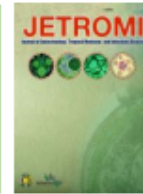**JETROMI**

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Type 1 Diabetes and Cytomegalovirus Infection: Cytokine and T Lymphocytes Profile in Pointe Noire, Congo

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Abstract

Background: Type 1 Diabetes (T1D) is an autoimmune disease characterized by the destruction of beta cells in the pancreatic islets of Langerhans. This study aimed to investigate the T lymphocyte pathway involved in cytomegalovirus (CMV) infection in T1D patients in the context of. in Pointe Noire

Method: We conducted an analytical case-control study over 6 months between June and November 2022. A total of 234 subjects were enrolled, including 68 (T1D +CMV+) cases, 62 (T1D +CMV-) cases, and 104 healthy subjects as the control group (healthy controls). The plasma concentrations of CD4, CD8, CD28, IL2, IL4, and IL10 were measured using ELISA. Linear regression analysis was performed to explore the correlation between T lymphocyte types (CD4, CD8, and CD28) and interleukins.

Results: In the case group, the average age was 20.85±0.63 years for (T1D+CMV+) cases, 21.88±4.07 years for (DT1+CMV-) and 31.95±2.13 years for healthy controls. Men were the majority in the study, representing 55.38%, with a male-to-female ratio of 1:2. Plasma concentrations of different types of lymphocytes were higher in the case group compared to the controls CD4 (7,21±0,23 vs 5,71±3,27 vs 2,07±0,14; p<0,0001); CD8 (13,73±0,91 vs 10,01±1,88 vs 1,27±0,14 p <0,0001); CD28 (45.95±2.18 vs 14.39±1.99 vs 7.97±1.96; p<0,0001); IL2 (1048.0±43.47 vs 252.0±10.91 vs 52.91±23.95; p<0,0001); IL4 (474.3±18.45 vs 279.3±169.2 vs 194.9±136.2); IL10 (275.0±134.0 vs 206.0±84.77 vs 44.62±7.22; p <0,0001), and (CD4, CD8, CD28, IL2, IL4, and IL10) showed significant elevation in the

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case group compared to the controls. The study also revealed a direct correlation between CD4 and CD28.

Conclusion: These findings suggest that CMV infection worsens T1D by promoting the increase in CD4, CD8, and CD28 lymphocytes as well as plasma concentrations of interleukins (IL2, IL4, and IL10), and no correlation was observed with CD8.

Keywords: Type 1 Diabetes, CMV Infection, Pro-Inflammatory Cytokines, T lymphocytes.

Abstrak

Latar Belakang: Diabetes Tipe 1 (T1D) adalah penyakit autoimun yang ditandai dengan penghancuran sel beta di pulau pankreas Langerhans. Penelitian ini bertujuan untuk menyelidiki jalur limfosit T yang terlibat dalam infeksi cytomegalovirus (CMV) pada pasien T1D dalam konteks. di Pointe Noire

Metode: Kami melakukan studi kasus-kontrol analitik selama 6 bulan antara Juni dan November 2022. Sebanyak 234 subjek terdaftar, termasuk 68 kasus (T1D + CMV +), 62 kasus (T1D + CMV-), dan 104 subjek sehat sebagai kelompok kontrol (kontrol sehat). Konsentrasi plasma CD4, CD8, CD28, IL2, IL4, dan IL10 diukur menggunakan ELISA. Analisis regresi linier dilakukan untuk mengeksplorasi korelasi antara jenis limfosit T (CD4, CD8, dan CD28) dan interleukin.

Hasil: Pada kelompok kasus, usia rata-rata adalah 20,85±0,63 tahun untuk kasus (T1D + CMV +), 21,88±4,07 tahun untuk (DT1 + CMV-) dan 31,95±2,13 tahun untuk kontrol sehat. Laki-laki adalah mayoritas dalam penelitian ini, mewakili 55,38%, dengan rasio laki-laki dan perempuan 1: 2. Konsentrasi plasma dari berbagai jenis limfosit lebih tinggi pada kelompok kasus dibandingkan dengan kontrol CD4 (7,21±0,23 vs 5,71±3,27 vs 2,07±0,14; p<0,0001); CD8 (13,73±0,91 vs 10,01±1,88 vs 1,27±0,14p <0,0001); CD28 (45,95±2,18 vs 14,39±1,99 vs 7,97±1,96; hal<0,0001); IL2 (1048,0±43,47 vs 252,0±10,91 vs 52,91±23,95; hlm<0,0001); IL4 (474,3±18,45 vs 279,3±169,2 vs 194,9±136,2); IL10 (275,0±134,0 vs 206,0±84,77 vs 44,62±7,22; p <0,0001), dan (CD4, CD8, CD28, IL2, IL4, dan IL10) menunjukkan peningkatan yang signifikan pada kelompok kasus dibandingkan dengan kontrol. Studi ini juga mengungkapkan korelasi langsung antara CD4 dan CD28.

