Tuberculosis 94 (2014) 482–493

Contents lists available at ScienceDirect

Tuberculosis

journal homepage: http://intl.elsevierhealth.com/journals/tube

IMMUNOLOGICAL ASPECTS

Altered CD8$^+$ T cell frequency and function in tuberculous lymphadenitis

Nathella Pavan Kumar [a, b, *], Rathinam Sridhar [c], Luke Elizabeth Hanna [b], Vaithilingam V. Banurekha [b], Mohideen S. Jawahar [b], Thomas B. Nutman [d], Subash Babu [a, d]

[a] National Institutes of Health—International Center for Excellence in Research, Chennai, India
[b] National Institute for Research in Tuberculosis, Chennai, India
[c] Government Stanley Medical Hospital, Chennai, India
[d] Laboratory of Parasitic Diseases, National Institutes of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA

ARTICLE INFO

Article history:
Received 21 February 2014
Received in revised form
27 May 2014
Accepted 15 June 2014

Keywords:
Tuberculosis
Lymphadenitis
CD8 T cells
Cytokines
Cytotoxic molecules

SUMMARY

CD8$^+$ T cells secreting Type1 and Type 17 cytokines and cytotoxic molecules play a major role in immunity and protection against pulmonary tuberculosis (PTB), although their role in tuberculous lymphadenitis (TBL) is not well known. To identify the distribution and function of CD8$^+$ T cells expressing Type1, Type 2 and Type 17 cytokines and cytotoxic molecules in TBL, we examined baseline and mycobacterial–antigen specific immune responses in the whole blood of individuals with PTB and compared them with TBL. TBL is characterized by elevated frequencies of baseline and mycobacterial-antigen stimulated CD8$^+$ T cells expressing Type 1 (IL-2 and TNFα) and Type 17 (IL-17A and IL-17F) cytokines in comparison to PTB individuals. In contrast, TBL individuals exhibited diminished frequency of CD8$^+$ T cells expressing perforin, granzyme B and CD107a. The blockade of IL-1R and IL-6R during antigenic stimulation resulted in significantly diminished frequencies of CD8$^+$ T cells expressing Type 1 and Type 17 cytokines in TBL. Therefore, our data suggest that TBL is characterized by an IL-1 and IL-6 dependent expansion of CD8$^+$ T cells expressing Type 1 and Type 17 cytokines as well as altered frequencies of cytotoxic molecules, reflecting an important association of these cells with the pathogenesis of TBL.

1. Introduction

Tuberculosis (TB) remains a huge public health problem with nearly 2 billion infections worldwide. 90% of infected individuals are able to resist overt disease (active TB) development and manifest only latent infection [1]. Although the lung is the most common site for *Mycobacterium tuberculosis* (Mtb) infection, dissemination of mycobacteria to other organs can occur when alveolar macrophages become infected with bacteria. The subsequent migration of activated macrophages to secondary lymphoid tissue for antigen presentation to CD4$^+$ helper T cells can facilitate spread of mycobacteria, yet the exact mechanism as to how Mtb leaves the lungs is poorly understood. Tuberculous lymphadenitis (TBL) is a common clinical presentation of extra-pulmonary tuberculosis and it is a local manifestation of the systemic disease [2].

T cell subsets can be differentiated into Th1, Th2, Th17 and other cytokine producing subsets. Both Th1 and Th17 cells have been implicated in the host defense against Mtb, largely by inducing macrophage activation and favoring the control of Mtb replication [3–5]. CD8$^+$ T cells are also major producers of Type 1 cytokines and have been shown to recognize and lyse Mtb infected macrophages [6,7]. Cytotoxic T lymphocytes play a central role in the immune system by eliminating various infected cells with the help of cytotoxic molecules [8]. CD8$^+$ T cells expressing perforin have been shown to mediate protection against Mtb infection in mice [9] and humans [10,11]. However, the role of other cytotoxic molecules, such as granzyme B and CD107a have not been explored in detail.

We have previously shown that TBL is characterized by an antigen – specific expansion of CD4$^+$ Th1 and Th17 cells [12]. Since TBL is felt to reflect a hematogenous disseminated form of TB, we postulated that CD8$^+$ T cells might also play a different role in TBL compared to PTB. To this end, we examined the frequencies of CD8$^+$

* Corresponding author. NIH-ICER, National Institute for Research in Tuberculosis, Chennai, India. Tel.: +91 44 28369766.
E-mail addresses: pavankumarn@nirt.res.in, nathellapavan@gmail.com (N.P. Kumar).

http://dx.doi.org/10.1016/j.tube.2014.06.007
1472-9792/

T cells expressing Type 1 and Type 17 cytokines and cytotoxic molecules in TBL, both at baseline and following mycobacterial antigen stimulation and have demonstrated that in TBL there is an expansion of Type 1 and Type 17 cytokines that is mediated in part by IL-1 and IL-6. In addition, TBL is characterized by a contraction in the $CD8^+$ T cell compartment expressing perforin, granzyme B and CD107a.

2. Materials and methods

2.1. Ethics statement

All individuals were examined as part of natural history study approved by the Institutional Review Board of the National Institute of Research in Tuberculosis (NCT01154959) and informed written consent was obtained from all participants.

2.2. Study population

We studied a group of 45 individuals with TB–20 with pulmonary TB and 25 with tuberculous lymphadenitis (Table 1). Individuals with pulmonary TB were diagnosed on the basis of sputum smear positivity for acid fast bacillus (AFB) by Ziehl Neelsen staining. Individuals with TBL were diagnosed on the basis of clinical examination showing the presence of enlargement of lymph nodes as well as fine needle aspiration cytology and direct microscopic identification for AFB. All individuals were HIV negative and did not differ significantly in age or gender distribution. All blood was collected prior to commencement of anti-tuberculous chemotherapy.

2.3. Antigens

TB antigens used were purified protein derivative (PPD; Serum Statens Institute), early secreted antigen – 6 (ESAT-6) and culture filtrate protein -10 (CFP-10) (both from Fitzgerald Industries Intl. Inc). Endotoxin levels were below the threshold of detection according to the manufacturer's datasheet. Final concentrations were 10 μg/ml for PPD, ESAT-6 and CFP-10 and 5 μg/ml for anti-CD3.

2.4. In Vitro culture

Whole blood cell cultures were performed to determine the intracellular levels of cytokines. Briefly, whole blood was diluted 1:1 with RPMI-1640 medium, supplemented with penicillin/streptomycin (100 U/100 mg/ml), L-glutamine (2 mM), and HEPES (10 mM) (all from Invitrogen) and distributed in 12-well tissue culture plates (Costar). The cultures were then stimulated with PPD, ESAT-6, CFP-10 or anti-CD3 or media alone in the presence of the co-stimulatory molecules, CD49d/CD28 at 37 °C for 6 h. Brefeldin A (10 μg/ml) was added after 2 h. After 6 h, centrifugation, washing and red blood cell lysis was performed. The cells were fixed using cytofix/cytoperm buffer (BD Biosciences) and cryopreserved at −80 °C. For neutralization experiments, whole blood was cultured in the presence of anti-IL-1R (5 μg/ml), anti-IL-6R (5 μg/ml) and anti TNFR1 (5 μg/ml) (R&D Systems) or isotype control antibody (5 μg/ml) (R&D Systems) at 37 °C for 1 h following which PPD was added and cultured for an additional 23 h.

Table 1
Study demographics.

	PTB	TBL
Study demographics		
No. of subjects recruited	20	25
Gender (M/F)	11/9	8/17
Median age (Range)	42 (19–64)	35 (18–45)
Smear grade (0/1+/2+/3+)	0/2/5/13	–
TB Lymphadenitis	–	25
Hematology profile		
Red blood cell count, $\times 10^6$ cells/μl	4.33 (2.93–7.66)	4.27 (3.3–5.62)
White blood cell count, $\times 10^6$ cells/μl	10,175.22 (6300–19,900)	7923 (3400–13,300)
Lymphocyte count, cells/mL	1737.72 (850.5–3315)	2049.84 (1102.5–4043.2)
Neutrophil count, cells/mL	7045.36 (4329.2–16,735.9)	4177.83 (1543.6–8804)
Monocyte count, cells/mL	853.8 (506–1631.8)	308.7 (267.9–904.8)
Eosinophil count, cells/mL	169.5 (74.2–409.2)	258.2 (102.4–939.6)
Basophil count, cells/mL	67.2 (31.4–387.8)	48.42 (18.9–106.4)
Platelet count, $\times 10^3$ platelets/μl	306.54 (146–595)	323.26 (159–542)

2.5. Ex vivo analysis

$CD8^+$ T cell memory subsets phenotyping was performed using CD45RA (Biolegend) and CCR7 (BD Pharmingen) antibodies. $CD8^+$ T cells were classified as naive ($CD45RA^+CCR7^+$), central memory ($CD45RA-CCR7^+$), effector memory ($CD45RA-CCR7-$) and TEMRA ($CD45RA^+CCR7-$).

