Altered spontaneous and antigen-specific frequencies of Th1 cells in filarial lymphedema

Study -5
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Introduction

CD4+ T cells differentiate into distinct subsets characterized by their functions and their cytokine profiles (Th1 or Th2 cells). Th1 cells produce high levels of interleukin 2 (IL-2) and gamma interferon (IFN-γ), which are strong inducers of cell-mediated immunity, [131,147]. The immune responses to filarial parasites have been well studied with respect to natural history, diagnosis and treatment; there is a relative paucity of information in terms of the mechanisms underlying the development of pathology. The two major independent components of lymphatic filarial disease are lymphangiectasia and inflammatory reactions around the adult worms [82,91]. It is clear that with patent infection, lymphangiectasia develops in the vicinity of adult worm nests [26]. It is also clear that the host adaptive immune and inflammatory response against the dead or dying worms and the subsequent release of parasite products and inflammatory mediators may lead to the irreversible lymphatic dysfunction and blockage [84,148]. This in turn manifests clinically as progressive lymphedema. In addition, significant lymphatic dysfunction has been shown to predispose infected individuals to secondary bacterial and fungal infections that trigger inflammatory reactions in the skin and subcutaneous tissue and that accelerates the progression of lymphedema and precipitates the development of elephantiasis [149,150].

The pathogenesis of lymphatic filarial disease is influenced by multiple factors, of both parasite and host origin. In terms of the host immunity, both arms of the host immune system - innate and adaptive – are thought to play major roles in the development of pathology. Studies
using animal models of lymphatic disease have clearly delineated an important role for T cells in the development of disease [32,151]. Moreover, T cells have also been shown to play a role in human lymphatic disease, with the presence of abnormal T cell infiltrates exhibiting a biased TCR repertoire at the site of inflammation [22,78] as well as altered chemokine receptor and activation marker expression in peripheral blood [152,153,154]. In terms of T cell responses, filarial disease has been associated with increased frequencies of CD4\(^+\) T cells expressing IFN\(\gamma\) in response to parasite but not non-parasite antigens, [155]. In addition, filarial disease is also associated with elevated production of both IFN\(\gamma\) and IL-2 and increased expression of the Th1 master transcription factor – T-bet – upon filarial antigen stimulation in peripheral blood mononuclear cells [79]. While the nature of immune responses to parasite antigens has been well characterized in patients with the asymptomatic (or subclinical) form of filarial infection [90].

Since Th1 cells are important in the pathological manifestations, we sought to determine the expression pattern of CD4\(^+\) T cells in filarial lymphedema patients, asymptomatic infected and uninfected individuals following stimulation with parasite and control antigens. We show that lymphatic disease in human LF is characterized by both increased spontaneous and antigen-driven CD4\(^+\) Th1 cells. Our findings reveal a role for subsets of CD4\(^+\) T cells in the development of overt filarial disease in Lymphatic Filariasis. In addition, our data also reveal that IL-1, IL-23 and TGF-\(\beta\) are cytokines that are important in regulation of these cell types in filarial disease.

**Study population**

We studied a group of 33 individuals with filarial lymphedema (hereafter CP), 25 clinically asymptomatic, filarial infected (hereafter INF) individuals and 15 uninfected,
endemic normal (hereafter UN) individuals in an area endemic for LF in Tamil Nadu, South India. All CP individuals were circulating filarial antigen negative by both the ICT filarial antigen test (Binax, Portland, ME) and the TropBio Og4C3 enzyme-linked immunosorbent assay (ELISA) (Trop Bio Pty. Ltd, Townsville, Queensland, Australia), indicating a lack of current active infection. The diagnosis of prior filarial infection was made by history and clinical examination as well as positive Brugia malayi antigen (BmA) -specific IgG4. BmA-specific IgG4 and IgG ELISA were performed exactly as described previously. All INF individuals tested positive for active infection by both the ICT filarial antigen test and the TropBio Og4C3 ELISA and had not received any anti-filarial treatment prior to this study. All UN individuals were circulating filarial antigen negative and without any signs or symptoms of infection or disease. There were no differences between the groups in terms of demographics or socio-economic status. All individuals were examined as part of clinical protocols approved by Institutional Review Boards of both the National Institutes of Allergy and Infectious Diseases and the National Institute for Research in Tuberculosis (NCT00375583 and NCT00001230), and informed written consent was obtained from all participants.

