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BACKGROUND TO THE INVENTION

[0001] The present invention relates to the use of molecules capable of inhibiting the binding between NGF and its receptor, TrkA. In particular, it relates to antibodies that, by blocking the biological activity of NGF, have a prolonged analgesic effect. Owing to the enduring analgesic effect thereof, they provide an advantageous therapy for pathologies with persistent forms of pain, known also as chronic pain, such as neuropathic pain.

STATE OF THE ART

[0002] The nociceptive signals afferent to the spinal cord are carried by the fibres Aδ 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 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 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 therefore different 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 painful stimulus transmission (Nakatsuka et al., 2002). From these observations it is therefore clear that the ATP receptors (especially those belonging to the P2X3 class) play a fundamental role in the pain pathways (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 on postsynaptic terminals in the spinal cord (Khakh, 2001). There is considerable evidence showing an involvement of the nerve growth factor (NGF) and its high-affinity receptor TrkA (Levi-Montalcini, 1987; Levi-Montalcini et al., 1996; Frade and Barde, 1998; Kaplan, 1998) in the molecular processes underlying the main kinds of "persistent" pain, indicating a

major therapeutic area (that of pain, with particular reference to the "tonic" forms), for the antibodies which 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 to the capsaicin algogenic stimulus (Shu and Mendell, 1999). From a functional standpoint, 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 typical of states of chronic pain (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 phospholipase C gamma ((PLCgamma, Chuang et al., 2001). The peripheral NGF levels 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 mast cells, fibroblasts and other cell types in the peripheral sites where inflammatory processes occur. In particular, mast cells appear to play a fundamental role (Woolf et al., 1996). As they 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 NGF/TrkA system appears to mediate mastocyte activation through an autocrine positive feedback mechanism which allows local amplification of the algogenic inflammatory signal.

[0003] High levels of NGF are also found in neurons, where this neurotrophin is apparently responsible for the modifications of the 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 demonstrate how, by blocking NGF, it is 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 generated significant interest in therapeutic approaches based on the reduction of NGF effects 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 was also genetically demonstrated. 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 nociceptive stimuli, 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 inventors with the characterisation of anti-NGF transgenic mice phenotype (AD11). In these animals, the ectopic expression of the anti-NGF antibody αD11 produces a functional block of NGF in adult age. Such block consistently translates into an increase in the latency time of the response to harmful heat stimuli (Capsoni et al., 2000; Ruberti et al., 2000). Numerous evidence indicates the system constituted by the nerve growth factor (NGF) and its high-affinity receptor TrkA as a possible target for pain therapy. For this reason, antibodies capable of neutralising the biological activity of the NGF/TrkA system by blocking the TrkA receptor may represent an important resource for pain therapy, in particular for persistent pain.

[0004] The authors of the present invention make use of antibodies (directed against the TrkA receptor) which are able to block the biological effects of NGF mediated by TrkA. The reagents MNAC13 is of particular interest.

[0005] The MNAC13 antibody is a mouse monoclonal antibody directed against the human TrkA receptor (Cattaneo et al., 1999; Pesavento et al., 2000), particularly effective in the inhibition of TrkA activation by NGF and the downstream biological functions, both *in vitro* and *in vivo* (Cattaneo et al., 1999; Pesavento et al., 2000). Anti-TrkA antibodies, including the MNAC13 antibody, having an antagonist activity preventing the functional activation of TrkA by NGF" are disclosed in EP 1.181.318. Derivatives of such antibody are also disclosed in WO2005/061540. However the therapeutic or preventive effect of such molecules on chronic pain is not disclosed.

[0006] The antibodies were characterised in detail from the point of view of the structure (Covaceuszach et al., 2001) and from the molecular interaction with the TrkA receptor (Covaceuszach et al., 2005). On the basis of such in-depth structural knowledge, by means of an innovative method a humanised version of

MNAC13 was generated (Hu-MNAC13), with the same features of antigen binding as the parental antibody (patent application WO2005/061540).

