Al-Nisour Journal for Medical Sciences

Manuscript 1155

The Role of Regulatory T Cells in Ischemic Heart Disease Patients

Duha Alaa Hassan

Hayfaa Mahmood Fahad

Saad Fadhel Nassar

Follow this and additional works at: https://journal.nuc.edu.iq/home

 Part of the Medical Sciences Commons

AL-NISOUR JOURNAL FOR MEDICAL SCIENCE 2025;7:73–78

Scan the QR to view
the full-text article on
the journal website

ORIGINAL STUDY

The Role of Regulatory T Cells in Ischemic Heart Disease Patients

Duha Alaa Hassan [a,*], Hayfaa Mahmood Fahad [a], Saad Fadhel Nassar [b]

[a] Department of Microbiology, College of Medicine, Al-Iraqia University, Baghdad, Iraq
[b] Department of Internal Medicine, College of Medicine, Al-Iraqia University, Baghdad, Iraq

Abstract

Background: Regulatory T cells (Tregs) represent 5–10% of the total T cell population and are known as potent suppressors of the immune system. They are crucial for managing immune activation, ensuring peripheral tolerance, and supporting tissue repair and overall homeostasis. These cells are characterized by the expression of specific biomarkers, including CD4+, FOXP3+, and CD25+. While CD4 and CD25 were initially used for their identification, the discovery of CD25 on other T cell types led to a more precise evaluation of Tregs through the assessment of the intracellular Foxp3 transcription factor.

Objective: The primary goal of this study was to determine the function of regulatory T cells in patients with ischemic heart disease by measuring the concentrations of their key markers (CD4+, CD25+, and FOXP3+).

Patients and Methods: This was a case-controlled study that included 45 patients with a clinical diagnosis of IHD and 45 healthy individuals as a control group. Of the patients, 30 (66.7%) were male and 15 (33.3%) were female. Samples were collected from a specialized cardiac center in Baghdad, Iraq, between October and December 2022. Serum levels of CD4, CD25, and FOXP3 were quantitatively measured using the Enzyme-Linked Immunosorbent Assay (ELISA) according to the manufacturer's protocol.

Results: The mean CD4 level was found to be significantly higher in the patient group compared to the control group. Similarly, the mean CD25 level was also significantly elevated in the IHD group. In contrast, the mean FOXP3 level was significantly lower among the patients compared to the healthy controls.

Conclusion: The study found that males were more commonly affected by IHD than females. It also revealed a notable imbalance in the T regulatory cell markers, with significantly higher levels of CD4+ and CD25+ and significantly lower levels of FOXP3+ in the patient group, highlighting their potential role in the disease pathology.

Keywords: Ischemic heart disease, Treg cells, CD4+, CD25+, FOXP3+

1. Introduction

Ischemic heart disease (IHD), a type of cardiovascular disease (CVD), results from a narrowing of blood arteries caused by plaque buildup on vessel walls. This plaque, which includes fat, cholesterol, calcium, and other substances, can restrict blood flow and reduce the heart's oxygen supply, often leading to chest discomfort and diminished patient activity. Furthermore, plaque can lead to blood clots and blocked blood flow, which may trigger a chest discomfort episode and result in death (Suryati & Suyitno, 2020).

The primary cause of ischemic cardiovascular diseases is atherosclerosis (Alisherovna et al., 2022). Atherosclerosis is an artery disease where plaques composed of lipids, inflammatory cells, and fibrous materials accumulate. Interventions that target the onset, progression, and stability of atherosclerotic lesions are crucial since this is the main reason for coronary artery disease (CAD) development (Silvis et al., 2021).

From naive CD4+ T cells, various lineages differentiate, including T helper type 1 (Th1), T helper type 2 (Th2), and T helper type 17 (Th17), as well as T regu-

Received 21 August 2025; accepted 17 September 2025.
Available online 4 October 2025

* Corresponding author.
E-mail address: duhaalaa1997@gmail.com (D. A. Hassan).

https://doi.org/10.70492/2664-0554.1155
2664-0554/© 2025 The Author(s). Al-Nisour University College.

latory (Treg) and T follicular helper (Tfh) cells. These effector lineages play a vital role in the development of atherosclerosis (Zhu & Zhu, 2020).

