Austin Journal of HIV/AIDS Research

Open Access

Austin Publishing Group

Research Article

Imbalance of T Helper and Regulatory Cells in Patients with Tuberculosis Co-infection Acquired Immunodeficiency Syndrome

Alimu Ayiguli[1#], Li Li[1,2,3#*], Zhong Xuemei[2#], Gong Hui[1], Abudureheman Zulipikaer[1], Yilamujiang Subinuer[1], Liu Ya[1] and Hairoula Dilare[1]

[1]Department of Clinical Research Center of Infectious Diseases (Pulmonary Tuberculosis), First People's Hospital of Kashi, Xin Jiang, China

[2]Department of Respiratory and Critical Care Medicine, First People's Hospital of Kashi, Xin Jiang, China

[3]State Key Laboratory of Pathogenesis, Prevention and Treatment of High Incidence Diseases in Central Asia, Xinjiang Medical University, China

[#]All authors contributed equally to this work

***Corresponding author:** Li Li, The First People's Hospital of Kashi. No. 120, Yingbin Avenue, Kashi, Xin Jiang, China

Received: April 08, 2022; **Accepted:** June 10, 2022; **Published:** June 17, 2022

Abstract

Background: To ensure that patients with Acquired Immunodeficiency Syndrome (HIV) and active tuberculosis(TB) co-infection (HIV-TB) can get timely treatment, we investigated T helper cells17 (Th17), Tregulatorycells(Treg) levels in the peripheral blood mononuclear cells (PBMCs) of the TB, HIV, HIV-TB patients provide a better consult for prediction of HIV-TB patients.

Methods: A total of 62 patients with TB, HIV, HIV-TB and 30 healthy peoples were included in our study. The PBMCs were isolated from the peripheral blood and the level of Th17 and Treg cells were tested by flow cytometry. Serum levels of cytokines Interleukin 17 (IL-17), Interleukin 6 (IL-6) and Interleukin 10 (IL-10) were determined by ELISA.

Results: Th17/Treg imbalance in HIV-TB patients, and is lower than HIV, TB and control group (Th17/Treg: 0.10±0.03 vs 0.25±0.08 vs 0.47±0.14 or 1.03±0.47, $p<0.05$). The expression of IL-10 in HIV-TB patients were significantly increased than HIV, T Band control groups, IL-17 and IL-6 were significantly decased (IL-10: 28.8±4.3 vs 23.6±3.6 vs 18.5±2.9 or 14.3±1.8pg/mL, $p<0.05$; IL-17: 17.1±3.6 vs 24.2±4.2 vs 28.3±5.8 or 40.9±6.8pg/mL, $p<0.05$; IL-6: 15.9±2.1 vs 23.8±3.4 vs 27.8±4.3 or 32.5±4.6pg/mL, $p<0.05$).

Conclusions: These studies revealed that Th17/Treg in HIV-TB patients was imbalanced, and suppressor T cell subsets are dominant, thus participate in immune pathogenesis of HIV-TB.

Keywords: Co-Infection AIDS with Tuberculosis; T helper Cells; Tregulatorycells; Interleukin; PBMC

Introduction

Pulmonary tuberculosis is common opportunistic infectious diseases in AIDS patients, which enable to patients, obtain dual infections of AIDS and pulmonary tuberculosis [1]. The incidence of HIV-TB patients is 30 times than normal population [2], and the risk of death is 2.87 times than ordinary tuberculosis patients [3]. $CD4^+$ T are command center of immune system, and T helper cell (Th17) and T regulatory cell (Treg) are a subset of $CD4^+$ T cells. Th17 mainly promotes the immune response, while Treg cells mainly suppress the immune response; they can transform each other during immune activities, there by maintaining the body's immune balance [4-5].

