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(54) Title: DIAGNOSIS OF MULTIPLE SCLEROSIS AND OTHER DEMYELINATING DISEASES

(57) Abstract

Methods of diagnosing demyelinating disease including multiple sclerosis (MS), subgrouping patients suffering from such disease including MS and/or monitoring the stage of activity of demyelinating disease including MS based on the detection, in a retroviral particle or an encapsidated virion–like particle, of the RGH virus–derived SEQ ID NO:1 or a subsequence or variant hereof and diagnostic agents comprising probes capable of hybridizing to such sequences, subsequences or variants, antibodies reacting with epitopes of proteins or peptides encoded by sequences, subsequences or variant and/or such epitopes for such methods are provided.

DIAGNOSIS OF MULTIPLE SCLEROSIS AND OTHER DEMYELINATING DISEASES

FIELD OF INVENTION

5 The present invention relates to a method of diagnosing demyelinating diseases, e.g. multiple sclerosis (MS) and chronic myelopathy, comprising detecting, in a sample material derived from a patient suspected of suffering from such a disease, the presence of an RGH virus nucleic acid (SEQ ID NO:1) or a subsequence or variant of said virus in a retroviral particle or an encapsidated virion-like particle by use of at least one

10 probe which specifically recognizes SEQ ID NO:1 or a subsequence or variant thereof, or by use of at least one primer which is capable of generating a nucleic acid fragment (SEQ ID NO:1) or a subsequence or variant thereof. There is also provided diagnostic agents for the diagnosis, subgrouping or monitoring of multiple sclerosis by the detection of the presence of SEQ ID NO:1 or a subsequence or variant thereof. The inven-

15 tion further relates to a method of diagnosing, subgrouping or monitoring multiple sclerosis comprising detecting the presence of an antibody reacting specifically with an epitope of a viral particle or an encapsidated virion-like particle containing SEQ ID NO:1 or a protein or a peptide encoded by SEQ ID NO:1 or by a subsequence or variant of said sequence.

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Within the scope of the invention is the combination of said methods and diagnostic agents with methods and diagnostic agents for the detection of other retroviruses such as an MSRV virus.

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GENERAL BACKGROUND

For several decades, the unexplained etiology of MS has been one of the great scientific challenges in medicine. MS is a demyelinating inflammatory disease affecting the

30 central nervous system. There is considerable evidence that MS is immune-mediated, although the antigen or antigens against which the immune response is directed have not yet been identified. MS is usually considered to be an autoimmune disease, but intensive research for several decades has not pinpointed the auto-antigen(s) in question. The extensive epidemiological studies of MS clearly indicate that, as well as ge-

netic factors, environmental agent(s) are involved, most probably virus(es). Virus(es) can initiate an autoimmune disease in various ways and can directly elicit the immune-mediated reaction causing the demyelination. MS also shares both clinical and histo-pathological features with retrovirus-mediated neurological diseases in animals and

5   human beings.

Currently, the MS diagnosis is also troublesome. Ever since Chargot recognized the characteristic clinical and pathologic features of the disease about a hundred years ago, no single test has been found consistently positive in 100% of clinically definite

10   MS. Presently, the MS diagnosis is usually based on three criteria:

- the appearance of lesions on magnetic resonance scans (MRI)
- the appearance of oligoclonal bands (OB) in analyses of IgG in the cerebrospinal fluid
15 - indications of demyelination in multimodality sensory evoked response studies (EP).

During recent years, several groups have performed research in order to develop inter alia diagnostic assays to be used in the diagnosis of multiple sclerosis, see e.g. WO

20   93/07259, WO 95/21256 and WO 97/06260. However, none of these patent applications have provided diagnostic assays with sufficiently high sensitivity and specificity to make them useful for clinical use.

Thus, a reasonably simple, straightforward diagnostic assay with a high sensitivity and

25   specificity would be a vast improvement on the present situation.

SUMMARY OF THE INVENTION

30   Accordingly, the present invention relates in a first aspect to methods of diagnosing demyelinating diseases including multiple sclerosis, subgrouping patients having a de-myelinating disease including multiple sclerosis and/or monitoring the stage or activity of demyelinating disease including multiple sclerosis, the methods comprising detect-ing, in a retroviral particle or an encapsidated virion-like particle present in a sample de-

rived from a patient suspected of suffering from demyelinating disease including multiple sclerosis, the presence of SEQ ID NO:1 or a subsequence or variant thereof. As used herein, the expressions "retroviral particle" and "encapsidated virion-like particle" include any kind of nucleic acid and/or peptide/protein fragment of such particles that are useful in

5 the methods and diagnostic agents of the invention.

In a further aspect the invention pertains to a diagnostic agent for the diagnosis, subgrouping or monitoring of demyelinating diseases including multiple sclerosis by the detection, in a sample material, of the presence of SEQ ID NO:1 or a subsequence or

10 variant thereof, the diagnostic agent comprising a probe which is capable of hybridizing specifically with SEQ ID NO:1 or with a subsequence or variant thereof that has a sequence identity with a similar part of SEQ ID NO:1 of at least 80%.

There is also provided a diagnostic agent for the diagnosis, subgrouping or monitoring of

15 demyelinating disease including multiple sclerosis by detection of the presence, in a sample material derived from a patient suspected of suffering from demyelinating disease including multiple sclerosis, of SEQ ID NO:1 or a subsequence or variant thereof, said agent comprising at least one primer that is capable of generating a nucleic acid fragment, the nucleotide sequence of which has a sequence identity with a similar part of SEQ ID NO:1

20 of at least 80%.

In yet other aspects of the invention there are provided a method of diagnosing, subgrouping or monitoring demyelinating disease including multiple sclerosis, the method comprising detecting the presence of a protein or a peptide encoded by SEQ ID NO:1

25 or by a subsequence or variant thereof, and a diagnostic agent for the diagnosis, subgrouping or monitoring of demyelinating disease including multiple sclerosis by detection of a peptide encoded by SEQ ID particle containing SEQ ID NO:1 or a peptide encoded by SEQ ID NO:1 or by NO:1 or by a subsequence or variant of said sequence, said diagnostic agent comprising an antibody reacting specifically with an epitope of a

30 protein or a peptide encoded by SEQ ID NO:1 or by a subsequence or variant of said sequence. There is also provided a method of diagnosing, subgrouping or monitoring demyelinating diseases including multiple sclerosis, the method comprising detecting the presence of an antibody reacting specifically with an epitope of a viral particle containing SEQ ID NO:1 or a peptide encoded by SEQ ID NO:1 or by a subsequence or

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variant of said sequence and a diagnostic agent for such a method that comprises an epitope of a viral a subsequence or variant of said sequence.

In a still further aspect, there is provided a method of isolating and/or purifying from a
5 sample material a retroviral particle or an encapsidated virion-like particle, the method comprising contacting the sample material with an antibody reacting specifically with an epitope of a protein or a peptide encoded by SEQ ID NO:1 or by a subsequence or variant thereof.

10 In further useful aspects, the invention pertains to the use of an isolated and/or purified RGH virus particle or encapsidated virion-like particle, or a fragment hereof, as a diagnostic agent, to an isolated and/or purified RGH virus particle or encapsidated virion-like particle, or a fragment hereof, for use as a medicament and to the use of an isolated and/or purified RGH virus particle or encapsidated virion-like particle, or a
15 fragment hereof, in the manufacturing of a medicament for the treatment or prevention of a demyelinating disease e.g by vaccination.

DETAILED DISCLOSURE OF THE INVENTION

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The present invention is based upon the finding that in a majority of multiple sclerosis patients investigated so far, nucleotide sequences related to a known endogenous retrovirus, RGH, have been identified in samples of retroviral particles or encapsidated virion-like particles. Noteworthy, all MS patients with active disease at the time of
25 sampling were RGH-positive. No similar sequences have been found in similar samples from healthy blood donors nor from patients with other autoimmune diseases. This virus, which has previously been described by Hirose et al., 1993, as two variants, RGH-1 (SEQ ID NO:2) and RGH-2 (SEQ ID NO:1), has not previously been associated with multiple sclerosis. The sequences found in the experiments described in Example
30 2 are 81%-95% identical with RGH-2. Within the scope of the invention are subsequences and variants of RGH-2 (SEQ ID NO:1). When in the present specification and claims reference is made to RGH, the reference is meant to include all subsequences or variants which have a sequence identity with a similar part of SEQ ID NO:1 of at

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least 80% as described and defined in the following. RGH-1 is an example of such a variant.

