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PROTECTIVE IMMUNITY AND VACCINATION IN
 ONCHOCERCIASIS AND LYMPHATIC FILARIASIS:

REPORT OF THE THIRTEENTH MEETING OF THE
 SCIENTIFIC WORKING GROUP ON FILARIASIS



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SUMMARY

Although there is little evidence for the occurrence of acquired immunological resistance to Onchocerca volvulus in humans, epidemiological and laboratory data support the view that host-protective responses do develop in human lymphatic filariasis and in animal models. In view of the new opportunities to use these acquired immune mechanisms and molecular biology to generate potential immunogens, a meeting of the TDR Scientific Working Group on Filariasis was convened to consider appropriate research priorities and to formulate strategies for advancing the prospect for filarial vaccines. The absence of appropriate experimental animal filarial infections, which can be manipulated to explore protective immunity and test candidate vaccines, was identified as a major constraint requiring urgent attention. Furthermore, immunoregulatory and immunopathogenic processes need to be examined in these models so that suitable

targets can be pursued for protection-induction without concomitant complications, such as immunological exacerbation of pathology or autoimmunity. Immunological correlates of in vivo events are also urgently needed, both from human studies and controlled laboratory models, so that appropriate antigens can be selected for practical in vitro generation. Intensive study of human endemic-area controls, uninfected and unaffected by prevailing filarial parasites, is considered to be an especially valuable approach in this regard. Exploration of experimental models permitting longitudinal evaluation of effects on parasites (for example, in implanted chambers) is also considered highly likely to yield important advances. At this point, in the absence of clearly delineated strategies, the induction of resistance mechanisms against any or all of the parasitic stages in humans must be retained as a potentially attractive goal. To achieve this goal, it was considered imperative to target the early developing juvenile forms as a means of determining immune destructive processes against these stages in vivo and to clone genes for important, probably surface or secretory/excretory, antigens. Overall, the conclusions emerged that significant advances in knowledge of the biology of filarial infections needed to be promoted in order to take advantage of contemporary biotechnology and that biotechnological advances had made available a broader range of experimentation and more sophisticated analytical tools for probing antifilarial immunity and creating practical prophylactic agents for human use.

1. INTRODUCTION

The thirteenth meeting of the Scientific Working Group (SWG) on Filariasis of the UNDP/WORLD BANK/WHO Special Programme for Research and Training in Tropical Diseases (TDR) was held at Woods Hole, MA, USA, and co-sponsored by the Edna McConnell Clark Foundation (EMCF), New York, NY, USA.

The purpose of the meeting was to review the clinical, epidemiological, and experimental information bearing on resistance and protective immunity to filarial infections, particularly onchocerciasis and lymphatic filariasis, and to determine what further research is needed to develop successful vaccines against these infections.

In opening the meeting, Dr A.S. Dissanaiké read a message from Dr Tore Godal, Director of TDR, who recalled that there had been two previous SWG meetings dealing with protective immunity and vaccination. The first was held in London in 1975 at the very beginning of TDR and the second was the fourth SWG meeting held in Geneva in 1979. He indicated that to date studies on protective immunity and vaccination had had a relatively low priority in the work of the Filariasis SWG but the time was now ripe to pay more attention to this subject for both onchocerciasis and lymphatic filariasis. The new immunological and molecular biology techniques for identifying and obtaining critical filarial antigens and the encouraging results of vaccine and protective immunity studies in other parasitic diseases, notably malaria and schistosomiasis, make the outlook promising.

Dr Joseph A. Cook, Director of the Program for Tropical Disease Research of the Edna McDonnell Clark Foundation, reviewed the Foundation's long-standing, continuing interest in research on protective immunity in schistosomiasis. The Foundation's new programme on preventing the two major infectious causes of blindness (trachoma and onchocerciasis) has led to its current commitment to support efforts directed at developing protective vaccines against onchocerciasis. The Foundation's approach to this problem was outlined in a strategic plan developed in 1984 and includes not only individual grant support but also provision of research materials (e.g., DNA libraries of

O. volvulus and supplies of infective third-stage Onchocerca spp.), a commitment to fund vaccine trial experiments in chimpanzees in the 1990s, after suitable candidate vaccines have been identified, and the sponsorship of meetings such as the present one in close cooperation with TDR.

The technical sessions of the meeting were held at the Marine Biological Laboratory in Woods Hole and chaired by Prof J.F. Williams, Chairman of the Steering Committee of the Filariasis SWG, with Dr E.A. Ottesen acting as Rapporteur.

2. SCIENTIFIC PRESENTATIONS: ABSTRACTS OF WORKING PAPERS AND CONTRIBUTORS' COMMENTS

2.1 Evidence for Naturally Developing Immunity to Filariasis

2.1.1 Onchocerciasis
Abstract (Dr B.O.L. Duke)

Natural resistance to O. volvulus occurs in all animal species so far tested except the human, the gorilla and the chimpanzee. There is no evidence of human racial immunity, and persons of any age appear susceptible to infection, but it is possible that females of child-bearing age may be slightly less susceptible than others. Occasionally, individuals in hyperendemic communities may remain apparently negative for O. volvulus infection or harbour unusually light infections. The clinical, parasitological and immunological status of such persons needs careful investigation to ascertain the reasons for their "immune" or "near-immune" condition.

Acquired immunity to superinfection is difficult to assess, because it is only at the microfilarial stage that parasite density can be measured with any degree of accuracy. The "plateau-ing" of microfilarial concentrations in the skin, which is often seen after 20 to 40 years (Duke and Moore, 1968), may reflect an antimicrofilarial immunity, but it more likely results from a balance between introduction of new parasites and natural death of adult worms at a given level of transmission.

In cases of sowda, there is an exaggerated immune response against microfilariae, whose clinical consequences are most distressing to the patient and should serve as a warning of the possible dangers of developing a vaccine directed against microfilariae (mf).

There is no evidence of acquired immunity to reinfection in humans. Persons whose infections have been radically cured by treatment with suramin but who continue to be exposed to heavy transmission in an endemic area rapidly become reinfected, and these infections may reach pre-treatment levels of intensity within four to nine years (Duke, 1968).

Important lines of research needed to develop a protective vaccine would appear to be: development of better (small) animal models for O. volvulus infection; determination of O. volvulus population dynamics; study of tissue damage resulting from the host immune response.

Contributors' comments

The epidemiology of O. volvulus infection in hyperendemic areas shows an age-specific prevalence rising progressively after the first few years of life to almost 100% by the age of 30-40 years; the prevalence of disease (ocular and dermal) rises continuously to almost 100% by the fifth and sixth decades of life. This pattern of infection prevalence is similar to that of the filaria

Mansonella ozzardi (which is generally nonpathogenic) but contrasts with Wuchereria or Brugia infections, which peak and then decline after the middle decades. Prevalence and clinical data from the rainforest and savanna areas of the Cameroon give little support to the notion that protection develops naturally in hyperendemic areas (Anderson et al., 1974). On the other hand, prevalence data may be misleading, and the failure of microfilarial densities in the skin to increase progressively with age in the adult population may be evidence of resistance to reinfection (Nelson, 1958). This is particularly the case in areas with very high levels of continuing transmission. The same phenomenon is seen in many other helminth infections, for example, in schistosomiasis where the age-related egg output data are much more reliable indicators of resistance than are prevalence data (Wilkins et al., 1987) [Nelson].

Similar epidemiological findings have been noted in the hyperendemic regions of West Africa currently involved in the Onchocerciasis Control Programme in West Africa (OCP), where it is estimated that only 2-3% of adults remain free of Onchocerca infection [Karam].

In mesoendemic areas, the percentage of individuals remaining "infection-free", despite exposure to infective larvae, appears higher. A strategy to define immunological parameters responsible for, or associated with, naturally occurring immunity/resistance to infection in mesoendemic areas of Guatemala was described. Its premise was that a population of individuals exposed to infection but clinically and parasitologically "negative" (as well as on multiple thorough examinations over a number of years and on Mazzotti tests) could be defined as putatively immune. Comparison of a broad range of immunological responses to Onchocerca parasites between such "immune" individuals and patients with O. volvulus infections has been partially completed and shows significant immunological differences in T-lymphocyte reactivity to parasite antigen (greater responsiveness in the putatively immune group) (Ward et al., in press). No consistent differences between antigens recognized by IgG or IgE antibodies have yet been defined using adult worm extracts in immunoblotting procedures; infective larval antigen extracts have not yet been examined [Nutman].

There are many variables that could undermine the interpretation of such an approach (e.g., the various immunoregulating mechanisms resulting in parasite-specific immunosuppression and the tolerizing effect of prenatal exposure to parasite antigen), and selecting epidemiologically matched individuals for putatively immune and nonimmune populations is difficult [Taylor]. Also, zooprophylaxis [i.e., the prevention or attenuation of disease in humans as a result of previous exposure to heterologous filariae of animal origin (Nelson, 1974; Townson et al., 1985)] could complicate interpretation of such immunoepidemiological studies [Nelson].

Although no specific antigens or immune responses have yet been identified as being associated with an immune state in humans, it has been noted that seroconversion [of the response to adult worm antigens measured by enzyme-linked immunosorbent assay (ELISA)] occurs one to two years before parasitological positivity (microfilariae in skin snips) can be detected [Karam].