Results

CP individuals exhibit altered baseline frequencies of Th1 cells

To determine the cytokine expressing phenotype of T cells in CP individuals at homeostasis, we measured the frequency of CD4⁺ T cells expressing different cytokines at baseline and compared them to INF and UN individuals. CP individuals exhibit significantly increased frequencies of CD4⁺ T cells expressing both Th1 (IL-2, TNF-α and IFNγ) in
Figure 5.1. Filarial lymphedema is associated with altered frequencies of CD4\(^+\) T cells expressing Th1 cytokines.

Figure 5.1.A. Filarial lymphedema is associated with altered baseline frequencies of CD4\(^+\) T cells expressing Th1 cytokines.

Figure 5.1.B. BmA

Figure 5.1.C. Mf
Figure 5.1.D. PPD

![Graph showing frequency of cytokines in PPD](image)

Figure 5.1.E. P/I

![Graph showing frequency of cytokines in P/I](image)

Figure 5.1. Filarial lymphedema is associated with altered frequencies of CD4+ T cells expressing Th1 cytokines. (A) Baseline frequencies of CD4+ T cell expressing Th1 (IL-2, TNF-α and IFN-γ) cytokines in CP, INF and UN individuals. (B, C, D, E) BmA, Mf, PPD and P/I induced frequencies of CD4+ T cell expressing Th1 (IL-2, TNF-α and IFN-γ) cytokines in CP, INF and UN individuals. The bars represent geometric means and 95% confidence intervals. P values were calculated using the kruskal wallis analysis.
comparison to INF and UN shown in figure 5.1.A. Thus, filarial lymphatic disease is associated with perturbations in the homeostatic frequency of cytokine producing T cells.

**CP individuals exhibit significantly higher antigen - specific frequencies of Th1 cells**

To determine the expression pattern of CD4⁺ T cells expressing TNF-α, IL-2 and IFN-γ in CP individuals, we measured the frequency of Th1 cells and compared them to INF and UN individuals at baseline and following stimulation with BmA, Mf, PPD and P/I. CP individuals exhibit significantly higher frequencies of Th1 cells following stimulation with BmA and Mf antigens in comparison to INF and/or UN individuals (figure 5.1.B, C). On the other hand, CP individual exhibited no significant difference in the frequency of Th1 cells in response to PPD and P/I (figure 5.1.D, E) in comparison to the other two groups. UN individuals exhibited significantly increased frequencies of filarial antigen - driven Th1 cells in comparison to INF individuals. Thus, filarial lymphatic disease is associated with expansion of baseline and antigen - stimulated Th1 cells, which is relatively filarial antigen - specific.

**CP individuals exhibit increased frequencies of baseline and filarial – antigen specific Th1 subsets**

To determine the role of different Th1 subsets in filarial lymphedema, we measured the frequency of CD4⁺ T cells expressing IL-2/IFNγ or IL-2/TNF-α or TNF-α/IFNγ in response to parasite and non-parasite antigens as well as to P/I and compared them to INF and UN individuals. CP individuals exhibited significantly increased baseline as well as BmA and Mf antigen - stimulated frequencies of Th1 cells co-expressing IL-2 and TNF-α in comparison to
Figure 5.2. IL-1, IL-6, TNFR and anti-IL-10 regulate the frequencies of Th1 cells in filarial lymphedema

Figure 5.2.A IL-1, IL-6, TNFR regulate the frequencies of Th1 cells in filarial lymphedema

Figure 5.2.B IL-10 and TGF-β regulate the frequencies of Th1 cells in filarial lymphedema

