Short Report

Development of anti-filarial antibodies in a group of expatriate mine-site workers with varying exposure to the disease

W. Melrose[1], J. Usurup[2], B. Selve[3], P. Pisters[4] and P. Turner[5] *[1]School of Public Health and Tropical Medicine, and [5]Centres for Partnerships in Health, James Cook University, Townsville, 4811 Australia; [2]Placer Niugini Porgera Joint Venture, Papua New Guinea; [3]Misima Mines Ltd, Papua New Guinea; [4]Department of Pathology, Townsville General Hospital, Townsville, Australia*

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Introduction

Thousands of American servicemen became infected with filariasis during World War II (WARTMAN, 1947) and a number of cases of filariasis among missionaries (MCQUAY, 1967), expatriate workers (CHARTERS, 1981; TURNER & USURUP, 1997) and travellers (BEAN *et al.*, 1992) have been reported in the literature. Despite this, lymphatic filariasis is seldom thought of as a threat to expatriates working in, or travellers to, filarial-endemic areas. This study investigated the development of IgG1 and IgG4 anti-filarial antibodies in a group of expatriate mine-site workers working for varying lengths of time in a filarial-endemic area.

Materials and Methods

Sera for this study had been collected during 1997 and 1998 for a previous study in which blood had been obtained from 96 male and 2 female expatriate mine-site employees on Misima Island, Papua New Guinea. The sera had been stored at −70°C. Length of employment of the employees ranged from 1 to 8 years with a roster arranged on a 'fly in, fly out' schedule with workers spending 14 days on the mine site followed by 7 days' leave. Misima Island is highly endemic for nocturnally periodic *Wuchereria bancrofti*, with 2 villages with >90% filarial antigenaemia located within 0·5 km of the mine site. A vector control programme was in place but mosquitoes were still common, especially in the wet season.

Previous studies have shown that antibodies of specific immunoglobulin isotypes are associated with certain stages of the disease. IgG1 antibodies are found in early infection (SIMONSEN *et al.*, 1996), IgG4 antibodies are associated with active infection and show less cross-reactivity with other parasites than other IgG isotypes, and increased IgG2, IgG3 and IgE antibodies are associated with chronic pathology (OTTESEN *et al.*, 1985; HUSSAIN *et al.*, 1987; KURNIAWAN *et al.*, 1993; WAMAE, 1994; SIMONSEN *et al.*, 1996). On the basis of this evidence it was decided that, since chronic pathology was not an issue in this study, assays for only IgG1 and IgG4 antibodies would be run. They were measured by ELISA using an antigen prepared from *Dirofilaria immitis* (KWAN-LIM *et al.*, 1990; TURNER *et al.*, 1993). Previous studies have shown that this assay system does not cross-react with other common intestinal nematodes (TURNER *et al.*, 1993). Pooled hyper-immune serum obtained from filarial-infected Papua New Guineans and assigned a nominal figure of 100 units was used to construct a standard curve. Pooled serum from blood donors who had never been in a filarial-endemic area was used as a negative control. Samples were considered positive if the optical density (OD) was >0·12, i.e., 2 standard deviations above the mean OD of the negative control sera. Sera were also tested for filarial antigen, using the monoclonal antibody-based ICT test (AMRAD/ICT, Sydney, Australia) and the Og4C3 antigen ELISA (TropBio, Townsville, Australia). Antigen tests have been shown to be very sensitive and specific in detecting circulating antigens from adult filarial worms (SIMONSEN & DUNYO, 1999). No attempt was made to detect microfilariae but microfilaraemia is exceedingly rare in expatriate cases. Among the several thousand cases reviewed by WARTMAN (1947) only 20 cases of microfilaraemia were found. It was therefore not considered worthwhile causing inconvenience by collecting night blood. As this was a retrospective study using stored serum samples, no clinical information was available.

Results

There was a sharp rise in the prevalence of IgG1 anti-filarial antibody in the second year of exposure but no significant change was seen in years 4–8 (Fig. 1). By contrast, however, there was a significant correlation ($P = 0{\cdot}0046$; r^2 0·8253) between the length of exposure and the level of IgG1 antibody (Fig. 2). Six subjects with 6–8 years of exposure had positive IgG4 antibody: 21, 7, 17, 12, 6 and 10 units, but there was no correlation between the level of antibody and exposure. None of the subjects, even among those who were IgG4 positive, was filarial antigen positive. When the subjects were subdivided into 'indoor workers' (those working in administration and assays laboratories) and 'outdoor workers' (those working at the open-cast mine site, in field parties and in open workshop facilities), there was a significant difference in the prevalence of IgG1 antibody between

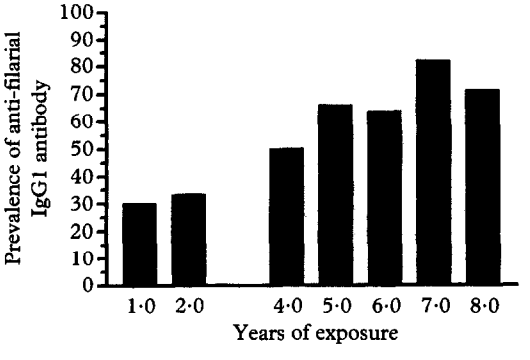

Fig. 1. Prevalence of IgG1 anti-filarial antibody in a group of expatriate mine-site workers in Papua New Guinea after different lengths of exposure.

