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Th17 and MAIT cell mediated inflammation in antipsychotic free schizophrenia patients

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ABSTRACT

The immune hypothesis of schizophrenia has gained significant popularity in recent years in schizophrenia research. Evidence suggests that the peripheral immune system communicates with central nervous system and the effect propagates through microglial and lymphocyte crosstalk, especially during neuro-inflammation. Although, there is previous literature indicating changes in lymphocyte population in schizophrenia, detailed studies with respect to T and B cells are scarce. Mucosal associated invariant T (MAIT) cells are functionally associated with the gut microbiome. The gut microbiome has been implicated in the pathogenesis of schizophrenia. However, there is no information on the frequency of MAIT cells in schizophrenia. Hence, we investigated changes in proportions of T cells, B cells and MAIT cells in peripheral blood mononuclear cells derived from antipsychotic-free patients with schizophrenia in comparison to healthy controls. In line with earlier reports, we noted perturbations in T_h17 cells. This study for the first time reports changes in frequencies of MAIT cells in a homogenous population of antipsychotic-free patients with schizophrenia. These changes, though not common across all patients nevertheless point to the fact that inflammation is prevalent in a significant subset of schizophrenia cases.

1. Introduction

Schizophrenia is a neuropsychiatric illness characterized by cognitive and social deficits affecting approximately 1% of the global population. The neurobiological basis of schizophrenia has been poorly defined, partly due to the complexity of the disease. Several theories such as dopamine (Howes and Kapur, 2009), glutamate (Gysin et al., 2007), inflammation (Najjar et al., 2013), infection (Yolken and Torrey, 1995), genetic (Henriksen et al., 2017), and many others have been proposed with each theory partially contributing to understanding of the pathogenesis of schizophrenia. Immune cells and neuronal cells share several receptors. For example, classic neuronal receptors such as dopamine receptors and glutamate receptors are found in lymphocytes (Buttarelli et al., 2011; Pacheco et al., 2007). Conversely, immune cell receptors such as toll-like receptors and chemokine receptors are expressed by neurons (Okun et al., 2011; Meucci et al., 2000). This leads to two-way interaction between neuronal and immune cells. Hence, any changes in immune cells due to inflammatory process or changes in neuronal signalling due to neuronal dysfunction can directly impact other pathways including dopamine or glutamate signalling each of which possibly contributes to pathogenesis of schizophrenia.

The pertinent role of the immune system in maintaining the neuronal architecture is well established (Ransohoff et al., 2015), which suggests that changes in immune functioning may play a key role in the pathogenesis of schizophrenia. Although neuro-inflammation is predominantly controlled and driven by microglia, increased blood brain barrier leakiness leads to recruitment of lymphocytes and macrophages (Najjar et al., 2017; Cai et al., 2018; Greene et al., 2018). The "Inflammation hypothesis" of schizophrenia suggests that there is an increased or uncontrolled microglial activity which contributes to neuronal damage and loss which is consistently reported in schizophrenia. There is also sufficient evidence to

Abbreviations: ANA, anti-nuclear antibodies; RF, rheumatoid factor antibodies; MAIT cells, mucosal associated invariant T cells; T_h1 cells, T helper-1 cells; T_h2 cells, T helper-2 cells; T_h17 cells, T helper-17 cells; DN MAIT, double negative ($CD4^-$ $CD8^-$) MAIT cells; PBMCs, peripheral blood mononuclear cells; SNP, single nucleotide polymorphism; HIPC, Human Immunology Project Consortium; NSAIDs, Non-Steroidal Anti-Inflammatory Drugs.

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indicate that in the event of inflammation, blood brain barrier leakiness allows homing of T cells into the central nervous system. Following this, microglia interact with imported T cells, thereby amplifying the neuro-inflammation through exaggerated production of pro-inflammatory molecules which in turn leads to escalated neuronal assault (Juckel et al., 2011; De Picker et al., 2017).

T cells are copious producers of cytokine and are key mediators of inflammation. T cells are classified into CD4 helper T (Th) cells and CD8 Cytotoxic T cells (CTLs). Upon activation, Th cells are differentiated into Th1, Th2, Th17 and regulatory T cells (Treg). Th1 and Th17 cells are pro-inflammatory in nature, Th2 cells are protective and drive humoral immunity, while T regulatory cells are immunosuppressive. Notably, the ratio of Th1/Th2 and Th2/Th17 are used to indicate the shift of immune balance (Romagnani, 1991; Romagnani, 1999; Kidd, 2003). Important role of T cells has consistently been reported in patients with schizophrenia, this included altered counts of various T cell subsets, T cell activation, impaired T cell functions as well as abnormal T cell receptor repertoires (Debnath, 2015).

Among T cells, MAIT cells are unconventional T cells first reported in 1999 (Tilloy et al., 1999). Subsequently, they were noted to be active in the intestinal mucosal region and were hence called as Mucosal associated invariant T cells (Treiner et al., 2003). These cells are characterized by the expression of a semi-invariant T cell receptor (Vα7.2-Jα33/12/20) which recognizes an evolutionarily conserved MHC-related protein 1 (MR-1). The MR-1 can directly recognise bacterial metabolites (Kjer-Nielsen et al., 2012). The MAIT cells also express CD161 and IL-18R, which aid in identifying them as a separate T cell population (Ussher et al., 2014; Xiao and Cai, 2017) by multiparametric flow cytometry analysis. These cells are further classified into DN MAIT (double negative), $CD4^+$ MAIT cells and $CD8^+$ MAIT cells, based on the presence or absence of CD4 and CD8 markers (Sugimoto et al., 2015). CD8/DN MAIT cell ratio is considered as a marker of senescence in MAIT cells (Novak et al., 2014).

Though a significant correlation between immune cells/cytokine expression and clinical scores in schizophrenia has been demonstrated in some studies, others reports failed to do so (Miller and Goldsmith, 2017). Further, the pathogenesis of schizophrenia has been correlated with several other contributing factors such as genetics, environmental factors and microbiome (Dean and Murray, 2005; Xu et al., 2015; Kanji et al., 2018). For example, G197A Single Nucleotide Polymorphism (SNP) in the NFAT protein leads to increased expression of IL-17 which is significantly associated with inflammation in schizophrenia (Subbanna et al., 2018). This SNP has been reported to be associated with enhanced autoimmunity (Arisawa et al., 2008; Nordang et al., 2009; Espinoza et al., 2011). Further, understanding the role of inflammation may help explain treatment resistance in schizophrenia (Lin et al., 1998).

