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D E C I S I O N
of 2 December 2004

Case Number: W 0016/04 - 3.3.8

Application Number: PCT/GR03/00014

Publication Number: WO 03/087373

IPC: C12N 15/12

Language of the proceedings: EN

Title of invention:

Production of recombinant fragments of muscle acetylcholine receptor and their use for ex vivo immunoadsorption of anti-ch receptor antibodies from myasthenic patients

Applicants:

Hellenic Pasteur Institute and
Association Française contre les Myopathies

Opponent:

-

Headword:

Muscle acetylcholine receptor/HELLENIC PASTEUR INSTITUTE

Relevant legal provisions:

PCT Art. 17.3(a)

PCT R. 13.1, 13.2, 40.1, 40.2(c), 40.2(e), 40.3

Keyword:

"Lack of unity a posteriori (yes)"

"Protest dismissed (yes)"

Decisions cited:

-

Catchword:

-



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International Application No. PCT/GR03/00014

D E C I S I O N
of the Technical Board of Appeal 3.3.8
of 2 December 2004

Applicants:

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Representative:

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Decision under appeal:

Protest according to Rule 40.2(c) of the Patent
Cooperation Treaty made by the applicants
against the invitation (payment of additional
fees) of the European Patent Office
(International Searching Authority) dated
8 August 2003.

Composition of the Board:

Chairman: L. Galligani
Members: T. J. H. Mennessier
M. B. Günzel

Summary of Facts and Submissions

- I. International patent application PCT/GR03/00014 (published as WO-A2-03/087373) was filed on 15 April 2003 with 10 claims:

Claims 1 to 5 read as follows:

"The procedure which consists of the production of a group of recombinant domains (large sequences of more than approximately 70 amino acids long; preferably about 200 amino acids long) of the human (or other primate) muscle nicotinic acetylcholine receptor (AChR) subunits alpha, beta, gamma, delta and epsilon, or mutant forms of said molecules (including substitutions of free cysteins by other amino acids and substitutions of the hydrophobic loops of the subunits corresponding to alpha128-142 by more hydrophilic sequences), or the alpha domain containing the P3A exon, the sum of which contains the major part of the extracellular domain of said receptor, and:

- a. each said subunit domain having the capacity (when permanently, preferably covalently, immobilized on insoluble carriers) of immunoadsorbing (eliminating) large fractions of anti-AChR antibodies from myasthenia gravis (MG) patients (for each subunit domain, these fractions exceed the 30% of the total anti-AChR antibodies in at least some MG sera, whereas the alpha subunit domain immunoadsorbs up to 94% of the anti-AChR antibodies from some MG sera.
- b. when said immunoadsorbents are used in combination or sequentially, their total absorbing capacity of MG antibodies, equals approximately the sum of the individually absorbed antibody fractions.

Since each subunit domain eliminates the corresponding to said domain fraction of the total anti-AChR antibodies, the combined use of all or several of these domains is needed in order to eliminate the maximum possible amount of the anti-AChR antibodies from the patients."

"2. The molecules of claim 1 (with the characteristics 1a and 1b), which consist of the extracellular N-terminal parts of the human AChR subunits alpha, beta, gamma, delta and epsilon or mutant forms of said molecules."

"3. The procedure that consists of the permanent (covalent) immobilization of the molecules of claims 1 and 2 on insoluble carriers, selected from several suitable matrixes known in the art including agaroses (for example CNBr-Sepharose), celluloses, porous glass, silica, resins, synthetic matrixes including acrylamide derivatives, methacrylamide derivatives or polystyrene derivatives etc, in various forms including beads, fibrous form, sheets or hollow fibers, with spacer arms or without, by approaches known in the art for immobilization of other ligands."

"4. The procedure which consists of using the permanently immobilized molecules of claim 3, separately or in combination (i.e. several or all of them together or sequentially), for *in vitro* elimination of anti-AChR antibodies (preferably the majority) from sera of MG patients."

"5. The use of the permanently immobilized molecules of claim 3, preferably in combination, and of the application of the procedure of claim 4, for therapeutic *ex vivo* elimination/apheresis of the majority of the anti-AChR antibodies from the blood of MG patients."

Claim 6 was dependent on claim 1 and covered embodiments thereof using eukaryotic expression systems for expressing or coexpressing the molecules referred to therein.

Claims 7 to 10 while containing a reference to claim 6 paralleled claims 2 to 5.

II. On 8 August 2003, the European Patent Office, acting as an International Searching Authority (ISA), invited the applicants to pay within a time limit of thirty days four additional search fees pursuant to Article 17(3)(a), Rule 40.1 and 40.3 PCT and issued, as an annex to the invitation, a communication relating to the results of the partial international search carried out on the invention first mentioned in claims 1 to 10.

