48 Titus, R.G. and Ribeiro, J.M.C. (1988) *Science* 239, 1306–1308
49 Titus, R.G. and Ribeiro, J.M.C. (1990) *Parasitology Today* 6, 157–160
50 Rossignol, P.A. *et al.* (1985) *Proc. Natl Acad. Sci. USA* 82, 7725–7727
51 Daniel, T.L. and Kingsolver, J.G. (1983) *J. Theor. Biol.* 105, 661–672
52 Day, J.F., Ebert, K.M. and Edman, J.D. (1983) *J. Med. Entomol.* 20, 120–127
53 Rossignol, P.A. and Rossignol, A.M. (1988) *Parasitology* 97, 363–372
54 Molyneux, D.H. and Jefferies, D. (1986) *Parasitology* 92, 721–736
55 Dye, C. (1990) *Parasitology* 101, 409–415
56 Macloskie, C. (1888) *Am. Nat.* 22, 884
57 Grossman, G.L. and Pappas, L.G. (1991) *J. Med. Entomol.* 28, 456–460
58 Jenni, L. *et al.* (1980) *Nature* 283, 383–385
59 Jefferies, D., Livesey, J.L. and Molyneux, D.H. (1986) *Acta Trop.* 73, 43–53
60 Yorke, W. and Macfie, J.W.S. (1924) *Ann. Trop. Med. Parasitol.* 18, 103–108

Human Filariasis

Immunological Tolerance: The Key Feature in Human Filariasis?

R.M. Maizels and R.A. Lawrence

Filariasis is a widespread tropical disease caused by a group of nematode parasites that can survive for many years in immunocompetent hosts. The paradox of filariasis has always been the inverse association between parasite density, in terms of circulating microfilariae in the blood, and severe pathology. In this review, Rick Maizels and Rachel Lawrence argue that microfilariae and adult parasites induce a form of immunological tolerance which prevents both parasite elimination and progression to disease. The breakdown of this unresponsiveness is seen as the critical step towards pathogenesis. However, not every exposed individual progresses through infection to disease. The authors discuss evidence for protective immunity acting on antigens from the mosquito-borne infective larva, and propose that this stage represents a vulnerable target outside the scope of tolerance and pathogenesis. Stage-specific larval antigens, to which asymptomatic hosts are known to respond, may therefore represent the most effective and safe choice for an anti-filarial vaccine.

Filarial nematode parasites cause the major tropical diseases of lymphatic filariasis and onchocerciasis which afflict some 90 million and 18 million people, respectively. A striking feature of these infections is the protracted and stable relationship reached between parasitized hosts and their burden of long-lived multicellular organisms. This balance serves to limit mortality directly caused by filarial worms but fails to prevent substantial morbidity and disfigurement in many individuals. Because individual parasites may survive for eight years or more[1], we argue here that antigenic variation is unlikely to be a primary immune evasion mechanism. Instead, filariae may induce a semi-permanent state of immunological unresponsiveness in which the host becomes effectively tolerant of the parasite burden. However, as the tolerant state breaks down the host may unleash an immune response which generates pathology and commits the individual to hypersensitivity reactions on further infection, exacerbating disease lesions.

The starting point for discussion will be the induction of specific immunological nonresponsiveness (tolerance) to filarial antigens and the progression from the asymptomatic state to pathological damage. The pathways leading to chronic disease remain undefined beyond the strongly implied involvement of the immune system itself[2,3]. This association places a strict constraint on vaccine development, in that candidate antigens must be dissociated from lesion-promoting immune responses. We suggest that the parasite stage-specific nature of induced tolerance and of anti-filarial immune responses can guide us to antigens that may fulfill this role.

Filarial-specific tolerance

In recent years it has been established that a profound state of hyporesponsiveness or immunological tolerance exists towards adult and microfilarial antigens in the large group of lymphatic microfilarial carriers who are asymptomatic[4–9]. More limited studies in onchocerciasis point to the same conclusion[10–12]. In animal models, the loss of antigen reactivity in T-cell proliferation assays is associated with the onset of microfilaraemia[13,14]. This finding holds true for adult- and microfilarial-derived antigens, but anti-L3 responses have yet to be investigated.

