J. Biosci., Vol. 19, Number 2, June 1994, pp 201–206. © Printed in India.

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

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MS received 3 August 1993; revised 2 April 1994

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 ($P < 0{\cdot}01$) at the first month post-treatment. About

Table 1. ELISA titres of IgG antibodies to DSSd antigen.

Group	Number tested	IgG titre
Nonendemic normals (NEN)	20	200 ± 126 (50 – 400)*
Endemic normals (EN)	20	2640 ± 1369 (400 – 6400)
Chronic filarial patients (CP)	20	6044 ± 3021 (1600 – 12800)
Asymptomatic microfilaraemic (AS) patients	23	230 ± 122 (100 – 400)
DEC-treated** (AS) patients	23	2365 ± 885 (800 – 4800)

*Range of IgG levels.

**6 months after treatment.

Table 2. IgG titre to DSSd antigen in asymptomatic microfilaraemic patients before and after DEC therapy (6 months).

Patient No	Age (years)	Sex	Titre	
			Pre-treatment	Post-treatment
1	20	Female	200	2400
2	17	Female	200	1600
3	15	Female	400	2400
4	10	Male	200	2400
5	30	Female	400	2400
6	46	Female	200	4800
7	25	Female	200	4800
8	22	Female	100	1600
9	26	Female	100	2400
10	52	Female	100	2400
11	32	Female	100	2400
12	14	Male	100	2400
13	50	Female	400	1600
14	25	Male	400	2400
15	35	Male	200	2400
16	23	Female	200	2400
17	18	Male	100	1600
18	16	Male	200	2400
19	54	Male	200	2400
20	21	Female	400	2400
21	30	Female	400	1600
22	32	Female	100	2400
23	27	Male	400	800

7 individuals (30%) were found to maintain the enhancement even after 14 months.

It may be mentioned that IgG levels to (water soluble) whole parasite extract of *S. digitata* were not affected at any time during post therapy. Pre-treatment

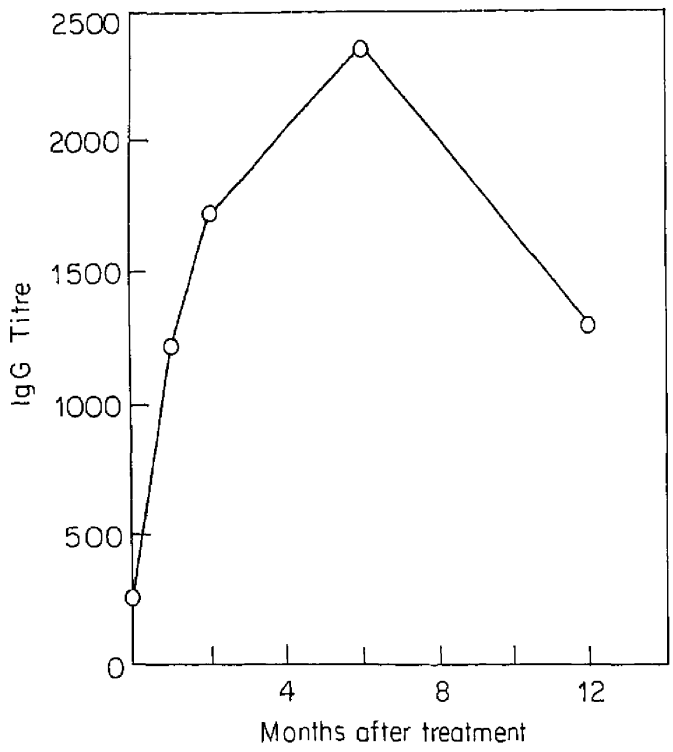

Figure 1. IgG levels to DSSd antigen in a group of asymptomatic microfilaremic carriers (n = 23) after DEC treatment. ELISA readings are shown as mean absorbance values of each individuals. Sera were used at a fixed dilution (1 :200).

ELISA reading (0·35 ± 0·11) did not register significant change (0·30 ± 0·11) at 3 month post treatment.

4. Discussion

The role of immunity to filariasis in humans is a matter of considerable interest at present (Philipp *et al* 1988; Selkirk *et al* 1992). The antibody response to various antigens have been suggested to be of importance in mediating protective immunity (Canlas *et al* 1984; Kazura *et al* 1986; Nanduri and Kazura 1989; Kurniwan *et al* 1990; Cheirmaraj *et al* 1991). Kazura *et al* (1986) detected higher IgG titres to a 25 kD *B. malayi* microfilarial antigen in a group of amicrofilaraemic humans compared to people with patent infection and indicated the protective nature of this antigen in clearing microfilariae. Cheirmaraj *et al* (1991) have detected filarial antigens with potential importance in protective immunity. Interestingly these authors demonstrated higher IgG levels to certain antigenic fractions in endemic normals compared to microfilaraemic and clinical filarial sera.

In the present work we have described the isolation of an antigenic fraction of *S. digitata* whose IgG response in asymptomatic microfilaraemic patients became dramatically elevated over the initial reduced level after DEC therapy. However

IgG level to whole parasitic extract remained unchanged by DEC treatment stressing the importance of using purified antigen in delineating immune response. The inability to detect enhanced antibody production to whole parasite after chemotherapy conforms well to earlier studies as mentioned in the introduction. The presence of innumerable antigenic determinants in the complex antigenic mixtures of parasite may "mask" the finer aspects of immune response. This is the first report in filariasis indicating that chemotherapy can induce augmented antibody production to an antigen whose response is otherwise suppressed in active infection. Similar enhancement of antibody response to a defined antigen in contrast to whole parasitic extract was reported in *Schistosomia mansoni* infected individuals following chemotherapy (Correa-Oliveria *et al* 1989). Antibodies to paramyosin, a candidate vaccine immunogen in schistosomiasis, rose above the pre-treatment levels.

The present antigen DSSd is isolated from the aqueous insoluble components of *S. digitata* antigenic mixtures. Since the majority of immunological studies were carried out using water soluble antigens, DSSd probably represent new epitopes which have been hitherto overlooked. The results presented here showing higher antibody levels to DSSd in endemic normals and chronic patients who are MF negative suggest the importance of this antigen especially in antimicrofilarial immunity. The significant induction of elevated response from the reduced level in microfilaraemic patients after drug therapy would indicate a novel role for this immune response in host-parasite interaction.

Acknowledgements

We would like to thank Dr S P Tripathy, Dr K Satyanarayana, for their interest and Mr A S Acharya for his assistance in the preparation of the manuscript.

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