

## BRIEF REPORT

# *Wolbachia* Endosymbiotic Bacteria of Filarial Nematodes. A New Insight into Disease Pathogenesis and Control

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Filarial nematodes are parasitic worms that cause some of the most devastating of all tropical diseases such as elephantiasis and river blindness. Studies on the inflammatory pathogenesis of filarial disease have shown that endotoxin-like activity derived from endosymbiotic *Wolbachia* bacteria is the major inflammatory stimulus of filarial nematodes. *Wolbachia* appear to have evolved as essential symbionts of their filarial nematode hosts. Antibiotic depletion of bacteria shows that they are required for normal fertility and development of the worm and may even protect the parasites from host immunity. In addition to the uncovering of a fascinating symbiotic relationship, this discovery means we can now consider using antibiotics as a new approach to the treatment of filarial diseases. © 2002 IMSS. Published by Elsevier Science Inc.

*Key Words:* Filariasis, *Wolbachia*, Pathogenesis, Antibiotic, Symbiosis.

### Introduction

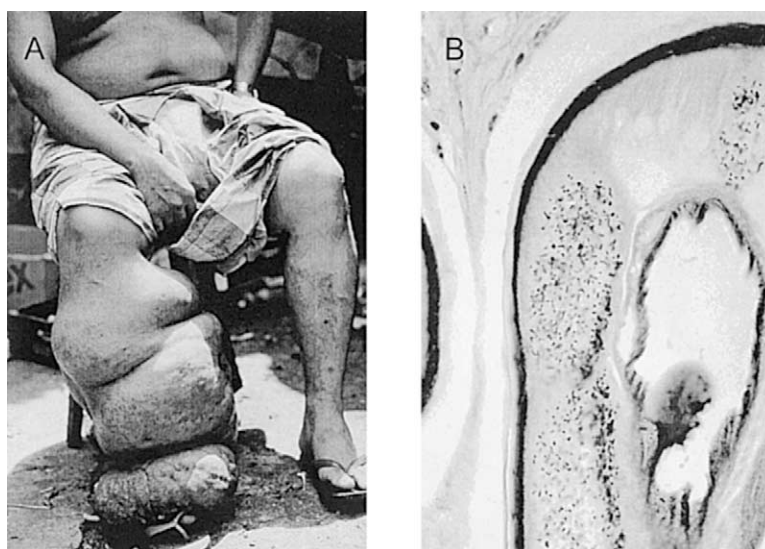
Filariasis is recognized as one of the world's most disabling diseases. More than 150 million people throughout some of the poorest communities in the world are infected with the filarial nematodes *Wuchereria bancrofti*, *Brugia malayi*, and *Onchocerca volvulus*, which are responsible for the majority of human filarial disease (1,2). Pathology of filariasis is associated with a diverse range of inflammatory conditions. In lymphatic filariasis, inflammatory pathology can present as acute inflammation characterized by recurrent attacks of adenolymphangitis associated with death of adult worms, or chronic inflammation associated with hydrocele, lymphedema, and elephantiasis (1) (Figure 1A). In onchocerciasis, pathogenesis is principally caused by the death of microfilariae and subsequent inflammation in the skin and eye (2). Inflammatory responses are also a feature of the adverse reactions to filarial chemotherapy.

### *Wolbachia* in the Inflammatory Pathogenesis of Filarial Disease

Recent studies have shown that the potent inflammatory activity of filarial nematodes is mediated by a bacterial endo-