Tregs, which constitute approximately 5–10% of all T cells, are potent inhibitors of immune activation. They promote peripheral tolerance, tissue healing, and homeostasis. Tregs perform their regulatory function by secreting anti-inflammatory cytokines, engaging in direct cell-to-cell interactions, and competing with effector T cells (Tregs) for interleukin-2 (IL-2), thus controlling Teff proliferation (Zhao et al., 2020). Treg deficiency accelerates atherosclerosis in preclinical models, while Treg therapy slows it down. Additionally, Tregs play a crucial role in regulating post-ischemic immune responses and promoting cardiac repair in Myocardial Infarction (MI) model organisms (Zhao & Mallat, June 2022). While pro-inflammatory Tregs are expanded and activated in patients with MI, observational studies have shown a decline in the number and activity of Tregs in these patients (Mallat & Binder, 2022).

Tregs are essential for controlling immunological activation and enforcing tolerance. Some Tregs are located in non-lymphoid tissues, where they regulate sterile inflammation and maintain tissue homeostasis (Zhao et al., 2022).

Tregs express the biomarkers CD4, FOXP3, and CD25. Initially, CD4 and CD25 surface markers were used to identify Tregs (CD4+CD25+ cells). However, because CD25+ was also found on other non-Treg T cells, the investigation of Tregs became more focused by assessing the intracellular expression of the Foxp3 transcription factor (Mostafa et al., 2021).

1.1. Protective impact of tregs in atherosclerosis

Sakaguchi first identified natural Tregs originating from the thymus in 1995. For their differentiation and functionality, Tregs constitutively express significant quantities of the transcription factor Foxp3 and the CD25 (IL-2 receptor beta-chain) protein (Joly et al., 2018). Multiple lines of evidence suggest that Tregs prevent atherosclerosis by limiting harmful immunoinflammatory reactions. Naive T cells in the periphery, such as in gut-associated lymphoid tissue (GALT), can also produce peripherally derived Tregs (pTregs), which maintain mucosal immunological tolerance and decrease autoimmune reactions. Low numbers of FOXP3+ Tregs have been found throughout atherosclerotic plaques (Depuydt et al., 2020).

Tregs' overexpression of IL-10 inhibits the development of atherosclerosis (Proto et al., 2018). The CD4+Foxp3+ Tregs in the aorta and secondary lymphoid organs can transform into dysfunctional Th1-like cells (Ali et al., 2020). As a result, IL-10 triggers the M1 macrophage to switch to the M2 phenotype, which reduces the production of IFN-, IL-1, and proteolytic enzymes while increasing collagen synthesis and smooth muscle cell (SMC) proliferation, thereby reducing the severity of atherosclerosis (Ou et al., 2018).

1.2. Biomarkers

CD4: (Cluster of Differentiation 4) CD4 is a glycoprotein that may be present on the surface of CD4 cells. CD4 cells, which include T helper cells, monocytes, macrophages, and dendritic cells, are crucial components of the human immune system (Bai et al., 2020).

CD4 functions as a T cell coreceptor and a key surface antigen for recognizing Th cells, in addition to being involved in signal transduction. Although much research has been done on using CD4 as a T cell surface marker to distinguish CD4+ Th cells, its role in signal transduction is still not fully understood (Tian et al., 2022). Mammals lack a substantial extracellular region; however, they do have a transmembrane domain and a crucial cytoplasmic tail with a conserved motif (CXC) that interacts with the tyrosine kinase Lck. Antigen-presenting cells (APCs) express the MHC class II complex, which interacts with the extracellular D1 and D2 regions of CD4. The Lck protein tyrosine kinase then noncovalently binds with the cytoplasmic tail of CD4 to initiate T cell activation (Tran et al., 2020).

Forked Box P3 (FOXP3): Foxp3 is a marker for human Tregs, which regulate the release of the anti-inflammatory cytokine IL-10 and are essential for controlling immune tolerance and anti-inflammatory responses (He et al., 2020).

FoxP3 belongs to the forkhead transcription factor family and is primarily found in the nucleus. Genome-wide investigations of FoxP3 target genes have shown that FoxP3 may bind to up to 700 different genes, as well as miRNAs that participate in transcriptional regulation, the T cell receptor (TCR) signaling pathway, and cell communication. This complexity emphasizes the crucial role FoxP3 plays in modifying transcriptional and receptor signaling networks throughout the development and maintenance of Treg cells (Deng et al., 2019).