The experiments were designed so as to detect only virion-like encapsidated RGH
5   RNA. Any RGH sequence which is co-packaged in a virion may also be detected.
Thus, if a patient is infected with another retrovirus such as a Human Immunodefi-
ciency Virus (HIV) or a Human T-cell Leukemia Virus (HTLV), then the RGH sequences
may be co-packed without being an indication of the presence of multiple sclerosis. In
cases where such an origin of the detected RGH sequence is suspected, it will be
10 necessary to further investigate whether the patient in question is in fact infected with
another retrovirus and thus that the presence of the RGH sequences may be the result
of the co-packing instead of being of diagnostic significance with respect to multiple
sclerosis. The invention provides methods and diagnostic agents useful in that re-
spect.
15

In one aspect, the present invention relates to a method of diagnosing a demyelinating
disease including multiple sclerosis, the method comprising detecting in a retroviral
particle or an encapsidated viral-like particle present in a sample material derived from
a patient suspected of suffering from a demyelinating disease such as multiple sclero-
20 sis, the presence of RGH nucleic acid or a subsequence or variant thereof. In the pres-
ent context, the expression "sample material" includes any tissue or body fluid mate-
rial from which the retroviral particles or encapsidated virion-like particles may be iso-
lated or purified, including, but not limited to, a cell free serum sample or a sample of
plasma from heparinized, EDTA-treated or citrate-treated blood, a sample of cerebro-
25 spinal fluid and supernatants of cell cultures such as of mononuclear cells from hepa-
rinized, EDTA-treated or citrate-treated blood. The retroviral particles can be isolated
and/or purified based upon e.g. size, density, charge or specific surface determinants.
Examples of such methods are centrifugation in gradient media such as self-forming
Optiprep gradients or antibody-based affinity isolation or purification methods.
30

As used herein, the term "diagnosing" is not meant as an absolute term implying that
the diagnosis of a demyelinating disease such as multiple sclerosis can be based solely
upon the detection of the presence of SEQ ID NO:1 or a subsequence or variant
thereof in the retroviral particles or encapsidated viral-like particles, but rather that the

presence or absence of such sequences can supplement other signs of the disease and thereby aid in the diagnosis of multiple sclerosis or any other demyelinating disease that can be diagnosed by the method of the invention. Based upon the results in Example 2 it appears that a positive result is highly indicative of the person having multi-

5   ple sclerosis. Actually, the present results indicate a diagnostic specificity of 100% whereas the diagnostic sensitivity is 24/33 = 72.7%. It is contemplated that when run as a routine analysis, the diagnostic specificity of the method according to the invention will be in the range of 80% to 100%, and the diagnostic sensitivity in the range of 50% to 100%, such as 66% to 90%, e.g. 75%-80%. The invention thus in

10 its broadest aspect relates to a method of diagnosing multiple sclerosis which has a diagnostic sensitivity in the range of 50% to 100%, such as 66% to 90%, e.g. 75%-80%.

In general, the method is performed on samples of plasma, serum, cerebrospinal fluid
15 or any other body samples from a person suspected of having a demyelinating disease including multiple sclerosis in order to aid in the differential diagnosis towards other diseases with similar symptoms. However, it may also be performed on samples from persons not suspected of having multiple sclerosis or other demyelinating disease, i.e. for epidemiological studies. It is possible that samples from apparently non-MS indi-

20 viduals may turn out to be positive. It is likely that such a result is indicative of a person who may later develop multiple sclerosis or indicative of the person being infected with another retrovirus, the presence of the RGH sequence being a result of co-packaging. However, it cannot be ruled out that such a result may constitute a truly false positive indicating that the diagnostic specificity is not 100%. In any event, the

25 method will be within the scope of the present invention.

It is possible that the subpopulation of the patients suffering from multiple sclerosis or other demyelinating disease which has a negative test result represents a subgroup having a common pathogenetic cause of their disease which is different from the sub-

30 population with the positive result which may indicate that these two subgroups should be given different medical treatments as this finding may indicate a difference in the effect of the various treatments and/or indicate a different prognosis. Therefore, it may be of medical interest to determine, for an individual multiple sclerosis patient, whether he or she is RGH positive or RGH negative. Accordingly, the invention relates

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in a further aspect to a method of subgrouping patients having multiple sclerosis com-
prising detecting the presence of SEQ ID NO:1 or a subsequence or variant thereof in
a retroviral particle or an encapsidated virion-like particle, the particle being isolated or
purified as described above.

5

By the term "subsequence" is meant a sequence which comprises at least 100, 150,
200, 300 or 400 nucleotides. By the term "variant" is meant a sequence which is not
identical to SEQ ID NO:1, but which has a sequence identity of at least 80%, 85%,
90%, 95%, 98%, 99% or 99.5%. These two terms may be combined, i.e. the inven-
10   tion comprises detection of a variant of SEQ ID NO:1 which has a sequence identity of
at least 80% with a subsequence of SEQ ID NO:1 of at least 100, 150, 200, 300 or
400 nucleotides. The region of the RGH sequence which is most unique for RGH com-
prises the *gag*, *pol* and *env* regions as well as the intervening sequences between *gag*
and *pol* and between *pol* and *env* which comprises nucleotides 500 to 8214 of SEQ ID
15   NO:1.

As used herein, the term "sequence identity" indicates that the sequence is compared
with a similar part of SEQ ID NO:1. The best possible alignment of the sequences is
made and then the degree of identity is calculated. A computer program such as GCG,
20   Wiconson Package, Version 9.1, Genetics Computer Group (GCG), 575 Science Drive,
Madison, Wiconsin, USA 53711 using the defaults proposed in the program Bestfit
with respect to gap weight (50), length weight (3), average match 10,000, average
mismatch (-9,000) may be useful in this respect. A DNA sequence variant of RGH is
defined as having a sequence identity with a similar part of SEQ ID NO:1 of at least
25   80%.

When the term "nucleotide" is used in the present specification, it includes DNA and
RNA as well as combinations of DNA and RNA, PNA, and combinations of PNA with
DNA or RNA and similar embodiments such as described in WO 97/06260.

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The calculation of amino acid sequence identity is preferably based on the sequence of
a mature protein or peptide, i.e. without taking any possible leader sequence or modi-
fications such as phosphorylations or glycosylations of the amino acids into considera-
tion. The definition of sequence identity is as described above with respect to nucleo-

tides and the preferred range of sequence identity is the same. Preferably, the length
of the peptides will be in the range of 7 to 100 amino acids, such as 10 to 30 amino
acids.

5   The presence of SEQ ID NO:1, subsequences or variants thereof may be detected by
use of at least one probe which specifically recognizes SEQ ID NO:1 or a subsequence
or variant thereof, or by use of at least one primer that is capable of generating a nu-
cleic acid fragment (SEQ ID NO:1) or a subsequence or variant thereof. The design of
such probes or primers and the principles are well known in the art, see Dieffenbach
10   et al., 1993.

Suitable oligonucleotides which are useful as primers or probes are selected on the ba-
sis of the RGH sequence with the aid of e.g. OLIGO (Rychlik and Rhoads, 1989) and
hybridization conditions which are likely to lead to the hybridization of the selected
15   oligonucleotides with SEQ ID NO:1 or a subsequence or variant. The oligonucleotides
usually have a length of 6-100 bp, such as 10-30 bp.

After nucleic acid amplification such as reverse transcription PCR (RT-PCR) has been
performed, an amplification product is identified e.g. by gel electrophoresis and the
20   specificity of the product is confirmed e.g. by dideoxy sequencing so that it is con-
firmed that the product has sequence identity with a similar part of SEQ ID NO:1 of at
least 80%, such as at least 85%, 90%, 95%, 98%, 99% or 99.5%. Alternative
methods of nucleic acid amplification could be the ligase chain reaction (LCR) or nu-
cleic acid sequenced based amplification (NASBA) (Barany, 1991; Romano et al.,
25   1997).

If the sequence is likely to be almost identical to RGH, hybridization to a long probe
may suffice, or the specificity of the amplification product could be confirmed by di-
gestion with a selection of restriction enzymes known to have sites within the ex-
30   pected sequence of the amplification product followed by e.g. agarose or acrylamide
gel electrophoresis.

After such an initial validation procedure, those primers which specifically recognize
SEQ ID NO:1 or a subsequence or variant thereof under the chosen hybridization con-

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ditions will be considered useful in the method of the invention. Examples of useful probes or primers are SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10 as described herein as well as the primers described in Hirose et al., 1993.

5

Although the invention is not limited thereto, it is presently preferred that the primers or probes are at least 20 nucleotides long and at least 90% identical to a similar region on SEQ ID NO:1 and the hybridization conditions are as described in Example 1.

10 Within the scope of this invention is a diagnostic agent for the diagnosis, subgrouping or monitoring of multiple sclerosis or other demyelinating disease by the detection, in a sample material, of the presence of SEQ ID NO:1 or a subsequence or variant thereof which comprises a probe capable of hybridizing specifically with SEQ ID NO:1 or a subsequence or variant thereof, or at least one primer capable of generating a nu-

15 cleic acid fragment (SEQ ID NO:1) or a subsequence or variant thereof. By the term "a diagnostic agent" is meant a means for detecting the presence of a nucleotide sequence, an epitope, a protein, a peptide or an antibody as the case may be. The fundamental principles as well as the more specific details with respect to the various different kinds of diagnostic agents are well known to the person skilled in the art of

20 producing diagnostic agents.

In general, the nucleotide sequence, epitope, peptide or antibody will be bound to a carrier or support as described below, and e.g. antibodies from the sample binding to the nucleotide sequence or epitope can be detected using a secondary antibody capa-

25 ble of binding to the first bound antibody and provided with a label as described below.

The substance used as label may be selected from any substance which is in itself detectable or which may be reacted with another substance to produce a detectable

30 product. Thus, the label may be selected from radioactive isotopes, enzymes, chromophores, fluorescent or chemiluminescent substances, and complexing agents.

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Examples of enzymes useful as labels are β-galactosidase, urease, glucose oxidase, carbonic anhydrase, peroxidases (e.g. horseradish peroxidase), phosphatases (e.g. al-kaline or acid phosphatase), glucose-6-phosphate dehydrogenase and ribonuclease.

