RESEARCH

Decreased programmed cell death ligand 2-positive monocytic myeloid-derived suppressor cells and programmed cell death protein 1-positive T-regulatory cells in patients with type 2 diabetes: implications for immunopathogenesis

Zhaoxiang Liu[1,*], Mingqiang Zhang[2,*], Xiaohu Shi[3,*], Wenhui Zhao[1], Chenxiang Cao[1], Lixia Jin[1], Yanlei Wang[1] and **Jianzhong Xiao[1]**

[1]Department of Endocrinology, Beijing Tsinghua Changgung Hospital, School of Clinical Medicine, Tsinghua University, Beijing, China
[2]Department of Respiratory and Critical Care Medicine, Beijing Tsinghua Changgung Hospital, School of Clinical Medicine, Tsinghua University, Beijing, China
[3]Department of Traditional Chinese Medicine, Peking Union Medical College Hospital, Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing, China

Correspondence should be addressed to J Xiao: xjza01150@btch.edu.cn

*(Z Liu, M Zhang and X Shi contributed equally to this work)

Abstract

Objectives: The activation of immune cells plays a significant role in the progression of type 2 diabetes. This study aimed to investigate the potential role of myeloid-derived suppressor cells (MDSCs) and T-regulatory cells (Tregs) in type 2 diabetes.
Methods: A total of 61 patients diagnosed with type 2 diabetes were recruited. Clinical characteristics were reviewed and peripheral blood samples were collected. We calculated the percentage of different cells. Frequencies of MDSC subsets refered to the percentage of G-MDSCs (CD15+CD33+CD11b+CD14-HLA-DR-/low) in CD45 positive cells and the percentage of M-MDSCs (CD14+CD15-CD11b+CD33+HLA-DR-/low) in lymphocytes plus monocytes.
Results: Frequencies of programmed cell death ligand 1-positive granulocytic MDSCs (PD-L1$^+$ G-MDSCs), programmed cell death ligand 2-positive monocytic MDSCs (PD-L2$^+$ M-MDSCs), PD-L2$^+$ G-MDSC, and programmed cell death protein 1-positive Tregs (PD-1$^+$Tregs) were decreased in patients with type 2 diabetes. The frequency of PD-1$^+$ Tregs was positively related to PD-L2$^+$ M-MDSCs (r = 0.357, P = 0.009) and negatively related to HbA1c (r = -0.265, P = 0.042), fasting insulin level (r = −0.260, P = 0.047), and waist circumference (r = −0.373, P = 0.005).
Conclusions: Decreased PD-L2$^+$ M-MDSCs and PD-1$^+$ Tregs may promote effector T cell activation, leading to chronic low-grade inflammation in type 2 diabetes. These findings highlight the contribution of MDSCs and Tregs to the immunopathogenesis of type 2 diabetes and suggest their potential as targets for new therapeutic approaches.

Key Words
- type 2 diabetes mellitus
- myeloid-derived suppressor cells
- T-regulatory cells

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Introduction

Diabetes is a global health concern owing to its high prevalence and related health problems (1). Type 2 diabetes mellitus is caused by a combination of genetic and environmental factors and is also recognized as a chronic, low-grade inflammatory disease characterized by increased circulating concentrations of inflammatory cytokines and the recruitment of immune cells, including macrophages and T cells, to metabolic tissues (2).

Myeloid-derived suppressor cells (MDSCs) represent a group of immature myeloid cells with potent immunosuppressive activity. During pathological processes such as cancer, chronic inflammation, and trauma, myeloid cells undergo a conversion to MDSCs, which exert immunosuppressive effects on T cells (3). There are two functional subsets of MDSCs, $Ly6G^{+}/Ly6C^{low}$ referred to as granulocytic MDSC (G-MDSC) and $Ly6G^{-}/Ly6C^{high}$ referred to as monocytic MDSC (M-MDSC) (4). MDSCs often express high levels of arginase 1 and produce nitric oxide and reactive oxygen species, resulting in the apoptosis of T cells, inhibition of T cell proliferation, as well as expansion of T-regulatory cells (Tregs) (4, 5).

Tregs play a critical role in maintaining immune homeostasis and preventing the development of autoimmune diseases by suppressing effector T cells and B cells (6). The immunosuppressive mechanisms employed by Tregs include the expression of cytotoxic T-lymphocyte antigen-4 (CTLA-4) to suppress costimulation, consumption of interleukin-2 (IL-2) through CD25) expression, and the secretion of anti-inflammatory cytokines such as IL-10 and transforming growth factor beta (TGF-β) to suppress inflammation (7). In addition to CTLA-4, Tregs also express high levels of programmed cell death 1 (PD-1) (8). It has been reported that programmed cell death ligand 1 (PD-L1) and programmed cell death ligand 2 (PD-L2) expressed on MDSCs play a critical suppressive role on T cells (9). The PD-1/PD-L1 or PD-L2 axis is also involved in Treg differentiation and function (10). It is hypothesized that the interaction between PD-L1 or PD-L2 on MDSCs and PD-1 on Tregs may mediate the key suppressive pathway targeting effector T cells.

While MDSCs have been extensively studied in cancer and autoimmune patients, the understanding of their role in type 2 diabetes is limited and contradictory. For patients with type 1 diabetes, autoimmune T cells mediate the destruction of insulin-producing beta cells in their pancreas. An increased frequency of MDSCs has been observed not only in the blood of type 1 diabetes patients but also in their relatives at risk for the disease (11). Data regarding the role of MDSCs in type 2 diabetes are conflicting. Chronic hyperglycemia has been shown to dysregulate T cell function (12). Studies have demonstrated reduced frequencies of Tregs but increased levels of proinflammatory subsets such as Th1 and Th17 cells in patients with type 2 diabetes, suggesting a natural skewing of T cells toward proinflammatory subsets (13, 14). However, other studies showed elevated frequencies of MDSCs in type 2 diabetes (15), indicating a potential immunosuppressive mechanism mediated by MDSCs.

In this study, we conducted a comparative analysis of two subsets of MDSCs, G-MDSCs and M-MDSCs, as well as T cell subsets, to investigate their frequencies, mutual relationship, and immunosuppressive mediators in the peripheral blood of patients with type 2 diabetes and healthy individuals. The aim was to explore the potential role of MDSCs and Tregs in type 2 diabetes.

