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# Homocysteine induced upregulation of cell surface molecules in human peripheral blood mononuclear cells and U937 monocyte like cells

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Hyperhomocysteinemia is recognised as an independent risk factor for atherosclerosis; however, its underlying mechanisms remain unclear. The chemotaxis and accumulation of leukocytes, including monocytes and T cells, are critical events in the initiation and progression of atherosclerosis. This study aimed to investigate the effects of homocysteine (Hcy) on activation markers in lymphocytes and monocytes by analysing cell surface molecules using human peripheral blood mononuclear cells (PBMCs) and U937 monocyte like cells. Flow cytometry was employed to assess the expression of CD3, CD11a, CD27, CD45, and HLA-DR in peripheral blood lymphocytes, as well as CD11a, CD11c, CD14, CD45, CD63, CD71, HLA-DR, and ICAM-1 (CD54) in peripheral blood monocytes following a 24-hour incubation with Hcy at concentrations of 5, 25, and 50 µg/mL. In addition, CD71 and ICAM-1 expression was evaluated in U937 cells after exposure to Hcy at 25, 50, 100, and 200 µg/mL. Treatment of PBMCs with Hcy resulted in a dose-dependent increase in CD11a,CD45, and HLA-DR expression on both lymphocytes and monocytes. Additionally, CD3 and CD27 were upregulated in lymphocytes, while CD11c, CD14, CD63, CD71, and ICAM-1 were upregulated in monocytes. Similarly, exposure of U937 cells to Hcy led to a significant, dose-dependent increase in CD71 and ICAM-1 expression. Homocysteine significantly enhances the expression of cell surface molecules involved in activation, inflammation, antigen presentation, and leukocyte extravasation in lymphocytes and monocytes. These findings suggest that homocysteine-induced activation of immune cells could play a role in the pathogenesis of inflammatory conditions such as atherosclerosis.

**Keywords**: Activation marker, Adhesion, Atherosclerosis, Flow cytometry, Immune cells, Inflammation

Elevated levels of homocysteine (Hcy), a condition known as hyperhomocysteinemia (HHcy), have been identified as an independent risk factor for cardiovascular diseases, including atherosclerosis. The pathophysiological mechanisms through which Hcy contributes to vascular diseases are multifactorial, involving endothelial dysfunction, oxidative stress, and inflammation[1]. Among these processes, the activation of monocytes and lymphocytes is crucial for the initiation and progression of atherosclerotic lesions[2,3].

Lymphocytes and monocytes express various cell surface molecules that act as activation markers, such as CD3, CD11a, CD27, CD45, and HLA-DR on lymphocytes, and CD11a, CD11c, CD14, CD45, CD63, CD71 (transferrin receptor), HLA-DR, and intracellular adhesion molecule-1 (ICAM-1) on monocytes[4]. The upregulation of these molecules facilitates leukocyte adhesion, migration, and antigen presentation, which are key processes involved in inflammation and atherogenesis. Previous studies have shown that Hcy modulates adhesion molecules and chemokines in endothelial cells and monocytes[5-8]. Hcy increases vascular cell adhesion molecule-1 (VCAM-1) expression in endothelial cells, promoting monocyte adhesion, and stimulates the release of monocyte chemoattractant protein-1 (MCP-1) and interleukin-8 (IL-8) in monocytes, enhancing leukocyte recruitment to inflammatory sites[9,10]. Despite these findings, the direct effects of Hcy on the expression of activation markers in both monocytes and lymphocytes remain insufficiently explored.

The U937 cell line, a human monocyte-like cell line, serves as a valuable model to investigate the molecular mechanisms of monocyte activation and differentiation. It has been demonstrated that

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U937 cells are effectively utilised to explore key immune responses, such as the regulation of mitochondrial dynamics and innate immune activity, particularly in the context of DDIT4L (DNA damage inducible transcript 4 like) overexpression[11]. Additionally, U937 cells have been used to investigate the cellular mechanisms of atherosclerosis, where FURIN (proprotein convertase subtilisin/kexin type 3, PCSK3) inhibition was found to influence monocyte migration, macrophage proliferation, and cytokine production[12]. Moreover, U937 cells have been employed to examine exosomal miRNA cargo, specifically miR-24, revealing its role in macrophage activation and intercellular communication[13]. In the current study, both human peripheral blood mononuclear cells (PBMCs) and U937 cells were used, as each model provides distinct advantages. Human PBMCs provide a more physiologically relevant context, while U937 cells offer a more standardized experimental approach, enabling a comprehensive exploration of the effects of Hcy on immune cell activation.

To the best of our knowledge, this is the first study to assess the dose-dependent effects of Hcy on the expression of several surface molecules in lymphocytes and monocytes. Specifically, it investigates the impact of Hcy on surface molecules CD3, CD11a, CD11c, CD14, CD27, CD45, CD63, CD71, HLA-DR, ICAM-1 (CD54) and CD71 that regulate leukocyte adhesion, migration, and antigen presentation in human PBMCs and U937 monocyte-like cells simultaneously. Furthermore, the study evaluates the modulation of these markers following increasing Hcy concentrations, providing insights into the dose-dependent role of Hcy in lymphocyte and monocyte activation within the context of inflammatory diseases, particularly atherosclerosis.

## Materials and Methods

### Flow cytometry analysis

PBMCs from healthy individuals were isolated by Ficoll density gradient method. Human PBMCs were incubated with Hcy at concentrations of 5, 25, and 50 µg/mL for 24 hours. U937 monocyte-like cells (American Type Culture Collection, Rockville, MD) were exposed to increasing concentrations of Hcy (25, 50, 100, and 200 µg/mL) for 24 hours.

The surface molecules of PBMCs and U937 cells were analysed following the immunofluorescence procedure recommended by Beckman Coulter. Briefly, PBMCs and U937 cells were incubated with 10µL FITC, PE labeled mouse anti-human monoclonal antibodies in the dark for 15 minutes at room temperature. A Beckman Coulter Navios Flow Cytometry Device (Miami, CA, USA) was used to assess the phenotype analysis of PBMCs and U937. For each analysis, 20000 cells were used. In lymphocyte analysis, CD3, CD11a, CD27, CD45 and HLA-DR expressions were evaluated. For the monocytes analysis, CD11a, CD11c, CD14, CD45, CD63, CD71, HLA-DR, and ICAM-1 (CD54) were used. For U937 cells, the expression of CD71 and ICAM-1 (CD54) were evaluated. MFI values were used to compare surface molecules.

