well microtiter plate. A most preferred form is a plastic well microtiter plate. In addition, the inert solid-phase

35 support material can be placed into a holder, including but not

limited to, a membrane sheet holder, a dot-blot apparatus, a microtiter plate, a column, and a filter. Preferred holders include a membrane sheet holder, a dot-blot apparatus and a microtiter plate.

5 The blocking buffers for use in the present invention to prevent non-specific binding can be any suitable blocking buffer including, but not limited to, goat serum, fetal calf serum, gelatin, low fat milk, and Tween-20 at various dilutions in an aqueous solution.

10 The washing solution for use in the present invention can be any suitable aqueous buffer including, but not limited to, phosphate buffered saline (PBS), tris(hydroxymethyl)amino methane (TRIS) and N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES). Such
15 aqueous buffers and their appropriate pHs are well known to those skilled in the art.

 The target-binding substance for use in the present invention is a substance which binds specifically to the target substance. Examples of target-binding substances include, but
20 are not limited to, antibodies. Preferred target-binding substances are antibodies to proteins of the membranous structures of the inner ear. More preferred target-binding substances are antibodies to a 28 kD antigen of the membranous structure of the inner ear in serum from
25 individuals having inner ear disease. Most preferred target-binding substances are antibodies to a 28 kD protein of the membranous structure of the inner ear in the serum of individuals having Meniere's disease.

 Any convenient indicator method can be used to
30 detect binding of a target-binding substance to a target substance. Such methods include, but are not limited to, the use of enzymes, enzyme cofactors, enzyme effectors, chromogenic substances, fluorogenic substances, chemiluminescent substances, bioluminescent substances, and
35 labeled antibodies. Preferred indicator methods are the

peroxidase-labeled antibody method and the alkaline phosphatase-labeled antibody method.

5 The present invention further comprises an assay kit for detecting target-binding substance in a biological sample comprising an inert solid-phase support material having target-binding substance immobilized thereon and may further contain reagents and a holder for the inert solid-phase support material. The kit may additionally contain equipment for safely containing the samples, a vessel for containing the reagents, a timing means, and a colorimeter, reflectometer, or standard against which a color change may be measured. The reagents, including the target substance coated particle and the detectable particle are preferably lyophilized. Most preferably, the coated particle, and the detectable particle are provided in lyophilized form in a single container.

10 For example, an immunoassay kit useful for measuring the target-binding substance in a biological sample can involve a "sandwich immunoassay". The kit contains a particle coated on its surface with the binding substance, a detectable particle capable of binding to the target-binding substance and a porous membrane having a pore size that prevents passage of the coated particle and allows passage of the detectable particle. The first step in the immunoassay is a binding step, and the second step is a detection step.

25 In the binding step, a solid phase particle or sphere coated with the target substance is combined in a solution with a sample containing the target-binding substance and reacted for a sufficient amount of time to allow the target substance and the target binding substance to interact. In the detection step, a detectable particle, such as a colored bead, coated with a substance that binds readily to the target-binding substance, such as protein A, protein G, a second antibody reactive to the target-binding substance, or a small synthetic affinity ligand is added to the suspension. The detectable particle binds to the target-binding substance complexed to the

target substance coated particle. The reaction mixture is then placed on a membrane having a pore size of sufficient dimension to exclude passage of target substance coated particle which have bound target-binding substance and, therefore, bound detectable particle. Those components which are complexed as target substance particle plus target-binding substance plus detectable particle are retained on the membrane while the other components pass through the pores.

The complex is detected either visually with the naked eye or using a conventional detector, such as a colorimeter or reflectometer, well known to those skilled in the art. In this sandwich immunoassay, the presence of detectable particles indicates the presence of target-binding substance in the sample.

The 28 kD antigen and the immunoassay method and kit described above will be further understood with reference to the following examples, which are in no way intended to limit the scope of the present invention.

Example 1: Isolation and Identification of 28 kD Protein from the Membranous Fraction of Retrocochlear Tissue
Protein Extraction

Proteins are extracted from the membranous fraction of retrocochlear tissue in accordance with the method of Suzuki, M. et al. *ORL*, 59:10-17, 1997 as follows. Briefly, forty Harley strain male guinea pigs (Sasco Co., Wilmington, MA) are anesthetized by intraperitoneal injection of a mixture of ketamine (70 mg/kg) and xylazine (70 mg/kg) and perfused with 0.01 M phosphate buffered saline (PBS), pH 7.4, through the left ventricle. Both temporal bones are removed and placed in crushed ice.

The membranous portion of the inner ear, including basilar membrane, organ of Corti, stria vascularis, spiral ligament and vestibular epithelium are dissected under a microscope. The neural portion of the inner ear, including the

spiral ganglion, cochlear nerve in the modiolus, and vestibular nerve in the temporal bone, the facial nerve and brain tissue are dissected under a microscope.

