



Preliminary Study of $V\delta 2^+$ $\gamma\delta$ T cell Distribution in Healthy Thai Adults and Older Adults

Yaquta Nur Mohamud, Asma Tahir, Kesara Na-Bangchang, and Wanna Chaijaroenkul*

Chulabhorn International College of Medicine, Thammasat University, Rangsit Center,
Pathum Thani 12120, Thailand.

*Corresponding author, E-mail: wn_ap39@yahoo.com

Abstract

Gamma delta ($\gamma\delta$) T cells, particularly the $V\delta 2^+$ subset, are critical components of the human immune system, playing a pivotal role in immunosurveillance against diverse pathogens and tumors. While the immune system undergoes well-documented age-related changes, collectively termed immunosenescence, the specific dynamics of $V\delta 2^+$ cells in aging populations, especially within particular ethnic groups, remain underexplored. This preliminary cross-sectional study aimed to compare the proportions of circulating $V\delta 2^+$ T cells between younger adults (18-55 years) and older adults (>55 years) among healthy Thai individuals. Thirty healthy Thai individuals were recruited, stratified into two age groups (n=15 each), and $V\delta 2^+$ T cell percentages were quantified by flow cytometry of isolated peripheral blood mononuclear cells. Statistical analysis, employing the Mann-Whitney U test due to non-normal data distribution, revealed a significantly lower median percentage of $V\delta 2^+$ T cells in older adults (1.75%, interquartile range [IQR]: 1.10%-2.29%) compared to younger adults (4.07%, IQR: 3.90%-5.24%), ($p = 0.002$). These findings indicate a significant age-related decline in circulating $V\delta 2^+$ T cells among healthy Thai individuals. This observed reduction holds potential implications for age-related immune competence, possibly increasing susceptibility to infectious diseases and certain cancers. The study underscores the necessity for further investigation into the functional consequences of this quantitative decline and highlights the importance of establishing population-specific immunological reference ranges.

Keywords: Gamma delta T cells ($\gamma\delta$ T cells), $V\gamma 2V\delta 2$ T cells, Immunosenescence, flow cytometry, Thai population

1. Introduction

Gamma delta ($\gamma\delta$) T cells represent a distinct and unconventional lymphocyte subset that contributes significantly to both innate and adaptive immune responses (Clark & Thomas, 2020; Tyler et al., 2015). Among the various $\gamma\delta$ T cell populations, $V\delta 2^+$ T cells constitute the predominant subset in human peripheral blood, particularly in adults, are commonly paired with the $V\gamma 9$ chain (Fonseca et al., 2020; Lawand et al., 2017). These cells play crucial roles in immunosurveillance by providing protection against bacterial and other infectious diseases and by contributing to anti-tumor immunity (Chen & Letvin, 2003; Davey et al., 2018). Their unique recognition capabilities, distinct from conventional $\alpha\beta$ T cells, position them as important mediators in the early stages of immune responses (Hu et al., 2023; Xu et al., 2019).

The human immune system undergoes considerable alterations with age, a process termed immunosenescence, which leads to a decline in immune function and increased susceptibility to various health challenges, including infection and cancer (Kallemeijn et al., 2017; Xu et al., 2019). While extensive research has documented age-related changes in $\alpha\beta$ T cell populations, the impact of aging on $\gamma\delta$ T cells, particularly the $V\delta 2^+$ subset, remains an evolving area of research (Clark & Thomas, 2020; Xu et al., 2019). Previous investigations have yielded mixed results regarding $V\delta 2^+$ T cell prevalence with advancing age; some studies report a decrease in the proportion of $\gamma\delta$ T cell, including $V\delta 2^+$ cells, often noted after the age of 35 or 45 (Hu et al., 2023; Jia et al., 2023; Kallemeijn et al., 2017). This age-related reduction is influenced by processes such as thymic involution, which leads to a gradual decline in peripheral $\gamma\delta$ T cell proportions from childhood to old age (Hu et al., 2023). Conversely, some studies suggest that the composition and function of $V\delta 2^+$ T cells may be largely preserved in older adults, indicating a potential resilience to age-related decline (Xu et al., 2019). This divergence underscores the need for further investigation into the precise dynamics of $V\delta 2^+$ T cells throughout the human lifespan.



