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Age-dependent immune profile in healthy individuals: an original study, systematic review and meta-analysis

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Abstract

Background The circulatory peripheral immune system is the most convenient approach for determining an individual's immune status. Due to various reasons, while previous studies have addressed the critical impact of age, most individual studies did not analyze immunosenescence in a systemic manner, which complicates the possibility of building a reference range for age-dependent immune profiles for effective immune monitoring. To address this gap, this study analyzed a group of healthy individuals to establish age-specific reference ranges of the healthy circulatory immune profile, and a systematic review and meta-analysis were conducted to validate the findings and create generalizable immune cell reference ranges.

Results Our study recruited a total of 363 healthy Taiwanese adults (median age 42 years [IQR 30, 62], age range 21 to 87 years, 43.3% male), including 158 under 40 years old, 127 between 40–64 years old, and 78 over 64 years old. Significant age-related alterations were observed in both adaptive and innate immune cell subsets. CD8+T cells decreased and CD4/CD8 ratio increased, with notable increases in NK cells. CD4+T cells were less impacted by aging, while CD8+T cells significantly lost CD28 and increased CD31 expression with age. A clear reverse trend in naïve and memory subsets of CD4+and CD8+T cells was observed. Detailed reference ranges for immune cell subsets in healthy Taiwanese adults were established. A systematic review included 7,425 adults and a meta-analysis of 12 eligible studies confirmed our findings in Taiwan, enhancing generalizability.

Conclusions Combined with previous studies and original data through a systematic review and meta-analysis, we highlighted and quantified significant immune profile differences between older and younger individuals. The sex and age-specific reference ranges for peripheral immune cell subsets can serve as a basis for effective immune monitoring of various aging-related illnesses.

Keywords Immunosenescence, Aging, Flow cytometry, Lymphocytes, Reference range

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Background

The human immune system, crucial for defending against infectious pathogens throughout life, continually adapts to the ever-changing environment by immune cell activation, proliferation, and differentiation. Over time, age-related changes in the immune system have been collectively defined as "immunosenescence" [1, 2]. While the term intuitively implies the progressive deterioration of the immune system with aging, it is being realized that the aging process has diverse effects on the immune system, and these changes are not always detrimental and degenerative [3]. For example, the decrease in naïve T cells, a well-characterized aging-related change in the immune system [4], is associated with decreased vaccine response [5] and increased COVID-19-associated mortality [6]. On the other hand, the emergence of T_{EMRA} cells (effector memory T cells with CD45RA re-expression) during aging could be viewed as a curtailed control of virus-specific T cell expansion to create resources for remaining immune cells [7].

Many previous reports from the literature have investigated the phenotypic characterization of circulatory immune cells in healthy humans to define the "healthy" immune system [8]. While some reports have established the reference values for peripheral blood lymphocytes [9–11], these reports failed to consider the potential effects of aging on the immune system. On the other hand, among studies that did consider age's effects on the immune system, they were frequently based on a smaller number of individuals and/or narrow age ranges, and some were based on frozen samples, potentially biased by the viability of immune cells [12, 13]. Furthermore, some studies focus on only specific cell types or fail to account for gender differences [14, 15], thereby overlooking immunosenescence beyond the main lymphocyte subsets [16]. These limitations hinder a comprehensive understanding of age-related changes in the circulatory human immune profile, complicating the definition of immune health across different ages.

In this study, we analyzed the peripheral circulatory T, B, NK cells, and monocyte differentiation subsets in healthy Taiwanese to define the reference range of immune cell populations across different age groups. Furthermore, we conducted a systematic literature review and meta-analysis, integrating our findings with existing research. The meta-analysis aims to corroborate and extend several key findings of age effects on immune cell composition from the literature.

Methods

Enrollment of participants and eligibility criteria

A total of 363 healthy adults, aged between 21 and 87 years old, were recruited from the Health management center of Far Eastern Memorial Hospital between January 2021 and December 2022.

The inclusion criteria were as follows: (1) age twenty years and older, (2) females weighing more than 45 kg, and males weighing more than 50 kg. To determine whether an individual is healthy, all participants were also examined to exclude the following conditions: (1) women who were pregnant or within six months postpartum (including after a miscarriage), (2) individuals who have had major surgery or received a blood transfusion within the last year, (3) individuals who have been vaccinated against measles, German measles, mumps, or polio (oral) with live attenuated vaccines within the last four weeks, (4) individuals currently diagnosed with or undergoing treatment for: syphilis, acquired immunodeficiency syndrome (AIDS), active tuberculosis, diabetes, cardiovascular diseases (arrhythmia, myocardial infarction, and those who have undergone cardiovascular surgery), gastrointestinal ulcer bleeding, kidney-related diseases (chronic renal failure), acute infections, contagious diseases, liver-related diseases (chronic hepatitis, cirrhosis), various cancers, autoimmune diseases (systemic lupus erythematosus, multiple sclerosis, rheumatoid arthritis), (5) participants with coagulopathy, known anemia with hemoglobin levels below 10 mg/dl, or recent trauma. Because the recruitment was performed along at the same time with physical checkups, individuals with incidental findings of illnesses were also excluded. All participants provided the informed consent, and this study was approved by the Institutional Review Board of the National Yang Ming Chiao Tung University (YM109137E) and the Far Eastern Memorial Hospital Research Ethics Committee (108,058-E).

Blood collection and immunophenotyping of peripheral immune cell subsets using flow cytometry

Peripheral blood samples (20 ml each) were collected for all participants before meals at Far Eastern Memorial Hospital. Each sample was processed using a C00162 Duraclone IM Count Tube containing an antibody mixture (Table S1) for staining, followed by adding A09777 VersaLyse buffer to lyze red blood cells. All samples were analyzed using a multicolor flow cytometer Beckman Coulter CYTOFLEX, and after optical scanning by the flow cytometer, data were analyzed using FlowJo software.

Three panels (Figure S1) were designed to identify key immune cell subsets and monocytes, including but not limited to: CD3 + T cells (CD3 +), CD4 + T cells (CD3 + CD4 +), CD8 + T cells (CD3 + CD8 +), B cells, and NK cells [17]. In the T-cell panel, subsets of CD4 + and CD8 + were determined using CD45RA and CD197 (CCR7) to identify naïve (CD197 + CD45RA +),

effector memory RA+(CD197-CD45RA+, T_{EMRA}), central memory (CD197+CD45RA-, T_{CM}), and effector memory (CD197-CD45RA-, T_{EM}) T cells. CD28 is an important costimulatory molecule during T-cell activation and proliferation [18]. CD57 is a surface glycoprotein and CD57+T cells exhibit impairment in replication capacity [19]. Past research has found a high correlation between the increase in CD57 and the decrease in CD28 with age [20]. CD31, also known as platelet endothelial cell adhesion molecule-1 (PECAM-1), is upregulated in naïve cells, and progressively downregulated during T cell differentiation [21]. Thus, we also evaluated the surface expression of CD28, CD57, and CD31 in CD4+and CD8+T cell subsets.

In the B-cell panel, B cells were identified as CD3-CD19+lymphocytes. Different subsets of B cell maturation were examined, including naïve (CD27-IgD+), memory (CD27+IgD- and CD27-IgD-), and transitional B cells (CD38highCD24+). In the NK cell, dendritic cell, and monocyte panel, three groups of NK cells were identified based on their expression of CD3- and different combinations of CD16 and CD56: total NK (CD16+or CD56+), CD56high NK (CD16-CD56+), and CD56low NK (CD16+CD56-). Additionally, three populations of myeloid cells were identified: CD14+CD16+monocytes, CD14+CD16- (conventional/M1) monocytes, and CD14-CD16+(non-conventional/M2). Dendritic cells (DC) were characterized as Lineage (CD3-CD19-CD20-CD16-CD56- HLA-DR+) PBMCs.