5 The membranous tissue and the neural tissue are each sonicated for 20 seconds in lysis buffer (100 mM NaCl, 10 mM Tris buffer (Sigma Chemical Company, St. Louis, MO), pH 7.6; 1 mM ethylenediaminetetraacetate (EDTA; Sigma Chemical Company, St. Louis, MO), pH 8.0; a surfactant including, but not limited to, 0.1% sodium dodecyl sulfate (SDS, Sigma Chemical Company, St. Louis, MO) and 1% Nonidet P-40 (Sigma Chemical Company, St. Louis, MO; 2 µg/ml aprotinin; and, 100 mg/ml phenylmethylsulfonyl fluoride (PMSF, Sigma Chemical Company, St. Louis, MO). The extracted membranous proteins and the extracted neural proteins are incubated at 0°C for 30 minutes and centrifuged at 10,000 rpm for 10 minutes. Each supernatant is filtered through a 0.22 µm filter (Millipore Co., Bedford, MA), boiled for five minutes in a boiling water bath and stored at -80 C.

Protein concentration is determined after electrophoresis in one-dimensional 12% SDS-polyacrylamide gels (SDS-PAGE) using molecular weight standards (Life Technology, Inc., Grand Island, NY.). A range of 1 µg/ml to 10 µg/ml of standard is loaded on the same gel and is compared with inner ear protein extract and with Raf-1 protein.

SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Samples are fractionated by SDS-PAGE using a 12% running gel and a 5% stacking gel in accordance with the method of Laemmli et al. Samples of membranous proteins, of neural proteins and of Raf-1 protein are each mixed 1:1 with 100 mM Tris, pH 6.8, 4% SDS, 0.2% bromphenol blue, 20% glycerol and 200 mM dithiothreitol and heated at 100°C. for three minutes. The gels are electrophoresed in a vertical electrophoresis apparatus (Life Technologies, Inc., Grand

Island, NY) at 90 volts for seven hours. The gels are fixed and stained with Coomassie brilliant blue (BioRad, Melville, NY) and are destained with 45% methanol, 10% acetic acid and 45% distilled water. Apparent molecular weights of the separated components are calculated by comparison with prestained molecular weight markers (Life Technologies, Inc., Grand Island, NY) electrophoresed in the same gel.

Western Blotting

Proteins, separated in 12% one-dimensional SDS-PAGE as described above, are electroblotted onto polyvinylidene difluoride (PVDF) membrane (BioRad, Melville, NY) using a BioRad Semi-Dry Transblot Cell (BioRad, Melville, NY) for 1 hour. The PVDF membrane is washed one time with 20 mM Tris, pH 7.5, 500 mM NaCl containing 0.025% Tween-20 (TTBS).

Amino Acid Sequencing

Samples separated in SDS-PAGE and are electroblotted onto PVDF membrane as described above. The band corresponding to the molecular weight of 28 kD is excised from the PVDF membrane and is microsequenced using automated Edman degradation (Applied Biosystems, Ragweed City, CA). Nineteen amino acids, having the sequence N'-IVQQFGFQRRASDDGKLTQ-C' (SEQ.ID.NO.:1), are identified with an initial yield of approximately 78.6 picomoles.

Sequence comparison with the Swiss Protein Database shows the microsequence of the 28 kD membranous inner ear protein to have 89.5% identity, 19 amino acid overlap, with residues 41-60 of human Raf-1 protein (SEQ.ID.NO.:2) and 78.9% identity with residues 41-60 of chicken Raf-1 protein (SEQ.ID.NO.:3) as shown in Table 1.

Table 1.

The 28 kD protein microsequence from guinea pig inner ear and residues 41-60 of Raf-1 protein from human and chicken

Amino Acid	40	50	Identity
28kD protein	IVQQFGFQR	RASDDGKLTQ	
Human Raf-1	 Y ..	 D	89.5%
Chick Raf-1	 Y ..	 ISD	78.9%

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Example 2: Preparation of Recombinant GST-Raf-1

A glutathione-s-transferase (GST)-Raf-1 construct, N-domain with residues of 1-149, (SEQ.ID.NO.:4) is provided by Dr. Mark Marshall (Indiana University, IN). An overnight culture is diluted 1:100 in fresh LB medium containing 100 µg/ml ampicillin and grown at 37°C to an OD₆₀₀ of 0.5. The culture is equilibrated to 30°C and 1 mM isopropyl-beta-D-thiogalacto-pyranoside (Sigma. Chemical Co., St. Louis, MO) is added to induce GST-Raf-1 expression. After three hours of induction, the cells are pelleted, resuspended in PBS buffer, and incubated overnight at -70°C. The lysate is sonicated and the clear lysate is tumbled with glutathione-Sepharose beads (Pharmacia Biotech Inc., Piscataway, NJ) for 30 minutes. The beads are washed four times with PBS and the GST-Raf-1 fusion protein is eluted with 10 mM reduced glutathione at pH 8.0. The GST-Raf-1 fusion protein is dialyzed against Tris-HCl buffer, pH 8.0, aliquoted, snap frozen, and stored at -80°C. The purity and concentration of the GST-Raf-1 fusion protein is determined by 12% SDS-PAGE.

