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Datasheet for the decision of 22 April 2008

Case Number: T 1720/06 - 3.3.08

Application Number: 97920236.3

Publication Number: 0937148

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Language of the proceedings: EN

Title of invention:
Histidine-tagged intimin and methods of using intimin to stimulate an immune response and as an antigen carrier with targeting capability

Applicant:
HENRY M. JACKSON FOUNDATION FOR THE ADVANCEMENT OF MILITARY MEDICINE

Headword:
Intimin adherence/HENRY JACKSON

Relevant legal provisions:
EPC Art. 56

Relevant legal provisions (EPC 1973):
-

Keyword:
"Main request, first, second and third auxiliary requests - inventive step (no)"

Decisions cited:
T 0351/98

Catchword:
-

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Case Number: T 1720/06 - 3.3.08

D E C I S I O N
of the Technical Board of Appeal 3.3.08
of 22 April 2008

Appellant: HENRY M. JACKSON FOUNDATION FOR THE ADVANCEMENT OF MILITARY MEDICINE
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Decision under appeal: **Decision of the Examining Division of the European Patent Office posted 13 June 2006 refusing European application No. 97920236.3 pursuant to Article 97(1) EPC 1973.**

Composition of the Board:

Chairman: L. Galligani
Members: P. Julià
C. Heath

Summary of Facts and Submissions

I. European patent application No. 97 920 236, published as WO 97/40161 with the title "Histidine-tagged intimin and methods of using intimin to stimulate an immune response and as an antigen carrier with targeting capability", was refused by the examining division pursuant to Article 97(1) EPC 1973 because the claimed subject-matter was considered not to fulfil the requirements of Article 56 EPC.

II. The applicant (appellant) filed an appeal against the decision of the examining division, paid the appeal fee and submitted a statement setting out the grounds of appeal.

III. The examining division did not rectify the contested decision and referred the appeal to the board of appeal (Article 109 EPC 1973).

IV. The board sent a communication as annex to the summons to oral proceedings stating its preliminary, non-binding opinion.

V. With letter dated 25 March 2008, the appellant replied to the board's communication and filed a new main request and auxiliary requests 1 to 3. A clean copy of the main request was filed on 16 April 2008.

VI. Oral proceedings took place on 22 April 2008.

VII. Claim 1 of the appellant's **main request** read as follows:

"1. Use of an intimin protein encoded by an *eae* gene, or a portion thereof which is capable of binding to epithelial cell lines and inducing anti-intimin antibodies that block the binding between the intimin protein and epithelial cell lines, for the preparation of a composition for promoting a protective immune response in a human or an animal against bacteria expressing an intimin protein."

Independent claims 2 and 3 were directed to further uses of an intimin protein or a portion thereof defined as in claim 1, in particular for the preparation of a composition for promoting a protective immune response against at least one antigen chemically, physically or recombinantly conjugated to said intimin or portion thereof (claim 2), or for the preparation of a composition for targeting the delivery to epithelial cells in a human or an animal of at least one antigen, at least one drug, or a combination thereof, conjugated to said intimin or portion thereof (claim 3).

Claims 4 to 6 related to particular embodiments of claims 1 to 3, wherein the intimin protein or portion thereof was further qualified as being enriched, purified or histidine-tagged. Claims 7 to 12 were directed to the use of anti-intimin antibodies that blocked the binding between an intimin protein and epithelial cells for the preparation of a composition for providing immune protection to a human or an animal against bacteria expressing an intimin protein. Claims 13 to 18 related to a method of preparing anti-intimin antibodies.

Independent claims 19 to 21 were directed to pharmaceutical compositions comprising an intimin protein or a portion thereof defined as in claim 1 (claim 19), at least one antigen, at least one drug, or a combination thereof conjugated to an intimin protein or a portion thereof as defined in claim 3 (claim 20), or anti-intimin antibodies as defined in claim 7 (claim 21). Independent claim 22 was directed to an intimin protein or a portion thereof defined as in claim 1 for promoting a protective immune response in a human or an animal against bacteria expressing an intimin protein. Independent claim 23 was directed to anti-intimin antibodies defined as in claim 7.

VIII. The **first auxiliary request** read as the main request except for a disclaimer ("*with the proviso that the method is not a therapeutic method*") introduced into the method of claim 13 for preparing the anti-intimin antibodies. The **second and third auxiliary requests** read as the first auxiliary request except for the fact that the intimin protein of claims 1, 19 and 22 was further characterized as being "isolated" (second auxiliary request) or "enriched or purified" (third auxiliary request).

