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Research Paper

Increase in Peripheral Blood Intermediate Monocytes is Associated with the Development of Recent-Onset Type 1 Diabetes Mellitus in Children

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Abstract

Monocytes play important roles in antigen presentation and cytokine production to achieve a proper immune response, and are therefore largely implicated in the development and progression of autoimmune diseases. The aim of this study was to analyze the change in the intermediate (CD14+CD16+) monocyte subset in children with recent-onset type 1 diabetes mellitus (T1DM) and its possible association with clinical parameters reflecting islet β-cell dysfunction. Compared with age- and sex-matched healthy controls, intermediate monocytes were expanded in children with T1DM, which was positively associated with hemoglobin A1C and negatively associated with serum insulin and C-peptide. Interestingly, the intermediate monocytes in T1DM patients expressed higher levels of human leukocyte antigen-DR and CD86, suggesting better antigen presentation capability. Further analysis revealed that the frequency of CD45RO+CD4+ memory T cells was increased in the T1DM patients, and the memory T cell content was well correlated with the increase in intermediate monocytes. These results suggest that expanded intermediate monocytes are a predictive factor for the poor residual islet β−cell function in children with recent-onset T1DM.

Key words: Children; Type 1 Diabetes Mellitus; Intermediate Monocytes; Islet Beta Cell Function; Memory T cells.

Introduction

Type 1 diabetes mellitus (T1DM) is an autoimmune disease in which the function of insulin-secreting pancreatic β-cells is impaired due to autoreactive immune cell-mediated destruction (insulitis). T1DM is characterized by dysregulation of blood glucose caused by β-cell insufficiency accompanied by increased hemoglobin A1C (HbA1c) [1, 2]. Several studies suggest that the development of T1DM is strongly associated with different immune cell subsets, including monocytes [3-5]. Specifically, an increase in the monocyte population has been shown to trigger β-cell destruction during insulitis [6].

Human peripheral monocytes act as antigen-presenting cells (APCs) to activate T cells during inflammatory conditions [7, 8], and to secrete cytokines shaping T-cell differentiation [9]. Monocytes are comprised of heterogeneous subgroups that can be classified as classical

(CD14++CD16–, 85%), intermediate (CD14+CD16+, about 5%), and non-classical (CD14dimCD16++, about 10%) based on the differential expression levels of CD14 (a lipopolysaccharide [LPS] receptor) and CD16 (FcγRIII) [10-12]. Increasing evidence has shown that intermediate monocytes exert an antigen-presenting function with a dendritic cell-like feature [13]. It has been documented that intermediate monocytes exhibit enriched expression in antigen-presenting-related factors such as major histocompatibility complex class II (MHC-II) subunits, CD74 (class II invariant chain), human leukocyte antigen (HLA)-DO, as well as CD40 [14]. Moreover, intermediate monocytes mediate inflammation and the pathogenesis of infections [15].

Intermediate monocytes have been shown to expand in many inflammatory and autoimmune conditions, including chronic kidney disease, active rheumatoid arthritis, coronary artery disease, and type 2 diabetes [16-19]. Significantly increased intermediate monocytes in patients with juvenile-onset T1DM were demonstrated to produce more tumor necrosis factor-alpha (TNF-α), an effective inflammatory factor [6, 10]. In fact, upon antigen stimulation, intermediate monocytes become the main producers of inflammatory factors, including interleukin (IL)-1α, IL-6, and TNF-α [20], and TNF-α has been shown to correlate with the severity of T1DM [6, 21-23].

The monocytes of diabetic patients are able to induce CD4+ T cells to produce proinflammatory cytokines such as interferon-gamma (IFN-γ), TNF-α, and IL-17 [24]. Monocytes were also found to promote the expansion of memory T cells in patients with human immunodeficiency virus (HIV) [25]. Respiratory monocytes enhance the responses of CD4+ memory T cells to mucosal immunization with recombinant adenovirus-based vaccines [26]. However, the action of the intermediate monocytes in children with recent-onset T1DM, and the consequences for memory T cell responses have not been fully investigated.

Here, we aimed to investigate the changes and functions of intermediate monocytes and to analyze the possible association of the change in the intermediate monocyte subset with clinical parameters reflecting islet β-cell insufficiency in children with recent-onset T1DM. The increase in this subset demonstrated a good correlation with worse residual islet β–cells function. We also found that children with recent-onset T1DM that had a higher intermediate monocyte population had a greater number of memory T cells, which secreted high levels of IL-2 and IFN-γ. Collectively, these results suggest that expansion of intermediate monocytes is a poor prognostic factor for the progress of T1DM in children.

Material and methods

Subjects

A total of 54 patients (30 boys, 24 girls; mean age 7.3 ± 3.9 years) with recent-onset T1DM were enrolled in this study between January 2015 and July 2016 from Beijing Children's Hospital. Patients with micro-vascular complications, and coexisting autoimmune, chronic, and acute inflammatory diseases were excluded from the study. The clinical characteristics of the patients are presented in Table 1. Forty-nine age- and sex-matched healthy children (26 boys, 23 girls; mean age 6.1 ± 3.9 years) were included as a healthy control (HC) group. Thirty-nine age- and sex-matched patients with Graves' disease (19 boys, 20 girls; mean age 7.6 ± 3.6 years) were included as a non-T1DM autoimmune control group. The study was approved by the ethics committee of Beijing Children's Hospital, which acts in compliance with ethical standards defined by the Declaration of Helsinki. Written consent for research purposes was provided by all participants and their parents or legal guardians.