Statistical analysis of the Taiwanese original data

After removing outliers that deviated more than three standard deviations from the raw data, we conducted a normality test using Q-Q plots. To explore age-related changes in healthy Taiwanese adults, we categorized 363 participants into three age groups: individuals under 40 years, those between 40–64 years, and those over 64 years. One-way Analysis of Variance (ANOVA) was used to compare the differences in absolute numbers among three age groups, with Tukey's Honestly Significant Difference (HSD) post-hoc test for further comparison. Differences between genders were analyzed by independent-sample t-test. Spearman correlation analysis was used to investigate the relationships between immune cell subsets and age. A spearman correlation matrix visualization was generated using the R package "corrplot". Variables are considered correlated only if the correlation coefficient (r) is greater than 0.2 or less than -0.2, and the *p*-value is less than 0.05. Finally, we conducted a non-parametric bootstrap to calculate reference range (percentile 2.5–97.5). All data synthesis and analysis in this study were conducted using STATA version 18 and R version 4.3.2. All statistical tests were two-tailed with a significance level of 5%.

Systematic review of the literature

The systematic review and meta-analysis of this study followed the PRISMA (Preferred Reporting Items for Systematic Review and Meta-Analysis) guidelines [22]. We conducted a comprehensive search of medical electronic databases, including PubMed, MEDLINE, Embase, and Web of Science, to identify relevant studies published from inception to November 2023. Keywords were organized using Medical Subject Headings (MeSH) such as "Lymphocytes," "Reference ranges," "Flow Cytometry," and "Healthy adults." (Table S2). Additionally, the reference lists of included studies were searched manually to increase the retrieval of studies. Two reviewers (STC, YFC) independently screened the full text of the identified studies based on the inclusion and exclusion criteria (Table S3). Eligible studies were selected to extract data, including sample size, country, and the age group of participants. Furthermore, specific details were extracted regarding the flow cytometry protocol used for measuring lymphocytes, such as the gating strategy, single or dual platform, and instrument used. Summary statistics, including mean, median, percentiles, and confidence intervals, were also extracted for absolute immune cell numbers and/or the percentage of immune cell subsets relative to the total numbers.

Meta-analysis of included studies and Taiwan's data

The meta-analysis included studies that provided data on both the dispersion (e.g., standard deviation) and central tendency (e.g., mean) of immune cell subsets estimation. Studies that only reported a wide age range ($\geq$ 40 years) were not included in the meta-analysis. Details for feasibility of including studies in a meta-analysis are presented in Table S4.

Two approaches of meta-analysis were utilized to assess the mean and normal ranges (2.5th-97.5th percentile) of immune cell numbers from the included studies and our results in Taiwan. First, corresponding to our data analysis, separate meta-analysis was conducted for three distinct age groups: individuals under 40 years old, those between 40–64 years old, and those over 64 years old. This approach allowed comparisons of immune cell numbers within similar age groups. Second, we combined data from all eligible studies and estimated the relationship between immune cell numbers and continuous age from 18 to 95 years old. This approach provided insights into how immune cell numbers change across the entire age spectrum. Both approaches of meta-analysis were conducted using absolute and percentage of immune cell numbers. Detailed information on the meta-analysis

methodology was provided in Additional file 1: Supplementary material. All meta-analysis in our study was conducted using R version 4.3.2.

Results

Significant differences in immune cell subsets across different age groups

A total of 363 healthy Taiwanese adults (median age 42 years [IQR 30, 62], age range 21 to 87 years, 43.3% male) were recruited, including 158 individuals under 40 years old, 127 between 40–64 years old, and 78 over 64 years old. The demographics and average distribution of immune cell subsets in each age group are detailed in Table 1.

Our data revealed a significant age-related increase in NK cells and CD4/CD8 ratio, along with a decrease in CD8+T cells and CD19+B cells (Table 1). In detail, naïve CD4+T cells (Fig. 1a) and naïve CD8+T cells (Fig. 1b) decreased as individuals aged, while the numbers of memory cell subsets like the CD8+effector memory RA+(T_{EMRA}) T cells (Fig. 1b), CD4+central memory (T_{CM}) T cells, CD4+effector memory (T_{EM}) T cells, and CD4+effector memory RA+(T_{EMRA}) T cells (Fig. 1a) were significantly increased with age. Although the numbers of CD4+T cells remained relatively stable, age-related reverse trends of naïve and memory T lymphocyte subsets were clearly demonstrated (Fig. 1e). In the older age group, there was also an increase in absolute numbers of NK cells, primarily attributed to a significant increase in $CD56_{low}$ NK cells (Fig. 1c). Lastly, although total CD19+B cells decreased with age (Fig. 1d), the decrease was not due to changes in the naive, memory, or transitional B cell subsets, which remained relatively stable.

Besides changes in the aforementioned T differentiation status, T cells exhibit many other phenotypic changes during aging. With increasing age, a significant increase in absolute numbers of CD8+CD28-CD57+T cells were observed, while CD8+CD31+T cells (Fig. 1b) decreased significantly with advancing age. Nevertheless, the numbers of CD4+CD31+T cells (p=0.59), CD4+CD28+T cells (p=0.55), and CD4+CD28null T cells (p=0.1) remained relatively stable across three age groups (Table 1).

As expected, the number of total monocytes increased with increasing age. Cell numbers of different monocyte subsets (M1, M2, M3) also showed tendencies to increase with age. Nevertheless, the number of dendritic cells was not affected by age.

Correlations between immune cell subsets and age

Since peripheral blood immune subsets showed significant differences between age groups, a Spearman correlation matrix was used to analyze all possible correlation coefficients between immune cell subsets and age, or between the subsets themselves (Figure S2).

The percentage of CD3+T cells showed a significant negative correlation with age (percentage: r=-0.34, p<0.0001), while their absolute numbers also decreased. There was a significant correlation between age and CD8+T cells (absolute: r=-0.27, p<0.0001; percentage: r=-0.38, p<0.0001), as were CD4/CD8 ratio (r=0.31, p<0.0001), NK cells (absolute: r=0.21, p<0.0001), and CD19+B cells (percentage: r=-0.23, p<0.0001). Again, CD4+T cells did not correlate with age in either absolute or percentage numbers. Finally, there was a strong correlation between age and naïve CD8+T cells (absolute: r=-0.76, p<0.0001).

Effects of gender on immunosenescence

In our study of 363 healthy adults, which comprising 157 males and 206 females across various age groups, we discovered significant gender-related differences in immune cell numbers. Notably, CD4/CD8 ratio was significantly higher in females than in males (p=0.017, Table S5). This distinction was also significant in the age group of 40–64 years (p=0.017, Table S7) and in those over 64 years (p=0.035, Table S8). Meanwhile, females showed a higher level of memory B cells (p=0.005, Table S5) and CD4+CD31+T cells (p<0.001, Table S5), while males demonstrated a higher level of monocytes (p=0.001, Table S5), CD14+CD16- (conventional/M1) monocytes (p=0.004, Table S5) and CD8+CD28-CD57+T cells (p=0.021, Table S5).

