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REF: Expediente 13-025-485

TÍTULO SOLICITADO: "PROTEÍNAS DE UNIÓN DE DOMINIO VARIABLE DUAL QUE SE UNEN A NGF Y AL MENOS A UN BLANCO ADICIONAL, Y MÉTODOS PARA HACER Y USAR DICHAS PROTEÍNAS DE UNIÓN"

PUBLICACIÓN: Gaceta 667 de 15 de mayo de 2013, N° de publicación 524.

PRIORIDAD: US 61/363-120 09/07/2010

SOLICITANTE: ABBVIE, INC

APODERADO: ALICIA LLORED A RICAURTE

TRÁMITE: SUSTENTACIÓN DE OPOSICIÓN

JOSÉ LUIS REYES VILLAMIZAR, mayor de edad, identificado como aparece al pie de mi firma, abogado en ejercicio, titular de la tarjeta profesional número 44655 del Consejo Superior de la Judicatura, obrando en calidad de apoderado de la sociedad **TECNOQUIMICAS S.A.**, dentro del trámite de la referencia, en ejercicio de lo dispuesto por el artículo 42 inciso 2º de la Decisión 486 de 2000, por medio del presente escrito, procedo a sustentar y complementar la oposición presentada el 12 de agosto de 2013, en los siguientes términos:

I. RATIFICACIÓN

La solicitud 13-025-485, referida a proteínas de unión multivalentes y multiespecíficas diseñadas, métodos para elaborarlas y, específicamente, sus usos en la prevención, el diagnóstico y/o el tratamiento de enfermedades, carece de claridad y unidad de invención, e incumple también los requisitos de novedad y

nivel Inventivo, especialmente a la luz de las revelaciones del documento US 2009/0304693 de Ghayur et al.

En primer lugar, se objetó la claridad, concisión y soporte de la descripción y de las reivindicaciones de la solicitud en trámite, debido a que los anticuerpos divulgados no definen la secuencia, ni sus características técnicas estructurales, por el contrario, intenta identificar la proteína por una fórmula general conocida de anticuerpos duales.

Se observó que la materia pretendida por las reivindicaciones es tan amplia que permite la protección de anticuerpos tipo Rituximab (que se unen a CD20), Bevacizumab (que se unen a VEGF) y Cetuximab (que se unen a EGFR) entre otros, conocidos en el estado de la técnica.

Se observó que la solicitud no cumple con el requisito de unidad de invención en las reivindicaciones 1 – 33 ya que indican proteínas de unión que se unen a diferentes antígenos o sitios de unión como NGF y MTX, o NGF y NKG2D o NGF e IGF1, etc.; cada una de las proteínas con sitio de unión a cada pareja obedece a un objeto inventivo diferente.

En cuanto a la materia revelada en las reivindicaciones 59 a 61, el uso de las proteínas de unión para el tratamiento de enfermedades divulgado no es patentable según la legislación vigente.

Por su parte, el documento US 2009/0304693 de Ghayur et al., se destaca por contener *la materia relevante* de las reivindicaciones debido a que este documento describe proteínas de unión capaces de unirse a un par de antígenos, que comprenden una cadena polipeptídica que comprende $VD-1(X1)n-VD2-C-(X2)n$, en donde;

VD1 es un primer dominio variable de la cadena pesada;

VD2 es un segundo dominio variable de la cadena pesada;

C es un dominio constante de la cadena pesada;

X1 es un conector con la condición que no sea CH1 (párrafo 0014);

X2 es una región Fc y n es 0 o 1 .

Se observa entonces, que la fórmula propuesta por la solicitud en trámite ha sido de uso recurrente en el estado de la técnica indicando incluso los sustituyentes comunes de cada uno de las cadenas variables.

Se identificó más específicamente que la proteína de unión liga a un par de antígenos que consisten de NGF (párrafo 0019) y, aunque no es específico, la anterioridad en mención enseña composiciones farmacéuticas para tratar una enfermedad, que comprenden la proteína de unión y metotrexato (MTX). En consecuencia, la solicitud no cumple con el requisito de novedad ya que no demuestra características técnicas esenciales novedosas e incluso reclama las mismas secuencias y proteínas de unión de anticuerpos conocidos en una fórmula también conocida de anticuerpos biespecíficos y bivalentes.

II. COMPLEMENTO DE INFORMACIÓN

Es importante desatacar que a la fecha, no existen modificaciones al capítulo reivindicatorio de la solicitud en estudio, sin embargo, en el presente documento se reitera que la solicitud en curso no cumple con los requisitos de patentabilidad.

Adicionalmente a lo ya expuesto, la presente solicitud de patente no cumple con lo establecido por la Decisión 486 como se puede ver de la materia descrita en la reivindicación 8, la cual menciona lo siguiente:

8. La proteína de unión de acuerdo con la reivindicación 1, 3, 6, 24 ó 91-94, en donde X1 es por lo menos uno de ID de SEQ No.; 1-27, o una secuencia basada en G/S.

Se observa que en ésta reivindicación se incluyen de 1 a 27 secuencias de cadena polipeptídica donde los dominios variables obedecen a aquellos de cadena del ligador; dichas secuencias sin embargo, ya habían sido descritas con anterioridad en el mismo documento citado de Gahyur et al., tal como se observa en la SEQ ID NO1:

For example, X1 is a linker with the proviso that X1 is not CH1. For example, X1 is a linker selected from the group consisting of AKTTPKLEEGEFSEAR (SEQ ID NO: 1); AKTTPKLEEGEFSEARV (SEQ ID NO: 2); AKTTPKLGG (SEQ ID NO: 3); SAKTPKLGG (SEQ ID NO: 4); SAKTTP (SEQ ID NO: 5); RADAAP (SEQ ID NO: 6); RADAAPTVS (SEQ ID NO: 7); RADAAAAGGPGS (SEQ ID NO: 8); RADAAA(G₄S)₄ (SEQ ID NO: 9); SAKTPKLEEGEFSEARV (SEQ ID NO: 10); ADAAP (SEQ ID NO: 11); ADAAPTVSIFPP (SEQ ID NO: 12); TVAAP (SEQ ID NO: 13); TVAAPSVFIFPP (SEQ ID NO: 14); QPKAAP (SEQ ID NO: 15); QPKAAPSVTLFPP (SEQ ID NO: 16); AKTTPP (SEQ ID NO: 17); AKTTPPSVTPLAP (SEQ ID NO: 18); AKTTAP (SEQ ID NO: 19); AKTTAPSVYPLAP (SEQ ID NO: 20); ASTKGP (SEQ ID NO: 21); ASTKGPSVFPLAP (SEQ ID NO: 22); GGGGSGGGSGGGGS (SEQ ID NO: 23); GENKVEYAPALMALS (SEQ ID NO: 24); GPAKELTPLKEAKVS (SEQ ID NO: 25); and GHEAAVMQVQYPAS (SEQ ID NO: 26). In an embodiment, X2 is an Fc region. In another embodiment, X2 is a variant Fc region.

(Párrafo 0013 de Gahyur et al.,)

Se observan las 26 primeras secuencias reivindicadas opcionales para X1 mientras que en el aparte de abajo se observan las mismas secuencias descritas en la solicitud (copia de la solicitud internacional WO 2012/006490 correspondiente a la solicitud colombiana en trámite, página 5)

proviso that X1 is not CL1. In an embodiment, X1 comprises AKTTPKLEEGEFSEAR (SEQ ID NO: 1); AKTTPKLEEGEFSEARV (SEQ ID NO: 2); AKTTPKLGG (SEQ ID NO: 3); SAKTPKLGG (SEQ ID NO: 4); SAKTTP (SEQ ID NO: 5); RADAAP (SEQ ID NO: 6);
25 RADAAPTVS (SEQ ID NO: 7); RADAAAAGGPGS (SEQ ID NO: 8); RADAAA(G₄S)₄ (SEQ ID NO: 9); SAKTPKLEEGEFSEARV (SEQ ID NO: 10); ADAAP (SEQ ID NO: 11); ADAAPTVSIFPP (SEQ ID NO: 12); TVAAP (SEQ ID NO: 13); TVAAPSVFIFPP (SEQ ID NO: 14); QPKAAP (SEQ ID NO: 15); QPKAAPSVTLFPP (SEQ ID NO: 16); AKTTPP (SEQ ID NO: 17); AKTTPPSVTPLAP (SEQ ID NO: 18); AKTTAP (SEQ ID NO: 19); AKTTAPSVYPLAP
30 (SEQ ID NO: 20); ASTKGP (SEQ ID NO: 21); ASTKGPSVFPLAP (SEQ ID NO: 22); GGGGSGGGSGGGGS (SEQ ID NO: 23); GENKVEYAPALMALS (SEQ ID NO: 24); GPAKELTPLKEAKVS (SEQ ID NO: 25); or GHEAAVMQVQYPAS (SEQ ID NO: 26). In an embodiment, the binding protein does not comprise X2.

