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Enriched and Highly Functional Mucosa-Associated Invariant T Cells in Follicular Fluid of Patients with Polycystic Ovary Syndrome

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Research Article

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Abstract

Background

The involvement of mucosa-associated atypical T (MAIT) cells in polycystic ovary syndrome (PCOS) has not been studied adequately, and their ability to serve as potential therapeutic targets remains unexplored. To investigate the immune profile of mucosa-associated atypical T (MAIT) cells in the localized inflammatory milieu of the ovaries of patients with polycystic ovary syndrome (PCOS) and determine whether they exacerbate the inflammatory manifestations of PCOS.

Methods

Follicular fluid samples (n = 38) were obtained from patients undergoing in vitro fertilization(IVF) treatment between May 2023 and December 2023, of which 18 were from healthy individuals (male infertility factors) and 20 were from patients with PCOS. Peripheral blood samples were collected from the patients before the ovum retrieval procedure. Mononuclear cells were extracted from the follicular fluid and peripheral blood of patients with PCOS and healthy controls. Flow cytometric techniques were used to analyze the abundance of MAIT cells and their cytokine production in both peripheral blood and follicular fluid and to explore the correlation between MAIT cells in these two compartments. The responses of MAIT cells to stimuli in patients with PCOS were detected in vitro. The association between clinical indices and the levels of MAIT cells and cytokines in the participants was analyzed.

Results

Circulating MAIT cells from patients with PCOS showed reduced frequency, inhibited proliferation, and were depleted by hyperactivation. MAIT cells were enriched in follicular fluid from patients with PCOS, expressed high levels of PD-1 and CD38, and secreted more interferon (IFN)-γ and interleukin (IL)-17A, suggesting that both MAIT1/MAIT17 phenotypes contribute to the ovarian inflammatory milieu in the infiltrate.

Conclusions

MAIT cells were enriched in the follicular fluid of patients with PCOS, while exhibiting significantly increased immunoreactivity and cytotoxicity. There was a correlation between MAIT cells and clinical symptoms in patients with PCOS, suggesting that ovarian infiltration of MAIT cells may be related to the inflammatory environment of the ovary.

1. Background

Polycystic ovarian syndrome (PCOS) is a disorder characterized by reproductive, endocrine, and metabolic abnormalities that significantly affect fertility in women of reproductive age. The incidence of PCOS has dramatically increased over the past three decades [1]. Traditional treatments, such as metformin and oral contraceptives, primarily address the complications of PCOS; however, they often offer limited long-term efficacy and are associated with serious side effects. Therefore, novel therapeutic targets need to be identified. Recent studies have increasingly highlighted chronic inflammation driven by immune dysfunction as a key risk factor in the pathogenesis of PCOS. Local immune cells in the ovary play a critical role in regulating granulosa cell survival and oocyte quality [2]. However, factors that trigger immune cell dysfunction are poorly understood. Therefore, investigating specific immune cell profiles and the associated inflammatory mediators is crucial for the development of targeted immunotherapies for PCOS.

Follicular fluid, formed mainly by the secretion of granulosa cells, zona pellucida cells, and oocytes and leakage of plasma components from capillaries into the antrum, provides an important microenvironment for follicular development and oocyte maturation [3]. Follicular fluid provides a medium for communication between oocytes and other cells [4]. High levels of interferon-γ (IFN-γ), interleukin (IL)-17A and tumor necrosis factor-α (TNF-α) in the follicular fluid of patients with PCOS promote granulosa cell apoptosis, which affects oocyte quality [4–7].

Mucosal-associated invariant T (MAIT) cells are a unique subset of T cells comprising 1–10% of circulating T cells and have gained increasing attention in recent years. These cells exhibit strong tissue-homing properties, with particularly high abundance in the liver and lungs [8]. MAIT cells express high levels of IL-12 and IL-18 receptors, which allow them to interact with antigen-presenting cells and contribute to immune responses against pathogens [9]. Upon activation, MAIT cells produce and secrete cytokines such as IFN-γ, TNF-α, and IL-17, playing essential roles in pro-fibrotic processes, tissue repair, and anti-tumor immunity [10–12]. A reduction in circulating MAIT cells has been linked to several endocrine and metabolic disorders, including endometriosis, obesity, and insulin resistance [13–16].

To date, it remains unclear whether MAIT cells are involved in the development of PCOS or whether they could serve as potential therapeutic targets for this condition. In this study, we assessed the abundance of MAIT cells, elucidated their cytokine production in both peripheral blood and follicular fluid, and explored the correlation between MAIT cells in these two components. We further demonstrated the heightened activity of MAIT cells in response to stimuli in patients with PCOS in vitro. Finally, we analyzed the association between clinical indices and MAIT cell and cytokine levels in the participants.

2. Methods

2.1 Study design and participants

Thirty-eight infertile women who underwent in vitro fertilization/ intracytoplasmic sperm injectionin (IVF/ICSI) between May 2023 and December 2023 in the reproductive center of Shanghai First Maternity

and Infant Hospital were included. Among these, 20 participants were diagnosed with PCOS and 18 were controls (Table 1). The diagnostic criteria for PCOS were based on the Rotterdam criteria (at least 2 of the following three criteria): chronic anovulation or oligomenorrhea, hyperandrogenemia, and polycystic ovaries. Women with bleeding intervals of 35 days to three months were categorized as having oligomenorrhea. Ultrasound manifestations of polycystic ovaries were defined as ovaries containing ≥ 12 sinus follicles measuring 2–9 mm in diameter or at least one ovary with a 10 cm increase in ovarian volume and no ovarian cysts. Exclusion criteria were as follows: (1) age > 40 years or < 20 years; (2) women with any contraindication to pregnancy; (3) women receiving other medications, such as metformin and growth hormone; and (4) other diseases similar to PCOS, such as thyroid disorders, hyperprolactinemia, androgen-secreting tumors, and Cushing's syndrome. The control group consisted of healthy women who received ICSI because of oligozoospermia in their male partners. Written informed consent was obtained from all the participants. This study was approved by the Ethics Committee of the Shanghai First Maternity and Infant Health Hospital (No. KS24436).