Given the notable effect of age and gender on the immune system, we established estimated reference ranges for immune cell subsets in Taiwanese males and females separately across different age groups, detailed in Table S6-S8.

Study selection for systemic review

The selection of studies was guided by the PRISMA (Preferred Reporting Items for Systematic Review and Meta-Analysis) guidelines, as shown in Figure S3. After reviewing the titles and abstracts of 236 articles found in the electronic database, 50 articles were selected for a thorough full-text review. 29 studies [8–13, 15, 16, 23–43] met the eligibility criteria for inclusion and the relevant data was extracted as shown in Table S9.

Due to insufficient data for estimation (e.g. only reported median) and/or wide age range, 18 studies [8, 9, 11, 12, 23, 25–30, 32, 33, 35, 37, 38, 41, 43] were excluded from the meta-analysis (Table S4). Data from 11 studies [10, 13, 15, 16, 24, 31, 34, 36, 39, 40, 42] and Taiwan's data were used: 12 studies for the meta-analysis of immune

Table 1 Demographics and immune cell subsets distribution of study population ($n = 363$) by three age groups

	<40 years old ($n = 158$)	**40–64 years old ($n = 127$)**	**>64 years old ($n = 78$)**	***p*-value**
Age, median (Q1, Q3)	29 (27, 34)	47 (43, 55)	70.5 (67, 76)	<0.001
Gender; Male, *n* (%)	72 (45.6%)	56 (44.1%)	29 (37.2%)	0.46
Immune cell subsets, mean (SD)				
Lymphocyte (cells/μL)	1942 (631)	1992 (668)	2069 (706)	0.38
CD3+T cells (%)	68.2 (7.0)	66.3 (7.4)[††]	60.3 (9.6)[**]	<0.001
CD3+T cells (cells/μL)	1324 (459)	1316 (452)	1253 (499)	0.52
CD4+T cells (%)	34.7 (6.5)	36.8 (7.3)	35.1 (8.7)	0.054
CD4+T cells (cells/μL)	682 (286)	735 (293)	731 (341)	0.26
Naïve T cells (cells/μL)	331 (178)	303 (186)[†]	221 (158)[**]	<0.001
Central memory T cells (T_{CM}), (cells/μL)	138 (79)[§]	174 (114)	198 (122)[**]	<0.001
Effector Memory T cells (T_{EM}), (cells/μL)	192 (99)[§]	236 (125)	271 (213)[**]	<0.001
Effector memory RA+T cells (T_{EMRA}), (cells/μL)	20 (22)	22 (27)[†]	40 (83)[*]	0.003
CD4+CD28+T cells (cells/μL)	624 (266)	659 (274)	642 (276)	0.55
CD4+CD28null T cells (cells/μL)	58 (56)	76 (86)	89 (193)	0.100
CD4+CD31+T cells (cells/μL)	292 (144)	277 (122)	280 (145)	0.59
CD8+T cells (%)	25.0 (5.6)[§§]	22.3 (6.7)[††]	18.3 (6.9)[**]	<0.001
CD8+T cells (cells/μL)	480 (175)	434 (170)[††]	382 (219)[**]	<0.001
Naïve T cells (cells/μL)	223 (115)[§§]	119 (80)[††]	31 (33)[**]	<0.001
Central memory T cells (T_{CM}), (cells/μL)	24 (33)	33 (51)	24 (38)	0.18
Effector Memory T cells (T_{EM}), (cells/μL)	153 (72)	167 (102)	182 (135)	0.088
Effector memory RA+T cells (T_{EMRA}), (cells/μL)	80 (83)	116 (106)[††]	145 (130)[**]	<0.001
CD8+CD31+T cells (cells/μL)	344 (142)[§§]	285 (107)	264 (154)[**]	<0.001
CD8+CD28-CD57+T cells (cells/μL)	43 (57)[§§]	68 (68)	84 (83)[**]	<0.001
CD4/CD8ratio	1.48 (0.54)[§§]	1.87 (0.82)[††]	2.30 (1.34)[**]	<0.001
CD19+B cells (%)	14.7 (4.4)	14.0 (4.5)[††]	11.4 (5.5)[**]	<0.001
CD19+B cells (cells/μL)	285 (129)	282 (150)[†]	237 (148)[*]	0.032
CD19+CD20+B cells (cells/μL)	280 (127)	279 (148)[†]	234 (147)[*]	0.036
Naïve B cells (cells/μL)	127 (70)	131 (94)	135 (96)	0.75
Memory B cells (cells/μL)	70 (43)	78 (48)	75 (51)	0.34
Transitional B cells (cells/μL)	9 (11)	7 (7)	9 (10)	0.16
NK cells (%)	14.3 (7.9)	14.5 (9.0)[††]	19.7 (10.4)[**]	<0.001
NK cells (cells/μL)	279 (146)	326 (211)[†]	403 (244)[**]	<0.001
NK cells/CD56 high NK cells (cells/μL)	11 (9)	12 (24)	7 (4)	0.14
NK cells/CD56 low NK cells (cells/μL)	268 (146)	314 (208)[†]	396 (243)[**]	<0.001
CD86+monocytes (cells/μL)	399 (151)[§]	400 (138)[†]	451 (119)	0.017
Monocytes (cells/μL)	457 (178)	453 (165)[†]	509 (131)	0.042
CD14+CD16- monocytes (M1), (cells/μL)	334 (131)	326 (120)	361 (98)	0.14
CD14+CD16+monocytes (cells/μL)	14 (17)	16 (24)	20 (14)	0.053
CD14-CD16- monocytes (M2), (cells/μL)	24 (37)	29 (49)	35 (19)	0.17
Dendritic cells (DC), (cells/μL)	267 (141)	263 (135)	257 (100)	0.86

[*] $p \le 0.05$: <40 years vs. >64 years

[**] $p \le 0.001$: <40 years vs. >64 years

[§] $p \le 0.05$: <40 years vs. 40–64 years

[§§] $p \le 0.001$: <40 years vs. 40–64 years

[†] $p \le 0.05$: 40–64 years vs. >64 years

[††] $p \le 0.001$: 40–64 years vs. >64 years

P-values were all determined with the Tukey's Honestly Significant Difference (HSD) post-hoc test

Table 2 Summary of immune cell subsets change associated with aging

Immune cell subsets	Result from meta-analysis	Result from original study in Taiwan[a]
CD3 + T cell number	↓ (Absolute) /↓ (Percentage)	— (Absolute) /↓ (Percentage)
CD4 + T cell number	↑ (Absolute) /↓ (Percentage)	—
Naïve T cell number	NP	↓
Central memory T cell (T_{CM}) number	NP	↑
Effector Memory T cell (T_{EM}) number	NP	↑
Effector memory RA + T cell (T_{EMRA}) number	NP	—
CD8 + T cell number	↓	↓
Naïve T cell number	NP	↓
Central memory T cell (T_{CM}) number	NP	—
Effector Memory T cell (T_{EM}) number	NP	—
Effector memory RA + T cell (T_{EMRA}) number	NP	↑
CD4/CD8 ratio	↑	↑
NK cell number	↑	↑
CD56high NK cell number	NP	↓
CD56low NK cell number	NP	↑
B cell number	↓	↓
Naïve B cell number	NP	—
Memory B cell number	NP	—
Transitional B cell number	NP	—

Changes are indicated with an up arrow (↑) for increase, a down arrow (↓) for decrease, and absence of changes with a horizontal bar (—)

NP Not provided in meta-analysis

[a] Results from original study in Taiwan were according to the spearman correlation matrix analysis as shown in Additional file 1: Figure S2

cell absolute numbers and 11 for the meta-analysis of the percentage numbers of immune cells.