Th17 cells are considered to have a significant inhibitory effect on the replication and expansion of HIV [6]. The main role of Treg is to inhibit the effect of T lymphocytes to prevent excessive autoimmune symptoms, thereby reducing the body resistance to external pathogens [7]. The Th17/Treg ratio is relatively stable under normal circumstances, but inflammation and other immune conditions can disrupt this balance [8]. For example, in the process of inflammation, TGF-β can promote the production of Treg cells and promote the differentiation of Th17 and other cells. In the late stage of inflammation, TGF-β can inhibit the immune response by inhibiting the proliferation of Treg cells, there for, the Th17/Treg balance is essential for maintaining normal immune function [9].

The importance of pro inflammatory cytokines, in support of HIV virus replication at sites of TB has been well established. Transcriptional activation of HIV virus by pro inflammatory cytokines TNF-α, interleukin (IL)-1 β, IL-6, and IL-8 is based on induction of nuclear factors [10]. IL-17 is the main effector cytokine of Th17, which can stimulate T cell activation, stimulate epithelial cells, endothelial cells and fibroblasts to produce a variety of cytokines, such as IL-6, IL-8, etc., thereby resisting extracellular bacteria, etc. Pathogen [11]. Interleukin 17 (IL-17) promotes cytokines and plays an important role in the inflammatory immune response.

There have been studies on the changes of Th17 and Treg cells in HIV/TB patients, but there are few studies on HIV-TB patients. Therefore, this study used flow cytometry and ELISA to detect the expression of Th17, Treg, $CD4^+$ T cells and related cytokines in the peripheral blood of HIV-TB patients for detailed analysis.

Materials and Methods

Study Participants

From January 2021 to August 2021, patients with a first diagnosis of HIV-TB, TB, and HIV at the First People's Hospital of Kashi were recruited for the study along with healthy controls. Patients and

Citation: Ayiguli A, Li L, Xuemei Z, Hui G, Zulipikaer A, Subinuer Y, et al. Imbalance of T Helper and Regulatory Cells in Patients with Tuberculosis Co-infection Acquired Immunodeficiency Syndrome. Austin J HIV/AIDS Res. 2022; 8(1): 1050.

controls were matched for age, sex, and body mass index (BMI). All subjects were required to complete a questionnaire regarding basic information and provide 5mL of peripheral blood for testing. Written informed consent was obtained from all participants. Inclusion criteria: (1) it matches the diagnostic criteria of the AIDS diagnosis and treatment guidelines formulated by the Chinese Medical Association, and has undergone clinical diagnosis and laboratory tests to confirm HIV antibody positive. Some individuals were on anti-retroviral treatment (2) it matches the diagnostic criteria for tuberculosis revised and issued in 2001, and is accompanied by significant Signs of tuberculosis poisoning, the tuberculosis culture was positive, and the imaging examination was confirmed as pulmonary tuberculosis. The actuve TB patients are being treated with drugs (3) the control group were Healthy people, which HIV seronegative and mycobacterium tuberculosis culture negative. (4) Non-pregnant patients; (5) Patients older than 15 years old.

Exclusion criteria

(1) Used glucocorticoids, Immunosuppressants; (2) Other immune system diseases and other chronic diseases.

Flow Cytometric Detection of Th17 and Treg Cells

Whole blood was diluted with an equal volume of PBS before layering on Ficoll-Paque PLUS in a 15mL tube. Tubes were then centrifuged or 25 min with the brakes off at 950g PBMCs were collected from the layer between the plasma and Ficoll liquid and washed twice with PBS. Cells were pelleted by centrifugation at 350g for 7 min and then re-suspended in complete PBS. Th17 and Treg cell antibodies were respectively added into PBMC and incubate at room temperature for 30 min in the dark. After washing twice with PBS, re-suspend in 500 μL PBS and transfer to flow tube for detection by flow cytometry. According to flow cytometry results, the percentage of Th17 and Treg cells among the groups was compared. Following antibodies for Flow Cytometry: TL17-PE (Bio Legend Inc. #317306), Foxp3-PE (Bio Legend Inc. #980804). Main instruments: Flow cytometry, biological safety cabinet, low-temperature and high-speed centrifuge (Thermo Fisher Scientific, USA).

