Parasitology International 58 (2009) 141–144

Contents lists available at ScienceDirect

Parasitology International

journal homepage: www.elsevier.com/locate/parint

The effect of HIV on filarial-specific antibody response before and after treatment with diethylcarbamazine in *Wuchereria bancrofti* infected individuals

Heidi H. Petersen [a], Nina O. Nielsen [b], Jesper Monrad [c], Stephen M. Magesa [d], Paul E. Simonsen [a,*]

[a] DBL-Centre for Health Research and Development, Faculty of Life Sciences, University of Copenhagen, Frederiksberg, Denmark
[b] Department of Epidemiology Research, Statens Serum Institut, Copenhagen, Denmark
[c] Danish Centre for Experimental Parasitology, Faculty of Life Sciences, University of Copenhagen, Frederiksberg, Denmark
[d] National Institute for Medical Research, Amani Medical Research Centre, Muheza, Tanzania

ARTICLE INFO

Article history:
Received 18 September 2008
Received in revised form 7 January 2009
Accepted 15 January 2009
Available online 25 January 2009

Keywords:
HIV
Wuchereria bancrofti
Lymphatic filariasis
Co-infection
Antibodies
Diethylcarbamazine

ABSTRACT

The effect of HIV on filarial-specific antibody response before and after treatment with diethylcarbamazine (DEC) was analysed by comparing two groups of *Wuchereria bancrofti*-infected adult individuals (positive for circulating filarial antigen) who were positive ($n=15$) or negative ($n=21$) for HIV co-infection. Prior to DEC treatment there was no significant difference in filarial-specific IgG1, IgG2, IgG4 and IgE antibody response between the HIV negative and the HIV positive group, while a five times (statistically significant) higher filarial-specific IgG3 response was observed in the HIV positive than in the HIV negative group. At 12 weeks after treatment with DEC, a significant decrease in filarial-specific IgG4 was observed in the HIV positive but not in the HIV negative group, indicating that DEC treatment had a stronger antifilarial effect in individuals co-infected with HIV. DEC treatment had no significant effect on the other classes of filarial specific antibodies, neither in the HIV negative or the HIV positive group.

1. Introduction

Worldwide, 33.2 million people were estimated to be infected with Human Immunodeficiency Virus (HIV) in 2007, with Sub-Saharan Africa being home to an estimated 22.5 million of these [1]. In many parts of Africa, HIV infection is common in areas, which are also endemic for *Wuchereria bancrofti* infection (the cause of lymphatic filariasis in Africa), and individuals may be concurrently infected with both *W. bancrofti* and HIV. In such areas it has been observed that the two infections may interact with each other. Thus, pregnant HIV positive women were more likely to have lymphatic filariasis than pregnant HIV negative women [2], individuals positive for *W. bancrofti* infection had a higher risk of being HIV positive compared to individuals negative for *W. bancrofti* infection [3], and individuals co-infected with *W. bancrofti* and HIV had significantly lower level of filarial-antigen stimulated release of IL-4 compared to HIV negative individuals infected with *W. bancrofti* [4]. Diethylcarbamazine (DEC) treatment was moreover observed to reduce the HIV viral load in HIV positive individuals who were concurrently positive for *W. bancrofti*, probably by acting indirectly through its effect on the filarial infection, while such effect was not seen in *W. bancrofti* negative individuals [5].

Further investigations are needed in order to better understand how filarial and HIV infections interact, and not least to understand the effect that DEC based filariasis control may have on the progression of HIV in areas where co-infections occur. In the present study we analyzed the potential impact of HIV on the filarial-specific antibody response in individuals with *W. bancrofti* infection, before and after treatment with DEC. The serum samples, which formed basis of the study, were collected as a part of a larger project on interactions between HIV infection, lymphatic filariasis and DEC carried out in Tanga Region, Tanzania, in 2002 [3–5].

2. Materials and methods

2.1. Study population and design

The field work was conducted in five villages in the coastal part of Tanga Region, north-eastern Tanzania. During a baseline survey in February/March 2002 [4], 858 adult volunteers were screened for *W. bancrofti* specific circulating filarial antigens (CFA) by using a rapid field test (Now®ICT Filariasis, Binax, USA) and for HIV infection by using two different rapid field tests (Hexagon HIV, Human, Germany and Uni-Gold HIV, Trinity Biotech, Ireland; the two tests gave the same result in all cases). Among these individuals, 398 (46.4%) and 68 (7.9%) were positive for CFA and HIV, respectively, and 29 (3.4%) were positive for both. All double-infected individuals who could subsequently be traced and who had no visible signs of AIDS were invited to

* Corresponding author. DBL-Centre for Health Research and Development, Faculty of Life Sciences, University of Copenhagen, Thorvaldsensvej 57, 1871 Frederiksberg C, Denmark. Tel.: +45 35 33 14 15; fax: +45 35 33 14 33.
E-mail address: pesi@life.ku.dk (P.E. Simonsen).

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doi:10.1016/j.parint.2009.01.003

participate in the present study. An age-matched group of similar size of the HIV negative/CFA positive individuals, who had no visible signs of lymphatic filariasis, were also invited to participate. A total of 36 individuals (15 positive for both HIV and CFA; 21 positive for CFA only) accepted the invitation, and participated fully in pre-treatment examination, treatment and 12 weeks post-treatment examination, and thus comprise the study individuals for the present study.

A venous blood sample was collected from each study individual at pre-treatment in May 2002 [5]. Immediately thereafter all individuals were randomly allocated to treatment with a single oral dose of diethylcarbamazine (DEC; 6 mg/kg body weight) or similar looking placebo tablets. A venous blood sample was again collected at 12 weeks post-treatment. The study individuals had not received treatment with DEC before inclusion into the study, and the placebo recipients received DEC treatment at completion of the 12 weeks study period. Permission and ethical clearance to carry out the study was granted by the Medical Research Coordinating Committee of the National Institute for Medical Research (NIMR) in Tanzania and the study was reviewed and approved by the Central Scientific Ethical Committee in Denmark.

