

Original Research

Received 01 June 2026

Revised 23 July 2026

Accepted 07 Aug 2026

Natural Resources for Human Health 2026, Vol. 6 (15s): 72–78

KEYWORDS:

Tuberculosis, CD4⁺ T cells, FoxP3, Tregs.

Alterations in CD4⁺ Expression, FoxP3, and Regulatory T Cells in Iraqi Patients with Pulmonary Tuberculosis: A Case–Control Study.

Mohammed J. Mohammed¹; Kawkab A. Hussain²; Haider H. Mohammed Ali³

¹ Department of Biology, College of Science, University of Karbala, Karbala, Iraq

² Department of Biology, College of Science, University of Karbala, Karbala, Iraq

³ Department of Biology, College of Science, University of Karbala, Karbala, Iraq

ABSTRACT:

Background: Effective control of *Mycobacterium tuberculosis* relies on CD4⁺ T cells, whereas regulatory T cells (Tregs) restrain excessive immune responses. Changes in the balance of these cell populations may contribute to tuberculosis pathogenesis. This study aimed to assess the CD4⁺ T-cell expression and Treg frequencies in patients with active tuberculosis and compared them with those in healthy controls. **Methods:** Blood samples were taken from the veins of 50 patients with tuberculosis and 50 apparently healthy controls, and the percentages of CD4⁺ T cells, FoxP3 molecules, and T reg cells were assessed by Flow Cytometry. **Results:** Patients with active tuberculosis had a significantly higher proportion of regulatory T cells (Tregs) than healthy controls, whereas CD4⁺ T-cell expression was significantly lower in Tuberculosis patients than in controls. **Conclusion:** Patients with tuberculosis exhibited weaker CD4⁺ T-cell responses and higher Treg frequencies, suggesting immune dysregulation during *Mycobacterium tuberculosis* infection. Although elevated Tregs may help maintain immune homeostasis and limit tissue damage, they may also suppress protective antimycobacterial immunity and contribute to bacterial persistence.

INTRODUCTION:

Mycobacterium tuberculosis is an aerobic, non-motile, slow-growing, acid-fast bacillus because its lipid-rich cell envelope contains mycolic acids, which resist acid–alcohol decolourisation and reduce drug penetration, causing tuberculosis, a chronic infectious disease mainly affecting the lungs (Jacobo-Delgado *et al.*, 2023). Most importantly, *Mycobacterium tuberculosis* is hazardous because it has the ability to survive inside macrophages, elude the host's immunity, establish a latent infection, and gradually destroy the lung. Treatment is prolonged for many months due to its slow replication, intracellular and granulomatous persistence, drug-tolerant bacterial populations, and the protective lipid (Warner *et al.*, 2025; Sarangi *et al.*, 2024).

Adaptive immunity, particularly CD4⁺ T-cell responses, is essential for protection against TB through secretion of interferon- γ and activation of infected macrophages (Sakai *et al.*, 2016). Nevertheless, active TB is frequently associated with functional exhaustion of CD4⁺ T cells rather than simple depletion of cell numbers. Chronic antigen exposure may induce impaired cytokine production, increased inhibitory receptor expression, and diminished effector function, ultimately compromising bacterial clearance (Jayaraman *et al.*, 2016). Concurrently, expansion of regulatory T cells (Tregs) expressing the transcription factor FoxP3 has been consistently reported in TB patients (Ahmed *et al.*, 2020). FoxP3 acts as a master regulator of regulatory T cells' suppressive activity and contributes to the inhibition of Th1-mediated antimycobacterial immunity through the secretion of IL-10 and TGF- β , the suppression of antigen-presenting cell function, and the expression of inhibitory molecules such as

CTLA-4 (Wing *et al.*, 2019). Although Tregs may protect against excessive tissue damage, excessive FoxP3-mediated immunosuppression may facilitate bacterial persistence and disease progression (Bozzano *et al.*, 2021).

