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Research Article

Inflammatory Cytokines and the Risk of Cardiovascular Complications in Type 2 Diabetes

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This study evaluates peripheral blood T lymphocyte expression of inflammatory and proinflammatory cytokines as well as T regulatory (Treg) (FOXP3+CD25+CD4+) cells in type 2 diabetes (T2DM). Participants included 40 T2DM and 30 healthy control subjects. Twenty-four patients had no complications while 16 were afflicted with coronary heart disease (CHD). Relative to healthy subjects, all T2DM patients showed a significant increase in expression of CD4+IFN-Υ+, CD4+TNF-α+, and CD4+IL-8+ T cells ($P < 0.001$) as well as CD4+IL-6+, CD4+IL-1β+, and IL-17+ T cells ($P < 0.05$) while the ratios of Treg/Th1(CD4+IFN-Υ+) and Treg/Th-17(CD4+IL-17+) cells were significantly decreased ($P < 0.05$ and $P < 0.01$). T2DM patients with CHD showed a significant increase in CD4+IFN-Υ+, CD4+TNF-α+, and CD4+IL-17+ T cells and a significant decrease in Treg/Th1 and Treg/IL-17 cells compared to T2DM patients without CHD ($P < 0.05$). In CHD-afflicted T2DM, HbA1c correlated positively with CD4+IFN-Υ+ T cells ($P < 0.01$), HDL correlated negatively with each of CD4+IL-8+ T cells and CD4+IL-17+ T cells ($P < 0.05$), and LDL correlated positively with CD4+IL-1β+ T cells ($P < 0.05$). *Conclusion*. This study shows that hyperglycemia and dyslipidemia correlate with increased inflammatory cytokine expression and suggests the involvement of T cells in the development of diabetes and its complications.

1. Introduction

Type 2 diabetes mellitus affects different components of the immune system, and most changes occurring in adipose tissue, B cells of pancreatic islet, liver, and levels of circulating cytokines leading to apoptosis and tissue fibrosis [1]. In inflammation, activation of monocytes occurs by increased level of cytokines and inflammatory markers that lead to complications [2]. These complications affect many organs and tissues causing retinopathy, nephropathy, and cardio vascular disease. In adipose tissues, immune cells, including monocytes, chemoattractant protein-1 (MCP-1), plasminogen activator inhibitor (PAI-1), and cytokines, undergo different abnormality in type 2 diabetes [1, 2]. In addition, TNF-α is released by the immune cells, its level is increased in obesity, and it plays a major role in insulin resistance [3]. Also, MCP, PAI, and IL-8, most of them are chemoattractive proteins that recruit immune cells to the site of inflammation in adipose tissue, when they are increased, they cause the release of acute phase proteins such as C-reactive protein (CRP) [4]. In addition, glucose and fat intake induce inflammation that promotes oxidative stress [2, 5]. Hyperglycemia and hyperlipidemia affect a number of pathways including aldose reduction pathway, advanced glycation end pathway (AGE), reactive oxygen intermediate pathway, and PKC pathway. These pathways when affected they lead to activation of inflammatory mediators and induce oxidative stress that promotes micro- and macrocomplications [3]. In normal individuals, insulin interacts with insulin receptors to activate two main pathways in cardio vascular tissues. These two pathways are phosphoinositide 3 kinase (PI3 K) pathway that inhibits atherogenesis and has antiatherogenic effect and MAPK pathway that promotes cellular growth and enhances atherogenesis. However, in case of insulin resistance, hyperglycemia and high free fatty acid lead to an increase in inflammatory cytokines and affect regulation of

PKC and MAPK activities [1, 3]. Inflammatory cytokines, such as TNF-α and IL-1β, stimulate I-kappa-β (IKβ) kinase-β and IKKα to induce activation of nuclear factor kappa β (NF-κb) that promote insulin resistance [6]. Moreover, high concentration of circulating IL-6 in obese people maintains insulin resistance, and act on liver to increase VLDL and decrease HDL [2]. However, IL-6 and IL-1β decrease with weight loss which improves insulin sensitivity [4].

Recent studies showed that an elevation in Th17 and Th1 subsets with a decrease in the CD4+CD25hi Tregs culminates in inflammation and insulin resistance [7, 8]. More recently, it was reported that Th17 is negatively related to plasma high-density lipoprotein (HDL) suggesting that HDL modulates T cell polarization in T2DM patients [9]. T2DM patients are skewed towards proinflammatory subsets [10]; however, the relationship of peripheral Th1, Th17, and Treg cells in T2DM with hyperglycemia and dyslipidemia as well as diabetes complications is not fully addressed. The present study gives an insight into potential treatment of T2DM by regulating diabetic Th1, Th17, and Treg cells.

