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Effect of SARS-CoV-2 seropositivity on antigen – specific cytokine and chemokine responses in latent tuberculosis

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ABSTRACT

SARS-CoV-2 and latent *Mycobacterium tuberculosis* infection are both highly co-prevalent in many parts of the globe. Whether exposure to SARS-CoV-2 influences the antigen specific immune responses in latent tuberculosis has not been investigated. We examined the baseline, mycobacterial antigen and mitogen induced cytokine and chemokine responses in latent tuberculosis (LTBI) individuals with or without SARS-CoV-2 seropositivity, LTBI negative individuals with SARS-CoV-2 seropositivity and healthy control (both LTBI and SARS-CoV-2 negative) individuals. Our results demonstrated that LTBI individuals with SARS-CoV-2 seropositivity (LTBI+/IgG +) were associated with increased levels of unstimulated and TB-antigen stimulated IFNγ, IL-2, TNFα, IL-17, IL-1β, IL-6, IL-12, IL-4, CXCL1, CXCL9 and CXCL10 when compared to those without seropositivity (LTBI+/IgG-). In contrast, LTBI+/IgG+ individuals were associated with decreased levels of IL-5 and IL-10. No significant difference in the levels of cytokines/chemokines was observed upon mitogen stimulation between the groups. SARS-CoV-2 seropositivity was associated with enhanced unstimulated and TB-antigen stimulated but not mitogen stimulated production of cytokines and chemokines in LTBI+ compared to LTBI negative individuals. Finally, most of these significant differences were not observed when LTBI negative individuals with SARS-CoV-2 seropositivity and controls were examined. Our data clearly demonstrate that both baseline and TB – antigen induced cytokine responses are augmented in the presence of SARS-CoV-2 seropositivity, suggesting an augmenting effect of prior SARS-CoV-2 infection on the immune responses of LTBI individuals.

1. Introduction

Tuberculosis (TB) remains of great public health significance in India, which accounts for a quarter of the 10 million global TB cases and 1.4 million TB deaths every year [1]. It is estimated that a quarter of the globe's population is affected by *Mycobacterium tuberculosis*. Only 5–15% of these people will develop active TB disease [1]. In India, more than one third of the population has latent TB infection (LTBI) [1]. COVID-19 is caused by SARS-CoV-2, the pandemic currently raging worldwide. As of February 24, 2021, 111.42 million people have been infected with SARS-CoV-2 and 2.47 million people have died [2]. Approximately 85% of infections are asymptomatic or mild and do not require any major intervention [3]. T TB and COVID-19 are both infectious diseases that attack mainly the lungs. Both diseases have similar symptoms such as cough, fever and difficulty breathing [4].

Recent studies have shown that SARS-CoV-2 infection elicits increased cytokine responses, including interleukin-1 (IL-1β), interferon- (IFN-γ), tumour necrosis factor- (TNF-α), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-10 (IL-10), which ultimately leads to severe inflammation and severe disease [5–7]. A recent study has indicated that individuals with latent TB are more susceptible for acquiring SARS-CoV-2 infection and develop more expeditious symptoms of severe

Abbreviations: LTBI, Latent tuberculosis infection; IL-1β, Interleukin-1β; IFN-γ, Interferon-γ; TNF-α, Tumour necrosis factor-α; IL-2, Interleukin-2; IL-4, Interleukin-4; IL-10, Interleukin-10.

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COVID-19 disease [8]. The relationship between TB and COVID-19 is presumably two way. TB which causes momentary immunosuppression, which could enhance the vulnerability of TB patients to COVID-19, and COVID could, consecutively, enhance vulnerability to TB [4]. Both $CD4^+$ and $CD8^+$ counts were markedly decreased, and the surviving T cells appeared to demonstrate "functional exhaustion" in COVID-19. This T cell depletion and dysfunction may promote the development of active TB in patients with LTBI [4]. However, there is a paucity of information on SARS-CoV-2 with latent TB coinfection.

We hypothesised that altered immunity due to seropositive SARS-CoV-2 infection could lead to altered immune responses in LTBI individuals. Herein, we examined the levels of cytokines and chemokines following baseline, TB antigen or mitogen stimulation in LTBI individuals with or without SARS-CoV-2 seropositivity, as well as in seropositive SARS-CoV-2 individuals with or without LTBI. Our data clearly demonstrate that seropositive SARS-CoV-2 infection modulates immune responses in LTBI.

2. Methods

2.1. Ethics statement

The study was approved by the Ethics Committees of NIRT (NIRT-INo: 2020010). Informed written consent was obtained from all participants. The study is part of the clinical study entitled, Study to evaluate the effectiveness of the BCG vaccine in reducing morbidity and mortality in elderly individuals in COVID-19 hotspots in India (NCT04475302).

2.2. Study population

Elderly persons, aged between 60 and 80 years, living in hotspots for SARS-Cov2 infection were recruited in the study period from July 2020 and September 2020 in Chennai, India. Informed written consent was obtained from the study participants. Individuals diagnosed as tuberculosis (TB) 6-months prior or were presently on anti-TB treatment were excluded from the study. We studied a group of 35 individuals with LTBI (confirmed by IGRA) and seropositive SARS-CoV-2 infection confirmed by SARS-CoV-2 specific IgG antibody (hereafter LTBI+/IgG +), 24 with LTBI without seropositive SARS-CoV-2 infection (negative for IgG and RT-PCR for SARS-CoV-2) (hereafter LTBI+/IgG-). As a control group, we also enrolled 39 individuals negative for LTBI and seropositive (hereafter LTBI-/IgG +) and 20 individuals negative for LTBI and seronegative and PCR negative (hereafter LTBI-/IgG-). The recruited individuals were negative for HIV or malignancy. Hematology was done using Act-5 Diff hematology analyzer (Beckman Coulter) for all study participants. Demographic details and hematology details were shown in Table.1. LTBI was diagnosed based on positive QuantiFERON TB Gold in tube (QGIT) test, with no signs and symptoms for active TB, any prior history of TB and normal chest radiographs. QGIT was done in accordance with manufacturer's instructions (Qiagen).