Cada una de las secuencias es idéntica en 100% a su correspondiente en el documento de anterioridad, razón por la cual, la materia es la misma objetándose de forma contundente el requisito de novedad de la solicitud en trámite.

De otro lado, los anticuerpos seleccionados y evidenciados en las secuencias, aparte de no ser novedosos, no incluyen efectos técnicos que permitan demostrar que superan en alguna medida a los anticuerpos de la anterioridad, es decir, su selección es la solución alternativa del problema técnico objetivo.

Por esta razón, se solicita tomar atenta nota en cuanto a que cualquier argumento a favor del nivel inventivo de modalidades particulares no merece prosperar, al ser una alternativa rutinaria de los anticuerpos descritos por la anterioridad citada; adicionalmete, se sabe que, para que se reconozca la unidad de invención, se ha determinado que la materia debe ser unida por un único concepto inventivo, el cual deberá ser nuevo e inventivo.

A pesar de lo anterior, y con el fin de que sea más claro el establecimiento de la falta de novedad y nivel inventivo de la solicitud en trámite, me permito presentar la solicitud americana US 2007/0092507 publicada por Balthasar et al., titulado "Anti-FCRN antibodies for treatment of auto/alloimmune conditions".

En dicho documento, se reclaman anticuerpos o inmunoglobulinas (IgG1) que se unen a metotrexate; estos anticuerpos pueden ser policlonales, monoclonales, quiméricos o humanizados, o fragmentos de unión al antígeno de los mismos. Además menciona la anterioridad que son útiles para la reducción de la concentración de IgGs patogénicos en individuos y por lo tanto usados en terapia contra enfermedades autoinmunes y aloinmunes, veamos:

Ind.). Both IVIG preparations are solvent/detergent-treated and are manufactured via cold ethanol fractionation of human plasma. Outdated human platelets were obtained from the American Red Cross (Buffalo, N.Y. and Salt Lake City, Utah). A murine antimethotrexate IgG1 monoclonal antibody (AMI) was generated and purified in our laboratory. Goat antihuman IgG (no cross-reactivity to goat and mouse serum proteins) and alkaline phosphatase—conjugated goat antimouse IgG (no cross-reactivity to goat and human serum proteins) were both obtained from Rockland (Gilbertsville, Pa.). Mouse antihuman IgG, fluorescein

En este caso, los anticuerpos IgG1 dirigidos contra metotrexato han sido empleados incluso como control, lo cual implica un grado de desarrollo en este sentido, y una necesidad de experimentación en esta área:

[0075] Also shown are results from incubation of the 293 cells with supernatant from cells that secrete murine monoclonal IgG1 antibodies directed against methotrexate (i.e., as a negative control). Incubation of the 293 cells with the anti-methotrexate antibody did not lead to a significant assay response. This is (again) consistent with the hypothesis that specific anti-hFcRn antibodies are responsible for the significant binding observed following incubation of cells with 11E4 & 1H5 supernatant.

De esta manera, habría sido obvio para el técnico versado en la materia combinar las enseñanzas de Balthasar et al., considerando la sugerencia de inmunoglobulinas anti-metotrexate con la sugerencia de obtener proteínas de unión multiespecífica de Ghayur, con el fin de mejorar la unión de dicha proteína de unión al metotrexate, y de esta forma marcar la unión de las inmunoglobulinas a células igualmente marcadas en un método para tratar una enfermedad tal como lo enseña Ghayur et al., (como se observa, se basa en doble marcaje para identificar el tratamiento a las células autoinmunes).

De otro lado, una consecuencia de la carencia de concisión y la falta de unidad de invención de la solicitud en trámite es la presencia de múltiples posibilidades de anticuerpos, los cuales, incluso son mencionados en una misma reivindicación bajo características explícitas, evidentemente demostrando que incumplen los criterios de patentabilidad; este es el caso de la reivindicación 89 que pretende proteger la siguiente materia:

89. La proteína de unión de acuerdo con las reivindicaciones 1, 3, 6, o 24, en donde los dominios variables de cadena pesada VD1 y/o VD2, si están presentes, comprenden el ID de SEQ No.: 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, o 90, respectivamente; y/o en donde los dominios variables de cadena liviana VD1 y/o VD2, si están presentes, comprenden el ID de SEQ No.: 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, o 91, respectivamente.

De acuerdo con lo anterior, el documento de Ghayur et al., enseña una proteína de unión donde los dominios variables de cadena pesada VD1 y VD2 independientemente pueden comprender tres CDRs que unen NGF:

[0088] In a full-length antibody, each heavy chain is comprised of a heavy chain variable region (abbreviated herein as HCVR or VH) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as LCVR or VL) and a light chain constant region. The light chain constant region is comprised of one domain, CL. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. Immunoglobulin molecules can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG 3, IgG4, IgA1 and IgA2) or subclass.

Si bien este documento aunque menciona las características generales de los anticuerpos de la solicitud, no enseña específicamente donde las secuencias de unión a NGF comprenden CDRs de la SEQ ID NO: 86:

86	AB118VH	VH - NGF (seq. 2)	EVQLVESGGGLVQPGGSLRLSCAASGFSLTNNNVNWRQA PGKGLEWVGGVWAGGATDYN SALKSRFTISRDN SKNTAYL QMNSLRAEDTAVYYCARDGGYSSSTLYAMD A WGQGT LVTV SS
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Sin embargo, en una publicación similar, el documento US 2009/0208490 titulado “molecules that are able to inhibit the binding between NGF and the TRKA receptor as analgesics with prolonged efect” de Pavone et al., publicado el 20 de agosto de 2009, incluye anticuerpos anti-NGF capaces de inhibir la unión entre NGF y TrkA, de bloquear la actividad biológica de TrkA para la preparación de un medicamento para el tratamiento o prevención de una enfermedad crónica, y menciona específicamente las secuencias de la solicitud.

Ahora bien, observábamos que una de las opciones de los anticuerpos duales es que la cadena pesada de CDRs comprenda la SEC ID NO. 86; esta secuencia, sin embargo es la misma secuencia divulgada por Pavone et al., en su secuencia SEQ ID NO:4:

SEQ ID No. 4 (VH, variable region of the heavy chain of Hu-αD11) :

H CDR1
EVQLVESGGGLVQP GGSLRLSCAASGFSLTNNNVNWRQAPGKGLEWV

H CDR2
GGVWAGGATDYN SALKSRFTISRDN SKNTAYLQMNSLRAEDTAVYYCA

H CDR3
RDGGYSSSTLYAMD A WGQGT LVTVSS

Teniendo en cuenta que los anticuerpos descritos por Pavone et al., se unen a NGF y presentan estructura idéntica a las CDRs (subrrayadas SEQ ID NO: 4) de la solicitud en estudio en su SEQ ID No: 86, se demuestra que las características por las cuales se reclaman los anticuerpos son conocidas en el estado de la técnica y por lo tanto habría sido obvio para una persona versada en la materia incluir dichas CDRs en la proteína de unión enseñada por Ghayur et al.

En conclusión, una persona versada en la materia habría derivado de forma evidente lo revelado en la solicitud en trámite, con las mismas características técnicas reclamadas bajo las enseñanzas del documento de Ghayur et al., en cuanto a la fórmula (la estructura y la funcionalidad del anticuerpo solicitado), las secuencias anti-NGF como Pavone et al., y las secuencias anti-MTX de Balthasar et al., descritas opcionalmente en las mismas reivindicaciones.

Por todo lo anterior, los anticuerpos reclamados no aportan características técnicas novedosas, adicionalmente, no se identifica ningún efecto técnico sorprendente o inesperado con respecto al arte anterior.