2. Materials and Methods

2.1. Patients. Subjects for this investigation included 40 patients with type 2 diabetes (Table 1), 22 males and 18 females (mean age 50 ± 2.1 years) in which the diabetes was diagnosed first time with fasting glucose test and 75 g glucose tolerance test. The mean duration of the disease was 5.2 ± 0.59 years. All patients were receiving antidiabetic tablets: 20 were on metformin (Glucophage) (500 mg twice a day), 18 were on gliclazide (Diamicron) (160 mg twice a day), and 2 were on repaglinide (NovoNorm) (4 mg three times a day). The average BMI in patients was (29.6 ± 1.39 Kg/m^2) while in healthy control subjects was (27.4 ± 3.7 Kg/m^2). All patients were controlled for hypertension. Sixteen of the patients had CHD and twenty four had no complications. Of all patients recruited, those with missing essential clinical data, current immunomodulating treatment (e.g., steroids, azathioprine, or cyclosporine A), a history of neoplastic disease, surgical or angioplastic intervention before the study, or acute respiratory or genitourinary infections were excluded. Demographic, anthropometric, and metabolic data as well as information on diabetes complications were extracted from the patients' medical records. The control cohort included 30 healthy subjects. Participation in this study was voluntary and conducted with institutional approval.

2.2. Chemistry Profile. The levels of total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, and glycated hemoglobin (A1C) were determined using standard laboratory methods. These assays were conducted in the main clinical laboratories of Mubarak hospital in Kuwait. Standard blood analyte detection equipment including the COBAS INTEGRA 400/700/800 biochemical analysis system (Roche Diagnostics, Indianapolis, IN, USA) was used for these measurements.

2.3. Intracellular Cytokines Analysis. Peripheral blood mononuclear cells (PBMC) were evaluated by 2-color flow cytometry for representation of CD4+ T cells producing interferon-gamma (IFN-Υ), tumor necrosis factor alpha (TNF-α), interleukin-8 (IL-8), ineterleukin-6 (IL-6), and ineterleukin-1beta (IL-1β).

Peripheral blood mononuclear cells (PBMC) were separated by Ficoll-paque (Pharmacia, Uppsala, Sweden) density gradient centrifugation. The cells were washed and suspended in RPMI 1640 medium (Gibco BRL, Gaithersburg, MD, USA) at density of 1×10^6 cells/mL. 200 μL cultures of PBMC in 96-well plates were incubated under humidified conditions for 6 hours at 37°C. The cells were stimulated in the presence of 50 ng/mL of phorbol 12-myristate 13-acetate (PMA, Sigma, St. Louis, MO), 1 ng/mL of ionomycin (Sigma), and 2 mM monensin (Sigma). Following CD4 cell surface staining for 15 min, cells were fixed for 15 min using a fix and perm cell permeabilization kit (Caltag Laboratories, Burlingame, CA, USA), permeabilized with the same kit, and stained intracellularly for 30 min at room temperature with monoclonal antibodies to IFN-Υ, TNF-α, IL-8, IL-6, Il-1α, and IL-1β provided by BD Pharmingen. After washing, cells were analyzed by flow cytometry. Two parameter analyses were undertaken on FC-500 (Beckman Coulter Corporation, Hialeah, FL, USA). Negative isotype controls were used to verify the staining specificity of the antibodies used.

2.4. Measurement of CD4+CD25+FOXP3+ T Cells. Peripheral blood mononuclear cells were stained for 30 min at 4°C with PC5-conjugated CD4 and FITC-conjugated CD25 mAbs (Beckman Coulter Corporation, Hialeah, FL, USA). After washing, cells were fixed with fixation/permeabilization solution and treated with permeabilization buffer according to the manufacturer's instructions (eBioscience, San Diego, CA, USA). They were then stained with PE-conjugated FoxP3 mAb (eBioscience) for 30 min at 4°C. After washing, cells were analyzed on FC-500 (Beckman Coulter Corporation, Hialeah, FL, USA). We calculated the ratio of regulatory T cells to peripheral blood mononuclear cells (PBMCs), gating PBMCs using the anti-CD4 antibody and the anti-CD25 antibody and regating these subpopulations using the anti-Foxp3 antibody. Negative isotype controls were used to verify the staining specificity of the antibodies used.

2.5. Statistical Analyses. Statistical analysis was performed using one way analysis of variance (ANOVA) to explore the differences in intracellular cytokines expression between groups. Tukey post hock multiple comparison analysis was performed to identify significant differences between any two groups. All analyses were performed using the SPSS for Windows statistical package version 17 (Norusis/SPSS, Inc.). The Pearson product-moment coefficients were determined in order to evaluate the statistical significance of correlations between cytokine expression, glycemic, or lipid parameters. Differences in outcome variable magnitude between subject groups or Pearson correlation between the above mentioned parameters were considered to be statistically significant at $P < 0.05$.

