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(57) Abstract

Vaccine preparations are provided comprising a major outer membrane protein from Chlamydia and a mucosal adjuvant such as chlorea Toxin or Heat labile enterotoxin. Such preparations provide protection from Chlamydia induced fertility.

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CHLAMYDIA VACCINES

The present invention relates to a vaccine formulation for the prevention of Chlamydia infections. In particular, to a formulation containing a recombinant or purified major outer membrane protein (MOMP) from Chlamydia trachomatis combined with a mutated heat-labile enterotoxin (mLT) from *E. coli* or cholera toxin (CT).

The gram-negative bacterium *Chlamydia trachomatis* is a common human pathogen; transmitted from human to human, it causes ocular and genital infections which can result in long term sequelae. Genital Chlamydial infections which are targeted by these vaccine preparations, are the most common bacterial sexually transmitted diseases (STDs) in the US. The infection exerts its most detrimental consequences in women, the cervix being the most commonly infected site although severe complications like endometritis, pelvic inflammatory diseases (PID) and salpingitis can result from ascending infections leading to infertility and ectopic pregnancy. It has been shown that, whereas a single episode of PID can result in an infertility rate of 6.1%, three or more episodes have led to an infertility rate of 54% (20).

The *C. trachomatis* species is serotyped into 15 serovars and STDs are caused by serovars D to K which cover 3 different serogroups (19), therefore a vaccine against Chlamydial STD should protect against multiple serovars that are more or less antigenically related. Vaccine trials performed in man and non-human primates using the whole organism as immunogen gave serovar-specific protection but some of the vaccinees developed more severe reactions upon reinfection (6). Several studies have demonstrated that the pathology associated with a Chlamydial infection is immunologically mediated (7); moreover a purified Chlamydia 57 kDa protein (Hsp60) was shown to elicit a pathology similar to that observed in animals previously infected (13, 14). These observations lead to the conclusion that protection against Chlamydia infections could be achieved by using a subunit vaccine.

For the design of a subunit vaccine, much interest has been focused on the 40 kDa major outer membrane protein (MOMP). This protein which was shown to function *in vitro* as a porin (4), is present during the whole life cycle of the bacteria (8) and is highly immunogenic in humans and animals. The MOMP displays 4 variable domains (VD) surrounded by five constant regions that are highly conserved among serovars (15, 22). *In vitro* and *in vivo* neutralizing B-cell epitopes have been mapped on these VDs (2, 23) whereas T-cell epitopes have been identified in both variable and constant domains (1, 16). The protein is produced with a signal sequence which is cleaved to produce the mature protein.

Immunisations with recombinant or purified MOMP followed by homotypic or heterotypic Chlamydia challenges have been performed in different animal models with variable effects on the parameters of the infection (3, 17, 18). In a heterotypic challenge experiment, Tuffrey *et al.* have shown that parenteral and mucosal immunisation with rMOMP, adsorbed on alhydrogel, did reduce the severity of the salpingitis and the duration of the lower genital tract colonization respectively. However the preparation conferred no protection against infertility resulting from the infection. In a recent study, using the same mouse model, we have shown that immunisation with a vaccine comprising 3D-MPL and QS21 or DQ and MOMP from serovar L2 is effective in conferring protection against infertility resulting from heterologous Chlamydial infection (12).

In this particular case, the presence of elevated MOMP-specific IgG2a ratios in the serum of immunised mice as well as the secretion of IFN-gamma upon *in vitro* restimulation of immune spleen cells has confirmed that protection is associated with an antigen-specific Th1-like immune response. Similarly, others have shown that adoptive transfer of a MoPn-specific Th1 clone enables infection to be resolved in nude mice, genitally infected with MoPn. The activation of a predominantly Th1-like subset is consistent also with the protective immune response to other intracellular pathogens such as Leishmania (9) and Mycobacterium (21).