Así, ni el concepto general plasmado en las reivindicaciones principales, ni las modalidades particulares incluidas en reivindicaciones dependientes que incluyen secuencias específicas, permiten demostrar la novedad ni el nivel inventivo de la solicitud, por tanto, se incumplen los requisitos de patentabilidad. En consecuencia, rogamos se analicen los argumentos expuestos con el rigor y detenimiento habituales y que en consecuencia se decrete, además de la negación de la solicitud en comento, el archivo del expediente que la contiene.

III PRUEBAS

- La publicación americana US 2007/0092507 publicado por Balthasar et al., titulado "Anti-FCRN antibodies for treatment of auto/alloimmune conditions". publicado el 26 de abril de 2007. Portada, páginas 1,10, 14, 18.
- El documento US 2009/0208490 publicado por Pavone et al., titulado "molecules that are able to inhibit the binding between NGF and the TRKA receptor as analgesics with prolonged effect", publicado el 20 de agosto de 2009, Páginas 1, 8, 10-12, 23.

Señor Superintendente,



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US 20070092507A1

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Balthasar et al. (43) **Pub. Date: Apr. 26, 2007**

(54) **ANTI-FCRN ANTIBODIES FOR TREATMENT OF AUTO/ALLO IMMUNE CONDITIONS**

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Related U.S. Application Data

(63) Continuation-in-part of application No. 10/914,403, filed on Aug. 9, 2004, now abandoned.

(60) Provisional application No. 60/762,151, filed on Jan. 25, 2006. Provisional application No. 60/493,901, filed on Aug. 8, 2003.

Publication Classification

(51) **Int. Cl.**
A61K 39/395 (2006.01)
C07K 16/42 (2006.01)
(52) **U.S. Cl.** **424/133.1; 530/387.2**

(57) **ABSTRACT**

Antibodies to heavy chain of human FcRn are provided which function as non-competitive inhibitors of IgG binding to FcRn. The antibodies may be polyclonal, monoclonal, chimeric or humanized, or antigen binding fragments thereof. These antibodies are useful for reducing the concentration of pathogenic IgGs in individuals and therefore used as a therapeutic tool in autoimmune and alloimmune conditions.

ANTI-FCRN ANTIBODIES FOR TREATMENT OF AUTO/ALLO IMMUNE CONDITIONS

[0001] This application claims priority to U.S. Provisional application No. 60/762,151, filed on Jan. 25, 2006, and is also a continuation-in-part of U.S. Non-provisional application Ser. No. 10/914,403, filed on Aug. 9, 2004, the disclosures of which are incorporated herein by reference.

[0002] This work was supported by Grant No. HL 067347 and AI60687 from the National Institutes of Health. The Government has certain rights in the invention.

FIELD OF THE INVENTION

[0003] The present invention relates generally to the field of autoimmune and alloimmune diseases.

BACKGROUND OF THE INVENTION

[0004] Humoral autoimmune and alloimmune conditions are mediated by pathogenic antibodies. Some examples of autoimmune diseases include immune neutropenia, myasthenia gravis, multiple sclerosis, lupus and immune thrombocytopenia (ITP).

[0005] ITP is primarily a disease of increased peripheral platelet destruction, where most patients develop antibodies that bind to specific platelet membrane glycoproteins. The anti-platelet antibodies effectively opsonize platelets, leading to rapid platelet destruction by cells of the reticulo-endothelial system (e.g., macrophages). Relative marrow failure may contribute to this condition, since studies show that most patients have either normal or diminished platelet production. In general, attempts to treat ITP include suppressing the immune system, and consequently causing an increase in platelet levels.

[0006] ITP affects women more frequently than men, and ITP is considered to be an autoimmune disease that is common in children and adults. The incidence is 1 out of 10,000 people. In the US, the incidence of ITP in adults is approximately 66 cases per 1,000,000 per year. An average estimate of the incidence in children is 50 cases per 1,000,000 per year. Internationally, childhood ITP occurs in approximately 10-40 cases per 1,000,000 per year.

[0007] This problem is significant because chronic ITP is one of the major blood disorders in both adults and children. It is a source of significant hospitalization and treatment cost at specialized hematological departments in the US and around the world. Each year there are approximately 20,000 new cases in the US, and the cost for ITP care and special therapy is extremely high.

[0008] Most children with ITP have a very low platelet count that causes sudden bleeding, with typical symptoms including bruises, small red dots on the skin, nosebleeds and bleeding gums. Although children can sometimes recover with no treatment, many doctors recommend careful observation and mitigation of bleeding and treatment with intravenous infusions of gamma globulin.

[0009] Intravenous administration of human immunoglobulin (IVIG) in large amounts has been shown to increase platelet counts in children afflicted with immune ITP, and IVIG has shown to be beneficial as a treatment for several other autoimmune conditions.

[0010] Many studies have investigated the mechanisms by which IVIG achieves effects in the treatment of autoimmune diseases. With regard to ITP, early investigations led to the conclusion that IVIG effects are mainly due to blockade of the Fc receptors responsible for phagocytosis of antibody-opsonized platelets. Subsequent studies showed that Fc-depleted IVIG preparations provided increases in platelet counts in some patients with ITP, and recently it was reported that IVIG effects are due to stimulation of FcγRIIb expression on macrophage cells, leading to inhibition of platelet phagocytosis. Such IVIG treatments, however, have substantial side effects and are very costly to develop and administer. Further, other therapies used for the treatment of autoimmune/alloimmune conditions other than IVIG include polyclonal anti-D immunoglobulin, corticosteroids, immuno-suppressants (including chemotherapeutics), cytokines, plasmapheresis, extracorporeal antibody adsorption (e.g., using Prosorba columns), surgical interventions such as splenectomy, and others. However, like IVIG, these therapies are also complicated by incomplete efficacy and high cost.

[0011] Recently, it has been proposed to raise anti-human FcRn antibodies in knock-out mice lacking the FcRn gene (Roopenian, 2002, U.S. publication no. 2002/128863). The author argues that high affinity antibodies that bind to the same epitope of FcRn as IgG would competitively inhibit the binding of pathogenic IgG to FcRn and therefore increase clearance. However, no such antibodies were demonstrated and therefore the efficacy of such antibodies is still in question. Moreover, owing to the high affinity of endogenous IgG to FcRn and to the high concentrations of endogenous IgG in blood, it is likely that competitive inhibition of FcRn would require very high doses and therefore may be associated with similar side effects as the current IVIG treatment.

[0012] Based on the state of the prior art, there is substantial need for the development of new therapies for autoimmune and alloimmune conditions that do not have the low potency and high cost of IVIG. It is therefore desirable to identify a safer and more effective alternative to IVIG for treatment for autoimmune and alloimmune conditions.

SUMMARY OF THE INVENTION

[0013] This invention provides compositions and methods for treatment of autoimmune and alloimmune conditions. The compositions of the present invention comprise agents which function non-competitively to inhibit the transport of IgG by FcRn receptors. Non-competitive receptor inhibitors, by definition, demonstrate inhibitory activity that is independent of the concentration of the receptor ligand (e.g., IgG). With regard to FcRn-mediated transport of IgG, non-competitive inhibition may be achieved by non-competitive binding of inhibitors to FcRn at physiological pH, and incomplete dissociation of the inhibitor-FcRn complex during the time-course of endosomal sorting and transit. Alternatively, or additionally, non-competitive inhibition may be achieved by binding to a site that is distant from, and/or not identical to, the ligand binding site. The antibodies of the present invention bind to the FcRn such that binding of pathogenic antibodies to the FcRn is inhibited thereby improving the clearance of the pathogenic antibodies from an individual's body. In one embodiment, the agents which binds to FcRn are polyclonal or monoclonal antibodies

[0044] An alternative to preparing monoclonal antibody-secreting hybridomas is to identify and isolate monoclonal antibodies by screening a recombinant combinatorial immunoglobulin library (e.g., an antibody phage display library) with FcRn or a fragment thereof.

[0045] Administration of the compositions of the present invention can be carried out by methods known to those skilled in the art. When the specific inhibitor of FcRn comprises an antibody, administration may be carried out by, for example, intravenous, intramuscular or subcutaneous injection, cannula or other methods known to those skilled in the art. Similarly, administration of small molecules effective to prevent binding of anti-platelet antibodies to FcRn receptors can be carried out by methods well known to those skilled in the art.

