Europäisches Patentamt
Beschwerdekammern

European Patent Office
Boards of Appeal

Office européen des brevets
Chambres de recours

Veröffentlichung im Amtsblatt	Ja/Nein
Publication in the Official Journal	Yes/No
Publication au Journal Officiel	oui/Non

Aktenzeichen / Case Number / N° du recours : T 499/88

Anmeldenummer / Filing No / N° de la demande : 82 300 947.7

Veröffentlichungs-Nr. / Publication No / N° de la publication : 59 598

Bezeichnung der Erfindung:
Title of invention:
Titre de l'invention : A process and apparatus for the recovery of immunoglobulins

Klassifikation / Classification / Classement : A23J 1/20

ENTSCHEIDUNG / DECISION

vom / of / du 11 January 1990

Anmelder / Applicant / Demandeur :

Patentinhaber / Proprietor of the patent / Titulaire du brevet : Unilever PLC

Einsprechender / Opponent / Opposant : Boehringer Mannheim

Stichwort / Headword / Référence : Immunoglobulins/UNILEVER

EPÜ / EPC / CBE Art. 56

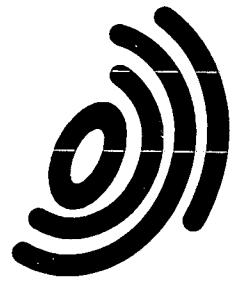
Schlagwort / Keyword / Mot clé : "Inventive step (no)"
"Obvious replacement of polyclonal antibodies by monoclonal antibodies"

Leitsatz / Headnote / Sommaire

Europäisches Patentamt
Beschwerdekammern

European Patent Office
Boards of Appeal

Office européen des brevets
Chambres de recours



Case Number : T 499 /88 - 3.3.1

D E C I S I O N
of the Technical Board of Appeal 3.3.1
of 11 January 1990

Appellants : (Proprietors of the patent)
Unilever PLC
Unilever House Blackfriars
P.O. Box 68
London EC4P 4BQ (GB)

Unilever N.V.
Burgemeester s'Jacobplein 1
P.O. Box 760
NL-3000 DK Rotterdam (NL)

Representative :

Respondent : (Opponent)
Boehringer Mannheim GmbH
Fabrik chem. und pharm. Produkte
Sandhoferstr. 116,
Postfach 31 01 20
D-6800 Mannheim 31

Representative :

Decision under appeal : Decision of Opposition Division of the European Patent Office of 23 June 1988, posted on 10 August 1988, revoking European patent No. 59 598 pursuant to Article 102(1) EPC.

Composition of the Board :

Chairman : R.W. Andrews
Members : U. Kinkeldey
G.D. Paterson

Summary of Facts and Submissions

I. European patent application No. 82 300 947.7, which was filed on 24 February 1982 and claimed priority from a British patent application filed on 26 February 1981, was granted as European patent No. 59 598 on 17 July 1985 with 16 claims, the independent Claims 1 and 2 reading as follows:

"1. A process for the recovery of immunoglobulins of high purity and potency from natural sources such as a milk and blood serum, characterised in that a source of immunoglobulins is contacted with an insoluble carrier material to which is bound a low-affinity monoclonal antibody specific to one or more of the immunoglobulins but not specific to any other common constituent of the source, the antibody binds immunoglobulin molecules and, following removal of the residue of the source, immunoglobulin molecules are released from the antibody.

2. A process for the recovery of immunoglobulins of high purity and potency from milk, characterised in that milk is contacted with an insoluble carrier material to which is bound a low-affinity monoclonal antibody specific to one or more of the immunoglobulins but not specific to any other common constituent of milk, the antibody binds immunoglobulin molecules and, following removal of the residue of the milk, immunoglobulin molecules are released from the antibody."

Independent Claims 7, 9, 12 and 16 relate to an immuno-adsorbent column or filter comprising a low-affinity monoclonal antibody, an apparatus for recovering from milk immunoglobulins of high purity and potency, a milk processing plant comprising said apparatus and a process

for the manufacture of an immunoadsorbent column or filter respectively.

