Long-term effect of prenatal exposure to maternal microfilaraemia on immune responsiveness to filarial parasite antigens

Cathy Steel, Archibald Guinea, James S McCarthy, Eric A Ottesen

Summary

To identify long-term effects of prenatal exposure to maternal filarial-parasite infection, we assessed lymphocyte responses in 21 Polynesian children born 17–19 years previously to mothers diagnosed as being microfilaraemic or infection-free.

All children lived on an island endemic for bancroftian filariasis but were free from infection at the time of study. While children (n=10) of infection-free mothers responded vigorously to microfilarial antigen with lymphocyte proliferation, production of interleukin 2 (IL-2), IL-5, IL-10, granulocyte macrophage colony-stimulating factor (GM-CSF), and interferon γ (IFN-γ), cellular hyporesponsiveness was seen in children (n=11) born to microfilaraemic mothers. The hyporesponsiveness appeared restricted to microfilarial antigens and did not extend to non-parasite antigens.

These findings suggest that hyporesponsiveness resulted from in-utero acquisition of tolerance to microfilarial antigens in chronically-infected mothers.

Lancet 1994; **343:** 890–93

See Commentary page 868

Introduction

The various clinical outcomes of filarial-parasite infection are believed to result from different patterns of immune responsiveness to filarial antigens. In lymphatic filariasis, outcomes include microfilaraemia in individuals who are usually asymptomatic and immunologically hyporesponsive to parasite antigens, episodic lymphatic inflammation with lymphoedema and elephantiasis in individuals who are usually amicrofilaraemic and highly reactive to parasite antigens, and no signs or symptoms of filarial disease in individuals who are resistant to or immune to infection.[1] These outcomes seem to be influenced by whether infection is acquired as a visitor to an endemic region or by a resident of that area since birth. In the visitor, infection is most often characterised by lymphatic inflammation, whereas in residents it is usually asymptomatic but with microfilariae circulating in the blood.[1,2]

While there are a number of explanations for these different host responses to infection—including variable patterns of parasite exposure and genetically determined differences in parasite-antigen processing—the most intriguing is that prenatal exposure to specific antigens, as a result of being born of a microfilaraemic mother, alters an individual's subsequent responsiveness. Evidence exists for in-utero exposure to filarial antigens in humans,[3,4] and in animals such exposure has been associated with altered immune responsiveness, in some cases affecting the outcome of infection.[5,6] While in certain human parasite infections (including filariasis, schistosomiasis, and toxoplasmosis) neonates born to infected mothers can have altered responses to parasite antigens,[7] it has not been determined whether such altered responsiveness is transient or long-lasting.

Our study looked for long-term effects of in-utero exposure to *Wuchereria bancrofti* infection on subsequent immune responsiveness to filarial antigens in two groups of young adults 17–19 years old from a Pacific island where bancroftian filariasis is endemic. As bancroftian filariasis had been investigated in the island population 17 years previously, the parasitological status of the mothers of these children was known. One group (n=11) was born to mothers microfilaraemic at the time of their birth, and the other (n=10) to mothers who had been amicrofilaraemic and free of infection.

Subjects and methods

21 young adults (aged 17–19) from the *W bancrofti*-endemic island of Mauke (Cook Islands) were selected because the filarial-infection status of their mothers had been assessed 17 years earlier (1975) as part of a larger study in which half of the island's population of 640 had been investigated.[8,9] Since microfilaraemia usually persists for years in untreated individuals, we assumed that the status of the mother determined in 1975 also defined her status during the previous 2 years (1973 and 1974). None of the mothers had received antifilarial treatment before the birth of the study subjects. In addition to their normal clinical and parasitological evaluations, all amicrofilaraemic mothers were tested for circulating filarial antigens[10] and found to be negative.

The 11 children (5 male, 6 female) of microfilaraemic mothers and the 10 children (3 male, 7 female) of amicrofilaraemic mothers had fathers whose microfilaraemia rates and densities at the time of their children's births were not significantly different. Furthermore, both groups of individuals were born into households that were geographically similar on this small, ten square-mile island. Medical history, physical and laboratory (haematology and clinical chemistry) examinations, were normal in all study patients. All were determined to be amicrofilaraemic by filtering 1 mL of blood through a 3 μ (pore size) Nuclepore filter (Pleasanton, CA, USA) and all were circulating-antigen negative.[10]