Analysis of antibody responses of patients with sowda has shown a striking IgG₃ response to a low-molecular-weight antigen from adult worm O. volvulus that is not found in patients with generalized onchocerciasis. Whether these individuals with the hyper-responsive sowda syndrome are also immune to reinfection has not been established, so that the relevance of this unique immune response to protective immunity is still uncertain [Parkhouse].

Because the major pathology of onchocerciasis results from a host response to microfilariae, in the development of a vaccine for onchocerciasis the

selection of L₃s, L₄s or young adults as targets for protective immune responses may be safer than aiming at microfilariae. This issue is still uncertain, however, and must be further explored, since an anti-microfilariae vaccine that would prevent production of microfilariae (a type of 'anti-fecundity vaccine') has great theoretical appeal [Nelson].

2.1.2 Lymphatic filariasis Abstract (Dr J.W. Kazura)

Cross-sectional surveys of residents of areas endemic for brugian or bancroftian filariasis generally indicate that patent infection increases in frequency until the age of 20-30 years and then remains constant or declines thereafter. In addition, a variable proportion of subjects over 30-40 years of age are amicrofilaraemic with no evidence of lymphatic disease. These surveys suggest, but do not prove, that resistance may develop in persons continually exposed to L₃-containing mosquitos. In order to define more clearly a population of individuals who have developed protective immunity, it is necessary to undertake:

- longitudinal studies in endemic areas of subjects who are amicrofilaraemic and have no evidence of disease; comparison should be made with age-matched persons who are microfilaraemic and reside in the same endemic area. These groups are ideally suited for evaluation of immunological correlates of protective immunity in humans.
- measurements of intensity of exposure to L₃, to be made concurrently.
- investigation of the role of genetic [e.g., human leukocyte antigen (HLA)] and maternal factors in immunity.

Contributors' comments

It is likely that immune individuals in endemic populations will be found among those who do not have microfilariae circulating in the blood. Such amicrofilaraemics may show lymphatic disease resulting from previous infection, acute adenolymphangitis from early developing infection (as seen among American servicemen in the Pacific in World War II and in transmigrants in Indonesia) or no signs or symptoms of filarial infection (i.e., "endemic normals"). Distinguishing clinically or by laboratory tests which of these amicrofilaraemics have protective immune responses is a problem [Partono].

A putatively infection-free group of individuals from a W. bancrofti endemic area (Cook Islands) was identified on the basis of clinical and parasitological findings, as well as by the absence of characteristic reactions after treatment with diethylcarbamazine (DEC) and by negative findings from a circulating parasite antigen assay. Detailed immunological comparison of these individuals with those infected with W. bancrofti showed greater quantitative responses (lymphocyte proliferation, IgG and IgE antibodies) in the putatively immune group. Qualitative analysis failed to identify a unique immunogen when responses to adult worm (B. malayi) antigen were assessed, but the immune group showed unique IgG antibody responsiveness to an antigen with a relative molecular mass (M_r) of 42 000 (42K) derived from infective third-stage larvae. Further analysis of this and other potentially protective immunogens from infective larvae is necessary [Ottesen].

2.2 Experimental Induction of Immunity to Filariae: Animal Models

2.2.1 Onchocerciasis Abstract (Dr N. Weiss)

Partial immunity to challenge infections has been shown in different

filarial models (e.g., Dirofilaria immitis, Acanthocheilonema viteae*) after the inoculation of live, irradiated third-stage larvae (L₃). However, the induction of protective immune responses is host-species dependent; for A. viteae, jirds, but not hamsters, are partially protected. The injection of dead larvae does not confer immunity to challenge. Protective antigens, therefore, seem to be parasite products released by live worms (L₃, moulting L₃, L₄). Although anti-surface antibodies to developing larvae can be detected in immune animals, there is no direct evidence that they are involved in effector mechanisms against developing larvae. For A. viteae it has been shown that effector mechanisms are directed only against developing, not vector-form, L₃. Their development in immune animals is arrested at the moult, as demonstrated by micropore chamber experiments.

Immunity to microfilariae has been more extensively studied in different laboratory models (only rarely in the natural host). Live microfilariae were shown to be better immunogens than dead microfilariae or parasite extracts. There is good evidence that microfilariae killing depends on antibody-dependent cellular cytotoxicity reactions for which an anti-surface antibody of a distinct isotype is required. Stage-specificity for surface antigens is species-dependent; common antigens are present on the surface of microfilariae and L₃ of Litomosoides carinii, whereas for A. viteae surface antigens are stage-specific.

Contributors' comments

Studies of O. lienalis in experimentally infected calves have shown that a proportion of female larvae fail to complete maturation and appear to be arrested in development. They may be in a dormant state but able subsequently to mature and replace adult female parasites that are no longer reproductively active. Equivalent studies are lacking in O. volvulus infections, but the phenomenon could be of importance in both chemotherapy and vaccination studies [Bianco].

Longitudinal studies of lymphocyte responsiveness in O. lienalis-infected cattle indicate that the responses to Onchocerca antigen are rapidly lost when the infection becomes patent (i.e., microfilariae identifiable in the skin). While this implies that microfilariae could induce a state of immunosuppression, calves exposed to microfilariae alone (by injection of parasites) exhibit high blastogenic responses, so that life-cycle stages other than microfilariae might be required to act synergistically to effect the immunosuppression seen in patent infections [Bianco].

Preliminary experiments on immune responses and protective immunity in six chimpanzees are planned in Liberia, using irradiated third-stage O. volvulus as immunogens. Studies to determine the optimal radiation dose for these larvae (assessed by determination of the maximal dose permitting the L₃-L₄ moult in vitro) are first being carried out [Prince].

2.2.2 Lymphatic filariasis

Abstract (Dr G. Higashi and Dr J.A. Yates [presented by Dr Yates])

Progress in the development of a small animal model suitable for vaccine studies in lymphatic filariasis has been slow. The jird (Meriones unguiculatus) is a reliable host for subperiodic B. malayi and B. pahangi and has proved useful in numerous studies of Brugia biology and physiology, as well as

* Previously called Dipetalonema viteae but recently reclassified (Bain et al., 1982).

immunology. Vaccination of inbred jirds with ^{60}Co radiation-attenuated B. malayi infective stage larvae (L_3) protected against homologous challenge given either subcutaneously or by the intraperitoneal (IP) route. Groups of jirds vaccinated subcutaneously with 15-kilorad-irradiated L_3 showed from 60% to 91% reduction in recovered worms after IP challenge infection, compared with infection in non-vaccinated control jirds, while a 75% reduction in mean worm burden was seen in jirds receiving subcutaneous challenge infections (Yates and Higashi, 1985).

A marked increase in anti-B. malayi antibody in vaccinated jirds was seen (by ELISA) immediately after challenge infection, and an immunofluorescence assay showed that L_3 s incubated in serum from vaccinated jirds were completely and uniformly covered with specific antibody. Eosinophil-rich granulomas containing moribund and dead larvae were recovered from vaccinated jirds but not from control jirds that had received only the challenge infection. Evaluation of these larva-containing granulomas by transmission electron microscopy revealed that they contained large numbers of eosinophils. Cuticular surfaces were covered with eosinophils; where cuticular degeneration was evident, the eosinophils had invaded the interior of the worm and degranulated. The granulomas also contained small numbers of neutrophils, lymphocytes and macrophages. These findings suggest that cells and specific antifilarial antibodies are involved in the immune mechanism (Yates and Higashi, 1986). This model of protective immunity in a Brugia-susceptible small rodent should be especially useful in characterization of immune mechanisms and for the identification and evaluation of filaria-protective immunogens.

Contributors' comments

Ultraviolet light has also been found effective in attenuating third-stage larvae for use as immunogens to B. pahangi in jirds. Such studies suggest that it is the excretory-secretory (ES) products or moulting fluid that contains the effective immunogen. Indeed, vaccination with ES products collected from third-stage larvae of B. pahangi in vitro has resulted in partial immunity (determined by decreased worm numbers and/or absence of circulating microfilariae) in immunized jirds. Further analysis of the mechanisms involved has been hampered by the difficulty of collecting such ES material and by its lability [Wong].

In cats B. pahangi microfilaraemia levels cannot be used to assess immunity, as they reflect neither the number of L_3 s inoculated nor the number of adult female worms found in the animals. Comparative studies of the effectiveness of different levels of ^{60}Co irradiation indicated that greater immunity could be achieved with 90-kilorad doses than with smaller doses. Indeed, low-level irradiation actually caused the immunizing L_3 s to initiate local disease in the lymphatics [Denham].

The mouse, in which B. malayi can not develop fully, may be useful for screening potential immunogens in vivo. L_3 s can be inoculated and recovered after intraperitoneal injection and their viability can be assessed. The mouse model, given the number of inbred strains available, also has potential for identification of the mechanisms responsible for larval killing in vivo [Hayashi].

2.3 In Vitro Antifilarial Killing Mechanisms Abstract (Dr B.M. Greene)

Microfilarial killing has been investigated using a variety of in vitro systems. In O. volvulus, opsonic antibodies obtained from infected persons promote adherence of granulocytes and killing of microfilariae. Eosinophils appear to be more efficient than neutrophils in killing microfilariae.

especially in the presence of a source of complement. Adherent peripheral blood mononuclear cells and lymphocytes from both normal and infected persons are not cytotoxic for microfilariae in this system. In W. bancrofti infection, individuals who are amicrofilaraemic or have elephantiasis demonstrate opsonic antibodies directed against the surface of microfilariae which promote attachment and killing. The predominant isotype binding to the surface of microfilariae is IgG, but IgM is also seen.

