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D E C I S I O N
of 23 April 2003

Case Number: T 1228/01 - 3.3.4

Application Number: 88908889.4

Publication Number: 0396552

IPC: C07K 15/14

Language of the proceedings: EN

Title of invention:
Method of preparation and use for zona pellucida antigens and antibodies for sterilization and contraception

Applicant:
ZONAGEN, INC.

Opponent:
-

Headword:
Zona pellucida antigens/ZONAGEN INC.

Relevant legal provisions:
EPC Art. 123(2)

Keyword:
"Allowability of amendments (no)"

Decisions cited:
T 0301/87, T 0081/87, G 0002/98, G 0001/93

Catchword:
-

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Case Number: T 1228/01 - 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.3.4
of 23 April 2003

Appellant: ZONAGEN, INC.
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Decision under appeal: **Decision of the Examining Division of the European Patent Office posted 26 June 2001 refusing European patent application No. 88 908 889.4 pursuant to Article 97(1) EPC.**

Composition of the Board:

Chairwoman: U. M. Kinkeldey
Members: M. R. J. Wieser
R. Moufang

Summary of Facts and Submissions

I. The appeal lies from the decision of the examining division to refuse under Article 97(1) EPC the European Patent Application No. 88 908 889.4, which was published as international application WO 89/03399 with the title "Method of preparation and use for zona pellucida antigens and antibodies for sterilization and contraception", because it contained subject-matter which extended beyond the content of the application as filed, contrary to the requirements of Article 123(2) EPC.

II. On 14 March 2003 the appellants filed a new "replacement main" and auxiliary request and withdrew their request for oral proceedings.

III. Claim 1 of the main request read:

"A pharmaceutical composition comprising:
a) a pharmacologically appropriate carrier; and
b) an immunogenically effective amount of a substantially pure polypeptide as produced by recombinant expression from encoding DNA in a transformed prokaryotic or eukaryotic host cell whereby the polypeptide is substantially free of native glycosylation, the polypeptide including an amino acid sequence encoded by the P3 coding sequence within the λgt11-P3 deposit available from ATCC 40378, wherein the polypeptide includes a specific antigenic determinant which on administration to an animal induces the production of antibodies capable of binding to the zona pellucida to cause temporary, reversible contraception."

Claim 1 of the auxiliary request differed therefrom insofar as the term "an animal" was replaced by "a cow, pig, cat, dog or human".

IV. In a communication dated 4 April 2003, issued pursuant to Article 11(2) of the rules of procedure of the boards of appeal, the board noted that they were not convinced that the claims of both requests filed on 14 March 2003 met the requirements of Articles 123(2), 56 and 83 EPC.

V. Oral proceedings were held on 23 April 2003 in the absence of the appellants, who had informed the board that they will not attend.

VI. The following documents are mentioned in this decision:

(12) DNA Sequence-The Journal of Sequencing and Mapping Vol. 4, 1994, pages 361 to 393

(13) The Journal of Biological Chemistry, Vol. 266, No. 11, 1991, pages 7214 to 7219

Both documents are post published and have been introduced into the proceedings by the appellants, document (12) with a letter dated 14 August 1995 and document (13) with a letter dated 14 March 2003.

VII. The submissions made by the appellants may be summarised as follows:

The newly filed claims were fully based on the application as filed. The P3 sequence information presented in figure 4 of the application, consisting of two partial sequences of a single, full-length clone, contained sequencing errors. The true P3 coding sequence, as disclosed in documents (12) and (13), was identically contained in the λgt11-P3 phage deposited

as ATCC 40378. A reference to this deposited phage was contained in the paragraph bridging pages 29 and 30 of the application as filed.

Article 123(2) EPC, being the sole ground for refusal of the application by the Examining Division, was considered to be the only subject of the appeal proceedings.

VIII. The appellants requested in writing that the decision under appeal be set aside and that claims 1 to 8 of the "Replacement Main Request" or claims 1 to 8 of the "First Auxiliary Request", filed 14 March 2003, be held allowable under Article 123(2) EPC, and that the case be remitted for further prosecution to the first instance.