The present invention provides a vaccine composition which is effective at the mucosal level in conferring protection against infertility resulting from Chlamydia infections. Advantageously, the vaccine is effective in the mucosa where Chlamydia infections are primarily associated. The vaccine may be administered by any known route, but is advantageously useful as an oral or intranasal vaccine

Accordingly the present invention provides a vaccine formulation comprising a recombinant or purified major outer protein (rMOMP) and a mucosal adjuvant. In particular, the vaccine contains MOMP from the serovar L2, F, D or E, but may additionally contain antigens from other serovars. Combination vaccines comprising MOMP from two or more serovars may be utilised. Preferred combination comprise MOMP from D and E serovars.

In preferred compositions of the invention, the mucosal adjuvant is a mutated LT (for example LT R192G) from *E. coli* or the cholera toxin (CT). Mutated LT R192G can be obtained from following the teaching of IPA PCT/US95/09005 published under No. 96/06627]. Cholera Toxin is available commercially from Swiss Serum, Bern.

The amount of protein in each vaccine is selected as an amount which induces an immunoprotective response without significant, adverse side effects in typical vaccinees. Generally it is expected that each dose will comprise 1-1000 μg of protein, preferably 2-100 μg, typically between 4-40 μg. An optimal amount for a particular vaccine can be ascertained by standard studies involving observation of appropriate immune responses in subjects.

Following an initial vaccination, subjects may receive one or several booster immunisations adequately spaced.

The formulations of the present invention may be used for both prophylactic and therapeutic purposes.

Accordingly in one aspect, the invention provides a method of treatment comprising administering an effective amount of a vaccine of the present invention to a patient.

In another aspect of the invention the vaccine may be administered intra nasally.

MATERIAL AND METHODS

Purified rMOMP production and formulation

The obtention and the use of the DNA construct pET15-MOMP for antigen production are described in the U.K. patent GB 9506863.1 published as PCT No. 96/31236. Purification of the protein was carried out under denaturing conditions using His.Bind resin (Novagen) as disclosed by the same patent; the LPS and the protein concentrations were measured in the final product using a *Limulus* amoebocyte lysate test (Coatest, Chromogenix) and the BCA method (BCA kit, Pierce) respectively. Doses of vaccine devoted to intra-nasal immunisation were prepared by mixing 10 μg mLT (obtained from SmithKline Beecham Biologicals) or CT (Swiss Serum, Bern) with 10 μg of rMOMP serovar F (rMOMPF) or L2 (rMOMPL2) in a final volume of 20 μl PBS.

Vaccination in the mouse model of salpingitis, fertility, sampling and immunological follow-up.

Groups of ten female C3H mice (6 weeks, Iffa Credo) were immunised at week 0 and 2 by intra-nasal administration of 20 μl of the vaccine formulation containing CT or mLT under Hypnorm (Janssen-Cilag) and Dormicum (Roche) anesthesia. The experimental challenge was carried out as following: at week 5, mice were given 2.5 mg progesterone intra peritoneally (Depo-Provera, Upjohn) and at week 6, they were infected by bilateral intrauterine inoculation with 5×10^5 inclusion forming units (IFU) *C. trachomatis* NI1 (serovar F) in 100 μl sucrose phospate glutamate buffer (SPG) or with 100 μl of a Mc Coy cell extract for the fertility positive control group.

At week 10, treated mice were cagged with males for 3 months for fertility assessment (1 male for 2 females per cage with weekly rotation of the males within

each group); the parameters used for estimating group's fertility were : F (number of mice which littered one time or more divided by the total number of mice), M (number of newborn mice (dead or alive) divided by the number of litters) and N (number of newborn mice (dead of alive) divided by the total number of mice).

