ISOLATION OF A cDNA ENCODING AN IgG IMMUNOREACTIVE ANTIGEN (ZINC FINGER PROTEIN) OF *STRONGYLOIDES STERCORALIS*

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Summary :

A full length cDNA encoding an IgG immunoreactive antigen of *Strongyloides stercoralis* is described. A clone containing 1,328 bp insert was selected following screening of *S. stercoralis* cDNA library with an IgG fraction obtained from a pool of 78 *S. stercoralis* positive human sera samples. The nucleotide sequence of the 1,328 bp insert was found to be 70.5 % A/T, reflecting a characteristic A/T codon bias of *S. stercoralis*. The nucleotide sequence of this insert identified a cDNA coding for a zinc finger protein. The conceptually translated amino acid sequence of the open reading frame for the IgG immunoreactive antigen of *S. stercoralis* encodes a 211 amino acid residue protein with an apparent molecular weight of 22.8 kDa and a predicted isoelectric point of 8.71. The diagnostic potential of this IgG immunoreactive antigen of *S. stercoralis* is also discussed.

KEY WORDS : *Strongyloides*, parasite, antigen.

Résumé : ISOLEMENT D'UN ADNc CODANT POUR UN ANTIGÈNE (PROTÉINE EN DOIGT DE ZINC) DE *STRONGYLOIDES STERCORALIS* RÉAGISSANT AVEC DES ANTICORPS IgG

La totalité de l'ADNc codant pour un antigène de Strongyloides stercoralis *réagissant avec des anticorps IgG est décrite. Un clone représentant 1 328 bp a été sélectionné à la suite de l'étude d'une banque d'ADNc de* Strongyloides stercoralis *à l'aide d'un pool de 78 sérums humains positifs à* S. stercoralis. *La séquence nucléotidique des 1 328 paires de bases est de 70,5 % A/T reflétant le biais caractéristique de* S. stercoralis *vers l'usage de codons riches en A/T. La séquence nucléotidique de cet ADNc révèle une homologie avec celles codant pour des protéines en doigt de zinc. La séquence en acides aminés qui a été déduite pour cet antigène caractérise une protéine de 211 acides aminés, d'une masse molaire apparente de 22,8 kDa ayant un point isoélectrique prévu de 8,71. L'utilisation potentielle de cet antigène pour le diagnostic de* S. stercoralis *est discuté dans la partie finale.*

MOTS CLÉS : Strongiloides, *parasite, antigène.*

Strongyloides stercoralis is endemic in tropical and subtropical areas worldwide and in the southeastern United States (for review see, Berk *et al.*, 1987; Liu & Weller, 1993; Grove 1996; Zaha *et al.*, 2000). *S. stercoralis* has unique characteristics, including its ability to replicate in the human host. This ability permits cycles of autoinfection, and a disposition to produce chronic disease. (Grove 1996; Mansfield *et al.*, 1996). Categorization of infection includes acute, chronicuncomplicated, and disseminated forms (Wehner & Kirsch, 1997). Clinical manifestations depend on the particular organs involved, 15-30 % of chronically infected people harbor asymptomatic gut infections (Wehner & Kirsch, 1997). On the other hand, in patients on chronic steroid therapy, hyperinfection can develop with the dissemination of larvae to extra-intestinal organs which results in mortality rates of up to 80 % (for review see, Woodring *et al.*, 1996; Siddiqui *et al.*, 2000a,b,c). The diagnosis of *S. stercoralis* infection is suspected in patients from endemic areas who have blood eosinophilia, and gastrointestinal or pulmonary symptoms (for review see, Liu & Weller, 1993; Wehner & Kirsch, 1997). A definitive diagnosis is established by demonstration of *S. stercoralis* larvae in stool, body fluids, or tissues (for review see, Liu & Weller, 1993; Wehner & Kirsch, 1997). A presumptive diagnosis of *S. stercoralis* infection can be achieved by serology.

The ELISA for the detection of serum IgG against crude extract of filariform larvae can be 85 % sensitive in detecting *S. stercoralis* infection (Neva *et al.*, 1981). However, the specificity of this ELISA is limited (Conway *et al.*, 1994; Siddiqui *et al.*, 1997). Therefore identification of bona fide *S. stercoralis* antigens is essential for the development of a sensitive and specific diagnostic test.

Following is a report on the isolation of a cDNA clone encoding a *S. stercoralis* antigen (zinc finger protein) which is recognized by the host's immune system in natural human infections. This antigen may have some diagnostic potential.

