Journal of Nematology 41(3):228–233. 2009.

Identification of a Secreted Fatty Acid and Retinol-Binding Protein (Hp-FAR-1) from *Heligmosomoides polygyrus*

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Abstract: Hp-FAR-1 is a major, secreted antigen of the parasitic nematode *Heligmosomoides polygyrus*, a laboratory mouse model frequently used to study the cellular mechanisms of chronic helminth infections. The DNA encoding Hp-FAR-1 was recovered by screening a fourth larval (L_4) *H. polygyrus* cDNA expression library using antibodies raised against L_4 stage excretory/secretory (E/S) proteins. Predictions of secondary structure based on the Hp-FAR-1 amino acid sequence indicated that an alpha-helix predominates in Hp-FAR-1, possibly with some coiled-coil conformation, with no beta-structure. Fluorescence-based ligand binding analysis confirmed that the recombinant Hp-FAR-1 (rHp-FAR-1) binds the fluorescent fatty acid analog 11-((5-[dimethylaminoaphthalene-1-sulfonyl)amino)undecanoic acid (DAUDA), and by competition oleic acid. RT-PCR amplification of the *hp-far-1* gene indicated that the gene is transcribed in all parasitic stages of the organism's life cycle. The presence of a secreted FAR protein in the well-defined laboratory model of *H. polygyrus* provides an excellent model for the further study and analysis of the *in vivo* role of secreted FAR proteins in parasitism, and supports the mounting evidence that secreted FAR proteins play a major role in nematode parasitism.

Key words: Heligmosomoides polygyrus, host-parasitic relationship, Hp-FAR-1, *hp-far-1*, lifecycle, molecular biology, nematode, retinol binding.

Parasite-induced immunomodulation by the nematode *H. polygyrus* has long been acknowledged (Behnke et al., 1992), yet very little progress has been made in the identification and isolation of specific effector molecules. This study began as an investigation into the E/S proteins released by the L_4 stage of *H. polygyrus*, in an attempt to identify the proteins secreted into the host tissue that are potentially in contact with host immune cells and could therefore potentially act as downregulators of host immunity.

In this paper, we report the isolation and molecular characterization of a fatty acid and retinol binding protein, Hp-FAR-1, which is an E/S product of the L_4 stage of *H. polygyrus*, and for which the encoding cDNA was isolated from a cDNA expression library using antiserum raised against L_4 E/S components. Expression of rHp-FAR-1 resulted in an isolated recombinant fusion protein that binds fatty acids with binding characteristics similar to those observed for previously characterized nematode FAR proteins (Basavaraju et al. 2003; Fairfax et al., 2009; Garofalo et al., 2003b).

The discovery of this protein in *H. polygyrus* is interesting in the context of the possible role of FAR proteins in immunomodulation, as it is hypothesized that FAR proteins may play a role in interfering with intracellular lipid signaling related to host defenses (Basavaraju et al., 2003; Garofalo et al., 2002; Garofalo et al., 2003a; Garofalo et al., 2003b, Kennedy et al., 1997; Fairfax et al., 2009). In addition, the *H. polygyrus* model for the study of FAR proteins may well prove useful, as the cellular characteristics of infection with *H. polygyrus* are well defined and documented (Liu et al., 2004; Anthony et al., 2006; Gustad and Robinson, 1996; Rausch et al., 2008). As such, the *H. polygyrus*/mouse model system provides a particularly valuable opportunity for the detailed study of the potential role of nematode FAR proteins in parasitism.

Materials and Methods

Preparation of H. polygyrus E/S proteins and anti-L_4 E/S hyperimmune serum: The E/S proteins from various stages of *H. polygyrus* were prepared as previously described (Robinson et al., 1997). Briefly, Swiss Webster mice were infected orally with approximately 650 *Hp*L_3. At two hundred hours (L_4 stage) and 14 days (adult stage) post infection, the small intestines were removed, slit open lengthwise, and the parasites extracted according to a modified Baermann technique (Robinson et al., 1997). L_3 larva, L_4 larva and adult *H. polygyrus* were placed into three separate wells of a 24-well tissue culture plate and submersed in phosphate-buffered saline (PBS, 10 mM sodium phosphate, 150 mM sodium chloride, pH 7.8) supplemented with 100 U/ml of penicillin and 100 μg/ml of streptomycin. The plates were incubated at 37°C with 5% CO_2 for 18 hours. The PBS was then removed, centrifuged, and the supernatant passed through a 0.45 μm filter to remove debris. The resulting filtrate was then concentrated by centrifugation through a centricon YM-10 (Millipore, Bedford, MA). The concentration of the resulting protein was estimated using an ND-1000 UV-Vis spectrophotometer (NanoDrop Technologies, Wilmington, DE). Anti-L_4E/S hyperimmune serum (HIMS) was prepared by injecting Swiss Webster mice three times with 150 μg of L_4 E/S protein.

