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Research Article

CTLA4 Gene Expression in Type 1 Diabetes Patients with CMV Infection in Pointe-Noire

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ABSTRACT

Background: Type 1 diabetes is an autoimmune disease characterized by the destruction of pancreatic cells.

Objective: The aim of this work was to measure CTLA4 gene expression in T1D patients with CMV infection.

Materials and Methods: We carried out an analytical case-control study over 6 months between June and November 2022. A total of 234 subjects were enrolled, of whom 68 T1D with positive CMV serology (T1D+CMV+) constituted the case group; 62 T1D with negative CMV serology (T1D+CMV-) constituted the diseased control group and 104 healthy subjects constituted the healthy control group. The determination of the plasma concentrations of CD4, CD8 and CD28 was carried out by ELISA. CTLA4 gene expression was measured by real-time PCR using the double delta technique. The correlation of CD4, CD8 and CD28 and the expression of the CTLA4 gene was studied by linear regression.

Results: The mean age in the different study groups was respectively: 20.85 ± 0.63 years for cases, 21.88 ± 4.07 years for T1+CMV- and 31.95 ± 2.13 years for healthy controls.

Plasma concentrations of different lymphocyte types were higher in the case group compared to controls (CD4: 7.21 ± 0.23 vs 5.71 ± 3.27 vs 2.07 ± 0.14, CD8: 13.73 ± 0.91 vs 10.01 ± 1.88 vs 1.27 ± 0.14; CD28: 45.95 ± 2. 18 vs 14.39 ± 1.99 vs 7.97 ± 1.96) with a statistically significant difference (p0.0001). The CTLA4 gene was overexpressed in the case group compared to the control group. The study of the correlation between CD4, CD8, CD28, and CTLA4 gene expression showed no direct relationship.

Conclusion: Our results showed that CMV infection could be an aggravating factor in T1D by promoting the over expression of the CTLA4 gene.

Keywords

CTLA4 gene, Type 1 diabetes, CMV infection.

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Introduction

Type 1 diabetes (T1D) is an autoimmune disease characterized by the destruction of pancreatic islet beta cells. This destruction is mainly mediated by CD4+ helper T cells and CD8+ cytotoxic T cells that induce cell death or apoptosis of β cells [1]. Recently, some researchers have questioned the autoimmune aspect of the disease. Type 1 helper T cells (TH1), producing key cytokines IFN-γ and TNF-α, were mainly detected in T1D-specific inflammation of the pancreatic islets [2]. The involvement of immune cells in the occurrence of T1D is well established. Several experimental and clinical arguments argue in favor of a contribution of immune system failures to the development of T1D. On the one hand, there are animal models such as the Non-Obese Diabetic Mouse (NOD), models that are certainly imperfect but reproduce several characteristics of human T1D. Experiments with models show the possibility to transmit the disease by transferring LT from a T1D mouse to a healthy mouse [3,4]. But other subsets of CD4+, CD8 T and CD28 T cells, producing e.g., IL-2, IL-4 and IL-10 that are likely to activate signaling pathways have also been found at student frequencies in the peripheral blood of T1D patients [2]. A variety of genetic and environmental factors contribute in influencing the pathogenesis of T1D. Some evidence has suggested that genes sensitive to T1D are associated with amplification of immune response and rate of disease progression [5]. This is the case of CTLA4 gene (Cytotoxic T-Lymphocyte associated protein 4), a T Helper cell receptor that functions as an immune checkpoint and a negative regulator of immune responses. It is expressed on activated T cells and CD28 and involved in the process of regulation and activation of T lymphocytes by antigen and immune presenting cells [6]. CTLA4 has been considered a permissive candidate gene involved in the etiology of autoimmune diseases. CTLA-4 plays a role in the regulation of T cell activity and interactions between T and B cells [7]. CTLA-4 consists of 4 exons and several polymorphisms have been described at the promoter region and exon 1 of this gene [8]. A single nucleotide polymorphism (SNP) present in the untranslated region 3' of exon1, leading to a substitution of an alanine for an amino acid threonine at position 49 (49A / G; rs231775), was associated with a predisposition T1D [8].