Table 1. Clinical characteristic of patients with type 1 diabetes.

Clinical parameter	Mean ± standard deviation	range of reference value
Age (years)	7.3±3.9	
Duration of diabetes (days)	19.5±9.1	
HbA1c (%)	11.8±2.5	4.0-6.0
Insulin (IU/ml)	3.2±2.3	6.0-27.0
C-peptide (ng/ml)	0.9±0.7	1.1-5.0

Sample collection

Serum and peripheral blood mononuclear cells (PBMCs) were isolated from ethylenediaminetetraacetic acid-treated blood samples collected from all participants. The PBMCs were separated by standard Ficoll–Hypaque density centrifugation at 1000 rpm for 20 min.

Blood measurements

The serum HbA1c level was determined by high-performance liquid chromatography using the Ark-8150 system (Arkray Inc. Kyoto, Japan). Fasting insulin and C-peptide levels were analyzed via immune chemiluminometric assays (Roche cobas E601 analyzer, Roche Diagnostics, USA). The intra- and inter-assay coefficients of variation were <5% and <10%, respectively.

Surface phenotypic marker expression

The absolute counts of total lymphocytes, monocytes, and neutrophils in the peripheral blood of

all subjects were measured by flow cytometry with a Guava easyCyte 8 system (Merck Millipore, USA). Cell-surface monocyte phenotypic analysis was performed after staining with human anti-CD14 [1] and anti-CD16 [1]. Fluorochrome-conjugated monoclonal antibodies allophycocyanin (APC)-CD40, phycoerythrin (PE)-CD86, APC-HLA-DR, PE-CD11b, APC-CD11c, APC-CD62L, PE-CD68, PE-CD4, peridinin chlorophyll protein complex (PerCP)-CD3, PE-CD45RO, APC-CCR7, fluorescein isothiocyanate (FITC)-CD40L, and PE-CD163 were purchased from BD Biosciences Pharmingen (Franklin Lanes, NJ, USA).

Intracellular staining

Isolated monocytes (10^5) and PBMCs were incubated with 100 ng/ml LPS (Sigma Aldrich), 5 ng/ml IL-2 (Cell Signaling Technologies), 50 ng/ml phorbol-12-myristate-13-acetate (PMA, Merck), and 1 μg/ml ionomycin (Sigma Aldrich) for 37°C, 5 h, respectively; and GolgiStop (BD Biosciences) was added for the final 3 h. Monocytes were stained with FITC-CD14 and PerCP-CD16 (Biolegend), and PBMCs were stained with FITC-CD45RO, PE-CD4, PerCP-CD3, and APC-CCR7 for 30 min. Cells were fixed and permeabilized using the protocol from the Cytofix/Cytoperm kit (BD Biosciences). Then, the monocytes were stained with anti-TNF-α (Biolegend) and anti-IL-6 (Biolegend), and the PBMCs were stained with anti-IFN-γ (BD Biosciences) and anti-IL-2 (BD Biosciences) for 30 min.

Expression of cell-surface markers was assessed using flow cytometry (FACSCalibur, BD, USA) after gating of live cells, and analyzed according to scatter characteristics using FlowJo software.

Statistical analysis

Pairwise comparisons of mean lymphocyte and monocyte cell counts among the HC, Graves, and diabetic groups were performed with the Student's *t*-test. Spearman's correlations were used to compare the associations among intermediate monocyte subsets, clinical parameters, and memory T cells in the T1DM group. The level of significance was set at $p < 0.05$. Significant differences were calculated with GraphPad Prism 4.0 using an unpaired two-tailed t-test.

Results

The increase of intermediate monocytes is specific to T1DM

The intermediate monocytes have been shown to contribute to the pathogenesis of pediatric T1DM, a typical autoimmune and inflammatory disease [6, 24]. In the present study, we sought to determine the phenotype and function of intermediate monocytes in children with recent-onset T1DM.

Our results showed that the percentage of total monocytes was increased in the children with T1DM compared with the age- and sex-matched HC and Graves' disease groups (Fig. 1A). The absolute count of monocytes in the children with T1DM was significantly higher than that in the HC group and Graves patients. However, there were no differences in the populations of lymphocytes and neutrophils among the T1DM, Graves, and HC groups. The absolute counts of total lymphocytes and neutrophils in the peripheral blood of T1DM patients showed no differences from those of the HC group.

When monocyte subsets were further examined, the proportion of each subset relative to total monocytes was also found to vary in the T1DM group (Fig. 1B). Compared with the age- and sex-matched HC group and patients with Graves, the ratio of intermediate monocytes was specifically increased in the T1DM children (Fig. 1C). Absolute cell counting also revealed a specific increase in the intermediate monocytes of children with T1DM (Table 2).