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Tolerance to filariasis does not shut down all immune responses. For example, IgE (Ref. 15) and IgG4 (Refs 16,17) antibody responses can be extremely strong, and infection is generally accompanied by high levels of circulating eosinophils[18]. These three facets of the immune response are now known to be promoted by one T-cell subset, the T_H2 cells, which exist in a reciprocally inhibitory network with a separate population of T_H1 cells[19]. T_H1 cells are the major contributors to blastogenic responses, *in vitro*, as well as to cell-mediated reactions, *in vivo*. Filarial tolerance may therefore more accurately be thought of as a partial tolerance[20] among the T_H1 subset rather than a broad ablation of all pathways of responsiveness.

Filarial tolerance is peripheral, clonal anergy

The most stringent tolerance is effected by pre- or neonatal elimination of most self-reactive T-cell clones in the early thymus, a process termed clonal deletion[21,22]. However efficient this process may be, there will remain a requirement for tolerance induction to antigens expressed only in nonthymic tissues or later in maturation, for cells that break through the thymus, and possibly for autoimmune responses precipitated by infectious organisms bearing host crossreactive determinants. This secondary level of tolerance operates primarily by inactivation rather than deletion, resulting in a state of clonal anergy[23].

Tolerance to filarial parasites can be established within a competent, mature immune system, and hence corresponds to T-cell anergy rather than clonal deletion. A simple view of T-cell anergy is that lymphocytes are exposed to antigen but fail to receive an accessory signal, often termed Signal 2 (Refs 23–25). Signal 2 may be a single co-stimulatory event such as the binding of the T-cell surface ligand CD28, or may be a more complex series of gates spanning the time period from initial CD4/CD8 binding to MHC to receipt of exogenous IL-2, each of which, if blocked, can lead to anergy[26,27]. Once anergized, T cells are unresponsive to specific antigen even if subsequently presented together with adequate co-stimulatory signal(s).

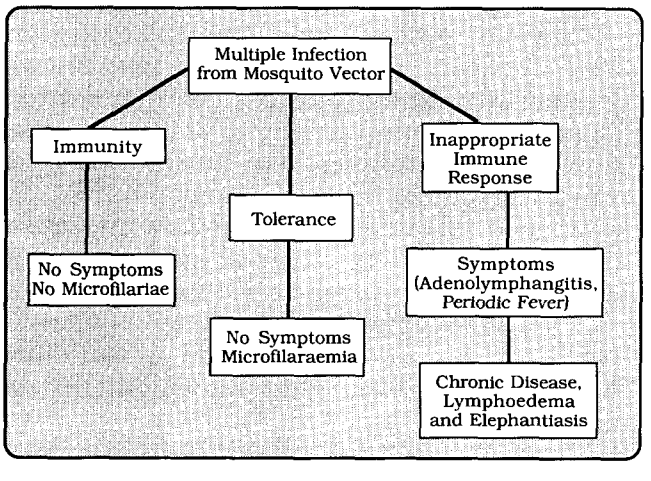

Fig. 1. An outline of the spectrum of filarial disease, based on that proposed by Ottesen[2]. All inhabitants of an endemic area are assumed to receive repeated bites from infected mosquitoes. At a fixed point in time, some individuals will be free of microfilariae in the peripheral blood and show no disease manifestations. This group will contain those with sub-threshold and pre-patent infections, together with people who show effective immunity. A second category consists of patent, microfilaraemic cases who do not suffer from severe filarial disease. These subjects are considered to be immunologically tolerant to adult worms and microfilariae. The third set comprises individuals with chronic disease manifestations (periodic fever, lymphadenitis, lymphangitis, hydrocoele, chyluria, oedema and elephantiasis). These persons are generally amicrofilaraemic and their pathology is attributed to intensified but inappropriately channelled immune responses.

Classic observations link peripheral tolerance to antigen presentation: soluble protein antigen is tolerogenic whereas aggregated antigen is immunogenic. Anergy tends to result when antigen is presented by cells other than conventional antigen-presenting cells (APC) (eg. pancreatic β cells, or chemically treated APC)[28]. This is explained as being due to either a lack of Signal 2 or a low-avidity interaction sufficient to bind the T-cell receptor (TCR) complex but insufficient to activate or to initiate endogeneous IL-2 production[29]. The consequences of inappropriate antigen presentation are illustrated by the protracted nonresponsiveness of T-cell clones exposed to Ag–MHC complexes inserted into artificial membranes[30]. Because tolerance induction is closely associated with antigen presentation, anergy can be maintained at a strictly antigen-specific level.