II. Notice of opposition was filed by

Boehringer Mannheim GmbH

requesting the revocation of the patent on the ground that the patent did not comply with Article 100(a) and (b) EPC, referring to the prior art documents

E1: Sapin et al in J.Immunol.Meth.9 (1975) pages 27-38,
E2: Jefferis et al in the same periodical 39 (1980) pages 355-362.

III. The Appellants (Proprietors of the patent) contested the alleged lack of inventiveness relying inter alia, but, in particular, on the argument that at the time of the priority date of the disputed patent, there was no common general knowledge relating to the use of monoclonal antibodies for the large scale purification process as claimed with the additional advantage of leaving the nutritional properties of the source of the immunoglobulins to be purified, namely milk, unchanged. This was a surprising and unexpected effect when using monoclonal antibodies in a large scale purification process. To support this argument the Appellants referred to a further publication

E3: Secher and Burke in Nature 285 (1980) pages 446-449.

IV. In a decision announced at the end of oral proceedings held on 23 June 1988 and posted on 10 August 1988 the

Opposition Division revoked the patent.

The Decision held that it was known from document E1 to provide polyclonal antibodies as specific immunoadsorbents for immunoglobulin fractions and to bind them to a carrier; many details concerning the adsorbtion, capacity, the elution conditions and the specificity of the adsorbent had been investigated. It was, furthermore, quite obvious that the described adsorbent material was of low affinity since elution of the adsorbed fraction was carried out with appreciable yields. Document E1 was therefore distinguished from the subject-matter of the present patent only by the type of antibodies, namely polyclonal in document E1 and monoclonal in the disputed patent. When weighing this document as the closest state of the art the technical problem underlying the alleged invention was to be seen in the provision of another process effectively yielding the desired immunoglobulins in high purity and potency.

Document E2 described monoclonal antibodies used to form insoluble complexes with immunoglobulin fractions and document E3 proposed the use of monoclonal antibodies for a large scale purification of human interferon. The replacement of polyclonal antibodies in an otherwise identical process by monoclonal antibodies was said to be obvious in the light of the information contained in document E1, combined with either document E2 or E3.

An objection under Article 100(b) EPC which had been raised by the Opponent but which had not been substantiated was rejected in the Opposition Division's decision.

V. On 10 October 1988 the Appellants filed a Notice of Appeal against this decision, together with payment of the appeal

fee. A Statement of Grounds was filed on 19 December 1988 by facsimile which was confirmed by letter received on 21 December 1988 by the European Patent Office.

The main arguments submitted were as follows:

The general premise which emerged from the decision that at the priority date of the present patent it was common general knowledge in the art that monoclonal antibodies could be used to replace conventional polyclonal antibodies in known processes was not accepted. Although the principle of monoclonal antibodies had been published six years before the claimed priority date, it was only to be expected that several years would elapse before this radically new technology became generally appreciated and workers in other laboratories and scientific institutions developed the necessary expertise to produce monoclonal antibodies for themselves, prior to investigating possible alternative uses. Attention was drawn to the publication date of document E3 which was close to the priority date of the present patent and which was said not to support any general premise that it was obvious to use monoclonal antibodies in any affinity chromatography procedure. The recovery of interferon as described in document E3 was solving a very specific problem because interferon is present in serum in extremely small amounts and the authors of document E3 did not suggest that their findings could be applied more generally. The contemplated "large scale purification of interferon" could not be compared to the scale of a recovery procedure for immunoglobulins as contemplated in the present patent. Rather, if the skilled man had contemplated the use of monoclonal antibodies in a process like the claimed one, he would have expected that although the purity of the recovered immunoglobulin might be higher, the overall yield would have suffered because the monoclonal antibody would be too specific to adsorb a

useful proportion of the total immunoglobulin content of the source material. Therefore, the immunoglobulin recovery processes claimed in Claims 1 and 2 of the patent were non-obvious.