Furthermore, the prevalence of $\gamma\delta$ T cell subsets can exhibit significant variability across different ethnic and geographical populations (Cairo et al., 2010; Esin et al., 1996). Studies comparing diverse populations have highlighted notable differences in circulating $\gamma\delta$ T cell percentages, suggesting that genetic and environmental factors may influence these immune cell populations (Cairo et al., 2010; Esin et al., 1996).

Despite the critical importance of $V\delta 2^+$ T cells in immune health, there is a scarcity of comprehensive data on their prevalence in specific Asian populations, such as healthy Thai adults. Understanding the baseline characteristics of these immune cells within a healthy Thai cohort is essential for establishing normal physiological ranges and for future comparative studies involving disease states.

2. Objectives

This preliminary cross-sectional study aims to determine the proportion of circulating $V\delta 2^+$ T cells in a healthy Thai population. The primary objective is to compare the proportion of $V\delta 2^+$ T cells between younger adults (18–55 years) and older adults (>55 years) within this population.

3. Materials and Methods

3.1 Study population

A preliminary cross-sectional study was conducted in a cohort of 30 healthy Thai individuals of both sexes. Participants were stratified into two age-defined groups: Group 1 (18–55 years; $n = 15$) and Group 2 (> 55 years; $n = 15$). Written informed consent was obtained from all participants prior to the collection of peripheral blood samples. The study was conducted between 3 November 2025 and 16 December 2025. Ethical approval was obtained from the Thammasat University Ethics Committee.

3.2 Inclusion and exclusion criteria

3.2.1 Inclusion Criteria:

Eligible participants were Thai adults aged 18 years or older. Both male and female participants were included. Participants were required to be free of serious medical conditions, including cancer, autoimmune diseases, chronic infectious diseases, or other disorders that could affect immune function. All participants were required to be willing to participate in the study and to provide written informed consent prior to enrollment.

3.2.2 Exclusion Criteria:

Participants were excluded if they had a history of infectious or chronic disease. Individuals who had used anti-inflammatory medications, undergone radiation therapy, received other medical treatments, or consumed herbal remedies or dietary supplements within two months prior to participation were also excluded. Female participants who were pregnant or breastfeeding at the time of the study were excluded.

3.3 Sample collection

A 10 mL venous blood sample was obtained from each participant using a sterile lithium heparin-anticoagulant tube. Samples exhibiting visible hemolysis at the time of collection were excluded from further analysis. All samples were processed within 2 hours collection.

3.4 Peripheral blood mononuclear cell (PBMC) isolation

PBMCs were separated from each blood sample within 2 hours of collection using Ficoll-Paque™ PLUS (Cytiva Sweden AB, Sweden). Briefly, each sample was diluted with an equal volume (1:1) of phosphate-buffered saline (PBS; 1×) and carefully layered over an equal volume of Ficoll-Paque in a 50 mL conical centrifuge tube. The diluted blood was centrifuged at $600 \times g$ for 30 min at 25 °C, after which the PBMC layer was collected and transferred to a new conical tube. The cells were then washed with 35 mL of PBS and centrifuged at $900 \times g$ for 10 min at 4 °C. After removing the supernatant, 13 mL of PBS was added, and the cells were counted. Based on the cell count, the required volume of medium was calculated. The cells were then centrifuged again at 4 °C for 5 min. The supernatant was discarded, and the PBMC

[253]



pellet was resuspended in the appropriate volume of RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) for subsequent staining and flow cytometric analysis.

3.5 Blood Cell Count

Following PBMC isolation, cell concentration and viability were determined using a hemocytometer and trypan blue dye exclusion. An aliquot of the cell suspension was mixed at a 1:1 with 0.4% trypan blue solution, and viable (unstained) cells were counted under a light microscope. The total cell number was recorded. Based on the cell count, the appropriate volume of RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) was calculated to achieve the desired cell concentration for subsequent flow cytometry staining.