Through interactions with other transcription factors, Foxp3 controls Treg differentiation, maintenance, and functional maturation. Additionally, it suppresses CD4+ T cell proliferation while upregulating the expression of CD25 and other Treg markers

and inhibiting IL-2 production. Peripheral CD4+ T cells and CD4 single positive (SP) cells are present in 10-15% of healthy mice (Ono, 2020).

FoxP3 directly targets only a small number of genes in the overall program of FoxP3-dependent gene expression, indicating that it indirectly controls a large portion of the transcriptional program by interacting with other transcription factors. In Treg cells, FoxP3 functions as both a transcriptional activator and a repressor of its target genes. FoxP3 and FoxP1 heterodimerize, enforcing FoxP3-mediated control of gene expression and coordinating regulatory T cell function (Konopacki et al., 2019).

CD25 marker: Soluble Cluster of Differentiation 25 (sCD25), also known as soluble Interleukin-2 receptor (sIL2R), is a sign of inflammation and a diagnostic indicator of immune system activation (Zahraini et al., 2022). It is regarded as the most visible indication of cellular activity. Several subsets of peripheral blood lymphocytes, including regulatory and resting memory T cells, inherently display it on their surface (Kasahara et al., 2022).

When T cells, B cells, and dendritic cells are activated, the CD25 receptor is cleaved, resulting in soluble CD25 (sCD25). The protease enzyme matrix metalloproteinase-9 (MMP-9), which is produced by T cells, macrophages, and dendritic cells stimulated by inflammatory conditions, removes the CD25 receptor from the cell membrane. Cleaving CD25 to create sCD25 can also reveal the proliferation and activation of T cells as well as the activity of IL-2 linked to its receptors (Webb et al., 2020).

2. Materials and methods

This case-controlled study included 45 patients clinically diagnosed with IHD and 45 healthy controls. The participants were between the ages of 40 and 73. The samples were collected during the period from 4 October to 31 December 2022 at the Ibn Al-Bitar Specialized Center for Cardiac Surgery in Iraq. Verbal consent was obtained from the patients for the process of collecting samples and conducting the research.

The patient group consisted of individuals in the Intensive Care Unit (ICU) and Catheter Lobbies. They all had Coronary Artery Disease Progression, had undergone angioplasty, and suffered from Diabetes Mellitus (DM) and Hypertension, with 90% of patients being smokers. All control individuals were basically healthy with no Coronary artery disease. All patients were taking drugs such as Aspirin, Insulin, Crestor, Heparin, and Brilinta. The study groups consisted of two groups: Ischemic Heart Disease patients included 45 patients (30 males, 15 females), and 45 healthy individuals (27 males, 18 females).

The entire sample was used in this investigation. Each patient's veins were pricked for five milliliters of blood, as were those of the healthy control groups. A sample was placed in a gel tube and allowed to coagulate for about 30 minutes. Serum was then isolated. The Enzyme-Linked Immune Sorbent Assay (ELISA) method was then used to quantitatively evaluate the serum levels of CD4, CD25, and FOXP3. ELISA testing was performed according to the manufacturer's recommendations (Mybiosource, USA). The optical densities of each well were measured with an ELISA reader at 450 nm, and the results for the concentrations of CD4, CD25, and FOXP3 were extrapolated from the standard curve.

2.1. Statistical analysis

Statistical analysis for this study was carried out using IBM SPSS versions 25.0. Data were checked for normal distribution. Homogeneity and randomization were calculated. For parametric data, mean and standard deviation, T-test, ANOVA table, and Pearson correlation were used to establish significant differences, while for non-parametric data, Pearson's chi-square test was also used.

2.2. Ethical approval

Experimental design was carried out based on the guidelines of the laboratory and according to the protocol approved by the College of Medicine/Department of Microbiology/Al-Iraqia University, Baghdad, Iraq, with the ethical clearance number 195 dated 12 September 2022.

3. Results

3.1. Baseline characteristics of study samples

A total of 90 participants (45 cases and 45 controls) were investigated. The ages of the study sample ranged from 40 to 73 years, with a mean of 57.89 ± 7.581 years. There were no significant differences in age or gender between the patient and control groups.

3.2. Comparison of immunological parameters among study's groups

The mean level of CD4 was significantly higher in the patient group compared to controls (1.31767 ± 0.129556 vs. 0.71433 ± 0.107324).

