Development of new immunological tools for the study of *Toxoplasma gondii*

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The invasion process in the Apicomplexa

Toxoplasma

Cryptosporidium

Plasmodium
**Cryptosporidium parvum**
- **sporozoite**
- **merozoite**

- **TRAP-C1**

- **CpMIC1**

**Toxoplasma gondii**
- **tachyzoite**

- **SAG5**

- **TgMIC14, TgMIC15**
  - (unpublished)

- **TgLCCL protein family**
  - (unpublished)
Identifying protein constituents
of the oocyst wall of *Toxoplasma gondii*

Production of oocyst-specific antibodies
- coprodiagnosis
- immunomagnetic separation (IMS)

mAb 3G4 (anti-oocyst wall) - mAb 4B6 (anti-sporocyst wall)

mice immunized with purified oocyst walls

major limitations of developed IMS

target proteins not identified

**Identification of putative oocyst wall proteins (OWPs)**

**Production of recombinant proteins in bacteria**

**Immunolocalization (IFA) with polyclonal antisera**

**Production of mAbs**

Gene cloning
The coccidian oocyst wall (1): ultrastructure

Physical-chemical properties of the oocyst wall

- **Resistant to**
  - sodium hypochlorite (<10%)
  - 2% potassium dichromate
  - 2% sulphuric acid
  - proteolytic enzymes

- **Impermeable to**
  - water-soluble substances

- **Permeable to**
  - lipid-soluble and gaseous substances

**O/I layers**
- proteins, lipids, carbohydrates

*T. gondii* early oocyst

*T. gondii* unsporulated oocyst

*Eimeria maxima*
The coccidian oocyst wall (2): molecular structure

*Toxoplasma gondii*

*Eimeria maxima*

*Cryptosporidium parvum*

**Tyrosine-rich proteins:** gam56 and gam82 (dityrosine-protein crosslink)

**Cysteine-rich proteins:** COWP family
The oocyst wall protein (OWP) family of *Toxoplasma gondii*
Production of polyclonal sera against putative TgOWPs

- 20.m (500)
- 25.m (463)
- 27.m (783)
- 41.m (452)
- 50.m (535)
- 59.m (641)
- 76.m (512)

* = bacterially expressed recombinant protein fragment
○ = synthetic peptide
Immunolocalization of putative TgOWPs
Production of monoclonal Abs against select TgOWPs

- mAb 1B4
- mAb 1G12

IgM isotype
Reactivity of mAb 1B4

A negative control

B

C

D
Conclusions

*Toxoplasma gondii* possesses a family of 7 cysteine-rich extracellular proteins structurally related to the *Cryptosporidium* oocyst wall protein COWP-1.

The genes of 3 putative TgOWPs have been cloned and shown to be expressed in partially sporulated *T. gondii* oocysts.

Polyclonal Abs raised against 6 of the putative TgOWPs were shown to react with the wall of *T. gondii* oocysts and/or sporocysts.

Two monoclonal Abs specific for the protein 59.m were shown to recognize the outer wall of *T. gondii* oocysts.
Future objectives

Cloning of the cDNAs encoding the remaining TgOWPs

Localization of individual TgOWPs by IFA and immunoEM

Production of monoclonal Abs directed against other members of the TgOWP family located in the outer layer of the oocyst wall

Assessment of the cross-reactivity of anti-TgOWPs mAbs with other coccidia (Hammondia hammondi, H. heydorni, Neospora caninum)

Set up of an immunofluorescence test for the coprodiagnosis of toxoplasmosis

Development of an immunomagnetic separation technique for the capture of T. gondii oocysts from environmental samples