METHODOLOGY:

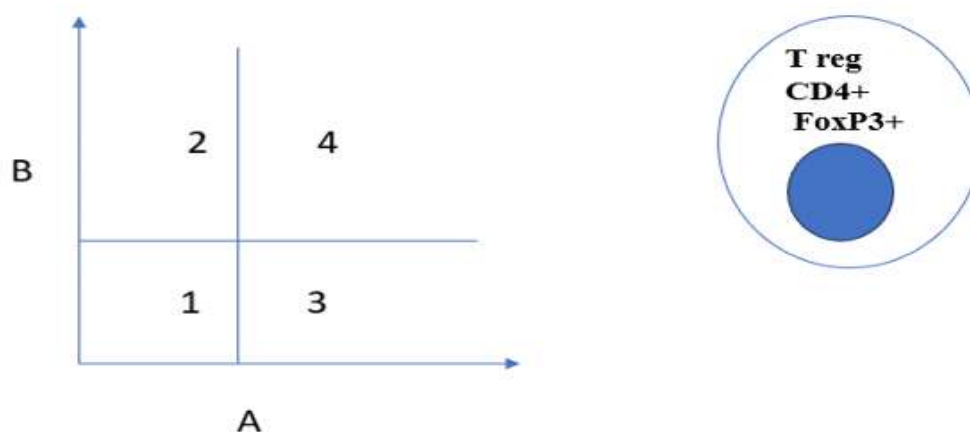
Study design and sample collection:

A case-control study was conducted among Tuberculosis patients from December 2024 to May 2025; blood samples were collected from patients attending the Advisory Clinic for Respiratory Diseases in Karbala and the National Tuberculosis Institute in Baghdad. Tuberculosis patients were diagnosed by a physician based on clinical signs and symptoms and, depending on lab results, by GeneXpert testing. The healthy controls were age- and sex-matched volunteers. The study included 50 patients with tuberculosis and 50 individuals without any autoimmune disease or other disease as the control group.

The Markers of CD4⁺ T cells and the FoxP3 molecule were measured using flow cytometry. Two mL of Peripheral blood was withdrawn by syringe from Tuberculosis patients and healthy volunteers and placed in an EDTA tube for flow cytometry.

100 μ L of anticoagulated whole blood (collected in EDTA or ACD tubes) was transferred into a 12 $\times$ 75 mm polystyrene tube and incubated with the appropriate fluorochrome-conjugated antibody according to the manufacturer's instructions. The samples were gently vortexed and incubated at room temperature in the dark for the recommended period. Subsequently, 100 μ L of Fixation Reagent (Reagent A) was added to each tube, followed by vortexing and incubation for 10 minutes at room temperature in the dark. After fixation, 1 mL of Permeabilization Reagent (Reagent B) was added, and the samples were vortexed and incubated for 20 minutes in the dark. The cells were then centrifuged at 300 $\times$ g for 5 minutes, the supernatant was discarded, and the pellet was resuspended in 1 mL of phosphate-buffered saline (PBS) for washing. Following a second centrifugation at 300 $\times$ g for 5 minutes, the supernatant was removed, and the cell pellet was finally resuspended in 300 μ L of PBS. For samples that were not analysed immediately, PBS containing 1% paraformaldehyde was used to preserve the cells. Finally, the prepared samples were analysed using a flow cytometer or stored at 2–8°C in the dark and analysed within 24 hours.

Samples were analysed using the gating strategy, as shown in Figure 1. The Treg cells (CD4⁺ FoxP3⁺) are found in the upper right quadrant,



- 1- Double negative for both A and B
- 2- Single cells exhibiting positivity for B
- 3- Single cells exhibiting positivity for A
- 4- Double positive for both A and B.

Figure 1: The methodology used to evaluate the outcomes of flow cytometry and the specific cells targeted in the investigation.

