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Immune response studies with *Wuchereria bancrofti* vespider allergen homologue (WbVAH) in human lymphatic filariasis

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Abstract

A homologue of *Brugia malayi* venom allergen (BmVAH) was cloned from the infective stages (L3) of *Wuchereria bancrofti*. Sequence analysis showed 90% sequence identity between WbVAH and BmVAH. Recombinant WbVAH was then expressed and purified. VAH from other nematode parasites is being evaluated as potential vaccine candidates. Because *W. bancrofti* infections are more prevalent than *B. malayi*, it will significantly benefit using *W. bancrofti* antigens for vaccine development. In this study, we have evaluated the human immune responses to rWbVAH in putatively immune individuals who live in the endemic regions (endemic normal, EN) to determine the vaccine potential of WbVAH. These responses were then compared to those in infected individuals (microfilaraemic, MF and chronic pathology, CP). Results show that EN subjects carry WbVAH-specific IgG1, IgG2, and IgG3 circulating antibodies. It is interesting to note that CP patients also carried antibodies against WbVAH that was mainly of the IgG3 isotype. Peripheral blood mononuclear cells (PBMC) from EN individuals responded strongly to rWbVAH by proliferating and secreting IFN- γ . PBMC from MF patients also proliferated in response to rWbVAH but secreted mainly IL-10. Thus, there was a clear dichotomy in the cytokine production by infected patients vs individuals who are putatively immune (EN). Although vaccine potential of WbVAH has not been established yet, our findings suggest that WbVAH mediated immune responses in EN individuals is primarily Th1-biased. Further vaccination studies are underway in animal models to determine the role of WbVAH in protective immunity against *W. bancrofti* and *B. malayi* infections.

Introduction

Lymphatic filariasis is a debilitating mosquito-borne helminthiasis caused by the parasites *Wuchereria bancrofti*, *Brugia malayi*, and *B. timori*. Nearly 80% of the infection in the endemic areas is due to *W. bancrofti* alone (Michael and Bundy 1997). The clinical presentations in infected individuals display a spectrum of disease. Complex host–parasite interaction and associated immune response is thought to be responsible for such a wide clinical spectrum (Ottesen 1984).

Although diethylcarbamazine (DEC), ivermectin, and albendazole are the commonly used drugs to treat lymphatic filariasis, they have the inherent disadvantage of requiring repeated and prolonged treatment for years leading to potential drug resistance (Schwab et al. 2006,

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2007). Despite the major advantages, these chemotherapeutic agents can cause hypersensitivity reaction due to dead or dying adult worms, which could worsen the lymphatic pathology. Therefore, alternative strategies like vaccination could be beneficial along with chemotherapy and vector control in eradicating filariasis from endemic areas (Grieve et al. 1995).

Vaccination with antigens derived from infective third-stage larvae are of particular interest as they are important for the establishment of infection and represent key targets of protective immunity (Blaxter et al. 1996; Gregory et al. 2000). For example, abundant larval transcript (ALT) gene (Gregory et al. 2000; Gnanasekar et al. 2004; Ramachandran et al. 2004), which, as the name suggest is abundantly expressed in L3 stages of the parasite, has been shown to provide significant levels of protection in animal models. Although the nature of protective immune responses is highly debated over several years (Peralta et al. 1999; Ravindran et al. 2000), the consensus is that the host immune responses play a major role in determining clinical manifestations of various groups (Frank and Grieve 1996; Helmy et al. 2000). In this respect, the endemic normal (EN) group, which resides in endemic areas and is constantly exposed to the infection without showing any symptoms of parasitemia (Frank and Grieve 1996; Peralta et al. 1999), is probably the most attractive group because they carry circulating antibodies that may be host-protective. Hence, the antigens identified from the infective stage and which are recognized by the putative immune individuals will be suitable targets for developing a vaccine against human lymphatic filariasis.