### Determination of homocysteine doses

In the preliminary study, cell death was detected after administering 400 µg/mL of Hcy; therefore, the maximum Hcy dose was set at 200 µg/mL, which is the highest concentration at which cell viability was maintained. Furthermore, varying concentrations of Hcy were analysed to assess their influence on surface activation markers in human PBMCs and U937 monocyte-like cells. As a result, concentrations demonstrating a more pronounced dose-response relationship were chosen to better elucidate Hcy′s dose-dependent effects.

### Selection of cell types

Lymphocytes and monocytes were selected for this study due to their central roles in immune responses and inflammation. Lymphocytes, including T and B cells, are critical in adaptive immunity and have been shown to be directly involved in the pathogenesis of atherosclerosis through their activation and interaction with endothelial cells. Monocytes, on the other hand, are key players in the innate immune system and are implicated in the initiation and progression of atherosclerotic lesions. Upon activation, monocytes differentiate into macrophages, which contribute to the inflammatory microenvironment within vascular tissues. Given their involvement in both inflammation and immune modulation, these cell types provide valuable insights into the effects of Hcy on immune cell activation and its potential role in inflammatory diseases such as atherosclerosis.

### Statistical analysis

Data analysis was conducted using Jamovi 2.3.21, and the results are presented as mean ± standard deviation (SD). The Shapiro-Wilk test was employed to assess normality. For non-normally distributed

variables, the Kruskal-Wallis test was applied, followed by the Mann-Whitney U test with Bonferroni adjustment for subgroup comparisons. A $P$-value < 0.05 was considered statistically significant.

## Results

### Hcy in human peripheral blood lymphocytes

Hcy exposure significantly and dose dependently increased the expression of various surface molecules on human peripheral blood lymphocytes. Specifically, Hcy upregulated CD3, CD11a, CD27, CD45, and HLA-DR, with higher doses leading to more pronounced expression of these markers. The most significant increase in marker expression was observed at the highest concentration of Hcy (50 µg/mL), where expression levels were significantly higher compared to both the control group and the lowest dose of Hcy (5 µg/mL). In addition, the 25 µg/mL dose of Hcy also significantly increased the expression of CD3, CD11a, and CD27 when compared to the control group (Fig. 1 & 2).

### Hcy in human peripheral blood monocytes

Similarly, Hcy exposure resulted in a significant, dose-dependent increase in the expression of several surface molecules on human peripheral blood monocytes. The markers CD11a, CD11c, CD14, CD45, CD63, CD71, HLA-DR, and ICAM-1 (CD54)

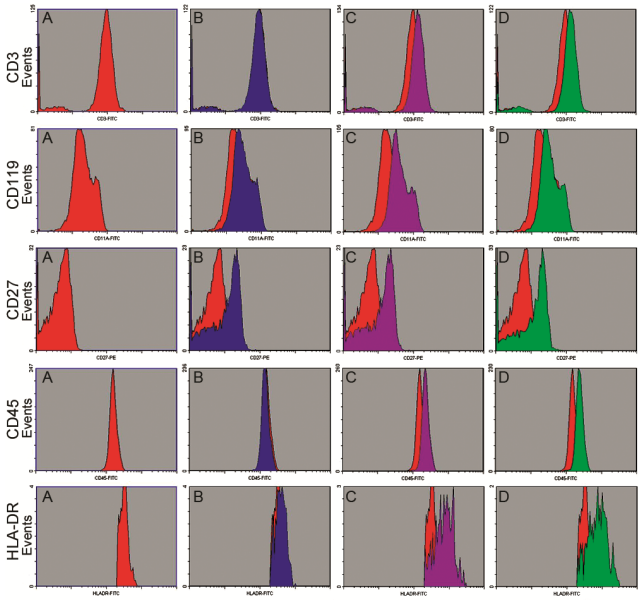


Fig. 1 — Flow cytometry histogram of CD3 (A), CD11a (B), CD27 (C), CD45 (D) and HLA-DR (E) surface molecules in human peripheral blood lymphocytes at different Hcy concentrations. [A-Control, B-Hcy 5 µg/mL, C-Hcy 25 µg/mL, D-Hcy 50 µg/mL]

