

Antibody response to a potentially protective antigen in human filariasis

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Antibody isotype (IgG and IgM) levels to a high molecular weight antigen (1.56×10^5 kDa) isolated from water-insoluble components of *Setaria digitata* were determined to be highly elevated in microfilariae-negative individuals of a *Wuchereria bancrofti* endemic area of Orissa, India. The highest IgM response was found in endemic normals. The prevalence of antibodies was minimum in microfilaraemic group and maximum in endemic normals of the population.

LYMPHATIC filariasis is one of the most prevalent parasitic diseases, estimated to have infected 78 million people globally. In India alone about 45 million people are infected primarily by *Wuchereria bancrofti*¹. It is a chronic debilitating disease of humans initiated by infective bites of mosquitoes *Culex quinquefasciatus*.

Much attention has been focused recently on the concept of protective immunity in filariasis^{2,3}. Various models of animal filariasis indicate that protective responses can be induced by exposure to live or irradiated parasites^{4,5}. However, very little is known about protective immunity in human filariasis and there is no direct evidence that such immunity exists in humans. On the other hand, epidemiological studies indicate that age-related microfilarial rates increase and then decrease in older-age classes of endemic population⁶. A proportion of adult residents remain amicrofilaraemic despite intensive exposure to infective larvae (L₃) of filarial parasites. Such disease-free adult residents (endemic normals) are believed to have developed protective immunity to filarial infection. It is presumed that host protective immunity might be effective against the acquisition of (new) infection. Identification of antigens associated with the resistance to infection under natural conditions will be of great importance in deciphering the complex mechanism of filarial protective immunity. In the present report we describe a high molecular weight antigen of *Setaria digitata* which stimulates increased antibody responses only in amicrofilaraemic individuals. Moreover, the IgM response to this antigen is considerably elevated, especially in endemic normals compared to infected individuals.

The study population is from *Wuchereria bancrofti* infected village, Baniatangi (Khurda district, Orissa), about 45 km away from Bhubaneswar, Orissa. Individuals were classified into different clinical groups, namely asymptomatic microfilaraemics (AS), filariasis

with chronic pathology elephantiasis and/or hydrocele (CP) and endemic normals (EN) as described earlier⁷. In a random survey of 318 people (age 3–75 years) in the village, AS and CP patients constituted 15.09 and 13.20% of the population, respectively. No attempt was made to include individuals with acute filarial symptoms (adenolymphangitis). Disease-free microfilariae (MF) negative adults (≥ 18 years, 21.83%) were regarded as endemic normals of the region.

Setaria digitata adults were collected from cattle in the local slaughterhouse. Aqueous-insoluble materials were obtained as a pellet by centrifuging the extracts of worms following homogenization and sonication in saline. The pellet was solubilized in the detergent NP-40 (0.5% NP-40 in 0.01 M Tris-HCl, pH 8.0) by keeping for 2 h at 25°C with occasional shaking. The extract was centrifuged again and ammonium sulphate (50%) was added to the supernatant, which was kept at 4°C overnight. The precipitate collected after centrifugation was dissolved in water and was dialysed against phosphate-buffered saline PBS (0.01 M phosphate, 0.15 M NaCl, pH 7.2). The dialysed material upon Sephadex G-200 column chromatography resolved into a major peak (designated as DSSd₁) with a molecular mass of 1.56×10^5 kDa, eluted after the void volume, followed by a minor peak.

Pooled sera were made by adding equal volumes of serum from individuals ($n = 40$ in each category) belonging to different categories of bancroftian filariasis. A control serum pool was also prepared from 20 residents of a nonfilarial region (Koraput district) of Orissa and was used as a nonendemic (NEN) sample. Levels of serum antibody to DSSd₁ were measured by ELISA using 96-well polystyrene plates. The wells were coated by incubation at 37°C (5 h) and then 4°C (overnight) with antigen (3 µg/ml) in bicarbonate buffer (pH 9.6). Next, the wells were blocked with 0.4% bovine serum albumin (BSA) at 37°C (1 h), then incubated (3 h) with diluted serum samples in Tween-20 (0.1% in phosphate buffer saline (PBS)). Final incubations were performed for 1 h at 37°C and 4°C (overnight) with peroxidase-conjugated rabbit anti-human IgG or IgM (Dakopatts) diluted to 1:1000 in 0.1% Tween-20, followed by colour development using orthophenylenediamine substrate. Absorbance was read at 492 nm using an ELISA plate reader (Bio-Rad).

