IL-1 and IL-23 mediated expansion of filarial – antigen specific Th17 and Th22 cells in filarial lymphedema

Study -6
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Introduction

Apart from Th1 and Th2 cells, newer subsets of CD4⁺ T cells have been recently described, including Th17 cells expressing IL-17 and Th22 cells expressing IL-22. Th17 cells are cells that express the prototypical cytokine -- IL-17 and are known to play a major role in defense against extracellular pathogens as well as being involved in a variety of inflammatory and autoimmune diseases [158]. Recently, Th17 cells have been classified into different subsets with each subset playing a specialized role in resistance to different infections [159]. Th22 cells are cells that express the prototypical cytokine -IL-22 and are thought to play an important role in protection against intestinal pathogens [160]. In addition, similar to Th17 cells, Th22 cells have also been linked to various inflammatory and autoimmune diseases [161]. TGF β, IL-1 β, IL-6 and IL-23 are major players driving Th17 and Th22 responses in mice and humans [162].

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 [78,163] as well as altered chemokine receptor and activation marker expression in peripheral blood [153,164,165]. In terms of T cell responses, filarial disease has been associated with increased frequencies of CD4⁺ T cells expressing IFNγ in response to parasite but not non-parasite
antigens [155] as well as markers of Type 17 responses [79]. However, very little is known about the regulation of Th17 or Th22 cells in filarial infections.

Since Th17 and Th22 cells are clearly important in the pathological manifestations of autoimmune and other diseases, we sought to determine the expression pattern of CD4\(^+\) Th17 and Th22 cells and their subsets in filarial infections. Our data reveal an important association of expansion of Th17 and Th22 cells with the presence of lymphedema in filarial infections. 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. 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.
Figure 6.1. Filarial lymphedema is associated with higher baseline and antigen-stimulated frequencies of Th17 and Th22 cells.

Figure 6.1.A

(A) A representative flow plot depicting the BmA and P/I stimulated frequency of CD4+ T cells expressing IL-17, IL-22 and IFNg in a CP individual. (B) Baseline as well as antigen and P/I stimulated frequencies of Th17 cells in CP (n=23), INF (n=25) and UN (n=15) individuals. (C) Baseline as well as antigen and P/I stimulated frequencies of Th22 cells. The data are depicted as scatter plots with each circle representing one individual and the line representing the geometric mean or as bar graphs with bar representing the geometric mean and 95% confidence intervals. The data for antigen or P/I stimulation are shown as net frequencies with the baseline frequencies subtracted. P values were calculated using the Kruskal-Wallis test with Dunn’s multiple comparisons (* p < 0.05, ** p < 0.01, *** p < 0.001).
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 significantly higher baseline and antigen-specific frequencies of Th17 and Th22 cells

To determine the expression pattern of CD4+ T cells expressing IL-17 and IL-22 in CP individuals, we measured the frequency of Th17 and Th22 cells and compared them to INF and UN individuals at baseline and following stimulation with BmA, Mf, PPD and P/I. The BmA and P/I induced expression of Th17 and Th22 cells is shown in a contour plot from a representative CP individual in Figure 6.1.A. As shown in Figure 6.1.B, CP individuals exhibit significantly higher frequencies of Th17 cells both at baseline and following stimulation with BmA and Mf antigens in comparison to INF and/or UN individuals. Similarly as shown in Figure 6.1.C, CP individuals exhibited significantly higher frequencies of Th22 cells at baseline and following BmA and Mf antigen stimulation. On the other hand, CP individual exhibited no significant difference in the frequency of Th17 and Th22 cells in response to PPD and P/I in comparison to the other two groups. Interestingly, INF individuals exhibited significantly increased frequencies of filarial antigen-driven Th17 and Th22 cells in comparison to UN individuals. Thus, filarial lymphatic disease is associated with expansion of baseline and antigen-stimulated Th17 and Th22 cells, which is relatively filarial antigen-specific.
Figure 6.2. Filarial lymphedema is associated with higher baseline and antigen-stimulated frequencies of Th17 and Th22 subsets.