Materials and methods

Study populations

Sixty-one patients diagnosed with type 2 diabetes mellitus were recruited from the Department of Endocrinology at Tsinghua Changgung Hospital between February 2019 and January 2020. The study population consisted of 39 men and 22 women, with an average age of (58.1 ± 14.1) years and a BMI of (26.90 ± 5.59) kg/m^2. The diagnosis of type 2 diabetes mellitus was based on the diagnostic criteria established by the World Health Organization (WHO) in 1999 (16). We excluded patients who had serious heart problems (New York Heart Association III/IV), liver problems (severe hepatic impairment or liver failure), lung problems (conditions that may predispose to hypoxemia), or kidney problems (primary nephrotic syndrome, glomerulonephritis, obstructive renovascular disease, nephrectomy, renal transplant, etc.), were pregnant, or had been diagnosed with type 1 diabetes mellitus, special type of diabetes, or gestational diabetes. Thirty-eight healthy individuals undergoing physical examination, including 20 men and 18 women, were included as controls. The control group had an average age of (56.9 ± 9.6) years and a BMI of (24.8 ± 4.68) kg/m^2. The healthy controls had not received any immunomodulatory or corticosteroid drugs.

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The patients with type 2 diabetes and healthy controls were matched for age, sex, and BMI to ensure comparability. This study protocol was approved by the ethics committee of Tsinghua Changgung Hospital (no. 20280-0-01), and all participants provided informed consent after being informed about the study background and objective.

Assessment of microvascular complications

Microvascular complications were evaluated in patients with type 2 diabetes mellitus. Diabetic kidney disease (DKD) was diagnosed based on the presence of albuminuria and/or a reduced estimated glomerular filtration rate (<60 mL/min/1.73 m^2) in the absence of signs or symptoms of other kidney diseases. Diabetic retinopathy (DR) status was assessed by fundus photography and graded by an experienced ophthalmologist. Diabetic peripheral neuropathy (DPN) was diagnosed based on the results of nerve conduction studies using standardized protocols.

Peripheral blood sampling and processing

Peripheral blood samples were collected from each patient within 24 h of admission to the hospital and from each healthy individual during the physical examination. Five milliliters of blood were centrifuged at 1200 × ***g*** for 5 min to obtain serum for further measurements. Another 5 mL of blood were heparinized and used to isolate peripheral blood mononuclear cells through density-gradient centrifugation using Ficoll (GE Healthcare) according to a previously described method (17).

Circulating T cell subpopulations

The phenotypes of T cells were determined using flow cytometry based on CD3 expression with forward and side scatter properties as previously described. The functional compartments of $CD4^+$ and $CD8^+$ T cells were phenotypically characterized as $CD3^+CD4^+CD8^-$ and $CD3^+CD4^-CD8^+$, respectively. Tregs were identified

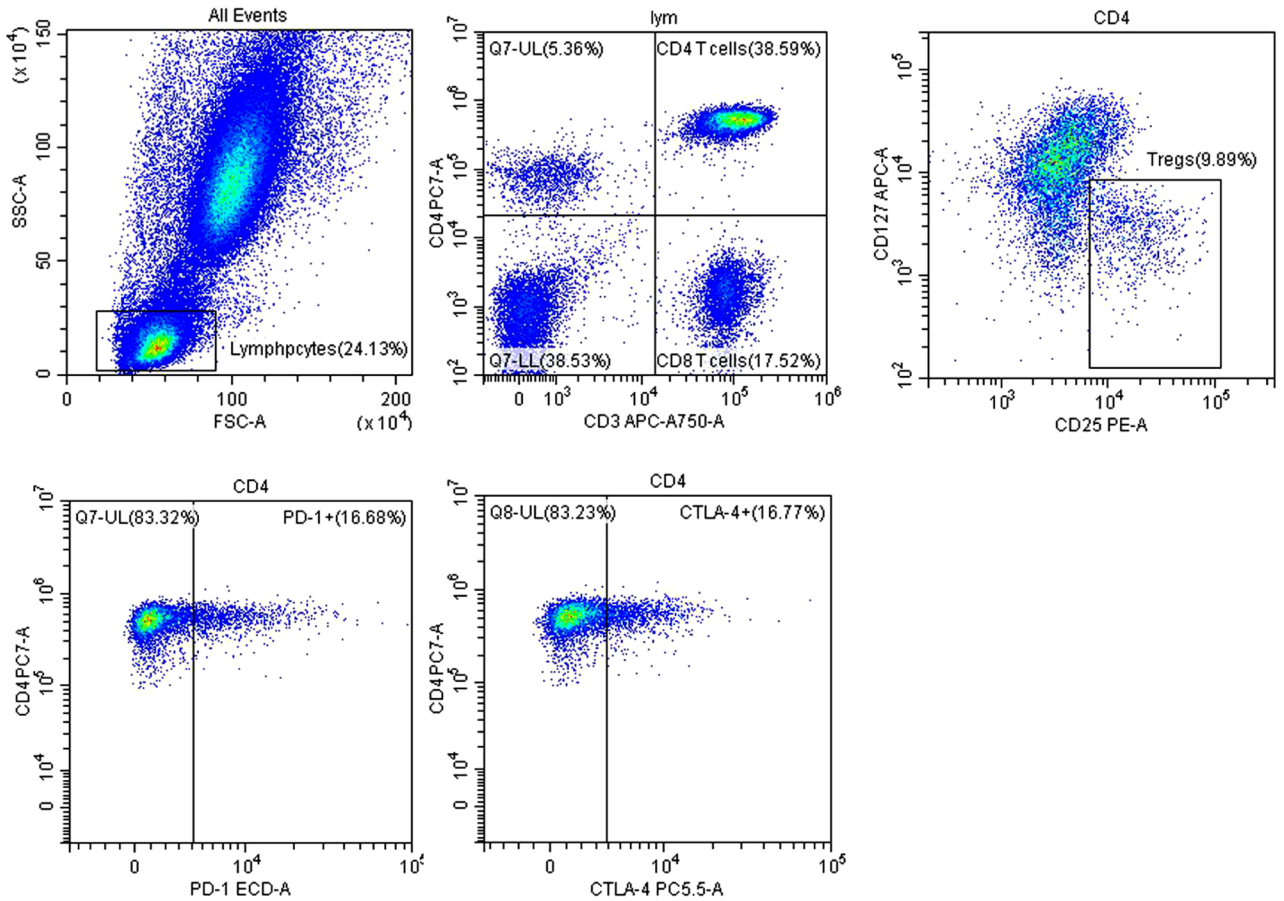

Figure 1
The strategies of flow cytometric analyses to identify T cell subpopulations in the circulation.