Parasite antigens used for this study were saline extracts of adult (BmA) and microfilarial (Mf) stages of the closely related filarial parasite, *Brugia malayi*.[11] Nonparasite antigens were streptolysin O (SLO; DIFCO, Detroit, MI, USA) and tuberculin-purified protein derivative (PPD; Connaught, Ontario, Canada). Serum antibody concentrations of BmA and Mf antigens were determined by standardised ELISA as described previously for IgG, IgG4, and IgE;[12] sera used for detecting antifilarial IgE antibodies were preabsorbed with Protein G Sepharose (Pharmacia; Piscataway, NJ, USA). Peripheral blood mononuclear cells (PBMC) were separated from 80 mL of heparinised blood by ficoll diatrizoate density centrifugation.[13] For proliferation assays, the cells were cultured at 1×10^5 cells/well in 96-well U-bottom plates as described.[12] Triplicate cell cultures were incubated with the previously defined optimal concentrations of antigens (1 μg/mL for Mf; 5 μg/mL for BmA; 10 μg/mL for PPD; 1/100 [final

Laboratory of Parasitic Diseases, Building 4 Room 126, National Institutes of Health, Bethesda, MD, USA (C Steel PhD, J S McCarthy FRACP, E Ottesen MD) **Health Department, Mauke, Cook Islands, South Pacific** (A Guinea MB)

Correspondence to: Dr Cathy Steel

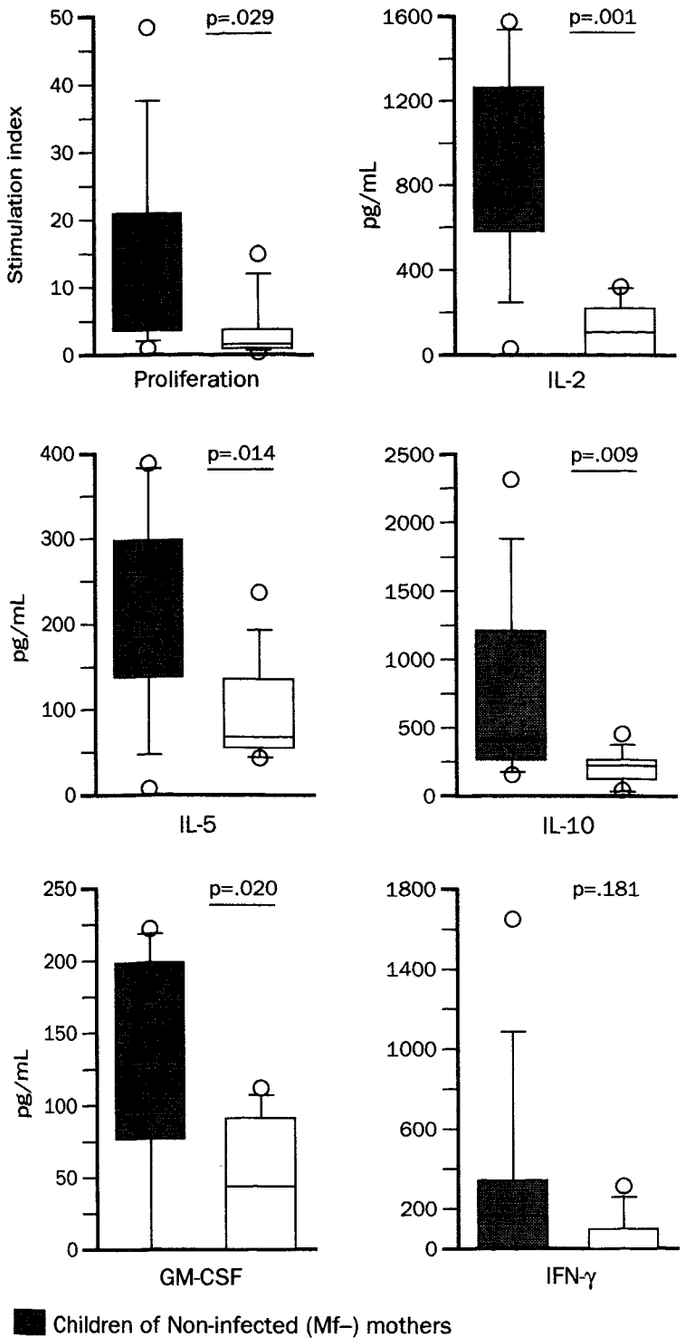

Figure: **PBMC proliferation and cytokine production responses to microfilarial antigen**