Received for publication July 29, 2009.
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The authors would like to thank Thomas R. Gustad, North Dakota State University, for his technical assistance. The research from the lab of Jennifer L. Bath is supported in part by the Fuglestad-Torsveit grant and in part by the Fargo Moorhead Economic Development Corporation, grant number 253031. The work from the lab of Michael Robinson was supported in part by an NIH/DHHS/NIAID/AREA grant number AI-47959. The research from the lab of Malcolm W. Kennedy is supported by the Wellcome Trust (UK), grant number WT083625MA.
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This paper was edited by Melissa Goellner Mitchum.

Cloning of hp-far-1 cDNA from L_4 H. polygyrus: To construct an *H. polygyrus* cDNA library, 114 hour post-infection L_4 *H. polygyrus* were obtained from infected Swiss Webster mice according to methods previously described (Robinson et al., 1997). mRNA was isolated using the Messenger RNA Standard Isolation Kit (Sigma Chemical Co., St. Louis, MO). Using the mRNA as the template, a cDNA library was produced utilizing the SuperScript Lambda System according to the manufacturer's instructions (GE Healthcare, Carlsbad, CA). Size-fractionated cDNA was ligated into the EcoR1 arms of Lambda-Ziplox (GE Healthcare) and packaged using the Packagene Lambda DNA Packaging system (Promega, Madison, WI). The lambda ZipLox cDNA library was screened using mouse anti- L_4E/S HIMS. Plasmids carrying positive cDNA inserts in lambda Ziplox after a second round of screening were auto-subcloned into the pZL1 plasmid by cre-loxP-mediated recombination in *E. coli* strain DH10BZIP, according to the manufacturer's protocol. Sequencing reactions for both strands of the isolated cDNA were prepared using a Sequitherm EXCEL II Long-Read Sequencing Kit-LC (Epicentre Biotechnologies, Madison, WI). The reactions were then electrophoresed using a LI-COR Automated DNA Sequencer (LI-COR, Inc., Lincoln, Nebr.). Sequence data have been submitted to GenBank under the accession code AY033652.

Sequence comparisons and secondary structure predictions: Sequence analysis was performed using programs available through the ExPASY molecular biology server (www.expasy.ch/tools). Translate Tool was used to translate the DNA nucleotide sequence to an amino acid sequence. The Signal P program (Nielsen et al., 1997), using neural networks trained on eukaryotic sequences, was used to predict the location of the signal peptide and its cleavage site. The PSORT II program (Horton and Nakai, 1997) was used to predict protein localization and sorting signals. The molecular weight and isoelectric point of Hp-FAR-1 were estimated using the ProtParam program (Gasteiger et al., 2005), and the secondary structure prediction of the sequence was performed using the PHD (Rost and Sander, 1993) and Jpred algorithms (Cuff et al., 1998). Sequence comparisons were made with a range of databases by using BLAST searches through the EBI World-Wide Web pages (http://www.ebi.a.uk/ebi-home.html).