Kesimpulan: Temuan ini menunjukkan bahwa infeksi CMV memperburuk T1D dengan mempromosikan peningkatan limfosit CD4, CD8, dan CD28 serta konsentrasi plasma interleukin (IL2, IL4, dan IL10), dan tidak ada korelasi yang diamati dengan CD8.

Kata kunci: Diabetes Tipe 1, Infeksi CMV, Sitokin Pro-inflamasi, T Limfosit.

1   Introduction

Type 1 Diabetes (T1D) is an autoimmune disease characterized by the destruction of beta cells in the pancreatic islets of Langerhans. This destruction is primarily mediated by CD4+ helper T lymphocytes and CD8+ cytotoxic T lymphocytes, which induce cell death or apoptosis of beta cells.[1] Recently, some researchers have challenged the purely autoimmune nature of the disease. T1DM-specific inflammation has been associated with type 1 helper T cells (TH1), which produce key cytokines such as IFN-γ and TNF-α.[2] The involvement of immune cells in the onset of T1D is well established. Several experimental and clinical arguments support the contribution of immune system failures to the development of T1D. Notably, animal models such as the Non-Obese Diabetic (NOD) mouse, though imperfect, reproduce several characteristics of human T1D. Experiments in these models have shown that it is possible to transmit the disease by transferring T1D mouse lymphocytes to healthy mice.[3-4] Other subsets of CD4+ T

lymphocytes, CD8 T lymphocytes, and CD28 T lymphocytes producing cytokines such as IL2, IL4, and IL10, which can activate signaling pathways, have also been found at high frequencies in the peripheral blood of DT1 patients.[5]

Indeed, pro-inflammatory cytokines IL1β, IFN-γ, and TNF-α play a significant role in T1D by activating inflammatory cascades and endoplasmic reticulum stress within β-cells, ultimately leading to cell apoptosis.[2] Human cytomegalovirus (CMV), a member of the herpesviridae family, is a ubiquitous pathogen that constantly infects 60 to 90% of the global population.[5] During active CMV infection, patients often experience immunological dysfunctions and autoimmune phenomena such as autoantibodies.[6] Multiple case reports describe primary, reactivating, or persistent CMV infections as potential triggers for autoimmune endocrine diseases, including T1D.[5]

It is important to bear in mind that the true cause of the disease, whether autoimmunity or inflammation, remains unknown to this day. Therefore, CD4+ lymphocytes-T can be divided into two groups based on their cytokine secretion (TH1 and TH2). IL2 cytokines secreted by TH1 cells activate cytotoxic-type reactions by stimulating cytotoxic CD8+ lymphocytes-T. Few studies in the sub-region have investigated the pathway of lymphocytes involved in the production of pro-inflammatory cytokines. The general objective of this study was to determine the lymphocyte pathway involved in the production of pro-inflammatory cytokines in T1D patients in the context of cytomegalovirus in Pointe Noire. This study aimed to investigate the lymphocyte T pathway involved in the production of pro-inflammatory cytokines in T1D patients in Pointe Noire in the context of CMV infection.

2   Methods

We conducted an analytical case-control study with prospective data collection. The study took place between June and November 2021, spanning 6 months. Our study population consisted of two groups: the cases, which included children with T1DM from the "Life of Children" program at the Adolph Sicé General Hospital in Pointe-Noire. We included all T1D patients aged 25 years or younger at the time of diagnosis. For the control group, we selected individuals with T1D who had a negative CMV serology (T1D+CMV-) and healthy blood donors without any signs of autoimmunity or autoimmune diseases (Healthy Controls). A survey form was used to gather sociodemographic data from the patients. Five milliliters of blood were collected from the elbow into both plain tubes and EDTA tubes. After centrifugation at 3000 rpm, the serum obtained from the plain tubes was used for the biochemical analyses. Molecular analysis was performed on the total blood collected in EDTA tubes.