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Example 3: Western Blot Immunochemistry Of Sera From Patients With Inner Ear Disease

Proteins are extracted from the guinea pig inner ear, separated in SDS-PAGE and electroblotted onto PVDF

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membrane as in Example 1. The PVDF membranes are washed one time with TTBS.

5 Sera from patients with various inner ear diseases, including Meniere's disease, (experimental sera) are provided by Drs. J. Bernstein (Department of Otolaryngology, State University of New York, Buffalo, NY) and Y. Yazawa (Department of Otolaryngology, Shiga University of Medical Science, Seta, Japan). The diagnosis of Meniere's disease is based on the AAO-HNS criteria. Sera from patients with no
10 inner ear diseases (control sera) is obtained from age matched patients. Sera are stored at -20°C.

For use with serum as target binding substance (1st antibody), nonspecific binding is blocked using 25% goat serum in TTBS containing 0.02% sodium azide for one hour at
15 room temperature. The PVDF membrane is incubated in serum diluted 1:50 in TTBS containing 25% goat serum and 0.02% sodium azide overnight at room temperature. The membrane is then washed three times in TTBS and incubated with peroxidase conjugated goat anti-human polyvalent immunoglobulin (Accurate Chemical and Scientific Corp.,
20 Westbury, NY) diluted 1:2,000 for two hours at room temperature. The PVDF membrane is washed twice with TTBS for ten minutes at room temperature. Immunoreactive bands are visualized using 0.05 M Tris-HCl, pH 7.6, containing
25 0.02% 3,3'-diaminobenzidine (Chemicon International, Inc. Temecula, CA) and 0.01% hydrogen peroxide.

As shown in Table 2, with 24 (53%) of 45 patients with various inner ear diseases show a positive Western blot, whereas 0 of 10 (0%) control patients show a positive Western
30 blot. Of 25 patients with Meniere's disease, 15 (60%) show positive Western blots.

Table 2.

Patients' diagnoses and Western blot immunochemistry of guinea pig inner ear protein probed from patients with various inner ear diseases.

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	n	Positive Western blot	
		n	%
Meniere's disease	25	15	60
Otosclerosis	6	3	50
Hearing loss and tinnitus (diagnosis undetermined)	6	3	50
PSNHL	2	2	100
Cogan's syndrome	1	0	0
Sudden deafness	1	0	0
Strial atrophy	2	0	0
Hereditary hearing loss	1	0	0
Syphilitic labyrinthitis	1	1	100
Control	10	0	0

Among the 10 positively reactive bands detected, a 28 kD band is the most common positive band found in sera from Meniere's disease patients with 7 out of 25 sera reactive.

10

As shown in Table 3, of 24 patients who show a least one positively reactive band, 10 show a positive reaction only in the membranous part, 1 only in the neural part and 13 in both parts.

Table 3

15

Differences in Western blotting results between the membranous part and the neural part of the inner ear.

	25 kD	28 kD	30 kD	32 kD	40 kD	42 kD	46 kD	52 kD	67 kD	79 kD
Membranous part	4	9	1	1	1	2	1	7	6	2
Neural part	0	0	2	1	1	2	1	6	4	0

The marked difference between the two parts of the inner ear is the 28 kD band which is detected only on the membranous part.

5 *Example 4: Presence of GST-Raf-1 (SEQ.ID.NO.:4) In Neural Inner Ear Proteins, Facial Nerve Proteins and Brain Tissue.*

Proteins extracted from the membranous portion of the inner ear, the neural portion of the inner ear, the facial nerve and the brain are electrophoresed and electroblotted onto
10 PVDF membrane as in Example 1.