III. The invitation to pay additional search fees stated the five "multiple inventions" to which the application was found to relate, namely:

"1. Claims: 1-10 (all partially)

Procedure consisting in the production of the extracellular N-Terminal part of the recombinant Alpha subunit (sequence ID no.1) or fragments thereof of human muscle nicotinic acetylcholine

receptor (AChR) and in the immobilization of said domain. Molecule and the use thereof separately or in combination with any other subunit of the AChR in the In Vitro elimination or Ex vivo elimination/apheresis of anti-AChR antibodies from the blood or sera of myasthenic patients."

"2. Claims: 1-10 (all partially)

Procedure consisting in the production of the extracellular N-Terminal part of the recombinant Beta subunit (sequence ID no.5) or fragments thereof of human muscle nicotinic acetylcholine receptor (AChR) and in the immobilization of said domain. Molecule and use thereof separately or in combination with any other subunit of the AChR in the In Vitro elimination or Ex Vivo elimination/apheresis of anti-AChR antibodies from the blood or sera of myasthenic patients."

"3. Claims: 1-10 (all partially)

Procedure consisting in the production of the extracellular N-Terminal part of the recombinant Gamma subunit (sequence ID no.2) or fragments thereof of human muscle nicotinic acetylcholine receptor (AChR), in the immobilization of said domain. Molecule and use thereof separately or in combination with any other subunit of the AChR in the In Vitro elimination or Ex vivo elimination/apheresis of anti-AChR antibodies from the blood or sera of myasthenic patients."

"4. Claims: 1-10 (all partially)

Procedure consisting in the production of the extracellular N-Terminal part of the recombinant Delta subunit (sequence ID no.4) or fragments thereof of human muscle nicotinic acetylcholine receptor (AChR), in the immobilization of said domain. Molecule and use thereof separately or in combination with any other subunit of the AChR in the In Vitro elimination or Ex vivo elimination/apheresis of anti-AChR antibodies from the blood or sera of myasthenic patients."

"5. Claims: 1-10 (all partially)

Procedure consisting in the production of the extracellular N-Terminal part of the recombinant Epsilon subunit (sequence ID no.3) or fragments thereof of human muscle nicotinic acetylcholine receptor (AChR), in the immobilization of said domain. Molecule and use thereof separately or in combination with any other subunit of the AChR in the In Vitro elimination or Ex vivo elimination/apheresis of anti-AChR antibodies from the blood or sera of myasthenic patients."

IV. The following documents are referred to in the present decision:

- (1) Tzartos S.J. et al., Society for Neuroscience Abstracts, Vol. 27, No. 1, 2001, Page 1278
- (2) Yun Yao et al., J. Biol. Chem., Vol. 277, No. 15, 12 April 2002, Pages 12613 to 12621

- (3) Masaharu Takamori and Takahiro Maruta, *Ther. Apher.*, Vol. 5, No. 5, 2001, Pages 340 to 350
- (4) Masaharu Takamori and Yoshihiko Ide, *Transfus. Sci.*, Vol. 17, No. 3, 1996, Pages 445 to 453
- (5) Socrates J. Tzartos et al., *Immunol. Rev.*, Vol. 163, 1998, Pages 89 to 120
- (6) Leslie Jacobson et al., *J. Neuroimmunol.*, Vol. 98, 1999, Pages 112 to 120

V. The reasons for the non-unity finding were indicated as being associated with an *a posteriori* objection to lack of inventive step.

The reasoning was as follows:

The human muscle nicotinic acetylcholine receptor (AChR) was well documented in the prior art. It consisted of five homologous subunits in adults. The alpha subunit had been characterised and its extracellular domain (alpha-(1-210)) had been expressed in the yeast *Pichia pastoris*. It contained the main immunogenic region (MIR), the major target for autoantibodies in myasthenia gravis. The use of an immobilised fragment/domain of the AChR for antigen-specific immunoadsorption therapy for myasthenia gravis was documented in the prior art. Reference was made to documents (1) to (4).

In view of the prior art, the problem underlying the application was defined as the provision of further fragments/domains of the muscle nicotinic acetylcholine receptor and their use in ex vivo or in vitro immunoabsorption of anti-AChR antibodies from myasthenic patients, the solution proposed being the five groups indicated above (cf section III, supra).

Due to the fact that the alpha subunit of the human AChR (and more particularly its extracellular domain) was known in the prior art, due to the essential difference in structure between the different solutions, and due to the fact that no other technical features could be distinguished which, in the light of the prior art, could be regarded as special technical features, there was no single inventive concept underlying the plurality of claimed inventions of the present application in the sense of Rule 13.1 PCT. Consequently, there was lack of unity among the different inventions.

- VI. On 30 October 2003, the applicants paid four additional search fees under protest pursuant to Rule 40.2(c) PCT and provided a reasoned statement to the effect of establishing that the international application complied with the requirement of unity of invention.

