Enhanced Mycobacterial Antigen–Induced Pro-Inflammatory Cytokine Production in Lymph Node Tuberculosis

Gokul Raj Kathamuthu,^{1,2*} Kadar Moideen,¹ Rathinam Sridhar,³ Dhanaraj Baskaran,² and Subash Babu^{1,4}

¹National Institutes of Health, National Institute for Research in Tuberculosis (NIRT), International Center for Excellence in Research, Chennai, India; ²National Institute for Research in Tuberculosis (NIRT), Chennai, India; ³Government Stanley Medical Hospital, Chennai, India; ⁴Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland

Abstract. Lymph node tuberculosis (LNTB) is characterized by the enhanced baseline and antigen-specific production of type 1/17 cytokines and reduced baseline and antigen-specific production of interleukin (IL)-1 β and IL-18 at the site of infection when compared with peripheral blood. However, the cytokine profile in the lymph nodes (LNs) of *Mycobacterium tuberculosis* culture–positive LNTB (LNTB+) and negative LNTB (LNTB–) has not been examined. To address this, we have examined the baseline and mycobacterial antigen–stimulated cytokine levels of type 1 (interferon gamma [IFN γ], tumor necrosis factor alpha [TNF α], IL-2), type 2 (IL-4, IL-5, and IL-13), type 17 (IL-17A, IL-17F, and IL-22), pro-inflammatory (IL-1 α , IL-1 β , IL-18, and granulocyte macrophage colony-stimulating factor [GM-CSF]), and regulatory cytokines (IL-10, transforming growth factor beta [TGF- β]) cytokines in the LN culture supernatants of LNTB+ and LNTB– individuals. We have observed significantly enhanced baseline levels of IL-13 and IL-10 and significantly reduced baseline levels of IL-4 and GM-CSF in LNTB+ individuals compared with LNTB– individuals. By contrast, we have observed significantly enhanced levels of type 1 (IFN γ , TNF α , and IL-2), type 17 (IL-17F and IL-22), and pro-inflammatory (IL-1 α and GM-CSF) cytokines and significantly reduced levels of TGF β in response to purified protein derivative, early secreted antigen-6, and culture filtrate protein-10 antigens in LNTB+ compared with LNTB– individuals. On phorbol 12-myristate 13-acetate/ionomycin stimulation, no significant difference was observed for any of the cytokines examined. Thus, our study revealed several interesting differences in the cytokine profiles of mycobacterial antigen–stimulated LN cultures in LNTB+ and LNTB– individuals. Therefore, we suggest the presence of mycobacteria plays a significant role in driving the cytokine response at the site of infection in LNTB.

INTRODUCTION

Lymph node tuberculosis (LNTB) is the most common form of extra-pulmonary tuberculosis. Extra-pulmonary tuberculosis comprises about 20% of all TB cases in India and LNTB constitutes nearly 35% of these cases.^{1–3} Extra-pulmonary tuberculosis has become more common since the advent of HIV infection. Lymph node tuberculosis is common in children; however, adults between the age of 20 and 40 years can also be commonly affected, with a predominant female bias. The clinical presentation depends primarily on the site of disease with LNTB in the cervical, axillary, and inguinal areas often presenting as non-tender swellings without any significant systemic symptoms.⁴ Lymph node tuberculosis is hard to diagnose because there are several other infectious and noninfectious diseases that can present with the same clinical profile. Excisional biopsy followed by bacterial culture has a high sensitivity for LNTB diagnosis, yet this procedure is invasive and not commonly performed. Fine-needle aspiration (FNA) biopsy is a much less invasive procedure, which provides a good clinical specimen for the initial pathologic assessment. The gold standard for diagnosis of LNTB still remains either culture (in solid or liquid media) or demonstration of the bacilli by nucleic acid amplification tests.⁵