Considering the significant differences among genetic and environmental factors globally, it is imperative to investigate the role of immune aberrations in the pathophysiology of schizophrenia. Compelling evidence towards this was obtained from imaging studies indicating significant microglial activity in schizophrenia. Indeed, there is a substantial increase in microglial activity in high-risk cases who later develop schizophrenia (Bloomfield et al., 2016). In addition, studies on peripheral immune cells, especially lymphocytes also provided robust evidence towards immunopathogenesis of schizophrenia. A recent meta-analysis of 16 studies probing quantitative changes in lymphocytes from schizophrenia cases suggested that there were significant changes in peripheral $CD4^+$ and $CD8^+$ T lymphocytes in patients who were not on anti-psychotics (Miller et al., 2013). Besides, Th17 cells have been implicated in schizophrenia pathogenesis (Debnath and Berk, 2014; Ding et al., 2014). Although, earlier studies from India have investigated plasma cytokine abnormalities in schizophrenia, they have not assessed the immunophenotypes that may be responsible for the cytokine changes (Kalmady et al., 2014; Subbanna et al., 2018). Additionally, antipsychotics are shown to have immuno-modulatory effects and the data on the effects antipsychotics on various lymphocyte populations are inadequate.

In this study, we hypothesized that significant alterations occur in the frequencies of peripheral lymphocyte subsets derived from antipsychotic-free schizophrenia patients in comparison to healthy controls. Hence, in the present study we investigated changes in frequency of T cells, B cells and MAIT cells in PBMCs derived from antipsychotic-free patients with schizophrenia, using multi-parametric flow cytometry to understand their potential role in schizophrenia.

2. Materials and methods

2.1. Subject selection

The methods adopted for recruiting and collecting samples from the patient ($n = 32$) and healthy control samples ($n = 24$) have been detailed in our previous study (Varun et al., 2018). Briefly, schizophrenia cases were identified by a qualified psychiatrist based on DSM-IV TR criteria (DSM-IV, 2000). The inclusion criteria for schizophrenia patients were subjects aged 18–45 years of both gender, attending the psychiatry clinic from January 2016 to June 2017 who were free of antipsychotics for at least 3 months. The schizophrenia cases were classified as either drug naïve or drug free. Drug naïve cases were defined as patients with schizophrenia who had never been treated with antipsychotics. Drug free cases were defined as patients with schizophrenia who had been free of antipsychotics for a minimum period of 3 months prior to enrolment into the study. The exclusion criteria for the patients were: any active medication; history of nicotine or alcohol dependence or opioid use; history of illness in the past 90 days such as infection or fever. Psychopathology scores were available for a subset of the schizophrenia patients using the Positive and Negative Syndrome Scale (PANSS) scale (Kay et al., 1987). Healthy controls were defined as healthy volunteers unrelated to the patients, aged 18–45 years of both gender without any history of psychiatric illness as assessed by an oral interview. The other exclusion criteria were similar to that of patient group as stated above. All the samples used for this study were also screened for presence of anti-nuclear antibodies and Rheumatoid Factor antibodies.

2.2. Informed consent

The study was approved by the Institutional Ethics Committee. Written informed consent for obtaining the blood sample for research was taken from all the study subjects.

2.3. Sample collection, processing and storage

Approximately 10 ml of blood was collected using EDTA vacutainer (BD; USA). The blood samples were processed using density gradient centrifugation as described previously (Basheer et al., 2018). In brief, the blood sample was centrifuged and the plasma was separated. The remaining blood sample was mixed with an equal volume of RPMI 1640 (Sigma-Aldrich, St. Louis, USA), overlaid on Histopaque (Sigma-Aldrich) and centrifuged at 400*g* at room temperature for 25 min. The buffy coat layer was washed in 1× PBS and transferred to a cryovial containing 90% FCS and 10% DMSO (Sigma-Aldrich). The cooling was performed by a slow cooling process, at the rate of −1 °C/min in a cryofreezing container with isopropanol, overnight at −80 °C (Nazarpour et al., 2012). The PBMCs were preserved in liquid nitrogen for a period of 2 weeks to 16 months until immunophenotyping was performed. The viability of the PBMCs ranged from 58 to 87%.

2.4. Immunophenotyping

Multi-parametric flow cytometry was performed after staining with relevant fluorescent monoclonal antibodies to quantify T cell and B cell subsets, as per the Human Immunology Project Consortium (HIPC) guidelines (Maecker et al., 2012; Finak et al., 2016). Briefly, the T cells were identified as $CD3^+$ positive live lymphocytes. They were subtyped into CD4 and CD8 cells. The CD4 cells were further classified into Th1, Th2 and Th17 using CXCR3 and CCR6 surface markers. CD38 and HLA-DR were used to identify activation of cells. B cells were identified as $CD3^-$ $CD19^+$ live lymphocytes. They were further classified as $CD27^-$ (Naïve B cells), $CD27^+$ (Memory B cells), $CD38^+$ $CD20^-$ (plasmablasts) and $CD38^+$ $CD24^{high}$ (Transitional B cells) surface marker expression. MAIT cells were immunophenotyped based on previous literature (Ussher et al., 2014; Xiao and Cai, 2017). Briefly, MAIT cells were identified as lymphocyte subtype with $V\alpha7.2^+$ $CD161^+$. MAIT cells were classified into $CD4^+$, $CD8^+$ or DN ($CD4^-$ $CD8^-$) MAIT cells. Each subtype was further assessed for expression of HLA-DR to indicate activation. The list of fluorochrome labelled antibodies used for this study is provided in Supplementary Table 1. The gating process followed for immunophenotyping is provided in Supplementary Fig. 1. The immunophenotyping data was acquired through FACS Verse flow cytometer (BD Biosciences, USA) with BD FACSuite interface (v1.0.5.3841). The data acquired were analyzed using FlowJo software (vX.0.7; Treestar Inc.; USA).

2.5. Statistical analysis

The data for each type of cell was calculated as a percentage of parent cell population and analyzed using R statistical package 3.5.0. The values obtained for each parameter was assessed for normality distribution and goodness of fit using the Shapiro-Wilk Test. For normal data, the parametric unpaired 't' test was used. For data which did not comply with normal distribution, the non-parametric Mann Whitney *U* test was used as described earlier (Basheer et al., 2018). Grubb's statistical test was used to identify the significant outliers ($p < 0.05$) in the data (Adikaram et al., 2014).

Li's test for multiple comparisons was performed. Li's method is a *p* value based two-step rejection procedure for testing multiple hypotheses (Li, 2008). Briefly, this test computes the probability (Li_p) that the derived *p* value is observed due to a chance, following multiple comparisons. A $Li_p > 0.05$ (reject hypothesis), suggests that the there is a true statistically significant difference and further adjustment of *p* value is not warranted.

