PN-ABM-686
79798
6.225

HUMAN ONCHOCERCIASIS: NEW IMMUNODIAGNOSTIC ASSAYS AND CONTROL MEASURES

In: BIOTECHNOLOGY FOR BIOLOGICAL CONTROL OF PESTS AND VECTORS. 1991. Karl Maramorosch, editor. CRC Press, Inc., Boca Raton, Florida

Ricardo Luján, Ph.D.*
Professor
Centro de Investigaciones en Enfermedades Tropicales
Instituto de Investigaciones
Universidad del Valle de Guatemala
11 calle 15-79, Zona 15, Vista Hermosa III
Apartado Postal No. 82
01901 Guatemala, Guatemala, C.A.

Eddie W. Cupp, Ph.D.
Professor of Medical Veterinary Medicine
Department of Entomology
College of Agriculture
The University of Arizona
Tucson, Arizona 85721 U.S.A.

---

* "This publication was supported in part under Grant No. DPE-5542-G-SS-7033-00, Program in Science and Technology Cooperation (PSTC), Office of the Science Advisor, U.S. Agency for International Development."

REC'D IN RAD/R
OCT 8 1992

cc: CDIE
BOSTID

Chapter 13

HUMAN ONCHOCERCIASIS: NEW IMMUNODIAGNOSTIC ASSAYS AND CONTROL MEASURES

Ricardo Luján and Eddie W. Cupp

TABLE OF CONTENTS

I. Introduction 180
- A. Biology of the Parasite 180
- B. Distribution and Vectors 180

II. Immunodiagnostic Assays 181
- A. Immunodiagnosis 181
- B. Molecular Biology 183

III. Control Measures 184
- A. Vector Control 184
- B. Chemotherapy 185
- C. Vaccine Development 186

References 187

I. INTRODUCTION

A. BIOLOGY OF THE PARASITE

Onchocerca volvulus, the causative agent of "river blindness" in Africa and Robles' disease in the New World, follows a pattern of morphogenesis and development generally similar to related taxa within the nematode superfamily Filarioidea.[1] Hematophagous arthropods (in this case members of the family Simuliidae — "blackflies") serve as intermediate hosts for development of the initial larval stages (L_1-L_3) after ingesting microfilariae (these embryonic forms of *O. volvulus* are found in the skin) from an infected human. Following development in the thoracic musculature, *O. volvulus* L_3s (infective stage larvae) migrate to the head where, during the course of bloodfeeding by the female, they escape into the skin by breaking through the terminal portions of the mouthparts. Because blackflies are telmophagous, i.e., the rasping action of their mouthparts causes the formation of tiny subdermal hemorrhages, infective larvae escape into an environment of pooled blood and hemolymph from the ruptured mouthparts of the vector. This method of transmission and infection is clearly distinct from that of mosquito-associated filariases.

Following infection of the human host, the L_3 molts to the fourth larval stage (L_4); prior to ecdysis, the worm enters a state of lethargus for 24 to 48 h and then escapes from the old cuticle. *In vitro* studies,[1] as well as those using infective stage larvae implanted into laboratory mice,[2] indicate that the L_3 to L_4 molt by *O. volvulus* occurs within 2 to 4 d. The L_4 is highly motile and presumably is the migratory stage, allowing the parasite to move to its final anatomical site for a final molt (L_4-juvenile) and subsequent growth to reproductive competence. Estimates for the length of time in which the parasite remains in the L_4 stage vary, ranging from 1 to $2^1/_2$ months.[1,3]

The prepatent period appears to be 12 to 15 months. Adult worms are usually encapsulated in connective tissue that forms subcutaneous nodules or that are located deeply within the body or along the pelvic region of the skeleton.[4] The nodule not only protects the adults but nourishes the parasite through a vascularization process which brings blood vessels adjacent to the worm's cuticle and into a central fibrin lake.[5] Females are multiply-inseminated by itinerant males.[6] Following fertilization, embryonation begins, ultimately producing unsheathed microfilarie which congregate in the uterus and from which they are expelled. Larvating eggs of *O. volvulus* readily bind a variety of lectins, however, neither *in utero* derived microfilariae nor those found in the skin do so,[7] suggesting a loss of surface antigenicity. Once inside the blackfly vector, the cuticular surface of the developing larva continues to change in an apparent attempt to also evade the arthropod's immune system.[8]

Based on classical taxonomic,[9,10] parasitological/entomological[1] and molecular evidence,[11] *O. volvulus* appears to be a relative "new" parasite of humans, having probably evolved from an antecedent bovid species. This assumption is based on the high degree of morphological and vectorial similarity among those species, the recent human cultural adaptation of domesticating and tending cattle, as well as the fact that no other species of *Onchocerca* naturally parasitizes primates. This unique evolutionary aspect has important ramification for control in that the reservoir for *O. volvulus* is limited to human hosts. It also poses problems in making accurate identifications of the parasite in the vector population where closely related *Onchocera* species of ungulates may occur and blackfly taxa are zoophilic.

B. DISTRIBUTION AND VECTORS

The Simuliidae, and in particular, certain members of the *Simulium damnosum* sibling species complex serve as vectors of *O. volvulus*. This parasite, which is the second leading cause of infectious blindness in the world, infects an estimated 11 million persons living in 32 countries; at least 663,000 persons are irreversibly blind as a result of chronic oncho-

cerciases, and some 85,583,780 persons are at risk of infection.[12] Thus, this is a vector-associated disease of major proportions.

The distribution of the parasite in Africa is brokered over a large part of its range through a well-balanced coevolutionary relationship within the *S. damnosum* complex, i.e., major savanna zone vectors are composed primarily of two chromosomally distinct cytospecies (*S. damnosum* sensu stricto; *S. sirbanum*) with three others (*S. yahense*, *S. soubrense*, and *S. squamosum*) serving as primary vectors in the rain forest.[13] Vector competency is partitioned, i.e., forest vectors are incompetent for the savanna form of *O. volvulus* and savanna vectors are incompetent for the forest form of the parasite.[14] Accurate identification of wild-caught adult flies is frequently difficult due to the lack of consistently distinct morphological characters of the various sibling taxa. Consequently, approaches involving the use of DNA probes,[15] cuticular hydrocarbons,[16] Malpighian tubule chromosomes,[17] and allozymes[18] have been suggested for differentiation, however, only the latter two techniques appear promising under field conditions in Africa. Several alpha-level species belonging to the *S. neavei* group serve as vectors in the highlands of East Africa.

Important New World vector taxa (where *O. volvulus* was probably introduced several hundred years ago) include *S. ochraceum* (Guatemala, Mexico), *S. exiguum* (Colombia, Ecuador), *S. metallicum* (Venezuela, Guatemala) and *S. oyapockense* s.l. (Brazil, Venezuela).[19] Microfilarial intake and subsequent infection of the hemocoel in *S. ochraceum* is regulated by a bucco-pharyngeal armature which shreds most of the ingested worms;[20] however, once microfilarie successfully bypass this barrier, infection of the flight muscles and development to the third larval stage is readily achieved.