However, these studies have yielded conflicting results [9,10], such inconsistency could be due to the weak effect of polymorphism on T1D. Human cytomegalovirus, a member of the herpesviridae family, is a ubiquitous pathogen that constantly infects 60–90% of the world's population [11]. During active CMV infection, patients often suffer from immunological dysfunctions and autoimmune phenomena such as autoantibodies [11]. Multiple case reports describe primary, reactivating or persistent CMV infections as potential triggers of autoimmune endocrine diseases such as T1D [12]. Few studies have talked about the expression of CTLA4 gene in T1D patients. These studies were more likely oriented in the polymorphism of this gene in the pathogenesis of T1D.

That is the context in we conducted this work with general objective of measure the expression of CTLA4 gene in T1D patients with cytomegalovirus infection with Pointe Noire.

Materials and Methods

Study Population

We conducted an analytical case-control study with prospective data collection. The study was conducted over 6 months between June and November 2021. Our study population was made up of children with type 1 diabetes from the *"LIFE OF CHILDREN"* program of the General Adolphe Sicé Hospital in Pointe-Noire for cases. We included all T1D patients up to 25 years old at the discovery. For the control group included type 1 diabetic subjects with negative CMV serology (T1D + CMV-) and blood donors without stigma of autoimmunity or autoimmune disease (healthy controls).

Blood Samples Collection

An investigation sheet was used to collect the patients' socio-demographic data. Five mL of blood was collected from the elbow on dry and EDTA tubes. After centrifugation at 3000 rpm of the dry tube, the serum obtained was used for biochemical analysis. Molecular analysis was performed on whole blood collected in EDTA tube.

Biochemical Analyses

Biochemical analyses were performed in duplicate using the same package of kits in each case. DONOV ELISA kits (Shanghai, China) were used to determine the respective plasma concentrations of CD4, CD8 and CD28.

Molecular Analyses

Measurement of CTLA4 Gene Expression

RNA Extraction

Total RNA was extracted from blood collected on EDTA tube using the Total RNA blood purification kit (Invitrogen, USA) following the manufacturer's recommendation. After elution, the RNA was stored at -80°C until use.

Amplification of the CTLA4 gene

Amplification was performed in one step including reverse transcription and real-time PCR using the "One Step TB PrimeScriptTM RT-PCR kit" (TAKARA BIO INC, China).

The reaction was performed in a MIC thermal cycler (Bio Molecular systems, Canada) according to the following program: reverse transcription (42°C/5min, 95°C/10sec) and PCR reaction (95°C/5sec and 60°C/34sec) over 40 cycles.

As the kit is not specific to the target gene, we added primers specific to CTLA4 and B2-microglobin with the following sequences: CTLA4 (5'-CTACCTGGGCATAGGCAACG-3' and 5'-CCCCGAACTAACTGCTGCAA-3) and β2-microglobulin (5'-TCTACTTTGAGTGCTGTCCATGT-3 and 5-AAGTTGCCAGCCCTCCTAGAG-3).

The analysis of relative gene expression was performed using the double Delta method (ΔΔCT) or Livek method. It consisted in the

calculation of Relative Quantification (RQ) using the following formula:

$$RQ = 2^{e-(Ct\ gene\ cible - Ct\ gène\ controle)}$$

A negative value refers to an under expression of the gene and a positive value to an overexpression relative to the control gene [13]. The expression of CTLA4 in each sample was performed in duplicate and the level was normalized with respect to β2-microglobulin.

Ethical Approval

This study was carried out in accordance with the guidelines of the Declaration of Helsinki and was approved by the Health Research Ethics Board (HREB) of Marie Madeleine Gombes Foundation of Pointe Noire.

Statistical analysis

Data were analyzed using GraphPad prism software version 7 (SPSS Inc., Chicago, IL, USA). Results are presented as a percentage and as an average ± Standard Deviation (SD). An exact Fischer test was used to compare categorical variables. Unmatched T-tests and analysis of variance (ANOVA) were used to compare data normally distributed between study groups. The 95% confidence interval (CI) was calculated. The values of p under 0.05 were considered significant.

Results

Socio-Demographic Characteristics of the Study Population

68 T1D + CMV + patients were enrolled including 72 (55.38%) men and 58 (44.61%) women with extremes ranging from 11 years to 34 years for the case group. The control subjects consisted of two groups: 62 T1D+CMV patients (42 (55.38%) males and 20 (44.61%) females) with ages ranging from 11 to 34 years and 104 healthy blood donors (28 females and 52 males) with ages ranging from 18 to 36 years. The mean age of all patients was 20.8 5 ± 0.63 respectively for T1D +CMV+, 21.88 ± 4.07 years for T1D+CMV- and 31.95 ± 2.13 for healthy controls. At the discovery of the disease, 69.4% of patients were between 15 and 25 years old. The average life age with T1D was 3.9 ± 2.02 years. All results are shown in Table 1.