Production of rHp-FAR-1: DNA encoding the Hp-FAR-1 protein was amplified by PCR for cloning purposes using PCR primers designed to omit the putative signal peptide from the N-terminus of the protein, as well as insert restriction sites onto the 5′ and 3′ ends of the resulting PCR product. The following oligonucleotide primers were used: forward primer (HpFAR-F1): 5′ CAC GGA TCC CCT ATC AAA AAA GCC GAA 3′ and the reverse primer (HpFAR-R1): 5′ CTC GAA TTC TCA GTT GCT TCC AAC TAG 3′. The PCR product that was obtained for Hp-FAR-1 was cloned directly into pGEX-6P-2 (GE Healthcare) in accordance with the manufacturer's instructions, and was then sequenced to verify the reading frame of the insert. The recombinant pGEX-6P-2 */hp-far-1* plasmid was maintained in *E. coli* JM109 (Promega), and the purified plasmid was then transformed into *E. coli* BL21(DE3) cells (Novagen, Gibbstown, NJ) for expression of the recombinant fusion protein. The expression and purification of the protein was performed in accordance with the manufacturer's instructions for high yield (GE Healthcare). Enzymatic cleavage of the GST tag from GST-Hp-FAR-1 was performed using PreScission Protease (GE Healthcare), according the manufacturer's instructions. The purity of the Hp-FAR-1 and GST-Hp-FAR-1 fusion proteins were confirmed by SDS-PAGE analysis on a 12% Tris-glycine polyacrylamide gel. The purified proteins were exhaustively dialyzed at 4°C against PBS using Slide-A-Lyzer Dialysis Cassettes with a molecular weight cutoff of 10 kDa (Pierce, Rockford, IL) and subsequently passed through a column of Extracti-Gel D resin (Pierce) to deplete any contaminating detergent.

Western Blotting: Soluble, purified fractions of Hp-FAR-1 and GST-Hp-FAR-1 fusion protein were run on a 12% Tris-glycine polyacrylamide gel (Bio-Rad, Carlsbad, CA) and subsequently transferred onto a PVDF membrane (Bio-Rad). After transfer, the membrane was blocked with 5% non-fat dry milk in TBS-Tween overnight, and then probed with either a 1:5,000 dilution of goat anti-rabbit GST primary antibody (GE Healthcare) or mouse anti-*H. polygyrus* L_4E/S HIMS. After three washes in TBS-Tween, the membrane was incubated with the appropriate alkaline phosphatase-conjugated secondary antibody (Promega) for 45 minutes. The blot was then washed three times with TBS-Tween, and the reaction was developed with Alkaline Phosphatase Conjugate Substrate Kit (Bio-Rad), according to the manufacturer's directions.

Spectrofluorimetry and fluorescence-based ligand-binding assays: Fluorescence emission spectra were recorded at 20°C with a SPEX FluorMax spectrofluorimeter (Spex Industries, Edison, NJ), using 2 ml samples in silica cuvettes. The fluorescent fatty acid analog 11-((5-dimethylaminonaphthalene-1-sulphonyl)amino)undecanoic acid (DAUDA) was obtained from Invitrogen/Molecular Probes (Eugene, OR) and oleic acid was obtained from Sigma. The monochromatic excitation wavelength used was 345nm. DAUDA was stored as a stock solution at approximately 10 mM in ethanol in the dark at −20° C and freshly diluted in PBS to approximately 1 μM by serial dilution in PBS for use in the fluorescence experiments. Oleic acid for competition studies was prepared as a stock solution in ethanol at approximately 10 mM and diluted 1:10, 1:100 and 1:1000 in PBS for use in the assays. For retinol binding analysis, retinol was dissolved in ethanol and added directly to the buffer containing Hp-FAR-1. The retinol binding assay was carried out as previously described

(Prior et al., 2001; Suire et al., 2001). The fluorescence titration data were analyzed using MicroCal Origin software.

RNA isolation and RT-PCR: RT-PCR was employed to determine the developmental stage specificity of *hp-far-1* mRNA transcription in L_3, L_4 and adult *H. polygyrus*. Total RNA from each life stage was isolated using the RNeasy mini kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. cDNA was synthesized utilizing the RNA template with oligo d(T) primers and the iScript cDNA Synthesis Kit (Bio-Rad) according to the manufacturer's instructions. The *hp-far-1* specific primers (HpFAR-F1 and HpFAR-R1, described above) were used for PCR amplification, and the first strand cDNAs served as templates.

Results

Isolation of an hp-far-1 cDNA: Primary immunoscreening of the *H. polygyrus* L_4 cDNA library with anti-L_4E/S HIMS yielded 13 positive clones. DNA sequencing showed that all of the clones contained identical HP-FAR-1-encoding cDNA sequences. The *hp-far-1* cDNA encodes a predicted open reading frame of 171 amino acids, with a 5′ start Met codon, a 3′ stop codon, and a 3′ poly(A) tail.