5   Enzymes are not in themselves detectable, but must be combined with a substrate to catalyze a reaction the end product of which is detectable. Thus, a substrate may be added to the reaction mixture resulting in a coloured, fluorescent or chemiluminescent product or in a colour change or in a change in the intensity of the colour, fluore-scence or chemiluminescence. Examples of substrates which are useful in the present

10 method as substrates for the enzymes mentioned above are H_2O_2, p-nitrophenylphos-phate, lactose, urea, β-D-glucose, CO_2, RNA, starch or malate. The substrate may be combined with e.g. a chromophore which is either a donor or acceptor.
Fluorescent substances which may be used as labels for the detection of the compo-nents as used according to the invention may be 4-methylumbelli-eryl-phosphate,

15 4-methylumbelliferyl-D-galactopyranoside, and 3-(p-hydroxyphenyl) propionic acid. These substances may be detected by means of a fluorescence spectrophotometer. Chemiluminescent substances may be peroxidase/eosin/EDTA, isoluminol/EDTA/H_2O_2 and a substrate therefor.

20 Chromophores may be o-phenylenediamine or similar compounds. These substances may be detected by means of a spectrophotometer.

Radioactive isotopes may be any detectable and in a laboratory acceptable isotope, e.g. ^{125}I, ^{131}I, ^{3}H, ^{32}P, ^{35}S or ^{14}C. The radioactivity may be measured in a

25 gamma-counter or a scintillation counter or by autoradiography followed by densito-metry.

Complexing agents may be Protein A, Protein G (which forms a complex with immuno-globulins), biotin (which forms a complex with avidin and streptavidin), and lectin

30 (which forms a complex with carbohydrate determinants, e.g. receptors). In this case, the complex is not in itself directly detectable, necessitating labelling of the substance with which the complexing agent forms a complex. The marking may be performed with any of the labelling substances described above.

As indicated above, this type of diagnostic agent normally comprises the nucleotide sequence or epitope bound to a carrier or support so that appropriate washing or other treatments as appropriate may be performed without risk of appreciable loss of the bound nucleotide sequence or epitope. The carrier or support is normally solid, and the

5   nucleotide sequence or epitope is bound to the carrier or support by any suitable mode of binding, such as hydrogen bonding, hydrophobic bonding, van der Waals' forces, covalent bonding, etc.

In an embodiment of the invention, the nucleotide sequence or epitope of the inven-

10 tion may be indirectly coupled to a solid support via a bridging compound or "linker". The linker, which is designed to link the solid support and the nucleotide sequence or the epitope, may be hydrazide, Protein A, glutaraldehyde, carbodiimide, or lysine.

The solid support employed is e.g. a polymer or it may be a matrix coated with a

15 polymer. The matrix may be of any suitable solid material, e.g. glass, paper or plastic. The polymer may be a plastic, cellulose such as specially treated paper, nitrocellulose paper or cyanogenbromide-activated paper, silicone, silica, or a polysaccharide such as agarose or dextran. Examples of suitable plastics are latex, a polystyrene, polyvinyl-chloride, polyurethane, polyacrylamide, polyvinylacetate and any suitable copolymer

20 thereof. Examples of silicone polymers include siloxane.

The solid support may be in the form of a tray, a plate such as a mitrotiter plate, e.g. a thin layer or, preferably, strip, film, threads, solid particles such as beads, including Protein A-coated bacteria, or paper.

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In a further aspect, the invention relates to a diagnostic agent which comprises an antibody as defined above, preferably a monoclonal antibody. The diagnostic agent may comprise the antibody coupled to a carrier or support. Alternatively, the diagnostic agent may be in the form of a test kit comprising an antibody as defined above in a

30 container. The diagnostic agent may be used in the diagnosis of multiple sclerosis, comprising contacting a sample of a body fluid, such as a blood sample or a sample of cerebrospinal fluid, with a diagnostic agent comprising an antibody as defined above, and determining the presence of any nucleotide sequence or epitope from the sample binding to the antibody. Optionally, the sample may have been purified by appropriate

means prior to contact with the diagnostic agent in order to increase the relative amount of nucleotide sequence or epitope present in the biological sample.

The diagnostic agent may be one which is suited for use in an agglutination assay in
5 which solid particles to which the antibody is coupled agglutinate in the presence of a nucleotide sequence or an epitope of the invention in the serum sample subjected to testing. In this type of testing, no labelling of antibody is necessary. For most uses it is, however, preferred that the antibody is bound to a carrier or support, using, e.g., the techniques described above in connection with diagnostic agents based on a nu-
10 cleotide sequence or epitope, and that the binding of nucleotide sequence or epitope from the sample to the diagnostic agent is detected using a secondary antibody which is capable of binding to the thus bound nucleotide sequence or epitope, the second antibody being provided with a label for the detection of bound secondary antibody. The substance used as label may be selected from any substance which is in itself de-
15 tectable or which may be reacted with another substance to produce a detectable product. Thus, the label may be selected from radioactive isotopes, enzymes, chromophores, fluorescent or chemiluminescent substances, and complexing agents, all of which are described in greater detail above.

20 In one preferred embodiment of the invention it is preferred that the antibody used in the diagnostic agent is a monoclonal antibody as this generally provides a higher precision and accuracy of the assay, at the same time possibly requiring less time to perform. Furthermore, a mixture of two or more different monoclonal antibodies may be employed as this may increase the detection limit and sensitivity of the test. The
25 monoclonal antibody may be obtained by the method described below. Antibodies possessing high avidity such as polyclonal antibodies may be selected for catching techniques.

The antibody used in the present method is preferably in substantially pure form puri-
30 fied according to suitable techniques well known in the art in order to improve the precision and/or accuracy of the assays of the invention.

The invention also encompasses novel probes and primers which are useful in said diagnostic agent, e.g. long probes as described above having a length of at least 100

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bp. When used for diagnostic or similar purposes the probe will usually be marked by means of e.g. a radioactive isotope, an enzyme such as peroxidase or alkaline phosphatase or be detectable by any other means.

5 Detection of virion-associated MSRV-RNA in serum of patients with multiple sclerosis has recently been published by Garson et al., 1998. In this paper it is indicated that it is presently not known whether MSRV itself is endogenous or exogenous. Although the present inventors do not want to be bound by any specific theory, it is possible that multiple retroviruses, e.g. RGH and MSRV virus, may be present simultaneously

10 or consecutively in multiple sclerosis patients. One possibility is that a general activation of endogenous retroviruses takes place in the disease process, another that MSRV stimulates the activation of RGH virus or that RGH stimulates the activation of MSRV virus.

15 It is thus not unlikely that RGH virus as well as MSRV virus may be present at the same time which may represent a means for increasing inter alia the diagnostic specificity and sensitivity of the test. Furthermore, the testing of the same samples for the presence of both viruses may represent a means for further investigating the pathogenesis of these viruses. If a general activation takes place it is likely that all the pa-

20 tients will be positive for both retroviruses at substantially the same time provided that for both retroviruses viral material is present in sufficient amounts to be detectable. Consequently, under these circumstances the diagnostic sensitivity of a test will be increased, if both viruses are searched for.

25 If, on the other hand, MSRV stimulates the activation of RGH virus it is likely that some of the patients positive for MSRV virus will be negative for RGH virus at the first investigation but at a later point in time change to be positive for RGH virus. Also, some of the patients tested positive for RGH virus will be negative for MSRV virus indicating that this virus is no longer present in detectable amounts. Such findings may

30 be useful in establishing the stage of the disease process and also with respect to determining the kind of medical treatment which should be given to the individual patient and/or determining the prognosis of the individual patient. Based upon the present results it seems that the presence of SEQ ID NO:1 or a subsequence or variant thereof

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indicates an active stage of disease. It is concievable that the same applies if is as-
sumed that RGH stimulates the activation of MSRV virus.

In a further embodiment, the invention relates to a method of monitoring the stage or
5   activity of multiple sclerosis or any other demyelinating disease, the method com-
prising detecting the presence of SEQ ID NO:1 or a subsequence or variant thereof in
a retroviral particle or an encapsidated virion-like particle that is present in a sample ma-
terial derived from a patient suffering from multiple sclerosis or another demyelinating
disease.
10
Within the scope of the present invention is a method of detecting SEQ ID NO:1 or a
subsequence or variant thereof which method further comprises detecting the pres-
ence of one or more non-RGH retrovirus(es), such as an HIV, HTLV, MSRV or HERV
virus, or a subsequence or variant of said retroviruses, in a retroviral particle, e.g. a
15   particle isolated or purified from cell free plasma samples as described above e.g. by
means of affinity purification. This method may also be useful for subgrouping or
monitoring patients having a demyelinating disease including multiple sclerosis.

One embodiment of the invention provides a diagnostic agent as described above
20   which comprises further sets of primers or probes, the individual set of probes each
being capable of hybridizing specifically with one other retrovirus, such as an HIV,
HTLV, MSRV or another retrovirus, or which individual set of primers is capable of
generating a nucleic acid fragment of a sequence coding for an HIV, HTLV, MSRV or
another retrovirus or a subsequence or variant thereof. Examples of such primers and
25   probes are well known within the art.

A preferred embodiment is a method wherein an MSRV virus is detected, said method
being accomplished by use of at least one probe which specifically recognizes an
MSRV virus or a subsequence or variant thereof, or a primer which is capable of gen-
30   erating an MSRV nucleic acid fragment or a subsequence or variant thereof. The terms
"subsequence" and "variant" are defined as outlined above with respect to RGH.