Relationship between intermediate monocytes and the remnant islet β–cell function in T1DM children

A decrease in serum insulin concentration and C-peptide is a typical pathophysiological change recognized in T1DM patients, and has been commonly used for evaluating the remnant islet β–cell function. HbA1c is a recognized standard clinical marker of glycemic control, and is significantly increased in T1DM. These clinical indices are therefore currently used to assess the condition of T1DM patients. Recent studies have also shown that the expansion of intermediate monocytes could trigger the destruction process of β-cell [6, 10]. Therefore, it is important to determine the relationship between the intermediate monocytes and clinical indices attributed to pancreas islet functions.

Table 2. Absolute counting of different types of cells.

Absolute number (cells/mL)	Lymphocytes ($\times10^6$)	Monocytes ($\times10^5$)	Neutrophils ($\times10^6$)	Memory T cells ($\times10^5$)	CD14++CD16- ($\times10^5$)	CD14+CD16+ ($\times10^4$)	CD14dimCD16++ ($\times10^4$)
HC	1.43±0.78	2.10±1.20	1.14±0.93	2.64±2.00	1.27±0.79	2.38±1.51	0.74±0.52
Graves	1.58±1.02	1.57±0.97	1.73±1.60	2.90±2.40	0.69±0.41	1.82±1.10	0.57±0.45
T1DM	1.77±0.83	2.91±1.77	1.38±0.82	4.88±2.97	1.40±1.03	6.87±6.30	1.24±0.99