The detailed characteristics of the included studies were described in Table S10. Our research included various geography setting: 9 studies in Asia [9, 13, 14, 16, 27, 29, 34, 38, 39], 5 in Middle East [15, 23–25, 40], 4 in Europe [8, 12, 26, 28], 4 in South America [10, 31, 35, 37], 3 in Africa [30, 33, 36], 1 in USA [41], and 1 in Australia [11]. These studies all focused on immune cell numbers in healthy adults using flow cytometry, totaling 17 studies [8, 11, 13, 16, 23, 24, 26, 27, 31, 32, 35, 36, 38–40, 42, 43] that employed CD45 versus side scatter and 3 studies [8, 9, 24] that used forward and side scatter as a gating strategy. The gating strategy in the remaining studies was not clearly defined. All studies had reported the type of anticoagulants used: 27 used EDTA, 1 used Acid Citrate Dextrose (ACD), and 3 used heparins.

Overall, a total of 7,245 healthy adults were involved in the systematic review, with 4,116 healthy adults aged between 18 and 95 years included in the meta-analysis.

Meta-analyses of age-dependent immune changes

The meta-analyses based on three age groups (see Table S11) showed a similar trend in both percentage and absolute immune cell numbers. We found that the pooled mean of CD3 + T cell decreased in numbers from 1517 cells/μ L (or 71.11%) in age < 40 years old to 1337 cells/μ L (or 61.13%) in age ≥ 65 years old; CD8 + T cell numbers decreased from 611 cells/μ L (or 27.79%) to 525 cells/μ L (or 23.88%); CD4/CD8 ratio increased from 1.54 to 1.9; B cell numbers decreased from 244 cells/μ L (or 12.99%) to 217 cells/μ L (or 8.83%); NK cell numbers increased from 305 cells/μ L (or 13.69%) to 355 cells/μ L (or 23.59%). Nevertheless, CD4 + T cells (either absolute numbers or percentages) showed no specific pattern across the three age groups.

The meta-analysis considering continuous age in years, showed that the relationship between immune cell numbers and age mirrors the pattern observed in the aggregated results across three age groups (see Fig. 2 and Table S12).

Most numbers of immune cell subsets changed with specific pattern across age, except for CD4 + T cells. From ages 18 to 94, the pooled mean of CD3 + T cell numbers decreased from 1512 cells/μ L (74.1%) to 1405 cells/μ L (62.7%) with advancing age (Fig. 2a and g); CD8 + T cell numbers decreased from 598 cells/μ L (or 28.4%) to 507 cells/μ L (or 23.9%) (Fig. 2c and i) corresponding with an

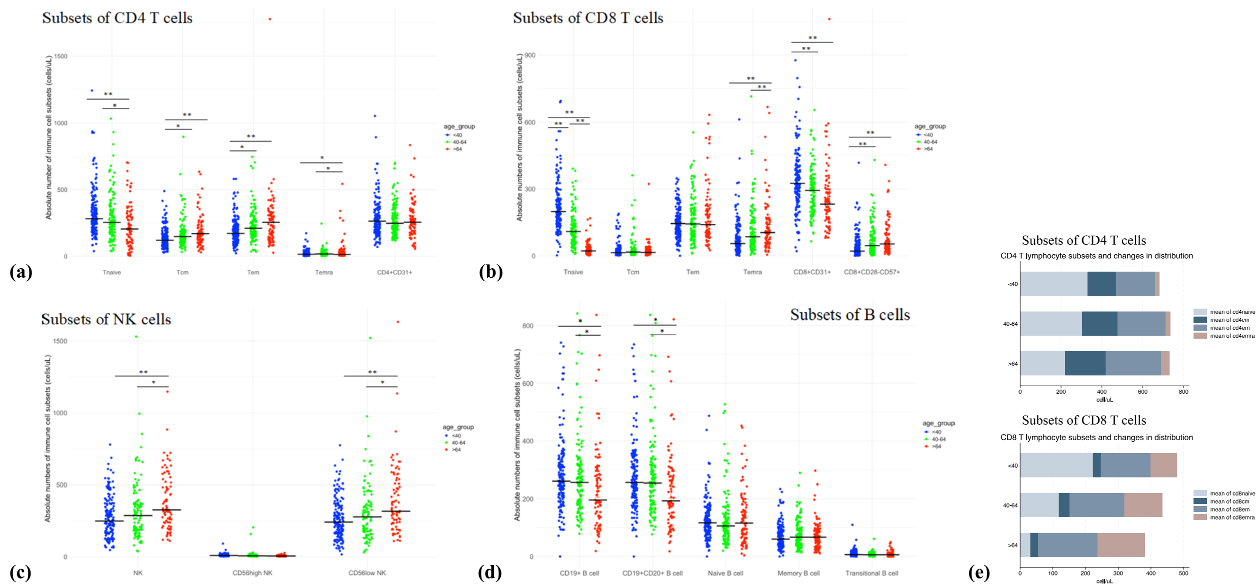

Fig. 1 Detailed comparison of absolute numbers of immune cell subsets in three age groups. **a** Subsets of CD4 T cells (**b**) Subsets of CD8 T cells. **c** Subsets of NK cells. **d** Subsets of B cells. **e** Comparison of CD4+T cell subsets and CD8+T cell subsets distribution across three age groups (blue: < 40 years old, green: 40–64 years old, red: > 64 years old). Black bars: medians. *P*-values were determined with the Tukey's Honestly Significant Difference (HSD) post-hoc test. $^{**}p \leq 0.001$ $^{*}p \leq 0.05$