One such antigen, VAL-1, a homologue of insect venom allergen identified earlier from *B. malayi* is shown to be a promising vaccine candidate in experimental animal models (Murray et al. 2001). One of our previous studies showed that EN individuals carry circulating antibodies against BmVAL-1 (designated in the study as BmVAH) (Gnanasekar et al. 2004). In this study, we have designated the *W. bancrofti* homologue protein as WbVAH because the VAH/VAL-1/ASP-1 family of proteins share significant similarity with hymenopteran venom allergens (VA). VAH family of proteins are cysteine rich secretory proteins (CRISP) and several homologues have been documented from a wide range of nematodes such as *Haemonchus contortus* (Rehman and Jasmer 1998), *Ancylostoma caninum* (Hawdon et al. 1996), *Ancylostoma duodenale* (Bin et al. 1999), *Onchocerca volvulus* (MacDonald et al. 2004), *Necator americanus* (Daub et al. 2000), *Toxocara canis* (Tetteh et al. 1999), *C. elegans*, *Ascaris*, and *Strongyloides* (Hawdon et al. 1999). Immunization studies using VAH from various parasite species (*B. malayi*, *A. caninum*, and *O. volvulus*) suggest that VAH is a potential vaccine candidate that can confer significant protection (Hawdon et al. 1996; Murray et al. 2001; MacDonald et al. 2004). A recent study also suggests that VAH is a potent adjuvant for other antigens (MacDonald et al. 2004). Therefore, VAH proteins appear to be clinically relevant.

Despite mass chemotherapeutic approaches and vector control measures, there is an increase in the prevalence of *W. bancrofti* infection in endemic areas. In this regard, developing a vaccine against this disease is a possibility. However, a major drawback is the lack of a suitable animal model for *W. bancrofti* infections. Another shortcoming is the lack of enough data in the literature analyzing human immune responses to antigens derived from *W. bancrofti* and thus the characteristics of the protective immune responses to *W. bancrofti* are poorly understood.

Infective L3 stages are most attractive as vaccine targets because killing L3s can block the infection and thus are preventive. It is interesting to note that L3 stages express high levels of VAH proteins. In this study, we have evaluated the immune responses to WbVAH in filarial patients because VAH appears to be an attractive target for vaccine development. After cloning, the WbVAH gene from the infective stages (L3) of *W. bancrofti*, we expressed recombinant WbVAH and evaluated the humoral and cellular immune responses to WbVAH in a cohort of

subjects residing in a *W. bancrofti* endemic area who are putatively immune individuals (EN) or patients who are clinically diagnosed with lymphatic filariasis (microfilaraemic, MF or chronic pathology, CP).

Materials and methods

Study population

Standardized histories were obtained and physical examinations were done during routine epidemiological surveys on subjects living in and around Vellore, India, an area endemic for *W. bancrofti* infections. Patients identified positive were then recruited through the National Filarial Control Units under the Directorate of Public Health (Vellore, India) after obtaining informed consent with protocols approved by Institutional Review Boards of the Anna University, Chennai. Parasitological examination of all individuals was done by detection of microfilariae in night blood smears. Heparinized blood was collected with prior consent from all individuals in the study. The study population was divided into three categories (EN, CP or MF) based on the detection of circulating parasites, antigens or by evaluating the clinical symptoms of the disease. Circulating microfilariae in the blood samples were identified as described previously (Gnanasekar et al. 2004) and the circulating filarial antigens were detected using a commercially available Og4C3 kit (Lalitha et al. 1998) and a WbSXP-based enzyme-linked immunosorbent assay (ELISA) (Rao et al. 2000). Individuals with no circulating antigen or microfilariae were considered as EN, whereas subjects with circulating microfilariae and/or circulating antigen as detected by Og4C3/WbSXP ELISA were considered as MF. Subjects showing lymphedema and other visual clinical symptoms of filariasis were classified into CP. Sera samples from non-endemic normal (NEN) individuals were a kind gift from Dr. Thomas B. Nutman, NIAID, USA.