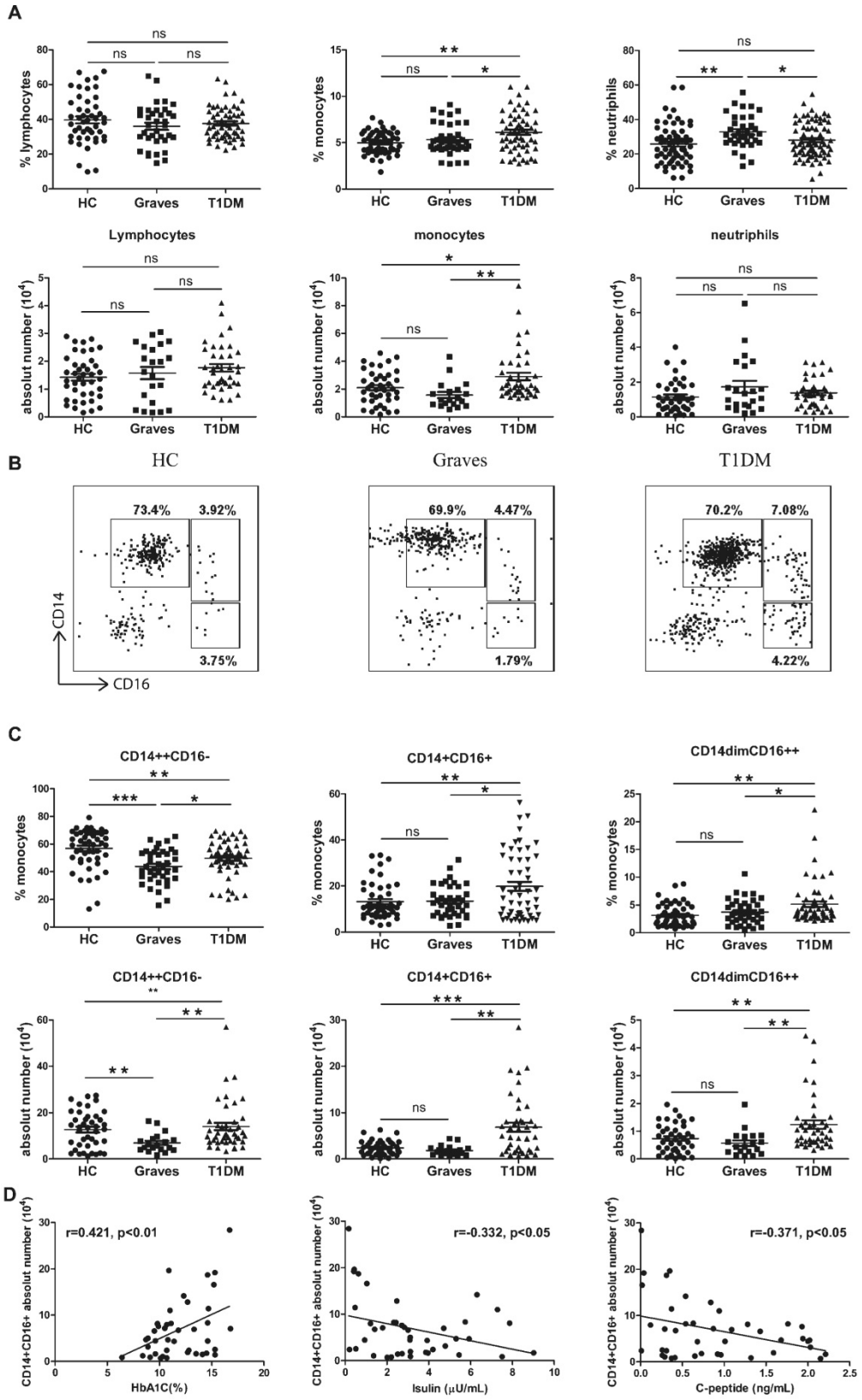

Figure 1. (A) The percentages of different cell types in the blood. (A) Left panel: the proportion (%) of total lymphocytes. Middle panel: the proportion (%) of total monocytes. Right panel: the proportion (%) of total neutrophils. (B) The whole blood samples were stained with antibodies against CD14 and CD16 molecules to identify the monocyte subsets as follows: CD14++CD16– (classical monocytes), CD14+CD16+ (intermediate monocytes), and CD14dimCD16++ (non-classical monocytes). (C) The percentages of the three monocyte subsets among blood monocytes. Left panel: the proportion (%) of CD14++CD16– cells. Middle panel: the proportion (%) of CD14+CD16+ cells. Right panel: the proportion (%) of CD14dimCD16++ cells. (D) Left panel: correlation of the HbA1c level with the absolute number of CD14+CD16+ monocytes ($r = 0.421$, $p < 0.01$). Middle panel: correlation of the insulin level with the absolute number of CD14+CD16+ monocytes ($r = –0.332$, $p < 0.05$). Right panel: correlation of the C-peptide level with the absolute number of CD14+CD16+ monocytes ($r = –0.371$, $p < 0.05$). Each dot represents a different individual; ns, $p > 0.05$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

All of the T1DM patients recruited in this study showed a dramatic increase in HbA1c (11.8% ± 2.5, reference value 4.0–6.0%), and a significant decrease in both the serum insulin concentration (3.2 ± 2.3 IU/ml, reference value 6.0–27 IU/ml) and serum C-peptide level (0.9 ± 0.7 ng/ml, reference value 1.1–5.0 ng/ml) (Table 1). Correlation analysis revealed that T1DM patients with higher HbA1c levels had greater numbers of peripheral blood intermediate monocytes (Fig. 1D, left panel). Moreover, an inverse correlation was observed between the absolute number of intermediate monocytes and the concentration of insulin and C-peptide, respectively, in T1DM children (Fig. 1D, middle and right panels). We also analyzed the correlation in HC, and found there is no correlation between intermediate and the islet β-cell function (Supplementary Figure 2A, 2B, 2C). The correlation between intermediate and the remnant islet β−cell function is specific in T1DM. These results imply that the enrichment of intermediate monocytes in T1DM has a destructive effect on the function of residual islet β-cells. Therefore, we next examined the phenotype and function of APCs in the intermediate monocyte subset in children with T1DM.

Phenotype of the intermediate monocytes in children with T1DM

To further characterize the expression of cell-surface markers related to inflammatory chemotaxis, and activation and phagocytosis of the CD14+CD16+ monocyte subset, we investigated the expression of CD11b, CD11c, CD68, CD62L, and CD163 monocytes from the T1DM patients, Graves patients, and HC group.

The integrin Mac-1 (CD11b) is one of the most well-studied leukocyte adhesion molecules mediating leukocyte adhesion and migration to regulate the inflammatory response [27]. We found that the intermediate monocyte population from the T1DM patients showed higher CD11b expression compared to those of the Graves and HC groups (Fig. 2A). CD62L is an adhesion molecule that is present on the surface of inflammatory monocytes, and its expression has been linked to the capacity of monocytes to preferentially migrate to the sites of inflammation [28]. The percentage of CD62L on intermediate monocytes was significantly higher in the T1DM group (Fig. 2C). Overall, these data showed the enhancement of monocyte inflammatory chemotaxis in children with T1DM.

To determine the changes in the activation of intermediate monocytes, we analyzed CD11c, a marker of activated monocytes/macrophages [27]. As expected, the expression of CD11c was significantly higher in the T1DM patients compared with that in the Graves and HC group, respectively (Fig. 2B).

To investigate possible differences in the phagocytosis of intermediate monocytes, we analyzed the expression of CD68 and CD163, a marker of tissue macrophages and a member of the scavenger receptor cysteine-rich family of proteins, respectively [29]. Specifically, intermediate monocytes from the T1DM patients showed higher surface levels of CD68 and CD163 than those derived from the Graves patients or HC group (Fig. 2D and E).