Figure 1 depicts the antibody levels to DSSd₁ in different dilutions of pooled sera. It can be seen clearly that AS serum among the filarial groups exhibited the lowest antibody response. Both EN and CP sera had high antibody levels. IgM levels were highest in EN serum, while CP serum had highest levels of IgG. Antibodies in control NEN serum were found to be negligible at 1:400 dilution. IgM and IgG levels of individual sera were determined (Figure 2). The mean antibody values and the extent of seropositivity (prevalence) are shown in Table 1 and Figure 3.

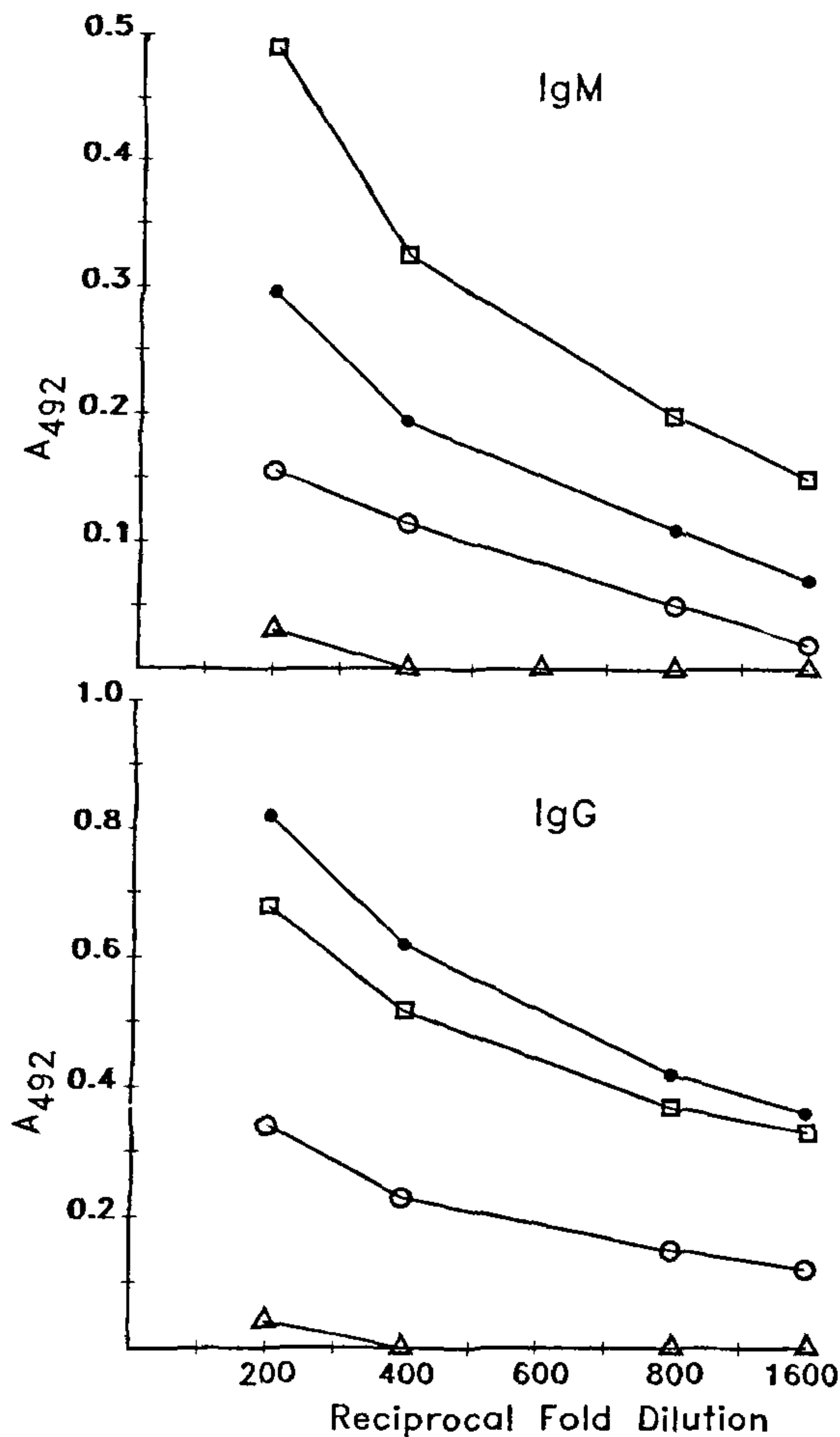


Figure 1. IgM and IgG antibody levels to DSSd₁ in pooled filarial sera chronic filariasis (●), asymptomatic microfilaraemic carriers (○), endemic normals (◻) and nonendemic normals (Δ).

The prevalence of DSSd₁-specific antibodies in filarial groups was evaluated (Figure 3). IgG and IgM prevalence in AS group is low; only 27.5 and 10%, respectively. The prevalence of these two isotypes in EN is 75 and 85% and in CP 100 and 32%, respectively. All individuals in CP are IgG-positive but only 32% of CP patients have IgM antibodies. EN as a group have the highest of prevalence for both isotypes.

An interesting approach to study the protective immunity is to analyse the immune response of endemic normal ('immune') individuals *vis-à-vis* infected persons. Endemic normals can be assumed as putatively immune since they fail to acquire a detectable parasite burden (MF load and/or filarial symptoms). Such an approach has led to the identification of a 43 kDa larval antigen that is recognized only by endemic normals of a

