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Parasite antigenaemia and IgG_4 antibodies to a filarial protease in an endemic human population in India

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Abstract

Levels of circulating filarial antigen (Og4C3) and IgG_4 antibodies to a filarial protease were determined in subjects of *Wuchereria bancrofti* exposed sera from Orissa, India. In addition to all individuals with antigenaemia (microfilaraemia), IgG_4 antibodies were also detected in some individuals without antigenaemia. A 2-year longitudinal follow-up indicated that IgG_4 seropositivity in asymptomatic amicrofilaraemics could be a risk factor for acquiring infection (antigenaemia).

Introduction

Infection with the filarial parasite *Wuchereria bancrofti* induces humoral and cellular immune responses in people living in filaria endemic regions. Individuals exposed to the infection have various anti-filarial antibodies among which the IgG_4 subclass was demonstrated to be associated with microfilaraemia (Hussain *et al.*, 1987). Bal & Das (1999) have reported on a metalloprotease (SdpI) isolated from the cattle parasite *Setaria digitata* which revealed a single band in gelatin-impregnated substrate gel and the specific IgG4 response in human filariasis. IgG from chronic filarial patients inhibited the proteolytic activity. An IgG subclass analysis indicated the dominance of IgG4 antibodies to the protease. SdpI-IgG_4 antibodies could be detected, in considerable numbers, in microfilariae negative chronic filariasis (46%, 23/50) and endemic normal individuals (40%, 20/50), in addition to the majority of microfilariae positive individuals. Antigen-based immune assay kits are available which detect circulating filarial antigen (active infection) in both microfilaraemic and amicrofilaraemic people (More & Copeman, 1990; Turner *et al.*, 1993; Weil *et al.*, 1996). The Og4C3 assay detects antigenic determinants expressed and secreted by *W. bancrofti* adult worms without cross-reactivity to *Brugia malayi* or other common intestinal helminths of humans (Chanteau *et al.*, 1994). The present paper attempts to correlate IgG_4 antibodies to a filarial protease with the presence of Og4C3 circulating filarial antigen in individual filarial serum and thereby detect a group of endemic normals that are IgG_4 positive but otherwise free from active infection (antigen negative) who could be at risk for acquiring antigenaemia.

Materials and methods

Sera from the adult (> 18 years) individuals were collected two years before the present investigation began from an area known to be endemic for *W. bancrofti* infection (Beuria *et al.*, 2001). The study area, which is rural, is about 40 km from Bhubaneswar and most of the inhabitants depend mainly on agriculture (paddy fields). The area is characterized by microfilariae prevalence of 12% and chronic filariasis (elephantiasis and hydrocele) constituted 20% of the population. Sera from young children (< 15 years) from the same village were also collected. Participants who had given their consent, or from their parents in the case of children, were included in the study. Each subject was examined for the symptoms of lymphatic filariasis and for microfilariae in Giemsa-stained smears prepared from finger prick blood (50 μl) collected between 2030–2330 h. Based on clinical and parasitological criteria of each donor, the sera were classified as follows: symptomatic chronic filariasis was identified as elephantiasis or hydrocele; asymptomatic microfilaraemic carriers if the donor was microfilaraemic but had no clinical symptoms, and asymptomatic

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amicrofilaraemics if the donor had no symptoms or microfilaraemia. To assess potential cross-reactivity, sera from individuals (n = 40) with parasitic diseases (leprosy, malaria, ascariasis) other than filariasis were used. Healthy control sera (n = 20) from non-filarial regions were also used.

The procedure for the detection of *W. bancrofti* circulating filarial antigen (CFA) in the serum was performed using a Og4C3 Trop Bio kit according to the manufacturer's recommendation (JCU Tropical Biotechnology Queensland, Australia, and catalogue no. 03-010-05). After boiling pretreatment each serum was tested in duplicate and the mean optical density values were used to determine the antigen concentration in units from the standard curve (relating optical density and antigen content of the seven standards enclosed in the kit). Serum samples with unit > titre 3 were considered CFA positive.

IgG4 antibody levels to the filarial protease were determined as described previously (Bal & Das, 1999). Enzyme-linked immunosorbent assay (ELISA) plates were coated with the protease (100 μl per well in alkaline buffer pH 9.2, containing 2 μg protein ml^{-1}). Plates were saturated with 0.5% bovine serum albumin in the buffer for 1 h at room temperature, washed three times with PBS-Tween 0.1% Tween 20, incubated in triplicate with human sera (1:100) for 3 h at 37 °C and then washed again. Mouse monoclonal antibody against human IgG4 (1:5000) was added overnight at 4 °C, followed by 2 h incubation with peroxidase conjugated goat antimouse Igs (1:7000) at room temperature. After washing with PBS-Tween, the presence of IgG_4 was detected with substrate (OPD containing H_2O_2). Adding a drop of 4 N sulphuric acid to stop the enzymatic reaction, the absorbance was read at 492 nm using an ELISA reader (Bio-Rad).

