Abstract:
The present invention is directed to anti-CGPR antibodies that are useful for the treatment of migraines and the use of anti-CGPR antibodies for the treatment of migraines.
TREATMENT OF MIGRAINE WITH ANTI-CGRP ANTIBODIES

FIELD OF THE INVENTION

The present invention is in the field of medicine. More specifically, the invention relates to antibodies to CGRP and the use of such antibodies for therapy and prophylaxis of migraines.

BACKGROUND OF THE INVENTION

Calcitonin gene related peptide (CGRP) is a 37 amino acid neuropeptide secreted by the nerves of the central and peripheral nervous systems. CGRP exists as highly homologous α and β isoforms in both human and rat although each is encoded by a distinct gene. The α and β isoforms of the CGRP peptides differ by three amino acids in humans and one amino acid in rats. The amino acid sequences of CGRP peptides are well conserved among species and are considered members of a family of peptides that includes amylin, calcitonin, and adrenomedullin.

CGRP is widely distributed in sensory nerves, both in the peripheral and central nervous system and displays a large number of different biological activities. When released from trigeminal and other nerve fibers, CGRP is thought to mediate its biological responses by binding to specific cell surface receptors.

CGRP receptors have been identified and pharmacologically evaluated in several tissues and cells, including brain, cardiovascular, endothelial and smooth muscle. Multiple CGRP receptors have been characterized based on distinct pharmacological properties.

These receptors are divided into at least two subtypes, denoted as CGRP1 and CGRP2 (Pharmacol. Rev 54:233-246, 2002).

Recently, Arulmozhi and colleagues have reported various theories on migraines (Vascular Pharmacology 43; 176-187, 2005). One of the theories proposes that the currently unknown triggers of migraine stimulate trigeminal nerves and ganglia that innervate cephalic tissue, giving rise to release of neuropeptide messenger molecules from axons on the vasculature. Release of these neuropeptides then activates a series of
events, a consequence of which is migraine pain. In addition, release of these neuropeptides changes vascular permeability resulting in subsequent leakage of plasma proteins in tissues innervated by stimulated trigeminal fibers. This leakage results in neurogenic inflammation which leads to migraines.

Of these neuropeptides, CGRP has been reported to play a role in migraines as CGRP is released upon stimulation of sensory nerves and has potent vasodilatory activity. (Vascular Pharmacology 43; 176-187, 2005). Further, the release of CGRP increases vascular permeability and subsequent plasma protein leakage (plasma protein extravasation) in tissues innervated by trigeminal nerve fibers upon stimulation of these fibers. (Vascular Pharmacology 43; 176-187, 2005). In addition, studies have reported that infusion of CGRP in patients who suffer from migraines has resulted in migraine-like symptoms. (Cephalagia 22(1): 54-61, 2002)

Historically, small molecule agonists of serotonin 5- HIWm receptors have been used as treatments for migraines. These so-called triptans are potent vasoconstrictors and have been shown to inhibit plasma protein extravasation due to stimulation of trigeminal nerve fibers in an experimental animal migraine model. In addition, doses of a triptan that decreased plasma protein extravasation also attenuated CGRP levels in the same experimental animal model. (Br. J. Pharmacology 99; 202-206, 1990; Neuropharmacology 30(11): 1193-1200, 1991)

Although triptans have been found to be efficacious, many patients who respond to triptan treatment suffer from recurrent headaches within several hours after treatment. Further, since triptans are potent vasoconstrictors, they are contraindicated in certain patient populations, such as populations of patients suffering from hypertension or suffering from ischemic heart disease. There is therefore a need for therapeutic compounds to prevent and/or treat migraines without unwanted side effects such as cardiovascular-related effects.

The compounds used in the methods of treatment encompassed by the present invention solve problems associated with cardiovascular and other unwanted side-effects by specifically binding CGRP itself rather than binding CGRP receptors located throughout the body. Furthermore, the anti-CGRP antibodies of the present invention preferentially bind CGRP over other homologous proteins, including those of the calcitonin protein family which further minimizes potential side effects. While there is
some research focused on developing CGRP receptor antagonists, this is the first report of compounds that block receptor activation by binding to a specific region on the CGRP peptide itself.

**SUMMARY OF THE INVENTION**

The present invention relates to a method of treating migraines comprising administering to a subject in need thereof, an effective amount of an anti-CGRP antibody that binds to and inhibits the ability of CGRP to bind to the CGRP receptor. The invention further provides for a method of preventing migraines comprising administering to a subject who has suffered at least one migraine previously, preferably a human, an effective amount of an anti-CGRP antibody that binds to and inhibits the ability of CGRP to bind to the CGRP receptor. In another embodiment, anti-CGRP antibodies are administered for reducing pain due to migraines.

The invention also embodies an anti-CGRP antibody that binds to and inhibits the ability of CGRP to bind to the CGRP receptor for use in the manufacture of a medicament for administration to a subject, preferably a human, for the treatment of migraines.

The invention further embodies a method of inhibiting binding of CGRP to a CGRP receptor in a patient suffering from migraine, comprising administration of an anti-CGRP antibody. In another embodiment, an anti-CGRP antibody of the invention is used in a method of inhibiting CGRP receptor activation in a patient suffering from migraine comprising administering an anti-CGRP antibody.

In one embodiment, the anti-CGRP antibodies of the invention specifically bind CGRP with an EC\textsubscript{50} of less than about 2.33 x 10^{-9} M, or less than about 2.09 x 10^{-9} M, or less than about 1.24 x 10^{-9} M or less than about 1.07 x 10^{-9} M.

In another preferred embodiment, the anti-CGRP antibodies of the invention have K\textsubscript{D} to CGRP of less than about 2.99 x 10^{-9} M, or less than about 2.21 x 10^{-9} M, or less than 1.50 x 10^{-10} M.

In an embodiment, the anti-CGRP antibodies of the invention will inhibit or prevent activation of the CGRP receptor. For example, in an in vitro assay, the anti-CGRP antibodies will preferably have an IC\textsubscript{50} of less than about 1.45 x 10^{-8} M, or less than about 5.49 x 10^{-9} M.
In another embodiment of the invention, an article of manufacture containing materials useful for the treatment of the disorders described above is provided. The article of manufacture comprises a container and a label. Suitable containers include, for example, bottles, vials, syringes, and test tubes. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition, preferably sterile, which is effective for treating the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The active agent in the composition is the anti-CGRP antibody. The label on, or associated with, the container indicates that the composition is used for treating the condition of choice. The article of manufacture may further comprise a second container comprising a pharmaceutically-acceptable buffer, such as phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

In another embodiment, the invention provides a pharmaceutical composition comprising an anti-CGRP monoclonal antibody of the invention. The pharmaceutical composition of the invention may further comprise a pharmaceutically acceptable carrier. In said pharmaceutical composition, the anti-CGRP monoclonal antibody of the invention is the active ingredient. Preferably the pharmaceutical composition comprises a homogeneous or substantially homogeneous population of an anti-CGRP monoclonal antibody of the invention. The composition for therapeutic use is sterile and may be lyophilized, optionally supplied with an appropriate diluent.

**SUMMARY OF THE FIGURES**

FIG. 1 shows the amino acid sequence of human α calcitonin gene related peptide (αCGRP).

FIG. 2 shows the amino acid sequence of human β calcitonin gene related peptide (βCGRP).

FIG. 3 show the DNA sequence of the human calcitonin gene

FIG. 4 shows an alignment of the amino acid sequence of calcitonin gene family peptides of human and rat species.
FIG. 5 shows the amino acid sequence of CDRs of the LCVR of various antibodies of the invention.

FIG. 6 shows the amino acid sequence of CDRs of the HCVR of various antibodies of the invention.

FIG. 7 shows the alignment of the amino acid sequence of LCVRs of various antibodies of the invention. The CDR domains are underlined in the first antibody sequence.

FIG. 8 shows the alignment of the amino acid sequence of HCVRs of various antibodies of the invention. The CDR domains are underlined in the first antibody sequence.

FIG. 9 shows the murine IgGl heavy chain constant chain (SEQ ID NO: 40) and the murine kappa light chain constant chain (SEQ ID NO: 41)

DETAILED DESCRIPTION

The invention presents anti-CGRP antibodies useful for the prevention and treatment of migraines.

Definitions

As used herein, the term "CGRP" refers to the human α and β isoforms of calcitonin gene-related peptide, collectively. "αCGRP refers to the α isoform (SEQ ID NO: 1) that is encoded by the calcitonin gene and results from alternative splicing of the calcitonin gene (SEQ ID NO: 3). βCGRP refers to the β isoform (SEQ ID NO: T) of calcitonin gene-related peptide.