Table 1. Baseline characteristics of participants.

Characteristic	PCOS (n=20)	HC (n=18)	P-value
Age (years)	32.95±4.35	33.67±2.99	0.60
BMI (kg/m2)	25.28 (20.62, 30.9)	20.81 (19.08, 22.2)	0.02
AMH (ng/mL)	6.18 (6, 6.47)	2.15 (1.66, 2.42)	0.0001
FSH (mIU/mL)	6.06 (5.03, 7.41)	7.19 (5.23, 9.9)	0.27
LH (mIU/mL)	5.99 (4.47, 6.53)	4.81 (3.44, 6.14)	0.10
E2 (pg/mL)	38.72±12.26	40.47±9.38	0.60
T (ng/mL)	0.34 (0.3, 0.4)	0.2 (0.17, 0.24)	0.00
Gn doses (IU)	2025 (1575, 3025)	1950 (1650, 2400)	0.76
Gn doses (days)	10 (8.5, 12)	9.5 (7.25, 11.5)	0.43
HCG day E2 (pg/mL)	2909.49±2385.91	1566.5±525.55	0.06
Retrieved oocytes (N)	10.47±6.35	7.28±3.21	0.06
MII (N)	8 (3, 11.5)	6 (3, 7)	0.29

2.2 Peripheral blood and follicular fluid samples collection

All participants underwent controlled ovarian stimulation following the standard procedure of the Reproductive Center. Follicular size and count and serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), progesterone (P), testosterone (T), and anti-Müllerian hormone

(AMH) were measured. Oocyte maturation was triggered when three or more dominant follicles reached a diameter of 18 mm. Oocyte retrieval was performed using transvaginal ultrasound-guided follicular aspiration 36–38 hours after stimulation. Approximately 5 mL of follicular fluid was collected. Fresh venous blood samples were obtained before oocyte retrieval. Mononuclear cells were isolated from both peripheral blood and follicular fluid by density gradient centrifugation at 400 xg for 20 min. Lymphocytes from the middle layer were carefully aspirated, washed, and stored in a cryopreservation medium containing 10% dimethyl sulfoxide (DMSO; Millipore Sigma) and 90% fetal bovine serum (FBS; Thermo Fisher Scientific).

2.3 Flow cytometry

PBMC (Peripheral blood mononuclear cells) were thawed and washed twice. PBMCs were cultured in RPMI1640 Glutamax (Thermo Fisher Scientific, Darmstadt, Germany) supplemented with 100 U/mL Penicillin (Sigma Aldrich), 100 µg/mL Streptomycin (Sigma Aldrich), 10% fetal bovine serum (Greiner-Bio one, Frickenhausen, Germany), non-essential amino acids (NEAA, Sigma Aldrich) and sodium pyruvate (Sigma Aldrich) at a concentration of $0.5x10^6$ cells/mL in 96-well U-bottom plates (Sigma Aldrich). Surface staining was performed by incubating the cells with the desired monoclonal antibody for 30 min in the dark at room temperature (20–25°C). Anti-human CD45 (clone 2D1, APC-Cy7, BD Pharmingen), anti-human CD3 (FITC, Biolegend), anti-human CD8 (clone RPA-T8, BB700, BD Pharmingen), anti-human CD161 (APC, Biolegend) and anti-human TCR Vα7.2 (PE Biolegend) were used to identify MAIT cells, while anti-human CD279 (PD-1) (clone MIH4, BV421, BD Pharmingen), anti-human CD38 (clone HIT2, Alexa Fluor 700, BD Pharmingen), anti-human HLA-DR (clone G46-6, BV605, BD Pharmingen) and anti-human TIGIT (clone VSTM3, Brilliant Violet 785™, Biolegend) were used to assess the activity of MAIT cells.

For IFN-γ, IL-17 and TNF-α detection, cells were harvested at the end of the culture period and stimulated with phorbol 12-myristate 13-acetate (PMA, 10 ng/mL, Sigma Aldrich) and ionomycin (1 µg/mL, Sigma-Aldrich) in the presence of Brefeldin A (BFA, BD Biosciences) in a 5% CO2 atmosphere at 37°C for 4 h. Then cells were stained with anti-human CD45 (clone 2D1, APC-Cy7, BD Pharmingen), anti-human CD3 (FITC, Biolegend), anti-human CD8 (clone RPA-T8, BB700, BD Pharmingen), anti-human CD161 (APC, Biolegend), and anti-human TCR Vα7.2 (PE Biolegend), followed by fixation and permeabilization (CytoFix/CytoPerm kit, BD Biosciences, Erembodegen, Belgium). Cells were stained intracellularly for IL-17A (clone N49-653, BV421, BD Pharmingen), IFN-γ (clone B27, BV605, BD Pharmingen), or TNF-α (PE/Cy7, Biolegend). Flow cytometry was performed using CytoFLEX (Beckman Coulter). Data were analyzed using the FlowJo software.

2.4 Functional MAIT cell assay

The frozen PBMCs were thawed and stimulated with or without recombinant IL-12 (10 ng/mL; PeproTech) or IL-18 (100 ng/mL; R&D Systems) for 48 h. Cells were then restimulated with PMA and ionomycin in the presence of Brefeldin A 4 h, followed by surface and intracellular staining as described above. MAIT cell cytokine production (IFN-γ, IL-17A, TNF-α) was analyzed via flow cytometry.