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MATERIALS AND METHODS

Sera and antibody purification

Seventy eight different sera samples were obtained from patients admitted to the James H. Quillen Veterans Affairs Medical Center, Mountain Home, TN (Siddiqui *et al.,* 1997). All of these patients had a significant number of *Strongyloides* larvae in their stool samples and were diagnosed with only *S. stercoralis* infection. The serology and parasite ova examinations of these 78 patients were negative for other parasites (Siddiqui *et al.,* 1997). These 78 sera samples from only *Strongyloides*-infected patients were pooled and presoaked with tissues homogenates of schistosomes, filarial parasites, hookworms and *Onchocerca*. The precleared sera was used to isolate an IgG fraction via affinity chromatography using immobilized Protein A in a hydrophilic matrix, Trisacryl GF2000 (Pierce, Rockford, IL). This IgG fraction was used to screen a directional *S. stercoralis* cDNA library.

cDNA library construction and screening

A cDNA library was constructed as follows: double-stranded, size-selected (> 0.4 kb), high molecular weight cDNA was prepared using oligo-dT primed *S. stercoralis* mRNA (L_3 larvae) which was isolated using magnetic beads (Dynal, Lake Success, NY). The cDNA ends were modified by ligation of linkers encoding *EcoR*I and *Xho*I sites and then cloned into compatible sites present in the multiple cloning region of the Uni-ZAP XR vector (Stratagene, La Jolla, CA). *S. stercoralis* cDNA expression library was screened with the IgG fraction as described earlier (Karcz *et al.,* 1991; Siddiqui *et al.,* 2000b). Primary screening yielded several positive clones, one such clone (16B-4) containing a 1.3 kb insert was selected because of its very high immunoreactivity. This clone was plaque-purified and subcloned into pBluescript. The 1.3 kB insert was sequenced using T3 and T7 primers at the Molecular Genetics Facility, Athens, GA.

Sequence analysis

The sequence was then compared with the GenBank database by BLAST analysis (Altschul *et al.,* 1997). The 1.3 kB insert contained the full-length cDNA for a zinc finger protein of *S. stercoralis* designated SsZFP. The SsZFP sequence has been deposited in GenBank (Accession # AF188206). Multiple sequence alignment of SsZFP with other sequences was performed via MULTALIN software (Corpet 1988).

RESULTS AND DISCUSSION

In our continual effort to improve the diagnosis of *S. stercoralis* we report the cloning of an immunoreactive antigen which may play a role in the deve-

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ggcacgaggagagaactagtctcgagttttttttctaatcaaccaaaattctttttata
atggaaaatcagcaacaagcaacagctagtgaactttgccgtgccggttgtggctttttt
 M  E  N  Q  Q  Q  A  T  A  S  E  L  C  R  A  G  C  G  F  F
ggatcagctactacagagggactttgttctaagtgttataaggattcaatcaaacgcaaa
 G  S  A  T  T  E  G  L  C  S  K  C  Y  K  D  S  I  K  R  K
catgataatgtccgtctttctccatcatcagtgcctcattcaggagcatcaacgtctgat
 H  D  N  V  R  L  S  P  S  S  V  P  H  S  G  A  S  T  S  D
gcatgttcttcgcgtaatgtagatcatgttgctgaacaaattagagaagttgtttctgca
 A  C  S  S  R  N  V  D  H  V  A  E  Q  I  R  E  V  V  S  A
tgtcagtcattaaaatcttctgacatcactcaaaagtcactcgaaaatgtaataagccaa
 C  Q  S  L  K  S  S  D  I  T  Q  K  S  L  E  N  V  I  S  Q
acatcggttgttgttccacatgtagaaaaggtagaccttgcatctgttcaaattccatca
 T  S  V  V  V  P  H  V  E  K  V  D  L  A  S  V  Q  I  P  S
agttctgtttcttcaaaaccatcaataggtaaatcatcctctgacaatcaaccaccaact
 S  S  V  S  S  K  P  S  I  G  K  S  S  S  D  N  Q  P  P  T
gacatttctgtaacaaagaaaacagtaaatcgttgtggaatgtgtaagaagaaagttggt
 D  I  S  V  T  K  K  T  V  N  R  C  G  M  C  K  K  K  V  G
ttgactggatttacatgtagatgtggtggactttattgtagtacacatagatatgattct
 L  T  G  F  T  C  R  C  G  G  L  Y  C  S  T  H  R  Y  D  S
gctcacgattgctcatttgactataggactactgaacgtgaacaaattgcaaagaacaac
 A  H  D  C  S  F  D  Y  R  T  T  E  R  E  Q  I  A  K  N  N
ccaacaattggattcaataaaattgaacgcatctgatgttaactcataatcaccacttttt
 P  T  I  G  F  N  K  I  E  R
aaaagttcaaatattgtactactattaatttttttgtcaaatgtatatctcctatgttaa
gctgtctctttttgaacaaaatttgtcattgatattgattatgtatttctaatttttttt
ttttgcgatacaacaatttttataatattaatattattgttcacattgttgtttgctgt
tcttgagaattagatttatacttttctttctactatttgaaattattctaactttggtga
tacttggagtgttccgatgcctcaaaaataattatatatattcacatatatatgagtact
ttttttttaattaccaaaaaagaagttcttctgtattaattatatagaaacagcggggaag
aatttttcttgatagaatgttctttgcttgttacatttaaccatatgtacatattatttt
ttactttataaatttttttttttgctttttaaatcaattatatatttctcttaattcaatg
taaacatttattattttttttaaagttaaagaagaaatcatttgtatgtttaatgtattc
tgtgatgtttttttttcttgttaataaagtaaaagtaaaaaatttatattaaaaaaaaaa
aaaaaaa
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Fig. 1. – Nucleotide and deduced amino acid sequence of the cDNA encoding a *Strongyloides stercoralis* zinc finger protein. Zinc finger domain AN1 is underlined (amino residues 152-192).

