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IMMUNOLOGICAL ASPECTS

Anti-tuberculosis treatment enhances the production of IL-22 through reducing the frequencies of regulatory B cell

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SUMMARY

IL-22 has been suggested to play an important role in immune response against *Mycobacterium tuberculosis* infection. However, the exact role of IL-22 in human tuberculosis (TB) infection remains unclear and the regulatory mechanism of IL-22 response in human TB is unknown. In this study, we observed that successful anti-tuberculosis treatment induced an enhanced and sustained *M. tuberculosis* antigen-specific IL-22 response, correlated with the decrease of the frequencies of $CD19^+CD5^+CD1d^+$ regulatory B cells. We also found that depletion of $CD19^+$ B cells significantly enhanced *M. tuberculosis* antigen-specific IL-22 production by peripheral blood mononuclear cells. More importantly, we observed that purified $CD19^+$ B cells, and more efficiently, $CD19^+CD5^+CD1d^+$ regulatory B cells, suppressed IL-22 production. In summary, we showed here for the first time that effective anti-tuberculosis treatment restores *M. tuberculosis* antigen-specific IL-22 response through a novel mechanism by reducing the frequencies of $CD19^+CD5^+CD1d^+$ regulatory B cells in human TB.

1. Introduction

IL-22 has been suggested to play an important role in immune response against tuberculosis (TB) infection, a deadly infectious disease caused by the infection of *Mycobacterium tuberculosis* (*MTB*). Accumulating evidence has suggested that Th22, an important subset of $CD4^+$ T cells characterized by the production of IL-22 and the expression of transcription factor AHR [1], play a crucial role in modulation of the immune response against microbial infection [2–5]. Indeed, we and others have recently shown that IL-22 may facilitate the limitation of replication of intracellular *MTB* [6–9], and our genetic study revealed a functional single nucleotide polymorphism (rs2227473) in the promoter region of IL-22 gene that is associated with susceptibility to TB [10]. On the other hand, it has been reported that IL-22 plays minor or no role in controlling aerosol infections with *MTB* and is dispensable for protective immunity during mice TB [11]. Therefore, the exact immune function particularly of host immunity or immunopathogenesis conferred by IL-22 during human *MTB* infection remains largely unknown.

Another uncertainty is how antigen-specific IL-22 response is regulated in human TB. While $CD4^+CD25^+$ regulatory T cells (Treg) has been convinced to play a role in inhibiting antigen-specific IFN-γ production during *MTB* infection [12–16], previous reports indicated that Treg did not affect IL-17 production, an IL-22 related cytokine [12]. In contrast, regulatory B cells had been reported to modulate Th17 differentiation [17]. In addition, we previously found that $CD19^+CD1d^+CD5^+$ B cells, a B cell subpopulation which is significantly increased in patients with active TB, inhibited IL-17 and IL-22 production of CD4 T cells activated by anti-CD3 and anti-CD28 antibodies [18]. The in vivo inhibitory effect of $CD19^+CD1d^+CD5^+$ B cells on Th17 response has recently suggested by Guo Y et al. in investigating the therapeutic effect of human mesenchymal stem cells on experimental autoimmune encephalomyelitis [19]. Considering the increasing appreciation of the role of $CD5^+$ B cells in regulating T cell immunity and inflammation

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[20–22], we hypothesed that $CD19^+CD1d^+CD5^+$ B cells might participate in regulating antigen-specific IL-22 response.

Anti-TB chemotherapy is highly effective with <5% relapse rate, and so any changes in cellular immune response occurring during chemotherapy may reflect the expression of protective immunity [23,24]. Therefore, in this study, we investigated IL-22 production in a cohort of individuals with active TB and tracked the dynamics of IL-22 production after these patients received routine anti-TB-treatment. We found that anti-TB treatment induced an enhanced and sustained *MTB* antigen-specific IL-22 response, which coincided with reduced frequency of $CD19^+CD1d^+CD5^+$ B cells during anti-TB treatment. Purified $CD19^+CD1d^+CD5^+$ B cells effectively inhibited *MTB* antigen-specific IL-22 production in vitro. Thus, our study provides a novel mechanism regulating the IL-22 immune response in human TB.

2. Materials and methods monitor

2.1. Patients and samples

A total of 61 newly diagnosed HIV negative pulmonary TB patients were recruited and followed-up in this study. All patients were sputum *MTB* culture-positive and were positive for sputum Acid- Fast Bacilli (AFB) smear microscopy test. The patients were divided into four groups per grading of the sputum AFB smear as described before [25]: AFB 1+: 1 to 9 AFB per 100 fields; AFB 2+: 10 to 99 AFB per 100 fields; AFB 3+: 1 to 10 AFB per field in 50 fields; and AFB4+: More than 10 AFB per field in 20 fields. All sputum specimens were digested and decontaminated of other bacteria by the standard *N*-acetyl-L-cysteine (NALC)-NaOH-sodium citrate method. An aliquot of the specimen was used for microscopical examination of Ziehl–Neelsen stained smears and the remainder was used for parallel testing with BACTEC TB 960 culture system as per the manufacturer's instructions. Sputum samples were classified according to the highest number of AFB per specimen. The detailed characteristics including sex, age, BCG vaccination, tuberculin skin test, chest X-ray findings, absolute number of CD4 T cells, and ratio of CD4 to CD8 T cells were listed in Table 1. Peripheral blood samples were collected by venipuncture from recruited patients before initiation of anti-tuberculosis treatment, and 3, 6, 9, 12 months thereafter during follow-up. The study was approved by the Institutional Review Board of Shenzhen Third People's Hospital, and informed consent was obtained from each participant.