2.6. Intracellular cytokine staining

The cells were thawed, washed and then stained with surface antibodies for 30–60 min. Surface antibodies used were CD3, CD4, CD8, Perforin, Granzyme B and CD107a. The cells were washed and permeabilized with BD Perm/Wash buffer (BD Biosciences) and stained with intracellular cytokines for an additional 30 min before washing and acquisition. Cytokine antibodies used were IFNγ, TNFα, IL-2, IL-4, IL-9, IL-10, IL-17A, IL-17F and IL-22. Cytotoxic molecules used were perforin, granzyme B and CD107a. Cytokines and cytotoxic molecules were stained in different panels. Eight-color flow cytometry was performed on a FACSCanto II flow cytometer with FACSDiva software v.6 (Becton Dickinson). The lymphocyte gating was set by forward and side scatter and 100,000 events were acquired. Gating for cytokine and cytotoxic molecule expression was done using FMO (fluorescence minus one) strategy. Data were collected and analyzed using Flow Jo software (version 10.0.5 TreeStar Inc). All data are depicted as frequency of the various cell populations expressing cytokine(s). Baseline values following media stimulation are depicted as baseline frequency while frequencies following stimulation with antigens are depicted as net frequencies (with baseline values subtracted).

2.7. Statistical analysis

Data analyses were performed using GraphPad PRISM (GraphPad Software, Inc.). Geometric means (GM) were used for measurements of central tendency. Statistically significant differences between two groups were analyzed using the nonparametric Mann–Whitney *U* test. Multiple comparisons were corrected using the Holm's correction.

3. Results

3.1. TBL is not associated with alterations in the frequencies of central memory, effector memory or TEMRA $CD8^+$ T cells

To determine if TBL is associated with alterations in the $CD8^+$ T cell compartment ex vivo, we examined the frequency of $CD8^+$ T cell naive and memory subset distribution in comparison to PTB.

Our data reveal that in TBL individuals there was an enhanced frequency of naïve CD8$^+$ T cells when compared to the PTB group ($p = 0.0367$) but no significant alterations in the other memory compartments (Figure 1).

3.2. TBL is associated with increased frequencies of antigen-stimulated CD8$^+$ T cells expressing Type 1, Type 2 and Type 17 cytokines

It has been established that mycobacteria-specific CD8$^+$ T cells are induced in response to Mtb infection and that these cells can recognize Mtb infected macrophages [6,13]. To determine the association between the frequencies of CD8$^+$ T cell subsets and TBL, we used multi-parameter flow cytometry to define the frequencies CD8$^+$ T cells expressing Type 1 (IFNγ, IL-2 or TNFα), Type 2 (IL-4, IL-9 or IL-10) and Type 17 (IL-17A, IL-17F or IL-22) cytokines at baseline and following stimulation with either mycobacterial antigens or anti-CD3 (Figure 2A). As shown in Figure 2B, there were significantly increased frequencies of CD8$^+$ T cells expressing Type 1 (IL-2 and TNFα) and Type 17 (IL-17A and IL-17F) cytokines at baseline in TBL compared to PTB. In response to PPD (Figure 2C), CFP-10 (Figure 2D) and ESAT-6 (Figure 2E), we observed significantly elevated frequencies of CD8$^+$ T cells expressing either IL-2 or TNFα or IL-17A or IL-17F in TBL compared to PTB individuals. On the other hand, no significant differences were seen in the frequencies of CD8$^+$ T cells expressing Type 2 cytokines at baseline or upon antigenic stimulation. Finally, there were no significant differences in the net frequencies of CD8$^+$ T cells expressing pro-inflammatory cytokines between the 2 groups (with the exception of CD8$^+$ T cells expressing IL-17A (Figure 2F)) following stimulation with anti-CD3, indicating that the increased frequency of pro-inflammatory cytokine expressing CD8$^+$ T cells induced in TBL individuals was predominantly antigen − specific.

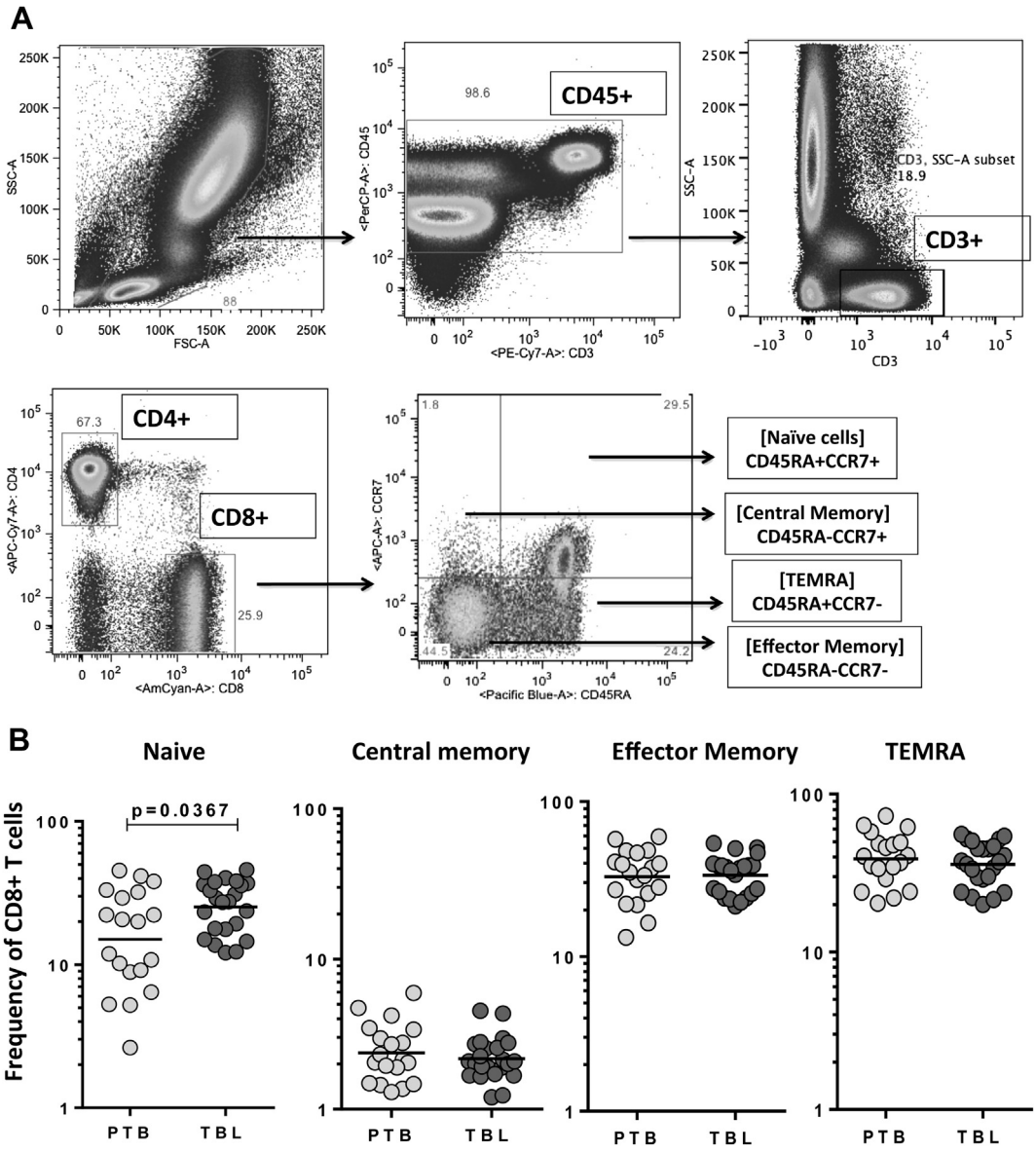

Figure 1. TBL is not associated with altered frequencies of CD8$^+$ T cell memory subsets. (A) The gating strategy for determining the frequencies of CD8$^+$ T cell naive and memory subsets from whole blood. (B) Ex vivo frequencies of naive (defined as CD45RA$^+$CCR7$^+$), central memory (defined as CD45RA−CCR7$^+$), effector memory (defined as CD45RA−CCR7−) and TEMRA (defined as CD45RA$^+$CCR7−) CD8$^+$ T cells in PTB ($n = 20$) and TBL ($n = 25$) individuals.

3.3. TBL is associated with decreased frequencies of CD8$^+$ T cells expressing perforin, granzyme B or CD107a following exposure to antigen

To determine the association of CD8$^+$ T cell expression of cytotoxic molecules with TBL, we measured the frequencies of CD8$^+$ T cells expressing perforin, granzyme B or CD107a at baseline and following stimulation with mycobacterial antigens (Figure 3A). There were significant differences observed in the frequency of CD8$^+$ T cells expressing perforin and granzyme B between TBL and PTB individuals at baseline (Figure 3B, C and D). In addition, as shown in Figure 3B, C and D, there were significantly diminished frequencies of CD8$^+$ T cells expressing perforin, granzyme B and CD107a following TB antigen stimulation in TBL compared to PTB individuals. Thus, TBL is associated with diminished frequencies of CD8$^+$ T cells expressing cytotoxic molecules.