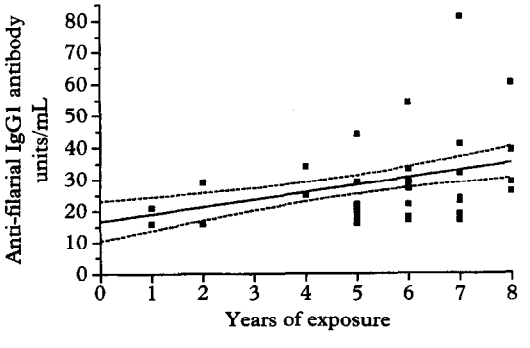

Fig. 2. Differences in levels of anti-filarial IgG1 antibody in a group of expatriate mine-site workers in Papua New Guinea after different lengths of exposure. Symbols show individual subjects, lines show mean antibody level for that year and 95% confidence interval.

the 2 groups. The indoor workers had a prevalence of 24% and outdoor workers 74% (Fisher's exact test $P = 0{\cdot}002$). None of the indoor workers was positive for IgG4 antibody.

Discussion

It is obvious from these results that there was a significant exposure to filariasis in the mine workers, resulting in the production of anti-filarial antibodies. In the case of IgG1 it is possible that the antibody response was due to exposure to in-coming infective larvae rather than infection with the adult parasite, as there is an active IgG1and little IgG4 response to infective larvae (KURNIAWAN-ATMADJA *et al.*, 1998). As previously noted, IgG4 is strongly associated with active filarial infection and it is possible that these subjects who were positive for IgG antibody had an adult worm *in situ*, but it is difficult to explain why they were filarial antigen negative as IgG4 positivity in indigenous populations is almost invariably associated with antigenaemia (TURNER *et al.*, 1993). It is known from previous work that unlike infected indigenous inhabitants, who have a down-regulated immune system against filarial antigens, expatriates mount a vigorous tissue response against the adult worm (WARTMAN, 1947) and it is possible that the filarial antigen is bound into an immune complex near the site of the worm and not liberated into the blood. It is also possible that the subjects' immune response killed the worm shortly after it became established and that the filarial antigen had been cleared from the blood before the person was tested. This study suggests that filariasis is a threat to expatriate workers and travellers especially if their work environment and lifestyle constantly expose them to mosquito bites. This possibility should be considered when investigating illness in returnees from filarial-endemic areas. There is no proven prophylaxis against filariasis and the only effective means of control is protection from mosquito bites. A follow-up study which will include collection of clinical data, testing for microfilaraemia, the use of scrotal ultrasound to detect adult worms (DREYER *et al.*, 1999) and the effects of treatment, is being planned.

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Short Report

Detection of ehrlichiae in African ticks by polymerase chain reaction

Philippe Parola[1], Veronique Roux[1], Jean-Louis Camicas[2], Issa Baradji[3], Philippe Brouqui[1] and Didier Raoult[1]* *[1]Unité des Rickettsies, CNRS UPRES A 6020, Université de la Méditerranée, Faculté de Médecine, 27 Bd Jean Moulin, 13385 Marseille Cedex 5, France; [2]Centre IRD (ex ORSTOM), Montpellier, France; [3]Laboratoire Central Vétérinaire, Bamako, Mali*

*Author for correspondence; phone +33 4 91 32 43 75, fax +33 4 91 83 03 90, e-mail didier.raoult@medecine.univ-mrs.fr

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In Africa, tick-borne ehrlichioses are considered among the most important livestock diseases (SONNENSHINE, 1993). In recent years, useful and rapid molecular tools such as sequence analysis of polymerase chain reaction (PCR) products have been developed for the detection and identification of microorganisms in arthropods (HIGGINS & AZAD, 1995). The aim of this work was (i) to select broad-spectrum specific primers that could amplify, by PCRs, DNA of all the known ehrlichiae, and (ii) to use them to detect ehrlichiae in ticks collected in Mali.

Two oligonucleotide primers EHR16SD (GGTAC CYACAGAAGAAGTCC) and EHR16SR (TAGC