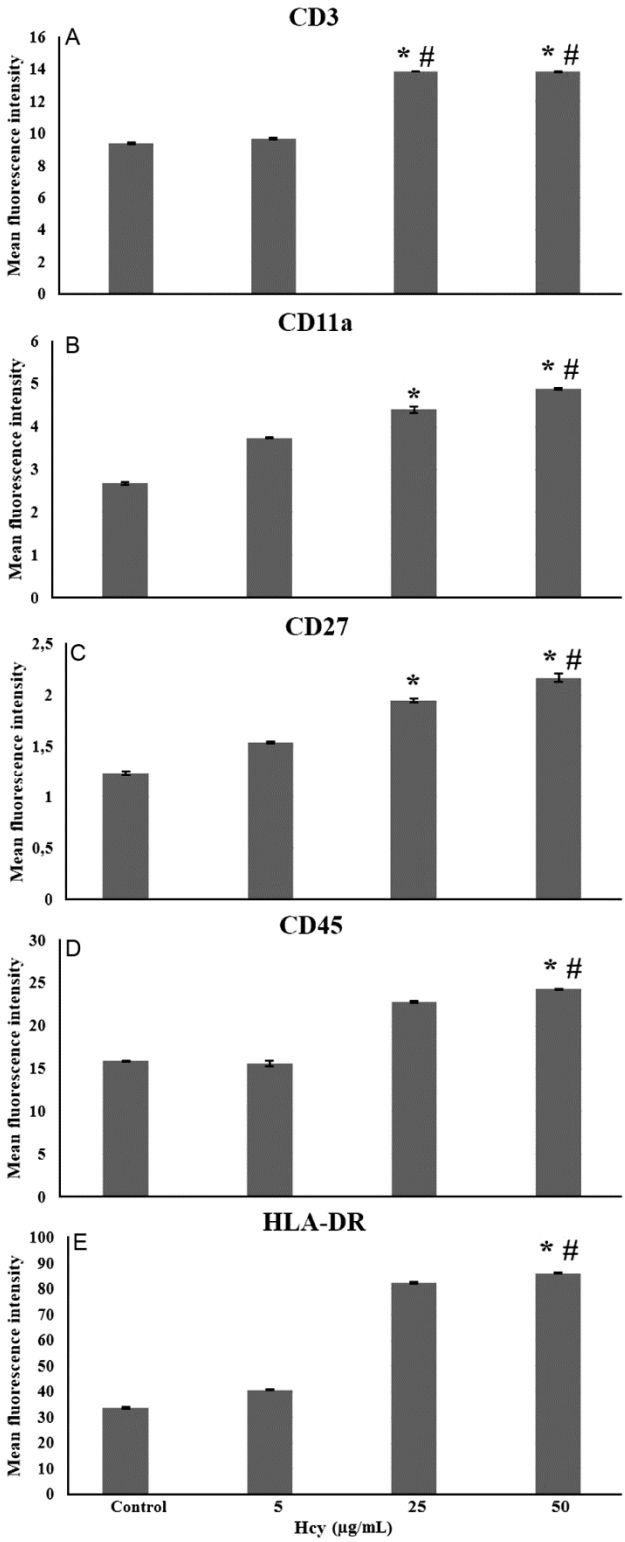


Fig. 2 — The effect of homocysteine on CD3 (A), CD11a (B), CD27 (C), CD45 (D) and HLA-DR (E) surface molecules in human peripheral blood lymphocytes. [*indicates a statistically significant difference compared to the control group; #compared to the 5 µg/mL group. Data are presented as mean ± SD. A $P$-value of <0.05 was considered significant]

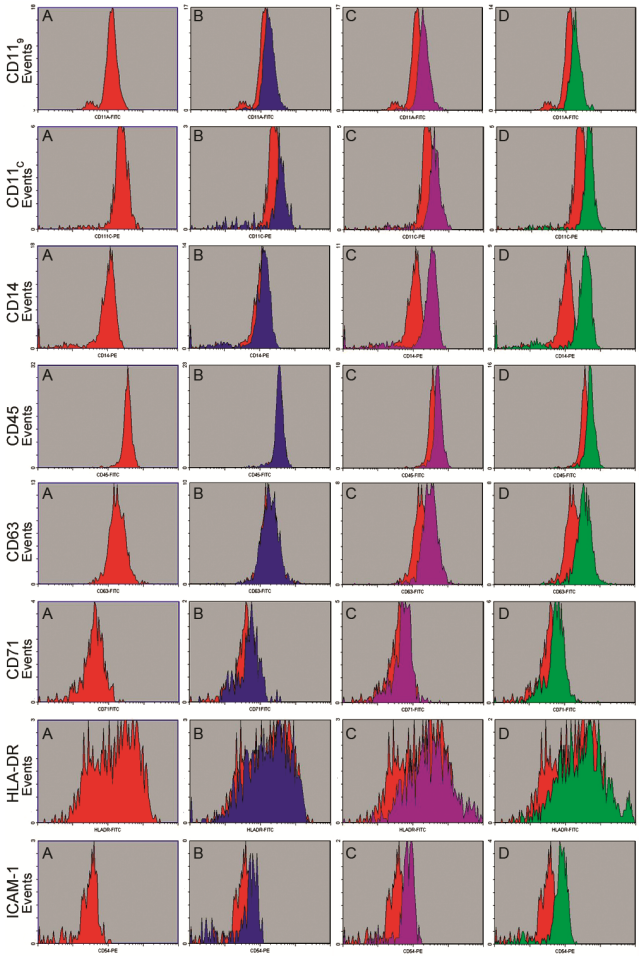


Fig. 3 — Flow cytometry histogram of CD11a (A), CD11c (B), CD14 (C), CD45 (D), CD63 (E), CD71 (F), HLA-DR (G) and ICAM-1(CD54) (H) surface molecules in human peripheral blood monocytes at different Hcy concentrations. [A-Control, B-Hcy 5 µg/mL, C-Hcy 25 µg/mL, D-Hcy 50 µg/mL]

were all upregulated in response to Hcy treatment. The most prominent increase in marker expression was observed at the 50 µg/mL concentration, where the expression was significantly higher compared to both the control group and the lowest dose of Hcy (5 µg/mL). Furthermore, the expression of all markers at the 25 µg/mL dose was significantly higher than that of the control group (Fig. 3 & 4).

**Hcy in U937 monocyte-like cells**

In U937 monocyte-like cells, a significant dose-dependent increase in the expression of CD71 and ICAM-1 was observed following Hcy exposure. CD71 expression was significantly elevated at concentrations of 50 µg/mL and above, with the highest expression levels observed at 200 µg/mL. ICAM-1 expression also exhibited a significant increase, with the highest expression observed at 200 µg/mL, where it was significantly higher compared to both the control group and the lowest dose of Hcy (25 µg/mL). Additionally, the 100 µg/mL dose of Hcy significantly increased ICAM-1 expression compared to the control group (Fig. 5 & 6).

## Discussion

The present findings demonstrate that Hcy significantly and dose-dependently upregulates the expression of activation markers on both lymphocytes and monocytes. Specifically, in human peripheral blood lymphocytes, Hcy increased the surface expression of CD3, CD11a, CD27, CD45, and HLA-DR. In monocytes, Hcy elevated the levels of CD11a, CD11c, CD14, CD45, CD63, CD71, HLA-DR, and

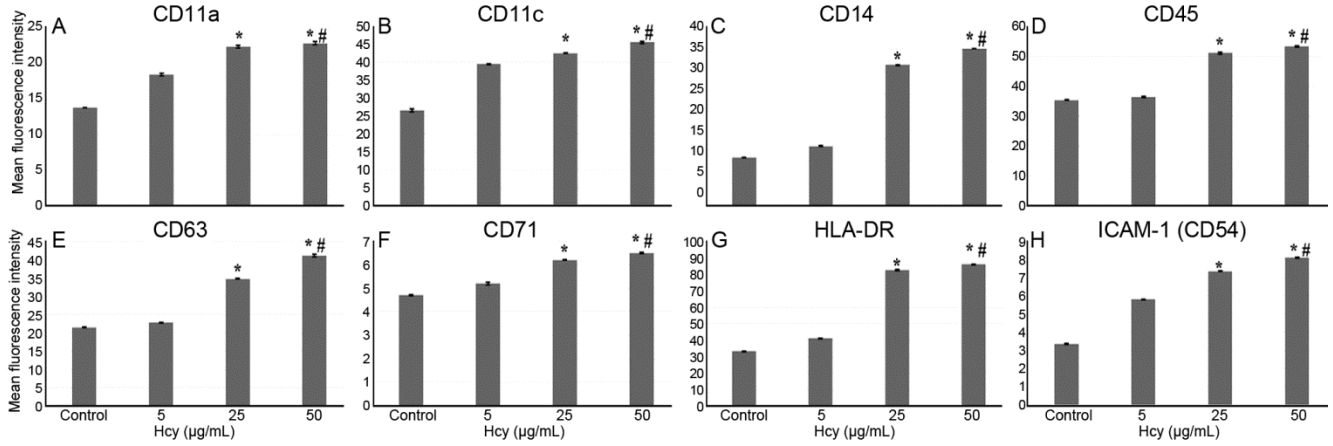


Fig. 4 — The effect of homocysteine on CD11a (A), CD11c (B), CD14 (C), CD45 (D), CD63 (E), CD71 (F), HLA-DR (G) and ICAM-1(CD54) (H) surface molecules in human peripheral blood monocytes. [*indicates a statistically significant difference compared to the control group; #compared to the 5 µg/mL group. Data are presented as mean ± SD. A *P*-value of <0.05 was considered significant]

