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Onchocerciasis: the Role of *Wolbachia* Bacterial Endosymbionts in Parasite Biology, Disease Pathogenesis, and Treatment

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INTRODUCTION

Most filarial nematodes, including those responsible for onchocerciasis, harbor an intracellular bacterial symbiont, *Wolbachia*. The presence of intracellular bacteria in *Onchocerca volvulus* was first reported by Kozek and Marroquin (45). In nematodes, *Wolbachia* bacteria are obligate mutualistic endosymbionts, in contrast to the case in most arthropod-*Wolbachia* associations, in which they are generally considered to be reproductive parasites due to their manipulation of host reproduction to enhance transmission (65). In *Onchocerca volvulus* all individual worms and all life cycle stages, contain the endosymbionts. They are present in all developmental stages and inhabit the lateral chords of adult worms and the reproductive system of females, where they are transmitted transovarially (68). *Wolbachia* bacteria are an important target for antifilarial therapy (66). Clearance of the endosymbionts by antibiotic treatment causes inhibition of worm development, blocks embryogenesis and fertility, and reduces viability (36). Our understanding of the biological basis of the symbiotic relationship is limited and is based on comparative genomics of *Wolbachia* and host nematodes. This has suggested that various biochemical pathways which are intact in *Wolbachia* but absent or incomplete in the nematode, including heme, nucleotide, and enzyme cofactor biosynthesis, are candidates for *Wolbachia*'s contribution to nematode biology (61).

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INTRODUCTION TO ONCHOCERCIASIS (RIVER BLINDNESS)

Parasitological and Epidemiological Features

Onchocerciasis is caused by the filarial nematode *Onchocerca volvulus*, which is transmitted by *Simulium* sp. black flies, intermediate hosts that require fast-flowing water for their breeding and development; the disease is thus restricted to areas adjacent to river systems. An estimated 37 million people in 34 countries in sub-Saharan Africa and South America are infected with the disease (4). The large adult female worms are contained within fibrous nodules or onchocercomas in subcutaneous or deeper tissues. Males migrate between nodules to inseminate the females, which when fertilized give birth to 1,000 to 3,000 microfilariae per day that migrate into the skin to be transmitted to their black fly vectors. In communities where the infection is highly endemic, disease prevalence increases with age up to the 30- to 40-year age group and is accompanied by an increasing prevalence of troublesome itching and chronic papular onchodermatitis. Older age groups then begin to acquire skin depigmentation, impaired vision, and blindness (69).

Disease Manifestations

The socioeconomic and public health importance of visual impairment, blindness, and the more widespread dermatitis are profound (4). The spectrum of disease manifestations ranges from asymptomatic/paucisymptomatic infection, or generalized onchocerciasis (GEO), to severe pathology presenting as visual impairment and blindness and acute and chronic skin disease. A large body of evidence supports host immunity to the microfilarial stage as the cause of pathology, with a hyporesponsive immunological state present in the majority of asymptomatic infections (35). The type and magnitude of the immune response and consequent clinical manifestations may be influenced by host genetic factors (48, 50).

Onchocerciasis is characterized by cutaneous and ocular pathology that occurs after the invasion and death of microfilariae in the skin and eye, while adult worms are enclosed in nodules (onchocercomas) in the subcutaneous and deeper tissues. Cutaneous pathology with troublesome itching is the most common manifestation in infected people, driving social stigma due to skin appearance (82) and accounting for 60% of the 1 million disability-adjusted life years (DALYs) for onchocerciasis (85). The spectrum of skin pathology manifestations is broad. The more common generalized form presents with subclinical or intermittent dermatitis (acute and chronic papular dermatitis) that may progress to skin hyperpigmentation or depigmentation (leopard skin) and atrophy with loss of elasticity (hanging groin). A less common but severe hyperreactive form (lichenified onchodermatitis or sowda), a feature of onchocerciasis common in certain geographical areas such as Yemen and Sudan, is characterized by pruritic hyperpigmented hyperkeratotic plaques, often asymmetrical and localized, associated with local lymphadenopathy (49).

Visual impairment and blindness represent the most severe pathological outcomes of onchocerciasis, with 500,000 and 270,000 cases estimated, respectively (85). Their incidence has been dramatically reduced in areas where control programs are implemented (59). The occurrence of ocular pathology varies between geographical locations, being more common in savannah areas of West Africa and Central Africa and in Latin America (6), and has been related to various factors, such as localization of nodules in the upper part of the body (60), vector species (3), microfilarial burdens (47), and parasite strain (88), and more recently to a higher *Wolbachia* load in the more virulent savannah strain (28). The most common ocular pathology involves the cornea, but other structures of the anterior segment and the posterior segment can also be affected. Corneal pathology begins with "fluffy" or "snow-flake" opacities (punctate keratitis), which later coalesce and may become hyperpigmented (sclerosing keratitis). In the anterior chamber dead microfilariae can cause uveitis with formation of sinechiae, cataract, and glaucoma. Posterior segment lesions include atrophy of the retinal-pigment epithelium, choroido-retinal scarring, subretinal fibrosis, and postneuritic optical atrophy (17).