Reasons for the Decision

Article 123(2) EPC

1. Claim 1 of both requests refers to a pharmaceutical composition comprising a "polypeptide including an amino acid sequence encoded by the P3 coding sequence within the λgt11-P3 deposit available from ATCC 40378".

2. Example 3 of the application as originally filed refers to the isolation of zona pellucida (ZP) protein cDNA clones. RNA is produced from frozen and pulverized rabbit ovaries. The total RNA obtained is purified and polyA-RNA is isolated by oligo (dT)-cellulose chromatography. Double stranded cDNA is synthesized from the isolated polyA-mRNA, provided with EcoRI linkers, treated with EcoRI and ligated to the λgt11 vector for the preparation of an expression library. The obtained plaques are plated and then transferred to

nitrocellulose paper for screening with polyclonal antibodies against rabbit ZP protein. Four clones, λgt11-S1, -P1, -P2 and -P3, are subcloned. The subclones are cloned into the M13 phage for sequencing of the cDNA. The results are shown in figure 4, where two P3 sequences, a 272 base fragment and a 484 base fragment, are disclosed. Further, it is demonstrated by northern blot analyses with total RNA from various tissues, using EcoRI digested cDNA probes from λgt11-P2 and -P3, that RNA for ZP proteins is present in the ovary but not in other tissues.

3. The deposition of the bacteriophages λgt11-P1 and λgt11-P3 with the American Type Culture Collection (ATCC) under the Deposit Accession No. 40377 and 40378 is mentioned on page 29, line 34 to page 30, line 3, of the application as filed. Deposit receipts, showing the deposits have been made in accordance with the requirements of Article 83 and Rule 28 EPC, have been submitted by the appellants on 8 July 1992. The date of the deposition is 8 October 1987, i.e. after the claimed priority date.

4. Example 4 refers to "Expression of ZP proteins by recombinant DNA". E. coli Y1089 is infected with the recombinant λgt11 phages of example 3. Transcription of the cloned gene in isolated lysogenic cultures is stimulated. The transformed cells are harvested and frozen. The cells are lysed upon thawing and a fusion protein containing the ZP protein is released (page lines 12 to 32).

On pages 30 to 33 a further expression strategy is described, wherein the ZP inserts of the recombinant λgt11 phages are isolated by EcoRI digestion and inserted into pEX plasmids. Finally, the example refers on page 33 to the expression of recombinant ZP DNA in yeast cells.

5. Example 5, relating to "Purification of recombinant Zona pellucida protein", describes the processing of proteins expressed according to the first embodiment described in example 4. Transformed E. coli cells are frozen and thawed, and the expressed protein, isolated as β-galactosidase fusion protein, is purified and analysed by SDS-PAGE and immunoblotting. The example explicitly refers to a SDS-PAGE immunoblot of a ZP antigen-fusion protein expressed by the P1 clone, which is shown in figure 6 (page 34, lines 18 to 20). Purification of an expression product of the P3 clone is not reported.

6. The P3 polypeptide of the present application is the rabbit ZP protein designated as ZBP in document (12), which is also designated ZP1 in other publications (see appellants letter of 19 November 1997, page 3). On page 2 of the letter of 25 March 1999, the appellants have stated that "The 272 base sequence corresponds to the portion of the rabbit ZPB sequence of figure 13 of Harris et al. beginning at amino acid residue 45", and that "The 484 base sequence corresponds to the portion of the rabbit ZPB sequence of figure 13 of Harris et al. which ends at the C-terminal double arginine. The stop codon TGA is at the end of the second complete line up from the end of the 484 sequence". (Harris et al. is document (12) in the present proceedings.)

On 14 March 2003, in a letter accompanying the new claim requests, the appellants informed the board that the P3 sequence information presented in figure 4 contained sequencing errors. The correct coding sequence and encoded polypeptide sequence were represented in figure 2 of document (13). The amino acid sequence indicated therein was identical to that appearing in figure 13 of document (12).