VI. In reply the Respondents filed a further prior art document

E4: EP-A-0 014 519

and argued that all the cited documents had to be judged as normal state of the art in the sense of Article 54(2) EPC because they were published prior to the priority date of the present patent and this was the only decisive fact with regard to the inventive step required by Article 56 EPC.

The Respondents emphasised that six years after the advent of the technique for producing monoclonal antibodies it was the obvious and logical step to replace in a known process polyclonal antibodies by monoclonal antibodies if any disadvantages had been observed with the use of polyclonal antibodies. Document E4 was submitted as a further example that the use of monoclonal antibodies for affinity chromatography was obvious. A monoclonal antibody specific for immunoglobulin G was disclosed in particular.

VII. During oral proceedings which took place on 11 January 1990, the Appellants accepted the relevance of document E4, and especially having regard to this document, they submitted two new sets of claims as their main and auxiliary requests respectively. Claim 1 of the main request is now limited having regard to document E4, by cancelling blood serum as one of the natural sources from which the immunoglobulins are to be recovered.

The main claim of the auxiliary request corresponds to Claim 2 of the main request. In this claim, in addition to the deletion of "blood serum" the substance "whey" is deleted. The main claims of both requests read as follows:

Main request

"1. A process for the recovery of immunoglobulins of high purity and potency from milk, characterised in that milk in the form of normal whole milk or skimmed milk or whey derived from normal whole milk, is contacted with an insoluble carrier material to which is bound a low-affinity monoclonal antibody specific to one or more of the immunoglobulins but not specific to any other constituent of milk, the antibody binds immunoglobulin molecules and, following removal of the residue of the milk, immunoglobulin molecules are released from the antibody."

Auxiliary request

"1. A process for the recovery of immunoglobulins of high purity and potency from milk, characterised in that the milk in the form of normal whole milk or skimmed milk is contacted with an insoluble carrier material to which is bound a low-affinity monoclonal antibody specific to one or more of the immunoglobulins but not specific to any other common constituent of milk, the antibody binds immunoglobulin molecules and, following removal of the residue of the milk, immunoglobulin molecules are released from the antibody."

The Appellants stated that the limitations in the newly filed main claims of both requests are allowable with

regard to Article 123(2) and (3) EPC since in the specification as originally filed milk is mentioned as one alternative for the sources from which the immunoglobulins can be recovered and the term "milk" is used to mean whole milk or any derivative of whole milk, such as skimmed milk or whey.

With regard to the inventive step of the new main claims of both requests, the Appellants contended that by further limiting the claims having regard to document E4, it is made clear that the purification of the immunoglobulins by a monoclonal antibody from blood serum is no longer claimed. The isolation of said immunoglobulins from milk is a quite different problem and thus inventive skill has to be acknowledged for the new claim.

The main claim of the auxiliary request is further limited by the deletion of whey as a source material. This limitation was considered necessary in the light of document E1, in which a defatted colostrum was used as source material.

A further argument put forward by the Appellants was that in document E4, a European patent of which one of the inventors and applicants is Professor Milstein, who was one of the persons who developed and published in 1975 the technique for producing hybridomas, the cell fusion product from which monoclonal antibodies are obtained, there is one example, namely Example 6, which described the purification of a monoclonal antibody against mouse immunoglobulin. To do so a complicated and conventional method is used instead of an immunoadsorbent method using a monoclonal antibody. Apparently even the father of the process for the production of monoclonal antibodies did not recognise the advantage of using monoclonal antibodies

in purifying immunoglobulins, which means that it was not at all obvious to do so.

The Respondents replied particularly to this point that the system for purifying a certain substance by a monoclonal antibody initially requires the production of that specific monoclonal antibody which had to be purified conventionally. It was evident that there cannot be already a monoclonal antibody for purifying this first monoclonal antibody.

The Appellants requested that the decision under appeal be set aside and that the patent be maintained on the basis of the newly filed claims.

The Respondents requested that the appeal be dismissed.

