

Internal distribution code:

- (A) [] Publication in OJ
(B) [] To Chairmen and Members
(C) [X] To Chairmen
(D) [] No distribution

D E C I S I O N
of 8 May 2003

Case Number: T 0446/99 - 3.3.8

Application Number: 88308124.2

Publication Number: 0306318

IPC: C12N 15/00

Language of the proceedings: EN

Title of invention:

Recombinant DNA-derived bordetella toxin subunit analogs

Patentee:

Amgen Inc.

Opponent:

Chiron Corporation

Aventis Pasteur Limited/Aventis Pasteur Limitée

Headword:

Bordetella toxin/AMGEN

Relevant legal provisions:

EPC Art. 56, 83

Keyword:

"Main request: sufficiency of disclosure (yes)"

"Inventive step (yes)"

Decisions cited:

-

Catchword:

-



Case Number: T 0446/99 - 3.3.8

D E C I S I O N
of the Technical Board of Appeal 3.3.8
of 8 May 2003

Appellant: Aventis Pasteur Limited/Aventis Pasteur
(Opponent 2) Limitée
1755 Steeles Avenue
Toronto
Ontario
M2R 3T4 (CA)

Representative: Weiss, Wolfgang
Weickmann & Weickmann
Patentanwälte
Postfach 86 08 20
D-81635 München (DE)

Respondent: Amgen Inc.
(Proprietor of the patent) One Amgen Center Drive
Thousand Oaks, CA 91320-1799 (US)

Representative: Voelker, Ingeborg
UEXKÜLL & STOLBERG
Patentanwälte
Beselerstrasse 4
D-22607 Hamburg (DE)

Party as of right: Chiron Corporation
(Opponent 1) 4560 Horton Street
Emeryville, California 94608-2917 (US)

Representative: Hallybone, Huw George
Carpmaels and Ransford
43 Bloomsbury Square
London WC1A 2RA (GB)

Decision under appeal: **Interlocutory decision of the Opposition
Division of the European Patent Office posted
23 February 1999 concerning maintenance of
European patent No. 0306318 in amended form.**

Composition of the Board:

Chairman: L. Galligani
Members: T. J. H. Mennessier
C. Rennie-Smith

Summary of Facts and Submissions

- I. Both opponents 1 and 2 lodged appeals against the interlocutory decision of the opposition division dated 23 February 1999, whereby the European patent No. 0 306 318, claiming the priority dates of 4 September 1987 and 17 August 1988, was maintained on the basis of the first auxiliary request (claims 1 to 19) then on file, in two versions, one for all designated states except ES and GR (non-ES, non-GR) and the other for ES and GR.
- II. The patent had been opposed (i), under Article 100(a) EPC, on the grounds that the invention was not new, did not involve an inventive step and was not susceptible of industrial applicability, and (ii), under Article 100(b) EPC, on the ground that the invention was not sufficiently disclosed (Article 83 EPC).
- III. In reply to the two statements of grounds of appeal, the patent proprietor (respondent) filed observations with a letter dated 24 January 2000.
- IV. With a letter dated 14 April 2000, opponent 1 withdrew its appeal, opponent 2 thus remaining the only appellant.
- V. On 30 January 2003, the board issued a communication pursuant to Article 11(2) of the Rules of Procedure of the Boards of Appeal indicating its preliminary and non-binding views on the matters of the case.
- VI. In reply thereto, the respondent filed with a letter dated 5 May 2003 a first auxiliary request, the claims

as maintained by the opposition division being its main request.

VII. **The claims (non-ES, non-GR) as maintained by the opposition division** consisted of 19 claims, of which, in addition to claim 1, claims 4, 10, 17, 18 and 19 were independent.

Claim 4 read:

"4. A polypeptide analog of S1 subunit of the Bordetella exotoxin, the polypeptide analog having an amino acid sequence which differs from the naturally occurring sequence of the S1 subunit by one or more amino acid residues in the region bounded by valine 7 and proline 14, inclusively, wherein arginine 9 has been replaced by lysine, which polypeptide analog (a) can elicit toxin-neutralizing levels of antibodies and (b) is free of enzymatic activities associated with toxin reactogenicity."

