Characterisation of a repeated DNA sequence specific for the Direction of Human lymphatic filarial parasite Wuchereria bancrofti and the enchancement of sensitivity by the Polymerase chain reaction

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Abstract:

A genomic library constructed in EMBL3 with Wuchereria bancrofti DNA partially digested with say I was differentially screened with 32p-labelled W.bancrofti and human total DNA, and a strongly reactive recombinant, EMBL3Wb34 was isolated. This clone contained an approximately 16 kb insert which showed some crosshybridization with Brugia malayi and Brugia pahangi DNA. However, a 969 bp subclone derived from EMBL3WB34, designed pWb 12, hybridized only with W.bancrofti DNA and was able to detect as little as 300 pg of W.bancrofti DNA. Furthermore, pWb 12 could detect DNA from a single infective larva or one microfilaria. It has a miderate copy number (450-700) and appears to be interspersed within the parasite genome. The nucleotide sequence contains 66 percent A+T and 34 percent G+C and shows no notable internal repeats. The sensitivity and specificity of the pWb 12 DNA probe for the detection of filarial infection was studied by comparing radiolabelled method, non-radiolabelled colorimetric and chemiluminescence methods of DNA hybridisations with the microscopical findings from thick blood films and was found that all DNA hybridisation assays were more sensitive than parasitological methods of diagnosis. Furthermore, the non-radiolabelled chemiluminescence assay of detection was evaluated as the best comparable to microscopy and radiolabelled methid of DNA hybridisation. For the enhancement of the sensitivity of DNA probe hybridisation assay, the polymerase chain reaction (PCR) was applied for specific amplification of W.bancrofti DNA. Accordingly, oligonucleotide primers were designed to amplify a 490 bp DNA fragment in the 5' end of the pWb 12 repeated DNA sequence in W. bancrofti. A single microfilatia in 100ul of blood or single microfilaria added to 1 ml of blood or a single L3 larva in a pool of 20 uninfected mowquitoes or 0.4 pg of W.bancrofti genomic DNA added to 100 ul human blood or serum can be detected by this PCR method. The parasite DNA in human blood and hydrocele samples, and in misquitoes were isolated free if aby PCR inhibitors by simple purification techniques. Detection of PCR products was carried out by agarose gel electrophoresis, followed by ethidium bromide staining and visualisation under ultra-violet illumination. The results indicate that the PCR method is rapid, more sensitive than that of DNA probes and routine microscopy, and species-specific.

Key Words : Wuchereria bancrofti/Wuchereria bancrofti-genetics/Filariasis/Polymerase Chain Reaction