Immune Responses and Pathogenesis of Onchocerciasis in Humans

The host inflammatory response to microfilariae and *Wolbachia* is thought to be the driver of onchocercal keratitis and dermatitis, while retinal lesions may result from autoimmune processes driven by cross-reaction between retinal and parasite proteins (53). Nematode-derived molecules such as proteases may also be involved (26). Consistent evidence shows that pathology is caused by the immune response to the parasite, in a balance between pro- and anti-inflammatory regulation of immune responses, modulated by host factors (genetic background and prenatal exposure). Studies on human onchocerciasis tend to classify patients into three groups. Patients with generalized onchocerciasis (GEO) represent the vast majority of infected subjects and are characterized by having weak or no skin inflammation despite high parasite burdens, while patients with severe chronic dermatitis (sowda) suffer from severe symptoms and present low microfilaria and adult burdens. A third, small subgroup of people living in areas of endemicity do not acquire detectable patent infection despite exposure to infective vector bites and have been termed "endemic normals" (EN) or "putatively immune" (PI). EN have been studied to shed light on the immune mechanisms involved in their supposed refractoriness to infection; however, their true classification as uninfected is difficult to prove and has been debated (75, 81). The majority of studies investigating the relationship between immune responses and pathogenesis of onchocerciasis compared GEO and sowda patients, correlating the low microfilarial loads and the strong T helper 2 (Th2) response to greater severity of pathological manifestations, as seen in sowda (1, 70). Nevertheless, this comparison could be misleading. Indeed, sowda is relatively rare compared to GEO, it is geographically confined, and it has been correlated with specific genetic polymorphisms (2, 31). Sowda is characterized by strong Th2 cytokine responses, high levels of anti-*O. volvulus* immunoglobulin G1 (IgG1) and IgG3 (isotypes involved in EN resistance to infection) and IgE, pronounced eosinophilia, increased circulating levels of eosinophil cationic protein, and increased delayed-type hypersensitivity (7, 9, 70, 72). Eosinophils are probably the effector cells involved in microfilarial killing in sowda. Eosinophils from these patients show high chemotactive responsiveness *in vitro*, and they were the major effector cells of immunity to *Onchocerca* microfilaria in a mouse model of onchocerciasis (18, 55). Taken together, these characteristics suggest that the mechanisms behind the pathogenesis of sowda and GEO might not lie on a continuum, with sowda being instead the result of a hyperreactive Th2 immune reaction to microfilaria, mirroring tropical pulmonary eosinophilia observed in lymphatic filariasis.

GEO is characterized by weak proliferative responses to filarial antigens, low levels of gamma interferon (IFN-γ), and increasing generalized Th2 responses with increasing severity of pathology, but the correlation between low microfilarial loads and severity of pathology in these patients is not clear-cut (70). IgG4 and IgE are the prominent antibody responses, with IgG4 possibly acting as a blocking isotype (20, 40). Vital nondegenerating microfilariae are not attacked by effector cells (granulocytes and mast cells) except after death either through natural attrition or following microfilaricidal treatment (12, 25, 86). In comparison to EN, who show a mixed Th1/Th2 response, both responses appear to be downregulated in infected people and partially restored after ivermectin (IVM) therapy (15, 27).

The majority of infected patients clearly have mechanisms

modulating the host response to prevent immune-mediated damage while allowing high parasite burdens. The fact that both arms (Th1 and Th2) of the immune response are restored after antimicrofilarial treatment shows that the hyporesponsiveness in generalized onchocerciasis is not simply due to a shift from Th1 to Th2 (15). Various mechanisms appear to be involved in the immune downregulation. Production of interleukin-10 (IL-10) has been repeatedly associated with hyporesponsiveness in onchocerciasis (27, 62). The additional neutralization of transforming growth factor β (TFG-β) enhanced but did not completely restore proliferation of peripheral blood mononuclear cells (PBMC) from microfilaridermic patients (15). Antigen-specific regulatory T cells (Tr1), first described in human infectious diseases in onchocerciasis, seem to play a key role in inducing peripheral tolerance by production of IL-10 and TGF-β and expression of cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) (57). Alternatively activated macrophages have been studied extensively in mouse models of filariasis (35), and recently macrophages with an alternative activation phenotype in onchocercomas have been described (44). Moreover, the antigen-presenting functions of dendritic cells have been found to be impaired by exposure to filarial parasites *in vitro* (58). This complex immune modulation has been attributed to molecules secreted by the parasite, such as antioxidants, proteases, cytokine homologues (TGF-β), glycoproteins (ES-62), and lipid mediators (prostaglandin E_2 [PGE_2]) (reviewed in reference 7), and to *in utero* exposure to the parasite (16). Genetic background has also been implicated in the diverse clinical outcomes after exposure, with polymorphisms of several immune response genes, including those for Fc gamma RIIa (CD32), IL-13, and HLA, associated with chronic and hyperreactive skin lesions (2, 13, 50, 71).