lopment of a serodiagnostic test. To obtain a cDNA clone for this antigen, we have first obtained an IgG fraction from a pool of 78 sera samples obtained from patients with *S. stercoralis* infection (Siddiqui *et al.,* 1997). The screening of *S. stercoralis* cDNA library with this IgG fraction yielded numerous clones. However, one positive clone (16B-4) showed very high immunoreactivity. This clone was selected and an insert of 1.3 kB was obtained following *EcoR*I and *Xho*I digestion. The nucleotide sequencing of this insert is shown in Figure 1. The search of GenBank identified this IgG immunoreactive antigen to be a zinc finger protein (Altschul *et al.,* 1997).

An open reading frame for SsZFP potentially encodes a 211 amino acid residue protein with an apparent molecular weight of 22.8 kDa and an isoelectric point of 8.71. The SsZFP sequence contains a typical poly (A) addition site (AATAAA) 22 bases upstream of poly (A) tail that is composed of 17 bases. Further, SsZFP sequence has a characteristic A/T bias (Moore *et al.,* 1996; Siddiqui *et al.,* 2000a,b,c), and is 70.5 % A/T. The full length sequence of SsZFP exhibits 42 % identity in amino acid composition with *Caenorhabditis elegans* zinc finger protein (Z71262). SsZFP also showed 32 % identity in amino acid sequence with mouse (AF062071) and human (PZNF216) zinc finger protein ZNF 216 (Fig. 2). The comparison of SsZFP sequence and those of free-living nematode (*C. elegans*), mouse and human zinc finger protein ZNF 216 suggest that all share a common ancestry (Fig. 2). The ZNF216 gene is highly conserved between human and mouse, contains two regions that show homology to the putative zinc finger domains of other proteins, and may be involved in autosomal recessive nonsyndromic hearing loss (Scott *et al.,* 1998). However, the function of SsZFP is yet to be elucidated.

Protein Families Data Base of Alignments and Hidden Markov Models (Pfam HMM) search revealed, SsZFP amino acid sequence contains a AN1-like (Linnen *et al.,* 1993) zinc finger at the C-terminus from amino residues 152-192 (Fig. 1). Seven distinct ABlock Motifs were also detected in the SsZFP sequence. More importantly, Transfac search (Quandt *et al.,* 1995) of the SsZFP nucleotide sequence showed the presence of four (nucleotides, 191-180, 450-461, 201B212, and 260-271) consensus sequences (NWWWATCATNNN) of *C. elegans* skn-1 motif, a maternal gene product (Blackwell *et al.,* 1994). In *C. elegans,* skn1 is usually required for embryonic endodermal and mesodermal specification and for maintaining differentiated intestinal cells postembryonically (Walker *et al.,* 2000), its role is *S. stercoarlis* is yet to be elucidated. Furthermore, The prediction for SsZFP according to the neural networks method for cytoplasmic/nuclear discrimination was found to be nuclear with 94 % reliability (Reinhardt & Hubbard, 1998).

Several immunodiagnostic assays for the detection of *S. stercoralis* have been tested over the years with

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          1                                                   50
    SsZFP  .MENQQQATA SELCRAGCGF FGSATTEGLC SKCYKDSIKR KHDNVRLSP.
 MouseZFP  MAQETNQTPG PMLCSTGCGF YGNPRTNGMC SVCYKEHLQR QQNSGRMSPM
 HumanZFP  MAQETNQTPG PMLCSTGCGF YGNPRTNGMC SVCYKEHLQR QQNSGRMSPM
    CeZFP  MENEQQQAQT APSCRAGCGF FGASATEGYC SQCFKNTLKR QQDTVRLTSP
Consensus  m.##q#Qa.. ..lCraGCGF %G...T#G.C S.C%K#.lkR qq#.vR$sp.

