

Molecular characterization of a cDNA encoding an excretory–secretory antigen from *Toxocara canis* second stage larvae and its application to the immunodiagnosis of human toxocariasis

ABSTRACT

The cDNA encoding an excretory–secretory antigen from the second stage larvae of *Toxocara canis* has been characterized. Sequence analysis revealed an open reading frame encoding a protein of 226 amino acid residues ($M_r=24\,398$). Sequence database searches showed similarities to regions corresponding to epidermal growth factor-like and lectin-like domains of the core proteins of vertebrate chondroitin sulfate proteoglycans, which are major components of the extracellular matrix. The *T. canis* core protein was expressed as a fusion protein with thioredoxin A using an *Escherichia coli* expression system, and then affinity purified on a metal affinity resin in the presence of 8 M urea. When the purified recombinant *T. canis* protein was used as an antigen, immunoblot analysis revealed the protein specifically reacted with sera from toxocariasis patients. The antigenic protein did not react with sera from patients with *Brugia malayi* infection, dirofilariasis, or ascariasis. In some cases of anisakiasis, cross-reactions were observed; however, the cross-reacting bands disappeared when anisakiasis sera preabsorbed with *Anisakis* antigen were used, indicating that the recombinant *T. canis* protein is very promising for use as an immunodiagnostic antigen for human toxocariasis.

Keyword: Excretory-secretory antigen; Human toxocariasis; Immunodiagnosis; Proteoglycan core protein; Recombinant antigen; Second stage larvae; *Toxocara canis*