The term "migraine" as used herein refers to "migraines without aura" (formerly "common migraine") and "migraines with aura" (formerly "classical migraines") according to the Headache Classification Committee of the International Headache Society (International Headache Society, 2004). For example, "migraines without aura", typically may be characterized as having a pulsating quality, a moderate or severe intensity, are aggravated by routine physical activity, are unilaterally located and are associated with nausea and with photophobia and phonophobia. "Migraines with aura"
may include disturbances in vision, disturbances in other senses, unilateral weakness, and in some instances difficulty with speech.

The term "antibody," in reference to an anti-CGRP antibody of the invention (or simply, "antibody of the invention"), as used herein, refers to a monoclonal antibody. A "monoclonal antibody" refers to an antibody that is derived from a single copy or clone, including e.g., any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced. In addition, a "monoclonal antibody" as used herein refers to a humanized antibody or a fully human antibody, unless otherwise indicated herein. Preferably a monoclonal antibody of the invention exists in a homogeneous or substantially homogeneous population. A "monoclonal antibody" can be an intact antibody (comprising a complete or full length Fc region), a substantially intact antibody, or a portion or fragment of an antibody comprising an antigen-binding portion, e.g., a Fab fragment, Fab' fragment or (Fab')2 fragment of a humanized or human antibody. "Monoclonal antibodies" of the invention can be produced using e.g., hybridoma techniques well known in the art, as well as recombinant technologies, phage display technologies, synthetic technologies or combinations of such technologies or other technologies readily known in the art.

A full-length antibody as it exists naturally is an immunoglobulin molecule comprised of four peptide chains, two heavy (H) chains (about 50-70 kDa when full length) and two light (L) chains (about 25 kDa when full length) interconnected by disulfide bonds. The amino terminal portion of each chain includes a variable region of about 100-110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function.

Light chains are classified as kappa or lambda and characterized by a particular constant region as known in the art. Heavy chains are classified as gamma, rrrm, alpha, delta, or epsilon, and define the antibody's isotype as IgG, IgM, IgA, IgD, and IgE, respectively and several of these may be further divided into subclasses (sub-isotypes) e.g., IgGi, IgG2, IgG3, IgG4, IgAi, and IgA2. Each heavy chain type is characterized by a particular constant region known in the art. The subunit structures and three-dimensional configurations of different classes of antibodies are well known in the art. Each heavy chain is comprised of an N-terminal heavy chain variable region (herein "HCVR") and a
heavy chain constant region. The heavy chain constant region is comprised of three domains (CH1, CH2, and CH3) for IgG, IgD, and IgA; and 4 domains (CH1, CH2, CH3, and CH4) for IgM and IgE. Each light chain is comprised of a light chain variable region (herein "LCVR") and a light chain constant region, CL. The HCVR and LCVR regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each HCVR and LCVR is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FRI, CDRI, FR2, CDRL2, FR3, CDR3, FR4. Herein the 3 CDRs of the heavy chain are referred to as "CDRHI, CDRH2, and CDRH3" and the 3 CDRs of the light chain are referred to as "CDRL1, CDRL2 and CDRL3." The CDRs contain most of the residues which form specific interactions with the antigen. CDR3 is typically the greatest source of molecular diversity within the antibody-binding site. Assignment of amino acids to each domain is in accordance with well-known conventions [e.g., Kabat, "Sequences of Proteins of Immunological Interest," National Institutes of Health, Bethesda, Md. (1991)]. The functional ability of an antibody to bind a particular antigen is largely influenced by the six CDRs.

The variable regions of each light/heavy chain pair form the antigen-binding sites of the antibody. Thus, an intact IgG antibody has two binding sites. Except in bifunctional or bispecific antibodies, the two binding sites are the same. As used herein, the "antigen-binding portion" or "antigen-binding region" or "antigen-binding fragment" refers interchangeably to that portion of an antibody molecule, within the variable region, which contains the amino acid residues that interact with an antigen and confer on the antibody its specificity and affinity for the antigen. This antibody portion includes the framework amino acid residues necessary to maintain the proper conformation of the antigen-binding residues. Preferably, the CDRs of the antigen-binding region of the antibodies of the invention will be of murine origin or substantially of murine origin with certain amino acids residues altered to improve a particular activity. Preferably, the framework regions of antibodies of the invention are of human origin or substantially of human origin (or have at least 95%, 97% or 99%, sequence identity with a particular human germ-line framework). In other embodiments, the antigen-binding region, or the CDRs of the antigen-binding region, can be synthetic and/or derived from other non-
human species including, but not limited to, rabbit, rat or hamster. In other embodiments, the antigen-binding region can be entirely of human origin or substantially of human origin with certain amino acids residues altered to improve a particular activity, e.g., affinity or specificity.

Furthermore, a "monoclonal antibody" as used herein can be a single chain Fv fragment that may be produced by joining the DNA encoding the LCVR and HCVR with a linker sequence. (See, Pluckthun, *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenberg and Moore eds., Springer-Verlag, New York, pp 269-315, 1994). It is understood that regardless of whether fragments or portions are specified, the term "antibody" as used herein includes such fragments or portions as well as single chain forms. As long as the protein retains the ability to specifically or preferentially bind its intended target (i.e., epitope or antigen), it is included within the term "antibody."

The term "humanized antibody" as used herein refers to an antibody comprising portions of antibodies of different origin, wherein at least one portion is of human origin. For example, the humanized antibody can comprise portions derived from an antibody of nonhuman origin with the requisite specificity, such as a mouse, and from an antibody of human origin, joined together chemically by conventional techniques (e.g., synthetic) or prepared as a contiguous polypeptide using genetic engineering techniques. In addition, a "humanized antibody" has CDRs that originate from a non-human antibody (preferably a mouse monoclonal antibody) while framework and constant region, to the extent it is present, (or a significant or substantial portion thereof, i.e., at least about 90%, 92%, 94%, 95%, 96%, 97%, 98% or 99%) are encoded by nucleic acid sequence information that occurs in the human germline immunoglobulin region (see, e.g., the International ImMunoGeneTics Database)

A "population of monoclonal antibodies," refers to a homogeneous or substantially homogeneous antibody population (i.e., at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, more preferably at least about 97% or 98% or most preferably at least 99% of the antibodies in the population would compete in an ELISA assay for the same antigen or epitope). Antibodies may or may not be glycosylated and still fall within the bounds of the invention. Monoclonal antibodies may be homogeneous if they have identical amino acid sequence although they may differ in a post-translational modification, e.g., glycosylation pattern.
The term "specifically binds" as used herein refers to the situation in which one member of a specific binding pair does not significantly bind to molecules other than its specific binding partner(s) as measured by a technique available in the art, e.g., competition ELISA or BIACORE assay. The term is also applicable where e.g., an antigen-binding domain of an antibody of the invention is specific for a particular epitope that is carried by a number of related proteins, in which case the specific antibody carrying the antigen-binding domain will be able to specifically bind to the various related proteins carrying the epitope. If the related proteins do not carry the particular epitope, the antigen-binding domain, will not bind to the proteins. Accordingly a monoclonal antibody of the present invention specifically binds to CGRP and not to other members of the calcitonin family of proteins.

The term "preferentially binds" as used herein refers to the situation in which an antibody binds a specific antigen at least about 20% greater than it binds a different antigen as measured by a technique available in the art, e.g., competition ELISA or K_d measurement with BIACORE assay. Similarly, an antibody may preferentially bind one epitope within an antigen over a different epitope within the same antigen.

The term "epitope" refers to that portion of a molecule capable of being recognized by and bound by an antibody at one or more of the antibody's antigen-binding regions. Epitopes often consist of a chemically active surface grouping of molecules such as amino acids or sugar side chains and have specific three-dimensional structural characteristics as well as specific charge characteristics. An "inhibiting epitope" and/or "neutralizing epitope" is an epitope, which when in the context of the intact molecule (in this case, CGRP) and when bound by antibody specific to the epitope, results in loss or diminution of a biological activity of the molecule or organism containing the molecule, in vivo or in vitro.

The term "antigenic epitope," as used herein, is defined as a portion of a polypeptide to which an antibody can specifically bind as determined by any method well known in the art, for example, by conventional immunoassays. Antigenic epitopes need not necessarily be immunogenic, but maybe immunogenic. An "immunogenic epitope," as used herein, is defined as a portion of a polypeptide that elicits an antibody response in
an animal, as determined by any method known in the art. (See, e.g., Geysen et al., Proc. Natl. Acad. Sci. USA 81:3998-4002 (1983)). For example, an epitope may be determined using the techniques as outlined in Example 1 or Example 2 discussed below. Briefly, in an ELISA or a BIACORE assay as outlined in Example 1 or Example 2, peptide fragments of differing size and sequence are synthesized and the antibodies of interest are tested for their ability to bind to the specific fragments.