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based on differential expression of CD25 and CD127 ($CD4^+CD25^{++}CD127^{-/low}$). The frequencies of cells expressing PD-1 and CTLA-4 were determined within each T cell subset, allowing for the identification of $PD\text{-}1^+$ CD4, $CTLA\text{-}4^+$ CD4, $PD\text{-}1^+$ CD8, $CTLA\text{-}4^+$ CD8, $PD\text{-}1^+$ Treg, and $CTLA\text{-}4^+$ Treg. Phenotypic analyses of T cells were performed using fluorescence-activated cell sorting (FACS) with a CytoFLEX instrument (Beckman Coulter, USA) and a panel of anti-human–specific antibodies against surface CD markers: CD3-APC-Alexa Fluor 750, CD4-PE-Cyanine7, CD25-PE-Cyanine7, CD127-Alexa Fluor 700, CD279 (PD-1)-PE, and CD152 (CTLA-4)-PerCP-eFluor 710 (all purchased from eBioscience, Invitrogen). Figure 1 illustrates a strategy of flow cytometric analyses to identify T cell subpopulations in the circulation.

Immunophenotyping of MDSC subsets by flow cytometry

The MDSC subsets, G-MDSCs ($CD15^+CD33^+CD11b^+CD14^-HLA\text{-}DR^{-/low}$) and M-MDSCs ($CD14^+CD15^-CD11b^+CD33^+HLA\text{-}DR^{-/low}$), were characterized by FACS using a CytoFLEX (Beckman Coulter) with a panel of the following fluorescent-labeled monoclonal antibodies: anti-CD15, anti-CD33, anti-CD14, anti-CD45, anti-CD11b, and anti-HLA-DR. All antibodies were purchased from eBioscience (Invitrogen). The frequencies of cells expressing PD-L1 and PD-L2 within the MDSC subsets were determined using fluorescent-labeled monoclonal antibodies CD274-APC and CD273-PE. This allowed for the identification of $PD\text{-}L1^+$ G-MDSCs, $PD\text{-}L1^+$ M-MDSCs, $PD\text{-}L2^+$ G-MDSCs, and $PD\text{-}L2^+$ M-MDSCs. The flow cytometric analyses strategies for identifying G-MDSCs and M-MDSCs in the peripheral blood are presented in Figures 2 and 3.

Analysis and statistics

Data management and analysis were performed using SPSS version 23.0 (SPSS Inc.) and GraphPad Prism 8.0 software (GraphPad Software). Normal distributive data were expressed as the mean ± s.d., while

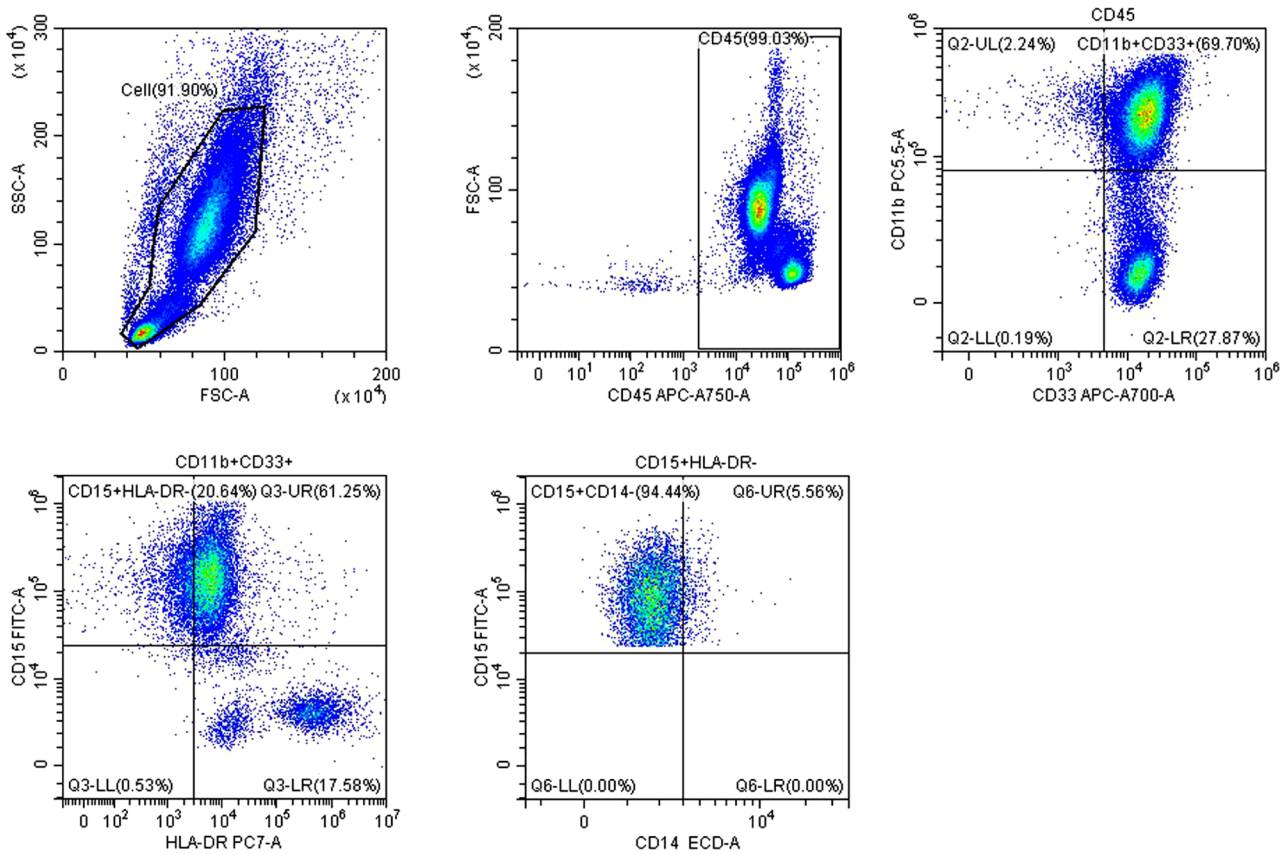

Figure 2
The strategies of flow cytometric analyses to identify G-MDSCs in the circulation. G-MDSCs, granulocytic myeloid-derived suppressor cells.