Little is known about effector mechanisms against infective larvae. Selective adherence of eosinophils to infective W. bancrofti larvae was demonstrated in one study. This phenomenon appeared to be IgG-dependent and was enhanced by the presence of fresh serum. No evidence of damage to larvae was seen. In another study, normal human buffy coat cells attached to infective B. malayi larvae in the presence of sera from normal or infected persons; however, sera from individuals with tropical pulmonary eosinophilia caused greatest adherence. Scanning electron microscopy suggested damage to the larvae.

The relevance of these in vitro observations to in vivo killing mechanisms is difficult to assess. While the potential importance of the various reactants studied in vitro can be determined, many of the in vitro model systems can be set up to demonstrate phenomena unlikely to be of importance in vivo. Therefore, until some strong correlations between in vivo protective immune responses and in vitro parasite killing mechanisms can be established, caution should be exercised in gauging the implications of such in vitro observations.

Contributors' comments

In the presence of cells, but not antibody, complement kills eggs, desheathed and unsheathed microfilariae, and infective larvae from a number of filarial species in vitro. C₃ receptors can be recognized on these filarial stages and the complement appears to be activated by the alternate pathway. While neutrophils and macrophages appear to have the greatest cytotoxic effects on lymphatic filarial larvae, mediators (undefined) have been recognized in eosinophil culture supernatants that activate macrophage-mediated cytotoxicity against B. malayi L₃s [Subrahmanyam].

A cytotoxic monoclonal antibody has recently been raised against B. malayi L₃s which recognizes filaria-specific antigens in the 20-45K range. Such antigens need further evaluation for their potential use as protective immunogens [Subrahmanyam].

A compromise between in vitro and in vivo observations on larval growth or destruction involves the use of micropore chambers implanted subcutaneously. These chambers provide a more physiological milieu for larval growth and survival than in vitro cultures and still permit analysis of host effector mechanisms. The infiltrating cell populations provoked by the parasites in these chambers differ from those in the peripheral blood or peritoneum and might reflect a situation closer to that found normally in vivo [Weiss].

An additional problem that influences interpretation of studies using third-stage larvae in vitro is the fact that larval development in vivo changes the parasite surface in a way that may alter the larval response to specific immune mechanisms [Weiss].

2.4 Successful Strategies in Veterinary Anti-helminth Vaccines Abstract (Dr R.B. Grieve)

Vaccines for prevention of canine hookworm infection and bovine lungworm infection became commercially available some time ago (Jarrett et al., 1960;

Miller, 1978). Both vaccines employed live, radiation-attenuated larvae and were especially feasible because they were intended for use in young animals for the prevention of clinical disease rather than infection. In addition, immunity against these diseases may have been relatively easy to achieve through vaccination because similar immunity eventually occurs with natural exposure to nonlethal levels of both infections.

The hookworm vaccine had several valuable attributes: (1) immunity persisted until age-related resistance developed; (2) maternal antibody or inter-current infection did not interfere with vaccine efficacy; (3) cross-protection occurred among various hookworm species. The hookworm vaccine is no longer available, however, for several reasons. First, adverse reactions were observed in hookworm-hypersensitive dogs when attenuated larvae reached the lungs. Second, the vaccine was judged expensive in comparison to available, effective anthelmintics, and this expense was compounded by both a limited product shelf-life and the irregular dispensing of vaccine in individual doses. Third, although the vaccine was intended for the prevention of clinical disease in young animals, any infection (evidenced by hookworm eggs in faeces) was (wrongly) perceived as a vaccine failure.

The lungworm vaccine is still used successfully in selected situations. Problems associated with shelf-life of this vaccine are obviated by the simultaneous dispensing of vaccine to many animals. However, since post-vaccination immunity is perpetuated by regular exposure to infective larvae, the vaccine is less successfully employed in hypoendemic or sporadically endemic areas.

The success of these anti-nematode vaccines should encourage the development of antifilarial vaccines. However, both the lack of biological relatedness of these organisms to filariae and, in contrast to filariasis, the marked degree of acquired immunity to these infections that develops under conditions of natural exposure necessitate caution in extrapolation of these results to filariasis.

On the other hand, a filarial nematode of veterinary importance, *D. immitis*, should serve as a good model for immunoprophylaxis of human filariasis. Radiation-attenuated larvae and chemically abbreviated infections have been used to produce variable levels of protective immunity against this parasite (Wong, et al., 1974; Blair and Campbell, 1981). Investigation of these protective immune responses currently involves studies on the biology of developing larvae, methods for obtaining antigens from living larvae, development of surrogate rodent hosts and optimization of levels of protection in dogs using chemically abbreviated infections. Progress relevant to human filariasis has been made in each of these areas.

Contributors' comments

There were two additional difficulties with veterinary anti-nematode vaccines that could be relevant to attempts to vaccinate humans against filariae. First, the vaccines were often not effective in very young animals and by the time their immune systems had matured enough to respond appropriately to the immunogen, the animals had already become infected. Second, different breeds of animals reacted differently to the vaccines, with consequent variations in vaccine efficacy [Ogilvie].

Chemically abbreviated *D. immitis* infections have also been studied in ferrets, where it was found that "trickle infections" with small numbers of L₃s (followed by ivermectin treatment) did not provide as high a level of immunity as did a single large (200) inoculum of L₃s followed by ivermectin. This observation suggests that natural exposure, even if followed by intermit-

tent chemotherapy, might not provide the necessary "biomass" (moulting fluid, ES antigen) to stimulate strong protective immune responses [Wong].

2.5 Successful Strategies in Schistosome Vaccine Development Abstract (Dr F.A. Sher)

Recent advances in the immunology of schistosomiasis suggest that vaccination against the human schistosomes may be feasible. For the first time, investigators have been able to partially protect laboratory hosts against challenge infection by immunization with purified or recombinant schistosome antigens. The two vaccination strategies employed have been the induction of antibodies against the schistosome surface and the stimulation of cell-mediated immunity against soluble, non-surface-derived parasite molecules. At the same time, field studies on human populations infected with Schistosoma mansoni or S. haematobium have confirmed the existence of acquired immunity in humans and provided preliminary evidence for resistance-associated immunological correlates.

While filarial parasites are inherently more difficult to study than schistosomes, vaccines could be developed against them using similar approaches. In particular, progress in schistosome research suggests that the identification of antigens in the infective L₃ or L₄ stages recognized specifically by immune human populations or laboratory hosts would be the most logical and direct approach to filarial vaccines.

Contributors' comments

In humans, therapeutic vaccines (i.e., vaccines that eliminate existing infection) may be a feasible alternative to prophylactic vaccines and may be more easily tested for efficacy than prophylactic vaccines. Target antigens of therapeutic vaccines are likely to be molecules of low immunogenicity that serve an essential function in the established worm. If the function of such molecules could be neutralized, existing immune responses might then be sufficient to eliminate the parasite. Enzymes of established worms are obvious candidates for therapeutic vaccines, and some encouragement to pursue this approach has been provided in other helminth infections by studies with the glutathione S-transferase enzyme in S. japonicum and with digestive proteases in hookworms [Mitchell].

2.6 Cloning the Genes and Producing the Critical Antigens for Protective Immunity Abstract (Dr M. Philipp)

Various factors governing resistance to reinfection with B. malayi larvae in Balb/c mice have been examined to provide baseline data for use of this model in studies of immunoprophylaxis (Carlow and Philipp, 1987). Soluble saline extracts of B. malayi infective larvae contain antigens that enhance the ability of Balb/c mice to reject intraperitoneal infection with these parasites. This effect was augmented (76% protection) when the antigens were injected subcutaneously with Freund's complete adjuvant (FCA).

Because of its operational rapidity and simplicity, the mouse model can be used to screen pools of recombinant parasite antigens for their protective capacity. For example, mice were immunized with a pool of five recombinant B. malayi antigens from a λ gt11 genomic DNA expression library. Animals received approximately 10 μ g of each recombinant antigen emulsified in FCA and administered twice at an interval of four weeks. Mice challenged four weeks after the second injection possessed serum antibodies reacting with third-stage larval extracts electroblotted onto nitrocellulose. Despite effective sensitization to parasite components, the immunized mice showed no enhanced

resistance compared to controls. Therefore, the five recombinant antigens tested are probably not protective in this model system.

Sensitization with living male or female adult worms, fourth-stage larvae or microfilariae of B. malayi, or infective B. pahangi larvae conferred substantial resistance to larval challenge. The finding that there are B. malayi protective antigens active in the mouse that are neither species- nor stage-restricted should facilitate access to more abundant sources of parasite material.

The antigenic crossreactivity found between surface antigens of O. cervicalis and O. volvulus microfilariae (of 68K and 14.5K, respectively) establishes a relevant connection between human and horse parasites and extends previous work showing crossprotection against O. lienalis microfilariae in mice stimulated by heterologous parasites (including O. volvulus) and by their crude extracts (Townson and Bianco, 1982a; Townson et al., 1984; Carlow et al., 1986; Carlow and Bianco, 1987a,b). For these reasons, what can be learned from the O. cervicalis-Balb/c mouse model about the protective properties of defined antigens may be pertinent to immune responses to O. volvulus microfilariae in the mouse, and perhaps in the human.