toxin-like activity derived from intracellular symbiotic bacteria (3,4) (Figure 1B). All pathogenic human filarial nematodes are infected with *Wolbachia* endosymbionts, which appear to have evolved a mutualistic association with their nematode host (5,6). Activation of innate inflammatory responses involves the pattern recognition receptors CD14 and TLR4 and can be inhibited by antagonists of lipid A (3,4). Endotoxin-like activity from these bacteria is the critical mediator of systemic inflammation following ivermectin treatment (3) and is also responsible for the inflammatory pathogenesis of ocular keratitis in a mouse model of onchocerciasis (7). In human filariasis, release of bacteria into the blood following anti-filarial treatment of worms is strongly associated with severe systemic inflammatory reactions in persons infected with *B. malayi* (8,9). This provides direct evidence that on death of the nematode, *Wolbachia* is liberated into the blood and exposed to the immune systems of the host. Bacterial products also appear to be exported from living worms and are responsible for the recruitment of neutrophils surrounding adult *O. volvulus* (10). Studies in animals infected with *B. malayi* have shown a further link between immune responses to *Wolbachia* and development of filarial pathology. Antibody responses to a *Wolbachia* surface protein (WSP) coincide with onset and duration of episodes of inflammatory lymphedema (11). This finding confirms that endosymbiont antigens are recognized by the acquired immune response and lends support to the idea that both innate and acquired immune responses

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**Figure 1.** A) Elephantiasis caused by *Brugia malayi*. (photograph, AE Bianco); B) *Wolbachia* distributed throughout the median and lateral cords of *Onchocerca volvulus*. Bacteria can be seen as numerous black particles scattered throughout the cord cells. Stained using Warthin-Starry method (photograph, BO Duke and the American Registry of Pathology).

to *Wolbachia* may contribute to filarial disease pathogenesis (12).

### **Wolbachia as a Target for Antibiotic Therapy**

Another major area of research to emerge from the discovery of the symbiosis of *Wolbachia* bacteria and filarial nematodes is the targeting of endosymbionts with antibiotic therapy as a novel strategy for the control of filarial parasites and disease. A number of studies in a variety of animal models of filariasis have shown that antibiotic targeting of *Wolbachia* with tetracyclines can have profound effects on the development, viability, and fertility of filarial parasites (13–15). Several weeks of treatment are required to clear bacterial infection although, once eliminated, worms appear unable to regain fertility and viability (16). The major effects of antibiotic treatment are 1) inhibition of development of infective larvae through to adult worms, 2) complete and long-term inhibition of embryogenesis with cytotoxicity of developing embryos and inhibition of transovarial transmission, and 3) eventual loss of viability, including adulticidal activity (13). Evidence to suggest that *Wolbachia* are targets of antibiotic therapy comes from experiments using *Acanthocheilonema viteae*, one of only two species of filarial parasites that appears to be free of *Wolbachia* infection. Antibiotic treatment fails to have any effect on *A. viteae* (14). Also, use of antibiotics known to be ineffective against rickettsial bacteria fail to clear *Wolbachia* and have no effect on filarial parasites (17). The recent finding that long-term antibiotic treatment can result in complete adulticidal activity in the cattle parasite *Onchocerca ochengi* under field conditions is an important observation that may hold promise for a safe and effective macrofilaricide (18). Recent trials in hu-

man onchocerciasis have also shown that doxycycline treatment can eliminate *Wolbachia* for long periods, leading to complete and long-term block of embryogenesis and sustained reductions in skin microfilariae following ivermectin treatment (19,20). In comparison with the tetracycline group of antibiotics, rifampicin treatment has been shown as more active in reducing worm motility, viability, and clearance of microfilaria (21). These studies have established the principle of targeting *Wolbachia* as a future control strategy. In addition to anti-filarial activity of antibiotics, it may be possible through clearance of endosymbionts to reduce or eliminate severe adverse reactions to anti-filarial chemotherapy. Should *Wolbachia* also contribute to the pathogenesis of acute or chronic filarial pathology, antibiotic therapy may then prevent or delay the onset of filarial disease.