Each box (shaded for children born to noninfected, amicrofilaraemic mothers; open for children born to infected, microfilaraemic mothers) is composed of three horizontal lines representing the 25th, 50th (median), and 75th percentiles of the responses. Vertical lines denote the 10th and 90th percentiles, and circles indicate values lying ouside these percentiles.

concentration] for SLO) or with media alone for 5 days at 37°C, 5% CO_2, after which 1 μCi/well of [^{3}H]TdR (NEN; Boston, MA, USA) was added. The cultures were harvested 18 h later, and results expressed as stimulation indices (SI = mean counts per minute [cpm] of stimulated cultures/cpm of unstimulated [media alone] cultures). For cytokine production, PBMC were cultured at 2×10^6 cells/well in 48-well plates (CoStar; Cambridge, MA, USA) in RPMI medium (Bio-Whittaker; Walkersville, MD, USA) with gentamicin (80 μg/mL), 10 mM HEPES buffer (GIBCO; Grand Island, NY, USA), 10% fetal calf serum (GIBCO), and the stimulating antigens described above. Supernatants were harvested at 2 days for interleukin 2 (IL-2) and IL-4 and at 5 days for IL-5, IL-10, interferon γ (IFN-γ), and granulocyte macrophage colony stimulating factor (GM-CSF). Cytokines were quantitated by ELISA. Details of reagent and methods used are available from the corresponding author.

Statistical comparisons used the Mann-Whitney U test for comparison of grouped data, Fisher's exact test for comparison of response rates, and Spearman rank test for analysis of correlations.

Results

PBMC responses to microfilarial antigen were strikingly different in the two groups (figure). Although children born to infection-free mothers generally showed vigorous lymphocyte reactivity to microfilarial antigens, those whose mothers had been infected and microfilaraemic at the time of their birth had comparatively low proliferative and cytokine-producing PBMC responses. Differences between the two groups were statistically significant for lymphocyte proliferation ($p = 0{\cdot}029$); production of cytokines IL-2 ($p = 0{\cdot}001$), IL-5 ($p = 0{\cdot}14$), IL-10 ($p = 0{\cdot}009$); and GM-CSF ($p = 0{\cdot}02$). For IFN-γ response to microfilarial antigen, the trend was in the same direction though there was heterogeneity and no statistically significant difference between the responses of the two groups. For the children of infected mothers, neither this heterogeneity nor the variations seen for other cytokine and proliferation responses were correlated to maternal microfilarial counts. IL-4 production was not detected in either group.

The apparent antigen specificity of this long-term hyporesponsiveness to microfilarial antigen in the children of infected, microfilaraemic mothers is shown in table 1. Cellular responses to the nonparasite antigen SLO were equivalent in both groups as were the responses to PPD (data not shown). Furthermore, despite the presence of shared epitopes in the adult and microfilarial antigen preparations,[14] a stage specificity in the hyporesponsiveness to parasite antigen among children born to microfilaraemic mothers was indicated by the finding that responses to adult filarial antigens in both groups of children were equivalent in all but one assay (IL-2 production [$p = 0{\cdot}002$, table 1]).

Long-term hyporesponsiveness to microfilarial antigens in the offspring of microfilaraemic mothers was restricted to proliferation and cytokine responses; it did not extend to serum antibody production, as both groups appeared to have equivalent serum antibody responses to both microfilarial and adult-stage antigens (table 2). Although not everyone produced specific IgE and IgG4 antibodies, the two study groups were equivalent in the proportion of those making such antibodies; curiously, of those children

		Proliferation*	IL-2	IL-5	IL-10	IFN-γ	GM-CSF
Non parasite	Children of non-infected mothers	11 0	562	1 1	741	582	99
Antigen (SLO)	Children of microfilaraemic mothers	5 8	835	0 08	692	224	48
Adult stage	Children of non-infected mothers	12 4	730	84	726	69	109
Filarial antigen	Children of microfilaraemic mothers	6 1	76 8†	141	335	45	44

*Proliferation expressed as stimulation index; all cytokines are expresssed as pg/mL; values are geometric means.
†Difference between groups is statisticaly significant ($p = 0$ 002); for all other comparisons, differences are not significant.

Table 1: **Cellular responses of study population to non-microfilarial antigens**

Antibody isotype	Children of non-infected mothers		Children of infected mothers	
	No of responders	Mean of responders	No of responders	Mean of responders
Response to MF Ag				
IgG	10/10	189 (40–934)	11/11	188 (58–517)
IgG4	3/10	504 (282–725)	6/11	1540† (729–6320)
IgE	5/10	101 (17–421)	6/11	56 (19–156)
Response to BmA				
IgG	10/10	545 (186–1913)	11/11	546 (172–1620)
IgG4	9/10	1201 (161–3310)	9/11	2580 (151–22640)
IgE	5/10	156 (27–792)	6/11	87 (34–253)

*Geometric mean (and range) of specific anti-microfilarial and anti-BmA antibody levels expressed in arbitrary units/mL.
†Difference between groups is statistically significant (p = 0 02).