The phrases "biological property" or "bioactivity," "activity" or "biological activity," in reference to an antibody of the present invention, are used interchangeably herein and include, but are not limited to, epitope/antigen affinity and specificity, ability to neutralize or antagonize an activity of CGRP in vivo or in vitro, IC50 in a cAMP assay or other in vitro activity assay, the in vivo stability of the antibody and the immunogenic properties of the antibody. Other identifiable biological properties of an antibody include, for example, cross-reactivity, (i.e., with non-human homologs of the targeted peptide, or with other proteins or tissues, generally), and ability to preserve high expression levels of protein in mammalian cells. The aforementioned properties or characteristics can be observed or measured or assessed using art-recognized techniques including, but not limited to, ELISA, competitive ELISA, BIACORE analysis, in vitro and in vivo neutralization assays without limit, receptor binding, and immunohistochemistry with tissue sections from different sources including human, primate, or any other source as the need may be.

The term "inhibit" or "neutralize" as used herein with respect to an activity of an antibody of the invention means the ability to substantially antagonize, prohibit, prevent, restrain, slow, disrupt, eliminate, stop, reduce or reverse the biological effects of CGRP binding to the CGRP receptor.

The term "isolated" when used in relation to a protein (e.g., an antibody) refers to protein that is identified and separated from at least one contaminant with which it is ordinarily associated in its natural source. Preferably, an "isolated antibody" is an antibody that is substantially free of other antibodies having different antigenic specificities (e.g., pharmaceutical compositions of the invention comprise an isolated antibody that specifically binds CGRP and is substantially free of antibodies that specifically bind antigens other than CGRP).
The terms "Kabat numbering" and "Kabat labeling" are used interchangeably herein. These terms, which are recognized in the art, refer to a system of numbering amino acid residues which are more variable (i.e., hypervariable) than other amino acid residues in the heavy and light chain variable regions of an antibody (Kabat, et al., Ann. NY Acad. Sci. 190:382-93 (1971); Kabat, et al., Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242 (1991)).

The terms "individual," "subject," and "patient," used interchangeably herein, refer to a subject, preferably human, suffering from a migraine or susceptible to migraines that would benefit from a decreased level or decreased bioactivity of CGRP.

As used herein, the terms "treatment", "treating", and the like, refer to obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a migraine or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for migraines and/or adverse affect attributable to migraines. "Treatment", as used herein, includes administration of an antibody of the present invention for treatment of migraines in a subject, particularly in a human, and includes: (a) preventing the migraine from occurring in a subject which may be predisposed to migraines; (b) inhibiting migraines, i.e., arresting its development; and (c) relieving migraines, i.e., alleviating symptoms or complications thereof. Dosage regimens may be adjusted to provide the optimum desired response (e.g., a therapeutic or prophylactic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation.

Further as used herein, a "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary to treat migraines, and/or to reduce pain due to migraines. A therapeutically effective amount of the antibody may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the antibody or antibody portion to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effect of the antibody, are outweighed by the therapeutically beneficial effects.
A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, a prophylactic dose is used in subjects prior to the onset of a migraine and/or prior to the onset of symptoms of migraines such as to prevent or inhibit the occurrence of a migraine.

A "therapeutically-effective" or "prophylactically-effective" amount is at least the minimal dose, but less than a toxic dose, of an active agent which is necessary to impart therapeutic benefit to a subject. Stated another way, a therapeutically-effective amount of an antibody of the invention is an amount which in a subject, treats conditions wherein the presence of CGRP causes or contributes to undesirable pathological effects or the decrease in CGRP levels results in a beneficial therapeutic effect in a subject, preferably a human, including, but not limited to, reduction in pain due to migraines, prevention of migraines and treatment of migraines.

Antibody Characterization

An antibody of the present invention will be characterized by its ability to specifically bind to CGRP. An antibody of the present invention preferably will be characterized by an EC50, as determined in an ELISA assay as in Example 1, of less than about 2.33 x 10^{-9} M, less than about 2.09 x 10^{-9} M, less than about 1.24 x 10^{-9} M, or less than about 1.07 x 10^{-9} M.

In another embodiment, the present invention relates to antibodies that specifically bind CGRP with high affinity. In one embodiment, the anti-CGRP antibodies have a Kd for CGRP of less than about 3.00 x 10^{-9} M. In another embodiment, the anti-CGRP antibodies have binding affinities of less than about 2.99 x 10^{-9} M or less than about 2.0 x 10^{-9} M. Also the antibodies of the present invention may have a Kd of less than about 1.5 x 10^{-10} M. Antibody affinities may be determined by techniques known in the art, e.g. as described in the examples herein below.

The antibodies of the present invention specifically inhibit or prevent activation of the CGRP receptor by binding CGRP. Antibodies that inhibit or prevent activation of the CGRP receptor may have an IC50 of less than about 15 x 10^{-9} M, or less than about 10 x 10^{-9} M, or less than about 5.5 x 10^{-9} M. IC50 of antibodies that inhibit or prevent activation of the CGRP receptor may be determined by various methods known in the art.
An IC₅₀ of an antibody of the present invention may be determined as outlined in Example 4.

In one embodiment, an antibody of the invention comprises a LCVR comprising a peptide with a sequence selected from the group consisting of SEQ ID NOs: 28-32 (Fig. 7) and/or a HCVR comprising a peptide with a sequence selected from the group consisting of SEQ ID NOs: 34-38 (Fig. 8). In another embodiment, an antibody of the invention comprises an LCVR polypeptide of a SEQ ID NO as shown in Table 1 herein and further comprises a HCVR polypeptide of a SEQ ID NO as shown in Table 1.

In another embodiment, an antibody of the invention comprises (i) a LCVR polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 28-32, and (ii) a HCVR polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 34-38. For example, an antibody of the invention comprising an LCVR polypeptide comprising an amino acid sequence of SEQ ID NO: 28, preferably further comprises a HCVR polypeptide comprising an amino acid sequence of SEQ ID NO: 34.

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<th>LCVR</th>
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In another embodiment, an antibody of the invention comprises a light chain CDR (CDRL) selected from the group consisting of SEQ ID NOs: 8-13 (Fig. 5). In another embodiment, an antibody of the invention comprises a heavy chain CDR (CDRH) selected from the group consisting of SEQ ID NOs: 14-22 (Fig. 6).

In another embodiment, an antibody of the present invention comprises a polypeptide comprising a CDRL selected from the group consisting of SEQ ID NOs: 8-13.
and a polypeptide comprising a CDRH selected from the group consisting of SEQ ID NOs: 14-22.

In another embodiment, an antibody of the present invention comprises a polypeptide comprising a CDRH1 of SEQ ID 8, a CDRH2 of SEQ ID 9, a CDRH3 of SEQ ID 10. In another embodiment, an antibody of the present invention comprises a polypeptide comprising a CDRH1 of SEQ ID 11, a CDRH2 of SEQ ID 12, and a CDRH3 of SEQ ID 13.

In another embodiment, the antibody of the present invention comprises a polypeptide comprising a CDRH1 of SEQ ID 14, a CDRH2 of SEQ ID 15, and a CDRH3 of SEQ ID 16.

In another embodiment, the antibody of the present invention comprises a polypeptide comprising a CDRH1 of SEQ ID 17, a CDRH2 of SEQ ID 15, and a CDRH3 of SEQ ID 18.

In another embodiment, the antibody of the present invention comprises a polypeptide comprising a CDRH1 of SEQ ID 18, a CDRH2 of SEQ ID 19, and a CDRH3 of SEQ ID 19.

In another embodiment, the antibody of the present invention comprises a polypeptide comprising a CDRH1 of SEQ ID 18, a CDRH2 of SEQ ID 20, and a CDRH3 of SEQ ID 19.

In another embodiment, the antibody of the present invention comprises a polypeptide comprising a CDRH1 of SEQ ID 18, a CDRH2 of SEQ ID 19, and a CDRH3 of SEQ ID 20.

In another embodiment, the antibody of the present invention comprises a polypeptide comprising a CDRH1 of SEQ ID 22, a CDRH2 of SEQ ID 23, and a CDRH3 of SEQ ID 24.

In another embodiment, the antibody of the present invention comprises a polypeptide comprising a CDRH1 of SEQ ID 8, a CDRH2 of SEQ ID 9, a CDRH3 of SEQ ID 10 and a polypeptide comprising a CDRH1 of SEQ ID 14, a CDRH2 of SEQ ID 15, and a CDRH3 of SEQ ID 16.

In another embodiment, the antibody of the present invention comprises a polypeptide comprising a CDRH1 of SEQ ID 8, a CDRH2 of SEQ ID 9, a CDRH3 of
SEQ ID 10 and a polypeptide comprising a CDRH1 of SEQ ID 17, a CDRH2 of SEQ ID 15, and a CDRH3 of SEQ ID 16.

In another embodiment, the antibody of the present invention comprises a polypeptide comprising a CDRL1 of SEQ ID 8, a CDRL2 of SEQ ID 9, a CDRL3 of SEQ ID 10 and a polypeptide comprising a CDRH1 of SEQ ID 18, a CDRH2 of SEQ ID 19, and a CDRH3 of SEQ ID 16.