Experimental cross-infections using *Simulium* spp. and *O. volvulus* from Africa and the New World (Guatemala, Venezuela) revealed that a nonreciprocal pattern exists intercontinentally between the vectors and the parasite,[21,22] with none of the vectors (*S. ochraceum*, *S. metallicum*, *S. damnosum* sensu lato) able to serve as competent intermediate hosts. However, both the Guatemalan and Venezuelan strains of *O. volvulus* were equally compatible with *S. metallicum* from either Guatemala (where this blackfly is a secondary vector) or Venezuela.[23] Thus partitioning of vector competence by this New World species complex does not appear to have taken place.

The pathology of human onchocerciasis varies considerably between the various endemic areas in Africa and the New World. For example, in West Africa, the prevalence of blindness and skin disease is more intense in the Sudan and Guinea savannas than in the rainforest and transition zones. Thus the "savanna strain" of *O. volvulus* is considered to be the most intrinsically pathogenetic;[24] however the magnitude of annual parasite transmission[25] and intensity of skin infection[26] are important epidemiological factors as well. In Latin America, particularly Guatemala, ocular pathology is variable but is associated primarily with intensity of skin infection,[27] i.e., the number of microfilariae per milligram of skin.

II. IMMUNODIAGNOSTIC ASSAYS

A. IMMUNODIAGNOSIS

Diagnosis of human onchocerciasis is important for determining the prevalence and intensity of infection, identifying individuals who may require drug treatment, evaluating the success in the application of a given treatment, and assessing the impact of control efforts. Diagnostic procedures used so far have relied mainly upon the identification of characteristic microfilariae in skin biopsies from infected individuals[28] or by palpation of subcutaneous nodules (onchocercomata) that develop around the adult worm.[29,30] Neither of these methods however, can detect early prepatent infections, nor the presence of adult worms that may exist occult or free of nodules.[30] Thus, present parasitological methods may not detect parasites because of the low sensitivity of these techniques, leading to an inaccurate diagnosis.

3

Immunodiagnostic assays, which are more sensitive and specific for detecting parasite products (antigenic fractions) rather than the organisms themselves in body fluids of infected individuals, have been developed as an alternative to parasitological diagnosis.[31] Polyvalent antisera,[32] as well as several monoclonal antibodies (MoAb) have been raised against different antigenic determinants of filarial parasites, including *O. volvulus*, and have been used in isotopic and nonisotopic assays for the detection of circulating parasite antigens.[33,34] The induction and selection of MoAb has been hampered, however, by the use of heterogeneous immunogens and by the cross-reactivity observed with phosphorylcholine, which is present in many preparations of helminth antigens. Nevertheless, serological reactivity [by MoAb] has been identified in low molecular weight (14 to 18 kDa) polypeptides of *O. volvulus*,[35] *O. volvulus* antigens have been detected also in other body fluids, such as breast milk.[36] In spite of the diverse applications of MoAb, "... the practical utility of these reagents [MoAb] to solve the diagnostic dilemmas [in filariasis including human onchocerciasis] has not yet been demonstrated".[37]

Filarial infections in humans induce both humoral and cellular immune response.[38] Detection of host antibodies, however, has been used more frequently because of the amplificative effect of this component of the immune system, and the easier application in serological studies on population-based surveys. A variety of immunodiagnostic methods, which have been applied for diagnosis of onchocerciasis, include the complement fixation,[39] indirect hemagglutination,[40] immunofluorescence,[41] ELISA,[42,43] and radio-allergosorbent[44] tests. The sensitivity and specificity for the diagnosis of onchocerciasis infections has been significantly improved over the traditional parasitological methods. Many problems still remain unsolved, especially in the high degree of cross-reactivity observed between onchocerciasis and other filarial and helminthic infections.[45] Most of the serologic assays have employed crude extracts of adult *O. volvulus* worms as antigens or extracts from related filarial parasites (heterologous antigens), counting on the high degree of cross-reactivity observed between these parasites.[46] Evidence, however, for species- and stage-specific reactions in human onchocerciasis has not yet been described.[47]

Attempts have been made to improve the quality of *O. volvulus* antigens used in isotopic tests, for example, by applying purification procedures such as affinity chromatography or isoelectric focusing to remove host globulins and other antigenic contaminants.[48] However, the real value of this method for increasing the specificity of the ELISA test remains to be evaluated in a large scale survey. Recently, gel filtration for purification of *O. volvulus* adult worm antigen yielded a 20 kDa surface antigenic fraction which, when radiolabeled, provided an assay with a sensitivity of 92% and a specificity of 98% in a seroepidemiological study of human onchocerciasis in Mexico.[49] This antigen, however, was not strictly species-specific since it detected high levels of antibodies in sera from Indians exposed to *Wuchereria bancrofti* in the New Delhi endemic area. Nevertheless, the specificity of serologic tests for onchocerciasis may be increased by focusing on the characterization of surface, somatic, and excreted antigens, and by identifying class or subclass-specific antibody response.

Modern methods for the characterization of surface antigens are based on the combination of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), with the most commonly used buffer system being that of Laemmli,[50] with the subsequent electrophoretical transfer of proteins onto nitrocellulose filter sheets, according to Towbin et al.[51] These procedures together with immunoassays employing isotopic and nonisotopic markers, have been used to characterize the reactive specificities of antigens and antibodies in several parasitic infections, including human onchocerciasis.[51,52,58]

Low molecular weight (LMW) antigens (ranging from 20 to 43 kDa) of adult *O. volvulus* worms have been shown by two-dimensional SDS-PAGE to be more species-specific than high molecular weight (HMW) antigens, which cross-react with sera from patients with other filariases.[53] Similar findings were obtained on immunoblotted antigens, where *Onch-*

ocerca-specific bands were observed in the 12- to 25-kDa range of phosphate-buffered saline (PBS)- and detergent-solubilized worm extracts.[54] Interestingly, comparable LMW antigens to adult worm surface proteins,[49,54,58] have been found to be present in other parasite stages, such as skin microfilariae.[55] Based on these results, a LMW (12.5 to 35.5 kDa) antigenic fraction was recently prepared by gel filtration of crude extracts of soluble, adult *O. volvulus* and used in an ELISA test to determine the prevalence of onchocerciasis in African villages. The specificity of the test was significantly improved when the LMW fractions were used as antigens, in comparison to the crude adult worm extracts. The assay could be used to evaluate onchocerciasis prevalence, especially when testing individuals 5 to 15 years of age.[59]

The specificity of serological tests for onchocerciasis may also be increased by focusing on the detection of class or subclass-specific antibodies. It appears that IgE antibodies are more species-specific than IgG antibodies in both human onchocerciasis and lymphatic filariasis.[44,60] In addition, IgG4 type antibodies are preferentially induced during filarial infections and can recognize *O. volvulus*[58,61] and lymphatic filariasis antigens[62-64] more specifically than any of the other classes or subclasses of antibodies. Clinico-immunological relations have been suggested between signs of the disease and class or subclass-specific responses, "but their broad applicability is unproven still".[37] Nevertheless, the specificity of ELISA and immunoblot tests for serological diagnosis of human onchocerciasis is improved when IgG4 or IgE responses are examined, even when whole worm soluble antigenic preparations are used.