RESULTS AND DISCUSSION

Expression of CD4 T cells in peripheral blood of the control and the patients

The results showed the percentage of CD4⁺ T lymphocytes in healthy controls and tuberculosis (TB) patients. The control group had a slightly higher mean CD4⁺ T cell percentage (96.38%) than the patient group (95.59%) (fig. 2). No significant differences at $P < 0.005$.

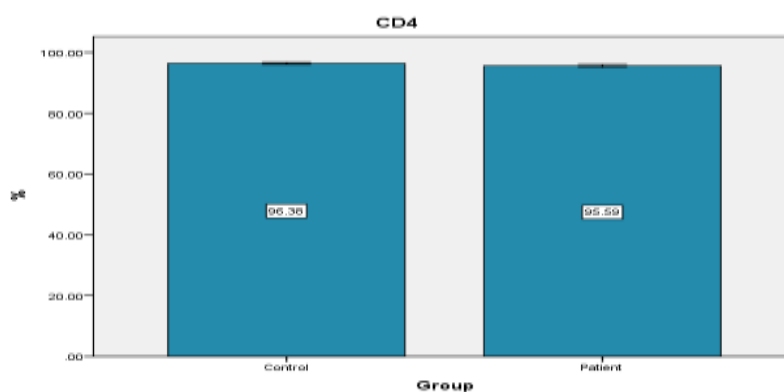


Fig.2 Percentage of CD4 T cells in Peripheral blood in the control and patient groups

The current study showed the distribution of CD4⁺ Mean Fluorescence Intensity (MFI) among healthy controls and tuberculosis patients. A marked decrease in CD4⁺ MFI was observed in the TB group (21170.83) compared with controls (34119.46), Figure 3, indicating a highly significant difference between the control groups ($P < 0.0001$).

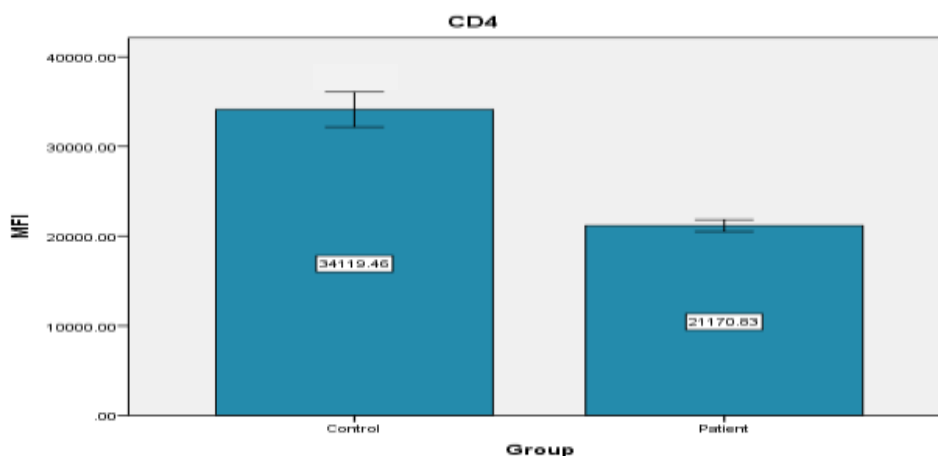


Fig.4: MFI of CD4 molecule on the T Lymphocyte in peripheral blood of the control and patient groups.

The immune function of T cells in Tuberculosis is primarily reflected in cytokines like IFN- γ , secreted by activated CD4⁺ T cells and CD8 T cells, and mice and humans with IFN- γ deficiencies or abnormalities in the IFN- γ receptor show increased susceptibility to TB (Maciag *et al.*, 2024). CD4⁺ T cells play an essential role in controlling Mtb infection and preventing disease progression (Flynn *et al.*, 2022; Morgan *et al.*, 2021). This is clearly demonstrated in HIV-infected individuals, where CD4⁺ T cell depletion significantly increases their susceptibility to active TB (Gupta *et al.*, 2013). T cell counts are known to change in response to *M. tuberculosis* infection. Severe TB patients typically experience a decrease in CD4⁺ T cells (Kapata *et al.*, 2013). The current study agrees with Marion *et al.* (2025), who noticed that individuals diagnosed with disseminated tuberculosis exhibited the lowest average CD4⁺ count as shown in Figure 3, denoting a remarkable correlation between advanced immunosuppression and the occurrence of severe forms of tuberculosis.