The applicants stated that they had proved in the application that the alleged five groups of inventions shared common technical features.

In particular, they had explained (i) that the essence of the invention consisted in the combination of the five subunits which were parts of a single molecule,

the AChR, (ii) that each subunit domain that they were using capable of immunoadsorbing a separate fraction of the pool of anti-AChR antibodies from each myasthenia gravis (MG) patient, and (iii) that the combined use of all or most of the subunit domains was needed for the elimination of the majority of the anti-AChR antibodies from the majority of the MG patients. These observations defined a new concept.

Therefore, the claims all referred to the combined production or use of the five domains together as part of a single molecule. The combined use of the five subunits of the human muscle nicotinic acetylcholine receptor in order to immunoadsorb the majority of the autoantibodies from patients afflicted by myasthenia gravis formed a basis for a technical relationship between the claims. This relationship involved technical features that defined a contribution which each of the inventions, considered as a whole, made over the prior art.

None of the cited documents was in relation to this concept. In particular, document (1) only mentioned the production of the extracellular domain of the alpha subunit of the human muscle nicotinic acetylcholine receptor in Pichia pastoris. Moreover, the document did not disclose the methodology used, it was silent about the capacity of that domain to bind large quantities of antibodies from patients afflicted by myasthenia gravis and ignored a possible use thereof as an immunoadsorbent.

VII. On 9 March 2004, the ISA transmitted the International Search Report, which had been established for the whole set of claims.

VIII. On the same date, the ISA communicated to the applicants the results of its review under Rule 40.2(e) PCT, which was essentially as follows.

Document (1) disclosed the production by Pichia pastoris of the extracellular part of the alpha subunit of the human muscle nicotinic acetylcholine receptor and other fragments thereof in a native conformation and in a quantity sufficient for structural analysis and therapeutic assays. Said subunit, known to contain the main immunogenic region, was capable of binding many sera from patients afflicted by myasthenia gravis. Thus, claim 1 was not new over document (1). The capability of the subunit recombinant domains of immunoadsorbing large fractions of autoantibodies from patients afflicted by myasthenia gravis as expressed in claim 1 was no more than a desideratum. As subunits of the human muscle nicotinic acetylcholine receptor other than the alpha subunit were known, their production in Pichia pastoris did not involve an inventive step over document (1).

Removal of myasthenia gravis autoantibodies using said subunit or fragments thereof had been disclosed in the prior art. It was also known that extracellular epitopes were present on the other subunits of the human receptor (cf document (5)). Therefore, a skilled person would have regarded it as obvious to remove antibodies specific to the human receptor in patients

afflicted by myasthenia gravis by using different fragments of subunits of the human receptor.

The proposal of a combined use of all or most of the extracellular domains of the five subunits of the human muscle nicotinic acetyl choline receptor in an immunoadsorption method to remove a majority of antibodies to said receptor in a patient afflicted with myasthenia gravis could not confer unity to claims which referred to a process for producing in Pichia pastoris a group of recombinant domains of the said receptor.

The applicants were invited to pay within one month the protest fee.

- IX. The protest fee was paid by the applicants on 31 March 2004, no further arguments being provided.

Reasons for the Decision

1. The protest is admissible.
2. The objection to lack of a *posteriori* unity of invention by the ISA is essentially based on the finding that the preparation in Pichia pastoris of the extracellular domain of the alpha subunit of the human muscle nicotinic acetylcholine (the human muscle nicotinic AChR) as a recombinant polypeptide, which has the native conformation of the molecule and is capable of binding to all conformation dependent monoclonal antibodies specific to the main immunogenic region of the domain, as well as to many sera containing

autoantibodies from patients afflicted by myasthenia gravis, is disclosed in document (1). Claim 1 lacks consequently novelty. The five inventions encompassed by claim 1 are no longer linked as to form a single general inventive concept.

3. The reasoning to be applied, when assessing whether, in a group of inventions claimed in one and the same international application, the inventions are so linked as to form a single general inventive concept as referred to in Rule 13.1 PCT, has to rely on the provision as set forth in Rule 13.2 PCT, according to which, **there shall be a technical relationship among those inventions involving one or more of the same or corresponding "special technical features"**, ie those features that define a contribution which each of the claimed inventions, considered as a whole, makes over the prior art.