IL-6, IL-10, IL-17 Expression Level Detection

Take 2mL of fasting venous blood from the patient, centrifuge (1000×g) and take the upper serum, and detect the expression of IL-17, IL-6 and IL-10 in serum by ELISA. The specific operation is in accordance with the instructions of the ELISA kit. IL-17, IL-6 and IL-10 Enzyme-Linked Immunosorbent Assay (ELISA) kits (Jianglai Biotechnology Company, Shanghai, China).

Statistical Analysis

SPSS 21.0 software (IBM Corp., Armonk, NY, USA) was used for statistical analyses. Quantitative data following a normal distribution are expressed as the mean ±SD. Data that had homogeneity of variance were compared by analysis of variance between multiple groups, followed by pair wise comparisons. The enumeration data was evaluated by chi-square test ($\chi 2$).The correlation between Th17/Treg and cytokines are use person related. According to the least significant difference test, $P<0.05$ was considered statistically significant. Bar graphs were generated using Graph Pad Prism 8.0 (Graph Pad Software, Inc., San Diego, CA, United States).

Results

Basic Information of the Study Participants

A total of 92 people were invited to participate in this research (Table 1). There were 24 patients in TB group, 21 patients in HIV group, 17 patients in HIV-TB group, and 30 healthy persons in control group. The gender, BMI, smoking status, job occupation and age of TB, HIV and HIV-TB patients were not statistically significant compared with control group (P>0.05).

Th17 and Treg Cell Levels in Peripheral Blood

Flow cytometry was performed to evaluate the Th17/Treg dynamics in all subjects. Th17 ($CD4^+IL\text{-}17$) and Treg ($CD4^+CD25^+Foxp3^+$) levels in HIV-TB, HIV, TB patients, and healthy controls (Figure 1). Treg level in HIV-TB patients were significantly higher than HIV, TB or control groups, and Th17, $CD4^+$T cells were significantly decased (Treg: 6.73±1.47% vs 5.63±1.53% vs 3.98±1.12% or 2.62±1.05%, p<0.05; Th17: 0.61±0.26% vs 1.19±0.33% vs 1.75±0.38% or 2.37±1.06%, p<0.05; Th17/Treg: 0.10±0.03 vs 0.25±0.08 vs 0.47±0.14 or 1.03±0.47, p<0.05; $CD4^+$: 36.2±4.21% vs 41.5±5.32% vs 52.9±6.9% or 60.5±8.1%, p<0.05) (Figure 2).

Expression of IL-17, IL-6 and IL-10 in Serum

The expression of IL-17, IL-6 and IL-10 were determined by ELISA in all groups (Figure 3). IL-10 in HIV-TB patients were

Table 1: Basic information of Study participants.

Variables	TB(n=24)	HIV(n=21)	HIV-TB(n=17)	Control(n=30)	P, χ2/F
Age (mean ± SD)	45.3±10.5	40.3±9.5	40.3±12.5	42.7±13.2	0.236 (4.213)
Sex, n (%)					0.356 (3.281)
Male	9 (37.50)	12 (57.15)	10 (58.82)	18 (0.60)	
Female	15(62.50)	9 (42.85)	7 (41.17)	12 (0.40)	
BMI(mean ± SD)	19.39 ± 2.13	19.31 ± 2.07	18.89 ± 1.87	20.13 ± 3.42	0.125 (4.372)
Smoking status, n (%)					0.149 (5.369)
Yes	9 (37.50)	11 (52.38)	7 (41.17)	20 (66.67)	
No	15 (62.50)	10 (47.61)	10 (58.82)	10 (33.33)	
Job occupation, n (%)					0.367 (1.995)
Farmers	10 (41.67)	9 (42.86)	7 (41.18)	12 (40.0)	
Retirees	8 (33.33)	7 (33.33)	5 (29.41)	9 (30.0)	
Other	6 (25.0)	5 (23.81)	5 (29.41)	9 (30.0)	