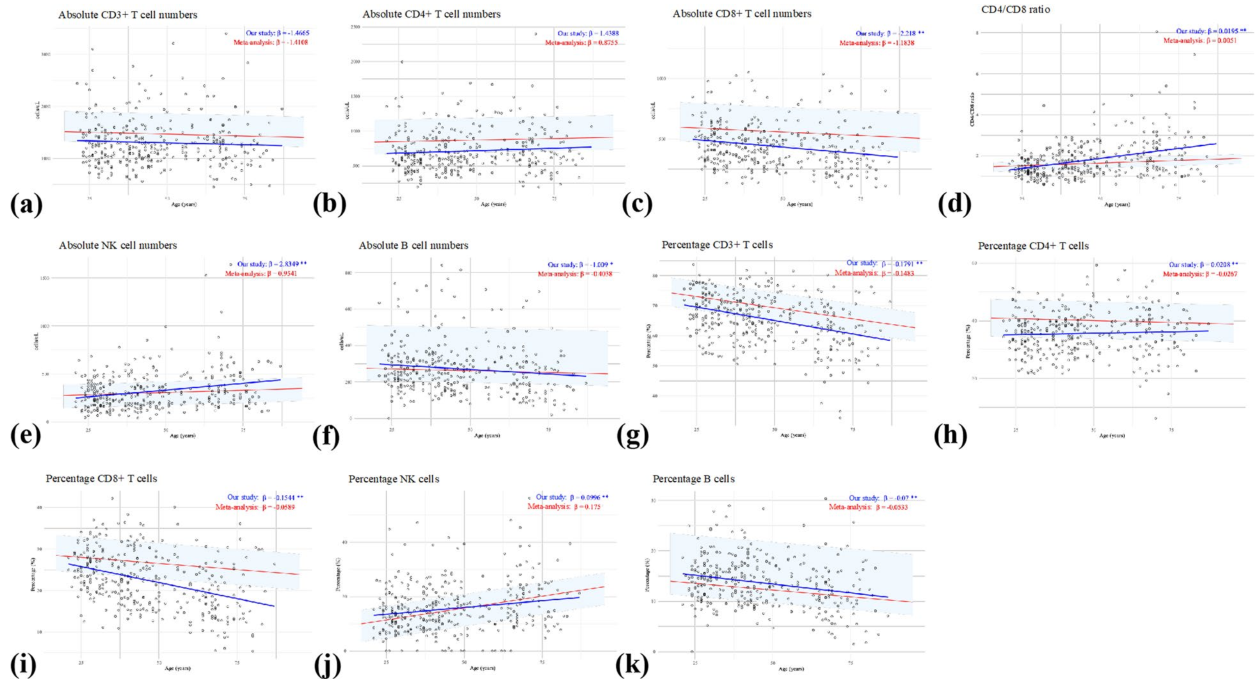

Fig. 2 Meta-analysis of absolute numbers and percentage of immune cell subsets according to continuous age (years), including the pooled mean (red line) and the pooled normal range in 2.5th -97.5.th percentile (skyblue region) with the liner curve (blue line) fitting of Taiwan's data (black hollow dots). $^{**}p \leq 0.001$ $^{*}p \leq 0.05$

increased CD4/CD8 ratio from 1.478 to 1.873 (Fig. 2d); NK cell increased from 279 cells/μ L (or 10.2%) to 352 cells/μ L (or 23.6%) (Fig. 2e and j); B cells decreased from 276 cells/μ L (or 13.9%) to 245 cells/μ L (or 9.8%) (Fig. 2f and k). Despite an increase in the absolute count of CD4+T cells from 843 to 910 cells/μL, their percentage

declined from 40.9% to 38.8% (Fig. 2b and h). This discrepancy in trends between absolute numbers and percentages suggests no specific age-related changes in CD4 + T cells. Lastly, the linear regression analysis of the Taiwanese immune cell subsets aligned with the normal range established by our meta-analysis, confirming the consistency of trends observed in Taiwan's data with the aggregated findings (see Fig. 2 and Table 2).

Discussion

In this study, we observed significant age-related changes in both adaptive and innate immune cell subsets among healthy adults. Key findings included a decrease in CD8 + T cells and an increase in the CD4/CD8 ratio with aging, accompanied by a notable increase in NK cells and a decrease in B cells. Interestingly, the numbers of CD4 + T cells appear less impacted by the aging process compared to CD8 + T cells and other subsets of immune cells. Additionally, our findings from Taiwan revealed a clear reverse trend of naïve and memory functional subsets in both CD4 + and CD8 + T cells. We also established a robust reference range of peripheral blood immune cell subsets in Taiwanese healthy adults. Finally, we complemented the original data with a systemic review and meta-analysis of 12 studies to estimate the normal ranges for major immune cell subsets. Our analyses concluded the key immunosenescence characteristics beyond our original data, significantly enhancing the generalizability of our findings and aligning the results with most studies in the meta-analysis. The results and analyses are of high translational and clinical value and can serve as a basis for implanting immune monitoring in preventive and therapeutic scenarios.

A clear reverse trend of naïve and memory functional subsets in both CD4 + and CD8 + T cells could be explained by a process known as thymic involution. Thymic involution significantly impacts cellular aging by reducing the number of naïve T cells and narrowing the diversity of the T-cell receptor (TCR) repertoire, which contributes to a weakened and exhausted immune system [44]. The age-related decrease in naïve T cells may partially explain the reduced capacity to respond to new antigens and the increased susceptibility to malignancies in older adults [45, 46]. Furthermore, as reviewed by Yu et al., the increase in highly differentiated memory T cell subsets may have pathogenic potential in cardiovascular diseases by releasing pro-inflammatory cytokines and cytotoxic mediators [47]. These findings highlight the critical role of lymphocyte numbers of changes in various age-related diseases and conditions. However, recent studies suggest that many age-related changes in the immune system may be beneficial adaptations to the normal aging process [3]. For example, thymic involution may be essential for minimizing energy expenditure in an organ that, while not crucial for survival, requires significant energy to maintain; similarly, an increase in memory subsets of lymphocytes may enhance the ability to combat pathogens more efficiently. Although immunosenescence may contribute to age-related diseases, its overall impact on aging remains a debated topic.

The finding that CD4 + T cells showed greater resistance to age-related phenotypic changes compared to CD8 + T cells aligned with results from prior studies [48, 49]. While the total number of CD4 + T cells remained relatively stable, we observed a significant change in the composition of memory subsets with aging. That included a decrease in naïve subsets and a corresponding increase in memory subsets in both CD4 + and CD8 + T cells with a more pronounced magnitude observed in CD8 + T cell. This may also explain why the ratio of CD4 + to CD8 + T cells significantly increased with age. However, the exact mechanisms involved remain unclear.

With aging and increased exposure to chronic antigens, previous research has also suggested an expansion of CD28 − CD57 + T cells, predominantly within the CD8 + compartment [20, 50], a trend that was also confirmed in our study. The CD8 + CD28 − CD57 + T cells are characterized as advanced-differentiated cells, with significantly shortened telomeres, reduced proliferation capacity, and a shorter replicative lifespan [50]. These characteristics may contribute to immune incompetence, susceptibility to persistent viral infections, and reduced response to anti-cancer treatment or vaccination in older adults [51]. Along with thymic involution, the age-related increase of these senescent subsets also results in a marked decrease in antigenic diversity in older individuals [52]. This evidence highlights the importance of monitoring CD8 + CD28 − CD57 + T cells in aging, and our data suggest that their decline is a typical feature of immune aging. Of note, many aforementioned aging-associated changes in T cells (especially CD8 +) have been associated with CMV infection [53]. CMV infection drives the expansion of CMV-specific memory T cells with aging, and these cells frequently reside in the late-differentiated CD28-CD57 + compartment. Despite numerous research, CMV's dynamic, bidirectional interaction with immunosenescence remains a poorly understood complex phenomenon [54].

Besides T cells, alterations in the numbers of NK cells are also a significant marker of immune aging. A remodeling of NK cell subsets characterized by a decrease in the number of the more immature $CD56_{high}$ NK cells and an increase of $CD56_{low}$ subsets was clearly shown. Few studies included in our systematic review have evaluated the numbers of $CD56_{high}$ and $CD56_{low}$ NK cells separately.

$CD56_{low}$ NK cells are mature subsets mainly involved in cytotoxic activities, while $CD56_{high}$ NK cells are progenitor subsets but primarily responsible for producing cytokines like TNF-α and IFN-γ [55]. During the aging process, $CD56_{low}$ NK cells continue to differentiate and increase the expression of CD57 and CD16 markers, activating receptors such as NKG2D, and experience telomere shortening [56]. Previous research based on single-cell RNA expression analysis also found the context of aging in NK cells, with expansion of low cytotoxic subsets and decreased virus defense responses [57]. In contrast to T cells, although we already know that the levels of CMV antibodies are closely associated with the aging of T cell immunity [48], whether CMV serostatus affects the numbers of NK cells is still controversial [56]. Overall, there is a great number of knowledges regarding the alterations in NK cells numbers and function as individuals age; however, there remains a gap in understanding the underlying factors that contribute to these changes.