2.3. Multiplex analysis on QGIT supernatants

Whole blood obtained from all the above mentioned 4 groups of individuals was incubated in vitro with media alone (unstimulated) or a cocktail of TB antigens (ESAT-6, CFP-10, TB 7.7 [TB Ag]) or mitogen (PHA) for 18 hrs., according to the manufacturer's instructions using the QGT kit (Qiagen). The baseline (unstimulated) or TB antigen or mitogen stimulated whole blood supernatants were then used to analyse the levels of array of cytokines and chemokine panel using a Human magnetic Luminex Assay Kit from R&D Systems. The cytokine parameters analysed were IFNγ, 5.7 pg/ml; IL-2, 3.6 pg/ml; TNFα, 12.4 pg/ml; IL-1α, 10.6 pg/ml; IL-1β, 3.5 pg/ml; IL-17A, 9 pg/ml; IL-6, 9.0 pg/ml; IL-12, 18.5 pg/ml; IL-4, 1.1 pg/ml; IL-5, 6.2 pg/ml; IL-13, 31.8 pg/ml and IL-10, 32.2 pg/ml. The following chemokines were measured in all the 4 group of individuals. CCL1, 1.4 pg/ml; CCL2, 5.9 pg/ml; CCL2, 5.9 pg/ml; CCL4, 103.8 pg/ml; CCL11, 21.6 pg/ml; CXCL1, 19.1 pg/ml; CXCL2, 21.1 pg/ml; CXCL9, 11.7 pg/ml; CXCL10, 2.6 pg/ml and CXCL11 21.6 pg/ml. The net cytokine and chemokine levels were calculated by TB antigen stimulation or mitogen minus unstimulated levels.

Table 1
Demographic and Hematology of the study population.

	LTB+/IgG+	LTB+/IgG-	LTB-/IgG+	LTB-/IgG-
Subjects Enrolled	n = 35	n = 24	n = 39	n = 20
Age in Years (Median)	64 (60–78)	65 (60–78)	63 (60–80)	66 (60–79)
Gender (M/F)	19/16	11/13	17/12	12/8
Height (Median)	156 cm	155 cm	159 cm	157 cm
Weight (Median)	55 Kg	63 Kg	63 Kg	62 Kg
Pulse rate (Median)	88	88	89	86
Systolic Blood Pressure (Median)	137	138	131	115
Diastolic Blood Pressure (Median)	80	80	80	78
SPOS% (Median)	95	98	97	98
RBC, g/dL	4.8 (3.9–6.5)	4.9 (3.49–6.06)	4.8 (3.56–8.03)	4.6 (3.8–6.04)
Hb, g/dL	13.9 (9.2–17.8)	13.5 (7.5–18.5)	14.28 (10–21.8)	12.11 (9.1–14.6)
Hematocrit, %	41.4 (6.2–48)	39.2 (22.3–53)	36.54 (26.94–45)	37.46 (23.2–51.5)
Platelet, 10^3/uL	241 (141–427)	268.0 (168–455)	324.72 (171–649)	333.50 (126–671)
WBC 10^3/mL	12.14 (7.1–21.2)	11.52 (7.2–19.1)	9.6 (5.3–12.9)	10.37 (6.9–15.4)
Neutrophil 10^3/mL	8.34 (4.8–15.05)	8.24 (4.8–15.05)	9.1 (4.2–11.3)	7.12 (4.44–12.28)
Lymphocyte 10^3/mL	3.47 (0.94–4.1)	3.55 (0.94–4.31)	4.55 (0.94–4.51)	2.01 (1.15–3.53)
Monocyte 10^3/mL	1.01 (0.45–1.23)	0.92 (0.58–1.9)	0.96 (0.28–1.2)	0.72 (0.22–1.32)
Eosinophil 10^3/mL	0.71 (0.22–3.44)	0.42 (0.15–1.4)	0.63 (0.21–3.21)	0.31 (0.07–1.95)
Basophil 10^3/mL	0.08 (0.01–0.22)	0.07 (0.03–0.15)	0.09 (0.01–0.22)	0.02 (0.01–0.15)

2.4. Statistical analysis

Central tendency were measured using Geometric means. Statistically significant differences between the groups LTBI+/IgG+, LTBI+/IgG-, LTBI-/IgG+ and LTBI-/IgG-.were analysed by means of Kruskal-Wallis test with Dunn's multiple comparisons. Geomean values were shown in Sup.Table.1. GraphPad PRISM version 9 (GraphPad Software, Inc., San Diego, CA, USA) was used for the data analysis.

3. Results

3.1. LTBI+/IgG-+ individuals are associated with elevated baseline or unstimulated levels of pro-inflammatory cytokines and altered levels of anti-inflammatory cytokines

We wanted to determine the influence of seropositive SARS-CoV-2 infection on baseline levels of pro and anti-inflammatory cytokines in LTBI individuals. As shown in Fig. 1A, LTBI+/IgG+ individuals exhibited elevated baseline or unstimulated levels of pro-inflammatory cytokines IFNγ, IL-2, TNFα, IL-17A, GM-CSF, IL-1α,IL-1β, IL-6 and IL-12 in comparison to LTBI+/IgG- individuals. In contrast, LTBI+/IgG+ individuals exhibited diminished baseline levels of the anti-inflammatory cytokine,IL-10 in comparison to LTBI+/IgG- individuals.