Biochemical analyses were performed in duplicate using the same set of kits in each case. DONOV ELISA kits from Shanghai, China, were used to determine the respective plasma concentrations of CD4, CD8, CD28, IL2, IL4, and IL10.

This study was conducted following the guidelines of the Helsinki Declaration and was approved by the Research Ethics Committee in Health (CERS) of the Marie Madeleine Gombes Foundation in Pointe-Noire.

Statistical Analyses

The data were analyzed using GraphPad Prism version 7 software (SPSS Inc., Chicago, IL, USA). Results are presented as percentages and mean ± standard deviation. The Fisher's exact test was used to compare categorical variables. Unpaired T-tests and analysis of variance (ANOVA) were used to compare normally distributed data between study groups. A 95% confidence interval (CI) was calculated. p-values less than 0.05 were considered significant.

3   Results

A total of 68 patients with T1D + CMV+ were enrolled, including 72 (55.38%) males and 58 (44.61%) females, with ages ranging from 11 to 34 years for the case group. The control subjects consisted of two groups: 62 patients with T1D + CMV (42 (55.38%) males and 20 (44.61%) females) aged 11 to 34 years and 104 healthy blood donors (28 females and 52 males) aged 18 to 36 years. The mean age of all patients was 20.85±0.63 years for T1D + CMV+; 21.88±4.07 years for (T1D+CMV-); and 31.95±2.13 years for healthy controls. At the time of disease discovery, 69.4% of the patients were between 15 and 25 years old. The mean duration of living with T1D was 3.9±2.02 years. All results are presented in Table I.

Table 1 Demographic Characteristics of Study Groups

Parameters	(T1D+CMV+) n=68	(T1D+CMV-) n=62	Control n=104	p-value
Age (yr)	20.85±0.63	21.88±4.07	31.95±2.13	**0.001**
Gender				
Male	42	42	76	
Female	14	20	28	
Weight (Kg)	57.82±1.46	61.58±7.76	72.00±2.82	0.36
Sizes (M)	1.57±0.02	1.76±0.04	1.75±0.01	0.36
BMI (Kg/M^2)	22.61±3.89	23.72±3.89	23.44±1.13	**0.007**

The concentrations of CD4, CD8, and CD28, as well as the plasma concentrations of IL2, IL4, and IL10. The results are presented in Table 2.

Table 2 Lymphocytes and Interleukins types between (T1D+CMV+) and (T1D+CMV-)

| Characteristic	n	(T1D+CMV+) (n=68)	(T1D+CMV-) (n= 62)	p-value	OR	95% CI
CD4	130	7.34 (1.85)	4.17 (1.80)	**0.001**	**3.10**	1.84, 6.41
CD8	130	14(7)	16 (7)	0.4	0.96	0.85, 1.05
CD28	130	47 (17)	18 (7)	**0.001**	**1.61**	1.38, 2.00
IL2	130	1043 (350)	689 (544)	**0.041**	**1.00**	1.00,1.01
IL4	130	483 (147)	285 (366)	**0.002**	**1.01**	0.99,1.03
IL10	130	283 (1.106)	127 (60)	0.60	**1.00**	1.00,1.02

Univariate regression

The concentrations of CD4, CD8, and CD28 using ELISA. Regardless of the control group considered, a statistically significant difference was observed with the case group for CD4 and CD28 (p<0.001). However, a statistically significant difference was only observed for CD8 when compared to healthy controls (p<0.001). The results are presented in Tables 2 and 3.

Table 3 Concentration of CD4, CD8, and CD28 lymphocytes between DT1+CMV- and healthy controls (Blood Donors).

| Characteristic	n	(T1D+CMV+) n=68	Control n=104	p-value	OR	95% CI
CD4	172	7.34 (1.85)	3.5 (2.30)	**0.001**	**2.16**	1.18, 3.64
CD8	172	13.8 (7.5)	4.4 (7.1)	0.4	1.17	1.10, 1.27
CD28	172	46.5 (18.4)	18.7 (18.8)	**0.001**	**1.10**	1.06, 1.15
IL2	172	1043(350)	390.2(508.2)	**0.001**	**1.00**	1.00, 1.01
IL4	172	483 (147)	285(366)	**0.001**	**1.00**	1.00, 1.01
IL10	172	283(1.106)	66.8 (45.2)	**0.001**	**1.04**	1.00, 1.01