Anti-Raf-1 specific monoclonal antibody is obtained from Transduction Laboratories (Lexington, KY). This anti-Raf-1 recognizes amino acids 162-378 of human Raf-1 protein (SEQ.ID.NO.:5). For use with anti-Raf-1 as target-binding substance (1st antibody), nonspecific binding is
15 blocked using 5% nonfat dry milk in 10 mM Tris, pH 7.5, 100 mM NaCl and 0.1% Tween-20 for one hour at room temperature. The PVDF membrane is incubated in anti-Raf-1 diluted 1:1000 in 5% nonfat dry milk in 10 mM Tris, pH 7.5,
20 100 mM NaCl and 0.1% Tween-20 overnight at room temperature. The membrane is then washed three times in TTBS and incubated with peroxidase-conjugated goat anti-mouse IgG (Sigma Chemical Co., St. Louis, MO). Immunoreactive bands are visualized using 0.05 M Tris-HCl,
25 pH 7.6, containing 0.02% 3,3'-diamino-benzidine (Chemicon International, Inc., Temecula, CA) and 0.01% hydrogen peroxide.

Anti-Raf-1 monoclonal antibody recognizes 115 kD and 74 kD proteins in extracts from the membranous portion of the inner ear (Fig. 1, Col. A), the neural portion of the inner ear Fig. 1, Col. B), the facial nerve (Fig. 1, Col. C) and the brain (Fig. 1, Col. D). In contrast, anti-Raf-1 monoclonal antibody recognizes the 28 kD protein (SEQ.ID.NO.:1) in extracts from the membranous portion of
30

the inner ear, but not in extracts from the neural portion of the inner ear, the facial nerve and the brain .

Example 5: Determination of Raf-1 Antibodies in Sera From Meniere's Patients

Proteins extracted from the membranous portion of the inner ear and recombinant GST Raf-1 protein are electrophoresed in SDS-PAGE and electroblotted onto PVDF membrane as in Example 1 for immunochemical analysis as in Example 3.

Sera from patients with symptomatic Meniere's disease are provided by Drs. J. Bernstein (Department of Otolaryngology, State University of New York, Buffalo, NY) and Y. Yazawa (Department of Otolaryngology, Shiga University of Medical Science, Seta, Japan). The diagnosis of Meniere's disease is based on the AAO-HNS criteria. Sera are stored at -20°C.

Figure 2A shows that sera from Meniere's disease patients reacts with the 28 kD antigen (SEQ.ID.NO.:1) extracted from the membranous portion of the inner ear of the guinea pig. Figure 2B shows that sera from the same Meniere's disease patients reacts with the 40 kD recombinant GST-Raf-1 (SEQ.ID.NO.:4).

Example 6: Reactivity of Sera From Meniere's Disease Patients with GST Raf-1 (SEQ.ID.NO.:4)

Sera from 27 Meniere's disease patients, provided as in Example 5, are assayed for antibodies against GST-RAF-1 (SEQ.ID.NO.:4) in ELISA.

One hundred microliters of GST-Raf-1 (SEQ.ID.NO.:4) containing 5 µg/µl in 0.1 M carbonate buffer, pH 9.6, are dispensed into each well of a polystyrene microtiter plate (Costa, Cambridge, MA) and incubated overnight at 4°C. The antigen coated plates are washed three times in PBS-0.05% Tween buffer and incubated with patient's

sera (1:40, 1:80, or 1:160 dilution) or with 0.1 M carbonate buffer, pH 9.6, as a control, overnight at 4°C. The plates are washed five times with PBS-0.05% Tween buffer and incubated overnight with c-chain specific anti human IgG antibodies (Sigma chemical Co., St. Louis, MO) at 4°C. The plates are washed five times in PBS-0.05% Tween buffer and citric acid-phosphate buffer, pH 5.0, containing 0.15 mg/ml of o-phenylenediamine (Sigma Chemical Co., St. Louis, MO) is added. The color is developed at room temperature and the reaction is stopped by 2.5 M sulfuric acid. The color is measured at 492 nm.

As shown in Figure 3, 16 of 27 sera obtained from Meniere's patients (59%) recognize GST-Raf-1 (SEQ.ID.NO.:4) in ELISA at dilutions of 1:40, 1:80 and 1:160. Seven of these sera are tested in Western blot analysis and show positive reactivity with the 28 kD band in membranous inner ear extracts of the guinea pig.

While the foregoing specification teaches the principles of the present invention, with examples provided for the purpose of illustration, it will be understood that the practice of the invention encompasses all of the usual variations, adaptation, modification or deletions as come within the scope of the following claims and their equivalents.

