<Original Article>

High Monocyte CD300e Expression in Patients with Acute Onset Type 1 Diabetes

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ABSTRACT

Type 1 diabetes (T1D) is characterized by immune-related pancreatic β-cell destruction. The CD300e antibody level transiently increases during the acute phase of fulminant T1D (FT1D). Here, we investigated the contribution of CD300e to T1D development. Monocytes were obtained from 20 patients with FT1D, 25 with acute onset T1D (AT1D), 14 with type 2 diabetes (T2D), and 17 healthy controls (HCs). Additionally, peripheral blood mononuclear cells (PBMCs) were obtained from 8 patients with FT1D, 12 with AT1D, 11 with T2D, and 9 HCs. Using flow cytometry, we analyzed CD300e expression in monocytes and myeloid dendritic cells in PBMCs. Tumor necrosis factor (TNF)-α was measured after the stimulation of monocytes with 3.3 ng/μL sphingomyelin (SM), a CD300e ligand. The percentage of CD300e-positive cells in the monocytes of patients with AT1D was higher than that in those of HCs ($p = 0.006$). The percentage of CD300e-positive cells was higher in female participants than in male participants, and was negatively correlated with the estimated glomerular filtration rate and age (sex $p = 0.023$, eGFR $p = 0.028$, age $p = 0.023$). There was no difference in SM-stimulated TNF-α production by monocytes among diabetic subtypes. We showed differences in CD300e expression in human monocytes with diabetes type, sex, age, and renal function. These results provide information on the physiological features of CD300e and insights into the role of CD300e in AT1D pathogenesis.

INTRODUCTION

Type 1 diabetes (T1D) is characterized by almost complete insulin depletion by pancreatic β-cell destruction [1]. Thus, insulin injection therapy is inevitable during the lifetime of T1D patients [1]. In general, autoimmune pancreatic β-cell destruction is the cause of T1D, which is different from the mechanism in type 2 diabetes (T2D), in that the latter is characterized by both increase in insulin resistance and non-autoimmune decrease in insulin secretion [1].

In Japan, T1D that develops in an acute course is further divided into two subtypes. One is typical acute onset T1D

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(AT1D), in which islet-associated autoantibodies are positive before and after the onset of hyperglycemia, and the time from onset of diabetic symptoms to diabetic ketoacidosis (DKA) is generally less than 3 months [1,2]. The other is fulminant T1D (FT1D), in which islet-associated autoantibodies are usually negative even at the onset of hyperglycemia, and it is characterized by the rapid progression to DKA, usually within a week from the onset of diabetic symptoms, due to rapid and severe destruction of pancreatic islets [2,3]. Mononuclear cellular infiltration to the islets and pancreas is observed in both subtypes, indicating that some types of immune reaction would contribute to pancreatic β-cell destruction in the development of T1D [4].

Recently, we reported that CD300e antibody increases during the acute, but not chronic, phase of FT1D using serological analysis and proposed that this antibody could be an early diagnostic marker [5]. This finding prompted us to investigate the contribution of CD300e to the development of T1D.

The CD300 family members, which are mainly expressed in myeloid cells [6], have recently attracted attention as novel regulators of immunity. CD300 family molecules are type1 transmembrane proteins composed of an immunoglobulin variable-like extracellular domain, a transmembrane domain, and a cytoplasmic tail [6-8]. In humans, the CD300 family consists of eight members (CD300a-h), divided into two functional groups, except for CD300g [7]. CD300b, CD300c, CD300d, CD300e, and CD300h have short cytoplasmic tails with basic transmembrane residues or leucine zipper-like sequences [7]. These transmembrane domains associate with immunoreceptor tyrosine-based activation motifs, such as DNAX-activating protein 12 (DAP12) and FcRγ chains, or adapter proteins containing phosphatidylinositol 3-kinase binding motifs, such as DAP10, which enables their activation [7]. In contrast, CD300a and CD300f exhibit inhibitory capacity through long cytoplasmic tails containing immunoreceptor tyrosine-based inhibition motifs [7]. Although the specific ligands for each CD300 member are still unknown, lipids such as phosphatidylserine, phosphatidylethanolamine, and sphingomyelin or adenoviruses have been indicated as candidates [7,9].