Tolerance induction selects for antigenic conservation

An interesting prediction of tolerance as a survival mechanism for filariae is that there will be selective pressure on parasites to conform, to share the tolerogenic antigens. Deviation, mutation and variation may all move the individual beyond the shelter of tolerance and towards immune elimination. There are good examples of antigenic conservation in filariae. Homologues of the major surface glycoprotein, gp29, from *Brugia* and *Wuchereria* show a remarkable degree of antigenic conservation[31,32] and an immunologically crossreactive molecule is also present in *Loa loa*[33]. The *B. malayi* and *B. pahangi* gp29 molecules are not distinguishable by one-dimensional peptide map techniques[34]. Surface glycoproteins from different *Onchocerca* species are also crossreactive and retain some reactivity with anti-*Brugia* antibodies[35]. Thus, the antigens most exposed to immune recognition show extraordinarily little divergence or species specificity, as we would predict.

A further highly conserved determinant is phosphorylcholine (PC), found in all filarial nematodes and many other pathogens[36,37]. PC evokes strong humoral responses which do not appear to be in any way protective. While PC may act simply as a decoy antigen, diverting the immune system into fruitless antibody production, more profound effects are

possible. For example, PC or the macromolecule bearing this determinant may directly interact with immune system cells to cause down-modulation of critical responses[20,38].

Pathology is immune-mediated

The pattern of filarial infections in human populations is typically spread over a wide spectrum ranging from individuals who appear free from infection or disease, through asymptomatic carriers, to patients with varying degrees of acute or chronic manifestations[2,39,40]. These conditions have generally been grouped into the three categories: 'endemic immunes', disease-free microfilaraemics and cases with chronic pathology (Fig. 1). While this classification is a useful guide to the status of a cross section of the population at any one time, it does not suggest the likely pathways along which chronic disease/immunity may develop over longer periods.

Studies on the pathogenesis of chronic lymphatic filarial disease have advanced our knowledge of the nature of the lesions. There is good (albeit circumstantial) evidence that the pathology has an immunological origin and that in disease the immune system becomes hyper-reactive to filarial antigens[2,3,39]. There is also a strong trend for disease manifestations to develop as a sequel to the asymptomatic microfilaraemic state, so much so that in Pondicherry, India, for example, the total prevalence of disease approximates the cumulative proportion of the population which has at any time previously been microfilaraemic[41]. It therefore becomes important to question what changes in the immune system provoke it to develop damaging hyper-responsiveness.

When tolerance breaks, immunopathology is initiated

A critical point from the above is that filarial infection imposes tolerance at a peripheral level and that this anergy is inherently unstable. Hyporesponsiveness will tend to break down, altering the immunological status and consequently the reactivity to filarial parasites of an infected individual. Tolerance will break in an increasing proportion of individuals over time, and an age-related rise in filarial responsiveness will be seen in the population, similar to that observed for disease manifestations. The breaking of tolerance may then be the key event in disease initiation and progression (Fig. 2).

Neonatal tolerance induction may play a contributory role, reinforcing the tendency for filariae to induce anergy. Transmigrants who move from non-endemic to endemic areas of Indonesia suffer far higher rates of clinical disease than either indigenous residents, or children who migrate before five years of age[3,42]. Conversely, children of microfilaraemic mothers are more likely to become infected; this is probably due to neonatal experience as there is no similar paternal effect[43]. Tolerization of the offspring of experimentally infected animals has been shown to increase susceptibility[44,45]. Tolerance