SEQUENCE LISTING

- (1) GENERAL INFORMATION:
- 5 (i) APPLICANT: Yoo, Tai-June
Cheng, Kuang-Chuan
- 10 (ii) TITLE OF INVENTION: Autoimmune Inner Ear Disease Antigen
and Diagnostic Assay
- (iii) NUMBER OF SEQUENCES: 6
- 15 (iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Jones & Askew
(B) STREET: 191 Peachtree Street, 37th Floor
(C) CITY: Atlanta
(D) STATE: Georgia
20 (E) COUNTRY: U.S.A.
(F) ZIP: 30303
- (v) COMPUTER READABLE FORM:
25 (A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
30 (A) APPLICATION NUMBER: US
(B) FILING DATE:
(C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
35 (A) NAME: Young, Leona G.
(B) REGISTRATION NUMBER: 37,266
(C) REFERENCE/DOCKET NUMBER: 25490-0100
- (ix) TELECOMMUNICATION INFORMATION:
40 (A) TELEPHONE: (404) 818-3700
(B) TELEFAX: (404) 818-3799
- (2) INFORMATION FOR SEQ ID NO:1:
- 45 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
50 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

22

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

5 (vi) ORIGINAL SOURCE:
(A) ORGANISM: Guinea Pig

(ix) FEATURE:

10 (A) NAME/KEY: Peptide
(B) LOCATION: 1..19
(D) OTHER INFORMATION: /note= "Guinea Pig Raf-1"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

15 Ile Val Gln Gln Phe Gly Phe Gln Arg Arg Ala Ser Asp Asp Gly Lys
1 5 10 15

20 Leu Thr Gln

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

25 (A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

35 (v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:

40 (A) ORGANISM: Homo sapiens

(ix) FEATURE:

45 (A) NAME/KEY: Peptide
(B) LOCATION: 1..19
(D) OTHER INFORMATION: /note= "Human Raf-1"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

5 Ile Val Gln Gln Phe Gly Tyr Gln Arg Arg Ala Ser Asp Asp Gly Lys
 1 5 10 15

 Leu Thr Asp

10 (2) INFORMATION FOR SEQ ID NO:3:

 (i) SEQUENCE CHARACTERISTICS:

 (A) LENGTH: 19 amino acids
 (B) TYPE: amino acid
15 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

 (ii) MOLECULE TYPE: peptide

20 (iii) HYPOTHETICAL: NO

 (iv) ANTI-SENSE: NO

 (vi) ORIGINAL SOURCE:

25 (A) ORGANISM: Chicken

 (ix) FEATURE:

 (A) NAME/KEY: Peptide
 (B) LOCATION: 1..19
30 (D) OTHER INFORMATION: /note= "Chicken Raf-1"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

35 Ile Val Gln Gln Phe Gly Tyr Gln Arg Arg Ala Ser Asp Asp Gly Lys
 1 5 10 15

 Ile Ser Asp

40

(2) INFORMATION FOR SEQ ID NO:4:

 (i) SEQUENCE CHARACTERISTICS:

 (A) LENGTH: 148 amino acids
45 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

 (ii) MOLECULE TYPE: peptide

50

(iii) HYPOTHETICAL: NO

 (iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:

5 (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

10

Met Glu His Ile Gln Gly Ala Trp Lys Thr Ile Ser Asn Gly Phe Gly
1 5 10 15

15

Phe Lys Asp Ala Val Phe Asp Gly Ser Ser Cys Ile Ser Pro Thr Ile
20 25 30

Val Gln Gln Phe Gly Tyr Gln Arg Arg Ala Ser Asp Asp Gly Lys Leu
35 40 45

20

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

25

Lys Gln Arg Thr Val Val Asn Val Arg Asn Gly Met Ser Leu His Asp
65 70 75 80

Cys Leu Met Lys Ala Leu Lys Val Arg Gly Leu Gln Pro Glu Cys Cys
85 90 95

30

Ala Val Phe Arg Leu Leu His Glu His Lys Gly Lys Lys Ala Arg Leu
100 105 110

Asp Trp Asn Thr Asp Ala Ala Ser Leu Ile Gly Glu Glu Leu Gln Val
115 120 125

35

Asp Phe Leu Asp His Val Pro Leu Thr Thr His Asn Phe Ala Arg Lys
130 135 140

Thr Phe Leu Lys
145

40

(2) INFORMATION FOR SEQ ID NO:5:

45

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 217 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