Table 1: Socio-demographic characteristics of our study groups.

Parameters	T1D+CMV+ n=68	T1D+CMV- n=62	Controls subjects n=104	p- value
Age	20.85 ± 0. 63	21.88 ± 4.07	31.95 ± 2. 13	**0.001**
Sex				
Men	42	42	76	
Women	14	20	28	
Weight	57.82 ± 1. 46	61.58 ± 7.76	72.00 ± 2. 82	0.36
Sizes	1.57 ± 0. 02	1.76 ± 0. 04	1.75 ± 0. 01	0.36
BMI	22.61 ± 3.89	23.72 ± 3.89	23.44 ± 1. 13	**0.007**

The results are presented under mean ± standard deviation. BMI: Body mass index, T1D+CMV: Type 1 diabetic patients with CMV infection, T1D+CMV-: Type 1 diabetic patients without CMV infection.

Determination of CD4, CD8 and CD28 Lymphocyte Concentrations

We measured CD4, CD8 and CD28 concentrations by ELISA. Whatever the control considered, a statistically significant difference was observed with the case group for CD4 and CD28 ($P<0.001$). On the other hand, a statistically significant difference was observed for CD8 only with the healthy controls ($P<0.001$). The results are shown in Tables 2 and 3 respectively.

Assessment of CTLA4 gene expression.

CTL4 gene expression was studied by real-time PCR using β2-microglobulin as a control gene. Expression was assessed by QR obtained by the double delta technique. Compared with the control gene β2-microglobulin, T1D+CMV+ patients expressed CTLA4 gene 32.6 times more than TD1+CMV- patients and 54.3 times more than healthy controls. The expression of the CTLA4 gene is represented in Table 4.

Study of the Correlation of the CTLA4 gene and CD8 and CD28 CD4

We tested whether the production of pro-inflammatory cytokines is correlated with CTLA4 gene expression. No statistical differences were observed between cytokine production and CTLA4 gene expression ($p >0.005$).

Discussion

The involvement of the immune system in the development of T1D has been demonstrated [2]. The aim of this study was to evaluate the expression of the CTLA4 gene in T1D patients with CMV infection from Pointe Noire.

The average age in our different study groups was 21, 22 and 32 years for cases, disease controls and healthy controls respectively. In his study in Gabon conducted on type 1 diabetic children, PAMBOU et al. found a mean age of 16 years [14]. The high mean age observed in our study can be explained by the fact that we included subjects older than 20 years, contrary to the Gabon study which was limited to patients aged 15 years or less.

The majority of patients in the case group, 69.4% had an age between 15-25 years at the time of discovery of the disease with an average age of life with T1D of about 4 years. Several authors in the literature have observed the same results, including studies conducted in Kenya and South Africa [15,16]. Men were the most represented in our study (55.38%) and sex ratio (M/F) was 1.2. Our data are consistent with those found in the literature [13-15]. This confirms the hypothesis that T1D affects more men compared to women. We noted significant differences between subjects in groups of case and control concerning BMI ($P< 0.007$). Our results

Table 2: CD4, CD8 and CD28 lymphocyte concentration between T1D+CMV+ and diseased controls (T1D+CMV-).

	Description			Regression uni-variable			
Characteristic	**T1D+CMV+, N=68[1]**	**T1D+CMV-, N = 62[1]**	**p-value[2]**	**N**	**OR[3]**	**95% CI[3]**	**p-value**
CD4	7.34 (1.85)	4.17 (1.80)	**<0.001**	130	**3.10**	1.84, 6.41	**<0.001**
CD8	14(7)	16 (7)	0.4	130	0.96	0.85, 1.05	0.4
CD28	47 (17)	18 (7)	**<0.001**	130	**1.61**	1.38, 2.00	**<0.001**

The results are presented under mean ± standard deviation. CD4: differentiation cluster 4, CD8: differentiation cluster 8, CD28: differentiation cluster 28, N: Mean, OR: Odds ratio, 95% CI: 95% confidence interval.

Table 3: Concentration of CD4, CD8 and CD28 lymphocytes between T1D+CMV- and healthy controls (blood donors).