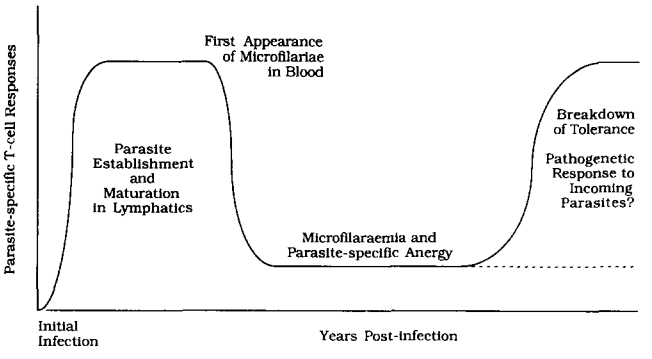

Fig. 2. A schematic time course of filarial infection in a human subject. Exposure from early childhood establishes a parasite population able to induce specific immunological unresponsiveness to adult and microfilarial antigens. Around the time that blood microfilariae appear, T-cell proliferative responses (T_H1-dominated) are ablated. This hyporesponsive state is intrinsically unstable and in many individuals it will eventually break down, leading to expression of strong immune responses. This nontolerant condition is associated with pathogenesis. The loss of tolerance may enable infected hosts to clear their worm load but, if subjected to continuing transmission, reactions to new waves of maturing parasites may continue to provoke pathology. Some individuals, particularly those exposed for the first time in adulthood, may show a shortened 'tolerant' phase and proceed more rapidly to pathology; others may remain in the asymptomatic carrier state for many years.

established early may be induced at a lower threshold, and is more likely to endure. Hence stronger tolerance in neonatally exposed individuals may retard the expression of pathogenetic immune responses and reduce the incidence of clinical disease.

Tolerance may be stage-specific

Anergy may be established to only one part of the parasite life cycle. Thus, microfilaraemics with bancroftian filariasis are found with high titres of antibody to the surface of infective larvae[18,46] although such individuals are universally devoid of anti-microfilarial surface antibody. The ability to mount strong responses to the L3 larvae in the face of adult- and microfilaria-directed tolerance may be an essential element of filariasis immunity, as discussed below. Responses to antigens from the adult and microfilarial stages may also be regulated at independent levels. In cat and rodent models, some animals may kill adult worms but retain circulating microfilariae, while others clear microfilariae but are unable to kill the adults[47,48].

Responses causing pathology are stage-specific

Filarial infections therefore anergize host responses to adult and microfilarial antigens. In lymphatic filarial infections, once individuals re-express their anti-filarial responses, they may be able to kill resident adults and microfilariae and thereby eliminate the source of immunopathogenesis. However, such people will continue to be exposed to new infective larvae from mosquito vectors. If incoming L3 larvae are able to mature through the L4 stage in the nontolerant host, they may act as a renewed target for aggressive reactions, generating acute clinical manifestations, lymphatic lesions, and, over

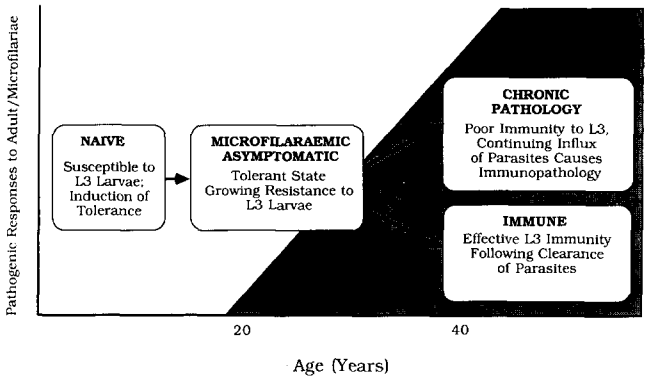

Fig. 3. A classification of endemic populations based on both immunological and parasitological status. Early in life, most or all residents of an endemic area will become infected. In many, a degree of tolerance will be established to adult and microfilarial antigens and an asymptomatic infected state will result. Meanwhile, such individuals are steadily increasing their experience of L3 infections and are building a protective immune response to L3 stages. Over time, the unresponsive state begins to break down and strong anti-adult and anti-microfilarial responses are expressed (shading). If this response fails to eliminate resident parasites, pathogenic lesions will develop. If resident worms are cleared, pathology may still ensue from reactions to new waves of incoming parasites, unless a strong level of anti-L3 immunity has already built up.

time, the cumulative damage of chronic disease (Fig. 2). Thus, it seems clear that post-L3 stages are responsible for lymphatic pathology.