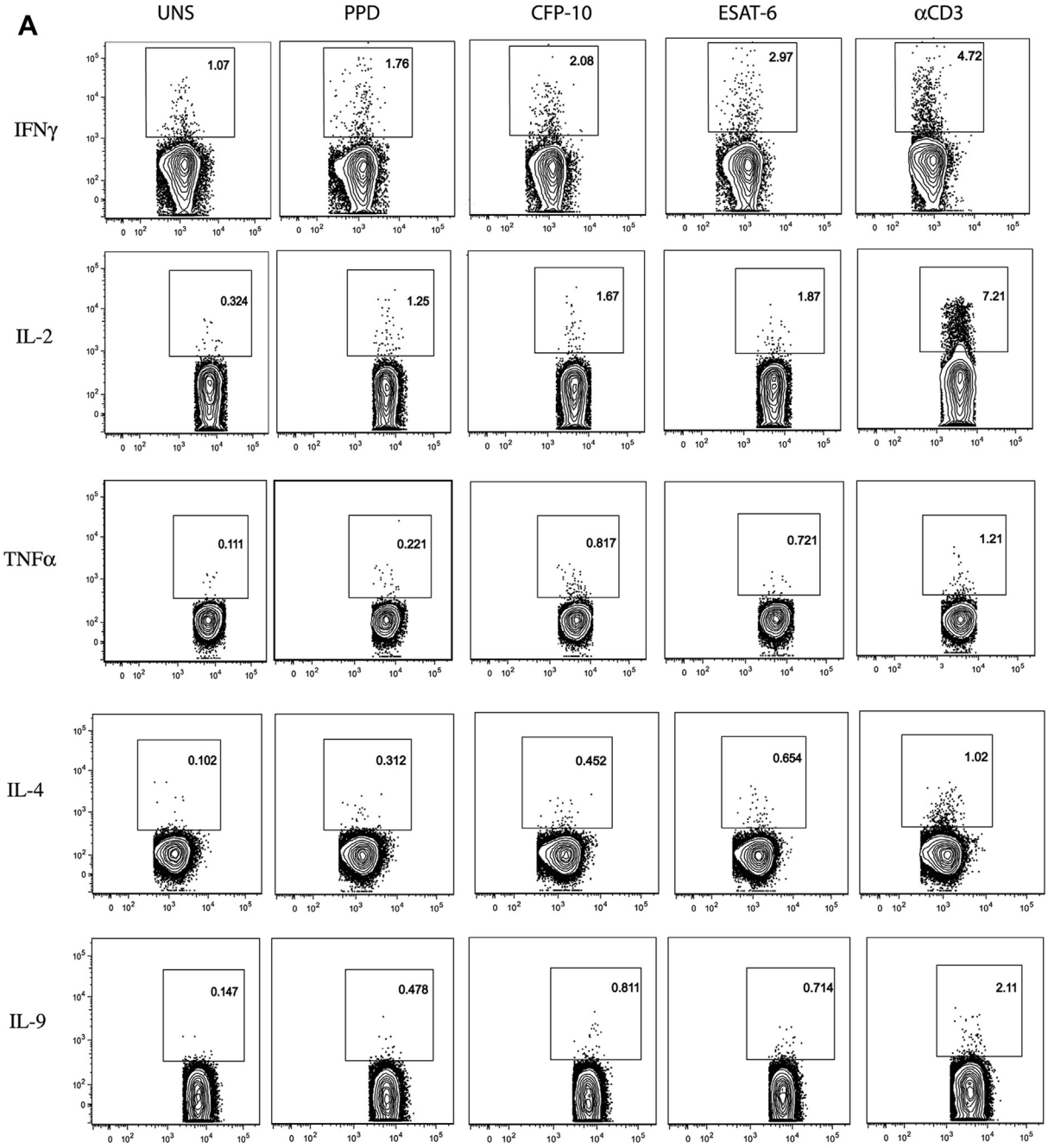

Figure 2. Elevated antigen-specific frequencies of Type 1 Type 2 and Type 17 cytokine secreting CD8$^+$ T cells in TBL. (A) A representative whole-blood intracellular cytokine assay flow data from a TBL individual showing expression of IFNγ, IL-2, TNFα, IL-4, IL-9, IL-10 IL-17A, IL-17F and IL-22 (B) The baseline frequency of CD8$^+$ T cells expressing Type 1 (IFNγ, IL-2, TNFα) or Type 2 (IL-4, IL-9 and IL-10) or Type 17 (IL-17A, IL-17F and IL-22) cytokines is shown as bar graphs with the bar representing the geometric mean of the frequency of CD8$^+$ T cells expressing the respective cytokine(s) and the error bar representing the 95% confidence interval in PTB ($n = 20$) and TBL ($n = 25$) individuals. (C, D, E) The net frequency of CD8$^+$ T cells expressing Type 1 (IFNγ, IL-2, TNFα) or Type 2 (IL-4, IL-9 and IL-10) or Type 17 (IL-17A, IL-17F and IL-22) cytokines in response to PPD (C), CFP-10 (D) and ESAT-6 (E) is shown in PTB and TBL individuals. (F) The net frequency of CD8$^+$ T cells expressing the different cytokines in response to anti-CD3 stimulation is shown in PTB and TBL individuals. Net frequencies were calculated by subtracting baseline frequencies from antigen − or anti-CD3 − stimulated frequencies. *P* values were calculated using the Mann–Whitney test.

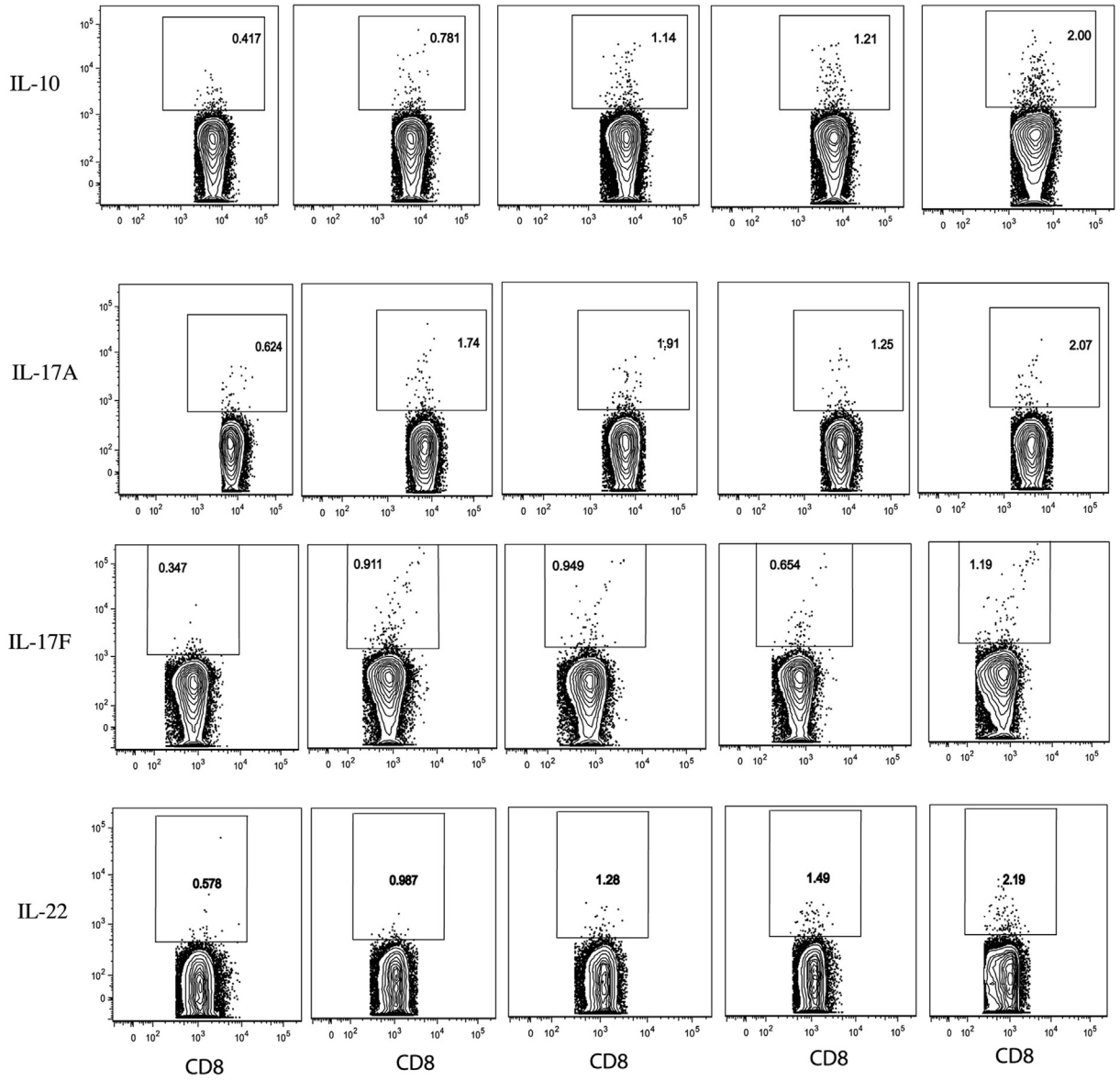

Figure 2. (*continued*).