In another embodiment, the antibody of the present invention comprises a polypeptide comprising a CDRL1 of SEQ ID 11, a CDRL2 of SEQ ID 12, a CDRL3 of SEQ ID 13 and a polypeptide comprising a CDRH1 of SEQ ID 22, a CDRH2 of SEQ ID 23, and a CDRH3 of SEQ ID 24.

In another embodiment, the antibody of the present invention comprises a polypeptide comprising a CDRL1 of SEQ ID 8, a CDRL2 of SEQ ID 9, a CDRL3 of SEQ ID 10 and a polypeptide comprising a CDRH1 of SEQ ID 18, a CDRH2 of SEQ ID 20, and a CDRH3 of SEQ ID 16.

In another embodiment, the antibody of the present invention comprises a polypeptide comprising a CDRL1 of SEQ ID 8, a CDRL2 of SEQ ID 9, a CDRL3 of SEQ ID 10 and a polypeptide comprising a CDRH1 of SEQ ID 18, a CDRH2 of SEQ ID 15, and a CDRH3 of SEQ ID 21.

In an embodiment of the invention, an anti-CGRP antibody useful in the methods described herein is one that competes with a monoclonal antibody comprising combinations of variable chains described in Table 1. In one embodiment these useful antibodies will bind the same epitope or an epitope that overlaps with an epitope bound by an antibody comprising the combination of variable chains described in Table 1. Competitive inhibition between antibodies may be measured by assays readily known to one of skill in the art, e.g. a competition ELISA assay. In one embodiment, the antibody is one that is competitively inhibited from binding CGRP by a competing antibody comprises two peptides with the sequences of SEQ ID 28 and SEQ ID 34. In another
embodiment, the antibody is one that is competitively inhibited from binding CGRP by a competing antibody comprises two peptides with the sequences of SEQ ID 29 and SEQ ID 35. In another embodiment, the antibody is one that is competitively inhibited from binding CGRP by a competing antibody comprising two peptides with the sequences of SEQ ID 30 and SEQ ID 36. In a further embodiment, the antibody is one that is competitively inhibited from binding CGRP by a competing antibody comprising two peptides with the sequences of SEQ ID 31 and SEQ ID 37. In an embodiment, the antibody is one that is competitively inhibited from binding CGRP by a competing antibody comprising two peptides with the sequences of SEQ ID 32 and SEQ ID 38. In another embodiment, the antibody is one that is competitively inhibited from binding CGRP by a competing antibody comprising two peptides with the sequences of SEQ ID 33 and SEQ ID 39. In a further embodiment, an antibody of the present invention will comprise the murine IgG1 heavy chain constant region (SEQ ID 40) and the murine kappa light chain constant region (SEQ ID 41).

In an embodiment of the invention, an anti-CGRP antibody useful in the methods described herein and useful for the manufacture of a medicament is one that is able to bind to a fragment of CGRP as well as the full length CGRP. In one embodiment, the anti-CGRP antibody binds to a fragment of CGRP comprising CGRP (29-37). In another embodiment, the anti-CGRP antibody of the invention binds to a fragment comprising CGRP (30-37). In another embodiment, the anti-CGRP antibody of the invention binds to a fragment comprising CGRP (31-37). In one embodiment, the anti-CGRP antibody of the invention binds to a fragment comprising CGRP (32-37).

In one embodiment of the invention, an anti-CGRP antibody useful to reduce or inhibit plasma protein extravasation binds to a fragment of CGRP as well as full length CGRP. In one embodiment, the anti-CGRP antibody useful to reduce or inhibit plasma protein extravasation binds to a fragment of CGRP comprising CGRP (29-37). In another embodiment, the anti-CGRP antibody useful to reduce or inhibit plasma protein extravasation binds to a fragment of CGRP comprising CGRP (30-37). In another embodiment, the anti-CGRP antibody useful to reduce or inhibit plasma protein extravasation binds to a fragment of CGRP comprising CGRP (31-37). In another embodiment, the anti-CGRP antibody useful to reduce or inhibit plasma protein extravasation binds to a fragment of CGRP comprising CGRP (32-37).
An antibody of the invention may further comprise a heavy chain constant region selected from the group consisting of human (or substantially of human origin) IgGi, IgG₂, IgG₃, IgG₄, IgA, IgE, IgM and IgD, preferably IgGi or IgG₄. An anti-CGRP monoclonal antibody of the invention may further comprise a human kappa or lambda light chain constant region. When the antibody is to be used as a therapeutic or preventative in a human, the constant region is preferably substantially or entirely of human origin.

**Humanized antibodies**

Preferably an antibody of the invention to be used for therapeutic purposes would have the sequence of the framework and constant region (if a constant region is included) derived from the mammal in which it would be used as a therapeutic so as to decrease the possibility that the mammal would illicit an immune response against the therapeutic antibody. Humanized antibodies are of particular interest since they are considered to be valuable for therapeutic application and avoid the human anti-mouse antibody response frequently observed with murine antibodies. Additionally, in humanized antibodies if the effector portion is included it is human so it may interact better with the other parts of the human immune system (e.g., destroy the target cells more efficiently by complement-dependent cytotoxicity or antibody-dependent cellular cytotoxicity). Also, injected humanized antibodies may have a half-life more like that of naturally occurring human antibodies than do e.g., murine antibodies, thereby allowing smaller and less frequent doses to be given.

Humanized forms of non-human (e.g., murine) antibodies include an intact antibody, a substantially intact antibody, a portion of an antibody comprising an antigen-binding site, or a portion of an antibody comprising a Fab fragment, Fab' fragment, F(ab')₂, or a single chain Fv fragment. Humanized antibodies preferably contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies may also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the amino acids in the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the amino acids in the FR regions

A humanized antibody of the present invention may comprise or be derived from a human germline light chain framework. In particular embodiments, the light chain germline sequence is selected from human VK sequences including, but not limited to, A1, A10, A11, A14, A17, A18, A19, A2, A20, A23, A26, A27, A3, A30, A5, A7, B2, B3, L1, L1O, L1I, L12, L14, L15, L16, L18, L19, L2, L20, L22, L23, L24, L25, L4/18a, L5, L6, L8, L9, O1, O1I, O12, O14, O18, O2, O4, and O8. In certain embodiments, this light chain human germline framework is selected from VI-II, VI-11, VI-13, VI-16, VI-17, VI-18, VI-19, VI-2, VI-20, VI-22, VI-3, VI-4, VI-5, VI-7, VI-9, V2-1, V2-11, V2-13, V2-14, V2-15, V2-17, V2-19, V2-6, V2-7, V2-8, V3-2, V3-3, V3-4, V4-1, V4-2, V4-3, V4-4, V4-6, V5-1, V5-2, V5-4, and V5-6. See PCT WO 2005/005604 for a description of the different germline sequences.

In other embodiments, a humanized antibody of the present invention may comprise or be derived from a human germline heavy chain framework. In particular embodiments, this heavy chain human germline framework is selected from VH1-18, VH1-2, VH1-24, VH1-3, VH1-45, VH1-46, VH1-58, VH1-69, VH1-8, VH2-26, VH2-5, VH2-70, VH3-11, VH3-13, VH3-15, VH3-16, VH3-20, VH3-21, VH3-23, VH3-30, VH3-33, VH3-35, VH3-38, VH3-43, VH3-48, VH3-49, VH3-53, VH3-64, VH3-66, VH3-7, VH3-72, VH3-73, VH3-74, VH3-9, VH4-28, VH4-31, VH4-34, VH4-39, VH4-4, VH4-59, VH4-61, VH5-51, VH6-1, and VH7-81. See PCT WO 2005/005604 for a description of the different germline sequences.

In particular embodiments, the light chain variable region and/or heavy chain variable region comprises a framework region or at least a portion of a framework region (e.g., containing 2 or 3 subregions, such as FR2 and FR3). In certain embodiments, at least FRH1, FRH2, FRH3, or FRH4 is fully human. In other embodiments, at least FRH1, FRH2, FRH3, or FRH4 is fully human. In some embodiments, at least FRH1, FRH2, FRH3, or FRH4 is a germline sequence (e.g., human germline) or comprises human consensus sequences for the particular framework. In other embodiments, at least FRH1,
FRH2, FRH3, or FRH4 is a germline sequence (e.g., human germline) or comprises human consensus sequences for the particular framework. In preferred embodiments, the framework region is a human framework region.