Table 1: Cardiovascular metabolite profiles for diabetic ($n = 24$) and control subjects ($n = 15$). Mean percentage of each subpopulation ± SEM shown.

	All type 2 diabetes (AD) ($n = 40$)	Diabetes without CHD ($n = 24$)	Diabetes with CHD ($n = 16$)	Healthy control subjects ($n = 30$)
Sex (M/F)	25/15	16/8	9/7	19/11
Age (years)	50.3 ± 21.4	49.8 ± 1.67	51.9 ± 2.1	50.1 ± 1.7
BMI (Kg/m^2)	29.6 ± 1.39*	29.4 ± 0.87*	30.44 ± 1.4*	27.4 ± 3.7
HbA1c (mmol/L)	7.98 ± 0.63*	8.20 ± 0.25*	8.7 ± 0.46*	5.6 ± 0.72
Total cholesterol (mmol/L)	4.3 ± 0.28	4.9 ± 0.17	4.29 ± 0.30	4.21 ± 34
HDL (mmol/L)	0.91 ± 0.07*	1.08 ± 0.06$^\Psi$	0.83 ± 0.07*	1.5 ± 0.05
LDL (mmol/L)	2.70 ± 0.23	1.90 ± 0.15	2.69 ± 0.27	2.07 ± 0.30
Triglycerides (mmol/L)	1.46 ± 0.21	2.66 ± 0.94*	2.1* ± 0.27	1.22 ± 0.17

$^*P < 0.05$ compared to normoglycemic control subjects.

$^\Psi P < 0.05$ compared to diabetes with CHD.

SEM: standard error of the mean.

Table 2: Absolute numbers and percentages of T helper cells in peripheral blood of diabetes patients and healthy control subjects. Values are given as mean ± SEM of cell density (cells/μL) and percentages within total lymphocytes of CD4+ T cells.

	All type 2 diabetes (AD) ($n = 40$)	Diabetes with CHD ($n = 16$)	Diabetes without CHD ($n = 24$)	Healthy control subjects ($n = 30$)
Cell density (cells/μL)	1025.2 ± 56.4	1041.1 ± 71.2	998.0 ± 49.8	1008.6 ± 39.2
% within total lymphocytes	45.5 ± 1.7	44.3 ± 0.9	45.8 ± 1.2	43.7 ± 0.9

Table 3: The ratios of Treg (CD4+CD25+FOXP3) cells to Th17 or Th1 in type 2 diabetes patients.

Ratios	All type 2 diabetes (AD) ($n = 40$)	Diabetes with CHD ($n = 16$)	Diabetes without CHD ($n = 24$)	Healthy control subjects ($n = 30$)
Treg/Th1 cells	0.351 ± 0.1*	0.313 ± 0.1*	0.131 ± 0.05$^{**\Psi}$	0.638 ± 0.08
Treg/Th17 cells	0.302 ± 0.04**	0.354 ± 0.05**	0.26 ± 0.03$^{***\Psi}$	0.654 ± 0.09

$^*P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ versus healthy control subjects.

$^\Psi P < 0.05$ versus diabetes without CHD.

3. Results

As shown in Table 1, measurement of BMI and serum cardiovascular metabolites revealed that relative to healthy control subjects, all T2DM patients group exhibited a significantly elevated BMI and HbA1c, while HDL was significantly reduced ($P < 0.05$). No significant differences in the absolute numbers or the percentages of CD4+ T cells in peripheral blood were observed between the study groups (Table 2). The expression of CD4+ T cells was not significantly different between T2DM groups under treatment with metformin, gliclazide, or repaglinide (data not shown). Relative to healthy subjects, all T2DM patients showed a significant increase in expression of CD4+IFN-Υ+, CD4+TNF-α+, CD4+IL-8+ T cells ($P < 0.001$) (Figure 1), as well as CD4+IL-6+, CD4+IL-1β+, and IL-17+ T cells ($P < 0.05$) (Figure 2); while Treg cells were significantly decreased ($P < 0.05$) (Figure 2). A similar pattern was observed in T2DM patients with or without CHD; T2DM patients with CHD showed a significant increase in CD4+IFN-Υ+, CD3+TNF-α+, and CD4+IL-17+ T cells compared to T2DM patients without CHD ($P < 0.05$) (Figures 1 and 2). Dot plots of FACS staining for IFN-γ, TNF-α, and IL-17 in gated CD4+ T cells from T2DM patients with and without CHD are shown in Figures 3(a), 3(b), and 3(c).