### **The Nature of Wolbachia and Nematode Symbiosis**

Although *Wolbachia* have been implicated in disease pathogenesis and hold promise as a new target for treatment, we know almost nothing of the molecular interaction between the bacteria and nematode. One example of a bacterial molecule, which may be important in the symbiotic relationship, is *Wolbachia* catalase, an enzyme that may protect both bacteria and nematode from oxidative damage (22). Evasion of immune-mediated damage would enable long-term survival of filarial nematodes in mammalian host. Interestingly, within the genus *Onchocerca* a single species, *O. flexuosa*, appears free of bacterial symbionts (11). The adult worm is unusually short-lived, surviving only 1 to 2 years in its red deer host (23) in contrast to 10 years or more for symbiont infected species (24).

Further studies on the molecular interaction in *Wolbachia* nematode symbiosis will be accelerated by the complete genome sequencing of bacteria from *B. malayi* and *O. volvulus*, currently underway. Together with on-going filarial genome projects (25), this will provide a framework with which to dissect molecular interaction between bacteria and nematode, which may eventually lead to the discovery of new drug targets. In the meantime, further studies are required to define optimal antibiotic and treatment regimes for different filarial infections. The benefits of using existing antibiotics include their relative inexpense and availability in many endemic areas, a crucial factor in developing countries where filariasis exists.