In recent years, the CD300 molecules have been shown to be associated with allergy, infectious disease, autoimmunity, and inflammation, but the detailed mechanism through which the CD300 molecules are involved in such diseases remains largely unknown.

In this study, we aimed to investigate the contribution of CD300e in the development of type 1 diabetes.

MATERIALS and METHODS

Participants selection

The present study was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) and recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals. Informed consent (Research Ethics Committee approval number 2704-3) for participation in the study was obtained from 20 patients with FT1D, 25 with AT1D, 14 with T2D, and 17 healthy controls (HCs) who were diagnosed based on the criteria of the Japan Diabetes Society, visiting at Osaka Medical and Pharmaceutical University Hospital. Patients with cancer, autoimmune diseases other than T1D, those taking steroids/immunosuppressants, and those who did not agree to participate in the study were excluded from the study.

Monocyte and peripheral blood mononuclear cell (PBMC) isolation

Fifteen milliliters of blood was collected from the patients in all groups and stored in vacuum blood collection tubes (Benoject II) containing EDTA2K (VP-DK050K, Catalog #61-9705-35; Terumo Corporation, Tokyo, Japan). Thereafter, monocyte isolation was performed using the negative selection technique and the EasySep™ Direct Human Monocyte Isolation Kit (STEMCELL Technologies, Vancouver, BC, Canada) following the manufacturer's protocol. Briefly, 12 *mL* of blood was mixed with Isolation Cocktail RapidSpheres™ (STEMCELL Technologies) in a 14 *mL*-polystyrene round bottom tube. EasySep™ Buffer (Catalog #20144; STEMCELL Technologies) was then added thrice to fully adhere non-monocyte cells to the magnets for monocyte isolation. The PBMCs were collected from the remaining 3 mL of blood (from eight patients with FT1D, 12 with AT1D, 11 with T2D, and nine HCs) using Ficoll®-Paque PREMIUM density gradient medium (GE Healthcare, Chicago, IL, USA).

Flow cytometry

Flow cytometry measurements were performed according to the method described previously [6] and in accordance with the procedure recommended by BD Biosciences (San Jose, CA, USA). $CD45^+CD14^+$ cells in the isolated monocytes and $CD45^+CD11c^+$ human leucocyte antigen (HLA)-DR^+ myeloid dendritic cells (mDCs) in the isolated PBMCs were identified, and the expression rate of CD300e was evaluated. The following fluorochrome conjugated anti-human monoclonal antibodies (all BD Pharmingen™ from BD Biosciences) were used for flow cytometry: PerCP-Cy™ 5.5 mouse anti-human CD45 (clone HI30; Catalog Number: 564106), FITC mouse anti-human CD14 (clone M5E2; 561712), Alexa Fluor® 647 mouse anti-human IREM-2 (CD300e; clone UP-H1; 566315), and Alexa Fluor® 647 mouse IgG2a, κ isotype control (clone G155-178; 5665357) for monocytes; PerCP-Cy™ 5.5 mouse anti-human CD45 (clone HI30; 564106), BV421 mouse anti-human CD11c (clone 3.9; 565806), APC-H7 mouse anti-human HLA-DR (clone G46-6; 561358), Alexa Fluor® 647 mouse anti-human IREM-2 (CD300e; clone UP-H1; 566315), and Alexa Fluor® 647 mouse IgG2a, κ iso-

type control (clone G155-178; 5665357) for mDCs. Next, the cells were washed with phosphate-buffered saline (PBS), and Human BP Fc Block™ (Catalog Number: 564219, BD Biosciences) was added; 10 min later, the cells were incubated with the respective fluorochrome-conjugated antibodies for 30 min on ice. Unbound antibodies were removed by washing the cells, whereas the antibodies reacting with cells were analyzed using a fluorescence-activated cell sorting (FACS) Aria™ Cell Sorter (BD Biosciences). The percentage of CD300e-positive cells and CD300e mean fluorescence intensity (MFI), that is, the average value of fluorescence absorbance, an index showing the fluorescence intensity per cell, were also evaluated using the FACS Aria™ Cell Sorter (BD Biosciences).