2.5 Statistics

All data are presented as mean ± SD, or n (%) values. Group comparisons between two independent groups were performed using the Mann–Whitney U test for non-normally distributed data or unpaired t-test for normally distributed data, as determined by the Shapiro–Wilk test. Comparisons involving more than two groups were conducted using one-way ANOVA followed by Tukey's honest significant difference (HSD) post hoc test for pairwise comparisons. Paired samples (peripheral blood vs. follicular fluid from the same patient) were analyzed using the Wilcoxon matched-pairs signed-rank test. Correlations between clinical parameters and immune markers were assessed using Spearman's rank correlation coefficient.

To account for multiple comparisons, the Benjamini–Hochberg false discovery rate (FDR) procedure was applied to the correlation analyses presented in Fig. 5. Only correlations with an FDR-adjusted q value < 0.05 were considered statistically significant. For all other analyses, no adjustment for multiple comparisons was made because each hypothesis test was pre-specified and independent. All statistical tests were two-tailed. A significance threshold of $P < 0.05$ was used, with the following annotation: *p < 0.05, **p < 0.01, ***p < 0.001. Analyses were performed using GraphPad Prism 9 (GraphPad Software, San Diego, CA, USA) and SPSS version 26.0 (IBM Corp., Armonk, NY, USA).

3. Results

3.1 Baseline characteristics of participants

The baseline characteristics of the patients with PCOS and healthy controls (HC) are summarized in Table 1. Patients with PCOS exhibited significantly higher BMI, AMH, and T levels than the HC group. No other significant differences were observed between the two groups except for BMI, AMH levels, and T.

3.2 Circulating MAIT cells are reduced but highly activated in patients with PCOS

PBMCs were isolated and analyzed for MAIT cells, defined as $CD3^+CD8^+$TCR $V\alpha7.2^+CD161^+$ cells. The frequency of MAIT cells was significantly lower in patients with PCOS than in HCs (Fig. 1A). However, the expression of the activation markers CD38 and TIGIT was markedly increased in the PCOS group (Fig. 1B, C). Additionally, the exhaustion marker PD-1 was upregulated in MAIT cells from patients with PCOS (Fig. 1D), indicating a hyperactivated state. HLA-DR expression did not differ between the groups (Fig. 1E).

Upon PMA/ionomycin stimulation, MAIT cells from patients with PCOS produced significantly higher levels of IFN-γ and IL-17A than those from HCs (Fig. 1F, G). No significant difference was observed in TNF-α secretion (Fig. 1H). These findings suggest that circulating MAIT cells in patients with PCOS are numerically reduced, but exhibit heightened activation and proinflammatory capacity.

3.3 MAIT cells were enriched and highly activated in the follicular fluid of patients with PCOS

Given that the follicle constitutes the fundamental functional unit of ovarian physiology (17), we next investigated whether the distribution and phenotypic characteristics of MAIT cells within the follicular microenvironment are altered in PCOS. The gating strategy for identifying follicular fluid MAIT cells is delineated in Fig. 2A. Notably, the proportion of MAIT cells relative to CD8 + T lymphocytes was significantly elevated in the follicular fluid of patients with PCOS compared to that of HCs (Fig. 2B), suggesting a potential preferential recruitment or local expansion of these cells within the ovarian niche. Phenotypic analysis revealed that follicular MAIT cells from the PCOS group exhibited a significantly heightened activation profile, as evidenced by the increased expression of CD38, PD-1, and TIGIT (Fig. 2C-E), while the expression of HLA-DR remained comparable between groups (Fig. 2F). Functionally, upon stimulation, MAIT cells isolated from the PCOS cohort demonstrated a markedly enhanced capacity for cytokine production, characterized by significantly elevated levels of the proinflammatory cytokines IFN-γ and IL-17A relative to those from HCs (Fig. 2G-I). These findings indicate that the ovarian follicular compartment in PCOS is characterized by an accumulation of MAIT cells with an activated and proinflammatory phenotype.

3.4 Paired comparison of MAIT cells in blood and follicular fluid

To further elucidate the compartment-specific enrichment of MAIT cells, we performed a paired analysis of peripheral blood and follicular fluid samples obtained from eight patients with PCOS. This intra-individual comparison revealed a significant compartmentalization, characterized by a markedly higher frequency of MAIT cells in the follicular fluid relative to paired peripheral blood (Fig. 3A). Concordantly, MAIT cells residing in the follicular microenvironment exhibited a more activated phenotype, with significantly elevated expression of PD-1 and CD38 compared to their circulating counterparts (Fig. 3B, C). Functionally, the follicular milieu was associated with enhanced effector responses, as MAIT cells derived from follicular fluid produced substantially higher levels of the proinflammatory cytokines IFN-γ and IL-17A upon stimulation (Fig. 3D, E). In contrast, the expression of TIGIT, HLA-DR, and the secretion of TNF-α remained comparable between the two anatomical compartments (Fig. 3F-H), suggesting a selective modulation of activation markers and cytokine profiles within the ovarian niche

3.5 MAIT cells in patients with PCOS exhibit enhanced cytokine production in response to IL-12/IL-18

Given the proinflammatory cytokine milieu characteristic of PCOS and the well-documented capacity of MAIT cells to respond to innate cytokine signals, we next evaluated their functional responsiveness to stimulation with IL-12 and IL-18. MAIT cells isolated from the follicular fluid of patients with PCOS exhibited a significantly heightened functional response compared to those from HCs. Specifically, upon cytokine stimulation, PCOS-derived MAIT cells demonstrated markedly increased production of both IFN-

γ (Fig. 4A) and IL-17A (Fig. 4B). These data indicate that MAIT cells residing in the PCOS ovarian microenvironment are hyperresponsive to innate inflammatory cues, suggesting a potential mechanism by which the local cytokine milieu primes these cells for enhanced effector functions.

3.6 MAIT cell activity correlates with endocrine indicators in PCOS

To investigate the potential clinical relevance of our findings, we performed correlation analyses between MAIT cell parameters and key clinical indicators in patients with PCOS using Spearman's rank correlation (Fig. 5A). BMI was positively correlated with both the frequency of MAIT cells and the levels of IFN-γ produced by these cells. Notably, serum anti-Müllerian hormone (AMH) concentrations exhibited widespread positive associations with multiple MAIT cell features, including their frequency, the production of IL-17A and IFN-γ, and the expression of activation markers CD38, TIGIT, PD-1, and HLA-DR. Additionally, the frequency of T cells was positively associated with IL-17A production and PD-1 expression on MAIT cells.