Contributors' comments

Sponsored by the EMCF, Prof Donelson and Dr Duke travelled to four onchocerciasis-endemic regions [Guatemala, Mali (savanna) and Cameroon (Touboro savanna area and Kumba forest area)] to perform nodulectomies for collection of parasites and preparation of cDNA libraries. These libraries have been constructed in bacteriophage λ gt11; the average cDNA insert size of 18 randomly selected clones was 1200 base pairs (bp) (range: 3000-500 bp). When screened with human patient sera or rabbit antisera raised against O. volvulus lysate, about 1 in 500 clones in the primary cDNA libraries was positive and about 1 in 700 in the amplified libraries. These libraries, as well as sheared genomic DNA libraries to be constructed in λ gt11, are available through the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, MD 20852, USA [Donelson].

The genomes of B. malayi, B. pahangi and D. immitis all are very AT rich (about 70-73%) and have about 80×10^6 bp. In the coding regions there appears to be approximately a 2:1 preference for AT base pairs in the third position (the degenerate or wobble position of the codon). A genomic library would require about $1-5 \times 10^6$ recombinants to have all epitopes expressed. This estimate assumes that there are not large numbers of intervening sequences to break up coding regions and their epitopes. In each L_3 there are about 2-5 ng of DNA. If an equal amount of RNA is assumed, then 20 000 L_3 s would yield 40-100 μ g of total RNA. Estimates indicate that about 5% would be mRNA (for making cDNA libraries), but difficulty in lysing L_3 s and degradation of the nucleic acids would probably reduce the mRNA yield [McReynolds].

One rational approach to identifying appropriate target antigens is to study functional proteins necessary for entry or maturation of the parasite within the host. Invasion by all three life-cycle stages is probably mediated by proteases elaborated by the parasite. By identifying the class of proteases active at each stage, advantage can be taken of consensus sequences of proteases of a given class to design molecular probes for cloning. Cloned proteases could then be used to test whether an induced immune response to them can interrupt parasite invasion, development or transmission [McKerrow].

Similarly, detailed study of the cellular and biochemical aspects of the reproductive biology of filarial nematodes could reveal targets for immunological or chemotherapeutic intervention. Preliminary examination of the

polypeptides associated with the reproductive tissues of male O. volvulus, O. gibsoni, Ascaris lumbricoides, D. immitis and Caenorhabditis elegans has revealed a high degree of epitope homology between sperm-associated proteins. Furthermore, this homology, at least for one of the peptides, extends to the DNA level. Such information suggests that a variety of nematode species have adopted similar reproductive strategies and that information collected on the biology and biochemistry in model systems may be highly relevant to human pathogens [Scott].

Approximately 100 cDNA clones representative of many distinct proteins have been isolated from adult B. pahangi in a λ gt11 library with human sera from an area in Indonesia endemic for B. malayi. About 10% of these clones encode proteins not recognized by individuals with patent infection. Many of the proteins isolated represent major structural components of the parasite unlikely to be appropriate targets of an immune response in a living worm. Such blind screening may therefore represent an inefficient use of time and money. More focused screening with a polyclonal antiserum raised to the major surface 29K glycoprotein of adult worms has led to the isolation of 20 clones [Selkirk et al., 1987]. This protein is expressed on L₄ and L₃ following infection of the vertebrate host. It appears to be highly conserved among the lymphatic filariae and is accessible to antibody binding in live worms [Selkirk].

Several cautionary notes must be sounded about generating libraries and screening for potential immunogens. First, protein synthesis is known to change as worms (adults, infective larvae and microfilariae) are cultured in different conditions (e.g., different temperatures), and little is known about the timing (and cues) for synthesis of selected molecules in any of the life-cycle stages [Selkirk]. Second, many of the immunoscreening protocols used to detect antigen-producing recombinant clones screen only for the IgG component of the antisera; binding of antigen-specific IgE, IgA, IgM or the IgG subclasses may also be very important [Scott]. Third, because of their acknowledged biological importance, the availability of gene libraries, the power of protein immunochemistry and the capacity to measure them in large quantities, proteins will dominate in the quest for a vaccine against filariae, although carbohydrate and glycolipid antigens, whose significance has already been well demonstrated in patients with cutaneous leishmaniasis, should not be neglected [Mitchell].

2.7 Provision of Larvae for Immunological Studies and Vaccine Development

2.7.1 Onchocerca spp. Abstract (Dr E.W. Cupp)

To produce large quantities of Onchocerca spp. infective larvae (L₃), it has been necessary first to develop and then to integrate various laboratory subsystems. These include methods and techniques for collection and cryopreservation of microfilariae, mass rearing and/or colonization of susceptible Simulium species, and infection, harvesting and cryopreservation of L₃s. Collectively, these newly developed techniques have made it possible to work successfully in the laboratory with the advanced larval stages of O. lienalis and O. volvulus.

However, the level of efficiency for the mass production of infective larvae requires improvement. Approaches currently being employed include the use of laboratory-derived S. yahense, a natural vector of O. volvulus in Liberia; the development of an in vitro membrane feeding method for per os infection of natural and surrogate vectors; and the selection of genetically defined laboratory strains of S. vittatum highly susceptible to infection with Onchocerca spp. These latter techniques, emphasizing the use of nearctic

Simulium species, will be coupled with in vitro methods currently being developed to induce morphogenesis of nodular O. volvulus microfilariae in the vector.

2.7.2 Brugia spp.

Abstract (Dr D.A. Denham)

In theory, large numbers of living infective larvae (L₃) can be produced in two ways. Although rationally it would seem that the only way to produce vast numbers is to culture microfilariae in vitro, the method currently being used is to passage microfilariae through an insect vector. However inefficient this approach seems, when compared to the problems of providing large numbers of L₃ of O. volvulus, the problems of providing L₃ of B. malayi and W. bancrofti pale in significance.

Fortunately, the mosquito Aedes aegypti, homozygous for the fm gene, is a laboratory vector easily colonized in large numbers and susceptible to infection with both B. malayi and W. bancrofti. The fm gene occurs in all wild populations of A. aegypti. It is safe to work with this strain in tropical areas because if the mosquito is accidentally released the recessive fm gene will be swamped by the dominant FM gene, which would make the progeny of any escapees resistant.

By hatching eggs and collecting pupae on a single day, 20 000 female mosquitos can be reared per week with relatively little effort. If large animals, such as cats, dogs or monkeys, infected with B. malayi are available, it is possible to feed several cages of mosquitos on the side of an anaesthetized host, but with hundreds of cages it is usually necessary to use some form of membrane feeding system with Brugia spp. microfilariae (obtained by lavaging the peritoneal cavities of jirds infected by intraperitoneal injection of L₃) suspended in fresh blood and fed to mosquitos through a membrane. The only source of large numbers of W. bancrofti microfilariae is human blood, and experience suggests that collection of such blood is a major hurdle in some endemic areas.

These obviously important constraints lend weight to the view that mass production of L₃ will only be achieved when the problem of growing microfilariae to L₃ in vitro is solved.

2.8 Immunoregulation as a Potential Modifier of Vaccine Efficacy or Inducer of Complications

Abstract (Dr T.R. Klei)

Vaccination of individuals against filariae may produce two major unacceptable outcomes. First, it may induce immune responses that initiate or accelerate the pathogenesis of lymphatic lesions and disease, in addition to those responsible for protective resistance. Studies on the immune status of human populations suggest that individuals showing signs of chronic lymphatic pathology have greater specific cellular immune responses and higher antibody titres to filarial antigens than do individuals without disease (Ottesen, 1984; Piessens et al., 1987). Similarly, animals sensitized to antigen prior to infection or receiving multiple infections develop more severe lymphatic lesions than do non-sensitized individuals (Klei et al., 1982; Denham and Fletcher, 1987). These observations suggest that a positive relationship exists between disease and specific immune responses. Second, the vaccine might fail if introduced into immunologically tolerant individuals or those with existing antigen-specific suppression of immune responses. Evidence from studies of human populations and from animal model systems suggests that concern about this possibility is warranted (Klei et al., 1986). Although it will be difficult to test these hypotheses in human populations, it is currently feasi-

ble to conduct experiments examining these possibilities in existing animal models of lymphatic filariasis.

Contributors' comments

Breast milk from women with onchocerciasis contains parasite antigens detectable with filaria-specific monoclonal antibodies. Antigen-containing milk and fractions prepared from the milk contain high-molecular-weight parasite antigens that inhibit antigen and mitogen-induced T-cell proliferation and that induce nonspecific suppressor T cells *in vitro*. This finding raises the possibility that oral feeding of parasite antigens in the neonatal period could modify immune responses to filariae during subsequent infection [Piessens].

2.9 Perspectives

Abstract (Dr F.A. Sher)

It has been said that since parasites survive the immune responses they induce, the antigens recognized in these responses must be neither good targets nor good inducers of protective immunity. Therefore, the best vaccine immunogens should be those antigens not recognized during chronic infection, since the response to these molecules may be lethal and therefore normally suppressed. It follows that targeted vaccine approaches should be designed to block parasite morphogenesis, migration or reproduction or to find a target antigen that is functionally and metabolically the "Achilles' heel" of the parasite (e.g., an enzyme required for normal physiological function).