WOLBACHIA AND THE INFLAMMATORY PATHOGENESIS OF ONCHOCERCIASIS

Wolbachia and the Inflammatory Response

Wolbachia and *Wolbachia*-derived molecules, as demonstrated in another filarial species, *Brugia malayi*, can be released from worms and come in contact with the host immune system after parasite death or release of excretory/secretory products (5, 8, 43). The characteristics of this interaction can be replicated *in vitro* by exposing innate immune cells to *Wolbachia*-containing parasite extracts, which, in contrast to extracts from aposymbiotic species (*Acanthocheilonema viteae* and *Loa loa*) or extracts from worms depleted of *Wolbachia* by antibiotic treatment of the host, mount a potent proinflammatory cytokine response (67, 76). Figure 1 illustrates *Wolbachia*-induced responses in different immune cell types. Experiments using *Wolbachia*-containing extracts of *O. volvulus* in a mouse model of onchocercal keratitis demonstrated that the presence of the bacteria was essential for neutrophil-mediated inflammation, opacity, and corneal haze (Fig. 2) (see "Role of *Wolbachia* in a Murine Model of Onchocercal Keratitis" below). Neutrophil activation is also observed during adverse reactions following treatment with diethylcarbamazine (DEC) and IVM, which include fever, generalized body pain, pruritus, edema, lymphoadenopathy, and in the case of DEC, which kills microfilaria much more rapidly than IVM, ocular inflammation (10). The occurrence and severity of adverse reactions correlate with microfilarial load (19) and the presence of *Wolbachia* DNA, proinflammatory cytokines, and neutrophil-derived antibacterial calprotectin levels in the blood after microfilaricidal treatment (43). Neutrophils are an abundant inflammatory component of the nodule tissues and are most numerous adjacent to adult worms. Following doxycycline depletion of *Wolbachia*, the neutrophil infiltrate of nodules is drastically reduced (8). This depletion of neutrophils does not depend on the presence of dead worms after treatment and suggests that intact viable parasites are a source of *Wolbachia* inflammatory products released via secretory or excretory processes. The role of *Wolbachia* in the pathogenesis of river blindness is illustrated in Fig. 2.

Role of *Wolbachia* in a Murine Model of Onchocercal Keratitis

Microfilariae invade both the anterior and the posterior segments of the eye (17). In the latter case, they cause uveitis and chorioretinitis, resulting in loss of vision. Due to ethical restrictions on the availability of ocular tissue from human cases of onchocerciasis, tissues from *Onchocerca* dermatitis have been studied, and these show microfilariae surrounded by neutrophils, eosinophils, or macrophages (12, 25). The likely explanation is that neutrophils surround recently dead and degenerating worms, whereas macrophages and eosinophils migrate to the site at later time points. Our findings in a murine model (23) show that neutrophils surround microfilariae in the cornea within 24 h, and immunogold labeling of the major *Wolbachia* surface protein shows neutrophils in close proximity to *Wolbachia* (Fig. 3). Using a similar mouse model of *O. volvulus* keratitis in which filaria-*Wolbachia* extracts were injected into the corneal stroma (24, 56), we demonstrated that endosymbiotic *Wolbachia* bacteria are essential for the pathogenesis of *O. volvulus* keratitis, as (i) *O. volvulus* from individuals depleted of *Wolbachia* by antibiotic treatment does not induce corneal inflammation, (ii) related filarial species containing *Wolbachia* induce keratitis, in contrast to filarial species lacking *Wolbachia*, and (iii) isolated *Wolbachia* bacteria induce neutrophil recruitment to the corneal stroma.

Wolbachia and Toll-Like Receptors in the Cornea

Toll-like receptors (TLR) are surface and endosomal receptors that are expressed in the cornea and respond to microbial products. TLR2 forms heterodimers with TLR1 or TLR6 to initiate signaling through adaptor molecules to induce nuclear factor kappa-light-chain enhancer of activated B cells (NF-κB) translocation to the nucleus and the production of proinflammatory and chemotactic cytokines (42). Our findings using gene knockout mice clearly demonstrate that *O. volvulus* extracts containing *Wolbachia* or isolated nematode or insect *Wolbachia* selectively activates TLR2 and TLR6 and the adaptor molecules myeloid differentiation primary response gene 88 (MyD88) and MyD88 adaptor-like molecule (Mal) (29). Figure 3 shows that corneal inflammation (neutrophil infiltration and increased corneal haze) is entirely dependent on activating TLR2, and the use of chimeric mice also shows that

