Onchocerca ochengi infections in cattle as a model for human onchocerciasis: recent developments

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SUMMARY

The bovine parasite *Onchocerca ochengi* is a nodule-dwelling filarial nematode, closely related to *O. volvulus*, the causal agent of human River Blindness, and, sharing with it, the same vector. This brief review, based on a presentation at the BSP Autumn Symposium 1999, describes recent work supported by the WHO Drug Development Research Macrofil programme and the Edna McConnell Clark Foundation vaccine development programme, to research the chemotherapy and immunology of onchocerciasis utilising this model system, with experimental infections in Liverpool and field infections in northern Cameroon. In a series of chemotherapeutic trials involving 10 compounds in 20 treatment regimes, the comparability of drug efficacy against *O. ochengi* with that described against *O. volvulus* has been demonstrated. Repeated, long-term treatment with oxytetracycline has been shown to be macrofilaricidal and the effect is hypothesized to be related to action on *Wolbachia* endobacteria, abundant in *O. ochengi*. Avermectins/milbemycins are not macrofilaricidal (even in high and repeated long-term treatments) but induce sustained abrogation of embryogenesis. In prospective, field exposure experiments with naive calves, prophylactic treatments with ivermectin and moxidectin prevented the development of adult worm infection, raising the possibility that drug-attenuated larval challenge infections may induce immunity. Putatively immune adult cattle exist in endemically exposed populations, and these have been shown to be significantly less susceptible to challenge than age-matched naive controls, whereas radically drug-cured, previously patently-infected cattle were not. Experimental infections with *O. ochengi* have revealed the kinetics of the immune response in relation to parasite development and demonstrate analogous responses to those reported in *O. volvulus* infection in humans and chimpanzees. In an immunization experiment with irradiated L_3 larvae, cattle were significantly protected against experimental challenge – the first such demonstration of the experimental induction of immunity in a natural *Onchocerca* host–parasite system. Taken collectively, these studies not only demonstrate the similarity between the host–parasite relationships of *O. ochengi* in cattle and *O. volvulus* in humans, but promise to advance options for the control of human onchocerciasis.

Key words: onchocerciasis, *Onchocerca ochengi*.

INTRODUCTION

The aim of this paper, which is based on a presentation at the BSP Autumn Symposium 1999, is to provide a brief review of aspects of *Onchocerca ochengi* relevant to its experimental use as a model system for *O. volvulus* and to outline some of the recent research results relating to its immunology, immunoprophylaxis and chemotherapy generated in drug and vaccine development projects supported, respectively, by the WHO and the Edna McConnell Clark Foundation. It does not aim to provide a comprehensive review on *O. ochengi*, and much recent, excellent research, notably on the epidemiology of the infection and its consequences for human onchocerciasis by Renz, Wahl, Achu-Kwi and Harnett, is not covered. Many of the results are currently the subject of papers submitted or in preparation and will be only briefly described as unpublished observations, although some of the findings are of significance. For earlier reviews readers are referred to Trees (1992) and Trees *et al.* (1998).

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BIOLOGY, PARASITOLOGY AND INFECTION

Experimental infections

Experimental infections of cattle have been achieved using either fresh L_3s, generated in surrogate British simuliids (McCall & Trees, 1989) by intrathoracic infection of dermal microfilariae (mf) collected and cryopreserved as previously described (Bianco *et al.* 1980; Ham *et al.* 1981), or cryopreserved L_3s, dissected from wild-caught *S. damnosum*, after engorgement on an *O. ochengi*-infected bait cow and cryopreserved with DMSO using a protocol

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Table 1. Summary of parasitological observations following experimental infections with *Onchocerca ochengi*

Calf	No. of L_3 inoculated	Earliest detection of nodules (days p.i.)	No. of nodules detected	Earliest detection of microfilariae (days p.i.)	Peak microfilarial density (per g skin)
704	350	ND*	ND	ND	ND
706	350	600	1	ND	ND
707	350	110	7	279	3330
708	350	209	4	404	7750
709	350	ND	ND	539†	ND
712	350	322	1	348	140
751	700	172	5	294	4000
752	700	187	4	368	2350
C2	1000	210	3	322	167
C9	1000	210	8	294	1230
C11	1000	154	9	294	290
C12	1000	210	2	ND	ND
C13	1000	210	4	382	167
C17	1000	154	16	322	100
C18	1000	182	3	ND	ND
C22	1000	182	4	382	32

* ND, not detected.
† Single biopsy identified positive by PCR.
Animals observed to 600 days post-infection (p.i.) except those inoculated with 1000 L_3s which were observed until 382 days p.i.

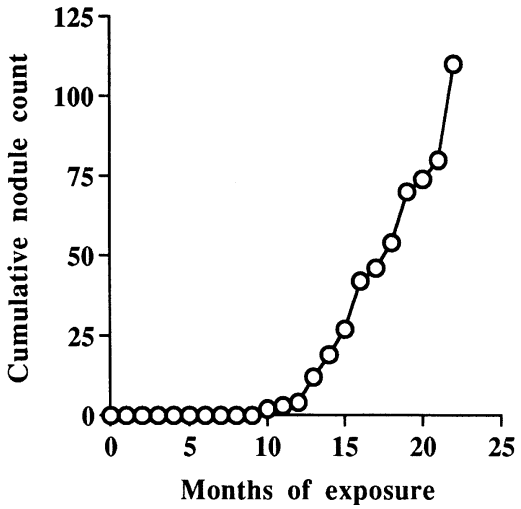


Fig. 1. The rate of acquisition of *O. ochengi* nodules in calves exposed to field challenge. The cumulative group total ($n = 14$) of nodules in naive calves exposed from birth to 22 months is shown.