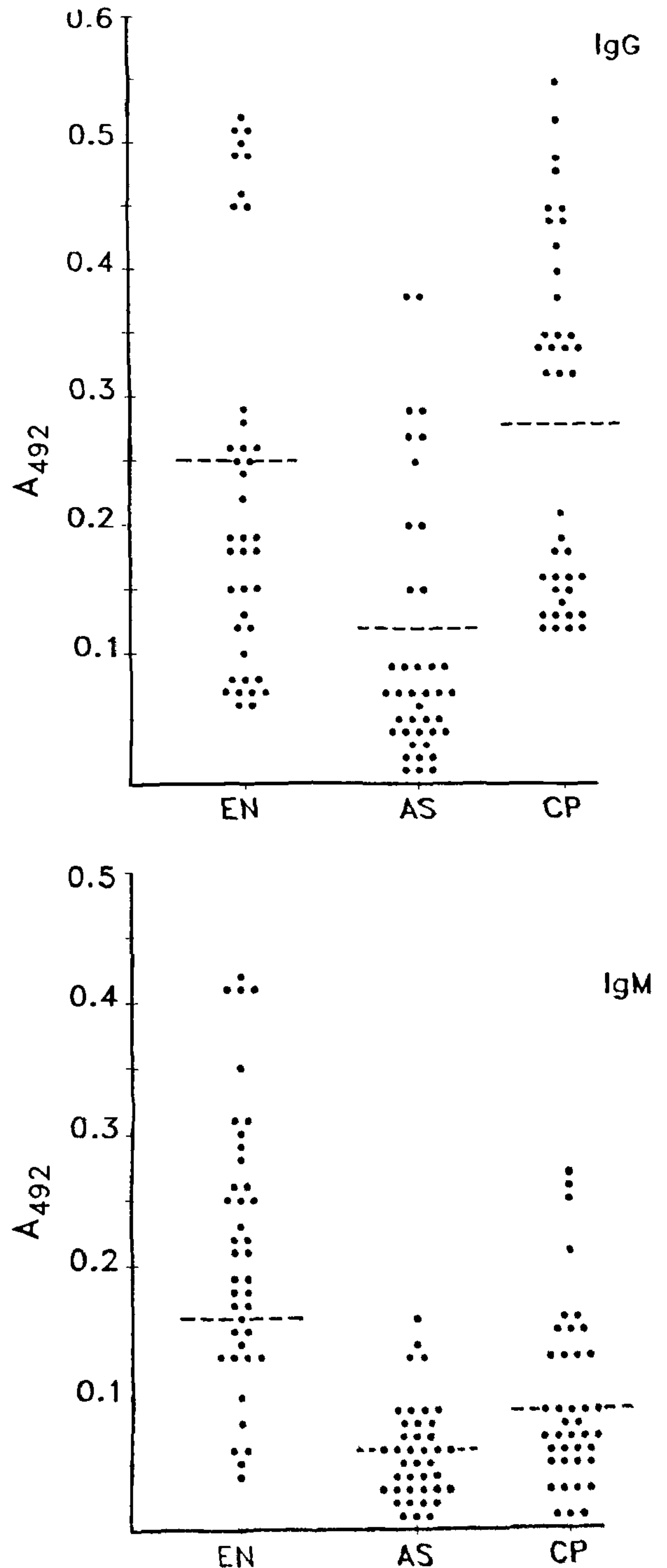


Figure 2. IgG and IgM levels to DSSd₁ antigens in filarial sera. The horizontal bar indicates the mean values.

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Table 1. EI ISA analysis of DSSD₁-specific antibody response Each sample ($n = 40$ in each filarial group) was tested in triplicate (at 1 400 dilution)

Group	IgM		IgG	
	OD 492 (range)	Percentage positive (+ n)	OD 492 (range)	Percentage positive (+ n)
Endemic normals	0.16 ± 0.08* (0.03–0.41)	85 (34)	0.25 ± 0.16* (0.07–0.51)	75(30)
Asymptomatic microfilaraemic	0.06 ± 0.03 (0.01–0.14)	10(4)	0.12 ± 0.10 (0.01–0.38)	27.5(11)
Chronic filariasis	0.09 ± 0.06** (0.01–0.27)	32.5(13)	0.28 ± 0.14* (0.12–0.58)	100(400)
Nonendemic normals	0.03 ± 0.03 (0–0.07)	0	0.04 ± 0.02 (0.02–0.09)	0