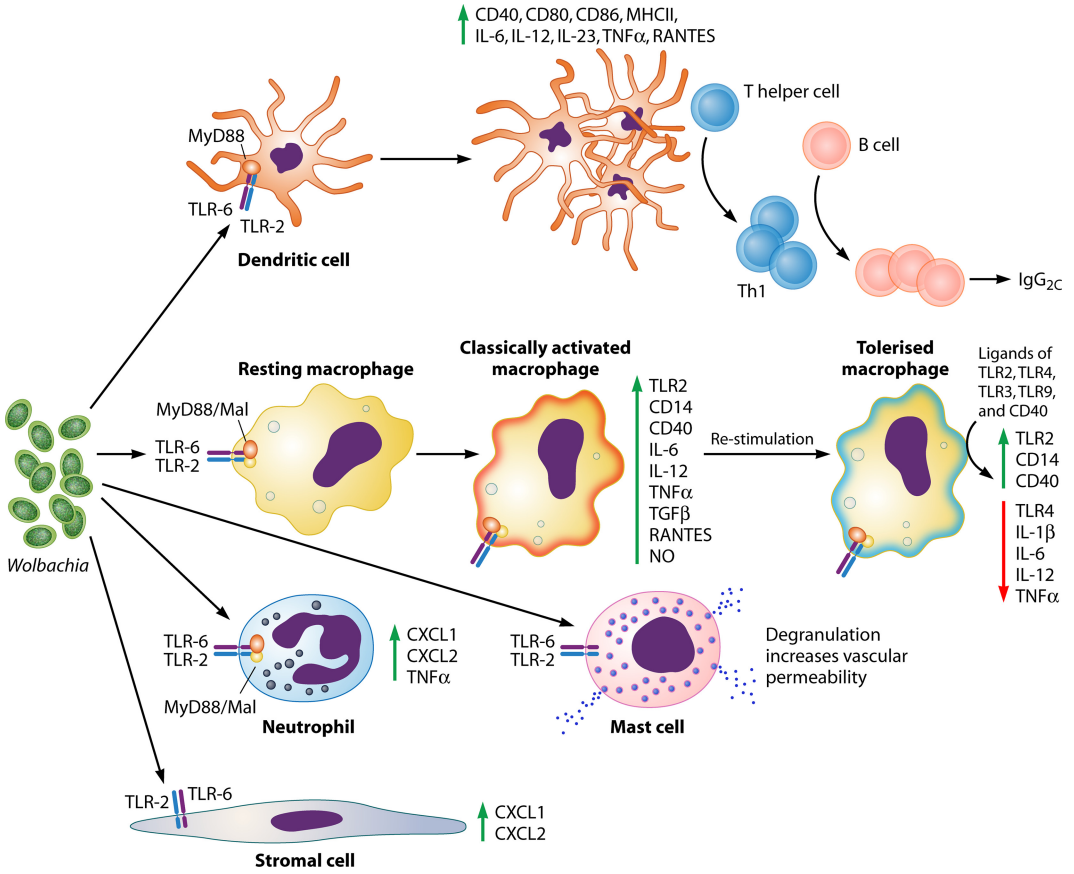

FIG. 1. *Wolbachia*-induced responses of specific cell types. *Wolbachia*-exposed dendritic cells are activated via the TLR2/6-MyD88-Mal pathway, as shown by enhanced expression of surface costimulatory molecules and produce proinflammatory cytokines, inducing a preferential type 1 (Th1) immune response (11, 77). Macrophages stimulated with *Wolbachia* or *Wolbachia*-containing but not *Wolbachia*-depleted filarial extracts enhance their surface expression of costimulatory molecules and produce proinflammatory cytokines and oxidative products. Macrophages can be homo- and heterotolerized by a subsequent stimulation, contributing to the immune downregulation characterizing the majority of filarial infections (29, 67, 76, 77). Neutrophils and corneal stromal cells are also able to interact with *Wolbachia* via the TLR2-MyD88 pathway, producing CXC chemokines and contributing to the inflammatory response to the parasite (22–24). Mast cells are stimulated by *Wolbachia* via TLR2 to degranulate and increase vascular permeability to facilitate establishment of infection (63). Abbreviations: TLR, Toll-like receptor; CD, cluster of differentiation; MHCII, major histocompatibility complex class II; IL, interleukin; TNF-α, tumor necrosis factor alpha; RANTES, regulated upon activation, normal T-cell expressed, and secreted; TGF-β, transforming growth factor β; NO, nitric oxide; MyD88, myeloid differentiation primary response gene (88); Mal, MyD88 adaptor-like.

TLR2 expressed on bone marrow-derived cells has an important role (22).

Taken together, these findings indicate that *Wolbachia* induces TLR2 activation in resident macrophages in the corneal stroma and produces proinflammatory cytokines and CXC chemokines, which mediate neutrophil recruitment from peripheral limbal vessels into the corneal stroma. Neutrophil responses to *Wolbachia* are also dependent on TLR2/MyD88, which mediate cytokine production by these cells and may contribute to degranulation and secretion of reactive oxygen species and matrix metalloproteinases, resulting in cell death and loss of corneal clarity (22, 24).