Analysis of covariance (ANCOVA) for 2 independent samples was used to correct for the effect of age. Pearson's correlation was used to assess correlation between age, duration of illness or psychopathology score (PANSS) and frequency of cell population. Spearman's rank correlation coefficient was used to determine the correlation between gender and frequency of cell population.

3. Results

The demographic details of the study subjects are summarized in Table 1. The data presented hereby are based on *p* values obtained after correcting for age as a covariate. T cell immunophenotyping revealed no observable difference in the overall percentages of $CD4^+$ T cells and $CD8^+$ T cells. Additionally, $CD3^+$ $CD4^+/CD8^+$ ratio did not differ between the controls and patients with schizophrenia ($p = 0.52$). Subtyping of $CD4^+$ T cells showed significantly elevated frequencies of T_h17 cells ($p = 0.045$) and activated T_h17 subset ($p = 0.005$) in the schizophrenia group (Fig. 1A–B). Comparison of the ratios of T_h1, T_h2 and T_h17 identified that T_h2/T_h17 ratio was significantly lower ($p = 0.03$) in schizophrenia, indicating a shift of balance towards increased T_h17 (Fig. 2A). The summarized results and statistics of immunophenotyping of T cells and their subtypes are presented in Supplementary Table 2A. B cell immunophenotyping did not reveal any significant difference between the two groups (Supplementary Table 2B). While total DN MAIT cells were significantly reduced ($p = 0.029$), the $HLA\text{-}DR^+$ DN MAIT cells were found to be upregulated in schizophrenia patients. In addition, $HLA\text{-}DR^+$ $CD4^+$ MAIT cells were found to be elevated in schizophrenia patients (Fig. 1C–F).

To establish unambiguous evidence that the statistically significant differences obtained in the study were not contributed by a potential outlier, the Grubbs' test was performed (Adikaram et al., 2014). Grubbs test is a statistical tool to identify a single probable outlier, which can potentially skew the represented data. Upon finding a significant outlier ($p < 0.05$), the statistics were repeated, by excluding the detected outlier. The test identified a potential outlier in each of the following datasets-$CCR6^+$ $CXCR3^+$ T cells, Th1, Activated Th1, Th2, Activated Th2, Activated Th17, Plasmablasts, Transitional B cells, Total MAIT cells, $CD4^+$ $HLA\text{-}DR^+$ MAIT cells, $HLA\text{-}DR^+$ DN MAIT cells. However, it was observed that the statistical differences remained valid following the Grubbs test (Supplementary Table 2).

Correction for multiple comparisons by Li's statistical test did not warrant any further adjustments for *p* values (Supplementary Table 2). Hence, unadjusted *p* values were used for interpretation of the data. Further, the usefulness of adjusted *p* value has been strongly debated, especially in exploratory and immunophenotyping studies (Rothman, 1990; Hashimoto et al., 2011; Basheer et al., 2018).

Further, we did not observe statistically significant correlation between the percentages of cells and age, gender or duration of illness (Table 2). Psychopathology scores were available for 21/32 patients and did not reveal any correlation with immune parameters.

4. Discussion

A plethora of literature exists on the immunophenotyping of PBMCs from schizophrenia patients with varied findings, globally (Craddock et al., 2007; Muller et al., 2000; Ozdin et al., 2017; Muller and Schwarz, 2010; Maino et al., 2007; Debnath and Berk, 2014). However, there are no studies on immunophenotypes among patients with schizophrenia from India. It is now well recognised that peripheral immune cells contribute to neuronal modelling directly or through microglial cells (Peruzzotti-Jametti et al., 2014). Indeed, studies have convincingly demonstrated that the peripheral immune cells such as lymphocytes and monocytes contribute to several functions of the central nervous system such as learning, formation of spatial memory and dendritic spine remodelling thus highlighting the importance of interrogating aberrations among peripheral immune cells in neuropsychiatric illnesses (Garre et al., 2017; Derecki et al., 2010). Although alterations in T_h1 and T_h2 cells were considered important in schizophrenia, recent evidence has implicated dysregulated T_h17 cells (Muller et al., 2000; Debnath and Berk, 2014). This view has been further refined to suggest a possible role for IL-23/IL-17 pathway through T_h17 as one of the central driving factors responsible for neuroinflammation in schizophrenia (Debnath and Berk, 2017). Further, aberrant microglial cell activation in the central nervous system leads to altered neuroplasticity, commonly observed in schizophrenia (De Picker et al., 2017).

In this study, we demonstrate changes in frequencies of T_h17 cells and activated Th17 cells in schizophrenia patients compared to controls. Alterations in the ratio of T helper subtypes are suggestive of changes in immune balance. For example, Th2 cells are anti-inflammatory in nature and drive humoral immunity in contrast to Th1 and Th17 cells which are pro inflammatory (Kidd, 2003). A decreased Th2/Th17 ratio indicating a shift in balance towards the pro-inflammatory phenotype was observed in our study, suggesting an inflammatory process similar to previously reported