The ratios between Treg (CD4+CD25+FOXP3)/Th1 cells and Treg/Th17 cells in T2DM were reduced relative to healthy subjects ($P < 0.05$ and 0.01, resp., in all T2D group; $P < 0.05$ and 0.01, resp., in T2DM with no complications; and $P < 0.01$ and 0.001, resp., in T2DM patients afflicted with CHD) (Table 3). Furthermore, the CHD-afflicted T2D exhibited lower ratios of Treg/Th1 cells and Treg/Th17 cells relative to T2D without CHD ($P < 0.05$).

Pearson product-moment analysis for relationships between variables in the present investigation revealed that all diabetic subject groups exhibited a significant positive correlation between CD4+IL-17 and each of CD4+IL-8+ and CD4+IL-1β+ T cells ($P < 0.05$); in addition, a significant positive correlation between Treg cells and CD4+IL-6+ ($P < 0.01$) and CD4+IL-1β+ T cells ($P < 0.05$) was observed in this group. Diabetes patients with CHD showed a significant positive correlation between CD4+IL-17+ and CD4+IL-1β+ T cells ($P < 0.05$). The healthy subject group showed a significant correlation between CD4+IL-17+ T cells and each of CD4+IFN-Υ+ and CD3+TNF-α+ T cells ($P < 0.05$) and between CD4+IL6+ and CD4+IL-1β+ T

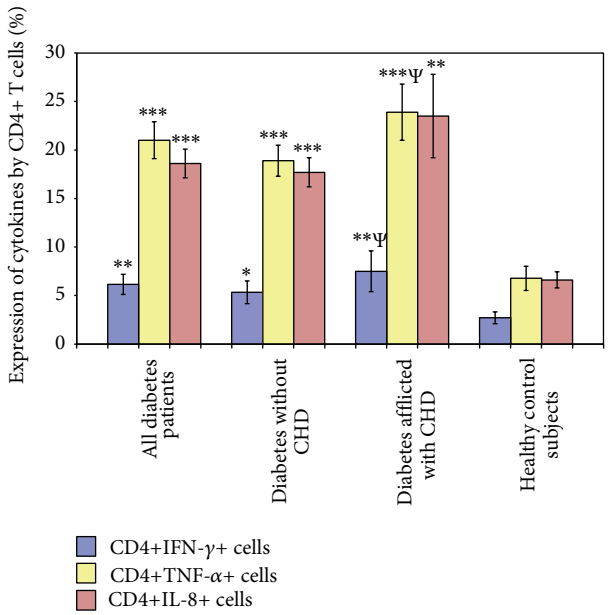

Figure 1: A significant increase in the expression of CD4+IFN-Υ+ ($P < 0.01$), CD4+TNF-α+, and CD4+IL-8+ T cells ($P < 0.001$) was shown in all T2DM patients versus control. The expression of CD4+IFN-Υ+ and CD4+TNF-α+ T cells was significantly higher in patients with CHD versus patients without CHD. PBMCs were stimulated by PMA and ionomycin for 6 h and analyzed for intracellular cytokines by flow cytometry. $^{*}P < 0.05$ versus healthy control subjects. $^{\Psi}P < 0.05$ versus diabetes without CHD.

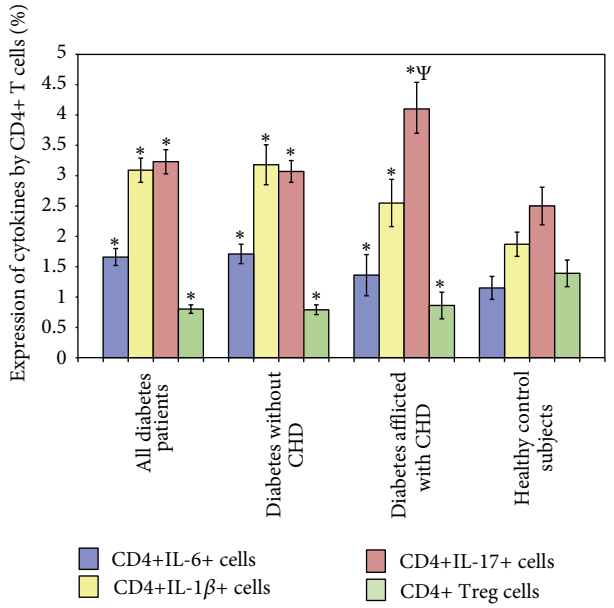

Figure 2: A significant increase in expression of CD4+IL-6+, CD4+IL-1β+, and IL-17+ T cells ($P < 0.05$) and a decrease in CD4+Treg cells ($P < 0.05$) were shown in all T2DM patients versus control. The expression of CD4+IL-17+ T cells was significantly higher in patients with CHD versus patients without CHD. $^{*}P < 0.05$ versus healthy control subjects. $^{\Psi}P < 0.05$ versus diabetes without CHD.