(A) Baseline as well as antigen and P/I stimulated frequencies of Th17 cells co-expressing IL-17 and IFNγ in CP (n=23), INF (n=25) and UN (n=15) individuals. (B) Baseline as well as antigen and P/I stimulated frequencies of Th17 cells co-expressing IL-17 and IL-22 in CP, INF and UN individuals. (C) Baseline as well as antigen and P/I stimulated frequencies of Th22 cells co-expressing IL-22 and IFNγ in CP, INF and UN individuals. The data are depicted as scatter plots with each circle representing one individual and the line representing the geometric mean or as bar graphs with bar representing the geometric mean and 95% confidence intervals. The data for antigen or P/I stimulation are shown as net frequencies with the baseline frequencies subtracted. P values were calculated using the Kruskal-Wallis test with Dunn’s multiple comparisons (* p < 0.05, ** p < 0.01, *** p < 0.001).
CP individuals exhibit increased frequencies of baseline and filarial – antigen specific Th17/Th22 subsets

To determine the role of different Th17/Th22 subsets in filarial lymphedema, we measured the frequency of CD4+ T cells expressing IL-17/IFNγ or IL-17/IL-22 or IL-22/IFNγ in response to parasite and non-parasite antigens as well as to P/I and compared them to INF and UN individuals. As shown in Figure 6.2.A, CP individuals exhibited significantly increased baseline as well as BmA and Mf antigen - stimulated frequencies of Th17 cells co-expressing IL-17 and IFNγ in comparison to INF and UN individuals. Similarly, as shown in Figure 6.2.B, CP individuals exhibited significantly increased frequencies of baseline as well as filarial - antigen specific frequencies of Th17 cells co-expressing IL-17 and IL-22 in comparison to IN and/or UN individuals. Finally, as shown in Figure 6.2.C, CP individuals exhibited significantly increased frequencies of baseline as well as filarial - antigen specific frequencies of Th22 cells co-expressing IL-22 and IFNγ in comparison to IN and/or UN individuals. Of interest, the frequencies of these Th17 and Th22 subsets did not differ significantly amongst the groups in response to PPD and P/I. Thus, filarial lymphatic disease is characterized by a filarial – antigen driven expansion of Th17/Th22 subsets.

IL-1 and IL-23 receptor blockade results in significantly decreased frequencies of Th17 and Th22 cells in CP individuals

To identify the role of cytokines in the regulation of Th17 and Th22 cells in filarial lymphedema, we measured the frequency of Th17 and Th22 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. As shown in Figure 6.3.A, CP individuals
Figure 6.3. Cytokines regulate the frequencies of Th17 and Th22 cells in filarial lymphedema.

(A) The net frequencies of Th17 cells following stimulation with BmA in the presence of blocking antibodies to IL-1R, IL-23R, TNFR or neutralizing antibodies to TGF-β or IL-10 in CP individuals (n=10). (B) The net frequencies of Th22 cells following stimulation with BmA in the presence of blocking antibodies to IL-1R, IL-23R, TNFR or neutralizing antibodies to TGF-β or IL-10 in CP individuals (n=10). Antigen-stimulated frequencies are shown as net frequencies with the baseline levels subtracted. Each line represents a single individual. P values were calculated using the Wilcoxon signed rank test.
exhibited significantly decreased net frequencies of Th17 cells following IL-1R, IL-23R and TNFR blockade and following TGF-β neutralization. On the other hand, CP individuals exhibited significantly decreased net frequencies of Th22 cells following IL-1R and IL-23R blockade and significantly increased net frequencies of Th22 cells following TGF-β and IL-10 neutralization (Figure 6.3.B). Thus, the filarial lymphatic disease is characterized by an IL-1 and IL-23 dependent expansion of Th17 and Th22 cells.

Discussion

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 [118]. Expansion of antigen-driven Th1 type CD4+ T cells have long been considered to be the hallmark of chronic pathology in filariasis [118]. More recently, the involvement of Th17 responses has also been implicated mostly based on cytokine mRNA measurements [79]. The role of Th2 responses in protection from or pathogenesis underlying filarial disease has not been well characterized either [118]. The role of other CD4+ T cell subsets remains to be elucidated.