[0046] For the elimination of pathogenic antibodies in the treatment of autoimmune and alloimmune conditions, the inhibitor antibody or antibodies of the present invention can be administered. It will be appreciated by those skilled in the art that the effects of the inhibitor antibodies on the elimination of pathogenic antibodies in a particular individual will likely be dependent on the dosing regimen, the pharmacokinetics of the inhibitor antibodies (i.e., the rate and extent of inhibitor distribution and elimination), the affinity of the inhibitor antibodies for FcRn, the transport capacity of FcRn and, potentially, on the turnover of the FcRn receptor. Animal studies presented herein have demonstrated that a model inhibitor antibody led to a dose-dependent, transient increase in IgG elimination in rats. It is believed that the transient nature of the effect may allow control of the duration of FcRn blockade, and may allow minimization of any risks associated with FcRn blockade (e.g., risk for infection).

[0047] The pH 6 to 8 independent and non-competitive inhibitors of the present invention should cause parallel decreases in the concentrations of endogenous pathogenic and non-pathogenic IgG antibodies. As such, the influence of high affinity, non-competitive inhibitors of FcRn on pathogenic antibody concentrations may be estimated based on the effects of the inhibitors on total serum concentrations of endogenous IgG. The FcRn inhibitors may be administered as single and/or multiple-doses. Generally, 1-2000 mg/kg, preferably 1-200 mg/kg, and a more preferably, 1-40 mg/kg may be administered to patients afflicted with autoimmune or alloimmune conditions, and these regimens are preferably designed to reduce the serum endogenous IgG concentration to less than 75% of pretreatment values. Intermittent and/or chronic (continuous) dosing strategies may be applied.

[0048] While the present invention is illustrated by way of the following examples, the examples are meant only to illustrate particular embodiments of the present invention and are not meant to be limiting in any way.

EXAMPLE 1

[0049] This example describes the general methods used. Female Sprague-Dawley rats, 200 to 225 g, were used for the in vivo analyses. Rats were instrumented with jugular vein catheters 2 days prior to treatment. 7E3, a murine antiglycoprotein IIb/IIIa (GPIIb/IIIa) monoclonal antibody, was produced from hybridoma cells obtained from American Type Culture Collection (Manassas, Va.). Hybridoma

cells were grown in serum-free media (Life Technologies®, Rockville, Md.) and antibodies were purified from the media using protein G chromatography. IVIG preparations were obtained from Baxter Healthcare® (Hyland Division, Glendale, Calif.) and Bayer M (Pharmaceutical Division, Elkhart, Ind.). Both IVIG preparations are solvent/detergent-treated and are manufactured via cold ethanol fractionation of human plasma. Outdated human platelets were obtained from the American Red Cross (Buffalo, N.Y. and Salt Lake City, Utah). A murine antimethotrexate IgG1 monoclonal antibody (AMI) was generated and purified in our laboratory. Goat antihuman IgG (no cross-reactivity to goat and mouse serum proteins) and alkaline phosphatase—conjugated goat antimouse IgG (no cross-reactivity to goat and human serum proteins) were both obtained from Rockland (Gilbertsville, Pa.). Mouse antihuman IgG, fluorescein isothiocyanate (FITC)—labeled antimouse IgG, and p-nitrophenyl phosphate were from Pierce® (Rockford, Ill.). Bovine serum albumin (BSA) and buffer reagents were obtained from Sigma® (St Louis, Mo.). Buffers were phosphate-buffered saline (PBS, pH 7.4), 0.02 M Na₂HPO₄ (PB), and PB plus 0.05% Tween-20 (PB-Tween).

[0050] Examples 2-5 illustrate the effect IVIG on anti-platelet antibody. These examples illustrate that IVIG is able to attenuate the effects of an antiplatelet antibody in a rat model of ITP in a dose-dependent manner, and that IVIG has a dramatic, and apparently nonspecific, effect on anti-platelet antibody clearance.

EXAMPLE 2

[0051] This example demonstrates that administration of IVIG clears anti-platelet antibodies in a rat model of IPT. Rats were dosed with IVIG (0.4, 1, or 2 g/kg) via the jugular vein catheter. Following IVIG dosing, a blood sample (0.15 mL) was withdrawn for a baseline measurement of platelet counts. Rats were then dosed with an anti-platelet antibody, 7E3, 8 mg/kg, and platelet counts were taken over 24 hours, using a Cell-Dyne 1700 multiparameter hematology analyzer (Abbott Laboratories®, Abbott Park, Ill.). Control animals were dosed with saline, followed by 7E3. The platelet nadir for each animal was the lowest observed platelet count. Platelet count data were normalized by the initial platelet count because of large interanimal variability in initial platelet counts. By normalizing the data, the effects of 7E3 and IVIG can be better compared between animals. Blood samples (0.15 mL) were taken for pharmacokinetic analysis at 1, 3, 6, 12, 24, 48, 96, and 168 hours after 7E3 dosing. 7E3 plasma concentrations were determined using an enzyme-linked immunosorbent assay (ELISA) as follows. Human GPIIb/IIIa was diluted 1:500 in PB, and added to Nunc Maxisorp plates (0.25 ml/well). Plates were incubated overnight at 4° C. Standards and samples were then added to the plate (0.25 ml/well) and allowed to incubate for 45 minutes at room temperature. Finally, p-nitro phenyl was added (4 mg/ml; in DEA) and the change in absorbance versus time was recorded with a SpectraMax Microplate reader. Plates were washed 3 times with PB-Tween between each step of the assay. Standards were made to final concentrations of 0, 1, 2, 2.5, 5, 10 and 20 ng/ml 7E3 in 1% mouse plasma. Intra-assay variability was <15% for quality control samples within the standard range curve.

[0052] At a dose of 8 mg/kg, 7E3 caused rapid and severe thrombocytopenia in the rats. As can be seen in FIG. 1,

TABLE 3

293 cell-associated fluorescence after incubation with human FITC-IgG and potential inhibitors						
FITC-IgG (μg/ml)						
	0	1	1	1	1	1
Positive clones	N/A	N/A	11E4	1H5	11F12	10E7
PH = 6	5222	253465	49904	49751	40230	39750
PH = 8	5302	10881				

N/A indicates not applicable.

[0072] These results indicate that the binding of human IgG to 293 cells expressing human FcRn is pH dependent, with much greater binding shown at pH 6.0 relative to that seen at pH 8.0. Culture supernatant from hybridomas secreting antibodies directed against the human FcRn light chain are able to inhibit the binding of human IgG to FcRn.

EXAMPLE 11

[0073] This example further demonstrates that the antibodies of the present invention are non-competitive inhibitors of IgG binding to FcRn. Binding of mouse IgG to 293 cells expressing hFcRn was determined in the presence or absence of the anti-hFcRn antibodies was determined as follows. 293 cells were incubated with PBS, with cell culture supernatant from two hybridomas that were identified as secreting anti-human FcRn light chain antibodies, and with cell culture supernatant obtained from cells secreting monoclonal anti-methotrexate mIgG1 (AMI, as a negative control). This incubation was performed in duplicate, with or without co-incubation with human IgG (1 mg/ml). Following this incubation, the cells were incubated with an anti-mouse IgG antibody labeled with FITC (i.e., to detect the presence of murine anti-FcRn antibody bound to human FcRn on the surface of the 293 cells). Cells were washed and cell associated fluorescence was assessed via a fluorometer. All incubations were performed at pH 7.4.

[0074] The results (FIG. 11) show significant binding of mouse IgG to 293 cells expressing hFcRn following the incubation of cells with culture supernatant from hybridoma cells (11E4 & 1H5 from Example 9). These binding data show that co-incubation with human IgG did not lead to a significant change in the assay response, which is consistent with “non-competitive” binding (i.e., where the apparent affinity of the anti-FcRn antibodies for hFcRn is not altered by the presence of the natural ligand—human IgG).

[0075] Also shown are results from incubation of the 293 cells with supernatant from cells that secrete murine monoclonal IgG1 antibodies directed against methotrexate (i.e., as a negative control). Incubation of the 293 cells with the anti-methotrexate antibody did not lead to a significant assay response. This is (again) consistent with the hypothesis that specific anti-hFcRn antibodies are responsible for the significant binding observed following incubation of cells with 11E4 & 1H5 supernatant.