Work in animal models of filariasis supports this view. First, repeated infection of cats and ferrets with infective larvae generates increasing oedema[49,50] and lymphatic damage in cats that have killed their adult worms is more extensive after repeated infection[49]. More recently, F. Medeiros, C. Baldwin and D.A. Denham (unpublished) have shown that cats that kill both adult worms and microfilariae have the most severe lymphatic pathology, while microfilaraemic cats have the least. From these data, it appears that either or both adult worms and microfilariae may be targeted by the immunopathogenic response.

Chronic lymphatic pathology may therefore occur either in individuals who have active infections or in those who are simply subject to continuing transmission. However, over time, the proportion with mature infection would be expected to decline and this is borne out by the low prevalence of microfilaraemia among those afflicted with chronic lymphoedema or elephantiasis-grade pathologies.

In onchocerciasis, the focus of pathogenetic immune responses is restricted to only one stage of the life cycle, namely the microfilaria. In this case, hosts that are unable to kill adult worms, either because of adult-specific anergy or due to the sequestered nature of the nodule, cannot eliminate the source of microfilarial production. Loss of tolerance to microfilarial antigens will then result in prolonged pathological consequences.

Protective immunity is stimulated by L3 larvae

Experimental work has clearly established that high levels of protective immunity (>90%) can be generated in animal models using radiation-attenuated infective larvae[51,52]. Moreover, irradiated larvae show a high degree of crossprotection between *Brugia* species[53] and even between filarial genera[54]. Significantly, dead L3 do not vaccinate effectively, indicating that the active immunogen is either a secreted antigen or a product associated with post-infective stages. Conversely, irradiated larvae must have lost their anergenic potential or the ability to survive beyond the minimum time to complete tolerogenesis.

Immunity also appears to be primarily directed against the L3 larvae rather than later stages[53,55]. The identification of the infective L3 stage as the target for immunity is supported by evidence from the cat–*B. pahangi* model, in animals which develop immunity after repeated infection[56,57]. When cats that have been subjected to multiple infections, and are solidly immune, are challenged with live L3 parasites, the majority of the larvae are killed within the first 24 hours[56].

Acquired resistance in humans resembles concomitant immunity

Immunity to filarial infection has generally been defined as the absence of evident infection or signs of disease in an individual who is subject to continuing parasite transmission. Although some subjects in this category can be identified[11,58], the immunity which protects the already-infected individual from further waves of parasites may be of more clinical and epidemiological importance. Such immunity is termed concomitant immunity.

Support for this view of the immune status of the infected population has recently emerged from studies on age-stratified populations in Papua New Guinea (PNG) and India. These suggest that a protective immunity to incoming infective larvae is acquired over a number of years of exposure to infected mosquitoes. In PNG, worm burdens were quantitated and found to increase in children and adolescents, while adults showed a stable worm load[59]. A similar conclusion has been drawn from a large-scale study of infection dynamics in Pondicherry in which the rate of gain of infection levelled off after adulthood[41,60].

In the PNG study, a correlation was established between the 'immune' adults, all of whom possessed antibody to the surface of L3 larvae, and the 'nonimmune' children who mostly did not. This result indicated that L3 stage-specific antigens may act as an effective target for a protective immune response both in infected and uninfected individuals[46].

One critical conclusion, supported by these studies, is that if anti-L3 and anti-adult immunity operate independently, as suggested above, then normal and infected individuals, although differing in their anti-adult responses, can still share effective anti-L3 immunity. Differential analyses of these groups may not therefore define the antigenic targets

of protective immunity which need to be identified for vaccine use. Rather, the distinction may be made between young children and adult age groups in a similar manner to that becoming established in schistosomiasis.

The state of concomitant immunity implied by these data is entirely consistent with tolerance, provided that the anergy is induced by L4, adult and microfilarial parasites and is specific to those stages. If so, anti-L3 immunity can be maintained in an apparently hyporesponsive, anergic patient, ie. the asymptomatic microfilaraemic. In this way, concomitant immunity can protect against superinfection while leaving resident parasites unharmed in the asymptomatic patient. One significant conclusion from this is that strong responses to the L3 itself are not associated with pathogenesis.