cells ($P < 0.01$). Furthermore, this group showed a positive correlation between Treg cells and each of CD4+IL-6+ ($P < 0.01$) and CD4+IL-1β+ T cells ($P < 0.05$) (Table 4).In CHD-afflicted T2DM, HbA1c correlated positively with CD4+IFN-Υ+ T cells ($P < 0.01$), HDL correlated negatively with each of CD4+IL-8+ T cells and CD4+IL-17+ T cells ($P < 0.05$), and LDL correlated positively with CD4+IL-1β+ T cells ($P < 0.05$) (Table 5). Furthermore, this group showed a negative correlation between HbA1c and the ratio of CD4+CD25+FOXP3+/Th17 cells.

4. Discussion

Immune activation may precede the manifestation of type 2 diabetes and CHD [11, 12]. In type 2 diabetes, impaired glucose signalling causes hyperglycaemia, which stimulates insulin secretion. This activates inhibitor kβ (IkB)/nuclear factor kβ (NFκb) [3] which also causes insulin resistance. Insulin itself is not proinflammatory; indeed, a major function of insulin in healthy individuals is to maintain cellular homeostatic activity that prevents dysregulated inflammation [13]. Nevertheless, sustained physiological hyperinsulinaemia activates multiple genes involved in inflammation [14]. The mitogen activated protein (MAP) kinase pathway is excessively stimulated [15] leading to excessive production of growth factors/inflammatory cytokines, contributing to accelerated atherosclerosis. In diabetes mellitus, sustained hyperglycemia results in the generation of reactive oxygen species, ultimately leading to increased oxidative stress and inflammation in vital tissues [16, 17].

In the present study, we have investigated immune activation in type 2 diabetes by evaluation of cytokine expression by peripheral blood lymphocytes. An immune activation state in type 2 diabetes is shown as the patients exhibited an increase in expression of inflammatory cytokines versus healthy control subjects; this included CD4+IFN-Υ+ T cells ($P < 0.01$), CD4+TNF-α+ and CD4+IL-8+ T cells ($P < 0.001$) as well as CD4+IL-6+, CD4+IL-1β+, and CD4+IL-17+ T cells ($P < 0.05$); while Treg cells were reduced compared to healthy subjects in all the diabetic groups ($P < 0.05$) (Figures 1 and 2). The CHD-afflicted T2DM group expressed higher levels of CD4+IFN-Υ+, CD4+TNF-α+, and CD4+IL-17+ T cells compared to diabetics without CHD ($P < 0.05$) (Figures 1 and 2).

These findings support the hypothesis that the low-grade systemic inflammation in T2DM is linked to the development of diabetic complications and also likely related to the pathogenesis of this disorder [18, 19]; however the mechanism of immune alteration was far not fully addressed.

The enhanced immune activation and inflammation in T2DM may be caused by the decreased immunosuppressive potentiality of Tregs and Treg/Th1 ratios (Figure 1, Table 3), and enhanced inflammatory Th17 and Th1 cells (Figures 1 and 2). Furthermore, it may provide one possible approach to prevent the development of diabetic complications by modification of CD4+ T cell subpopulations.

Evidence supports that macrophage activation and accumulation is one of the major causes for the development of

Table 4: Pearson product-moment analysis of correlations between lymphocyte expression of intracellular cytokines and CD4+CD25+ FOXP3+ cells in All type 2 diabetes patients (T2DM), CHD-afflicted T2DM, and in healthy control subjects.