EXAMPLE 12

[0076] This example describes the generation of monoclonal antibodies with specificity for human FcRn. The

peptide sequence GEEFMNFDLKQGT (Invitrogen Corp., Carlsbad, Calif.), selected from the primary sequence of the human FcRn heavy chain, was conjugated with keyhole limpet hemocyanin (KLH) (Pierce Biotechnology Inc., Rockford, Ill.), emulsified in Freund’s incomplete adjuvant (Sigma Chemical) and used to repetitively immunize six Balb/c mice (Harlan, Indianapolis, Ind.). Animals were bled from the saphenous vein 7-10 days after immunization, and antisera were assessed for activity in inhibiting the binding of human IgG to cells expressing human FcRn with a cell binding assay. Briefly, serum samples from the animals were incubated with 293 cells expressing human FcRn and with 50 μg/ml FITC-labeled human IgG, at pH 6, 37° C., for 2 hours. The mixture was then centrifuged at 250 g for 5 minutes and the cells were washed with PBS at pH 6. After centrifugation, the cells were resuspended in PBS at pH 7.4. Fluorescence was assessed by fluorometry. The excitation and emission wavelength were set at 494 and 520 nm respectively. The animal associated with the anti-sera demonstrating maximum inhibition of the fluorescence signal was selected for use as a splenocyte donor. Spleen cells were obtained and fused with murine SP20 myeloma cells (ATCC, Manassas, Va.). Briefly, the mouse was sacrificed with ketamine (150 mg/kg) and xylazine (30 mg/kg), and the spleen was rapidly removed using aseptic technique. Splenocytes were teased out of spleen tissue with the use of sterile 22-gauge needles, suspended in RPMI 1640, and fused with SP20 cells by centrifugation with polyethylene glycol, using standard techniques (e.g., as described in: Harlow E and Lane D. 1988. Antibodies: A laboratory manual. New York: Cold Spring Harbor Laboratory). Fused cells were selected through application of HAT selection medium (Sigma Chemical) and cloned by the method of limiting dilution. Tissue culture supernatant was assayed for inhibition of the binding of human IgG to 293 cells expressing human FcRn, using an approach modified from that described above. Thirty-two viable hybridoma clones were identified, and tissue culture supernatant was obtained from the culture of each clone to screen for the presence of anti-human FcRn antibodies. Briefly, tissue culture supernatants from all the viable clones were incubated with human FcRn-transfected 293 cells and 30 μg/ml FITC-labeled human IgG at pH 6, 37° C., for 2 hours. After incubation, the cells were washed and then resuspended in PBS at pH 7.4. Sample fluorescence was analyzed by fluorometry. From the 32 viable potential anti-human FcRn clones, 2 positive clones were identified. These clones were 1D6 and 11C1 (FIG. 13). One-way ANOVA revealed significant differences in assay response (p<0.0001), and the assay responses for the 2 positive clones were found to be significantly different from that of the control (p<0.01 for each clone, Dunnett multiple comparison test). Additionally, this assay revealed that AMI antibody, which is a negative control, murine IgG1 antibody with high affinity for methotrexate, failed to inhibit the binding of human IgG to the human FcRn-transfected 293 cells.

EXAMPLE 13

[0077] In order to test whether 1D6 or 11C1 bind to human FcRn (hFcRn) non-competitively (i.e., with respect to human immune globulin), culture supernatant from W8 (control), 1D6, and 11C1 were incubated with hFcRn-transfected 293 cells at pH 7.4 and 37° C. for 2 hours with or without the presence of 1 mg/ml of pooled human



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(54) **MOLECULES THAT ARE ABLE TO INHIBIT
THE BINDING BETWEEN NGF AND THE
TRKA RECEPTOR AS ANALGESICS WITH
PROLONGED EFFECT**

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(57) **ABSTRACT**

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Use of an anti-NGF antibody capable of inhibiting the binding between NGF and TrkA, capable of blocking the biological activity of TrkA for the preparation of a medicament for treating and/or preventing chronic pain.

**MOLECULES THAT ARE ABLE TO INHIBIT
THE BINDING BETWEEN NGF AND THE
TRKA RECEPTOR AS ANALGESICS WITH
PROLONGED EFFECT**

BACKGROUND TO THE INVENTION

[0001] The present invention relates to the use of molecules that are capable of inhibiting the binding between NGF and its receptor, TrkA. In particular, it relates to antibodies for one of the two molecules that, by blocking the biological activity of NGF, have a prolonged analgesic effect. Owing to the enduring analgesic effect thereof they are an advantageous therapy for pathologies with persistent forms of pain, known also as chronic pain, such as but not limited to neuropathic or oncological pain.

STATE OF THE ART

[0002] The nociceptive signals afferent to the spinal cord are carried by the fibres A δ and C, the cell bodies of which (primary sensitive neurons) are located in the spinal dorsal ganglia (DRG). The primary sensitive neurons release glutamate together with ATP as an excitatory neurotransmitter, and various other substances such as substance P and CGRP (calcitonin-gene-related-peptide), (Hunt and Mantyh, 2001). The release of these excitatory neurotransmitters is controlled by various classes of receptors present on the afferent terminals, including those that are sensitive to capsaicin (vanilloid receptors, VR1), those activated by GABA, those activated by ATP itself and those activated by cannabinoids (CB1) (Sivilotti and Nistri, 1991; Hunt and Mantyh, 2001; Khakh, 2001; Morisset et al., 2001). One of the physiopathological mechanisms whereby chronic pain occurs is allodynia, i.e. the transformation of stimuli that are not normally painful into painful sensations. This phenomenon involves various ionic currents and thus various channels of the "ligand-gated" type, including the receptor for the capsaicin, VR1, and the ionotropic receptors for ATP (Khakh, 2001). The simultaneous activation of the receptors for VR1 and of those for ATP on spinal nociceptive interneurons generates a considerable accumulation of the excitatory synaptic signals with reinforcement of the transmission of the painful stimulus (Nakatsuka et al., 2002). On this basis, it is therefore clear that the ATP receptors (especially those belonging to the class P2X3) play a fundamental role in the pain paths (Burnstock, 2001). These receptors are present on the peripheral nerve terminals activated by algogenic stimuli, on the cell bodies of the neurons in the DRGs and on the presynaptic terminals thereof as well as naturally on postsynaptic terminals in the spinal cord (Khakh, 2001). There is considerable evidence that shows that the system constituted by the nerve growth factor (NGF) and by the high-affinity receptor thereof, TrkA (Levi-Montalcini, 1987; Levi-Montalcini et al., 1996; Frade and Barde, 1998; Kaplan, 1998) plays a fundamental role in the molecular processes underlying the main forms of "persistent" pain. This indicates a main therapeutical area (the one of pain, with particular reference to the "tonic" forms), for the antibodies that block the NGF/TrkA system (Levine, 1998). The development of sensitive nociceptive neurons depends greatly on NGF, and the responses of the adult nociceptors are modulated by the same factor (Julius and Basbaum, 2001). In particular, NGF exerts acute sensitisation of the capsaicin algogenic stimulus (Shu and Mendell, 1999). From a functional point of view, the nociceptive neurons, following