[0007] The currently available therapies for the treatment of neuropathic pain, caused by a primary lesion or by a dysfunction of the nervous system, for treatment of oncological pain, and for numerous other forms of persistent pain (also of an inflammatory nature) have been found to be of limited effectiveness. There is a clear need to identify and develop new molecules with analgesic activity, with different mechanism of action compared with drugs currently used in therapy, in order to solve side effects related problems. The international patent application WO 02/20479 discloses small synthetic molecules which, by inhibiting the TrkA receptor, have potential analgesic activity. Nevertheless, the effect of these molecules on certain pain models has not been demonstrated. Furthermore, when compared with antibodies, small molecules have the drawback of being more likely to penetrate the haematoencephalic barrier, with the possibility of serious side effects. In fact, the cholinergic neurons of the basal forebrain, a neuronal population affected by various forms of progressive neurodegeneration, including Alzheimer's disease (Saper et al., 1985), express the TrkA receptor and depend on NGF for correct functioning (Holtzman et al., 1992). The international patent application WO 01/78698 proposes the use of an NGF antagonist for preventing or treating chronic visceral pain, but not neuropathic or oncological pain. Although the application states that the antagonist can bind both NGF and the TrkA receptor, it is not demonstrated that upon binding of the antagonist to TrkA the receptor is functionally blocked.

[0008] Based on the ability of MNAC13 antibody to block the biological activity of NGF/TrkA, the antibody and its humanised versions were tested in various animal models of persistent pain, in particular in the "Chronic Constriction Injury" model (CCI, chronic constriction injury of the sciatic nerve), for assessment of chronic pain of neuropathic nature (Bennett and Xie, 1988).

SUMMARY OF THE INVENTION

[0009] The object of the present invention is the use of an anti-TrkA antibody that is able to inhibit the binding between NGF and TrkA, for the preparation of a medicament for the treatment of neuropathic pain.

[0010] Suitably the antibody blocks the biological activity of TrkA i.e. is an antagonistic antibody.

[0011] A molecule that blocks the biological activity of TrkA refers to a molecule that acts as an antagonist in terms of the NGF binding to the TrkA receptor, and which can be defined as a synthetic molecule or a monoclonal antibody or a biological/synthetic derivative thereof which:

i) binds to TrkA; and

ii) inhibits the binding of NGF to the "native" TrkA receptor expressed on the surface of living cells; and

iii) blocks the biological activity deriving from NGF binding to the same TrkA receptor.

[0012] 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 the functional neutralisation of biological consequences downstream of the activation process: second messengers, new gene expression, phenotypic and functional modifications both at cell and system level. The molecule of the invention 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).

[0013] As noted above antagonistic TrkA antibodies are disclosed in EP 1181318 and in WO 2005/061540.

[0014] Therefore it is an object of the invention the use of an anti-TrkA antibody capable of inhibiting the binding between NGF and TrkA for the preparation of a medicament for treating and/or preventing neuropathic pain. Suitably the antibody is capable of blocking the biological activity of TrkA.

[0015] There is also provided as an aspect of the invention a method of treatment and/or prevention of neuropathic pain in a subject comprising administering to the subject an effective amount of an anti-TrkA antibody thereby to treat and/or prevent neuropathic pain in said subject. There is also provided a kit comprising a composition containing an anti-TrkA antibody together with instructions directing administration of said composition to a subject in need of treatment

and/or prevention of neuropathic pain thereby to treat and/or prevent neuropathic pain in said subject.

[0016] In an aspect of the invention the variable region of the antibody light chain comprises the complementarity determining regions (CDRs) having the sequence selected from aa. 24 to aa. 33 of SEQ ID No.1; from aa. 49 to aa. 55 of SEQ ID No. 1; from aa. 88 to aa. 96 of SEQ ID No. 1. The variable region of the antibody light chain may, for example, comprise the sequence of SEQ ID No.1.

CDR L1 CDR L2
DIVLTQSPAIMSASLGEEVTLTCSASSSVSYMHWYQQKSGTSPKLLIYTTSNLASGVPSRFSGSGSGTFY
CDR L3
SLTISSVEAEDAADYYCHQWSSYPWTFGGGTKLEIK (SEQ ID No 1).

[0017] In an aspect of the invention the variable region of the antibody heavy chain comprises 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. 66 of SEQ ID No. 2; from aa. 99 to aa. 112 of SEQ ID No. 2. The variable region of the antibody light chain may, for example, comprise the sequence of SEQ ID No.2.

CDR H1 CDR H2
EVKLVESGGGLVQPGGSLKLSCAASGFTFSTYTMSWARQTPEKRLEWVAYISKGGGSTYYPDTVKGRFTI
CDR H3
SRDNAKNTLYLQMSSLKSEDTALYYCARGAMFGNDFFFPMDRWGQGTSVTVSS (SEQ ID No 2).

[0018] 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.

[0019] Alternatively the antibody may comprise two light chains and two heavy chains.