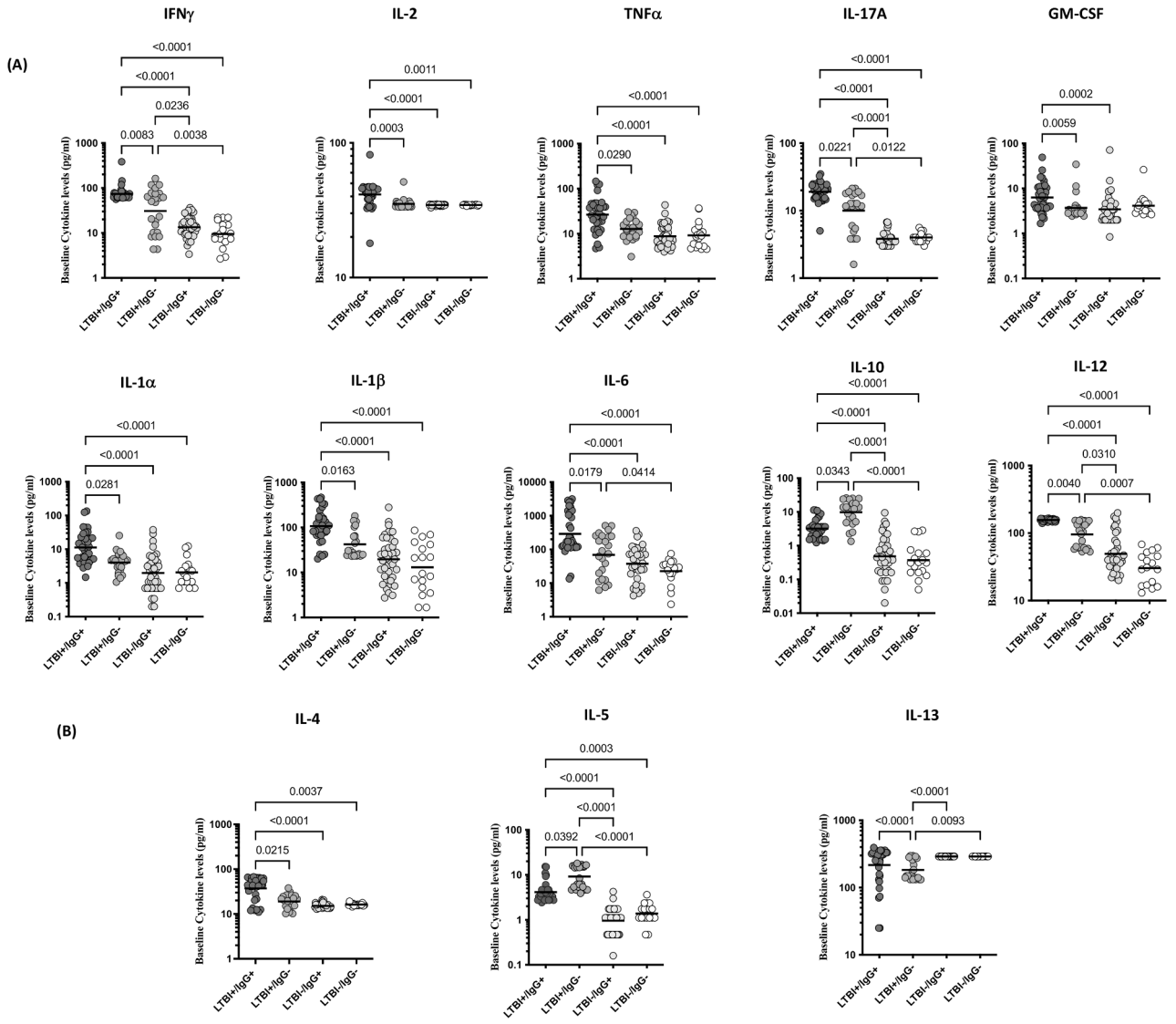

Fig. 1. LTBI+/IgG+ individuals are associated with elevated baseline or unstimulated levels of pro-inflammatory cytokines and altered levels of anti-inflammatory cytokines. (A) The plasma levels of pro-inflammatory cytokines were measured in LTBI+/IgG+ (n = 35) and LTBI+/IgG- (n = 24) individuals, LTBI-/IgG+ (n = 24) and LTBI-/IgG- (n = 20) individuals at unstimulated or baseline. (B) The plasma levels of anti- inflammatory cytokines were measured in LTBI+/IgG+ (n = 35) and LTBI+/IgG- (n = 24) individuals, LTBI-/IgG+ (n = 24) and LTBI-/IgG- (n = 20) individuals at unstimulated or baseline. p values were calculated using the Kruskal-Wallis test with Dunn's post-hoc for multiple comparisons.

As shown in Fig. 1B, LTBI+/IgG+ individuals exhibited elevated baseline or unstimulated levels of anti-inflammatory cytokines IL-4 and IL-13 in comparison to LTBI+/IgG- individuals. In contrast, LTBI+/IgG+ individuals exhibited diminished baseline levels of anti-inflammatory cytokine IL-5 in comparison to LTBI+/IgG- individuals.

As shown in Fig. 1A, LTBI+/IgG+ individuals exhibited elevated baseline or unstimulated levels of pro-inflammatory cytokines IFNγ, IL-2, TNFα, IL-17A GM-CSF, IL-1α, IL-1β IL-6, IL-10 and IL-12 in comparison with LTBI-/IgG+ individuals. Similarly, as shown in Fig. 1B, LTBI+/IgG+ individuals exhibited elevated baseline or unstimulated levels of anti-inflammatory cytokines IL-4 and IL-5 in comparison to LTBI-/IgG+ individuals. Those who are LTBI-/IgG- individuals exhibited diminished levels of pro and anti-inflammatory cytokines in comparison with other 3 groups of individuals. Thus, LTBI with seropositive SARS-CoV-2 infection is associated with increased levels of most cytokines at baseline.