* $P < 0.01$ compared to asymptomatic microfilaraemics

** $P < 0.01$ compared to endemic normal.

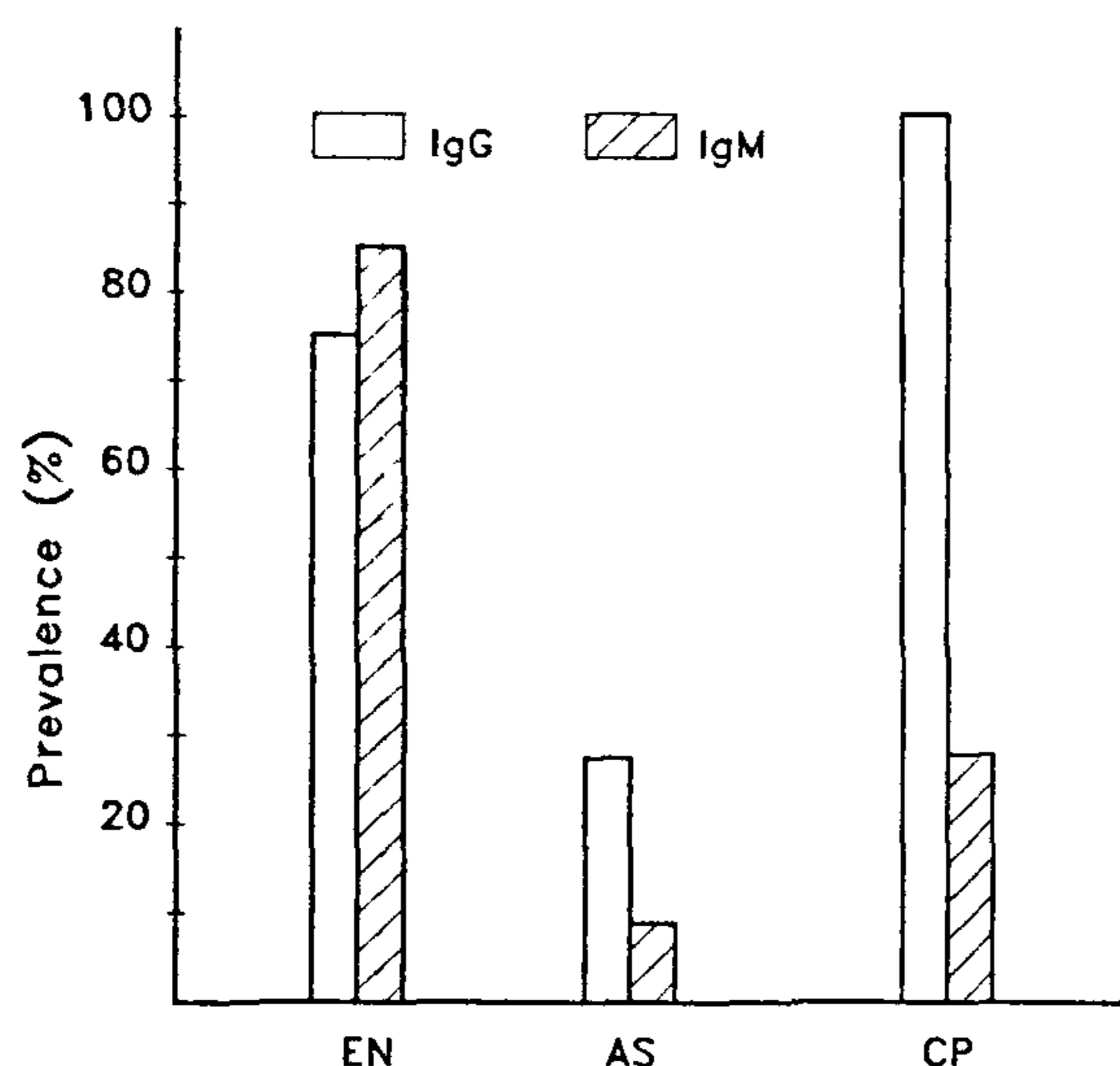


Figure 3. The prevalence of antibodies to DSSD₁ in filarial endemic region. Isotypic seropositivity was calculated at 1 400 dilution with respect to nonendemic normals ($> \text{mean} + 3 \text{SD}$; $n=20$). The number of sera in each filarial group was 40

filarial endemic region⁸. Similarly, in schistosomiasis, infection-free individuals have higher levels of antibodies to paramyosin than the infected persons⁹. Interestingly, paramyosin has also been shown as a protective antigen in *B. malayi* infected animals^{10,11}. Several other filarial antigens with protective potential have been identified and characterized^{12–14}, but studies on human immune responses are limited.

Two notable findings of the present report are: (1) the depressed antibody responses to DSSD₁ in microfilaraemic carriers AS group and (2) the elevated antibody response in EN, even higher than in the CP group. This is also reflected in antibody prevalence, which was found to be low in AS, rising to higher values in microfilaraemics EN and CP groups. These observa-

tions become more marked in comparison to NEN individuals (Figure 1) and suggest that the antibody response that was measured here is specific to filarial infection. The antigen DSSD₁ was isolated from the aqueous-insoluble materials of filarial parasite which are normally discarded. In this sense this antigen might constitute a hitherto undetected novel target.

The presence of increased antibody levels in microfilaraemic individuals gives rise to the possibility that DSSD₁ antigen could be a target of antimicrofilarial immunity in the course of natural infection.

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