Construction of WbVAH expression vector

Full-length WbVAH were cloned in prokaryotic expression vector pRSET A, using insert specific primers (forward primer 5'-cgcggatccatgttacttttcgtcatattt-3' containing *Bam*HI site and reverse primer 5'-cccaagcttcactttctgcacaatccaga-3' containing *Hind*III site) that were designed from GenBank accession no. AAD16985. Primers were synthesized at IDT DNA technologies. Using insert specific primers, the VAH gene was amplified by polymerase chain reaction (PCR) from *W. bancrofti* L3 cDNA library. PCR parameters were 95°C of denaturation for 30 s, 55°C of primer annealing for 30 s, 72°C of primer extension for 30 s, and the cycle was repeated for 30 times. A final extension of 5 min was performed at 72°C before storing the samples at 4°C. PCR products obtained were digested with *Bam*HI and *Hind*III enzymes and ligated to similarly digested T7 expression vector pRSET A. Insert DNA was sequenced to ensure authenticity of the cloned nucleotide sequence on both the strands.

Recombinant protein expression and purification

For expression, salt inducible *Escherichia coli* GJ1158 cells (Bangalore Genei, India) were transformed with recombinant plasmid and allowed to grow. When the cultures reached an optical density of 0.6 at 600 nm, 0.3 M NaCl was added to the cultures to induce gene expression and the cultures were incubated for 3 h (Fig. 1). Total cell proteins were analyzed by 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and the presence of histidine-tagged recombinant protein was confirmed by immunoblotting with anti-histidine tag antibodies (anti-HisG, Invitrogen, San Diego, USA). Subsequently, the histidine-tagged recombinant protein was purified from pelleted bacteria using chelating sepharose fast flow (Amersham Biosciences, Uppsala, Sweden) as per manufacturer's recommendations.

Humoral immune response to recombinant WbVAH protein in an endemic population

Levels of WbVAH-specific antibodies in sera from human subjects were studied by ELISA as previously described (Cheirmaraj et al. 1992). Briefly, polyvinyl microtiter plates (NUNC, Germany) were coated with rWbVAH protein at a concentration of 1 µg/ml at 4°C and blocked with 3% bovine serum albumin in 0.01 M phosphate buffered saline (PBS) at 37°C for 2 h. After blocking the plates with blocking buffer, diluted (1:100) serum samples in 0.01 M PBS was added and the plates were incubated at 37°C for 2 h. After further washes, the plates were incubated with peroxidase-conjugated affinity-purified goat anti-human IgG (GibcoBRL) for 1 h at 37°C, washed, and developed with a substrate containing H₂O₂ and 3,3',5,5'-tetramethylbenzidine (TMB) (Sigma Chemical, USA) after 10 min at room temperature. The optical density (OD) was measured at 490 nm.

ELISA to determine isotype-specific antibodies

ELISA was carried out as described by Cheirmaraj et al. 1992 with few modifications using biotin-conjugated mouse monoclonal anti-human IgG1, IgG2, IgG3, and IgG4 and avidin horse radish peroxidase (HRPO) conjugate (Sigma Chemical, USA). The wells of a polystyrene microtiter plate (NUNC, Germany) were coated with rWbVAH (at a concentration of 1 µg/50 µl) in 0.06 M carbonate buffer pH 9.6 and incubated at 4°C overnight and blocked with 100 µl of 2% BSA at 37°C for 1 h; 50 µl of diluted sera (1:100) were added and incubated at 37°C for 1 h.

Wells were washed and further incubated with biotinylated monoclonal mouse anti-human IgG isotype antibodies (IgG1 at 1:1,000; IgG2 at 1:15,000; IgG3 at 1:5,000; and IgG4 at 1:15,000) at 37°C for 1 h; 50 µl of avidin peroxidase conjugate (1:20,000) (Sigma Chemical, USA) was added and incubated for 30 min. After final washing, each well was incubated with 50 µl of substrate consisting of *O*-phenylene diamine dihydrochloride (OPD) (Sigma Chemical, USA) and H₂O₂ in citrate phosphate buffer, pH 5.0. The reaction was stopped after 15 min by adding 5 N HCl. OD₄₉₀ values were read in an ELISA reader.