Univariate regression

The production of pro-inflammatory cytokines is correlated with the CD4 T-cell pathway. Our data showed a significant difference between the concentration of CD4 and that of IL10 with a negative correlation factor (p < 0.01 and r = -0.20) (Figure 1)
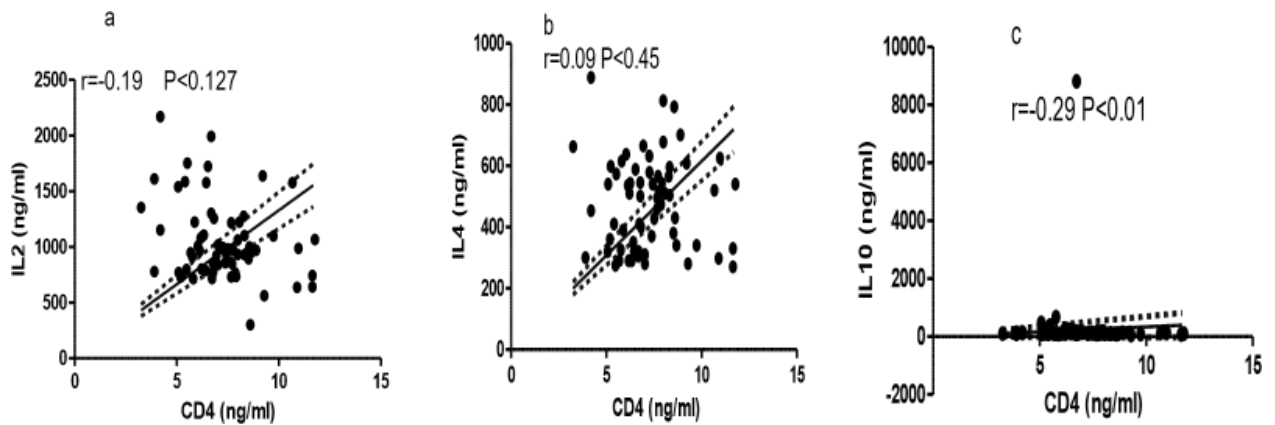
Figure 1 Correlation between the production of pro-inflammatory cytokines and the expression of the CTLA4 gene: a) Correlation between CD4 and IL2; b) Correlation between CD4 and IL4; c) Correlation between CD8 and IL10.

The production of pro-inflammatory cytokines is correlated with the CD8 T-cell pathway. Our data did not show any difference between the concentration of CD8 and that of IL2, IL4, and IL10 (Figure 2).
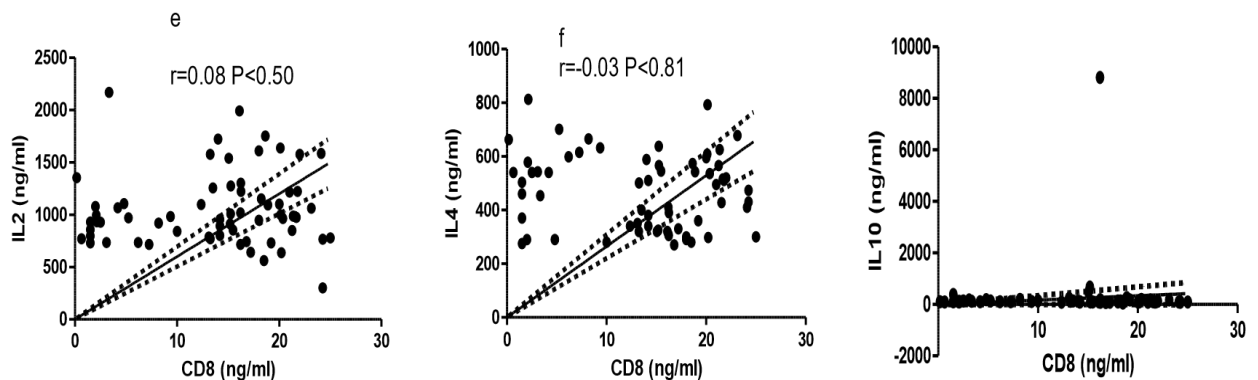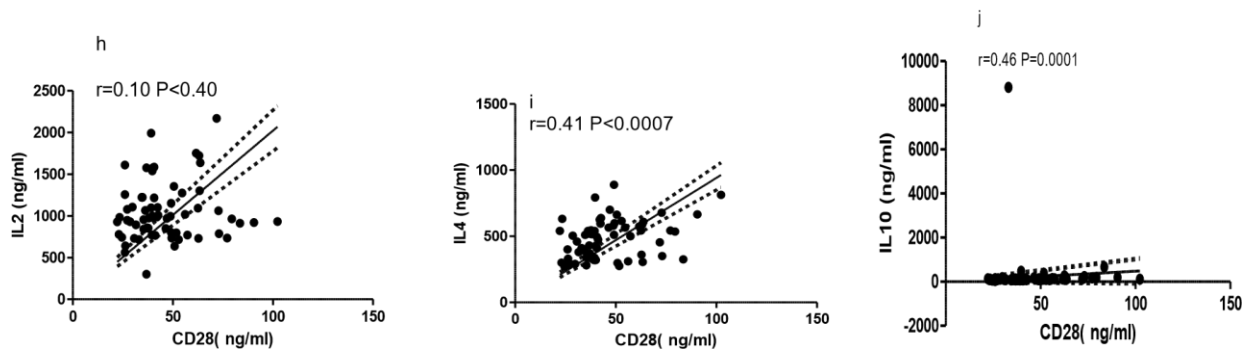