To further dissect these relationships, multivariate linear regression analysis was conducted (Fig. 5B). This analysis confirmed that CD38 expression, along with IL-17A and IFN-γ production by MAIT cells, were independently and significantly associated with AMH levels. Furthermore, IL-17A production emerged as a significant positive correlate of testosterone (T) levels.

Collectively, these correlative data suggest a potential interplay between MAIT cell-driven proinflammatory responses and key endocrinological features of PCOS, namely elevated AMH and androgen levels. This raises the possibility that MAIT cells may contribute to the pathogenesis of PCOS by modulating the ovarian follicular environment and steroidogenesis, although further mechanistic studies are warranted to establish causality.

4. Discussion

While circulating MAIT cells were diminished in patients with PCOS, a starkly different picture emerged within the ovarian follicle. Here, we found that MAIT cells were not only enriched in the follicular fluid but also exhibited a markedly activated and pro-inflammatory phenotype. This local MAIT cell population displayed elevated expression of activation (CD38) and inhibitory (PD-1, TIGIT) receptors and produced significantly higher levels of the inflammatory cytokines IFN-γ and IL-17A. The positive correlation of these MAIT cell features with serum AMH and testosterone levels implicates them in the hormonal dysregulation central to PCOS, highlighting a potential link between local immune activation and ovarian dysfunction.

MAIT cells contribute to insulin resistance and impair glucolipid metabolism by inducing inflammatory responses in adipose tissues, depending on the major histocompatibility complex class I (MR1) [17]. In a study examining the immunophenotype of blood MAIT cells in obesity, Lu et al. found that MAIT cells secrete higher levels of IL-17 in obese individuals with type 2 diabetes (characterized by disordered

insulin sensitivity) than in those without insulin resistance, indicating a link between MAIT cells and impaired glucose metabolism [15]. Nieuwdorp et al. found that amino acid treatment elevated the frequency of MAIT cells and improved glycemic markers in peripheral blood [18]. The authors also suggested that flavin biosynthesis intermediates contribute to glycemic control by regulating the function of MAIT cells. PCOS is often accompanied by obesity and insulin resistance; therefore, we hypothesized that MAIT may play a role in the pathogenesis of PCOS. In this study, the number of MAIT cells decreased in the blood but increased in the follicular fluid of patients with PCOS. Interestingly, similar phenomena have been reported for other diseases. MAIT cells in community-acquired pneumonia are reduced in the peripheral blood but are enriched in the alveolar fluid [19]. In contrast, MAIT cells are reduced and activated in the peripheral blood of children with active tuberculosis but do not accumulate in the alveolar fluid [20]. The number of circulating MAIT cells in patients with acute appendicitis is significantly lower than that in healthy subjects, whereas it is increased in the inflamed appendix [21]. Following appendectomy, the number of MAIT cells in blood rapidly returns to normal, suggesting that MAIT cells are temporarily recruited to inflamed tissues in a reversible manner [21]. Several hypotheses have been proposed to explain the decreased frequency of circulating MAIT cells, including their recruitment to inflammatory tissues, overactivation leading to exhaustion, and apoptosis [22]. The precise mechanisms underlying the decrease in MAIT cells in the peripheral blood of patients with PCOS require further exploration.

As expected, the MAIT cell phenotype was significantly altered in patients with PCOS. CD38, a single-chain transmembrane glycoprotein, is a T-cell activation marker [23] that balances nicotinamide adenine dinucleotide (NAD+) levels and exhibits NADase activity [24]. High CD38 expression has been previously reported in colitis and inflammatory bowel disease (IBD) [25, 26], and we found similar results on the surface of MAIT in peripheral blood. TIGIT, an immune checkpoint, regulates downstream immune responses by inhibiting glucocorticoid receptor activity [27]. TIGIT activates regulatory T-cells and inhibits dendritic cell activity [28, 29]. In our study, TIGIT expression was higher in the peripheral blood of patients with PCOS than in healthy controls. PD-1, a classic marker of immune cell exhaustion, was also highly expressed in the MAIT cells of patients with PCOS, leading us to hypothesize that MAIT cells were depleted due to overactivation. We further characterized the MAIT cell phenotype in the local immune environment of the ovary by analyzing the follicular fluid. The activity and function of MAIT cells are significantly enhanced in both the peripheral blood and follicular fluid of patients with PCOS, which is characterized by increased expression of activation markers (CD38) and exhaustion markers (TIGIT and PD-1). The decrease in circulating MAIT cells may be due to their recruitment to the site of inflammation where they exert immune effects. To test this hypothesis, we paired follicular fluid and peripheral blood samples from eight patients with PCOS. MAIT cells expressed higher levels of the hyperactivation and exhaustion markers CD38 and PD-1 in follicular fluid than in peripheral blood. These findings suggest that MAIT cells are more depleted by overactivation in follicular fluid than in peripheral blood.

Depending on immune function, MAIT cells can be classified into RORγt – T-bet+ MAIT1 and RORγt+ MAIT17 cells. Specific subpopulations of MAIT cells have been shown to have different roles in chronic airway inflammation, with MAIT type 1 cells producing IFN-γ to protect the airway and MAIT type 17 cells

producing IL-17A to promote inflammation [30]. MAIT1 and MAIT17 cells can phenotypically and functionally interconvert when MAIT cells respond to different stimuli, such as acute infections or organ transplantation [31]. This plasticity indicates that MAIT cells play a dominant role in mucosal immunity. In this study, we detected the coexistence of IFN-γ- and IL-17A-secreting MAIT cells, suggesting that MAIT cells in patients with PCOS tend to function in a mixed MAIT1/17 state. Dysregulated levels of T cells and IFN-γ disrupt the mechanisms that regulate collagenase and elastase expression, leading to the degradation of extracellular matrix proteins and contributing to follicular rupture and ovulation [32]. Elevated IL-17a disrupts follicular atresia, impairs apoptosis, and inhibits oocyte maturation [33]. Follicular fluid contains high levels of IL-12 and IL-18 [34, 35], and our study confirms that these cytokines further enhance the ability of MAIT cells from patients with PCOS to secrete IFN-γ and IL-17A, thereby exacerbating ovarian inflammation.