Expression of FoxP3-expressing cells in the peripheral blood of the control and patients

The result showed a lower percentage of FoxP3-expressing cells in tuberculosis patients than in healthy controls. This may indicate migration of these cells to the lungs, where the infection is located. The control group had a mean of 23.29%, whereas the patient group had a mean of 16.90%.

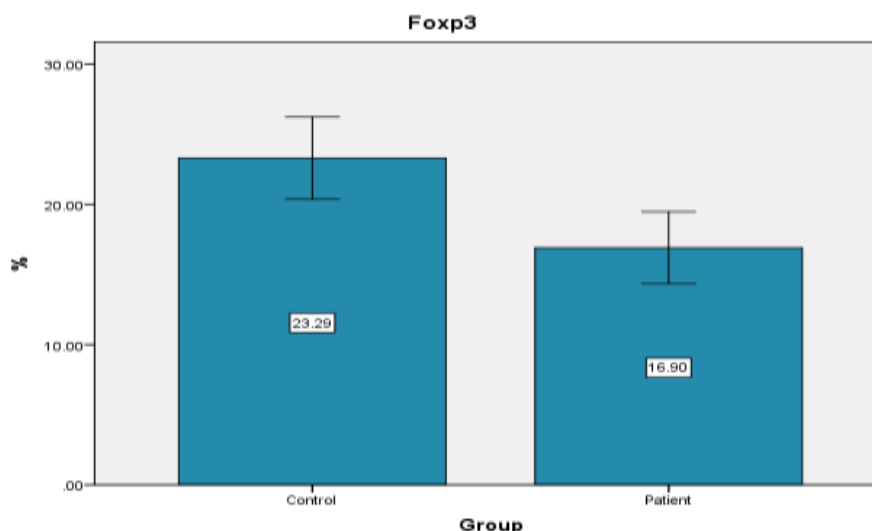


Fig. 4: shows the percentage of FoxP3-expressing cells in the peripheral of the control and patient groups.

The results showed the mean fluorescence intensity (MFI) of FoxP3 between healthy controls and tuberculosis patients. The control group showed a mean FoxP3 of 8911.29, whereas the patient group showed a higher mean value of 9588.61.