B. MOLECULAR BIOLOGY

It appears that one of the major problems in immunodiagnosis of human onchocerciasis (the specificity of the test) can be partly solved when antigens of low molecular mass are utilized instead of whole worm soluble antigenic preparations. Stage-specific surface, somatic, and excreted antigens may be of particular value for accurate serodiagnosis. A problem still remains, however, in providing a constant, reliable source of adult worms and key larval stages to prepare large enough quantities of these antigens. Currently, obtaining antigens is dependent on preparation from live or frozen parasites, but worldwide there is a limited availability of *O. volvulus* parasites since there is currently no suitable laboratory model for human onchocerciasis. Therefore, alternative antigen sources have to be developed, either from other *Onchocerca* species which display a high degree of cross-reactivity or by cloning DNA sequences for specific *O. volvulus* antigens.

Recombinant DNA technology can be extremely useful for filariasis research since purified reagents can be produced in large quantities.[65] Recent applications of this technology have been aimed at detecting *O. volvulus* infections in human beings and blackflies, differentiating *O. volvulus* from other helminths or filarial parasites,[66,67] distinguishing forest from savanna strains of *O. volvulus*,[68] expressing stage-specific antigens (L_3),[69] and producing proteins that may serve as specific antigens in serodiagnosis or that might be involved in developing a vaccine against *O. volvulus*. Recently, a recombinant cDNA clone derived from microfilariae-producing female *O. volvulus* and expressing a released product of 16 to 17 kDa, has been identified to be specific for the serodiagnosis of onchocerciasis, since sera of patients with other filariases did not recognize this antigen.[70]

This aspect of the review has focused primarily on immunodiagnosis of *O. volvulus* infections in humans by detecting antigen products of the parasite or host antibodies in body fluids. Other human filarial agents such as *Wuchereria bancrofti, Brugia malayi, or Loa loa*, or nonhuman filarial pathogens, such as *Dirofilaria immitis, B. pahangi, Acanthocheilonema viteae*, and others, have not been included due to space limitations. Much information, however, has been derived from these other systems and have contributed significantly to the development of accurate immunodiagnosis for human onchocerciasis. Modern immunodiagnostic assays which rely on isotopic (e.g., RIA, IRMA, RIPEGA) or

nonisotopic (e.g., ELISA, IFA) markers have more than an acceptable sensitivity. The major problem for diagnosis of human onchocerciasis has been the extensive cross-reactive antigenicity observed between *Onchocerca* spp., other filarial parasites, and other helminths. For this reason, each serological test has an inadequate specificity which is not suitable for epidemiological studies, follow-up of infected or treated patients, or for surveillance of control areas. The introduction of immunoblotting techniques and determination of class and subclass-specific antibodies has provided a significant improvement on the specificity of immunodiagnosis over the preceding assays using crude soluble antigens. The limited source and complexity of *O. volvulus* antigens, however, is a serious constraint to the development of sensitive and specific assays. This problem may be overcome by recombinant cDNA expression products, which can be a substitute for the traditional serological assays.

III. CONTROL MEASURES

A. VECTOR CONTROL

Systematic attempts at controlling human onchocerciasis date back to the 1940s when DDT became available as an effective blackfly larvicide.[71] By instituting successful control of blackfly vectors using this organochlorine, transmission of *O. volvulus* was temporarily curtailed or eliminated in several parts of Africa[12,72] and Guatemala.[73] More recently, a multinational program aimed at interrupting transmission of *O. volvulus* by members of the *Simulium damnosum* sibling species complex was instituted in a large geographic zone of the savannahs of West Africa.[74] The Onchocerciasis Control Program (OCP), funded by the World Bank and administered by the World Health Organization, is scheduled for a 20-year operational period (1974 to 1994).

At its inception, because of the lack of an appropriate mass chemotherapeutic agent, the strategy of the OCP was oriented exclusively to vector control using extensive applications of organophosphate larvicides.[75] After a 10-year period of highly successful blackfly control using temephos and chlorphoxim, transmission of *O. volvulus* had been reduced below the accepted levels of 100 *O. volvulus* infective stage larvae (L_3)/person/year in 90% of the treatment area. In so doing, a 97% reduction in infections among children was noted, indicating that a cohort of approximately three million children were free from risk of ocular onchocerciasis.

However, attendant with the success of this program, several entomological and epidemiological problems emerged which have threatened its overall operational well-being. Chief among these are insecticide resistance[76,77] and reinvasion of areas previously sanitized by larviciding,[78] others include lack of suitable means for precise identification of the vector stages of *O. volvulus* "strains" and related bovine *Onchocerca* species, as well as the need to accurately separate savanna vector species adults (*S. damnosum* s.s., *S. sirbanum*) from forest forms at the interface of these major geobotanical zones. In response, the original research-operational goals of the OCP were expanded to include a drug discovery component, development of alternative *in vivo* and *in vitro* systems for experimental drug screening, and ancillary research focusing on modern identification methods for both vector and parasite. A territorial extension of the vector control program was also instituted to provide adequate insecticide coverage in westward areas believed to be the source of invading flies.[79] Molecular biologic approaches, including the evaluation of current methodologies for vaccine research,[65] were also independently formulated to diversify control possibilities.

In response to the occurrence of resistance in larval populations of important vector cytospecies (*S. sirbanum*, *S. damnosum* s.s.),[80] attention was directed toward the discovery of alternative larvicides which had little or no deleterious impact on aquatic nontarget organisms.[81] Because of cross-resistance to organophosphorus (OP) insecticides, permethrin (a pyrethroid) and carbosulfan (a carbamate) received strong consideration as potential

replacements, even though the prospects of resistance, nontarget toxicity, and carry (distance below application with 100% effect) remain as potential or real operational problems.

Because of its specific mode of action and rapidity of toxicity for medically important, nematocerous Diptera[82] and the low potential for selection of resistance,[83] the H-14 serotype of *Bacillus thuringiensis*[84] has been used extensively in combatting the spread of OP-resistance in both forest and savanna populations of *S. damnosum* sensu lato.[85] For economical and operational reasons, the initial use of *Bacillus thuringiensis* H-14 was restricted primarily to the dry season. Implementation during this time was based on decreased river discharge, hence less material was required to treat larval sites. A flowable concentrate formulation was applied weekly by air to large rivers and was alternated with other insecticides (usually chlorphoxim) in the wet season in an attempt to curtail expansion of temephos resistance.[86] Smaller water courses, i.e., with flow rates ≤ 50 m^3/s, in areas populated by resistant forms were treated throughout the year. In implementing *Bacillus thuringiensis* H-14 as a response to resistance, over one million liters of material was used by the OCP between 1981 and 1985.[87]

The *Bacillus thuringiensis* H-14 serotype is the first commercially available biological control agent for blackflies.[88] As such, it has reached a relatively wide market, in part, because of overlap with mosquito control. In contrast, the operational control potential of other simulid pathogens appears to be relatively low, due to the inability to effectively mass produce and deliver promising entomopathogens.[89,90] Indeed, operational, wide-scale application of *Bacillus thuringiensis* H-14 is still less cost effective than traditional insecticides because of excess bulk, formulations requirements and variability in concentration of active ingredient, a biotechnological problem currently being addressed.[91]

OP-resistance gene flow within *Simulium soubrense/sanctipauli* populations in the Ivory Coast has been followed using cytogenetic techniques.[92] A chromosomal rearrangement (inversion A) on the long arm of chromosome II (IIL-A) served as a marker to trace the spread of resistance eastward from the Bandama river. The use of this inversion marker was also later verified for populations of *S. sanctipauli* from the Ivory Coast, Ghana, and Mali.[93] However, while serving as an excellent phenotypic marker, the actual genetic/biochemical mechanism(s) for temephos/chlorphoxim detoxification is still under study. For example, total esterase activity seen in larvae collected from areas where resistance occurs appeared to be predictive although the high degree of enzyme heterogeneity among individual larvae in both resistant and susceptible populations made this interpretation difficult.[94]