          51                                                  100
    SsZFP  SSVPHSGAST SDACSSRNVD HVAEQIREVV SACQSLKSSD ITQKSLENVI
 MouseZFP  GTASGSNSPT SDSASVQRAD AGLNNC.EGA AGSTSEKSRN VPVAALPVTQ
 HumanZFP  GTASGSNSPT SDSASVQRAD TSLNNC.EGA AGSTSEKSRN VPVAALPVTQ
    CeZFP  VVSPSSMAAT SSALKSEP.. .......... ......SSVD MCMKAAVSVS
Consensus  ...p.S.a.T Sda.ss...d .......e.. ....s.kS.# ...kal..v.

          101                                                 150
    SsZFP  SQTSVVVPHV EKVDLASVQI PSSSVSS.KP SIGKSSSDNQ PPTDISVTKK
 MouseZFP  QMTEMSISRE DKITTPKTEV SEPVVTQPSP SVSQPSSSQS EEKAPELPKP
 HumanZFP  QMTEMSISRE DKITTPKTEV SEPVVTQPSP SVSQPSTSQS EEKAPELPKP
    CeZFP  DETA...... .KMDCEDIIN VCDQINDDSV TVAESTAPTT ITVDVPVPVK
Consensus  ..T....... .K.d...... ....!...sp s!..ss.... ...d..vpkk

          151                                                 200
    SsZFP  TVNRCGMCKK KVGLTGFTCR CGGLYCSTHR YDSAHDCSFD YRTTEREQIA
 MouseZFP  KKNRCFMCRK KVGLTGFDCR CGNLFCGLHR YSDKHNCPYD YKAEAAAKIR
 HumanZFP  KKNRCFMCRK KVGLTGFDCR CGNLFCGLHR YSDKHNCPYD YKAEAAAKIR
    CeZFP  KANRCHMCKK RVGLTGFSCR CGGLYCGDHR YDQAHNCQFD YKTMERETIR
Consensus  k.NRC.MCkK kVGLTGF.CR CGgL%Cg.HR Yd.aH#C.%D Ykt.ere.Ir

          201         214
    SsZFP  KNNPTIGFNK IERI
 MouseZFP  KENPVVVAEK IQRI
 HumanZFP  KENPVVVAEK IQRI
    CeZFP  KNNPVVVSDK VQRI
Consensus  K#NPv!v.#K !#RI
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Fig. 2. – Alignment of the *Strongyloides stercoralis* zinc finger protein with zinc finger proteins ZNF 216 other species. The alignment was performed with MULTALIN software. SsZFP = *Strongyloides stercoralis* zinc finger protein (AF188206); CeZFP = *Caenorhabditis elegans* zinc finger protein ZNF 216 (Z71262); Mouse ZFP = *Mus musculus* zinc finger protein ZNF 216(AF062071); Human ZFP =*Homo sapiens* zinc finger protein ZNF 216 (PZFN216). Consensus levels: high = 90 % low = 50 %. Consensus symbols: ! is anyone of IV, $ is anyone of LM, % is anyone of FY, # is anyone of NDQEBZ.

limited success. These assays include skin testing with larval extracts, indirect immunofluorescence using fixed larvae, radioallergosorbent testing for specific IgE and gelatin particle agglutination (for review see, Liu & Weller, 1993; Grove, 1996). An ELISA for detecting the serum IgG against a crude extract of the filariform larvae of *S. stercoralis* is also available (for review see, Liu & Weller, 1993; Grove, 1996). The sensitivity and specificity of this ELISA can be improved to about 85 % if the serum samples are preincubated with *Onchocerca* antigens before testing (for review see, Gam *et al.,* 1987; Genta, 1989; Liu & Weller, 1993; Conway *et al.,* 1994; Grove, 1996). The ELISA is available only at specialized centers and a very limited number of clinical diagnostic laboratories. Furthermore this ELISA shows extensive cross-reactivity with hookworms, filaria, schistosomes, *Paragonimus,* and *Echinococcus*; and cannot be used in over 1/3 of the world population which is known to be infected with these parasites (for review see, Liu & Weller, 1993; Grove, 1996, Siddiqui *et al.,* 2000b). On a practical basis, this test is unlikely to be available for a wider use because a constant supply of the *S. stercoralis* larval tissue is needed to obtain the crude antigen preparation and abundant supply of *Onchocerca* antigens is also required for presoaking of sera samples, before performing an ELISA detection test.

A rational way to develop a detection system for *S. stercoralis* is to first identify highly specific and immunoreactive antigens of the parasite and then exploit their immunodiagnostic potential (Siddiqui *et al.,* 1997, 2000b; Ramachandran *et al.,* 1998). SsZFP may turn out to be an important antigen for diagnostic purposes because it is highly immunoreactive, however, we have not yet exhaustively studied its cross-reactivity with other helminth infections. We are now generating recombinant SsZFP to study its specificity for *S. stercoralis.*

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