chronic inflammation, develop alterations in the frequency and duration of their action potential. These phenomena regress by blocking endogenous NGF leading to a significant attenuation of the hyperexcitability that is typical of chronic painful states (Djouhri et al., 2001). NGF action in defining the pain threshold in adult nociceptors is mediated by the TrkA receptor, also through modulation of the response mediated by the VR1 receptor present on the nociceptive terminals. The TrkA-dependent potentiation of the VR1 response is thought to occur through the intracellular transduction pathway of the gamma version of phospholipase C (PLCgamma, Chuang et al., 2001). The levels of peripheral NGF are increased in inflammatory processes, while the administration of exogenous NGF has a hyperalgesic effect on rats and produces muscular pain in humans. Furthermore, NGF produces hypersensitisation to heat stimulation in humans and mammals in general. NGF is released by mastocytes, fibroblasts and other cell types in the peripheral sites where inflammatory processes occur. In particular the mastocytes appear to play a fundamental role (Woolf et al., 1996). As these cells produce NGF and at the same time express functional TrkA receptors on their surface (Nilsson et al., 1997), they are able to respond to NGF itself in the presence of lysophosphatidylserine (Horigome et al., 1993; Kawamoto et al., 2002). As a result, the system NGF/TrkA appears to mediate mastocyte activation through a positive feedback autocrine mechanism allowing the local amplification of the algogenic inflammatory signal. High levels of NGF are also found in neurons, where this neurotrophin is apparently responsible for the modifications of nerve fibres, associated with pain (Harpf et al., 2002). In certain forms of cancer, the excess of NGF facilitates the growth and infiltration of nerve fibres with induction of oncological pain (Zhu et al., 1999). Recent experimental studies show that, by blocking NGF, it could be possible to significantly reduce the formation of neuromas, responsible for neuropathic pain, without damaging the cell bodies of the lesioned neurons (Kryger et al., 2001). These results elicited significant interest in therapeutic approaches based on the reduction of the effects of NGF for the treatment of chronic pain (Saragovi and Gehring, 2000). In recent years, the involvement of the NGF/TrkA system in the molecular processes of pain transduction has also been demonstrated on a genetic base. In particular, mutations of the TrkA gene (localised on the chromosome 1q21-q22) are responsible for a hereditary recessive autosomic syndrome known as CIPA ("congenital insensitivity to pain with anhydrosis"), characterised by recurrent episodic fever, anhydrosis, absence of reaction to stimuli that cause pain, mental retardation and a tendency to self-mutilation (Indo et al., 1996; Saragovi and Gehring, 2000; Indo, 2001; Indo et al., 2001). Further confirmation of the involvement of NGF in the nociceptive response was recently obtained by the work on phenotype characterisation of anti-NGF transgenic mice (AD11). In these animals, the ectopic expression of the anti-NGF antibody α D11 produces a functional block of NGF in adult. This block translates in a consistent manner into an increase in the latency time of the response to harmful heat stimuli (Capsoni et al., 2000; Ruberti et al., 2000). Antibodies that are able to neutralise the biological activity of the NGF/TrkA system by blocking the ligand or the receptor may represent an important resource in pain therapy, in particular for persistent forms of pain. In this context, a very recent publication demonstrates that treatment with a neutralising anti-NGF antibody produces significant pain reduction in a

[0012] An anti-NGF molecule that blocks the biological activity of TrkA is defined as a molecule that acts as an antagonist in terms of the NGF binding with the TrkA receptor and comprises: synthetic molecule or monoclonal antibody or a biological/synthetic derivative thereof that:

- [0013] i) binds to TrkA;
- [0014] ii) inhibits the binding of NGF to the “native” TrkA receptor expressed on the surface of living cells (“native” meaning “in the natural in vivo conformation”); and
- [0015] iii) blocks the biological activity that derives from the NGF binding with the same TrkA receptor.

[0016] The term “blocking the biological activity” does not simply mean blocking activation of the receptor, defined as blocking the conversion process of the receptor itself into an “active” state but also functional neutralisation of the biological consequences that are downstream of this activation process: second messengers, new gene expression, phenotypical and functional modifications. The molecule is not only able to block TrkA in a classic in vitro test (test of neuritic growth in PC12 cells), but also in vivo (functional block of the cholinergic neurons of the basal forebrain and block of the nociception in a classic “hot plate” test).

[0017] It is an object of the invention the use of an anti-NGF antibody capable of inhibiting the binding between NGF and TrkA for the preparation of a medicament for treating and/or preventing chronic pain. Preferably the antibody is able to recognise and bind to a NGF molecule domain containing the aa. 41-49 region of human or rat NGF: EVNINNSVF (SEQ ID No. 9), more preferably the domain contains also the as 23-35 region: GDKTTATDIKGKE (SEQ ID No. 10). More preferably the antibody is capable of blocking the biological activity of TrkA.

[0018] There is also provided as an aspect of the invention a method of treatment and/or prevention of chronic pain in a subject comprising administering to the subject an effective amount of an anti-NGF antibody thereby to treat and/or prevent chronic pain in said subject. There is also provided a kit comprising a composition containing an anti-NGF antibody together with instructions directing administration of said composition to a subject in need of treatment and/or prevention of chronic pain thereby to treat and/or prevent chronic pain in said subject.

[0019] In a preferred aspect the variable region of the antibody light chain comprises at least one, more preferably two, most preferably three of the complementarity determining regions (CDRs) having the sequence selected from aa. 24 to aa. 34 of ID No. 1; from aa. 50 to aa. 56 of SEQ ID No. 1; from aa. 89 to aa. 97 of SEQ ID No. 1.

[0020] In a further preferred aspect the variable region of the antibody light chain comprises essentially the sequence of SEQ ID No. 1.

(VL, SEQ ID No. 1):
L CDR1
DIQMTQSPASLSASLGFTVTIECRASEDIYNALAWYQKPGKSPQLLIY
L CDR2
NTDTLHTGVPSRFSFGSGSGTQYSLKINSLSQSEDVASYFCQHYFHYPTF
L CDR3
GGGKLELK

[0021] In a preferred aspect the variable region of the antibody heavy chain comprises at least one, more preferably two, most preferably three of the complementarity determin-

ing regions (CDRs) having the sequence selected from aa. 26 to aa. 35 of SEQ ID No. 2; from aa. 50 to a. 65 of SEQ ID No. 2; from aa. 98 to aa. 111 of SEQ ID No. 2.

[0022] In a further preferred aspect the variable region of the antibody heavy chain comprises essentially the sequence of SEQ ID No. 2.

(VH, SEQ ID NO 2):
H CDR1
QVQLKESGPGLVQPSQTLSTCTVSGFSLTNNVNWVRQATGRGLEWMG
H CDR2
GVWAGGATDYNALKSRLLTITRDTSKQVFLKMHSLQSEDTATYYCAR
H CDR3
DGGYSSSTLYAMDAGQGTTVTVSA

[0023] The antibody may be in single chain form and comprises a light chain variable region and a heavy chain variable region joined by a linker.

[0024] Alternatively the antibody may comprise two light chains and two heavy chains.

[0025] In a preferred aspect of the invention the anti-NGF antibody is a human or humanised antibody. The skilled in the art shall select the proper humanisation method to design the antibody, a preferred method is the method as disclosed in WO 2005/061540.

[0026] Briefly, a “humanized” variant of the antibody variable region was obtained by grafting the Complementarity Determining Regions (CDRs) of the rat antibody on to a human immunoglobulin framework. The complete structural information obtained from X-ray diffraction studies concerning the Fab fragment of the αD11 antibody was exploited to select an acceptor framework of human origin. Two different criteria were adopted to minimize structural differences between the rat αD11 and the acceptor human antibody: i) level of primary structure homology, ii) level of tridimensional structure similarity. After choosing the framework, the replacement of human residues by rat counterparts was minimized to reduce the potential immunogenicity of the resulting humanized antibody.

[0027] Exemplary humanised antibodies comprise a light chain variable region which is a humanised derivative of SEQ ID No 1 (a rat origin sequence). Exemplary humanised antibodies comprise a heavy chain variable region which is a humanised derivative of SEQ ID No 2 (a rat origin sequence).

[0028] In a preferred aspect of the invention the variable region of the humanised antibody light chain comprises essentially the sequence of SEQ ID No. 3.

SEQ ID NO 3 (VL, variable region of the light chain of Hu-αD11):
L CDR1
DIQMTQSPSSLSASVGDRTITCRASEDIYNALAWYQKPGKAPKLLIY
L CDR2
NTDTLHTGVPSRFSFGSGSGTDYTLTISSLQPEDFATYFCQHYFHYPT
L CDR3
FGGQTKVEIK

[0029] In a preferred aspect of the invention the variable region of the humanised antibody heavy chain comprises essentially the sequence of SEQ ID No. 4.

SEQ ID No. 4 (VH, variable region of the heavy chain of Hu- α D11):

H CDR1

EVQL VESG GGLVQP GGSRL SC AASGFSLTNNVNWVRQA PGKGLEWV

H CDR2

GGVWAGGATDYNALKSRFTI SRD NSKNTAYLQMNSL RAEDTA VYYCA

H CDR3

RDGGYSSSTLYAMDAGQGT LVTVS S

[0030] The above described humanized variable regions were cloned into appropriate expression vectors into a human IgG1 or IgG4 isotype format and transfected into mammalian cell lines to allow for expression, purification and pharmacological characterization.

[0031] Different variants of Hu- α D11 (complete IgG: heavy chain light chain) were finally produced (differing because of different constant parts).

[0032] In a preferred aspect of the invention the humanised antibody light chain has essentially the sequence of SEQ ID No. 8.