Aside from NK cells, other subsets of innate immune cells showed significant age-related changes. There was an increase in the absolute number of monocytes, while dendritic cells remained unchanged. This aligned with the general expectation that peripheral cells of innate immunity may be better preserved with age compared to the adaptive immune system [3, 41], while their changes contribute to an age-dependent increase in chronic inflammation, or "inflammaging" [58].

Compared to other immune cells, conflicting results have been shown regarding peripheral B cells. B cells are antigen-presenting cells (APCs) essential for humoral immunity by secreting antibodies and modulating T cell activation. Our original data showed an age-dependent decrease in total B cell number, but subset homeostasis was unchanged. One study included in our review has found that B cell subsets are increased in the peripheral blood of healthy elderly adults [24], while others have not [12, 16]. However, functional changes in B lymphocytes associated with aging have been reported. Frasca et al. [59] observed that although the frequencies of influenza vaccine-specific memory B cells and plasmablasts were similar in young and elderly adults, older individuals exhibited a diminished fold-increase in serum titers following vaccination. This suggests a compromised ability in older adults to differentiate from memory B cells to plasma cells. Consequently, older individuals may show a reduced capacity to generate antibodies even after immunization, underlining a critical aspect of immunosenescence. Longitudinal studies involving standardized methods for B cell count and functional analysis will be critical to uncovering additional effects of aging on B cell immunity.

Our study also demonstrated distinct age-dependent changes in innate and adaptive immunity between males and females. The significant increase in the CD4/CD8 ratio, memory B cells, and CD4 T cell subsets in females and the increase in monocyte numbers in males suggests hormonal influences. Previous reports [60] indicate that estrogen enhances both cell-mediated and humoral immune responses, and genes associated with adaptive immunity are elevated in post-pubertal females [61]. Recently, a study investigating the epigenome signature of aging revealed that 15 times more monocyte epigenetic loci were activated in men than women. In contrast, B cell-specific loci were significantly inactivated in men [62]. Additionally, our results revealed an increased CD8+CD28-CD57+T cells in males compared to females. As a double-edged sword, the faster aging of the T cell compartment in males may benefit autoimmune conditions but also contribute to increased immunosenescence and risk of malignancy [61].

Our original study includes a detailed analysis of immune cell subsets and employs a robust systematic review and meta-analysis to integrate existing related research, thereby increasing the overall confidence and validation of our results. Evidently, age and gender differences should be considered when interpreting immune profiles in clinical practice when immune monitoring is necessary. Therefore, establishing a reference range is crucial for accurately assessing diverse patients in clinical settings, particularly for managing age-related immune changes in geriatrics and immunocompromised hosts. For example, the number of circulatory naïve T cells could predict the efficacy of influenza vaccination [63] and severity of COVID-19 infection [6]. Measuring immunosenescence provided a better prediction of mortality [64] than using well-established cardiovascular disease risk factors.

Nevertheless, several limitations should be noted. First, we only examined immune cell subsets in peripheral blood and did not analyze tissues like the spleen, lymph nodes, or interstitial mucosa, where most immune cells are located. Additionally, we did not investigate genetic factors or CMV serology status, which could affect immune responses based on prior studies. Nevertheless, given the high prevalence rate of CMV infection in the overall population, a large comprehensive study investigating a large population of only CMV-uninfected individuals will be difficult to execute. Lastly, estimations of immune cell numbers can vary depending on the gating strategy used and can also differ between laboratories, potentially introducing heterogeneity into meta-analyses.

Not all elderly individuals experience age-related diseases, as evidenced by the healthy older adults in our

study and, remarkably, by centenarians [65]. Research has shown that centenarians exhibit well-balanced levels of inflammatory and anti-inflammatory responses and infections do not necessarily shorten their lifespan. Future studies should try to monitor an individual's immune system over its lifespan, focusing on the association between the immune system and ideal aging, which involves maintaining a dynamic and resilient balance in response to various events over time. In contrast, an inability to adapt could lead to maladaptation, manifesting as increased severity of age-related diseases and increased mortality in both acute and chronic healthcare settings.

Conclusions

Since healthy aging involves maintaining a dynamic equilibrium of the immune system with the environment, it becomes essential to identify phenotypic changes in circulating immune cells across age in healthy adults. Examining a total of 363 healthy Taiwanese adults, this study thus established detailed reference ranges of peripheral immune subsets for different age groups to provide values for clinical decision-making and contribute to the effective management of various age-related diseases. Besides novel observations, results were also validated through a rigorous systematic review and meta-analysis.

Abbreviations

CMV	Cytomegalovirus
EDTA	Ethylenediaminetetraacetic Acid
DC	Dendritic Cells
HLA-DR	Human Leukocyte Antigen—DR isotype
IFN-γ	Interferon-gamma
TNF-α	Tumor Necrosis Factor-alpha
NK	Natural Killer) cells
PBMCs	Peripheral Blood Mononuclear Cells
PECAM-1	Platelet Endothelial Cell Adhesion Molecule-1
Q-Q plots	Quantile-Quantile plots
RA	Retinoic Acid

Supplementary Information

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Supplementary Material 1.

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Authors' contributions

YLC and YFC designed and initiated the study. YLC, IYC, and RWH recruited study populations. IYC and RWH carried out experiments. STC analyzed the experiments. MRL assisted in the analysis of experiments. AHL, YTF, and TOY helped with the execution and analysis of the study. STC, YFC, and YLC interpreted the data and wrote the manuscript. All authors contributed to reading and completing the manuscript and have approved the final version.

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Data availability

The authors will provide the raw data supporting the conclusions of this article upon request without restriction.

Declarations

Ethics approval and consent to participate

All participants provided informed consent, and the study received approval from the Institutional Review Board of the National Yang Ming Chiao Tung University (YM109137E) and the Far Eastern Memorial Hospital Research Ethics Committee (108058-E).

Consent for publication

All authors have given their approval for the publication of this final version of the manuscript.

Competing interests

The authors declare no competing interests.

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References

1. Nikolich-Zugich J. The twilight of immunity: emerging concepts in aging of the immune system. Nat Immunol. 2018;19(1):10–9.
2. Pawelec G, Larbi A, Derhovanessian E. Senescence of the human immune system. J Comp Pathol. 2009;142(Suppl 1):S39–44.
3. Fulop T, Larbi A, Dupuis G, Le Page A, Frost EH, Cohen AA, Witkowski JM, Franceschi C. Immunosenescence and inflamm-aging as two sides of the same coin: friends or foes? Front Immunol. 2017;8:1960.
4. Goronzy JJ, Fang F, Cavanagh MM, Qi Q, Weyand CM. Naive T cell maintenance and function in human aging. J Immunol. 2015;194(9):4073–80.
5. Crooke SN, Ovsyannikova IG, Poland GA, Kennedy RB. Immunosenescence and human vaccine immune responses. Immun Ageing. 2019;16:25.
6. Rydyznski Moderbacher C, Ramirez SI, Dan JM, Grifoni A, Hastie KM, Weiskopf D, Belanger S, Abbott RK, Kim C, Choi J et al.: Antigen-Specific Adaptive Immunity to SARS-CoV-2 in Acute COVID-19 and Associations with Age and Disease Severity. Cell 2020, 183(4):996–1012 e1019.
7. Akbar AN, Henson SM. Are senescence and exhaustion intertwined or unrelated processes that compromise immunity? Nat Rev Immunol. 2011;11(4):289–95.
8. Rovati B, Mariucci S, Poma R, Tinelli C, Delfanti S, Pedrazzoli P. An eight-colour flow cytometric method for the detection of reference values of lymphocyte subsets in selected healthy donors. Clin Exper Med. 2014;14(3):249–59.
9. Chng WJ, Tan GB, Kuperan P. Establishment of adult peripheral blood lymphocyte subset reference range for an Asian population by single-platform flow cytometry: influence of age, sex, and race and comparison with other published studies. Clin Diagn Lab Immunol. 2004;11(1):168–73.