Human PBMC response to WbVAH

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized venous blood by gradient centrifugation over lymphocyte medium (Lymphoprep; Nycomed Pharma AS, Oslo, Norway). The cells were washed and the viability was determined by trypan blue dye exclusion method. The cells were then cultured in round-bottomed microtiter plates at a concentration of 0.2×10^6 cells/well in RPMI 1640 (Sigma, St. Louis, USA) supplemented with gentamicin (80 µg/ml) (Ranbaxy Laboratories, India), 25 mM HEPES (USB, Amersham Pharmacia, UK), 2 mM glutamine (USB, Amersham Pharmacia, UK), and 10% human AB serum or FCS and stimulated with a soluble crude extract of BmA (10 µg/ml), ConA (10 µg/ml) or medium alone as negative control. A dose response study using rWbVAH was carried out with PBMCs from normal healthy individuals to determine the optimum proliferative doses from 1 to 20 µg/ml. Cultures were performed in triplicate wells in a humidified atmosphere of 5% CO₂ at 37°C for 72 h. Cell proliferation was determined by an MTT assay using the formula: stimulation index (SI)=absorbance recombinant stimulate/absorbance unstimulated.

Cytokine assays

Separate cultures were set up for cytokine assay. Briefly, PBMCs were washed and plated onto 24-well flat-bottomed tissue culture plates (Costar) at 4×10^6 cells/ml in a volume of 1 ml of RPMI 1640 supplemented with 10% fetal calf serum (Gibco-BRL) and 80 µg/ml gentamicin. PBMCs were then stimulated with or without rWbVAH at 10 µg/ml. Supernatants were harvested at 48 h or 96 h after stimulation and stored at -70°C before use. Capture ELISAs (Pierce Biotechnology, Rockford, IL) were performed to detect IFN-γ (for Th1 responses) and

IL-10 (for Th2 responses) in culture supernatants as described previously (Mahanty et al. 1996). All values were derived from the interpolation of standard curves run simultaneously on every plate. The sensitivities of the assays for IFN- γ and IL-10 were 39 pg/ml, respectively.

Statistical analysis

Statistical analysis was performed using XL STAT software v.7.5.2 (Kovach Computing Services, Anglesey, UK). Statistical significance between comparable groups was evaluated using appropriate non-parametric tests with the level of significance.

Results

Expression of rWbVAH

The rWbVAH cloned in pRSET A was expressed as histidine-tagged fusion protein. The molecular mass of the recombinant fusion protein (with the histidine tag) was approximately 27 kDa (Fig. 1a). SDS-PAGE analysis showed that expressed rWbVAH consisted of over 10% of the total *E. coli* proteins. Subsequently, the recombinant protein was purified using metal affinity column chromatography (Fig. 1a) and the size was confirmed by immunoblotting using anti-His antibody (Fig. 1b).

Humoral immune response to recombinant WbVAH protein in an endemic population

ELISA results presented in Fig. 2a show that significant ($P<0.01$) amounts of anti-rWbVAH IgG antibodies were present in the sera of EN, MF, and CP individuals compared to sera from NEN individuals. Further analyses suggest that EN individuals carry significantly ($P<0.05$) higher titers of anti-rWbVAH IgG antibodies in their sera compared to individuals in the MF or CP group.

Isotype profiles to rWbVAH in human sera

Because WbVAH IgG antibodies were significantly increased in all groups of subjects except NEN, further analyses were performed to determine the isotype of WbVAH IgG antibodies. These analyses showed that majority of the WbVAH IgG antibodies in the sera of EN and MF individuals were of IgG1, IgG2, and IgG3 isotypes. However, IgG2 levels in EN sera were slightly higher compared to its IgG1 or IgG3 isotypes, whereas antibodies in the sera of CP patients were mainly of IgG3 isotype (Fig. 2b). WbVAH IgG4 isotype of antibodies were barely detectable in all three groups (EN, MF or CP) of subjects.