For the CD300e antibody, an isotype control Alexa Fluor® 647 mouse IgG2a, κ isotype control (clone G155–178; 5665357) was used and a line was drawn where 95 % of cells were negative. In addition, CD300eMFI was standardized using fluorescent beads as an indicator.

Stimulation of monocytes with sphingomyelin (SM)

We prepared an SM-stimulating culture of monocytes using the method described previously [6]. Briefly, frozen monocytes from the patients in all groups were thawed, washed, and resuspended in Roswell Park Memorial Institute medium (Gibco™ RPMI 1640 Medium, GlutaMAX™ Supplement, Catalog Number: 61870036; Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10 % fetal bovine serum (SIGMA 176012; Sigma-Aldrich, St. Louis, MI, USA) and 1 % PCG (Gibco™ Penicillin-Streptomycin 10,000 *U/mL*, Catalog Number: 15140122) to a density of 1.0 × 10^6 *cells/mL*. Next, the cells were stimulated with 3.3 *ng/μL* SM (from chicken egg yolk, ≥ 95 %, Catalog Number: S0756; Sigma-Aldrich), positive control [0.1 *pg/μL* lipopolysaccharide (LPS); obtained from *E. coli* O55 by ultracentrifugation, Catalog Number: 128–05171; FUJIFILM Wako Pure Chemicals, Osaka, Japan], or negative control (medium only) medium in a 3590 Polystyrene Flat Bottom 96–well High Bind EIA/RIA Clear Microplate (Corning Life Sciences, Tewksbury, MA, USA) for 24 h at 37 °C in 5 % CO_2. After culturing, the cells were observed under a CK40 Culture Microscope (Olympus, Tokyo, Japan); then, the supernatants were immediately cryopreserved at – 80 °C. In addition, 200 *μL* of 0.4 % trypan blue solution (Gibco™, Thermo Fisher Scientific) diluted 2–fold with PBS was gently added to the plate after collection, and after 30 s, the liquid was gently aspirated and removed. The survival state of the monocytes remaining on the plate surface was promptly confirmed under the CK40 Culture Microscope (Olympus). In three cases of FT1D and one HC, the concentration of tumor necrosis factor (TNF)-α in the supernatant after cell culture and LPS stimulation was 50 *pg/mL* or less. These cases were excluded from further analyses because we considered that the cells were not alive following evaluation using trypan blue staining.

Measurement of TNF-α production

To determine cytokine production, we used the Human TNF-α Quantikine enzyme-linked immunosorbent assay (ELISA) Kit (Catalog number: DTA00D; R&D Systems, Minneapolis, MN, USA) following the manufacturer's protocol. TNF-α secretion in cell supernatants was measured using the Varioskan™ LUX multimode microplate reader (Thermo Fisher Scientific).

Statistical analyses

We used JMP Statistical Discovery software (SAS Institute, Cary, NC, USA) for statistical analyses. The background for each group is shown as the median interquartile range (IQR). Group differences between pairs, as well as the percentage of CD300e-positive cells, CD300e MFI, and SM-stimulated TNF-α/Control TNF-α ratio, were assessed using the Wilcoxon test with Bonferroni correction. The factors associated with CD300e expression were assessed using both univariate and multivariate analyses. Results with $p < 0.05$ were considered statistically significant.

RESULTS

Participant background

The background of all participants is shown in **Table 1**. There were no differences in age, sex, hemoglobin A1c and blood glucose levels, diabetes duration, estimated glomerular filtration rate (eGFR), and body mass index among the three patient groups (i.e., FT1D, AT1D, and T2D). The C-peptide index (CPI), which indicates insulin secretory capacity [10], was 0.00 (IQR; 0.00,0.05) for FT1D, 1.58 (IQR; 1.01,2.03) for T2D, and 0.00 (IQR; 0.00,0.09) for AT1D. CPI was significantly higher in T2D than in FT1D or AT1D ($p < 0.0001$; **Table 1A**) but no significant difference was observed between FT1D and AT1D.