10. Moreno-Galván M, Palafox A. CD4+ CD8+ T cell reference values in the Mexico City population. Clin Vaccine Immunol. 2013;20(2):306–8.
11. Brotchie J, Chapple P, Came N, Liew D, Juneja S. Reference ranges of peripheral blood lymphoid subsets by 10-colour flow cytometry based on 69 healthy adults from Australia. Pathology. 2016;48(6):631–4.
12. Jentsch-Ullrich K, Koenigsmann M, Mohren M, Franke A. Lymphocyte subsets' reference ranges in an age- and gender-balanced population of 100 healthy adults–a monocentric German study. Clin Immunolog (Orlando, Fla). 2005;116(2):192–7.
13. Choi J, Lee SJ, Lee YA, Maeng HG, Lee JK, Kang YW. Reference values for peripheral blood lymphocyte subsets in a healthy korean population. Immune Netw. 2014;14(6):289–95.
14. Xia Y, Liu A, Li W, Liu Y, Zhang G, Ye S, Zhao Z, Shi J, Jia Y, Liu X, et al. Reference range of naïve T and T memory lymphocyte subsets in peripheral blood of healthy adult. Clin Exp Immunol. 2022;207(2):208–17.
15. Shahal-Zimra Y, Rotem Z, Chezar J, Shochat T, Ross L, Pickholtz I, Rabizadeh E. Lymphocyte Subset Reference Ranges in Healthy Israeli Adults. Isr Med Assoc J. 2016;18(12):739–43.
16. Sun HQ, Kang X, Chen XC, Cai LL, Li YR, Yu JH, Wu C, Deng XL. Immunosenescence evaluation of peripheral blood lymphocyte subsets in 957 healthy adults from 20 to 95 years old. Exp Gerontol. 2022;157:11.
17. Finak G, Langweiler M, Jaimes M, Malek M, Taghiyar J, Korin Y, Raddassi K, Devine L, Obermoser G, Pekalski ML, et al. Standardizing flow cytometry immunophenotyping analysis from the human immunophenotyping consortium. Sci Rep. 2016;6:20686.
18. Zhang H, Watanabe R, Berry GJ, Nadler SG, Goronzy JJ, Weyand CM. CD28 signaling controls metabolic fitness of pathogenic T cells in medium and large vessel vasculitis. J Am Coll Cardiol. 2019;73(14):1811–23.
19. Pangrazzi L, Reidla J, Carmona Arana JA, Naismith E, Miggitsch C, Meryk A, Keller M, Krause AAN, Melzer FL, Trieb K, et al. CD28 and CD57 define four populations with distinct phenotypic properties within human CD8(+) T cells. Eur J Immunol. 2020;50(3):363–79.
20. Czesnikiewicz-Guzik M, Lee WW, Cui D, Hiruma Y, Lamar DL, Yang ZZ, Ouslander JG, Weyand CM, Goronzy JJ. T cell subset-specific susceptibility to aging. Clin Immunol. 2008;127(1):107–18.
21. Briceño O, Peralta-Prado A, Garrido-Rodríguez D, Romero-Mora K, Chávez-Torres M, de la Barrera CA, Reyes-Terán G, Ávila-Ríos S. Characterization of CD31 expression in CD4+ and CD8+T cell subpopulations in chronic untreated HIV infection. Immunol Lett. 2021;235:22–31.
22. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, Shamseer L, Tetzlaff JM, Akl EA, Brennan SE, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ. 2021;372:n71.
23. Al-Mawali A, Pinto AD, Al Busaidi R, Al-Zakwani I. Lymphocyte subsets: reference ranges in an age- and gender-balanced population of omani healthy adults. Cytom Part A. 2013;83A(8):739–44.
24. Al-Thani A, Hamdi WS, Al-Marwani A, Alnaqdy A, Sharafeldin H. Reference ranges of lymphocyte subsets in healthy Qatari adults. Biomark Med. 2015;9(5):443–52.
25. Alamooti AA, Ardalan FA, Abdolahi A, Zeidi M, Firouzjaie F. Determination of Lymphocyte Subsets Reference Values in Healthy Iranian Men by a Single Platform Flow Cytometric Method. Cytom Part A. 2010;77A(9):890–4.
26. Bisset LR, Lung TL, Kaelin M, Ludwig E, Dubs RW. Reference values for peripheral blood lymphocyte phenotypes applicable to the healthy adult population in Switzerland. Eur J Haematol. 2004;72(3):203–12.
27. Choong ML, Ton SH, Cheong SK. Influence of race, age and sex on the lymphocyte subsets in peripheral blood of healthy Malaysian adults. Ann Clin Biochem. 1995;32(6):532–9.
28. García-Dabrio MC, Pujol-Moix N, Martinez-Perez A, Fontcuberta J, Souto JC, Soria JM, Nomdedéu JF. Influence of Age, Gender and Lifestyle in Lymphocyte Subsets: Report from the Spanish Gait-2 Study. Acta Haematol. 2012;127(4):244–9.
29. Jiao Y, Qiu Z, Xie J, Li D, Li T. Reference ranges and age-related changes of peripheral blood lymphocyte subsets in Chinese healthy adults. Sci China C Life Sci. 2009;52(7):643–50.
30. Klose N, Coulibaly B, Tebit DM, Nauwelaers F, Spengler HP, Kynast-Wolf G, Kouyaté B, Kräusslich HG, Böhler T. Immunohematological reference values for healthy adults in Burkina Faso. Clin Vaccine Immunol. 2007;14(6):782–4.
31. Kokuina E, Breff-Fonseca MC, Villegas-Valverde CA, Mora-Díaz I. Normal Values of T, B and NK Lymphocyte Subpopulations in Peripheral Blood of Healthy Cuban Adults. MEDICC Rev. 2019;21(2–3):16–21.
32. Liu W, Xu J, Pu Q, Lan M, Zhang X, Gu Y, Wang Y, Zheng F, Qian J, Fan C, et al. The reference ranges and characteristics of lymphocyte parameters and the correlation between lymphocyte parameters and routine health indicators in adults from China. Immun Ageing. 2022;19(1):42.
33. Naluyima P, Eller LA, Ouma BJ, Kyabaggu D, Kataaha P, Guwatudde D, Kibuuka H, Wabwire-Mangen F, Robb ML, Michael NL, et al. Sex and urbanicity contribute to variation in lymphocyte distribution across ugandan populations. PLoS ONE. 2016;11(1):16.
34. Qin L, Jing X, Qiu Z, Cao W, Jiao Y, Routy J-P, Li T. Aging of immune system: Immune signature from peripheral blood lymphocyte subsets in 1068 healthy adults. Aging. 2016;8(5):848–59.
35. Rudolf-Oliveira RCM, Gonçalves KT, Martignago ML, Mengatto V, Gaspar PC, de Moraes ACR, da Silva RM, Bazzo ML, Santos-Silva MC. Determination of lymphocyte subset reference ranges in peripheral blood of healthy adults by a dual-platform flow cytometry method. Immunol Lett. 2015;163(1):96–101.
36. Sahmoudi K, El Allam A, El Fakihi S, Tahoune H, Sadak A, El Hafidi N, Bourkadi J, El Aouad R, Seghrouchni F. Moroccan lymphocyte subsets reference ranges: age, gender, ethnicity, and socio-economic factors dependent differences. J Immunoassay Immunochem. 2020;41(3):281–96.
37. Valiathan R, Deeb K, Diamante M, Ashman M, Sachdeva N, Asthana D. Reference ranges of lymphocyte subsets in healthy adults and adolescents with special mention of T cell maturation subsets in adults of South Florida. Immunobiology. 2014;219(7):487–96.
38. Wong WS, Lo AWI, Siu LP, Leung JNS, Tu SP, Tai SW, Lam SC, Wong KF. Reference ranges for lymphocyte subsets among healthy Hong Kong Chinese adults by single-platform flow cytometry. Clin Vaccine Immunol. 2013;20(4):602–6.
39. Xu D, Wu Y, Gao C, Qin Y, Zhao X, Liang Z, Wang Y, Feng M, Zhang C, Liu G, et al. Characteristics of and reference ranges for peripheral blood lymphocytes and CD4 + T cell subsets in healthy adults in Shanxi Province, North China. J Int Med Res. 2020;48(7):300060520913149.
40. Yaman A, Çetiner S, Kibar F, Tasova Y, Seydaoglu G, Dündar IH. Reference ranges of lymphocyte subsets of healthy adults in Turkey. Med Princ Pract. 2005;14(3):189–93.
41. Yi JS, Rosa-Bray M, Staats J, Zakroysky P, Chan C, Russo MA, Dumbauld C, White S, Gierman T, Weinhold KJ, et al. Establishment of normative ranges of the healthy human immune system with comprehensive polychromatic flow cytometry profiling. PLoS ONE. 2019;14(12):18.
42. Zhang K, Wang F, Zhang M, Cao X, Yang S, Jia S, Wang L, Luo J, Deng S, Chen M. Reference ranges of lymphocyte subsets balanced for age and gender from a population of healthy adults in Chongqing District of China. Cytometry B Clin Cytom. 2016;90(6):538–42.
43. Torres AJL, Cisneiros P, Guedes R, Grassi MFR, Meyer R, Bendicho MT, Lopes T, Félix G, Netto EM, Brites C, et al. Lymphocyte subset reference intervals in blood donors from northeastern Brazil. An Acad Bras Cienc. 2015;87(2):1019–25.
44. Thomas R, Wang W, Su DM. Contributions of age-related thymic involution to immunosenescence and inflammaging. Immun Ageing. 2020;17:2.
45. Nikolich-Žugich J. Aging of the T cell compartment in mice and humans: from no naive expectations to foggy memories. J Immunol. 2014;193(6):2622–9.
46. Fülöp T, Dupuis G, Witkowski JM, Larbi A. The role of immunosenescence in the development of age-related diseases. Rev Invest Clin. 2016;68(2):84–91.
47. Yu HT, Park S, Shin EC, Lee WW. T cell senescence and cardiovascular diseases. Clin Exp Med. 2016;16(3):257–63.
48. Chiu YL, Shu KH, Yang FJ, Chou TY, Chen PM, Lay FY, Pan SY, Lin CJ, Litjens NHR, Betjes MGH, et al. A comprehensive characterization of aggravated aging-related changes in T lymphocytes and monocytes in end-stage renal disease: the iESRD study. Immun Ageing. 2018;15:27.
49. Weinberger B, Lazuardi L, Weiskirchner I, Keller M, Neuner C, Fischer KH, Neuman B, Würzner R, Grubeck-Loebenstein B. Healthy aging and latent infection with CMV lead to distinct changes in CD8+ and CD4+ T-cell subsets in the elderly. Hum Immunol. 2007;68(2):86–90.