50

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

25

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

5

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

15

Gly Phe Arg Cys Gln Thr Cys Gly Tyr Lys Phe His Glu His Cys Ser
1 5 10 15

Thr Lys Val Pro Thr Met Cys Val Asp Trp Ser Asn Ile Arg Gln Leu
20 25 30

20

Leu Leu Phe Pro Asn Ser Thr Ile Gly Asp Ser Gly Val Pro Ala Leu
35 40 45

Pro Ser Leu Thr Met Arg Arg Met Arg Glu Ser Val Ser Arg Met Pro
50 55 60

25

Val Ser Ser Gln His Arg Tyr Ser Thr Pro His Ala Phe Thr Phe Asn
65 70 75 80

Thr Ser Ser Pro Ser Ser Glu Gly Ser Leu Ser Gln Arg Gln Arg Ser
85 90 95

26

Thr Ser Thr Pro Asn Val His Met Val Ser Thr Thr Leu Pro Val Asp
 100 105 110
 5 Ser Arg Met Ile Glu Asp Ala Ile Arg Ser His Ser Glu Ser Ala Ser
 115 120 125
 Pro Ser Ala Leu Ser Ser Ser Pro Asn Asn Leu Ser Pro Thr Gly Trp
 130 135 140
 10 Ser Gln Pro Lys Thr Pro Val Pro Ala Gln Arg Glu Arg Ala Pro Val
 145 150 155 160
 Ser Gly Thr Gln Glu Lys Asn Lys Ile Arg Pro Arg Gly Gln Arg Asp
 165 170 175
 Ser Ser Tyr Tyr Trp Glu Ile Glu Ala Ser Glu Val Met Leu Ser Thr
 180 185 190
 20 Arg Ile Gly Ser Gly Ser Phe Gly Thr Val Tyr Lys Gly Lys Trp His
 195 200 205
 Gly Asp Val Ala Val Lys Ile Leu Lys
 210 215
 25

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 648 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 30

(ii) MOLECULE TYPE: protein
 35

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal
 40

(vi) ORIGINAL SOURCE:
 (A) ORGANISM: Homo sapiens
 45

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

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

Val Gln Gln Phe Gly Tyr Gln Arg Arg Ala Ser Asp Asp Gly Lys Leu
35 40 45

	Thr	Asp	Pro	Ser	Lys	Thr	Ser	Asn	Thr	Ile	Arg	Val	Phe	Leu	Pro	Asn	
	50						55					60					
5	Lys	Gln	Arg	Thr	Val	Val	Asn	Val	Arg	Asn	Gly	Met	Ser	Leu	His	Asp	
	65				70					75						80	
	Cys	Leu	Met	Lys	Ala	Leu	Lys	Val	Arg	Gly	Leu	Gln	Pro	Glu	Cys	Cys	
10				85						90					95		
	Ala	Val	Phe	Arg	Leu	Leu	His	Glu	His	Lys	Gly	Lys	Lys	Ala	Arg	Leu	
				100					105					110			
15	Asp	Trp	Asn	Thr	Asp	Ala	Ala	Ser	Leu	Ile	Gly	Glu	Glu	Leu	Gln	Val	
			115					120					125				
	Asp	Phe	Leu	Asp	His	Val	Pro	Leu	Thr	Thr	His	Asn	Phe	Ala	Arg	Lys	
	130						135					140					
20	Thr	Phe	Leu	Lys	Leu	Ala	Phe	Cys	Asp	Ile	Cys	Gln	Lys	Phe	Leu	Leu	
	145				150						155					160	
	Asn	Gly	Phe	Arg	Cys	Gln	Thr	Cys	Gly	Tyr	Lys	Phe	His	Glu	His	Cys	
25				165						170					175		
	Ser	Thr	Lys	Val	Pro	Thr	Met	Cys	Val	Asp	Trp	Ser	Asn	Ile	Arg	Gln	
			180						185					190			
30	Leu	Leu	Leu	Phe	Pro	Asn	Ser	Thr	Ile	Gly	Asp	Ser	Gly	Val	Pro	Ala	
		195						200					205				
	Leu	Pro	Ser	Leu	Thr	Met	Arg	Arg	Met	Arg	Glu	Ser	Val	Ser	Arg	Met	
	210					215						220					
35	Pro	Val	Ser	Ser	Gln	His	Arg	Tyr	Ser	Thr	Pro	His	Ala	Phe	Thr	Phe	
	225				230						235					240	
	Asn	Thr	Ser	Ser	Pro	Ser	Ser	Glu	Gly	Ser	Leu	Ser	Gln	Arg	Gln	Arg	
40				245						250					255		
	Ser	Thr	Ser	Thr	Pro	Asn	Val	His	Met	Val	Ser	Thr	Thr	Leu	Pro	Val	
			260						265					270			
45	Asp	Ser	Arg	Met	Ile	Glu	Asp	Ala	Ile	Arg	Ser	His	Ser	Glu	Ser	Ala	
		275						280					285				
	Ser	Pro	Ser	Ala	Leu	Ser	Ser	Ser	Pro	Asn	Asn	Leu	Ser	Pro	Thr	Gly	
	290					295						300					
50	Trp	Ser	Gln	Pro	Lys	Thr	Pro	Val	Pro	Ala	Gln	Arg	Glu	Arg	Ala	Pro	
	305				310						315					320	
	Val	Ser	Gly	Thr	Gln	Glu	Lys	Asn	Lys	Ile	Arg	Pro	Arg	Gly	Gln	Arg	