Determination of the MOMP-specific humoral response

Sampling and quantification of antibody (Ab) responses by ELISA were performed on individual animals as disclosed in the patent GB 9506863.1 supra with some modifications. Vaginal secretions were collected at weekly intervals from week 3 until week 7 by repeated flushing and aspiration of 50 µl PBS, diluted 1:4 in PBS containing 0.5% BSA and 0.1% Tween 20 and analyzed for rMOMP-specific secretory IgA or IgG antibodies. Since the concentration of specific Ab can be affected by variations in fluide recovery during the lavage, total IgA were also quantified but only in the first experiment. Since we detected little or no variation in total Ab level (not shown) between analyzed mice, subsequent vaginal washing were devoted to MOMP-specific IgA analysis only. In order to assess the effectiveness of the intra-nasal immunisation, CT-specific IgA and IgG were also determined in the samples from the first experiment. Titers were determined arbitrarly as the reciprocal of the sample dilution corresponding to an optical density of 1 at 492 nm and mice that displayed at least once a titer higher or equivalent to 4 were considered to be positive for antigen-specific IgA .

Blood samples were collected at week 6 (week of the challenge) and sera were analysed for the presence of rMOMP-specific IgG. In the first experiment, CT-specific IgG were also determined in the serum in order to make sure of the effectiveness of the intra-nasal immunisation; therefore, microtiter plates were precoated with 0.5 µg of CT (Swiss Serum, Bern) per well and then processed as described in patent GB 9506863.1.

Determination of the MOMP-specific cellular response

Two groups of five female C3H mice (6 weeks, Iffa Credo) were immunised at week 0 and 2 by intra-nasal administration of 20 μl of the vaccine formulation containing mLT under Hypnorm (Janssen-Cilag) and Dormicum (Roche) anesthesia; negative control groups were sham-immunised with the mLT only following the same procedure. Animals from group 1 and 2, and those from corresponding controls were bled for serological analysis and sacrificed on day 9 and 19 after the second boost respectively; spleens were aseptically removed, pooled and single cell suspension were prepared for restimulation with 1μg/ml rMOMP serovar L2 or with 4 μg/ml Concanavalin A (Boerhinger Mannheim) as a positive control; unrestimulated cultures were used as negative control of the cellular activation.

For the measurement of cell proliferation, triplicates cultures were set up in round bottom 96-well culture plates using $5x10^4$ responder cells per well in 200 μl of RPMI 1640 with 10% foetal calf serum (FCS, Gibco-BRL); after 72 hours of incubation at 37°C in 7% CO_2, supernatants (SN) were recolted while cells were pulsed for 18 h with 1 μCi of tritiated thymidine (Amersham) per well, harvested onto glass-fiber (Skatron), air dried and counted for beta emission by standard liquid scintillation. The stimulation index (SI) which is the mean of antigen or ConA-stimulated T-cell uptake of tritiated thymidine for triplicate wells divided by the mean of unstimulated T-cell uptake for triplicate wells, was calculated for each group.

IFN-gamma was determined in culture SN using a commercial ELISA kit (Duoset, Genzyme). For cells obtained at day 9 after boosting, 72 h culture SN of the lymphoproliferative assay pooled per triplicate were used while for those obtained at day 19, 48 h culture SN from 24-well plates especially established for that purpose ($5x10^6$ cells per ml of RPMI 1640 containing 10% FCS) were used.

RESULTS

Evidence that mucosal immunisation with rMOMP combined with CT or mLT can afford protection against infertility caused by Chlamydial challenge is given by the first two experiments described below. As these experiments were primarily designed for evaluation of systemic immunisation (not shown), the negative and positive control groups were subcutaneously treated with adjuvants other than CT or mLT; rMOMP-naive animals (negative control groups) were infected to ascertain the effect of the challenge on the fertility while rMOMP-immunised animals (positive control groups) were sham-infected in order to take into consideration the alteration of the fertility that could result from the manipulation of the animals during intrauterine inoculation.

A third experiment was set up in order to characterize the cellular activation evoked by rMOMP adjuvanted with mLT wherein the negative control group consisted in mice intra nasally sham-immunised with mLT alone.