	Description			Regression uni-variable			
Characteristic	**T1D+CMV+, N = 68[1]**	**Healthy controls, N = 104[1]**	**p-value[2]**	**N**	**OR[3]**	**95% CI[3]**	**p-value**
CD4	7.34 (1.85)	3.5 (2.30)	**<0.001**	172	**2.16**	1.18, 3.64	**<0.001**
CD8	13.8 (7.5)	4.4 (7.1)	0.4	172	1.17	1.10, 1.27	**<0.001**
CD28	46.5 (18.4)	18.7 (18.8)	**<0.001**	172	**1.10**	1.06, 1.15	**<0.001**

Table 4: CTLA4 gene expression between T1D+CMV+ and controls.

Patients	**EXPRESSION OF GENE CTLA4**							
	About Expression				**Under expression**			
	N	**∑ct CTLA4**	**∑Ct β 2-micro**	**RQ**	**N**	**∑Ct CTLA4**	**∑Ct β 2-micro**	**RQ**
T1D+CMV+	66	16	28	65,2	2	29	28	-4.43
T1D+CMV-	9	22	28	32,6	53	31	28	-2.43
Normal controls	1	26	28	10,9	102	33	28	-043

The results are presented as mean ± standard deviation, ∑ct CTLA4: Mean CT gene CTLA4, ∑ Ct β 2-micro: Mean Ct gene β 2-microglobulin, RQ: Relative quantification.

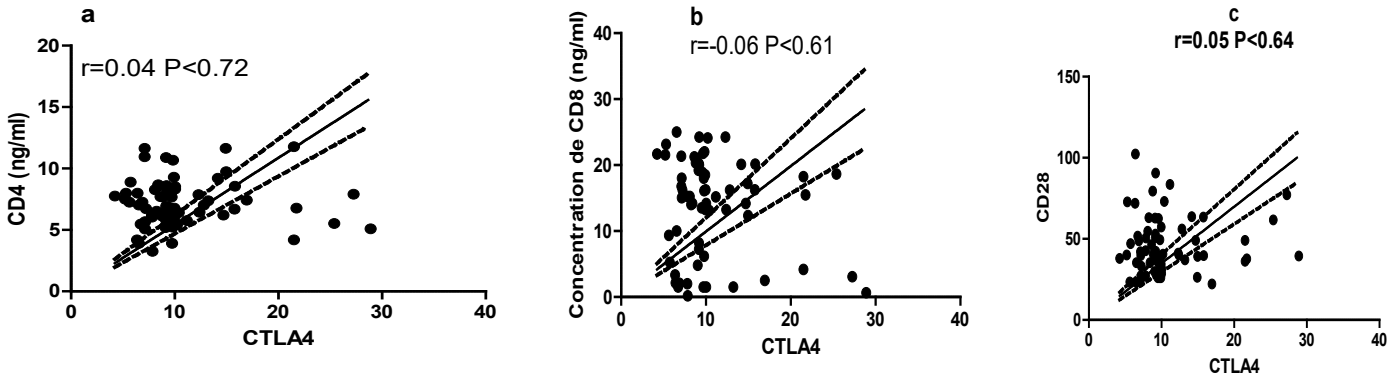

Figure 1: Correlation between the production of pro-inflammatory cytokines and the expression of CTLA4 gene: a) Correlation between CD4 and CTLA4; *b) Correlation between CD28 and CTLA4; c) Correlation between CD8 and CTLA4.*

are consistent with those of YIMANGOU et al. [17] who found similar results showing that the BMI of T1D subjects was lower than that of controls. These data would be explained by the fact that T1D is a diabetes of the lean subject in underweight compared to the group of healthy controls.

The plasma concentration of different types of CD4, CD8 and CD28 lymphocytes was measured. A statistically significant difference was observed between subjects in the T1D+CMV+ group and those in the two control groups (p<0.0001). Our data corroborate those found in the literature [15,18]. They are consistent with the study conducted by Wargner et al. which returned to a high CD28 concentration in T1D patients since CD28 is a co-stimulation pathway for TCD4 lymphocytes. In addition, blocking this pathway could serve as a therapeutic approach in the treatment of T1D [18].

These results can be explained by the fact that T1D is an autoimmune disease caused by the breakdown of the immune system's tolerance between self and non-self. Indeed, TCD4 and TCD8 lymphocytes thus activated change their differentiation pathways to an effector-type phenotype thus participating in the destruction of cells β islets of Langerhans [19-23].