Given the heightened activation of MAIT cells in the follicular fluid of patients with PCOS, we next investigated their clinical relevance by correlating them with key endocrine markers. Serum AMH, a reliable marker of ovarian reserve, and circulating testosterone (T), a driver of ovarian inflammation and apoptosis, are both central to PCOS pathogenesis [36, 37]. Our analysis revealed that IL-17A-producing MAIT cells were positively correlated with both AMH and T levels, while IFN-γ-producing MAIT cells correlated with AMH. These findings suggest that pro-inflammatory MAIT cells in the follicular microenvironment may exacerbate PCOS symptoms by influencing androgen and AMH pathways, potentially linking local immune activation to hormonal dysregulation.

This study has several strengths. First, we measured the frequency, immunophenotype, and function of MAIT cells in the circulation and follicular fluid, and compared these characteristics in paired samples. Most previous studies have examined only the frequency of MAIT cells in the peripheral blood of patients with PCOS [38]. Secondly, we used flow cytometry rather than ELISA to analyze the cytokines secreted by MAIT cells. This approach accurately reflects the functional status of MAIT cells and avoids potential interference from cytokines released by other immune cells. However, the limitations of our study should also be considered. First, follicular fluid is obtained from a surgical ovulation retrieval procedure following medication to promote ovulation rather than natural ovulation in a physiological state. Second, although we observed a correlation between IL-17A- or IFN-γ-secreting MAIT cells and clinical parameters (AMH and T levels) in patients with PCOS, we did not investigate the potential influence of other cytokines secreted by MAIT cells on these clinical parameters. Future studies should explore whether MAIT cells in their natural state contribute to the pathogenesis of PCOS by secreting inflammatory factors.

5. Conclusions

In summary, our study demonstrates that MAIT cells are reduced in the circulation but enriched in the follicular fluid, where they are functionally enhanced in patients with PCOS. The cytokines IL-17A and IFN-γ secreted by MAIT cells are significantly correlated with serum AMH and T levels. These findings suggest that MAIT cells may be recruited from the circulation to the ovary, where they potentially

influence AMH and testosterone levels, possibly via interactions with granulosa cells. Further mechanistic studies are required to establish causality.

Abbreviations

AMH
Anti-Müllerian hormone
BMI
Body mass index
DMSO
Dimethyl sulfoxide
E2
Estradiol
FBS
Fetal bovine serum
FSH
Follicle-stimulating hormone
HC
Healthy controls
ICSI
Intracytoplasmic sperm injection
IFN-γ
Interferon-gamma
IL
Interleukin
IVF
In vitro fertilization
LH
Luteinizing hormone
MAIT
Mucosa-associated invariant T
PBMC
Peripheral blood mononuclear cell
PCOS
Polycystic ovary syndrome
PD-1
Programmed cell death protein 1
PMA
Phorbol 12-myristate 13-acetate
T

Testosterone
TCR
T cell receptor
TIGIT
T cell immunoreceptor with Ig and ITIM domains
TNF-α
Tumor necrosis factor-alpha

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Shanghai First Maternity and Infant Hospital (Approval No. KS24436). All procedures performed were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments. Written informed consent was obtained from all individual participants included in the study.

Consent for publication

Not applicable. This manuscript does not contain any individual person's data in any form (including individual details, images, or videos).

Availability of data and materials

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Y.F. and X.Z. conceptualized the study. J.L., Y.Z., M.Q. and X.Z. were in charge of patient recruitment. Y.Z. performed the experiment and statistical analysis. Y.Z. and Q.W. prepared the draft manuscript. Y.Z., Q.W. X.Z. and Y.F. revised the manuscript. All authors contributed to the critical revision and approved the publication of the final version. All authors had full access to all of the data in the study and had final responsibility for the decision to submit for publication.

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Authors' information (optional)