modified from Lowrie (1983) (Lustigman, personal communication). The infections were produced in the course of experiments to study the kinetics of the immune response and to determine the immunity conferred by immunization with irradiated L_3s (Graham, Trees, Collins, Taylor, Moore, Guy & Bianco, unpublished observations; Graham, Trees, Lustigman & Bianco, unpublished observations). The parasite intensity, time to appearance of nodules and time to first mf detection from a series of infections following a single subcutaneous inoculation at the umbilicus in 2–3 month old calves are shown in Table 1. Infections with 700 or 1000 L_3s led to adult infections in all cattle with a median (and range) time to nodule detection of 182 days (154–210, $n = 10$). Of six cattle inoculated with 350 L_3s, four developed detectable nodules, and in a fifth skin mf were detected by PCR (method of Meredith *et al.* 1991) but not by parasitological means. For all infected cattle, the minimum mf prepatent period was 279 days. There was a trend towards increased nodule number with increased L_3 dose and cryopreserved L_3s were not obviously less infective than freshly dissected L_3s. For 10 animals each inoculated with 1000 cryopreserved L_3s, the worms recovered at 382 days represented 0·83% of the number inoculated. Nodules were distributed over the ventral abdomen and upper hind leg. All of 50 nodules dissected from cattle inoculated with 1000 L_3s contained only one female worm with numbers of males varying from 0 to 3. Of 50 female worms, 17 were fecund at the time of examination and in all cases but one, male worms were present in their nodules. All nodules were intradermal and, after careful post-mortem dissection, no nodules were found in deeper sites. The anatomical distribution of nodules and of mf, and the numbers of worms in nodules was in accord with the properties in naturally infected cattle, except the latter may often carry large numbers of males in some nodules (Trees *et al.* 1992; Wahl *et al.* 1994).

O. ochengi *infections do not cause disease*

Whilst *O. ochengi* infections in cattle serve as an excellent parasitological analogue of *O. volvulus* in humans, the dermal and ocular pathology associated

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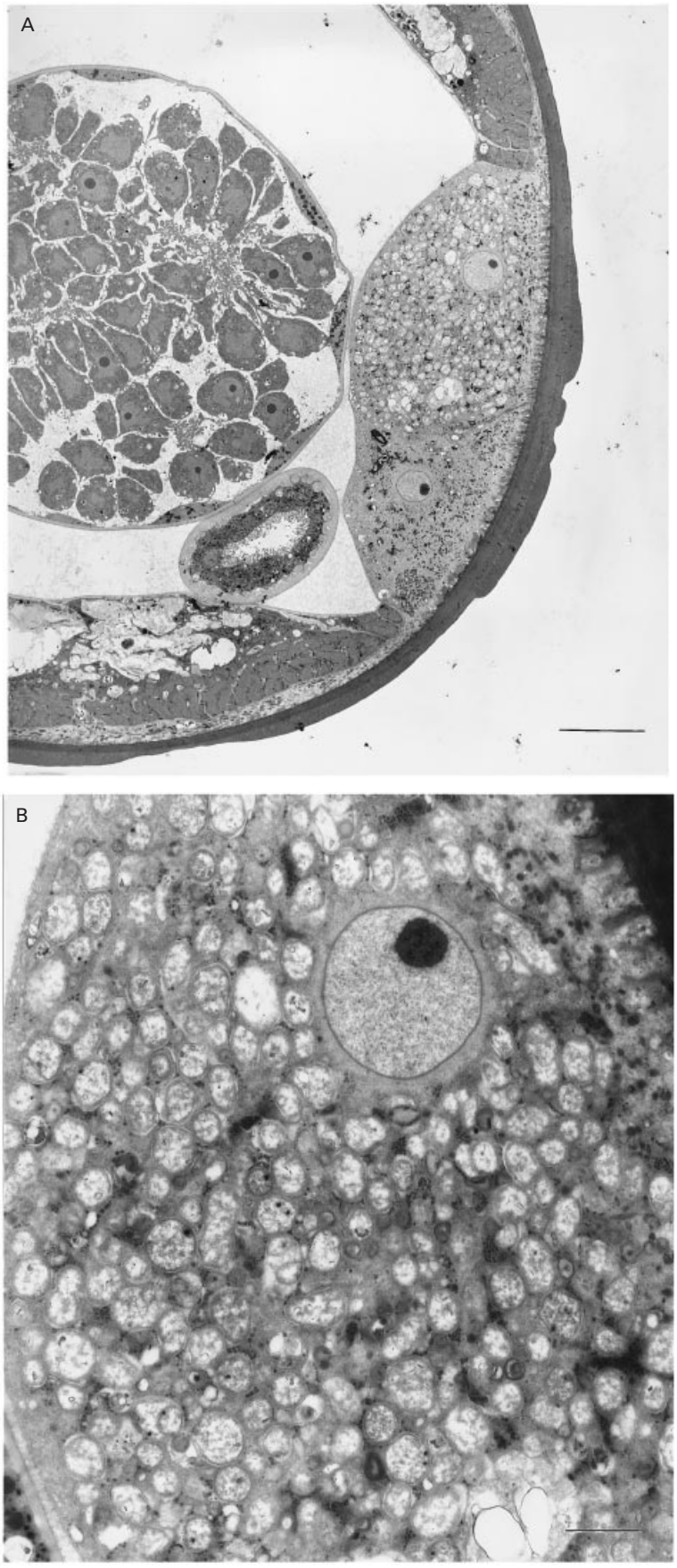


Fig. 2. (A) *Wolbachia* bacteria are abundant in one hypodermal cell in this transverse section of a male *O. ochengi*. The other hypodermal cell appears uninfected (bar, 10 μm). (B) Higher power of a male *O. ochengi* showing packed bacteria in the hypodermal cells, the nucleus of which is prominent (bar, 2 μm).