Claim 18 was directed to a method of producing such an analog.

Claim 10 and **claim 19** were directed, respectively, to a vaccine comprising such an analog and a method of producing such a vaccine.

Claim 1 and **claim 17** were directed, respectively, to a DNA molecule encoding such an analog and a method of producing such a DNA molecule.

The set of claims for ES and GR contained corresponding method claims.

VIII. Oral proceedings took place on 8 May 2003. They were attended by the appellant and the respondent.

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

- (D1) Alfredo Nicosia et al., Proc. Natl. Acad. Sci. USA, Vol. 83, No. 13, July 1986, Pages 4631 to 4635;
- (D2) Camille Locht and Jerry M. Keith, Science, Vol. 232, No. 4755, 6 June 1986, Pages 1258 to 1264;
- (D5) W. Neal Burnette et al., Bio/Tech., Vol. 6, June 1988, Pages 699 to 706;
- (D12) Rino Rappuoli et al., Tibtech, July 1991, Vol. 9, No. 7, Pages 232 to 238;
- (D18) Makoto Tamura et al., Biochemistry, Vol. 21, No. 22, 26 October 1982, Pages 5516 to 5522;
- (D19) Juan L. Arciniega et al., Infect. Immun., Vol. 59, No. 1, October 1991, Pages 3407 to 3410;
- (D26) Hiroko Sato et al., Infect. Immun., Vol. 55, No. 4, April 1987, Pages 909 to 915;
- (D27) Hiroko Sato et al., Infect. Immun., Vol. 46, No. 2, November 1984, Pages 422 to 428;

- (D31) W. Neal Burnette et al., *Biologicals*,
Vol. 21, No. 1, March 1993, Pages 12 and 13;
- (D36) W. Neal Burnette, in "Vaccine Research and
Developments", Vol. 1, Wayne C. Koff and
Howard R. Six Editors, Marcel Dekker Inc.,
New York, 1992, Pages 143 to 189;
- (D39) Sheena M. Loosmore et al., *Infect. Immun.*,
Vol. 58, No. 11, November 1990, Pages 3653
to 3662.

X. The appellant's submissions in writing and during oral proceedings, insofar as they are relevant to the present decision, can be summarized as follows:

*Claims as maintained by the opposition division:
sufficiency of disclosure, novelty and inventive step
of the subject-matter of claim 4*

Producing analogs of S1 subunit of the Bordetella exotoxin using site-directed mutagenesis and testing said analogs could be performed without any burden. Nevertheless, none of the analogs structurally defined as in claim 4 and theoretically encompassed within that claim could (i) be capable on their own of eliciting toxin-neutralizing levels of antibodies and (ii) be free of enzymatic activity associated with toxin reactogenicity. Such analogs were not disclosed in the patent. In post-published documents, the inventor himself had admitted that no analog was capable on its own of eliciting toxin-neutralising levels of antibodies. Therefore, the requirements of Article 83 EPC were not met.

Novelty was not an issue. The person skilled in the art, on the basis only of either of documents (D1) or (D2), would have regarded it as "logical" to replace the arginine at position 9 by a lysine. Thus, without the exercise of inventive skill, he/she would have prepared analogs structurally and functionally defined as in claim 4. Therefore, the requirements of Article 56 EPC were not met.

- XI. The respondent's submissions in writing and during oral proceedings, insofar as they are relevant to the present decision, can be summarized as follows:

*Claims as maintained by the opposition division:
sufficiency of disclosure, novelty and inventive step
of the subject-matter of claim 4*

There was no requirement that the claimed analogs always elicited toxin-neutralizing levels of antibodies. They only had to have the capability of doing so. In any case, analogs could be regarded as intermediate products compared to the whole holotoxin. "Eliciting toxin-neutralizing levels of antibodies" was to be interpreted as "providing immunoprotection". "Free of enzymatic activity associated with toxin reactogenicity" should be interpreted in the light of the description as exhibiting little or no ADP-ribosyltransferase activity. Therefore, the analogs of the invention were sufficiently disclosed. They were also undoubtedly susceptible of industrial applicability and new over the cited prior art. Neither of documents (D1) and (D2) contained an incentive to replace

arginine at position 9 by lysine. Therefore, the claimed invention involved an inventive step.