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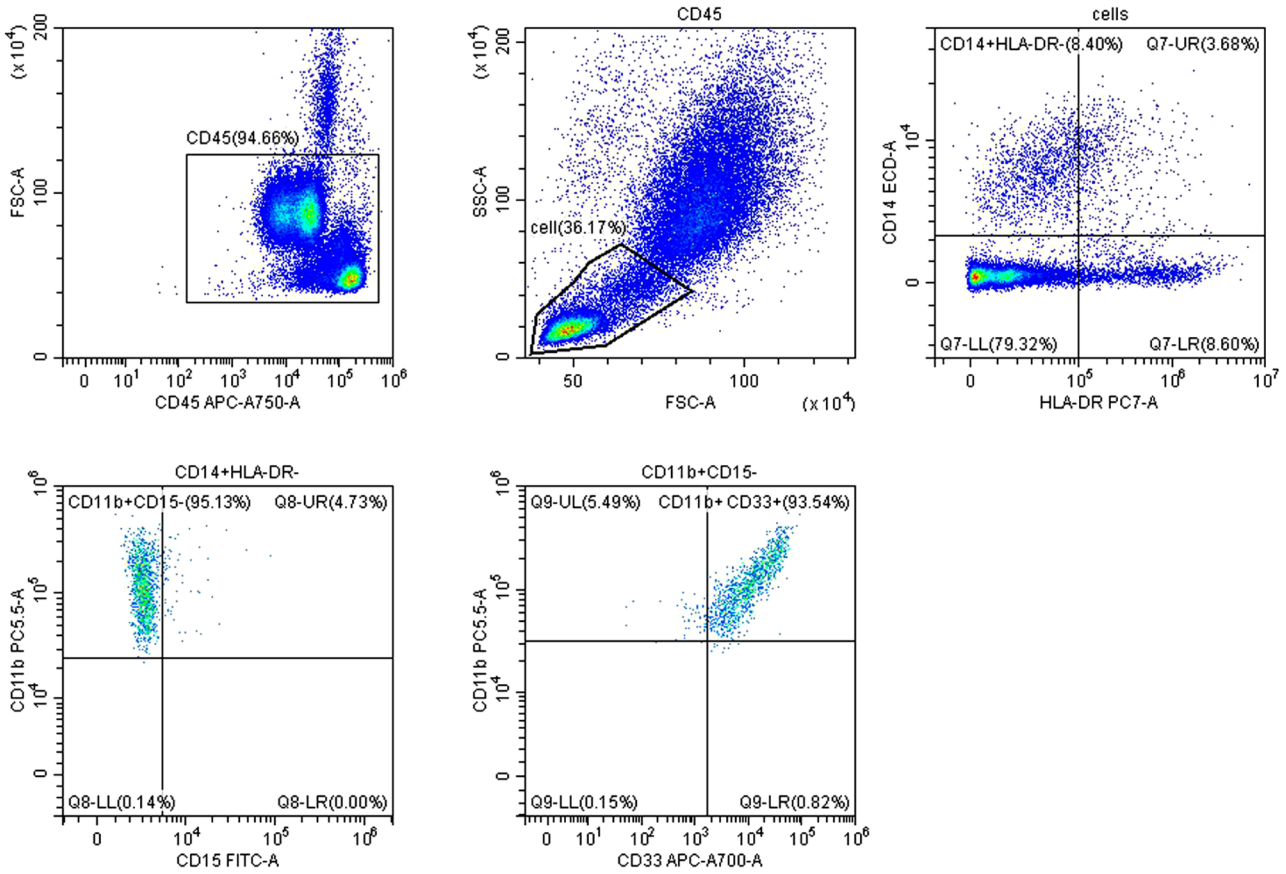

Figure 3
The strategies of flow cytometric analyses to identify M-MDSCs in the circulation. M-MDSCs, monocytic myeloid-derived suppressor cells.

non–normally distributed data were presented as median (quartiles). Paired *t*-test were used for paired samples with a normal distribution, and Wilcoxon matched-pair signed-rank tests were employed for paired samples with an abnormal distribution. Unpaired *t*-tests were performed for unpaired samples with normally distributed data, and Mann–Whitney *U* tests were used for samples with an abnormal distribution. Categorical variables were expressed as proportions and analyzed using the chi-square test. Pearson correlation analysis was conducted to evaluate the relationship between T cell subsets, MDSC subsets, and clinical variables. Statistical significance was set at $P < 0.05$.

Results

The frequencies of peripheral blood T cell subsets and MDSC subsets in type 2 diabetes were analyzed using flow cytometry. Figures 4 and 5 depict the flow cytometric analyses of Tregs, PD-1$^+$ Tregs, CTLA-4$^+$ Tregs, PD-1$^+$CD4$^+$ T cells, CTLA-4$^+$CD4$^+$ T cells, PD-1$^+$CD8$^+$ T cells, and CTLA-4$^+$CD8$^+$ T cells. It was observed that the frequencies of Tregs ($P < 0.001$) and PD-1$^+$ Tregs ($P = 0.012$) were decreased in the peripheral blood of patients with type 2 diabetes compared to healthy controls (Fig. 6 and Table 1). However, the distribution of CTLA-4$^+$ Tregs, PD-1$^+$CD4$^+$ T cells, CTLA-4$^+$CD4$^+$ T cells, PD-1$^+$CD8$^+$ T cells, and CTLA-4$^+$CD8$^+$ T cells was similar between patients with type 2 diabetes and healthy controls.

Flow cytometric analyses of G-MDSCs and M-MDSCs (Figs. 7, 8 and Table 2) revealed that patients with type 2 diabetes exhibited lower frequencies of PD-L1$^+$ G-MDSCs ($P < 0.001$), PD-L2$^+$ M-MDSCs ($P = 0.003$), and PD-L2$^+$ G-MDSCs ($P = 0.049$) in the peripheral blood when compared to healthy controls. However, there were no significant differences between patients with type 2 diabetes and healthy controls in terms of the frequencies of total G-MDSCs ($P = 0.932$) and total M-MDSCs ($P = 0.341$) in the peripheral blood.

Correlations between the frequencies of peripheral blood T cell subsets and MDSC subsets in type 2 diabetes