CD4+ T cells that express the prototypical cytokine IL-17 is emerging as an important regulatory of inflammation and fibrosis [166]. IL-17 expression has been implicated in the pathogenesis of pulmonary fibrosis [167], chronic allograft rejection [168], myocardial
fibrosis [169] and hepatic fibrosis [170]. Moreover, Th17 cells are clearly associated with the development of pathology in other parasitic infections, including schistosomiasis [171,172], leishmaniasis [173,174] and toxoplasmosis [175]. Similarly, although Th22 cells (that express the prototypical cytokine IL-22) are thought to play an anti-inflammatory role in certain settings [176], they have been implicated in the pathogenesis of a variety of inflammatory diseases, including psoriasis [177], systemic lupus erythematosus [178] and rheumatoid arthritis [179]. Our study identifies a novel association of Th17 and Th22 cells with the presence of lymphatic pathology in filarial infections. Our study provides the first detailed examination of Th17 cell subsets as well as Th22 cells in filarial infections and reveals that both these subsets of CD4+ T cells are up regulated in an antigen - specific manner in filarial infection. In addition, the modulation of Th17 and Th22 cells are also highly antigen - specific since control antigen or polyclonal stimulation did not alter the frequencies of these cells. Our data also reveal a concordance in the response of different Th17 subsets in filarial infections. Thus, Th17 and Th22 subsets co-expressing IFNγ are all modulated in a similar fashion by filarial antigen in filarial infection denoting the similarity of Th17 and Th22 subset responses. Since Th17 cells are expressed at significantly higher frequencies in infected as well as chronic pathology individuals compared to uninfected individuals, we speculate that this enhanced induction Th17 subsets could reflect their role in promoting the development of pathology. Our data also suggests an important association of Th22 cells with the pathogenesis of lymphatic filarial disease. The exact mechanism by which IL-17 and IL-22 potentially mediate pathogenic responses in filarial infections needs to be elucidated, including postulated mechanisms such as neutrophilic inflammation and induction of matrix metalloproteinases [166].
Our study also provides evidence for an important role for upstream cytokines in the regulation of Th17 and Th22 cells in filarial infections. We have previously shown that filarial infection and/or disease is associated with high systemic levels of IL-1β, TNF-α, IL-23, TGF-β and IL-10 [180]. While it is well known that TGF-β along with IL-6, IL-1β and IL-23 plays a major role in the regulation of Th17 cells [181], the identity of the cytokines involved in the regulation of Th22 cells is not well defined. It has been proposed that IL-6 in the absence of TGF-β is an important inducer of Th22 cells [160]. Our data clearly reveal that IL-1, IL-23, TGF-β and TNF-α play an important role in the expansion of Th17 cells in filarial pathology. On the other hand, while IL-1 and IL-23 act as promoters of Th22 expansion in filarial pathology, TGF-β and IL-10 act as down modulators of this response. Thus, TGF-β has a diametrically opposite effect on Th17 and Th22 induction - it promotes Th17 expansion but diminishes Th22 expansion. Surprisingly, IL-10 also appears to play a role in the regulation of Th22 cells. While IL-10 blockade has been previously shown to modulate both Th1 cytokine production and T cell proliferation in infected individuals [44], to our knowledge this is the first report on a putative role for IL-10 in the regulation of Th22 cells. IL-1 and IL-23 appear to be common factors in the induction of both Th17 and Th22 responses and IL-1-IL-23-IL-17/IL-22 cytokine axis is possibly an important pathway in the inflammation driven fibrosis in filarial lymphedema.

Our study therefore, highlights an important role for CD4⁺ T cell subsets in the regulation of immune responses in filarial infections and reveals an unexpected elevation in the circulating frequencies of Th17 and Th22 cells. Since fibrotic pathology is the final, common outcome of many chronic inflammatory diseases, our study of Th17 and Th22 cells in filarial disease holds important implications for other inflammatory diseases as well. Our
data suggest that targeting the IL-17/IL-22 pathway or its upstream inducers would hold promise in ameliorating pathological disease manifestations in filarial infections and other pathologies of similar etiology.