				325					330				335				
	Asp	Ser	Ser	Tyr	Tyr	Trp	Glu	Ile	Glu	Ala	Ser	Glu	Val	Met	Leu	Ser	
				340					345					350			
5																	
	Thr	Arg	Ile	Gly	Ser	Gly	Ser	Phe	Gly	Thr	Val	Tyr	Lys	Gly	Lys	Trp	
			355					360					365				
10	His	Gly	Asp	Val	Ala	Val	Lys	Ile	Leu	Lys	Val	Val	Asp	Pro	Thr	Pro	
		370					375					380					
	Glu	Gln	Phe	Gln	Ala	Phe	Arg	Asn	Glu	Val	Ala	Val	Leu	Arg	Lys	Thr	
	385					390					395					400	
15	Arg	His	Val	Asn	Ile	Leu	Leu	Phe	Met	Gly	Tyr	Met	Thr	Lys	Asp	Asn	
				405						410					415		
	Leu	Ala	Ile	Val	Thr	Gln	Trp	Cys	Glu	Gly	Ser	Ser	Leu	Tyr	Lys	His	
20				420					425					430			
	Leu	His	Val	Gln	Glu	Thr	Lys	Phe	Gln	Met	Phe	Gln	Leu	Ile	Asp	Ile	
			435					440					445				
25	Ala	Arg	Gln	Thr	Ala	Gln	Gly	Met	Asp	Tyr	Leu	His	Ala	Lys	Asn	Ile	
		450					455					460					
	Ile	His	Arg	Asp	Met	Lys	Ser	Asn	Asn	Ile	Phe	Leu	His	Glu	Gly	Leu	
	465				470						475					480	
30	Thr	Val	Lys	Ile	Gly	Asp	Phe	Gly	Leu	Ala	Thr	Val	Lys	Ser	Arg	Trp	
				485						490					495		
	Ser	Gly	Ser	Gln	Gln	Val	Glu	Gln	Pro	Thr	Gly	Ser	Val	Leu	Trp	Met	
35				500					505					510			
	Ala	Pro	Glu	Val	Ile	Arg	Met	Gln	Asp	Asn	Asn	Pro	Phe	Ser	Phe	Gln	
			515					520					525				
40	Ser	Asp	Val	Tyr	Ser	Tyr	Gly	Ile	Val	Leu	Tyr	Glu	Leu	Met	Thr	Gly	
		530					535					540					
	Glu	Leu	Pro	Tyr	Ser	His	Ile	Asn	Asn	Arg	Asp	Gln	Ile	Ile	Phe	Met	
	545					550					555					560	
45	Val	Gly	Arg	Gly	Tyr	Ala	Ser	Pro	Asp	Leu	Ser	Lys	Leu	Tyr	Lys	Asn	
				565						570					575		
	Cys	Pro	Lys	Ala	Met	Lys	Arg	Leu	Val	Ala	Asp	Cys	Val	Lys	Lys	Val	
				580					585					590			

[illegible]

CLAIMS

We claim:

5 1. An antigen of the membranous structure of the inner ear, wherein the antigen is reactive with antibodies from patients having Meniere's disease.

10 2 The antigen of claim 1, wherein the antigen is a protein or peptide selected from the group consisting of extracts of membranous inner ear proteins, fractions of extracts of membranous inner ear proteins, proteins purified from extracts of membranous inner ear proteins, recombinant proteins or peptides and synthesized proteins or peptides.

15 3. The antigen of claim 1, wherein the antigen is a protein or peptide having an amino acid sequence selected from the group consisting of SEQ.ID.NO.:1, SEQ.ID.NO.:2 and SEQ.ID.NO.:4.

20 4. A method of detecting Meniere's disease in an animal or human comprising the steps of:

25 (a) incubating a biological sample from the animal or human with a target substance under conditions sufficient to bind a target-binding substance in the biological sample to the target substance, wherein the target substance is a 28 kD antigen or a nucleic acid molecule encoding a 28 kD antigen of the membranous structures of the inner ear of a mammal and

30 (b) detecting the target-binding substance bound to the target substance.

5. The method of claim 4, wherein the target substance is a protein or peptide having an amino acid sequence selected from the group consisting of SEQ.ID.NO.:1, SEQ.ID.NO.:2 and SEQ.ID.NO.:4.

6. The method of claim 4, wherein the target-binding substance is an antibody.

7. The method of claim 4, wherein the target-binding substance is a nucleic acid probe.

8. The method of claim 4, wherein the amount of target-binding substance in the biological sample is quantitated.

9. The method of claim 4, wherein the biological sample is a biological fluid from an animal or human having symptoms of an autoimmune inner ear disease.

