INCREASED SERUM LEVELS OF ANTIFILARIAL (IgA) ANTIBODIES IN PATIENTS WITH TROPICAL PULMONARY EOSINOPHILIA

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ABSTRACT

Tropical Pulmonary Eosinophilia (TPE) is diagnosed on the basis of high peripheral eosinophilia associated with clinical symptoms and signs. Elevated levels of total and antifilarial immunoglobulins is one of the characteristic features of TPE. Ten clinically diagnosed TPE patients and ten controls were compared for their anti-filarial and anti-ascaris antibody levels of classes IgG, IgM and IgA. While, IgG antibodies exhibited considerable cross reactivity between Ascaris and Filarial antigens, IgM antibodies showed nonspecific binding to filarial antigens. However, IgA antibodies were found to discriminate between TPE and control sera better than IgG and IgM antibodies.

Introduction

Tropical pulmonary eosinophilia (TPE) is a well defined clinical syndrome with prominent laboratory findings of high peripheral eosinophilia and an elevated total and specific filarial IgE and IgG. Ottesen et al. elegantly demonstrated specific allergic sensitisation of TPE patients to filarial antigens. Early studies of Danaraj et al. measured anti-filarial complement fixing antibodies. Recent studies of Ottesen et al., Hussain et al. measured IgG and IgE classes of antifilarial antibodies in TPE; little attention has been paid to the antibody response of IgA and IgM isotypes. IgA antibodies may be of special interest in this syndrome as TPE has predominant pulmonary features and IgA is the predominant immunoglobulin class in mucosal secretion. Present study describes the level of IgG, IgM and IgA antifilarial antibodies and examines the specificity of the antibody response.

Materials and Methods

Study Population

Ten TPE patients, with nocturnal cough and wheeze, peripheral eosinophil count above 2000 cells/ml and 10 normal healthy individuals were included in the study.

Antigens

Saline sonicated extracts from human filarial worm B. malayi (BmA), cattle filarial worm Setaria digitata (SeA), dog filarial worm Dirofilaria immitis (DiA), and a common intestinal helminth Ascaris lumbricoidis (AsA), were standardised for protein content and were used as antigens in ELISA.

ELISA

The plates were sensitized by incubating 0.2 µg antigen in 0.1 ml carbonate buffer (pH 9.6) in each well. Briefly the test was carried out as follows:-

The plates were washed with Tween-20 PBS (0.025 M, pH 7.2, containing 0.1% tween-20) and then incubated sequentially with (i) serum dilution (1/400 for IgM, 1/1600 for IgA and 1/6400 for IgG), (ii) anti IgA and anti IgM conjugates at 1/2500 dilution and anti IgG conjugate at 1/1000 dilution (Cappel Labs., USA) and (iii) substrate (Ortho-phenylene diamine (OPD) 0.02% with 0.003% H2O2). The plates were washed with PBS-tween after each incubation. All incubations were carried out at 37°C for 30 minutes. Reaction was terminated with 8 N H2SO4 and O.D. was measured in multiscanner (Flow Laboratories, USA).

Statistical Analysis

Arithmetic means were calculated for each group of observations and the group differences were calculated with either dependent or independent 't' test.

Results and Discussion

The TPE patients had higher mean levels of antibodies of all isotypes (G, A and M) when compared with levels in the normal healthy control sera. The response in TPE patients was higher for all isotypes...
The above figures represent mean optical densities (± standard deviation) against B. malayi (BmA), D. immitis (DiA), S. digitata (SaA) and Ascaris lumbricoides (AsA) adult somatic antigens. Three pairs of points for each antigen represent antibodies of isotypes IgG (G), IgA (A), and IgM (M). The open circles represent mean (± S.D.) for TPE sera and closed circles represent mean (± S.D.) for control sera.
and to all filarial antigens used. notwithstanding the species of filarial worm. (Fig. 1-4.)

The mean IgG antibody level was significantly higher in TPE sera than in control sera. This finding corroborates with findings in earlier studies showing elevated anti-filarial IgG titres in TPE\(^3\). Further, significantly elevated levels of IgG were observed in TPE sera when a non-filarial antigen (ascaris) was used. It may be argued that the significant levels of anti-ascaris antibody in TPE may have resulted as a response to past or present ascaris infection. But, this is an unlikely explanation as the control subjects too were from the same area and hence, were exposed to ascaris antigens to the same extent. Also, there is no study suggesting that TPE patients are more prone to ascaris infection. Considering these arguments, it is more probable that the elevated anti-ascaris IgG seen in TPE sera may be due to cross reactivity between ascaris and filarial antigens.

Similar cross reactivity between filarial and ascaris antigens was seen to a much lesser extent in the case of IgA and IgM antibody responses. While in TPE sera, there was no significant elevation of IgM and IgA to ascaris antigen, anti-filarial IgA and IgM were detected in significantly higher quantity in patients' sera. However, mean anti-filarial IgM levels were higher than anti-ascaris IgM levels even in control subjects implying thereby that IgM also was reacted less with ascaris antigen. Higher binding to filarial antigen in controls may have resulted from non-specific binding of anti-IgM enzyme conjugate to filarial antigen; or specific IgM sensitisation to filarial antigen may be higher in normal healthy controls. In either case the capacity of IgM antibodies to discriminate between TPE and controls appears to be limited.

Considering cross reactivity of IgG antibodies and non-specific binding in IgM antibody assay. IgA anti-filarial antibodies appear to be the most specific. Apart from IgA, isotype, the antifilarial IgE response has been shown to be high and discriminatory in TPE and other filarial infections\(^5,6\). IgE antibodies are present in very small quantities and as such to quantify specific IgE in sera requires sophisticated techniques. In contrast, IgA antibodies are present in relatively abundant quantities and can be measured using simpler and faster assays such as an ELISA or immunofluorescence assay. Hence, it would be useful to probe further into the specificity of IgA antibodies in TPE and filariasis.

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