Table 2: **Serum antibody reactivity to Mf and adult (BmA) Ag in children of noninfected and infected mothers**

who did make IgG4 antibodies, those born of infected mothers made significantly more (p = 0·02) to Mf antigen than those born of non-infected mothers, but since the number of such children was small, the implications of this finding are uncertain.

Discussion

Although the long-term immunological outcome of in-utero exposure to filarial infection had not previously been defined, the concept that chronic maternal infection can lead to prenatal sensitisation by filarial antigens has been recorded earlier both directly and indirectly. The most direct evidence, albeit an uncommon observation, was finding microfilariae in the peripheral or umbilical cord blood of children born to mothers with bancroftian filariasis[15] or in the skin of newborns of mothers with onchocerciasis.[3] More indirect evidence has been provided by the finding of filaria-specific antibodies of isotypes that do not cross the placental barrier (IgM and IgE) in cord blood of infants born to infected mothers;[4] the isotype of these specific antibodies and their relative concentrations in the cord blood make it clear that the sensitised fetus, not the mother, was responsible for their production. Similarly, indirect evidence for in-utero sensitisation in other parasite infections has been provided by the demonstration of cord-blood lymphocytes that react with the idiotypes of maternal antibodies in neonates born of mothers with either *Trypanosoma cruzi* or *Schistosoma mansoni* infection.[16]

While it has been shown in experimental animals that in-utero exposure to filarial parasites is capable by altering subsequent immune responses in an antigen-specific manner,[17] the immunological or clinical consequences of such in-utero exposure are not well defined in humans. Of probable relevance are the recent epidemiological findings in Haiti that young children whose mothers were microfilaraemic at the time of study were 2–3 times more likely to be microfilaraemic than children with amicrofilaraemic mothers; reduced proliferation responses of whole-blood microcultures to filarial antigen suggested altered immune responsiveness in these children, but the specific nature of such changes was not explored.[7]

The results of the present study show profound immunological alterations following in-utero exposure to infection. These responses appear restricted to the cellular immune system (proliferation and cytokine production) to microfilariae without extending to adult-stage filarial antigens, to nonfilarial antigens, or to specific antifilarial antibody responses. Remarkably, these alterations lasted 17 to 19 years after in-utero exposure.

Differences between the two study groups other than in-utero exposure to microfilariae must be considered. First, even though the people studied had been born long before the present evaulation, the infection status of their mothers was known from clinical and immunological evaluations 17 years previously. Second, although environmental exposure to mosquito-borne filarial larvae was not quantified for each of the study subjects, it can be inferred to be essentially the same for the two groups, since living conditions on this small island were similar both at birth and at the time of the present study. Third, although the possibility that genetic differences between the two groups affected immune responsiveness to microfilarial antigens cannot be discounted, previous MHC class I antigen studies in this population[18] and current analysis of the MHC class II region (HLA-DQA1 and DQB1)[19] in these study subjects (data not shown) have indicated equivalent distribution of alleles. Finally, although children may have been exposed to filarial antigens in the milk of infected mothers (all children were breast-fed), the parasite antigens and specific antibodies that have been identified in maternal milk in some parasitic infections[20] have yet to be shown to induce subsequent alterations in the immune responses of the offspring.

Although changes in the immunue system of children of infected mothers appear similar to the filaria-specific hyporesponsiveness described in infected individuals with asymptomatic microfilaraemia in this and other populations,[21–24] observations suggest different underlying aetiologies. Specifically, chronically infected individuals have marked elevations of filaria-specific IgG4 antibody[25] and their PBMC, when stimulated with parasite antigen, show relative increases in Th2 cytokines (IL-4, IL-5, and IL-10) production[26] suggesting an active immunological cross-regulation response that inhibits pro-inflammatory responses to the parasite. By contrast, hyporesponsive offspring of infected mothers in our study do not show a distinction between antigen-induced responses for Th1 and Th2 cytokines, but rather a global cellular hyporesponsiveness. Similarly, IgG4 responses in these two study groups were equivalent for adult filarial antigens and showed only minimal differences for Mf antigens.