In general, humanized antibodies may be produced by obtaining nucleic acid sequences encoding the HCVR and LCVR of an antibody, e.g., a murine antibody or antibody made by a hybridoma, which binds a CGRP epitope of the invention, identifying the CDRs in said HCVR and LCVR (nonhuman), and grafting such CDR-encoding nucleic acid sequences onto selected human framework-encoding nucleic acid sequences. Optionally, a CDR region may be optimized by mutagenizing randomly or at particular locations in order to substitute one or more amino acids in the CDR with a different amino acid prior to grafting the CDR region into the framework region. Alternatively, a CDR region may be optimized subsequent to insertion into the human framework region using methods available to one of skill in the art. Preferably, the human framework amino acid sequences are selected such that the resulting antibody is likely to be suitable for in vivo administration in humans. This can be determined, e.g., based on previous usage of antibodies containing such human framework sequence. Preferably, the human framework sequence will not itself be significantly immunogenic.

Alternatively, the amino acid sequences of the frameworks for the antibody to be humanized may be compared to those of known human framework sequences the human framework sequences to be used for CDR-grafting and selected based on their comprising sequences highly similar to those of the parent antibody, e.g., a murine antibody which binds CGRP. Numerous human framework sequences have been isolated and their sequences reported in the art. This enhances the likelihood that the resultant CDR-grafted humanized antibody, which contains CDRs of the parent (e.g., murine) or optimized CDRs of the parent antibody grafted onto selected human frameworks (and possibly also the human constant region) will substantially retain the antigen binding structure and thus retain the binding affinity of the parent antibody. To retain a significant degree of antigen binding affinity, the selected human framework regions will preferably be those that are expected to be suitable for in vivo administration, i.e., not immunogenic.

In either method, the DNA sequence encoding the HCVR and LCVR regions of the preferably murine anti-CGRP antibody are obtained. Methods for cloning nucleic acid sequences encoding immunoglobulins are well known in the art. Such methods may,
for example, involve the amplification of the immunoglobulin-encoding sequences to be cloned using appropriate primers by polymerase chain reaction (PCR). Primers suitable for amplifying immunoglobulin nucleic acid sequences, and specifically murine HCVR and LCVR sequences have been reported in the literature. After such immunoglobulin-encoding sequences have been cloned, they will be sequenced by methods well known in the art.

After the CDR-encoding sequences are grafted onto the selected human framework encoding sequences, the resultant DNA sequences encoding the "humanized" variable heavy and variable light sequences are then expressed to produce a humanized Fv or humanized antibody that binds CGRP. The humanized HCVR and LCVR may be expressed as part of a whole anti-CGRP antibody molecule, i.e., as a fusion protein with human constant domain sequences whose encoding DNA sequences have been obtained from a commercially available library or which have been obtained using, e.g., one of the above described methods for obtaining DNA sequences, or are in the art. However, the HCVR and LCVR sequences can also be expressed in the absence of constant sequences to produce a humanized anti-CGRP Fv. Nevertheless, fusion of human constant sequences onto the variable region is potentially desirable because the resultant humanized anti-CGRP antibody may possess human effector functions.

Methods for synthesizing DNA encoding a protein of known sequence are well known in the art. Using such methods, DNA sequences which encode the subject humanized HCVR and LCVR sequences (with or without constant regions) are synthesized, and then expressed in a vector system suitable for expression of recombinant antibodies. This may be effected in any vector system which provides for the subject humanized HCVR and LCVR sequences to be expressed as a fusion protein with human constant domain sequences and to associate to produce functional (antigen binding) antibodies or antibody fragments.

Human constant domain sequences are well known in the art, and have been reported in the literature. Preferred human constant light chain sequences include the kappa and lambda constant light chain sequences. Preferred human constant heavy chain sequences include human IgGi, human IgG2, human IgG3, human IgG4, and mutated versions thereof which provide for altered effector function, e.g., enhanced in vivo half-life, reduced Fc receptor binding, altered deamidation profile and the like.
If present, human framework regions are preferably derived from a human antibody variable region having sequence similarity to the analogous or equivalent region of the antigen binding region donor (i.e., the parent antibody). Other sources of framework regions for portions of human origin of a humanized antibody include human variable consensus sequences (see e.g., Kettleborough, CA. et al. Protein Engineering 4:773-783 (1991); Carter et al, WO 94/04679. For example, the sequence of the antibody or variable region used to obtain the nonhuman portion can be compared to human sequences as described in Kabat et al. Sequences of Proteins of Immunological Interest, Fifth Edition, NTH, U.S. Government Printing Office (1991). In one embodiment, the framework regions of a humanized antibody chain are derived from a human variable region having at least about 60% overall sequence identity, preferably at least about 70% overall sequence identity and more preferably at least about 85% overall sequence identity, with the variable region of the nonhuman donor. A human portion can also be derived from a human antibody having at least about 65% sequence identity, and preferably at least about 70% sequence identity, within the particular portion (e.g., FR) being used, when compared to the equivalent portion (e.g., FR) of the nonhuman donor.


**Human Antibodies**

human immunoglobulin loci into transgenic animals, e.g., mice in which the endogenous immunoglobulin genes have been partially or complete inactivated. Upon immunization, e.g., with an antigen comprising an immunogenic epitope of the invention, a full repertoire of human antibody production is obtained, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly and antibody repertoire. This approach is described, for example, in U.S. Pat. Nos. 5,545,807; 5,545,806; 5,569,825; 5,589,369; 5,591,669; 5,625,126; 5,633,425; 5,661,016, and in the following scientific publications: Marks et al., BioTechnology 10:779-783, 1992; Lonberg et al., Nature 368: 856-859, 1994; Morrison, Nature 368: 812-13, 1994; Fishwild et al., Nature Biotechnology 14:845-51, 1996; Neuberger, Nature Biotechnology 14: 826 (1996); Lonberg and Huszar, Intern. Rev. Immunol. 13: 65-93 (1995) and Jobkobovits et al., Proc. Natl. Acad. SciL USA, 90:2551, 1993.

Human immunoglobulin genes introduced into the mouse thus creating transgenic mice capable of responding to antigens with antibodies having human sequences are also described in Bruggemann et al. Proc. Nat'l. Acad. SciL USA 86:6709-6713 (1989). There are several strategies that exist for the generation of mammals that produce human antibodies. In particular, there is the "minilocus" approach in which an exogenous Ig locus is mimicked through the inclusion of pieces (individual genes) from the Ig locus (see, e.g., U.S. Pat. Nos. 5,545,807, 5,545,806, 5,625,825, 5,625,126, 5,633,425, 5,661,016, 5,770,429, 5,789,650, and 5,814,318, 5,612,205, 5,721,367, 5,789,215), YAC introduction of large and substantially germline fragments of the Ig loci [See Mendez et al. Nature Genetics 15:146-156 (1997), Green and Jakobovits J. Exp. Med. 188:483-495 (1998)], and introduction of entire or substantially entire loci through the use of microcell fusion (see European Patent Application No. EP 0 843 961 Al).

Any transgenic mouse capable of responding to immunization with antibodies having human sequences may be used to produce an anti-CGRP antibody of the invention when using methods available to one skilled in the art, e.g., when such mouse is immunized with a polypeptide comprising an immunogenic epitope of the invention.

Monoclonal antibodies may be made using the hybridoma method widely known in the art (see e.g., Kohler et al., Nature, 256:495, 1975) or may be made by recombinant DNA methods (e.g., as in U.S. Patent No. 4,816,567). Generally, a hybridoma can be produced by fusing a suitable immortal cell line (e.g., a myeloma cell line such as
SP2/0) with antibody producing cells of the immunized animal. The antibody producing cell, preferably those of the spleen or lymph nodes, are obtained from animals immunized with the antigen of interest. The fused cells (hybridomas) can be isolated using selective culture conditions, and cloned by limiting dilution. Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the antigen. Preferably, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an in vitro binding assay, such as radioimmunoassay (RIA) or ELISA. Cells which produce antibodies with the desired binding properties can be selected by a suitable screening assay. Methods for such isolation and screening are well known in the art.

Other suitable methods of producing or isolating antibodies of the invention can be used, including, for example, methods which select a recombinant antibody (e.g., single chain Fv or Fab) from a library, or which rely upon immunization of transgenic animals (e.g., mice) capable of producing a repertoire of human antibodies (see e.g., Jakobovits et al., Proc. Natl. Acad. ScL USA, 90:2551-2555, 1993; Jakobovits et al, Nature, 362:255-258, 1993; Lonberg et al., U.S. Patent Number 5,545,806; Surani et al., U.S. Patent Number 5,545,807).