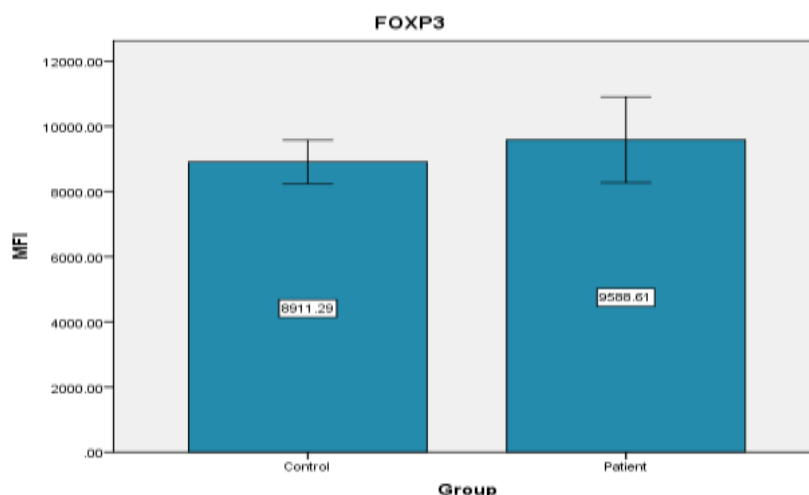


Fig.5: MFI of FoxP3 molecule on T regs

Forkhead Box P3 (FoxP3) is a master transcription factor that governs immune regulatory pathways by promoting anti-inflammatory gene expression and suppressing pro-inflammatory responses. Therefore, reduced FoxP3 expression may reflect impaired transcriptional control of immune regulation, leading to dysregulated immune responses (Wang *et al.*, 2025). The observed reduction in FoxP3 expression in tuberculosis patients is consistent with several previous studies that demonstrate altered regulation of this key transcription factor during chronic infection, as shown in Figure 4. Our result is compatible with a study conducted by Burl *et al.* (2007) performed a study of FoxP3 gene expression in TB contacts. They found that non-

infected contacts had lower levels of FoxP3 mRNA in blood than Mtb-infected contacts; they hypothesised that this may be due to the sequestering of Tregs in the lungs at early stages of infection and their subsequent reappearance in the periphery.

Expression of Tregs in peripheral blood in the control and the patient groups.

The result showed that TB patients had a higher mean Treg percentage (10.63%) than healthy controls (8.81%), suggesting enhanced regulatory immune activity in patients with tuberculosis.

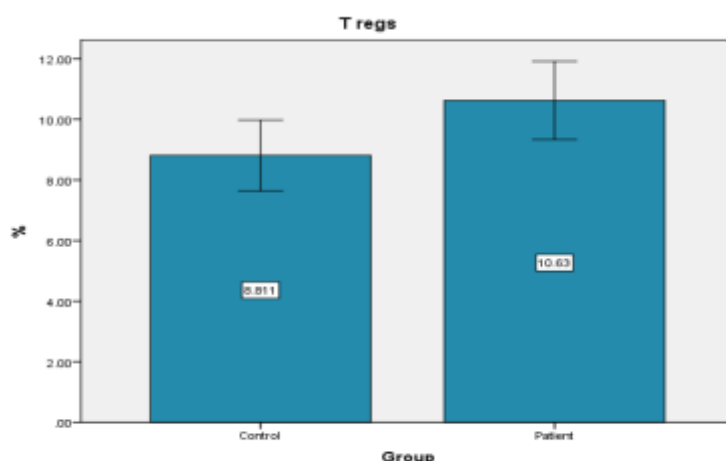


Fig. 6: Percentage of Tregs in peripheral blood in the control and patient groups.

The result demonstrated an increase in Treg mean fluorescence intensity (MFI) in tuberculosis patients (10.63) compared with healthy controls (8.81).

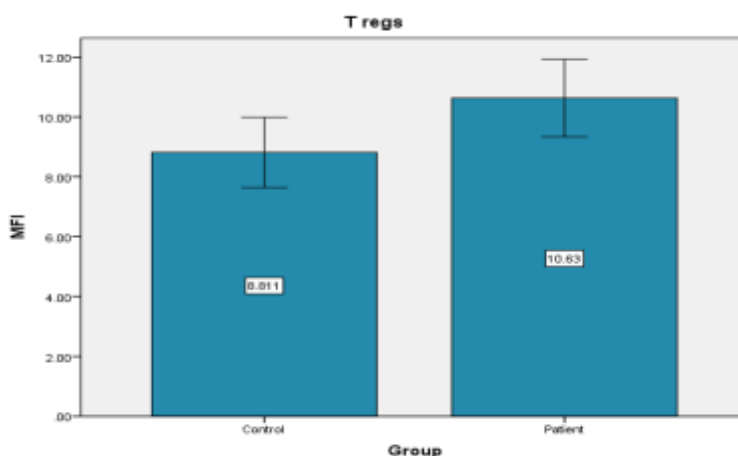


Fig. (7): MFI of T reg cells in the peripheral blood of the control and patient groups

The present study showed an increase in the percentage of regulatory T cells (Treg) in tuberculosis patients compared with controls, as shown in Figure (6). Tregs play a central role in immune response suppression and maintenance of immune homeostasis, and their expansion in tuberculosis is consistently associated with insufficient host defence (Ahmed and Vyakarnam, 2020). The higher Treg levels seen in female patients also seem to point to sex-based differences in immune regulation. This finding is supported by modern immunological evidence that females often have enhanced regulatory feedback, partly due to X-linked immune gene expression and endocrine factors, which is able to amplify Treg responses (Márquez *et al.*, 2020).