Analysis of CD300e expression using flow cytometry

The percentage of CD300e-positive cells in the monocytes of the FT1D, AT1D, T2D, and HC groups was 95.4 (IQR; 92.0,97.4), 96.3 (IQR; 94.8,98.2), 94.9 (IQR; 90.2,97.3), and 93.3 (IQR; 90.5,96.3), respectively, with the percentage in AT1D being significantly higher than that in HC ($p = 0.006$) , and there was no significant differences in HC vs FT1D ($p = 0.293$) and HC vs T2D ($p = 0.513$) (**Fig. 1A, B**). The percentage of CD300e-positive cells in mDCs in the FT1D, AT1D, T2D, and HC groups was 99.7 (IQR; 98.6,100.0), 99.6 (IQR; 89.6,100.0), 99.9 (IQR; 99.4,100), and 99.8 (IQR; 99.6,100), respectively, with no significant differences among the four groups (**Fig. 1C, D**). The CD300e MFI in monocytes was 838.8 (IQR; 680.990.2) in FT1D, 958.1 (IQR; 808.3,1068.6) in AT1D, 999.0 (IQR; 825.1,1062.4) in T2D, and 811.3 (IQR; 758.1,978.7) in HC, with no significant differences among the four groups (**Fig. 2A, B**). The CD300e MFI in mDCs was 907.3 (IQR; 826.2,980.9) in FT1D, 915.7

Table 1 Characteristics of the participants.

Characteristics	FT1D (N = 20)	AT1D (N = 25)	T2D (N = 14)	HC (N = 17)	
Age (*y*)					
Median (IQR)	59.5 (51.0-74.0)*	45.0 (37.5-63.0)	57.0 (54.0-73.5)*	37.0 (30.5-43.5)	*$p < 0.001$ vs HC
Sex					
Number of females (%)	11.0 (55.0)	15.0 (60.0)	10.0 (71.4)	9.0 (52.9)	
HbA1c (%)					
Median (IQR)	7.75 (7.60-8.10)*	7.40 (6.90-8.05)*	7.30 (7.05-8.20)*	5.00 (4.85-5.25)	*$p < 0.001$ vs HC
BG (mg/dL)					
Median(IQR)	145.0 (122.8-248.8)*	180.0 (132-226.0)*	146.5 (112.5-181.5)*	98.0 (92.5-113.5)	*$p < 0.001$ vs HC
Diabetes duration (*y*)					
Median (IQR)	11.0 (3.0-17.0)*	15.0 (11.0-22.5)*	14.0 (8.0-19.3)*	0.0 (0.0-0.0)	*$p < 0.001$ vs HC
CPI					
Median(IQR)	0.000 (0.000-0.053)*	0.000 (0.000-0.090)*	1.579 (1.008-2.025)	No data	*$p < 0.001$ vs T2D
eGFR (*mL/min/1.73 m²*)					
Median (IQR)	77.50 (60.25-86.75)	85.00 (69.00-96.50)	74.00 (55.75-92.75)	80.20 (75.75-88.90)	
BMI (*kg/m²*)					
Median (IQR)	22.7 (19.7-25.8)	22.2 (20.3-24.0)	25.3 (19.7-31.2)**	20.0 (19.3-23.4)	**$p < 0.005$ vs HC

Data are median (interquartile range) or n (%). FT1D, fulminant type 1 diabetes; AT1D, acute onset type 1 diabetes; T2D, type 2 diabetes; HC, healthy controls; HbA1c, hemoglobin A1c; BG, blood glucose; CPI, C-peptide index; eGFR, estimated glomerular filtration rate; BMI, body mass index.