7. Document (13) is a scientific paper published three and a half years after the priority date of the present application, naming one of the designated inventors as author. The document discloses the isolation, cloning and sequencing of a full-length cDNA (rc55) encoding the major rabbit ZP glycoprotein having a molecular weight of 55kDa. The predicted amino acid sequence thereof consists of 540 amino acids including a putative signal peptide of 18-24 residues (abstract). On page 7215, in the passage bridging left and right column, RNA isolation and preparation of a gene expression library is disclosed. By using the method also described in example 3 of the present application, a 1700bp clone is isolated, whose identity is verified by matching the deduced amino acid sequence thereof with the NH_2-terminal amino acid sequence from a rabbit ZP protein of 55kDa. The 1700bp EcoRI insert (rc55) is subcloned, transformed into JM101 and sequenced. The resulting nucleotide sequence and the deduced NH_2-terminal amino acid sequence are shown in figure 2. On page 7215, right column, third paragraph, it is further disclosed, that the EcoRI insert is subcloned into pEX2 and expressed as fusion protein.

8. Document (12), a scientific paper published seven years after the claimed priority date, reports of the cloning and characterization of ZP genes and cDNAs from a variety of mammalian species. The paper describes the presence of all three major zona pellucida gene families, ZPA, ZPB and ZPC, within individual mammalian species.

On page 362, right column, reference is made to document (13) as being the first disclosure reporting the cloning of the gene coding for **rabbit** ZPB protein. Figure 13 on pages 385 to 386, shows a comparison of the ZPB deduced amino acid sequences from rabbit, cat,

pig and human and a consensus sequence derived therefrom. The sequence of the rabbit protein, consisting of 540 amino acids, is identical to the one of figure 2 in document (13). On page 391, right column, third paragraph, it is stated that the rabbit ovarian cDNA libraries were constructed in the phage λgt10.

9. The application as originally filed does not contain information concerning the size or complete DNA sequence of the P3 insert of λgt11-P3.

From a comparison of the nucleotide- and deduced amino acid sequences in figure 2 of document (13) and figure 13 of document (12) with the sequences "P3 272" and "P3 484" in figure 4 of the present application, it is apparent that the sequences differ. This is acknowledged by the appellants in the letter of 14 March 2003. Moreover, no information concerning the actual expression product of the "P3 coding sequence" is contained in the application.

In addition it is found that neither document (12) nor (13) refer to the deposited phage now contained in claim 1.

10. In the letter of 14 March 2003, the appellants argued that the application discloses cloning, sequencing and expression of the rc55 cDNA of document (13), i.e. the 1700 bp EcoRI restriction fragment described therein. They referred to the similarity between the methods of example 3 and document (13), and relied in this respect to the following two passages of the application:

(i) page 22 of the description was considered to identify 55 kD as "the relevant molecular weight", and

(ii) according to page 28, it was shown that the P3 insert of the deposited λgt11 clone is an EcoRI restriction fragment.

11. These two citations from the description are not considered by the board to be able to substantiate appellants' line of argumentation.

11.1 As mentioned in point (9), the application does not contain any information concerning the size of the P3 insert and does accordingly not allow any speculation concerning the molecular weight of the encoded polypeptide. On page 22 of the application the molecular weights of the major **pig** ZP polypeptides are said to be approximately 35, 55 and 80 kD. The molecular weights of the major **rabbit** ZP polypeptides however are described to be 50, 75 and 85 kD. Since P3 is a rabbit ZP protein, the board, contrary to the appellants, does not see a basis in the cited passage for 55 kD being "the relevant molecular weight" of the application.

11.2 Page 27, lines 12 to 20 of the application reads: "The cDNA was methylated at the EcoRI sites before ligation to EcoRI linkers. The linkered cDNA was then treated with EcoRI enzyme and purified by chromatography on Biogel P50 (BioRad) and ligated to the λgt11 arms obtained from Strategene according to procedures described therewith. Using this procedure approximately 5 x 10 plaques were obtained for the 6 week and 8 month old rabbit libraries and 1 x 10^7 plaques were obtained for the 12 week rabbit library." Consequently, it cannot be deduced from the mere fact that the P3 insert of the deposited λgt11 clone is an EcoRI restriction fragment, that it is exactly the 1700 bp EcoRI insert coding for a 55 kD rabbit ZP glycoprotein disclosed in document (13).

12. In the letter of 14 March 2003, appellants conclude, with regard to the formulation used in claim 1, that "the reference in the new claims to the deposited sequence ensures that the sequence is correct".