Adolescents in this study are currently free from filarial infection, so it appears that hyporesponsiveness to microfilaria-stage antigens need not necessarily affect resistance to infective-stage parasites. While it is possible that the hyporesponsiveness reflects constant exposure to low doses of mosquito-borne parasite antigen that act to maintain by direct suppression a clonal anergy to antigens first encountered in utero, such hyporesponsiveness might also reflect a permanent deletion in responsive lymphocyte populations. Exposure to parasite antigens early enough in development for them to be recognised as self could lead to clonal deletion of microfilaria-responsive cells. Alternatively, the fact that antigen-specific anergy can develop when antigens are presented by MHC class II molecules in the absence of appropriate costimulatory signals from the antigen-presenting cells (APCs)[27] raises the possibility that microfilarial antigens presented before the development of the appropriate receptor complexes on the APCs could be subject to specific tolerance by this mechanism as well.

Prenatal determinants limiting the capacity of an individual to respond to parasite antigens later in life probably play a major role in explaining the different clinical and immunological outcomes of infection—not

only those within endemic populations[1,22,28] but also those between expatriate and endemic populations.[2,29,30] A practical implication is in the development of vaccines to control such infections: there must be an awareness that capability of immune systems to respond to immunogens that appear ideal in animal models might differ crucially between naive individuals and those born in endemic areas. Similarly, the findings imply that if further study shows an advantage for individuals to be fully responsive to those antigens to which they have developed tolerance in utero, a control strategy including treatment programmes targeting women at or near child-bearing age might be particularly appropriate.

We thank Dr Marianne Cannon, Kura Guinea, Akekaro Chapman, Vaine Papa, Tini Vaine Keu, Terepai Tuakana, and Jack Vaine Keu for help in the clinical aspects of this study; Robert Poindexter for laboratory assistance; Dr Peter Zimmerman for DQA1 and DQB1 analysis; the Cook Island Health Department and the Mauke Island Council, particularly Tautara Purea; Dr Thomas Nutman for valuable suggestions; and Ms Sheryl Rathke for editorial assistance. This investigation received financial support from the UNDP/World Bank/WHO Special Programme in Research and Training in Tropical Diseases (TDR).

References

1 Ottesen EA. Filariasis now. *Am J Trop Med Hyg* 1989; **41:** 9–17.

2 Wartman WB. Filariasis in American armed forces in World War II. *Medicine* 1947; **12:** 181–89.

3 Prost A, Gorim de Ponsay E. Importance epidemiologique du parasitisme neo-natal par microfilaires d'*Onchocerca volvulus*. *Tropenmed Parastiol* 1979; **30:** 477–81.

4 Weil GJ, Hussain R, Kumaraswami V, Tripathy SP, Phillips KS, Ottesen EA. Prenatal allergic sensitization to helminth antigens in offspring of parasite-infected mothers. *J Clin Invest* 1983; **71:** 1124–29.

5 Haque A, Cuna W, Pestel J, Capron A, Bonnel B. Tolerance in rats by transplacental transfer of *Dipetalonema viteae* microfilariae: recognition of putative toleragen(s) by antibodies that inhibit antigen-specific lymphocyte proliferation. *Eur J Immunol* 1988; **18:** 1167–72.

6 Klei TR, Blanchard DP, Coleman SU. Development of *Brugia pahangi* infections and lymphatic lesions in male offspring of female jirds with homologous infections. *Trans R Soc Trop Med Hyg* 1986; **80:** 214–16.

7 Lammie PJ, Hitch WL, Walker Allen EM, Hightower W, Eberhard ML. Maternal filarial infection as risk factor for infection in children. *Lancet* 1991; **337:** 1005–06.

8 Weller PF, Ottesen EA, Heck L, Tere T, Neva FA. Endemic filariasis on a Pacific island I. Clinical, epidemiologic and parasitologic aspects. *Am J Trop Med Hyg* 1982; **31:** 942–52.

9 Ottesen EA, Weller PF, Lunde MN, Hussain R. Endemic filariasis on a Pacific island II. Immunologic aspects: immunoglobulin, complement, and specific antifilarial IgG, IgM, and IgE antibodies. *Am Trop Med Hyg* 1982; **31:** 953–61.

10 Weil GJ, Jain DC, Santhanam S, et al. A monoclonal antibody-based enzyme immunoassay for detecting parasite antigenemia in bancroftian filariasis. *J Infect Dis* 1987; **156:** 350–55.