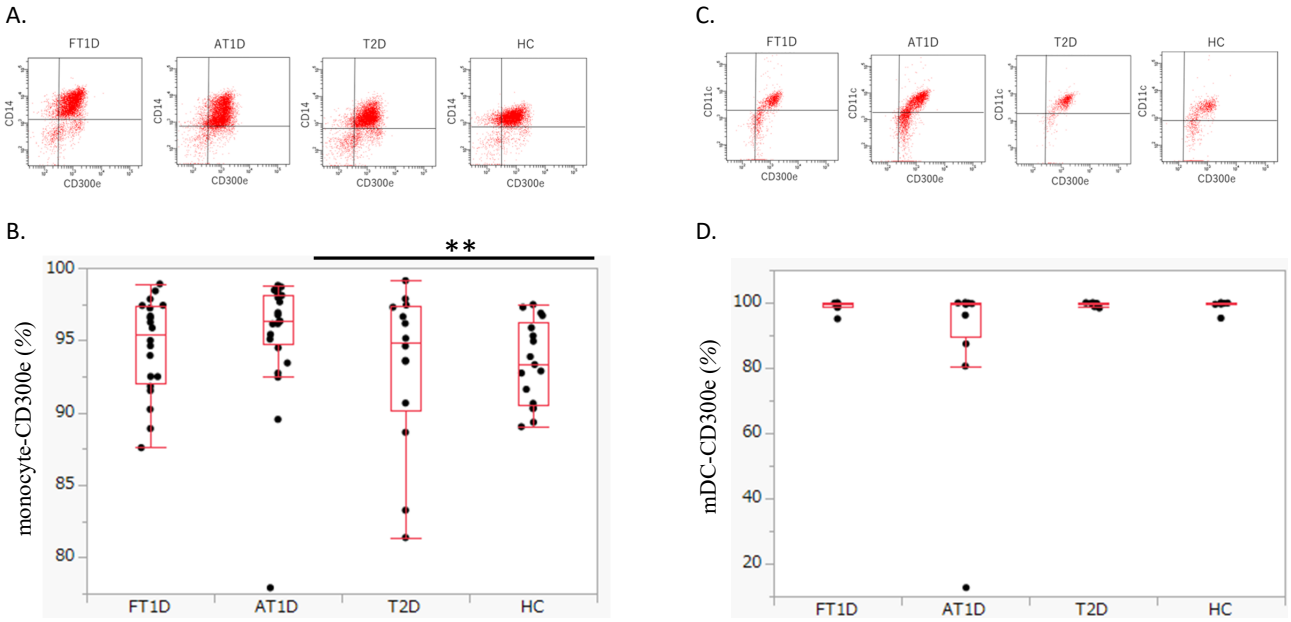

Figure 1 Percentage of CD300e-positive cells in each group.
A, Monocyte flow cytometry diagram. Human monocytes were stained with CD45, CD14, and CD300e and assessed using flow cytometry. CD45-positive cells were extracted, and the fluorescence absorbance of CD14 (vertical axis) and CD300e (horizontal axis) is shown. Contour plots represent the expression of CD300e on CD45+ and CD14+ monocyte subsets. B, The percentage of CD300e-positive cells in monocytes was higher in AT1D than in HC. C, mDCs flow cytometry diagram. PBMCs were treated with CD45, HLA-DR, CD11c, and CD300e and evaluated using flow cytometry. CD45+ and HLA-DR+ cells were extracted, and the fluorescence absorbance of CD11c (vertical axis) and CD300e (horizontal axis) is shown. Contour plots represent the expression of CD300e on CD45+, HLA-DR+, and CD11c+ mDC subsets. D, No significant differences were found in the percentage of CD300e-positive cells in mDCs between FT1D, AT1D, T2D, and HC.

(IQR; 740.5,1074.5) in AT1D, 959.1 (IQR; 865.9,1155.3) in T2D, and 795.4 (IQR; 718.2,967.4) in HC, with no significant differences among the four groups (**Fig. 2C, D**).

Factors associated with CD300e expression

Table 2 shows the possible factors associated with the percentage of CD300e-positive cells and CD300e MFI in monocytes. The univariate analysis showed that female patients had a higher percentage of CD300e-positive cells in monocytes than male patients [sex p = 0.040, hazard ratio (HR) (95 % CI) = – 0.969 (– 1.894 to – 0.044)]. In addition, the multivariate analysis showed that the percentage of CD300e-positive cells was higher in AT1D than in HC and in female patients than in male patients. Furthermore, the percentage of CD300e-positive cells showed a negative correlation with eGFR and age [AT1D p = 0.027, HR (95 % CI) = 1.593 (0.186 to 3.000); sex p = 0.023, HR (95 % CI) = –1.068 (–1.981 to – 0.155); eGFR p = 0.028, HR (95 % CI) = –0.049 (–0.093 to 0.005); age p = 0.023, HR (95 % CI) = –0.086 (–0.160 to –0.012)]. As for the factors associated with the CD300e MFI of monocytes (**Table 2**), both univariate and multivariate analyses showed that female patients had a higher monocyte CD300e MFI than male patients [univariate: sex p < 0.001, HR (95 % CI) = –69.729 (–107.840 to –31.619); multivariate: sex p < 0.001, HR (95 % CI) = – 69.881 (–109.826 to –29.935)].