The occurrence of OP resistance has proven costly to the overall fiscal, operational and epidemiological aspects of the OCP.[95] In developing a resistance management scheme, current approaches involve not only the use of *Bacillus thuringiensis* H-14 to reduce the frequency of resistant genotypes but the selection of chemical larvicides to which there is less likelihood of cross-resistance or that exhibit negative cross-resistance.[80] However, the latter approach is not without difficulty due to possible "omnipotent" detoxification mechanisms controlled at other genetic loci.[96]

B. CHEMOTHERAPY

The chemotherapy of human onchocerciasis has seen a dramatic change in the past 5 years as a result of the introduction of ivermectin, a potent microfilaricidal drug.[97] Of particular importance is the fact that ivermectin produces substantially fewer clinical reactions than drugs used previously.[98] Prior to the advent of this drug, treatments were problematic due to the nephrotoxicity of the only available macrofilaricide (Suramin) and the adverse side-reactions associated with microfilarial-killing by diethylcarbamazine, a piperazine derivative.[99] As a broad spectrum antiparasitic agent, ivermectin is free of these major problems: reported side effects including edema of the limbs,[100,101] and face,[101] and occasional headache, fever,[102] and postural hypotension.[100] Because of its safety, ivermectin provides the first

opportunity for implementation of chemotherapy programs integrating mass treatment with vector control. Indeed, as of January 1989, more than 120,000 individual doses had been administered without severe adverse reactions in various WHO-sponsored field trails.[97] With its recent registration (as Mectizan) for human consumption, ivermectin is currently being dispensed in the OCP area of West Africa.[103] In addition to its treatment value for *O. volvulus*, a single dose of ivermectin is highly effective against *Ascaris lumbricoides*, *Strongyloides stercoralis* and *Trichuris trichiura* with relatively good activity against hookworm (probably *Nectar americanus*).[104] Thus the clinical implications of a chemotherapy-based program for human onchocerciasis control can also be expected to have a major impact on reducing enteric roundworm burdens as well.

The demonstration that a single treatment with ivermectin substantially reduced the uptake of *O. volvulus* microfilariae for periods of 4 to 10 months by both forest[105,106] and savanna[107] vectors in West Africa suggested that in addition to its favorable clinical properties this drug could be used to interrupt transmission as well. Because of its ability to prevent vector infection by ridding the skin of microfilariae for epidemiologically important periods of time, it has been suggested that ivermectin would be particularly effective where parasite transmission is limited by seasonality, discrete ecological boundaries, or vector inefficiency.[105] This latter possibility was explored in Guatemala where it was shown that two treatments, given at 7-month intervals to a highly infectious group of volunteers, resulted in almost complete suppression of developing or infective larvae in *S. ochraceum* for a 6-month period.[108] Also, the overall decline of vector infection was an order of magnitude lower than pretreatment levels.

Studies have begun in Africa and Guatemala to measure the effects of community-wide treatment on the annual transmission potential[12] of *O. volvulus*. Preliminary results from investigations using one[109] or two[110] community-wide treatments are encouraging and demonstrate a downturn in *S. damnosum* s.l. infection with developing larvae, hence L_3 production. Data from Guatemala, where four 6-monthly treatments have been completed in three communities,[111] indicate that the basic reproductive rate (R_0) of the parasite can be drastically lowered with sequential ivermectin treatment, thereby possibly reaching the threshold for continued transmission using chemotherapy alone.[107] These findings have broad ramifications for the planning and implementation of future control programs, particularly in the African savannas. With the availability of modern molecular techniques to specifically identify *O. volvulus* L_3s in zoophilic blackfly vectors,[113] it should now be possible to critically evaluate similar studies in the savanna when multiple treatments are given on a scheduled basis. In doing so, the role of vector control in concert with ivermectin chemotherapy can be determined so that cost-effective protection can be established at the community level.

C. VACCINE DEVELOPMENT

The development of an *O. volvulus* synthetic vaccine is an emerging area which will require careful evaluation. Factors which must be considered include the social, political, and economic realities of vaccine delivery after identifying and cloning genes for protective immunogens,[114] as well as possible difficulties in demonstrating the efficacy of a prophylactic vaccine because of the chronic nature of this parasitism.[115] The spread of AIDS throughout Africa and Latin America also poses potential problems when considering a vaccine for a chronic helminth infection such as human onchocerciasis[116] because of the former's effect on immunoresponsiveness.

Nevertheless, techniques in molecular genetics and immunology have been successfully implemented in surveying the *O. volvulus* genome for potentially useful antigens. For example, several cDNA fusion products that are recognized by human sera from endemic areas have been isolated and characterized.[69,117] One of these, paramyosin, is a major immunogen in filarial infections, exhibiting a high degree of amino acid homology between *O. volvulus*

and *D. immitis*.[118] As a corollary to the search for protective antigens, recent field observations in Guatemala of persons living in an endemic zone suggest that some level of natural immunity to *O. volvulus* occurs within a subset of that population; the protective mechanism appears to be associated with greater lymphocyte responsiveness involving production of interleukin-2.[119]

The recent development of several field and laboratory techniques should also prove complementary in further identifying potentially useful clones/fusion products. These include the use of *in situ* DNA-mRNA hybridization to determine the tissue location of genes encoding specific antigens,[120] greater access to specific stages of the parasite's life cycle, including infective stage larvae,[121] *in vitro*-cultured L_3s[122] and adult worms.[123] These techniques, when coupled with the usefulness of cryopreservation of live worms,[124] should provide added means to pursue the vaccine discovery process. However, as reviewed by Gutteridge,[125] while vaccines are potentially safer, cheaper, and more prophylactically efficacious than drugs, the latter will continue to be needed for some time to come.

