Enhanced antibody response to a detergent-soluble antigen in human filariasis after treatment with diethylcarbamazine

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Abstract. An antigen fraction has been isolated from the water insoluble component of cattle filarial parasite Setaria digitata by detergent NP-40 solubilization, precipitation with ammonium sulphate and fractionation on sephadex G-100. Immunoglobulin G response to the isolated antigenic fraction was selectively suppressed in asymptomatic microfilaraemic people in comparison to the amicrofilaraemic groups of endemic normals and chronic patients. However, treating microfilaraemic people with diethylcarbamazine enhanced the antibody levels by 10-fold. These results suggest that active infection suppresses the response induced by the isolated antigenic fraction which is elevated after clearance of microfilariae.

Keywords. Lymphatic filariasis; chemotherapy; immunity; purified antigen.

1. Introduction

Filariasis brings out a wide spectrum of clinical manifestations in people living in endemic regions. Three major groups are asymptomatic microfilaraemic carriers (AS), individuals without any clinical symptoms but harbouring microfilariae (MF); chronic patients (CP), people exhibiting overt clinical symptoms like elephantiasis or hydrocele who are generally amicrofilaraemic; and endemic normals (EN) who are permanent residents in endemic regions but remain if possible free from infection as judged clinically and parasitologically. Such wide variation in the clinical spectrum is believed to be due at least partly to differential immune responses exhibited by the host to the parasite. Indeed, people with patent filarial infection i.e. the AS individuals are immunologically hyporesponsive to the parasite (Piessen et al 1980; Nutman et al 1987). Diethylcarbamazine (DEC), the common antifilarial drug in usage, rapidly clears microfilariae from the circulation in vivo. Alterations in immune reactivity of filarial patients after DEC therapy have been observed mostly in restoring cellular immune responsiveness of these subjects. Numerous studies on the other hand have shown that the drug therapy does not lead to any significant change in filarial antibody levels (Lammie et al 1988; Weil et al 1988; Dissanayake 1989). However, we wish to report here about marked increase in IgG level to a detergent-soluble filarial antigenic (DSSd) fraction following DEC treatment in microfilaraemic patients.

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2. Materials and methods

Individuals living in the filariae (*Wuchereria bancrofti*) endemic villages (Pure/Khurda districts) of Orissa, India provided the sera (Beuria and Das 1992) which were collected by finger prick between 20 : 30 and 24 h and microfilaraemic status was determined. Twenty three MF positive carriers of both sexes aged 10–54 years (median, 25) wishing to participate in chemotherapy were given DEC orally for 12 days at 6 mg/kg body wt. All the patients were checked to be amicrofilaraemic at one month after DEC treatment The sera were collected at 15 days, 1, 2, 3, 6 and 12 month after treatment. Sera (kindly provided by Dr S S S Mohapatra) from normal individuals living in non-filarial regions (Jeypore/Koraput) of Orissa were used as non-endemic samples.

Adult filariae parasite, *Setaria digitata* (both sexes) were collected from the cattle in the local slaughter house. The worms were ground, homogenized, sonicated in saline (Beuria and Das 1992) and centrifuged in the cold to collect the insoluble pellet. The pellet was solubilized in 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. It was centrifuged again and to the supernatant was added ammonium sulphate (50%), kept at 4°C for overnight. The precipitate collected after centrifugation was dissolved in water, dialyzed against PBS (0·01 M phosphate, 0·15 M NaCl, pH 7·2) and chromatographed on sephadex G-100 column (0·9 × 35 cm). The last eluting peak which constituted about 40% of total amount loaded is referred as the detergent soluble antigen (DSSd) of *S. digitata*.

The antibody levels were measured by indirect ELISA. The ELISA plates were coated with 5 μg/ml of filarial antigen (Das et al 1992). Horse radish peroxidase conjugated rabbit antihuman IgG (1 : 1000 dil, Dakopatts, Denmark) was used to determine IgG levels in the sera. The titre denotes the reciprocal of the serum dilution at which the absorbance at 492 nm is higher than 0·07.

3. Results

Initial determination of IgG levels to DSSd in the filarial sera at a fixed dilution (1/200) indicates the suppressed antibody response in microfilaraemic people in comparison to both EN and CP. The titre of DSSd antibodies (table 1) confirmed this. Both EN and CP groups exhibited high antibody titre, the latter possessing the highest level. The titre of AS group was almost similar to that of non-endemic normals who are residents of non-filarial regions of Orissa.

The effect of DEC treatment on the antibody response in twenty three AS patients was evaluated. DEC treated individuals exhibited 10-fold increase in DSSd titre above the pre-treatment levels. The increased titre became similar to that in endemic normals (table 1). The change in titre in individual sera is shown in table 2. An enhancement in the titre was noticed in the majority of AS people though the magnitude varied. Out of 23 cases, only 1 individual exhibited marginal (two times) enhancement and in all the other cases antibody enhancement ranged from 4 to 24 times. The increase in antibody levels began from the first month, persisted up to six month and then declined around one year after DEC therapy (figure 1). IgG level was significantly enhanced \((P < 0·01)\) at the first month post-treatment. About