Single chain antibodies, and humanized (CDR-grafted) antibodies, as well as CDR-grafted single chain antibodies, and the like, comprising portions derived from different species, are also encompassed by the present invention and the term "antibody". The various portions of these antibodies can be joined together chemically by conventional techniques, synthetically, or can be prepared as a contiguous protein using genetic engineering techniques. For example, nucleic acids encoding a chimeric or humanized chain can be expressed to produce a contiguous protein. See e.g., U.S. Patent No. 4,816,567; European Patent No. 0,125,023 Bl; U.S. Patent No. 4,816,397; European Patent No. 0,120,694 Bl; WO 86/01533; European Patent No. 0,194,276 B1; U.S. Patent No. 5,225, 539; European Patent No. 0,239,400 B1 and U.S. Patent Nos. 5,585,089 and 5,698,762. See also, Newman, R. et al. BioTechnology, 10:1455-1460, 1993, regarding primatized antibody, and Ladner et al., U.S. Patent No. 4,946,778 and Bird, R.E. et al., Science, 242:423-426, 1988, regarding single chain antibodies.

In addition, functional portions of antibodies, including antigen-binding portions of humanized, human or single chain antibodies, can also be produced. Functional
portions of the foregoing antibodies retain at least one antigen-binding function and/or biological function or bioactivity of the full-length antibody from which they are derived. Preferred functional portions retain an antigen-binding function of a corresponding full-length antibody (e.g., the ability to bind a mammalian mature form of CGRP). Particularly preferred functional portions or fragments retain the ability to inhibit one or more functions or bioactivities characteristic of a mammalian mature CGRP, such as a binding activity, a signaling activity, and/or stimulation of a cellular response. For example, in one embodiment, a functional portion or fragment can inhibit the interaction of mature CGRP with its receptor, resulting in the inhibition of one or more receptor-mediated functions.

Antibody portions or fragments capable of binding to mature CGRP include, but are not limited to, Fv, Fab, Fab' and F(ab')
2 fragments and are encompassed by the invention. Such fragments can be produced by enzymatic cleavage or by recombinant techniques. For instance, papain or pepsin cleavage can generate Fab or F(ab')
2 fragments, respectively. The smallest antigen-binding fragment is the Fv, which consist of the HCVR and the LCVR domains. The Fab fragment consists of the HCVR-CHl and LCVR-CL domains covalently linked by a disulfide bond between the constant regions. To overcome the tendency of non-covalently linked HCVR and LCVR domains in the Fv to dissociate when co-expressed in a host cell, a so-called single chain (sc) Fv fragment (scFv) can be constructed, in which a flexible and adequately long polypeptide links either the C-terminus of the HCVR to the N-terminus of the LCVR or the C-terminus of the LCVR to the N-terminus of the HCVR. The most commonly used linker has been a 15-residue (Gly4Ser)3 peptide, but other linkers are also known in the art. Antibodies can also be produced in a variety of truncated forms using antibody genes in which one or more stop codons has been introduced upstream of the natural stop site.

be successful alternatives to classical hybridoma technology (recent reviews: Little M. et al, Immunology Today, 21:364-70, 2000;).

The antibodies of the present invention can be expressed using DNA recombinant techniques and using a wide variety of host expression systems known in the art that can include prokaryotic (bacterial) and eukaryotic expression systems (such as yeast, baculovirus, plant, mammalian and other animal cells, transgenic animals, and hybridoma cells), as well as phage display expression systems. An example of a suitable bacterial expression vector is pUC1 19 and a suitable eukaryotic expression vector is a modified pcDNA3.1 vector with a weakened DHFR selection system. Other antibody expression systems are also known and are contemplated herein. (Sambrook, Fritsch, and Maniatis (Eds.), Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., 1989; Ausubel, et al (Eds.) Current Protocols in Molecular Biology, Greene Publishing Associates, 1989).

**Therapeutic Uses for the Antibody**

CGRP has been reported to play a role in the pathophysiology of migraines. (Cephalagia 24; 611-622, 2004) Migraines are caused by currently unknown triggers which stimulate trigeminal nerves and ganglia that innervate cephalic tissue, giving rise to release of neuropeptide messenger molecules from axons on the vasculature. Release of these neuropeptides then activates a series of events, a consequence of which is migraine pain. Further, release of these neuropeptides is thought to change vascular permeability resulting in subsequent leakage of plasma proteins in tissues innervated by stimulated trigeminal fibers. This leakage is one component of neurogenic inflammation which through an unknown mechanism may also lead to migraines. (Vascular Pharmacology 43; 176-187, 2005). CGRP may play a role in migraines because CGRP is released upon stimulation of sensory nerves and has potent vasodilation activity. Further, in an experimental animal model of migraine, CGRP increased vascular permeability and subsequent plasma protein leakage (plasma protein extravasation) in tissues innervated by trigeminal nerve fibers upon stimulation of these fibers. In addition, studies have reported that infusion of CGRP in patients who suffer from migraines has resulted in migraine-like symptoms. (Cephalagia 22(1): 54-61, 2002)
The present invention provides a pharmaceutical composition comprising an anti-CGRP antibody used to decrease circulating levels of free CGRP which is useful for the treatment or prevention of migraines in subjects, preferably humans. As demonstrated in Example 5, an anti-CGRP antibody of the present invention reduces protein plasma extravasation in an experimental in vivo migraine model. Briefly, an anti-CGRP antibody is administered intravenously to a rat, followed by fluorescein isothiocyanate dye labeled bovine serum albumin (FITC-BSA) which serves as a marker of protein extravasation. After 10 minutes, the left trigeminal ganglion is stimulated and the amount of FITC-BSA is measured in the dural membranes, representing the amount of protein that has leaked from the vasculature. As demonstrated in Example 5, an anti-CGRP antibody is able to reduce the amount of protein extravasation when administered in an experimental in vivo migraine model. Reduction in the amount of protein extravasation in this model is an indication of the ability to treat migraines which may be caused by a release of these neuropeptides which changes vascular permeability resulting in subsequent leakage of plasma proteins in tissues innervated by stimulated trigeminal fibers.

Further the present invention provides a pharmaceutical composition used to decrease circulating levels of free CGRP which is useful for the treatment or prevention of migraines in subjects comprising an anti-CGRP antibody which binds to fragments of CGRP. For instance in Example 3, the anti-CGRP antibody described in Example 5 was tested to determine the minimal epitope required for binding to CGRP. As shown in Table 4, the results of Example 3 indicate that the antibody effective in reducing plasma protein extravasation, binds within a region of CGRP (29-37).

**Pharmaceutical Composition**

An antibody of the invention can be incorporated into pharmaceutical compositions suitable for administration to a subject. The compounds of the invention may be administered alone or in combination with a pharmaceutically acceptable carrier, diluent, and/or excipients, in single or multiple doses. The pharmaceutical compositions for administration are designed to be appropriate for the selected mode of administration, and pharmaceutically acceptable diluents, carrier, and/or excipients such as dispersing agents, buffers, surfactants, preservatives, solubilizing agents, isotonicity agents, stabilizing agents and the like are used as appropriate. Said compositions are designed in

A pharmaceutical composition comprising an anti-CGRP monoclonal antibody of the present invention can be administered to a subject at risk for or exhibiting migraines as described herein using standard administration techniques including oral, intravenous, intraperitoneal, subcutaneous, pulmonary, transdermal, intramuscular, intranasal, buccal, sublingual, or suppository administration.

The route of administration of an antibody of the present invention may be oral, parenteral, by inhalation, or topical. Preferably, the antibodies of the invention can be incorporated into a pharmaceutical composition suitable for parenteral administration. The term parenteral as used herein includes intravenous, intramuscular, subcutaneous, rectal, vaginal, or intraperitoneal administration. Peripheral systemic delivery by intravenous or intraperitoneal or subcutaneous injection is preferred. Suitable vehicles for such injections are straightforward in the art.

The pharmaceutical composition typically must be sterile and stable under the conditions of manufacture and storage in the container provided, including e.g., a sealed vial or syringe. Therefore, pharmaceutical compositions may be sterile filtered after making the formulation, or otherwise made microbiologically acceptable. A typical composition for intravenous infusion could have a volume as much as 250-1000 ml of fluid, such as sterile Ringer's solution, physiological saline, dextrose solution and Hank's solution and a therapeutically effective dose, (e.g., 1 to 100 mg/mL, or more) of antibody concentration. Dose may vary depending on the type and severity of the disease. As is well known in the medical arts, dosages for any one subject depends upon many factors, including the patient's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. A typical dose can be, for example, in the range of 0.001 to 1000 mg; however, doses below or above this exemplary range are envisioned, especially considering the aforementioned factors.