[0020] In a preferred aspect of the invention the anti-TrkA 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. Exemplary humanised antibodies comprise a light chain variable region which is a humanised derivative of SEQ ID No 1 (a mouse origin sequence). Exemplary humanised antibodies comprise a heavy chain variable region which is a humanised derivative of SEQ ID No 2 (a mouse origin sequence).

[0021] In a preferred aspect of the invention the variable region of the human-

ised antibody light chain comprises the sequence from aa. 1 to aa. 106 of SEQ ID No. 3.

[0022] In a more preferred aspect the humanised antibody light chain has the sequence of SEQ ID No. 3.

DIVLTQSPSSLSASVGDRVTITCSASSSVSYMHWYQQKPGQAPKLLIYTTSNLASGVPSRFSGSGSGTDY
*TLTISSLQPEDVATYYCHQWSSYPWTFGGGTKVEIK*RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP
REAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR
GEC hMNAC13 Vk hCk (SEQ ID No. 3).

[0023] In a preferred aspect of the invention the variable region of the humanised antibody heavy chain comprises the sequence from aa. 1 to aa. 123 of SEQ ID No. 4 .

[0024] In a more preferred aspect the humanised antibody heavy chain has a sequence selected from SEQ ID No. 4, SEQ ID No. 5, SEQ ID No. 6.

EVQLLESGGGLVQPGGSLRLSCAASGFTFSTYTMSWARQAPGKGLEWVAYISKGGGSTYYPDTVKGRFTI
*SRDNSKNTLYLQMNSLRAEDSAVYYCARGAMFGNDFFFPMDRWGQGTLVTVSS*ASTKGPSVFPLAPSSKS
TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNH
KPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK
FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR
EPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK
SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK hMNAC13 VH hIgG1 (SEQ ID No. 4).

EVQLLESGGGLVQPGGSLRLSCAASGFTFSTYTMSWARQAPGKGLEWVAYISKGGGSTYYPDTVKGRFTI
*SRDNSKNTLYLQMNSLRAEDSAVYYCARGAMFGNDFFFPMDRWGQGTLVTVSS*ASTKGPSVFPLAPSSKS
TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNH
KPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK
FNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR
EPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK
SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK hMNAC13 VH hIgG1 (N297A) (SEQ ID No. 5).

EVQLLESGGGLVQPGGSLRLSCAASGFTFSTYTMSWARQAPGKGLEWVAYISKGGGSTYYPDTVKGRFTI
*SRDNSKNTLYLQMNSLRAEDSAVYYCARGAMFGNDFFFPMDRWGQGTLVTVSS*ASTKGPSVFPLAPCSRS
TSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRVESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNW
YVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQ
VYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRW
QEGNVFSCSVMHEALHNHYTQKSLSLSLGK hMNAC13 VH hIgG4 (SEQ ID No. 6).

[0025] Italics: variable regions, Bold: mutations in the mouse sequence in the humanization process, Underlined: CDRs.

[0026] 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.

[0027] The anti-TrkA antibody of the invention is suitably administered systemically. Systemic administration can be performed by injection, e.g. continous 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 antibody can be performed by local administration eg intra-articular injection or subcutaneous, intramuscular injection in the vicinity of affected tissues.

[0028] The anti-TrkA 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) as appropriate containing appropriate buffers and molarity modifiers eg phosphate, salt and/or dextrose.

[0029] Treatment regimen i.e. dose, timing and repetition, can be represented by single or repeated administrations (eg 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-TrkA antibody has a long duration of action. In particular the clinical effect of the antibody extends following administration may be as long as 21days as determined from animal studies. Furthermore preliminary data implies that anti-TrkA 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 fol-

lowing its administration. 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.

[0030] A suitable dose of the anti-TrkA antibody will typically range from 0.1mg/kg to 10mg/kg body weight

[0031] Novel antibodies and compositions containing them disclosed herein are claimed as an aspect of the invention.

[0032] Non-limitative embodiments of the present invention will now be disclosed, with particular reference to the following figures:

FIGURE 1: Effect of the anti-TrkA monoclonal antibody MNAC13 (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 I.P. 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 are expressed in terms of absolute value (grams) of the threshold force for the hindpaw ipsilateral to lesion. The values are subjected to statistical analysis by means of analysis of 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 are significantly different from the controls, from day 4 to day 14.

FIGURE 2: Effect of the anti-TrkA monoclonal antibody MNAC13 (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 I.P. 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) are 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 were significantly different from the controls from day 4 to day 14.