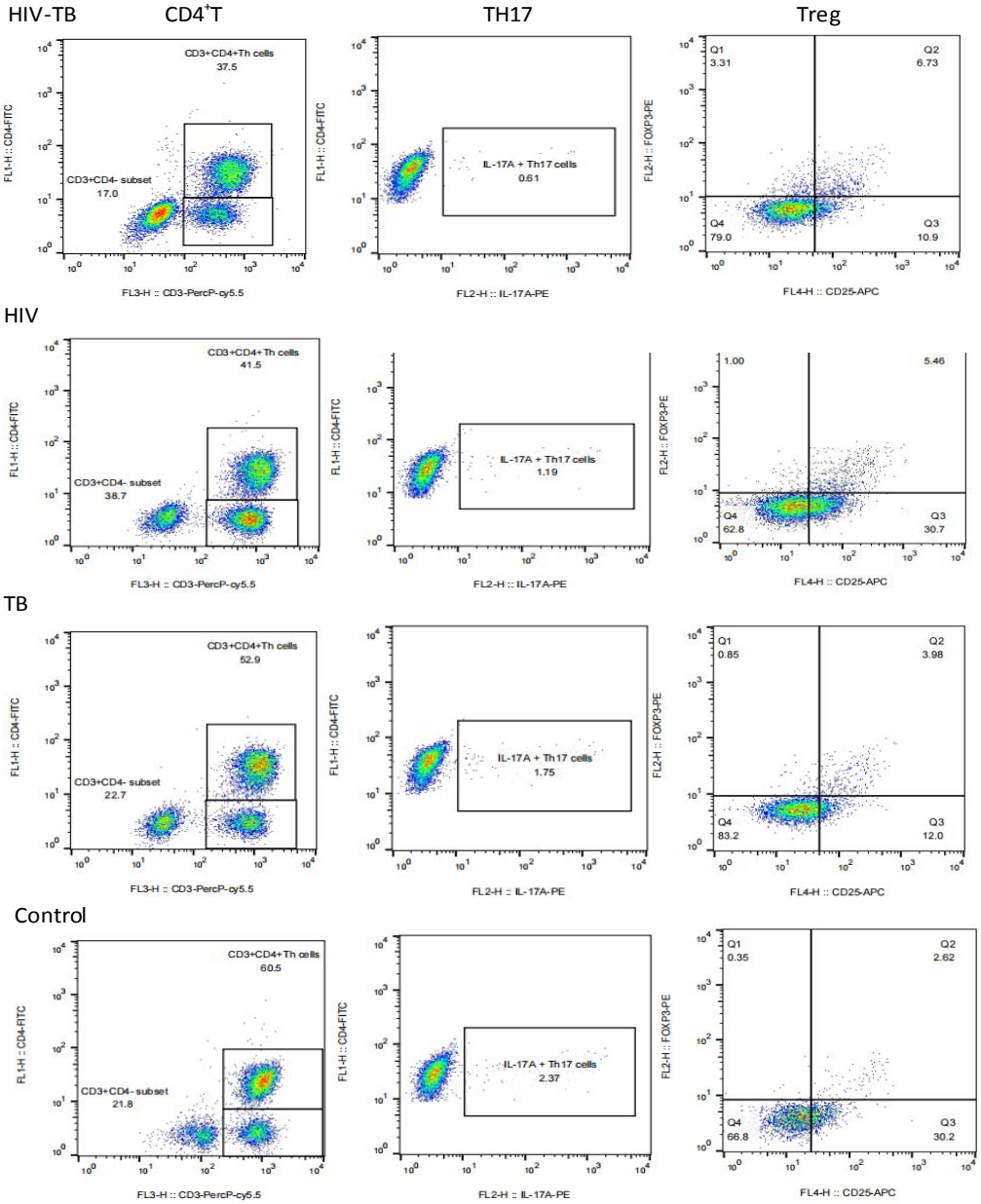

Figure 1: Flow cytometric diagram of Th17, Treg cells in peripheral blood.

significantly increased than HIV, TB or control groups, and IL-17, IL-6 were significantly decased (IL-10: 28.8±4.3 vs 23.6±3.6 vs 18.5±2.9 or 14.3±1.8pg/mL, $p<0.05$; IL-17:17.1±3.6 vs 24.2±4.2 vs 28.3±5.8 or 40.9±6.8pg/mL, $p<0.05$; IL-6: 15.9±2.1 vs 23.8±3.4 vs 27.8±4.3 or 32.5±4.6 pg/mL, $p<0.05$).