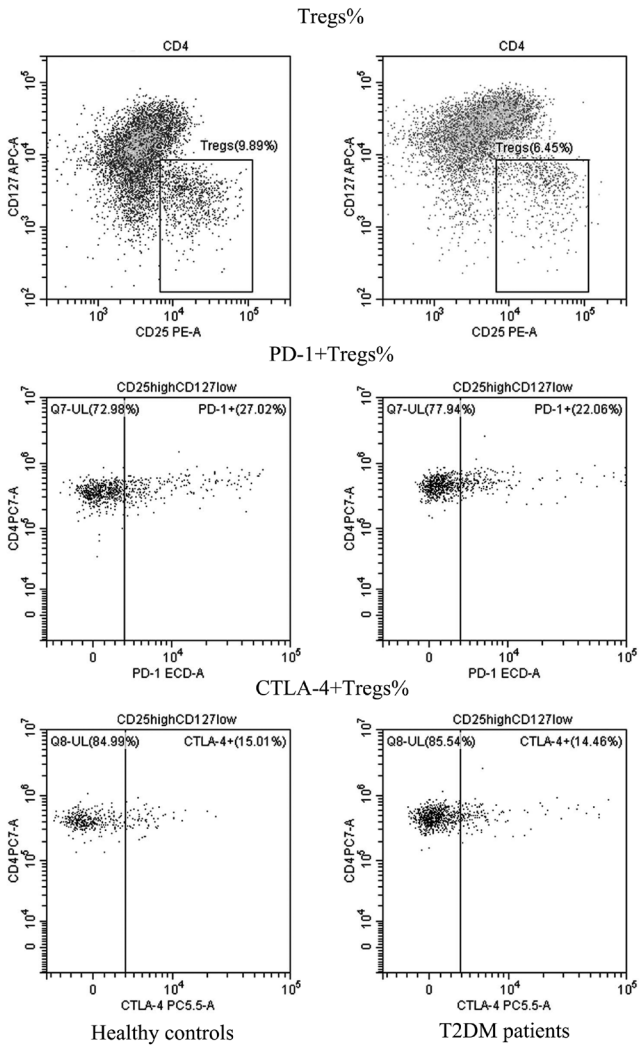

Figure 4
Flow cytometric analyses of Tregs, PD-1$^+$Tregs, and CTLA-4$^+$ Tregs in patients with T2DM and healthy controls. CTLA-4, cytotoxic T-lymphocyte antigen-4; PD-1, programmed cell death protein 1; T2DM, type 2 diabetes mellitus; Tregs, T-regulatory cells..

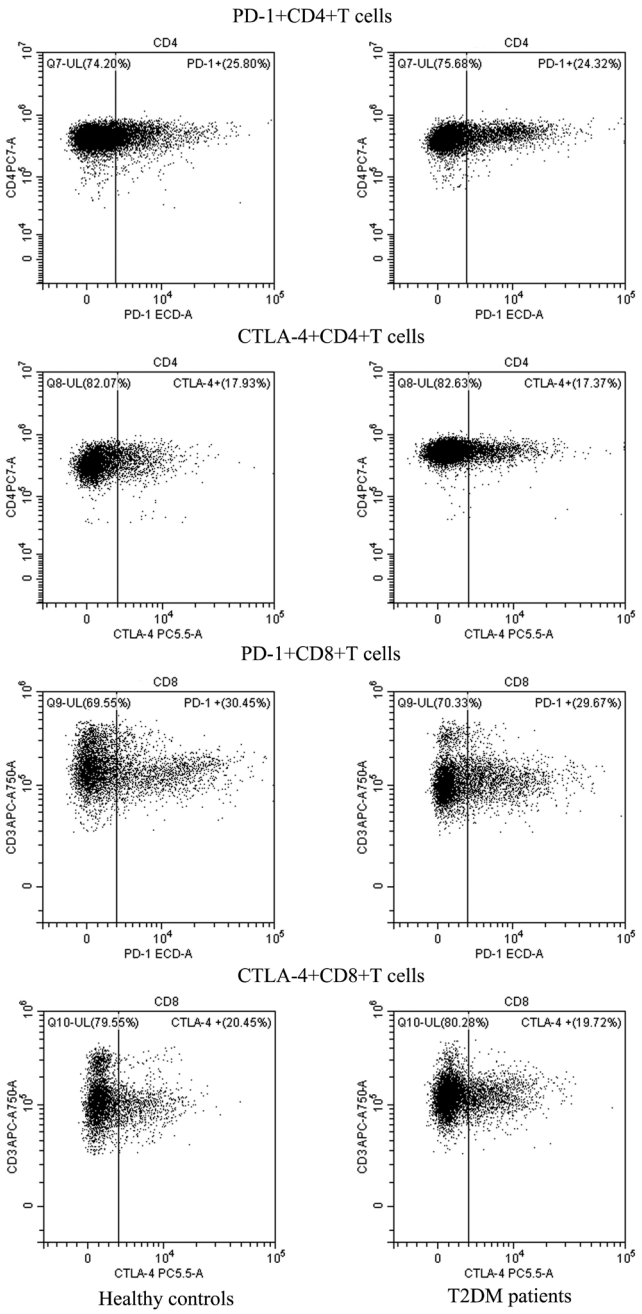

Figure 5
Flow cytometric analyses of PD-1$^+$CD4$^+$ T cells, CTLA-4$^+$CD4$^+$ T cells, PD-1$^+$CD8$^+$ T cells, and CTLA-4$^+$CD8$^+$ T cells in patients with T2DM and healthy controls. CTLA-4, cytotoxic T-lymphocyte antigen-4; PD-1, programmed cell death protein 1; T2DM, type 2 diabetes mellitus; Tregs, T-regulatory cells..

were examined in this study. The analysis revealed a positive association between the frequency of PD-1$^+$ Tregs and the frequency of PD-L2$^+$ M-MDSCs ($r=0.357$, $P=0.009$). Other T cell subsets were not related to the frequencies of MDSC subsets.

The relationship between clinical variables and the frequencies of PD-1$^+$ Tregs and MDSCs was also investigated. The results presented in Tables 3 and 4 demonstrated that the frequency of PD-1$^+$ Tregs in the peripheral blood exhibited negative associations with HbA1c level ($r=-0.265$, $P = 0.042$), fasting insulin level ($r=-0.260$, $P=0.047$), and waist circumference ($r=-0.373$, $P=0.005$). These findings suggest that lower frequencies of PD-1+Tregs are associated with higher levels of HbA1c, fasting insulin, and waist circumference, which are indicators of insulin resistance and disease progression in type 2 diabetes. On the other hand, no significant associations were observed between the