Level of L3 immunity may determine pathological sequelae

In the nontolerant host who has cleared resident parasites, the progression to pathology may depend on how effective the immune response is at preventing new waves of larvae from becoming established (Fig. 3). We suggest that, once tolerance is broken, individuals will fall into two groups: (1) those with an immune response to incoming L3 that is strong enough to block infection and development will appear as 'immune' endemic normals; they will have moderate anti-adult responses as a consequence of the release of L3 somatic antigens and they will not have antibody to microfilariae, and (2) those with inadequate anti-L3 defences who allow larvae to gain entry to the lymphatics and to mature to a later stage; they will develop acute and chronic lesions as a result of their continuous reaction against maturing parasites.

In this model, the longer the period of tolerance persists, the more opportunity the host has to build up stringent anti-L3 immunity and block the pathogenetic events once tolerance breaks. However, there is no indication as to how long anti-L3 immunity may last in the absence of boosting by natural transmission. Epidemiologically, this is a critical problem. If immunity wanes rapidly and repeated exposure to infective larvae is necessary to maintain protection, persons living in areas where transmission has been controlled may be subject to an increased risk of pathological reactions if control ceases and infection is reintroduced.

Vaccination against filariasis with L3 antigens

Vaccination seeks to establish long-lasting immunity against invading larval parasites, but within the context of recent evidence for natural development of protective immunity in human populations and subject to the stricture that candidate vaccines must be free from any association with lesion promoting responses[52]. The hypothesis set out here is that protective immunity is elicited by stage-specific L3 antigens and operates independently of the immune responses to adult worms or microfilariae, and that the status of adult responsiveness (tolerant or reactive) does not impinge on protective immunity.

The implications of this hypothesis for vaccine development are most hopeful. First, stage-specific L3 antigens can be used in vaccines without fear of exacerbating disease. Second, L3 antigens could be used to boost immunity to new infections in currently infected individuals, or in cases recently cleared of infection by diethylcarbamazine or ivermectin. In contrast, antigens expressed by L4 or adult parasites (or even determinants shared by all life cycle stages), if introduced into an asymptomatic individual, might precipitate disease by breaking the tolerant state. These propositions can now be tested in a suitable animal model, in order that the appropriate target for vaccine development can be identified, and that immuno-prophylaxis may perhaps be developed as a major strategy in the eradication of the human filarial diseases.

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References

1 Leeuwin, R.S. (1962) *Trop. Geogr. Med.* 14, 355–360
2 Ottesen, E.A. (1984) *Trans. R. Soc. Trop. Med. Hyg.* 78 (Suppl), 9–18
3 Partono, F. (1982) *Southeast Asian J. Trop. Med. Public Health* 13, 275–279
4 Ottesen, E.A., Weller, P.F. and Heck, L. (1977) *Immunology* 33, 413–421
5 Piessens W.F. *et al.* (1980) *J. Clin. Invest.* 65, 172–179
6 Piessens, W.F. *et al.* (1980) *N. Engl. J. Med.* 302, 833–837
7 Piessens, W.F. *et al.* (1980) *Am. J. Trop. Med. Hyg.* 29, 563–570
8 Nutman, T.B., Kumaraswami, V. and Ottesen, E.A. (1979) *J. Clin. Invest.* 79, 1516–1523
9 Piessens, W.F., Wadee, A.A. and Kurniawan, L. (1987) in *Filariasis (Ciba Symposium 127)* (Evered, D. and Clark, S., eds), pp 164–179, John Wiley & Sons
10 Gallin, M. *et al.* (1988) *J. Immunol.*140, 1999–2007
11 Ward, D.J. *et al.* (1988) *J. Infect. Dis.* 157, 536–543
12 Luty, A.J.F. *et al.* (1990) *Trop. Med. Parasitol.* 41, 371–375
13 Prier, R.C. and Lammie, P.J. (1988) *Infect. Immun.* 56, 3052–3057
14 Levia, L.E. and Lammie, P.J. (1989) *J. Immunol.* 142, 1304–1309
15 Hussain, R. *et al.* (1981) *J. Immunol.* 127, 1623–1628
16 Ottesen, E. A. *et al.* (1985) *J. Immunol.* 134, 2707–2712
17 Kwan-Lim, G-E., Forsyth, K.P. and Maizels, R.M. (1990) *J. Immunol.* 145, 4298–4305
18 Wong, M.M. and Guest, M.F. (1969) *Trans. R. Soc. Trop. Med. Hyg.* 63, 796–800
19 Mossman, T.R. and Coffman, R.L. (1989) *Annu. Rev. Immunol.* 7, 145–173
20 Mitchell, G.F. *et al.* (1976) *Int. Arch. Allergy Appl. Immunol.* 52, 64–78
21 Kappler, J.W., Roehm, N. and Marrack, P. (1987) *Cell* 49, 273–280
22 MacDonald, H.R. *et al.* (1988) *Nature* 332, 40–45
23 Schwartz, R.H. (1990) *Science* 248, 1349–1356
24 Jenkins, M.K. *et al.* (1987) *Proc. Natl Acad. Sci. USA* 84, 5409–5413
25 Mueller, D.L., Jenkins, M.K. and Schwartz, R. (1989) *Annu. Rev. Immunol.* 7, 445–480
26 Waldmann, H. *et al.* (1989) *Cold Spring Harbor Symp. Quant. Biol.* 54, 885–892
27 Ramansee, H-G., Krochewski, R. and Frangoulis, B. (1989) *Nature* 339, 541–544
28 Jenkins, M.K. and Schwartz, R.H. (1987) *J. Exp. Med.* 165, 302–319