3.5 Flow cytometry

For flow cytometric analysis, 100 μ L of the PBMC suspension in RPMI-1640 medium was dispensed into two Eppendorf tubes, with one serving as an unstained control. To both tubes, 44 μ L of fetal calf serum (FCS) was added to reduce nonspecific binding. PE-conjugated anti-human CD3 antibody (BioLegend, clone HIT3a) and FITC-conjugated anti-human TCR V δ 2 antibody (BioLegend, clone B6) were added only to the test tube according to the manufacturer's instructions. Both tubes were incubated in the dark on ice for 15 min. Following incubation, 200 μ L of 2% FCS/PBS was added, and the cells were centrifuged at 600 \times g for 2 min at 4 $^{\circ}$ C. The supernatant was carefully discarded without disturbing the cell pellet. This wash step was repeated three times. After the final wash, the cells were fixed in 2% formaldehyde or resuspended in 2% FCS. Flow cytometry was performed to analyze cell populations, and the percentage of $\gamma\delta$ T cells (V δ 2 $^{+}$) was determined. Flow cytometry data were analyzed using quadrant gating. Gates were set based on the unstained control to define positive and negative populations. CD3 and TCR V δ 2 expression were analyzed using the PE and FITC channels, respectively. The CD3 $^{+}$ V δ 2 $^{+}$ double-positive population in the upper-right quadrant was identified as $\gamma\delta$ T cells. The percentage of $\gamma\delta$ T cells among PBMCs was calculated for each sample and used for subsequent analysis.

3.6 Statistical analysis

All analyses were performed using R version 4.3.0. Continuous variables (age, body weight, height, BMI, and $\gamma\delta$ T cells [V δ 2 $^{+}$ subset]) were summarized as median and interquartile range (IQR). Comparisons between the two age groups (18–55 vs. >55 years) were performed using the Mann–Whitney U test. A p-value < 0.05 was considered statistically significant.

4. Results and Discussion

4.1 Results

4.1.1 Demographic characteristics of study participants by age group

A total of 30 healthy participants were enrolled in the study and divided into two age groups: Group 1 (18–55 years) and Group 2 (>55 years). All participants were healthy and had no history of chronic or infectious disease. The median age was 40.0 years (range: 31.0–51.0) in Group 1 and 62.0 years (range: 55.0–70.0) in Group 2. No statistically significant differences were observed between the two groups in terms of body weight, height, or body mass index (BMI) (**Table 1**). These findings indicate that the groups were comparable with respect to these baseline characteristics.

Table 1 Demographic characteristics of study participants by age group

variables	Group 1 (18–55 years) Median (Range)	Group 2 (55 years) Median (Range)	p-value
Age (years)	40 (31–51)	62 (55–70)	<0.001
Weight (kg)	62 (47–80)	64 (47–88)	0.818
Height (cm)	160 (156–174)	158 (149–178)	0.328
BMI (kg/m 2)	23.43 (18.82–32.45)	23.70 (18.83–36.60)	0.787

[254]



4.1.2 Comparison of $V\delta 2^+ \gamma\delta$ T cell percentages between age groups

The percentage of $V\delta 2^+$ T cells was the primary immunological outcome of this study. Normality was assessed using the Shapiro–Wilk test. The data were not normally distributed in either group (Group 1: $W = 0.600$, $p < 0.001$; Group 2: $W = 0.772$, $p = 0.002$). Therefore, the Mann–Whitney U test was used for between-group comparison.

Group 1 (younger adults, 18–55 years) exhibited substantially higher percentages of $V\delta 2^+$ T cell, with a median of 4.07% (IQR: 3.90–5.24%). Values ranged from 2.29% to 21.45%, spanning more than a 9-fold range. In contrast, Group 2 (older adults, >55 years) showed lower values with a median of 1.75% (IQR: 1.10–2.29%). The range in this group was narrower (0.40–7.50%), with a maximum value that was approximately one-third of that observed in the younger group (**Figure 1**). This difference was statistically significant ($p = 0.002$).