We studied the expression of the CTLA4 gene between the different groups. The evaluation of expression was made using the RQ obtained against the control gene β2-microglobulin. We noticed an overexpression of the CTLA4 gene which was 53.3 times more expressed in the group of cases compared to the group of sick controls and 32.6 times compared to the group of healthy control. Our data are consistent with studies conducted by Gunavathy et al. as well as Nikman et al. [24,25]. There are several reasons for this: i) Failure of the immune system because the CTLA4 gene is an immune checkpoint to moderate the immune response by acting as a switch and inhibiting the action of self-reactive T cells, ii) Our study was conducted as part of a CMV infection, a study conducted by Atandi et al. [26] in 2021 to reveal that CMV was responsible for hypoinsulinemia in T1D patients so we can establish the same link in this study. The study conducted by Weitz et al. [27] in 2011 showed that the persistence of CMV infection contributes to a co-stimulation of the polymorphism of the CTLA4 gene including the CTLA4+49A / G mutation which is strongly involved in the occurrence of T1D. This fact would show that CMV is an aggravating factor for T1D and would promote the overexpression of the CTLA4 gene in T1D.

The study of the correlation between CTLA4 and CD4, CD8 and CD28 did not show a direct link between the CTLA4 gene and CD4, CD8 and CD28 lymphocyte concentrations. Our data are contrary to those obtained in the literature [28-36]. Indeed, Klak et al. [28] having worked on several genes observed a statistically significant difference between the different study groups.

Conclusion

In this study, the authors observed that cytomegalovirus infection is an aggravating factor of T1D because it promotes overexpression of the CTLA4 gene and increases in CD4, CD8 and CD28 plasma concentration.

Thanks

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References

1. Atkinson MA, Maclaren NK. The pathogenesis of insulin-dependent diabetes mellitus. N.Engl.J. Med. 1994; 331: 1428-1436.
2. Waugh K, Snell-Bergeon J, Michels A, et al. Increased inflammation is associated with islet autoimmunity and type 1 diabetes in the Diabetes Autoimmunity Study in the Young (DAISY). PloSone. 2017; 12: e0174840.
3. Kent SC, Chen Y, Bregoli L, et al. Expanded T cells from pancreatic lymph nodes of type 1 diabetic subjects recognize an insulin epitope. Nature. 2005; 435: 224-228.
4. Hoglund P, Mintern J, Waltzinger C, et al. Initiation of autoimmune diabetes by developmentally regulated presentation of islet cell antigens in the pancreatic lymph nodes. J. Exp. Med. 1999; 189: 331-339.
5. Field LL. Genetic linkage and association studies of type 1 diabetes: challengesand rewards. Diabetologia. 2002; 45: 21-35.
6. Walunas TL, Bakker CY, Bluestone JA. CTLA4 ligation blocks CD28-dependent T cell activation. J. Exp. Med. 1996; 183: 2541-2550.
7. Lane P. Regulation of T and B cell responses by modulating inter-actions between CD28/CTLA4 and their ligands, CD80 and CD86. Ann. N. Y. Acad. Sci. 1997; 815: 392-400.
8. Kumar N, Kaur G, Kanga U, et al. CTLA4+49G allele associates with early onset of type 1 diabetes in North Indians. Int J Immunogenet. 2015; 42: 445-452.
9. Payne F, Cooper JD, Walker NM, et al. Interaction analysis of the CBLB and CTLA4 genes in type 1 diabetes. Journal of Leukocyte Biology. 2007; 8583.
10. Chen Y, Chen S, Gu Y, et al. CTLA-4 +49 G/A, a functional T1D risk SNP, affects CTLA-4 level in Treg subsets and IA-2A positivity, but not beta-cell function. Sci Rep. 2018; 4: 8.
11. Kenneson A, Cannon MJ. Review and meta-analysis of the epidemiology of congenital cytomegalovirus (CMV) infection. Rev Med Virol. 2007; 17: 253-276.
12. Marc S Horwitz, Nora Sarvetnick. Virus and autoimmunity, Annales de l'Institut Pasteur/ Actualités. 1996; 7: 2.
13. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001; 25: 402-408.
14. Pambou Damiens A, Ganga –Zandzou PS, Tsoucka Ibounde E, et al. Type 1 diabetes mellitus in Gabon: A study of epidemiological aspects. Int J Pediatr Adolesc Med. 2019; 6: 87-91.
15. Palmer T, Waliaula C, Shannon G, et al. Understanding the Lived Experience of Children With Type 1 Diabetes in Kenya: Daily Routines and Adaptation Over Time. Qual Health Res. 2022; 32: 145-158.
16. Pirie FJ, Jairam V, Paruk IM, et al. High frequency of hypoglycaemia in patients with type 1 diabetes mellitus attending a tertiary diabetes clinic in Durban, South Africa. Diabetes Res Clin Pract. 2019; 155: 107783.
17. Lontchi-Yimagou E, Dasgupta R, Anoop S, et al. An atypical form of diabetes among individuals with low BMI. Diabetes Care. 2022; 45: 1428-37.
18. Wagner DH Jr. Overlooked Mechanisms in Type 1 Diabetes Etiology: How Unique Costimulatory Molecules Contribute to Diabetogenesis. Front Endocrinol (Lausanne). 2017; 23; 8: 208.
19. Silva DG, Daley SR, Hogan J, et al. Anti-islet autoantibodies trigger autoimmune diabetes in the presence of an increased frequency of islet-reactive CD4 T cells. Diabetes. 2011; 60: 2102-11.
20. Seyfarth J, Mütze N, Antony Cruz J, et al. CD4+ T-Cells With High Common γ Chain Expression and Disturbed Cytokine Production Are Enriched in Children With Type-1 Diabetes. Front. Immunol. 2019; 10: 820.