29 Sprent, J., Gao, E-K. and Webb, S.R. (1990) *Science* 248, 1357–1363
30 Quill, H. and Schwartz, R.H. (1987) *J. Immunol.* 138, 3704
31 Maizels, R.M. *et al.* (1985) *Trop. Med. Parasitol.* 36, 233–237
32 Morgan, T.M. *et al.* (1986) *Parasitology* 93, 559–569
33 Egwang, T.G. *et al.*(1988) *Mol. Biochem. Parasitol.* 31, 263–272
34 Maizels, R.M. *et al.* (1989) *Mol. Biochem. Parasitol.* 32, 213–228
35 Bradley, J.E. *et al.* (1989) *Mol. Biochem. Parasitol.* 34, 197–208
36 Maizels, R.M., Burke, J. and Denham, D.A. (1987) *Parasite Immunol.* 9, 49–66
37 Gualzata, M., Weiss, N. and Heusser, C.H. (1986) *Exp. Parasitol.* 61, 95–102
38 Lal, R.B. *et al.* (1990) *Am. J. Trop. Med. Hyg.* 42, 56–64
39 Ottesen, E.A. (1980) *Springer Semin. Immunopathol.* 2, 373–385
40 Partono, F. (1987) in *Filariasis (Ciba Symposium 127)* (Evered D. and Clark, S., eds), pp 15–31, John Wiley & Sons
41 Bundy, D.A.P., Grenfell, B.T. and Rajagopalan, P.K. (1991) in *Immunoparasitology Today* (Ash, C. and Gallagher, R., eds), pp 71–75, Elsevier Trends Journals, Cambridge
42 Partono, F. (1984) *Trans. R. Soc. Trop. Med. Hyg.* 78, 9–12
43 Lammie, P.J. *et al.* (1991) *Lancet* 337, 1005–1006
44 Haque, A. and Capron, A. (1982) *Nature* 299, 361–363
45 Schrater, A.F., Spielman, A. and Piessens, W.F. (1983) *Am. J. Trop. Med. Hyg.* 32, 1306–1308
46 Day, K.P., Gregory, W.F. and Maizels, R.M. (1991) *Parasite Immunol.* 13, 277–290
47 Denham, D.A. *et al.* (1972) *Int. J. Parasitol.* 2, 401–407
48 Storey, N., Wakelin, D. and Behnke, J.M. (1985) *Parasite Immunol.* 7, 349–358
49 Rogers, R. *et al.* (1975) *Am. J. Trop. Med. Hyg.* 69, 77–84
50 Hines, J.A. *et al.* (1985) *Lymphology* 18, 173–174
51 Maizels, R.M. and Selkirk, M.E. (1988) in *The Biology of Parasitism* (Englund, P.T. and Sher, A., eds), pp 285–308, Alan R. Liss
52 Selkirk, M.E., Maizels, R.M. and Yazdanbakhsh, M. *Immunobiology* (in press)
53 Oothuman, P. *et al.* (1979) *Parasite Immunol.* 1, 209–216
54 Storey, D.M. and Al-Mukhtar, A.S. (1982) *Tropenmed. Parasitol.* 33, 23–24
55 Yates, J.A. and Higashi, G.I. (1985) *Am. J. Trop. Med. Hyg.* 34, 1132–1137
56 Denham, D.A. *et al.* (1983) *Parasitology* 86, 11–18
57 Denham, D.A. and Fletcher, C. (1987) in *Filariasis (Ciba Symposium 127)* (Evered, D. and Clark, S., eds), pp 225–230, John Wiley & Sons
58 Freedman, D.O., Nutman, T.B. and Ottesen, E.A. (1989) *J. Clin. Invest.* 83, 14–22
59 Day, K.P. *et al. Am. J. Trop. Med. Hyg.* (in press)
60 Vanamail, P. *et al.* (1989) *Trans. R. Soc. Trop. Med. Hyg.* 83, 689–693

