Analysis of Wucheraria bancrofti Microfilarial antigens and the cloning and chacterization of its action gene

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Abstract:

Several antigens from the microfilarial stage of Wuchereria bancrofti have been identified using immunoblots of microfilarial antigens and screening with immune sera and Tropical pulmonary eosinophilia (TPE) sera. This analysis revealed an array of antigens with apparent molecular weights of 14kD, 35kD, 42kD, 63kD, 88kD, 97kD, and 200kD. Among these only the 14kD and 42kD antigens were consistently recognized by most of the immune sera. A 132kD antigen was recognized only by TPE sera. Analysis of rabbit immune sera revealed that the 42kD antigen was shared by two developmental stages of W. bancrofti, namely L3 and mF. This antigen could become a potential vaccine candidate. The 14kD antigen seems specific for the microfilarial stage and therefore could be a diagnostic marker for active infection. The 132kD antigen could aid in the diagnosis of TPE. Screening of a genomic DNA library of W.bancrofti in EMBL3 with the actin gene of Setaria digitata yielded a clone with an insert size of approximately 13kb. This clone contained the entire actin gene, including the 5' and 3'flanking regions. The swquences around the 5' and 3' splice sites were fairly conserved when compared to the eukaryotic consensus sequences and those of parasitic nematodes. The coding region contained five exons encoding 376 aminoacids and four introns ranging in size from 109 - 190bp. There were no new intron positions when compared to the positions described to date. Only position 170 - 1 was common to three filarial species Onchocerca volvulus, S.digitata and W.bancrofti, the only three filarial species in which the actin gene has been characterized. The W.bancrofti actin aminoacid sequence showed a high degree of homology to the actins of many organisms from varied taxonomic groups, but the highest homology was observed with the free-living nematode Plectus acuminatus. This suggests that P. acuminatus may bare a close evolutionary relationship to W.

bancrofti. At the 5' flanking region, a putative mRNA initiation site and a potential `TATA' box with the sequence TATAAA could be identified, though a sequence similar to the ` CAAT ` box could not be identified. At the 3' flanking region, a potential polyadenylation signal with the sequence ATTAAA could be identified. The G+C content of the entire gene including the 5' and 3' flanking region was 37.2 percent, whilw the G+C content of the coding region was 45.18 percent. The introns had an AT content of 70.33 percent. The codon usage revealed a 63.76 percent preference for T or A in the third position. A Southern blot analysis of W.bancrofti genomic DNA indicated that the actin gene is found as a single copy. A detailed analysis of the aminoacid residues revealed that W. bancrofti actin closely resembles the cytoplasmic actins of certebrates.