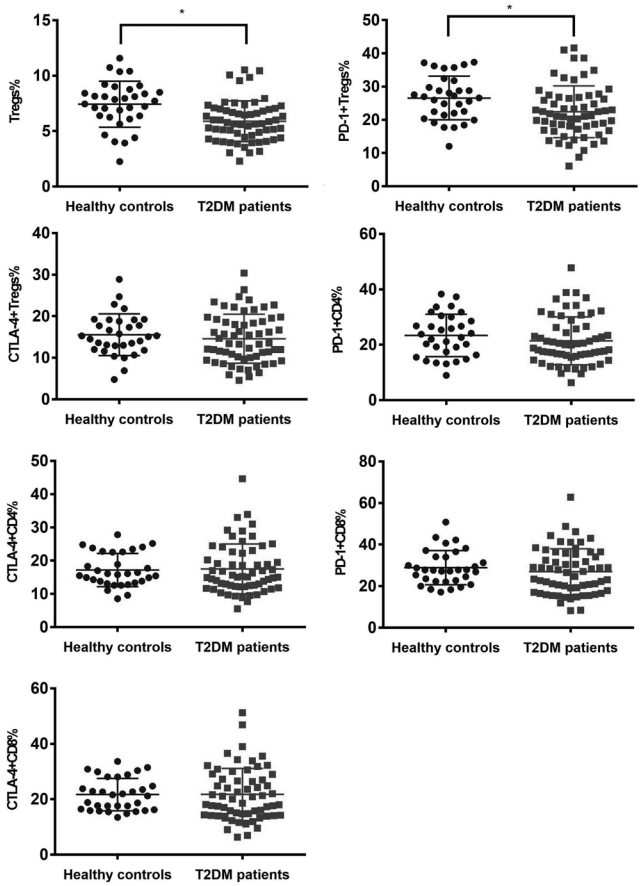

Figure 6
Different frequencies of T cells in the peripheral blood between patients with T2DM and healthy controls (*$P < 0.05$). T2DM, type 2 diabetes mellitus.

frequencies of MDSC subset cells in the peripheral blood and these metabolic indexes. Additionally, among patients who were taking metformin, a lower frequency of PD-L2$^+$ G-MDSCs was observed compared to those who were not taking metformin. However, no significant differences in the. frequencies of Tregs and MDSCs were observed between patients with different microvascular complications such as DKD, DR, and DPN and patients without these complications.

Discussion

The role of the immune system in type 2 diabetes has been increasingly recognized, as it affects insulin secretion, blood glucose levels, and chronic inflammation. Previous studies have shown imbalances in the ratios of Treg and Th17 cells and Treg and Th1 cells in patients with type 2 diabetes, indicating a skewed

Table 1 Frequencies (%) of peripheral blood T cell subsets in the peripheral blood between patients with type 2 diabetes and healthy controls.

T cell subsets	Healthy controls ($n = 38$)	Type 2 diabetes ($n = 61$)	*P*
Tregs	7.43 ± 2.07	5.89 ± 1.84	**<0.001**[a]
PD-1$^+$ Tregs	26.58 ± 6.57	22.44 ± 7.78	**0.012**[a]
CTLA-4$^+$ Tregs	15.56 ± 5.01	14.60 ± 5.88	0.435
PD-1$^+$ CD4	23.38 ± 7.64	21.45 ± 8.63	0.290
CTLA-4$^+$ CD4	17.19 ± 5.00	17.54 ± 7.49	0.815
PD-1$^+$ CD8	28.89 ± 8.19	26.95 ± 11.02	0.340
CTLA-4$^+$ CD8	21.70 ± 5.83	21.77 ± 9.43	0.967

[a]$P < 0.05$.

balance between anti-inflammatory and pro-inflammatory T cell subsets (13). In line with these findings, our study also revealed decreased frequencies of Tregs and PD-1$^+$ Tregs in patients with type 2 diabetes compared to healthy controls, suggesting a loss of T cell homeostasis that may contribute to tissue-specific and systemic inflammation as well as impaired glucose metabolism.

Elevated fasting insulin levels and waist circumference are indicators of insulin resistance,

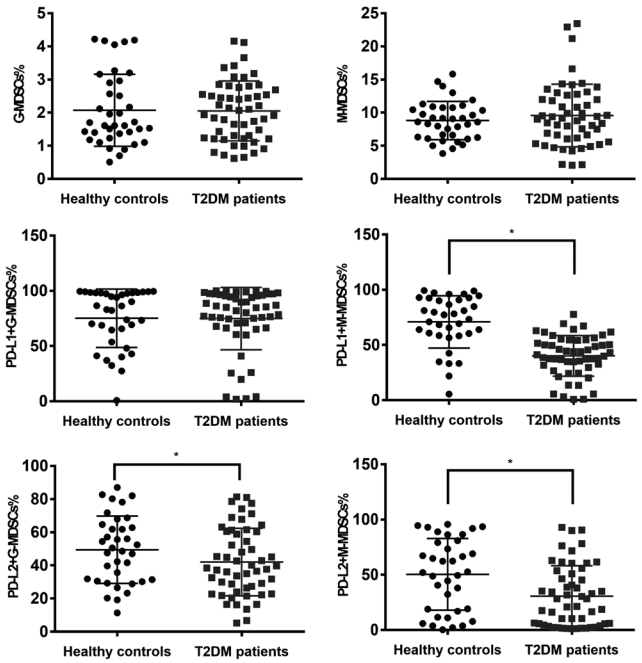

Figure 7
Different frequencies of G-MDSC and M-MDSC cells in the peripheral blood between patients with T2DM and healthy controls (*$P < 0.05$). G-MDSCs, granulocytic myeloid-derived suppressor cells; M-MDSCs, monocytic myeloid-derived suppressor cells; T2DM, type 2 diabetes mellitus.

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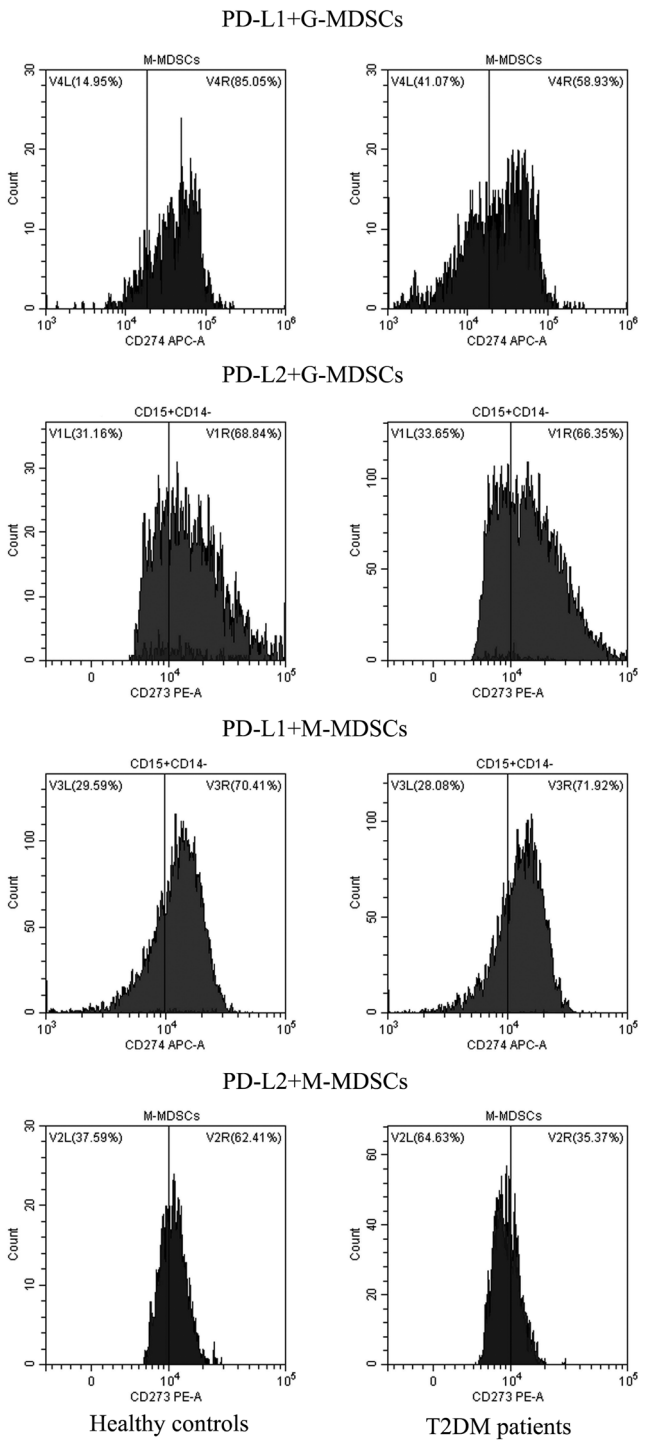