VIII. At the end of the oral proceedings the Chairman announced the decision of the Board that the appeal is dismissed.

Reasons for the Decision

1. The appeal complies with Articles 106 to 108 and Rule 64 EPC and is, therefore, admissible.

2. The main claims of the main and auxiliary requests respectively, submitted during the oral proceedings, are amended as described above in paragraph VII. In the description of the patent in column 1, lines 4-14 (cf. also page 1, paragraph 2 of the published patent application) the sources are identified from which immunoglobulins may be recovered. One of the sources is milk and the term "milk" is then defined as whole milk or any derivative of whole milk, such as skimmed milk or whey, in liquid or in solid form as long as such solid

form is soluble or dispersible in water, the whole milk or derivative thereof containing biologically active immunoglobulins. The newly submitted main claims are restricted to one of the alternatives described in the patent. Amendments of this kind do not contravene the requirements of Article 123(2) and (3) EPC. The newly submitted main claims are therefore allowable having regard to the above-mentioned Article.

3. **Novelty** (Article 54 EPC)

The Respondents have not contested the novelty of the newly submitted main claims and it is also the opinion of the Board that none of the documents in the proceedings discloses a process having all the features of said claims, which, therefore, are novel.

4. **Inventive step** (Article 56 EPC)

4.1 Document E1 relates to the recovery of immunoglobulins, namely normal human IgA, IgM and IgG fragments by polyacrylamide bead immunoadsorbents, wherein the human IgA was isolated from human colostrum which had been defatted and from which the casein had been precipitated by acidification. This colostrum was contacted with an insoluble carrier material to which was bound an antibody which had been made monospecific to one of the immunoglobulins but not specific to any other constituent of the colostrum. The respective antibodies were made monospecific by successive passages on several glutaraldehyde immunoadsorbents, then, following this purification step of the antiserum, pure antibodies were obtained using immunoadsorbents. The purified antibodies were bound to polyacrylamide beads, activated with glutaraldehyde, whereby an antibody immunoadsorbent was formed. This antibody-immunoadsorbent, being monospecific

to a certain immunoglobulin was used to purify certain immunoglobulins from a given source, for example human colostrum.

These immunoadsorbents using monospecific polyclonal antibodies have remarkable advantages compared to purification methods not making use of polyclonal monospecific antibodies. For example the linking of pure antibodies to polyacrylamide beads with glutaraldehyde is a simple procedure, the immunoadsorbents can be used for more than a year without any noticeable change in results, the immunoadsorbents are particularly easy to handle and, furthermore, the binding capacity of these immunoadsorbents are as a rule satisfactory; for example for 34 mg of a pure antibody fixed to the polyacrylamide beads, 10 mg of a certain immunoglobulin was eluted. Thus, this purification method allows a simple and rapid purification of large quantities of immunoglobulins which are difficult to isolate by classical methods.

Nonetheless, there remain some disadvantages of the described method, such as contamination of the desired immunoglobulin by traces of other globulins, for example, albumin.

A further inconvenience of using immunoadsorbents as described for the isolation of immunoglobulins could be the use of an acid pH for the elution. Even though no modifications of the eluted immunoglobulins were noted by electrophoresis or immunoelectrophoresis, it is possible that certain physical, chemical or biological properties are changed by this acid treatment.

4.2 From reading document E1, the skilled person would conclude that a problem to be solved is to increase the specificity of the antibody used while simultaneously

providing an antibody with low affinity so that the physical, chemical or biological properties of the immunoglobulin to be purified are maintained.

4.3 In order to solve this problem the main claim of the patent in suit suggests modifying the process for the recovery of immunoglobulins described in document E1 by replacing the monospecific polyclonal antibody by a low affinity monoclonal antibody specific to one or more of the immunoglobulins but not specific to any other constituents of milk.

4.4 From the data disclosed in the patent specification in columns 7 to 10, line 4, it seems plausible that monoclonal antibodies having the claimed properties have been successfully isolated and selected by using cell fusion methods known in the art and disclosed in the specification in a sufficiently detailed manner. Moreover, the assays for biological activity of the recovered immunoglobulins, after elution from the immunoadsorbent column having the monoclonal antibody bound thereto, show that bovine immunoglobulin fully retains its biological activity.