Letters

Viruses of Parasitic Protozoa

In their article *Viruses of Parasitic Protozoa*[1], A.L. and C.C. Wang note that 'In 1972 Mattern and colleagues[2] made the first observation by electron microscopy of virus-like particles in certain strains of *Entamoeba histolytica*'. The authors dismiss our work on the basis that there was no follow up to this study and therefore it was difficult to ascertain whether these particles were indeed viruses. In so doing, the authors overlook the first unquestionable evidence provided for the existence of viruses in protozoa. Obviously, it will come as a surprise to the authors to learn that eight papers appeared between the years 1972 and 1977 on our studies of viruses in *E. histolytica*. A careful reading of our first publication[3] reveals that we provided unequivocal, classical evidence for the existence of viruses in these protozoa. The viruses passed through a 0.22 μm filter, caused lysis of susceptible strains of *E. histolytica*, could be continuously passaged in these susceptible strains and possessed the morphological characteristics of known viruses, one icosahedral in form and the other filamentous in form. Later, we described a beaded virus[4]. We also described an icosohedral virus in *E. hartmanni*. Unlike the RNA viruses of *Trichomonas vaginalis* and *Giardia intestinalis*, described by the authors, those of *E. histolytica* appear to be double-stranded DNA.

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References

1 Wang, A.L. and Wang, C.C. (1991) *Parasitology Today* 7, 76–80
2 Mattern, C.F.T., Diamond, L.S. and Daniel, W.A. (1972) *J. Virol.* 9, 342–358
3 Diamond, L.S., Mattern, C.F.T. and Bartgis, I.L. (1972) *J. Virol.* 9, 326–341
4 Mattern, C.F.T. *et al.* (1977) *J. Virol.* 23, 685–691

Second International School: Parasite–Host–Environment

In 1987, the First International School was held in Varna, Bulgaria and, because of the success of this meeting, a second school was held in Sofia from 28 May–1 June 1991. It was organized by members of the Institute of Parasitology of the Bulgarian Academy of Sciences, under the Presidency of Professor Olga Poljakova-Krusteva. The aim of these meetings was to bring together young scientists and experts from a variety of countries and this was achieved with a very friendly atmosphere and a high level of individual participation. Some 70 individuals from ten countries attended, including 16 invited speakers.

The full texts of the lectures are published in the conference proceedings. These are available for US$20.00 from Professor Olga Poljakova-Krusteva, Institute of Parasitology, Bulgarian Academy of Sciences, Academician G. Bonchev Street, Block 25, 1113 Sofia, Bulgaria. (Bank account: Institute of Parasitology, 621/424 291 300-0, Bulgarian Foreign Trade Bank, Sofia.)

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Reply

The definition of a virus[1] has undergone drastic changes in the past 20 years. What was once known as a 'filterable infectious agent' must now include viruses, mycoplasmas and plasmids, as well as viroids. An observation of virus-like particles (VLP) in *Entamoeba* under electron microscopy does not provide unquestionable evidence for the presence of a virus, nor can it link the VLP to the 'filterable infectious agent'. The VLPs seen in *Entamoeba* in 1972 can still be claimed to be the first viruses found in protozoa if more supporting data can be provided even as late as today.

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Reference

1 Fraenkel-Conrat, H., Kimball, P.C. and Levy, J.A. (1988) *Virology* (2nd edn), Prentice Hall