IX. The following documents are cited in the present decision:

D1: CA-A-2 078 716 (publication date: 22 March 1994);

D2: WO-A-96/00233 (publication date: 4 January 1996);

D3: M.L. McKee, "Adherence of Enterohemorrhagic *Escherichia coli* to human epithelial cells: the role of intimin", Dissertation submitted to the Faculty of the Department of Microbiology and Immunology Graduate Program of the Uniformed Services University of the Health Sciences F. Edward Hèbert School of Medicine, Bethesda, MD (USA), 1995;

D22: P. Sherman et al., Infection and Immunity, 1991, Vol. 59, No. 3, pages 890 to 899.

X. The appellant's arguments insofar as relevant to the present decision may be summarized as follows:

Main request and first auxiliary request
Articles 54 and 56 EPC

Document D1 disclosed an avirulent bacterial carrier strain transformed with an intimin expression vector for use as a vaccine. However, there was no disclosure of making use of the intimin protein for preparing a vaccine. The document did not disclose which of the many components of the transformed bacterial carrier was responsible for inducing a protective immune response and there was no disclosure as to whether the intimin protein alone, i.e. in the absence of any component of the avirulent bacterial carrier, was able to elicit a protective immune response. Document D1 only contemplated the use of the intimin protein for the preparation of anti-intimin antibodies which were further used in the detection and diagnosis of enterohemorrhagic *Escherichia coli* (EHEC) infection.

Document D3 disclosed the production of antisera against histidine-tagged intimin protein. However, there was no disclosure of the use of an intimin protein for promoting a protective immune response against bacterial infection nor of the use of anti-intimin antibodies for providing passive immune protection. Although document D3 taught that intimin was a likely candidate as a component in an ideal EHEC vaccine, this ideal EHEC vaccine included inactivated *E. coli* heat-stable Shiga-like toxin (SLT) or an immunogen eliciting a protective response against SLT. Hence, contrary to the teachings of the application, which contemplated the use of intimin alone, document D3 taught that intimin alone was not sufficient to elicit a protective response against EHEC. Moreover, the suggestion of a potential or a possibility worth investigating did not constitute an actual disclosure of a medical use.

Multiple molecules were known at the priority date of the application to be involved in bacterial adherence. There was confusion in the art with regard to the role of intimin in bacterial EHEC adherence since there were several documents disclosing contradictory data and conclusions. In particular, the prior art referred *inter alia* to a 94 kDa outer membrane protein (document D22) and to an *E. coli* EHEC adhesin different from intimin. This adhesin was disclosed in document D2 (published shortly before the first priority date of the present application) which, based on the results reported in the prior art, also stated that a molecule other than intimin was the primary adhesin of EHEC *E. coli* for epithelial cells. Thus, no consensus was

present in the art as to which experimental results were flawed and which were not.

In fact, even the results reported in document D3 contributed to this confusion and casted doubts on the role of intimin in bacterial EHEC adherence. In particular, the in-frame deletion mutant disclosed in this document showed the same log jam adherence pattern as the EHEC and EPEC *E. coli* strains and both the parent and the mutant strains competed equally well for attachment sites in the intestine of a known mouse intestinal colonization model.

It was thus not obvious to a skilled person, unbiased towards any of these prior art documents, which results to believe in and which ones to disregard. The choice of intimin out of all other possible vaccination targets was therefore not obvious. There was no indication in the prior art that blocking intimin would be as effective or even more effective in preventing bacterial EHEC infection than blocking any other of these potential target molecules. The selection of a prior art document related to intimin as closest prior art involved hindsight. There were post-published documents on file showing that the choice of intimin was not a random one, since the administration of an intimin protein indeed induced a protective immune response in an animal.

The present case was similar to the situation leading to the decision T 351/98 of 15 January 2002, where the board considered that there was no clear identification of the agent(s) responsible for AIDS because the prior art proposed that AIDS could be caused by a fungal

infection, a mutant hepatitis B virus (HBV), a prion-like agent or a retrovirus. Even though the fungal infection and the HBV mutant were identified and published earlier (and in less corroborated journals) than the publications identifying the retrovirus, these earlier publications were considered nevertheless to contribute to the confusion in the art and the later publication on retrovirus not to be sufficient to dispel this confusion.

