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Humoral immune response to filarial antigens in chyluria

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Abstract. Humoral immune parameters like total immunoglobulins and specific antibody levels in serum were studied in filarial chyluria patients. Mean serum IgG was significantly reduced in this group compared to normal controls, while IgA and IgM levels remained comparable to controls. Anti-filarial antibody titre as measured by enzyme-linked immunosorbent assay also was significantly reduced. However, the total and specific IgE antibody titre was similar to that of controls. Specific IgE contents of the patients' sera could be related to their microfilaraemic status.

Keywords. Chyluria; humoral immunity; IgE.

Introduction

Chyluria is one of the many clinical manifestations of filariasis.It is the commonest of the chylous complications of lymphatic obstruction. Several studies in the past have analysed the cellular and humoral immune parameters of lymphatic filariasis patients (Desowitz *et al.,* 1976; Paranjape, 1981). Until recently, there have been very few published reports on the status of cellular and humoral immunity in patients with chyluria. We have in our previous experiments observed a reduction in general cellular immune response (Raja *et al.,* 1983). The present paper summarises our findings regarding the total and specific immunoglobulin levels and reaginic antibody response in these patients.

Materials and methods

Patients for the study were chosen from those attending the Filariasis Clinic of Government General Hospital, Madras. All the patients had the complaint of passing milky urine. Five of the patients had circulating microfilaria in their blood, 6 of the

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Abbreviations used: SRID, Single radial immunodiffusion; ELISA, enzyme linked immunosorbent assay; PRIST, paper radio immunosorbent test; DiA, *Dirofilaria immitis;* BmA, *Brugia malayi;* SeA, *Setaria digitata;* RAST; radio allergo sorbent test.

patients had other clinical manifestations of filariasis like lymphadenitis, lymphangitis and hydrocele and 7 did not have any evidence of filariasis. The male: female ratio was approximately 5:1 and they were spread over a broad age group ranging from 18 yrs to 50 yrs. Lymphangiography was done for all the patients. Dilated tortuous lymphatics and retrograde flow at several sites were observable.

Total protein estimation

Total protein estimation in serum was done according to Lowry *et al.* (1951).

Immunoglobulin estimation

IgG, IgA and IgM in serum were estimated by single radial immuno diffusion (SRID) following Rowe *et al.* (1970). Serum IgE was estimated by paper radio-immunosorbent test (PRIST). (Kit from Pharmacia Diagnostics, New Jersey, USA).

Specific antibody measurement

Specific antihelminthic antibodies in serum of class IgG were measured by enzyme linked immunosorbent assay (ELISA). Helminthic antigens *Dirofilaria immitis* (DiA), *Brugia malayi* (BmA), and *Setaria digitata* (SeA) were used. DiA and BmA were saline extracts of the adult worms obtained from the Laboratory of Parasitic Diseases, NIAID, NIH, USA. SeA was an extract of the adult worms in tissue culture medium RPMI 1640.

The sera from normals and patients were tested at a dilution of 1/800. The assay was done according to the method of Voller *et al.* (1976) with slight modifications as reported elsewhere (Narayanan *et al.,* 1983).

Specific IgE antibodies were measured by radio-allergo sorbent test (RAST) using BmA as the antigen (Hamilton *et al.,* 1981).

Results

Immunoglobulins *G, A* and *M* in 17 chyluria sera have been measured using SRID. Twenty eight normal sera were also included as controls. The results are presented in table 1. Total proteins, IgA and IgM, all remained comparable in chyluria and normal control sera. The mean IgG levels in the serum of chyluria patients was 790 ± 446 mg/dl, whereas that of normal subjects was 1295 ± 414 mg/dl. This reduction in the IgG levels was statistically significant ($P < 0{\cdot}001$).

Anti-helminthic IgG antibody levels were estimated using ELISA. Figure 1 shows the mean antibody titres in the patients' sera and that of endemic controls using BmA antigen. The mean antibody levels in the patient group was lower than the endemic controls. Similar reduction was seen also with heterologous antigens of DiA and SeA (figure 2).

Serum IgE levels have been estimated using PRIST in 15 patients and 10 normal subjects. Geometric mean of total IgE level in the patient group was approximately 10 times higher than the normal control group. BmA specific IgE levels in the patients as measured by RAST were comparable to the normal control sera with mean values of

Table 1. Total proteins and immunoglobulins in chyluria sera.

Patients S. No.	IgG (mg/dl)	IgA (mg/dl)	IgM (mg/dl)
1	837	195	177
2	804	446	163
3	609	428	83
4	609	184	163
5	364	148	55
6	773	180	76
7	185	267	150
8	287	850	55
9	520	108	42
10	358	37	138
11	499	50	376
12	1803	286	142
13	1484	213	165
14	1377	203	213
15	1097	66	190
16	914	85	225
17	914	96	169
Mean ± S.D.	790 ± 446	226 ± 199	151 ± 80
Mean ± S.D. NHS (28)	1295 ± 414	172 ± 72	192 ± 95
t	3·85	1·29	1·46
p	< 0·001	> 0·20	≃ 0·20

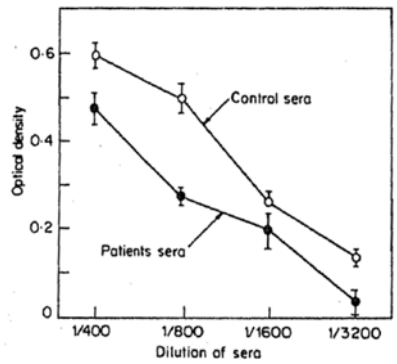

Figure 1. Anti BmA antibody levels of chyluria patients and control subjects.