REFERENCES

1. **Cupp, E. W.,** Human onchocerciasis developmental biology of the parasite, in *Proc UpJohn WHO/OCP Symp On hocerciasis/Filariasis*, Conder, G A and Williams, J F, Eds, Kalamazoo, MI, 1986, 1
2. **Bianco, A. E., Mustafa, M. B., and Ham. P. J.,** Fate of developing larvae of *Onchocerca lienalis* and *O volvulus* in micropore chambers implanted into laboratory hosts, *J Helminthol*, 63, 218, 1989
3. **Bianco, A. E. and Muller, R.,** Experimental transmission of *Onchocerca lienalis* to calves, *Parasitology* (Suppl), 1982, 349
4. **Duke, B. O. L.,** Onchocerciasis deep worm-bundles close to hip joints, *Trans R Soc Trop Med Hyg*, 64, 791, 1970
5. **George, G. H., Palmieri, J. R., and Connor, D. H.,** The onchocercal nodule interrelationships of adult worms and blood vessels, *Am J Trop Med Hyg*, 34, 1144, 1985
6. **Schulz-Key, H. and Karam, M.,** Periodic reproduction of *Onchocerca volvulus*, *Parasitology Today*, 2, 284, 1986
7. **Paulson, C. W., Jacobson, R. H., and Cupp, E. W.,** Microfilarial surface carbohydrates as a function of developmental stage and ensheathment status in six species of filarids, *J Parasitol*, 74, 743, 1988
8. **Ham, P. J., Smail, A. J., and Groeger, B. K.,** Surface carbohydrate changes on *Onchocerca lienalis* larvae as they develop from microfilariae to the infective third-stage in *Simulium*, *J Helminthol*, 62, 195, 1988
9. **Muller, R.,** Identification of *Onchocerca*, in *Problems in the Identification of Parasites and Their Vectors*, Vol 17, Taylor, A E R and Muller, R, Eds, Blackwell Scientific, Oxford, 1979, 175
10. **Bain, O.,** Le genre *Onchocerca* hypotheses sur son evolution et de dichotomique des especes, *Ann Parasitol*, 56, 503, 1981
11. **Meredith, S. E. O., Unnasch, T. R., Karam, M., Piessens, W. F., and Wirth, D. F.,** Cloning and characterization of an *Onchocerca volvulus* specific DNA sequence, *Mol Biochem Parasitol*, 36, 1, 1989
12. **World Health Organization,** WHO Expert Committee on Onchocerciasis, Tech Rep Ser, 752, Geneva, 1987
13. **Dunbar, R. W. and Vajime, C. G.,** Cytotaxonomy of the *Simulium damnosum* complex, in *Black Flies—The Future for Biological Methods in Integrated Control*, Laird, M, Ed, Academic Press, London, 1981, 31
14. **Duke, B. O. L., Lewis, D. J., and Moore, P. J.,** *Onchocerca-Simulium* complexes I Transmission of forest and Sudan-savanna strains of *Onchocerca volvulus*, from Cameroon, by *Simulium damnosum* from various West African biochimatic zones, *Ann Trop Med Parasitol*, 60, 318, 1966
15. **Post, R. J.,** DNA probes for vector identification, *Parasitology Today*, 1, 89, 1985
16. **Philips, A., Walsh, J F., Garms, R., Molyneux, D. H., Milligan, P., and Ibrahim, G.,** Identification of adults of the *Simulium damnosum* complex using hydrocarbon analysis, *Tropenmed Parasitol*, 36, 97, 1985
17. **Procunier, W. S. and Post, R. J.,** Development of a method for the cytological idientification of man-biting sibling species within the *Simulium damnosum* complex, *Trop Med Parasitol.*, 37, 49, 1986.

9

18 **Thomson, M. C., Davies, J. B., and Wilson, M. D.**, A portable allozyme electrophoresis kit used to identify members of the *Simulium damnosum* Theobald complex (Diptera Simuliidae) in the field, *Bull Entomol Res*, 79, 685, 1989

19 **Shelley, A. J.**, Vector aspects of the epidemiology of onchocerciasis in Latin America, *Annu Rev Entomol*, 33, 337, 1988

20 **Omar, M. S. and Garms, R.**, The fate and migration of microfilariae of a Guatemalan strain of *Onchocerca volvulus* in *Simulium ochraceum* and *S metallicum* and the role of the buccopharyngeal armature in the destruction of microfilariae, *Tropenmed Parasitol*, 26, 183, 1975

21 **DeLeon, R. and Duke, B. O. L.**, Experimental studies on the transmission of Guatemalan and West African strains of *Onchocerca volvulus* by *Simulium ochraceum*, *S metallicum* and *S callidum*, *Trans R Soc Trop Med Hyg*, 60, 735, 1966

22 **Duke, B. O. L.**, *Onchocerca-Simulium* complexes VI Experimental studies on the transmission of Venezuelan and West African strains of *Onchocerca volvulus* by *Simulium metallicum* and *S exiguum* in Venezuela, *Ann Trop Med Parasitol*, 64, 421, 1970

23 **Takaoka, H., Tada, I., Hashiguchi, Y., Baba, M., Kokenaga, M., Onofre, J., Convit, J., and Yarzabal, L.**, A cross compatibility study of Guatemalan and North Venezuelan *Onchocerca volvulus* to *Simulium metallicum* from two countries, *Jpn J Parasitol*, 35, 35, 1986

24 **Budden, F. H.**, Comparative study of ocular onchocerciasis in savanna and rainforest, *Trans R Soc Trop Med Hyg*, 57, 64, 1963

25 **Thylefors, B., Philippon, B., and Prost, A.**, Transmission potentials of *Onchocerca volvulus* and the associated intensity of onchocerciasis in a Sudan-savanna area, *Tropenmed Parasitol*, 29, 346, 1978

26 **Remme, J., Dadzie, K. Y., Rolland, A., and Thylefors, B.**, Ocular onchocerciasis and intensity of infection in the community I West African savanna, *Trop Med Parasitol*, 40, 340, 1989

27 **Brandling-Bennett, A. D., Anderson, J., Fuglasang, H., and Collins, R.**, Onchocerciasis in Guatemala. Epidemiology in fincas with various intensities of infection, *Am J Trop Med Hyg*, 30, 970, 1981

28 **Taylor, H. R., Munoz, B., Keyvan-Larijani, E., and Greene, B. M.**, Reliability of detection of microfilariae in skin snips in the diagnosis of onchocerciasis, *Am J Trop Med Hyg*, 41, 467, 1989.

29 **Shulz-Key, H. and Karam, M.**, Quantitative assessment of microfilariae and adults of *Onchocerca volvulus* in ethanol-fixed biopsies and nodules, *Trans R Soc Trop Med Hyg*, 78, 157, 1984

30 **Nnochiri, E.**, Observations on onchocercal lesions in autopsy specimens in Western Nigeria, *Ann Trop Med Parasitol*, 58, 89, 1964

31 **Hamilton, R. G.**, Application of immunoassay methods in the serodiagnosis of human filariasis, *Rev. Infect Dis*, 7, 837, 1985

32 **Ouaissi, A., Koumemeni, L.-E., Haque, A., Ridel, P.-R., Saint André, P., and Capron, A.**, Detection of circulating antigens in onchocerciasis, *Am J Trop Med. Hyg*, 30, 1211, 1981

33 **Weiss, N.**, Monoclonal antibodies as investigative tools in onchocerciasis, *Rev Infect. Dis*, 7, 826, 1985.

34 **Des Moutis, I., Ouaissi, A., Grzych, J. M., Yarzábal, L., Haque, A., and Capron, A.**, *Onchocerca volvulus* Detection of circulating antigen by monoclonal antibodies in human onchocerciasis, *Am J Trop Med. Hyg*, 32, 533, 1983

35. **Cabrera, A. and Parkhouse, R. M. E.**, Isolation of an antigenic fraction for diagnosis of onchocerciasis, *Parasite Immunol*, 9, 39, 1987

36 **Petralanda, I., Yarzábal, L., and Piessens, W. F.**, Parasite antigens are present in breast milk of women infected with *Onchocerca volvulus*, *Am J Trop Med Hyg.*, 38, 372, 1988.

