# *Wolbachia*: Potential targets for control of filariases

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Filariae are responsible for devastating diseases in man, including blindness (onchocerciasis) and elephantiasis (lymphatic filariasis), with 150 million infections worldwide[1]. Onchocerciasis is mainly restricted to Africa (inhabits 99% of the infected people)[2], whereas lymphatic filariasis (LF) is widespread and is a major cause of clinical morbidity of humans in the tropics and subtropics, afflicting over 120 million people worldwide[3]. Further, more than 1.1 thousand million people (20% of the world's population) now live in areas where they are at the risk of infection[2]. India alone accounts for 40% of the global prevalence of infection[4].

LF is caused by the blood-borne filarial parasites *Wuchereria bancrofti*, *Brugia malayi* and *B. timori*. These filarial parasites have a complex life cycle and require a mosquito vector for transmission. Infection is initiated by the infective third-stage larvae (L3) via the bite of an infected mosquito. The L3 then migrate to the lymphatics and develop through two further moults to become adult worms (commonly named macrofilariae); following mating the adult female produces an abundance of microfilaria (Mf or Larvae 1) which circulate in the blood stream of the infected host. When ingested by a susceptible mosquito, the Mf migrate to the thoracic muscles and develop through further two moults to become L3, which are transmitted to a new host when the infected mosquito next takes a blood meal[5].

The world community has made it a goal to interrupt transmission and eliminate LF globally as a public health problem by the year 2020, in accordance with resolution WHA50.29 of the Fiftieth World Health Assembly[2,3]. Similarly, the elimination of onchocerciasis as a public health problem in 11 west African countries has been targeted by 2002 (ref. 2). Therefore, control programmes have been launched worldwide with an emphasis on community-wide treatment. The present antifilarias like diethylcarbamazine (DEC), ivermectin and albendazole are mainly targeted at mature Mf and have low macrofilaricidal activity[3,6,7]. Many of the adult parasites, therefore, survive after the chemotherapy and lead to reappearance of Mf several months after treatment. Thus, there is a pressing need for new antifilarial drugs that have macrofilaricidal efficacy and/or that show total and long-lasting suppression of embryo production, to complement currently available microfilaricides. Under these circumstances, *Wolbachia*, the bacterial endosymbiont of the filarial parasites, show great promise as targeting of these bacteria might offer a novel alternative that could have effects on both adult worms and Mf.

## What is *Wolbachia*?

*Wolbachia* is a group of intracellular bacteria, discovered in the seventies[8–11] with the advent of electron microscopy. They belong to the order Rickettsiales and are closely related to the genera *Ehrlichia*, *Cowdria* and *Anaplasma*[12]. They are widespread in the arthropods and important insect pests as well as disease vectors. They are also present in filarial nematodes[13]. It was speculated that these bacteria might be related to the *Wolbachia* symbionts of vector insects and might contribute to the pathogenesis of filarial disease and offer a novel target for chemotherapy[8–11]. However, this fascinating symbiosis between the filarial nematodes and *Wolbachia* remained ignored for a long time, until later identified in the dog heart worm, *Dirofilaria immitis*, as a close relative of *Wolbachia* complex[14].

*Wolbachia* have been detected in majority of filarial species analysed so far, (including the major human filarial parasites *W. bancrofti*, *B. malayi* and *Onchocerca volvulus*)[11,15]; however, the rodent filaria *Acanthochelonema vitae*[16] and the deer parasite *O. flexuosa*[17,18] are devoid of these bacteria. Recently, the bacteria have been visualized by immunohistology using antibodies against bacterial catalase[17] and heat shock protein 60 (hsp60)[19]. The bacteria are widespread in female worms than males and the principal mode of transmission is via the eggs of females[15].

Phylogenetic analysis has shown that all filarial *Wolbachia* are closely related and in general form a group separate from *Wolbachia* of arthropods[15]. The phylogenetic patterns suggest a long coevolutionary history and reciprocal coadaptation between filarial worms and their *Wolbachia* symbionts. Further, treatment with bacteriostatic drug – tetracycline has been shown to inhibit reproduction and development in filarial nematodes harbouring *Wolbachia*. Thus studies support the possibility that *Wolbachia* is necessary to the host nematode and it seems to play an important role in the development, viability and fertility of filarial nematodes; however, the molecular basis of these interactions is unknown[12]. Having remained dormant for about 25 years since their discovery, these bacteria have emerged in an 'epidemic' throughout the filarial research community. The central question now is how the mutualistic symbiotic association between this bacteria and its filarial nematode host can be exploited to control this debilitating and disfiguring disease.

