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22 October 2009

(54) Title: A NOVEL NEOSPORA CANINUM VACCINE

A

NC	MKLEFFTLVLAVSAVAENACVQKAFMDIEIDGESAGRIVLELRGDVWEKTVKNTIGLEF	60
TG	VL A G R Y D H I E IA	60
NC	KYKGSTFHRVIADFMIGGDFENNINGTGCHSTYGPREFEDENFTLKHDRVISMANAGPNT	120
TG	V I P R D D E	120
NC	NGSQFFITTVKTEWLDGRHVVFCKITNDSWFTVQALREALGSSGGRFSKIATITDGLL	178
TG	A TE G V E	178

Fig. 1

(57) Abstract: Neospora caninum is the causal agent of bovine neosporosis which results in high levels of abortion. The present study determined the protective efficacy of two Neospora antigens- Neospora cyclophilin (NcCyP) and NcSRS2. The ability of native NcCyP to upregulate mouse IFN γ was also confirmed in this study. Recombinant NcCyP or NcSRS2 were tested either alone or in combination and formulated with adjuvant ImmuMax-SR and CpG. Female BALB/c mice (n=15) of 10-12 weeks of age were immunized s.c. twice in a 2-week interval with vaccines containing either NcCyP alone, NcSRS2 alone, NcCyP plus NcSRS2, or non-recombinant bacterial antigen (NR) in 2 separate trials. All mice were challenge-infected 3 weeks following the booster immunization and necropsied 3 weeks after the challenge infection. Brain and serum were collected and Nc-specific DNA sequence in brain tissue and antibodies in serum were analyzed by PCR or ELISA/Western blotting. Results showed that mice vaccinated with rNcCyP, rNcSRS2, or both rNcCyP and rNcSRS2 responded with high levels of NcCyP or NcSRS2 specific antibodies. Overall, mice received vaccines formulated with either rNcCyP or rNcCyP and rNcSRS2 had a higher (p<0.01) percent protection when compared to the mock- or non-vaccinated mice. The groups immunized with rNcSRS2 alone exhibited slightly lower levels of protection, which was higher (p<0.05) than that of the non-vaccinated group but did not differ (p=0.06) from that of the mock-vaccinated group. The results of the present study indicate that NcCyP is a highly efficacious vaccine candidate which may be useful in protection against Neospora infection.

A. CLASSIFICATION OF SUBJECT MATTER*A61K 39/002(2006.01)i, A61K 48/00(2006.01)i, A61P 37/04(2006.01)i, A61P 33/02(2006.01)i*

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 A61K 39/002, A61K 48/00, A61P 37/04, A61P 33/02

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 practicable, search terms used)

CA, PubMed, Delphion "(Neospora caninum OR N. caninum) AND (cyclophilin OR NcCyP OR NcSRS2 OR T. gondii)"

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TUO, W. et al. "Identification and Characterization of Neospora caninum Cyclophilin That Elicits Gamma Interferon Production." Infect. Immun. Aug 2005, Vol.73, No.8, Pages 5093-5100.	1-3, 8-11
Y		4-7
Y	JENKINS, M. C. et al. "Advances and Prospects for Subunit Vaccines against Protozoa of Veterinary Importance." Vet. Parasitol. 2001, Vol.101, Pages 291-310.	4-7
A		1-3, 8-11
Y	STASKA, L. M. et al. "Identification of Vaccine Candidate Peptides in the NcSRS2 Surface Protein Neospora caninum of by Using CD4+ Cytotoxic T Lymphocytes and Gamma Interferon-Secreting T Lymphocytes of Infected Holstein Cattle." Infect. Immun. Mar. 2005, Vol.73, No.3, Pages 1321-1329.	4-5
A		1-3, 6-11
A	INNES, E. A. et al. "Comparative Host-Parasite Relationships in Ovine Toxoplasmosis and Bovine and Strategies for Vaccination." Vaccine. Mar. 2007, Vol.25, Pages 5495-5503.	1-11



Further documents are listed in the continuation of Box C.



See patent family 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 application or patent 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 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

Date of the actual completion of the international search

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Date of mailing of the international search report

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Name and mailing address of the ISA/KR

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2009/033437**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 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. ☒ Claims Nos.: 12-15
because they relate to subject matter not required to be searched by this Authority, namely:
The subject matter of claims 12-15 pertains to methods for treatment of the animal by therapy, thus relates to a subject matter which this International Searching Authority is not required, under Article 17(2)(a)(i) of the PCT and Rule 39.1(iv) of the Regulations under the PCT to search.
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 No. III Observations where unity of invention is lacking (Continuation of item 3 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 and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.