2.2. Cells preparation and in vitro stimulation

Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood as described previously [18]. PBMCs were then cryopreserved in liquid nitrogen. To evaluate the *MTB* antigen-specific IL-22 response, PBMCs were thawed and cultured in a 96-well plate, with 2×10^5 cells/well, in the absence or presence of heat-killed *MTB* lysate (20 μg/mL). After 72 h, the culture supernatants were harvested, and cytokines (IL-22, IFN-γ, and IL-17) levels were determined.

2.3. Flow cytometry

Monoclonal antibodies against human CD3(SK7), CD4(SK3),CD14(M5E2) CD8(SK1), CD19(SK7), CD1d(CD1d42), CD5(UCHT2), CD56(B159), TCRαβ(IP26), IFN-γ(25723.11); IL-17(BL168), IL-22(22URTI), and isotype-matched control immunoglobulins were obtained from BD Biosciences. The absolute number of CD4 T cells and ratio of CD4 to CD8 T cells were routinely determined in our clinical laboratory using china Food and Drug Administration approved reagents from BD bioscience. Flow cytometry analysis of surface markers staining were performed as described previously [18]. For intracellular cytokine analysis, cells were incubated with *MTB* lysate (20 μg/mL) for 72 h in the presence of Brefeldin A (10 μg/mL; Sigma–Aldrich) for the last 6 h. After stimulation, cells were harvested and subjected to surface and intracellular staining as previously described [18]. At least 0.2 million events were acquired using a FACS Canto (Becton Dickinson, San Jose, CA, USA) and analyzed using FACSDiva software (version 5.0.2; BD Biosciences).

2.4. Separation and in vitro functional assay of B cells

PBMCs preparation from whole blood and subsequent B cell subpopulation separation were performed as described previously [18]. Briefly, depletion of $CD19^+$ B cells from fresh or frozen PBMCs was performed using human CD19 Microbead (Miltenyi, Germany). Depletion and cell sorting of $CD19^+CD1d^+CD5^+$ B cells and $CD19^+CD1d^+CD5^-$ B cells were done using BD FACSAria II cell sorter (BD Biosciences, CA, USA). The purities of sorted $CD19^+CD1d^+CD5^+$ B cells and $CD19^+CD1d^+CD5^-$ B cells were over than 95%.

To assess the inhibitory effect of B cells on IL-22 production by PBMCs, we first cultured PBMCs depleted of B cells in the same protocol for PBMCs in vitro stimulation as described above and

Table 1
The demographics of the study population ($n = 61$) before anti-TB treatment.

	Positivity *n* (%)	Sex (M/F)	Age (years)*	Absolute number of CD4 T cells/μL*	Ratio of CD4/CD8*	$CD19^+$ B cells (%)*	$CD19^+CD1d^+CD5^+$ (%)*
Sputum MTB culture	61 (100)	39/22	31.9 ± 11.5	461.4 ± 236.9	1.8 ± 0.8	7.6 ± 3.3	19.8 ± 10.1
Sputum AFB grading							
1+	24 (39.3)	14/10	29.6 ± 9.6	468.7 ± 141.5	1.9 ± 0.7	7.3 ± 3.5	18.8 ± 11.2
2+	8 (13.1)	4/4	32.9 ± 14.2	487.1 ± 224.8	2.1 ± 0.8	7.4 ± 2.1	20.9 ± 9.2
3+	8 (13.1)	5/3	33 ± 12.7	369.9 ± 76.9	1.9 ± 1.4	8.2 ± 2.1	20.7 ± 9.6
4+	21 (34.5)	16/5	32.9 ± 12.5	414.7 ± 173.4	1.7 ± 0.6	7.8 ± 3.8	19.2 ± 9.7
BCG vaccination status							
Vaccinated	29 (47.5)	21/8	26.5 ± 8.5†	467.1 ± 141.5	1.8 ± 0.6	7.8 ± 3.6	18.3 ± 11.4
Non-vaccinated	32 (52.5)	18/14	34.3 ± 12.1	486.3 ± 263.5	1.9 ± 1.0	7.3 ± 2.9	20.6 ± 8.6
Tuberculin skin test							
Induration ≥15 mm	42 (68.8)	28/16	32.4 ± 11.2	490.6 ± 253.7	1.9 ± 0.6	7.8 ± 3.7	19.6 ± 11.2
Induration <15 mm	19 (31.2)	13/6	29.8 ± 12.2	398.3 ± 186.5	1.8 ± 1.1	7.7 ± 2.1	18.1 ± 6.8
Findings on chest X-ray							
With cavity	32 (52.5)	22/10	32.7 ± 10.9	450.9 ± 178.8	1.7 ± 0.8	7.5 ± 2.5	21.4 ± 9.6‡
Without cavity	29 (47.5)	17/12	30.4 ± 12.1	473.4 ± 292.7	1.7 ± 0.9	7.8 ± 3.9	16.1 ± 9.1

* Data were expressed as mean ± SD.
† $p < 0.05$, vaccinated vs. Non-vaccinated.
‡ $p < 0.05$, with cavity vs. without cavity.

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