Sequence and secondary structure analysis: The PSORT II program predicts that the leader is involved in directing the protein to a secretory compartment in the synthesizing cell. The predicted amino acid sequence includes a hydrophobic leader peptide that is predicted by the SignalP program (Bjellqvist et al., 1994) set for eukaryotic sequences to be cleaved between Ser-16 and Thr-17 to give a mature polypeptide, according to Compute pI/Mw, of 17,553.37 Da with a theoretical isoelectric point of 8.96. The isoelectric point of the polypeptide with the leader sequence was estimated to be 9.11, and the size of the unmodified protein 19,414.81 Da. A consensus Casein Kinase II phosphorylation site at residues 45-48 was identified (Figure 1) and, similar to known FAR proteins from other nematodes, secondary structure analysis of Hp-FAR-1 predicts a predominantly alpha-helical conformation. The algorithms applied to the entire alignment predicted 72% helix, 28% loop structures, but no β/extended structure (Figure 1). In addition, the COILS program (Lupas et al., 1991) produced a strong prediction for coiled-coil structure in a section of Hp-FAR-1, although not as strong as that observed for other nematode FAR-1 proteins (Garofalo et al., 2003b).

Purification of recombinant Hp-FAR-1: Screening of rHp-FAR-1 expressed from the pZL1 plasmid within *E. coli* by western blot analysis using anti-L_4E/S HIMS, demonstrates the specificity of the antibodies raised against L_4E/S protein to the rHp-FAR-1 protein (Figure 2A). The protein was also expressed as a GST fusion protein in *E. coli*. The GST-Hp-FAR-1 protein was solubilized with the majority of the protein extracted within the soluble fraction. SDS-PAGE analysis demonstrates purified GST-Hp-FAR-1 and post-enzymatic cleavage of purified Hp-FAR-1 (Figure 2B). Western blot analysis using an anti-GST antibody was positive for the GST-Hp-FAR-1 fusion protein at the predicted size of approximately 42 kDa. Likewise, the cleaved fusion, resulting in free Hp-FAR-1 with no GST was not recognized by the anti-GST primary antibody (Figure 2C).

Ligand binding by Hp-FAR-1: Figure 3A shows that rHp-FAR-1 bound the fluorophore-tagged fatty acid DAUDA to produce a significant blue shift in its peak emission from 543 nm to 484 nm, which is unusually large for lipid transporter proteins but typical for FAR proteins (Basavaraju et al. 2003; Garofalo et al. 2002; Garofalo et al. 2003b; Prior et al. 2001), and indicative of a highly apolar binding site. Figure 3B shows that the addition of oleic acid to Hp-FAR-1:DAUDA complexes displaced DAUDA from the protein's binding site very efficiently, and complete replacement was observed at a ligand:competitor ratio of approximately 1:9. Figure 3C shows binding of retinol by Hp-FAR-1 which is indicated by a substantial increase in fluorescence emission intensity when retinol is added to a solution of Hp-FAR-1 in buffer.

Life stage transcription of the hp-far-1 gene: RT-PCR analysis of L_3, L_4, and adult cDNA was performed to assess the developmental stage-specific transcription of *hp-far-1*. RT-PCR was performed using *hp-far-1*-specific primers and resulted in the correct size band on agarose gel electrophoresis, from each sample analyzed, of approximately 513 bp (Figure 4).

Discussion

In this study we demonstrate the identification and isolation of an Hp-FAR-1 fatty acid and retinol binding

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MLRLGFLALLIVCVCSTPIKKAEDIPQEVREVLPENVVQLILSFTPAEKKVIEEFFNNWE 60
-HHHHHHHHHHHHHHH-----HHHHHHHHHHHHHHHHHHHHH---HHHHHHHHHHHH--

KFKTEDEALNFFKEKSPSLYAKIENLREILKKKVATLSPESKAFFDKVQSSLKDLHKQIL 120
----HHHHHHHHHHHHHHHHHHHHHHHHHHHHHH----HHHHHHHHHHHHHHHHH---

VGDAPSLDMFREVLRKHVDTYKALSADSKKELKKTFPIAARVMSKLVGSN           170
------HHHHHHHHHHHHHHH----HHHHHHHHH-HHHHHHHHHH---
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Fig. 1. A consensus casein kinase II phosphorylation site at residues 45-48 (underlined) and secondary structure analysis of Hp-FAR-1 predicts a predominantly alpha-helical conformation. The algorithms applied to the entire alignment predicted 72% helix, 28% loop structures, but no β/extended structure.

