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Artigo / Article

Correlation of IL-6 and IL-10 production following bone marrow transplantation with donor cytokine gene polymorphisms

Correlação da produção de IL-6 e IL-10 seguindo o transplante de medula óssea com os polimorfismos de genes de citocinas do doador

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Several candidate gene studies have demonstrated that genetic polymorphisms in cytokine genes contribute to variations in the levels of cytokines produced and this variation may influence the occurrence and severity of complications after stem cell transplantation (HSCT). In this work we compared the serum concentrations of TNF-α , IFN-γ , IL-6, IL-10, and TGF-β1 in 13 recipients following HSCT with the TNF^{-308}*, IFNG*$^{+874}$*, IL6*$^{-174}$*, IL10*$^{-1082,-819,-592}$*, and TGFB1*$^{+869,+915}$ *polymorphisms. Serum cytokine levels were assessed using commercial ELISA kits for TNF-α, IFN-γ , IL-6, IL-10, and TGF-β1 (BioSource®, Nivelles, Belgium, Europe). Donor/recipient genotypes for these cytokine polymorphisms were analyzed by polymerase chain reaction-sequence-specific primer (PCR-SSP) with the Cytokine Genotyping Primers Kit (One Lambda , Canoga Park, CA, USA). We found correlation between the levels of IL-6 and IL-10 concentrations following HSCT and the IL6*$^{-174}$ *and IL10*$^{-1082,-819,-592}$ *polymorphisms, but not for other cytokines investigated in this study. Those with genotypes associated with low production of IL-6 and IL-10 produced lower levels of these cytokines than those with genotypes associated with high or intermediate production of these cytokines (P < 0.05). Rev. Bras. Hematol. Hemoter. 2008;**30**(6):475-479.*

Key words: *Cytokine; polymorphism; GVHD; haematopoietic stem cell transplantation.*

Introduction

Complex networks of cytokines dynamically interact homeostatically to regulate immune responses and other biological pathways. Variations in cytokine levels have been correlated with disease susceptibility and outcomes of transplantation.[1-8]

Investigating genetic host factors and immune responses may help to understand associations with complications after stem cell transplantation (HSCT), such as graft-versus-host disease (GVHD). Several candidate gene studies have demonstrated that genetic polymorphisms in cytokine genes contribute to variations in the levels of cytokines produced and this variation may influence the occurrence and severity of post-transplantation complications. [9-21]

In this work we compared the serum concentrations of TNF-α, IFN-γ , IL-6, IL-10, and TGF- β1 in 13 recipients following HSCT with the *TNF*$^{-308}$, *IFNG*$^{+874}$, *IL6*$^{-174}$, *IL10*$^{-1082,-819,-592}$, and *TGFB1*$^{+869,+915}$ polymorphisms.

Patients and Method

Patients

Thirteen consecutive patients, who had undergone allogeneic HLA-identical HSCT in our center from April to December 2000 and had serum samples available, were

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enrolled in this study after informed consent. Characteristics of the patients are listed in Table 1. Transplanted individuals were followed up during 15 weeks after transplantation. This study was performed after approval of the Research Ethics Committee of the University Hospital of Campinas State University.

Transplantation procedures

All of the patients were prepared for HSCT according to appropriate protocols for their underlying and other diseases, as described elsewhere.[22] A summary of pretreatment preparative regimens and GVHD prophylaxis is shown in Table 1. Grading of acute and chronic GVHD was performed using the Glucksberg *et al*.[23] and Atkinson *et al*.[24] criteria, respectively.

Table 1. Patient's characteristics (N = 13)

Patient	Age/Sex	Diagnosis	Conditioning Regimen	Cells Source	Acute GVHD prophylaxis	Acute/ Chronic GVHD	Outcome (days)
1	44/F	CML	CY+BU	PBPC	CsA+MTX	IV/-	125 D
2	32/F	CML	CY+BU	PBPC	CsA+MTX	III/0	248 A
3	25/M	AA	CY	BM	CsA+MTX	0/0	237 A
4	37/M	CML	CY+BU	BM	CsA+MTX	0/0	194 A
5	37/M	AA	CY+BU	BM	CsA+MTX	0/0	115 D
6	42/M	CML	CY+BU	PBPC	CsA+MTX	II/-	39 D
7	32/M	CML	CY+BU	PBPC	CsA+MTX	0/E	383 A
8	43/M	CML	CY+BU	PBPC+BM	CsA+MTX	0/E	148 A
9	30/F	CML	CY+BU	PBPC	CsA+MTX	0/L	100 A
10	30/M	CML	CY+BU	PBPC	CsA+MTX	IV/-	104 D
11	30/M	AML	CY+BU	PBPC	CsA+MTX	0/0	133 D
12	26/M	AA	CY	BM	CsA+MTX	0/0	165 A
13	42/F	AA	CY	BM	CsA+MTX	II/-	70 D

