Maternal filarial infection: association of anti-sheath antibody responses with plasma levels of IFN-γ and IL-10

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SUMMARY

Maternal filarial infection influences the risk of acquiring infection and development of immunity in children. Here we have analysed the blood samples of 60 mothers (24 infected and 36 uninfected) and their corresponding cord bloods to assess the impact of maternal infection on the anti-sheath antibodies and cytokine production in neonates born from them. About 69·4% of non-infected mothers and their cord bloods showed the presence of anti-sheath antibodies, while only 16·6% of the cord bloods from infected mothers were positive for it. The IL-10 level was significantly high in cord bloods of infected mothers compared with non-infected mothers. At the same time the IL-10 level was also observed to be remarkably high in cord bloods of both infected and non-infected mothers negative for anti-sheath antibody. In contrast, IFN-γ levels were significantly high in cord bloods of non-infected mothers compared with infected mothers and the increment was prominent in cord bloods of both infected and non-infected mothers positive for anti-sheath antibody. The study reveals that the presence or absence of anti-sheath antibodies in association with cytokines skews the filarial specific immunity to either Th1 or Th2 responses in neonates. This may affect the natural history of filarial infection in early childhood.

Key words: filariasis, anti-sheath antibodies, cytokines, maternal filarial infection.

INTRODUCTION

Human lymphatic dwelling filarial parasites undergo several stages of development within their host and each developmental stage of the parasite produces many overlapping as well as stage-specific antigens. The ability of the parasite to survive, reproduce and cause chronic disease depends on its success to evade the protective responses of the host. On the other hand host genetic polymorphism and several environmental factors play crucial roles in modulating the infection outcome of an individual. Recently, maternal filarial infection has also been considered as one of the risk factors for increased susceptibility and facilitates the persistence of parasites in the offspring. However, the existence and the nature of protective immunity in human filariasis continues to be a subject of intense debate. Even though there is no broad consensus on functional immunity against larval and adult-stage parasites, anti-microfilarial immunity has been demonstrated to be mediated by antibodies to the microfilarial sheath (Ravindran *et al*. 1990). The prevalence of anti-sheath antibodies was observed to be highest in younger children and is inversely correlated with CFA (circulating filarial antigens). This suggests that sheath antibodies appear early during infection well before onset of microfilaraemia and may have a crucial role in maturation of adult filarial worms (Simonsen and Meyrowitsch, 1998; Ravindran *et al*. 2000). Since the first exposure of an individual to filarial antigen takes place *in utero*, maternal filarial infection is presumed to play an important role in the outcome of infection. The impact of this exposure on host susceptibility and development of anti-filarial immune responses following challenge with infective larvae is not clear. Epidemiological studies have provided evidence that children born to infected mothers are more susceptible to acquiring infection compared with children born to infection-free mothers (Malhotra *et al*. 2003, 2006; Rajan 2007). It has been described that soluble parasite antigens, maternal helminth-specific anti-idiotypic antibody and/or maternally derived cytokines may influence neonatal sensitization to parasite antigens (Carlier and Truyens, 1995). There is increasing evidence that pre-natal T-cell priming occurs via transplacental exposure to antigens and such primary sensitization affects the maturation of the post-natal immune system (Steel *et al*. 1994; Holt, 1995; Prescott *et al*. 1998). However, the mechanism by which maternal filarial infection sensitizes offspring is still not fully understood. It is also not exactly known how maternally conferred

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immunity affects the evolution of parasite-specific T-cell immunity and susceptibility to infection during childhood. In general, a Th1 type of immune response (high IFN-γ) has been associated with the absence of active filarial infection while a Th2 type of T-cell response (high IL-10) has been observed in the infected group (Maizels *et al*. 1993; Freedman, 1998). These differential cytokine productions could influence the survival of the parasite and the disease outcome. Although anti-microfilarial immunity in human lymphatic filariasis is believed to operate through antibodies to the microfilarial sheath, the biological role of anti-sheath antibodies in susceptibility to infection among the offspring has not been explored. Earlier, we demonstrated that the transplacental transfer of circulating filarial antigens and pre-natal sensitization to filarial antigens developed *in utero* (Bal *et al*. 2010). The present investigation attempted to determine the extent of influence of maternal filarial infection on the development of anti-sheath antibodies in the offspring and to establish the relationship between anti-sheath antibodies with the polarized T-cell responsiveness in cord blood from infected and uninfected mothers.