The mean level of the immunological parameter CD25 was also significantly higher in the IHD group compared to the control group (1.30562 ± 0.154170 vs. 0.58302 ± 0.089259).

Table 1. Baseline characteristics of the study sample (n=90).

Characteristics	Study groups (IHD)			
	Cases (Yes, n=45)	Control (No, n=45)	Total (n=90)	Significancy
Age (years)				
Mean ± SD	57.29 ± 7.516	58.49 ± 7.683	57.89 ± 7.581	*t*= -0.749, df: 88, *P*= 0.456[a]
Range (min-max)	33 (40–73)	30 (40–70)	33 (40–73)	
Age (In groups)				
≤ 46	3 (6.7)	5 (11.1)	8 (8.9)	x^2: 7.204, df: 4, *P* = 0.125 [b]
47–53	14 (31.1)	4 (8.9)	18 (20)	
54–60	13 (28.9)	17 (37.8)	30 (33.3)	
61–67	11 (24.4)	15 (33.3)	26 (28.9)	
> 67	4 (8.9)	4 (8.9)	8 (8.9)	
Gender				
Female	15 (33.3)	18 (40)	33 (36.7)	x^2: 0.431, df: 1 *P* = 0.512 [b]
Male	30 (66.7)	27 (60)	57 (63.3)	

Table 2. Mean comparison of immunological parameter of CD4 among study's groups (n=90).

Immunological Parameters (Mean ± SD)	Study groups (IHD) (n=90)		Mean differences	Significance [a]
	Cases (n = 45)	Control (n = 45)		
CD4	1.312878 ± 0.102548	0.72100 ± 0.095323	0.607778	*t*= 29.120, df:88, *P*= 0.000

However, the mean level of the immunological parameter FOXP3 was significantly lower in the patient group compared to the control group (0.64424 ± 0.111555 vs. 1.24971 ± 0.122511).

4. Discussion

Coronary atherosclerotic disease (CAD) is characterized by the accumulation of lipids and fibrous material in the coronary circulation, which can lead to conditions like myocardial infarction (MI) and unstable angina pectoris. The current study's finding of increased CD4 T cell accumulation in atherosclerotic lesions is consistent with previous research.

CD4+ T cells also express the T-cell interleukin-2 alpha receptor (sIL-2Ra), or CD25, at significantly higher levels in patients with an active disease compared to healthy individuals. The present study's

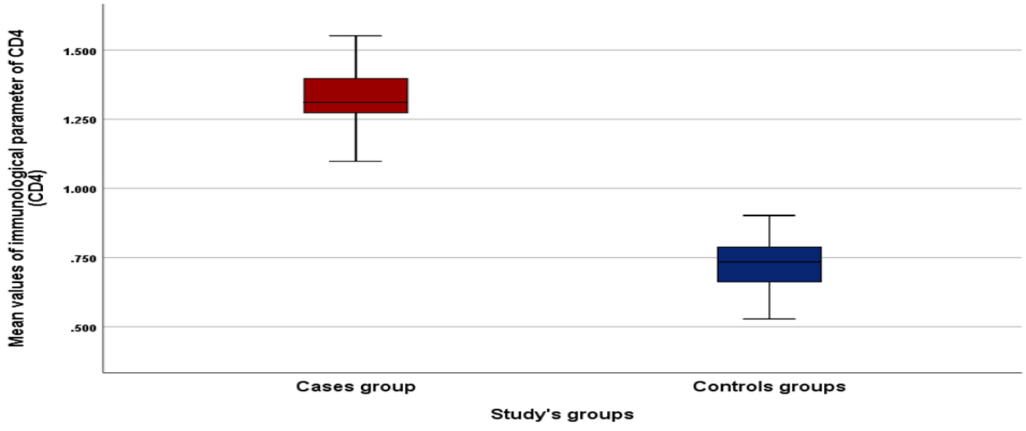

Fig. 1. Mean comparison of immunological parameter of CD4 among study's groups (n=90).

Table 3. Mean comparison of immunological parameter of CD25 among study's groups (n=90).

Immunological Parameters (Mean ± SD)	Study groups (IHD) (n=90)		Mean differences	Significance [a]
	Cases (n = 45)	Control (n = 45)		
CD25	1.30562 ± 0.154170	0.58302 ± 0.089259	**0.722600**	*t*= 27.210, df:88, *P*= 0.000

a: Unpaired T-Test.