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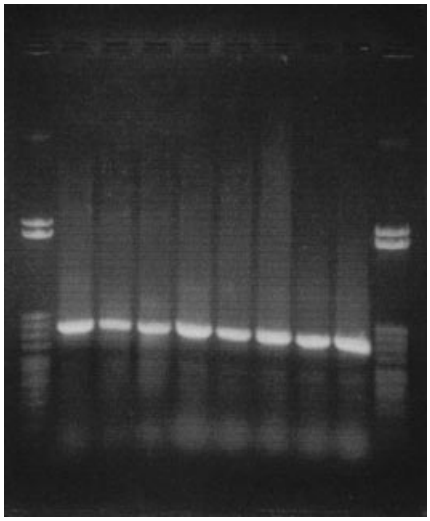


Fig. 3. *Wolbachia*-specific amplification product in each of four male (left) and four female *O. ochengi*. PCR using nested eubacterial and *Wolbachia*-specific 16S rDNA (see text). The amplified product has an estimated size of 766 bp.

with the latter is not observed in *O. ochengi* infections of cattle. Not only has dermatitis not been observed, but we have failed to provoke Mazzotti-like reactions in infected cattle treated with diethylcarbamazine (Renz, Bronsvoort & Trees, unpublished observations). Microfilariae have been found in bovine eyes (Daiber & Renz, unpublished observations) but no pathology has been associated with their presence. The lack of pathology and clinical signs may be a result of an inherently reduced pathogenicity, a lower parasite intensity (mf densities in bovine skin are one or two $\log_{10}$ increments lower than those seen in human onchocerciasis), a difference in the host's immune response, all of which may indicate a better adapted host–parasite relationship. Perhaps an added factor is that cattle do not live long enough to develop clinical signs.

Experimental field exposure reveals the kinetics of nodule acquisition and insusceptible individuals

The experimental infection data are consistent with field data, where, under conditions of high but seasonal challenge, calves become patently infected with nodules as early as 7 months and with mf as early as 10 months of age (Achu-Kwi *et al*. 1994; Tchakouté *et al*. 1999). The rate of acquisition of nodules under ongoing field exposure is shown in Fig. 1 (Gilbert, Langworthy, Tanya, Renz & Trees, unpublished observations). We have not continued experiments for long enough to determine if the rate of increase in nodule density slows, although an earlier cross-sectional study found that there was a progressive increase in group nodule density with age in three age cohorts up to > 8 years of age (Trees *et al*. 1992). In experiments involving exposure of calves from birth to natural challenge, individuals exhibit highly variable parasite loads, and there are some apparently insusceptible individuals (one of six and two of 14 after 21 and 22 months of exposure respectively; Tchakouté *et al*. 1999; Gilbert, Langworthy, Renz, Tanya & Trees, unpublished observations). These proportions are consistent with 3/30 cattle of > 8 years old maintained for life in an endemic area and in which infection was not detectable (Trees *et al*. 1992) and similar proportions of apparently uninfected cattle from the endemic area of the Adamawa reported by Wahl *et al*. (1994 and 1998). These individuals conform parasitologically and in terms of history of exposure with the endemic normals or putative immunes (PIs) recognized in human populations exposed to *O. volvulus*. Unlike human PIs, we have been able to test the susceptibility of such cattle to exposure – see later.

Drugs freely penetrate the nodule and adult male worms

The intradermal nodule of *O. ochengi* comprises a well developed capsule enclosing a matrix in which coiled worms lie. Detailed studies on nodule vascularization, such as done for *O. volvulus* (George, Palmieri & Connor, 1985; Smith *et al*. 1988) have not been conducted but extensive vascularization is obvious in nodule sections. *In vivo* studies have shown that drugs (suramin and ivermectin) readily penetrate into the matrix and nodule wall and achieve concentrations equivalent to or greater than those in plasma and skin (Cross *et al*. 1997). *In vitro* experiments with isolated male worms have demonstrated that ivermectin is readily taken up, predominantly across the cuticle (Cross, Renz & Trees, 1998). Transmission and scanning EM studies show the cuticle of female worms to be invested with an electron-dense coat (of unknown origin) into which cuticle lamellae project (Determann *et al*. 1997; Cross, Renz & Trees, 1998). The male cuticle exhibits a pitted and honey-combed surface appearance (Cross, Renz & Trees, 1998). These structures presumably serve to increase the interface with the surrounding medium and may facilitate molecular exchange across the cuticle.

O. ochengi *contains* Wolbachia-*like bacteria*

Intracellular rickettsia-like bacteria in filariae are currently the subject of much interest and molecular phylogeny has shown them to be closely related to the *Wolbachia* of insects (see review of Taylor & Hoerauf, 1999). In *O. ochengi*, *Wolbachia* are abundant (Determann *et al*. 1997) and can be especially concentrated in hypodermal cells of adults (see Fig. 2). Partial sequencing of the 16S rDNA of the *O. ochengi Wolbachia* (AF 172401) showed that in 858 nucleotides only one base differed from the hom-

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ologous sequence of the bacteria in *O. volvulus* (AF 069069) (Langworthy *et al.* 2000).We have investigated the prevalence of *Wolbachia* infection *O. ochengi* collected from cattle in northern Cameroon by polymerase chain reaction (PCR) analysis of DNA from individual worms using a nested PCR with primers for eubacterial and *Wolbachia*-specific 16S rDNA (Taylor, Bilo & Cross, unpublished observations). All of 89 male worms and 105 female worms were shown to harbour the infection – Fig. 3 (Whittle, McGarry, Cross, Taylor and Trees, unpublished observations). This prevalence study should be extended to populations elsewhere, but the results are consistent with a relationship of mutual dependence between worm and bacterium.