SEQ ID 8, Hu- α D11 Vk human Ck

DIQMTQSP SLSASVGDRTVITCRASEDIYNALAWYQQKPGKAPKLLIY

NTDTLHTGVPSRFSGSGSGTDYTLTISSLQPEDFATYFC FGQGTKVE

IKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWVKVDNALQ

SGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPV

TKSFNRGEC

(Italics = variable regions; Bold = mutations in the rat sequence in the humanization process; Underlined = CDRs)

[0033] In a preferred embodiment, the humanised anti-NGF heavy chain has essentially one of the following 3 sequences:

SEQ ID NO 5, Hu-antiNGF (VH) human IgG1

EVQL VESG GGLVQP GGSRL SC AASGFSLTNNVNWVRQA PGKGLEWV

GGVWAGGATDYNALKSRFTI SRD NSKNTAYLQMNSL RAEDTA VYYCA

RDGGYSSSTLYAMDAGQGT LVTVS SASTKGPSVFPLAPSSKSTSGGTAA

LGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVFPSS

SLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVF

LFPPPKDITLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP

REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG

QPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY

KTTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLS

SLSPGK

SEQ ID NO 6, Hu- α D11 (VH) human IgG1* (IgG1 with N297A mutation, as described by Bolt et al., 1993)

EVQL VESG GGLVQP GGSRL SC AASGFSLTNNVNWVRQA PGKGLEWV

GGVWAGGATDYNALKSRFTI SRD NSKNTAYLQMNSL RAEDTA VYYCA

RDGGYSSSTLYAMDAGQGT LVTVS SASTKGPSVFPLAPSSKSTSGGTAA

-continued

LGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVFPSS

SLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVF

LFPPPKDITLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP

REEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG

QPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY

KTTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLS

SLSPGK

SEQ ID NO 7, Hu- α D11 (VH) human IgG4

EVQL VESG GGLVQP GGSRL SC AASGFSLTNNVNWVRQA PGKGLEWV

GGVWAGGATDYNALKSRFTI SRD NSKNTAYLQMNSL RAEDTA VYYCA

RDGGYSSSTLYAMDAGQGT LVTVS SASTKGPSVFPLAPSSKSTSESTAA

LGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVFPSS

SLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPEFLGGPSVFLFP

PKPKDITLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE

QFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPR

EPQVYITLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT

TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSL

SLGK

(Italics = variable regions; Bold = mutations in the rat sequence in the humanization process; Underlined = CDRs; N297A mutation to abolish glycosilation site).

[0034] In a preferred aspect the molecules of the invention are used for the preparation of a medicament for the pain of the chronic inflammatory type, preferably caused by pancreatitis, kidney stones, headaches, dysmenorrhoea, musculoskeletal pain, sprains, visceral pain, ovarian cysts, prostatitis, cystitis, interstitial cystitis, post-operative pain, migraine, trigeminal neuralgia, pain from burns and/or wounds, pain associated with trauma, neuropathic pain, pain associated with musculoskeletal diseases, rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, periarthritic pathologies, oncological pain, pain from bone metastases, pain from HIV.

[0035] Alternatively the pain is a neuropathic pain or an oncological pain.

[0036] According to International Association for the Study of Pain (IASP, www.iasp-pain.org <<http://www.iasp-pain.org/>>), pain is generally defined as “An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage or both”. The essential element in all forms of pain is the activation of specialized high-threshold receptors and nerve fibers to warn the organism of potential tissue damage. The involvement of inflammatory cells and processes is a common element in many pain states. The term “acute pain” means immediate, generally high threshold, pain brought about by injury such as a cut, crush, burn, or by chemical stimulation. The term “chronic pain,” as used herein, means pain other than acute pain. It is understood that chronic pain often is of relatively long duration, for example, months or years and can be continuous or intermittent.

[0037] The anti-NGF antibody is suitably administered systemically. Systemic administration of the anti-NGF anti-

body can be performed by injection, e.g. continuous intravenous infusion, bolus intravenous infusion, subcutaneous or intramuscular injection. Alternatively other forms of administration (e.g. oral, mucosal, via inhalation, sublingually, etc.) may also be used. Local delivery of the antibody can be performed by local administration eg intra-articular injection or subcutaneous, intramuscular injection in the vicinity of affected tissues.

[0038] The anti-NGF antibody will suitably be formulated in a pharmaceutical composition appropriate for the intended route of administration. Solutions for injection will suitably contain the antibody dissolved or dispersed in an aqueous medium (eg water for injection) containing appropriate buffers and molarity modifiers eg phosphate, salt and/or dextrose.

[0039] Treatment regimen i.e. dose, timing and repetition, can be represented by single or repeated administrations (e.g. injections) of the product by the chosen administration route. The interval of dose administration can be subject to modifications depending on the extent and duration of the clinical response, as well as the particular individual and the individual clinical history. Suitably the anti-NGF antibody has a long duration of action. In particular the clinical effect of the antibody extends following administration as long as 21 days as determined from animal studies. Furthermore, preliminary data imply that anti-NGF antibodies may manifest clinical benefit for a longer period than that in which its presence can be detected in a relevant biological matrix such as serum or plasma following its administration.

[0040] In light of the intended long duration of action (i.e. an effect suitably lasting at least one week, or preferably at least two weeks eg at least three weeks or at least four weeks), suitably the antibody may be administered to subjects at a frequency of not more than once per week eg not more than once per two weeks or once per three weeks or once per four weeks.

[0041] A suitable dose of the anti-NGF antibody will typically range from 0.1 mg/kg to 10 mg/kg body weight

[0042] Novel antibodies and compositions containing them disclosed herein are claimed as an aspect of the invention.

[0043] Non-limitative embodiments of the present invention will now be disclosed, with particular reference to the following figures:

[0044] FIG. 1: BIAcore analysis of the binding of the α D11 anti-NGF antibody to mouse NGF (m-NGF) and recombinant mouse proNGF (rm-proNGF). The α D11 anti-NGF antibody was immobilized on flow cell 2, while flow cell 1 was left as a blank. Each curve is obtained subtracting the background signal (measured in cell 1) to the signal measured in cell 2. The Surface Plasmon Resonance signal gives the amount of surface-bound component at each stage and is expressed in resonance units (RU).

[0045] For m-NGF binding, the immobilization of the antibody was of 3000 resonance units (RU) in the experiment of panel A and of 6000 RU in the experiment of panel B. The injected concentrations of the m-NGF are indicated on top of each curve. From a complete analysis of the data, the affinity parameters were evaluated, and resulted to be the following. $KA=3.55 \cdot 10^{-11}$ 1/M; $KD=2.8 \cdot 10^{-12}$ M (χ^2 value of 0.123).

[0046] For rm-proNGF binding (panel C), the immobilization of the antibody was of 3000 RU.

[0047] The injected concentrations of the rm-proNGF are indicated on top of each curve. A kinetic analysis of the data allowed to evaluate the following parameters: $KA=1.2 \cdot 10^9$ 1/M; $KD=1.9 \cdot 10^{-9}$ M (χ^2 value of 0.09).

[0048] FIG. 2: Effect of Fab α D11 (alphaD11) and Fab Hu- α D11 (Hu-alphaD11) anti-NGF antibodies on the formalin-evoked pain (phase 2 of the formalin test: 15-40 min. Phase 2 corresponds to inflammation-related pain). Mice were subcutaneously injected with 5% formalin in the dorsal portion of the right hindpaw.

[0049] Treatment consisted in antibody injection (Fab alphaD11 or Fab Hu-alphaD11 vs either mock Fab or saline) performed (in the same paw as for formalin) 45 min, before formalin injection and testing (single dose of each antibody: 12.5 μ g). Each experimental group included at least 8 animals. Statistical analysis of data showed a significant analgesic effect of anti-NGF treatment (both for the parental and the humanized version of the antibody) that was clearly specific for the second phase (inflammatory) of pain response (time spent in licking): The effect of anti-NGF antibodies (both parental and humanized versions) is statistically different (ANOVA) with respect to either saline (** $p<0.01$) or mock Fab treatment (# $p<0.05$).