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### References

- Nutman TB, editor. Lymphatic filariasis. London: Imperial College Press;2000.
- Ottesen EA. Immune responsiveness and the pathogenesis of human onchocerciasis. *J Infect Dis* 1995;171:659–671.
- Taylor MJ, Cross HF, Bilo K. Inflammatory responses induced by the filarial nematode *Brugia malayi* are mediated by lipopolysaccharide-like activity from endosymbiotic *Wolbachia* bacteria. *J Exp Med* 2000; 191:1429–1436.
- Brattig NW, Rathjens U, Ernst M, Geisinger F, Renz A, Tischendorf FW. Lipopolysaccharide-like molecules derived from *Wolbachia* endobacteria of the filaria *Onchocerca volvulus* are candidate mediators in the sequence of inflammatory and anti-inflammatory responses of human monocytes. *Microbes Infect* 2000;2:1147–1157.
- Taylor MJ, Hoerauf A. *Wolbachia* bacteria of filarial nematodes. *Parasitol Today* 1999;15:437–442.
- Casiraghi M, Anderson TJ, Bandi C, Bazzocchi C, Genchi C. A phylogenetic analysis of filarial nematodes: comparison with the phylogeny of *Wolbachia* endosymbionts. *Parasitology* 2001;122:93–103.
- Saint Andre A, Blackwell NM, Hall R, Hoerauf A, Brattig NW, Volkmann LR, Taylor MJ, Ford L, Hise AG, Lass JH, Diaconu E, Pearlman E. The role of the endosymbiotic *Wolbachia* bacteria in the pathogenesis of river blindness. *Science* 2002;295:1892–1895.
- Cross HF, Haarbrink M, Egerton G, Yazdanbakhsh M, Taylor MJ. Severe reactions to filarial chemotherapy and the release of endosymbiotic *Wolbachia* into blood. *Lancet* 2001;358:1873–1875.
- Keisur PB, Reynolds SM, Awadzi K, Ottesen EA, Taylor MJ, Nutman TB. Bacterial endosymbionts of *Onchocerca volvulus* in the pathogenesis of posttreatment reactions. *J Infect Dis* 2002;185:805–811.
- Brattig NW, Buttner DW, Hoerauf A. Neutrophil accumulation around *Onchocerca* worms and chemotaxis of neutrophils are dependent on *Wolbachia* endobacteria. *Microbes Infect* 2001;3:439–446.
- Punkosdy GA, Dennis VA, Lasater BL, Tzertzinis G, Foster JM, Lamie PJ. Detection of serum IgG antibodies specific for *Wolbachia* surface protein in rhesus monkeys infected with *Brugia malayi*. *J Infect Dis* 2001;184:385–389.
- Taylor MJ, Cross HF, Ford L, Makunde WH, Prasad GB, Bilo K. *Wolbachia* bacteria in filarial immunity and disease. *Parasite Immunol* 2001;23:401–409.
- Taylor MJ, Bandi C, Hoerauf AM, Lazdins J. *Wolbachia* bacteria of filarial nematodes: a target for control? *Parasitol Today* 2000;16:179–180.
- Taylor MJ. *Wolbachia* bacteria of filarial nematodes in the pathogenesis of disease and as a target for control. *Trans R Soc Trop Med Hyg* 2000;94:596–598.
- Taylor MJ, Hoerauf A. A new approach to the treatment of filariasis. *Curr Opin Infect Dis* 2001;14:727–731.
- Hoerauf A, Nissen-Pahle K, Schmetz C, Henkle-Duhrsen K, Blaxter ML, Buttner DW, Gallin MY, Al-Qaoud KM, Lucius R, Fleischer B. Tetracycline therapy targets intracellular bacteria in the filarial nematode *Litomosoides sigmodontis* and results in filarial infertility. *J Clin Invest* 1999;103:11–18.
- Hoerauf A, Volkmann L, Nissen-Paehle K, Schmetz C, Autenrieth I, Buttner DW, Fleischer B. Targeting of *Wolbachia* endobacteria in *Litomosoides sigmodontis*: comparison of tetracyclines with chloramphenicol, macrolides and ciprofloxacin. *Trop Med Int Health* 2000; 5:275–279.
- Langworthy NG, Renz A, Mackenstedt U, Henkle-Duhrsen K, de Bronsvort MB, Tanya VN, Donnelly MJ, Trees AJ. Macroparasiticide activity of tetracycline against the filarial nematode *Onchocerca ochengi*: elimination of *Wolbachia* precedes worm death and suggests a dependent relationship. *Proc R Soc Lond B Biol Sci* 2000;267:1063–1069.
- Hoerauf A, Volkmann L, Hamelmann C, Adjei O, Autenrieth IB, Fleischer B, Büttner DW. Endosymbiotic bacteria in worms as targets for a novel chemotherapy in filariasis. *Lancet* 2000;355:1242–1243.
- Hoerauf A, Mand S, Adjei O, Fleischer B, Büttner DW. Depletion of *Wolbachia* endobacteria in *Onchocerca volvulus* by doxycycline prevents reappearance of microfilaridermia after ivermectin treatment. *Lancet* 2001;357:1415–1416.
- Townson S, Hutton D, Siemienka J, Hollick L, Scanlon T, Tagboto SK, Taylor MJ. Antibiotics and *Wolbachia* in filarial nematodes: antifilarial activity of rifampicin, oxytetracycline, and chloramphenicol against *Onchocerca gutturosa*, *Onchocerca lienalis*, and *Brugia pahangi*. *Ann Trop Med Parasitol* 2000;94:801–816.
- Henkle-Duhrsen K, Eckelt VH, Wildenburg G, Blaxter M, Walter RD. Gene structure, activity and localization of a catalase from intracellular bacteria in *Onchocerca volvulus*. *Mol Biochem Parasitol* 1998;96:69–81.
- Kläger S. Das fortpflanzungsverhalten von *Onchocerca volvulus* und verwandter arten: spermienbildung, insemination und fekundität sowie deren änderungen in alternden wurmpopulationen (Dissertation). Tübingen, Germany: Fakultät für Biologie, Eberhard-Karls-Universität Tübingen;1989.
- Karam M, Schulz-Key H, Remme J. Population dynamics of *Onchocerca volvulus*. *Acta Trop* 1987;44:445–457.
- Williams SA, Lizotte-Waniewski MR, Foster J, Guiliano D, Daub J, Scott AL, Slatko B, Blaxter ML. The filarial genome project: analysis of the nuclear, mitochondrial and endosymbiont genomes of *Brugia malayi*. *Int J Parasitol* 2000;30:411–419.