3.4. Expansion of Type 1 and Type 17 cytokine expressing CD8$^+$ T cells in TBL is dependent on IL-1 and IL-6

Since Type 17 cytokines are dependent on the upstream cytokines – IL-1, IL-6 and TNF-α, we wanted to examine the role of signaling through these cytokines in the expansion of Type 1 and Type 17 cytokine secreting T cells in TBL. To this end, we stimulated whole blood from TBL individuals with PPD in the presence of blocking antibodies for IL-1R or IL-6 or TNFR1 and measured the changes in frequencies of CD8$^+$ T cells expressing Type 1 (IFNγ, IL-2 or TNF-α) and Type 17 (IL-17A, IL-17F or IL-22) cytokines. As shown in Figure 4, blockade of IL-1R resulted in significantly decreased PPD-stimulated expansion of CD8$^+$ T cells expressing IFNγ, IL-2 and IL-17A in TBL individuals. Similarly, blockade of IL-6R resulted in significantly decreased PPD-stimulated expansion of CD8$^+$ T cells expressing IL-17A and IL-17F in TBL individuals (Figure 4). Finally, blockade of TNFR1 had no effect on the PPD – induced production of Type 1 and Type 17 cytokines in CD8$^+$ T cells. These data suggest that IL-1R and IL-6R-mediated signalling plays a significant role in the expansion of the T cell response in TBL.

4. Discussion

TB is primarily a disease of the lung, with pulmonary TB accounting for at least 70% of active tuberculosis cases with the remaining being extra-pulmonary disease. The most common form of extra-pulmonary TB is tuberculous lymphadenitis, that accounts for 30–35% of all extra-pulmonary TB cases most of which are manifested by infection of the cervical lymph nodes [14]. Moreover, it is important to understand the immunological underpinnings of TBL as diagnosis and treatment is highly challenging in this form of TB [15].

CD4$^+$ T cells producing Type 1 cytokines (e.g. IFNγ, IL-2 and TNFα) are important for the protective immune responses to *M. tuberculosis* and appears to require mycobacterial antigen driven specificity [16]. While antigen specific CD8$^+$ T cells and NK cells also produce IFNγ during *M. tuberculosis* infection, they cannot compensate for a lack of CD4$^+$ T cells [11,16,17]. Another important feature of CD8$^+$ T cells is their ability to perform cytotoxicity or specific killing of target cells. The key feature of cytotoxic CD8$^+$ T cells is their expression of cytotoxic molecules such as perforin,

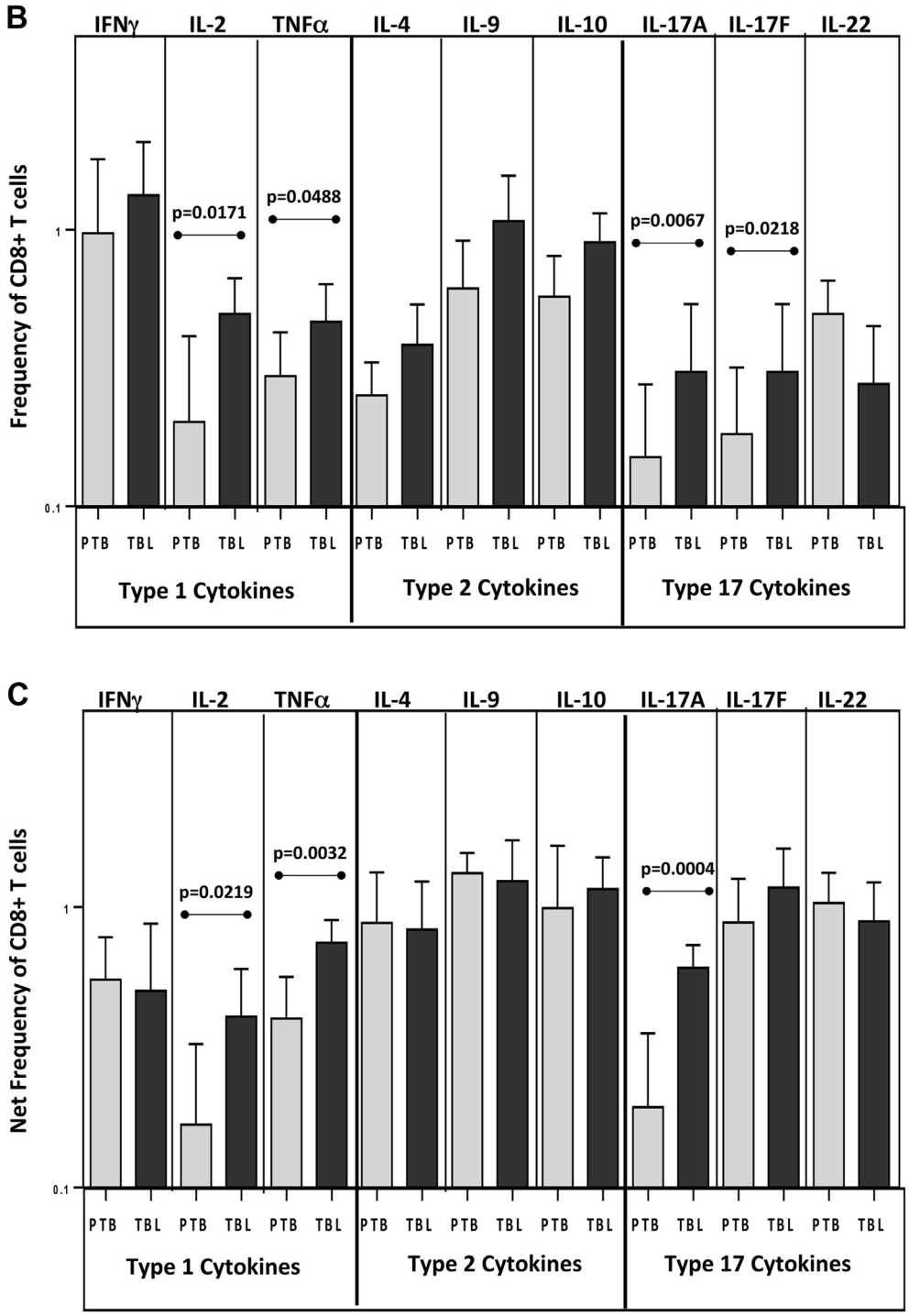

Figure 2. (*continued*).

granzyme B and CD107a. $CD8^+$ T cells may modulate phagocyte activity or produce granulysin that may be directly cytotoxic to the mycobacteria [10,18]. These cells also have the capacity to activate macrophage defenses through the secretion of IFNγ and TNF-α that can also help in eliminating the bacteria through a granule exocytosis pathway [19].

Our study sought to explore the distribution and function of $CD8^+$ T cells expressing cytokines or cytotoxic molecules in TBL in contrast to pulmonary TB. The examination of cytokines produced by $CD8^+$ T cells reveals two interesting features. First at baseline, there were significant differences in the frequencies of Type 1 or Type 17 secreting $CD8^+$ T cells in PTB compared to TBL, indicating that TBL significantly alters the homeostatic regulation of $CD8^+$ T cells. In contrast, polyclonal stimulus (anti-CD3) did not reveal any major difference in the frequency of Type 1 and Type 17 cytokines secreting $CD8^+$ T cells, indicating that the intrinsic potential of T cells to produce cytokines in TBL individuals is not altered. Second, the expansion of Type 1 and Type 17 cytokines in TBL individuals is relatively pathogen specific, because mycobacterial antigen stimulation appears to play a key role in driving the expansion of Type 1