3.2. LTBI+/IgG+ individuals are associated with elevated TB- antigen stimulated levels of pro-inflammatory cytokines and altered levels of anti-inflammatory cytokines

We wanted to determine the influence of seropositive SARS-CoV-2 infection on TB-antigen stimulated levels of pro and anti-inflammatory cytokines in LTBI individuals. As shown in Fig. 2A, LTBI+/IgG+ individuals exhibited elevated TB-antigen stimulated levels of pro-inflammatory cytokines IFNγ, IL-2, TNFα, IL-17A, IL-1β, IL-6 and IL-12 in comparison to LTBI+/IgG- individuals. In contrast, LTBI+/IgG + individuals exhibited diminished TB-antigen stimulated levels of IL-10 in comparison to LTBI+/IgG- individuals. As shown in Fig. 2B, LTBI+/IgG+ individuals exhibited elevated TB-antigen stimulated levels of anti-inflammatory cytokines IL-4 and IL-13 in comparison to LTBI+/IgG- individuals. In contrast, LTBI+/IgG+ individuals exhibited diminished TB-antigen stimulated levels of anti-inflammatory cytokine IL-5 in comparison with LTBI+/IgG- individuals.

As shown in Fig. 2A, LTBI+/IgG+ individuals exhibited elevated TB-antigen stimulated levels of pro-inflammatory cytokines IFNγ, IL-2,

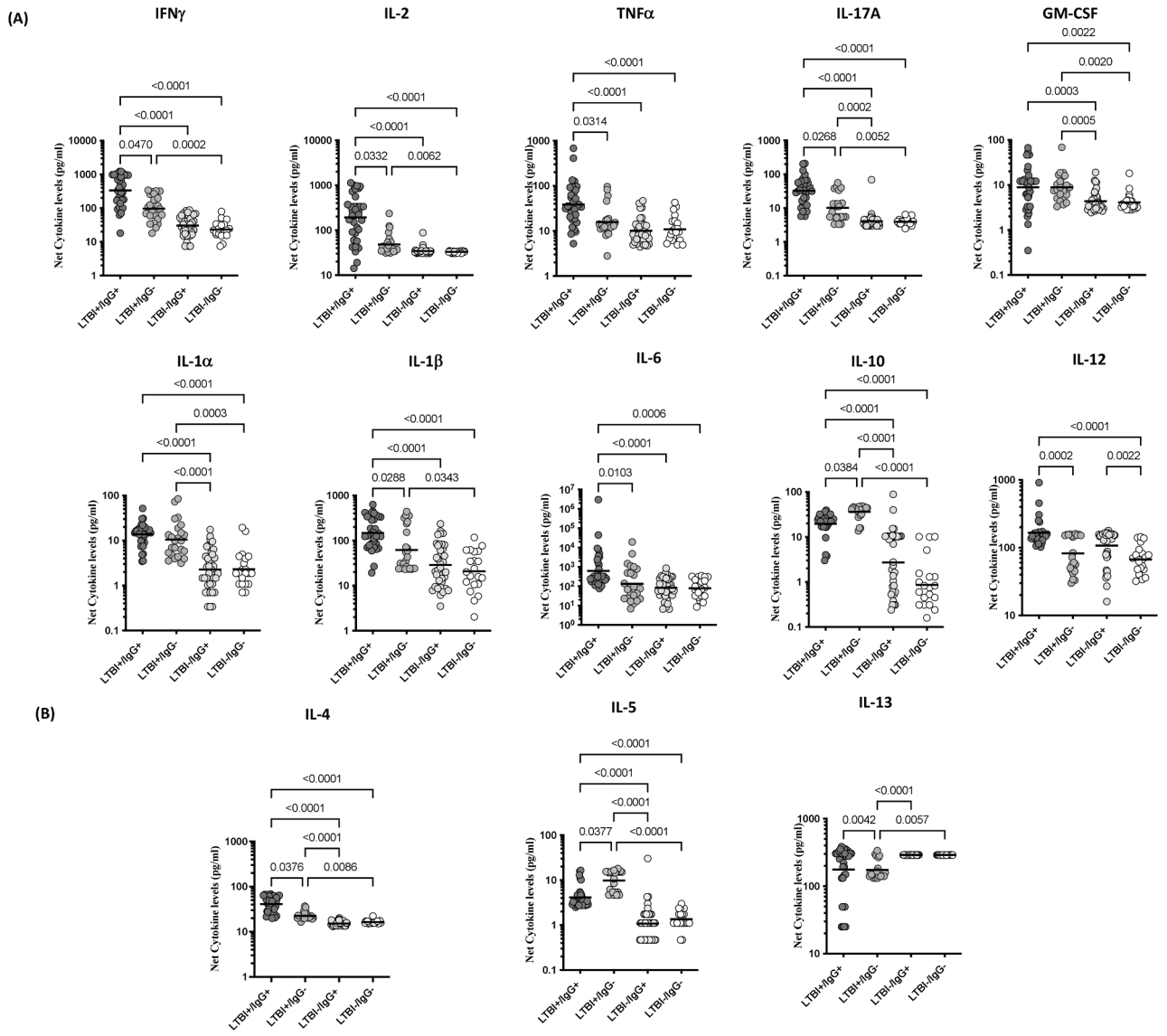

Fig. 2. LTBI+/IgG+ individuals are associated with elevated TB antigen stimulated levels of pro-inflammatory cytokines and altered levels of anti-inflammatory cytokines. (A) The plasma levels of pro-inflammatory cytokines were measured in LTBI+/IgG+ (n = 35) and LTBI+/IgG- (n = 24) individuals, LTBI-/IgG+ (n = 24) and LTBI-/IgG- (n = 20) individuals upon TB antigen stimulation. (B) The plasma levels of anti- inflammatory cytokines were measured in LTBI+/IgG+ (n = 35) and LTBI+/IgG- (n = 24) individuals, LTBI-/IgG +(n = 24) and LTBI-/IgG- (n = 20) individuals upon TB antigen stimulation. The data are represented as scatter plots with each circle representing a single individual. p values were calculated using the Kruskal-Wallis test with Dunn's post-hoc for multiple comparisons.