In theory, there are certain advantages to a vaccine based on the induction of cell-mediated immunity. Such immunity would bypass the evasion strategy of coating parasite surfaces to prevent recognition by host antibodies; it would create localized tissue responses, thereby avoiding risks of systemic immune complex disease, and it would involve peptide (T-cell recognized) epitopes that can be readily synthesized by either biochemical or recombinant DNA methods.

Studies in schistosomiasis are instructive for filarial vaccine development. Most important for the development of anti-schistosome vaccines has been the availability of good laboratory *in vivo* models, an abundant supply of parasite material from all stages of the life cycle and the availability of careful epidemiological studies to test the relevance of findings from studies in animals. The less important contributors to the development of anti-schistosome vaccines thus far have included *in vitro* immunology and gene cloning technology.

For filarial vaccines the ideal target appears to be the infective L₂ stage of the parasite. There are two 'ideal' approaches to the development of filarial vaccines. The first involves an epidemiological strategy that would:

- identify a subgroup of exposed individuals who appear immune to infection;
- characterize L₂-specific immune responses and antigens recognized by this putatively resistant population;
- use recombinant DNA (genomic or L₂-cDNA expression libraries) to synthesize the relevant epitopes;
- conduct limited vaccine trials in nonhuman primate models and, if successful, in humans.

The other ideal approach would be the "animal model strategy" (where susceptible small animal hosts exist, e.g., Brugia spp.). This approach would involve the following steps:

- development of attenuated L₃ vaccine models;
- characterization of effector mechanisms;
- development of a crude, nonliving vaccine in which the same effector mechanism is induced;
- identification and synthesis of the relevant protein antigen(s);
- tests of the vaccine in small animal models, primates and humans.

3. CONSENSUS STATEMENTS ON SPECIFIC ISSUES

3.1 Does Resistance to Filariae Occur or Develop Naturally in Humans? What Are the Mechanisms Underlying It? (Rapporteur: Dr J. Kazura)

3.1.1 Onchocerciasis

3.1.1.1 Background

Two categories of immunity to O. volvulus may be considered. The first is the natural immunity or, conversely, the innate susceptibility, of the mammalian host to infection. Studies to date indicate that only humans and higher apes are susceptible to infection by O. volvulus L₃s that develop eventually into fecund adult worms. Racial, sexual or age-related differences in innate susceptibility have not been described. The second category, acquired immunity, has received relatively more attention. This concept refers to the situation in which previous infection with O. volvulus confers an enhanced ability to eliminate one or more stages of the organism when superinfection takes place. Measurements of the prevalence and intensity of infection rely primarily on the presence of microfilariae in the skin, as the proportion of persons in endemic areas who have nodules only or microfilariae detected only in the eyes is usually quite low. To date, there are no reliable indicators of adult worm burden, whether by enumeration of palpable nodules or by detection of circulating antigens.

In infected populations living in hyper- or mesoendemic areas, it has been noted that the mean concentration of microfilariae in the skin usually rises steadily until adult life and then levels off between the ages of 20-40 years. This phenomenon is sometimes ascribed to the development of protective immunity, although it may alternatively be a result of the parasite population reaching a steady state between the number of adult worms dying and the number of newly introduced infective larvae that reach maturity.

To provide information on the development of resistance to reinfection, infected West African patients who had been treated with a course of suramin sufficient to kill adult worms and who continued to live in an area of ongoing transmission, were re-examined at intervals after treatment. Treatment with the drug reduced their skin microfilarial counts to near zero within two years of completing the course. Thereafter, the patients were exposed to continuing transmission at the same high level as before treatment. Within four to nine years of completing the course of suramin treatment, their infections had built up again to pre-treatment levels of intensity, and some had reached even higher levels. Although it cannot be said for certain that no male or female worms survived the suramin treatment, these observations suggest that little or no residual immunity to O. volvulus reinfection was induced by their initial infections.

Studies in other endemic areas of West Africa have provided some evidence that a small proportion of individuals may develop resistance. In Mali, children followed by serial skin-snip examination have been observed to convert from microfilariae-positive to microfilariae-negative. In addition, cross-sectional surveys in hyperendemic and mesoendemic areas show that 2% to 3% of adults are disease-free and microfilariae are either undetectable or found at very low levels in their skin.

In Guatemala, a group of persons with no clinical or parasitological evidence of infection and with negative Mazzotti tests have also been identified as possibly being resistant to infection (Ward et al., 1987). These individuals are currently thought to represent a small proportion of the exposed population, and although they manifest multiple humoral and cellular immune responses to *O. volvulus*, the role of these responses in resistance to the parasite has not been defined.

3.1.1.2 Recommendations for studying protective immunity to human onchocercal infection

(a) Guidelines for determination of protective immunity

The following is a suggested set of clinical and parasitological considerations to be taken into account in defining protective immunity in individuals who are apparently infection-free:

- (i) duration of residence in the endemic area, intensity of exposure to infective larvae and prior antifilarial therapy; concomitant entomological support to include an estimation of the amount of contact the subject has with L₃-containing *Simulium*;
- (ii) clinical examination for nodules and skin and eye lesions;
- (iii) microfilariae to be sought in at least four skin snips taken from an area of the body where microfilariae are expected to be present and also in the cornea and anterior chamber of the eye (with the aid of a slit-lamp);
- (iv) if the above evaluation suggests that the subject is not infected (i.e., at least three years' residence, with exposure to infection and no evidence of clinical disease or microfilariae), a provocative dose of DEC (50 mg orally) should be administered; subjects who do not develop a positive reaction after the Mazzotti test* can then be defined operationally as "resistant".

Serial examination (e.g., every one to three years) of immunological parameters of such individuals should confirm whether or not they are truly protected against infection. Such resistant subjects in a population must be compared, in the initial evaluation only, with those in an age-matched group who are microfilariae-positive (and thereby deemed to be at least "less resistant") in order to identify the immunological parameters that might determine resistance.

* Because of its potential danger, however, a Mazzotti test should never be performed in a subject who has not been thoroughly evaluated both clinically and parasitologically and found to be negative.

(b) Selection of study population

Stable human populations in which longitudinal surveys are possible would be ideal for such investigations. Preferably there should be no possibility of concomitant infection with other human filariae, only a single Simulium vector species and no concurrent intervention with mass chemotherapy or vector control. Some areas of Latin America would be especially suitable for such studies, but local epidemiological and entomological support are crucial.

(c) Alternative approaches to assessing protective immunity

It is also possible to evaluate acquired protective immunity to reinfection in a large population after administration of an effective macrofilaricidal drug (see 3.1.1.1). Similar criteria to those described above should be used to assess resistance. Obviously, such studies are not possible until an effective macrofilaricidal agent safe for large-scale use becomes available.

(d) Ethical considerations

Microfilariae-positive subjects should not be included in long-term studies of the type envisaged above. They should be offered appropriate drug therapy. At the end of longitudinal studies, appropriate chemotherapeutic or vector intervention should be planned for the entire community. However, putatively immune individuals (found to be microfilariae-negative and free of other evidence of infection) would normally not receive medication and could thus be followed in longitudinal studies on a voluntary basis without any ethical compromise.

3.1.1.3 immunological correlates: genetic background of resistant subjects; role of maternal status in determining protective immunity

These issues will be discussed below, under lymphatic filariasis [section 3.1.2.2.(c-f)].

3.1.2 Lymphatic filariasis

3.1.2.1 Background

Cross-sectional surveys of residents in areas endemic for brugian and bancroftian filariasis generally indicate that patent infection increases in frequency until the age of 20-30 years and remains constant or decreases in later life. In addition, a variable proportion of subjects older than 30-40 years of age are amicrofilaraemic and have no clinical evidence of lymphatic disease. These surveys suggest, but do not prove unequivocally, that some protection against infection develops in subjects who are presumably exposed to L₃-containing mosquitos (Partono, 1982).

Proof of the existence of protective immunity in such studies has been difficult to obtain because:

- (i) Exposure of individuals to mosquitos containing infective third-stage larvae (L₃) may be variable and difficult to quantify.
- (ii) Multiple parasite stages (L₃, L₄, adult worms, microfilariae) exist in infected humans and although resistance to any or all of these stages may develop, the only index of parasite burden that can be accurately quantified is the number of microfilariae in the blood-stream.

- (iii) Geographic differences in the intensity of transmission and possible use of antifilarial drugs make it difficult to generalize from the results of any single study of acquired resistance.
- (iv) Key antigens involved in the acquisition of resistance have not yet been identified or isolated.
- (v) Other infections (e.g., malaria, other chronic helminth infections) commonly coexist with lymphatic filariasis. These, as well as lymphatic filariasis itself, may induce either generalized or specific immune suppression and thus make it difficult to identify the relevant antigens and associated immune responses.
- (vi) Genetically determined differences in immune responsiveness influence the host's ability to develop protective immune responses, as has been described in several animal models of chronic helminth infection.