## *Wolbachia* in the pathogenesis of filarial disease

The pathogenesis of filarial disease is characterized by acute and chronic inflammation. Inflammatory responses are thought to be generated either by the parasite, the immune response or opportunistic infection[15]. The presence of large number of bacteria in the parasite tissues in a variety of developmental stages suggests that, either on death of the parasite or through a variety of secretory/excretory mechanisms, the release of bacteria and/or their products might contribute to the inflammatory pathology associated with human filarial disease. The most direct situation in which death of parasites leads to inflammatory responses is in the adverse reaction to chemotherapy. The increase in proinflammatory cytokines and inflammatory mediators following treatment and the clinical presentation of fever, headache, lethargy hypotension and so on, bear many simi-

larities to responses induced by bacterial inflammatory mediators[1,15].

It has been recently reported[20] that bacterial lipopolysaccharides (LPS), present in soluble extracts of the human filarial parasite *B. malayi*, induce potent inflammatory response, including tumour necrosis factor (TNF)-α, interleukin (IL)-1β, and nitric oxide (NO) from macrophages. Extracts from the rodent filaria, *A. vitae*, which is not infected with this endosymbiont failed to induce any inflammatory responses from macrophages, suggesting that the source of bacterial LPS in extracts of *B. malayi* is the *Wolbachia* endosymbiont. Thus, the presence of symbiotic *Wolbachia* in all of the major pathogenic filariae of humans[15] and the apparently exclusive induction of proinflammatory responses by bacterial LPS in *B. malayi* extracts suggest that *Wolbachia* LPS may be one of the major mediators of inflammatory pathogenesis in filarial nematode disease[20].

## *Wolbachia* involvement in the immune response to filariasis

Bacterial antigens and molecules will be present in most, if not all, crude extracts of filarial antigen, as forewarned by Kozek and Figueroa[11]. This might influence the interpretation of a variety of cellular and antibody responses currently thought to be specific to filarial worms – these responses could in fact be cross-reactions to conserved bacterial molecules or, perhaps more interestingly, be specific to *Wolbachia* themselves. In particular, the presence of bacterial endotoxin or other inflammatory mediators is likely to influence the type and magnitude of several components of the immune response, in addition to the induction or potentiation of inflammatory responses. The presence of bacteria and their products needs to be taken into consideration in the interpretation of the majority of molecular, biochemical and immunological investigations[15,21]. The recent information on *Wolbachia* offers a different view of filariasis – as a disease caused by both nematode and bacterial parasites[15].

## *Wolbachia* as a target for chemotherapy

The *Wolbachia* endosymbionts of filarial parasites are found to provide a novel target for antibiotic-based chemotherapy. Evidence from work on animals has showed that these endobacteria in filariae are targets for chemotherapy, since their depletion by tetracycline led to degeneration and sterility of adult worms. Tetracycline treatment had no effect on the development and fertility of *A. vitae*, which are free from *Wolbachia*, suggesting that the antibiotic has no direct activity on nematodes. Dose–response curves have shown that when tetracycline was used for too short a time or at a dose insufficient to deplete *Wolbachia*, worm development and fertility were unaffected. Tetracycline treatment for at least two weeks is required for prophylactic effects, and at least four weeks are necessary to evoke a block of fertility, i.e. degeneration of early embryonic stages. These findings[19,22,23] raised the question whether or not tetracycline therapy could be applied to human filariasis.

Recently, effectiveness of targeting *Wolbachia* in human onchocerciasis with respect to worm fertility and survival has been investigated[1]. With doxycycline (Vibramycin, Pfizer; 100 mg orally per day) for 6 months, it has been shown that targeting of *Wolbachia* in *O. volvulus* leads to sterility of adult worms to an extent not seen with drugs used against onchocerciasis. This study has shown successful endobacterial targeting in the treatment of human filariasis. As with animal filarial, *Wolbachia* seem essential for worm fertility in humans. By contrast with ivermectin, a drug that mainly affects mature Mf, doxycycline totally suppresses normal embryonic development during the early phase, i.e. the oocyte/morula stage. Doxycycline is a registered drug and is readily available. Besides, similar to targeting epicoplast endosymbionts in malaria[24], doxycycline targets metabolic pathways unique to *Wolbachia* and therefore causes little harm to mammalian hosts[1].

Penicillin, gentamycin, erythromycin, azithromycin, ciprofloxacin and chloramphenicol were found to be ineffective against *Wolbachia* in the *L. sigmodontis* model and did not display antifilarial activity. However, rifampicin showed clear prophylactic activity in *L. sigmodontis* and *B. pahangi* infection and onchocerciasis. The antifilarial properties of rifampicin merit further investigation and the efficacy of a combination treatment (tetracycline/rifampicin) and antibiotics plus antifilarial treatment needs to be evaluated[12].