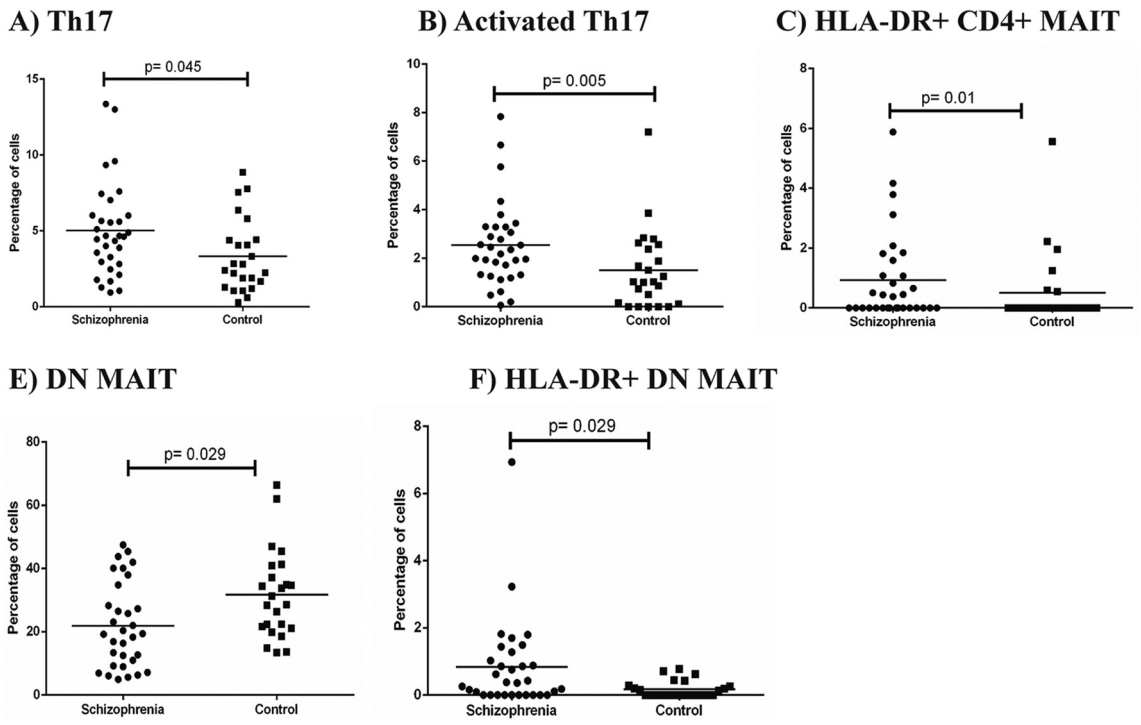

Fig. 1. Scatter plot representing data for percentage of cells found to be significantly dysregulated in this study between schizophrenia patients and healthy controls. Horizontal bars depict mean. X-axis indicates the samples. Y-axis indicates the percentage of cells from parent population. A) T_h17 cells. B) Activated T_h17 cells. C) HLA-DR$^+$ CD4$^+$ MAIT cells. D) DN MAIT cells. E) HLA-DR$^+$ DN MAIT cells.

observations (Ding et al., 2014). Though drug naïve and drug free schizophrenia cases had differences in treatment history, we did not observe any significant difference in frequencies of T cells or their subtypes (Supplementary Table 3).

Although B cells have been implicated in autoimmunity in schizophrenia (Ezeoke et al., 2013) the findings of this study did not reveal any statistically significant alterations in B cells or subsets between schizophrenia patients and healthy controls. In the present study stringent selection criteria were used for patients, to rule out confounding factors such as nicotine or alcohol abuse unlike previous studies. These factors may have probably caused the alterations in B cells reported earlier (Ganguli and Rabin, 1993; Steiner et al., 2010). Interestingly, B cell subsets did not significantly differ between drug naïve or drug free cases except for transitional B cells which were found to be decreased in drug free cases. The B cell subsets in general showed a trend of decrease in drug free cases in comparison to drug naïve cases. These findings are consistent with a previously reported observation that there is a marginal decrease in B cell counts following antipsychotic therapy (Steiner et al., 2010). Further, it should be noted that this study assessed changes in frequencies, and hence it is unknown if there are any functional changes in the B cells.

We have investigated the role of MAIT cells for the first time in schizophrenia. MAIT cells were chosen for immunophenotyping analysis based on the accumulation of circumstantial evidence. MAIT cells are predominantly CD8$^+$ cells that can produce IL-6 and IL-17 which are reported to be increased in schizophrenia (Chase et al., 2016; Coulter et al., 2017; Debnath and Berk, 2017; Ghazarian et al., 2017). Microbiome studies on schizophrenia have consistently indicated an association with *Lactobacillus species*, (Nguyen et al., 2018) which has been suggested to modulate MAIT cell activity (Johansson et al., 2016). MAIT cells thus may serve as a possible link for understanding the interaction between the microbiome and immune system in schizophrenia.

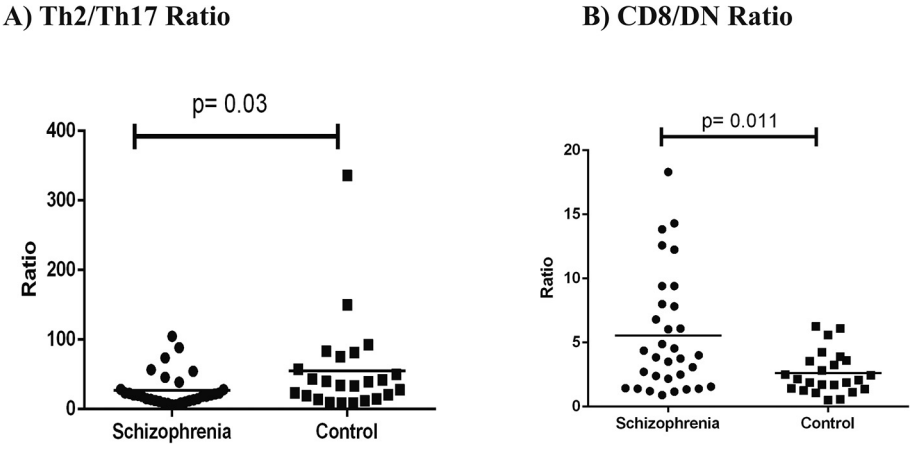

Fig. 2. Scatter plots representing ratio of cells. Horizontal bar represents the mean value. X-axis indicates the samples. Y-axis indicates the ratio of percentage of cells from parent population. A) T_h2/T_h17 ratio. B) CD8/DN MAIT ratio.

Table 1
Demographic data of schizophrenia patients and controls recruited for the study.

Parameter[a]	Schizophrenia patients	Healthy controls	Statistics
Number of subjects	n = 32	n = 24	–
Age	21–45 years	19–35 years	z = 1.89;
(In years)	(31.55 ± 4.97)	(28.75 ± 3.72)	$p = 0.057$
Gender	20/12	14/10	$\chi^2 = 0.099$;
(Male/female)			$p = 0.75$
Drug status	22 drug naïve	–	–
	10 drug-free		
Duration of illness reported	6–130	–	–
(In months)	(14.84 ± 22.01)		
History of smoking, nicotine/alcohol dependence, opioid abuse	Nil	Nil	–
Anti-nuclear antibodies (1:80 dilution)	Nil	Nil	–
RF antibodies	Nil	Nil	–

[a] The PANSS clinical scores were available only for 21/32 patients. The Scale for the Assessment of Positive Symptoms (SAPS) was 40.38 ± 18.6 and Scale for the Assessment of Negative Symptoms (SANS) was 41.66 ± 26.9.

In this study, DN MAIT cells were found to be decreased in schizophrenia patients in comparison to control samples. MAIT cell subsets did not significantly differ between drug naïve or drug free cases. There is evidence to suggest that MAIT cells vary by age, but are not strongly different in reproductive age (Walker et al., 2014; Novak et al., 2014). Similar findings were also observed in our study (Fig. 3). It has also been previously suggested that a decrease in CD8/DN MAIT cell ratio is reflective of senescence (Novak et al., 2014). However, in this study the CD8/DN MAIT cell ratio was higher ($p = 0.011$) in patients, indicating persistence as opposed to senescence (Fig. 2B). The precise molecular basis for these observations is unclear at present.