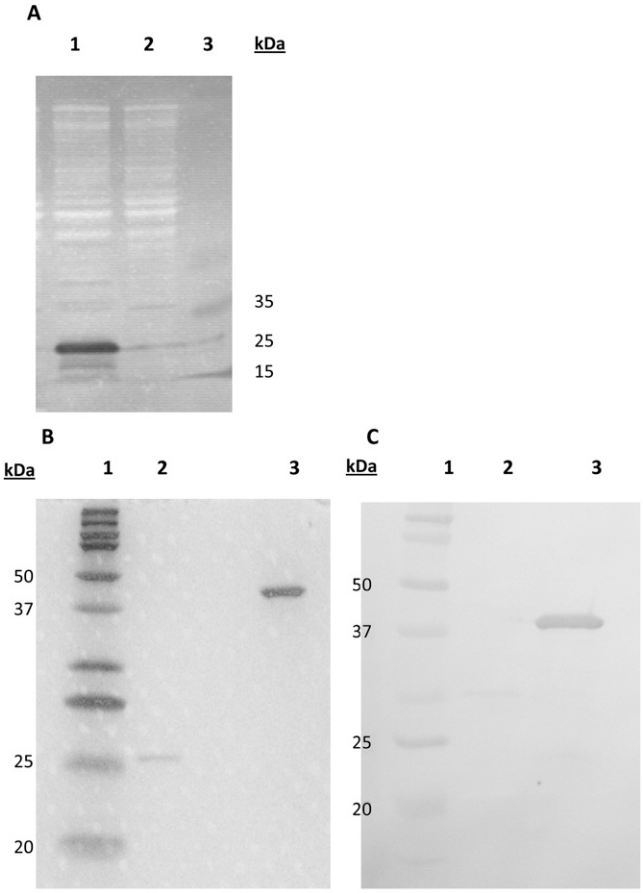

FIG. 2. SDS-PAGE and western blot analysis of recombinant Hp-FAR-1 samples. A) Western blot analysis of recombinant Hp-FAR-1 expressed in the pZL1 vector in *Escherichia coli* DH10B, demonstrating specificity of mouse anti-L_4E/S HIMS for rHp-FAR-1. Lane 1: positive result for pre-purified recombinant Hp-FAR-1 expressed in *E. coli* DH10B; Lane 2: negative control, pre-purified protein extract from pZL1 vector without Hp-FAR-1 insert in *E. coli* DH10B; Lane 3: molecular weight marker. B) SDS-PAGE analysis of purified rHp-FAR-1 and GST-Hp-FAR-1. Lane 1: broad range molecular weight markers, Lane 2: purified Hp-FAR-1, Lane 3: purified GST-Hp-FAR-1. C) Western blot analysis probed with anti-GST antibody. Lane 1: broad range molecular weight marker; Lane 2: negative result for rHp-FAR-1 with no GST fusion protein; Lane 3: positive result for GST-Hp-FAR-1 fusion protein at the predicted size of approximately 42 kDa.

protein from *H. polygyrus*. This protein shows similarity in its binding characteristics to previously described nematode FAR proteins as well as similarity in its primary and secondary structures (Kennedy et al., 1997; Basavaraju et. al., 2003; Garofalo et al., 2003b).

Several previous studies have examined the presence of FAR proteins from non-tissue dwelling stages of parasitic nematodes (Basavaraju et. al, 2003; Prior et al., 2001) as well as free-living nematodes (Garofalo et al., 2003b), however, have not analyzed the parasite life stages for the production of a FAR protein during stages in which the parasite is embedded within the host tissue, potentially in direct contact with mediators of host immunity. Because of the possibility of variability in transcriptional and translational rates, as well as the antigenicity and immunogenicity of Hp-FAR-1 as compared to