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Figures

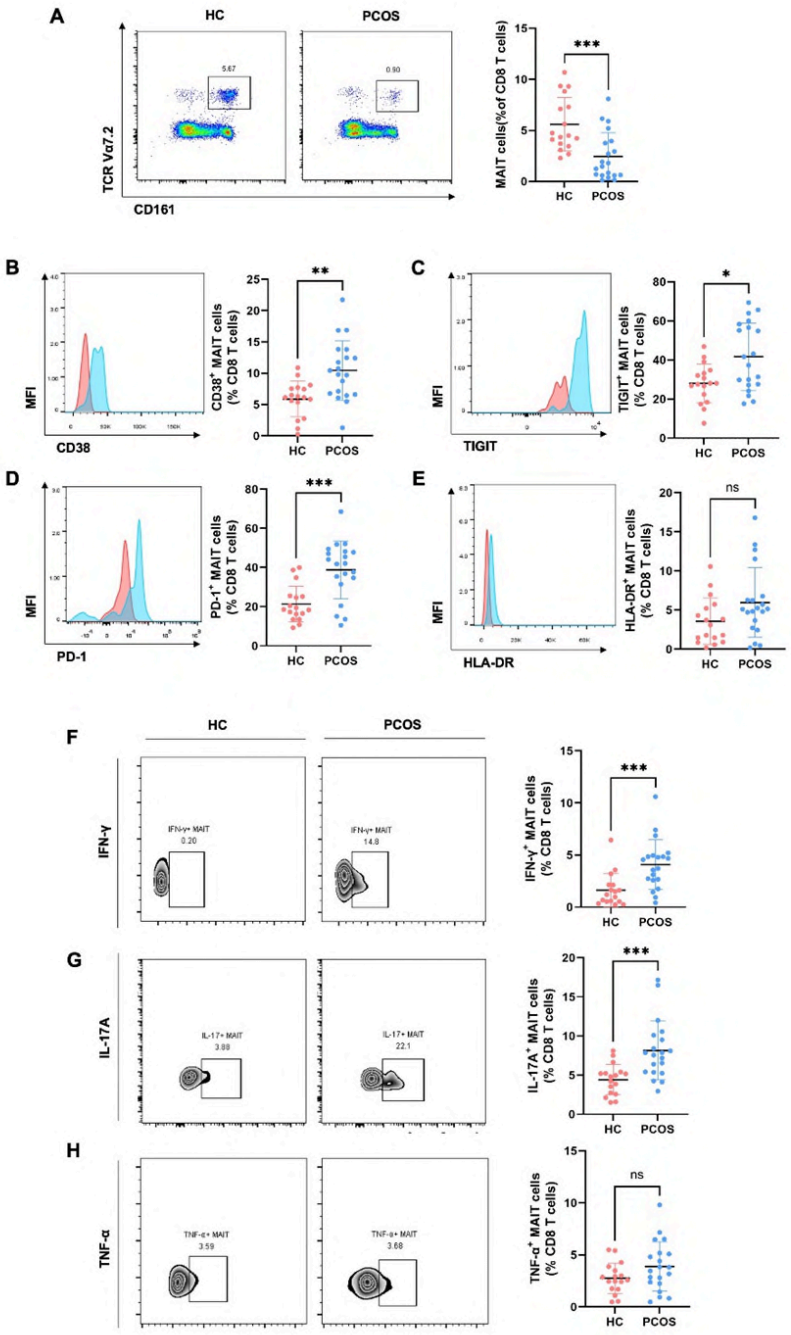


Figure 1

Abundance and activation of MAIT cells in the circulation of HC and PCOS participants.

(A) The percentage of MAIT cells among CD8 + T cells in the peripheral blood of 17 HCs and 20 patients with PCOS. (B-E) Representative percentages of CD38-expressing cells, TIGIT-expressing cells, PD-1-expressing cells and HLA-DR-expressing cells in the MAIT cell population. (F-H) Percentages of IFN-γ, IL-

17A or TNF-α producing MAIT cells. Symbols represent individual subjects and horizontal lines represent median values. Abbreviations: PCOS, Polycystic Ovary Syndrome; HC, healthy control; MAIT, mucosal-associated invariant T; CD38, Cluster of Differentiation 38; TIGIT, T cell immune receptor with immunoglobulin and ITIIM domain; PD-1, programmed death-1; HLA-DR, human leukocyte antigen-DR; IFN, interferon; IL, interleukin; TNF, tumor necrosis factor. *p<0.05, **p<0.01, ***p<0.001.

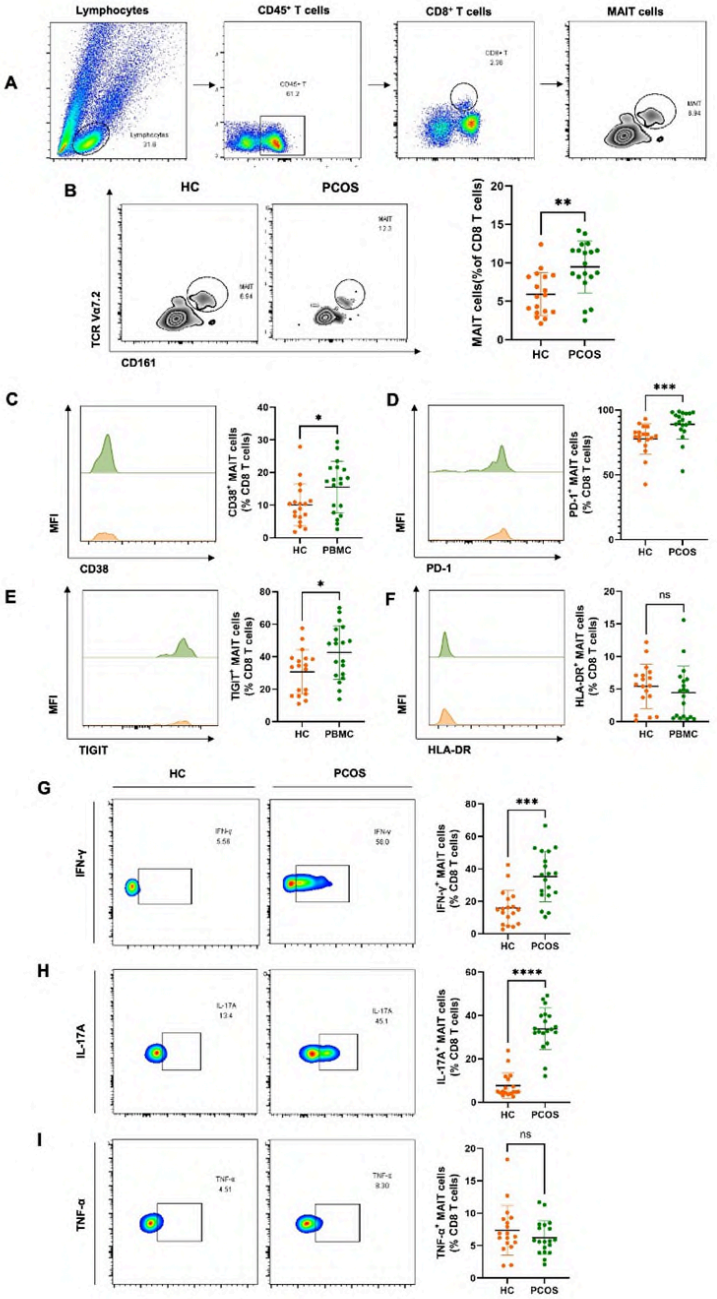


Figure 2

Abundance and activation of MAIT cells in the follicular fluid of HC and PCOS participants.