However, very few studies have examined the utility of antigen-specific cytokine responses in the lymph nodes (LNs) as a diagnostic biomarker. The most well-studied antituberculosis immunity is the T cell–mediated response, which is known to be crucial against *Mycobacterium tuberculosis* (Mtb)

infection. Type 1/pro-inflammatory cytokines such as interferon gamma (IFN γ) and tumor necrosis factor alpha (TNF α) are able to activate either macrophages or other phagocytes to confer host protection by limiting bacterial growth. Tumor necrosis factor alpha is also essential for maintaining granuloma formation (early phase of infection), a well-organized assembly consisting of both innate and adaptive cells.⁶ Interleukin (IL)-2 is secreted by numerous immune cells and is also a key component of protective immunity.⁷ Similarly, type 17 (IL-17A, IL-17F, and IL-22) cytokines, especially IL-17A, are essential in host protection against extracellular pathogens through the induction of other cytokines and chemokines.⁸ Furthermore, IL-22 has a defensive role in mucosal sites, by inducing antimicrobial proteins to mediate protection against extracellular pathogens.⁹ Collectively, published data suggest that both type 1 and type 17 cytokines are crucial in providing host protective immune responses against pulmonary tuberculosis (PTB).¹⁰

Apart from type 1/17 cytokines, several other effector molecules are able to contribute to host protection against TB disease. One such is the IL-1 family (including IL-1 α , IL-1 β , and IL-18) of cytokines, which contribute to diverse physiological functions such as host defense against bacterial and viral infection and activate the immune system (especially type 1 cytokines).¹¹ Likewise, the pro-inflammatory cytokine, GM-CSF, has the potential ability to combat the bacterial growth in vitro.¹² Natural killer (NK) T cells are the major source of GM-CSF, which is also produced by many other cell types.¹³ By contrast, the regulatory cytokines (IL-10 and TGF β) have a deleterious effect on host immunity and help in establishing chronic infection.¹⁴ Thus, studying the T-cell effector cytokines produced on site-specific Mtb infection might offer new and potential immunotherapy targets for vaccine assessment. Therefore, we wanted to examine the function of T cells producing type 1, type 2, and type 17 cytokines and immune regulatory (IL-10 and TGF β), IL-1 family (IL-1 α and IL-1 β), as well as pro-inflammatory (IL-18 and

* Address correspondence to Gokul Raj Kathamuthu, National Institutes of Health, National Institute for Research in Tuberculosis, International Center for Excellence in Research, National Institute for Research in Tuberculosis, No. 1, Mayor Sathyamoorthy Road, Chetpet, Chennai-600031, E-mail: gokul.r@nirt.res.in

GM-CSF) cytokines in the Mtb antigen-stimulated LN cell culture supernatants of positive LNTB (LNTB+) and negative LNTB (LNTB-) individuals. Our findings have shown that enhanced type 1, type 17 (except IL-17A), IL-1 α , GM-CSF, and diminished TGF β cytokines were associated with LNTB+ individuals.

METHODS

Ethics. The present study was approved by the National Institute of Research in Tuberculosis (NIRT) Institutional Ethics Committee (NIRTIEC2010007), and written informed consent was received from all the study participants.

Patient characterization. A total of 18 LNTB+ and 10 LNTB- individuals were recruited in the study, and their demographics are listed in Table 1. All LNTB individuals were positive for interferon gamma release assay and diagnosed on the basis of excision biopsy showing positivity in liquid culture for Mtb or by histopathological diagnosis. All the study participants were HIV negative and not under any steroid treatment. Both LNTB+ and LNTB- individuals were given antituberculosis chemotherapy for 6 months and were successfully cured as determined by the disappearance of LN enlargement by X-ray and computed tomography (CT) scans. Therefore, LNTB- individuals are also LNTB patients, who happen to have negative bacterial cultures.

Isolation of LN. Excision biopsy was performed to collect the infected LNs, and biopsy material was meticulously transferred in the Roswell Park Memorial Institute (RPMI)-1640 medium, which was used for this study. The LNs were carefully transferred onto a petri dish and washed with the RPMI-1640 medium to remove the blood stains. The samples were dissected into minute pieces using sterilized surgical blade and forceps, and then treated with liberase (collagenase I and II, 0.1 mg/mL), deoxyribonuclease (0.1 mg/mL) enzyme (Roche Diagnostics, Indianapolis, IN), incubated for 20–30 minutes, and homogenized using a syringe plunger. The samples were then dissolved in the RPMI medium and filtered using an 80–100- μ m filter (Becton Dickinson [BD] Biosciences, Franklin Lakes, NJ), inverted, and spun at 2,600 rpm for 10 minutes at 4°C. The supernatant was discarded, and the samples were once again washed with the RPMI medium. Finally, the cells were counted manually using trypan blue (Bio-Whittaker™, Walkersville, MD) and a hemocytometer and further used for in vitro culture stimulation.