ATTRIBUTES AS A MODEL SYSTEM

High degree of genetic and antigenic similarity with O. volvulus

Some of the attributes of *O. ochengi* as a model have been previously described (Trees, 1992). The close morphological and biological similarities between *O. ochengi* and *O. volvulus* (Bain, 1981), which include sharing the same vector (Wahl, Ekale & Schmitz, 1998), are confirmed by molecular phylogenetic studies (Xie, Bain & Williams, 1994). There is extensive conservation between the two species such that there is a high degree of antibody cross-reactivity to native or recombinant antigens (Hoch *et al.* 1994; Graham *et al.* 1999). This cross-reactivity has potentially important epidemiological consequences and field studies have provided evidence that exposure to *O. ochengi* infective stages cross-protects humans against *O. volvulus* (Wahl *et al.* 1998).

Multiple infection allows sequential evaluations of worm status

An important practical aspect of *O. ochengi* infection in cattle as an experimental system is that multiple infection is common in adult cattle exposed to natural infection in endemic areas. Thus in chemotherapeutic trials, for example, sequential nodulectomies from the same individual are possible over a period of months or years. This, in turn, requires that nodules are identified at the commencement of experiments involving chemotherapy, since it is impractical to prevent exposure to continuing infection and, hence, nodule acquisition, in experiments of long duration under field conditions. Nodules to be examined during the course of therapeutic experiments are pre-designated by mapping their location, marking the location with tattoo ink (three spots at the angles of a triangle enclosing each nodule), and subcutaneously implanting adjacent to each nodule a microchip transponder which carries a unique, ten digit signal. The location of each designated nodule can be subsequently determined by reference to a hide map and percutaneous scanning with the transponder detector to confirm the nodule identity and position, followed by shaving to reveal the tattoo marks.

CHEMOTHERAPY AND CHEMOPROPHYLAXIS

Methods for drug efficacy studies

Efficacy trials of potential macrofilaricides are conducted to a standard design. Naturally infected cattle of 3–5 years of age, each of which has > 20 palpable *O. ochengi* nodules, are recruited and assigned to treatment groups (usually three per group) to match for weight and nodule number. Nodules for subsequent examination are pre-designated (as described above) and, at each sampling, four are excised under local anaesthesia from each animal providing 12 nodules per treatment group at each time point. Skin biopsies are taken simultaneously and occasionally at other times and mf densities determined as previously described (Renz *et al.* 1995). Experiments usually last 9 months and may continue for 2 years.

Following nodulectomy under local anaesthesia, worms can be expressed from cut nodules and immediately obtained clean enough for viability estimates without recourse to collagenase digestion. In therapeutic studies the viability of worms is determined in three ways (triple assay) as described by Renz *et al.* (1995); by motility assessment, ability to reduce tetrazolium (MTT) to formazan (Comley *et al.* 1989*a*, *b*) and a quantitative and qualitative assessment of embryogenesis (Schulz-Key, 1988). MTT reduction is standardized per unit length (10 mm) of worm.

The search for macrofilaricidal agents

There is currently no compound safe enough for mass application to radically cure human infections with adult *O. volvulus* and the development of such a drug is an objective of the MACROFIL programme of the Drug Development Research division of WHO/TDR. *O. ochengi* infections in naturally infected cattle are being used as a tertiary screen for the development of a human macrofilaricide (Renz *et al.* 1995; Trees *et al.* 1998). This project, a collaboration between the Universities of Liverpool and Hohenheim and the Institut de Recherche Agricole pour le Developpement (IRAD), Cameroon, is based near Ngaoundere at the IRAD station at Wakwa. The activities of a number of drugs have been studied, some to validate the model system and others as lead research compounds. Results are summarized in Table 2, updated and adapted from Trees *et al.* (1998). Melarsomine and parenteral UMF-078 (a flubendazole derivative)

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Table 2. Summary of compounds tested for activity against *O. ochengi* in cattle

Compound	Dose	Route*	Frequency	Activity†		Note
				Mf	Adult	
Ivermectin	200 μg/kg	SC	×1	+	−	Renz *et al.* (1995)
	500 μg/kg/m‡	SC	×7	+	−	By 24 months; Bronsvoort *et al.* (unpublished)
Doramectin	500 μg/kg/d‡	SC	×7	+	−	By 24 months; Bronsvoort *et al.* (unpublished)
Moxidectin	500 μg/kg	SC	×1	+	−	In progress
	500 μg/kg/m	SC	×6	+	−	
Suramin	10 mg/kg/d	IV	×6	±	−	By 137 days; Renz *et al.* (1995)
	17 mg/kg/w‡	IV	×7	±	−	By 6 months; Tchakouté *et al.* (unpublished)
Melarsomine	4 mg/kg alternate days	IV	×3	+	+	Tchakouté *et al.* (unpublished)
CG 20376	20 mg/kg	PO	×1	+	−	Renz *et al.* (1995)
WR 251993	140 mg/kg	SC	×1	−	−	
	35 mg/kg	SC	×1	−	−	
UMF 078	150 mg/kg	IM	×1	+	+	Bronsvoort *et al.* (unpublished)
	50 mg/kg	IM	×1	±	±	
	150 mg/kg	IA	×1	+	−	
	50 mg/kg	IA	×1	±	−	
	150 mg/kg/d	PO	×2	+	−	Toxic
Oxytetracycline	10 or 20 mg/kg	IM	×13	+	+	Treatment intermittent over 6 months

* SC, subcutaneous; IV, intravenous; PO, *per os*; IA, intra-abomasal; IM, intramuscular.
† + for adults denotes effect characterized by reduction in nodule size, calcification or disappearance of worms or elimination of worm motility and ability to reduce MTT and for mf complete or almost complete absence from skin biopsies; ± means partial effects or total effect on only some worms; −, none of the foregoing effects.
‡ m, month; d, day; w, week.

were macrofilaricidal (Tchakouté, Renz, Tanya & Trees, unpublished observations; Bronsvoort, Renz, Tanya, Ekale & Trees, unpublished observations). Both compounds are potentially too toxic for mass human application and the development of UMF-078 has been abandoned. Nonetheless, the results showed that death of worms was followed by the resolution of intradermal nodules within months. As an effective macrofilaricide against *O. ochengi*, which is adequately tolerated by cattle, melarsomine provides a useful research tool to investigate immunity in drug-cured cattle (see later).