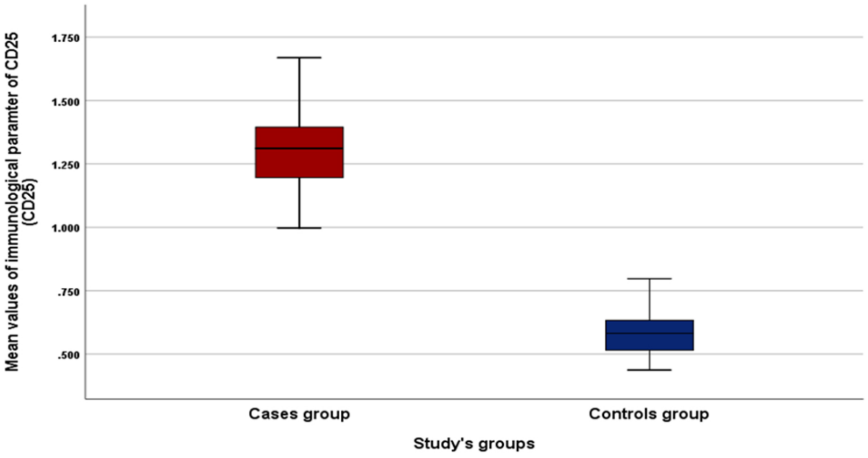

Fig. 2. Mean comparison of immunological parameter of CD25 among study's groups (n=90).

Table 4. Mean comparison of immunological parameter of FOXP3 among study's groups (n=90).

	Study groups (IHD) (n = 90)			
Immunological Parameters (Mean ± SD)	Cases (n = 45)	Control (n = 45)	Mean differences	Significance [a]
FOXP3	0.64424 ± 0.111555	1.24971 ± 0.122511	-0.605467	*t*= -24.513, df:88, *P*= 0.000

a: Unpaired T-Test.

results, which show significantly higher CD25 levels in the IHD group, align with this observation. However, the use of Foxp3 for more specific analysis of Tregs became necessary because CD25 is expressed on other non-Treg T cells.

Human regulatory T cells, identified by the FoxP3 marker, are believed to play a role in the pathogenesis of atherosclerosis by inhibiting pro-atherogenic immune mechanisms. The depletion of Foxp3+ Tregs has been shown to result in a significant increase in CD4 T cell accumulation within atherosclerotic lesions. This is consistent with our findings that the mean level of FOXP3 was significantly lower in the patient group.

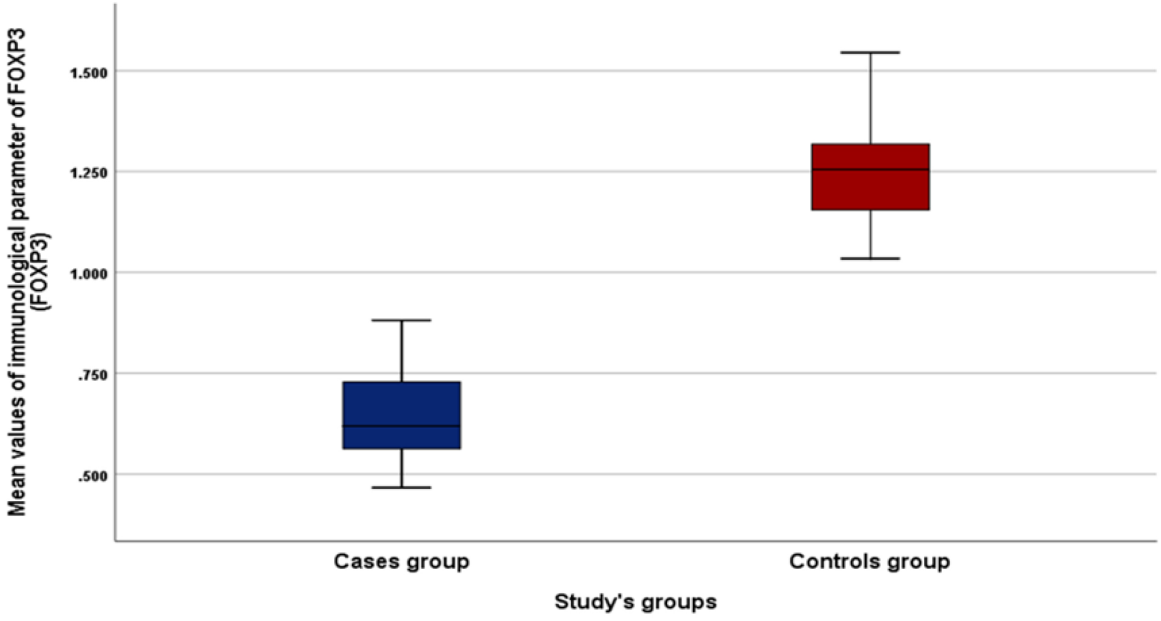

Fig. 3. Mean comparison of immunological parameter of FOXP3 among study's groups (n=90).