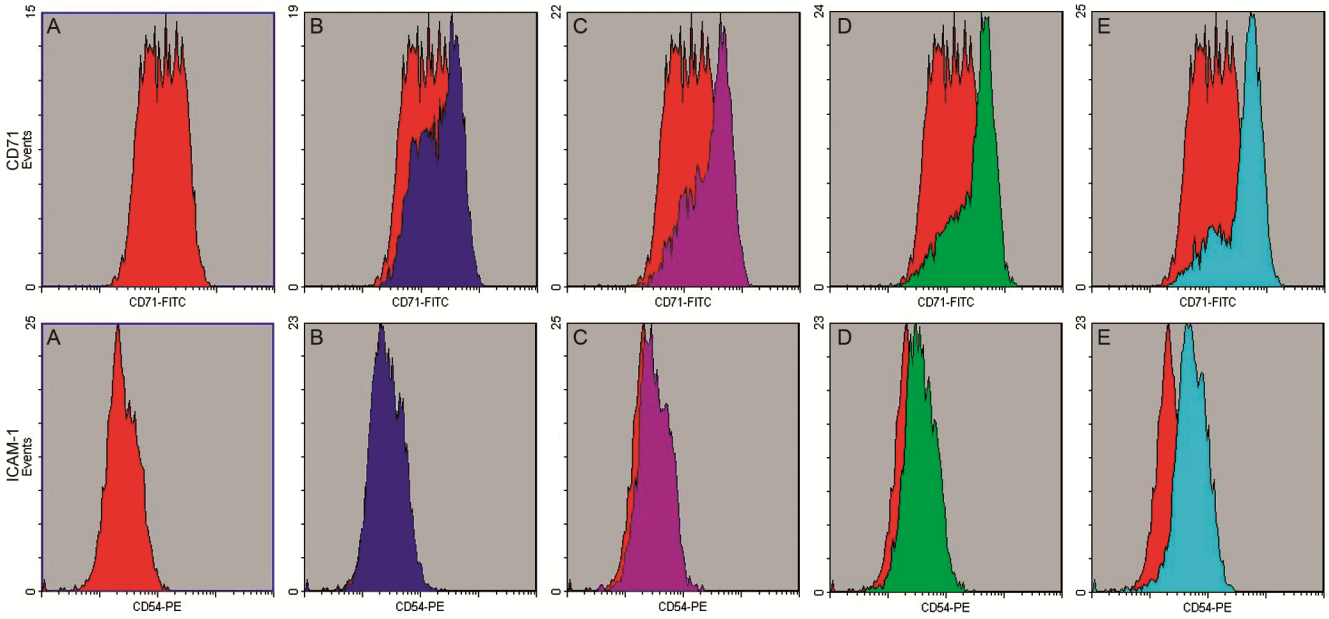


Fig. 5 — Flow cytometry histogram of CD71 (A) and ICAM-1(CD54) (B) surface molecules in U937 monocyte-like cells at different Hcy concentrations. [A-Control, B-Hcy 25 µg/mL, C-Hcy 50 µg/mL, D-Hcy 100 µg/mL, E-Hcy 200 µg/mL]

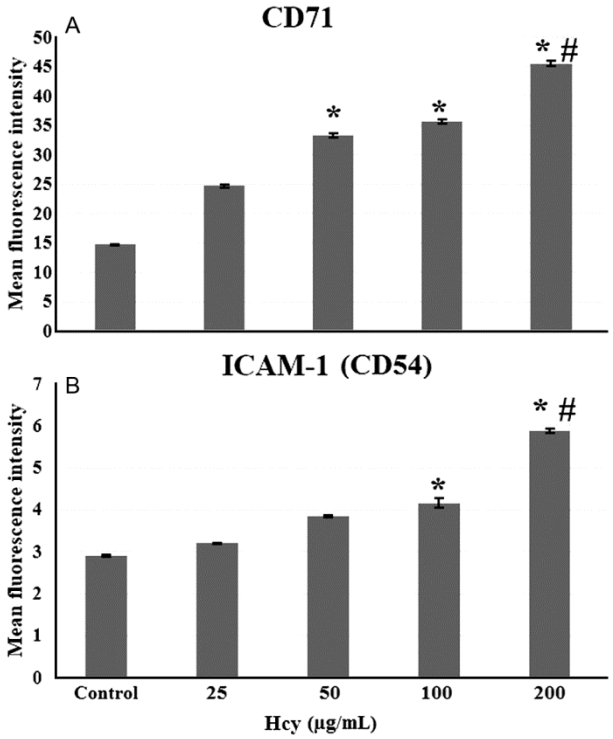


Fig. 6 — The effect of homocysteine on CD71 (A) and ICAM-1(CD54) (B) surface molecules in U937 monocyte-like cells. [*indicates a statistically significant difference compared to the control group; #compared to the 25 µg/mL group. Data are presented as mean ± SD. A *P*-value of <0.05 was considered significant]

ICAM-1. Furthermore, in U937 monocyte-like cells, Hcy enhanced the expression of CD71 and ICAM-1. These results suggest that Hcy promotes a pro-inflammatory phenotype in immune cells, potentially contributing to chronic inflammatory conditions such as atherosclerosis and autoimmune diseases.