Avermectins/milbemycins and their action against adult Onchocerca

It has been difficult in human studies to determine the macrofilaricidal activity, if any, of ivermectin, since assessments depend on once-only, retrospective assays of inherently aged worm populations. Most studies on *O. volvulus* have concluded that ivermectin is not macrofilaricidal (Chavasse *et al.* 1992; Kläger, Whitworth & Downham, 1996), although there is a profound abrogation of embryogenesis which is potentially reversible but in a proportion of worms, after repeated treatments, may be irreversible (see Plaisier *et al.* 1995). We have been able, in *O. ochengi*-infected cattle, to study the activity of ivermectin and some newer avermectins, in a protocol which permits pre- as well as post-treatment worm evaluation, in addition to comparisons with concurrent controls. Single or repeated high dose treatment with ivermectin (Table 2) caused sustained abrogation of embryogenesis and accumulation of dead mf *in utero*, analogous to findings with *O. volvulus*, but worms were not killed up to 2 years post-treatment (Renz *et al.* 1995; Bronsvoort, Renz, Tanya, Ekale & Trees, unpublished observations).

The milbemycin compound moxidectin ('Cydectin', Fort Dodge) also exhibits a potent effect on adult embryogenesis and recent results indicate that 2 years after six monthly treatments at 500 μg/kg normal embryogenesis was not restored. Following a single treatment at 500 μg/kg, effects were less marked. However, neither regime was macrofilaricidal.

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Oxytetracycline is macrofilaricidal

Our interest in the effect of tetracycline against *O. ochengi* was initially aroused following the resolution of all nodules in a heavily infected animal which was undergoing a prolonged course of antibiotic treatment for the incidental dermatophytic infection, streptothricosis. A growing awareness of the existence of intracellular bacteria in filarial nematodes (which had first been described in *O. volvulus* in 1977 by Kozek & Marroquin) prompted us to mount a controlled trial. This has demonstrated that intermittent treatment with oxytetracycline over a 6-month period completely eliminated *O. ochengi* infections and led to resolution of all nodules by 9 months post treatment (Langworthy *et al*. 2000). Death of worms occurred after the destruction of intracellular, *Wolbachia*-like bacteria, visualized by EM. It is not possible to rule out direct drug action against the worm but the sequence of events in which bacteria were eliminated before lethal effects on worms were observed suggests that bacterial death was causally related to worm death. Whether this is due to toxic products of bacterial degradation or due to the elimination of some essential function provided by the bacteria is not clear, but the 100% prevalence of bacterial infection in worms (see above) suggests a relationship of mutual dependency. Other accumulating evidence of the effects of antibiotics on filarial nematodes, indicates that these effects are due to indirect effects on the bacteria rather than direct efficacy against the worms (Hoerauf *et al*. 1999). In other filarial nematodes sublethal effects have been described and a macrofilaricidal effect has not been observed (Bosshardt *et al*. 1993; Bandi *et al*. 1999; Hoerauf *et al*. 1999). This may be partly related to the much shorter period of treatment used in other host–parasite systems. Much research is needed to confirm and extend these findings and to determine whether an efficacious regime using antibiotics is practicable, but these results provide the most promising lead yet in the search for a safe macrofilaricide against *Onchocerca* spp.

Avermectins are prophylactic against O. ochengi

Administration of ivermectin to whole communities on an annual basis is the principal tool of the African Programme for Onchocerciasis Control, APOC (Molyneux & Davies, 1997) which, by suppressing mf, aims to eliminate onchocerciasis as a public health problem. Since humans will be treated under conditions of continuing exposure to infection, we were interested to determine whether ivermectin was efficacious against L_3/L_4 stages. An experiment with *O. volvulus* in chimpanzees had suggested a partial effect against L_3 larvae but not against later stages (Taylor *et al*. 1988). Accordingly a prospective controlled study was conducted against natural infection in Cameroon. It showed conclusively that monthly treatment of growing calves with ivermectin at 200 μg/kg or at 500 μg/kg completely prevented infection, as determined by nodule development, up to 21 months of exposure, at which time 5/6 untreated calves had acquired a total of 54 *O. ochengi* nodules (Tchakouté *et al*. 1999). A second experiment has confirmed and extended these results with both ivermectin and moxidectin (at 150 μg/kg and 200 μg/kg doses respectively) and at 1 month and 3 month treatment intervals (Gilbert, Langworthy, Bronsvoort, Tanya, Renz & Trees, unpublished observations). These studies are not designed to provide a chemoprophylactic regime for mass human use. Their significance is twofold. Firstly, they indicate that if the annual ivermectin treatments carried out under APOC are timed to coincide with times of maximum transmission, a double benefit will accrue – namely prophylaxis as well as clearance of existing mf burdens. Secondly, they raise the possibility that chemoprophylactic attenuation of L_3/L_4 larvae will immunize individuals and protect against subsequent challenge (see later).

IMMUNOLOGY AND IMMUNITY

These studies have been carried out as part of the Edna McConnell Clark Foundation programme to develop a vaccine for human onchocerciasis. They have involved collaboration between Liverpool and IRAD, together with many laboratories involved in the EMCF programme. With respect to *O. ochengi* the objectives have been to (1) generate experimental infections and study the immune responses involved; (2) to determine if immunity to *O. ochengi* exists naturally; (3) to determine if immunization is possible with irradiated L_3s; (4) and finally to determine if recombinant antigens can immunize against field challenge. The first three objectives have been achieved and a major experiment to address the fourth is currently underway.