## *Wolbachia* genome consortium

*Wolbachia* genome research is progressing steadily under the auspices of the WHO-sponsored 'Filarial Genome Project' (FGP)[25]. Further, the '*Wolbachia* genome consortium[26] has been established with the objective of providing an organizational structure for scientific interactions and collaborations focused on investigating the genome of *Wolbachia*. Progress in *Wolbachia* genome research might provide clues to answer several unsolved questions. The molecular mechanisms that *Wolbachia* uses to manipulate host reproduction could be exploited to control insect pests and disease vectors. It could provide a set of molecular targets for the diagnosis and control of filarial diseases and for aiding investigations into the pathogenesis of these diseases.


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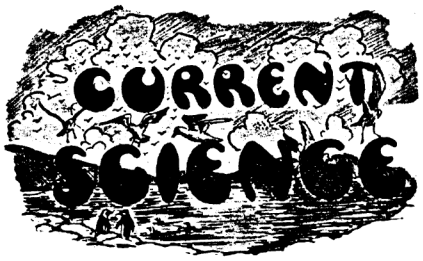


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### The artificial preparation of the male sex hormone

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The male sex hormone may be defined as a chemical compound produced in the testicle, and which in the male organism promotes the growth and function of the sex organs and glands, and also the development and maintenance of the secondary sex characteristics and sex instinct. The discovery of this hormone resulted from successful experiments on castrated male animals, in which the atrophy of the sex characteristics and organs was cured by implantation of the testicles of other adult animals. The first experiments in this direction date as far back as 1849, i.e. long before there existed a science of hormones, when Berthold (Göttingen) successfully implanted fresh testicles into capons.

In 1929 Gallagher, Koch and Moore (Chicago) succeeded for the first time in preparing a really effective testicular extract which exhibited, in castrated animals, effects similar to those formerly obtained by grafting fresh testicles. These investigators also worked out the first practical biological test for the detection of the male sex hormone. It is the so-called capon test, which was subsequently improved by Funk, Laqueur and others, and which is based on the principle that the stuned comb of a capon increases in size by the injection of the male sex hormone, such increase being roughly proportional to the quantity of hormone injected. We call a capon unit the quantity of hormone which, with a definite technique, produces an increase of about 20% in the surface area of the comb.

With the help of this method, Butenandt (Göttingen) isolated in 1931 a male sex hormone in crystalline form from the urine of men; the injection into a capon of 0.3 to 0.4 milligrammes of the said hormone, in fractional doses, in the course of a few days produces a 20% increase in the surface area of the comb. The isolation of this hormone, called androsteron, is extremely laborious and up to the beginning of 1933 only 25 mg of it had been isolated, for which quantity 50,000 liters of urine were required. Butenandt was able to establish that androsteron is a saturated oxyketone having the formula $C_{19}H_{30}O_2$ or $C_{18}H_{28}O_2$, and possessing four rings, although an exact chemical investigation was not possible at that time owing to the difficulty of obtaining sufficient quantities of the hormone. It was, however, possible to form a hypothetical picture of the probable structural formula of androsteron on the basis of the knowledge of the follicular hormone (theelin, oestrin) acquired in the meantime. . . .

From a clinical point of view, it is interesting to note that with capons in which too small a portion of testicle has been preserved for the stunted comb to be able to grow, temporary injections of androsteron cause a prolonged growth of the comb. In completely castrated capons, on the contrary, the comb stops growing on cessation of androsteron treatment, whereupon a gradual atrophy of the comb to its initial size takes place. Such effects have already been observed following the administration of testicular extracts. In certain cases of testicular hypofunction, androsteron can act as a 'hormone fillip' to stimulate the inactive generative glands into new activity. Investigations with mammals in that connection will be of great importance.

Furthermore, in castrated male rats, it was possible to obtain with androsteron a complete cytologic regeneration of the atrophied seminal vesicles (positive test according to Loewe-Voss). Finally the 'wedding dress' picture of the male small fish called *Rhodeus amarus*, which is obtainable with testicular extracts, could also be produced with androsteron. All the experiments which have been carried out in the past with the various extracts exhibiting the action of the male sex hormone and especially with testicular extracts, will be repeated with synthetic androsteron, which will subsequently also be tested clinically. These experiments will show whether androsteron, or any of its derivatives possessing stronger physiological properties, can completely play the role of the male sex hormone.