MATERIALS AND METHODS

Study population

Blood samples ($n = 60$) from mothers and their cords were collected at the time of uncomplicated delivery from the O & G Department of the District Hospital, Khurda of Orissa, India. Healthy pregnant women come for delivery from neighbouring villages known to be highly endemic for bancroftian filariasis having a microfilaria rate of about 12% (Beuria *et al*. 2003). Informed consent was obtained from each study subject before their enrolment. None of the mothers had symptoms of clinical filariasis at the time of admission. The study was approved by the institutional review boards of the Human Ethical Committee of the Regional Medical Research Centre (ICMR), Bhubaneswar. Venous blood samples were collected from mothers before delivery. Umbilical cord blood samples from neonates were collected immediately after birth. Maternal and cord samples were collected in differently sized tubes to avoid the chance of mislabelling. The haemoglobin alkaline denaturation test was used in order to exclude the admixture of maternal blood contamination with the fetal blood samples (Sepulveda *et al*. 1999). Sera were stored at −70 °C until further use. The microfilarial status of mothers and cord blood samples of offspring was checked by filtration of a 1 ml sample through a Nucleopore membrane.

Immunoperoxidase assay

Anti-sheath antibodies to microfilaria sheath of *Wuchereria bancrofti* were detected by indirect immunoperoxidase assay (IPA) as described by Ravindran *et al*. (2000). Briefly, acetone-fixed microfilariae purified from peripheral blood using 3 μm polycarbonate membrane (Nucleopore Corporation, USA) were used for the assay. The antigen slides were pre-treated for 20 min with 0·5% H_2O_2 in methanol to inactivate endogenous peroxidase activity. Approximately 15–20 μL of serum (1:5 diluted in PBS) were applied to the slides which were incubated in a humid chamber for 2 h at 37 °C. The slides were then washed 3 times with PBS and 15 μL of goat anti-human polyvalent IgG-peroxidase conjugate (sigma) were added to the spots for detecting bound antibody activity. After washing the slides, the reaction was visualized using a light microscope after staining the slides with the substrate, diaminobenzidine (50 mg 100 mL^{-1}) in Tris-HCl buffer, pH 8·6, with 1 μL mL^{-1} H_2O_2. All the samples were tested using microfilariae of the same donor for uniformity and standard positive and negative controls were taken for batch testing.

Detection of circulating filarial antigen (CFA) assay

Detection of CFA was carried out in serum samples using the Og4C3 enzyme-linked immunosorbent assay test kit (JCU Tropical Biotechnology, Queensland, Australia) according to the manufacturer's instructions. A serum sample from each individual was tested and the optical density values were used to determine the antigen concentration in units from the standard curve prepared using 7 standard antigens supplied in the kit. Serum samples with an antigen unit of 128 (> titre of standard no. 3) were considered as antigen positive.

Cytokine assays

Cytokine levels (IL-10 and IFN-γ) in plasma were measured by using ELISA kits and expressed in pg/ml by interpolation from the standard curve as described by the manufacturer's instructions. Briefly, the capture antibody was diluted in PBS to a concentration of 1 μg mL^{-1} and 100 μL were added to each well of the ELISA plate. The plate was sealed and incubated overnight at room temperature. The plate was washed 3 times with PBST and blocked with 1% BSA (PBS) and incubated for 1 h at room temperature. The plate was again washed and the standard and serum samples were added to a dilution of 1:5 and the plate was incubated for 2 h at room temperature. The detection antibody was added to each well after washing and incubated for 2 h at room temperature. The plate was washed and 100 μL of avidin-HRP conjugate were added to each well at a dilution of 1:2000 and incubated for 30 min at room temperature. The plate was again washed and 100 μL of ABTS substrate solution were added and the

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Table 1. Prevalence of anti-sheath antibodies and circulating filarial antigen (CFA) in pairs of mother and respective cord blood samples

	Maternal			Cord blood		
		Anti-sheath antibodies			Anti-sheath antibodies	
Infection status	*N*	Positive	Negative	*N*	Positive	Negative
CFA Positive	24	4 (16·6%)	20 (83·4%)	10	2 (20%)	8 (80%)
CFA Negative	36	25 (69·4%)	11 (30·5%)	50	27 (54%)	23 (46%)

colour development was monitored with an ELISA plate reader.