3.1.2.2 Recommendations for studies of Protective Immunity in Human Lymphatic Filariasis

- (a) Guidelines for evaluation of protective immunity
 - (i) The study population should be stable, with little movement and known durations of residency.
 - (ii) Clinical examination for lymphatic disease in the extremities and genitalia is mandatory.
 - (iii) Parasitological assessment of microfilaraemia should be done by Nuclepore filtration of blood at a time of day consistent with microfilarial periodicity.
 - (iv) Subjects who have had long-term exposure to infection but who have no evidence of clinical disease and are amicrofilaraemic should receive a provocative dose of DEC (8 mg per kg body weight in one day). Those who exhibit no local inflammatory reactions attributable to filarial infection (e.g., a painful cord in an extremity or the genitalia) may then be considered "resistant".
 - (v) Serial examinations should be performed at least once a year to confirm the parasitological and clinical status of such resistant individuals.
 - (vi) The importance of concurrent entomological studies (biting densities, infection rates in mosquitos, presence of animal filariae in the vector) and information on the behaviour patterns of resistant and nonresistant control subjects cannot be overemphasized. Such information is necessary to determine accurately whether apparent resistance is due to differences in exposure to L₃ larvae or to enhanced parasite rejection by the host.
- (b) Ethical considerations

The same principles are applicable as discussed with regard to onchocerciasis in section 3.1.1.2. (d).

(c) Immunological correlates of resistance

Comparative studies of T- and B-cell responses and in vitro effector mechanisms shown to be relevant in vivo in animal models should be carried out with sera and cells from subjects deemed resistant and nonresistant. Production of purified antigens and a greater understanding of the biology of filarial parasites will be required before further developments can be expected in this area.

(d) Circulating antigen assays

These assays should distinguish infection-free individuals from amicro-filaraemic persons with cryptic infections and should quantify adult-worm loads.

(e) Maternal factors

The importance of maternal factors (e.g., sensitization or development of tolerance to parasite antigens, placental transfer of antifilarial antibodies) in determining resistance in offspring should be explored. Such investigations should include immunological studies of pregnant and lactating women, cord blood and the newborn.

(f) Genetic factors

The role of genetic factors [e.g., Type I and Type II major histocompatibility complex (MHC) antigens] in determining resistance and immune responses to defined parasite antigens needs further study. Familial studies would provide a pragmatic approach to this problem.

(g) Lymphatic disease

It is not clear whether lymphatic inflammation (either acute adenolymphangitis or chronic obstructive disease) and resistance are mechanistically related. Immune reactivity to specific parasite antigens and apparent resistance should therefore be analysed with regard to the development of lymphatic disease. Detailed serial clinical and immunological examinations will be necessary.

3.1.3 Possible use of animal filariasis to study protective immunity relevant to human populations

Several animal filarial infections may provide insights into the development of resistance in field situations. Vector-host interactions can be more accurately and more easily quantified, adult worm burdens directly measured and serial immunological studies performed without danger to humans. Suitable field situations for such studies include O. gibsoni-infected cattle in Australia and South-East Asia and Onchocerca spp. infection in horses or cattle in Europe and North America.

3.2 What Has Been Learned from Animal and In Vitro Models that Could be Relevant to Producing an Antifilarial Vaccine for Humans?

(Rapporteur: Dr. A.E. Bianco)

3.2.1 Animal models

It is recognized that animal experiments have provided the most conclusive evidence to date that acquired resistance can be induced against filarial parasites. Resistance manifested against different life-cycle stages will have distinct consequences that should be carefully analysed with the available

models of filarial infection. The types of experimental vaccines that have been, or might be, examined can be broadly classified as:

- prophylactic vaccines directed against L₃, L₄ and immature adult worms;
- therapeutic vaccines directed against adult worms;
- transmission-blocking vaccines directed against microfilariae;
- disease-alleviating vaccines intended to abrogate pathological processes.

Hindering the pursuit of these objectives is the paucity of information on the developmental biology of filarial nematodes. This major constraint on vaccine development should be a key area for research in animal model systems.

3.2.1.1 Lymphatic filariasis

(a) Resistance to infective and developing larvae

The most desirable objective is to elicit host protection against incoming filarial larvae. Resistance to such larvae has been clearly demonstrated in several model infections, including Dirofilaria spp. in dogs (Wong et al., 1974) and monkeys (Wong, 1974); L. carinii in jirds (Storey and Al-Muhktar, 1982) and rats (Rao et al., 1977); A. viteae in jirds (Tanner and Weiss, 1981) and mice (Cass et al., 1979); and Brugia spp. in mice (Hayashi et al., 1984a,b), jirds (Yates and Higashi, 1985), cats (Oothuman et al., 1979), dogs (Ah et al., 1974) and monkeys (Wong et al., 1969).

The only reliable assessment of resistance has been the recovery of larvae or adult worms at necropsy. Because sterile immunity has rarely been achieved, neither patency rates nor the relative levels of microfilaraemia have proved meaningful indices of host protection.

The optimal procedure for eliciting protection has consistently been the sensitization of animals with live, developing, but attenuated, larvae. The efficiency of a given immunization schedule apparently depends more on the host species than on the parasite. Irradiated larvae have proved the most effective agents to engender resistance in fully permissive hosts (e.g., jirds and cats for Brugia), but the degree of attenuation in larval growth and moulting for optimal stimulation of the immune system has not been adequately studied. Chemically abbreviated primary infections have also been highly effective in stimulating resistance (Blair and Campbell, 1981; Chusattayanaond and Denham, 1984), and there is some evidence that secretory products are a source of protective antigens (Rajasekariah et al., 1987). Nonliving parasites or extracts have generally failed to confer resistance in fully permissive hosts, although they may do so in animals that are semi-permissive for parasite development (e.g., L. carinii in albino rats [Mehta et al., 1981]).

Resistance to developing larvae has been conferred in passive and adoptive transfer experiments using the Brugia/mouse system (Vickery et al., 1983; Hayashi et al., 1984b). This system opens the way for detailed analyses of the mechanisms of resistance, which, current evidence suggests, involve T cells and possibly serum components. The possibility that blocking antibodies could influence survival of developing larvae should also be investigated.

There has been little progress as yet in defining either the inducers or targets of host protection. Molecules generated by live, developing larvae may be worthy of special consideration. Antibody probes or T-cell clones derived

from resistant experimental animals may ultimately play an important role in the identification of the relevant parasite molecules.

The induction or exacerbation of lymphatic pathology is a potential hazard of vaccination that must be carefully examined. For example, cats vaccinated with irradiated B. pahangi larvae exhibit acute lymphoedema following a challenge infection (Oothuman et al., 1979). Molecular vaccines may or may not elicit similar reactions, and appropriate models of lymphatic pathology (jirds, cats, ferrets and nude mice with Brugia spp.) should be used in such evaluations and assessed with both noninvasive (e.g., xeroradiography) and invasive procedures.

Protection against developing stages has been demonstrated in Brugia and Dirofilaria infections following vaccination with heterologous species (Wong, 1974; Oothuman et al., 1979). Similarly, non-L₃ Brugia stages may stimulate resistance to infective larvae. These models offer an opportunity to utilize material derived from stages or species of filariae that may be more readily available than the target organism. The findings, however, also emphasize that the influence of resistance against stages other than the primary target of vaccination should not be neglected.

The duration of protection following vaccination has not been evaluated to date in any of the models of lymphatic filariasis. Rodents may not be suitable for long-term studies of this kind, but cats offer a potentially valuable model. Vaccinated animals exposed to transmission under natural conditions will be an important model to monitor protection under conditions approximating those of a field trial in humans.

It is felt that the widest possible use should be made of the available animal models in identifying potential targets of protective immunity by studying the development of larval filariae. For empirical screening of candidate vaccine components, a model that permits rapid and reliable quantification of parasites (e.g., Brugia infection in the peritoneal cavity of rodents) is required. Attempts to identify parasite components responsible for the protection afforded by irradiated larval vaccines might be best achieved in fully permissive hosts, such as the jird, cat or Presbytis monkey. Strains of mice that can be protected by vaccination, as well as strains that can not, may provide further tools for identifying protective antigens, as has recently been described in schistosomiasis (Smith et al., 1986).

(b) Resistance to adult worms

There is little evidence from animal studies that the resistance conferred by vaccination is directed against adult worms. According to one recent study (Kazura et al., 1986) B. malayi female worms in jirds are preferentially killed following immunization with microfilarial antigens, but there is no evidence that this killing occurs after maturation is complete. Vaccinated animals transplanted with mature adult worms would permit an analysis of protective effects exerted against the adult stage.

(c) Resistance to microfilariae

Several hosts may be protected against microfilariae by vaccination (Wong, 1964; Haque et al., 1978a; Grove et al., 1979; Mehta et al., 1981; Wenk and Wegerhof, 1982), and even nonliving parasite extracts can exert such a state of resistance (Haque et al., 1978b; Storey and Mettias, 1980; Kazura and Davis, 1982). A lower priority is attached to vaccines directed against microfilariae of the lymphatic filariae, but antigens from microfilariae may be

of value in protection against other life-cycle stages (Kazura et al., 1986). One possibility is that antimicrofilarial antibodies could inhibit parasite development in the intermediate host. Transmission-blocking immunity has received considerable attention in malaria, but its relevance to the control of filarial transmission needs to be explored in greater depth. Also, other forms of transmission-blocking immunity merit further study. For example, anti-fecundity vaccines can interfere with parasite mating or parturition (Marti and Murrell, 1986). The feasibility of this approach is suggested by a report that immune serum can suppress microfilarial release by gravid adult female worms in vitro (Haque et al., 1978a).

(d) New models

Because of the availability of animal models most of the current research on lymphatic filariae is carried out with Brugia spp. In view of the importance of W. bancrofti as a public health problem, encouragement should be given to developing the Presbytis model for W. bancrofti and W. kalimantani infections (Palmieri et al., 1983; Campbell et al., 1986). This monkey is also susceptible to Brugia (Mak et al., 1984), thereby providing a possible single primate system for the testing of vaccines against both genera of lymphatic filariae.