A number of different MSRV virus sequences which may be useful in the generation of
probes and primers as well as specific probes and primers for the detection of MSRV

15

virus are described in WO 97/06260 which is hereby incorporated by reference. A
useful sequence which is present in a retroviral particle from at least some patients
infected by the MSRV virus and/or in whom the MSRV virus has been reactivated, can
be selected from the group consisting of nucleotides having sequences identical, par-
5   tially or in total, or equivalent to the sequence defined in SEQ ID NO: 1, SEQ ID NO:
40, SEQ ID NO: 46, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 56,
SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61,
SEQ ID NO: 62, SEQ ID NO: 88, SEQ ID NO: 89 the sequences of the gene *pol* of
MSRV, the gene *env* of MSRV, the gene *gag* of MSRV and the sequences defined in
10   paragraphs (d)-(g) of claim 13 of WO 97/06260.

Under the circumstances where the RGH nucleotide sequence(s) is not a co-packaging
phenomenon, it is likely that the sequence codes for at least one protein or peptide. In
these cases an antibody which binds specifically to such a protein or peptide may be
15   raised in an individual or may be produced in an animal by conventional methods well-
known to the person skilled in the art. Within the scope of the invention are mono-
clonal as well as polyclonal antibodies raised against such RGH-encoded antigens. It
may be possible to detect a protein or a peptide encoded by SEQ ID NO:1 or a subse-
quence or variant of said sequence in plasma e.g. by means of an antibody reacting
20   specifically with an epitope of such a protein or peptide. Within the scope of the in-
vention is a peptide encoded by SEQ ID NO:1 or by a subsequence or variant of said
sequence, an antibody reacting specifically with such a peptide as well as a method of
detecting such a peptide in plasma and a diagnostic agent comprising such an anti-
body.
25

Purification of a protein or a peptide encoded by SEQ ID NO:1 or a subsequence or
variant of said sequence from plasma samples may be a useful means of purification.
Examples of procedures which may be useful for the purification include:
(i) immunoprecipitation or affinity chromatography with antibodies, (ii) affinity chro-
30   matography with a suitable ligand, (iii) other chromatography procedures such as gel
filtration, ion exchange or high performance liquid chromatography or derivatives of
any of the above, (iv) electrophoretic procedures such as polyacrylamide gel electro-
phoresis, denaturing polyacrylamide gel electrophoresis, agarose gel electrophoresis

16

and isoelectric focusing, (v) any other specific solubilization and/or purification techniques.

The invention further relates to a method of diagnosing, subgrouping or monitoring
5   multiple sclerosis or any other demyelinating disease, the method comprising detecting
the presence of an antibody reacting specifically with an epitope of one or more proteins encoded by SEQ ID NO:1 or a subsequence or variant of said sequence. Apart
from being useful in the diagnosis of multiple sclerosis, the presence of e.g. an increase in the antibody titer may be indicative of an activation or reactivation of the
10 disease process which may have implications with respect to treatment or prognosis.

Possible protein sequences of RGH are known from Hirose et al., 1993. Based upon
the diagnostic significance of the presence of an RGH nucleotide sequence in a retroviral particle, it is likely that it will also be possible to detect in plasma or serum samples
15 or in samples of cerebrospinal fluid an antibody reacting specifically with an epitope of
one or more proteins encoded by SEQ ID NO:1 or by a subsequence or variant of said
sequence and thereby provide or aid the diagnosis, subgrouping or monitoring of multiple sclerosis.

20 Epitopes of a protein or a peptide encoded by SEQ ID NO:1 or a subsequence or variant of said sequence may be deduced by methods well known in the art, cf. WO
97/06260. The epitope may constitute a consecutive subsequence of a protein or of a
peptide encoded by SEQ ID NO:1 or a subsequence or variant hereof, or be an epitope
which is present in a more spatial configuration e.g. including amino acids which are
25 positioned with some distance apart in the linear outline of the sequence of a protein
or a peptide. The epitope can also - entirely or partially - consist of compounds associated with one or more of the amino acids, should they be modified in various ways,
e.g. by glycosylation. The peptide comprising an epitope can, e.g., be synthesized by
the conventional Merrifield synthesis technique and may be coated or bound to a suit-
30 able carrier in order to provide a diagnostic agent useful for the detection of an antibody against an epitope of a peptide encoded by SEQ ID NO:1. Experiments for detecting an antibody reacting specifically with an epitope of a peptide encoded by SEQ
ID NO:1 or a subsequence or variant of said sequence are outlined in Example 4.

Within the scope of the invention is a diagnostic agent for the diagnosis, subgrouping or monitoring of multiple sclerosis or any ohter demyelinating disease by the detection of an antibody reacting specifically with an epitope of a protein or a peptide encoded by SEQ ID NO:1 or a subsequence or variant of said sequence as well as such a diag-

5   nostic agent which further comprises an epitope from one or more other retroviruses, such as an HIV, HTLV, MSRV or HERV virus epitope.

A useful epitope which is recognized by at least some sera from patients infected by the MSRV virus and/or in whom the MSRV virus has been reactivated, can be selected

10 from the group consisting of peptides encoded by any open reading frame found in MSRV or subsequences or variants thereof. As examples such peptides may have sequences identical, partially or in total, or equivalent to the sequence defined in the sequences SEQ ID NO: 39, SEQ ID NO:41 to SEQ ID NO: 44, SEQ ID NO: 63 and SEQ ID NO: 87 in WO 97/06260.

15

The combined test of the presence of an antibody reacting specifically with an epitope from an RGH protein or peptide and an antibody reacting specifically with an epitope of a peptide from at least one other retrovirus may provide supplemental information which is useful in the diagnosis of multiple sclerosis or another demyelinating disease,

20 the determination of disease stage, selection of medical treatment and the determination of the prognosis of the individual patient as outlined above with respect to diagnostic agents comprising probes or primers. Further, the presence of e.g. an increase in the antibody titer against MSRV may be indicative of an activation or reactivation of the disease process which may have implications with respect to treatment or progno-

25 sis.

It is concievable that an isolated and/or purified RGH virus particle is useful as a diagnostic agent for the purposes as described herein. Accordingly, in an interesting aspect of the invention, there is provided the use of such particles as a diagnostic agent.

30

Further concievable uses of an isolated and/or purified RGH virus particle according to the invention is its use as a medicament e.g. for the treatment or prevention of demyelinating diseases including multiple sclerosis or its use in the manufacturing of a medicament for the treatment of such diseases.

The invention is further illustrated in the following, non-limiting examples.

EXAMPLE 1

5

Establishment of RGH-producing cell lines from peripheral blood from MS patients

The following is a description of variants of the human endogenous retrovirus RGH as the retrovirus produced by spontaneously formed B-lymphoblastoid cell lines from peri-
10 pheral blood mononuclear cells from several MS patients.

Spontaneously formed B-lymphoblastoid cell lines from peripheral blood mononuclear cells from MS patients produce type C retrovirus-like particles in addition to the expression of Epstein-Barr virus (EBV) proteins and occasionally mature EBV particles
15 (Sommerlund et al., 1993; Munch et al., 1995).

The retrovirus particles possess reverse transcriptase activity and share a few antigenic determinants with HTLV-1, but are distinct from the known retroviruses at the antigenic level (Christensen et al., 1997). The B-lymphoblastoid cell lines were estab-
20 lished from venous blood from MS patients. The cell lines described in Table 1 arose spontaneously after long-term cultivation. The MS patients were from the Neurology Dept., Aarhus University Hospital; the Dermatology Dept., Marselisborg Hospital, Aarhus; the MS Hospital in Ry or the Dept. of Internal Medicine, Middelfart Hospital (all in Denmark). All blood samples were obtained with informed consent. Blood sam-
25 ples were obtained by drawing blood into Venoject VT100H tubes containing 150 u.s.p.u. sodium heparine (Meda, Denmark). The heparinized blood was subjected to Ficoll-Isopaque density gradient centrifugation. For establishing long-term cultured, spontaneously transformed cell lines, mononuclear cells were isolated, seeded at 20×10^6 cells/5 ml and cultivated in RPMI 1640 (Seromed) supplemented with 200 i.u./ml
30 penicillin (Leo), 0.2 mg/ml streptomycin (Rosco), 290 mg/ml glutamine (Sigma),
10 mM HEPES (Bioproduct) and 10% heat inactivated human serum (serum pool obtained from the blood bank at Skejby Hospital, Denmark) in Falcon Primaria bottles. After an initial period, the cells were subcultured three times a week at a density of 0.5×10^6 cells/ml. The cell lines arose spontaneously after long-term cultivation (more

than two months) and are continuously growing. The established cell lines were sup-
plemented with 10% heat inactivated fetal calf serum (Whitaker) instead of human
serum. The human origin of the line was confirmed by RFLP mapping and HLA-DQb
typing. The cell lines were tested for mycoplasmal contamination and found negative
5   by repeated analyses (standard Hoechst stain).

Table 1

MS B-lymphoblastoid cell lines				
Patient No.	Sex	Age, years	Diagnosis	Duration, years
MS1533	M	30	Chronic progressive myelopathy	7
MS1844	F	40	Benign MS	11
MS1845	M	33	Relapsing-Remitting MS	2
MS1851	F	24	Primary progressive MS	2

Expression of RGH variants in retroviral particles in B-lymphoblastoid cell lines estab-
lished from MS patients

30   The retrovirus produced by the spontaneously formed B-lymphoblastoid cell lines from
peripheral blood mononuclear cells from several MS patients can be identified at the
nucleic acid level by concentration and purification of the retroviral particles, isolation
of the genomic RNA in the retroviral particles, RT-PCR with the genomic RNA as tem-
plate and subsequent sequencing of the obtained amplicons. Retroviral particles were
35   purified from cell line supernatants by ultracentrifugation in self-forming Optiprep gra-
dients.