FIGURE 3: Effect of the anti-TrkA monoclonal antibody MNAC13 (2 doses: 0.9 and 2 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 I.P. 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 in terms of the absolute value (grams) of the threshold force for the hindpaw ipsilateral to lesion. The values were subjected to statistical analysis by means of analysis of 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 MNAC13 were significantly different from the controls up to the last day of observation (31), from day 5 (greater dose of antibody) or from day 7 (lesser dose).

FIGURE 4: Effect of the anti-TrkA monoclonal antibody MNAC13 (2 doses: 0.9 and 2 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 I.P. 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 control, mouse immunoglobulins were used (IgG, 2 mg/kg). Results were expressed as a % (ratio between the threshold force for the hindpaw ipsilateral to lesion and that corresponding to the contralateral hindpaw).The corresponding absolute values were subjected to statistical analysis by means of analysis of 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 MNAC13 were significantly different from the controls until the last day of observation (31), from day 5 (greater dose of antibody) or from day 7 (lesser dose).

METHODS

Production of monoclonal antibodies

[0033] The monoclonal antibody MNAC13 (variable region light chain SEQ ID No. 1; variable region heavy chain SEQ ID No. 2) may be produced from a hybridoma supernatant, according to standard methods, disclosed above (Galfre and Milstein, 1981; Cattaneo et al., 1988; Cattaneo et al., 1999). The supernatant containing each antibody was subjected to precipitation (29% ammonium sul-

phate), followed by dialysis against PBS 1X (Spectra-Por 12/14K membrane, Spectrum) and affinity chromatography on sepharose protein G column (4-Fast Flow, Amersham Biosciences). Elution occurred by means of a low pH (HC1 5 mM) solution that was neutralised upon collection. The final eluate was concentrated (Amicon Ultra-15, 50K, Millipore) to obtain preparations of purified antibody in concentrations between 1 and 5mg/ml. As far as the humanised versions (IgGl human) of the antibody (Hu-MNAC13) is concerned, they were also purified as disclosed above, starting from the supernatants of stably transfected CHO cell lines, which are stable cotransfectants for the heavy chain (pVH/CMVexpress) and the light chain (pVL/CMVexpress) of each antibody. Vectors are known in the art, i.e as disclosed in WO 02/096458. The stable cotransfecting clones were obtained through double selection with G418 and mycophenolic acid.

Experiments in murine pain models

[0034] The animals were treated and handled in accordance with the guidelines of the IASP Ethical Committee and the Italian national law (DL116/92, application of European Direction 86/609/EEC) on the use of animals in research. Every necessary effort was made to minimise the suffering of the animals and to use the minimum amount of animals required to produce reliable scientific data.

Sciatic nerve surgery

[0035] Male CD1 mice, weighing approximately 35 g, were anaesthetised (intraperitoneal injection with 500 mg/kg chloral hydrate), the sciatic nerve of the right hind paw was exposed to be subjected to undergo loose ligature by means of stitching thread according to the chronic constriction lesion model (CCI) of the sciatic nerve, disclosed by Bennett and Xie (1988). The loose ligature of the sciatic nerve, inside the upper portion of the thigh, induced peripheral mononeuropathy characterised by thermal/mechanical allodynia and hyperalgesia. By ligation of the nerve at 3 different but close points, the neuropathy was fully developed 3 days following the lesion and lasted for 2-3 months.

Pharmacological treatment

[0036] From the third day following the lesion, anti-TrkA (MNAC13) antibodies were administered in an entire form (Mab) that were diluted in saline solution (vehicle), as indicated in Table I. As controls mouse immunoglobulin was used (IgG), in the same dose as the blocking antibodies (at the greater dose if 2 doses were used), or saline solution. Each experimental group included N=10 animals (unless explicitly stated otherwise).

Table I: Administration protocols and measurement of mechanical allodynia.

Antibody	Dose	Administration i.p.	Allodynia measurement
MNAC 13	50 µg/mouse =1.4 mg/kg	4, at days 3,4,5,6 after lesion	Days 3 to 14
MNAC 13	70 µg/mouse = 2 mg/kg	8, at days 3,4,5,6,7,8,9,10 after lesion	Days 3 to 31
MNAC 13	30 µg/mouse = 0.9 mg/kg		

Mechanical allodynia was measured by means of a plantar dynamic aesthesiometer (Ugo Basile), as indicated in Table I. Day 3 was considered the baseline.

[0037] The same protocols were used to assess the analgesic action of the humanised versions of the antibody MNAC13.