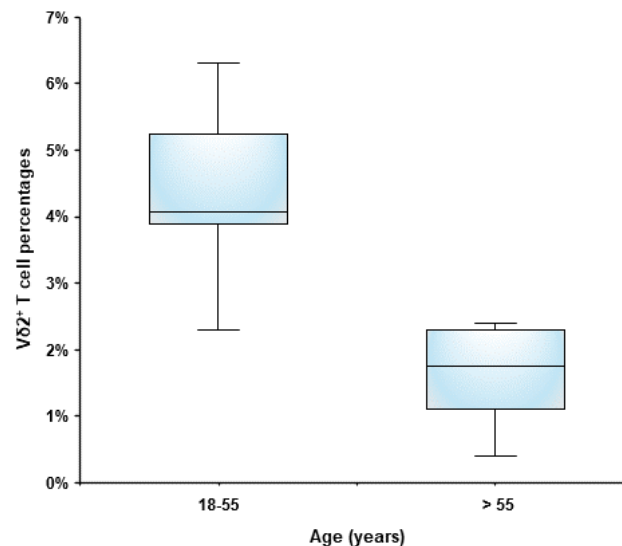


Figure 1 Box plots showing the percentages of $V\delta 2^+$ T cells in younger (18–55 years, $n = 15$) and older (>55 years, $n = 15$) healthy adults. Boxes represent the median and interquartile range. The older group shows significantly lower $V\delta 2^+$ T cell percentages than the younger group ($p = 0.002$).

4.2 Discussion

This preliminary cross-sectional study revealed a significantly lower median percentage of circulating $V\delta 2^+$ T cells in healthy Thai older adults (>55 years) than in younger adults (18–55 years), indicating an age-related decline in this critical immune cell subset within the Thai population.

These findings align with several studies that report a decrease in $\gamma\delta$ T cells, including $V\delta 2^+$ subsets, with advancing age. For instance, Kallemeijn et al., (2017) observed a significant decrease in $V\delta 2^+$ cells and $V\gamma 9/V\delta 2$ cell populations starting around age 40–50. Similarly, Jia et al., (2023) reported that, in a Chinese cohort, $V\delta 2$ T cells, while dominant in younger individuals, accounted for less than half of $\gamma\delta$ T cells after age 45, suggesting a decline. (Hu et al., 2023) even proposed age 45 as a checkpoint for $\gamma\delta$ T cell aging, based on changes in the $V\delta 2/V\delta 1$ ratio. Another study highlighted age as having a significant impact on the levels of $V\delta 2$ cell levels across different racial groups (Cairo et al., 2010). This age-related reduction in $V\delta 2^+$ T cell prevalence might contribute to immunosenescence, the general decline of immune function with age, potentially impacting the ability of older individuals to respond to infections and maintain immune surveillance (Kallemeijn et al., 2017).

Some studies suggest that $V\delta 2^+ +$ T cells may be more resilient to age-related decline than other T cell subsets. For example, Tan et al., (2016) reported that the composition and functionality of $V\delta 2^+$ T cells were not significantly altered in older adults, suggesting that their functionality may be preserved despite

[255]



aging.(Xu et al., 2019) also suggested $V\delta^+$ T cells might exhibit resistance to senescence hallmarks like telomere length and epigenetic profiles. While these studies suggest resistance to functional alterations, our data specifically point to a decrease in proportion, adding a new layer to the understanding of $V\delta^+$ T cell dynamics in aging.

A key strength of this study is its focus on a healthy Thai population. As highlighted in previous studies, the prevalence of $V\delta^+$ T cells varies considerably across different ethnic and geographical populations (Cairo et al., 2010; Esin et al., 1996). Our findings provide valuable baseline immunological data specific to the Thai population. Cairo et al., (2010) demonstrated differences in $V\delta^+$ cell levels between Caucasian and African American donors, while Fonseca et al., (2020) reported data from Caucasian Portuguese cohort and highlighted the scarcity of comprehensive reviews addressing factors that influence $\gamma\delta$ T cell representation in peripheral blood. Our study provides crucial baseline data for the Thai population, which has been largely underrepresented in such immunological surveys, thereby addressing a significant gap in the literature.

$V\delta^+$ T cells are important components of the innate immune system and acting as a first line of defense against a wide array of pathogens, particularly bacterial infections. They can recognize non-peptidic phosphorylated antigens (such as HMB-PP from bacteria) and rapidly respond by producing cytokines and exerting direct cytotoxic effects (Tan et al., 2016). A reduction in the number of these cells could therefore diminish the capacity for early immune responses, leaving older individuals more vulnerable to infections. These findings align with observations that elderly individuals often exhibit a higher susceptibility to severe disease outcomes, which some studies suggest may be partly attributable to lower levels of $V\delta^+$ T cells in the context of systemic inflammation (Carissimo et al., 2020). This observation suggests that a decrease in $V\delta^+$ T cells may contribute to the increased burden of infectious diseases observed in aging population.