CML = chronic myeloid leukemia; AA = aplastic anemia; AML = acute myeloid leukemia
CY = cyclophosphamide; BU = busulfan; PBPC = peripheral blood progenitor cells; BM = bone marrow; CsA = cyclosporine; MTX = methotrexate; L = limited; E = extensive; D = dead; A = alive

Table 2. Distribution of cytokine gene polymorphisms in recipients and donors of hematopoietic stem cell transplantation

Gene	Genotype	Predicted phenotype	Recipients (N = 13)	%	Donors (N = 13)	%
TNF $^{-308}$	G/G	low	11	84.6	11	84.6
	G/A or A/A	high	2	15.4	2	15.4
IFNG $^{+874}$	A/A	low	3	23.1	2	15.4
	T/A	intermediate	7	53.8	9	69.2
	T/T	high	3	23.1	2	15.4
IL6 ^{174}	C/C	low	0	0.0	1	7.7
	G/G or G/C	high	13	100.0	12	92.3
IL10 $^{-1082, -819, -592}$	ACC/ACC or ACC/ATA or ATA/ATA	low	4	30.8	3	23.1
	GCC/ACC or GCC/ATA	intermediate	6	46.2	6	46.2
	GCC/GCC	high	3	23.1	4	30.8
TGFB1 $^{+869, +915}$	CG/CC or CC/CC or TC/TC or TC/CC	low	0	0	0	0
	TG/CC or CG/CG or TG/TG	intermediate	2	15.4	5	38.5
	TG/TG or TG/CG	high	11	84.6	8	61.5

Cytokine gene polymorphism analysis

Cytokine genotypes were determined blind to the clinical outcome of transplantation for the 13 recipients and their donors. DNA was extracted and purified from whole blood collected in 5% EDTA using the Puregene DNA Isolation Kit (Gentra Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. Donor/recipient genotypes for the *TNF*$^{-308}$, *IFNG*$^{+874}$, *IL6*$^{-174}$, *IL10*$^{-1082,-819,-592}$, and *TGFB1*$^{+869,+915}$ polymorphisms were analyzed by polymerase chain reaction-sequence-specific primer (PCR-SSP) with the Cytokine Genotyping Primers Kit (One Lambda , Canoga Park, CA, USA). All amplifications were performed according to the manufacturer's recommendations. The PCR products were then visualized by electrophoresis in 2% agarose gel. Individuals were grouped into the predicted low, high, or intermediate producer phenotypes for these cytokines according to their genotypes as defined previously.[25-29]

Measurement of serum cytokines

Serum cytokine levels were assessed using commercial Enzyme Linked-Immuno-Sorbent Assay (ELISA) kits for TNF-α, IFN-γ, IL-6, IL-10, and TGF-β1 (BioSource®, Nivelles, Belgium, Europe). Assays were carried out as previously described.[6]

Statistical Analysis

Mean cytokine levels were calculated at different time intervals: preconditioning, post conditioning and weekly after HSCT until 15 weeks, or death or relapse of the patient and expressed as means ± SEM (standard error mean). The cytokine levels were compared with the donor and recipient polymorphisms for these cytokines, using a t-test for independent samples (2-tailed). Statistical analysis was conducted using SPSS software version 10.0 (SPSS Chicago, IL), using $P < 0.05$ as significant.

Results

Clinical outcome

Acute GVHD was seen in five patients: Grade II was recorded in two patients, Grade III in one patient and Grade IV in two patients. Three patients developed chronic GVHD, one with limited GVHD and the other two with extensive GVHD (Table 1). All patients developed bacterial infection and 5 patients presented viral infection and/or the viral disease after transplantation.

Correlation of the cytokine level production following HSCT with the cytokine polymorphisms

We previously reported the cytokine levels among 13 patients who received their transplants from HLA-identical siblings for treatment of hematologic malignancies in our centre.[6] Table 2 demonstrates the distribution of cytokine gene polymorphisms in recipients and donors of HSCT.

In this study, we compared the levels of cytokines with the receptor and donor cytokine genotypes. We found correlation between the levels of IL-6 and IL-10 following HSCT and the *IL6*$^{-174}$ and *IL10*$^{-1082, -819, -592}$ polymorphisms, but not for the other cytokines investigated in this study (Figures 1 and 2). Those with genotypes associated with low production of IL-6 and IL-10 produced lower levels of these cytokines than those with genotypes associated with high and/or intermediate production of these cytokines ($P < 0.05$).