Statistical analysis

[0038] The results were expressed in 2 different ways, both as an absolute value of the threshold force value (in grams) that was sufficient for the animal to retract the hind leg that is ipsilateral to the lesion, or in percentage value, as the ratio between the absolute values of the hind legs (ipsilateral/contralateral). 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 $p < 0.01$.

Model of chronic inflammatory Pain

[0039] Adjuvant induced arthritis is elicited in male Lewis rats (175-200g, 7-8 weeks) by injection of 0.1 ml of *Mycobacterium butyricum* in mineral oil into the base of the tail. (Taurog et al., 1988; Devesa et al., 2005). On day 14 arthritic rats are qualified for the study if they show symptoms of the disease, measured as presence of redness, an increase of both hind paw oedema, and an increase in the vocalization after flexion of the ankle.

[0040] MNAC13 antibody (2 mg/kg in sterile saline vehicle) is administered twice intravenously, at 14 and 20 days after induction of arthritis. Indomethacin (3 mg/kg) is used as a reference compound and administered orally every day starting from 14 days and up to 20 days after disease induction. Control animals

do not receive any treatment. The level of statistical significance was determined by analysis of variance (ANOVA) followed by Dunnett's t-test for multiple comparisons. P values of $p < 0.05$ (*) or $p < 0.01$ (**) were taken as significant. Data represent mean ± S.E.M. (n = 7). *$p < 0.05$;

**$p < 0.01$ compared with control group (arthritic rats).

RESULTS

Neuropathic pain

[0041] The results on the CCI model showed that the blocking antibody MNAC13 (Fig. 1 and Fig. 2) had a significant analgesic effect. In particular, a similar result was observed for the two antibodies at the 1.4 mg/kg dose. As shown in Fig. 3 and Fig. 4, they started to have an analgesic effect from the second day of administration (day 4), reaching the maximum effect around day 6, keeping substantially the same analgesic efficacy for the entire duration of the observation until day 14. Expressing the result in percentage terms (ratio between the threshold force for the hindpaw ipsilateral to the lesion and that corresponding to the controlateral hindpaw), as in Fig. 4, it can be stated that for each of the two blocking antibodies, the maximum percentage value was around 60%, being around 40% for the control groups (IgG and saline).

[0042] When the animals were observed for 4 weeks, up to day 31, administration of the antibody MNAC13 (Fig. 5 and Fig. 6) revealed a two-phase effect. The first phase of analgesic efficacy (from day 3 to day 17, i.e. until a week after the last injection) was characterised by a maximum effect around days 11-12 (Fig. 5). This effect was clear for both the doses used (0.9 and 2 mg/kg) although the analgesic efficacy of the lesser dose always remained lower than that of the greater dose. After a reduction of the effect to day 17 (nevertheless still statistically distinguishable from the controls), a second analgesic phase was observed with an increase in the effect up to day 31. The final percentage (day 31) was close to 70% and 65% for the doses of 2 and 0.9 mg/kg, respectively, compared with the percentage value of the control groups around 40%

[0043] (Fig. 6). Two phases in the analgesic action of MNAC13 can thus be distinguished: the first ("pharmacological" effect), that comprises the treatment period and the first week after the last injection of antibody (the week during which the effect diminishes, parallel to the haematic concentration of the antibody); the second, which identifies a long-term effect, probably requiring new gene tran-

scription gene expression, which is an effect that gives MNAC13 the unique feature (in the field of neuropathic pain) of being a "disease-modifying" active principle, i.e. capable of modifying in depth the course of the disease, unlike the products currently used in this therapeutical context, which demonstrate a simple pharmacological effect on the symptoms. Substantially identical results to those illustrated above were obtained when instead of the antibody MNAC13, some humanised versions were used (dose used: 2 mg/kg for each antibody), confirming that the latter have the same analgesic properties as the parental version. The antibody was humanised with the method of WO2005/061540, both at the light (SEQ ID No. 3) and the heavy chain (SEQ ID No. 4) variable regions. To construct whole humanised antibodies, different constant regions were utilised, as above described (SEQ ID No. 3-6).

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Patentkrav

1. Anti-TrkA antistof, som er i stand til at hæmme bindingen mellem NGF og TrkA til anvendelse i en fremgangsmåde til behandling og/eller forebyggelse af neuropatisk smerte, hvori den variable region af den lette antistofkæde omfatter alle tre komplementaritetsbestemmende regioner (CDR) med sekvensen valgt fra aa. 24 til aa. 33 af SEQ ID No.1; fra aa. 49 til aa. 55 af SEQ ID No. 1; fra aa. 88 til aa. 96 af SEQ ID No. 1, og hvori den variable region af den tunge antistofkæde omfatter alle tre komplementaritetsbestemmende regioner (CDR) med sekvensen valgt fra aa. 26 til aa. 35 af SEQ ID No.2; fra aa. 50 til aa. 66 af SEQ ID No. 2; fra aa. 99 til aa. 112 af SEQ ID No. 2.