Figure 5.2 (A) IL-1, IL-6R and TNFR neutralization significantly decreases the frequencies of IL-2 following stimulation with BmA in CP individuals (n=10). IL-1, IL-6R neutralization significantly increases the frequencies of TNF-α following stimulation with BmA in CP individuals (n=10). (B) IL-2 neutralization significantly increases the frequencies of IL-2, TNF-α following stimulation with BmA in CP individuals (n=10). Each line represent a single individual; p values were calculated using the Wilcoxon signed rank test.
INF and UN individuals. Similarly CP individuals exhibited significantly increased frequencies of baseline as well as filarial - antigen specific frequencies of Th1 cells co-expressing IL-2 and TNF-α and IFN-γ in comparison to INF and/or UN individuals (figure 5.1.A). CP individuals exhibited significantly increased frequencies of filarial - antigen specific frequencies of Th1 cells co-expressing IL-2 and IFNγ in comparison to INF and/or UN individuals (figure 5.1.B, C). Of interest, the frequencies of these Th1 subsets did not differ significantly amongst the groups in response to PPD and P/I (figure 5.1.D, E). Thus, filarial lymphatic disease is characterized by a filarial – antigen driven expansion of Th1 subsets.

**IL-1 and IL-23 receptor blockade exhibited decreased frequencies of Th1 cells in CP individuals**

To identify the role of cytokines in the regulation of Th1 cells in filarial lymphedema, we measured the frequency of Th1 cells CP individuals (n=10) following in vitro blockade or neutralization of IL-1, IL-23, TGF-β, IL-10 and TNF signaling and concomitant stimulation with BmA. CP individuals exhibited significantly decreased net frequencies of IL-2 where as TNF-a shows increased frequencies of following IL-1R, IL-6R and TNFR blockade (figure 5.2.A) and following TGFβ neutralization (figure 5.2.B). Thus, the filarial lymphatic disease is characterized by an IL-1 and IL-23 dependent expansion of Th1 cells.

**Discussion**

T cells have also been shown to play a role in human lymphatic disease, with the presence of abnormal T cell infiltrates exhibiting a biased TCR repertoire at the site of
inflammation [22,78] as well as altered chemokine receptor and activation marker expression in peripheral blood [152,153,154]. In terms of T cell responses, filarial disease has been associated with increased frequencies of CD4⁺ T cells expressing IFN-γ in response to parasite but not non-parasite antigens.

Filarial infection is characterized by a diverse set of clinical manifestations including an asymptomatic (or subclinical) form seen among the majority of infected people [90]. Although adaptive immune responses, especially T cell responses, are clearly important in the progression of asymptomatic infection to overt filarial disease, the nature of these T cell responses are still poorly characterized. Dysregulation of CD4⁺ T cell mediated immune activation, however, can lead to the development of tissue inflammation and pathology [146]. Expansion of antigen-driven Th1 type CD4⁺ T cells have long been considered to be the hallmark of chronic pathology in filariasis [146]. Individuals living in filariasis endemic areas that remain uninfected, especially those with lymphedema, display anti-filarial immune responses that are biased in the Th1 direction [156]. PBMC responses in these individuals are characterized by intense proliferative responses and IL-2 and IFN-γ secretion when stimulated with parasite antigens [157]. The first seminal study that identified a direct role for adaptive immunity in pathology was from a study reporting that PBMC from individuals with chronic lymphatic pathology made significantly higher levels of IL-2 and IFN-γ in response to parasite antigens compared to the asymptomatic-infected individuals [155]. A major hallmark of longstanding filarial infection (especially of the asymptomatic or subclinical variety) is the downregulation of parasite antigen driven Th1 differentiation. This is manifested by a significantly lower production of IFN-γ and IL-2 upon filarial antigen stimulation in asymptomatic-infected compared to diseased individuals [155].
Our study also provides evidence for an important role for upstream cytokines in the regulation of Th1 cells in filarial infections. Our data clearly reveal that IL-1, IL-6R, TGF-β plays an important role in the expansion of Th1 cells in filarial pathology. IL-10 blockade has been previously shown to modulate both Th1 cytokine production and T cell proliferation in infected individuals [44], IL-1 and IL-6R appear to be common factors in the induction of Th1 responses and IL-1, IL-6R cytokine axis is possibly an important pathway in the inflammation driven fibrosis in filarial lymphedema.

Our study therefore, highlights an important role for CD4+ Th1 cell subsets in the regulation of immune responses in filarial infections and reveals an unexpected elevation in the circulating frequencies of Th1 cells. Since fibrotic pathology is the final, common outcome of many chronic inflammatory diseases, our study of Th1 cells in filarial disease holds important implications for other inflammatory diseases as well.