Experiment 1

In the first experiment (table 1), intra-nasal immunisation with rMOMPF+CT was evaluated for its protective effect against infertility caused by Chlamydial infection (homotypic challenge). Analysis of the humoral immune response just before challenge revealed that all the mice displayed CT-specific IgG in their serum and CT-specific IgG and IgA in their vaginal secretions, but no detectable rMOMP-specific IgG or IgA responses in the same prelevements, respectively. However, after challenge, this group displayed values of the F and N fertility parameters which reached 77 and 66 %, respectively, of those of the postive control group, while the negative control group was nearly completely infertile (14 % of the F and 13% of the N values recorded in the positive control group).

Experiment 2

In the second experiment (tables 2 and 3), groups of mice were intra-nasally immunised either with rMOMPF combined with CT, or with rMOMPL2 combined with CT or mLT; in addition to the negative and positive control groups described above, a sham-immunised control group, intra-nasally treated with CT alone, was included in the experiment. As observed in the first experiment, intra-nasal administration of rMOMPF+CT did not induce any detectable humoral rMOMPF-specific response, neither in the sera collected just before challenge (IgG response), nor in the vaginal secretions collected weekly from boosting immunisation to challenge (IgA response). On the contrary, intra-nasal administration of rMOMPL2 combined with CT or mLT induced an antigen-specific humoral response in some of the animals: 1 and 3 out of 10 mice, respectively, were found to be IgG positive when analyzing sera collected just before challenge, while 5 and 7 out of 10 mice, respectively, were found to be IgA positive at least in one of the vaginal washes collected every weeks from boosting immunisation to challenge. Infection did not boost the MOMP-specific IgA response as shown by analysis performed one week after challenge.

When compared with the positive control sham-infected group, fertility in the negative control group was nearly completely abolished, indicating the specific effect of the Chlamydial infection. Fertility of the mucosally treated groups revealed that immunisation with rMOMPF or rMOMPL2 combined with CT gave similar level of protection (63 or 75 % respectively of the F, and 81 or 58 % of the N values recorded in the positive control group). Immunisation with rMOMPL2 combined with mLT gave the best level of protection, with the F value identical and the N value higher (150%) than those recorded in the positive control group. Administration of CT alone also seemed to reduce the infertility level, but to a lesser extent than the rMOMP+CT formulations with 40 % of the F and 35 % of the N values recorded in the positive control group.

Experiment 3

The cellular activation induced by the antigen formulated with mLT was analysed through cell proliferation and IFN-gamma secretion upon antigen-specific restimulation.

When tested at day 9 and 19 days after the boost, spleen cells from groups immunised with the antigen developed strong specific proliferative immune response (38% and 108% of the positive control respectively) while those from control animals that were sham-immunised with mLT alone did not respond to *in vitro* restimulation (table 4 and 5).

Spleen cells collected at both timepoints and restimulated with the antigen displayed IFN-gamma concentrations in their culture supernatants which were in the range of those restimulated during the same period with 4 μg/ml of Con A. On the other hand, cells isolated from sham-vaccinated animals and cultured with the antigen produced relatively low levels of IFN-gamma when compared with their counterpart cultured with ConA (table 4 and 5).

When looking at the humoral response, we were unable to detect nor rMOMP-specific IgG in pools and individual sera, neither rMOMP-specific IgA in pools and individual vaginal washings and that for prelevements made at both timepoints.

These data show that mucosal administration of rMOMP, when combined with CT or mLT, elicits protection (either homotypic or heterotypic) against infertility caused by a Chlamydial challenge. The fact that the protection cannot be correlated with local rMOMP-specific IgA argues for the existence of immune protective mechanism(s) different from a specific secretory antibody response. Results from the later experiment suggest that, in mouse, intra nasal administration of rMOMP combined with mLT induce a specific Th1 T cell immune response which could be responsible for the protection observed.