Figure 2 Correlation between the production of pro-inflammatory cytokines and the expression of the CTLA4 gene: d) Correlation between CD8 and IL2; e) Correlation between CD8 and IL4; f) Correlation between CD8 and IL10.

The correlation study between CD28 concentrations and IL2, IL4, and IL10 cytokines showed significant differences, respectively, with $p < 0.0007$ and 0.0001 (Figure 3).

Figure 3 Correlation between the production of pro-inflammatory cytokines and the expression of the CTLA4 gene: g) Correlation between CD28 and IL2; h) Correlation between CD28 and IL4; i) Correlation between CD28 and IL10.

4   Discussion

The involvement of the immune system in the onset of T1D is well established. The mean age in our different study groups was 21, 22, and 32 years for cases, disease controls, and healthy controls, respectively. In their study in Gabon on T1D children, PAMBOU et al. (2019) found a mean age of 16 years.[7-14] The higher average age observed in our study can be explained by the fact that we included subjects over 20 years old, unlike the Gabonese study, which was limited to patients aged 15 years or younger. The majority of patients in the case group, 69.4%, were between 15 and 25 years old at the time of disease discovery, with an average duration of 4 years living with DT1. Several authors in the literature have observed similar results, including studies conducted in Kenya and South Africa.[8.9] Men were the most represented in our study (55.38%), with a male-to-female ratio of 1.2. Our data are consistent with those found in the literature,[10,11] confirming the hypothesis that T1D affects men more than women. We noted significant differences between the subjects in the case and control groups regarding body mass index (BMI) ($p < 0.007$). Our results are consistent with those of YIMANGOU et al.; (2015),[12] who found similar results showing that the BMI of T1D subjects was lower than that of the healthy controls. These data could be explained by the fact that T1D is a lean-type diabetes, and T1D subjects tend to be underweight compared to healthy controls.

The results of our study showed significant differences between CD4, CD8, and CD28 T lymphocytes in T1D subjects compared to the control group. The plasma concentrations of CD4, CD8, and CD28 T lymphocytes in our study subjects were significantly higher compared to the control group ($p < 0.0001$, OR: 3.10; OR: 0.96; OR: 1.61 for (T1D+CMV+) vs. (T1D+CMV-); and $p < 0.0001$, OR: 2.16 for (T1D+CMV+) vs. healthy controls, respectively). Our data corroborate with those found in the literature.[13,14] This can be explained by the fact that T1D is an autoimmune disease caused by the breakdown of tolerance, leading to activated CD4, CD8, and CD28 T lymphocytes, which then change their differentiation pathway to an effector phenotype, thus participating in the destruction of pancreatic β-cells.[15,16] It is also important to consider the role of CMV; multivariate analysis showed that the odds ratio was relatively