54 ng/ml for patients and 77 ng/ml for controls. The level of specific IgE was less in microfilaraemic patients than the microfilaria negative group (table 2).

Discussion

Total serum IgG levels in chyluria patients was significantly lower than that of normal subject The total serum proteins, IgM and IgA levels of chyluria sera were within the

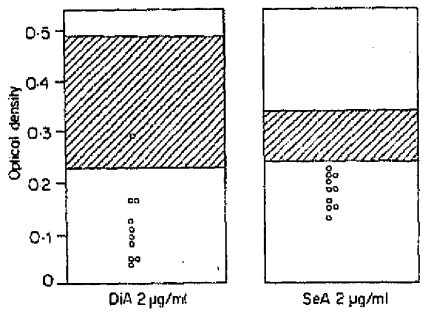

Figure 2. Levels of antifilarial antibody to heterologous antigens in chyluria sera.
Antibody levels have been compared at a dilution of 1/1600 of the sera. Hatched area represents the mean ± S.D. of the normal sera.

Table 2. Total and specific IgE in chyluria sera.

S. No.	Total IgE ng/ml	BmA specific IgE ng/ml
1*	2880	27
2	2400	58
3*	2016	80
4	5760	52
5	9120	43
6	3240	28
7*	2400	80
8	3024	14
9*	8640	65
10†	8160	62
11†	7680	66
12	3366	66
13	11040	55
14	18720	254
15	5640	57
GM for patients	5057	54
GM for 10 controls	636	77
GM for mf+ve group	3780	37
GM for mf −ve group	6620	73

* Microfilaria positive patients.
† Microfilariamic status could not be ascertained.

normal range. Many investigators have reported, either an increase in the IgG level of sera of filarial patients (Desowitz *et al.,* 1976; Saha *et al.,* 1979) or normal level of IgG (Paranjape, 1981). In our patients, the mean serum IgG was significantly reduced than in normals. But out of the 17 patients studied, only 5 showed drastic reduction in IgG, whereas the others remained within the normal limits. Date *et al.* (1981) had also reported normal IgG levels in chyluria patients. The reduction in the level of IgG observed in some patients could be due to the loss of proteins from retroperitonial lymphatics into the urinary tract occurring as a result of lymphatic urinary tract communication. This was evidenced by the lymphangiograms. Similar bulk loss of immunoglobulins into the gastro-intestinal tract and resultant hypogammaglobu-

linaemia in serum was encountered in patients with intestinal lymphangiectasia (Strober *et al.,* 1967). In 4 of the chyluria patients' urine analysed, considerable quantity of IgG and other proteins were detected, strengthening the possibility that the leakage may be the main causative factor for hypogammaglobulinaemia of serum. However, additional factors like decreased synthesis or increased catabolism may also play a role.

Nonavailability of adult *Wuchereria bancrofti* for antigen preparation, necessitated the use of other antigens prepared from human (BmA) and non-human worms (DiA and SeA) which have been shown to cross-react extensively with *W. bancrofti* (Takahashi and Sato, 1976; Dissanayake and Ismail, 1980; Tandon *et al.,* 1981). These antigens could be obtained comparatively easily from animal hosts. Mean antifilarial antibody levels in general in these patients were significantly reduced in comparison with endemic normal controls. The controls themselves showed detectable level of antibody activity because they were also sensitized to the endemic helminthic infections. But the response shown by the chyluria patients was far lower than the controls themselves, which was in accordance with the reduced serum IgG levels. However, reaginic sensitization to the filarial parasite was not hampered in chyluria patients. Mean total IgE was 10 times more than that of the normals and antigen specific IgE remained similar to the normals. Such non-specific potentiation of IgE response has been reported earlier in other helminthic diseases (Turner *et al.,* 1979). A distinct relationship could be discerned between filarial specific IgE and parasitological status of the patients. Mean BmA specific IgE was 73 ng/ml in the microfilariae negative group and 37 ng/ml in the microfilariae positive group.

The decreased IgE level in the microfilariamic patients could be due to the absorption of the specific IgE onto the surface of the microfilariae. Evidence for such an absorption has been shown by Mehta *et al.* (1980), where they have demonstrated the adherence of spleen cells to the microfilariae in the presence of immune serum and that the active principle in the immune serum responsible for cellular adhesion was IgE. The reduction may also be due to a specific suppression of production of IgE in the presence of microfilariae. Similar low level of specific IgE had been observed in other non-chyluria microfilariaemics also (Hussain *et al.,* 1981).

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