Antigens. Mycobacterial antigens used in the study were purified protein derivative (PPD) (Statens Serum Institute), recombinant early secreted antigen-6 peptide pools (ESAT-6) and culture filtrate protein-10 peptide pools (CFP-10) (both from Biodefense and Emerging Infections Research Resources Repository (BEI resources), National Institute of

Allergy and Infectious Diseases [NIAID], NIH). In addition, HIV Gag peptide pools (HIVPP) were used as negative controls (AIDS Reagent Program, Division of AIDS, NIAID, NIH). The final concentrations of antigens are 10 μ g/mL for PPD, 10 μ g/mL for ESAT-6 pp, CFP-10 pp, and HIV Gag peptide pools. Phorbol 12-myristate 13-acetate (PMA)/ionomycin (I) (P/I) (Calbiochem, San Diego, CA) was used as a positive control stimulus with the final concentrations of 12.5 and 125 ng/mL.

In vitro LN culture. Lymph node culture supernatants were used to measure the different cytokine responses. The harvested LNs were diluted with the RPMI-1640 medium supplemented with penicillin/streptomycin (100 U/100 mg/mL), L-glutamine (2 mM), and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (10 mM) (Invitrogen, San Diego, CA) and equally dispersed (2 mL/well, i.e., 2 million cells/well) in 12-well tissue culture plates (Costar; Corning, Inc., Corning, NY). The samples were stimulated with the following antigens: unstimulated (UNS), PPD, ESAT-6, CFP10, HIVPP, and P/I and incubated at 37°C in 5% CO₂ for 18 hours. After the incubation, the cells were transferred into sterile 50-mL falcon tubes and spun at 2,600 rpm for 10 minutes at 4°C. The culture supernatants were carefully transferred into 2-mL screw cap tubes and stored at –80°C.

Lymph node culture ELISA. Fifty microliters of LN culture supernatants was used to measure the following cytokines using DuoSet ELISA kits purchased from R&D systems (Minneapolis, MN). Type 1 cytokines IFN γ , TNF α , and IL-2; type 17 cytokines IL-17A, IL-17F, and IL-22; type 2 cytokines IL-4, IL-5, and IL-13; regulatory cytokines IL-10, TGF β , and IL-1 family; and pro-inflammatory cytokines IL-1 α , IL-1 β , IL-18, and GM-CSF. The UNS values were represented as baseline cytokines, whereas the net cytokines were represented for different antigenic (PPD, ESAT-6 PP, CFP-10 PP, HIV GAG PP, or PMA/I) stimulations (with the baseline cytokine values subtracted).

Statistics. Statistical analysis was performed using GraphPad PRISM 6 (GraphPad Software, Inc., San Diego, CA). The geometric means were used to measure the central tendency. Nonparametric Mann-Whitney *U* test were performed to calculate the statistical significance between two different groups. receiver operating characteristic (ROC) analysis was performed to measure the significant correlation among different cytokines analyzed.

RESULTS

Hematological parameters. The leukocyte and differential counts were performed for LNTB+ and LNTB- individuals using the Ac.T-5 Diff hematology analyzer (Beckman Coulter, Brea, CA). Positive lymph node tuberculosis individuals had significantly lower percentages of lymphocytes compared with LNTB- individuals. No other significant differences in hematological parameters were observed between the two groups (Table 2).