37. **World Health Organization**, Report of the Steering Committee of the Scientific Working Group on Filariasis, WHO TDR/FIL/SC-SWG(83-88)/88 3 1, 1988

38 **Greene, B. M., Gbakima, A. A., Albiez, E. J., and Taylor, H. R.**, Humoral and cellular immune responses to *Onchocerca volvulus* infection in humans, *Rev Infect Dis*, 7, 789, 1985

39 **Tanaka, H., Fujita, K., Sasa, M., Tagawa, M., Naito, M., and Kurokawa, K.**, Cross-reactions in complement fixation test among filarial species, *Jpn J Exp Med*, 40, 47, 1970

40. **Tada, I. and Ikeda, T.**, Indirect hemagglutination test in onchocerciasis with special reference to the utility of dried blood smears taken on filter papers, *J Kanazawa Med Univ.*, 1, 79, 1976.

41. **Collins, W. E., Campbell, C. C., Collins, R. C., and Skinner, J. C.**, Serologic studies on onchocerciasis in Guatemala using fixed-tissue sections of adult *Onchocerca volvulus*, *Am J. Trop Med Hyg*, 29, 1220, 1980

42 **Bartlett, A., Bidwell, D. E., and Voller, A.**, Preliminary studies on the application of the enzyme immunoassay in the detection of antibodies in onchocerciasis, *Tropenmed Parasitol*, 26, 370, 1975

43 **Luján, R., Collins, W. E., Stanfill, P. S., Campbell, C. C., Collins, R. C., Brogdon, W., and Huong, A. Y.**, Enzyme-linked immunosorbent assay (ELISA) for serodiagnosis of Guatemalan onchocerciasis: comparison with the indirect fluorescent antibody (IFA) test, *Am J. Trop. Med Hyg*, 32, 747, 1983.

44 **Weiss, N., Hussain, R., and Ottesen, E. A.**, IgE antibodies are more species-specific than IgG antibodies in human onchocerciasis and lymphatic filariasis, *Immunology*, 45, 129, 1982

45 **Voller, A. and De Savigny, D.**, Diagnostic serology of tropical parasitic diseases, *J Immunol Methods*, 46, 1, 1981

46. **Williams, J. F., El Khalifa, M., Mackenzie, C. D., and Sisley, B.**, Antigens of *Onchocerca volvulus*, *Rev Infect Dis*, 7, 831, 1985

47 **Schulz-Key, H., Albiez, E. J., and Buttner, D. W.**, Isolation of living adult *Onchocerca volvulus* from nodules, *Tropenmed Parasitol*, 28, 428, 1977

48 **Marcoullis, G and Grasbeck, P.**, Preliminary identification and characterization of antigen extracts from *Onchocerca volvulus*, *Tropenmed Parasitol*, 27, 314, 1976

49 **Philipp, M., Gómez-Priego, A., Parkhouse, R. M., Davies, M. W., Clark, N. W., Ogilvie, B. M., and Beltran-Hernández, F.**, Identification of an antigen of *Onchocerca volvulus* of possible diagnostic use, *Parasitology*, 89, 295, 1984

50 **Laemmli, U. K.**, Cleavage of structural proteins during the assembly of the head of bacteriophage T4, *Nature (London)*, 227, 680, 1970

51 **Towbin, H., Staehelin, T., and Gordon, J.**, Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets procedure and some applications, *Proc Nat Acad Sci U S A*, 76, 4350, 1979

52 **Hussain, R., Kaushal, N. A., and Ottesen, E. A.**, Comparison of immunoblot and immunoprecipitation methods for analyzing cross-reactive antibodies to filarial antigens, *J Immunol Methods*, 84, 291, 1985

53 **Lobos, E. and Weiss, N.**, Identification of non-crossreacting antigens of *Onchocerca volvulus* with lymphatic filariasis serum pools, *Parasitology*, 93, 389, 1986

54 **Cabrera, Z. and Parkhouse, R. M. E.**, Identification of antigens of *Onchocerca volvulus* and *Onchocerca gibsoni* for diagnostic use, *Mol Biochem Parasitol*, 20, 225, 1986

55 **Taylor, D. W., Goddard, J. M., and McMahon, J. E.**, Surface components of *Onchocerca volvulus*, *Mol Biochem Parasitol*, 18, 283, 1986

56 **Lucius, R., Buttner, D. W., Kirsten, C., and Diesfeld, H. J.**, A study on antigen recognition by onchocerciasis patients with different clinical forms of disease, *Parasitology*, 92, 569, 1986

57 **Lucius, R., Prod'Hon, J. Kern, A., Hebrard, G., and Diesfeld, H. J.**, Antibody responses in forest and savanna onchocerciasis in Ivory Coast, *Tropenmed Parasitol*, 38, 194, 1987

58 **Parkhouse, R. M. E., Cabrera, Z., and Harnett, W.**, *Onchocerca* antigens in protection, diagnosis and pathology, in *Filariasis* (Ciba Foundation Symposium 127), John Wiley & Sons, Chichester, 125, 1987

59 **Weiss, N. and Karam, M.**, Evaluation of a specific enzyme immunoassay for onchocerciasis using a low molecular weight antigen fraction of *Onchocerca volvulus*, *Am J Trop Med Hyg*, 40, 261, 1989

60 **Weiss, N., Speiser, F., and Hussain, R.**, IgE antibodies in human onchocerciasis Application of a newly developed radioallergosorbent test (RAST), *Acta Trop*, 38, 356, 1981

61 **Weil, G. J., Ogunrinade, A. F., Chandrashekar, R., and Kale, O. O.**, IgG4 subclass antibody serology for onchocerciasis, *J Infect Dis*, 161, 549, 1990

62 **Ottesen, E. A., Skvaril, F., Tripathy, S. P., Poindexter, R. W., and Hussain, R.**, Prominence of IgG4 in the IgG antibody response to human filariasis, *J Immunol*, 134, 2707, 1985

63 **Hussain, R., Poindexter, R. W., Ottesen, E. A., and Reimer, C. B.**, Use of monoclonal antibodies to quantify subclasses of human IgG II Enzyme immunoassay to define antigen specific (anti-filarial) IgG subclass antibodies, *J Immunol Methods*, 94, 73, 1986

64 **Hussain, R., Grogl, M., and Ottesen, E. A.**, IgG antibody subclasses in human filariasis Different subclass recognition of parasite antigens correlates with different clinical manifestations of infection, *J. Immunol*, 139, 2794, 1987

65 **Greene, B. M. and Unnasch, T. R.**, Molecular biologic approaches to research in onchocerciasis, *J Infect Dis*, 154, 1024, 1986

66 **Perler, F. B. and Karam, M.**, Cloning and characterization of two *Onchocerca volvulus* repeated DNA sequences, *Mol Biochem Parasitol*, 21, 171, 1986

67 **Shah, J. S., Karam, M., Piessens, W. F., and Wirth, D. F.**, Characterization of an *Onchocerca*-specific DNA clone from *Onchocerca volvulus*, *Am J Trop Med Hyg*, 37, 376, 1987

68 **Erttmann, K. D., Unnasch, T. R., Greene, B. M., Albiez, E. J., Boateng, J., Denise, A. M., Ferraroni, J. J., Karam, M., Schulz-Key, H., and Williams, P. N.**, A DNA sequence specific for forest form *Onchocerca volvulus*, *Nature (London)*, 327, 415, 1987

69. **Unnasch, T. R., Gallin, M. Y., Soboslay, P. T., Erttmann, K. D., and Greene, B. M.**, Isolation and characterization of expression cDNA clones encoding antigens of *Onchocerca volvulus* infective larvae, *J Clin Invest*, 82, 262, 1988