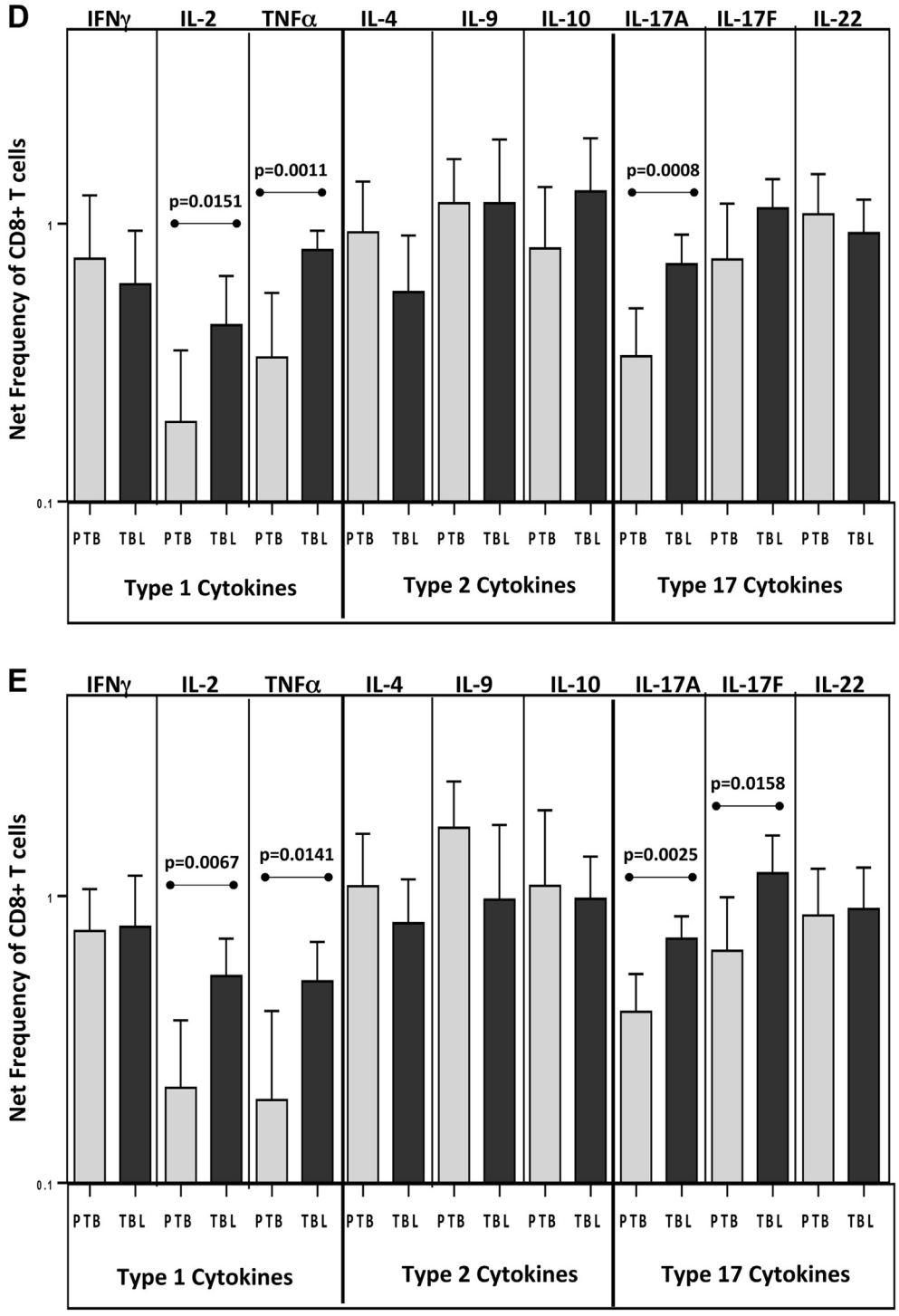

Figure 2. (*continued*).

or Type 17 cytokine expressing $CD8^+$ T cells in TBL individuals. This is true for crude antigen (PPD) as well as the RD1-specific recombinant antigens (CFP-10 and ESAT-6). Moreover, the heightened expansion in response to the RD1 antigens confirms that this response is specific to Mtb and not induced by either cross-reactivity or pre-exposure to other environmental mycobacteria. Our study reflects an important association of expanded frequencies of $CD8^+$ T cells with the pathogenesis in TBL and suggests that heightened frequencies of these could potentially lead to enhanced severity of disease. Our findings also suggest that this expanded population could possibly contribute to severity and dissemination.

Type 17 cytokines, most notably IL-17A and IL-17F have been shown to play an important role in mediating immunity to both extra – and intra – cellular bacteria, including Mtb [11,17]. However, IL-17 was also shown to mediate immune pathology in animal models of autoimmune diseases and infections, suggesting that IL-17 could also promote pathology in tuberculosis [20]. Our data

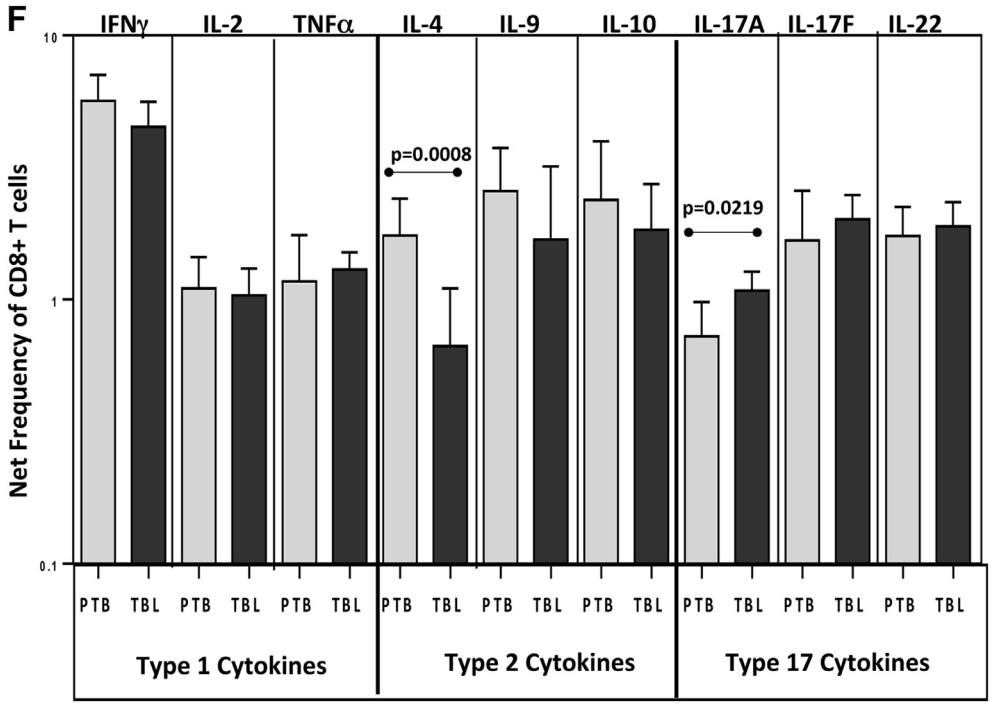

Figure 2. (*continued*).