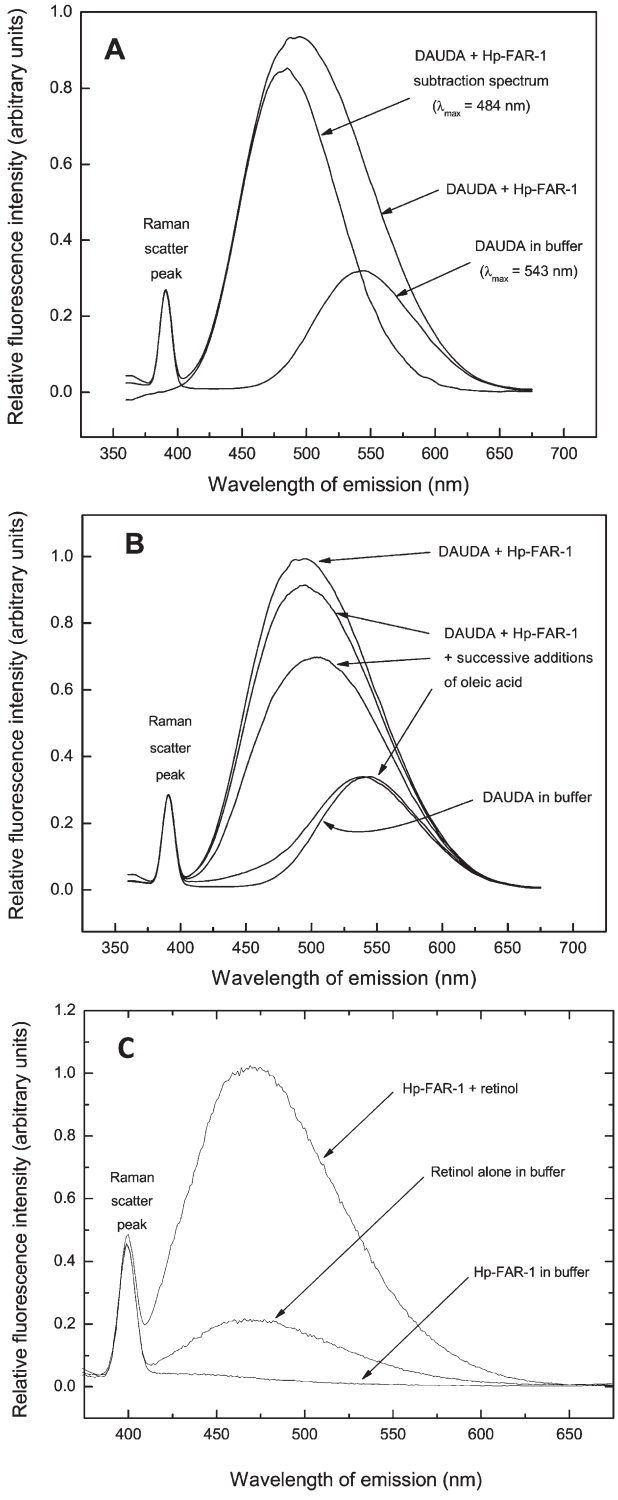

FIG. 3. Ligand binding by rHp-FAR-1. Protein was mixed with environment-sensitive fluorescent ligands DAUDA (panel A), oleic acid (panel B) or retinol (panel C). A) Binding of DAUDA by Hp-FAR-1 indicated by a substantial increase in fluorescence emission by DAUDA and the subtraction spectrum showing a significant blue shift in peak emission. B) Highly efficient displacement of DAUDA from rHp-FAR-1 by successive additions of oleic acid. The concentration of compounds in the cuvette were approximately 1 μM DAUDA, and approximately 0.08 μM, 0.9 μM, and 9 μM oleic acid in the successive additions. C) Binding of retinol by rHp-FAR-1 indicated by a substantial increase in fluorescence emission intensity when retinol is added to a solution of Hp-FAR-1 in buffer.