Discussion

HIV/TB infection is mutually promote disease rapid development and deterioration, and leads to the rapid death of patients. The immune cells of $CD4^+$ T cells play important role in the human body against tuberculosis infection. It is well established that AIDS virus destroys $CD4^+$ T cells, which progressively damages the immune system. Recent studies have suggested that an imbalance between Th17 and Treg subsets of $CD4^+$ T cells are related in infections, cancers, and autoimmune diseases [12]. Research has shown that AIDS virus multiplies in T cells, and was attacked T cells, affects the differentiation of T cells, and Lead to decrease in the number of $CD4^+$ T cells and functional damage [13].

The number of $CD4^+$ T cells is an indicator of immune system function. Th17 and Treg cells are newly discovered $CD4^+$ T cell subsets [14]. Th17/Treg ratio and $CD4^+$ T cell levels are related to the development of the disease. Previous studies have shown in patients with acute respiratory distress syndrome, as the condition gradually deteriorates, $CD4^+$ T cells and Th17/Treg gradually decreases [15-16]. Study has found that the replication of AIDS virus in tuberculosis patients [13]. Our study shows that Th17/Treg imbalance of HIV-TB patients, and it is lower than HIV, TB and healthy controls, the

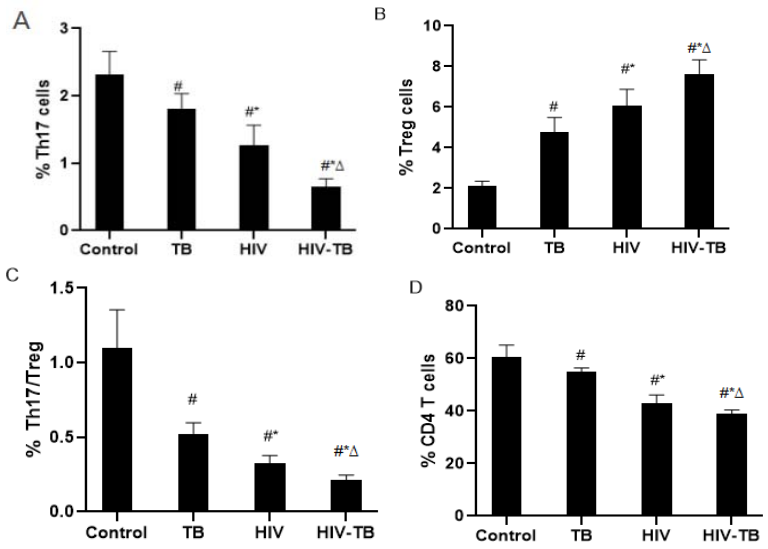

Figure 2: Expression Th17, Treg, CD4+T cells in peripheral blood of each group.
Note: #P < 0.05 compared with the control group; *P < 0.05 compared with the TB group ; ΔP < 0.05 compared with the HIV group. A is the content of Th17 cells in peripheral blood, B is the content of Treg cells in peripheral blood, C is Th17/Treg, D is the content of CD4 T cells in peripheral blood.