Second and third auxiliary requests
Articles 54 and 56 EPC

Document D1 did not teach or suggest the enrichment or purification of the intimin protein from the avirulent bacterial carrier strain (transformed with an intimin-expression vector) prior to the preparation of the vaccine. Even if an isolated or enriched intimin protein preparation could contain contaminants that were also components of an avirulent carrier bacterium as that disclosed in document D1, these contaminants were to be, if at all, in a different abundance than in the avirulent carrier bacterium.

XI. The appellant (applicant) requested that the decision under appeal be set aside and that a patent be granted on the basis of the main request filed on 16 April 2008 or auxiliary requests 1 to 3 filed on 25 March 2008.

Reasons for the Decision

General procedural considerations

1. In view of the reasons given under Article 56 EPC by the examining division in the contested decision and the fact that all requests on file are clearly affected by a negative decision on this article, it is considered to be expedient to deal directly with the substance and patentability of these requests, in particular with Article 56 EPC, rather than to enter into an extensive analysis of their compliance with the formal requirements of *inter alia* Articles 123(2) and 84 EPC.

Main request and first auxiliary request
Article 56 EPC

The disclosure of the application

2. The application discloses the use of an intimin protein or a portion thereof as defined in claim 1 for promoting a protective immune response in a human or an animal against bacteria expressing an intimin protein (cf. Section VII *supra* and *inter alia* page 7, lines 12 to 16 and claims 15 to 18 of the published application). This disclosure is supported by the experimental evidence provided in the examples of the application.

3. Example I reports the cloning of the *eae* (*E. coli* attach and efface) gene from the EHEC strain 86-24 (serotype 0157:H7) using PCR primers derived from a composite of two known EHEC *eae* sequences (strains CL8 and 933). The example discloses the construction of

plasmids encoding His-tagged intimin, such as plasmid pEB313 which encodes a His-tagged *eae* fragment of 101 kDa (RIHisEae) having 900 out of the 935 predicted amino acids (cf. page 19 to page 31 and Figures 1 and 2). Examples II and III disclose, respectively, the production of large scale His-tagged intimin using the plasmids of Example I (cf. page 31 to page 33) and the purification of enriched His-tagged intimin defined as "*consisting solely of intimin or portions or intimin, optionally tagged with histidine*" (cf. pages 34 and 35).

4. Example IV discloses *in vitro E. coli* adherence assays to HEp-2 and HCT-8 cells (cf. pages 35 to 36) and the construction of an *eae* in-frame deletion mutant of the EHEC strain 86-24 designated 86-24*eae*Δ10 (cf. pages 36 to 38). Whereas the wild-type 86-24 strain interacts *in vitro* with HEp-2 and HCT-8 cells, the 86-24*eae*Δ10 mutant strain is unable to adhere to HEp-2 cells but the adherence is fully restored when transformed with plasmids containing the *eae* gene. When added exogenously to HEp-2 cells, RIHisEae is also able to complement the HEp-2 cell binding defect of the mutant strain. Similarly, plasmids encoding the intimin fusion protein also complement the 86-24*eae*Δ10 mutant strain for attachment *in vitro*. According to the application, these results demonstrate that intimin alone complements the *eae* mutation (cf. pages 39 and 40).

5. Example V discloses the *in vivo* role of intimin in intestinal colonization, attaching and effacing (A/E) lesion formation, and EHEC-mediated colitis and diarrhea using a gnotobiotic piglet infection model. Whereas there is no evidence that the 86-24*eae*Δ10 mutant strain causes A/E lesions and colonizes the

intestine of inoculated piglets, piglets inoculated with the wild-type 86-24 parent strain develop diarrhea and have intimate bacterial adherence and A/E lesions. Inoculation of the mutant strain together with plasmids complementing the in-frame deletion results in adherence to mucosal enterocytes and A/E lesions. Similar experiments in a colostrum-deprived newborn calf model show that intimin is necessary to provoke A/E lesions as well as to evoke *E. coli* O157:H7 strain 86-24 mediated diarrhea (cf. page 41 to 43). Example VI reports the recognition of His-tagged intimin by convalescent immune sera tested from hemorrhagic colitis patients (cf. page 43).