**Articles of Manufacture**
In another embodiment of the invention, an article of manufacture containing materials useful for the treatment or prevention of the disorders or conditions described above is provided. The article of manufacture comprises a container and a label. Suitable containers include, for example, bottles, vials, syringes, and test tubes. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition of the invention which is effective for preventing or treating the disorder or condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The active agent in the composition is an anti-CGRP antibody of the invention. The label on or associated with, the container indicates that the composition is used for treating the condition of migraines. The article of manufacture may further comprise a second container comprising a pharmaceutically-acceptable buffer, such as phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

Example 1: BINDING OF ANTI-CGRP ANTIBODIES TO HUMAN AND RAT CGRP

To determine the reactivity of anti-CGRP antibodies obtained, an ELISA is used to determine binding of anti-CGRP antibodies to CGRP peptides or peptide fragments from human and rat origin. Peptides are dried onto a Greiner MultiBind microtiter plate (655 061) by dispensing 60 µL per well of a 5 µg/ml peptide concentration in water (dry the plate overnight at 37°C). The plates are washed 2 times with wash buffer (0.02 M Tris, 0.15 M NaCl, 0.1% Twccn 20, pH 7.40) and are blocked using 180 µL/well of Pierce Casein/PBS. The plates are incubated at room temperature (RT) for 1 hour. The plates are washed once, 50 µL of anti-CGRP antibody (diluted in casein/PBS) is added per well in duplicate, and the plates are incubated at RT for 1 hour. The plates are washed 3 times and bound anti-CGRP antibody is detected using a labeled secondary antibody (goat anti-mouse IgG kappa specific—HRP at 1:200 dilution) at 50 µL per well and incubation at RT for 1 hour. The plate are washed 4 times and 50 µL of OPD substrate (5 mg OPD
tablet dissolved in 12.5 ml of 0.1 M Na₂HPO₄, 0.05 M citric acid, pH 5.0 then add 5 µL of 30% H₂O₂) is dispensed. The reaction is stopped with 100 µL of 1 N HCl after 10 minutes. Absorbance at 490 nm (A₄₉₀) is read using an appropriate ELISA plate reader. The degree of binding is proportional to the A₄₉₀signal. The EC50 for each peptide is calculated using SigmaPlot regression (sigmoidal with Hill, 4 parameter). Table 2 demonstrates that an anti-CGRP antibody binds in a dose-dependent fashion to all 4 forms of CGRP tested.

Table 2: ELISA results

<table>
<thead>
<tr>
<th>Peptide</th>
<th>EC₅₀ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human αCGRP</td>
<td>1.24</td>
</tr>
<tr>
<td>Human βCGRP</td>
<td>1.07</td>
</tr>
<tr>
<td>Rat αCGRP</td>
<td>2.33</td>
</tr>
<tr>
<td>CGRP (8-37)</td>
<td>2.09</td>
</tr>
</tbody>
</table>

Example 2: Affinity Measurements

The affinity (K_D) and K_on and K_off rates of anti-CGRP Fabs and monoclonal antibodies of the present invention are measured using a BIAcore Biosensor 2000, and data are analyzed with BIAevaluation (v. 4.1) software using a 1:1 binding with mass transfer model with a global fit. Goat-anti mouse kappa (Southern Biotech) are coupled via free amine groups to carboxyl groups on flow cells 1, 2, 3, and 4 of a CM5 biosensor chip (BIAcore) using N-ethyl-N-dimethylaminopropyl carbodiimide and N-hydroxysuccinimide (EDC/NHS) following the manufacturer's instructions. Flow cells 1, 2, 3, 4, 2-1, 3-1, and 4-1 are monitored with a flow rate of 100 µL/min. All reagents are diluted to the desired concentration using HBS-EP (10 mM HEPES, pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.005% Surfactant P20). Specific mouse monoclonal or Fab
antibodies are captured on flow cell 2, 3, and 4 to yield a total of 800 to 1200 resonance units (RU; results reflect flow cells 2-1, 3-1, and 4-1) using a flow rate of 10 µL/min. Peptide (e.g., amylin, adrenomedullin, human α-CGRP (α-hCGRP), rat α-CGRP (α-rCGRP)) is then injected at a flow rate of 100 µL/min over flow cells 1, 2, 3, and 4 at increasing concentrations (0.39 x10^3 M to 50 nM) followed by a regeneration step using glycine-HCl (pH 1.5) between each cycle. One cycle includes an antibody capture step with a 1 minute dissociation period, an antigen capture step (X nM peptide) with a 10-20 minute dissociation period, and a regeneration step. A control injection containing no peptide (buffer only) serves as a control for baseline subtraction. Binding is determined at 25° C. "ND" indicates experiment was not done. α-hCGRP represents human α-CGRP, β-hCGRP represents human β-CGRP, and α-rCGRP represents rat α-CGRP. When tested in the above assay, the anti-CGRP Fab 2B5, the monoclonal antibody C71 13 (Sigma-Aldrich, St. Louis, MO) and the monoclonal antibody CGRPc1 have binding affinities characteristics as listed in Table 3. Neither monoclonal antibody nor Fab bound to amylin or adrenomedullin (tested at 500 nM). The anti-CGRP monoclonal antibodies and the anti-CGRP Fab tested specifically bind to rat and human α-CGRP and human β-CGRP.
Example 3: Determination of the Minimal Epitope of an Anti-CGRP Antibody

The minimal epitope required for binding of an anti-CGRP antibody of the present invention is determined following the procedure as outlined in Example 2. Briefly, α-rCGRP fragments differing in size are synthesized and are tested in the BIACORE assay as in Example 2. The α-rCGRP fragments are numbered with respect to the full length CGRP. For example, α-rCGRP (32-37) refers to a CGRP fragment that only contains amino acids 32-37 when compared to the full length CGRP peptide (1-37). Affinity measurements are conducted at 25°C. As demonstrated in Table 4, the minimal epitope required for binding by this particular anti-CGRP antibody is the α-rCGRP (32-37).
However, the minimal epitope to retain K similar to full length CGRP, is α-rCGRP (30-37). As above, α-rCGRP represents rat αCGRP. "NB" indicates that no binding was detected.

<table>
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<tr>
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<th>$k_{on}$ (1/Ms)</th>
<th>$k_{off}$ (1/s)</th>
<th>$K_D$ (M)</th>
</tr>
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<tr>
<td>α-rCGRP (1-37)</td>
<td>1.69E+06</td>
<td>1.72E-04</td>
<td>1.02E-10</td>
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<tr>
<td>α-rCGRP (29-37)</td>
<td>1.23E+06</td>
<td>2.49E-04</td>
<td>2.02E-10</td>
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<td>α-rCGRP (30-37)</td>
<td>1.88E+06</td>
<td>3.10E-04</td>
<td>1.65E-10</td>
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<tr>
<td>α-rCGRP (31-37)</td>
<td>1.45E+06</td>
<td>8.37E-04</td>
<td>5.77E-10</td>
</tr>
<tr>
<td>α-rCGRP (32-37)</td>
<td>3.21E+06</td>
<td>5.40E-03</td>
<td>1.68E-09</td>
</tr>
<tr>
<td>α-rCGRP (33-37)</td>
<td>NB</td>
<td>NB</td>
<td>NB</td>
</tr>
</tbody>
</table>

TABLE 4

EXAMPLE 4: Inhibition of CGRP-induced induction of cAMP in human SK-N-MC cells expressing the CGRP receptor

In this assay, SK-N-MC cells, which are a neuroepithelioma cell line endogenously expressing the CGRP receptor, are cultured in MEM containing 10% FBS, IX MEM non-essential amino acids, IX 100 mM MEM Sodium Pyruvate, IX Pen/Strep, IX 200 mM L-glutamine, and detached using enzyme-free cell dissociation buffer. Cells are stimulated using 15,000 cell/well in Stimulation Buffer (HBSS (with Mg and Ca), 5 mM HEPES, 0.1 % BSA, 100 µM Ascorbic acid) - PBS (1:2) containing 0.5 mM isobutylmethylxanthine (IBMX). Human CGRP is used to stimulate cAMP (final concentration 5 nM), with an anti-CGRP antibody (Fab or monoclonal antibody) added in varying concentrations. Cells are incubated for 1 hour with the quantitation of cAMP measured using homogeneous time-resolved fluorescence (HTRF). CGRP(8-37) is used as an internal control for inhibition and is included in each experiment. In addition, a mouse IgGl (mlgGl) and a Fab control are included as negative controls. The relative IC50 concentrations were calculated using a 4 parameter curve fit model, plotting the concentration of inhibitor (nM) versus the percentage of inhibition obtained from the assay with each reagent. Results for antibodies (monoclonal or Fab) to CGRP are listed in Table 2, controls in table 3.
The results indicate, the monoclonal C71 13, Fab 2B5, the monoclonal CGRP-Cl and CGRP(8-37) peptide, a known inhibitor resulted in IC₅₀'s of 1.01 x 10⁻⁸ M, 1.45 x 10⁻⁸ M, 5.49 x 10⁻⁸ M, and 1.34 x 10⁻⁸ M, respectively. All three anti-CGRP reagents completely inhibited CGRP-induced cAMP increases, whereas the negative controls (mTgGl and Fab control) had no effect. CGRP(8-37) completely inhibited the increase in cAMP, demonstrating the assay performed correctly. These results in Table 5 demonstrate that anti-CGRP antibodies (Fab or monoclonal antibody) effectively and specifically inhibit CGRP-induced increases in cAMP.