[0050] FIG. 3: Effect of the anti-TrkA monoclonal antibody MNAC13 (1.4 mg/kg) and anti-NGF monoclonal antibody α D11 (1.4 mg/kg) on neuropathic pain: mechanical allodynia measured by means of a plantar dynamic aesthesiometer; CD1 mice subjected to chronic constriction of the sciatic nerve; the antibodies are injected LP. at days 3, 4, 5, 6 after lesion of the sciatic nerve. Observation period: from day 3 to day 14. As a negative control, both saline (sal) and mouse immunoglobulins (IgG, 1.4 mg/kg) were used. Results were expressed in terms of absolute value (grams) of the threshold force for the hindpaw ipsilateral to lesion. The values were subjected to statistical analysis by means of an analysis of the variance (ANOVA) for repeated measurements, in which both the "treatment" factor and the repeated measurement (days) were significant with $p<0.01$. The animals treated with anti-TrkA or anti-NGF are significantly different from the controls from day 4 to day 14.

[0051] FIG. 4: Effect of the anti-TrkA monoclonal antibody MNAC13 (1.4 mg/kg) and anti-NGF α D11 antibody (1.4 mg/kg) on neuropathic pain: mechanical allodynia measured by means of a plantar dynamic aesthesiometer; CD1 mice subjected to chronic constriction of the sciatic nerve; the antibodies were injected LP. at days 3, 4, 5, 6 after lesion of the sciatic nerve. Observation period: from day 3 to day 14. As a control, both saline (sal) and mouse immunoglobulins (IgG, 1.4 mg/kg) were used. Results were expressed as a percentage, % (ratio between the threshold force of the hindpaw ipsilateral to lesion and that corresponding to the contralateral hindpaw). The corresponding absolute values were subjected to statistical analysis by means of an analysis of the variance (ANOVA) for repeated measurements, in which both the "treatment" factor and the repeated measurement (days) were significant with $p<0.01$ (at least). The animals treated with anti-TrkA or anti-NGF were significantly different from the controls from day 4 to day 14.

[0052] FIG. 5: Comparison between the effects of the anti-TrkA monoclonal antibody MNAC13 (2 doses: 0.9 and 2 mg/kg) and those of the anti-NGF monoclonal antibody α D11 (2 mg/kg dose), on neuropathic pain: mechanical allodynia measured by means of a plantar dynamic aesthesiometer; CD1 mice subjected to chronic constriction of the sciatic nerve; the antibodies were injected LP. at days 3, 4, 5, 6, 7, 8, 9, 10 after lesion of the sciatic nerve. Observation period: from day 3 to day 31. As a negative control, mouse immunoglobulins were used (IgG, 2 mg/kg). Results were expressed

1-24. (canceled)

25. An anti-NGF antibody capable of inhibiting binding between HGF ND TrkA for use in the treatment of chronic pain.

26. Method of treatment or prevention of chronic pain in a subject comprising administering to the subject an effective amount of an anti-NGF antibody capable of inhibiting binding between HGF ND TrkA to treat chronic pain in said subject.

27. (canceled)

28. Method of treatment or prevention of chronic pain as in claim 26 wherein the antibody is able to recognize and bind to a NGF molecule domain containing the aa. 41-49 region of human or rat NGF: EVNINNSVF (SEQ ID No. 9).

29. Method of treatment or prevention of chronic pain as in claim 26 wherein the antibody is able to recognize and bind to a NGF molecule domain containing the aa 23-35 region: GDKTTATDIKGKE (SEQ ID No. 10).

30. Method of treatment or prevention of chronic pain as in claim 26 wherein the antibody is capable of blocking the biological activity of TrkA.

31. Method of treatment or prevention of chronic pain as in claim 26 wherein the variable region of the antibody light chain comprises at least one of the complementarity determining regions (CDRs) having the sequence selected from aa. 24 to aa. 34 of SEQ ID No. 1; from aa. 50 to aa. 56 of SEQ ID No. 1; from aa. 89 to aa. 97 of SEQ ID No. 1.

32. Method of treatment or prevention of chronic pain as in claim 26 wherein the variable region of the antibody light chain comprises at least two of the complementarity determining regions (CDRs) having the sequence selected from aa. 24 to aa. 34 of SEQ ID No. 1; from aa. 50 to aa. 56 of SEQ ID No. 1; from aa. 89 to aa. 97 of SEQ ID No. 1.

33. Method of treatment or prevention of chronic pain as in claim 26 wherein the variable region of the antibody light chain comprises all of three complementarity determining regions (CDRs) having the sequence selected from aa. 24 to aa. 34 of SEQ ID No. 1; from aa. 50 to aa. 56 of SEQ ID No. 1; from aa. 89 to aa. 97 of SEQ ID No. 1.

34. Method of treatment or prevention of chronic pain as in claim 26 wherein the variable region of the antibody light chain comprises essentially the sequence of SEQ ID No. 1.

35. Method of treatment or prevention of chronic pain as in claim 26 wherein the variable region of the antibody heavy chain comprises at least one of the complementarity determining regions (CDRs) having the sequence selected from aa. 26 to aa. 35 of SEQ ID No. 2; from aa. 50 to aa. 65 of SEQ ID No. 2; from aa. 98 to aa. 111 of SEQ ID No. 2.

36. Method of treatment or prevention of chronic pain as in claim 26 wherein the variable region of the antibody heavy chain comprises at least two of the complementarity determining regions (CDRs) having the sequence selected from aa. 26 to aa. 35 of SEQ ID No. 2; from aa. 50 to aa. 65 of SEQ ID No. 2; from aa. 98 to aa. 111 of SEQ ID No. 2.

37. Method of treatment or prevention of chronic pain as in claim 26 wherein the variable region of the antibody heavy chain comprises all of three of the complementarity determining regions (CDRs) having the sequence selected aa. 26 to aa.

35 of SEQ ID No. 2; from aa. 50 to aa. 65 of SEQ ID No. 2; from aa. 98 to aa. 111 of SEQ ID No. 2.

38. Method of treatment or prevention of chronic pain as in claim 26 wherein the variable region of the antibody heavy chain comprises essentially the sequence of SEQ ID No. 2.

39. Method of treatment or prevention of chronic pain as in claim 26 wherein the antibody is in single chain form and comprises a light chain variable region and a heavy chain variable region joined by a linker.

40. Method of treatment or prevention of chronic pain as in claim 26 wherein the antibody comprises two light chains and two heavy chains.

41. Method of treatment or prevention of chronic pain as in claim 26 wherein the anti-NGF antibody is a human or humanized antibody.

42. Method of treatment or prevention of chronic pain as in claim 41 wherein the variable region of the humanized antibody light chain comprises essentially the sequence of SEQ ID No. 3.

43. Method of treatment or prevention of chronic pain as in claim 41 wherein the variable region of the humanized antibody heavy chain comprises essentially the sequence of SEQ ID No. 4.

44. Method of treatment or prevention of chronic pain as in claim 41 wherein the humanized antibody light chain has essentially the sequence of SEQ ID No. 8.

45. Method of treatment or prevention of chronic pain as in claim 41 wherein the humanized antibody heavy chain has essentially a sequence selected from SEQ ID No. 5, SEQ ID No. 6, SEQ ID No. 7.

46. Method of treatment or prevention of chronic pain as in claim 26 wherein the pain is of the chronic inflammatory type.

47. Method of treatment or prevention of chronic pain as in claim 46 wherein the chronic pain is caused by pancreatitis, kidney stones, headaches, dysmenorrhea, musculoskeletal pain, sprains, visceral pain, ovarian cysts, prostatitis, cystitis, interstitial cystitis, post-operative pain, migraine, trigeminal neuralgia, pain from burns and/or wounds, pain associated with trauma, neuropathic pain, pain associated with musculoskeletal diseases, rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, periarticular pathologies, oncological pain, pain from bone metastases, pain from HIV.

48. Method of treatment or prevention of chronic pain as in claim 26 wherein the pain is neuropathic pain.

49. Method of treatment or prevention of chronic pain as in claim 26 wherein the pain is oncological pain.

50. Method of treatment or prevention of chronic pain as in claim 26 wherein the antibody has a long duration of action.

51. Use of an anti-NGF antibody capable of inhibiting the binding between NGF and TrkA for the preparation of a medicament for treating and/or preventing chronic pain.

52. A kit comprising a composition containing an antibody capable of inhibiting binding between NGF and TrkA together with instructions directing administration of said composition to a subject in need of treatment of chronic pain thereby to treat chronic pain in said subject.

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