Beyond their role in anti-pathogen immunity, $V\delta^+$ T cells are also recognized for their potent anti-tumor function. They can directly kill tumor cells and also influence other immune cells to enhance anti-cancer responses (Mensurado et al., 2023). While further research is needed to directly link reduced $V\delta^+$ T cell percentages in aging with specific declines in anti-tumor immunity within the Thai population, a lower baseline number of these cells could theoretically translate into a compromised ability to detect and eliminate nascent tumor cells, potentially contributing to the increased cancer incidence observed in older adults. Consequently, $V\delta^+$ T cells have become an important focus in the development of immunotherapeutic approaches (Sanz et al., 2022).

This study has several limitations. The relatively small sample size ($n=30$) in this preliminary study limits the generalizability of the findings and necessitates validation in larger cohorts. In addition, the cross-sectional nature of the study, while identifying an age-related difference, does not allow longitudinal assessment of changes in $V\delta^+$ T cell percentages within individuals. Therefore, it cannot distinguish between cohort effects and true aging effects. Additionally, while addressing a critical data gap for the Thai population, recruitment was limited to healthy Thai individuals residing in Bangkok and its surrounding metropolitan areas. This geographic specificity may limit the generalizability of the findings to the Thai population as a whole. Consequently, this study could not provide insights into the functional status or responsiveness of these cells. Such insights would require assays such as expansion or activation to fully evaluate their immunological competence in future studies.

5. Conclusion

This preliminary cross-sectional study demonstrates a significant age-related decline in circulating $V\delta^+$ T cells among healthy Thai adults, with older individuals exhibiting a substantially lower proportion than their younger counterparts. This finding not only enriches the understanding of immunosenescence within a previously underrepresented ethnic population but also aligns with global literature indicating a general age-related decline in certain $\gamma\delta$ T cell subsets. The observed reduction in $V\delta^+$ T cells carries critical implications, potentially suggesting a diminished capacity for early immune responses against pathogens and compromised anti-tumor surveillance in older Thai adults, thereby contributing to the

[256]



heightened susceptibility to infections and cancer prevalent in aging populations. Given the study's limitations, including its modest sample size and cross-sectional design, future research is imperative. This should encompass larger, longitudinal studies incorporating comprehensive functional analyses to fully elucidate the clinical impact of these age-related changes on immune health and to establish robust immunological reference ranges specific to the aging Thai population.

6. Acknowledgements

Kesara Na-Bangchang was supported by the National Research Council of Thailand under the Research Team Promotion Grant (2567) and by the Thailand Science Research and Innovation Fundamental Fund Fiscal Year 2025.