TNFα, IL-17A, GM-CSF, IL-1α, IL-1β, IL-6, IL-10 in comparison to LTBI-/IgG+ individuals. Similarly, as shown in Fig. 2B, LTBI+/IgG+ individuals exhibited elevated TB-antigen stimulated levels of anti-inflammatory cytokines IL-4 and IL-5 in comparison to LTBI-/IgG+ individuals. Those who are LTBI-/IgG- individuals exhibited diminished levels of pro and anti-inflammatory cytokines in comparison with other 3 groups of individuals. Thus, LTBI with seropositive SARS-CoV-2 infection is associated with increased levels of most pro and anti-inflammatory cytokines upon TB antigenic stimulation.

3.3. LTBI+/IgG+ individuals are associated with no significant differences in mitogen stimulated levels of pro-inflammatory and anti-inflammatory cytokines

We wanted to determine the influence of seropositive SARS-CoV-2 infection on mitogen stimulated levels of pro and anti- inflammatory cytokines in LTBI individuals. As shown in Fig. 3A and 3B, LTBI+/IgG+ individuals exhibited no significant differences in mitogen stimulated pro and anti-inflammatory cytokine levels in comparison with other 3 groups of individuals. Thus, LTBI with seropositive SARS-CoV-2 infection is associated with no significant differences in pro and anti-inflammatory cytokines upon mitogen stimulation.

3.4. LTBI+/IgG+ individuals are associated with elevated baseline or unstimulated levels of chemokines

We wanted to determine the influence of seropositive SARS-CoV-2 infection on baseline levels of chemokines in LTBI individuals. As shown in Fig. 4, LTBI+/IgG+ individuals exhibited elevated baseline or unstimulated levels of CXCL1, CXCL9 and CXCL10 in comparison to LTBI+/IgG- individuals. LTBI-/IgG+ individuals exhibited elevated baseline or unstimulated levels of CC chemokines CCL1, CCL3, CCL4, CCL11 and CXC chemokines CXCL1, CXCL10 and CXCL11 in comparison to LTBI-/IgG+ individuals. Those who are LTBI-/IgG- individuals

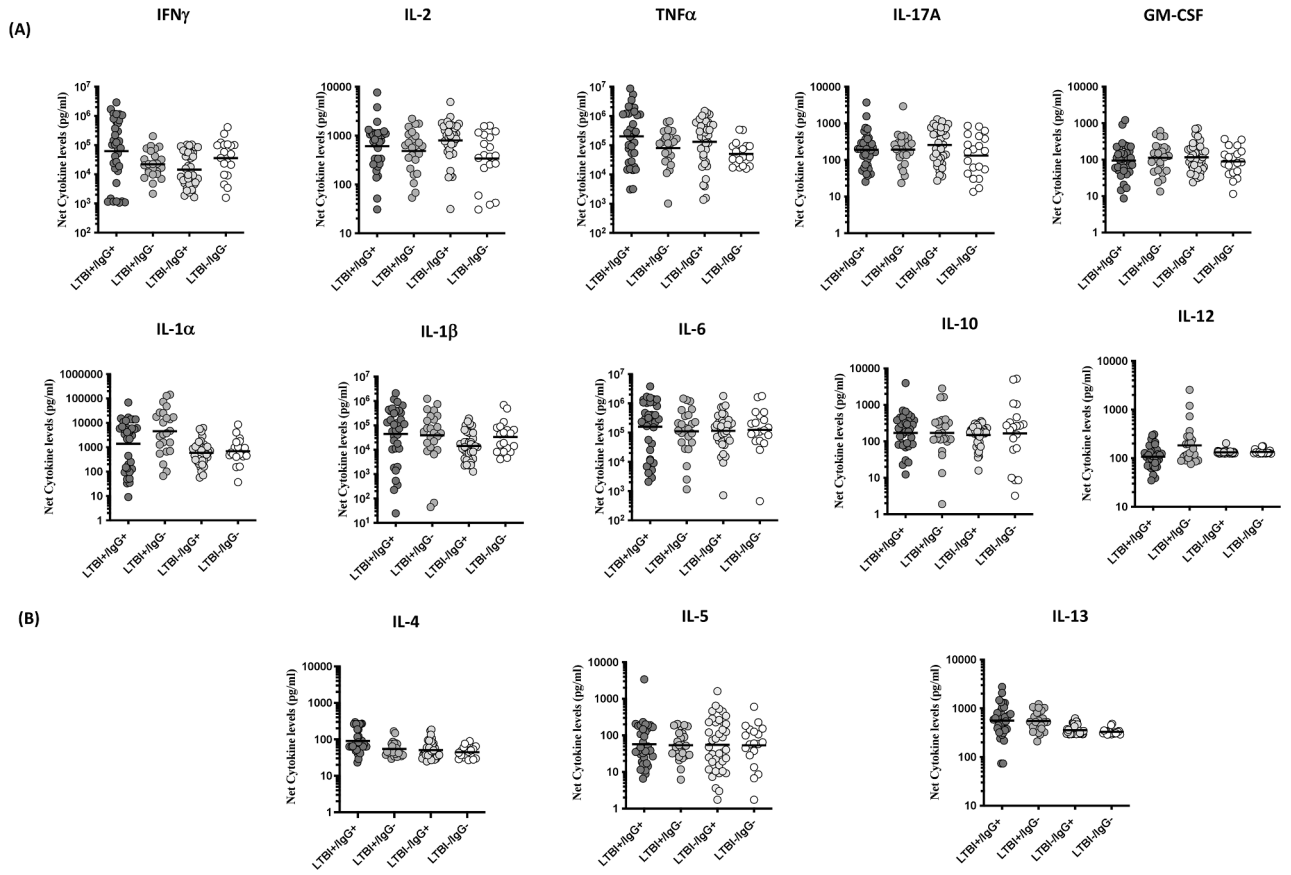

Fig. 3. LTBI+/IgG+ individuals exhibit no significant differences in mitogen stimulated levels of pro and anti-inflammatory cytokines. (A) The plasma levels of pro-inflammatory cytokines were measured in LTBI+/IgG+ (n = 35) and LTBI+/IgG- (n =24) individuals, LTBI-/IgG+(n = 24) and LTBI-/IgG- (n = 20) individuals upon mitogen stimulation. (B) The plasma levels of anti- inflammatory cytokines were measured in LTBI+/IgG+ (n = 35) and LTBI+/IgG- (n = 24) individuals, LTBI-/IgG+(n = 24) and LTBI-/IgG- (n = 20) individuals upon mitogen stimulation. The data are represented as scatter plots with each circle representing a single individual. p values were calculated using the Kruskal-Wallis test with Dunn's post-hoc for multiple comparisons.