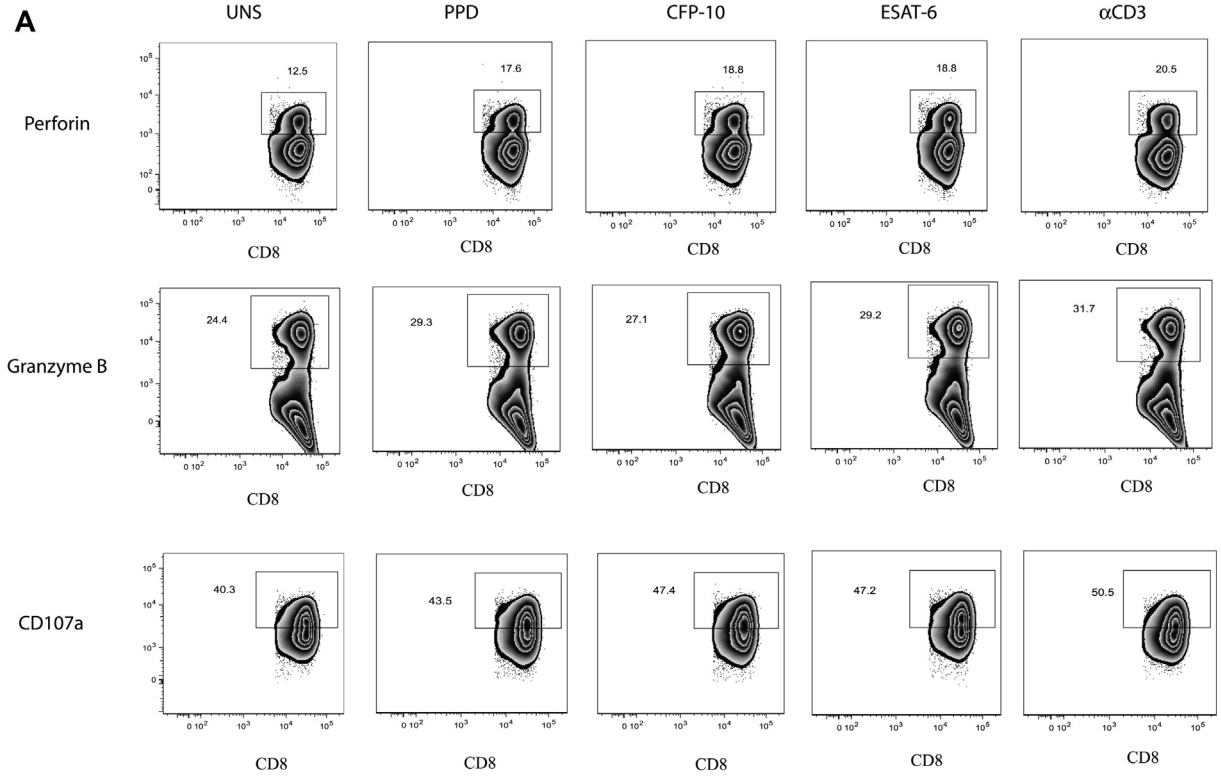

Figure 3. Diminished antigen-specific frequencies of perforin, granzyme B and CD107a cells in TBL. (A) A representative whole-blood assay flow data from a TBL individual showing expression perforin, granzyme B and CD107a. The frequencies of $CD8^+$ T cells expressing Perforin (B) or Granzyme B (C) or CD107a (D) in PTB ($n = 20$) and TBL ($n = 25$) individuals ex vivo or following stimulation with TB antigens or anti-CD3 were estimated by flow cytometry. Spontaneous frequencies are shown as scatter plots and stimulated frequencies as bar graphs. The bars represent geometric means and 95% confidence intervals. Spontaneous frequencies are shown as raw frequencies and the stimulated frequencies are shown as net frequencies with the spontaneous frequency subtracted. *P* values were calculated using the Mann–Whitney *U* test.

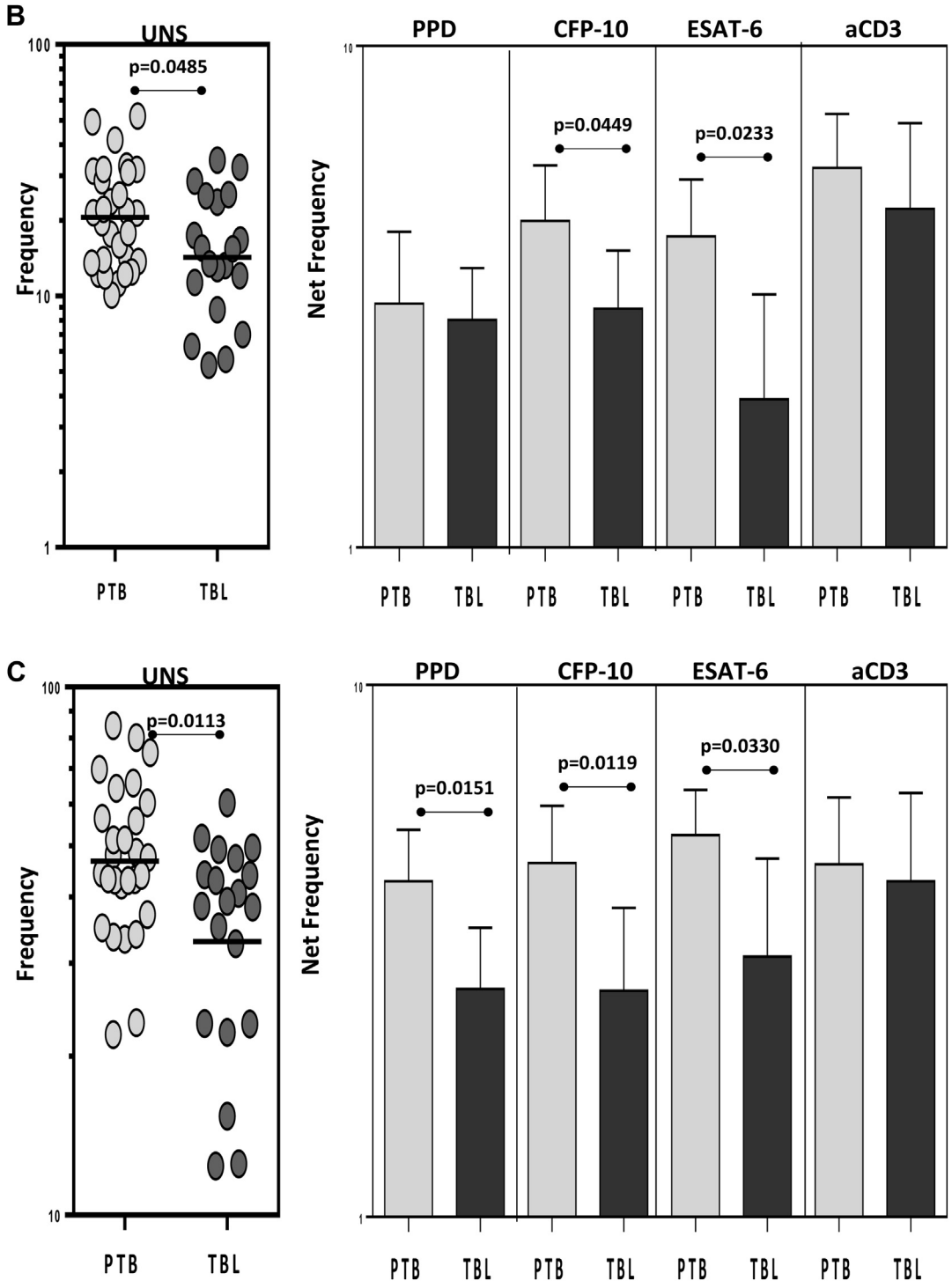

Figure 3. (*continued*).

suggest that TBL influences the expression pattern of TB antigen – specific $CD8^+$ T cells producing IL-17A, IL-17F and IL-22 and could potentially augment immune-mediated pathology in individuals with TBL. Therefore, an exaggerated Type 17 response, similar to the Type 1 response, occurs in active TBL individuals. This study also confirms that the response of $CD8^+$ T cells in TBL is very similar to that of $CD4^+$ T cells that we reported previously [12]. Whether these findings are the result of lymphoid tissue priming occurring more efficiently in TBL or whether it is the consequence of some intrinsic difference between the two disease states needs to be explored further.

Perforin/granzyme-induced apoptosis is a pathway used by cytotoxic lymphocytes to eliminate infected cells [21]. Perforin is a cytolytic protein found in the granules of cytotoxic T lymphocytes and NK cells and has a role in the control of intracellular bacterial infections, such as *M. tuberculosis* [22]. Perforin establishes pores in the cytoplasmic membrane, allowing entry of granzyme B, which in turn mediates apoptosis of Mtb infected cells. However, it has been

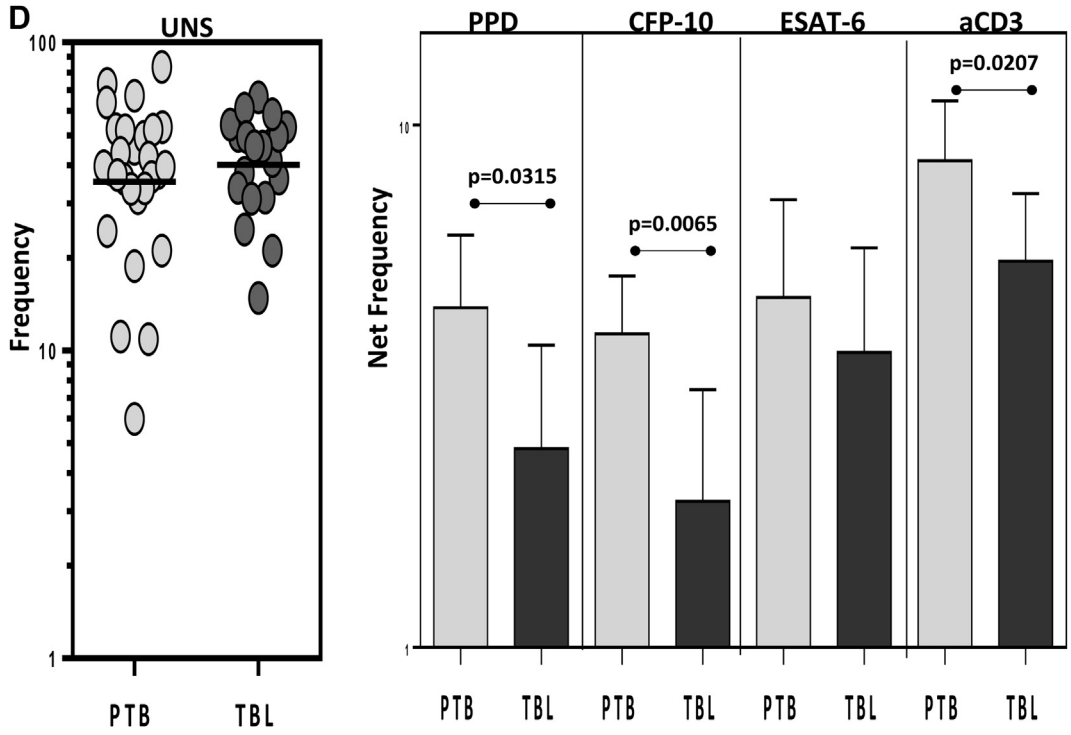

Figure 3. (*continued*).