The activity of MAIT cells is functionally associated with gut microbiome (Treiner et al., 2003). The majority of MAIT cell population are contributed by $CD8^+$ MAIT cells and DN MAIT cells, both of which have similar cytokine expression profiles (Brozova et al., 2016). The observation of a significant downregulation of DN MAIT cells in schizophrenia patients with concurrent observation of increased activation of DN MAIT cells is consistent with the fact that T cells undergo significant activation induced apoptotic death (Zhan et al., 2017). DN MAIT cells are shown to be depleted in SLE and in other inflammatory disorders similar to our observations (Cho et al., 2014; Touch et al., 2018). Thus, we speculate that the reduced DN MAIT cells indicate ongoing inflammation, probably mediated by the gut microbiome. Earlier reports have demonstrated an association between gut microbiome and schizophrenia (Schwarz et al., 2018). This is further strengthened by the fact that subjects in our study were screened for potential confounding factors such as Anti-nuclear antibodies, RF antibodies, nicotine or alcohol dependence, infections and opioid use (Table 1).

The lack of correlation between the immunophenotypes and age, gender, duration of illness or Psychopathology scores (PANSS) emphasises the fact that all cases of schizophrenia do not present with inflammation. By definition, a trait marker reflects the possible role of the marker in question in the development of the disorder, while a state marker correlates with the status of clinical symptomatology in the patients (Chen et al., 2006). Thus, we propose that the altered frequencies of T cells and MAIT cell subsets may possibly represent trait markers as opposed to state markers. Though, the meta-analysis by Miller et al., suggested the CD4/CD8 ratio as a possible state marker for schizophrenia, we did not find any significant alterations.

The findings of this study complement another recently reported study from a different set of patients from this centre, showing significant changes in plasma IL-1β, IL-6, IL-17F and IL-22 (Subbanna et al., 2018). The immunophenotyping panels recommended by HIPC guidelines do not allow for differentiation between T_h17 from T_h22 based on the surface markers CCR6 and CXCR3 (Finak et al., 2016). Thus, considering the previously reported increase in IL-22 plasma levels in patients with schizophrenia, a limitation of the present study is that the alterations in T_h22 cells were not investigated.

Several clinical trials have investigated the effect of antipsychotics in combination with Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) especially for treatment resistant schizophrenia cases. However, not all patients were found to benefit from the adjunctive therapy (Nitta et al., 2013). This highlights the importance of identifying the sub-group of schizophrenia patients with inflammation who may benefit from such therapy. However, there are no definitive markers for detecting inflammation in schizophrenia patients. The Th17 cell frequency has been suggested as a promising marker for inflammation. Our study, in addition to Th17 cells, reveals that the frequency of MAIT cells may serve as an important marker of inflammation in schizophrenia.

5. Conclusion

In conclusion, the multiparametric flow cytometry based immunophenotyping of PBMCs employed in this study reveals a substantial disturbance in percentages of T_h17 and MAIT cells, both of which are potentially inflammatory. The finding that these changes are not common across, but rather seen in a subgroup of schizophrenia cases, suggests that these patients may be possible candidates for anti-inflammatory therapy. Further, this study for the first time demonstrates a possible association of schizophrenia with MAIT cells, thereby providing a possible mechanism for the involvement of microbiome.

Table 2
Correlation analysis[a].

	Th 17	Activated Th 17	$CD8^+$ T cells	$CD4^+$ HLA-DR^+ MAIT cells	DN MAIT	DN HLA-DR^+ MAIT cells
Age (R^2)	0.002	0.017	0.116	0.116	0.0003	0.032
Gender (r_s; p value)	0.293; 0.102	0.255; 0.158	0.258; 0.152	0.23; 0.202	0.248; 0.170	0.298; 0.097
Duration of illness (R^2)	0.0503	0.001	0.014	0.236	0.0052	0.0194

[a] Statistically significant correlation was not observed against any variables tested.

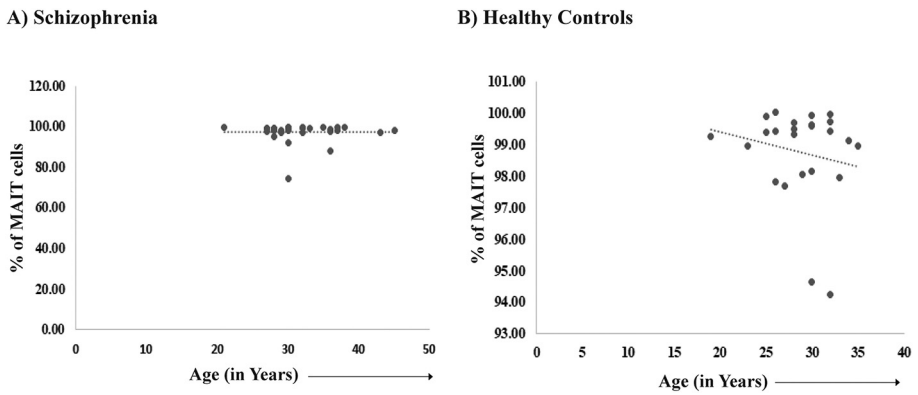

Fig. 3. Correlation of frequency of MAIT cells with age. X-axis represents Age (in years) Y-axis represents percentage of Total MAIT cells from parent population. Dotted line represents the linear correlation between age and percentage of total MAIT cells. A) Schizophrenia. B) Healthy controls.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.schres.2019.08.013.

Author contributions

VCN was involved in formulating methodology of the study, sample collection, processing and immunophenotyping experiments and wrote the first draft of the manuscript. MMV was involved in planning of immunophenotyping assays and analysis; and contributed significantly to the manuscript. RN helped VCN with flow cytometry experiments and contributed to editing of the manuscript. SV and GV coordinated the patient recruitment process. RR, GV, SV, MD and VR contributed to analysis of results and review of the study. All the authors contributed to editing and revision of the manuscript. The final submitted version of this manuscript has been read and approved by all the authors.

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Declaration of competing interest

The authors declare no conflict of interest, financial or otherwise.

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References

Adikaram, K.K., Hussein, M.A., Effenberger, M., Becker, T., 2014. Outlier detection method in linear regression based on sum of arithmetic progression. ScientificWorldJournal 2014, 821623.

Arisawa, T., Tahara, T., Shibata, T., Nagasaka, M., Nakamura, M., Kamiya, Y., Fujita, H., Nakamura, M., Yoshioka, D., Arima, Y., Okubo, M., Hirata, I., Nakano, H., 2008. The influence of polymorphisms of interleukin-17A and interleukin-17F genes on the susceptibility to ulcerative colitis. J. Clin. Immunol. 28, 44–49.