2. Anti-TrkA antistof til anvendelse i en fremgangsmåde til behandling og/eller forebyggelse af neuropatisk smerte ifølge krav 1, hvori den variable region af den tunge antistofkæde omfatter sekvensen af SEQ ID No. 2.

3. Anti-TrkA antistof til anvendelse i en fremgangsmåde til behandling og/eller forebyggelse af neuropatisk smerte ifølge krav 1 eller 2, hvori antistoffet er i enkeltkædeform og omfatter en variabel region af let kæde og en variabel region af tung kæde samlet med en binding.

4. Anti-TrkA antistof til anvendelse i en fremgangsmåde til behandling og/eller forebyggelse af neuropatisk smerte ifølge krav 1 eller 2, hvori antistoffet omfatter to lette kæder og to tunge kæder.

5. Anti-TrkA antistof til anvendelse i en fremgangsmåde til behandling og/eller forebyggelse af neuropatisk smerte ifølge ethvert af krav 1 til 4, hvori anti-TrkA antistoffet er et humant eller humaniseret antistof.

6. Anti-TrkA antistof til anvendelse i en fremgangsmåde til behandling og/eller forebyggelse af neuropatisk smerte ifølge krav 5, hvori den variable region af den lette kæde af humaniseret antistof omfatter sekvensen fra aa. 1 til aa. 106 af SEQ ID No. 3.

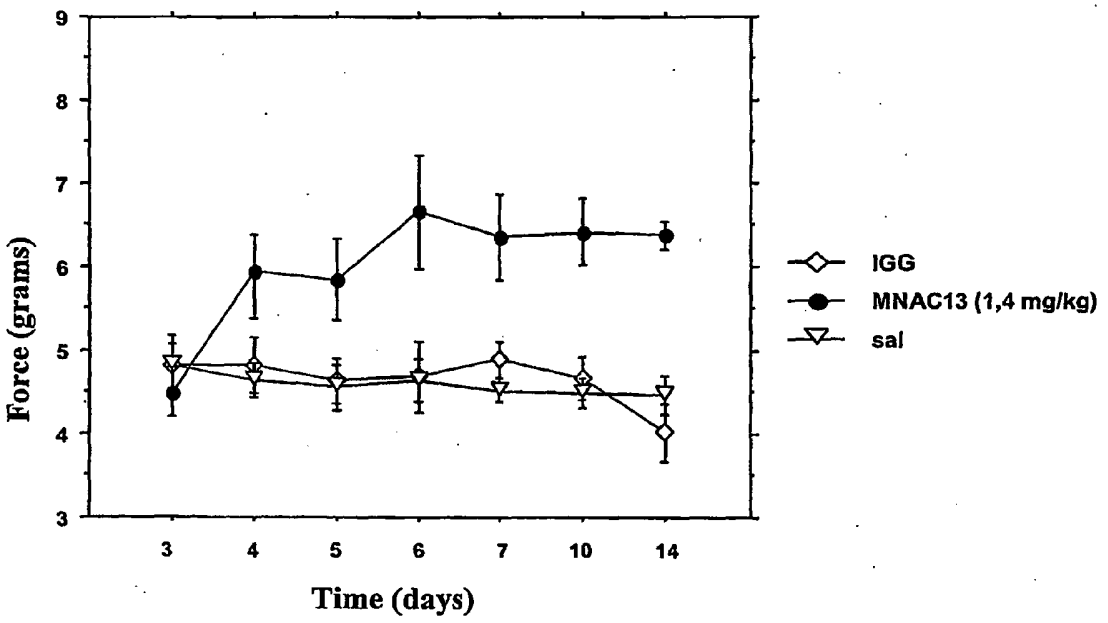
7. Anti-TrkA antistof til anvendelse i en fremgangsmåde til behandling og/eller forebyggelse af neuropatisk smerte ifølge krav 5 eller 6, hvori den variable region af den tunge kæde af humaniseret antistof omfatter sekvensen fra aa. 1 til aa. 123 af SEQ ID No. 4.

