Molecular Cloning of OVL3.C1, Marker of Putatively Immunity in Onchocerciasis

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ABSTRACT

The molecular cloning of OVL3.C1, a marker of putatively immunity in onchocerciasis is described. OVL3.C1 gene (EMBL GenBank accession number: AJ272105) was identified and isolated from a lambda Uni-ZAP-R cDNA expression library derived from *O. volvulus* infective larvae (L3) mRNA using putatively immune serum. The 798 bp insert possesses an open reading frame (ORF) of 288 bp which codes for a 10.5 kd protein corresponding to 95 amino acid residues. Flanked by 206 and 214 bp untranslated segments respectively at 3' and 5' ends, the ORF possesses the initiator codon and a stop codon These suggest that the ORF contains the entire coding sequence. The deduced amino acid sequence of the gene showed no similarity with known *O. volvulus* protein but was homologous to a portion of mitochondrial DNA thereby testifying the novelty of the gene. Western blotting of total *O. volvulus* antigen extract using plague-purified antibodies from putatively immune subjects revealed a parent protein of 72.7 kd which is higher than the predicted 10.5 kd protein. This indicates the immuno-relevance of the antigen while suggesting post-translational modifications undergone by the native protein. The expression and further characterization of the clone is currently going on.

Key words: ***Onchocerca volvulus***, vaccines, cloning, antigen.

INTRODUCTION

Considerable clinico epidemiological and experimental evidence have been presented in support of the development of protective immunity against human onchocerciasis (Ottessen, 1984, Titanji, 200). Prominent amongst these indicators is the presence of about 5% of the endemic population of hyperendemic onchocerciasis villages who despite long-term exposure of more than a decade, do not develop overt signs of infection under conditions when a majority of the residents do. Such individuals have been termed putatively immune (Ward *et al*, 1988; Elson *et al*, 1994). All of the antigens which induce such protective immunity have not yet been identified, although a number of candidates have been proposed (Bianco *et al*, 1991 and Titanji, 2000). The aim of the present investigation was to identify and clone additional markers of protective immunity against human onchocerciasis. To achieve this objective sera from putatively immune an onchocerciasis patients were employed to screen and expression gene library constructed from *O. volvulus* infective larvae (L3) messenger RNA.

MATERIALS AND METHODS

Sera from 18 putatively immune subjects and their age/sex matched counterparts (Titanji *et al* 1999) suffering from onchocerciasis were pooled separately and employed for library secreening. Fifty microliters of sera were taken from each vial, combined, thoroughly mixed then depleted of anti *E. coli* antibodies according to the following procedure. *E. coli* lysate was prepared as described by Sombrook *et al*.(1989). Briefly, single colonies of *E. coli* (Y1090) were cultured in 50 ml LB medium pH 7.4. to saturation at 37°C. Cultures were centrifuged at 8.000 g for 10 min at 4°C and pellets resuspended in phosphate-Buffered-Saline (PBS) containing 1.0 mM protease inhibitor, Phenylmethylsulfonylfluoride (PMSF). Following a centrifugation step, pellets were frozen then mixed with 2.0 g alumina and ground into a paste as described by Scopes (1987). Ten mililiters of ice-cold PBS containing PMSF (1.0mM) was added to the paste and following centrifugation 5.0 mg of supernatant (lysate) protein was stirred in centrifuge tubes while adding drop-wise, 3.0 ml of 2.5% gluteraldehyde solution to form a gel (immunosorbent). Antisera were diluted 1:10m in PBS-3% bovine serum albumin (BSA) and mixed with the gel. Mixtures were rocked gently for 1h and then centrifuged. Supernatants were repeatedly depleted of *E. coli* antibodies using fresh immunosorbent.

OVL3 library Screening

An expression library constructed in lambda Uni ZAP-R using mRNA from the infective larvae (L3)of *O. volvulus* was kindly donated by Professor Steven Williams of the Smiths College Massachusettes USA. The titre of the library was 10^8-10^{11} plaque forming units (pfu's)/ml.

A kit from Clontech was employed for screening according to the instructions of the manufacturer, except that the blocked nitrocellulore filters were first reacted with the absorbed antiserum described above. After washing off the unbound sera the filters were reacted with a rabbit antibody to human IgG (Sigma),then with biotinylated goat anti rabbit IgG. Detection of bound antibodies was done using avidin-biotinylated horseradish peroxidase complex and its substrate solution, 4-chloro-1-naphtol.

DNA Sequencing

The recombinant plasmid pBluescript SK-was excised in vivo (Stratagene) from the lambda Uni-AP-R recombinant, extracted by alkaline lysis the Sephaglas TM Band Prep kit (Pharmacia). The recombinant was then custom-sequenced by dideoxy-chain termination method of Sanger et al (1977).