6. Example VII refers to the administration of His-tagged intimin for promoting a protective immune response. Methods of administration are discussed and reference is made to the injection of His-tagged intimin into cow udders and to nucleic acid vaccines (cf. pages 44 to 47). Example VIII discloses the preparation of multiple vaccines by conjugation of antigens from various pathogens to His-tagged intimin (cf. pages 47 to 53). Example IX describes known techniques for generating antibodies against intimin and methods for testing the adherence-blocking ability of anti-intimin antibodies (cf. page 53 to 78). None of these examples report any experimental data on the actual immune response obtained or on the adherence-blocking ability of the anti-intimin antibodies.

Selection of the closest prior art

7. In line with the case law, which defines the closest prior art as a document disclosing "*subject-matter*

conceived for the same purpose or aiming at the same objective as the claimed invention and having the most relevant technical features in common" (cf. "Case Law of the Boards of Appeal of the EPO", 5th edition 2006, I.D.3.1, page 121), the board considers the closest prior art to be represented by document D3.

8. Document D3 establishes an *in vitro* model of EHEC adherence using HEp-2 and HCT-8 cells (cf. pages 48 to 88) and describes the isolation and cloning of the *eae* gene from the EHEC strain 86-24 (cf. pages 88 to 102). Reference is also made to the *eae* gene from the EHEC *E. coli* serotype O157:H7 strain CL8 (cf. page 97, second paragraph), which is the same EHEC strain used in document D1. Document D3 discloses the construction of the in-frame deletion mutant 86-24*eae*Δ10 (cf. pages 102 to 110) and, using this mutant (with or without complementing plasmids) and the wild-type EHEC strain, reports studies on the *in vitro* and *in vivo* role of intimin in EHEC adherence. For the *in vivo* studies a gnotobiotic piglet infection model and a mouse colonization model are used (cf. pages 110 to 122). The document further reports the construction of His-tagged intimin (RIHisEae) and the production of antibodies thereto (cf. pages 122 to 135). The recognition of intimin by the immune sera from hemorrhagic colitis patients is also disclosed (cf. page 135 to 140). It shows also that the anti-intimin antibodies block the adherence of EHEC strains to HEp-2 cells (cf. paragraph bridging pages 130 to 135). Except for the references in the present application to the studies performed in newborn calf, the disclosure of document D3 is identical to the experimental evidence (Examples I to VI) of the application (cf. points 3 to 5 *supra*).

9. Based on these results and on further evidence reported in the prior art, such as a study showing that an eae mutated EPEC strain caused diarrhea in 4 of 11 volunteers as compared to 11 of 11 individuals who received the wild-type strain (cf. page 23, first full paragraph and page 164, lines 7 to 12), document D3 concludes that "*intimin is a likely candidate as a component in an ideal EHEC* ***vaccine***" (in bold by the board) (cf. page 164, line 1). Thus, this document is in the board's judgement the best springboard for the evaluation of inventive step.

10. The appellant argues that there was confusion in the art with regard to the role of intimin in bacterial EHEC adherence and that the selection of document D3 as the closest prior art requires hindsight knowledge of the application. In the appellant's view, the present situation is similar to this leading to the decision T 351/98 (*supra*), where hindsight was used in the opponents' choice of the closest prior art because of the confused nature of the state of the art (cf. Section X *supra*).

11. This line of argument is not found convincing by the board. It is true that the prior art refers to the complexity of bacterial EHEC adherence. Document D3 itself states that "*three EHEC factors have been implicated in intimate adherence to epithelial cells and the capacity to cause A/E lesion in vivo*" and refers to the "*genetic complexity of EHEC adherence*" (cf. page 153, last paragraph to page 155, first paragraph). However, document D3 clearly identifies and derives the "***pivotal*** *role*" of intimin in intimate

adherence of EHEC to the intestinal epithelium from the very same experimental evidence as this of the present application (cf. page 151, last paragraph). Even more important is the fact that sound reasons are also given in document D3 for explaining the discrepant results of the earlier prior art referred to by the appellant, namely the presence in the earlier described *eae* insertion-deletion mutants of polar effects on genes downstream of the *eae* gene which encode (hypothetical) additional factors. These factors may not be required *in vivo* nor directly involved in EHEC adherence *in vitro* but they may be nevertheless required for localization or presentation of intimin by the bacterium *in vitro* (cf. page 151, last paragraph to page 153, second full paragraph).