Thus, the appellants infer that the reference to a deposited phage in a claim, whose correct deposit is mentioned in the application as originally filed, is an implicit disclosure of a part of a nucleotide sequence contained in said phage, although this sequence is not disclosed *per se*.

13. The boards of appeal in decision T 301/87 (OJ EPO 1990, 335) had to decide with regard to the entitlement of a patent to a claimed priority date, if the reference to a sequence in a priority document, and the corresponding deposition of a strain containing the sequence in a recombinant form, establishes by implication priority for the operatively important part of the sequence (see point 6 of the reasons for the decision). The competent board decided that this cannot be accepted. Although a whole recombinant plasmid and its incorporated sequence was in toto disclosed in the alleged priority document, in consequence of the deposition and corresponding description of some characteristics of this incorporated sequence, the same does not apply to component parts within these entities which are not disclosed in the priority document.

In their decision the board referred to the earlier decision T 81/87 (OJ EPO 1990, 250) where it was emphasised that the subject-matter of the claims must be clearly identifiable in the previous application as a whole and must relate to the same invention when it comes to priority. It was further stated that elements which are to be recognised as essential only later on, are not part of the disclosure. The board in T 301/87 took the view that if an entity itself is disclosed to

the skilled person, this does not necessarily mean that a component part is also disclosed for the purpose of priority if this cannot be envisaged directly and unambiguously as such, and requires considerable investigation to reveal its identity.

Present claim 1 refers to a polypeptide encoded by the operatively important part ("the P3 coding sequence") of a cDNA insert of a deposited phage, derived from total RNA of rabbit ovaries according to example 3 (see point 2 above). Besides the information that the P3 insert of the deposited λgt11 clone is an EcoRI restriction fragment, the application as originally filed does not contain any information about the size and structure of the P3 insert, or of the operatively important part thereof, the "P3 coding region", allegedly contained therein.

14. Although the decisions quoted above deal with the question of entitlement to priority, this board takes the view that the conclusion reached therein may also apply when the allowability of amendments in the light of the requirements of Article 123(2) EPC is examined.

The Enlarged Board of Appeal, in the decision G 1/93 (OJ EPO 1994, 541), stated that the underlying idea of Article 123(2) EPC was that an applicant should not be allowed to improve his position by adding subject-matter not disclosed in the application as filed, which would give him an unwarranted advantage and could be damaging to the legal security of third parties relying on the content of the original application.

With regard to the priority right, the Enlarged Board stated in the decision G 2/98 (OJ EPO 2001, 413), that a narrow and strict interpretation of the concept of "the same invention" referred to in Article 87(1) EPC, equating it with the concept of "the same subject-

matter" referred to in Article 87(4) EPC is perfectly consistent with Articles 4F and 4H of the Paris Convention, and is also an requirement to guarantee the legal security of third parties (point 8.1 of the reasons for the decision).

While an amendment, in order to be allowable under Article 123(2) EPC, must rely on subject-matter explicitly or implicitly disclosed in the application as originally filed, the right of priority for a later application is determined by, and limited to, what is explicitly or implicitly disclosed in the priority application.

Since the required standard of correspondence with an earlier document, the application as originally filed in the one case and the priority document in the other case, is the same, i.e. explicit or implicit disclosure, the board concludes that the findings of decision T 301/87 can be applied to the present situation.

15. Thus, the disclosure in the application as originally filed of the deposition of the recombinant bacteriophage λgt11-P3, is not considered to be a basis within the requirements of Article 123(2) EPC for the disclosure of a DNA sequence designated as "the P3 coding sequence" which allegedly is contained in said bacteriophage, but which as such is not disclosed in the application as originally filed.

The board comes to the conclusion that the introduction of the term "the polypeptide including an amino acid sequence encoded by the P3 coding sequence within the λgt11-P3 deposit available from ATCC 40378" into

claim 1 of both requests, results in information which is neither explicitly, i.e. directly and unambiguously, nor implicitly, derivable from that originally disclosed in the application.

Therefore, claim 1 of the replacement main request and of the first auxiliary request do not meet the requirements of Article 123(2) EPC.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar: The Chairwoman:

G. Rauh U. M. Kinkeldey