XII. The appellant requested that the decision under appeal be set aside and the patent be revoked.

XIII. The respondent requested that the decision under appeal be set aside and the patent maintained as in that decision save for an amended page 8 of the description as filed during the oral proceedings.

Reasons for the Decision

Claims as maintained by the opposition division

Article 83 EPC

1. The preparation by use of site-specific mutagenesis of analogs having an amino acid which differs from the naturally occurring sequence of the S1 subunit by one or more amino acid residues in the region bounded by valine 7 and proline 14, inclusively, wherein arginine 9 is replaced by lysine, and testing such analogs for the properties (a) and (b) as recited in claim 4 can be performed, on the basis of the disclosure made in the patent, by the person skilled in the art without any difficulties or undue burden. This is indeed admitted by the appellant which however argues that it would not be possible to succeed in obtaining analogs actually displaying the required activities a) and b). This in its view amounts to a lack of enablement.

2. A S1-derivative, referred to as "4-1", is disclosed in the patent which differs, in the region bounded by valine 7 and proline 14, inclusively, from the naturally occurring S1 subunit and from the corresponding recombinant S1 subunit, referred to as "the rS1 subunit", in that arginine 9 has been replaced by lysine. It further differs therefrom in that the native aspartylaspartyl residues at positions 1 and 2 of its amino terminus have been replaced by methionylvalyl residues (see page 14 to 16 and Figure 7 in the patent specification).

3. The immunological properties and enzymatic activities of the said derivative "4-1" have been investigated in a comparative study involving the rS1 subunit and 7 other S1-derivatives differing from derivative "4-1" only in that the region bounded by valine 7 and proline 14, inclusively, has been mutated differently.

4. The results of this investigation are summarized in Table 2 on page 12 of the patent specification with the indication, for each of the derivatives tested and for the rS1 subunit, of a binding to antibody 1B7 (see the column in the middle) and the presence of ADP-ribosyltransferase activity (see the right-hand column). As reflected by the table, both the derivative 4-1 (which is produced by clone pPTXS1 (6A-3/4-1)) and the rS1 subunit (which is produced by clone rPTXS (pPTXS1/1)) were proved to be capable of binding to antibody 1B7. In contrast, as also indicated in Table 2, not the rS1 subunit but only derivative 4-1 exhibited little or no ADP-ribosyltransferase activity.

5. In the board's judgment, on the basis of such results, the person skilled in the art would have considered derivative "4-1" able to elicit toxin-neutralizing levels of antibodies (see point 6, *infra*) and to be free of enzymatic activities associated with toxin reactogenicity (see point 7, *infra*). This finding is based on the following considerations:
 6. Immunological properties of derivative "4.1":
 - 6.1 An experiment is reported on page 8, lines 23 to 37 in the patent specification (see also Figure 6) in which mice were immunized with crude unmutated rS1 subunit, and subjected to intracerebral challenge with the Bordetella pertussis mouse virulent strain 18323. Mortality was scored for as long as 45 days post-challenge. An increase in survival time for rS1-immunized animals relative to unimmunized controls was observed. Further, a number of mice receiving adjuvanted rS1 were **completely protected** against challenge, the adjuvanted rS1 eliciting dose-responsive protection.
 - 6.2 Achievement of a complete protection means that rS1 subunit had elicited in the immunized mice toxin-neutralizing levels of antibodies.
 - 6.3 From the description (see page 10, lines 5 to 30, in the patent specification), the person skilled in the art would have known that, as proved using truncated versions of the mature S1 molecule, the antigenic epitope that binds antibody 1B7, a monoclonal antibody known to neutralize pertussis toxin biological activities and to passively protect mice against

intracerebral challenge with virulent B. pertussis (cf documents (D26) and (D27)), lies at least partially within the region bounded by valine 7 and proline 14, inclusively.