5. Conclusion

In conclusion, this study found that males were more frequently affected by IHD than females. We also found that the mean levels of CD4 and CD25 were significantly higher in the IHD group compared to controls, while the mean level of FOXP3 was significantly lower. These findings highlight the importance of T regulatory cells and their markers in understanding the immune response in ischemic heart disease.

References

Suryati, T. & Suyitno. (2020) Prevalence and risk factors of the ischemic heart diseases in Indonesia: a data analysis of Indonesia basic health research (riskesdas) 2013. *Public Health of Indonesia*, 6(4), 138–144. DOI: https://dx.doi.org/10.36685/phi.v6i4.366.

Alisherovna, K.M., Toshtemirovna, E.M., Totlibayevich, Y.S., *et al.* (2022) Effectiveness of Statins in the Prevention of Ischemic Heart Disease. *International scientific research journal*, 3(10), 979–2776.

Silvis, M.J., Demkes, E.J., Fiolet, A.T., *et al.* (2021) Immunomodulation of the NLRP3 Inflammasome in Atherosclerosis, Coronary Artery Disease, and Acute Myocardial Infarction. *Journal of Cardiovascular Translational Research*, 14, 23–34. https://doi.org/10.1007/s12265-020-10049-w.

Zhu, X. & Zhu, J. (2020) CD4 T Helper Cell Subsets and Related Human Immunological Disorders. *Int J Mol Sci.*, Oct 28, 21(21), 8011. 10.3390/ijms21218011.

Zhao, T.X., Newland, S.A., & Mallat, Z. (2020) 2019 ATVB plenary lecture: interleukin-2 therapy in cardiovascular disease: the potential to regulate innate and adaptive immunity. *Arterioscler Thromb Vasc Biol*, 40, 853–864. https://doi.org/10.1161/ATVBAHA.119.312287.

Zhao, T. & Mallat, Z. (June 2022) Adapting treatments for adaptive immunity in ischaemic heart disease. *Cardiovascular Research*, 118(9), e66–e68, https://doi.org/10.1093/cvr/cvac074.

Mallat, Z. & Binder, C.J. (2022) The why and how of adapative immune responses in ischaemic cardiovascular disease. *Nat Cardiovasc Res*, 1, 431–444. https://doi.org/10.1038/s44161-022-00049-1.

Zhao, T.X., Sriranjan, R.S., Tuong, Z.K., *et al.* (2022) Regulatory T-cell response to low-dose interleukin-2 in ischemic heart disease. *NEJM evidence*, 1(1), EVIDoa2100009.

Mostafa, G., Ibrahim, H.M., El Gendy, Y.G., *et al.* (2021) Frequency of CD4+CD25+Foxp3+ regulatory T cells (Tregs) in peripheral blood of pediatric patients with SARS CoV-2 Infection. *Egypt J Pediatr Allergy Immunol*, 19(2), 79–88.

Joly, A.L., Seitz, C., Liu, S., *et al.* (2018) Alternative splicing of FOXP3 controls regulatory T cell effector functions and is associated with human atherosclerotic plaque stability. *Circ. Res.*, 122, 1385–1394.

Depuydt, M.A.C., Prange, K.H.M., Slenders, L., *et al.* (2020) Microanatomy of the human atherosclerotic plaque by single-cell transcriptomics. *Circ. Res.*, 127, 1437–1455.

Proto, J.D, Doran, A.C, Gusarova, G., *et al.* (2018) Regulatory T cells promote macrophage efferocytosis during inflammation resolution. *Immunity*, 49, 666–677.

Ali, M., Lurwan, M., Halliru, S.N., *et al.* (2020) Role of T-Helper cells (CD4+ T Cells) in human immune system against some microbial infection: A mini review. *Int J Clin Microbiol Biochem Technol*, 3, 026–029. 10.29328/journal.ijcmbt.1001012.