Positive lymph node tuberculosis individuals are associated with altered cytokine responses in UNS LN supernatants. To determine the baseline cytokine profile, we measured the UNS LN supernatant levels of type 1 (IFN γ , TNF α , and IL-2), type 17 (IL-17A, IL-17F, and IL-22), type 2 (IL-4, IL-5, and IL-13), regulatory (IL-10 and TGF β), IL-1 family, and pro-inflammatory cytokines (IL-1 α , IL-1 β , IL-18, and GM-CSF) in LNTB+ and LNTB- individuals (Figure 1A). Among the different cytokines analyzed, we observed significantly decreased levels

TABLE 1
Demographics of study population

Study demographics	LNTB+	LNTB–	<i>P</i> value*
Number of subjects recruited (<i>n</i>)	18	10	–
Gender (M/F)	7/11	3/7	–
Median age in years (range)	29 (18–51)	35 (18–40)	NS (<i>P</i> = 0.5864)
Lymph node culture grade (0/1+/2+/3+)	0/16/2/0	0/0/0/0	–

LNTB– = culture negative lymph node tuberculosis; LNTB+ = culture positive lymph node tuberculosis; NS = non significant.

* Calculated using the Mann-Whitney test.

TABLE 2
Hematological profile of study population

Hematological profile	LNTB+	LNTB-	P value*
Whole blood cells ($10^9/L$)	7.417 (6.4–11.3)	7.2 (3.8–10.3)	NS
Red blood cells ($10^6/L$)	4.5161 (3.94–5.72)	4.4 (4.61–5.41)	NS
Lymphocytes (%)	28.01 (22.8–43.4)	32.87 (23.9–44.8)	0.0105
Monocytes (%)	7.8 (3.9–11.7)	6.83 (4.5–10.5)	NS
Eosinophils (%)	3.12 (1.1–11)	3.34 (2–6.3)	NS
Basophils (%)	1.05 (0.2–2.6)	0.93 (0.3–1.5)	NS
Neutrophils (%)	54.461 (42.9–71.3)	46.03 (43.8–63.3)	NS
Platelets ($10^3/L$)	321.4 (199–482)	297.9 (163–654)	NS
Hemoglobin (g/dL)	11.94 (8–16)	11.02 (10.7–13.6)	NS

LNTB- = negative lymph node tuberculosis; LNTB+ = positive lymph node tuberculosis.
Bold value indicates $P < 0.05$.

* Calculated using the Mann-Whitney test.

of IL-4 ($P = 0.0171$) and GM-CSF ($P = 0.0004$) and significantly enhanced levels of IL-13 ($P = 0.0081$) and IL-10 ($P = 0.0097$) in LNTB+ individuals when compared with LNTB- individuals. However, no significant differences in the levels of either type 1, type 17, or IL-1 family, and pro-inflammatory cytokines were found between the two study groups. Thus, LNTB+ individuals are associated with altered UNS cytokine levels.

Positive lymph node tuberculosis individuals are associated with enhanced type 1 and type 17 cytokines on TB antigen (PPD, ESAT-6 PP, and CFP-10 PP) stimulation in LN supernatants. To determine the mycobacterial antigen stimulated cytokine profile in LNTB+, we measured the levels of those cytokines following stimulation with TB antigens (PPD, ESAT-6 PP, and CFP-10 PP) in LNTB+ and LNTB- individuals (Figure 1B and Supplemental Figure 1A and B). Among the cytokines analyzed, significantly increased levels of IFN γ ($P = 0.0130$ in PPD, $P = 0.0466$ in ESAT-6 PP, and $P = 0.0043$ in CFP-10 PP), TNF α ($P = 0.0446$ in PPD, $P = 0.0129$ in ESAT-6 PP, and $P = 0.0069$ in CFP-10 PP), IL-2 ($P = 0.0311$ in PPD, $P = 0.0037$ in ESAT-6 PP, and $P = 0.0140$ in CFP-10 PP), IL-17F ($P = 0.025$ in PPD and $P = 0.0111$ in ESAT-6 PP), IL-22 ($P < 0.0001$ in PPD, $P = 0.0135$ in ESAT-6 PP, and $P = 0.0009$ in CFP-10 PP), IL-1 α ($P = 0.0002$ in PPD, $P < 0.0001$ in ESAT-6 PP, and $P = 0.0002$ in CFP-10 PP), and GM-CSF ($P = 0.0009$ in PPD, $P = 0.0024$ in ESAT-6 PP, and $P = 0.0002$ in CFP-10 PP) were observed in LNTB+ individuals in comparison with LNTB- individuals.