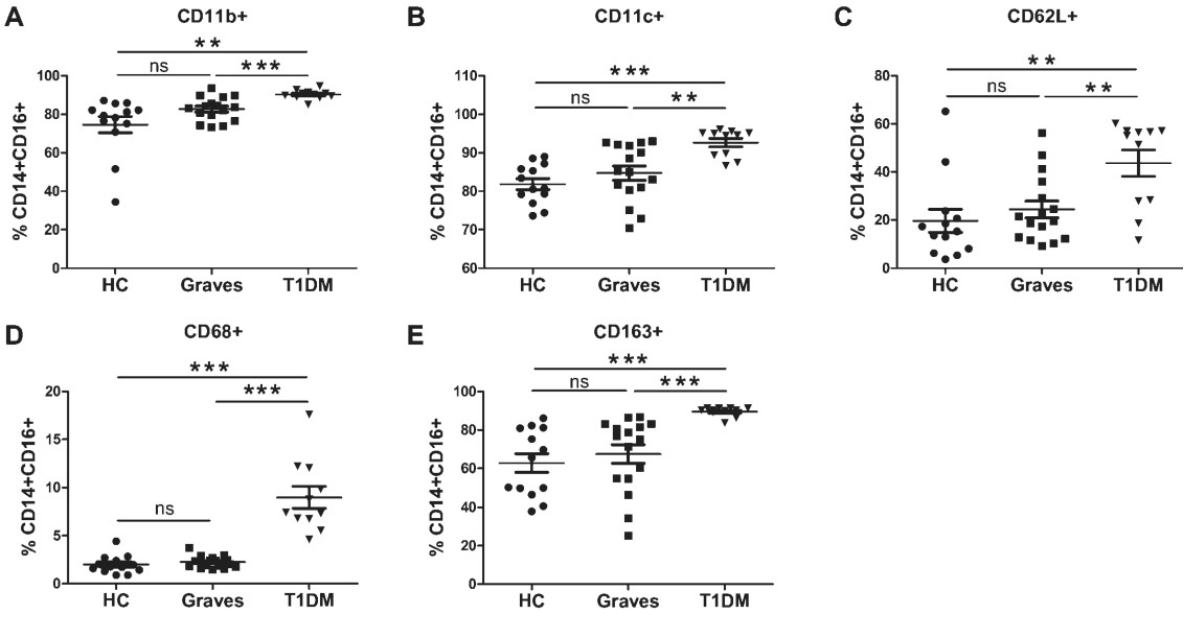

Figure 2. The phenotype of CD14+CD16+ monocytes in the healthy controls (HC), patients with Graves' disease, and children with type 1 diabetes mellitus (T1DM). Expression of the surface markers (A) CD11b, (B) CD11c, (C) CD62L, (D) CD68, and (E) CD163 on monocytes. Each dot represents a different individual; ns, $p > 0.05$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Antigen-presenting and proinflammatory function of the intermediate monocytes in children with T1DM

Because the changes of intermediate phenotypes were associated with the function of intermediate monocytes, we evaluated changes in the antigen-presenting function of this cell subset. The intermediate monocytes express high levels of MHC class II processing and presentation genes, and have been linked to antigen presentation in rheumatoid arthritis [30]. Therefore, we assessed the levels of molecules related to antigen presentation and co-stimulation on the intermediate monocytes of the children with T1DM.

HLA-DR expression suggests the antigen processing and presentation capability of the expanded subset [31]. The intermediate monocytes in T1DM patients were found to have significantly increased HLA-DR expression compared with those of the patients with Graves or the HC group (Fig. 3A). CD86 molecules are up-regulated in resting monocytes after induction by the CD40-CD40L interaction [32]. Therefore, we assessed the levels of molecules related to such co-stimulation (CD86) in the intermediate monocytes. The intermediate subset from patients with T1DM was also found to express significantly higher level of CD86 than those of the patients with Graves or the HC group, indicating the high antigen-presenting capability of this subset in T1DM (Fig. 3B).

LPS priming could prevent diminished T-cell IFN-γ production but had little effect on T-cell proliferation. Cros et al. [33] found that isolated human intermediate subset cells treated with LPS produced higher levels of TNF-α, IL-1β, and IL-6. Similarly, in our study, we detected strikingly increased production of TNF-α by the circulating intermediate monocytes from the T1DM subjects (Fig. 3C), suggesting a systemic alteration in the function of a subset of monocytes/macrophages. Intermediate monocytes derived from the recent-onset T1DM subjects were found to have specifically increased numbers of TNF-α–secreting cells compared with those derived from patients with Graves or the HC group. Interestingly, we also observed an increased number of IL-6-secreting cells in the intermediate monocytes from patients with T1DM compared with those of patients with Graves or HC (Fig. 3D).

Relationship between monocytes and memory T cells in children with T1DM

Monocytes may promote the expansion of memory T cells in patients with HIV [25]. Furthermore, respiratory monocytes enhance the responses of CD4+ memory T cells to mucosal immunization with recombinant adenovirus-based vaccines [26]. Therefore, we tested whether memory T cells were increased in T1DM. As shown in Fig. 4A and 4B, the percentage and absolute count of memory T cells were higher in the T1DM group than in the Graves and HC groups. We further investigated the relationship between the absolute numbers of intermediate and memory T cells. As shown in Fig. 4C, T1DM patients with higher numbers of intermediate cells also had a greater number of memory T cells. Our data demonstrated that there is no correlation between classical (or non-classical) and memory T cells in T1DM (Supplementary Figure 1B, 1C). This phenomenon is specific exist in intermediate and memory T cells. And we also analyzed the correlation between intermediate and memory T cells in HC and Graves (Supplementary Figure 2D, Supplementary Figure 3E), and found this correlation exists only in T1DM.