- 6.4 The rS1 subunit and the derivative "4-1", which have both been obtained recombinantly, only differ, apart from the aminoterminal substitution, in that, in the analog, arginine 9 has been replaced by lysine. Replacement and aminoterminal substitution have not affected the recognition by antibody 1B7 (see Table 2 on page 12 in the patent specification). Thus, the board considers that the person skilled in the art would have concluded that derivative "4-1" would have behaved *in vivo* as the rS1 subunit and, therefore, would have elicited, upon immunization of mice, toxin-neutralizing levels of antibodies.
- 6.5 The appellant argues that, in view of the statement also found on page 8 (see lines 37 and 38) in the patent specification, which reads: "Later studies have not confirmed immunoprotection against intracerebral challenge with B. pertussis mouse virulent strain 18323.", the results of the afore-mentioned immunoprotection experiments cannot be trusted and are only artefacts. The board notes that said statement does not contradict the finding that the rS1 subunit can provide complete immunoprotection but merely illustrates the inherent variability of results of experiments involving the mouse intracerebral challenge assay, a variability about which the inventor has expressed concerns in the post-published document (D5)(see page 704).

- 6.6 It is not denied that, in post-published documents cited by the parties, doubts have been expressed whether the recombinant S1 subunit was capable of eliciting mouse-protective antibodies on its own (see, for example, document (D31), page 12, which itself refers to document (D5)). However, positive results have been provided in the patent and, as these particular results have not been contradicted by any expert opinion, they cannot be disregarded. Moreover, post-published document (D19) has corroborated the capability of the mutant S1 subunit having arginine 9 replaced by lysine to protect on its own at least part of the tested mice from an aerosol challenge with B. Pertussis (see Table 2 on page 3408).
- 6.7 Notwithstanding these remarks, one should pay attention to the precise wording used in claim 4 which does not read "which polypeptide analog **elicits** toxin-neutralizing levels of antibodies" but which polypeptide analog can **elicit** toxin-neutralizing levels of antibodies" (emphasis added by the board). That wording is obviously intended to take into account situations where, although the analog tested has the capability of eliciting the antibodies, the expected elicitation or effect thereof is not or is only poorly observed, due to the uncertainty inherent in biological tests such as the mouse intracerebral challenge assay or because the conformational state of the analog might not permit in some instances the correct recognition of the protective epitope by the immune system of the immunized animals. In this last respect, it is to be noted that the protective epitope recognized by antibody 1B7 has been hypothesized to be composed of regions which are not contiguous in the primary

structure of the antigen (see document (D12), page 235) which means that this epitope may be conformational.

7. Enzymatic activities associated with toxin reactogenicity exhibited by derivative "4.1":
 - 7.1 The appellant argues that the wording of claim 4 requires that the analogs of the invention do not retain **any** enzymatic activity associated with toxin reactogenicity and that such analogs are not disclosed in the patent.
 - 7.2 The board notes that such a restriction is not regarded as an absolute prerequisite in the description. As pleaded by the respondent, the ADP-ribosyltransferase being regarded as a major marker of toxin activity, the intention was to prepare analogs exhibiting **little or no** ADP-ribosyltransferase, a reduction of that activity by a factor of at least 5000 being regarded as satisfactory (see page 12, lines 44 to 52 in the patent specification). The board considers that requiring that the analog be **absolutely** free of **any** enzymatic activities - which, indeed, is not claimed - would amount to an exaggerated and even unpracticable requirement.
 - 7.3 Therefore, the person skilled in the art would have concluded that derivative "4-1", which is shown in the patent to exhibit not only little or no ADP-ribosyltransferase, this activity being reduced by a factor of at least 5000, but also little or no detectable glycohydrolase activity, this activity being reduced by a factor of at least 50 to 100 (see page 12, lines 44 to 51, in the patent specification), is