(A-B) MAIT cell percentages among CD8 + T cells in the follicular fluid from 17 HCs and 20 patients with PCOS. (C-F) Representative percentages of CD38-expressing, TIGIT-expressing, PD-1-expressing, and HLA-DR-expressing cells in the MAIT cell population. (G-I) Percentages of IFN-γ, IL-17A or TNF-α producing MAIT cells. Symbols represent individual subjects and horizontal lines represent median values. Abbreviations: PCOS, Polycystic Ovary Syndrome; HC, healthy control; MAIT, mucosal-associated invariant T; CD38, Cluster of Differentiation 38; TIGIT, T cell immune receptor with immunoglobulin and ITIIM domain; PD-1, programmed death-1; HLA-DR, human leukocyte antigen-DR; IFN, interferon; IL, interleukin; TNF, tumor necrosis factor. *p<0.05, **p<0.01, ***p<0.001.

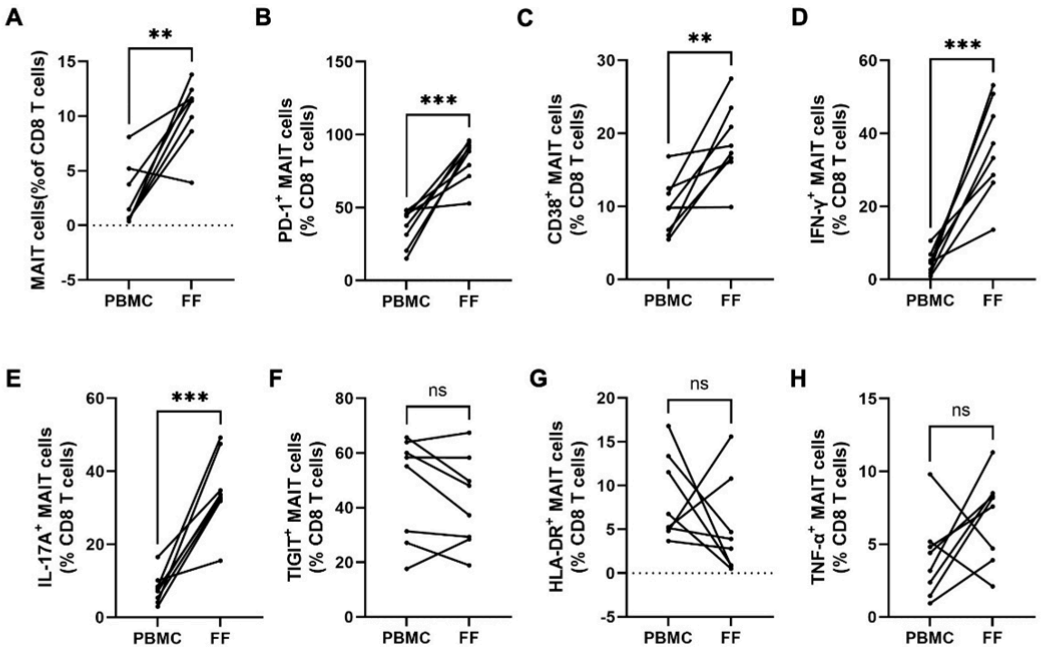


Figure 3

Paired samples of peripheral blood and follicular fluid were obtained from 8 patients with PCOS.

The percentages of total MAIT cells among (A) CD8 + T cells, (B) PD-1+MAIT cells, (C) CD38+MAIT cells, (F) TIGIT cells, and (G) HLA-DR cells were determined using flow cytometry. The expression of IFN-γ (D), IL-17A (E) and TNF-α (H) in the MAIT cell population was determined using intracellular flow cytometry after stimulation with PMA and IM. Symbols represent individual patients. Abbreviations: PCOS, Polycystic Ovary Syndrome; HC, healthy control; MAIT, mucosal-associated invariant T; CD38, Cluster of Differentiation 38; TIGIT, T cell immune receptor with immunoglobulin and ITIIM domain; PD-1,

programmed death-1; HLA-DR, human leukocyte antigen-DR; IFN, interferon; IL, interleukin; TNF, tumor necrosis factor. *p<0.05, **p<0.01, ***p<0.001.