50. Onyema OO, Njemini R, Forti LN, Bautmans I, Aerts JL, De Waele M, Mets T. Aging-associated subpopulations of human CD8+ T-lymphocytes identified by their CD28 and CD57 phenotypes. Arch Gerontol Geriatr. 2015;61(3):494–502.
51. Huff WX, Kwon JH, Henriquez M, Fetcko K, Dey M. The evolving role of CD8(+)CD28(-) immunosenescent T cells in cancer immunology. Int J Mol Sci. 2019;20(11):2810.
52. Effros RB. Telomere/telomerase dynamics within the human immune system: effect of chronic infection and stress. Exp Gerontol. 2011;46(2–3):135–40.
53. Sansoni P, Vescovini R, Fagnoni FF, et al. New advances in CMV and immunosenescence. Exp Gerontol. 2014;55:54–62. https://doi.org/10.1016/j.exger.2014.03.020.
54. Muller L, Di Benedetto S. Immunosenescence and Cytomegalovirus: Exploring Their Connection in the Context of Aging, Health, and Disease. Int J Mol Sci. 2024;25(2):753.
55. Michel T, Poli A, Cuapio A, Briquemont B, Iserentant G, Ollert M, Zimmer J. Human CD56bright NK Cells: An Update. J Immunol. 2016;196(7):2923–31.
56. Brauning A, Rae M, Zhu G, Fulton E, Admasu TD, Stolzing A, Sharma A. Aging of the immune system: focus on natural killer cells phenotype and functions. Cells. 2022;11(6):1017.
57. Zheng Y, Liu X, Le W, Xie L, Li H, Wen W, Wang S, Ma S, Huang Z, Ye J, et al. A human circulating immune cell landscape in aging and COVID-19. Protein Cell. 2020;11(10):740–70.
58. Franceschi C, Zaikin A, Gordleeva S, Ivanchenko M, Bonifazi F, Storci G, Bonafe M. Inflammaging 2018: An update and a model. Semin Immunol. 2018;40:1–5.
59. Frasca D, Diaz A, Romero M, Blomberg BB. The generation of memory B cells is maintained, but the antibody response is not, in the elderly after repeated influenza immunizations. Vaccine. 2016;34(25):2834–40.
60. Kronzer VL, Bridges SL Jr, Davis JM 3rd. Why women have more autoimmune diseases than men: An evolutionary perspective. Evol Appl. 2021;14(3):629–33.
61. Klein SL, Flanagan KL. Sex differences in immune responses. Nat Rev Immunol. 2016;16(10):626–38.
62. Marquez EJ, Chung CH, Marches R, Rossi RJ, Nehar-Belaid D, Eroglu A, Mellert DJ, Kuchel GA, Banchereau J, Ucar D. Sexual-dimorphism in human immune system aging. Nat Commun. 2020;11(1):751.
63. Wagar LE, Gentleman B, Pircher H, McElhaney JE, Watts TH. Influenza-specific T cells from older people are enriched in the late effector subset and their presence inversely correlates with vaccine response. PLoS ONE. 2011;6(8):e23698.
64. Alpert A, Pickman Y, Leipold M, Rosenberg-Hasson Y, Ji X, Gaujoux R, Rabani H, Starosvetsky E, Kveler K, Schaffert S, et al. A clinically meaningful metric of immune age derived from high-dimensional longitudinal monitoring. Nat Med. 2019;25(3):487–95.
65. Santoro A, Bientinesi E, Monti D. Immunosenescence and inflammaging in the aging process: age-related diseases or longevity? Ageing Res Rev. 2021;71:101422.

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