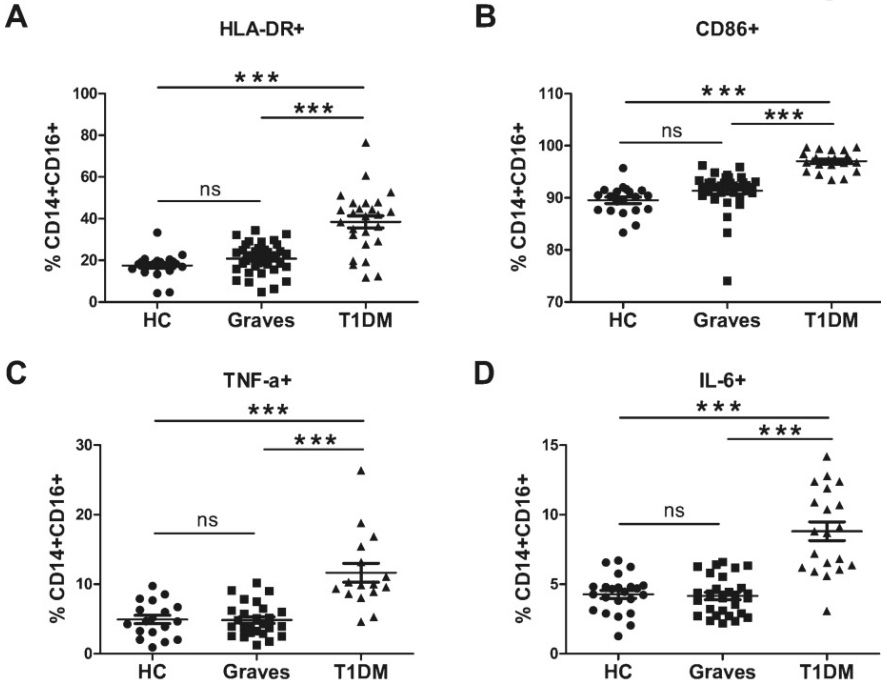

Figure 3. The antigen-presenting function of CD14+CD16+ monocytes. (A) Expression of the surface markers (A) HLA-DR and (B) CD86 on monocytes. Levels of the cytokines (C) TNF-α and (D) IL-6 secreted by CD14+CD16+ monocytes. Each dot represents a different individual; ns, $p > 0.05$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Furthermore, CD40 expressed on monocytes provides the co-stimulatory signals necessary for T cell activation and survival [34]. CD40L is a cell-surface interaction molecule that is expressed by T cells, and its interaction with CD40 plays a key role in the adaptive immune response [35]. Our data demonstrated that intermediate monocytes of patients with T1DM expressed higher levels of CD40 than those of patients with Graves or the HC group (Fig. 4D). Moreover, compared with the HC or Graves patients, a significantly higher percentage of memory T cells from children with T1DM exhibited CD40L expression (Fig. 4E). Our data showed that T1DM patients with higher percentage of CD40+ in intermediate cells also had a greater percentage of CD40L+ in memory T cells. This correlation remains unchanged when absolute number of cells was considered (Fig. 4F and 4G). This relationship does not exist in HC or Graves (Supplementary Figure 3A, 3B, 3C, 3D).