CD3, CD11a, CD27, CD45, and HLA-DR in lymphocytes are involved in critical immune functions such as T-cell activation, co-stimulation, and antigen presentation[14]. In monocytes, the expression of CD11a, CD11c, CD14, CD45, CD63, CD71, HLA-DR, and ICAM-1 reflects enhanced cellular adhesion, migration, and immune response, which are key processes in inflammation and atherosclerosis development[15]. CD3, as a key component of the T-cell receptor (TCR) complex, is essential for antigen recognition and signal transduction, the activation of T lymphocytes, and facilitating T-cell-mediated immune responses[16]. CD11a (LFA-1) facilitates T-cell interactions with antigen-presenting cells, while CD71, a crucial molecule for iron uptake, is associated with increased cellular proliferation, which may further exacerbate immune activation[17-19]. CD11c contributes to cell-cell interactions by mediating the binding of monocytes and neutrophils to the endothelium and promoting their transmigration[20]. Both CD11a and CD11c, as integrins, are essential for leukocyte adhesion and migration, which are fundamental processes in inflammation and vascular pathology[21,22]. The increased expression of CD14, a co-receptor for the detection of bacterial lipopolysaccharide, indicates heightened monocyte sensitivity to inflammatory

stimuli, potentially amplifying immune responses[23]. CD27 serves as a co-stimulatory molecule on T-cells and is found on the surface of resting T-cells, along with other lymphoid cells like B and NK cells. The co-stimulatory signaling through CD27 is involved in a strong T-cell-mediated immune response and plays a critical role in the survival of central memory T-cells[24,25]. Similarly, CD45 is an essential regulator in the activation of T-cell receptor signaling, primarily by modulating the stimulation of Src family protein-tyrosine kinases. CD45 deficiency leads to T- and B-lymphocyte dysfunction, resulting in profound combined immune deficiency and contributing to autoimmune diseases, cancer, and infections[26]. CD63 is a tetraspanin membrane protein predominantly localised in late endosomes and lysosomes the endosomal system and on the cell surface, where it regulates intracellular trafficking, endocytosis, and exosome biogenesis[27]. In resting T cells, CD63 is sequestered in the cytoplasm and relocates to the plasma membrane upon activation, where it enhances antigen-presenting cell-mediated co-stimulatory signaling, thereby amplifying and sustaining T-cell activation[28]. CD63 has also been implicated in the regulation of monocyte fusion, a process that may contribute to inflammation-related events[29]. Elevated levels of human leukocyte antigen (HLA)-DR, a major histocompatibility complex class II molecule, suggest an enhanced capacity for antigen presentation, facilitating T-cell activation and perpetuating the inflammatory cascade[30,31]. Conversely, the reduced expression of HLA-DR on monocytes is associated with immune dysfunction[32,33]. ICAM-1 is involved in leukocyte adhesion to endothelial cells, a pivotal step in the pathogenesis of atherosclerosis[34-36].

In the current study, the dose-dependent increase in the expression of key activation markers in lymphocytes and monocytes suggests that Hcy significantly enhances immune cell activation, highlighting its role in inflammatory processes. Specifically, the upregulation of CD3 and HLA-DR in lymphocytes, along with CD11a and CD45, indicates stronger antigen recognition and T-cell activation, while the increase in CD11c, CD14, CD63 and CD71 on monocytes highlights an amplification of inflammatory responses. CD11a and CD11c's involvement in leukocyte adhesion and migration, alongside CD45′s modulation of Src kinases, further suggests that Hcy may intensify the adhesion and transmigration of immune cells, leading to an exacerbated inflammatory reaction. The dose-dependent nature of these effects supports that Hcy not only enhances immune activation but also aggravates the inflammatory processes by altering immune cell behaviour, potentially contributing to the pathogenesis of various inflammatory and autoimmune diseases.

These findings are consistent with previous studies indicating that Hcy plays a pivotal role in modulating immune responses by inducing the expression of adhesion molecules and chemokines in vascular cells[5-8]. Specifically, Hcy has been shown to upregulate VCAM-1 in endothelial cells, thus promoting the adhesion of monocytes to the endothelium[37]. In a similar context, Hcy has been reported to trigger apoptosis in endothelial cells from the human umbilical vein, accompanied by elevated levels of ICAM-1, VCAM-1, endothelin-1, and MCP-1, along with a rise in the production of reactive oxygen species[9]. Consistently, ciliary neurotrophic factor (CNTF) expression is markedly upregulated during Hcy-mediated damage to vascular endothelial cells. This upregulation activates the JAK2-STAT3 signaling pathway, resulting in elevated levels of IL-6 and pro-inflammatory mediators such as IL-1β, ICAM-1, VCAM-1, and NLR family pyrin domain-containing 3 (NLRP3)[38]. Furthermore, Hcy stimulates the production of monocyte chemoattractant protein-1 (MCP-1) and interleukin-8 (IL-8), promoting the recruitment of leukocytes to sites of inflammation[10,39,40]. In addition, Hcy has been demonstrated to induce the expression of MCP-1 and IL-8 in human aortic endothelial cells, which further facilitates leukocyte migration and accumulation at sites of vascular injury[41]. The activation of nuclear factor-kappa B (NF-κB) by Hcy also contributes to the upregulation of MCP-1 in THP-1 macrophages, thereby enhancing monocyte chemotaxis and promoting inflammatory responses[42]. HHcy has been found to elevate the expression of IL-6 and NF-κB p65/RelA, contributing to a pro-inflammatory state. Moreover, it has been reported that HHcy can exacerbate hypertension-induced arterial damage through the activation of this signaling pathway[43]. These findings collectively suggest that Hcy-induced endothelial dysfunction and inflammatory signaling contribute to a pro-inflammatory microenvironment, potentially exacerbating vascular complications and promoting the pathogenesis of atherosclerosis.

Increased expression of immune activation markers has also been observed in chronic diseases, including cancer, diabetes, and neurodegenerative diseases.

These cell surface markers can influence cancer progression by modulating tumor associated immune responses and cellular interactions[44-47]. Similarly, in diabetes, altered expression of immune and endothelial surface markers reflects immune dysfunction and endothelial injury, contributing to the progression of diabetic complications, such as kidney disease[48-50]. Furthermore, the modulation of surface molecules plays a significant role in central nervous system disorders, such as Alzheimer's disease, epilepsy, stroke, multiple sclerosis, depression, and Parkinson's disease, by influencing neuroinflammation and neurodegeneration[51]. Given its ability to modulate cell surface molecules, the findings of this study suggest that Hcy may contribute to the pathogenesis of metabolic, neurodegenerative, and malignant disorders through immune activation.

To investigate monocyte-specific responses, this study utilised the U937 cell line, a human monocyte-like model that provides a system for exploring the molecular mechanisms involved in monocyte differentiation and activation[11-13]. Unlike THP-1-derived macrophages, which demonstrate a heightened responsiveness to M1 stimuli, U937 cells exhibit greater responsiveness to anti-inflammatory (M2) stimuli, making them particularly valuable for studying macrophage polarisation in the context of alternative immune responses[52]. Moreover, U937-derived macrophages have been utilised to examine the effects of various treatments on macrophage polarisation, such as the modulation of pro-inflammatory cytokines in the context of Kawasaki disease[53]. In addition, U937 cells serve as a relevant model for examining the formation of foamy macrophages during atherogenesis[54]. Taken together, these findings underscore the potential of U937 cells in elucidating key immune-related mechanisms.