8. Anti-TrkA antistof til anvendelse i en fremgangsmåde til behandling og/eller forebyggelse af neuropatisk smerte ifølge ethvert af krav 5 til 7, hvori den lette kæde af humaniseret antistof har sekvensen af SEQ ID No. 3.

9. Anti-TrkA antistof til anvendelse i en fremgangsmåde til behandling og/eller forebyggelse af neuropatisk smerte ifølge ethvert af krav 5 til 8, hvori den tunge kæde af humaniseret antistof har en sekvens valgt blandt SEQ ID No. 4, SEQ ID No. 5, SEQ ID No. 6.

10. Anti-TrkA antistof til anvendelse i en fremgangsmåde til behandling og/eller forebyggelse af neuropatisk smerte ifølge ethvert foregående krav, hvori antistoffet har lang virkningstid.

11. Kit omfattende en sammensætning indeholdende et anti-TrkA antistof til anvendelse i en fremgangsmåde til behandling og/eller forebyggelse af neuropatisk smerte ifølge ethvert af krav 1 til 9 sammen med instruktioner, der angiver indgivelse af sammensætningen til en patient med behov for behandling og/eller forebyggelse af neuropatisk smerte for derved at behandle og/eller forebygge neuropatisk smerte hos patienten.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Time (days)
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Fig. 1