Immune responses are down-regulated at patency in experimental infections

Cattle were infected with single-pulse infections with 350 or 700 L_3 larvae as briefly described above. This infection permitted the serial observation of synchronous infections and the determination of immunological events in relation to the parasite life-cycle (Graham, Trees, Collins, Taylor, Moore, Guy & Bianco, unpublished observations). Antigen-specific proliferation and *ex vivo* cytokine production by peripheral blood mononuclear cells (PBMCs) followed infection and was maintained throughout the prepatent period but both were down-regulated following the onset of microfilarial patency. Antigen-specific antibody production remained elevated throughout the post-infection (p.i.) period to

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600 days p.i. The results are consistent with evidence from human infections of cellular hyporesponsiveness in association with patent infections (Soboslay *et al*. 1997) and provide evidence of analogous immune responses in *O. ochengi* infections of cattle and *O. volvulus* infections of humans.

Putative-immune (PI) cattle are significantly protected against heavy challenge infections but radically drug-cured cattle are not

The existence of aparasitotic individual cattle reared in endemic areas has already been referred to above and the analogy made with PI humans. We sought to test whether such cattle were indeed resistant to parasite challenge by re-exposing them, together with age-matched, non-infected controls imported from a non-endemic area. Additionally, we sought to determine if previously patently-infected cattle are resistant to re-infection after radical drug treatment with a macrofilaricide (melarsomine). These experiments are difficult to undertake in humans, but would enhance understanding of protective immunity in onchocerciasis. Cattle were exposed for 19 months in an area of high natural transmission (estimated Annual Transmission Potential of between 2800 and 25300 depending on proximity to simuliid breeding site). PI cattle did develop palpable nodules but far fewer ($P = 0{\cdot}0002$) than naive or treated cattle; previously infected, melarsomine-treated cattle were fully susceptible to challenge infection and nodule loads were not statistically different from those in naive controls (Tchakouté, Bianco & Trees, unpublished observations). These results are consistent with observations on re-infection dynamics of *O. volvulus* in humans cured with suramin or stibocaptate (Duke, 1968).

Immunization with irradiated L_3 larvae induces significant protection

The use of chamber models has previously indicated that protection can be induced against L_3 larvae of *O. volvulus* implanted in mice (Lange *et al*. 1993; Taylor *et al*. 1994) and in calves, immunization by live *O. volvulus* L_3 larvae and subsequent challenge with *O. ochengi* L_3s showed a reduction in the numbers of *O. ochengi* L_3s developing to adults in vaccinated calves compared with naive controls (Achu-Kwi, Renz & Enyong, unpublished observations). The latter experiment was designed to be analogous to natural conditions, where cross-transmission of *O. ochengi* L_3s to humans is associated with reduced susceptibility to *O. volvulus* infection (Renz *et al*. 1994; Wahl *et al*. 1998). By the classical method of using irradiated larvae, attempted immunization of chimpanzees had given equivocal results due to limitations of the experimental system (Prince *et al*. 1992). Finding PI cattle in endemic areas and the results of the exposure experiment involving PI cattle (above) suggested that reduced susceptibility to *O. ochengi*, possibly immune-mediated, existed naturally and hence might be induced experimentally. Using irradiated *O. ochengi* L_3 larvae cattle were immunized then challenged under controlled conditions in Liverpool (Graham, Trees, Lustigman & Bianco, unpublished observations). Immunized cattle were significantly protected against challenge with 1000 *O. ochengi* L_3 larvae and adult worm burdens were very significantly reduced ($P < 0{\cdot}01$). This experiment illustrated that it was possible to induce and measure immunity to *O. ochengi* and has led to a current field trial to evaluate the efficacy of selected recombinant antigens to induce protection.

CONCLUSIONS AND FUTURE RESEARCH

As research continues it is clear that the biological and molecular similarities between *O. ochengi* and *O. volvulus* extend to functional aspects of their response to drugs and the hosts' immune responses to them. The possibility of inducing experimental infections of *O. ochengi* in cattle, although logistically challenging, has provided opportunities to study the kinetics of immune responses and relate them to stages in parasite development in the host, and to conduct precise infection and challenge experiments. The prevalence of naturally infected animals harbouring multiple infections is enabling studies in Africa to identify chemotherapeutic agents, and the high rate of endemic transmission is enabling field challenge experiments to assess chemo- or immunoprophylactic regimes within practicable time-frames. Current and future research will seek to examine the potential of antibiotics as macrofilaricides, the relationship of *Wolbachia* and *Onchocerca*, and the efficacy of other compounds arising from the DDR programme of WHO. A major field challenge trial is underway to determine the protection conferred by eight recombinant antigens, identified and selected by several laboratories participating in the EMCF vaccine development programme. The results of that will be known in 2001, as will those of a second field challenge experiment in which the immunity of calves reared under ivermectin and moxidectin chemoprophylaxis will be assessed. Concurrent with the advances in our knowledge of the parasitology of *O. ochengi*, developments in bovine immunology and the availability of reagents to assay antibody isotypes, cytokines and T cell subsets means that this host–parasite system can provide a highly comparable and experimentally malleable model for *O. volvulus* which can contribute to the fundamental understanding of onchocerciasis in humans.

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REFERENCES

ACHU-KWI, D., DAIBER, W. H., RENZ, A., WAHL, G. & WANJI, S. (1994). Prepatency period and some aspects of the epizootiology of *Onchocerca ochengi* infestation in cattle in the Adamawa Plateau, Cameroon. *Parasite* **1**, 10–12.