U937 cells, being a monocytic cell line, allowed for the isolation of monocyte-specific responses without the confounding influence of other immune cell types present in PBMCs. In this context, the direct effects of Hcy on monocyte activation were examined, as demonstrated by the upregulation of CD71 and ICAM-1. The dose-dependent increase in these markers in U937 cells confirmed that Hcy has a direct impact on monocyte activation, further supporting the findings observed in human monocytes. By integrating primary immune cells and a monocyte-like cell line, this study also provided a comprehensive assessment of Hcy-induced immune activation. While PBMCs ensured physiological relevance, U937 cells offered a more controlled experimental setting. Together, these findings suggest that Hcy plays a significant role in modulating immune cell activation across different cell types. Moreover, they highlight the potential for Hcy to influence both innate and adaptive immune responses, which may contribute to the development of inflammatory conditions associated with cardiovascular diseases.

## Conclusion

This study demonstrates that elevated homocysteine levels enhance activation marker expression in monocytes and lymphocytes, thereby promoting a pro-inflammatory state. These findings elucidate the mechanisms by which hyperhomocysteinemia contributes to the progression of atherosclerosis and inflammatory, metabolic, neurodegenerative and malignant diseases. Future research should focus on identifying potential therapeutic strategies to mitigate these effects, particularly those targeting immune modulation and endothelial function.

## Ethics statement

This study was conducted in accordance with the Declaration of Helsinki and received prior approval from the Ethics Committee of Zonguldak Bülent Ecevit University (approval number: 2025/04-10, date: 26.02.2025).

## Conflict of interest

The authors declare no competing interests.

## References

1 Koklesova L, Mazurakova A, Samec M, Biringer K, Samuel SM, Büsselberg D, Kubatka P & Golubnitschaja O. Homocysteine metabolism as the target for predictive medical approach, disease prevention, prognosis, and treatments tailored to the person. *EPMA J,* 12 (2021) 477. doi.org/10.1007/s13167-021-00263-0

2 Groh L, Keating ST, Joosten LAB, Netea MG & Riksen NP. Monocyte and macrophage immunometabolism in atherosclerosis. *Semin Immunopathol,* 40 (2018) 203. doi.org/10.1007/s00281-017-0656-7

3 Saigusa R, Winkels H & Ley K. T cell subsets and functions in atherosclerosis. *Nat Rev Cardiol,* 17 (2020) 387. doi.org/10.1038/s41569-020-0352-5

4 Janeway CA Jr, Travers P & Walport M. Immunobiology: The immune system in health and disease (*Garland Science*, New York), 2001.

5 Xu Y, Xu Y, Yu Z & He D. Homocysteine modulates CXCL10/CXCR3 axis activity to induce endothelial dysfunction. *Cell Mol Biol (Noisy-le-grand),* 70 (2024) 197.doi.org/10.14715/cmb/2024.70.2.28

6 Postea O, Koenen RR, Hristov M, Weber C & Ludwig A. Homocysteine up-regulates vascular transmembrane chemokine CXCL16 and induces CXCR6+ lymphocyte

recruitment in vitro and in vivo. *J Cell Mol Med,* 12 (2008) 1700. doi.org/10.1111/j.1582-4934.2008.00223.x

7 Wang G, Woo CW, Sung FL, Siow YL & O K. Increased monocyte adhesion to aortic endothelium in rats with hyperhomocysteinemia: role of chemokine and adhesion molecules. *Arterioscler Thromb Vasc Biol,* 22 (2002) 1777. doi.org/10.1161/01.atv.0000035404.18281.37

8 Prasad K. Atherogenic effect of homocysteine, a biomarker of inflammation and its treatment. *Int J Angiol,* 33 (2024) 262. doi.org/10.1055/s-0044-1788280

9 Tian X, Zhao L, Song X, Yan Y, Liu N, Li T, Yan B & Liu B. HSP27 inhibits homocysteine-induced endothelial apoptosis by modulation of ROS production and mitochondrial caspase-dependent apoptotic pathway. *Biomed Res Int,* 2016 (2016) 4847874. doi.org/10.1155/2016/4847874

10 Fu J & Zhu J. Relationship among serum homocysteine, intercellular adhesion molecule-1, monocyte chemoattractant protein-1, and visual impairment in diabetic macular edema. *J Coll Physicians Surg Pak,* 32 (2022) 57. doi.org/10.29271/jcpsp.2022.01.57

11 Michalski C, Cheung C, Oh JH, Ackermann E, Popescu CR, Archambault AS, Prusinkiewicz MA, Da Silva R, Majdoubi A, Viñeta Paramo M, Xu RY, Reicherz F, Patterson AE, Golding L, Sharma AA, Lim CJ, Orban PC, Klein Geltink RI & Lavoie PM. DDIT4L regulates mitochondrial and innate immune activities in early life. *JCI Insight,* 9 (2024) e172312. doi.org/10.1172/jci.insight.172312

12 Chua R, Wang L, Singaraja R & Ghosh S. Functional and multi-omics effects of an optimized CRISPR-mediated FURIN depletion in U937 monocytes. *Cells,* 13 (2024) 588. doi.org/10.3390/cells13070588

13 Choi SY, Hong SH & Lee HJ. Differential expression and sorting of exosomal microRNAs upon activation of the human monocyte-like cell line U937. *Biochem Biophys Res Commun,* 610 (2022) 147.doi.org/10.1016/j.bbrc.2022.04.048

14 Wu Z, Zheng Y, Sheng J, Han Y, Yang Y, Pan H & Yao J. CD3+CD4-CD8- (Double-Negative) T cells in inflammation, immune disorders and cancer. *Front Immunol,* 13 (2022) 816005.doi.org/10.3389/fimmu.2022.816005

15 Patel VK, Williams H, Li SCH, Fletcher JP & Medbury HJ. Monocyte subset recruitment marker profile is inversely associated with blood ApoA1 levels. *Front Immunol,* 12 (2021) 616305.doi.org/10.3389/fimmu.2021.616305

16 Yang H, Parkhouse RM & Wileman T. Monoclonal antibodies that identify the CD3 molecules expressed specifically at the surface of porcine gammadelta-T cells. *Immunology,* 115 (2005) 189.doi.org/10.1111/j.1365-2567.2005.02137.x