20

1.3-2 litres of suspension cultures were centrifuged at 4°C for 30 minutes at
2500 × g. The cell-free supernatant was aspirated, layered on a cushion of 4 ml of
50% Optiprep in NaCl/Hepes (Nycomed) in 60 ml tubes and ultracentrifuged at 4°C for
2 hours at 45,000 × g (Beckmann ultracentrifuge). Optiprep is an iodinated, nonionic

5   density gradient medium (Nycomed Pharma, Norway) which, in contrast to sucrose,
maintains intact retroviral particles.

The cushion and proximal overlaying retrovirus-containing medium was mixed, filtered
through a 0.45 µm filter (Acrodisc 32, Gelman Sciences) to remove any cellular de-

10 bris, adjusted to 20% Optiprep and centrifuged in 11.2 ml Optiseal (Beckmann) tubes
in a Beckmann NVTi rotor at 364,000 × g for 3.5 hours. 400-500 µl fractions (12
drops) were collected after centrifugation. RNA was purified from the 5-6 fractions
containing RT activity. Poly-A RNA was purified by lysing these fractions directly in 5
volumes of lysis-binding buffer (Dynal, Norway), adding 100 µl of beads/sample

15 (mRNA Direct kit, Dynal, Norway) and following the manufacturer's instructions. If not
used directly, the RNA-coupled beads were stored in 80% EtOH at -80°C. Before use,
each RNA sample was treated in a 20 µl final volume with amplification grade DNAse
(Life Technologies) according to the manufacturer's instructions. After cooling on ice,
the buffer was exchanged with 10 µl of DEPC-treated ddH$_2$O, and 2 µl aliquots were

20 used for each cDNA synthesis.

Using RT-PCR on retroviral RNA templates isolated from cell culture supernatants as
described above, with subsequent sequencing of the cloned amplicons, several frag-
ments with homology to RGH were identified (Hirose et al., 1993) in the retroviral par-

25 ticles produced by the cell lines indicated in Table 1 (MS1533, MS1844, MS1845 and
MS1851).

RT-PCR was performed using the GeneAmp RNA PCR kit (Perkin Elmer) according to
the manufacturer's suggestions, except that 1 unit of Taq polymerase was used per

30 reaction. All relevant controls were included. First strand cDNA synthesis was primed
with random hexamers, in conjunction with the bead-oligo-dT complexed RNA tem-
plate.

21

Primer sets for the PCR amplification of *gag* and *env* were as follows:

5'-CTTTTATTACCCAATCTGCTCCCGAYAT-3' (SEQ ID NO:3)

5   5'-TTAGTGGTGGACAGTCTCTTTTCCARTG-3' (SEQ ID NO:4) (45°C), (Medstrand et al., 1992)

5'-ATTTTATTACCCAATCTGCTCCAAACAT-3' (SEQ ID NO:5)

10   5'-AGGTGAGTTGAACAGTCTGATTTTTA-3' (SEQ ID NO:6) (50°C)

5'-CGTTTACATATCACTCCCTTCCTAGTCTCTGT-3' (SEQ ID NO:7)

5'-GCATTAACCTTGACTATGTCTTTAGCTCCAG-3' (SEQ ID NO:8) (50°C).

15

5'-GATCCTCCCCACTGGGTTCACCATT-3' (SEQ ID NO:9)

5'-GGAAGTATTGGAGGGTGCCCTGCC-3' (SEQ ID NO:10) (60°C).

20   All reactions were run in a Perkin Elmer DNA Thermocycler. Conditions for first strand synthesis: 5 minutes at room temperature, 45 minutes at 42°C, and 5 minutes at 95°C, followed by cooling to 4°C. PCR: one cycle for 2 minutes at 95°C was followed by 45/50 cycles with annealing temperatures as indicated above: 1 minute at 94°C, 2 minutes annealing, 3 minutes at 72°C with 10 seconds extension. This was followed

25   by a final extension for 7 minutes at 72°C. PCR products were analyzed by agarose gel electrophoresis, and each product was cloned in pUC using the SureClone Ligation kit (Pharmacia) and sequenced using the ABI Prism kit (Applied Biosystems) on an automatic sequenator (ABI 377).

30   5-30 clones from each product were analyzed for each positive RT-PCR. Clones were sequenced in both orientations and appeared to be variants of RGH-2.

EXAMPLE 2

Expression of RGH variants in retroviral particles in peripheral blood from MS patients

5 The retrovirus produced by the spontaneously formed B-lymphoblastoid cell lines from
peripheral blood mononuclear cells from MS patients can also be identified at the nu-
cleic acid level in clinical samples, i.e. peripheral blood from a large proportion of MS
patients, by concentration and purification of the retroviral particles in the plasma or
serum fraction, isolation of the genomic RNA in the retroviral particles, RT-PCR with
10 the genomic RNA as template, and subsequent sequencing of the obtained amplicons.

RGH sequence variants at particle level were demonstrated to be associated with MS
in vivo by performing RT-PCR analysis on clinical specimens, i.e. blood samples from
MS patients, from patients with autoimmune diseases, and from healthy controls.
15 RNA extracted from particles isolated from plasma was assayed by RT-PCR with sub-
sequent sequencing of the cloned amplicons.

All blood samples were obtained with informed consent from either healthy volunteers
or from patients at the Neurology Dept., Aarhus University Hospital; the Dermatology
20 Dept., Marselisborg Hospital, Aarhus; the MS Hospital in Ry; or the Dept. of Internal
Medicine, Middelfart Hospital (all in Denmark). Blood samples were drawn at the re-
spective clinics into VT-100SCPDA Venoject tubes containing 1.4 ml of CPD-adenine
solution (Meda, Denmark) and delivered by hand immediately after drawing. To avoid
contamination from nucleic acids, the samples were processed immediately without
25 any freeze-thawing procedures (plasma obtained by standard separation is not entirely
cell-free and freeze-thawing will disrupt remaining cells and release intracellular nucleic
acids).

All samples were processed in the inventors' laboratory. Cell- and debris-free plasma
30 samples were obtained from the citrate blood samples (40 ml of blood → appr. 10 ml
of plasma). After the initial separation by centrifugation at room temperature for 15
min. at 500 x g, the plasma was aspirated and subjected to another centrifugation at
4°C for 30 minutes at 1000 x g to pellet any remaining cells. The cell-free plasma was
aspirated and samples layered on 50% Optiprep, ultracentrifuged and filtered as de-

scribed in Example 1. The retrovirus/Optiprep mixture was directly lysed in 5 volumes
of lysis-binding buffer (Dynal, Norway) and poly-A RNA was purified on 50 l of
beads/sample using the mRNA-Direct kit (Dynal, Norway) as described in Example 1.

5   If not used directly, the RNA-coupled beads were stored in 80% EtOH at -80°C. Be-
fore use, each RNA sample was treated in a 20 µl final volume with amplification
grade DNAse (Life Technologies) according to the manufacturer's instructions. After
cooling on ice, the buffer was exchanged with 10 µl of DEPC-treated ddH$_2$O, and 2 µl
aliquots were used for each cDNA synthesis.

10

The RT-PCR conditions were as described for RT-PCR in Example 1. PCR products
were analysed by agarose gel electrophoresis, and each product was cloned in pUC
using the SureClone Ligation kit (Pharmacia) and sequenced using the ABI Prism kit
(Applied Biosystems) on an automatic sequenator (ABI 377). 5-30 clones from each

15 product were analyzed for each positive RT-PCR. The results of the analyses, including
characterization of the patients included in the study, are shown in Table 2.

RGH sequence variants in particles were thus demonstrated in cell-free plasma sam-
ples from 24 of 33 MS patients. The sequences were absent in all of 29 cell-free

20 plasma samples from patients with autoimmune diseases as well as in all of 20 cell-
free plasma samples from healthy controls. The expression of RGH sequence variants
in particles is thus specific for MS. All MS patients with active disease at the time of
sampling were RGH-positive.

25 A large subgroup of MS cases is thus associated with the production in the blood of
retroviral particles containing RGH sequence variants.

24

Table 2

All MS patients had clinically definite MS, the MS type and disease activity is indicated. Chronic progressive MS with no stationary phase, and exacerbations in relapsing/remitting MS were defined as active MS. RR: relapsing-remitting, SP: secondary
5 progressive, PP: primary progressive, SLE: systemic lupus erythematosus.