<table>
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<tr>
<th>reagent</th>
<th>Rel IC₅₀ (nM)</th>
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<tr>
<td>C7113</td>
<td>10.15</td>
</tr>
<tr>
<td>2B5</td>
<td>14.57</td>
</tr>
<tr>
<td>CGRP Cl</td>
<td>5.49</td>
</tr>
<tr>
<td>CGRP (8-37)</td>
<td>13.48</td>
</tr>
<tr>
<td>mIgG1 control</td>
<td>&gt;133</td>
</tr>
<tr>
<td>Fab control</td>
<td>&gt;90</td>
</tr>
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**EXAMPLE 5: Rat Dural Plasma Protein Extravasation (PPE) Model**

The rat PPE model is a well established pre-clinical model to evaluate the efficacy of compounds for the treatment of migraine. The aim of the study was to determine whether the blocking of CGRP would result in inhibition of stimulation-induced protein extravasation.

A 2 mg/mL solution of an anti-CGRP antibody (Ab) is prepared in saline solution. All subsequent dilutions are made with saline. A 2 mg/mL solution of monoclonal isotype control antibody (IgG) is also prepared in saline.

Male Sprague-Dawley rats from Harlan Laboratories (250 to 350 g) are anesthetized with Nembutal (60 mg/kg, ip.) and placed in a stereotaxic frame (David Kopf Instruments) with the incisor bar set at —2.5 mm. Following a mid-line sagittal scalp incision, two pairs of bilateral holes are drilled through the skull (3.2 mm
posteriorly, 1.8 and 3.8 mm laterally, all coordinates referenced to bregma). Pairs of stainless steel stimulating electrodes (Rhodes Medical Systems Inc), insulated except at the tips, are lowered through the holes in both hemispheres to a depth of 9.2 mm below the dura. Test compounds or saline vehicle are administered intravenously via the femoral vein (1 mL/kg). Eight minutes later a solution of fluorescein isothiocyanate (FITC) dye-labeled bovine serum albumin (BSA) (FITC-BSA, Sigma A9771 Lot# 122K7460) (20 mg/kg, iv.), is injected into the femoral vein to function as a marker for protein extravasation. Ten minutes following dosing with test compound or vehicle, the left trigeminal ganglion is stimulated for 5 minutes at a current intensity of 1.0 πiA (5 Hz, 5 ms duration) with a Model S48 Grass Instrument Stimulator. Five minutes following stimulation, the rats are killed by exsanguination with 40 mL of saline. The exsanguination also rinses residual FITC/BSA out of the blood vessels. The top of the skull is removed to collect the dural membranes. The membrane samples are removed from both hemispheres, rinsed with water, and spread flat on microscope slides. The slides are dried for 15 minutes on a slide warmer and cover-slipped with a 70% glycerol/water solution.

A fluorescence microscope (Zeiss) equipped with a grating monochromator and a spectrophotometer are used to quantify the amount of FITC-BSA dye in each dural sample. The microscope is equipped with a motorized stage interfaced with a personal computer. This facilitates the computer-controlled movement of the stage, with fluorescence measurements at 25 points (500 μm steps) on each dural sample. The extravasation induced by electrical stimulation of the trigeminal ganglion is an ipsilateral effect (i.e. occurs only on the side of the dura in which the trigeminal ganglion is stimulated). This allows the other (unstimulated) half of the dura to be used as a control. The extravasation ratio (i.e. the ratio of the amount of extravasation in the dura from the stimulated side compared to the unstimulated side) is calculated. Animals dosed with vehicle alone or an ineffective dose of the test compound have an extravasation ratio of approximately two, while totally effective treatments result in a ratio of approximately one. The results of Table 5 indicate that an anti-CGRP antibody inhibits protein extravasation in the rat PPE model in a dose dependant fashion.

TABLE 5
<table>
<thead>
<tr>
<th>Treatment (iv)</th>
<th>Extravasation Ratio (s.e.m.)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline vehicle</td>
<td>1.80 (0.07)</td>
<td>3</td>
</tr>
<tr>
<td>Anti-CGRP Ab (2.0 mg/kg)</td>
<td>1.05 (0.05)</td>
<td>3</td>
</tr>
<tr>
<td>Anti-CGRP Ab (0.2 mg/kg)</td>
<td>1.24 (0.16)</td>
<td>3</td>
</tr>
<tr>
<td>Anti-CGRP Ab (0.02 mg/kg)</td>
<td>1.97 (0.13)</td>
<td>3</td>
</tr>
<tr>
<td>IgG control Ab (2.0 mg/kg)</td>
<td>1.84 (0.07)</td>
<td>3</td>
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CLAIMS

1. A method of treating migraines comprising: administering an effective amount of an anti-CGRP antibody which binds to and inhibits the ability of CGRP to bind to the CGRP receptor to a subject suffering from a migraine.

2. A method of preventing migraines comprising: administering an effective amount of an anti-CGRP antibody which binds to and inhibits the ability of CGRP to bind to the CGRP receptor to a subject who has suffered at least one previous migraine.

3. A method of reducing pain due to migraines comprising administering an effective amount of an anti-CGRP antibody which binds to and inhibits the ability of CGRP to bind to the CGRP receptor to a subject suffering from a migraine.

4. The use of an anti-CGRP antibody which binds to and inhibits the ability of CGRP to bind to the CGRP receptor in the manufacture of a medicament for treating migraines.

5. The use of an anti-CGRP antibody which binds to and inhibits the ability of CGRP to bind to the CGRP receptor in the manufacture of a medicament for preventing migraines.

6. The use of an anti-CGRP antibody which binds to and inhibits the ability of CGRP to bind to the CGRP receptor in the manufacture of a medicament for treating pain due to migraine.

7. A method of inhibiting neurogenic protein extravasation in a human suffering from a migraine comprising administering an effective amount of anti-CGRP antibody to said human.

8. A method of inhibiting binding of CGRP to a receptor in a human suffering from a migraine comprising administering an effective amount of an anti-CGRP antibody.

9. A method of inhibiting CGRP receptor activation comprising administering an effective amount of an anti-CGRP antibody to a human suffering from a migraine.

10. A method of inhibiting CGRP activity comprising administering an amount of an anti-CGRP antibody effective to neutralize CGRP in a subject suffering from a migraine.

11. The method of any one of the previous claims, wherein the antibody is to human CGRP.
12. The method of any one of the previous claims 1-11, wherein said anti-CGRP antibody inhibits CGRP binding of a competing antibody comprising a polypeptide selected from the group consisting of SEQ ID NO's:

   a. 28-33 and
   b. 34-39.

13. The method of claim 12, wherein said competing antibody comprises SEQ ID's 33 and 39.

14. The method of any one of the previous claims 1-17, wherein the antibody has a Kd of no greater than 2.99 x 10^-9 M.

15. The method of any one of the previous claims 1-14, wherein the antibody is of a type selected from the group consisting of murine, humanized and human antibodies.

16. The method of any one of the previous claims 1-14, wherein said antibody is suitable for parenteral, oral, intranasal, subcutaneous, aerosolized or intravenous administration in a human or animal.
**INTERNATIONAL SEARCH REPORT**

**A. CLASSIFICATION OF SUBJECT MATTER**

INV. A61K39/395 C07K16/28 A61P25/06

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

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

C07K

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)

EPO-Internal, BIOSIS, EMBASE, WPI Data, Sequence Search

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

<table>
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<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate of the relevant passages</th>
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**Date of the actual completion of the international search**

21 May 2007

**Date of mailing of the international search report**

11/06/2007

**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**

Bernhardt, Wiebke

Form PCT/ISA/210 (second sheet) (April 2008)
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<td>WO 2005/100360 A (BOEHRINGER INGELHEIM INT [DE]; BOEHRINGER INGELHEIM PHARMA [DE]; MUELL) 27 October 2005 (2005-10-27) page 1; claims 1-10</td>
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<td>LITTLE M ET AL: &quot;Of mice and men: hybridoma and recombinant antibodies&quot; IMMUNOLOGY TODAY, ELSEVIER PUBLICATIONS, CAMBRIDGE, GB, vol. 21, no. 8, 1 August 2000 (2000-08-01), pages 364-370, XP004215163 ISSN: 0167-5699 page 367; figure 3</td>
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</table>
This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. **Claims Nos**
   - **X**
   - because they relate to subject matter not required to be searched by this Authority, namely
   - Although claims 1-3 and 7-10 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. **Claims Nos**
   - because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out specifically.

3. **Claims Nos**
   - because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 64(a).

**Box III** Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. **As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims**

2. **As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee**

3. **As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid specifically claims Nos**

4. **No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims, it is covered by claims Nos**

**Remark on Protest**

- The additional search fees were accompanied by the applicant's protest
- No protest accompanied the payment of additional search fees
<table>
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<td>wo 2005100360 A</td>
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