Human T cell response to rWbVAH

It has been demonstrated that cellular responses to mitogens do not differ among the various clinical groups of lymphatic filariasis. However, hyporesponsiveness to adult parasite soluble crude extract antigen (BmA) is a characteristic feature of patent filarial infection. To examine whether purified rWbVAH behaves similarly to BmA, PBMCs were obtained from EN, MF, and CP individuals and were stimulated with 10 μ g/ml of rWbVAH or 10 μ g/ml of BmA or ConA. As shown previously (King et al. 1992; Baize et al. 1997), BmA-induced proliferative responses was diminished in MF (geometric mean (GM) SI=1.67, $P<0.05$) patients compared to either the EN (GM SI=4.62, $P<0.05$) or CP (GM SI=4.02, $P<0.05$) individuals (Fig. 3). In contrast, rWbVAH induced higher proliferative responses in PBMCs collected from EN (GM SI=3.37, $P<0.05$) subjects compared to those from MF (GM SI=1.88, $P<0.05$) and CP (GM SI=1.64, $P<0.05$) individuals (Fig. 3). ConA induced significant proliferation of PBMCs collected from all groups.

Cytokine levels

Levels of IFN- γ (marker for Th1 immune response) and IL-10 (marker for Th2 immune responses) were determined in the PBMC culture supernatants stimulated with rWbVAH. Levels of IFN- γ (Fig. 4a) was significantly higher ($P<0.05$) (365.5 pg/ml) in the culture supernatants of PBMCs from EN compared to similarly stimulated PBMC from MF (169.6 pg/ml) and CP individuals (91.9 pg/ml) ($P<0.05$). It is interesting to note that IL-10 levels (Fig. 4b) were significantly ($P<0.05$) elevated in the culture supernatants of rWbVAH-stimulated PBMC from MF individuals (224 pg/ml) compared to CP (197 pg/ml) and EN (160.5 pg/ml) individuals. Cytokine levels in the culture supernatants of unstimulated control cultures were below detection limits.

Discussion

WbVAH cloned in this study shares significant sequence similarity with *B. malayi* VAH, *A. caninum* ASP-1, and *O. volvulus* ASP-1 family of proteins. ASP was initially identified from *A. caninum* as a major secretory protein important for its parasitism (Hawdon et al. 1996). Subsequently, its homologues from different parasites such as *A. ceylanicum* (Goud et al. 2004), *O. volvulus* (MacDonald et al. 2004), *N. americanum* (Fujiwara et al. 2005), and *B. malayi* (Murray et al. 2001) were cloned and studied in detail. Apart from its protective capability, ASP from *O. volvulus* has been shown to induce angiogenesis when injected in the cornea of mice (Tawe et al. 2000). However, a true function for this molecule still remains unidentified. Several studies reaffirm the ASP family of proteins as strong candidates for vaccination.

Protective immunity to filarial parasites have been demonstrated in both human and animal models (Zang et al. 2000). However, the mechanism of protective immune response in the humans is largely unknown. This information is especially important for developing an effective vaccine against lymphatic filariasis due to *W. bancrofti* because there are very limited animal models available.