3.2.1.2 Onchocerciasis

(a) Resistance to infective and developing larvae

No experiments to test vaccination against developing forms of Onchocerca spp. have yet been reported. While a prophylactic vaccine is highly desirable in onchocerciasis, the difficulties of achieving this goal stem largely from the limitations of available models.

Only chimpanzees have proved susceptible to the human parasite O. volvulus, and infective larvae for vaccination trials are in very limited supply. A major concern in testing vaccines in the chimpanzee is the limited number of options for assessing resistance, even if sufficient animals were available. Worm recoveries to assess the success of challenge infection cannot be made because of a prohibition on experiments terminating with sacrifice of the animal. Assessment of protection based on the development of patent infections is likely to succeed only in the case of sterile immunity. Indirect measurements, using currently available technology, such as assays that measure levels of circulating antigen (Forsyth et al., 1984), are also too insensitive.

A possible alternative is to assess killing of larvae implanted into chimpanzees within micropore chambers, a method of documented value in other models, including A. viteae (Cass et al., 1979), B. pahangi (Chandrashekar et al., 1985), B. malayi (Chandrashekar, 1986) and D. immitis infections. Pilot vaccination studies might be conducted in mice, since development of infective larvae to the early fourth stage has already been demonstrated within chambers in Mastomys (Strote, 1985) and inbred strains of mice.

The bovine parasite O. lienalis offers an attractive model for O. volvulus, since it is more readily available and the only other Onchocerca species which has been transmitted to and studied in experimental animals (Bianco et al., 1981; Bianco and Muller, 1982). Quantification of worm recoveries is difficult in cattle, but the chamber method should be applicable to permit studies more detailed than those possible in chimpanzees. Development of the infective larvae to fourth-stage worms appears to be similar in chambers implanted into cattle or mice, allowing bovine and murine models to be studied in parallel.

O. gibsoni from cattle offers a third model for testing vaccines and has the advantage of adult worms occurring in nodules, as do O. volvulus worms in humans. This infection has not been transmitted under laboratory conditions, but vaccinated cattle may be exposed to transmission and evaluated for adults in nodules collected post-mortem.

In view of the severe constraints on vaccine testing in onchocerciasis, a rational approach is absolutely essential to increase the chances of success. Priority should be given to defining putative targets for immune attack through studies both of the developmental biology of filariae and of the immune responses in patients and animals with onchocerciasis.

A. viteaa infection in rodents may also be of relevance, since this is an easily maintained, well-documented model with larvae that mature in the subcutaneous tissues.

(b) Resistance to adult worms

Adult Onchocerca may be transplanted into rodents and thereby offer a model to analyse resistance to this stage in a small animal host. Males of O. gutturosa (another cattle species) survive for several weeks in the mouse peritoneal cavity (Townson et al., 1981) and may be stored prior to use by cryopreservation techniques (Townson and Ham, 1986).

(c) Resistance to microfilariae

Because O. volvulus microfilariae are the principal inducers of disease, special caution will be required in the development of a vaccine directed against this stage; however, studies on resistance to this stage in experimental animals do offer a number of advantages.

- The availability of microfilariae of Onchocerca species from domestic animals permits the most rigorous studies currently possible with parasites of this genus.
- Monovalent vaccines against microfilariae should not necessarily induce host responses that lead to disease, and resistance directed against microfilariae may reduce adult worm fecundity, particularly if differences between the uterine and skin forms of microfilariae can be exploited in vaccine development.
- Trials with ivermectin have demonstrated that the destruction of microfilariae in humans can be achieved without serious adverse reactions (Greene et al., 1985).
- Antigens (or epitopes) conserved between microfilariae and other stages may be used in the development of prophylactic or therapeutic vaccines.
- Incomplete protection induced against developing larvae may lead to patent infections with undiminished levels of microfilariae in the skin. Vaccines directed against microfilariae would reduce their levels in proportion to the efficacy of immunization.
- Rabbit models are currently available to study onchocercal ocular pathology (Duke and Garner, 1975; Donnelly et al., 1984). These results should be verified by comparison with findings in affected human eyes, and models to examine dermal reactions should be developed and used to monitor vaccine-induced adverse reactions for any prospective Onchocerca vaccine.

The animal studies so far conducted have revealed that:

- Acquired resistance to O. lienalis microfilariae may be induced in inbred strains of mice and in the natural bovine hosts (Townson and Bianco, 1982a,b; Carlow et al., 1986).
- Parasite extracts confer a significant degree of resistance, but living microfilariae consistently engender 90% levels of protection (Townson et al., 1984; Carlow and Bianco, 1987b).
- Serum can transfer resistance to microfilariae in calves, and the thymus dependence of the response has been demonstrated using T-cell deprived mice (Townson et al., 1984; Carlow and Bianco, 1987b).
- Antigen derived from heterologous stages and species of Onchocerca afford protection, suggesting that immunity may not be strictly stage- or species-specific (Carlow and Bianco, 1987a; Townson et al., 1985). The crossprotection demonstrated between O. volvulus and animal parasites should be explored to identify antigens of potential relevance for human vaccines.

3.2.2 In vitro models

There have been numerous in vitro studies on the antiparasite effects mediated by serum and cellular components against many species of filariae. The abundance of mechanisms reported and the inconsistencies in the results obtained cast doubt on the validity of these assays in predicting in vivo effector mechanisms with confidence.

However, in vitro assessment of immune function induced in infected or vaccinated hosts (humans and laboratory animals) appears to be a far more promising application of in vitro techniques in filarial infections. Measurements of lymphocyte responsiveness both in model infections and in humans may offer an approach for assessing exposure to infection, induction of immunological responses following vaccination and/or development of active infections. Further studies will be necessary to explore these important potential applications of in vitro model systems.

3.3 How Should the Development, Screening, "Packaging" and Testing of Potential Immunogens be Carried Out?

(Rapporteur: Dr R.M. Maizels)

3.3.1 Identification of potential vaccine antigens

Because of major gaps in knowledge of basic parasite biology, rational selection of a small number of potential immunogens is difficult. Furthermore, very different objectives exist for the different types of possible vaccine strategies, viz. prophylactic (for sterile immunity), anti-disease (for clinical immunity), therapeutic (for current infection) and transmission-blocking vaccines. The varying requirements of these different potential vaccines suggest that no life-cycle stage of either onchocercal or lymphatic parasites should be excluded at present as a possible source of protective antigens. Furthermore, carbohydrate, as well as protein, specificities may be extremely important in protective immunity; in such instances, the production of anti-idiotypic antibodies to specific monoclonal antibodies represents a direct route of 'internal image' synthesis for large-scale production. Finally, since the targets and mechanisms of the damaging immunopathological responses have not been identified, no antigen should be ruled out as a protective immunogen, although any candidate now selected could carry with it a risk of exacerbation, rather than prevention, of disease.

3.3.1.1 Immunobiological approach

The immunobiological approach to identifying candidate protective antigens centres around known biological features of the parasite that may be recognized or blocked by an effective immune response, e.g., surface antigens, secreted proteins and physiologically important molecules. The testing of known surface proteins of *Brugia* spp. (including shared larval/adult glycoproteins and developmentally expressed microfilarial antigens) appears appropriate, but there is no compelling evidence that filariae are killed in vivo by immune recognition of surface antigens, so that this approach is not assured of success.

A number of ES molecules also provide potential vaccine targets. These include proteases and other enzymes which presumably aid the L₃ and L₄ stages of lymphatic filariae and *Onchocerca* spp. in their migration within the body. Moulting fluids, regulatory hormones and pheromones should also be considered as potential vaccine target molecules, as well as other critical enzymes defined, perhaps, by parasite biochemists studying filarial metabolism. Since there are now many examples, even in helminths, where shed somatic constituents or other "unlikely" proteins prove as effective as protective immunogens, there is no a priori basis for ignoring any antigen as a vaccine candidate.

A major weakness, however, in selecting immunogens on a biological basis is the lack of information available to correlate in vitro observations with the development of infective larvae in vivo. For example, the contrast between *Onchocerca* L₃s, which moult within two days, and the L₃s of *Brugia* and *Wuchereria*, which first migrate to the lymphatics and do not become L₄s until the second week, may have important implications that demand careful analysis in both in vivo and in vitro systems.

3.3.1.2 Immunoepidemiological approach

A second approach for selection of potential vaccine molecules is the careful comparison of immune reactivity in resistant and susceptible individuals. (In human onchocerciasis this is problematic because true resistance may not exist, although there is clearly a group of clinically immune people who remain disease-free if not completely parasite-free in the face of significant exposure.) Generally these studies will be serological rather than T-cell oriented, as the problems of conducting a comparative analysis of T-cell recognition in defined groups, where only rare antigens may be differentially recognized, are considerable. Nevertheless, potentially important T-cell antigens can be overlooked if antibody binding is used as the sole measure of immunogenicity.

This same general approach to definition of protective immunogens can be taken with the various attenuated larval vaccine models now available. The serological and cellular specificities in rodents receiving irradiated larvae should be examined in the light of data showing that immunity can be both actively and passively transferred to naïve recipients. Again it is clear that further studies must be conducted in these vaccination models, as neither the mechanism of successful parasite elimination nor the target antigens for protective immunity have been defined. The question of when the protective antigens are synthesized by the developing or mature parasite will be critical for the design of gene cloning strategies.