exhibited diminished levels of pro and anti-inflammatory cytokines in comparison with other 3 groups of individuals. Thus, LTBI with seropositive SARS-CoV-2 infection is associated with increased levels of most of the chemokines at baseline.

3.5. LTBI+/IgG+ individuals are associated with elevated TB-antigen stimulated levels of chemokines

We wanted to determine the influence of seropositive SARS-CoV-2 infection upon TB antigen stimulated levels of chemokines in LTBI individuals. As shown in Fig. 5, LTBI+/IgG+ individuals exhibited elevated TB-antigen stimulated levels of CCL1, CXCL1, CXCL9 and CXCL10 in comparison to LTBI+/IgG- individuals. LTBI+/IgG+ individuals exhibited elevated TB antigen stimulated levels of CC chemokines CCL1, CCL11 and CXC chemokines CXCL1, CXCL9, CXCL10 and CXCL11 in comparison to LTBI-/IgG+ individuals. Those who are LTBI-/IgG- individuals exhibited diminished levels of pro and anti-inflammatory cytokines in comparison with other 3 groups of individuals. Thus, LTBI with seropositive SARS-CoV-2 infection is associated with increased levels of most of the chemokines upon TB-antigen stimulation..

3.6. LTBI+/IgG+ individuals are associated with no significant differences in mitogen stimulated levels of chemokines

We wanted to determine the influence of seropositive SARS-CoV-2 infection on mitogen stimulated levels of CC and CXC chemokines in LTBI individuals. As shown in Fig. 6, LTBI+/IgG+ individuals exhibited no significant differences in mitogen stimulated chemokine levels in comparison with other 3 groups of individuals. Thus, LTBI with seropositive SARS-CoV-2 infection is associated with no significant differences in chemokines upon mitogen stimulation.

4. Discussion

Cytokines and chemokines play an important role in host resistance to TB infection [9]. In addition, cytokines and chemokines are crucial players in COVID-19 and their plasma levels are linked with disease severity [3]. A recent clinical report revealed that $CD4^+$ T-cell deficiency associated with COVID-19 could lead latent infection progress to active tuberculosis [10]. A study from China, reported that COVID-19 patients had significant diminution of T-cell lymphocyte counts [11] and $CD4^+$ and $CD8^+$ counts were drastically decreased [12]. This T cell depletion and dysfunction may promote the development of active TB from LTBI [10]. Co-infection of SARS-CoV-2 and *M. tuberculosis* could aggravate or reactivate the disease that are related with the respective pathogens [10,13]. The coinfection in macrophages may enhance the secretion of pro- and anti-inflammatory cytokines as well as chemokines and hence act as a key player in pathogenesis [14]. *M. tuberculosis* infection induces elevated cytokine and chemokine levels during active disease pathogenesis [15]. Hence, co-infections of SARS-CoV-2 with *M. tuberculosis* may induce higher levels of cytokine and chemokine secretion which could subsequently re-activate the LTB infection to active TB.

Our data reveal three salient features of seropositive SARS-CoV-2/LTBI coinfection. First, seropositive SARS-CoV-2 infection enhances the baseline and TB-antigen induced production of cytokines and