	IFN-Υ	TNF-α	IL-8	IL-6	IL-1β
All T2DM					
CD4+IL-17+ cells	$r = -0.173$	$r = -0.072$	$r = 0.309^{*}$	$r = 0.256$	$r = 0.358^{*}$
CD4+CD25+FOXP3+ cells	$r = -0.096$	$r = -0.189$	$r = 0.143$	$r = 0.276^{*}$	$r = 0.308^{*}$
CD4+CD25+FOXP3+/Th1 cells	$r = -0.333^{**}$	$r = -0.234$	$r = 0.249$	$r = -0.188$	$r = 0.186$
T2DM with CHD					
CD4+IL-17+ cells	$r = 0.362$	$r = 0.189$	$r = 0.699$	$r = 0.706$	$r = 0.842^{*}$
CD4+CD25+FOXP3+ cells	$r = 0.079$	$r = 0.385$	$r = 0.558$	$r = 0.603^{*}$	$r = 0.770$
CD4+CD25+FOXP3+/Th1 cells	$r = -0.658^{*}$	$r = -0.441$	$r = 0.361$	$r = -0.181$	$r = 0.176$
T2DM without CHD					
CD4+IL-17+ cells	$r = 0.370^{*}$	$r = -0.122$	$r = 0.261$	$r = 0.175$	$r = 0.318^{*}$
CD4+CD25+FOXP3+ cells	$r = 0.175$	$r = 0.077$	$r = -0.122$	$r = 0.190$	$r = 0.028$
CD4+CD25+FOXP3+/Th1 cells	$r = -0.317^{*}$	$r = -0.212$	$r = 0.269$	$r = -0.177$	$r = -0.072$
Healthy control					
CD4+IL-17+ cells	$r = 0.640^{*}$	$r = 0.595^{*}$	$r = 0.164$	$r = 0.849^{**}$	$r = 0.652^{**}$
CD4+CD25+FOXP3+ cells	$r = 0.385$	$r = -0.007$	$r = 0.453$	$r = 0.698^{**}$	$r = 0.575^{*}$
CD4+CD25+FOXP3+/Th1 cells	$r = -0.539^{*}$	$r = -0.726^{**}$	$r = -0.223$	$r = -0.139$	$r = -0.210$

*Significant direct or inverse correlation ($P < 0.05$) and **significant direct or inverse correlation ($P < 0.01$).

Table 5: Pearson product-moment analysis of correlations between lymphocyte expression of intracellular cytokines and glycemic and lipid parameters in CHD-afflicted T2DM patients.

	HbA1C	HDL	LDL
All T2DM			
CD4+IFN-Υ+ T cells	$r = -0.097$		
CD4+IL-8+ T cells		$r = -0.037$	
CD4+IL-17+ T cells		$r = 0.039$	
CD4+IL-1β+ T cells			
CD4+CD25+FOXP3+/Th17 cells	$r = 0.188$		$r = -0.153$
T2DM with CHD			
CD4+IFN-Υ+ T cells	$r = 0.975^{**}$		
CD4+IL-8+ T cells		$r = -0.838^{*}$	
CD4+IL-17+ T cells		$r = -0.801^{*}$	
CD4+IL-1β+ T cells			$r = 0.903^{*}$
CD4+CD25+FOXP3+/Th17 cells	$r = -0.616^{*}$		
T2DM without CHD			
CD4+IFN-Υ+ T cells	$r = -0.068$		
CD4+IL-8+ T cells		$r = 0.053$	
CD4+IL-17+ T cells		$r = -0.064$	
CD4+IL-1β+ T cells			$r = -0.286$
CD4+CD25+FOXP3+/Th17 cells	$r = 0.242$		
Healthy control			
CD4+IFN-Υ+ T cells	$r = 0.391$		
CD4+IL-8+ T cells		$r = 0.531$	
CD4+IL-17+ T cells		$r = 0.179$	
CD4+IL-1β+ T cells			$r = 0.241$
CD4+CD25+FOXP3+/Th17 cells	$r = 0.237$		

*Significant direct or inverse correlation ($P < 0.05$) and **significant direct or inverse correlation ($P < 0.01$).