Western blotting

To identify the parent protein of OVL3.C1, plaque-purified antibodies were used to probe *O. volvulus* total antigen extract. *E. coli* (XL.1 Blue) culture was infected with lambda Uni-ZAP-R recombinant and plated on LB agar plates. Plates were incubated at 42°C for 3 h during which the phages entered the lytic phase. Plates were transferred to 37°C and overlaid with dry nitrocellulose filters (Satorious) previously saturated with 10 mM Isopropylthiogalactopyranoside (IPTG) (Sigma), an inducer of the Lac operon. Filters were then lifted and washed in Tris-Buffered-Saline (TBS) pH 8.0 followed by 1h blocking step using 0.2% Bovine serum albumin (BSA). Filters were incubated overnight at 4°C in *E. coli* antibody-depleted serum from putatively immune subjects then washed five times in TBS-Tween 20. Antibodies were eluted by incubating filters in 5.0 ml glycine-HCl buffer pH 2.5. Each elution step was done for 6 min at room temperature. The pH of the eluted antibodies was then corrected to 8.0 using 1.0 M Tris and BSA added to 1.0 mg/ml to stabilize the eluted antibodies. Using the Clontech kit, plague purified antibodies were used to probe *O. volvulus* total antigen extract previously separated by SDS-PAGE and transferred onto nitrocellulose filters.

RESULTS AND DISCUSION

Amongst a pool of clones isolated and plague purified, OVL3.C1 was selected for its ability to react with putatively immune serum. Analysis of the clone reveals that it is a 798 bp insert with the longest open reading

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Histidine rich OvL3.C1 open reading frame. 95aminoacids

 215 atggaatttcccagcacgtgctcgactgcttacaaacaaattcc
       M  E  F  S  Q  H  V  L  D  C  L  Q  T  N  S
 260 agagctgttcggcgcgttcggaattttgatccaagaatggaagta
       R  A  V  R  R  V  R  N  F  D  P  R  M  E  V
 305 attcacttttgttggtaaacaaatgcgcaacgatatatttttat
       I  H  F  L  L  V  N  K  C  A  T  I  Y  F  Y
 350 atttaccaggttctaagtttcgttcgttacattttgctaattcat
       I  Y  Q  V  L  S  F  V  R  Y  I  L  L  I  H
 395 ctttttcaagaatttgtacaaaattttcaatccaccactcgtat
       L  F  Q  E  F  V  Q  N  F  S  I  H  H  S  Y
 440 tatcacaaatatcacaataaacacatgaacctacaccaccacaac
       Y  H  K  Y  H  N  K  H  M  N  L  H  H  H  N
485 catctgcatctgcattaa 502
      H  L  H  L  H  *
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Fig 1: Nucleotide sequence of OVL3.C1 gene.

frame (ORF) of 288 bp (fig 2) corresponding to 95 amino acid residues (10.5 kd) including both the postulated initiator codon and the stop codon. This is flanked by 206 bp and 214 bp untranslated segments at 3' and 5' ends respectively (Fig 1). These observations indicate that OVL3.C1 may be comprised of the entire gene length.

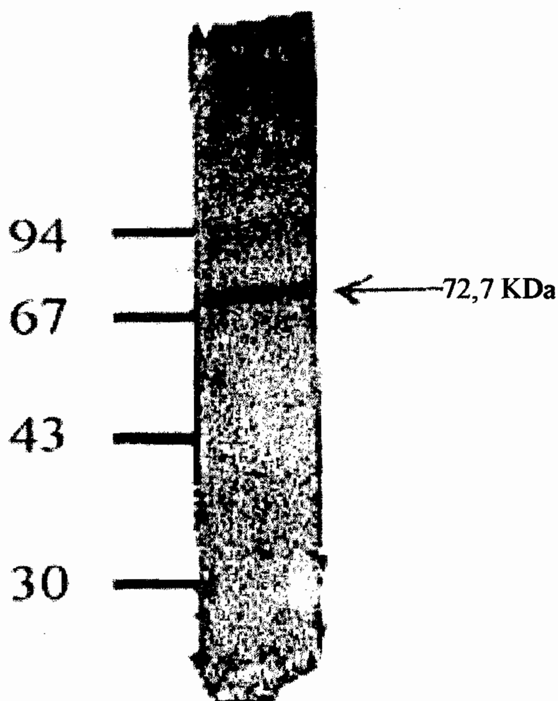

Fig 2: Western blot pattern of OVL3.C1. The immuno blot was probed with plaque-purified antibodies from an onchocerciasis serum pool. Arrow indicates parent protein, horizontal bars the molecular weights of reference proteins

Computer analysis of the nucleotide and deduced amino acid sequences of the putative protein coding region in all the nucleic acid and protein data bases revealed homologies with a portion of the mitochondrial DNA but with no known *O. volvulus* gene. This testifies the novelty of the gene. The Gene was assigned an EMBL Genbank accession number of AJ272105.

In order to identify the parent proteins of OVL3.C1, plague-purified antibodies were used to probe *O. volvulus* total antigen extract by Western blotting. The blot revealed a heavier protein band of 72.7 kd (fig. 3) although the ORF of the inserted DNA predicts a protein of about 10.5 kd. This increase in size may be due to post-translational modifications undergone by the native protein such as N-glycosylation that was shown to increase dramatically the apparent molecular weight of *Trypanosoma brucei* invariant surface protein from 5.0 kd to 100 kd. (Nolan *et al.,* 1997). Also these results indicate that the clone may not contain the complete ORF encoding the native OVL3.C1.

The function of OVL3.C1 is yet to be determined. However, the fact that it is homologous to a region of mitochondrial DNA suggests that it could be involved in energy-production processes. Also, because the clone reacted with putatively immune serum indicates its immuno-relevance. The expression and further characterization of OVL3.C1 is currently going on.

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