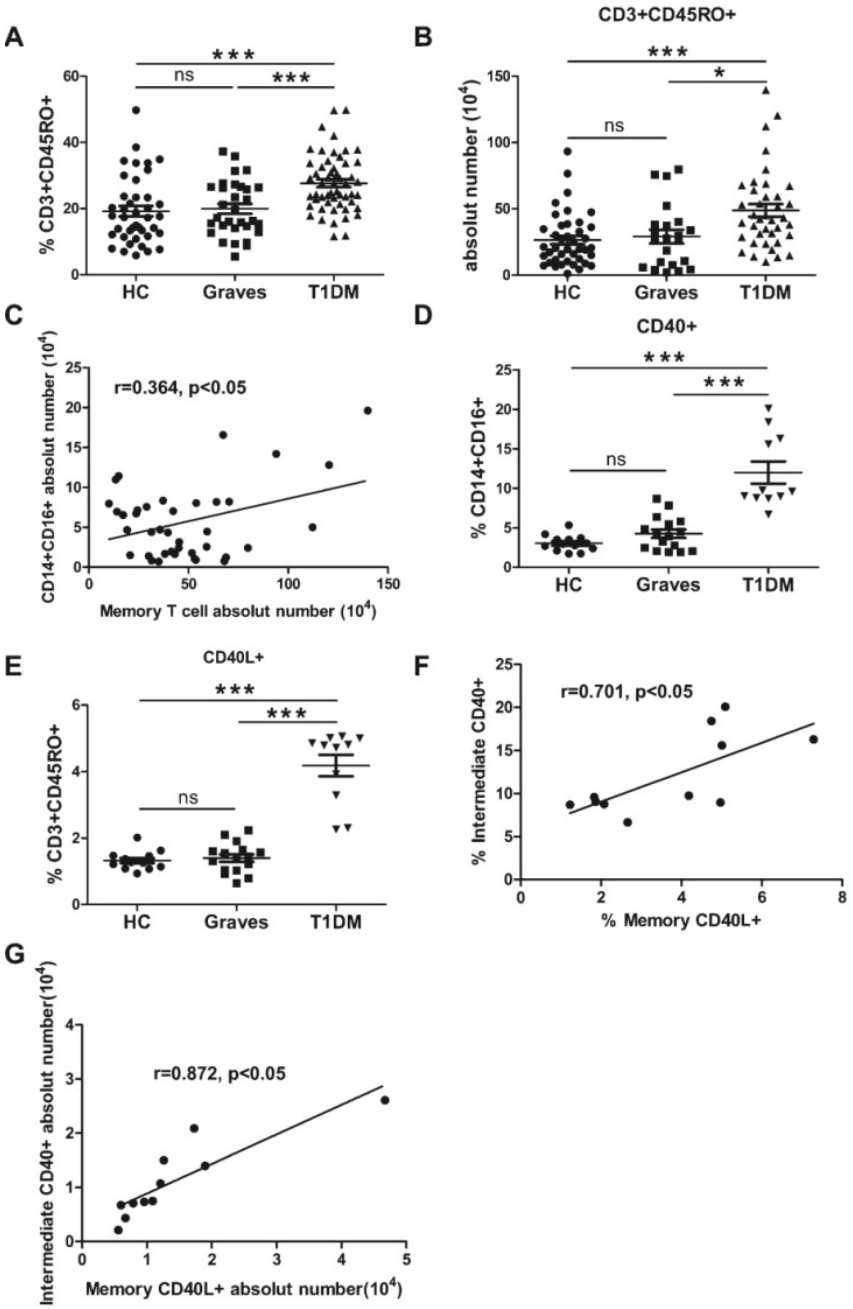

Figure 4. (A) Proportion (%) of memory T cells among blood lymphocytes. (B) Absolute number of memory T cells. (C) The correlation of the absolute number of memory T cells with the absolute number of CD14+CD16+ monocytes (r = 0.364, p < 0.05). (D) Expression of CD40 in CD14+CD16+ monocytes. (E) Expression of CD40L in memory T cells. Each dot represents a different individual; ns, p > 0.05; (F) The correlation of the percentage of CD40L+ memory T cells with the percentage of CD40+CD14+CD16+ monocytes (r = 0.701, p < 0.05); (G) The correlation of the absolute number of CD40L+ memory T cells with the absolute number of CD40+CD14+CD16+ monocytes (r = 0.872, p < 0.05); *, p <0.05; **, p < 0.01; ***, p < 0.001.

Effector and central memory T cells in children with T1DM

To specifically evaluate which type of memory T cells increased in T1DM, we used the expression of CCR7 to separate out the memory T cells. We demonstrated that the proportion of CCR7+CD45RO+ central memory CD4+ T cells from patients with T1DM was increased as compared to that of patients with Graves and the HC, respectively (Fig. 5A). Central memory T cells secrete the proinflammatory cytokine IL-2. IL-2 secretion analysis showed that the central memory T cells from patients with T1DM expressed higher levels of IL-2 (Fig. 5C). Furthermore, a higher frequency of CD4+ T cells from patients with T1DM presented a CCR7-CD45RO+ effector memory phenotype than those of patients with Graves and the HC (Fig. 5B). Moreover, the effector memory cells from patients with T1DM expressed the major effector cytokine IFN-γ (Fig. 5D).

Discussion

Through investigation of 54 recent-onset T1DM children aged from 1 to 11 years, we found that intermediate monocytes were increased compared with the healthy cohort. This finding is in line with previous reports showing an expansion of the intermediate monocyte population and a reduction of classical monocytes in juvenile T1DM patients whose disease duration was 5 years [6]. This

is also consistent with data showing that the CD16+ subset of monocytes is expanded in some autoimmune diseases and may be involved in the induction of the inflammatory immune response [36-38].

Recent gene profiling analyses suggest that the intermediate subset plays a critical role in antigen presentation [39, 40]. A series of studies showed that the antigen presentation ability is tightly associated with HLA-DR and CD86 expression, suggesting an important role of HLA-DR and CD86 in the antigen presentation process [31, 32]. We also demonstrated that the expression levels of CD86 and HLA-DR were increased in the intermediate monocytes of T1DM patients. Monocytes are producers of proinflammatory cytokines, including TNF-α, IL-1, IL-6, and IL-12 [33, 39, 41, 42]. In our study, the intermediate monocytes of children with T1DM were found to secrete higher levels of IL-6 and TNF-α than those of the HC group. Previous studies have shown that a higher HbA1c level was associated with higher production of TNF-α [43], and that monocytes mediated the endothelial damage in chronic kidney disease patients by secreting cytokines [44]. In our study, an increased level of HbA1c correlated well with the increased absolute number of the intermediate subset. The levels of insulin and C-peptide are reliable clinical indicators for the competent function of β–cell [45]. Our data demonstrated that the absolute number of intermediate monocytes was negatively correlated with the concentrations of C-peptide and insulin. Collectively, these results suggest that expanded intermediate monocytes might play a detrimental role for β–cell function in children with T1DM.