BAIN, O. (1981). Le genre *Onchocerca*: hypothèses sur son évolution et clé dichotomique des espèces. *Annales de Parasitologie* **56**, 503–526.

BANDI, C., McCALL, J. W., GENCHI, C., CORONA, S., VENCO, L. & SACCHI, L. (1999). Effects of tetracycline on the filarial worms *Brugia pahangi* and *Dirofilaria immitis* and their bacterial endosymbionts *Wolbachia*. *International Journal for Parasitology* **29**, 357–364.

BIANCO, A. E., HAM, P. J., EL SINNARY, K. & NELSON, G. S. (1980). Large-scale recovery of *Onchocerca* microfilariae from naturally infected cattle and horses. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **74**, 109–110.

BOSSHARDT, S. C., McCALL, J. W., COLEMAN, S. U., JONES, K. L., PETIT, T. A. & KLEI, T. R. (1993). Prophylactic activity of tetracycline against *Brugia pahangi* infection in jirds (*Meriones unguiculatus*). *Journal of Parasitology* **79**, 775–777.

CHAVASSE, D. C., POST, R. J., LEMOH, P. A. & WHITWORTH, J. A. G. (1992). The effect of repeated doses of ivermectin on adult female *Onchocerca volvulus* in Sierra Leone. *Tropical Medicine and Parasitology* **43**, 256–262.

COMLEY, J. C. W., REES, M. J., TURNER, C. H. & JENKINS, D. C. (1989*a*). Colorimetric quantitation of filarial viability. *International Journal for Parasitology* **19**, 77–83.

COMLEY, J. C. W., TOWNSON, S., REES, M. J. & DOBINSON, A. (1989*b*). The further application of MTT-formazan colorimetry to studies on filarial worm viability. *Tropical Medicine and Parasitology* **40**, 311–316.

CROSS, H. F., BRONSVOORT, B. M., WAHL, G., RENZ, A., ACHU-KWI, D. & TREES, A. J. (1997). The entry of ivermectin and suramin into *Onchocerca ochengi* nodules. *Annals of Tropical Medicine and Parasitology* **4**, 393–401.

CROSS, H. F., RENZ, A. & TREES, A. J. (1998). *In vitro* uptake of ivermectin by adult male *Onchocerca ochengi*. *Annals of Tropical Medicine and Parasitology* **6**, 711–720.

DETERMANN, A., MEHLHORN, H. & GHAFFAR, F. A. (1997). Electron microscope observations on *Onchocerca ochengi* and *O. fasciata* (Nematoda: Filaroidea). *Parasitology Research* **83**, 591–603.

DUKE, B. O. L. (1968). Reinfections with *Onchocerca volvulus* in cured patients exposed to continuing transmission. *Bulletin of the World Health Organization* **39**, 307–309.

GEORGE, G. H., PALMIERI, J. R. & CONNOR, D. H. (1985). The onchocercal nodule: interrelationship of adult worms and blood vessels. *American Journal of Tropical Medicine and Hygiene* **34**, 1144–1148.

GRAHAM, S. P., WU, Y. K., HENKLE DUEHRSEN, K., LUSTIGMAN, S., UNNASCH, T. R., BRAUN, G., WILLIAMS, S. A., McCARTHY, J., TREES, A. J. & BIANCO, A. E. (1999). Patterns of *Onchocerca volvulus* recombinant antigen recognition in a bovine model of onchocerciasis. *Parasitology* **119**, 603–612.

HAM, P. J., TOWNSON, S., JAMES, E. R. & BIANCO, A. E. (1981). An improved technique for the cryopreservation of *Onchocerca* microfilariae. *Parasitology* **83**, 139–146.

HOCH, B., WAHL, G., ENYONG, P., LUDER, C. G., HARNETT, W. & RENZ, A. (1994). Onchocerciasis of cattle and man: serological recognition of parasite specific and cross-reactive antigens. *Parasite* **1**, 14.

HOERAUF, A., NISSEN-PÄHLE, K., SCHMETZ, C., HENKLE-DÜHRSEN, K., BLAXTER, M. L., BÜTTNER, D. W., GALLIN, M. Y., AL-QAOUD, K. M., LUCIUS, R. & FLEISCHER, B. (1999). Tetracycline therapy targets intracellular bacteria in the filarial nematode *Litomosoides sigmodontis* and results in filarial infertility. *Journal of Clinical Investigation* **103**, 11–18.

KLÄGER, S., WHITWORTH, J. A. G. & DOWNHAM, M. D. (1996). Viability and fertility of adult *Onchocerca volvulus* after 6 years of treatment with ivermectin. *Tropical Medicine and Health* **1**, 581–589.

KOZEK, W. J. & MARROQUIN, H. F. (1977). Intracytoplasmic bacteria in *Onchocerca volvulus*. *American Journal of Tropical Medicine and Hygiene* **26**, 663–678.

LANGE, A. M., YUTANAWIBOONCHAI, W., LOK, J. B., TRPIS, M. & ABRAHAM, D. (1993). Induction of protective immunity against larval *Onchocerca volvulus* in a mouse model. *American Journal of Tropical Medicine and Hygiene* **49**, 783–788.

LANGWORTHY, N. G., RENZ, A., MECKENSTEDT, U., HENKLE-DÜHRSEN, K., BRONSVOORT, B. M. DEC., TANYA, V. N., DONNELLY, M. J. & TREES, A. J. (2000). Macrofilaricidal activity of tetracycline against the filarial nematode, *Onchocerca ochengi*: elimination of *Wolbachia* precedes worm death and suggests a dependent relationship. *Proceedings of the Royal Society London B* (in press).

LOWRIE, R. C. (1983). Cryopreservation of third-stage larvae of *Brugia malayi* and *Dipetalonema viteae*. *American Journal of Tropical Medicine and Hygiene* **32**, 767–771.