In chronically infected, untreated individuals, there is also an ongoing adaptive immune response, due to repeated invasion of microfilariae into the corneal stroma and consistent worm degeneration and release of *Wolbachia*. Upon infiltration, eosinophils and macrophages combine to cause permanent tissue damage, which manifests as corneal opacification, loss of vision, and blindness. We found TLR2-dependent *Wolbachia* activation of dendritic cells and T-cell production of IFN-γ but not IL-4 or IL-5 (11). IFN-γ also has an indirect role in enhancing proinflammatory and chemotactic cytokine production and thereby increasing neutrophil recruitment to the corneal stroma (21). Together, these findings demonstrate that TLR2 governs the host response to *Wolbachia* at several levels, including systemic and corneal responses through activation of innate and adaptive immunity.

Identification of a *Wolbachia* TLR2/TLR6 Ligand

The TLR2/TLR6 heterodimer is activated by diacylated lipoproteins. Biochemical removal of lipid and protein from native parasite extracts eliminates all inflammatory activity. Two lipoproteins were consistently predicated from database mining as candidate TLR2/TLR6 ligands, *Wolbachia* peptidoglycan-associated lipoprotein (wBmPAL) and type IV secretion system-VirB6. To examine the response of wBmPAL, corneas of C57BL/6, TLR1$^{-/-}$, TLR2$^{-/-}$, and TLR6$^{-/-}$ mice