Statistical analysis

The significance of the difference between group responses was determined using chi-squared test. The level of significance of cytokine levels was determined by Student's *t*-test.

RESULTS

The prevalence of CFA and anti-sheath antibodies were calculated from 60 pairs of mother and respective cord bloods as shown in Table 1. At the time of delivery all mothers included in this study were negative for both microfilaria and symptoms of filariasis. An overall CFA prevalence among mothers was noted to be 40% (24/60) whereas in cord blood it was only 16·6% (10/60). The prevalence of anti-sheath antibodies in mothers and their respective cord bloods was found to be similar i.e. 48·3% (29/60). However, 38·3% (23/60) of cord blood samples were negative for both CFA and anti-sheath antibodies, and only 3·3% (2/60) of cord blood samples were positive for both parameters. Interestingly, all the cord blood samples from anti-sheath antibody-positive mothers (69·45%) were positive for anti-sheath antibodies indicating trans-placental transfer of the antibodies. Only 16·6% (4/24) of cord blood from infected mothers were anti-sheath antibodies positive. Of these 4 cord bloods 2 were CFA positive and 2 were CFA negative. All the cord bloods showing anti-sheath antibody positivity were from the mothers with anti-sheath antibody positivity irrespective of infection status. There was a very significant inverse relationship (chi-squared test $P<0·001$) between the presence of CFA and absence of anti-sheath antibodies in mothers, but the association between the two parameters was not significant (chi-squared test $P=0·0817$) among the cord blood samples of infected and uninfected mothers.

The plasma levels of the cytokines IL-10 and IFN-γ were measured in the mothers and their respective cord blood samples and the results are shown in Figs 1 and 2. Fig. 1a shows that cord blood samples of infected mothers had significantly higher IL-10 than cord bloods of uninfected mothers ($P=0·007$). We analysed further the association of anti-sheath antibodies and levels of cytokines in mothers and their respective cord blood samples. Elevated levels of IL-10 were observed in anti-sheath antibody-negative cord bloods compared with anti-sheath antibody-positive cord bloods irrespective of the infection status of mother. Cord blood samples from infected mothers that were anti-sheath anti-body-negative had significantly higher levels of IL-10 than cord blood samples that were anti-sheath antibody-positive (Fig. 1b). In contrast the levels of IFN-γ were found to be significantly higher in CFA-negative cord blood than in CFA-positive cord blood samples ($P<0·02$) as shown in Fig. 2a. Elevated levels of IFN-γ were observed in anti-sheath antibody-positive cord blood samples compared with anti-sheath antibody-negative cord blood samples, irrespective of the infection status of the mothers (Fig. 2b). No significant difference was observed in plasma levels of IFN-γ in the presence or absence of anti-sheath antibodies of infected and uninfected mothers. Increased levels of IL-10 and down-regulation of IFN-γ have been detected in anti-sheath antibody-negative cord blood of children born to filarial-infected mothers.

DISCUSSION

Women harbouring filarial infection during their childbearing age have the possibility of exposing the developing fetus to filarial antigens *in utero* and owing to that there might be a change in the immunity and susceptibility to infection during their offsprings' early childhood. Since the mothers residing in highly filarial endemic areas are continuously exposed to infective larvae, there is a chance that the cord blood cells are also exposed to parasitic products during fetal life. According to the available literature reports, besides genetic predisposition, neonatal immune responses reflect influences of the *in utero* exposure of the fetus to filarial antigens, and the consequent immune priming indicates that the maternal environment plays a crucial role in the outcome of infection (Kohler *et al*. 2008). Earlier studies have shown that *in utero* exposure to filarial antigen occurs in human