4.5 The process of the patent in suit is the same as that described in document E1 except for the fact that the monospecific polyclonal antibodies of the prior art process are replaced by monoclonal antibodies. With respect to the presence of or absence of an inventive step for the subject-matter of Claim 1 in accordance with the main request, the key question to be considered is whether this replacement was obvious to the skilled person in the light of the above-defined problem.

4.6 One can assume that the scientists who developed the process for purifying immunoglobulins described in

document E1 were facing the same problem as defined above. The best possible solution available at the time when the authors of document E1 were working on this problem was to use monospecific polyclonal antibodies. This kind of antibody is produced as described in detail in document E1 by raising antisera in certain mammals, such as sheep or rabbits, by injecting intradermally the antigen against which antibodies are desired. The injections are repeated monthly and after six months antisera were obtained which were not monospecific. Since, however, the authors of document E1 even at that time recognised that specificity of the antibody was very important, they made the antibodies contained in the antisera as specific as possible, namely monospecific as already described above.

4.7 With the advent of monoclonal antibodies in 1975 by the famous milestone work of Köhler and Milstein, an opportunity was provided to overcome the disadvantages of monospecific polyclonal antibodies. The difference between monospecific polyclonal antibodies and monoclonal antibodies is that monoclonal antibodies are derived from only one single cell fusion product, called a hybridoma, which in turn is formed from one single B-cell clone, whereas polyclonal antibodies are produced by a multiplicity of B-cell clones, and thus may represent a multiplicity of individual antibodies, which only after their recovery from the animal's blood are made monospecific by the above described purification procedures. This difference between the processes to produce both types of antibodies ensures that monoclonal antibodies are **per definitionem** monospecific whereas polyclonal antibodies are not.

4.8 It was, therefore, proposed, for example in document E2, to use monoclonal antibodies instead of polyclonal ones for the routine quantitation of human immunoglobulin G

(IgG) since it was considered that monoclonal antibodies could be used to select and standardise reagents for specificity and affinity. A correlation coefficient of 0.979 was obtained for parallel determinations using monoclonal antibodies and polyclonal antisera (cf. page 355, Summary and first paragraph).

4.9 Document E3 discloses the use of monoclonal antibodies for large-scale purification of human leukocyte interferon. Like the immunoglobulins to be purified according to the patent in suit interferon is a protein which is readily denatured and therefore the problem in purifying interferon is comparable with the problem of purifying immunoglobulins. Purification of interferon by using monoclonal antibodies was very successful according to document E3, although the interferon to be purified was present only at less than 1% in a given source (cf. Summary on page 446).

4.10 In document E4, a new technique is described to prepare hybridomas wherein a rat myeloma cell line was used as fusion partner instead of mouse cell lines commonly used in the state of the art. Monoclonal antibodies produced by these cell fusion products are said to have various applications in therapeutics and particularly in diagnostics, and also in such procedures as affinity chromatography. Another type of use is exemplified by the use of antibody against a naturally occurring substance such as a protein for the purification of that substance (cf. the paragraph bridging pages 9 and 10). Thus document E4 clearly recognises and discloses the broad advantages of using monoclonal antibodies in this field.

4.11 Example 6 of document E4 describes the preparation of a suspension containing a specified amount of antibody per ml by producing cell fusion products by the method

disclosed in Example 2 and allowing the cells to grow until they reach the stationary phase. The antibodies were purified by a conventional and complicated method comprising adding ammonium sulphate to the suspension to produce 50% saturation and collecting the resulting precipitate. The precipitate is dissolved in a minimum volume of phosphate buffered saline and the solution is dialysed against the same medium to produce a purified antibody preparation. According to two further variants of this procedure the antibodies are purified either by continuing the above-cited procedure using DEAE chromatography or immunoadsorbents, for example anti-rat immunoglobulin, or alternatively the procedure described is replaced by the use of membrane filters.