17 Lacouture C, Chaves B, Guipouy D, Houmadi R, Duplan-Eche V, Allart S, Destainville N & Dupré L. LFA-1 nanoclusters integrate TCR stimulation strength to tune T-cell cytotoxic activity. *Nat Commun,* 15 (2024) 407.doi.org/10.1038/s41467-024-44688-3

18 Pacella I, Pinzon Grimaldos A, Rossi A, Tucci G, Zagaglioni M, Potenza E, Pinna V, Rotella I, Cammarata I, Cancila V, Belmonte B, Tripodo C, Pietropaolo G, Di Censo C, Sciumè G, Licursi V, Peruzzi G, Antonucci Y, Campello S, Guerrieri F, Iebba V, Prota R, Di Chiara M, Terrin G, De Peppo V, Grazi GL, Barnaba V & Piconese S. Iron capture through CD71 drives perinatal and tumor-associated Treg expansion. *JCI Insight,* 9 (2024) e167967.doi.org/10.1172/jci.insight.167967

19 Chiappelli F. CD71: Role in permafrost immunity. *Bioinformation,* 20 (2024) 208.doi.org/10.6026/973206300200208

20 Córbi AL & Lopéz-Rodríguez C. CD11c integrin gene promoter activity during myeloid differentiation. *Leuk Lymphoma,* 25 (1997) 415.doi.org/10.3109/10428199709039028

21 Shen Y, Nakajima H, Zhu J & Wu W. Integrin β2 regulates titanium particle-induced inflammation in macrophages: In vitro aseptic loosening model. *Mol Med Rep,* 31 (2025) 25.doi.org/10.3892/mmr.2024.13390

22 Hou L, Voit RA, Sankaran VG, Springer TA & Yuki K. CD11c regulates hematopoietic stem and progenitor cells under stress. *Blood Adv,* 4 (2020) 6086.doi.org/10.1182/bloodadvances.2020002504

23 Ghozali M, Matahari M, Cahyadi AI, Agustini SD, Ghrahani R, Reniarti L, Setiabudiawan B & Panigoro R. Inflammatory monocyte subsets correlation with iron levels in low vitamin D pediatric transfusion-dependent thalassemia. *J Inflamm Res,* 18 (2025) 421.doi.org/10.2147/JIR.S476688

24 Izawa K, Martin E, Soudais C, Bruneau J, Boutboul D, Rodriguez R, Lenoir C, Hislop AD, Besson C, Touzot F, Picard C, Callebaut I, de Villartay JP, Moshous D, Fischer A & Latour S. Inherited CD70 deficiency in humans reveals a critical role for the CD70-CD27 pathway in immunity to Epstein-Barr virus infection. *J Exp Med,* 214 (2017) 73.doi.org/10.1084/jem.20160784

25 Honikel MM & Olejniczak SH. Co-stimulatory receptor signaling in CAR-T cells. *Biomolecules,* 2022;12:1303. doi.org/10.3390/biom12091303

26 Rheinländer A, Schraven B & Bommhardt U. CD45 in human physiology and clinical medicine. *Immunol Lett,* 196 (2018) 22. doi.org/10.1016/j.imlet.2018.01.009

27 Pols MS & Klumperman J. Trafficking and function of the tetraspanin CD63. *Exp Cell Res,* 315 (2009) 1584. doi.org/10.1016/j.yexcr.2008.09.020

28 Pfistershammer K, Majdic O, Stöckl J, Zlabinger G, Kirchberger S, Steinberger P & Knapp W. CD63 as an activation-linked T cell costimulatory element. *J Immunol,* 173 (2004) 6000.doi.org/10.4049/jimmunol.173.10.6000

29 Champion TC, Partridge LJ, Ong SM, Malleret B, Wong SC & Monk PN. Monocyte subsets have distinct patterns of tetraspanin expression and different capacities to form multinucleate giant cells. *Front Immunol,* 9 (2018) 1247. doi.org/10.3389/fimmu.2018.01247

30 Nguyen TH, Kumar D, Prince C, Martini D, Grunwell JR, Lawrence T, Whitely T, Chappelle K, Chonat S, Prahalad S, Briones M & Chandrakasan S. Frequency of HLA-DR+CD38hi T cells identifies and quantifies T-cell activation in hemophagocytic lymphohistiocytosis, hyperinflammation, and immune regulatory disorders. *J Allergy Clin Immunol,* 153 (2024) 309. doi.org/10.1016/j.jaci.2023.07.008

31 Verstegen NJM, Hagen RR, Kreher C, Kuijper LH, Dijssel JVD, Ashhurst T, Kummer LYL, Palomares Cabeza V, Steenhuis M, Duurland MC, Jongh R, Schoot CEV, Konijn VAL, Mul E, Kedzierska K, van Dam KPJ, Stalman EW, Boekel L, Wolbink G, Tas SW, Killestein J, Rispens T, Wieske L, Kuijpers TW, Eftimov F, van Kempen ZLE, van Ham SM, Ten Brinke A & van de Sandt CE. T cell activation

markers CD38 and HLA-DR indicative of non-seroconversion in anti-CD20-treated patients with multiple sclerosis following SARS-CoV-2 mRNA vaccination. *J Neurol Neurosurg Psychiatry,* 95 (2024) 855. https://doi.org/10.1136/jnnp-2023-332224

32 Wu HP, Shih CC, Lin CY, Hua CC & Chuang DY. Serial increase of IL-12 response and human leukocyte antigen-DR expression in severe sepsis survivors. *Crit Care,* 15 (2011) R224.doi.org/10.1186/cc10464

33 Tschaikowsky K, Hedwig-Geissing M, Schiele A, Bremer F, Schywalsky M & Schuttler J. Coincidence of pro- and anti-inflammatory responses in the early phase of severe sepsis: Longitudinal study of mononuclear histocompatibility leukocyte antigen-DR expression, procalcitonin, C-reactive protein, and changes in T-cell subsets in septic and postoperative patients. *Crit Care Med,* 30 (2002) 1015.doi.org/10.1097/00003246-200205000-00010

34 Guerra-Espinosa C, Jiménez-Fernández M, Sánchez-Madrid F & Serrador JM. ICAMs in immunity, intercellular adhesion and communication. *Cells,* 13 (2024) 339.doi.org/10.3390/cells13040339

35 Ma VP, Hu Y, Kellner AV, Brockman JM, Velusamy A, Blanchard AT, Evavold BD, Alon R & Salaita K. The magnitude of LFA-1/ICAM-1 forces fine-tune TCR-triggered T cell activation. *Sci Adv,* 8 (2022) eabg4485.doi.org/10.1126/sciadv.abg4485