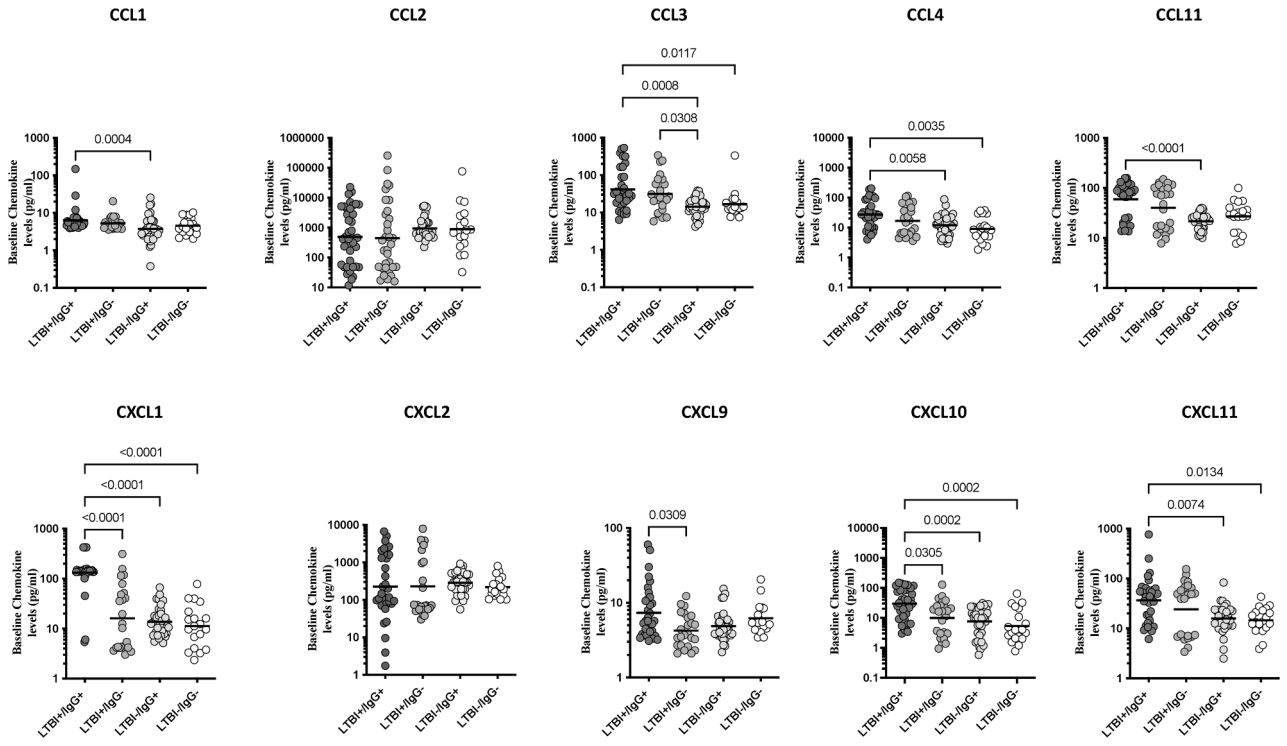

Fig. 4. LTBI+/IgG+ individuals are associated with elevated baseline or unstimulated levels of chemokines. The plasma levels of CC and CXC chemokines were measured in LTBI+/IgG+ (n = 35) and LTBI+/IgG- (n = 24) individuals, LTBI-/IgG +(n = 24) and LTBI-/IgG- (n = 20) individuals at unstimulated or baseline. The data are represented as scatter plots with each circle representing a single individual. p values were calculated using the Kruskal-Wallis test with Dunn's post-hoc for multiple comparisons.

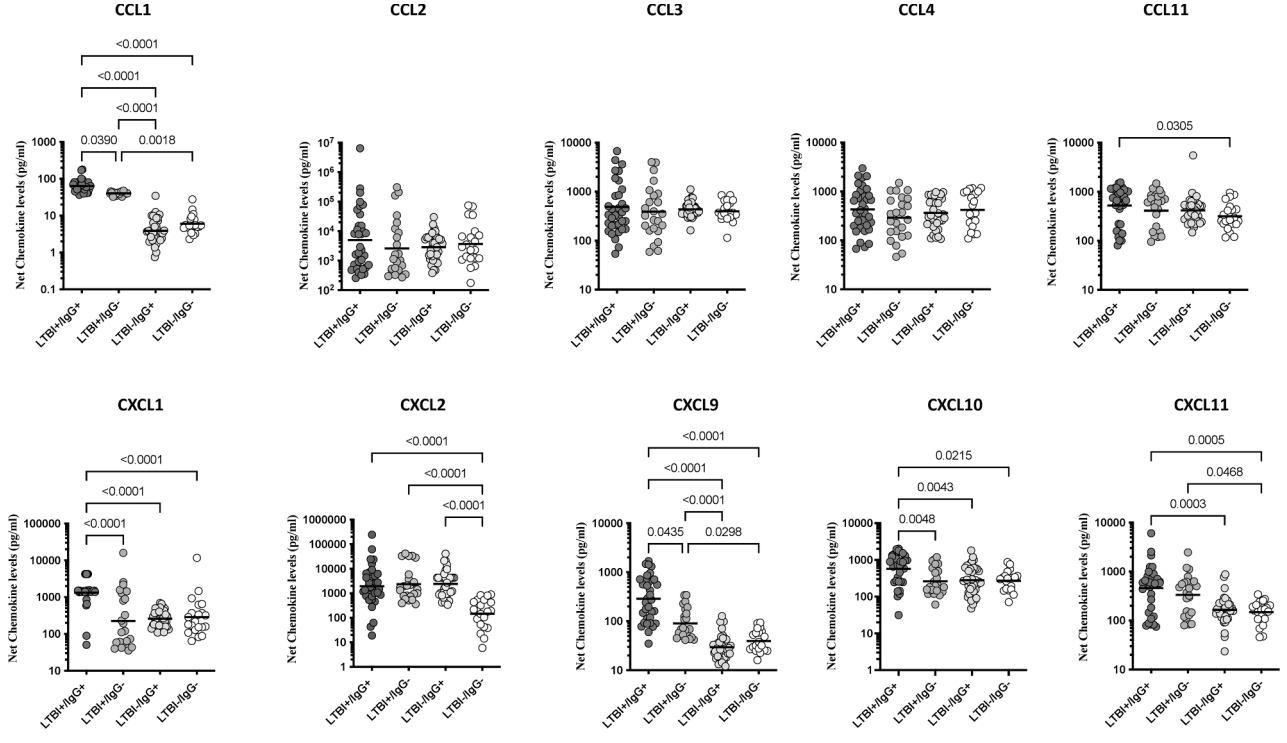

Fig. 5. LTBI+/IgG+ individuals are associated with elevated upon TB antigen stimulated levels of chemokines. The plasma levels of CC and CXC chemokines were measured in LTBI+/IgG+ (n = 35) and LTBI+/IgG- (n = 24) individuals, LTBI-/IgG+ (n = 24) and LTBI-/IgG- (n = 20) individuals upon TB antigen stimulation. The data are represented as scatter plots with each circle representing a single individual. p values were calculated using the Kruskal-Wallis test with Dunn's post-hoc for multiple comparisons.