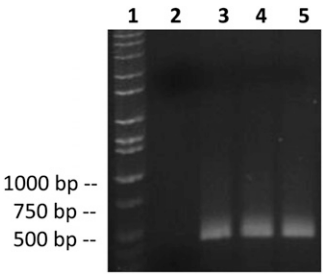

FIG. 4. Transcription of Hp-FAR-1 in L_3, L_4 and adult *Heligmosomoides polygyrus* using Hp-FAR-1 specific primers. RT-PCR was performed on mRNA isolated from the L_3, L_4, and adult stages of *H. polygyrus*. Lane 1: DNA ladder; Lane 2: no template control; Lane 3: L_3 *H. polygyrus* cDNA; Lane 4: L_4 *H. polygyrus* cDNA; Lane 5: Adult *H. polygyrus* cDNA.

other proteins, we performed RT-PCR to detect the presence of the *hp-far-1* transcript. Our results clearly demonstrate the transcription of the *hp-far-1* gene from the infective L_3 larval stage of *H. polygyrus*, as well as the L_4 and adult stages of the parasite, providing a context by which a protein could theoretically regulate host immunity based on the sequestration or delivery of small lipid ligands, as previously speculated (Basavaraju et al., 2003; Garofalo et al., 2002; Prior et al., 2001).

In addition, the high degree of conservation in the lipid-binding characteristics of FAR proteins, and their presence at the host:parasite interface, across multiple families of parasitic nematodes lends support to the likelihood that this nematode-restricted family of proteins may play a crucial role in the life cycle, and possibly directly in the parasitism of their host. Together, these attributes indicate a potential for FARs as targets for the development of vaccines or chemotherapeutic agents, as it may be possible to target multiple parasitic nematodes with a single, broadly cross-reacting vaccine or chemotherapeutic. In addition, while only the molecular structure of a free living *C. elegans* FAR protein has been solved (Jordanova et al., 2009), the structure of FAR proteins are clearly unlike that of any family of lipid-binding proteins, and also distinct from any protein known from mammals, increasing the possibility that they may present useful drug targets (Fairfax et al., 2009).

The identification of a highly conserved FAR protein in *H. polygyrus* alone adds to the mounting evidence that FAR proteins may play a crucial role in nematode parasitism. Potentially more significant, however, is the fact that the cellular responses of *H. polygyrus* infection have been widely studied and well-characterized, and given that *H. polygyrus* is a widely accepted and utilized model for chronic nematode infections, the ability to study FAR proteins in the context of the *H. polygyrus*/mouse infection model provides a tractable and well-characterized system for the study of both the *in vivo* role of FAR proteins in nematode infections, as well as preclinical testing of chemotherapeutics and vaccines.

LITERATURE CITED

Anthony, R. M., Urban, J. F., Jr., Alem, F., Hamed, H. A., Rozo, C. T., Boucher, J., Van Rooijen, N., and Gause, W. C. 2006. Memory TH2 cells induce alternatively activated macrophages to mediate protection against nematode parasites. Nature Medicine 12:955–960.

Basavaraju, S., Zhan, B., Kennedy, M. W., Liu, Y. Y., Hawdon, J., and Hotez, P. J. 2003. Ac-FAR-1, a 20 kDa fatty acid- and retinol-binding protein secreted by adult *Ancylostoma caninum* hookworms: gene transcription pattern, ligand binding properties and structural characterisation. Molecular and Biochemical Parasitology 126:63–71.

Behnke, J. M., Barnard, C. J., and Wakelin, D. 1992. Understanding chronic nematode infections: evolutionary considerations, current hypotheses and the way forward. International Journal for Parasitology 22:861–907.

Bjellqvist, B., Basse, B., Olsen, E., and Celis, J. E. 1994. Reference points for comparisons of two-dimensional maps of proteins from different human cell types defined a pH scale share isoelectric points correlate with polypeptide compositions. Electrophoresis 15:529–539.

Cuff, J. A., Clamp, M., Siddiqui, A., Finlay, M., and Barton, G. J. 1998. JPred: a consensus secondary structure prediction server. Bioinformatics 14:892–893.

Fairfax, K. C., Vermeire, J. J., Harrison, L. M., Bungiro, R. D., Grant, W., Husain, S. Z., and Cappello, M. 2009. Characterisation of a fatty acid and retinol binding protein orthologue from the hookworm *Ancylostoma ceylanicum*. International Journal for Parasitology 39:1561–1571.

Garofalo, A., Klager, S. L., Rowlinson, M. C., Nirmalan, N., Klion, A., Allen, J. E., Kennedy, M. W., and Bradley, J. E. 2002. The FAR proteins of filarial nematodes: secretion, glycosylation and lipid binding characteristics. Molecular and Biochemical Parasitology 122:161–170.