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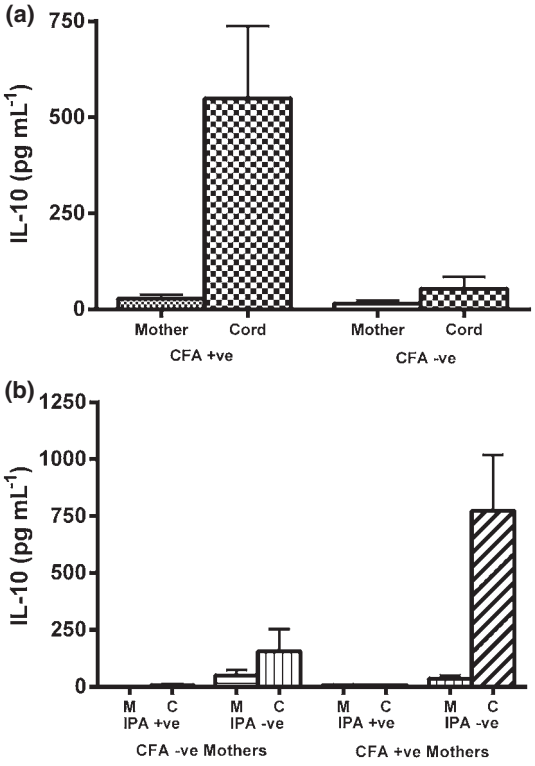

Fig. 1. Plasma levels of IL-10. (a) In mothers and their respective cord blood samples obtained from newborns of women with (CFA + ve) or without (CFA − ve) filarial infection. (b) The groups were further stratified according to anti-sheath antibody positivity (IPA + ve) and negative (IPA − ve) status. Bars represent geometric mean ± S.E. values. M, mothers; C, cord bloods.

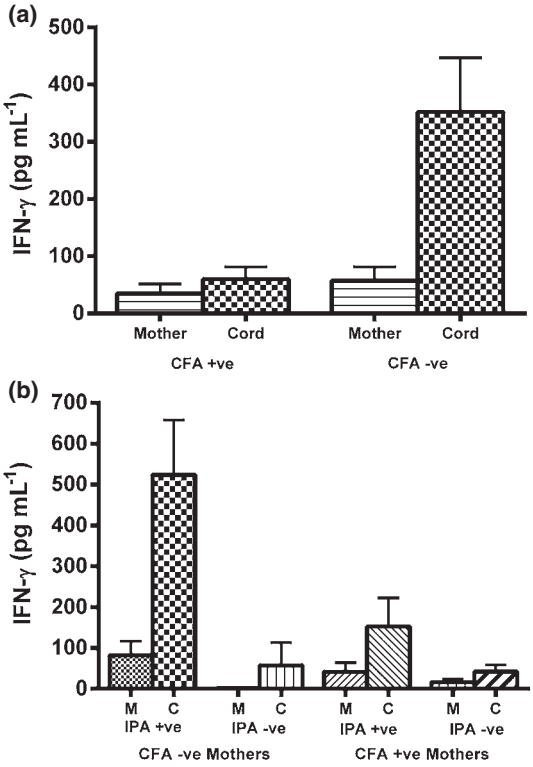

Fig. 2. Plasma levels of IFN-γ. (a) In mothers and their respective cord blood samples obtained from newborns of women with (CFA + ve) or without (CFA − ve) filarial infection. (b) The groups were further stratified according to anti-sheath antibody positivity (IPA + ve) and negative (IPA − ve) status. Bars represent geometric mean ± S.E. values. M, mothers; C, cord bloods.

filariasis and children born to infected mothers are at greater risk of acquiring infection than the offspring of uninfected mothers (Lammie *et al*. 1991; Malhotra *et al*. 1997; Bal *et al*. 2010). The mechanism that protects some of the infants from developing infection and allows others to acquire infection is not known. However, a strong inverse association between sheath antibody positivity and the markers of filarial infection has been demonstrated (Simonsen *et al*. 2008).

The present study has shown that 69% of cord bloods of infection-free mothers were positive for anti-sheath antibodies. This finding is at par with the previous observation where it was reported that 78% of children below 10 years of age were positive for anti-sheath antibodies (Ravindran *et al*. 2000). In a situation like this the transfer of said antibody from mother to offspring could help the neonates to remain infection free in the early period of their life by inhibiting the development of infective larvae. Further continuous exposure to infective larvae may induce production of anti-sheath antibodies, which could adversely affect further development of the incoming filarial larvae, keeping the host amicrofilaraemic. It is to be noted that the transfer of antibodies from mother to neonates opens up an interesting possibility in the understanding of the natural history of filarial infection and disease among filaria-endemic populations. About 38·3% of cord blood samples in the present study were negative for anti-sheath antibodies. This is because the majority of the samples were collected from infected mothers and the rest were from infection-free mothers, who might not have been sufficiently exposed to infection. The absence of anti-sheath antibodies in infected mothers (except 16·6%) and their respective cord bloods suggests that these neonates could develop established filarial infection in the event of exposure to infective larvae in a later period of life. However, the mechanisms by which maternal infections sensitize offspring are still not properly understood.