36 Kantari-Mimoun C, Barrin S, Vimeux L, Haghiri S, Gervais C, Joaquina S, Mittelstaet J, Mockel-Tenbrinck N, Kinkhabwala A, Damotte D, Lupo A, Sibony M, Alifano M, Dondi E, Bercovici N, Trautmann A, Kaiser AD & Donnadieu E. CAR T-cell entry into tumor islets is a two-step process dependent on IFNγ and ICAM-1. *Cancer Immunol Res,* 9 (2021) 1425.doi.org/10.1158/2326-6066.CIR-20-0837

37 Silverman MD, Tumuluri RJ, Davis M, Lopez G, Rosenbaum JT & Lelkes PI. Homocysteine upregulates vascular cell adhesion molecule-1 expression in cultured human aortic endothelial cells and enhances monocyte adhesion. *Arterioscler Thromb Vasc Biol,* 22 (2002) 587.doi.org/10.1161/01.atv.0000014221.30108.08

38 Zhang L, Xiang Y, Cao C, Tan J, Li F & Yang X. Ciliary neurotrophic factor promotes the development of homocysteine-induced vascular endothelial injury through inflammation mediated by the JAK2/STAT3 signaling pathway. *Exp Cell Res,* 440 (2024) 114103.doi.org/10.1016/j.yexcr.2024.114103

39 Zeng X, Dai J, Remick DG & Wang X. Homocysteine mediated expression and secretion of monocyte chemoattractant protein-1 and interleukin-8 in human monocytes. *Circ Res,* 93 (2003) 311.doi.org/10.1161/01.RES.0000087642.01082.E4

40 Ravi R, Kumaraswamy A, Chauhan P & Subramaniam Rajesh B. Exogenous administration of hydrogen sulfide alleviates homocysteine induced inflammation in ARPE-19 cells. *Exp Eye Res,* 212 (2021) 108759.doi.org/10.1016/j.exer.2021.108759

41 Poddar R, Sivasubramanian N, DiBello PM, Robinson K & Jacobsen DW. Homocysteine induces expression and secretion of monocyte chemoattractant protein-1 and interleukin-8 in human aortic endothelial cells: implications for vascular disease. *Circulation,* 103 (2001) 2717.doi.org/10.1161/01.cir.103.22.2717

42 Wang G, Siow YL & O K. Homocysteine induces monocyte chemoattractant protein-1 expression by activating NF-kappaB in THP-1 macrophages. *Am J Physiol Heart Circ Physiol,* 280 (2001) H2840.doi.org/10.1152/ajpheart.2001.280.6.H2840

43 Zhang L, Li Z, Xing C, Gao N & Xu R. Folate reverses NF-κB p65/Rela/IL-6 level induced by hyperhomocysteinemia in spontaneously hypertensive rats. *Front Pharmacol,* 12 (2021) 651582.doi.org/10.3389/fphar.2021.651582

44 Dunne MR, Phelan JJ, Michielsen AJ, Maguire AA, Dunne C, Martin P, Noonan S, Tosetto M, Geraghty R, Fennelly D, Sheahan K, Ryan EJ & O'Sullivan J. Characterising the prognostic potential of HLA-DR during colorectal cancer development. *Cancer Immunol Immunother,* 69 (2020) 1577.doi.org/10.1007/s00262-020-02571-2

45 Rao D, Lu H, Wang X, Lai Z, Zhang J & Tang Z. Tissue-derived exosome proteomics identifies promising diagnostic biomarkers for esophageal cancer. *Elife,* 12 (2023) e86209.doi.org/10.7554/eLife.86209

46 D'Aprile S, Denaro S, Pavone AM, Giallongo S, Giallongo C, Distefano A, Salvatorelli L, Torrisi F, Giuffrida R, Forte S, Tibullo D, Li Volti G, Magro G, Vicario N & Parenti R. Anaplastic thyroid cancer cells reduce CD71 levels to increase iron overload tolerance. *J Transl Med,* 21 (2023) 780.doi.org/10.1186/s12967-023-04664-9

47 Sharygin D, Koniaris LG, Wells C, Zimmers TA & Hamidi T. Role of CD14 in human disease. *Immunology,* 169 (2023) 260.doi.org/10.1111/imm.13634

48 Zaragoza-García O, Briceño O, Villafan-Bernal JR, Rojas-Delgado HU, Gutiérrez-Pérez IA, Morales-Martínez C, Rodriguez-Reyes RR & Guzmán-Guzmán IP. sCD14 as a biomarker for the progression of kidney disease among patients with diabetes. *J Diabetes Complications,* 39 (2025) 108980.doi.org/10.1016/j.jdiacomp.2025.108980

49 Restrepo BI, Twahirwa M & Jagannath C. Hyperglycemia and dyslipidemia: Reduced HLA-DR expression in monocyte subpopulations from diabetes patients. *Hum Immunol,* 82 (2021) 124.doi.org/10.1016/j.humimm.2020.11.005

50 Al-Qaissi A, Papageorgiou M, Deshmukh H, Madden LA, Rigby A, Kilpatrick ES, Atkin SL & Sathyapalan T. Effects of acute insulin-induced hypoglycaemia on endothelial microparticles in adults with and without type 2 diabetes. *Diabetes Obes Metab,* 21 (2019) 533.doi.org/10.1111/dom.13548

51 Cacabelos R, Torrellas C, Fernández-Novoa L & López-Muñoz F. Histamine and immune biomarkers in CNS disorders. *Mediators Inflamm,* 2016 (2016) 1924603.doi.org/10.1155/2016/1924603

52 Nascimento CR, Rodrigues Fernandes NA, Gonzalez Maldonado LA & Rossa Junior C. Comparison of monocytic cell lines U937 and THP-1 as macrophage models for in vitro studies. *Biochem Biophys Rep,* 32 (2022) 101383. doi.org/10.1016/j.bbrep.2022.101383

53 Guo MM, Huang YH & Kuo HC. Corticosteroid effects on IL-10 and IL-1β in U937-derived macrophages: A model for Kawasaki disease-associated inflammation. *Cytokine,* 185 (2025) 156809.doi.org/10.1016/j.cyto.2024.156809

54 Gu Q, Liu J & Shen LL. FXR activation reduces the formation of macrophage foam cells and atherosclerotic plaque, possibly by down regulating hepatic lipase in macrophages. *FEBS Open Bio,* 15 (2025) 311.doi.org/10.1002/2211-5463.13925