- essentially free of enzymatic activities associated with toxin reactogenicity.
8. The person skilled in the art would have immediately inferred from Table 2 that substitution in the S1-derivatives of methionylvalyl residues for the aspartylaspartyl residues at positions 1 and 2 of the amino terminus of the native S1 subunit had no impact on the capability of binding to antibody 1B7 and exhibiting ADP-ribosyltransferase activity. Such a substitution had been made for convenience of cloning only, as confirmed later on in document (D36) (see page 159), the resulting analog being referred to in the patent specification as "1-4" (see page 13, lines 13 to 16). The skilled person, knowing that this analog differs from derivative "4-1" only by the inclusion of the native aspartylaspartyl residues at positions 1 and 2, would have expected the analog to bind to antibody 1B7 and exhibit little or no ADP-ribosyltransferase activity just as derivative "4-1" does. Thus, analog "1-4" would also be expected to elicit toxin-neutralizing levels of antibodies and be free of enzymatic activities associated with toxin reactogenicity.
9. This conclusion is directly confirmed by the experiments reported on pages 13 and 14 in the patent specification and Figure 11. In these experiments, semi-recombinant holotoxins (B oligomer plus either the rS1 subunit or analog "1-4") were examined for their ability to elicit a clustering response in Chinese hamster ovary. Concentrations of at least 10 to 25 mg of holotoxin containing analog "1-4" were necessary to elicit the response which was obtained with

concentrations as low as 0,25 to 0,30 ng/ml with holotoxin containing the rS1 subunit or commercial pertussis toxin. These results indicate for analog "1-4" a drastic reduction of the cytotoxic effect associated with the enzyme activities of the S1 subunit.

10. Therefore, an analog is described in the patent specification which has both the required structural and functional technical features recited in claim 4.

11. The appellant does not deny that analogs of the invention, when used in combination with other subunits part of the toxin, can elicit toxin-neutralizing levels of antibodies, a fact which has been corroborated in post-published documents (see for example, document (D39), page 3661). Nevertheless, the appellant argues that the preparation of a complete holotoxin comprising an analog of the invention is not sufficiently disclosed in the patent.

12. The board does not consider that a detailed disclosure of how to prepare such an holotoxin should be contained in the description in order that the requirements of Article 83 EPC be met, since it has been established therein that the analogs are capable of eliciting toxin-neutralizing antibodies and general guidance is provided to the person skilled in the art for such preparation (see page 3, lines 46 to 53 and page 4, lines 24 to 38 in the patent specification). This is to be read with the background knowledge of the structure of the toxin and the subunit re-assembly experiments described in prior art document (D18). Nevertheless, the board notes that ample details are provided on page 26, lines 42 to 52 in the patent specification,

which may be useful for the preparation of semi-recombinant holotoxins.

13. For these reasons, the board considers that the analog as defined in claim 4 is sufficiently disclosed. This conclusion applies *de facto* to the other claimed aspects of the invention, as all remaining claims refer to this analog. Thus, the requirements of Article 83 EPC are met by the claims as maintained by the opposition division.

Article 57 EPC

14. The objection raised by the appellant was directly linked to its contention that analogs as referred to in the claims could not be made.
15. As the requirements of Article 83 EPC are considered to be met, the objection becomes groundless. In the board's judgment, the analog of claim 4 is susceptible of industrial application in the field of medicine, this judgment applying *de facto* to the other claimed aspects of the invention (see point 12, *supra*). Thus, the requirements of Article 57 EPC are met by the claims as maintained by the opposition division.

Article 54 EPC

16. Novelty was no longer objected to by the appellant at the oral proceedings. In the board's judgment, there are no documents on file which affect the novelty of the claimed subject-matter. Thus, the requirements of Article 54 EPC are met by the claims as maintained by the opposition division.

Article 56 EPC

17. The appellant refers to documents (D1) and (D2) as closest prior art.

18. Document (D1), which basically reports on the cloning and sequencing of the pertussis toxin genes, including the gene encoding the S1 subunit, provides a comparison of the amino acid sequence of that subunit with the amino acid sequence of the fragment A of cholera toxin, which uses the same NAD substrate for the ADP-ribosylation of different protein targets. Figure 7 (see page 4635) illustrates the homologies found between the first 100 amino acid residues of the subunit S1 of the pertussis toxin and the first 98 amino acid residues of cholera toxin fragment A. A number of homology regions are identified, one of them being within the 7 amino acid region referred to in claim 4 (Tyr8-Arg9-Tyr10-Asp11-Ser12-Arg13-Pro14 in the native S1 subunit). Amino acids Tyr8, Arg9, Asp11, Ser12, Arg13 and Pro14 are common to both toxins. It is suggested "that the homologous regions of the two proteins may be those interacting with NAD" (see the last but one concluding statement on page 4635).