21. Diana J, Simoni Y, Furio L, et al. Crosstalk between neutrophils, B-1a cells and plasmacytoid dendritic cells initiates autoimmune diabetes. Nat Med. 2013; 19: 65-73.
22. Bian MLO, Haigh D, Munster M, et al. Reactivated CD4+Tm cells of T1D patients and siblings display an exaggerated effector phenotype with heightened sensitivity to activation-induced cell death. Diabetes. 2015; 64: 2161-2171.
23. Yamina H, François-Xavier M, Hang-Phuong P, et al. A unique CD8+ T lymphocyte signature in pediatric type 1 diabetes. Journal of Autoimmunity. 2016; 73: 54-63.
24. Gunavathy N, Asirvatham A, Chitra A, et al. Association of CTLA-4 and CD28 Gene Polymorphisms with Type 1 Diabetes in South Indian Population. Immunol Invest. 2019; 48: 659-671.
25. Niknam A, Karimi MH, Yaghobi R, et al. The Association Between Viral Infections and Co-stimulatory Gene Polymorphisms in Kidney Transplant Outcomes. Jundishapur J Microbiol. 2016; 17; 9: e31338.
26. BATCHY AA, BOUMBA LMA, ELENGA-BONGO C, et al. Human Cytomegalovirus Infection Associated with Low Insulin Secretion in a Type 1 Diabetic Population in Pointe Noire. Med Clin Res Open Access. 2022; 3: 1-5.
27. Weitz M, Kiessling C, Friedrich M, et al. Persistent CMV infection correlates with disease activity and dominates the phenotype of peripheral CD8+ T cells in psoriasis. Exp Dermatol. 2011; 20: 561-567.
28. Klak M, Gomółka M, Kowalska P, et al. Type 1 diabetes: genes associated with disease development. Cent Eur J Immunol. 2020; 45: 439-453.
29. Silva DG, Daley SR, Hogan J, et al. Anti-islet autoantibodies trigger autoimmune diabetes in the presence of an increased frequency of islet-reactive CD4 T cells. Diabetes. 2011; 60: 2102-11.
30. Seyfarth J, Mütze N, Antony Cruz J, et al. CD4+ T-Cells With High Common γ Chain Expression and Disturbed Cytokine Production Are Enriched in Children With Type-1 Diabetes. Front. Immunol. 2019; 10: 820.
31. Diana J, Simoni Y, Furio L, et al. Crosstalk between neutrophils, B-1a cells and plasmacytoid dendritic cells initiates autoimmune diabetes. Nat Med. 2013; 19: 65-73.
32. Curran M, Campbell J, Drayson M, et al. Type 1 diabetes impairs the mobilisation of highly-differentiated CD8+T cells during a single bout of acute exercise. Exerc Immunol Rev. 2019; 25: 64-82.
33. Tai Y, Wang Q, Korner H, et al. Zhang L, Wei W. Molecular Mechanisms of T Cells Activation by Dendritic Cells in Autoimmune Diseases. Front Pharmacol. 2018; 9: 642.