Similarly, TGF β ($P = 0.0072$ in PPD, $P = 0.0025$ in ESAT-6 PP, and $P = 0.0452$ in CFP-10 PP) levels were diminished and IL-10 ($P = 0.0238$ in PPD) levels were enhanced in LNTB+ individuals when compared with LNTB- individuals. However, no significant differences in the levels of type 2 cytokines (IL-4, IL-5, and IL-13) or IL-1 β and IL-18 were found between LNTB+ and LNTB- individuals. Hence, tuberculous lymphadenitis (TBL) is associated with elevated levels of type 1, type 17, IL-1, or pro-inflammatory (IL-1 α and GM-CSF) and regulatory cytokines (IL-10) in the LNTB+ individuals on stimulation with different TB (PPD, ESAT-6 pp, and CFP-10 PP) antigens.

No significant differences in cytokine production on HIV GAG PP and P/I stimulation. To understand the positive (P/I) or non-TB specific or negative control (HIV GAG PP) antigen stimulated cytokine profile, we again measured the different cytokine levels in LNTB+ and LNTB- individuals. As shown in Figure 1C, we did not observe any significant difference in the LN supernatants for any of those cytokines analyzed on stimulation with P/I. Similarly, as shown in Supplemental

Figure 1C, the non-TB specific or negative antigen control stimulated levels did not exhibit any significant differences (except IL-4 [decreased in LNTB+] and IL-13, increased in LNTB+) among the panel of cytokines analyzed between the LNTB+ and LNTB- individuals. Therefore, there are no significant differences observed in LN supernatants following HIV Gag PP or P/I stimulation between LNTB+ and LNTB- individuals.

Baseline and TB antigen stimulated type 1, type 17, regulatory, and IL-1 family or pro-inflammatory cytokines clearly distinguish LNTB+ from LNTB- individuals. To understand the discriminatory power of different cytokines which distinguish LNTB+ from LNTB- individuals, we performed ROC analysis of type 1 (IFN γ , TNF α , and IL-2), type 17 (IL-17A, IL-17F, and IL-22), type 2 (IL-4, IL-5, and IL-13), regulatory (IL-10 and TGF β), IL-1 family, and pro-inflammatory cytokines (IL-1 α , IL-1 β , IL-18, and GM-CSF) in UNS, different TB (PPD, ESAT-6 PP, and CFP-10 PP) or non-TB (positive P/I or negative, HIV Gag PP) specific antigen stimulation (Supplemental Figure 2A–E). In UNS, IL-4, IL-13, GM-CSF, and IL-10 exhibited significant discriminatory power along with high area under the curve values, sensitivity, and specificity (Supplemental Figure 2A). Similarly, in different antigenic (PPD, ESAT-6 PP, and CFP-10 PP) stimulations, IFN γ , TNF α , IL-2, IL-17F (not for CFP-10 PP), IL-22 IL-1 α , GM-CSF, and TGF β were shown to exhibit clear discrimination and significance (Supplemental Figure 2B–D). However, in the HIV GAG PP stimulation, only IL-4, IL-13, and IL-1 α were found to have potential discrimination (Supplemental Figure 2E). Hence, we demonstrate that type 1/type 17 along with IL-1 family or pro-inflammatory (IL-1 α and GM-CSF) and regulatory (TGF β) cytokines could potentially serve as discriminatory markers between the LNTB+ and LNTB- infection.

DISCUSSION

It is postulated that type 1, type 17, and pro-inflammatory cytokines are protective against TB infection and disease.^{15,16} However, the role of these cytokines in the context of immunity to the extra-pulmonary form of TB is less studied. We have previously reported that the expansion of mono- and multi-functional Th1 and Th17 cells in response to TB antigens is associated with LNTB. Similarly, CD8⁺ T cells expressing type 1/17 cytokines were significantly enhanced in LNTB when compared with PTB.^{17,18} Furthermore, we have previously shown that LNTB was associated with significantly diminished systemic or mycobacterial antigen stimulated levels of pro-inflammatory cytokines (IL-1 β and IL-18) but not type 1/17 cytokines in comparison with LTB individuals. Similarly, we have also shown that LNTB individuals exhibit significantly elevated levels of type 1/type 17 cytokines and diminished baseline and antigen-specific levels of pro-inflammatory cytokines (IL-1 β and IL-18) in the whole blood/LN.^{19,20} Despite all these studies, the cytokine profile in the LNs of culture confirmed LNTB+ individuals in comparison to culture negative LNTB- has not been investigated. Hence, we sought to explore those cytokine levels in the site-specific infected LN samples.