Chronic antigen exposure during *Mycobacterium tuberculosis* infection promotes the expansion and functional activation of Tregs, causing an immunosuppressive environment that favours pathogen persistence (Wang *et al.*, 2025). However, Tregs inhibit T-cell activation through multiple pathways, including secretion of inhibitory cytokines such as IL-10 and TGF- β , as well as modulation of antigen-presenting cell function and co-stimulatory signalling (Chia *et al.*, 2024).

Statistical Analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS), version 22. Data were presented as the mean $\pm$ standard error of the mean (SEM). Statistical significance was determined at various levels of probability: *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001.

CONCLUSION:

The study found that patients with tuberculosis had significantly lower CD4 T-cell levels and a higher frequency of regulatory T cells (Tregs) than healthy controls. This suggests impaired cell-mediated immunity alongside increased immune regulation during *Mycobacterium tuberculosis* infection, possibly contributing to bacterial persistence and disease progression.

EXCLUSION CRITERIA

Patients with HIV infection, autoimmune diseases, chronic inflammatory diseases, malignancy, Asthma, or those receiving immunosuppressive therapy were excluded from the study.

Inclusion Criteria

Newly diagnosed Pulmonary TB patients

ETHICAL APPROVAL:

The current study was approved by the Research Ethics Committee of the College of Science, University of Kerbala, as documented in the approval letter (No. 0048CSE, dated 17/6/2025).

ACKNOWLEDGEMENTS:

I am grateful to the staff of the biology department, College of Science, University of Kerbala, Iraq, for their support and assistance in finishing my studies and overcoming difficulties.

REFERENCES:

- [1] **Ahmed, A.**, & Vyakarnam, A. (2020). Emerging patterns of regulatory T cell function in tuberculosis. *Clinical and Experimental Immunology*, 202(3), 273–287.
- [2] **Bozzano, F.**, Marras, F., & De Maria, A. (2021). Immunology of tuberculosis. *Mediterr J Hematol Infect Dis*, 13(1).
- [3] **Burl S**, Hill PC, Jeffries DJ, Holland MJ, Fox A, Lugos MD, Adegbola RA, Rook GA, Zumla A, McAdam KP, Brookes RH. (2007). FoxP3 gene expression in a tuberculosis case contact study. *Clin Exp Immunol*. Jul;149(1):117-22.
- [4] **Chia, J.**, E., Rousseau, R., P., Ozturk, M, Poswayo, S. K., L., Lucas, R, Brombacher, F, Parihar, &, & S., P. (2024). The divergent outcome of IL-4R α signalling on Foxp3 T regulatory cells in listeriosis and tuberculosis. *Frontiers in Immunology*, 15, Article 1427055.
- [5] **Daniel L. Barber** . S. G., Sallin, M. A., Kauffman, K. D., Sakai, S., Ganusov, V. V., & Barber, D. L. (2019). The rate of CD4 T cell entry into the lungs during *Mycobacterium tuberculosis* infection is determined by partial and opposing effects of multiple chemokine receptors. *Infection and Immunity*, **87**(6), e00841-18.
- [6] **Flynn, J. L.**, & Chan, J. (2022). *Immune cell interactions in tuberculosis*. *Cell*, 185(25), 4682–4702.
- [7] **Gupta, Ravindra K.**, Lawn, Stephen D., Bekker, Linda-Gail, Caldwell, Jennifer, Kaplan, Robin, & Wood, Robin. (2013). Impact of human immunodeficiency virus and CD4 count on tuberculosis diagnosis: *Analysis of city-wide data from Cape Town, South Africa*. *The International Journal of Tuberculosis and Lung Disease*, 17(8), 1014–1022.
- [8] **Jacobo-Delgado, Y. M., Rodríguez-Carlos, A., Serrano, C. J., & Rivas-Santiago, B.** (2023). *Mycobacterium tuberculosis* cell-wall and antimicrobial peptides: A mission impossible? *Frontiers in Immunology*, 14, Article 1194923.
- [9] **Jayaraman, P.**, Jacques, M. K., Zhu, C., Steblenko, K. M., Stowell, B. L., Madi, A., Anderson, A. C., Kuchroo, V. K., & Behar, S. M. (2016). TIM3 mediates T cell exhaustion during *Mycobacterium tuberculosis* infection. *PLoS Pathogens*, 12(3), e1005490