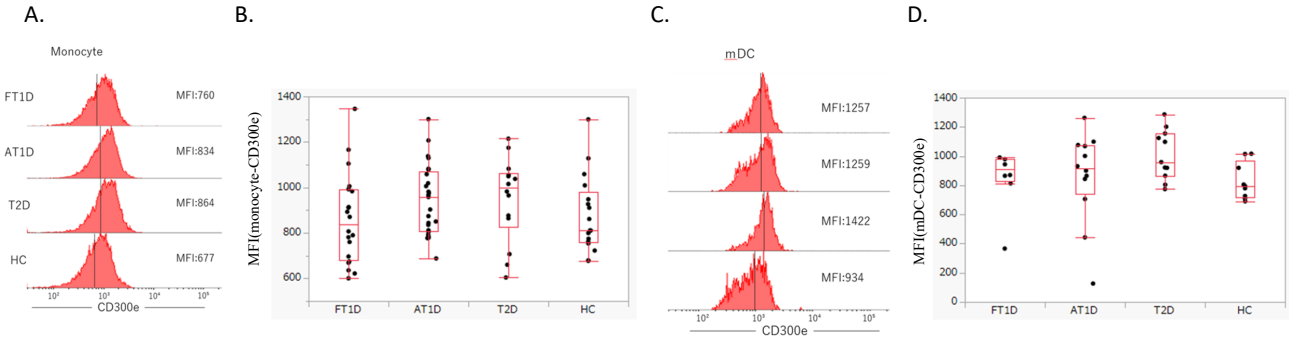

Figure 2 CD300e MFI of each group.

A, Histograms of concatenated monocytes and boxplots of the MFI of CD300e in monocytes. The numbers represent the MFI of CD300e expression. B, No significant differences were found in monocyte MFI between FT1D, AT1D, T2D, and HC. C, Histograms of concatenated mDCs and boxplots of the MFI of CD300e in mDCs. The numbers represent the MFI of CD300e expression. D, No significant differences were found in mDC MFI between FT1D, AT1D, T2D, and HC.

**, p < 0.01 (pairwise Wilcoxon test).

Table 2 Univariate and multivariate analyses of the relationship between the percentage of CD300e-positive cells and CD300e MFI in monocytes and various parameters.

	Percentage of CD300e-positive cells in monocytes		Percentage of CD300e-positive cells in monocytes		CD300e MFI in monocytes		CD300e MFI in monocytes	
	Univariate		Multivariate		Univariate		Multivariate	
	HR (95 % CI)	*P*	HR (95 % CI)	*P*	HR (95 % CI)	*P*	HR (95 % CI)	*P*
FT1D			1.336 (- 0.447 to 3.119)	0.140			- 14.161 (- 92.198 to 63.877)	0.718
AT1D			1.593 (0.186 to 3.000)	0.027			52.495 (- 9.081 to 114.072)	0.094
T2D			- 0.036 (- 1.992 to 1.919)	0.971			45.034 (- 40.539 to 130.606)	0.297
Age (*y*)	- 0.037 (- 0.095 to 0.022)	0.215	- 0.086 (-0.160 to - 0.012)	0.023	0.044 (- 2.522 to 2.611)	0.973	1.241 (- 1.989 to 4.472)	0.446
Sex (Male)	- 0.969 (- 1.894 to - 0.044)	0.040	- 1.068 (- 1.981 to - 0.155)	0.023	- 69.729 (- 107.840 to -31.619)	<0.001	- 69.881 (-109.826 to -29.935)	<0.001
HbA1c (%)	- 0.169 (- 0.779 to 0.441)	0.583	- 0.537 (- 1.476 to 0.402)	0.258	- 6.973 (- 33.492 to 19.546)	0.602	- 39.376 (- 80.478 to 1.726)	0.060
BG (*mg/dL*)	- 0.006 (- 0.020 to 0.007)	0.360			- 0.345 (- 0.925 to 0.234)	0.239		
CPI	- 0.884 (- 2.451 to 0.683)	0.263			24.646 (- 39.944 to 89.236)	0.448		
eGFR (*mL/min/1.73 m²*)	-0.025 (- 0.062 to 0.012)	0.186	- 0.049 (- 0.093 to 0.005)	0.028	0.535 (- 1.095 to 2.165)	0.515	0.744 (- 1.180 to 2.667)	0.443
BMI (*kg/m²*)	- 0.005 (- 0.235 to 0.225)	0.967			8.308 (- 1.499 to 18.114)	0.096		