Figure 8

Flow cytometric analyses of G-MDSCs and M-MDSCs using anti-CD274 (PD-L1) and anti-CD273 (PD-L2) in the peripheral blood of patients with T2DM and healthy controls. G-MDSCs, granulocytic myeloid-derived suppressor cells. M-MDSCs, monocytic myeloid-derived suppressor cells; PD-L1, programmed cell death ligand 1; PD-L2, programmed cell death ligand 2; T2DM, type 2 diabetes mellitus.

which is a key factor in the development of type 2 diabetes. The negative association between the frequency of PD-1$^+$ Tregs and HbA1c level, fasting insulin level, and waist circumference in our study suggests that decreased PD-1$^+$ Tregs may be involved in insulin resistance and the progression of type 2 diabetes.

MDSCs have emerged as universal regulators of immune function in many pathologic conditions, by inhibiting activation of effector T cells and inducing the expansion of Tregs. However, the role of MDSCs in type 2 diabetes remains unclear, and conflicting findings have been reported. In murine models of autoimmune diabetes, MDSCs were able to prevent the destruction of pancreatic islets and postpone diabetes onset (18). In that study, cytokine-induced MDSCs (1×10^7 cells) were adoptively transferred into streptozotocin-induced mice. The adoptive transfer of cytokine-induced MDSCs into streptozotocin-induced mice normalized the glomerular filtration rate and decreased fibronectin production in the renal glomerulus, ameliorating renal fibrosis and thus providing a promising treatment for renal fibrosis and the prevention of diabetic nephropathy (19). However, Whitfield-Larry *et al.* observed an increased frequency of MDSCs in the peripheral blood of patients with type 1 diabetes, but these native MDSCs were not maximally suppressive compared to cytokine-induced MDSCs *in vitro*. Interestingly, the frequency of MDSCs was decreased within the pancreatic islets of mice with diabetes (20). In our study, we did not observe differences in the frequencies of M-MDSCs and G-MDSCs between patients with type 2 diabetes and healthy controls. But frequencies of PD-L1$^+$ G-MDSC, PD-L2$^+$ M-MDSC, and PD-L2$^+$ G-MDSC decreased in the peripheral blood of patients with type 2 diabetes, which might reflect a reduced ability of these immune cells to regulate inflammation and immune responses. These findings suggest that MDSCs may play a complex role in type 2 diabetes, which may vary depending on the stage of the disease, the microenvironment of the cells, and the tissue source of the samples obtained.

Obesity, which is associated with chronic inflammation, has been linked to increased numbers of M-MDSCs in the peripheral blood of obese subjects. Tissue-infiltrating MDSCs may play a crucial role in controlling obesity-associated inflammation (21). Treatment with metformin in type 2 diabetes with ovarian cancer has been reported to reduce the frequency of circulating MDSCs and enhance the antitumor activity of circulating CD8$^+$ T cells, concomitant with

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Table 2 Frequencies (%) of peripheral blood MDSC subsets in the peripheral blood between patients with type 2 diabetes and healthy controls.

MDSC subsets	Healthy controls ($n = 38$)	Type 2 diabetes ($n = 61$)	*P*
G-MDSCs	9.32 ± 4.91	9.23 ± 4.05	0.932
PD-L1$^+$ G-MDSCs	70.88 ± 23.66	40.16 ± 18.39	<0.001[a]
PD-L2$^+$ G-MDSCs	51.52 (30.90, 67.13)	42.02 ± 20.33	0.049[a]
M-MDSCs	8.81 ± 2.89	9.58 ± 4.71	0.341
PD-L1$^+$ M-MDSCs	75.17 ± 26.39	74.88 ± 28.16	0.961
PD-L2$^+$ M-MDSCs	50.46 ± 32.48	30.75 ± 27.42	0.003

[a]$P < 0.05$.

longer overall survival (22). In our study, we did not observe differences in the total frequencies of M-MDSCs and G-MDSCs based on whether patients with type 2 diabetes were overweight or taking metformin. However, patients taking metformin had a lower frequency of PD-L2$^+$ G-MDSCs.

The PD-1–PD-L1 axis has been implicated in T cell exhaustion and Treg expansion driven by MDSCs in the context of tumors and chronic obstructive pulmonary disease (23, 24). In this study, we found a positive relationship between PD-1$^+$ Tregs and PD-L2$^+$ M-MDSC, suggesting a potential role for the PD-1–PD-L2 axis in the development of type 2 diabetes. Downregulation of the PD-1–PD-L2 axis may contribute to the dysregulation of T cell homeostasis in patients with type 2 diabetes.

Our study contributes to the growing body of evidence implicating immune dysregulation in the pathogenesis of type 2 diabetes. The imbalances observed in T cell subsets and MDSC subsets may play a role in

Table 3 Relationship between clinical variables of patients with type 2 diabetes and the frequencies of Tregs and PD-1$^+$ Tregs in the peripheral blood (Pearson correlation analysis and independent-sample *t*-test.