In order to correlate the transfer of anti-sheath antibodies from mother to cord blood with the *in utero* cytokine environment, we have measured plasma levels of cytokines in mothers and their respective cord blood samples. The increased levels of both cytokines in cord bloods compared with the respective mothers further implies *in utero* sensitization. It is known that neonatal T-cells require a much lower dose of antigen than adult T-cells to become activated, tolerated or biased towards the Th2 type phenotype (Pit *et al*. 2000). Similarly, immunological memory established by pre-natal sensitization is long-lived and the T- and B-cell

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responses detected in the cord blood preparations are not due to maternal lymphocytes that have passed into the fetal circulation (Malhotra *et al*. 1999). Therefore, it is speculated that susceptibility to filarial infection may not strictly be due to the expression of filarial-specific immune responses but may be a function of the cytokine environment in which the parasite survives and develops (Lammie, 2002).

The high level of IL-10 in CFA-positive cord blood compared with CFA-negative cord blood in our study suggests that *in utero* sensitization has skewed the immune response towards Th2 type. The high level of IL-10 associated with the absence of anti-sheath antibodies might be favouring the survival of the parasite during children's early exposure to infection; earlier findings have demonstrated that microfilaraemic subjects respond to parasite antigens by producing a set of suppressing cytokines (IL-10 and TGF-β) that may facilitate persistence of the parasite within humans (King *et al*. 1993). In addition to this, *in utero* exposure to filarial antigen perhaps leads to subtle down-regulation of the expression of Th1-associated responses, thus promoting the establishment of patent infection (Lammie, 2002). From all those observations it is tempting to predict that high levels of IL-10 with an absence of anti-sheath antibodies in the cord bloods of infected mothers could be an evolutionary adaptation for parasite survival in their offsprings' early exposure to infection.

Furthermore, the increased level of IFN-γ in CFA-negative cord bloods compared with CFA-positive cord bloods may skew the response towards a Th1 type of response. A prospective study initiated at birth has shown that helminth-specific T-cell immunity acquired *in utero* is maintained until 10–14 months of age in the absence of infection (Malhotra *et al*. 1999). The high level of IFN-γ with anti-sheath antibody positivity in CFA-negative cord bloods may restrict parasite survival and growth, subsequently giving protection to the newborn. Evidence of *in utero* sensitization to *Ascaris lumbricoides* infection indicates that induction of a host immune response starts before birth, while absence of sensitization could be suggestive of acquisition of immune responsiveness in later life (Guadalupe *et al*. 2009). However, it has not been resolved whether the apparent association between various pre-natal risk factors and susceptibility to infection is a result of immunotolerance leading to an inability to generate protective immunity (Lammie *et al*. 1991; King *et al*. 1992) or reflects the difference in exposure to mosquito-borne infective larvae (Alexander *et al*. 1998; Simonsen *et al*. 2002). The current study has provided evidence for the existence of Th1 or Th2 type of environments that were decided *in utero*, and that can play an important role in determining the infection outcome of the children in their later period of life.

A long-term follow-up of 2 groups of children – born from mothers positive or negative for anti-sheath antibodies in their cord bloods – can reveal the sequence of events taking place in natural filarial infection. It may give some indication as to why children born to infected mothers are more susceptible to filarial infection. From the current observations we can conclude that anti-sheath antibody negativity and a high IL-10 cytokine profile (dominant Th2) may be responsible for providing a conducive environment for the infective larvae to survive and persist in children during post-natal exposure to infection. In contrast, anti-sheath antibody positivity and high IFN-γ skewed the response towards a Th1 type of response that could give protection to the offspring against post-natal infection.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest concerning the work reported in this paper.

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