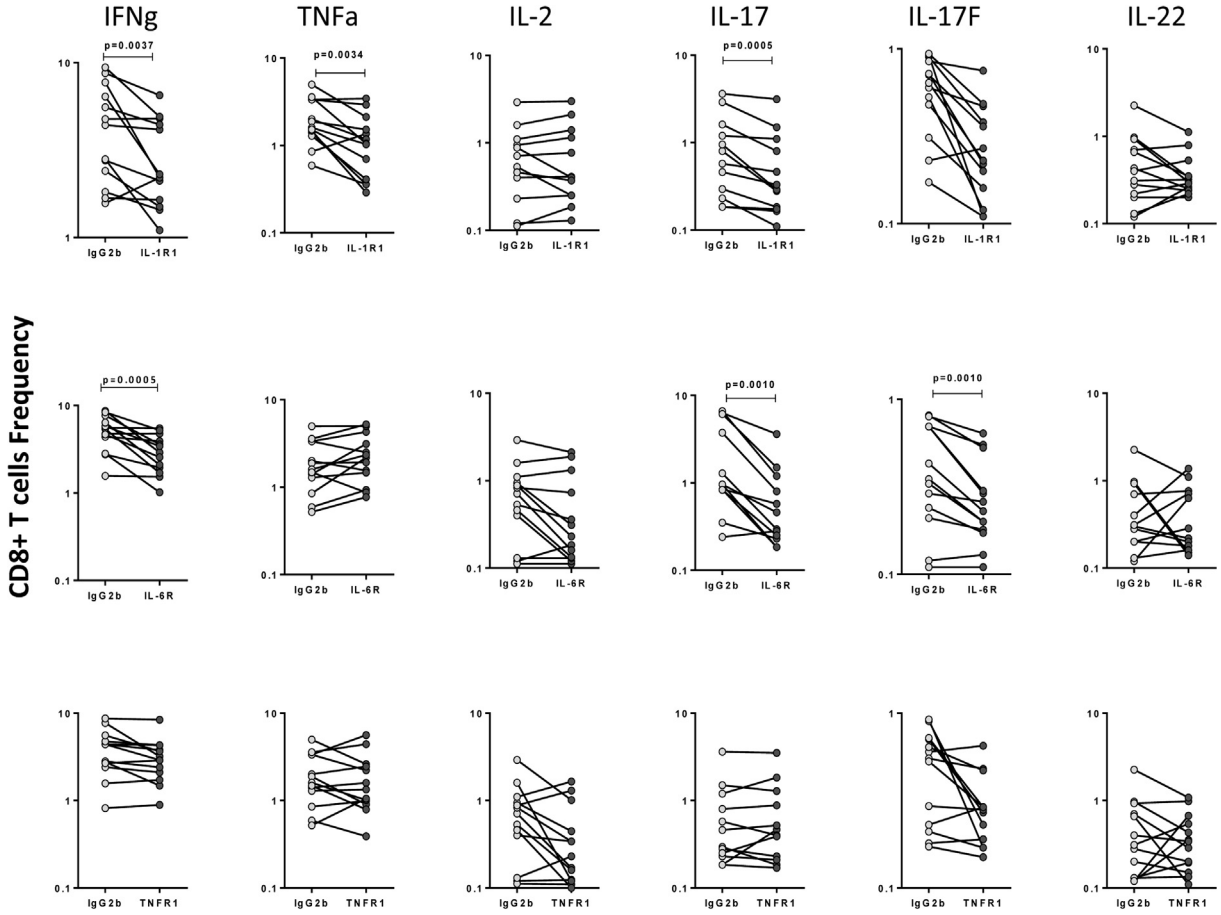

Figure 4. Blockade of IL-1R and IL-6R but not TNFR1 significantly diminishes the frequencies of cytokine expressing $CD8^+$ T cells in TBL. Whole blood from TBL individuals was stimulated with PPD (10 mg/ml) in the presence of anti-IL-1R Ab or anti-IL-6R or anti-TNFR1 or isotype controls for and the frequencies of $CD8^+$ T cells expressing IFNγ, IL-2, TNF-α, IL-17, IL-17F and IL-22 were measured by flow cytometry. Results are shown as line graphs with each line representing a single TBL individual ($n = 15$). Results are shown as net cytokine production over media control. *P* values were calculated using the Wilcoxon signed rank test.

shown that mice deficient with either perforin or granzyme B do not exhibit increased susceptibility to TB infection [23,24]. Other studies have shown that an apoptotic environment may be unfavorable to the mycobacteria [18,25]. CD107a (LAMP-1) is a marker for degranulation of activated $CD8^+$ T cells and it has been shown that CD107a is upregulated on the cell surface upon activation of $CD8^+$ T cells [26,27]. Our data reveals a decrease in the frequencies of $CD8^+$ T cells expressing perforin and granzyme B in TBL when compared with PTB individuals at baseline. Similarly, the frequency of TB antigen stimulated $CD8^+$ T cells expressing perforin, granzyme B and CD107a was also significantly diminished in TBL on comparison to PTB. This observation can be interpreted in two different but not mutually exclusive ways – one, PTB being a multibacillary form of TB induces higher frequencies of cytotoxic T cells and the potential mechanism for the higher frequency of $CD8^+$ T cells expressing lytic molecules in PTB may due to the higher antigen load [28]. The other explanation is that cytotoxic $CD8^+$ T cells in TBL individuals are primarily present in the lymph nodes and therefore are observed at reduced frequencies in the peripheral circulation. It is intriguing to note the differences in the patterns of distribution of $CD8^+$ T cells expressing cytokines versus those expressing cytotoxic potential.

One potential mechanism for increase in $CD8^+$ T cells subsets expressing Type 1 and Type 17 could be increased expression of pro-inflammatory cytokines in TBL. The common pro – inflammatory cytokines that are known to influence the induction of Type 1 and Type 17 immunity are IL-1, IL-6 and TNF-α, apart from IL-12 and IL-23 [29]. IL-1, a pro-inflammatory cytokine, released primarily from macrophages and many other cell types, plays a central role in the regulation of immune and inflammatory responses to infections [30]. In animal models it has been clearly shown that IL-1 has critical importance for the host control of Mtb infection, given that mice deficient in IL-1R succumb rapidly to low-dose aerosol infection with Mtb [31]. IL-6 has a vital role in the modulation and maintenance of the IL-17-producing cells in response to Mtb infection in mice [32]. TNFα is known to have a key role in Mtb infection by stimulating the migration of immune cells to the infection site, contributing to the granuloma formation and controlling the disease progression [33]. In murine models, neutralization of TNF in a low-dose Mtb infection leads to active TB and reactivation of infection [33–35] and TNF inhibitors in humans have been shown to cause reactivation of disease [36]. Our data shows that blockade of IL-1R and IL-6R prior to stimulation with Mtb antigen impaired the expansion of $CD8^+$ T cell subsets expressing Type 1 and Type 17 cytokines in TBL individuals. Our data also reveal an interesting dichotomy on the effect of IL-1 and IL-6 on Th17 cells – while IL-1 predominantly regulates IL-17A production, IL-6 has a more global effect on Th17 cells affecting the production of IL-17A, IL-17F and IL-22. Our data, however, failed to reveal any significant difference in the capacity of TNFα to modulate Type 1 or 17 cytokines and therefore, suggest that TNF-α, unlike IL-1 and IL-6, plays only a minor role in the active expansion of $CD8^+$ T cell responses in TBL.

Our data overall suggest that TBL is characterized by IL-1R and IL-6R dependent expansion of Type 1 and Type 17 cytokines, indicating that IL-1 and IL-6 receptor blockers could be of potential use therapeutically to ameliorate disease or shorten the duration of treatment in TB lymphadenitis.

Acknowledgments

We thank the staff of Department of Clinical Research and the Department of Social Work, NIRT especially Ms Kalaiselvi and Government Stanley Hospital, Chennai, for valuable assistance in recruiting the patients for this study, R. Anuradha, V. Gopinath and Jovvian George of the NIH-ICER for technical assistance.

Funding: This work was funded by the Division of Intramural Research, NIAID, NIH.

Competing interests: None declared.

Ethical approval: This study was approved by the ethical committee of NIRT.