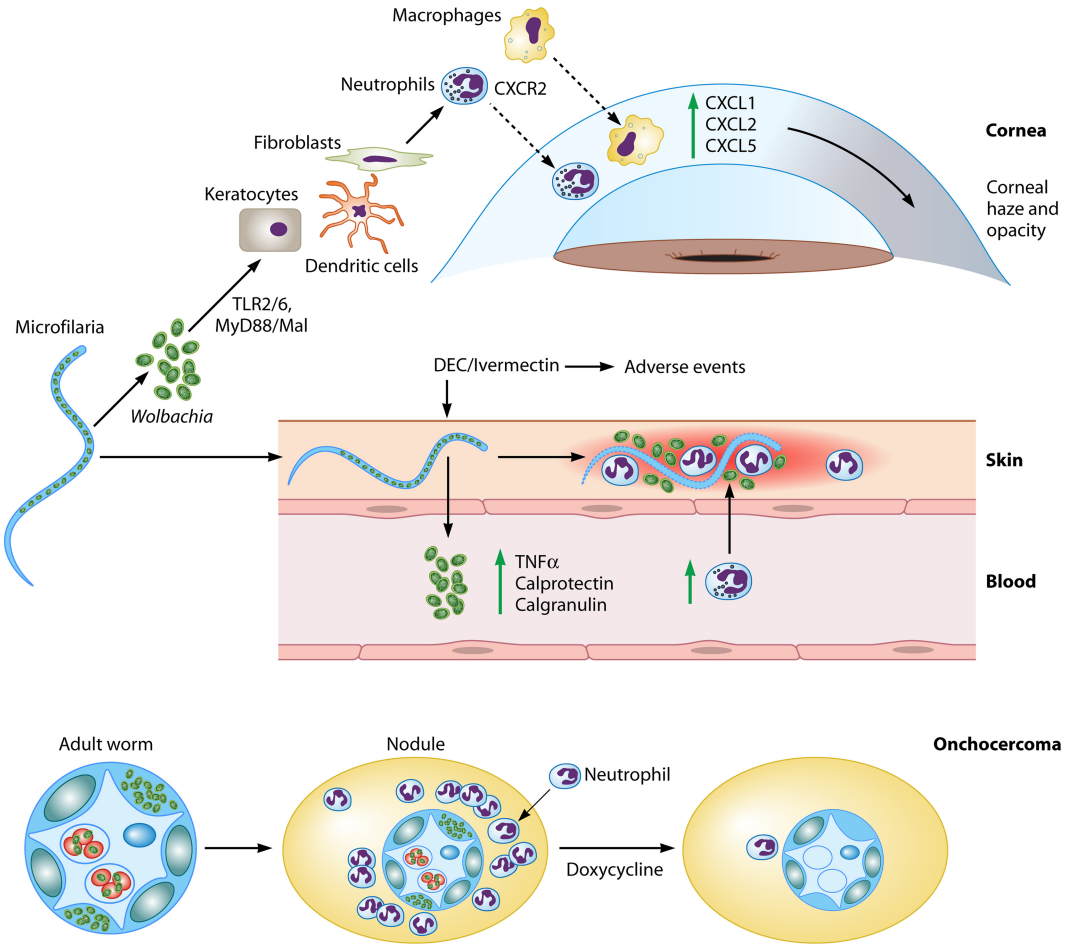

FIG. 2. Role of *Wolbachia* in river blindness. *Wolbachia* release after microfilaria death in the cornea causes corneal edema and opacity by inducing neutrophil and macrophage infiltration and activation in the corneal stroma that are dependent on TLR2-MyD88 activation and production of CXC chemokines. Keratocytes and bone marrow-derived cells in the corneal stroma can initiate this response, which is then perpetuated by inflammatory cells (22–24, 29, 56, 77). When large loads of *Wolbachia* bacteria are released from microfilariae after microfilaricidal treatment, this induces cutaneous and systemic side effects such as fever, tachycardia, hypotension, lymphadenopathy, and pruritus. In the skin, neutrophils are the first cells to be recruited and activated, inducing dermal inflammation. At a systemic level, adverse events correlate with microfilarial loads and are associated with *Wolbachia* DNA and whole bacterial levels in blood, proinflammatory cytokines, neutrophilia, and antibacterial peptides (calprotectin and calgranulin) (25, 43, 52, 80). The presence of *Wolbachia* is associated with neutrophil infiltration in the cornea, skin, and onchocercomas (8, 22, 23). Abbreviations: TLR, Toll-like receptor; MyD88, myeloid differentiation primary response gene (88); TNF-α, tumor necrosis factor alpha; DEC, diethylcarbamazine.

were injected with synthetic diacylated peptides of wBmPAL, and corneal inflammation was examined as before. We found that TLR2$^{-/-}$ and TLR6$^{-/-}$ corneas had significantly impaired neutrophil infiltration, indicating that the interaction between *Wolbachia* lipoproteins and TLR2/6 heterodimers on resident cells in the corneal stroma induces the early stages of *O. volvulus* keratitis (77).

Predicted Sequence of Events in *O. volvulus*/*Wolbachia*-Induced Keratitis

Taken together, studies using the mouse model of ocular onchocerciasis are consistent with the following sequence of events (Fig. 3): (i) the inflammatory response to *Wolbachia* is initiated after death and degeneration of microfilariae and release of bacteria into the corneal stroma; (ii) *Wolbachia* bacteria activate TLR2/6 and MyD88 on resident cells in the cornea, including resident fibroblasts and bone marrow-derived macrophages and dendritic cells; (iii) these cells produce proinflammatory and chemotactic cytokines (21, 22, 29); (iv) neutrophils migrate in a CXCR2-dependent manner through the stromal matrix to the site of microfilarial degradation and release of *Wolbachia* (22); (v) as neutrophils also express TLR2/6 and MyD88, they ingest *Wolbachia* and produce proinflammatory and chemotactic cytokines (22), which stimulate further neutrophil infiltration; and (vi) neutrophil degranulation and secretion of cytotoxic products such as nitric oxide, myeloperoxidase, and oxygen radicals have a cytotoxic effect on resident cells in the cornea, including fibroblasts and corneal endothelium, resulting in corneal edema and further loss of corneal clarity.

Alternative Roles for *Wolbachia*-Mediated Inflammation in Establishment of Infective Larvae

In addition to driving inflammatory components of disease pathogenesis and inflammatory adverse reactions to drugs, the