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Table 1 (Experiment 1)

Group No. Immunisation/ infection schedule	rMOMP-specific IgG geometric mean titer (serum)	rMOMP-specific IgA positive mice (vaginal washes)	F: proportion of fertile mice	N: mean nber of newborn per mouse	M: mean litter size
G2 (NEGATIVE CTRL) - (sc) MPL+QS21+SB62/ Infected	<100	ND	1/8	0.8	4
G3 (POSITIVE CTRL) rMOMP L2 (sc) MPL+QS21+SB62/Sham-infected	23900	ND	9/10	6.2	3.6
G8 rMOMP F (in) CT/Infected	<100	0/10	7/10	4.1	3.4

SC: subcutaneous
IN: intra-nasal

TABLE 2 (Experiment 2)

Group	Mouse number	Formulation	Route	IgA week 3 (vaginal	IgA week 4 washes)	IgA week 5	IgA week 6*	IgA week 7**	IgG week 6 (serum)
G1	1 to 10	- DQ	SC	ND	ND	ND	ND	ND	ND
G2	1 to 10	rMOMP L2 MPL+DQ	SC	ND	ND	ND	ND	ND	9000 (GMT)
G3	1	rMOMPL2 mLT	IN	8	<4	<4	<4	<4	<100
	2			<4	<4	<4	<4	<4	<100
	3			85	>108	140	8	>162	3400
	4			10	22	14	5	10	260
	5			17	<4	<4	<4	<4	<100
	6			<4	<4	110	<4	45	<100
	7			<4	<4	<4	<4	13	<100
	8			<4	<4	<4	<4	<4	<100
	9			<4	<4	<4	9	<4	<100
	10			18	22	7	<4	<4	120
G4	1 to 10	CT	IN	ND	ND	ND	ND	ND	ND
G5	1	rMOMPL2 CT	IN	<4	<4	4	<4	<4	<100
	2			<4	<4	<4	<4	<4	<100
	3			43	400	140	<4	16	170
	4			<4	<4	<4	<4	<4	<100
	5			<4	<4	<4	<4	<4	<100
	6			<4	<4	<4	<4	<4	<100
	7			6	13	10	<4	<4	<100
	8			5	<4	5	<4	<4	<100
	9			<4	30	<4	<4	<4	<100
	10			<4	<4	<4	<4	<4	<100
G5	1 to 10	rMOMP F CT	IN	<4 for all 10	<4 for all 10	<4	<4	<4	<100 for all 10

* Day of challenge ** Post challenge

Table 3 (Experiment 3)

Group No. Immunisation/infection schedule	rMUMP IgG Geometric mean titer (serum)	IgA positive mice (vaginal washes)	Fertue mice	N.mean nber of newborn per mouse
G1 (NEGATIVE CTRL) - (sc/sc) DQ MPL/DQ MPL Infected	<100	ND	1/9	0.2
G2 (POSITIVE CTRL) rMOMP L2 (sc/sc) DQ MPL/DQ MPL Sham-infected	9000	ND	8/8	6
G3 rMOMP L2 (in/in) mLT/mLT Infected	473 (on the 3 positive mice only)	7/10	10/10	9.1
G4 - (in/in) CT/CT Infected	ND	ND	4/10	2.1
G5 rMOMP L2 (in/in) CT/CT Infected	<100	5/10	6/8	3.5
G6 rMOMP (in/in) CT/CT Infected	<100	0/10	5/8	4.9

Table 4 (Experiment 3)

Cellular response analysed 9 days after boost immunization.

Group N° Immunization schedule: formulation (route)	Mean cpm ($5\ 10^4$ cells/well) - *ConA* *rMOMP*	Stimulation Index - *ConA* *rMOMP*	γ-IFN (pg/ml) $2.5\ 10^5$ C/ ml - *ConA* *rMOMP*
G1 - mLT (IN) (sham-imm)	897 35672 2516	1 40 2	<20 863 137
G2 rMOMPL2 mLT (IN)	516 30002 11517	1 58.1 22.3	<20 610 572

Table 5 (Experiment 3)

Cellular response analysed 19 days after boost immunization.