7. References

- Cairo, C., Armstrong, C. L., Cummings, J. S., Deetz, C. O., Tan, M., Lu, C., ... & Pauza, C. D. (2010). Impact of age, gender, and race on circulating $\gamma\delta$ T cells. *Human immunology*, *71*(10), 968-975. <https://doi.org/10.1016/j.humimm.2010.06.014>
- Carissimo, G., Xu, W., Kwok, I., Abdad, M. Y., Chan, Y. H., Fong, S. W., ... & Ng, L. F. (2020). Whole blood immunophenotyping uncovers immature neutrophil-to-VD2 T-cell ratio as an early marker for severe COVID-19. *Nature communications*, *11*(1), Article 5243. <https://doi.org/10.1038/s41467-020-19080-6>
- Chen, Z. W., & Letvin, N. L. (2003). $V\gamma 2V\delta 2+$ T cells and anti-microbial immune responses. *Microbes and infection*, *5*(6), 491-498. [https://doi.org/10.1016/S1286-4579\(03\)00074-1](https://doi.org/10.1016/S1286-4579(03)00074-1)
- Clark, B. L., & Thomas, P. G. (2020). A Cell for the Ages: Human $\gamma\delta$ T Cells across the Lifespan. *International journal of molecular sciences*, *21*(23), Article 8903. <https://doi.org/10.3390/ijms21238903>
- Davey, M. S., Willcox, C. R., Hunter, S., Kasatskaya, S. A., Remmerswaal, E. B. M., Salim, M., ... & Willcox, B. E. (2018). The human Vdelta2 (+) T-cell compartment comprises distinct innate-like Vgamma9 (+) and adaptive Vgamma9 (-) subsets. *Nat Commun*, *9*(1), Article 1760. <https://doi.org/10.1038/s41467-018-04076-0>
- Esin, S., Shigematsu, M., Nagai, S., Eklund, A., Wigzell, H., & Grunewald, J. (1996). Different percentages of peripheral blood $\gamma\delta+$ T cells in healthy individuals from different areas of the world. *Scandinavian journal of immunology*, *43*(5), 593-596. <https://doi.org/10.1046/j.1365-3083.1996.d01-79.x>
- Fonseca, S., Pereira, V., Lau, C., Teixeira, M. D. A., Bini-Antunes, M., & Lima, M. (2020). Human peripheral blood gamma delta T cells: report on a series of healthy Caucasian Portuguese adults and comprehensive review of the literature. *Cells*, *9*(3), 729. <https://doi.org/10.3390/cells9030729>
- Hu, Y., Hu, Q., Li, Y., Lu, L., Xiang, Z., Yin, Z., ... & Wu, Y. (2023). $\gamma\delta$ T cells: origin and fate, subsets, diseases and immunotherapy. *Signal transduction and targeted therapy*, *8*(1), Article 434. <https://doi.org/10.1038/s41392-023-01653-8>
- Jia, Z., Ren, Z., Ye, D., Li, J., Xu, Y., Liu, H., ... & Yin, Z. (2023). Immune-ageing evaluation of peripheral T and NK lymphocyte subsets in Chinese healthy adults. *Phenomics*, *3*(4), 360-374. <https://doi.org/10.1007/s43657-023-00106-0>
- Kallemeijn, M. J., Boots, A. M. H., van der Klift, M. Y., Brouwer, E., Abdulahad, W. H., Verhaar, J. A., ... & Langerak, A. W. (2017). Ageing and latent CMV infection impact on maturation, differentiation and exhaustion profiles of T-cell receptor gammadelta T-cells. *Scientific Reports*, *7*(1), Article 5509. <https://doi.org/10.1038/s41598-017-05849-1>
- Lawand, M., Déchanet-Merville, J., & Dieu-Nosjean, M. C. (2017). Key features of gamma-delta T-cell subsets in human diseases and their immunotherapeutic implications. *Frontiers in immunology*, *8*, Article 761. <https://doi.org/10.3389/fimmu.2017.00761>



- Mensurado, S., Blanco-Dominguez, R., & Silva-Santos, B. (2023). The emerging roles of $\gamma\delta$ T cells in cancer immunotherapy. *Nature reviews Clinical oncology*, 20(3), 178-191.
<https://doi.org/10.1038/s41571-022-00722-1>
- Sanz, M., Mann, B. T., Chitrakar, A., & Soriano-Sarabia, N. (2022). Human V δ 2 T cells and their versatility for immunotherapeutic approaches. *Cells*, 11(22), Article 3572.
<https://doi.org/10.1038/s41571-022-00722-1>
- Tan, C. T. Y., Wistuba-Hamprecht, K., Xu, W., Nyunt, M. S. Z., Vasudev, A., Lee, B. T. K., ... & Larbi, A. (2016). V δ 2+ and α/Δ T cells show divergent trajectories during human aging. *Oncotarget*, 7(29), Article 44906. <https://doi.org/10.18632/oncotarget.10096>
- Tyler, C. J., Doherty, D. G., Moser, B., & Eberl, M. (2015). Human V γ 9/V δ 2 T cells: innate adaptors of the immune system. *Cellular immunology*, 296(1), 10-21.
<https://doi.org/10.1016/j.cellimm.2015.01.008>
- Xu, W., Monaco, G., Wong, E. H., Tan, W. L. W., Kared, H., Simoni, Y., ... & Larbi, A. (2019). Mapping of γ/δ T cells reveals V δ 2+ T cells resistance to senescence. *EBioMedicine*, 39, 44-58.
<https://doi.org/10.1016/j.ebiom.2018.11.053>