References

[1] Corbett EL, Watt CJ, Walker N, Maher D, Williams BG, Raviglione MC, Dye C. The growing burden of tuberculosis: global trends and interactions with the HIV epidemic. Arch Intern Med 2003;163:1009–21.
[2] Kent DC. Tuberculous lymphadenitis: not a localized disease process. Am J Med Sci 1967;254:866–74.
[3] Chackerian AA, Perera TV, Behar SM. Gamma interferon-producing CD4+ T lymphocytes in the lung correlate with resistance to infection with mycobacterium tuberculosis. Infect Immun 2001;69:2666–74.
[4] Flynn JL, Chan J, Triebold KJ, Dalton DK, Stewart TA, Bloom BR. An essential role for interferon gamma in resistance to mycobacterium tuberculosis infection. J Exp Med 1993;178:2249–54.
[5] Orme IM, Cooper AM. Cytokine/chemokine cascades in immunity to tuberculosis. Immunol Today 1999;20:307–12.
[6] Lazarevic V, Flynn J. $CD8^+$ T cells in tuberculosis. Am J Respir Crit Care Med 2002;166:1116–21.
[7] Kamath AB, Woodworth J, Xiong X, Taylor C, Weng Y, Behar SM. Cytolytic $CD8^+$ T cells recognizing CFP10 are recruited to the lung after mycobacterium tuberculosis infection. J Exp Med 2004;200:1479–89.
[8] Stinchcombe JC, Griffiths GM. Secretory mechanisms in cell-mediated cytotoxicity. Annu Rev Cell Dev Biol 2007;23:495–517.
[9] Woodworth JS, Wu Y, Behar SM. Mycobacterium tuberculosis-specific $CD8^+$ T cells require perforin to kill target cells and provide protection in vivo. J Immunol 2008;181:8595–603.
[10] Bruns H, Meinken C, Schauenberg P, Harter G, Kern P, Modlin RL, Antoni C, Stenger S. Anti-TNF immunotherapy reduces $CD8^+$ T cell-mediated antimicrobial activity against mycobacterium tuberculosis in humans. J Clin Invest 2009;119:1167–77.
[11] Cooper AM. Cell-mediated immune responses in tuberculosis. Annu Rev Immunol 2009;27:393–422.
[12] Kumar NP, Sridhar R, Banurekha VV, Nair D, Jawahar MS, Nutman TB, Babu S. Expansion of pathogen-specific mono- and multifunctional Th1 and Th17 cells in multi-focal tuberculous lymphadenitis. PLoS One;8: e57123.
[13] Pathan AA, Wilkinson KA, Wilkinson RJ, Latif M, McShane H, Pasvol G, Hill AV, Lalvani A. High frequencies of circulating IFN-gamma-secreting CD8 cytotoxic T cells specific for a novel MHC class I-restricted Mycobacterium tuberculosis epitope in M. tuberculosis-infected subjects without disease. Eur J Immunol 2000;30:2713–21.
[14] Mohapatra PR, Janmeja AK. Tuberculous lymphadenitis. J Assoc Physicians India 2009;57:585–90.
[15] Handa U, Mundi I, Mohan S. Nodal tuberculosis revisited: a review. J Infect Dev Ctries;6: 6–12.
[16] Barry 3rd CE, Boshoff HI, Dartois V, Dick T, Ehrt S, Flynn J, Schnappinger D, Wilkinson RJ, Young D. The spectrum of latent tuberculosis: rethinking the biology and intervention strategies. Nat Rev Microbiol 2009;7:845–55.
[17] North RJ, Jung YJ. Immunity to tuberculosis. Annu Rev Immunol 2004;22: 599–623.
[18] Stenger S, Hanson DA, Teitelbaum R, Dewan P, Niazi KR, Froelich CJ, Ganz T, Thoma-Uszynski S, Melian A, Bogdan C, Porcelli SA, Bloom BR, Krensky AM, Modlin RL. An antimicrobial activity of cytolytic T cells mediated by granulysin. Science 1998;282:121–5.
[19] Harisinghani MG, McLoud TC, Shepard JA, Ko JP, Shroff MM, Mueller PR. Tuberculosis from head to toe. Radiographics 2000;20:449–70. quiz 528-449, 532.
[20] Khader SA, Cooper AM. IL-23 and IL-17 in tuberculosis. Cytokine 2008;41: 79–83.
[21] Trapani JA, Smyth MJ. Functional significance of the perforin/granzyme cell death pathway. Nat Rev Immunol 2002;2:735–47.
[22] Pitabut N, Sakurada S, Tanaka T, Ridruechai C, Tanuma J, Aoki T, Kantipong P, Piyaworawong S, Kobayashi N, Dhepakson P, Yanai H, Yamada N, Oka S, Okada M, Khusmith S, Keicho N. Potential function of granulysin, other related effector molecules and lymphocyte subsets in patients with TB and HIV/TB coinfection. Int J Med Sci;10: 1003–1014.
[23] Cooper AM, D'Souza C, Frank AA, Orme IM. The course of mycobacterium tuberculosis infection in the lungs of mice lacking expression of either perforin- or granzyme-mediated cytolytic mechanisms. Infect Immun 1997;65: 1317–20.
[24] Laochumroonvorapong P, Wang J, Liu CC, Ye W, Moreira AL, Elkon KB, Freedman VH, Kaplan G. Perforin, a cytotoxic molecule which mediates cell

necrosis, is not required for the early control of mycobacterial infection in mice. Infect Immun 1997;65:127–32.
[25] Ernst WA, Thoma-Uszynski S, Teitelbaum R, Ko C, Hanson DA, Clayberger C, Krensky AM, Leippe M, Bloom BR, Ganz T, Modlin RL. Granulysin, a T cell product, kills bacteria by altering membrane permeability. J Immunol 2000;165:7102–8.
[26] Aktas E, Kucuksezer UC, Bilgic S, Erten G, Deniz G. Relationship between CD107a expression and cytotoxic activity. Cell Immunol 2009;254:149–54.
[27] Savolainen LE, Koskivirta P, Kantele A, Valleala H, Pusa L, Tuompo R, Westerlund-Wikstrom B, Tuuminen T. Cytotoxic response persists in subjects treated for tuberculosis decades ago. BMC Infect Dis;13: 573.
[28] Lewinsohn DA, Heinzel AS, Gardner JM, Zhu L, Alderson MR, Lewinsohn DM. Mycobacterium tuberculosis-specific $CD8^+$ T cells preferentially recognize heavily infected cells. Am J Respir Crit Care Med 2003;168:1346–52.
[29] Khader SA, Pearl JE, Sakamoto K, Gilmartin L, Bell GK, Jelley-Gibbs DM, Ghilardi N, deSauvage F, Copper AM. IL-23 compensates for the absence of IL-12p70 and is essential for the IL-17 response during tuberculosis but is dispensable for protection and antigen-specific IFN-gamma responses if IL-12p70 is available. J Immunol 2005;175:788–95.
[30] Juffermans NP, Florquin S, Camoglio L, Verbon A, Kolk AH, Speelman P, van Deventer SJ, van Der Poll T. Interleukin-1 signaling is essential for host defense during murine pulmonary tuberculosis. J Infect Dis 2000;182:902–8.
[31] Mayer-Barber KD, Barber DL, Shenderov K, White SD, Wilson MS, Cheever A, Kugler D, Hieny S, Caspar P, Nunez G, Schlueter D, Flavell RA, Sutterwala FS, Sher A. Caspase-1 independent IL-1beta production is critical for host resistance to mycobacterium tuberculosis and does not require TLR signaling in vivo. J Immunol;184: 3326–3330.
[32] Jones GW, McLoughlin RM, Hammond VJ, Parker CR, Williams JD, Malhotra R, Scheller J, Williams AS, Rose-John S, Topley N, Jones SA. Loss of CD4+ T cell IL-6R expression during inflammation underlines a role for IL-6 trans signaling in the local maintenance of Th17 cells. J Immunol;184: 2130–2139.
[33] Mohan VP, Scanga CA, Yu K, Scott HM, Tanaka KE, Tsang E, Tsai MM, Flynn JL, Chan J. Effects of tumor necrosis factor alpha on host immune response in chronic persistent tuberculosis: possible role for limiting pathology. Infect Immun 2001;69:1847–55.
[34] Chakravarty SD, Zhu G, Tsai MC, Mohan VP, Marino S, Kirschner DE, Huang L, Flynn J, Chan J. Tumor necrosis factor blockade in chronic murine tuberculosis enhances granulomatous inflammation and disorganizes granulomas in the lungs. Infect Immun 2008;76:916–26.
[35] Botha T, Ryffel B. Reactivation of latent tuberculosis infection in TNF-deficient mice. J Immunol 2003;171:3110–8.
[36] Keane J, Gershon S, Wise RP, Mirabile-Levens E, Kasznica J, Schwieterman WD, Siegel JN, Braun MM. Tuberculosis associated with infliximab, a tumor necrosis factor alpha-neutralizing agent. N Engl J Med 2001;345:1098–104.