11 Gusmao R d'A, Stanley AM, Ottesen EA. *Brugia pahangi:* immunologic evaulation of the differential susceptibility to filarial infection in inbred Lewis rats. *Exp Parasitol* 1981; **52:** 147–59.

12 Steel C, Lujan-Trangay A, Gonzalez-Peralta C, Zea-Flores G, Nutman TB. Immunologic responses to repeated ivermectin treatment in patients with onchocerciasis. *J Infect Dis* 1991; **164:** 581–87.

13 Boyum A. Isolation of mononuclear cells and granulocytes from human blood. Isolation of granulocytes by combining centrifugation and sedimentation at 1 g. *Scand J Clin Lab Invest* 1968; **21** (suppl): 77–89.

14 Kaushal NA, Hussain R, Ottesen EA. Excretory-secretory and somatic antigens in the diagnosis of human filariasis. *Clin Exp Immunol* 1984; **56:** 567–76.

15 Raghavan NGS. Congenital filariasis. *Bull Nat Soc Ind Mal Mosq Dis* 1958; **6:** 147–54.

16 Eloi-Santos SM, Novato-Silva E, Maselli VM, Gazzinelli G, Colley DG, Correa-Oliveira R. Idiotypic sensitization in utero of children born to mothers with schistosomiasis or Chagas' Disease. *J Clin Invest* 1989; **84:** 1028–31.

17 Haque A, Capron A. Transplacental transfer of rodent microfilariae induces antigen-specific tolerance in rats. *Nature* 1982; **299:** 361–63.

18 Ottesen EA, Mendell NR, MacQueen JM, Weller PF, Amos DB, Ward FE. Familial predisposition to filarial infection-not linked to HLA-A or -B locus specificities. *Acta Tropica* 1981; **38:** 205–16.

19 Zimmerman PA, Carrington MN, Nutman TB. Exploiting structural differences among heteroduplex molecules to simplify genotyping the DQA1 and DQB1 alleles in human lymphocyte typing. *Nucleic Acids Res* 1993; **21:** 4541–47.

20 Petralanda I, Yarzabal L, Piessens WF. Parasite antigens are present in breast milk of women infected with *Onchocerca volvulus*. *Am J Trop Med Hyg* 1988; **38:** 372–79.

21 Ottesen EA, Weller PF, Heck L. Specific cellular immune unresponsiveness in human filariasis. *Immunology* 1977; **33:** 413–21.

22 King CL, Kumaraswami V, Poindexter RW, et al. Immunologic tolerance in lymphatic filariasis: diminished parasite-specific T and B lymphocyte precursor frequency in the microfilaremic state. *J Clin Invest* 1992; **89:** 1403–10.

23 Nutman TB, Kumaraswami V, Ottesen EA. Parasite-specific anergy in human filariasis: insights after analysis of prasite antigen-driven lymphokine production. *J Clin Invest* 1987; **79:** 1516–23.

24 Piessens WF, McGreevy PB, Piessens PW, et al. Immune responses in human infections with *Brugia malayi:* specific cellular unresponsiveness to filarial antigens. *J Clin Invest* 1980; **65:** 172–79.

25 Ottesen EA, Skvaril F, Tripathy SP, Poindexter RW, Hussain R. Prominence of IgG4 in the IgG antibody response to human filariasis. *J Immunol* 1985; **134:** 2707–12.

26 King CL, Mahanty S, Kumaraswami V, et al. Cytokine control of parasite-specific anergy in human lymphatic filariasis: preferential induction of a regulatory T helper type 2 lymphocyte subset. *J Clin Invest* 1993; **92:** 1667–73.

27 Gimmi CD, Freeman GJ, Gribben JG, Gray G, Nadler LM. Human T-cell clonal anergy is induced by antigen presentation in the absence of B7 costimulation. *Proc Natl Acad Sci USA* 1993; **90:** 6586–90.

28 Nutman TB, Kumaraswami V, Pao L, Narayanan PR, Ottesen EA. An analysis of in vitro B cell immune responsiveness in human lymphatic filariasis. *J Immunol* 1987; **138:** 3954–59.

29 Klion AD, Massougbodji A, Sadeler B-C, Ottesen EA, Nutman TB. Loiasis in endemic and nonendemic populations: immunologically mediated differences in clinical presentation. *J Infect Dis* 1991; **163:** 1318–25.

30 Partono F, Purnomo, Pribadi PW, Soewarta A. Epidemiological and clinical features of *Brugia timori* in a newly established village, Karakuak, West Flores, Indonesia. *Am J Trop Med Hyg* 1978; **27:** 910–15.