MFI, mean fluorescence intensity.

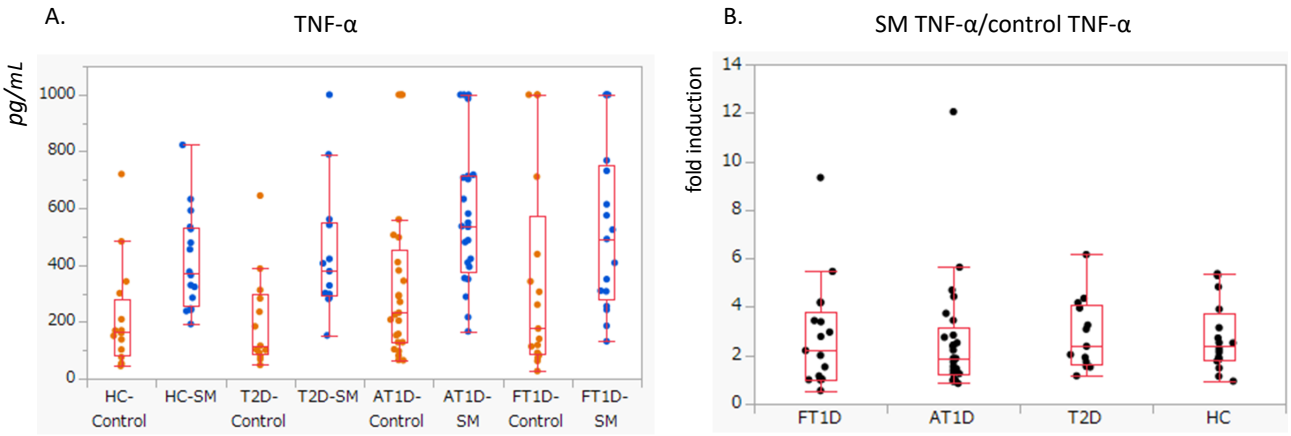

Figure 3 Human monocytes were stimulated using plate-coated SM or vehicle.
The levels of TNF-α released into the culture supernatants were measured using ELISA. A, TNF-α concentration in the supernatant of HC, T2D, AT1D, and FT1D cell cultures. B, No significant differences were found in SM TNF-α/control TNF-α between FT1D, AT1D, T2D, and HC.
TNF-α, tumor necrosis factor-α; SM, sphingomyelin.

Culture of monocytes with SM

As shown in Figure 3A, the variation in TNF-α secretion between individuals was considerable; therefore, we used the SM TNF-α/control TNF-α ratio to compare differences between the groups. The SM TNF-α/control TNF-α ratios were 2.2 (IQR; 1.0,3.8), 1.9 (IQR; 1.3,3.1), 2.4 (IQR; 1.6,4.1), and 2.4 (IQR; 1.8, 3.7) for the FT1D, AT1D, T2D, and HC groups, respectively, with no significant differences among them (**Fig. 3B**).

DISCUSSION

In the present study, we first found a higher percentage of CD300e-positive cells in the monocytes of patients with AT1D than in those of HCs using flow cytometry. The multivariate analysis also showed that the percentage of CD300e-positive cells in monocytes was independently associated with the type of diabetes. This is the first report on the positive association of CD300e expression with autoimmune disease development; however, the same association has been reported for CD300f and CD300a [9]. For example, the lack of CD300f expression has been shown to exacerbate the development of autoimmune diseases in mice [11].