Ou, H.X., Guo, B., Liu, Q., *et al.* (2018) Regulatory T cells as a new therapeutic target for atherosclerosis. *Acta Pharmacologica Sinica*, 39, 1249–1258. DOI:10.1038/aps.

Bai, J., Zhang, L., Zhao, Z., *et al.* (2020) Expression of melatonin receptors and CD4 in the ovine thymus, lymph node, spleen and liver during early pregnancy. *The journal of cells, Molecules, Systems and technologies*, 160, 52–63. doi/10.1111/imm.13180.

Tian, H., Xing, J., Tang, X., *et al.* (2022) Cluster of diferentiation antigens: essential roles in the identifcation of teleost FSH T lymphocytes. *Marine Life Science & Technology*, 4, 303–316 https://doi.org/10.1007/s42995-022-00136-z.

Tran, H.B., Risky, P.N., Padgett, S.R.M., *et al.* (2020) Molecular characterization of cobia (Rachycentron canadum) CD4 homologues revealed the frst evidence of soluble CD4 in FSH. *Fish Shellfsh Immuno*, 99, 239–242.

He, X., Liang, B., & Gu, N. (2020) Th17/Treg Imbalance and Atherosclerosis. *Hindawi Disease Markers*, 2020, 8, Article ID 8821029, https://doi.org/10.1155/2020/8821029.

Deng, G., Song, X., Fujimoto, S., *et al.* (2019) Foxp3 post-translational modifications and Treg suppressive activity. *Frontiers in immunology*, 10, 2486. https://doi.org/10.3389/fimmu.2019.02486.

Ono, M. (2020) Control of regulatory T-cell differentiation and function by T-cell receptor signalling and Foxp3 transcription factor complexes. *Journals of cells, molecules, systems and teqnologies*, 160, 24–37. doi:10.1111/imm.13178.

Konopacki, C., Pritykin, Y., Rubtsov, Y., *et al.* (2019) Transcription factor Foxp1 regulates Foxp3 chromatin binding and coordinates regulatory T cell function. *Nat Immunol*, 20, 232–42.

Zahraini, H., Tambunan, B.A., & Semedi, B.P. (2022) Soluble Cluster of Differentiation 25 (sCD25) as a Predictor of Mortality of COVID-19 Patients in Surabaya, Indonesia. *Indian Journal of Forensic Medicine & Toxicology*, 16(1).

Kasahara, K., Sasaki, N., Amin, H.Z., *et al.* (2022) Depletion of Foxp3+ regulatory T cells augments CD4+ T cell immune responses in atherosclerosis-prone hypercholesterolemic mice. *Heliyon*, 8(7), e09981.

Webb, B. J., Peltan, I. D., Jensen, P., *et al.* (2020) Clinical criteria for COVID-19-associated hyperinflammatory syndrome: a cohort study. *The Lancet Rheumatology*, 2(12), e754–e763.

Gisterå, A. & Hansson, G.K. (2017) The immunology of atherosclerosis. *Nat Rev Nephrol*, 13, 368–380.

Krishnaswamy, J.K., Alsén, S., Yrlid, U., *et al.* (2018) Determination of T follicular helper cell fate by dendritic cells. *Front Immunol*, 9, 2169.

Dekkema, G.J., Abdulahad, W.H., Bijma, T., *et al.* (2019) Urinary and serum soluble CD25 complements urinary soluble CD163 to detect active renal anti-neutrophil cytoplasmic autoantibody-associated vasculitis: a cohort study. *Nephrology Dialysis Transplantation*, 34(2), 234–242.

Jackowska, P., Chałubiński, M., Łuczak, E., *et al.* (2019) The influence of statin monotherapy and statin-ezetimibe combined therapy on FoxP3 and IL 10 mRNA expression in patients with coronary artery disease. *Advances in Clinical and Experimental Medicine*, 28(9).

Foks, A.C., Lichtman, A.H., & Kuiper, J. (2015) Treating atherosclerosis with regulatory T cells. *Arteriosclerosis, thrombosis, and vascular biology*, 35(2), 280–287. https://doi.org/10.1161/ATVBAHA.114.303568.

Saigusa, R., Winkels, H., & Ley, K. (2020) T cell subsets and functions in atherosclerosis. *Nature Reviews Cardiology*, 17(7), 387–401.