70 **Lobos, E., Altmann, M., Mengod, G., Weiss, N., Rudin, W., and Karam, M.**, Identification of an *Onchocerca volvulus* cDNA encoding a low-molecular-weight antigen uniquely recognized by onchocerciasis patient sera, *Mol Biochem. Parasitol*, 39, 135, 1990

71. **Jamnback, H.**, The origins of blackfly control programmes, in *Blackflies: The Future for Biological Methods in Integrated Control*, Laird, M, Ed, Academic Press, London, 1981, 71

72 **Walsh, J. F., Davies, J. B., and Cliff, B.,** World Health Organization Onchocerciasis Control Programme in the Volta River Basin, in *Blackflies: The Future for Biological Methods in Integrated Control*, Laird, M., Ed., Academic Press, London, 1981, 85

73 **Lea, A. O. and Dalmat, H. T.,** A pilot study of area larval control of blackflies in Guatemala, *J. Econ. Entomol.*, 48, 378, 1955

74 **Davies, J. B., LeBerre, R., Walsh, J. F., and Cliff, B.,** Onchocerciasis and *Simulium* control in the Volta River Basin, *J. Am. Mosq. Cont. Assoc.*, 38, 466, 1978

75 **Philippon, B.,** Problems in epidemiology and control of West African onchocerciasis, in *Black Flies*, Kim, K. C. and Merritt, R. W., Eds., Pennsylvania State University, University Park, 1986, 363

76 **Guillet, P., Escaffre, H., Ouedraogo, M. M., and Quilleveré, D.,** Mise en evidence d'une résistance au témephos dans le complexe *Simulium damnosum* (*S. sanctipauli* et *S. soubrense*) en Côte d'Ivoire, *Cah. ORSTOM Ser. Entomol. Med. Parasitol.*, 18, 291, 1980

77 **Kurtak, D.,** Insecticide resistance in the Onchocerciasis Control Programme, *Parasitology Today*, 2, 19, 1986

78 **Cheke, R. A. and Garms, R.,** Reinfestations of the southeastern flank of the Onchocerciasis Control Programme by wind borne vectors, *Phil. Trans. R. Soc. London Ser. B*, 302, 471, 1983

79 **Baker, R. H. A., Baldry, D. A. T., Boakye, D., and Wilson, M.,** Measures aimed at controlling the invasion of *Simulium damnosum* Theobald s.l. (Diptera: Simuliidae) into the Onchocerciasis Control Programme Area. III. Searches in the Upper Niger Basin of Guinea for additional sources of flies invading south eastern Mali, *Trop. Pest Manag.*, 33, 336, 1987

80 **Kurtak, D., Meyer, R., Ocran, M., Ouedraoogo, M., Renaud, P., Sowadogo, R. O., and Téle, B.,** Management of insecticide resistance in control of the *Simulium damnosum* complex by the Onchocerciasis Control Programme, West Africa: potential use of negative correlation between organophosphate resistance and pyrethroid susceptibility, *Med. Vet. Entomol.*, 1, 137, 1987

81 **Kurtak, D. C., Grunewald, J., and Baldry, D. A. T.,** Control of blackfly vectors of onchocerciasis in Africa, in *Black Flies*, Kim, K. C. and Merritt, R. W., Eds., Pennsylvania State University, University Park, 1986, 341

82 **Goldberg, L. J. and Margalit, J.,** A bacterial spore demonstrating rapid larvicidal activity against *Anopheles sergentii*, *Uranotaenia unguiculata*, *Culex univittatus*, *Aedes aegypti*, and *Culex pipiens*, *Mosq. News*, 37, 355, 1977

83 **Van Rie, J., McGaughey, W. H., Johnson, D. E., Barnett, B. D., and Van Mellaert, H.,** Mechanisms of insect resistance to the microbial insecticide *Bacillus thuringiensis*, *Science*, 247, 72, 1990

84 **Barjac, H. de,** Une nouvelle variéte de *Bacillus thuringiensis* très toxique pour les moustiques: *B. thuringiensis* var. *israelensis* serotype 14, *C. R. Acad. Sci. Ser. D.*, 286, 797, 1978

85 **Lacey, L. A., Escaffre, H., Philippon, B., Seketeli, A., and Guillet, P.,** Large river treatment with *Bacillus thuringiensis* (H-14) for the control of *Simulium damnosum* s.l. in the Onchocerciasis Control Programme, *Z. Tropenmed. Parasitol.*, 33, 97, 1982

86 **Lacey, L. A. and Undeen, A. H.,** Microbial control of blackflies and mosquitoes, *Annu. Rev. Entomol.*, 31, 265, 1986

87 **Copplestone, J. F. and Dobrokhotov, B.,** Biological control of vectors, in *Tropical Disease Research. A Global Partnership*, Maurice, J. and Pearce, A. M., Eds., UNDP/World/Bank/WHO Special Programme for Research and Training in Tropical Diseases, 8th Programme Rep., World Health Organization, Geneva, 1987, 125

88 **Molloy, D., Ed.,** Biological control of blackflies with *Bacillus thuringiensis* var. *israelensis* (serotype 14), *Misc. Publ. Entomol. Soc. Am.*, 12, 1982

89 **Lacey, L. A. and Undeen, A. H.,** The biological control potential of pathogens and parasites of blackflies, in *Black Flies*, Kim, K. C. and Merritt, R. W., Eds., Pennsylvania State University, University Park, 1986, 327

90 **Molloy, D. P.,** The ecology of blackfly parasites, in *Black Flies*, Kim, K. C. and Merritt, R. W., Eds., Pennsylvania State University, University Park, 1986, 315

91 **Pearson, D. and Ward, O. P.,** Effect of culture conditions on growth and sporulation of *Bacillus thuringiensis* subsp. *israelensis* and development of media for production of the protein crystal endotoxin, *Biotechnol. Lett.*, 10, 451, 1988

92 **Meredith, S. E. O., Kurtak, D., and Adiamah, J. H.,** Following movements of resistant populations of *Simulium soubrense/sanctipauli* (Diptera: Simuliidae) by means of chromosome inversions, *Trop. Med. Parasitol.*, 37, 290, 1986

93 **Post, R. J. and Kurtak, D.,** Identity of the OP-insecticide resistant species in the *Simulium sanctipauli* subcomplex, *Ann. Soc. Belge Med. Trop.*, 67, 71, 1987

94. **Magnin, M., Kurtak, D., and Pasteur, N.,** Charactérisation des estérases chez des larves du complexe *Simulium damnosum* résistantes aux insecticides organophosphores, *Cah. ORSTOM Ser. Ent. Med. Parasitol.*, 15, 57, 1988