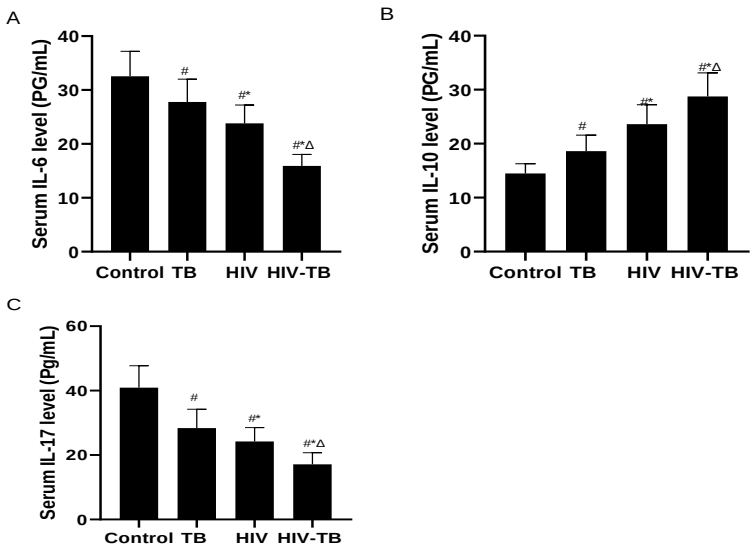

Figure 3: Expression of cytokines in serum of each group.

results similar to above study. TB patients have weakened immunity, and AIDS virus are easy attack CD4+ T cells, leading Treg/Th17 imbalance, and this imbalance accelerate, aggravation of AIDS and tuberculosis infection.

Our study has shown that Th17/Treg ratio in peripheral blood was positively correlated with IL-6 and IL-17, and negatively correlated with IL-10. This may be related to the following reasons: (1) Th17 is a newly discovered that subset of T cells, and can secrete IL-17. Cytokines such as IL-6 and IL-23 can promote the differentiation of T cells into Th17; thereby exerting immune effects [17-18]. Therefore, Th17 cell levels directly affect IL-17 and IL-6 levels. (2) Studies have found that IL-10 is the main effector cytokine of Treg cells, which can inhibit the proliferation and activation of effector T cells, and the level is elevated in patients with immunosuppressive state [19-20]. Therefore, IL-10 level in the peripheral blood of HIV-TB patients is elevated.

Conclusion

This study through laboratory tests found that Th17/Treg ratio was imbalanced in HIV-TB patients, and IL-6, IL-17 were reduced ,and IL-10 were increased. Suppressor T cell subsets are dominant, thus participating in the immune pathogenesis of HIV-TB. Further mechanism research in later period.

Authors' Contributions

All authors participated in the design, interpretation of the studies

and review of the manuscript; AA and LL participated analysis of the data. GH, AZ, YS, LY, HD and AA conducted the experiments, LL supplied critical reagents, AA and ZXM wrote the manuscript.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

This work was supported by The Tianshan Youth Project of Xinjiang Uyghur Autonomous Region (2020D14013). State Key Laboratory of Pathogenesis, Prevention and Treatment of High Incidence Diseases in Central Asia, (SKL-HIDCA-2020-KS1. State Key Laboratory of Pathogenesis, Prevention and Treatment of High Incidence Diseases in Central Asia (SKL-HIDCA-2020-10.

Ethics

This project was approved by the Ethics Committee of First People's Hospital of Kashi.