4. Claim 1 is directed to a process for preparing recombinant domains of more than 70 amino acids of alpha, beta, gamma, delta and epsilon subunits of primate muscle nicotinic AChRs (and mutants thereof). The wording of the claim is such that it does not necessarily imply that the claimed "procedure" is limited to the preparation of any specific domain of the said subunits nor to the **simultaneous** preparation of domains of the five distinct subunits. Indeed, as clearly deducible from dependent claim 6, the process of claim 1 covers both the separate expression of the molecules or the co-expression of two or more molecules. Claim 2 is directed to the "molecules of claim 1", these being the extracellular N-terminal parts of the human AChR subunits alpha, beta, gamma, delta and

epsilon or mutant forms thereof. This claim cannot possibly be interpreted as being directed to a composition where all molecules are together, but as a claim with a number of alternatives ("or" claim). The same applies to claim 7 which refers back to claim 6. All the other claims refer to the recombinant subunit domains obtained by the "procedure" of claim 1 and to their **separate** or **combined** use (cf eg claims 4 and 9: "separately and in combination"; claims 5 and 10: "preferably in combinations").

5. In view of the above considerations, it can be said that claim 1 covers the following five embodiments, namely:
- (i) a process for preparing a recombinant domain of the **alpha** subunit of a primate muscle nicotinic AChR, separately expressed or co-expressed with one or more domains of the same subunit or of other subunits,
 - (ii) a process for preparing a recombinant domain of the **beta** subunit of a primate muscle nicotinic AChR separately expressed or co-expressed with one or more domains of the same subunit or of other subunits,
 - (iii) a process for preparing a recombinant domain of the **gamma** subunit of a primate muscle nicotinic AChR separately expressed or co-expressed with one or more domains of the same subunit or of other subunits,
 - (iv) a process for preparing a recombinant domain of the **delta** subunit of a primate muscle nicotinic AChR separately expressed or co-expressed with one or more domains of the same subunit or of other subunits, and

- (v) a process for preparing a recombinant domain of the **epsilon** subunit of a primate muscle nicotinic AChR separately expressed or co-expressed with one or more domains of the same subunit or of other subunits.
6. The question to be answered is whether there exists between these five embodiments of the invention a technical relationship involving one or more of the same or corresponding technical features that define a contribution which each of the claimed embodiments, considered as a whole, makes over the prior art.
7. This question can be reformulated as follows, it being noticed that co-expression of one or more domains cannot be taken into account as a possible special technical feature because this is not an exclusive feature of the claims (cf point 4, supra): "Can any of the following features be seen as a "special technical feature" providing the required unitary link:
- (a) The indication of the selected domains and their capability of binding autoantibodies from patients with myasthenia gravis?
 - (b) The fact that the domains are recombinant, ie produced in a recombinant expression system?
 - (c) Their capacity of immunoadsorbing anti-AChR antibodies from patients with myasthenia gravis?
 - (d) The fact that, when used in combination or sequentially, their total absorbing capacity equals approximately the sum of the individually absorbed antibody fractions?"

8. The five subunits alpha, beta, gamma, delta and epsilon of the human muscle nicotinic AChR and their ability to bind antibodies present in MG sera were known in the art (cf eg documents (5) and (6)). Thus, feature a) cannot be seen as a "special technical feature" providing the unitary link.

9. The production of the domains in a recombinant expression system, eg expression in the yeast Pichia pastoris, is **not new** at least in respect of the production of the extracellular N terminal domain of the alpha subunit. In fact, document (1) which is part of the state of the art discloses the use of the yeast Pichia pastoris for the preparation of the extracellular domain of the human muscle nicotinic AChR alpha subunit which is obtained in a native conformation and is shown to bind to "all" conformational dependent monoclonal antibodies specific to the main immunogenic region of that domain, as well as to many sera from patients afflicted by myasthenia gravis. Thereby, the use in the preparation of a domain of the alpha subunit of a primate AChR of one of the preferred expression systems, namely the yeast Pichia pastoris (which had been proved in document (2) to be an expression system appropriate for the preparation of the extracellular domain of the murine nicotinic AChR), was disclosed in the state of the art. Thus, feature b) cannot be seen as a "special technical feature" providing the unitary link.

10. Also feature (c) cannot be seen as a "special technical feature" providing the unitary link, because, being the production of a recombinant domain of the alpha subunit recognised by myasthenic autoantibodies known from

document (1) (cf point 9 above) and being the removal of autoantibodies in patients with myasthenia gravis by immunoadsorption (cf eg document(3)), the said feature makes no inventive contribution over the prior art.

11. As for feature (d), this cannot be seen as a **technical** feature, but merely as a result to be ideally achieved (or "desideratum") which does not necessarily result from performing the procedure as claimed. Thus, it cannot serve the purpose of providing unity among a group of separate embodiments which are not otherwise linked.
12. Thus, there is no special technical feature which defines a contribution which one embodiment of claim 1 makes over the prior art and, thus, the five embodiments of claim 1 are to be regarded as five distinct inventions.
13. Therefore, it has to be concluded that the application lacks unity, contrary to the requirement of Rule 13.1 PCT.
14. Therefore, the invitation to pay four additional search fees was justified.

Order

For these reasons it is decided that:

The protest under Rule 40.2(c) is dismissed.

The Registrar:

The Chairman:

A. Wolinski

L. Galligani