The objective of this study was to dissect the immune responses to WbVAH in individuals exposed or infected with *W. bancrofti*. Analysis of the humoral immune responses to WbVAH in patients exposed (EN) or infected (MF and CP) with *W. bancrofti* showed that EN individuals carry significant levels of anti-WbVAH antibodies when compared to CP, MF or NEN individuals. These findings were consistent with what we reported earlier for BmVAH (Gnanasekar et al. 2004). However, the isotype profile of IgG antibodies showed that anti-WbVAH antibodies were predominantly of the IgG1 and IgG2 isotypes followed by IgG3 in the sera of EN individuals, whereas infected individuals (MF and CP) carry predominantly IgG3 antibodies followed by IgG2 and IgG1 isotypes. These observations were surprisingly different with the previously reported isotype profiles seen with either BmVAH or OvVAH (Murray et al. 2001; MacDonald et al. 2004) where the most predominant isotype in infected and protective individuals were IgG3 followed by IgG4 with very little IgG1 or IgG2 isotype. Although, BmVAH and WbVAH share significant sequence similarity, differences in their ability to induce differing isotype antibody responses in EN individuals is difficult to explain at this time.

Antigen-specific hyporesponsiveness is the hallmark of patent filarial infections. However, the patient still responds to certain mitogens and specific antigens (Dimock et al. 1996; Mahanty et al. 1996). In the present study, we evaluated the responses of PBMCs from CP and MF individuals to rWbVAH and compared these responses to those from EN individuals. Similar to the antibody responses, rWbVAH induced high levels of PBMC proliferation in EN individuals when compared to MF or CP. All EN individuals responded nearly at the same levels. However, studies by Murray et al. 2001 showed that BmVAH induced significant

proliferative responses only in some patients. Although we did not observe such a difference, it is interesting to note that in their studies (both humoral and proliferative responses), Murray et al. 2001 used samples obtained from Indonesia, which is endemic for *B. malayi*.

Comparison of results from our studies with that of BmVAH studies by Murray et al. 2001 also suggests that WbVAH and BmVAH might be mounting different immune responses in the respective infections caused by either *W. bancrofti* or *B. malayi*. The observed differences in immune responses between homologous antigens from *B. malayi* and *W. bancrofti* are not fully understood now and needs further evaluation. Comparison of immune responses between other candidate vaccine antigens from *W. bancrofti* and *B. malayi* will also help understand whether these immune differences are restricted to VAH alone or also extend to other antigens. Earlier studies have reported increased lymphocyte proliferation in the endemic group in response to *Seteria digitata* antigens than those of microfilaraemics or clinical filarial cases (Dalai et al. 1998). Similarly, endemic normal individuals from *W. bancrofti* endemic area also carry circulating antibodies that recognizes soluble antigens prepared from microfilariae and infective larvae. In addition, peripheral blood cells from these endemic normal individuals also proliferate in response to the soluble antigens (Dasgupta et al. 1984). Similar observations were recently made by Krithika et al. 2005 who reported significant lymphoproliferative responses against *Brugia* mf S-7 antigens in endemic normal individuals compared to MF or CP individuals. Results from our study support previous observations that EN individuals carry antibodies and cells that specifically respond to *W. bancrofti* antigens; in this case WbVAH, a potential vaccine candidate.

To dissect the type of cellular immunity induced by WbVAH in filarial patients, we studied the cytokine profile (IFN- γ for Th1 or IL-10 for Th2 type immunity) of PBMCs as read out. Our results clearly showed that IFN- γ levels (i.e., Th1 type of immunity) were higher in EN individuals compared to either MF or CP patients when stimulated with rWbVAH. Earlier studies have shown that Th1 cytokines predominate in EN and to a relative prominence in patients with elephantiasis (Ravichandran et al. 1997). Similarly in supportive of our data, studies with OvASP-1 from another filarial species induced predominant IFN- γ secretion from PBMC of infected and putatively immune individuals or endemic normal individuals (MacDonald et al. 2004). Recent studies with a putative vaccine antigen rBm-SL3 also stimulated increased secretion of IFN- γ by PBMCs from EN compared with those from CP and MF (Dabir et al. 2006). On the other hand, IL-10 levels were significantly elevated in MF individuals when compared with chronic and putatively immune individuals. Indeed, in one study, the levels of IL-10 mRNA in MF were correlated with their reduction in proliferative capacity (Ravichandran et al. 1997). MF individuals tend to have increased levels of both IL-10 protein and mRNA compared to other disease groups and often their PBMC secrete very high levels of spontaneous IL-10 production in vitro (570 pg/ml) (Mahanty and Nutman 1995). Our results presented in this study suggest that WbVAH primarily induces a Th1 type response in EN individuals characterized by high levels of WbVAH-specific IgG2 antibodies, significantly higher levels of IFN- γ secretion by PBMC, and low levels of IL-10 secretion. Thus, a Th1-biased immune response to WbVAH may have some relevance to protection, as EN individuals are considered putatively immune individuals. Further vaccination studies with WbVAH in experimental models will help understand the role of WbVAH in protection.