It is possible that the expansion of intermediate monocytes would result in the release of more proinflammatory cytokines, which would eventually lead to a detrimental effect on β–cells. It has been documented that continuous autocrine TNF-α stimulation of CD14+ monocytes drives the cells to differentiate into dendritic cells, which in turn incessantly produce TNF-α and other inflammatory factors [6]. Studies have also shown that intermediate monocytes are able to induce $CD4^+$ T cells to produce IFN-γ, TNF-α, and IL-17 in other autoimmune diseases [6, 46].

Based on our results and previous reports, it seems likely that intermediate CD14+CD16+ monocytes, which produce abundant TNF-α, may play an important role in promoting the inflammatory response in T1DM [10]. Moreover, some authors have demonstrated that TNF-α provides a unique vantage point from which to characterize the cellular niches maintaining memory T cells [47]. Therefore, these correlations suggest that intermediate monocytes and memory T cells have a relationship to a certain degree. Given this background, we evaluated the correlation of the absolute numbers of memory T cells and intermediate monocytes in patients with T1DM, and also found a strong positive association between the two subsets in the peripheral blood. We further found that the percentage of proinflammatory memory T cells was expanded in children with T1DM. Moreover, the CD40-CD40L interaction has been proposed to play an essential role in mediating the interaction between T cells and APCs [48, 49]. We also found that CD40 and CD40L were increased in the intermediate monocytes and memory T cells of children

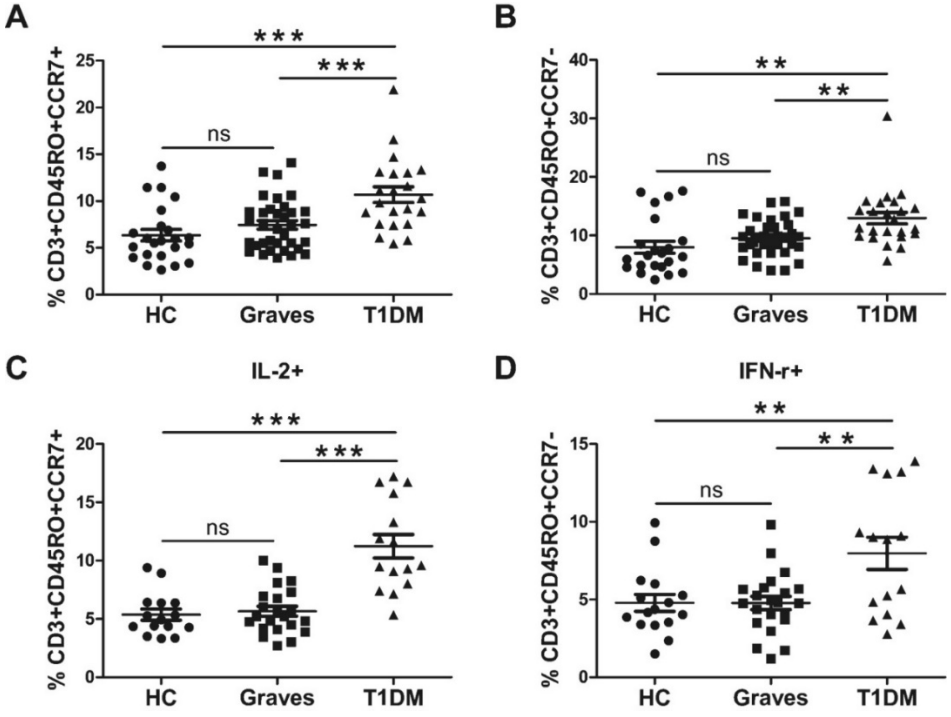

Figure 5. (A) Proportion (%) of central memory T cells. (B) Proportion (%) of effector memory T cells. (C) Level of IL-2 secreted by central memory T cells. (D) Level of IFN-γ secreted by effector memory T cells. Each dot represents a different individual; ns, $p > 0.05$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

with T1DM, respectively. And both of them were positively correlated. Our data demonstrated that the correlation between intermediate and memory T cells is specific in T1DM. Moreover, these cells secreted high levels of IL-2 and IFN-γ. Thus, our hypothesis is that intermediate monocytes can promote the progress of T1DM in children and may be an indicator of pre-clinical T1DM.

In conclusion, the intermediate monocyte population was found to be expanded in pediatric patients with T1DM. As these cells were shown to have proinflammatory activity, they are likely to be implicated in the impaired function of β–cells, with deleterious consequences for the development of T1DM. Further characterization of the function of intermediate monocytes could pave the road for improving understanding of the relationships between these proinflammatory cells and the destruction of β–cells in children with T1DM.

Supplementary Material

Supplementary figures.
http://www.ijbs.com/v13p0209s1.pdf

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Author Contributions

Conceived and designed the experiments: JG, CG, XN; performed the experiments: XR, WM, CS, XC, HZ, BC, XL; analyzed the data: XR, WM; contributed reagents/materials/analysis tools: JG, XN, CG, and DW; wrote the paper: XR, WM, JG, CG, CS.

Competing Interests

The authors have declared that no competing interest exists.

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