- [10] **Kapata, N.**, & P (2013). Chanda-Kapata, and C. Michelo. "The Social Determinants of Tuberculosis and Their Association with TB/HIV Co-infection in Lusaka, Zambia." *Medical Journal of Zambia*, vol. 40, no. 2, pp. 48–54.
- [11] **Maciag, K.**, Plumlee, C. R., Cohen, S. B., Gern, B. H., & Urdahl, K. B. (2024). Reappraising the Role of T Cell-Derived IFN- γ in Restriction of *Mycobacterium tuberculosis* in the Murine Lung. *The Journal of Immunology*, 213(3), 339–346.
- [12] **Marion, N.** Owono, M., Boulingui, C. M., Ngue Mebale, M. E., & Bouyou Akotet, M. K. (2025). Clinico-radiological characteristics and lethality of HIV-tuberculosis coinfection in the Infectiology ward of the Libreville University Hospital, Gabon. *Southern African Journal of Infectious Diseases*, 40(1), 695.
- [13] **Márquez, E. J.**, Chung, C.-H., Marches, R., Rossi, R. J., Nehar-Belaid, D., Eroglu, A., Mellert, D. J., Kuchel, G. A., Banchereau, J., & Ucar, D. (2020). Sexual dimorphism in human immune system ageing. *Nature Communications*, 11, Article 751.
- [14] **Morgan, J.**, K. Muskat, R. Tippalagama, A. Sette, J. Burel, and C. S. Lindestam Arlehamn, (2021). Classical CD4 T Cells as the Cornerstone of Antimycobacterial Immunity, *Immunological Reviews* 301, no. 1: 10–29.
- [15] **Sakai, S.**, Kauffman, K. D., Sallin, M. A., Sharpe, A. H., Young, H. A., Ganusov, V. V., & Barber, D. L. (2016). CD4 T cell-derived IFN- γ plays a minimal role in control of pulmonary *Mycobacterium tuberculosis* infection and must be actively repressed by PD-1 to prevent lethal disease. *PLoS Pathogens*, 12(5), e1005667.
- [16] **Sarangi, A.**, Singh, S. P., Das, B. S., Rajput, S., Fatima, S., & Bhattacharya, D. (2024). Mycobacterial biofilms: A therapeutic target against bacterial persistence and generation of antibiotic resistance. *Heliyon*, 10(11), Article e32003.
- [17] **Wang, L.**, Liang, Y., Zhao, C., Ma, P., Zeng, S., Ju, D., Zhao, M., Yu, M., & Shi, Y. (2025). Regulatory T cells in homeostasis and disease: molecular mechanisms and therapeutic potential. *Signal Transduction and Targeted Therapy*, 10(1), 345.
- [18] **Warner, D. F.**, Barczak, A. K., Gutierrez, M. G., & Mizrahi, V. (2025). *Mycobacterium tuberculosis* biology, pathogenicity and interaction with the host. *Nature Reviews Microbiology*, 23(12), 788–804.
- [19] **Wing, J. B.**, Tanaka, A., & Sakaguchi, S. (2019). Human FOXP3+ regulatory T cell heterogeneity and function in autoimmunity and cancer. *Immunity*, 50(2), 302–316.