Results and Discussion

Filarial antigenaemia (Og4C3) and IgG_4 levels were determined in 213 human filarial sera from adult individuals (table 1). Control sera (*Ascaris* − 20, malaria − 10, leprosy − 10 and 20 healthy non-endemic sera) were negative in the Og4C3 antigen assay as well as to SdpI-IgG_4 emphasizing the filarial specificity of SdpI-IgG_4 antibodies. All sera with CFA positivity (96) were IgG_4 positive indicating a significant association between the prevalence of antigenaemia and IgG_4 antibodies ($P < 0.001$). IgG_4 seropositivity was also detected in sera that were antigen negatives. Of 117 antigen negative sera, 34 (29%) were found to be IgG_4 positive. When arranged in different groups of filariasis (table 2) it was found that all (n = 47) microfilaraemic carriers were antigen and IgG_4 positive. Individuals (n = 34) who were CFA negative but IgG_4 positive belonged to the asymptomatic amicrofilaraemic (24), hydrocele (8) and elephantiasis (2) groups. IgG_4 seropositivity was also measured in endemic normal children (n = 81). IgG_4 positivity was detected in 13 of 61 CFA (21.31%) negative children in addition to 20 CFA positive sera. A group of adult asymptomatic amicrofilaraemics with (n = 7) and without (n = 22) IgG_4 positivity was longitudinally followed for 2 years. All the individuals followed for two years were CFA negative at the start of the two-year period. Individuals without IgG_4 positivity remained normal after 2 years of follow-up. In contrast, antigenaemia was observed in all seven subjects having IgG_4 positivity, and two individuals even became antigenaemic after 1 year (fig. 1). There was also a parallel increase in IgG_4 antibody levels in these individuals who recently acquired antigenaemia and the level of IgG_4 remained static in the subjects who remained normal throughout the study.

IgG4 as a serodiagnostic tool has been used in filarial infections (Das *et al.*, 1992; Turner *et al.*, 1993; Egwang *et al.*, 1994; Chanteau *et al.*, 1995). Apart from whole parasite extracts, well-defined antigens capable of inducing IgG_4 are also known. These are paramyosin (Langy *et al.*, 1998), SXPI (Dissanayake *et al.*, 1994) and the present filarial protease SdpI. Recently, a recombinant antigen based IgG_4 assay was reported to be useful for *B. malayi* detection (Rahmah *et al.*, 2001). The present paper demonstrated that IgG_4 is detected not only in all the antigen and microfilariae positive samples, but also in some sera from individuals who are free from these two parameters. The prevalence of IgG_4 was found to be higher than that of antigenaemia. The significance of an elevated IgG_4 response in individuals who are free from infection is of interest. Further research is needed to find out if such individuals, at least in the case of asymptomatic amicrofilaraemics, represent the initial prepatent stage and would acquire infection later. IgG_4

Table 1. The prevalence of circulating filarial antigen (CFA) and IgG_4 to a filarial protease (SdpI) in individuals exposed to *Wuchereria bancrofti* infection.

		SdPI IgG_4	
CFA	No. tested	No. positive	No. negative
Positive	96	96	0
Negative	117	34	83

Table 2. Filarial antigenaemia and IgG_4 positivity to SdpI in different groups of human filariasis and control sera.

		CFA (Og4C3)		IgG_4	
Group	No. studied	Positive	Negative	Positive	Negative
Asymptomatic amicrofilaraemics	98	29	69	53	45
Asymptomatic microfilaraemics	47	47	0	47	0
Hydrocele	43	17	26	25	18
Elephantiasis	25	3	22	5	20

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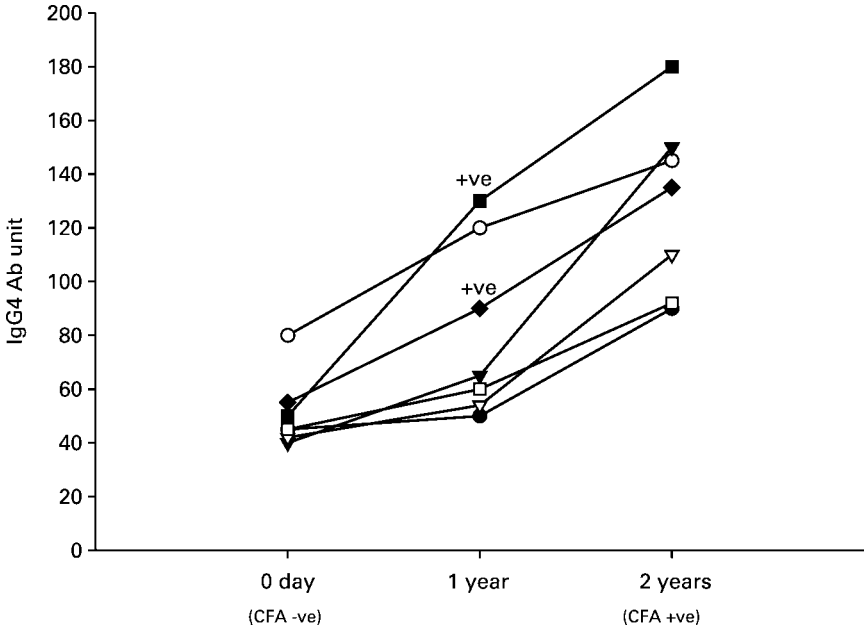

Fig. 1. IgG4 antibody unit levels and circulating filarial antigen (CFA) status in a group of endemic normals (n = 7) having IgG4 positivity followed up for two years. Two individuals acquired antigenaemia (marked + ve) after one year.

antibodies could be markers for early (pre-patent) infections. As noted in the longitudinal study, the acquisition of antigenaemia in IgG_4 seropositive individuals suggests such a possibility.

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