Clinical variables		*R* (Tregs%)	*P*	*R* (PD-1$^+$ Tregs %)	*P*
Age (years)		−0.164	0.215	0.111	0.405
Duration (years)		0.157	0.235	0.054	0.687
BMI (kg/m^2)		−0.104	0.433	−0.116	0.380
Waist circumference (cm)		−0.015	0.915	−0.373	0.005[a]
HbA1c (%)		−0.152	0.249	−0.265	0.042[a]
Fasting insulin (mU/L)		0.057	0.669	−0.260	0.047[a]
Fasting glucose (mmol/L)		0.185	0.161	−0.153	0.248
HOMA-IR		−0.063	0.635	−0.209	0.112
Clinical variables	*n*	**Tregs** (%)	*P*	**PD-1$^+$ Tregs** (%)	*P*
Gender			0.820		0.050
Men	39	5.9 ± 1.83		21.03 ± 7.58	
Women	20	5.81 ± 1.97		25.28 ± 7.97	
Metformin			0.095		0.163
Yes	31	5.51 ± 1.51		23.84 ± 7.85	
No	28	6.32 ± 2.13		20.95 ± 7.83	
BMI (kg/m^2)			0.802		0.519
<24	16	5.83 ± 2.02		23.59 ± 7.54	
≥24	42	5.96 ± 1.82		22.11 ± 8.18	
Diabetic kidney disease			0.784		0.346
Yes	18	5.99 ± 1.77		20.99 ± 8.40	
No	41	5.85 ± 1.92		23.12 ± 7.70	
Diabetic retinopathy			0.753		0.300
Yes	9	6.07 ± 1.70		25.01 ± 9.88	
No	50	5.86 ± 1.91		22.01 ± 7.53	
Diabetic peripheral neuropathy			0.526		0.460
Yes	34	5.76 ± 1.80		21.81 ± 7.11	
No	25	6.07 ± 1.97		23.37 ± 8.95	

[a]$P < 0.05$.

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Table 4 Relationship between clinical variables of patients with type 2 diabetes and the frequencies of peripheral blood MDSC subsets.

Clinical variables		***R*** (PD-L1⁺ G-MDSCs%)	***P***	***R*** (PD-L2⁺ M-MDSCs%)	***P***	***R*** (PD-L2⁺ G-MDSCs%)	***P***
Age (years)		0.311	0.026	0.074	0.605	0.050	0.725
Duration (years)		0.119	0.405	−0.145	0.308	−0.003	0.983
BMI (kg/m^2)		−0.014	0.920	0.134	0.349	−0.151	0.290
Waist circumference (cm)		0.019	0.897	0.075	0.609	−0.135	0.354
HbA1c (%)		−0.048	0.740	0.001	0.993	−0.172	0.228
Fasting insulin (mU/L)		−0.201	0.157	−0.064	0.657	0.090	0.531
Fasting glucose (mmol/L)		−0.027	0.853	−0.031	0.832	−0.193	0.175
HOMA-IR		−0.067	0.639	0.040	0.781	−0.221	0.119
Clinical variables	*n*	**PD-L1⁺ G-MDSCs** (%)	*P*	**PD-L2⁺ M-MDSCs** (%)	*P*	**PD-L2⁺ G-MDSCs**(%)	*P*
Gender			0.930		0.797		0.041[a]
Men	34	40.36 ± 20.16		30.03 ± 27.66		45.87 ± 19.46	
Women	17	40.85 ± 15.63		32.18 ± 28.46		33.39 ± 21.01	0.035[a]
Metformin			0.446		0.079		
Yes	27	38.62 ± 21.23		32.93 (6.01, 61.57)		35.90 ± 16.88	
No	24	42.66 ± 15.32		13.46 (2.17, 46.11)		48.25 ± 22.82	0.546
BMI (kg/m^2)			0.930		0.403		
<24	14	41.00 ± 18.54		25.30 ± 23.61		45.05 ± 21.39	
≥24	36	40.48 ± 19.14		32.74 ± 29.49		40.96 ± 20.57	0.454
Diabetic kidney disease			0.711		0.453		
Yes	16	43.50 ± 16.18		15.59 (2.34, 55.37)		44.95 ± 17.53	
No	35	39.16 ± 19.70		28.35 (6.01, 46.40)		40.23 ± 22.01	0.484
Diabetic retinopathy			0.585		0.199		
Yes	8	43.87 ± 10.99		48.03 ± 40.10		36.95 ± 23.97	
No	43	39.90 ± 19.74		27.53 ± 23.99		42.59 ± 20.17	0.327
Diabetic peripheral neuropathy			0.172		0.746		
Yes	29	43.64 ± 17.11		29.64 ± 27.05		39.22 ± 19.91	
No	22	36.41 ± 20.09		32.21 ± 29.03		44.99 ± 21.62	

[a]$P < 0.05$.

insulin resistance, chronic inflammation, and disease progression in type 2 diabetes. It is important to note several limitations of our study. First, while we observed lower frequencies of PD-L1⁺ G-MDSCs, PD-L2⁺ M-MDSCs, PD-L2⁺ G-MDSCs, and PD-1⁺ Tregs in the peripheral blood of patients with type 2 diabetes compared to healthy controls, the underlying mechanisms by which MDSCs and Tregs contribute to type 2 diabetes need further investigation. Secondly, our sample size was small, and larger studies are needed to confirm our results. Additionally, it would be valuable to analyze the frequencies of T cell subsets and MDSC subsets in various locations, such as pancreas tissues, lymphoid tissues, and adipose tissues, to gain a comprehensive understanding of their correlations in the context of type 2 diabetes. Future research should involve clinical studies combined with cell and animal models to elucidate the interaction between MDSCs and Tregs mediated by the PD-1–PD-L2 axis in patients with type 2 diabetes. Furthermore, the potential differential functions of MDSCs in patients with type 1 diabetes and type 2 diabetes, as well as the relationship between obesity, metformin use, and MDSCs, warrant further investigation.

Conclusion

Our study revealed decreased frequencies of PD-L1⁺ G-MDSCs, PD-L2⁺ M-MDSCs, PD-L2⁺ G-MDSC, and PD-1⁺ Tregs in patients with type 2 diabetes compared to healthy controls. We observed a positive correlation between PD-1⁺ Tregs and PD-L2⁺ M-MDSCs and a negative association between PD-1⁺ Tregs and HbA1c, fasting insulin level, and waist circumference. These findings suggest that the decreased frequencies of PD-L2⁺M-MDSCs and PD-1⁺Tregs may contribute to the activation of effector T cells and the development of chronic low-grade inflammation in type 2 diabetes.

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Our results indicate that MDSCs and Tregs play a role in the immunopathogenesis of type 2 diabetes and could be potential targets for novel therapeutic approaches. However, further studies are needed to fully understand the specific functions of MDSCs and Tregs in the context of type 2 diabetes.

Declaration of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Data availability

All datasets analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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