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Fig. 1.

Expression of WbVAH in *E. coli* GJ1158 host. **a** Total protein extracts from WbVAH and control pRSET A were solubilized in 1×SSB, separated on a 12% SDS-PAGE gel, and stained with Coomassie brilliant blue dye; 50 µg of protein was loaded in each of the respective lanes. Lane M molecular weight marker; lane 1 vector-uninduced; lane 2 vector-induced; lane 3 0.3 M NaCl WbVAH-uninduced; lane 4 WbVAH-induced at 3 h; lane 5 purified protein eluted at 200 mM imidazole. **b** Total protein extracts after inducing the recombinant clone WbVAH with NaCl were separated on 12% SDS-PAGE, transferred to nitrocellulose membrane, and probed with mouse monoclonal anti-Histidine antibody (1:2,000) followed by incubation with goat anti-mouse antibody conjugated with alkaline phosphatase (1:30,000) and developed with NBT and BCIP. Lane P anti-His antibody; lane M marker

Fig. 2.

Levels of WbVAH-specific IgG antibodies in the sera of various clinical groups of lymphatic filariasis. Wells were coated with rWbVAH and sera collected from EN, MF, and CP patients were added at 1:100 dilution. **a** Levels of total IgG reactivity were determined by ELISA. Sera from 10 subjects were tested in each group. Each dot in the scatter plot indicates individual sera. Total IgG reactivity with WbVAH was significantly higher in EN ($P<0.05$) when compared to CP or MF groups. **b** Isotype analysis of the WbVAH-specific IgG antibodies in the various clinical groups was determined by isotype-specific ELISA for IgG1, IgG2, IgG3, and IgG4. Levels of IgG1, IgG2, and IgG3 antibodies were significantly higher ($P<0.05$) in EN individuals. CP individuals had higher levels of IgG3. IgG1, IgG2, and IgG3 antibodies were also present in sera from MF individuals. However, levels were comparatively lower than EN and CP individuals. It is interesting to note that IgG4 levels were consistently low in all individuals.

Fig. 3.

Lymphoproliferative responses to WbVAH. PBMCs from EN, MF, and CP individuals were cultured at 0.2×10^6 cells/ml and stimulated with either recombinant WbVAH, BmA or ConA. Cells were incubated for 72 h in 5% CO₂ environment. Lymphocyte proliferation was measured by Cell Titre96[®] aqueous non-radio-active cell proliferation assay kit (MTT). Results are expressed as the SI. *Vertical bar* denotes the geometric mean of all 10 samples from each group. *EN* endemic normal, *CP* chronic pathology, *MF* microfilaraemic

Fig. 4.

IFN- γ and IL-10 responses to rWbVAH in human PBMCs. PBMCs collected from EN, MF, and CP individuals were cultured at 4×10^6 cells/ml and stimulated with rWbVAH (10 μ g/ml) for 96 h (a) or 48 h (b) at 37°C. After incubation, culture supernatants were collected and levels of IFN- γ and IL-10 were measured using the ELISA. Cells cultured in media alone were used as controls. Results show that IFN- γ levels (a) were significantly increased ($P < 0.05$) in EN individuals compared to CP or MF, whereas IL-10 levels (b) were significantly elevated in MF individuals compared to the EN and CP groups. Bars represent the geometric mean of 10 samples