12. It is important to note that this relevant prior art was not available to the authors of document D2, which is relied upon by the appellant to show that there was confusion in the art as regards adhesins. Document D2 has a priority date of 24 June 1994 and only refers to prior art published earlier than document D3 (cf. page 3, line 23 to page 4, line 1 of document D2). Hence, the conclusions of document D2 are based on earlier and, in a way superseded, prior art already addressed by document D3. Contrary to appellant's argument, document D2 cannot therefore lead the skilled person to disregard intimin in EHEC adherence or to add further confusion in the art with regard to its role in EHEC adherence.

13. The board considers that the present situation is different from that of decision T 351/98 (*supra*). In this decision it was considered that the identification

by three different groups at the forefront of the AIDS field of three independent retroviruses made the skilled person to face a more confusing picture on the source and origin of the disease since these three retroviruses were not known to be variants of the very same virus at the priority date of the contested patent (cf. points 70 and 71 of the Reasons). In the present case, even though the complexity of bacterial EHEC adherence is acknowledged in the prior art, the pivotal role of intimin is clearly established. Therefore, prior art concerned with intimin, in particular document D3, represents a valid and appropriate starting point for the assessment of the inventive step.

Differences between the application and the closest prior art

14. According to the appellant, document D3 discloses the use of intimin as ***a*** component in an EHEC vaccine whereas the application discloses the use of intimin as ***the*** component in an EHEC vaccine (cf. Section X *supra*).

15. Even leaving aside the board's doubts as to whether or not the application actually excludes further components in an intimin EHEC vaccine (see claim 2 which contemplates the use of intimin with at least one additional antigen for which a protective immmune response is expected to be obtained, cf. Section VII *supra*), the board considers that the presence of other components in the intimin EHEC vaccine is disclosed in document D3 only as a possible option, even though a preferred one. The presence of inactivated Shiga-like toxin (SLT) in an ideal EHEC vaccine might only confer additional protection, since the immune response against intimin is expected to block the initial step

in infection (the intimate association of EHEC with the intestinal mucosa) required for an efficient EHEC toxin delivery to the gut mucosa and ultimately to the blood system (cf. page 164, lines 2 and 3 and page 164, line 20 to page 165, line 7). The presence of "*other* ***putative*** *adhesins ... as additional vaccine candidates*" (bold by the board) or in "*a cocktail of multiple EHEC components*" might only be contemplated once the role of these putative adhesins is "*firmly established*" (cf. page 165, lines 7 to 10). This is certainly not done in document D3. Hence, although other EHEC vaccines with additional components are mentioned in document D3, intimin is nevertheless clearly identified as a pivotal, essential component of all these EHEC vaccines in the same sense as understood by the appellant, i.e. **the** component in the EHEC vaccine.

16. It is also worth mention that there are no experimental data whatsoever in document D3 or in the application to back up the protective immune response promoted by using intimin as a vaccine. This protective response is only derived from similar circumstantial evidence in both documents (cf. points 3 to 5 and 8 *supra*). In the absence of appropriate experimental comparative data, no conclusions can be drawn from the proposed presence of other components in an intimin vaccine. Neither the improvement provided thereby nor the sufficiency of intimin alone as alleged, respectively, by document D3 or the appellant, can be taken into account for the formulation of the technical problem to be solved.

Technical problem to be solved

17. In the light of the above considerations, the technical problem to be solved is considered to be the provision of means for the generation of a protective immune response against EHEC. The proposed solution, namely the use of intimin as a vaccine, is essentially based on the suggestion put forward in document D3. The supporting experimental evidence is also essentially that already described in document D3. Post-published documents on file show that immunization by intimin indeed promotes this protective immune response. The relevant question in relation to inventive step is whether the skilled person faced with said problem and starting from document D3 would have considered the suggestion made therein as a plausible solution which could be put into practice with a reasonable expectation of success.

Obvious and reasonable expectation of success

18. As stated in point 15 above, the selection of intimin as a component in an EHEC vaccine is obvious from the evidence shown in document D3. This document refers to three reasons for such a selection, namely i) the fact that sera from HC patients recognize EHEC intimin and that *in vivo* studies with volunteers show different effects for both the parent EPEC (diarrhea) and the mutated (non-diarrhea) strains, ii) the finding that in the absence of the *eae* gene no A/E lesions are found in gnotobiotic piglets and that iii) an immune response against intimin would advantageously block the initial step in infection required for toxin delivery by EHEC to gut mucosa (cf. pages 164 and 165). These reasons

further provide the skilled reader with a reasonable expectation of success as well.