19. Document (D2) also reports on the nucleotide sequence and genetic organisation of the pertussis toxin gene. The S1 subunit is compared not only to the cholera A subunit but also to the E. coli heat labile toxin A subunit. Two regions with significant homology are identified, one of them being the region defined in claim 4 (see Figure 3 on page 1261). The suggestion is made that "the NAD-binding function of the ADP-

ribosylating enzymes is dependent more on the secondary and tertiary structures than on the primary structures". There is no definite and unambiguous identification of the active sites for the ADP-ribosylation in the S1 subunit. The document ends with the speculative statement that "By comparison to other toxin genes with similar biochemical functions, and by physical identification of the active sites (..) for the ADP-ribosylation in the subunit, it is now possible to modify [those] sites by site-directed mutagenesis of the B. pertussis genome. Those modifications could abolish the pathobiological activities of pertussis toxin without hampering its immunogenicity and protectivity. Alternatively, by knowing the DNA sequence it will be possible to map protective epitopes."

20. The appellant argues that, on the basis of either of documents (D1) or (D2), the person skilled in the art would have regarded it as **"logical"** to replace arginine in the S1 subunit at position 9 by lysine.
21. The technical problem solved by the invention may be regarded as the provision of recombinant analogs of the S1 subunit which lack enzymatic activity while retaining that protective epitope which is recognized by the antibody 1B7 and plays an important role in most of the biological activities of the pertussis toxin. The solution to this problem as proposed in the patent is the provision of analogs as defined in claim 4.
22. Solving the problem would have required the person skilled in the art, as a first step to identify a region important for the ADP-ribosyltransferase

activity and establish the precise location of the epitope, and, as a second step, to ensure that the adequate modifications of that region and epitope were investigated.

23. On the basis only of a comparison of amino acid sequences, each of documents (D1) and (D2) points to regions of the S1 subunit amino acid sequences regarded as potential candidates for portions of the molecule playing a role in the ADP-ribosyltransferase activity. Among these regions is the region referred to in claim 4. In the absence of any experimental evidence that this region was actually involved in that enzymatic activity, the person skilled in the art would have found no incentive to select that particular region. Even if, by chance, he/she had selected it, he/she would also have found no guidance as to the minimal modification to be carried out. He/she would not have been in a position to decide which of the 6 common amino acids (Tyr8, Arg9, Asp11, Ser12, Arg13 and Pro14 in the S1 subunit) should be replaced and which amino acid should be used as a substitute for the one or more positions to be changed. If, by way of hypothesis, the person skilled in the art had (which is not accepted by the board) found some indication to select position 9 in the amino acid sequence as being the critical position, he/she would have had no particular reasons to select lysine for the replacement. Moreover, neither of those documents contains any guidance as to the location of the epitope recognized by antibody 1B7.

24. Therefore, the board considers that the person skilled in the art, aware of documents (D1) and (D2), would have had to exercise inventive skill in order to arrive at the proposed solution to the stated technical problem.

25. Thus, the board concludes that the invention of claim 4 involves an inventive step, that conclusion also applying *de facto* to the rest of claims (see point 12, *supra*). Thus, the requirements of Article 56 EPC are met by the claims as maintained by the opposition division.

Description

26. A discrepancy existed between the statement, found in claim 4 and each of the other independent claims, according to which "the polypeptide analog [having] an amino acid sequence which **differs from the naturally occurring sequence of the S1 subunit by one or more amino acid residues** in the region bounded by valine 7 and proline 14, inclusively, wherein arginine 9 has been replaced by lysine," (emphasis added by the board) and description page 8 as accepted by the opposition division which stated that "[M]odification of the valine 7 through proline 14 region, including **substitution and/or deletion** of one or more amino acids, results in S1 analog products" (emphasis added by the board). The respondent requests that the term "and/or deletion" be deleted.

27. As the requested amendment results in an appropriate adaptation of the description to the claims as maintained by the opposition division, which is necessary for a correct determination of the extent of protection as foreseen in Article 69 EPC, the board regards said amendment as acceptable.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.
2. The patent is maintained as requested by the respondent.

The Registrar:

The Chairman:

A. Wolinski

L. Galligani