4.12 As set out in paragraph VII above, the Appellants argued that even Professor Milstein was not aware of the possibility of purifying immunoglobulins (antibodies in the case of Example 6) by a similar process to the one claimed in the patent in suit. In fact he used conventional methods. Thus this method could not have been obvious.

4.13 In the Board's opinion this argument is not convincing because in a case where a specific antibody has been produced by a hybridoma for the first time there are no monoclonal antibodies available to purify these initially produced monoclonal antibodies. Therefore, when a certain monoclonal antibody has been produced for the first time, the use of a conventional method to purify this monoclonal antibody is necessary. It has further to be mentioned that in the second variant of the purification method described above the possibility of an immunoadsorbent method was contemplated, although this immunoadsorbent naturally cannot contain a monoclonal antibody which does not yet exist. Rather an anti-rat immunoglobulin was fixed to the

immunoadsorbent material which at least gives a certain degree of purification although not to the optimum degree which can be achieved using a monoclonal antibody. This alternative variant, however, gives a hint that the inventors of document E4 thought about the possibility of an immunoadsorbent and one can assume that Professor Milstein would have used a monoclonal antibody as the immunoadsorbent if this monoclonal antibody had been available.

4.14 The disclosures of documents E2, E3 and E4 summarised above in the Board's view clearly reflect a common general knowledge of skilled men in the art in 1980 as to the use of monoclonal antibodies in place of monospecific polyclonal antibodies for immunopurification. Since these documents do not contain any information about any disadvantages in using the monoclonal antibodies, in the Board's judgment at the priority date of the patent it did not require an inventive step for a skilled man to replace monospecific polyclonal antibodies by monoclonal antibodies. The argument put forward by the Appellants that the overall yield would suffer because the monoclonal antibody would be too specific to adsorb a useful proportion of the total immunoglobulin content of the source material seems to be irrelevant, because it is exactly the aim when tailoring antibodies for purification purposes to increase the specificity of the antibodies. This becomes clear from the disclosure of document E1 in which polyclonal antibodies were made monospecific. The next logical step of this development is the use of monoclonal antibodies. The higher the specificity of an antibody, the more quantitative is the binding to an immunoglobulin and thereby the purification result is improved. It is not the case that the specificity of an antibody may cause disadvantages in the sense stated by the Appellants but rather its affinity. This fact was

recognised by the Appellants who, therefore, used an antibody of low affinity in their purification processes.

4.15 It follows from the above that the replacement of monospecific polyclonal antibodies in an immunopurification process by monoclonal antibodies is the desired, logical and obvious step in solving the problem to improve a purification process as described in the closest prior art, namely document E1. Claim 1 of the main request does not, therefore, comply with the requirements of Article 56 EPC.

4.16 The main claim of the auxiliary request differs from Claim 1 of the main request insofar as whey as a source material has been excluded from the protection sought. In the Board's opinion this difference is not decisive and therefore the same reasons apply for the evaluation of obviousness of this claim as for Claim 1 of the main request. It is agreed that by deleting whey as a source material the claim is limited with respect to document E1 insofar as defatted and decaseinated colostrum may have the characteristics of whey. However, the remaining source material "normal whole milk or skimmed milk" does not differ from the colostrum mentioned in document in E1 in such a way, as to make an inventive step more plausible. The argument submitted by the Appellants during the oral proceedings that normal milk and skimmed milk as a source for the purification of the desired immunoglobulins is more difficult to handle because the concentration of the desired immunoglobulins is lower in this source than in colostrum is not convincing because, for example, in document E3 a protein, namely interferon, was purified effectively from a source containing less than 1% of it by an immunoadsorbent containing monoclonal antibodies.

Thus, the subject-matter of the main claim of the auxiliary request also lacks an inventive step as required by Article 56 EPC.

5. Since the main claims of both requests are not allowable, both requests must be rejected.

Order

For these reasons, it is decided that:

The appeal is dismissed.

The Registrar: The Chairman:

S. Fabiani R.W. Andrews

00714