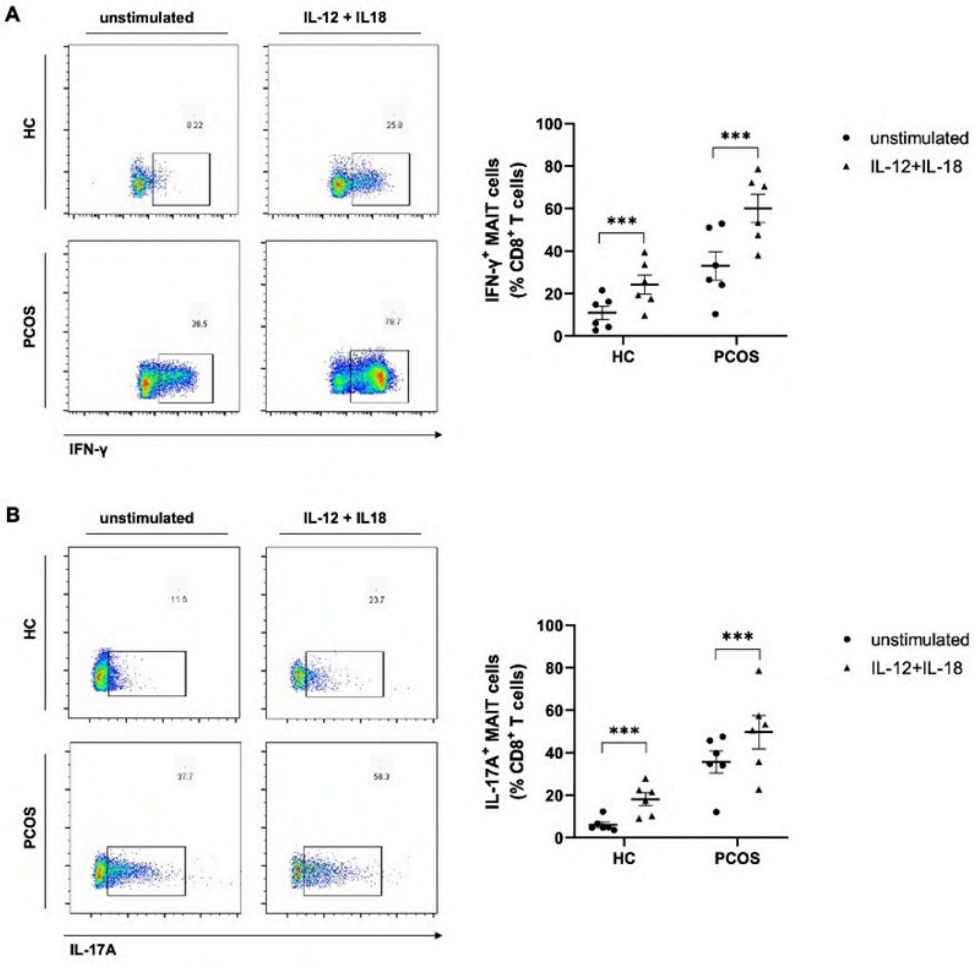


Figure 4

Proinflammatory cytokines produced by MAIT cells in response to stimuli in the follicular fluid of patients with PCOS.

Expression of (A) IFN-γ and (B) IL-17A in MAIT cells after stimulation with IL-12/IL-18. Abbreviations: PCOS, Polycystic Ovary Syndrome; MAIT, mucosal-associated invariant T; IFN, interferon; IL, interleukin.

*p<0.05, **p<0.01, ***p<0.001.

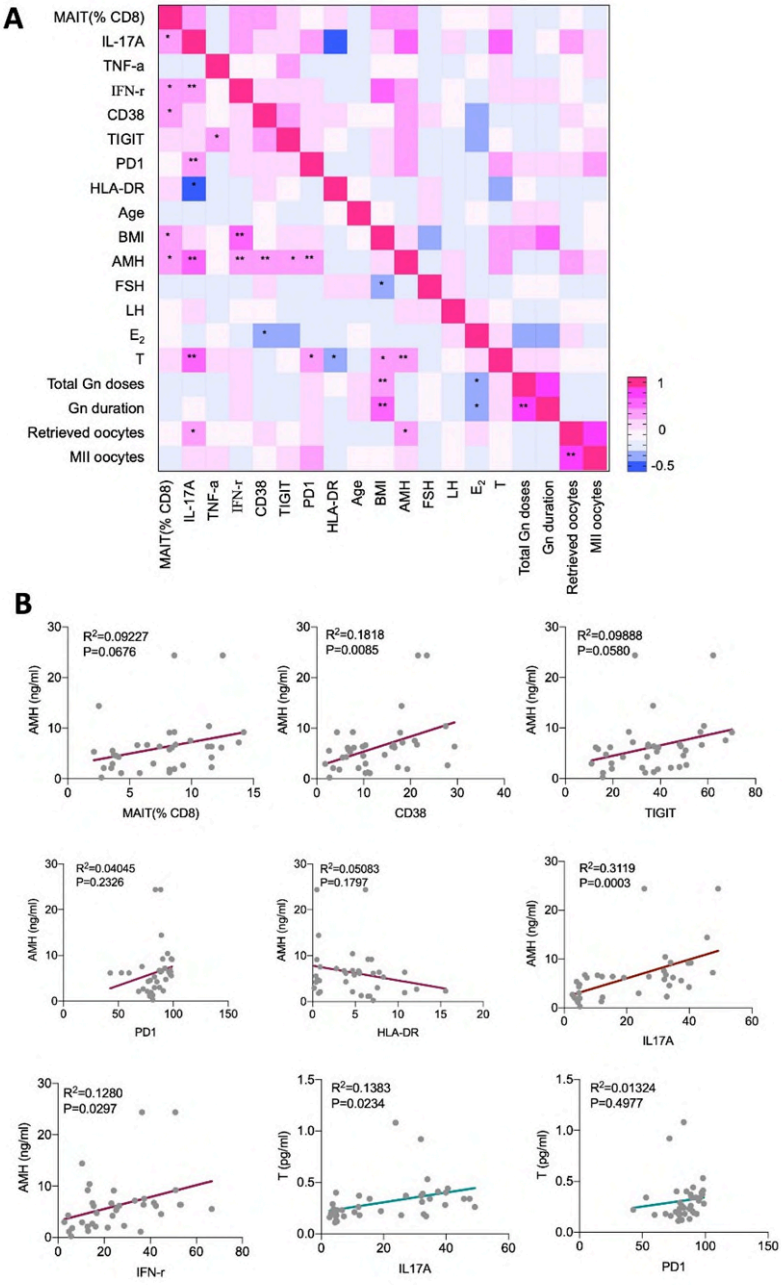


Figure 5

Association between MAIT cells and clinical index.

(A) Heat map represents the association. Red color represents a positive correlation, and blue color represents a negative correlation. *P<0.05, **P<0.01, represent the significance of the correlation coefficient. (B) Linear regression analysis between MAIT cells and AMH or T level. Abbreviations: BMI, body mass index; AMH, anti-Müllerian hormone; FSH, Follicle-stimulating Hormone; LH, Luteinizing Hormone; E2, Estradiol; T, testosterone; Gn, gonadotropins; HCG, Human Chorionic Gonadotropin; MII, Metaphase II.