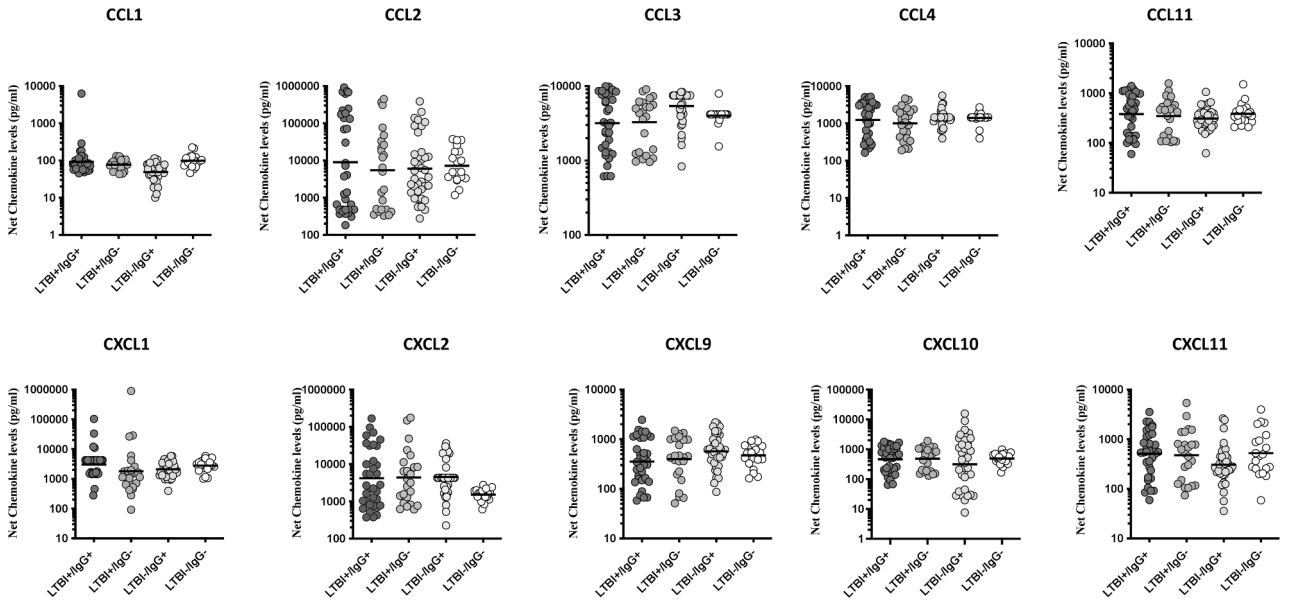

Fig. 6. IgG+/LTBI + individuals exhibit no significant differences in mitogen stimulated levels of chemokines. The plasma levels of CC and CXC chemokines were measured in LTBI+/IgG+ (n = 35) and LTBI+/IgG- (n = 24) individuals, LTBI-/IgG+ (n = 24) and LTBI-/IgG- (n = 20) individuals upon mitogen stimulation. The data are represented as scatter plots with each circle representing a single individual. p values were calculated using the Kruskal-Wallis test with Dunn's post-hoc for multiple comparisons.

chemokines typically associated with not only resistance to development of active TB disease (such as IFNγ, IL-2, TNFα, IL-17, IL-1β, IL-6, IL-12, CCL1, CXCL1, CXCL9 and CXCL10) but also cytokines and chemokines associated with susceptibility to active disease (such as IL-4, IL-13 and IL-10). Second, mitogen stimulation did not demonstrate these significant differences indicating that there is no significant intrinsic deficit in the ability of whole blood cells to produce cytokines/chemokines in these two groups of individuals. Finally, this induction of the immune response is dependent on LTBI as LTBI negative individuals failed to exhibit any significant difference in cytokine/chemokine production at baseline or following TB-antigen or mitogen stimulation. Our study also examined the role of seropositive SARS-CoV-2 infection in modulating the cytokine/ chemokine responses of LTBI individuals in comparison to healthy control (LTBI negative and SARS-CoV-2 negative) individuals. Our data clearly reveal that there is no compromise in the ability of LTBI individuals to mount Type 1, Type 17, pro-inflammatory and other cytokines responses at baseline or following TB antigen-stimulation. Our data also reveals that the LTBI individuals are not compromised in their ability to mount enhanced chemokine responses in comparison to healthy control individuals. Thus, SARS-CoV-2 seropositivity does not affect the cytokine and chemokine responses of LTBI individuals.

Thus, our study reveals that LTBI individuals with seropositive SARS-CoV-2 infection are not compromised in their ability to mount cytokine and chemokine response and if anything, mount even stronger responses. The main limitation of the study is that it is cross-sectional study and cannot infer causal relationship. Nevertheless, our study is strengthened by the inclusion of different groups of LTBI+/- and SARS-CoV-2 IgG+/- individuals in a sufficiently large sample size and the exhaustive list of cytokines and chemokines examined.

5. Conclusion

Our data suggest that COVID-19 can modulate the levels of cytokines and chemokines at baseline and upon TB antigenic stimulation, which has an impact on immunological function of LTBI. Whether this translates to clinical influences on active TB development remains to be determined.

Author Contributions

Designed the study (S.B., C.P); conducted experiments (N.P.K., R.A., A.N., N.S., R.M.R, V.V); acquired data (R.A., N.P.K.); analyzed data (R. A., N.P.K.); contributed reagents and also revised subsequent drafts of the manuscript (S.B., C.P.); responsible for the enrolment of the participants and also contributed to acquisition and interpretation of clinical data (C.P.); wrote the manuscript (R.A., S.B.). All authors read and approved the final manuscript.

CRediT authorship contribution statement

Anuradha Rajamanickam: Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization, Supervision, Writing – review & editing. **Nathella Pavan Kumar:** Methodology, Formal analysis, Investigation, Data curation, Visualization, Supervision. **Padmapriyadarsini Chandrasekaran:** Conceptualization, Resources, Writing – review & editing, Project administration, Funding acquisition. **Arul Nancy:** Investigation. **P.K. Bhavani:** . **Nandhini Selvaraj:** Investigation. **Kushiyasri Karunanithi:** . **Saravanan Munisankar:** . **R. Srinivasan:** . **Rachel Mariam Renji:** Investigation. **Shanmuga Priya kumaravadivelu:** . **Vijayalakshmi Venkatramani:** Investigation. **Subash Babu:** Conceptualization, Validation, Resources, Writing – original draft, Visualization, Writing – review & editing, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

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