19. In fact, the claimed use of intimin is based on the same technical evidence as this of document D3 (cf. point 8 *supra*). In line with the case law, the same standard must apply to both the application and the prior art, i.e. if the evidence of the former is enough for supporting the claimed use, so must it also be for the latter to convey the skilled person a reasonable expectation of success. The more so since the actual expectations are rather modest, namely "*immunization ... also means decreasing the ability of the pathogens to colonize the gastrointestinal tract and decreasing the severity of an infection*", wherein "*the precise degree of protection is unimportant to quantitate in practicing the invention*" (cf. page 16, last two paragraphs of the published application).

20. In arguing against a reasonable expectation of success, the appellant further put forward that similar log jam adherence patterns for the in-frame deletion mutant and the parental EHEC strains and their similar attachment to intestine of a mouse colonization model casted doubts and uncertainty on the role of intimin in bacterial EHEC adherence (cf. Section X *supra*).

21. Log jam adherence is described in document D3 as an additional pattern of adherence of EHEC to HCT-8 cells, wherein the bacteria are attached to and lined up at the junctions between these cells. Contrary to the well-described fluorescence actin staining (FAS) and localized adherence (LA) phenotype to HEp-2 cells by EHEC strains, the log jam phenotype attachment is

LA/FAS negative and limited to HCT-8 cells. This log jam pattern is observed among intestinally-derived pathogenic and nonpathogenic *E. coli* strains. Although all strains remain log jam positive, only *E. coli* carrying the *eae* locus are FAS positive, whereas the in-frame deletion mutant is LAS/FAS negative (cf. page 78 to page 84). Document D3 concludes that "*this phenotype does not appear to be associated with virulence*" and it "*may represent a basal adherence mechanism that allows a variety of E. coli to bind to and colonize the human intestine whether or not the organism expresses additional specific adhesive factors*" (cf. paragraph bridging pages 150 and 151). This information does not cast any doubts on the role of the *eae* locus in EHEC virulence and it certainly does not raise any confusion on the proposal to use the product of the *eae* locus (intimin) in a vaccine against EHEC virulence.

22. Similarly, although both parent and mutant strains competed equally well for attachment sites in the intestine of streptomycin-treated mice (cf. paragraph bridging pages 119 and 122), this result is not considered to be surprising in view of the fact that even the laboratory strain *E. coli* K12 is capable of colonizing the intestines of these mice (cf. page 156, full paragraph). This result appears to cast more doubts on the animal model used than on the actual role of the *eae* locus in bacterial EHEC adherence.

Conclusion

23. From all the above, it is concluded that claim 1 of the main request and of the first auxiliary request does not fulfil the requirements of Article 56 EPC.

Second and third auxiliary requests
Article 56 EPC

24. These requests define the intimin protein or portion thereof as being "isolated" (second auxiliary requests) or "enriched or purified" (third auxiliary request) (cf. Section VIII *supra*). The isolation and purification of intimin is described in the application under the heading "Isolating and Purifying His-tagged Intimin" (cf. pages 17 to 19) and further in Examples II and III (cf. pages 31 to 35). The use of His-tagged intimin for promoting a protective immune response is contemplated in the application as a preferred embodiment and Example VII describes the administration of RIHisEae (cf. page 12, lines 5 to 6, 12 to 13 and 19 to 20 and pages 44 and 45 of the published application).

25. In the Section "Material and Methods" under the heading "Expression and purification of fusion proteins" document D3 describes the purification to homogeneity of a His-tagged intimin (RIHisEae) using an immobilized metal chelate affinity chromatography (Ni-NTA, Nickel nitrilo-tri-acetic acid resin), which is a widely used technique in the art because of its efficiency and ease of use (cf. page 44, first paragraph and page 130, lines 6 to 7). In the next paragraph under the heading "Immunization of mice and rabbits", document D3 discloses the use of RIHisEae for injection in BALB/cJ

mice and New Zealand white rabbits (cf. page 44, second paragraph and page 130, full paragraph). The resulting antibodies specifically recognize the EHEC intimin and are capable of blocking the adherence of the parental EHEC strain to HEp-2 cells (cf. paragraph bridging pages 130 to 135 and page 135 first full paragraph).

26. The additional features introduced into the second and third auxiliary requests are thus contemplated in document D3. Therefore, they do not represent an inventive contribution over this prior art and consequently, none of these requests fulfils the requirements of Article 56 EPC.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar: The Chairman:

A. Wolinski L. Galligani