RGH positive by RT-PCR on plasma					
Type	No. of patients	Sex	Age, years	Diagnosis	Duration, years
Multiple	6	F	16 - 50	RR (2 active)	1 - 11
Sclerosis	2	M	50 - 52		9 - 15
n = 24					
	5	F	34 - 56	SP (2 active)	4 - 20
	7	M	40 - 72	(2 active)	9 - >20
	4	F	33 - 47	PP (3 active)	3 - 7
RGH negative by RT-PCR on plasma					
Multiple	1	F	35	RR	10
Sclerosis	1	M	30		1
n = 9					
	1	F	34	SP	15
	3	M	40 - 50		9 - 15
	2	F	31 - 36	PP	2 - 5
	1	M	36		6
Healthy	13	F	24 - 53		
Control	7	M	27 - 34		
n = 20					
Patient	3	F	56 - 62	Diabetes mellitus	
Control	5	M	50 - 63		
n = 29					
	1	M	68	Hairy cell leukemia	
	1	F	42	Prurigo nodularis Hide	
	2	F	35 - 61	Colitis ulcerosa	
	2	M	31 - 32		
	1	F	52	SLE	
	1	F	55	Arteritis temporalis	
	1	F	53	Rheumatoid arthritis	
	4	M	47 - 61		
	1	F	63	Thyreotoxicosis	
	1	M	55		
	1	M	47	Sarcoidosis	
	1	M	50	Collagenosis	
	1	F	58	Polymyositis	
	1	F	76	Myelomatosis	
	1	M	58	Leukemia/lymphoma	
	1	F	72	Polymyalgia rheumatoides	

25

EXAMPLE 3

Characterization of expressed RGH sequence variants

5   In Example 1 and Example 2 expressed RGH retroviral sequences could be identified in
retroviral particles produced by spontaneously formed B-lymphoblastoid cell lines from
peripheral blood mononuclear cells from several MS patients (Example 1) and in clini-
cal samples, i.e. the particulate fraction of plasma from peripheral blood from a large
proportion of MS patients (Example 2) by RT-PCR with *gag* and *env* primers on geno-
10   mic RNA from retroviral particles.

RGH belongs to the RTVL-H/HERV-H (priming of transcription with tRNA^{His}) family of
endogenous retroviruses. This family of type-C retroviruses is related to the onco-
retroviruses human T-cell leukemia virus (HTLV-1/-2), to bovine leucosis virus (BLV),
15   and to ERV-9. A genomic RTVL-H clone which potentially encodes functional proteins
has been described (Wilkinson et al., 1991).

RGH particles as such have not been reported previously. Two RGH clones have been
described: RGH-1 and RGH-2. The published RGH-1 clone is 4869 bp long and en-
20   compasses a partial *pol* and an *env* 3'LTR region and thus lacks *gag*, whereas the pub-
lished RGH-2 clone is 8715 bp long and includes the complete coding potential: 5'LTR
gag-pol-env 3'LTR. They are present in about 100 copies/haploid genome (Hirose et
al., 1993). The majority of cDNA clones isolated from MS patient material are closely
related to the full-length clone RGH-2 as they contain *gag* sequences.
25
Each and every one of the genomic RGH copies in each and every individual person's
genome represents a DNA sequence variant of RGH as defined above, with the poten-
tial of being transcribed to an RNA sequence variant.

30   Examples of RGH sequence variants are listed in Table 3. The sequence variants were
obtained using the RT-PCR strategy outlined in Example 1 and Example 2 (Table 3).
Examples of expressed RGH sequence variants are listed in Table 3 below.

Table 3

SEQ ID NO:11	CCCAATGCAA	CTAATCCCAA	ATCTTCCTCC	TTTCCCTCCC	GCCTGTCCCC	TCAGTACCAA
	CCCCAAGCGT	CGCTGAGTCT	TTCTAATCTT	CCTTTTCTAC	AGACCCATCT	GACCTCTCCC
	CTCCTCACCA	GGTCGAGCTA	GGTCCCAATT	CTTCTTCAGC	CTCCACTCCT	CCACCCTATA
	ATCCTTTTAT	CACCTCCCCT	CCTCACACCT	GGTCCAGCTT	ACAGTTTTCGT	TCTGCGACTA
	GCCTTCCCCC	ACCTGCCCAG	CAATTTCCTC	TTAAAAAGGT	GG	
SEQ ID NO:12	TCCAGTGCAA	CTCATCCCAA	ATGTTCCTTC	TTTCCCTCCC	ATCTGTCCCC	TCAGTACCAA
	CCCCAAGCGT	CACTGAGTCT	TTCTAATCTT	CCTTTTGTAC	AGACCCATCT	GACCTCTCCC
	TTCCTCCCCA	GGTCGTCCTC	GCCAGGCCGA	GCTAGGTCCC	AATTCTTCCT	CAGCCTCCAT
	TCCTCCACGC	TGTAATCTTT	TTATCACCTC	CCCTCCTCAC	ACCTGGTCCG	GCTTACAGTT
	TCGTTCTGTG	ACTAGCCCTC	CCCCACCTGC	CCAGCAATTT	ACTCTTAAAA	AGGCGG
SEQ ID NO:13	CCCGACATTA	AATAAAGCTC	CAAAAATTAG	ATTCCAGCCC	GGAAACCCCA	CAACAGGACT
	TAACCTCACC	TTCAAGGTGT	ACAATAATAG	AGAGGAGTCT	CAATTCTTGC	CTCTGCTGTG
	AGAGAAACCC	CAGCCACATC	TCCAGCACAC	AAGAACTTCA	AAATGCCT	
SEQ ID NO:14	CCAAACATTA	AATAAAACTC	CAAAAATTAA	ATTCCGGCCC	TCAAACCCCA	CAACAAGGCT
	TAATTAACCT	CACCTTCAAG	GTGTTCAATA	ACAGAGTAGA	GGCAACCAAG	TAGCAATGTA
	TTTCTAAGTT	GCAATTCCTT	ACCTCCACTG	TAAGACAAAC	CCCAGCCACA	TCTCCAGCAC
	ACAAGAACTC	CAAATGCCCG				
SEQ ID NO:15	CCAGAATAAA	GCTGTGTCCA	TCGGACAGCC	AGCCTAATCC	CTCCTCTTCC	TCCTGGAAGT
	CACAAGTATT	CTCCCCTACT	TCCCTTAAAC	TCACTCGTAT	TTCTGAAGAA	CAGTAATAGC
	CCTTATGAGC	CTAATACATC	CCTTCATTCT	ATTAGATCTG	TTCGTCCTTA	CCCTACTTTT
	TGCAACAGGG	CTTTACGAAG	TCACCCTNAC	CACTTGGCCT	GATCCCCAAA	AAACTAGTCA
	ACCCTACTAT	CTTCTGTCTA	GTCATACTCC	TATTCTCCAT	TCTCAANTAC	TTATAAATGC
	CCTACTCTTG	TTTCCACTGC	TGGTTTANAC	TGTTTCTTCA	AGCCATCACA	GCTGATATCT
	CTTGGTGCTA	TCCCGCAACT	GCCACTCTTA	ACTCCCTCTT	AGAGTGGATA	GATGATCTTT
	GC					
SEQ ID NO:16	CCAGAATAAA	GCTCTGTCCA	TCAGACAGCC	AGCCTAATCC	CTCCTCTTCC	TCCTGGAAGT
	CGCAAGTACT	CTCCCCTACT	TCCCTTAAAC	TCACTCATAT	TTCTGAAGAA	CAGTAATAAC
	CCTTATGAGC	CTAATACATC	CCTTCATTCT	GTTAGGTCTA	TTCGTCCTTA	CCCTACTTTT
	TGCAACGGGG	CTTTATGACG	TCACCCCCAC	CACTTAGGCT	GAGCCCCAAA	AAACTGTCA
	TCCCTACTAT	TTTCTGTCTA	GTCATACTCC	TATTCTCTGC	TCTTAACTAC	TTATAAATGC
	CCTACTCTTG	TTTACACTGC	CGGTTTACAC	TGTTTCTTCA	AGCCATCACA	GCTGATATCT
	CTTGGTGCTA	TCCCCAAACC	ACTACTCTTA	TTCCCTCTTA	GAGTGGGTAG	ATGATCTTTG
	C					

EXAMPLE 4

The present invention also relates to diagnosis and prognosis by immunological means
such as characterization of the RGH-associated antibody response or characterization
of RGH-associated antigens in samples from patients as well as other immunological
aspects such as production of RGH-specific antibodies and RGH encoded peptide anti-
gens for diagnostic and purification purposes.

RGH encoded antigens

RGH encoded peptide antigens can be acquired by different means, e.g. by chemical
synthesis of peptides deduced from the nucleotide sequence or by purification from
different sources such as e.g. various expression systems based on RGH nucleic acid
sequences or by purification from patient samples or patient derived cell cultures. As
an example, Optiprep-purified RGH particles from 800 ml supernatant from various cell
lines are separated by 4-20% SDS-PAGE, transferred to a membrane, cut in strips and
screened against a panel of sera from MS patients as well as controls thus providing a
means for selecting suitable epitopes for further investigation.

Having defined peptides with an amino acid sequence derived from the known se-
quences of RGH, e.g. the *gag* and *env* regions, RGH subsequences or sequence vari-
ants, the peptide comprising an epitope can be chemically synthesized, e.g. by the
conventional Merrifield synthesis technique.

Peptides or proteins may be produced in commercially available prokaryotic or eukary-
otic expression systems. Typical prokaryotic expression systems are based on *E. coli*
whereas typical eukaryotic systems are based on fungal cells including yeast cells, or
other eukaryotic cells such as e.g. baculovirus-controlled expression in insect cells. A
prokaryotic expression system (GST Gene Fusion System, Pharmacia Biotech) is de-
scribed in the following: Based on the sequence of RGH, RGH subsequence or sequen-
ce variant: two complementary oligonucleotides with suitable cloning sites at the 5'
and 3' end are synthesized. The oligonucleotides typically correspond to sequences
encoding 10 - 30 amino acids. The oligonucleotides are annealed by denaturation. The

28

annealed linear double-stranded DNA fragment produced in this way is processed with the appropriate restriction enzymes and cloned in an appropriate vector. The fragment/vector clone is transformed into a suitable *E. coli* host and expression is induced by adding IPTG to a growing culture. The expressed peptide/protein has a glutathione

5   tag, which enables the purification of the expressed peptide/protein on a glutathione sepharose column. The tag is subsequently removed by protease digestion.