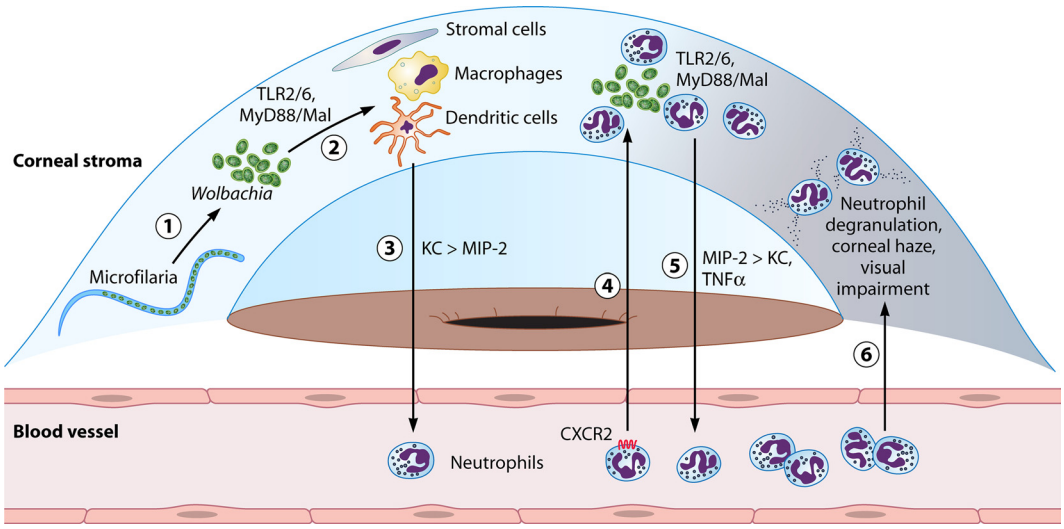

FIG. 3. Predicted sequence of events in *O. volvulus*/*Wolbachia*-induced keratitis. *Wolbachia* released from dying microfilariae in the corneal stroma (1) activates resident cells, including fibroblasts, dendritic cells, and macrophages (2). These cells produce chemokines MIP-2 and predominantly KC (3). KC induces a CXCR2-dependent neutrophil migration to the corneal stroma, where neutrophils are also activated via TLR2/6 by *Wolbachia* (4). Neutrophils produce additional chemokines, predominantly MIP-2, inducing further neutrophil migration (5). Neutrophils degrade the corneal matrix, causing corneal haze and visual impairment (6). Abbreviations: TLR, Toll-like receptor; MyD88, myeloid differentiation primary response gene (88); Mal, MyD88 adaptor-like; KC, keratinocyte-derived chemokine; MIP, macrophage-inflammatory protein 2; TNF-α, tumor necrosis factor alpha; DC, dendritic cell.

stimulation of inflammation by *Wolbachia* appears to have been exploited by the rodent filaria *Litomosoides sigmodontis* to facilitate its infection of the mammalian host (63). Exposure of mice to infective bites from the mite intermediate host results in a chemokine (C-C motif) ligand 17 (CCL17)-dependent infiltration of mast cells into the skin, which degranulate and increase vascular permeability. The recruitment of mast cells and subsequent increase in vascular permeability facilitate the entry of infective third-stage larvae into the host, leading to higher worm burdens in mice deficient in CCL17 than in wild-type controls. Vascular permeability could be blocked by chemical inhibition of mast cell degranulation and was dependent upon the presence of *Wolbachia* and TLR2. Thus, in this case the parasite appears to exploit the *Wolbachia*-mediated induction of inflammatory cells to its own advantage to promote its establishment in the mammalian host.

WOLBACHIA AS A TARGET FOR CHEMOTHERAPY

In Vitro and *In Vivo* Animal Studies

Following the resurgence of *Wolbachia* in filarial research, an obvious priority was to determine whether targeting the bacteria with antibiotics could provide an alternative approach to the treatment and control of onchocerciasis. Initial studies screened a series of antibiotics against *Onchocerca* spp. *in vitro* and in animal models, which showed that tetracyclines and rifamycins were the most potent drug classes to result in adult parasite death (73, 74). Macrofilaricidal activity was first demonstrated in a species infecting cattle, *O. ochengi*, in which a protracted course of oxytetracycline resulted in the elimination of *Wolbachia*, killing of adult worms, and resolution of nodules at 9 months posttreatment (46).

Human Field Trials with Antiwolbachial Drugs

Currently the treatment and control of onchocerciasis rely on a single drug, ivermectin. This is used in mass drug administration (MDA) either annually or biannually or for individual treatment every 3 to 6 months. It is effective at reducing microfilarial loads but is only marginally effective against adult worms and so requires sustained delivery for more than 15 to 17 years in order to interrupt transmission (14, 69).

Doxycycline was the first drug used in trials in human onchocerciasis to deplete *Wolbachia*. First, open trials performed in Ghana administered doxycycline at a daily dose of 100 mg for 6 weeks. This treatment resulted in *Wolbachia* depletion of more than 90% (38), which in later studies has become an empirical threshold for a reduction in bacteria that will lead to death of the worms (13, 78). After 4 to 6 months, embryogenesis was interrupted (33, 38), and patients showed sustained amicrofilaridermia after a single additional dose of the microfilaricidal drug IVM, in contrast to patients who had received IVM only (32). This study was terminated too early to allow assessment of a macrofilaricidal effect, which is seen only after approximately 2 years following treatment with doxycycline. Such an effect was, however, observed in further double-blind, placebo-controlled, randomized trials in Ghana, after administration of 200 mg/day of doxycycline for 4 or 6 weeks or of 100 mg/day for 5 weeks, whereby the highest dose showed a macrofilaricidal effect of up to 60% (70% if worms that had been newly acquired during the observation period were subtracted), in contrast to the 50% (60% after subtraction of newly acquired worms) seen after the 4-week or the 5-week dose (36, 37). The female worms that were still alive at the time of nodulectomy (extirpation of the onchocercomata) were sterile.

Importantly, both sterilizing and macrofilaricidal effects are due to doxycycline alone, as could be shown in a parallel study from Cameroon (79), where doxycycline monotherapy was administered without subsequent IVM, in contrast to trials in Ghana that had used IVM after 200-mg doxycycline regimens. Doxycycline efficacy without IVM had also been observed in the trial using doxycycline at 100 mg/day for 5 weeks (37).

Based on these trials, doxycycline at 200 mg/day for 6 weeks is recommended for patients in whom the highest possible macrofilaricidal activity is desired and who have moved away from areas with ongoing transmission. In those areas where reinfection will be frequent, single-dose IVM yearly is easier to comply with. However, exceptions should be made if the patient is suffering from severe skin disease, as this will resume a few months after IVM since this drug does not lead to a sustained interruption of embryogenesis. If doxycycline treatment aims at permanently clearing microfilariae (the inducers of pathology) from the skin of a patient, a regimen of 4 weeks of doxycycline at 200 mg/day will be sufficient (30).