95. **Walsh, J. F., Philippon, B., Hendrick, J. E. E., and Kurtak, D. C.,** Entomological aspects and results of the Onchocerciasis Control Programme, *Trop Med Parasitol*, 38, 57, 1987
96. **Roush, R. T.,** Designing resistance management programs How can you choose?, *Pestic Sci*, 26, 423, 1989
97. **Taylor, H. R. and Greene, B. M.,** The status of ivermectin in the treatment of human onchocerciasis, *Am J Trop Med Hyg*, 41, 460, 1989
98. **Greene, B. M., Brown, E. R., and Taylor, H. R.,** Use of ivermectin in humans, in *Ivermectin and Abamectin*, Campbell, W C, Ed, Springer-Verlag, New York, 1989
99. **Mackenzie, C. D., Boland, M., El Sheikh, H., and Dick, W.,** Adverse reactions to chemotherapy in onchocerciasis in *Proc Uppsala WHO/OCP Symp Onchocerciasis/Filariasis*, Conder, G A and Williams, J F, Eds, Kalamazoo, Mich, 1986, 153
100. **DeSole, G., Awadzi, K., Remme, J., Dadzie, K. Y., Ba, O., Giese, J., Karam, M., Keita, F. M., and Opoku, N. O.,** A community trial of ivermectin in the onchocerciasis focus of Asubende, Ghana II Adverse reactions, *Trop Med Parasitol*, 40, 375, 1989
101. **Zea-Flores, R. and Richards, F., Jr.,** personal communication, 1990
102. **Rothova, A., van der Lelij, A., Stilma, S. A., Wilson, W. R. and Barbe, R. F.,** Side effects of ivermectin in treatment of onchocerciasis, *Lancet*, 8650, 1439, 1989 (Vol 1)
103. **Bradshaw, H.,** Onchocerciasis and the Mectizan donation programme, *Parasitology Today*, 5, 63, 1989
104. **Freedman, D. O., Zierdt, W. S., Luján, A., and Nutman, T. B.,** The efficacy of ivermectin in the chemotherapy of gastrointestinal helminthiasis in humans, *J Infect Dis*, 159, 1151, 1989
105. **Cupp, E. W., Bernardo, M. J., Kiszewski, A. E., Collins, R. C., Taylor, H. R., Aziz, M. A., and Greene, B. M.,** The effects of ivermectin on transmission of *Onchocerca volvulus*, *Science*, 231, 740, 1986
106. **Prod'Hon, J., Lardeux, F., Bain, O., Hébrard, G., and Prud'Hom, J. M.,** Ivermectine et modalité de la reduction de l'infection des simulies dans un foyer forestier d'onchocercose humaine, *Ann Parasitol Hum Comp*, 62, 590, 1987
107. **Bissan, Y., Yingtain, P., Doucoure, K., Doumbo, O., Dembele, D., Ginoux, J., Cozettes, P., and Ranque, P.,** L'ivermectine (MK-933) dans le traitement de l'onchocercose, son incidence sur la transmission d'*Onchocerca volvulus* en savane soudanienne au Mali, *Med Afr Noire*, 33, 81, 1986
108. **Cupp, E. W., Ochoa, A. O., Collins, R. C., Ramberg, F. R., and Zea-Flores, G.,** The effects of multiple ivermectin treatment on infection of *Simulium ochraceum* (Diptera Simuliidae) with *Onchocerca volvulus* (Filarioidea Onchocercidae), *Am J Trop Med Hyg*, 40, 501, 1989
109. **Remme, J., Baker, R. H. A., DeSole, G., Dadzie, K. Y., Walsh, J. F., Adams, M. A., Alley, E. S., and Avissey, H. S. K.,** A community trial of ivermectin in the onchocerciasis focus of Asubende, Ghana I Effect on the microfilarial reservoir and the transmission of *Onchocerca volvulus*, *Trop Med Parasitol*, 40, 367, 1989
110. **Trpis, M., Childs, J. E., Fryauff, D. J., Greene, B. M., Williams, P. N., Muñoz, B. E., Pacque, M. C., and Taylor, H. R.,** Effect of mass treatment of a human population with ivermectin on transmission of *Onchocerca volvulus* by *Simulium yahense* in Liberia, West Africa, *Am J Trop Med Hyg*, 42, 148, 1990
111. **Cupp, E. W.,** personal observations, 1990
112. **Anderson, R. M.,** Epidemiological models and predictions, *Trop Geo Med*, 40, 530, 1988
113. **Harnett, W., Chambers, A. E., Renz, A., and Parkhouse, R. M. E.,** An oligonucleotide probe specific for *Onchocerca volvulus*, *Mol Biochem Parasitol*, 35, 119, 1989
114. **Ogilvie, B. M.,** Vaccines around which corner?, *Immunol Lett*, 19, 245, 1988.
115. **Mitchell, G. F.,** Problems specific to parasite vaccines, *Parasitology*, 98, S19, 1989
116. **Parkhouse, R. M. E. and Harrison, L. J. S.,** Antigens of parasitic helminths in diagnosis, protection and pathology, *Parasitology*, 99, S5, 1989
117. **Donelson, J. E., Duke, B. O. L., Moser, D., Zeng, W., Erondu, N. E., Lucius, R., Renz, A., Karam, M., and Flores, G. Z.,** Construction of *Onchocerca volvulus* cDNA libraries and partial characterization of the cDNA for a major antigen, *Mol Biochem Parasitol*, 31, 241, 1988
118. **Limberger, R. J. and McReynolds, L. A.,** Filarial paramyosin cDNA sequences from *Dirofilaria immitis* and *Onchocerca volvulus*, *Mol Biochem Parasitol*, 38, 271, 1990
119. **Ward, D. J., Nutman, T. B., Zea-Flores, G., Portocarrero, C., Luján, A., and Ottesen, E. A.,** Onchocerciasis and immunity in humans enhanced T cell responsiveness to parasite antigen in putatively immune individuals, *J Infect Dis*, 157, 536, 1988
120. **Arasu, P., Nutman, T. B., Steel, C., Mulligan, M. M., Abraham, D., Tuan, R. S., and Perier, F. B.,** Human T-cell stimulation, molecular characterization and *in situ* mRNA localization of a *Brugia malayi* recombinant antigen, *Mol Biochem Parasitol*, 36, 223, 1989
121. **Cupp, E. W., Bernardo, M. J., Kiszewski, A. E., Trpis, M., and Taylor, H. R.,** Large scale production of the vertebrate infective stage (L_3) of *Onchocerca volvulus* (Filarioidea Onchocercidae), *Am J Trop Med Hyg*, 38, 596, 1988

122 **Cupp, M. S., Cupp, E. W., and Poulopoulou, C.,** The use of cell-conditioned medium for the *in vitro* culture of *Onchocerca* spp larvae (Nematoda Filarioidea), *Trop Med Parasitol*, 41, 20, 1990

123 **Duke, B. O. L.,** Special requirements for use of adult *Onchocerca volvulus* in molecular biology, in *The Onchocerca Nodule and the Adult Filariae Normal Structure, Changes During Chemotherapy and Optimal Recovery of Worm Material*, Schulz-Key, H, Ginger C D, Duke, B O L, and Buttner, D W, Eds, *Trop Med Parasitol*, 39 (Suppl 4), 463, 1988

124 **Townson, S.,** The development of a laboratory model for onchocerciasis using *Onchocerca gutturosa in vitro* culture, collagenase effects, drug studies and cryopreservation, in the *Onchocerca Nodule and the Adult Filariae Normal Structure, Changes During Chemotherapy and Optimal Recovery of Worm Material*, Schulz-Key, H, Ginger, C D, Duke, B O L, and Butner, D W, Eds, *Trop Med Parasitol*, 39 (Suppl 4), 475, 1988

125 **Gutteridge, W. E.,** Parasite vaccines versus anti-parasite drugs rivals or running mates?, *Parasitology*, 98, S87, 1989

14