Group N° Immunization schedule: formulation (route)	Mean cpm (5 10^4 cells/well) - *ConA* *rMOMP*	Stimulation Index - *ConA* *rMOMP*	γ-IFN (pg/ml) 5.0 10^6 C/ ml - *ConA* *rMOMP*
G1 - mLT (IN) (sham-imm)	4379 20712 5890	1.0 4.7 1.3	<20 4348 <20
G2 rMOMPL2 mLT (IN)	1481 22234 24166	1.0 15.0 16.3	<20 5826 1790

Claims:

1. A vaccine comprising the major outer membrane protein (MOMP) for Chlamydia trachomatis and a mucosal adjuvant.

2. A vaccine as claimed in claim 1 wherein the adjuvant is selected from mLT or CT.

3. A vaccine as claimed in claim 1 wherein the outer membrane protein is selected from serovar - D to K or L.

4. A vaccine as claimed in claim 3 wherein the outer membrane is selected from F, L2, D or E.

5. A vaccine as claimed herein comprising a MOMP from two or more serovars.

6. A vaccine as claimed herein wherein the outer membrane protein is produced in E.Coli by recombinant DNA technology.

7. A vaccine as claimed herein wherein the mucosal adjuvant is LT holotoxin where arginine at position 192 is substituted with glycine (mLT R192 G).

8. A vaccine as claimed herein wherein the MOMP is the full length mature protein, devoid of the signal sequence.

9. A vaccine as claimed herein adapted for oral, or intranasal administration.

10. A process for the production of a vaccine comprsing admixing a mucosal adjuvant with a MOMP from Chlamydia trachomatis.

11. A method of treating a patient suffering from or susceptible to chlamydia trachmalis infectsion comprsing the administration of a safe and effective amount of a vaccine as claimed herein.

12. Use of a mucosal adjuvant and an MOMP from Chlamydia trachomatis for the manufacture of vaccine for the treatment or prevention of Chlamydia trachomatis infections.

INTERNATIONAL SEARCH REPORT

ternational Application No
PCT/EP 97/07282

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K39/39 //(A61K39/39,39:118)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 31235 A (PASTEUR MERIEUX SERUMS & VACCINS) 10 October 1996 see the whole document ---	1,12
X	VILLENEUVE A ET AL: "Cholera toxin B subunit overcome the H-2 restriction for neutralizable epitopes of the major outer membrane protein of Chlamydia trachomatis." THE 9TH INTERNATIONAL CONGRESS OF IMMUNOLOGY;MEETING SPONSORED BY THE AMERICAN ASSOCIATION OF IMMUNOLOGISTS AND THE INTERNATIONAL UNION OF IMMUNOLOGICAL SOCIETIES, SAN FRANCISCO, CALIFORNIA, USA, JULY 23-29, (1995) 585, XP002063817 see abstrat 3472 --- -/--	1-12

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

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Date of the actual completion of the international search	Date of mailing of the international search report
29 April 1998	20. 05. 1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Moreau, J

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

ernational Application No
PCT/EP 97/07282

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 96 16178 A (LEBENS M. ET AL.) 30 May 1996 see the whole document	1-12
A	WO 96 31236 A (SMITHKLINE BEECHAM BIOLOGICALS) 10 October 1996 cited in the application see the whole document	1-12
A	WO 96 06627 A (THE ADMINISTRATORS OF THE TULANE EDUCATIONAL FUND) 7 March 1996 cited in the application see the whole document	1-12
P,X	WO 97 02836 A (ORAVAX) 30 January 1997 see the whole document	1-12

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 97/07282

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/EP 97/07282

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Remark : Although claim11 is directed to a method of treatment of the human/animal body , the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

Information on patent family members

ernational Application No
PCT/EP 97/07282

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		EP 0837692 A	29-04-98