3.3.2 Screening in animal models

Animal models are essential both for a biological understanding of these parasites and for testing any potential vaccine product. For *Brugia*, recent developments in mouse models offer an economical protocol for detecting immuni-

ty to L₃s and early L₄s. The Brugia mouse model gives a rapid read-out at ten to 14 days post-challenge but is clearly not appropriate for assessing immunity to later, maturing stages. Microfilarial immunity can be readily assessed in a mouse model involving intravenous transfer of microfilariae.

A further test system is available in full-term Brugia infections in jirds; and, if desired, cat or primate hosts could be vaccinated. There is little scope in any of the Brugia models to account for genetic variation (e.g., MHC) in susceptibility or pathogenic responsiveness among individuals, since even the Balb/c mouse used in that model is only relatively susceptible and clears all developing parasites after about 14 days.

Screening for immunity to W. bancrofti in a natural host model system is unlikely to be possible outside a small number of primate hosts. A painstaking search for a small animal host for this parasite has not been successful and needs to be continued. For the moment, the hope is that the close antigenic relationship between W. bancrofti and B. malayi will lead to recognition of shared protective antigens.

In onchocerciasis, antimicrofilarial immunity can be tested in mice with O. lienalis or other ungulate parasites. Close antigen homologies between humans and animal species can be invoked to justify these systems, although O. lienalis and O. volvulus microfilariae differ sharply, for example, in the pathological responses they evoke in animal hosts. No rodent model for L₃-induced infection with Onchocerca has yet been developed; the best option currently available to screen putative Onchocerca protective immunogens is the use of O. gibsoni in cattle.

At times, the use of natural rodent filariae to screen for protective immunogens may prove appropriate. Where antigens, such as enzymes or reproductive products common to many nematode species, are to be tested, a more direct test can be undertaken in A. viteae or L. carinfi.

Since each currently available model shares particular features with human filarial infections, the models chosen for study must be selected to reflect the questions being asked or the probes available.

3.3.3 Vaccine technology

Techniques in recombinant DNA cloning are developing so rapidly that to recommend particular methodological approaches is unwise. Nevertheless, where libraries are being constructed for use in many laboratories, as with the available adult O. volvulus cDNA libraries described above (Section 2.6), λ gt11 should be used as a standard for screening. Both antibody screening and the use of nucleic acid probes from C. elegans or phylogenetically more distant organisms are clearly important.

It is a moot point whether cDNA libraries should be made from the relatively scarce O. volvulus L₃s. The fact that inadequate data are currently available on the expression of protective antigens by vector-derived L₃s vis-à-vis post-host-invasion L₃s or L₄s and the fact that the relevant antigen genes are likely to be available from an adult-derived genomic DNA library suggest that the first L₃s available should be utilized:

- to raise polyclonal antisera in rabbits and specific monoclonal antibodies in mice to both irradiated and nonirradiated L₃s of O. volvulus; such antisera would provide useful probes for later screening of recombinant antigens;

- to identify proteins and other antigens of L₃ and post-L₃ developing larvae by surface and metabolic labelling and by collection of radioactive ES and moulting fluid; parallel studies with an animal Onchocerca species would be ideal;
- to test existing monoclonal antibodies raised to adult Onchocerca or L₃s of other filariae by immunofluorescence on O. volvulus tissue sections.

3.3.4 Packaging and testing

At the present stage of filarial vaccine development it is premature to consider in detail the packaging and testing of a possible vaccine. Delivery systems such as vaccinia are now undergoing rapid development and the field will probably change markedly in the coming years. Similarly, it would be idle to design human testing protocols in the absence of any information about the details of a possible filarial vaccine, be it prophylactic or therapeutic.

3.3.5 Recommendations

- Serological reagents should be used to identify protective antigens and, equally importantly, targets of pathogenic responses.
- A wide range of surface, secreted and somatic antigens will need to be considered as vaccine candidates, and those that are currently well characterized should be tested in appropriate model systems.
- The developmental biology and immunology of filarial infective larvae should be investigated, initially with B. malayi and animal Onchocerca, subsequently with O. volvulus and W. bancrofti.
- The mechanisms and kinetics of irradiated larval vaccine systems should be elucidated to define the nature and expression of putatively protective larval antigens.
- Continued efforts should be made to construct small animal models for studying immunity to L₃s of Wuchereria and Onchocerca.

3.4 How Are Immunoregulating Mechanisms, Immunomodulators or Immunopotentiators Likely to Affect Development of a Successful Antifilarial Vaccine? (Rapporteur: Dr G.J. Weil)

3.4.1 Potential adverse effects of vaccines

Antifilarial vaccines may have greater potential for producing adverse side-effects than other types of vaccines because of the prominent immunopathology associated with these infections and the complexity of the organisms. Such problems may be especially likely if attenuated parasites or crude antigen mixtures are used in vaccines. If possible, defined antigens, produced by recombinant or synthetic methods, should be used in antifilarial vaccines.

3.4.1.1 Increased pathology

Numerous antigen-specific and nonspecific immunosuppressive mechanisms have been described in patients with patent filarial infections. These mechanisms may decrease immunopathological reactions in the host (Grove and Forbes, 1979; Mehta et al., 1980; Ottesen, 1984; Mistry and Subrahmanyam, 1985; Narayanan et al., 1986). Immunization during established infection may alter balanced immunological networks in such a way as to increase immunopathology

with or without providing immune protection. This possibility could be investigated with existing animal models. Immunization with crude parasite extracts prior to infection has been shown to increase pathological reactions in the B. pahangi-jird model (Klei et al., 1982). Live, attenuated filarial vaccines have also been directly associated with pathological reactions (Oothuman et al., 1979). Further studies along these lines would be helpful in confirming and extending these findings.

Antimicrofilarial immunity has been extensively studied in filariasis. Its association with pulmonary hypersensitivity reactions (lymphatic filariae and D. immitis infections) and with sowda (O. volvulus) would seem to make this approach less attractive for vaccine development, but this remains a moot point.

3.4.1.2 Immune enhancement

Vaccination may induce antibodies that block antiparasite effector mechanisms (Gryznych et al., 1984). On the other hand, drug therapy in filariasis seems to have an immunostimulant effect. The observation that T-cell responses to filarial antigens are enhanced following diethylcarbamazine therapy (Piessens et al., 1981) should be confirmed in humans and animals and extended to ascertain whether resistance to reinfection is stimulated concomitantly.

3.4.1.3 Autoimmunity

Filarial parasites are complex organisms that may share structural and metabolic features with their hosts. Antigenic mimicry may be one of their survival strategies. The absence of host responses to certain parasite antigens in natural infections may result from normal suppression of anti-self-immunity. Induction of immunity to such antigens by vaccination could result in autoimmune disease.

3.4.2 Miscellaneous factors affecting vaccine efficacy

The correct choice of immunogen is no guarantee of vaccine efficacy. Factors such as dose, route and adjuvants have important influences on qualitative aspects of the immune response and may be critical in providing protective immunity to parasitic infections. In addition, host factors, such as age, nutritional status and co-infection with other parasitic and non-parasitic agents, may affect individual responses. Such factors are important for all vaccines, but one that might have special relevance for filarial infections is the infection status of the mother. Recently reported animal studies have indicated that the outcome of filarial infections (microfilarial patency and lymphatic pathology) can be influenced by the presence of infection in the mother during gestation (Haque and Capron, 1982; Schraeter et al., 1983; Klei et al., 1986). The relevance of these observations for human lymphatic filariasis and onchocerciasis is suggested by studies of migrants: increased severity and an accelerated disease course were documented in individuals first exposed to infection during adult life (Partono, 1982). Reports demonstrating human fetal immune responses to filarial antigens (W. bancrofti) (Dissanayake and de Silva, 1980; Weil et al., 1983), transplacental migration of microfilariae (W. bancrofti and O. volvulus) (Raghavan, 1958; Brinkman et al., 1976), and soluble filarial antigen in the milk of mothers infected with O. volvulus, all suggest that pre- and perinatal exposure to filarial antigens does occur in humans. The influence of such exposure on subsequent immune responses and the development of disease is unknown. Further studies should be performed in humans or appropriate animal models to investigate the significance of pre- and perinatal exposure to filarial antigens.

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5. LIST OF WORKING PAPERS

CUPP, E.W. Protective immunity and vaccination in onchocerciasis and lymphatic filariasis. Provision of Onchocerca spp. larvae.

DENHAM, D.A. Provision of larvae for immunological studies/vaccine development.

DUKE, B.O.L. Evidence for naturally developing immunity to filariae - onchocerciasis.

GREENE, B.M. Antifilarial effector mechanisms.

GRIEVE, R.B. Successful strategies in veterinary anti-nematode vaccines.

HIGASHI, G. and YATES, J. Brugia malayi: L₃ radiation - attenuation vaccine studies in jirds.

KAZURA, J.W. Naturally developed immunity in human lymphatic filariasis.

KLEI, T.R. Immunoregulation as a potential modifier of vaccine efficacy or inducer of complications in lymphatic filariasis.

PHILIPP, M. ET AL. Identification of putative protective antigens of Brugia malayi infective larvae and Onchocerca microfilariae using murine models.

SHER, F.A. Successful strategies in schistosome vaccine development.

WEISS, N. ET AL. Experimental induction of immunity to non-lymphatic filariae.

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