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ScienceDirect
Infection, Genetics and Evolution 7 (2007) 155–160
Infection, Genetics and Evolution
www.elsevier.com/locate/meegid

Polymorphism of gp15/400 allergen gene of *W. bancrofti* from different regions of India endemic for lymphatic filariasis

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Received 23 May 2006; received in revised form 17 July 2006; accepted 18 July 2006
Available online 12 September 2006

Abstract

Nematode polyprotein allergens (NPA) are lipid binding/transport molecules that elicit elevated levels of IgE response in the infected host, leading to Th2 type of immune response. They also transport arachidonic acid and its metabolites that are known to be involved in the action of antifilarial drug, Diethylcarbamazine and hence are of great significance for the control of lymphatic filariasis. We investigated the polymorphism of gp15/400 polyprotein of 35 isolates of lymphatic filarial parasite *Wuchereria bancrofti* collected from different geographic locations of India. The repeat sub-unit of the gene was found to be highly conserved in all the isolates with only two nucleotide synonymous changes at positions 286 (A-G) and 337 (C-T). Since this molecule is highly conserved and has multifarious roles in the survival and pathogenesis of the parasite it has good potential as a target for drug, immunodulation tool and immunotherapy development.

Keywords: NPA; gp15/400; *Wuchereria bancrofti*; Polymorphism; Evolution

1. Introduction

The nematode polyproteins are allergen antigens (NPAs) involved in binding small lipids and transportation to muscles and gonads via coelomic cavity (Kennedy, 2000). First characterized in *Ascaris suum* and *A. lumbricoides* and named ABA-1 protein of *Ascaris*, they have been found in worms belonging to numerous species (e.g. *Onchocerca ostertagi*, *Dictyocaulus viviparus*, *A. suum*, *D. imitis*) since then (de Graaf et al., 1995) and have no counterparts in mammals. They are produced in the gut of parasites as large precursor protein complex comprising of 10–50 tandemly repeated polypeptide units, depending upon the species and have a short hydrophobic leader sequence. The repeat units get cleaved at cleavage sites (Arg-Arg-Lys-Arg) of subtilisin serine protease at the C-terminal into functionally similar repeat sub-units of approximately 15 kDa. The allergen, found in all stages of the parasite (Kennedy and Qureshi, 1986), was located as the most abundant protein species in the body fluid of parasites and secreted into that of infected animals (Christie et al., 1992; Kennedy and Qureshi, 1986; McGibbon et al., 1990; Spence et al., 1993; Tomlinson et al., 1989). Very importantly, nematode parasites are unable to synthesize their own complex lipids and derive them entirely from the hosts. A homologue of this polyprotein, called ladder or gp15/400 protein, has been found in filarial nematodes, which is also a complex of approximately 400 kDa with 20 tandemly arranged repeats sub-units of 132 amino acid residues and encoded by a single gene. gp15/400 protein of *Brugia malayi* is associated with the surface and also distributed in all tissues of the parasite (Tweedie et al., 1993). Similar to its counterparts in other nematodes, it is associated with elevated levels of IgE antibodies, thereby inducing allergic type-II (Th2) response in elephantiasis cases (Hussain et al., 1992) and exhibiting strong genetic control of immune response (Kennedy et al., 1990; Allen et al., 1995).

Paxton et al. (1993) have reported that the repeat sub-unit of the gp15/400 gene was highly conserved between brugian filarial parasites, *B. malayi* and *B. pahangi*, but exhibited small degree of divergence in *Wuchereria bancrofti* with 21 nucleotides (and 7 amino acids) variation (94% identity) and great degree of divergence in *Dirofilaria immitis* with only 57% identity. However, information on the diversity in the structure of lymphatic filarial antigens sequenced to date is extremely limited, both between closely related species and (presumably)

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doi:10.1016/j.meegid.2006.07.006

between different strains of the same species (Paxton et al., 1993). We therefore sequenced the repeat units of gp15/400 protein gene from 35 *W. bancrofti* isolates collected from different geographic areas of India in order to see the extent of nucleotide polymorphism, compared its nucleotide sequence with that of other filarial, non-filarial and parasitic nematodes and examined the evolutionary relationship in relation to its host inhabitation and parasitic life.

2. Materials and methods

2.1. Study areas, sample collection and purification of microfilaria and DNA

A total of 35 blood samples were collected from microfilaria (mf) carriers residing in locations representing different geographic regions of India (Hoti et al., 2003). Eighteen of these were from Pondicherry town in southern peninsular India, one each from two villages near Pondicherry (Chinnanergunam and Athipakkam, Tamil Nadu state), three from Rajahmundry town in southern state of Andhra Pradesh, five from Jagadalpur town in Chattisgarh state (central India) and seven from Varanasi town in northern region (Uttar Pradesh state) of India. While Varanasi and Kozhikode are highly endemic for bancroftian filariasis, the other locations are moderately endemic (Sabesan et al., 2000). Five milliliters of venous blood was collected from each mf carrier during night hours (20.00–22.00 h), after obtaining written consent from him/her. EDTA (final concentration 1 mM) was added to the blood sample to prevent it from clotting and the sample was stored at 4 °C. The mf were separated from the blood samples by membrane filtration technique (Dennis and Kean, 1971) followed by Percoll-Sucrose gradient centrifugation technique (Chandrasekar et al., 1984). The genomic DNA from mf was extracted following the method described earlier (Hoti et al., 2003).