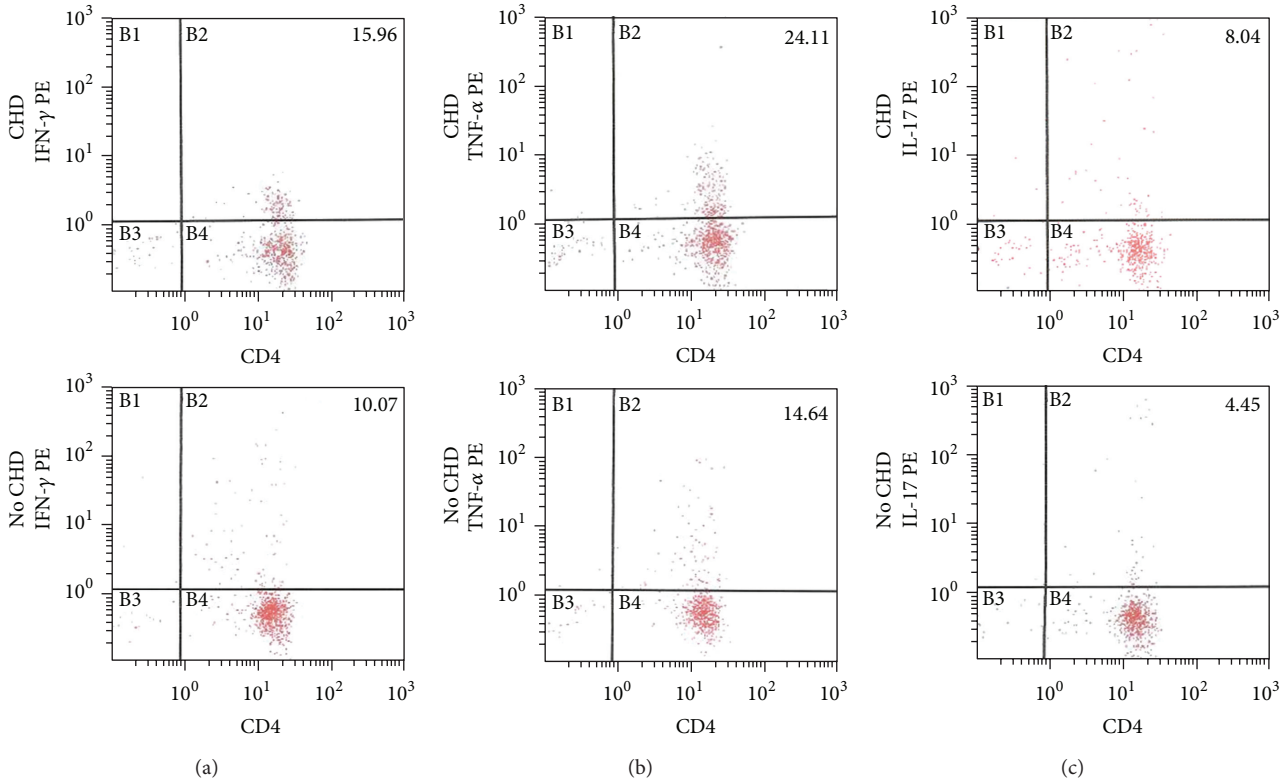

Figure 3: Elevated expression ofintracellular cytokines: IFN-γ, TNF-α, and IL-17 by PBMCs from T2DM patients with coronary heart disease (CHD) or without CHD. Representative FACS staining for IFN-γ, TNF-α and IL-17 in gated CD4+ T cells are shown in (a), (b), and (c).

diabetic complications [20, 21]. Thus, it is speculated that the decreased Tregs and its ratio to Th17 and Th1 cells may display less immunosuppressive effect on monocytes/macrophages and likely drive these cells to an inflammatory state, which may contribute to the occurrence of complications. In the present study, a positive correlation between Tregs and the enhanced expression of IL-6 on CD4+ T cells was observed in T2DM patients ($P < 0.05$) as well as in healthy subjects ($P < 0.01$) (Table 4). A recent investigation showed that IL-6 enhanced the level of Tregs in mice [22]; however with the enhanced IL-6 T cell expression in T2DM, the levels of Treg cells were significantly lower than those of controls in the present study (Table 4). These supports suggesting that the significantly decreased Tregs and increased Th17 and Th1 cells in T2DM patients may be involved in diabetes complications. In addition, in CHD-afflicted T2DM, HbA1c correlated positively with CD4+IFN-ϒ+ T cells ($P < 0.01$), HDL correlated negatively with CD4+IL-8+ T cells and CD4+IL-17+ T cells ($P < 0.05$), and LDL correlated positively with CD4+IL-1β+ T cells ($P < 0.05$) (Table 4). On the other hand, HbA1C correlated negatively with Treg/Th17 ratio ($r = -0.616$) (Table 5) in CHD-afflicted T2DM, suggesting that HbA1C is a factor in T cell polarization in CHD in T2DM. These findings show that hyperglycemia and dyslipidemia correlate with increased inflammatory cytokine expression which may play a role in development of CHD in T2DM patients.

In conclusion, this study gives insight into the understanding of the alteration of CD4+ T cells in T2DM patients and suggests the involvement of T cells in the development of diabetes and its complications, which may provide a possible therapeutic approach.

Conflict of Interests

The authors do not have a direct financial relation with the commercial identity mentioned in the paper that might lead to a conflict of interest.

References

[1] G. L. King, "The role of inflammatory cytokines in diabetes and its complications," *Journal of Periodontology*, vol. 79, no. 8, pp. 1527–1534, 2008.

[2] S. E. Shoelson, L. Herrero, and A. Naaz, "Obesity, inflammation, and insulin resistance," *Gastroenterology*, vol. 132, no. 6, pp. 2169–2180, 2007.

[3] M. Y. Donath and S. E. Shoelson, "Type 2 diabetes as an inflammatory disease," *Nature Reviews Immunology*, vol. 11, no. 2, pp. 98–107, 2011.