RGH-derived antigens may be purified by different methods from patient or cell culture samples e.g. from retroviral particles purified according to the methods outlined in Ex-

10 amples 1 and 2. The RGH-derived antigens can be purified by e.g size-dependent separation, charge-dependent separation, separation based on hydrophobicity or affinity chromatography and using methods based on antibodies e.g. as outlined in Harris and Angal, 1990 and Harlow and Lane, 1988.

15 The purified RGH-derived peptide or protein is useful for e.g. generation of antibodies and as a binding component in an ELISA.

Characterization of the RGH-associated antibody response

20 The RGH-associated antibody response is most easily characterized e.g. by using RGH-derived peptides purified as outlined above. Serologic reactivity can e.g. be characterized as follows:

a) Serologic reactivity characterized by a standard peptide ELISA: Sera can be ob-

25 tained by standard procedures and the peptide may be coupled to a carrier as outlined in Harlow and Lane, 1988, or used for passive coating of microtiter plates. Free binding sites are then blocked and the immuno-reactivity of the sera is determined in a standard indirect ELISA. All sera are assayed as ten-fold dilution series in duplicate.

30 An RGH-associated antibody response was demonstrated to be associated with MS by performing peptide ELISAs on clinical specimens, i.e. blood samples from MS patients, from patients with autoimmune diseases and from healthy controls.

29

All serum samples were obtained from blood samples as described in Example 2, except for the type of Venoject tubes, which in this case was VT-100UX Venoject without additions. After the initial separation by centrifugation at room temperature for 15 min. at 500 x g, the serum was aspirated, aliquoted into 1 ml portions and stored at -
5   70°C.

The peptides were synthesized by Genosys Biotechnologies (Europe) Ltd., London Road, Pampisford, Cambridge, UK, at more than 80% purity and used for passive coating of FluoroNunc MaxiSorp microtiter trays (NalgeNunc International, Life Tech-
10 nologies, Denmark) at a concentration of 0.1 µg or 1 µg/100 µl/well in coating buffer (15 mM Na_2CO_3, 35 mM $NaHCO_3$, 0.2% NaN_3, pH 9.6). The plates were incubated in a wet chamber at room temperature for 48 hrs, washed 3 times with 200 µl/well TBS-Tween (20 mM Tris-HCl, 137 mM NaCl, 0.05% Tween 20) and incubated with 100 µl serum/well for 24 hrs at 4°C. The sera were diluted at 1:20, 1:25, 1:250 and 1:500
15 in TBS-Tween. The sera were aspirated and the plates were washed three times in TBS-Tween before adding 100 µl bionylated rabbit anti-human IgG (Dako E0482)/well diluted 1:1000 in TBS-Tween. The plates were washed 3 times with TBS-Tween and 100 µl/well of Eu-labelled streptavidin (1244-363; LKB, Wallac, Turku, Finland) was added at 1:1000 dilution in TBS-Tween supplemented with 25 µM EDTA. 200 µl/well
20 of 10 µM diethylene-triaminepentaacetic acid (LKB, Wallac), 0.1% Triton X-100, 15 µM 2-naphtoyltrifluoroacetone in 0.1 M acetate, pH 3.2, was added. After gently shaking the plates for 5 minutes, the fluorescence was measured using a time-resolved plate fluorometer (LKB, Wallac).

25 Examples of RGH-encoded synthetic peptide antigens used in the ELISA are shown in Table 4 below:

Table 4. RGH-encoded synthetic peptide antigens

SEQ ID NO:17	GLDLLTAEKGGLCIFLNE
SEQ ID NO:18	STHLAATSRAPGTLAQG
SEQ ID NO:19	QPGLVYDNIKKLKERDQKL
SEQ ID NO:20	VLQAKVDSLAAVVLQNCR

30

SEQ ID NO:21	YKKPSPVHGSFGSNPET
SEQ ID NO:22	FITQSAAPNIKIKLQKLNSGP
SEQ ID NO:23	LQNRCGLDLLTAEKGGLCI
SEQ ID NO:24	DNIKKLKERDQKLANQAS

Table 5 summarizes the results for the peptides shown in Table 4.

Table 5. Testing of peptide antigens shown in Table 4

5

Equal numbers of MS sera and control sera were tested. MS: sera from MS patients; C: sera from healthy controls and from control patients with other autoimmune diseases; n: number of sera; DF: dilution factor; MS:C: average fluorescence of MS sera relative to control sera; ER Elisa ratio: individual readings relative to mean of controls; 10 MS:C >1 and/or ER >1 indicates a higher MS than control response.

	DF	n	MS:C	ER(C) >1	ER(MS) >1
SEQ ID NO:17	1:20	40	0.9	36%	30%
	1:200	56	0.8		
SEQ ID NO:18	1:25	50	1.2	33%	39%
	1:250	11	1.5		
SEQ ID NO:19	1:25	50	1.3	37%	55%
	1:250	11	1.5		
SEQ ID NO:20	1:25	50	1.1	27%	37%
	1:250	11	1.4		
SEQ ID NO:21	1:25	40	1.1	28%	32%
	1:250	40	1.0		
SEQ ID NO:22	1:25	40	1.2	30%	45%
	1:250	40	1.2		
SEQ ID NO:23	1:25	40	1.1	28%	23%
	1:500	40	1.0		
SEQ ID NO:24	1:25	40	1.2	21%	35%
	1:500	40	1.1		

b) In the experiments outlined in Example 3, the RGH sequence variants were found in
particles from plasma samples from all patients with active disease at the time of
sampling. This indicates that the production of RGH into the bloodstream is associated
with disease activity. The activity of the MS disease may be monitored by following

5   the changes in RGH production either by establishing a quantitative RT-PCR in essence
as outlined in Example 3 but with the addition of either an extra, competing template
or by the addition of primers amplifying a household transcript (as outlined in PCR pro-
tocols, a guide to methods and applications. edited by Micael A. Innes, D. H. Gelfand,
J. J. Sninsky, T. J. White, Academic Press, Inc. Harcourt Brace Jovanovich, Publish-

10 ers, 1990) or by following the changes in seroreactivity towards retroviral antigens
(both IgM and IgG). An MS attack or severe progression is thus reflected in an in-
crease in RGH transcripts or by an increase in serologic reactivity towards RGH vari-
ants.

15 c) Serologic reactivity characterized by SDS-PAGE followed by Western blotting:

Antibody reactivities towards RGH-derived antigens can be examined by standard
Western blotting procedures. It is also possible to perform inhibition studies by prein-
cubation of antibody and antigen before application to the blot.

20
Characterization of RGH-associated antigens in samples from patients

RGH-derived antigens can be purified, identified, and characterized from patient sam-
ples, e.g. by methods as outlined above. Specifically, the antigens can be identified by

25 using methods based on antibodies, e.g. RGH-specific antibodies, e.g. derived as out-
lined below. Ultimately, the purified RGH-derived antigens can be characterized by
protein sequencing, e.g. as outlined in Harris and Angal, 1990.

RGH-specific antibodies

30
The source of RGH-specific antibodies can be e.g. patient serum samples and serum
samples from immunized animals as well as ascitic fluid from animals inoculated by
hybridoma cells or growth medium from cultured hybridoma cells. The antibody
sources all contain various amounts of impurities which can be removed by different

32

protein purification methods as described above, e.g. by affinity chromotography using RGH-derived peptides. Useful standard techniques for immunization of animals and for production of hybridoma cells is described in e.g. Hudson and Hay, 1980. Antibody reactivity may be characterized as outlined above.

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CLAIMS

1. A method of diagnosing a demyelinating disease including multiple sclerosis, the method comprising detecting, in a retroviral particle or an encapsidated virion-like particle present
5 in a sample derived from a patient suspected of suffering from multiple sclerosis, the presence of SEQ ID NO:1 or a subsequence or variant thereof.

2. A method of subgrouping patients having a demyelinating disease including multiple sclerosis, said method comprising detecting, in a retroviral particle or an encapsidated vi-
10 rion-like particle derived from a patient suspected of suffering from a demyelinating disease including multiple sclerosis, the presence of SEQ ID NO:1 or a subsequence or variant thereof.

3. A method of monitoring the stage or activity of a demyelinating disease including multi-
15 ple sclerosis, the method comprising detecting, in a retroviral particle or an encapsidated virion-like particle derived from a patient suspected of suffering from a demyelinating disease including multiple sclerosis, the presence of SEQ ID NO:1 or a subsequence or variant thereof.

20 4. A method according to any of claims 1-3 wherein the detection is accomplished by using at least one probe that specifically recognizes SEQ ID NO:1 or a subsequence or variant thereof that has a sequence identity with a similar part of SEQ ID NO:1 of at least 80%.

5. A method according to any of claims 1-3 wherein the detection is accomplished by using
25 at least one primer that is capable of generating a nucleic acid fragment, the nucleotide sequence of which has a sequence identity with a similar part of SEQ ID NO:1 of at least 80%.

6. A method according to any of claims 1-3 which further comprises the detection of the
30 presence in a retroviral particle of one or more other retroviral nucleotide sequences including a nucleotide sequence selected from the group consisting of a sequence derived from a Human Immunodeficiency Virus (HIV), a sequence derived from a Human T-cell Leukemia Virus (HTLV), a sequence derived from a Multiple Sclerosis Retrovirus (MSRV) and a sequence derived from Human Endogenous Retrovirus-K (HERV-K).
35

7. A method according to claim 6 wherein the detection of the nucleotide sequence of the other retrovirus or retroviruses is accomplished by using one or more probes or sets of primers which is useful for recognizing a nucleotide sequence specific for one retrovirus.