2.2. Preparation of serum

At each sampling time 5 ml of venous blood were collected in non-heparinized vacutainer tubes for preparation of serum. The blood was left overnight at 4 °C to clot. Serum was collected the following day and kept frozen at −20 °C until use. In the laboratory in Denmark, a mixture of 0.3% TNBP (tri(n-butyl)phosphate) (Sigma®) and 1 % Tween 80 (polyoxyethylenesorbitan) (Sigma®) was added to all serum samples (13 µl per 1 ml of serum) in order to inactivate lipid-enveloped viruses [6].

2.3. Measurement of filarial-specific antibodies

The serum samples were examined for filarial-specific IgG1, IgG2, IgG3, IgG4 and IgE antibodies by an indirect enzyme linked immunosorbent assay (ELISA). Antigen was prepared from *Brugia pahangi* adult worms and the ELISA's were carried out as reported previously [7]. Prior to measurement of filarial-specific IgE, sera were absorbed with a commercial 1:1 suspension of protein A agarose beads in phosphate buffered saline (Kem-En-Tec A/S, Denmark) at a ratio of 50:140 to remove IgG4-blocking antibodies [8]. Optimal dilutions of antigen, serum and conjugate were determined by titration. Each serum sample was tested in duplicate and the arithmetic mean OD value calculated. Pre- and post-treatment specimens from the same individual were tested on the same ELISA plate. A positive control serum included on all plates was used to adjust for minor plate-to-plate variation in OD values.

Table 1
Characteristics of the study individuals according to infection status.

	Number of individuals	Mean age in years (range)	Number of males/females	Number given DEC/placebo
All Individuals	36	39.8 (19–63)	15/21	19/17
HIV+ group*	15	42.3 (25–56)	4/11	9/6
HIV− group	21	38.0 (19–63)	11/10	10/11

*Mean CD4% = 24.5; mean HIV load = 4.17±0.92 genome equivalents/ml.

2.4. Quantification of W. bancrofti-specific CFA, HIV viral load and CD4 cells

CFA was measured in serum using the TropBio ELISA kit (TropBio Pty. Ltd., Townsville, Australia), and the HIV viral load was determined in serum using an RT-PCR, as described previously [4]. Samples with more than 32 antigen units and 40 genome equivalents/ml, respectively, were considered positive in these tests. In all cases, the quantitative tests confirmed the CFA and HIV status previously determined by use of the rapid field tests. Due to considerable individual variation in CFA level among untreated CFA positive individuals, the effect of treatment on CFA is expressed as the mean of the individual reductions in CFA units for each group. CD4 T-cells were quantified in thin blood smears as previously described [5].

2.5. Statistical analysis

The OD values from individual test sera were normalized by $\log_{10}+1$ transformation. Geometric mean OD values in different groups of individuals at the same time were statistically compared by independent sample *t*-test, whereas pre- and post-treatment OD values from the same group of individuals were compared by paired *t*-test. Differences in age and sex composition were analysed by independent sample *t*-test and χ^2-test, respectively. Associations were analysed by t-test on Pearson's correlation coefficient. *P*-values below 0.05 were considered statistically significant.

3. Results

Table 1 shows the characteristics of the study individuals. Of the 36 individuals, 15 (42%) were males. The mean age was 39.8 years (range 19–63 years), and there was no significant difference in age between males and females. All individuals were CFA positive. Twenty-one (58%) of these were HIV negative, while 15 (42%) were HIV positive.

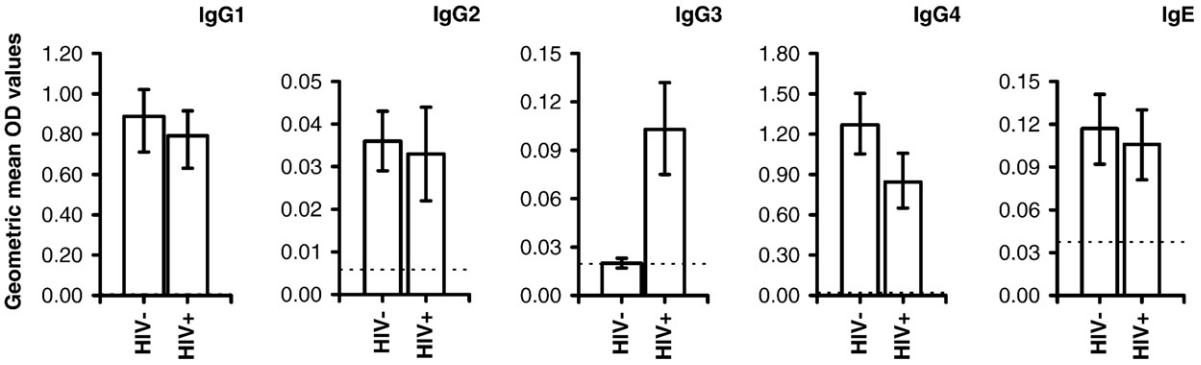

Fig. 1. Geometric mean OD values (±SE) of filarial-specific IgG1, IgG2, IgG3, IgG4 and IgE in serum from *W. bancrofti* infected individuals with (HIV+) or without (HIV−) HIV co-infection. The stippled lines indicate the cut-off between a negative and positive test (calculated for each antibody type as the geometric mean plus two standard deviations of OD values from five individuals from Denmark who had never been exposed to lymphatic filariasis).