McCALL, P. J. & TREES, A. J. (1989). The development of *Onchocerca ochengi* in surrogate temperate Simuliidae, with a note on the infective larva. *Tropical Medicine and Parasitology* **40**, 295–298.

https://doi.org/10.1017/S0031182099005788 Published online by Cambridge University Press

MEREDITH, S. E. O., LANDO, G., GBAKIMA, A. A., ZIMMERMAN, P. A. & UNNASCH, T. R. (1991). *Onchocerca volvulus*: application of the polymerase chain reaction to identification and strain differentiation of the parasite. *Experimental Parsitology* **73**, 335–344.

MOLYNEUX, D. H. & DAVIES, J. B. (1997). Onchocerciasis control: moving towards the millenium. *Parasitology Today* **13**, 418–425.

PLAISIER, A. P., ALLEY, E. S., BOATIN, B. A., VAN OORTMARSSEN, G. J., REMME, H., DE VLAS, S. J., BONNEUX, L. & HABBEMA, J. D. F. (1995). Irreversible effects of ivermectin on adult parasites in onchocerciasis patients in the Onchocerciasis Control Programme in West Africa. *Journal of Infectious Diseases* **172**, 204–210.

PRINCE, A. M., BROTMAN, B., JOHNSON, E. H., SMITH, A., PASCUAL, D. & LUSTIGMAN, S. (1992). *Onchocerca volvulus*: immunization of chimpanzees with X-irradiated third stage (L_3) larvae. *Experimental Parasitology* **74**, 239–250.

RENZ, A., ENYONG, P. & WAHL, G. (1994). Cattle, worms and zooprophylaxis. *Parasite* **1**, 4–6.

RENZ, A., TREES, A. J., ACHU-KWI, D., EDWARDS, G. & WAHL, G. (1995). Evaluation of suramin, ivermectin and CGP 20376 in a new macrofilaricidal drug screen, *Onchocerca ochengi* in African cattle. *Tropical Medicine and Parasitology* **46**, 31–37.

SCHULZ-KEY, H. (1988). The collagenase technique: how to isolate and examine adult *Onchocerca volvulus* for the evaluation of drug effects. *Tropical Medicine and Parasitology* **39**, 423–440.

SMITH, R. J., COTTER, T. P., WILLIAMS, J. F. & GUDERIAN, R. H. (1988). Vascular perfusion of *Onchocerca volvulus* nodules. *Tropical Medicine and Parasitology* **39**, 418–421.

SOBOSLAY, P. T., GEIGER, S. M., WEISS, N., BANLA, M., LUDER, C. G. K., DREWECH, C. M., BATCHASSI, E., BOATIN, B. A., STADLER, A. & SCHULTZ-KEY, H. (1997). The diverse expression of immunity in humans at distinct states of *Onchocerca volvulus* infection. *Immunology* **90**, 592–599.

TAYLOR, H. R., TRPIS, M., CUPP, E. W., BROTMAN, B., NEWLAND, H. S., SOBOSLAY, P. T. & GREENE, B. M. (1988). Ivermectin prophylaxis against experimental *Onchocerca volvulus* infection in chimpanzees. *American Journal of Tropical Medicine and Hygiene* **39**, 86–90.

TAYLOR, M. J., VAN ES, R. P., SHAY, K., FOLKAR, S. G., TOWNSON, S. & BIANCO, A. E. (1994). Protective immunity against *Onchocerca volvulus* and *O. lienalis* infective larvae in mice. *Tropical Medicine and Parasitology* **45**, 17–23.

TAYLOR, M. J. & HOERAUF, A. (1999). *Wolbachia* bacteria of filarial nematodes. *Parasitology Today* **15**, 437–442.

TCHAKOUTÉ, V. L., BRONSVOORT, M., TANYA, V., RENZ, A. & TREES, A. J. (1999). Chemoprophylaxis of *Onchocera* infections: in a controlled, prospective study ivermectin prevents calves becoming infected with *O. ochengi*. *Parasitology* **118**, 195–199.

TREES, A. J. (1992). *Onchocerca ochengi*: mimic, model or modulator of *O. volvulus*? *Parasitology Today* **8**, 337–339.

TREES, A. J., WAHL, G., KLÄGER, S. & RENZ, A. (1992). Age-related differences in parasitosis may indicate acquired immunity against microfilariae in cattle naturally infected with *Onchocerca ochengi*. *Parasitology* **104**, 247–252.

TREES, A. J., WOOD, V. L., BRONSVOORT, M., RENZ, A. & TANYA, V. N. (1998). Animal models – *Onchocerca ochengi* and the development of chemotherapeutic and chemoprophylactic agents for onchocerciasis. *Annals of Tropical Medicine and Parasitology* **1**, S175–S179.

WAHL, G., ACHU-KWI, M. D., MBAH, D., DAWA, O. & RENZ, A. (1994). Bovine onchocercosis in North Cameroon. *Veterinary Parasitology* **52**, 297–311.

WAHL, G., EKALE, D. & SCHMITZ, A. (1998). *Onchocerca ochengi*: assessment of the *Simulium* vectors in North Cameroon. *Parasitology* **116**, 327–336.

WAHL, G., ENYONG, P., NGOSSO, A., SCHIBEL, J. M., MOYOU, R., TUBBESING, H., EKALE, D. & RENZ, A. (1998). *Onchocerca ochengi*: epidemiological evidence of cross-protection against *Onchocerca volvulus* in man. *Parasitology* **116**, 349–362.

XIE, H., BAIN, O. & WILLIAMS, S. A. (1994). Molecular phylogenetic studies on filarial parasites based on 5s ribosomal spacer sequences. *Parasite* **1**, 141–151.

https://doi.org/10.1017/S0031182099005788 Published online by Cambridge University Press