Increased percentage of CD300e-positive cells was confirmed in the monocytes but not in the mDCs of AT1D patients in this study. CD300e is suggested to be an immunostimulatory receptor [6,7], therefore, increased percentage of CD300e-positive cells in monocytes may contribute autoimmunity of AT1D. Further investigation is required to determine whether macrophages and DCs involved in autoimmunity in AT1D also maintain high CD300 expression after differentiation from monocytes [12,13], because the presentation of β-cell peptides by antigen-presenting cells is critical for the initiation of autoimmunity in AT1D [14]. Recently, several viruses have been shown to affect the expression of CD300 family members [15,16]. For example, increased CD300e expression in monocytes is observed in patients with mild and moderate severe acute respiratory syndrome-coronavirus 2 infection [17]. In addition, enterovirus infections are critical environmental factors in the development of AT1D [18,19]. Although an association between enteroviruses and the CD300 family has not yet been reported, latent enterovirus infection in patients with AT1D might have influenced the high percentage of CD300e-positive cells.

In contrast to patients with AT1D, the patients with FT1D showed no significant difference from HCs regarding CD300e expression. However, no clear explanation for this finding was found in the present study. Although severe immune cell infiltration has been observed in patients with FT1D [20,21], this was detected at disease onset [3]. As insulitis in AT1D is not transient and lasts for some time after disease onset, the high percentage of CD300e-positive cells might have been observed in only patients with AT1D.

The multivariate analysis showed that the percentage of CD300e-positive cells in monocytes was higher in female participants than in male participants and showed negative correlations with eGFR and age. This is the first study to show the relationships between CD300e expression and sex, age, and renal function. It might be associated with increased immunoreactivity in women, as shown in other autoimmune diseases, such as AT1D, systemic lupus erythematosus, scleroderma (systemic sclerosis), Sjogren's syndrome, autoimmune thyroid diseases, rheumatoid arthritis, and myasthenia gravis [22,23]. The negative correlation between CD300e

and age observed in the present study may be a factor explaining the decline in both adaptive and innate immunity with aging [24-26]. A previous study indicated that the increase in the expression of CD300a in natural killer cells with aging contributed to the decrease in the immune function of the elderly individuals [27]. This study showed a negative correlation between CD300e and eGFR. Normal renal function is essential for maintaining homeostasis in the immune system [28]. Decreased renal function, also indicated by reduced eGFR, induces a chronic inflammatory state and dysfunction of immune cells, increasing the risk of cardiovascular disease and infections [28]. In this study, CD300e was coupled to even subtle changes in eGFR, suggesting that CD300e sensitively reflects the relationship between the kidney and immune system. Furthermore, CD300e might be a predictor of chronic kidney disease (CKD) progression. This is because glomerular and tubular damage caused by monocyte derived macrophages progresses CKD [28].

We found no difference in SM-stimulated reactivity of monocytes among diabetes subtypes. Recently, cardiolipin, lipid A, phosphatidic acid, phosphatidylcholine, phosphatidyl ethanolamine, phosphatidylglycerol, phosphatidylinositol, phosphatidylserine, and SM have been identified as CD300 family ligands [9]. At present, SM is the only verified ligand for CD300e [6], and the binding of SM to CD300e transduces immune signals such as TNF-α, interleukin (IL)-6, and IL-8 via DAP12 [6,29]. However, it also acts as a ligand for CD300b and CD300f [9,30]. CD300b functions as an immunostimulatory receptor [31,32] and CD300f as an immunosuppressive receptor [33]. As SM also acts on CD300b and CD300f, it is possible that the SM-stimulated culture test failed to detect differences between groups. This issue (i.e., stimulus reactivity) should be clarified using a CD300e-specific agonist, such as the CD300e antibody, in future research.

In conclusion, we showed that patients with AT1D had a higher percentage of CD300e-positive cells in monocytes than HCs. In addition, the percentage of CD300e-positive cells in monocytes was higher in female participants than in male participants and was negatively correlated with eGFR and age. Thus, this study proposed physiological features of CD300e and also suggests that CD300e might be involved in the pathogenesis of AT1D.

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Conflict of interest

The authors have no potential conflicts of interest to declare.

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