References

1. Nawaid Hussain Khan, Mikashmi Kohli, Kartik Gupta, Bimal Kumar Das, Ravindra Mohan Pandey, et al. HIV Drug Resistance Mutations in Patients with HIV and HIV-TB Coinfection After Failure of First-Line Therapy: A Prevalence Study in a Resource-Limited Setting. J Int Assoc Provid AIDS Care. 2019; 18: 2325958219849061.
2. Shah I, Poojari V, Meshram H. Multi-Drug Resistant and Extensively-Drug Resistant Tuberculosis. Indian J Pediatr. 2020; 87: 833-839.
3. A Bamford, A Turkova, H Lyall, C Foster, N Kleinll, et al. Paediatric European network for treatment of AIDS (PENTA) guidelines for treatment of paediatric HIV1 infection 2015: optimizing health in preparation for adult life. HIV Medicine. 2018; 19: e1-e42.
4. Z Wang, C Friedrich, S C Hagemann, W H Korte, N Goharani, et al. Regulatory T cells promote a protective Th17-associated immune response to intestinal babcterial infection with C. Rodentium. Mucosal Immunol. 2014; 7: 1290-1301.
5. Ling Li, Zhi-Yao He, Xia-Wei Wei, Yu-Quan Wei, et al. Recent advances of biomaterials in biotherapy. Regen Biomater. 2016; 3: 99-105.
6. C J Kim, A Nazli, O L Rojas, D Chege, Z Alidina, et al. A role for mucosal IL-22 production and Th22 cells in HIV-associated mucosal immunopathogenesis. Mucosal Immunol. 2012; 5: 670-680.
7. Jose SZ, Lu LF, Rudensky AY. Regulatory T cells: mechanisms of differentiation and function. Annu Rev Immunol. 2012; 30: 531-534.
8. Banesa de Paz, Catuxa Prado, Mercedes Alperi-López, Francisco J Ballina-García, Javier Rodriguez-Carrio, et al. Effects of glucocorticoid treatment on CD25FOXP3+ population and cytokine-producing cells in rheumatoid arthritis. Rheumatology (Oxford). 2012; 51: 1198-1207.
9. Lee GR. The Balance of Th17 versus Treg Cells in Autoimmunity. Int J Mol Sci. 2018; 19(3): 730-736.
10. Z Toossi, J L Johnson, R A Kanost, M Wu, H Luzze, et al. Increased replication of HIV-1 at sites of Mycobacterium tuberculosis infection: potential mechanisms of viral activation. J Acquir Immune Defic Syndr. 2001; 28(1): 1-8.
11. Nathella Pavan Kumar, Kadar Moideen, Vaithilingam V Banurekha, Dina Nair, Subash Babu. Modulation of Th1/Tc1 and Th17/Tc17 responses in pulmonary tuberculosis by IL-20 subfamily of cytokines. Cytokine. 2018; 108: 190-196.
12. Ilona Kryczek, Shuang Wei, Linhua Zou, Saleh Altuwaijri, Wojciech Szeliga. Th17 and regulatory T cell dynamics and the regulation by IL-2 in the tumor microenvironment. J Immunol. 2007; 178: 6730-6733.
13. Yan S L, WeiJ S. Effects of Th17/Treg cell imbalance on HIV replication in patients with AIDS complicated with tuberculosis. Exp Ther Med. 2018; 15(3): 2879-2883.
14. Hu Y, Zhang J, Li X. Penicillium marneffei infection: An emerging disease in mainland China. Mycopathologia. 2013; 175(1-2): 57-67.
15. Giorgio Basile, Isidora Paffumi, Anna Grazia D'Angelo, Paolo Figliomeni, Maria Despina Cucinotta. Healthy centenarians show high levels of circulating interleukin-22 (IL-22). Arch Gerontol Geriatr. 2012; 54: 459-461.
16. Engelhardt KR, Grimbacher B. Mendelian traits causing susceptibility to mucocutaneous fungal infections in human subjects. J Allergy Clin Immunol. 2012; 54: 459-461.
17. Kanwar B, Favre D, McCune JM.Th17 and regulatory T cells: implications for AIDS pathogenesis. Curr Opin HIV AIDS. 2010; 5: 151-157.
18. Masayuki Fukui, Kikuko Shinjo, Masayuki Umemura, Satoko Shigeno, Tetsuya Harakuni, et al. Enhanced effect of BCG vaccine against pulmonary Mycobacterium tuberculosis infection in mice with lung Th17 response to mycobacterial heparin-binding hemagglutinin adhesin antigen. Microbiology and Immunology. 2015; 59: 735-743.
19. Guo-qiang Wang, Cai-ling Yang, Dong-fang Yue, Li-hong Pei, Hua Zhong, et al. The changes and its significance of Th17 and Treg cells and related cytokines in patients with tuberculosis pleurisy. Allergy Asthma Clin Immunol. 2014; 10: 28-35.
20. Yawara Kawano, Oksana Zavidij, Jihye Park, Michele Moschetta, Katsutoshi Kokubun et al. Blocking IFNAR1 inhibits multiple myeloma–driven Treg expansion and immunosuppression. J Clin Invest. 2018; 128: 2487-2499.