Basheer, S., Venkataswamy, M.M., Christopher, R., Van Amelsvoort, T., Srinath, S., Girimaji, S.C., Ravi, V., 2018. Immune aberrations in children with Autism Spectrum Disorder: a case-control study from a tertiary care neuropsychiatric hospital in India. Psychoneuroendocrinology 94, 162–167.

Bloomfield, P.S., Selvaraj, S., Veronese, M., Rizzo, G., Bertoldo, A., Owen, D.R., Bloomfield, M.A., Bonoldi, I., Kalk, N., Turkheimer, F., Mcguire, P., De Paola, V., Howes, O.D., 2016. Microglial activity in people at ultra high risk of psychosis and in schizophrenia: an [(11)C]PBR28 PET brain imaging study. Am. J. Psychiatry 173, 44–52.

Brozova, J., Karlova, I., Novak, J., 2016. Analysis of the phenotype and function of the subpopulations of mucosal-associated invariant T cells. Scand. J. Immunol. 84, 245–251.

Buttarelli, F.R., Fanciulli, A., Pellicano, C., Pontieri, F.E., 2011. The dopaminergic system in peripheral blood lymphocytes: from physiology to pharmacology and potential applications to neuropsychiatric disorders. Curr. Neuropharmacol. 9, 278–288.

Cai, H.Q., Catts, V.S., Webster, M.J., Galletly, C., Liu, D., O'donnell, M., Weickert, T.W., Weickert, C.S., 2018. Increased macrophages and changed brain endothelial cell gene expression in the frontal cortex of people with schizophrenia displaying inflammation. Mol. Psychiatry. https://doi.org/10.1038/s41380-018-0235-x [Epub ahead of print].

Chase, K.A., Cone, J.J., Rosen, C., Sharma, R.P., 2016. The value of interleukin 6 as a peripheral diagnostic marker in schizophrenia. BMC Psychiatry 16, 152.

Chen, Y., Bidwell, L.C., Norton, D., 2006. Trait vs. state markers for schizophrenia: identification and characterization through visual processes. Curr. Psychiatr. Rev. 2, 431–438.

Cho, Y.N., Kee, S.J., Kim, T.J., Jin, H.M., Kim, M.J., Jung, H.J., Park, K.J., Lee, S.J., Lee, S.S., Kwon, Y.S., Kee, H.J., Kim, N., Park, Y.W., 2014. Mucosal-associated invariant T cell deficiency in systemic lupus erythematosus. J. Immunol. 193, 3891–3901.

Coulter, F., Parrish, A., Manning, D., Kampmann, B., Mendy, J., Garand, M., Lewinsohn, D.M., Riley, E.M., Sutherland, J.S., 2017. IL-17 production from T helper 17, mucosal-associated invariant T, and gammadelta cells in tuberculosis infection and disease. Front. Immunol. 8, 1252.

Craddock, R.M., Lockstone, H.E., Rider, D.A., Wayland, M.T., Harris, L.J., Mckenna, P.J., Bahn, S., 2007. Altered T-cell function in schizophrenia: a cellular model to investigate molecular disease mechanisms. PLoS One 2, e692.

De Picker, L.J., Morrens, M., Chance, S.A., Boche, D., 2017. Microglia and brain plasticity in acute psychosis and schizophrenia illness course: a meta-review. Front Psychiatry 8, 238.

Dean, K., Murray, R.M., 2005. Environmental risk factors for psychosis. Dialogues Clin. Neurosci. 7, 69–80.

Debnath, M., 2015. Adaptive immunity in schizophrenia: functional implications of T cells in the etiology, course and treatment. J. NeuroImmune Pharmacol. 10, 610–619.

Debnath, M., Berk, M., 2014. Th17 pathway-mediated immunopathogenesis of schizophrenia: mechanisms and implications. Schizophr. Bull. 40, 1412–1421.

Debnath, M., Berk, M., 2017. Functional implications of the IL-23/IL-17 immune axis in schizophrenia. Mol. Neurobiol. 54, 8170–8178.

Derecki, N.C., Cardani, A.N., Yang, C.H., Quinnies, K.M., Crihfield, A., Lynch, K.R., Kipnis, J., 2010. Regulation of learning and memory by meningeal immunity: a key role for IL-4. J. Exp. Med. 207, 1067–1080.

Ding, M., Song, X., Zhao, J., Gao, J., Li, X., Yang, G., Wang, X., Harrington, A., Fan, X., Lv, L., 2014. Activation of Th17 cells in drug naive, first episode schizophrenia. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 51, 78–82.

DSM-IV, A. P. A. T. F. O, 2000. Diagnostic and Statistical Manual of Mental Disorders, 4th edition text revision. American Psychiatric Association, Washington, DC (DSM-IV-TR).

Espinoza, J.L., Takami, A., Nakata, K., Onizuka, M., Kawase, T., Akiyama, H., Miyamura, K., Morishima, Y., Fukuda, T., Kodera, Y., Nakao, S., Japan Marrow Donor, P., 2011. A genetic variant in the IL-17 promoter is functionally associated with acute graft-versus-host disease after unrelated bone marrow transplantation. PLoS One 6, e26229.

Ezeoke, A., Mellor, A., Buckley, P., Miller, B., 2013. A systematic, quantitative review of blood autoantibodies in schizophrenia. Schizophr. Res. 150, 245–251.

Finak, G., Langweiler, M., Jaimes, M., Malek, M., Taghiyar, J., Korin, Y., Raddassi, K., Devine, L., Obermoser, G., Pekalski, M.L., Pontikos, N., Diaz, A., Heck, S., Villanova, F., Terrazzini, N., Kern, F., Qian, Y., Stanton, R., Wang, K., Brandes, A., Ramey, J., Aghaeepour, N., Mosmann, T., Scheuermann, R.H., Reed, E., Palucka, K., Pascual, V., Blomberg, B.B., Nestle, F., Nussenblatt, R.B., Brinkman, R.R., Gottardo, R., Maecker, H., Mccoy, J.P., 2016. Standardizing flow cytometry