There has been some skepticism as to whether the current doxycycline regimens are deliverable via community-directed interventions adopted for IVM-based control strategies. Wanji and colleagues, however, proved the skeptics wrong when they showed that more than 97% of ~13,000 people from villages where the infection is endemic who started doxycycline completed a 6-week regimen after community-directed explanation and organization of the delivery (community-directed treatment) (84). Current follow-up studies are aimed at assessing the impact of community-directed doxycycline administration (which comprised, on average, about 74% of the eligible population having started the treatment) on community microfilarial load and thus its impact on transmission.

Even if doxycycline should be administered in restricted areas at the health district level, the problem of contraindication for children and pregnant women remains. The best class of existing registered antibiotics would be macrolides, since they can be given during pregnancy and childhood. Therefore, following promising results by others (54) on the ability of azithromycin to deplete *Wolbachia*, trials on onchocerciasis (and also lymphatic filariasis) were undertaken. Unfortunately, azithromycin did not fulfill its promise and led to neither *Wolbachia* depletion nor antifilarial effects (34). This is in contrast to the case for rifampin, which had shown almost equivalent efficacy in the *L. sigmodontis* mouse model regarding *Wolbachia* depletion and inhibition of larval development (83) and led to considerable depletion of *Wolbachia* and interruption of embryogenesis when administered for 4 weeks to onchocerciasis patients (64). However, this human trial covered only a small number of patients and is currently being repeated in a placebo-controlled manner, from which more precise data on the extent of *Wolbachia* copy number reduction and antifilarial effects will be obtained. Importantly, since rifampin has shown synergistic activity with doxycycline, allowing a reduction of treatment time by 50% in the *L. sigmodontis* mouse model (A. Hoerauf et al., unpublished results), the current trials also involve combination regimens with doxycycline and rifampin using reduced time frames and doses as part of the regimen refinement objective of the Anti-*Wolbachia* (A-WOL) Consortium program (www.A-WOL.com) (see below).

Future Prospects for Use of Antiwolbachial Treatment in the Control and Elimination of Onchocerciasis

Although human trials of antiwolbachial therapy have delivered superior efficacy compared with existing antionchocercal drugs through permanent sterilization and macrofilaricidal activity against adult *O. volvulus*, the prolonged treatment regimens (4 to 6 weeks) and contraindications for doxycycline (pregnancy and age <8 years) remain barriers to the widespread use of these approaches in current control strategy scenarios. The desire to determine whether alternative drugs or combinations could deliver regimens more compatible with MDA approaches led to the formation of the A-WOL Consortium. The A-WOL Consortium product portfolio seeks to deliver (i) optimized regimens of existing drugs for rapid deployment in restricted settings now, (ii) the identification of alternative drugs and combinations which are effective in shorter time frames and safe for children and in pregnancy, and ultimately (iii) narrow-spectrum antiwolbachial drugs which hopefully will ensure that the goal of eliminating onchocerciasis can be ultimately achieved.

Regimen refinement trials are aimed at optimizing regimens of existing antiwolbachial drugs in combination and in reduced time frames for delivery in restricted settings where the urgent deployment of alternative treatment might be warranted. This might include areas where there is evidence for suboptimal efficacy and potential resistance to existing drugs may occur (51), areas where coendemicity of *Loa loa* and the risk of serious adverse events prevent introduction of IVM-based strategies, or where sustained delivery of IVM is compromised. It may also provide a useful tool in program endgame situations where a test-and-treat strategy to identify and treat residual infection could be used if the aim is to achieve elimination of the infection. The macrofilaricidal and permanent sterilization outcomes of antiwolbachial therapy should also drastically reduce the time frames of control programs compared with annual or biannual IVM delivery, which requires at least 15 to 17 years before transmission is interrupted (14).

A comprehensive drug screening strategy has been used against libraries of registered drugs, focused anti-infective drugs, and larger diversity-based synthetic and natural product libraries to identify existing and novel compounds active against *Wolbachia*, which conform to the A-WOL Consortium target product profile for drugs compatible with MDA control programs. Target discovery approaches have also been advanced to provide a core list of essential genes and enzyme targets in pathways validated as important to the *Wolbachia*-nematode symbiosis (39, 41, 61, 87).

The targeting of *Wolbachia* with antibiotics has already delivered a superior efficacy for individual treatment and has achieved the "holy grail" of a safe macrofilaricidal therapy that has so far eluded the field of onchocerciasis chemotherapy. The goal of our ongoing research is to deliver an antiwolbachial therapy that is compatible with MDA programs to sustain and enhance the achievements in onchocerciasis control and deliver the means for elimination of onchocerciasis.

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Continued next page

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