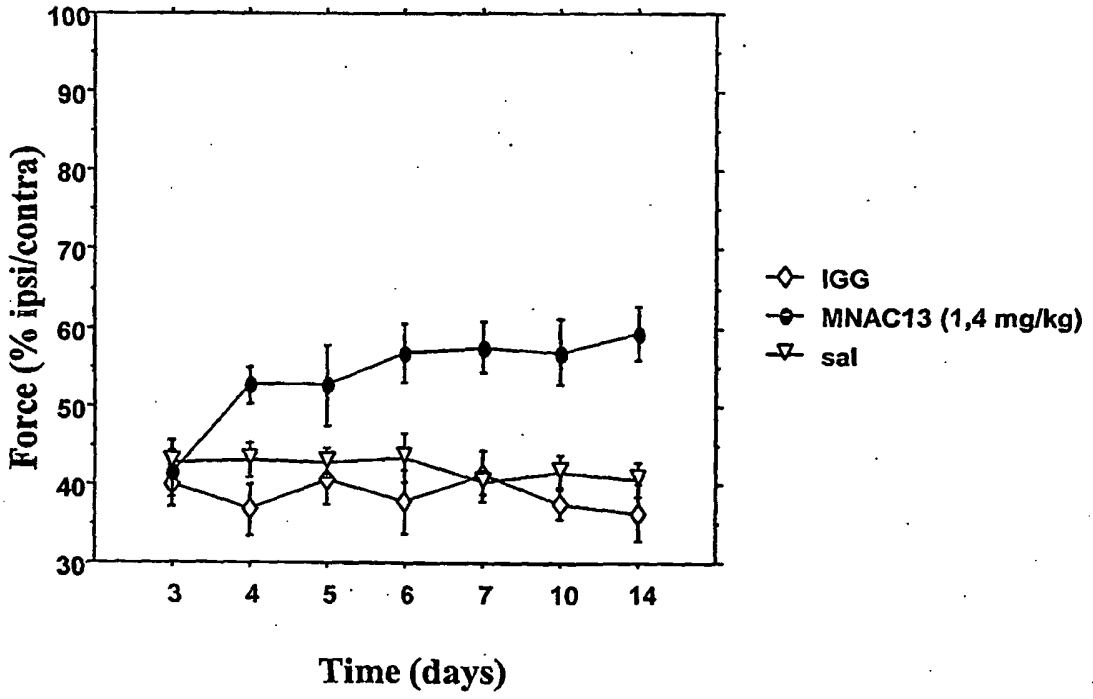
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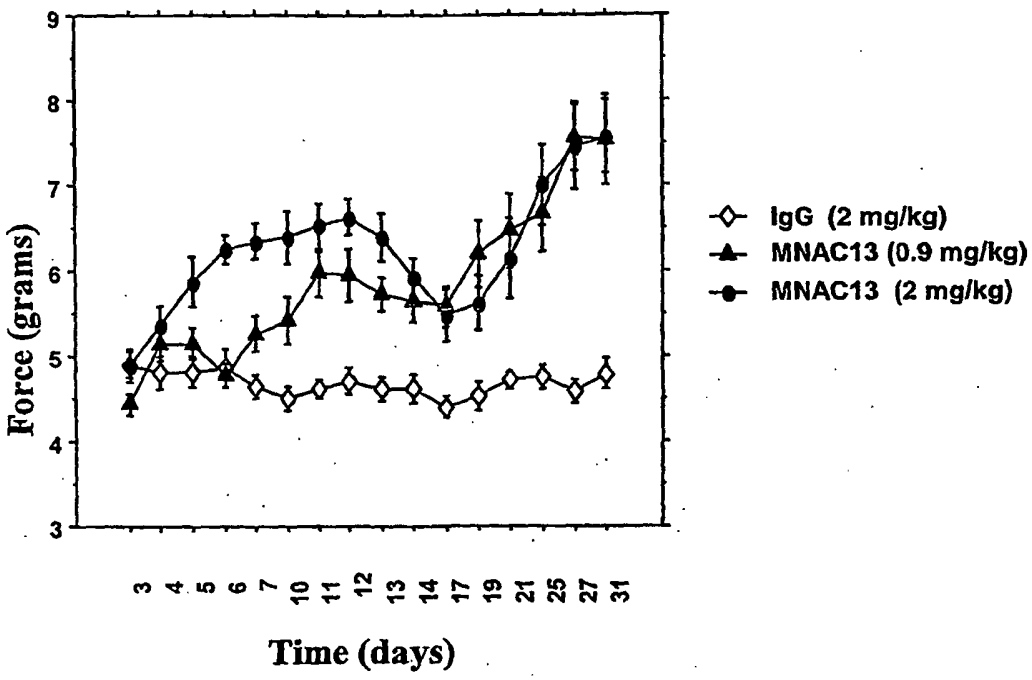
Fig. 2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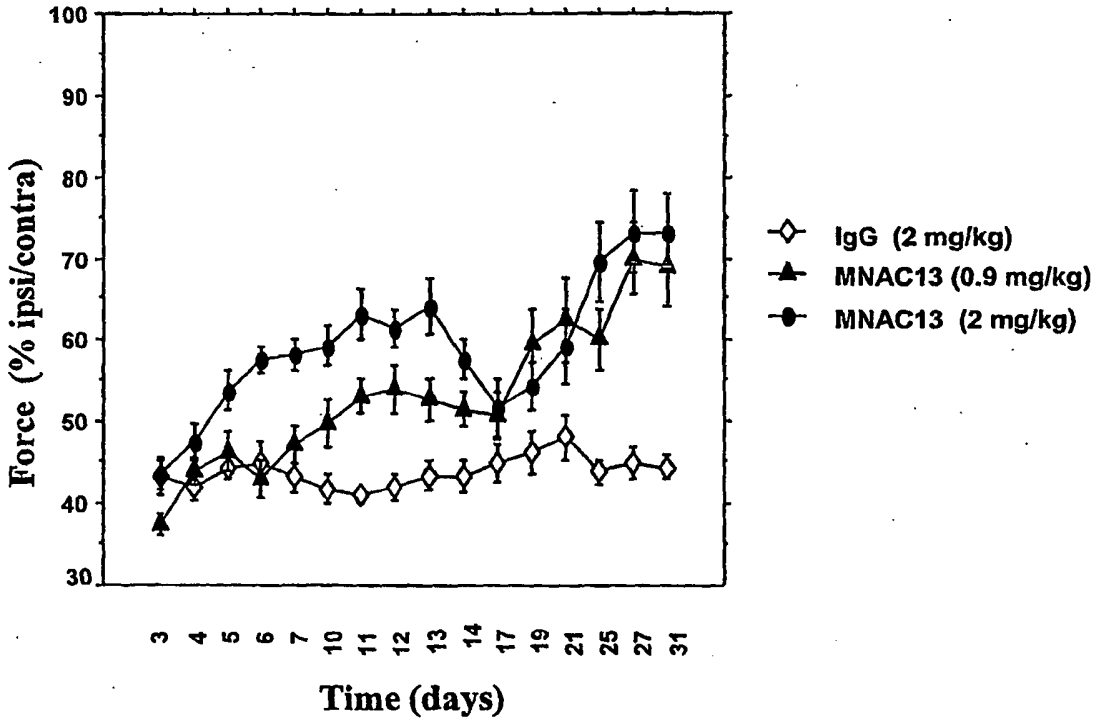
MNAC13 (2 mg/kg)

Fig. 3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Time (days)
IgG (2 mg/kg)
MNAC13 (0.9 mg/kg)
MNAC13 (2 mg/kg)

Fig. 4