higher, indicating that (T1D+CMV+) subjects have a 3 times higher chance of having elevated plasma concentrations of CD4, CD8, and CD28 T lymphocytes. This could be explained by the fact that pancreatic damage is more severe in T1D+CMV (+) subjects than in T1D+CMV (-) subjects, as CMV has a lytic action on the pancreatic β-cells responsible for insulin production.[17] CMV could be involved in accelerating the pancreas's failure to compensate for insulin resistance through at least two possible mechanisms. By directly influencing pancreatic cells, it could act indirectly by influencing the immune system, which, in turn, would affect the pancreas. According to the first possibility, CMV could infect and reside in pancreatic cells without causing cytopathic effects but still influence insulin production directly after repeated reactivations.[18] Furthermore, Hiemstra et al., in their study, demonstrated that CMV can be associated with the loss of tolerance of T cells to the GAD65 autoantigen, leading to a molecular mimicry mechanism resulting in autoimmunity, which would explain the increased elevation of CD4, CD8, and CD28 T lymphocyte concentrations in patients with CMV infection.[19]

In addition, we measured the plasma concentrations of IL2, IL4, and IL10 in our study groups. The plasma concentration of IL2 in our study subjects was significantly higher compared to the control group (p < 0.0001). Several scientific authors in the literature have found similar results.[21,22] This can be explained by the constant hyperglycemia present in T1D, chronic inflammation of pancreatic β-cells, and the activation of CD4 T lymphocytes, with possible consequences on the long-term course and severity of type 1 diabetes. IL2 may contribute to activating the TH2 stimulation pathway, which is highly cytotoxic.[21]

The plasma concentration of IL4 in our study subjects was significantly higher compared to the control group (p < 0.0001). Our data are in agreement with those of Ukah et al. (2017). This could be explained by the breakdown of immune system tolerance, especially with the expansion of CD4 T lymphocytes, which in turn secrete IL4, stimulating the proliferation of Th2 cells (auto-amplification) and inhibiting that of Th1 cells.[21]

The plasma concentration of IL10 in our study subjects was significantly higher compared to the control group (p < 0.0001). Our data are in agreement with those of LU et al. (2020). A high concentration of IL10 could be due to the breakdown of immune tolerance mechanisms because IL10 plays a role in reducing the immune system's action. This also indicates the severity of the disease in our patients.[23] Furthermore, CMV infection of pancreatic β-cells induces the release of pro-inflammatory cytokines and increased cellular immunogenicity in the pancreas, which suggests that T1D patients with infection have more severe pancreatic damage than those without infection.

Finally, we conducted a study of the correlation between pro-inflammatory cytokines and interleukins to identify the lymphocyte activation pathway. The correlation study between CD4 and IL2, IL4, and IL10 showed a significant difference between CD4 and IL10 with a negative

correlation factor (r = -0.29, p < 0.001). These data suggest that the production of IL10 may be related to the increased CD4 concentration. However, the analysis did not show any difference between CD4 and the other cytokines in our study, suggesting no direct or indirect relationship between the production of IL4 and IL10 and the increased CD4 concentration. Our data are similar to those of some authors regarding the CD4 and IL10 relationship.[19,20] These differences could be explained by the age of our study population and the methods used. Several studies used Western blot techniques for cytokine expression and flow cytometry to quantify CD4, suggesting an indirect link between increased CD4 and IL10. This shows that our patients follow the TH2 activation pathway. Literature data suggest that IL10's role is to decrease the immune system's effect because, in the context of T1D, the immune system is deficient due to the breakdown of tolerance mechanisms. IL10 could be a biomarker in assessing the severity of the disease.[23] Finally, our study was conducted in the context of CMV infection.

The correlation study between CD8 and IL2, IL4, and IL10 did not show a direct link between CD8 and interleukins. These data show that there is no direct link between the activation of CD8 and the secretion of interleukins. The high concentration of CD8 is not directly related to interleukins, but rather to an autoimmune mechanism.[17]

The correlation study between CD28, IL2, IL4, and IL10 showed a direct link between CD28 and interleukins, particularly (r = 0.10, p < 0.40 for IL2; r = 0.41, p < 0.0007 for IL4; and r = 0.46, p < 0.0001 for IL10). These data show that the high concentration of CD28 is closely related to IL2 and IL10. This can be explained by the fact that CD28 is a co-stimulator for the expansion of CD4 T lymphocytes.[23]

5   Conclusion

In this study, the authors observed that CMV is an aggravating factor of T1D because it promotes an increase in the concentration of CD4, CD8, and CD28 T lymphocytes, and there is a link between the elevation of immune cell concentrations and pro-inflammatory cytokines. A direct link has been established between CD28 and IL4 and IL10. A more in-depth study of this pathway could be a therapeutic approach for patients.

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