2.2. PCR reaction condition and sequencing

Single repeat unit of polyprotein allergen (gp15/400) gene of isolates of *W. bancrofti* from different geographical regions was amplified using gene specific primers (Wbgp15f-5′-TGGCTT-ACGGATGCCCAAAAGG-3′and Wbgp15r-5′-ACTTAGATG-CGTCCGAAAATAG-3′) (Paxton et al., 1993). The PCR mixture consisted 100 ng of purified DNA, 5 μl of 10× buffer, 20 pmol of each primer, 20 mM dNTP, 2.5 mM $MgCl_2$ and 2 units of Dynazyme II Ext Taq DNA polymerase (Finnzymes, Finland), in reaction volume of 50 μl. Amplification was carried out in a Master Cycler Gradient (Eppendorff, USA) and the temperature cycles consisted of an initial denaturation at 96 °C for 4 min followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min and extension at 72 °C for 2 min with a final extension step of 72 °C for 7 min. The amplified products were resolved in 1.5% Agarose gel, stained in ethidium bromide and observed under UV transilluminator. The amplified product was purified using nucleotide removal kits (Qiagen, German) and sequenced in an automated DNA sequencer (ABI 7000). The nucleotide sequences of the isolates have been deposited in the GenBank and have the accession numbers from DQ 321502 to DQ321536. The sequences of the NPA gene of other nematodes were obtained from the GenBank (Fig. 4). The sequences of *W. bancrofti* gp15/400 sub-unit, which are 396 bp in length were aligned with those of other nematodes using BioEdit programme (Hall, 1999), and edited by trimming off those that extended beyond the length of the former. The dendrogram was constructed using the sequences thus edited employing MEGA 3.1 programme (Kumar et al., 2004). MEGA 3.1 (Molecular Evolutionary Genetic Analysis) programme is an integrated tool for automatic and manual sequence alignment, inferring phylogenetic trees, mining web-based databases, estimating rates of molecular evolution, and testing evolutionary hypotheses. Pairwise DNA matrices were generated using the Kimura two-parameter model and phylogenetic analyses, using program and tree topologies were inferred by the unweighted pair-group method with arithmetic mean (UPGMA) with the Kimura two-parameter distance matrices. Bootstrap resampling (1000 data sets) of the multiple alignment tested the statistical robustness of the trees.

3. Results and discussion

NPAs, apart from binding small lipids, have also been reported to bind to haeme and divalent metal ions, arachidonic acid and its metabolites, lyso-platelet activating factor, lysophospholipids and retinoids, as also sequestering pharmacologically active lipids (Kennedy, 2000). The binding property of these proteins with arachidonic acid and its metabolites and platelet activating binding factor are of great significance to lymphatic filarial parasites as Diethylcarbamazine, the only available antifilarial drug, acts through interfering with the arachidonic acid pathway (Maizels and Denham, 1992). Principally, our interest in the polymorphism of this gene stems from facts that it is involved in (a) pathogenesis of lymphatic filariasis (Paxton et al., 1993) and hence may have potential to serve as effective components for immunodiagnostics and immunomodulation for controlling pathology, (b) transport of Arachidonic acid and metabolites (Kennedy, 2000), which in turn are involved in the action of antifilarial drug, Diethylcarbamazine and hence polymorphism of this gene, if exists, would affect the disease outcome of the infection and the response of the parasite to DEC treatment, and lastly (c) transports lipids from host to parasite (Barrett et al., 1997) and has no counterpart in mammals (Kennedy, 2000) and therefore forms a potential drug/vaccine candidate. In fact recent studies have shown that it is a potential candidate for vaccine development (McGibbon and Lee, 1990; Vercauteren et al., 2004).

Amplification using gp15/400 gene specific primers (Paxton et al., 1993) yielded a ladder like profile on gel with amplicons ranging from approximately 400–1200 bp, in multiples of 400 bp (Fig. 1) from all the *W. bancrofti* isolates collected from different geographic locations of India. A faint band of 1600 bp was also observed in the case of some isolates. But, upon repetition of amplification, it was found in all the isolates. This is similar to that reported for Indonesian isolate of *W. bancrofti* and other filarial nematodes, *B. malayi* and *B. pahangi*, by other