5   8. A diagnostic agent for the diagnosis, subgrouping or monitoring of a demyelinating disease including multiple sclerosis by the detection of the presence, in a sample material derived from a patient suspected of suffering from demyelinating disease including multiple sclerosis, of SEQ ID NO:1 or a subsequence or variant thereof, said agent comprising a probe that is capable of hybridizing specifically with SEQ ID NO:1 or with a subsequence or
10   variant thereof that has a sequence identity with a similar part of SEQ ID NO:1 of at least 80%.

9. A diagnostic agent for the diagnosis, subgrouping or monitoring of a demyelinating disease including multiple sclerosis by detection of the presence, in a sample material derived
15   from a patient suspected of suffering from demyelinating disease including multiple sclerosis, of SEQ ID NO:1 or a subsequence or variant thereof, said agent comprising at least one primer that is capable of generating a nucleic acid fragment, the nucleotide sequence of which has a sequence identity with a similar part of SEQ ID NO:1 of at least 80%.

20   10. A diagnostic agent according to claim 8 and 9.

11. A diagnostic agent according to any of claims 8-10 which further comprises a probe or primer which is useful for the detection of the presence in a retroviral particle or an encapsidated virion-like particle of one or more other retroviral nucleotide sequences including a
25   nucleotide sequence selected from the group consisting of a sequence derived from a Human Immunodeficiency Virus (HIV), a sequence derived from a Human T-cell Leukemia Virus (HTLV), a sequence derived from a Multiple Sclerosis Retrovirus (MSRV) and a sequence derived from Human Endogenous Retrovirus-K (HERV-K).

30   12. A method of diagnosing, subgrouping or monitoring demyelinating disease including multiple sclerosis, the method comprising detecting the presence, in a sample material derived from a patient suspected of suffering from demyelinating disease including multiple sclerosis, of a protein or a peptide encoded by SEQ ID NO:1 or by a subsequence or variant of said sequence.

35

13. A method according to claim 12 which further comprises detecting the presence of a protein or peptide encoded by one or more other retroviral nucleotide sequences including a nucleotide sequence selected from the group consisting of a sequence derived from a Human Immunodeficiency Virus (HIV), a sequence derived from a Human T-cell Leukemia

5 Virus (HTLV), a sequence derived from a Multiple Sclerosis Retrovirus (MSRV) and a sequence derived from Human Endogenous Retrovirus-K (HERV-K).

14. A diagnostic agent for the diagnosis, subgrouping or monitoring of demyelinating disease including multiple sclerosis by detection, in a sample material derived from a patient

10 suspected of suffering from demyelinating disease including multiple sclerosis, of a protein or a peptide encoded by SEQ ID NO:1 or by a subsequence or variant of said sequence, said diagnostic agent comprising an antibody reacting specifically with an epitope of a peptide encoded by SEQ ID NO:1 or by a subsequence or variant of said sequence.

15 15. A diagnostic agent according to claim 14 which further comprises an antibody useful for detecting an epitope selected from the group consisting of an epitope of a Human Immunodeficiency Virus (HIV), an epitope of a Human T-cell Leukemia Virus (HTLV), an epitope of a Multiple Sclerosis Retrovirus (MSRV) and an epitope of a Human Endogenous Retrovirus-K (HERV-K).

20 .

16. A method of diagnosing, subgrouping or monitoring demyelinating disease including multiple sclerosis, the method comprising detecting, in a sample material derived from a patient suspected of suffering from demyelinating disease including multiple sclerosis, the presence of an antibody reacting specifically with an epitope of a protein or a peptide en-

25 coded by SEQ ID NO:1 or by a subsequence or variant of said sequence.

17. A method according to claim 16 which further comprises detecting the presence of an antibody useful for detecting an epitope selected from the group consisting of an epitope of a Human Immunodeficiency Virus (HIV), an epitope of a Human T-cell Leukemia Virus

30 (HTLV), an epitope of a Multiple Sclerosis Retrovirus (MSRV) and an epitope of a Human Endogenous Retrovirus-K (HERV-K).

18. A diagnostic agent for the diagnosis, subgrouping or monitoring of demyelinating disease including multiple sclerosis by the detection, in a sample material derived from a pa-

35 tient suspected of suffering from demyelinating disease including multiple sclerosis, of an

38

antibody reacting specifically with an epitope of a protein or a peptide encoded by SEQ ID NO:1 or by a subsequence or variant of said sequence, said diagnostic agent comprising an epitope of a peptide encoded by SEQ ID NO:1 or by a subsequence or variant of said sequence.

5

19. A diagnostic agent according to claim 18 which further comprises an epitope selected from the group consisting of an epitope of a Human Immunodeficiency Virus (HIV), an epitope of a Human T-cell Leukemia Virus (HTLV), an epitope of a Multiple Sclerosis Retrovirus (MSRV) and an epitope of a Human Endogenous Retrovirus-K (HERV-K).

10

20. A method of isolating or purifying, from a sample material, a retroviral particle or an encapsidated virion-like particle, the method comprising contacting said sample material with an antibody reacting specifically with an epitope of a protein or a peptide encoded by SEQ ID NO:1 or by a subsequence or variant thereof.

15

21. A method according to any of claims 1-7 comprising that, prior to the detection, in a retroviral particle or an encapsidated virion-like particle, of the presence of SEQ ID NO:1 or a subsequence or variant thereof, said retroviral particle or encapsidated virion-like particle is isolated or purified using the method of claim 20.

20

22. A diagnostic agent according to any of claims 8-11 for use in a method according to claim 21.

23. A method according to claim 12 or 13 comprising that, prior to the detection of a protein or a peptide encoded by SEQ ID NO:1 or by a subsequence or variant thereof, a retroviral particle or an encapsidated virion-like particle comprising said said sequence, subsequence or variant, is isolated or purified using the method of claim 20.

25

24. A diagnostic agent according to claim 14 or 15 for use in a method according to claim 23.

30

25. Use of an isolated and/or purified RGH virus particle or encapsidated virion-like particle, or a fragment hereof, as a diagnostic agent.

26. An isolated and/or purified RGH virus particle or encapsidated virion-like particle, or a fragment hereof, for use as a medicament.

27. Use of an isolated and/or purified RGH virus particle or encapsidated virion-like parti-
5 cle, or a fragment hereof, in the manufacturing of a medicament for the treatment of a de-
myelinating disease including multiple sclerosis.

10

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tcctccacgc tgtaatcttt ttatcacctc ccctcctcac acctggtccg gcttacagtt   240
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Gln Lys Leu


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<400> 23
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INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER IPC 6 C12Q1/70 C07K14/15	

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6   C12Q   C07K

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HIROSE ET AL.: "PRESENCE OF env GENES IN MEMBERS OF THE RTVL-H FAMILY OF HUMAN ENDOGENOUS RETROVIRUS-LIKE ELEMENTS" VIROLOGY, vol. 192, 1993, pages 52-61, XP002085610 cited in the application	20
Y	the whole document	1-19, 21-27
Y	CHRISTENSEN ET AL.: "CHARACTERIZATION OF RETROVIRUSES FROM PATIENTS WITH MULTIPLE SCLEROSIS" ACTA NEUROL. SCAND. SUPPL.169, vol. 95, 1997, pages 49-58, XP002085611 cited in the application the whole document	1-19, 21-27

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[X]	Further documents are listed in the continuation of box C.	[X]	Patent family members are listed in annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 July 1999	09/08/1999

Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Hagenmaier, S

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	PERRON H ET AL: "MOLECULAR IDENTIFICATION OF A NOVEL RETROVIRUS REPEATEDLY ISOLATED FROM PATIENTS WITH MULTIPLE SCLEROSIS" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 94, July 1997 (1997-07), pages 7583-7588, XP002062853 See page 7587 left column, third paragraph the whole document	1-19, 21-27
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Y	WO 94 11514 A (ASTA MEDICA AG ;DIERICH MANFRED (AT); VOGETSEDER WERNER (AT)) 26 May 1994 (1994-05-26) the whole document	6,11,13, 15,17,19
A	PERRON ET AL.: "ISOLATION OF RETROVIRUS FROM PATIENTS WITH MULTIPLE SCLEROSIS" LANCET, vol. 337, 1991, pages 862-863, XP000673661 the whole document	
A	WO 95 21256 A (BIO MERIEUX ;PERRON HERVE (FR); MALLET FRANCOIS (FR); MANDRAND BER) 10 August 1995 (1995-08-10) cited in the application the whole document	
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A	EP 0 222 310 A (WISTAR INST) 20 May 1987 (1987-05-20) the whole document	
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A	EP 0 326 395 A (HOPE CITY) 2 August 1989 (1989-08-02) the whole document	
A	ANDERSSON ET AL.: "RETROELEMENTS IN THE HUMAN MHC CLASS II REGION" TRENDS IN GENETICS, vol. 14, no. 3, March 1998 (1998-03), pages 109-114, XP002085613 the whole document	
P,X	CHRISTENSEN ET AL.: "EXPRESSION OF SEQUENCE VARIANTS OF ENDOGENOUS RETROVIRUS RGH IN PARTICLE FORM IN MULTIPLE SCLEROSIS" LANCET, vol. 352, 26 September 1998 (1998-09-26), page 1033 XP002085614 the whole document	1-27

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			JP	8511170 T	26-11-1996
			NO	953925 A	04-12-1995
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