Garofalo, A., Kennedy, M. W., and Bradley, J. E. 2003a. The FAR proteins of parasitic nematodes: their possible involvement in the pathogenesis of infection and the use of *Caenorhabditis elegans* as a model system to evaluate their function. Medical Microbiology and Immunology 192:47–52.

Garofalo, A., Rowlinson, M. C., Amambua, N. A., Hughes, J. M., Kelly, S. M., Price, N. C., Cooper, A., Watson, D. G., Kennedy, M. W., and Bradley, J. E. 2003b. The FAR protein family of the nematode *Caenorhabditis elegans* - Differential lipid binding properties, structural characteristics, and developmental regulation. Journal of Biological Chemistry 278:8065–8074.

Gasteiger, E., Hoogland, C., Gattiker, A., Duvaud, S., Wilkins, M. R., Appel, R. D., and Bairoch, A. 2005. Protein identification and analysis tools on the ExPASy server. Pp. 571–607. *in* J. M. Walker, ed. The Proteomics Protocols Handbook. New York: Humana Press.

Gustad, T., and Robinson, M. 1996. Memory TH2 cells induce alternatively activated macrophages to mediate protection against nematode parasites. Parasite Immunology 18:87–93.

Horton, P., and Nakai, K. 1997. Better prediction of protein cellular localization site with the k nearest neighbors classifier. Proceedings of the 8th International Conference on Intelligent Systems for Molecular Biology 5:147–52.

Jordanova, R., Groves, M. R., Kostova, E., Woltersdorf, C., Liebau, E., and Tucker, P. A. 2009. Fatty acid- and retinoid-binding proteins have distinct binding pockets for the two types of cargo. Journal of Biological Chemistry 18:35818–26.

Kennedy, M. W., Garside, L. H., Goodrick, L. E., McDermott, L., Brass, A., Price, N. C., Kelly, S. M., Cooperi, A. C., and Bradley, J. E. 1997. The Ov20 Protein of the parasitic nematode *Onchocerca volvulus*. The Journal of Biological Chemistry 272:29442–29448.

Liu, Z., Liu, Q., Pesce, J., Anthony, R. M., Lamb, E., Whitmire, J., Hamed, H., Morimoto, M., Urban, J. F., Jr., Gause, W. C., and Anthony, R. 2004. Requirements for the development of IL-4-producing T cells during intestinal nematode Infections: what it takes to make a Th2 cell *in vivo*. Immunological Reviews 201:57–74.

Lupas, A., Van Dyke, M., and Stock, J. 1991. Predicting coiled coils from protein sequences. Science 252:1162–1164.

Nielsen, H., Engelbrecht, J., Brunak, S., and von Heijne, G. 1997. Identification of prokaryotic and eukaryotic signal peptides and prediction of their cleavage sites. Protein Eng. 10:1–6.

Prior, A., Jones, J. T., Blok, V. C., Beauchamp, J., McDermott, L., Cooper, A., and Kennedy, M. W. 2001. A surface-associated retinol- and fatty acid-binding protein (Gp-FAR-1) from the potato cyst nematode *Globodera pallida*: lipid binding activities, structural analysis and expression pattern. Biochemical Journal 356:387–394.

Rausch, S., Huehn, J., Kirchhoff, D., Rzepecka, J., Schnoeller, C., Pillai, S., Loddenkemper, C., Scheffold, A., Hamann, A., Lucius, R., and Hartmann, S. 2008. Functional analysis of effector and regulatory T cells in a parasitic nematode infection. Infection and Immunity 76 (5): 1908–1919.

Robinson, M., Gustad, T., Erickson, M. R., Ferguson, J. L., and David, C. S. 1997. Non-specific binding of IgG1 to *Heligmosomoides polygyrus*: adult worm homogenate superantigen is a target for immunoglobulin-induced inhibition. Parasite Immunology 19:469–474.

Rost, B., and Sander, C. 1993. Prediction of protein secondary structure at better than 70% accuracy. Journal of Molecular Biology 232:584–599.

Suire, S., Stewart, F., Beauchamp, J., and Kennedy, M. W. 2001. Uterocalin, a lipocalin provisioning the preattachment equine conceptus: fatty acid and retinol binding properties, and structural characterization. Biochemical Journal 356:369–376.