[4] J. Spranger, A. Kroke, M. Möhlig et al., "Inflammatory cytokines and the risk to develop type 2 diabetes: results of the prospective population-based European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam study," *Diabetes*, vol. 52, no. 3, pp. 812–817, 2003.

[5] A. Dey and J. Lakshmanan, "The role of antioxidants and other agents in alleviating hyperglycemia mediated oxidative stress

and injury in liver," *Food & Function*, vol. 4, no. 8, pp. 1148–1184, 2013.

[6] M. A. McArdle, O. M. Finucane, R. M. Connaughton, A. M. McMorrow, and H. M. Roche, "Mechanisms of obesity-induced inflammation and insulin resistance: insights into the emerging role of nutritional strategies," *Frontiers in Endocrinology*, vol. 4, article 52, 2013.

[7] S. Winer, Y. Chan, G. Paltser et al., "Normalization of obesity-associated insulin resistance through immunotherapy," *Nature Medicine*, vol. 15, no. 8, pp. 921–929, 2009.

[8] M. Feuerer, L. Herrero, D. Cipolletta et al., "Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters," *Nature Medicine*, vol. 15, no. 8, pp. 930–939, 2009.

[9] C. Zeng, X. Shi, B. Zhang et al., "The imbalance of Th17/Th1/Tregs in patients with type 2 diabetes: relationship with metabolic factors and complications," *Journal of Molecular Medicine*, vol. 90, no. 2, pp. 175–186, 2012.

[10] M. Jagannathan, M. McDonnell, Y. Liang et al., "Toll-like receptors regulate B cell cytokine production in patients with diabetes," *Diabetologia*, vol. 53, no. 7, pp. 1461–1471, 2010.

[11] H. Kolb and T. Mandrup-Poulsen, "An immune origin of type 2 diabetes?" *Diabetologia*, vol. 48, no. 6, pp. 1038–1050, 2005.

[12] L. F. van Gaal, I. L. Mertens, and C. E. de Block, "Mechanisms linking obesity with cardiovascular disease," *Nature*, vol. 444, no. 7121, pp. 875–880, 2006.

[13] P. L. Hooper and P. L. Hooper, "Inflammation, heat shock proteins, and type 2 diabetes," *Cell Stress and Chaperones*, vol. 14, no. 2, pp. 113–115, 2009.

[14] D. K. Coletta, B. Balas, A. O. Chavez et al., "Effect of acute physiological hyperinsulinemia on gene expression in human skeletal muscle in vivo," *American Journal of Physiology—Endocrinology and Metabolism*, vol. 294, no. 5, pp. E910–E917, 2008.

[15] K. Cusi, K. Maezono, A. Osman et al., "Insulin resistance differentially affects the PI 3-kinase- and MAP kinase-mediated signaling in human muscle," *Journal of Clinical Investigation*, vol. 105, no. 3, pp. 311–320, 2000.

[16] S. Neri, S. Calvagno, B. Mauceri et al., "Effects of antioxidants on postprandial oxidative stress and endothelial dysfunction in subjects with impaired glucose tolerance and type 2 diabetes," *European Journal of Nutrition*, vol. 49, no. 7, pp. 409–416, 2010.

[17] H. Kaneto, N. Katakami, M. Matsuhisa, and T.-A. Matsuoka, "Role of reactive oxygen species in the progression of type 2 diabetes and atherosclerosis," *Mediators of Inflammation*, vol. 2010, Article ID 453892, 11 pages, 2010.

[18] T. Mandrup-Poulsen, "IAPP boosts islet macrophage IL-1 in type 2 diabetes," *Nature Immunology*, vol. 11, no. 10, pp. 881–883, 2010.

[19] C. M. Larsen, M. Faulenbach, A. Vaag et al., "Interleukin-1-receptor antagonist in type 2 diabetes mellitus," *The New England Journal of Medicine*, vol. 356, no. 15, pp. 1517–1526, 2007.

[20] M. F. Lopes-Virella and G. Virella, "The role of immune and inflammatory processes in the development of macrovascular disease in diabetes," *Frontiers in Bioscience*, vol. 8, pp. s750–s768, 2003.

[21] S. Wueest, R. A. Rapold, D. M. Schumann et al., "Deletion of Fas in adipocytes relieves adipose tissue inflammation and hepatic manifestations of obesity in mice," *Journal of Clinical Investigation*, vol. 120, no. 1, pp. 191–202, 2010.

[22] M. Fujimoto, M. Nakano, F. Terabe et al., "The influence of excessive IL-6 production in vivo on the development and function of Foxp3+ regulatory T cells," *Journal of Immunology*, vol. 186, no. 1, pp. 32–40, 2011.