immunophenotyping analysis from the human immunophenotyping consortium. Sci. Rep. 6, 20686.
Ganguli, R., Rabin, B.S., 1993. CD5 positive B lymphocytes in schizophrenia: no alteration in numbers or percentage as compared with control subjects. Psychiatry Res. 48, 69–78.
Garre, J.M., Silva, H.M., Lafaille, J.J., Yang, G., 2017. CX3CR1(+) monocytes modulate learning and learning-dependent dendritic spine remodeling via TNF-alpha. Nat. Med. 23, 714–722.
Ghazarian, L., Caillat-Zucman, S., Houdouin, V., 2017. Mucosal-associated invariant T cell interactions with commensal and pathogenic bacteria: potential role in antimicrobial immunity in the child. Front. Immunol. 8, 1837.
Greene, C., Kealy, J., Humphries, M.M., Gong, Y., Hou, J., Hudson, N., Cassidy, L.M., Martiniano, R., Shashi, V., Hooper, S.R., Grant, G.A., Kenna, P.F., Norris, K., Callaghan, C.K., Islam, M.D., O'Mara, S.M., Najda, Z., Campbell, S.G., Pachter, J.S., Thomas, J., Williams, N.M., Humphries, P., Murphy, K.C., Campbell, M., 2018. Dose-dependent expression of claudin-5 is a modifying factor in schizophrenia. Mol. Psychiatry 23, 2156–2166.
Gysin, R., Kraftsik, R., Sandell, J., Bovet, P., Chappuis, C., Conus, P., Deppen, P., Preisig, M., Ruiz, V., Steullet, P., Tosic, M., Werge, T., Cuenod, M., Do, K.Q., 2007. Impaired glutathione synthesis in schizophrenia: convergent genetic and functional evidence. Proc. Natl. Acad. Sci. U. S. A. 104, 16621–16626.
Hashimoto, K., Ashwood, P., Corbett, B.A., Kantor, A., Schulman, H., Van De Water, J., Amaral, D.G., 2011. In search of cellular immunophenotypes in the blood of children with autism. PLoS One 6.
Henriksen, M.G., Nordgaard, J., Jansson, L.B., 2017. Genetics of schizophrenia: overview of methods, findings and limitations. Front. Hum. Neurosci. 11, 322.
Howes, O.D., Kapur, S., 2009. The dopamine hypothesis of schizophrenia: version III—the final common pathway. Schizophr. Bull. 35, 549–562.
Johansson, M.A., Bjorkander, S., Mata Forsberg, M., Qazi, K.R., Salvany Celades, M., Bittmann, J., Eberl, M., Sverremark-Ekstrom, E., 2016. Probiotic lactobacilli modulate Staphylococcus aureus-induced activation of conventional and unconventional T cells and NK cells. Front. Immunol. 7, 273.
Juckel, G., Manitz, M.P., Brune, M., Friebe, A., Heneka, M.T., Wolf, R.J., 2011. Microglial activation in a neuroinflammational animal model of schizophrenia—a pilot study. Schizophr. Res. 131, 96–100.
Kalmady, S.V., Venkatasubramanian, G., Shivakumar, V., Gautham, S., Subramaniam, A., Jose, D.A., Maitra, A., Ravi, V., Gangadhar, B.N., 2014. Relationship between Interleukin-6 gene polymorphism and hippocampal volume in antipsychotic-naive schizophrenia: evidence for differential susceptibility? PLoS One 9, e96021.
Kanji, S., Fonseka, T.M., Marshe, V.S., Sriretnakumar, V., Hahn, M.K., Muller, D.J., 2018. The microbiome-gut-brain axis: implications for schizophrenia and antipsychotic induced weight gain. Eur. Arch. Psychiatry Clin. Neurosci. 268, 3–15.
Kay, S.R., Fiszbein, A., Opler, L.A., 1987. The positive and negative syndrome scale (PANSS) for schizophrenia. Schizophr. Bull. 13, 261–276.
Kidd, P., 2003. Th1/Th2 balance: the hypothesis, its limitations, and implications for health and disease. Altern. Med. Rev. 8, 223–246.
Kjer-Nielsen, L., Patel, O., Corbett, A.J., Le Nours, J., Meehan, B., Liu, L., Bhati, M., Chen, Z., Kostenko, L., Reantragoon, R., Williamson, N.A., Purcell, A.W., Dudek, N.L., Mcconville, M.J., O'hair, R.A., Khairallah, G.N., Godfrey, D.I., Fairlie, D.P., Rossjohn, J., Mccluskey, J., 2012. MR1 presents microbial vitamin B metabolites to MAIT cells. Nature 491, 717–723.
Li, J., 2008. A two-step rejection procedure for testing multiple hypotheses. Journal of Statistical Planning and Inference 138, 1521–1527.
Lin, A., Kenis, G., Bignotti, S., Tura, G.J., De Jong, R., Bosmans, E., Pioli, R., Altamura, C., Scharpe, S., Maes, M., 1998. The inflammatory response system in treatment-resistant schizophrenia: increased serum interleukin-6. Schizophr. Res. 32, 9–15.
Maecker, H.T., Mccoy, J.P., Nussenblatt, R., 2012. Standardizing immunophenotyping for the human immunology project. Nat Rev Immunol 12, 191–200.
Maino, K., Gruber, R., Riedel, M., Seitz, N., Schwarz, M., Muller, N., 2007. T- and B-lymphocytes in patients with schizophrenia in acute psychotic episode and the course of the treatment. Psychiatry Res. 152, 173–180.
Meucci, O., Fatatis, A., Simen, A.A., Miller, R.J., 2000. Expression of CX3CR1 chemokine receptors on neurons and their role in neuronal survival. Proc. Natl. Acad. Sci. U. S. A. 97, 8075–8080.
Miller, B.J., Goldsmith, D.R., 2017. Towards an immunophenotype of schizophrenia: progress, potential mechanisms, and future directions. Neuropsychopharmacology 42, 299–317.
Miller, B.J., Gassama, B., Sebastian, D., Buckley, P., Mellor, A., 2013. Meta-analysis of lymphocytes in schizophrenia: clinical status and antipsychotic effects. Biol. Psychiatry 73, 993–999.
Muller, N., Schwarz, M.J., 2010. Immune system and schizophrenia. Curr. Immunol. Rev. 6, 213–220.
Muller, N., Riedel, M., Ackenheil, M., Schwarz, M.J., 2000. Cellular and humoral immune system in schizophrenia: a conceptual re-evaluation. World J Biol Psychiatry 1, 173–179.
Najjar, S., Pearlman, D.M., Alper, K., Najjar, A., Devinsky, O., 2013. Neuroinflammation and psychiatric illness. J. Neuroinflammation 10, 43.
Najjar, S., Pahlajani, S., De Sanctis, V., Stern, J.N.H., Najjar, A., Chong, D., 2017. Neurovascular unit dysfunction and blood-brain barrier hyperpermeability contribute to schizophrenia neurobiology: a theoretical integration of clinical and experimental evidence. Front Psychiatry 8, 83.