Interestingly, our analysis revealed that type 1/type 17 (except IL-17A) cytokines were significantly increased in the LN supernatants of LNTB+ individuals. This observation suggests that the altered levels of cytokine production were antigen or pathogen specific because a similar trend was not observed in the UNS supernatants of the same individuals. Type 1/17 cytokines (IFN γ and TNF α or IL-17) are arguably

A Unstimulated **B PPD** **C P/I**

FIGURE 1. Baseline, purified protein derivative (PPD), and Phorbol 12-myristate 13-acetate/ionomycin (P/I) stimulated cytokine production in positive lymph node tuberculosis (LNTB+) and negative lymph node tuberculosis (LNTB-) individuals. The (A) unstimulated, (B) PPD and, (C) P/I stimulated culture supernatant levels of type 1 (interferon gamma [IFN γ], tumor necrosis factor alpha [TNF α], and interleukin [IL]-2), type 17 (IL-17, IL-17F, and IL-22), type 2 (IL-4, IL-5, and IL-13), regulatory (IL-10 and TGF β), IL-1 family (IL-1 α and IL-1 β), and other pro-inflammatory (IL-18 and GM-CSF) cytokines were measured in lymph node cell suspensions of LNTB+ and LNTB- individuals. Each circle represents a single individual, and the bars represent the geometric mean values. Net cytokine levels were calculated by subtracting the baseline levels from the antigen-induced levels for each individual. *P* values were calculated using the Mann-Whitney *U* test.

well-established surrogate markers involved in the host protection by delivering the counter mechanisms against tuberculosis pathogenesis.^{10,21} In addition, high levels of IFN γ have been correlated with disease severity, and they diminish after the completion of successful anti-TB treatment.²² Individuals with IFN γ deficiency have disseminated form of TB infections with high bacterial burden.^{23,24} On the whole, these observations are consistent with our findings where we have shown increased IFN γ expression in the LNTB+ individuals on stimulation with different Mtb antigens. Elevated IFN γ levels had been associated with increased rates of disease progression and may inversely correlate with protection. Thus, a concerted action of multiple cytokines might be necessary for protection.²⁵ Tumor necrosis factor alpha, a key type 1 cytokine, is crucial in the granuloma biogenesis. Higher TNF α levels were observed in the peripheral blood mononuclear cells (PBMCs) or whole blood culture supernatants or systemic levels of PTB or PTB diabetes mellitus comorbidity.^{26,27} Similar responses were seen in the LN culture supernatants of LNTB individuals.²⁰ It is well known that IL-2 can promote T cell-dependent immune responses, and its deficiency results in exaggerated bacterial load.⁷ Our study results demonstrate that LNTB+ individuals have enhanced IL-2 levels on Mtb antigen stimulation and, therefore, the capacity to activate antimycobacterial responses. In general, IL-2 activation results in the proliferation of NK, memory, and newly activated effector cells, which are required for the host immunity.²⁸ Elevated levels of Type 1 cytokines could indicate disease severity or reflect the presence of antigen load in LNTB+ individuals.