10. The method of claim 4, wherein the biological sample is a biological fluid from a patient having symptoms of Meniere's disease.

11. The method of claim 4, wherein the biological fluid is serum.

12. An assay kit for detecting Meniere's disease in a patient comprising:

(a) a solid phase having a 28 kD target substance bound thereto which reacts with a target-binding substance in a biological fluid from an animal or human having symptoms of Meniere's disease and

(b) means for detecting binding of the target binding substance to the target substance.

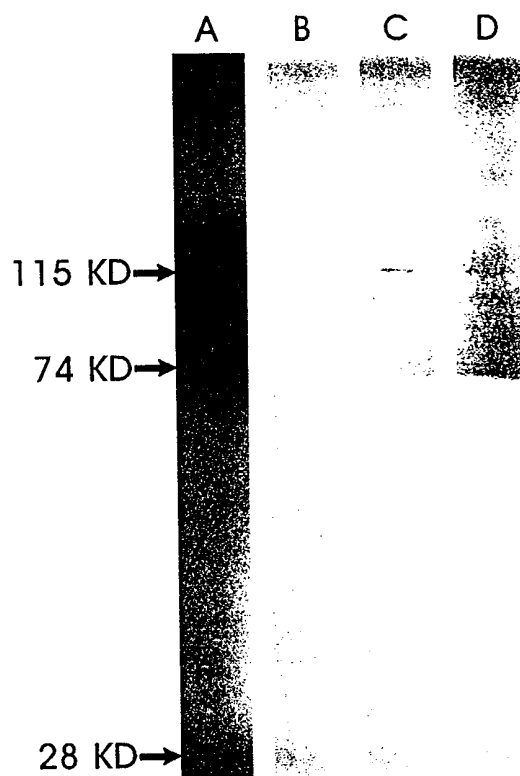
13. The assay kit of claim 12, wherein the target substance is a protein or peptide selected from the group consisting of extracts of membranous inner ear proteins, fractions of extracts of membranous inner ear proteins, proteins or peptides purified from extracts of membranous inner ear proteins, recombinant proteins or peptides and synthesized proteins or peptides.

14. The assay kit of claim 12, wherein the target substance is a protein or peptide having an amino acid sequence selected from the group consisting of SEQ.ID.NO.:1, SEQ.ID.NO.:2 and SEQ.ID.NO.:4.

1/3

FIGURE 1

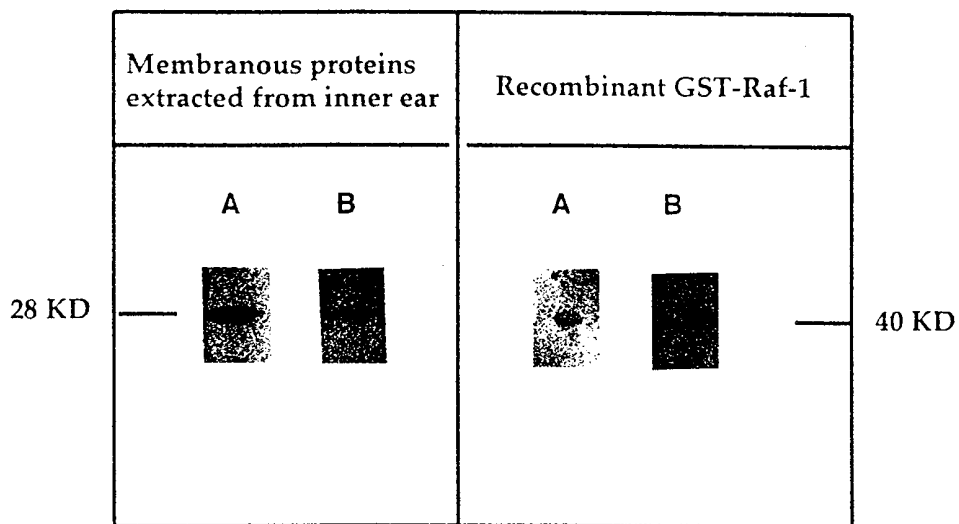
Western blot of protein extracts of membranous portion of the inner ear (Column A), neural portion of the inner ear (Column B), facial nerve (Column C) and brain (Column D) incubated with anti-GST-RAF-1 serum.



2/3

FIGURE 2

Western blot of membranous proteins extracted from the inner ear of the guinea pig (left panel) and of GST-Raf-1, SEQ.ID.NO.:4. (right panel) reacted with sera from Meniere's disease patients.



3/3

FIGURE 3

Summary of ELISA results demonstrating the reactivity of sera from Meniere's disease patients with GST-Raf-1 (SEQ.ID.NO.:4).