Nazarpour, R., Zabihi, E., Alijanpour, E., Abedian, Z., Mehdizadeh, H., Rahimi, F., 2012. Optimization of human peripheral blood mononuclear cells (PBMCs) cryopreservation. Int J Mol Cell Med 1, 88–93.
Nguyen, T.T., Kosciolek, T., Eyler, L.T., Knight, R., Jeste, D.V., 2018. Overview and systematic review of studies of microbiome in schizophrenia and bipolar disorder. J. Psychiatr. Res. 99, 50–61.
Nitta, M., Kishimoto, T., Muller, N., Weiser, M., Davidson, M., Kane, J.M., Correll, C.U., 2013. Adjunctive use of nonsteroidal anti-inflammatory drugs for schizophrenia: a meta-analytic investigation of randomized controlled trials. Schizophr. Bull. 39, 1230–1241.
Nordang, G.B., Viken, M.K., Hollis-Moffatt, J.E., Merriman, T.R., Forre, O.T., Helgetveit, K., Kvien, T.K., Lie, B.A., 2009. Association analysis of the interleukin 17A gene in Caucasian rheumatoid arthritis patients from Norway and New Zealand. Rheumatology (Oxford) 48, 367–370.
Novak, J., Dobrovolny, J., Novakova, L., Kozak, T., 2014. The decrease in number and change in phenotype of mucosal-associated invariant T cells in the elderly and differences in men and women of reproductive age. Scand. J. Immunol. 80, 271–275.
Okun, E., Griffioen, K.J., Mattson, M.P., 2011. Toll-like receptor signaling in neural plasticity and disease. Trends Neurosci. 34, 269–281.
Ozdin, S., Sarisoy, G., Boke, O., 2017. A comparison of the neutrophil-lymphocyte, platelet-lymphocyte and monocyte-lymphocyte ratios in schizophrenia and bipolar disorder patients - a retrospective file review. Nord J Psychiatry 71, 509–512.
Pacheco, R., Gallart, T., Lluis, C., Franco, R., 2007. Role of glutamate on T-cell mediated immunity. J. Neuroimmunol. 185, 9–19.
Peruzzotti-Jametti, L., Donega, M., Giusto, E., Mallucci, G., Marchetti, B., Pluchino, S., 2014. The role of the immune system in central nervous system plasticity after acute injury. Neuroscience 283, 210–221.
Ransohoff, R.M., Schafer, D., Vincent, A., Blachere, N.E., Bar-Or, A., 2015. Neuroinflammation: ways in which the immune system affects the brain. Neurotherapeutics 12, 896–909.
Romagnani, S., 1991. Type 1 T helper and type 2 T helper cells: functions, regulation and role in protection and disease. Int J Clin Lab Res 21, 152–158.
Romagnani, S., 1999. Th1/Th2 cells. Inflamm. Bowel Dis. 5, 285–294.
Rothman, K.J., 1990. No adjustments are needed for multiple comparisons. Epidemiology 1, 43–46.
Schwarz, E., Maukonen, J., Hyytiainen, T., Kieseppa, T., Oresic, M., Sabunciyan, S., Mantere, O., Saarela, M., Yolken, R., Suvisaari, J., 2018. Analysis of microbiota in first episode psychosis identifies preliminary associations with symptom severity and treatment response. Schizophr. Res. 192, 398–403.
Steiner, J., Jacobs, R., Panteli, B., Brauner, M., Schiltz, K., Bahn, S., Herberth, M., Westphal, S., Gos, T., Walter, M., Bernstein, H.G., Myint, A.M., Bogerts, B., 2010. Acute schizophrenia is accompanied by reduced T cell and increased B cell immunity. Eur. Arch. Psychiatry Clin. Neurosci. 260, 509–518.
Subbanna, M., Shivakumar, V., Talukdar, P.M., Narayanaswamy, J.C., Venugopal, D., Berk, M., Varambally, S., Venkatasubramanian, G., Debnath, M., 2018. Role of IL-6/RORC/IL-22 axis in driving Th17 pathway mediated immunopathogenesis of schizophrenia. Cytokine 111, 112–118.
Sugimoto, C., Konno, T., Wakao, R., Fujita, H., Fujita, H., Wakao, H., 2015. Mucosal-associated invariant T cell is a potential marker to distinguish fibromyalgia syndrome from arthritis. PLoS One 10, e0121124.
Tilloy, F., Treiner, E., Park, S.H., Garcia, C., Lemonnier, F., De La Salle, H., Bendelac, A., Bonneville, M., Lantz, O., 1999. An invariant T cell receptor alpha chain defines a novel TAP-independent major histocompatibility complex class Ib-restricted alpha/beta T cell subpopulation in mammals. J. Exp. Med. 189, 1907–1921.
Touch, S., Assmann, K.E., Aron-Wisnewsky, J., Marquet, F., Rouault, C., Fradet, M., Mosbah, H., Consortium, M, Isnard, R., Helft, G., Lehuen, A., Poitou, C., Clement, K., Andre, S., MetaCardis Consortium, 2018. Mucosal-associated invariant T (MAIT) cells are depleted and prone to apoptosis in cardiometabolic disorders. FASEB J., fj201800052RR https://doi.org/10.1096/fj.201800052RR [Epub ahead of print].
Treiner, E., Duban, L., Bahram, S., Radosavljevic, M., Wanner, V., Tilloy, F., Affaticati, P., Gilfillan, S., Lantz, O., 2003. Selection of evolutionarily conserved mucosal-associated invariant T cells by MR1. Nature 422, 164–169.
Ussher, J.E., Klenerman, P., Willberg, C.B., 2014. Mucosal-associated invariant T-cells: new players in anti-bacterial immunity. Front. Immunol. 5, 450.
Varun, C.N., Raju, R., Venkataswamy, M.M., Ravi, V., Varambally, S., 2018. Procalcitonin and C - reactive protein as peripheral inflammatory markers in antipsychotic drug-free schizophrenia patients. Asian J. Psychiatr. 35, 11–14.
Walker, L.J., Tharmalingam, H., Klenerman, P., 2014. The rise and fall of MAIT cells with age. Scand. J. Immunol. 80, 462–463.
Xiao, X., Cai, J., 2017. Mucosal-associated invariant T cells: new insights into antigen recognition and activation. Front. Immunol. 8, 1540.
Xu, K., Schadt, E.E., Pollard, K.S., Roussos, P., Dudley, J.T., 2015. Genomic and network patterns of schizophrenia genetic variation in human evolutionary accelerated regions. Mol. Biol. Evol. 32, 1148–1160.
Yolken, R.H., Torrey, E.F., 1995. Viruses, schizophrenia, and bipolar disorder. Clin. Microbiol. Rev. 8, 131–145.
Zhan, Y., Carrington, E.M., Zhang, Y., Heinzel, S., Lew, A.M., 2017. Life and death of activated T cells: how are they different from Naïve T cells? Front. Immunol. 8, 1809.