Like type 1 cytokines, other immune markers may have the potential ability to provide the host protection against Mtb. In vaccine-induced models, IL-17– or IL-17RA–deficient mice have diminished Th1 cytokine responses.²⁹ In HN878 Mtb infection, IL-17–deficient mice are more susceptible and have substantial defects in the localization of T cells and development of lung lymphoid follicles inside the TB granuloma.^{30,31} Our data reveal the presence of increased IL-17F in LNTB+ individuals, demonstrating that IL-17F might be correlated with the immune response against TB. Likewise, elevated IL-22 production has been reported at disease sites of active TB patients.³² This is delineated in our study as well, wherein we observe elevated IL-22 levels in LNTB+ compared with LNTB– individuals. Interleukin-1 family of cytokines is one of the most prominent factors in host defense against Mtb. A previous study suggested that IL-1–deficient mice have higher susceptibility rate when exposed to TB infection.³³ Interleukin-1 is very important for the fibroblast formation and indeed could have a role in granuloma formation.³⁴ Our data demonstrate the enhanced levels of IL-1 α on stimulation with different TB antigens in the supernatants of LNTB+ individuals. Most importantly, priming by IL-1 cytokine increases the granzyme B expression, antigen-specific cytotoxic T-cell function, and IFN γ production capacity of antigen-specific CD8⁺ T cells.³⁵ Therefore, increased IL-1 α levels could reflect the increased host immunity in TBL. Our data also reveal enhanced GM-CSF levels in response to TB antigens in LNTB+ individuals. We speculate that the increased level of this cytokine could contribute to the activation of leukocytes in LNTB disease because GM-CSF–deficient mice are highly susceptible to the infection.^{36–38} Surprisingly, unlike our previous data, two important cytokines (IL-1 β and IL-18) that had exhibited

decreased levels systemically as well as following TB antigen stimulation in blood and LN did not exhibit any significant difference between LNTB+ and LNTB– individuals.

Elevated Th2 responses play a vital function in TB susceptibility, as type 2 cytokines (IL-4, IL-5, and IL-13) can downregulate Th1 cell-mediated immunity and inhibit macrophage activation and autophagy.^{39,40} The present study did not show any differential effect of TB antigen stimulation on type 2 cytokines between LNTB+ and LNTB– individuals, making it unlikely that type 2 cytokine contribute to disease pathogenesis in LNTB. Both IL-10 and TGF β are the most important immunoregulatory cytokines capable of suppressive function. Increased IL-10 levels were reported in lungs and spleen of PTB and IL-10 acts by inhibiting Th1/Th17 immune responses and reducing the antimicrobial efficacy.⁴¹ TGF β , the other mediator of immune suppression, is expressed at high levels in TB patients in response to Mtb antigens and is associated with diminished T-cell proliferation and production.⁴² Our data reveal increased levels of IL-10 and decreased levels of TGF β in the culture supernatants of LNTB+ individuals. Thus, our data support a scenario wherein reduction in TGF β levels could support enhanced pro-inflammatory cytokine responses in the affected LNs and perhaps enhanced pathology.

Finally, ROC analysis of the data revealed the importance of a variety of cytokines in discriminating between the LNTB+ and LNTB– individuals. Thus, TNF α , IFN γ , IL-2, IL-1 α , IL-22, GM-CSF, and TGF β are the potential cytokines that could discriminate between the respective study groups either at baseline or following antigen stimulation. This could be linked to the higher frequency of Mtb antigen-specific T cells that are highly detectable in the infection site in TB-positive but not in TB-negative individuals.^{43,44}

Overall, our data suggest that the expression of type 1/17, IL-1 α , and GM-CSF cytokines in the site of infection probably indicates an association of these cytokines with the immune response in LNTB. However, our study has some limitations, including small sample size, lack of data availability on bacterial burdens, lack of non-TB LN samples, and no way to determine causality. Nevertheless, our study provides important insights into the cytokine profile at the site of infection in this understudied form of TB.

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Authors' addresses: Gokul Raj Kathamuthu, Kadar Moideen, and Subash Babu, National Institutes of Health, National Institute for Research in Tuberculosis, International Center for Excellence in Research (NIH-NIRT-ICER), Chennai, India, E-mails: gokul.r@nirt.res.in, kadar.m@nirt.res.in, and sbabu@niaid.nih.gov. Rathinam Sridhar, Government Stanley Medical Hospital, Government Hospital of Thoracic Medicine, Chennai, India, E-mail: srihema.1964@gmail.com. Dhanaraj Baskaran, National Institute for Research in Tuberculosis, Chennai, India, E-mail: baskar.d@nirt.res.in.

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