Discussion and Conclusion

It has been hypothesized that there is a correlation between polymorphisms in the *TNF, IFNG*, *IL6*, *IL10*, and *TGFB1* genes and the differential production of the respective cytokines. It has also been shown that some of these polymorphisms affect transplant outcomes.[9-21]

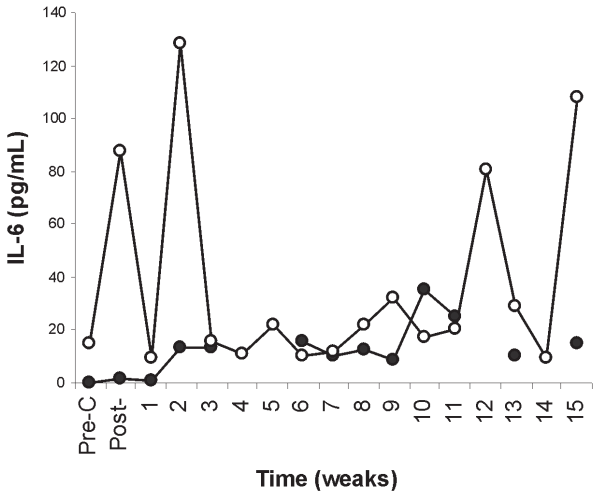

Figure 1. Kinetic profiles of serum IL-6 in patients with genotypes relative to low (closed circles) and high (open circles) production of this cytokine. Cytokine levels were expressed as mean ± SEM. pre-C = preconditioning week; post-C = post-conditioning week.

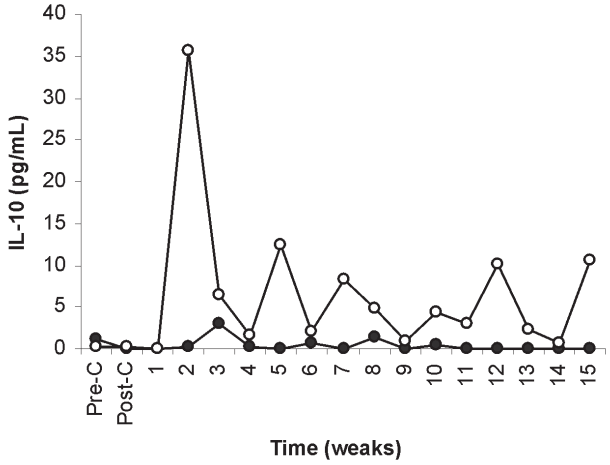

Figure 2. Kinetic profiles of serum IL-10 in patients with genotypes relative to low (closed circles) and high and/or intermediate (open circles) production of this cytokine. Cytokine levels were expressed as mean ± SEM. pre-C = preconditioning week; post-C = post-conditioning week.

In this study, we confirmed this correlation for IL-6 and IL-10 that are considered very important participants in the immune response in transplantation. Interleukin-6 is a pleiotropic cytokine with a central role in the host defense. Although it had been described as a pro-inflammatory cytokine, its anti-inflammatory and immunosupressor properties were recently reported.[30] The role of IL-6 in the differentiation of T-helper lymphocytes has been discussed[31] and a better understanding may clarify its participation in immunology mechanisms observed in the transplant outcome.

Interleukin-10 is an important immunoregulatory cytokine in humans produced by monocytes, macrophages, B cells, T cells and mast cells.[32] It inhibits production of pro-inflammatory cytokines including TNF-α, IL-1, IL-6 and IL-8.[33] Considering that these cytokines play an important role in the pathogenesis of GVHD[8], IL-10 could be an essential regulator of immune response after transplantation.

Due to the special situation of stem cell transplantation, where many cytokines participate and interact, we would suggest the importance of a prospective study involving more patients and other cytokines in stem cell transplantation.

Resumo

Estudos de vários genes candidatos têm demonstrado que polimorfismos genéticos em genes de citocinas contribuem com variações nos níveis de citocinas produzidas e esta variação pode influenciar a ocorrência e gravidade de complicações após o transplante de células-tronco hematopoéticas (TCTH). Neste trabalho comparamos as concentrações séricas de TNF-α, IFN-γ, IL-6, IL-10 e TGF-β1 em 13 receptores seguindo o TCTH com os polimorfismos

*TNF^{-308}, $IFNG^{+874}$, $IL6^{-174}$, $IL10^{-1082,-819,-592}$ e $TGFB1^{+869,+915}$. Os níveis séricos de citocinas foram medidos usando-se kits comerciais de ELISA para TNF-α , IFN-γ , IL-6, IL-10 e TGF-β1 (BioSource®, Nivelles, Belgium, Europe). Os genótipos de doadores/receptores para estes polimorfismos de citocinas foram analisados pela reação em cadeia da polimerase com sequências específicas de primer (PCR-SSP) com o kit Cytokine Genotyping Primers (One Lambda, Canoga Park, CA, USA). Encontramos correlação entre os níveis de IL-6 e IL-10 seguindo o TCTH e os polimorfismos $IL6^{-174}$ e $IL10^{-1082,-819,-592}$, mas não para outras citocinas investigadas neste estudo. Aqueles com genótipos relativos à baixa produção de IL-6 e IL-10 produziram mais baixos níveis destas citocinas que aqueles com genótipos relativos à produção alta e/ou intermediária destas citocinas (P < 0,05). Rev. Bras. Hematol. Hemoter. 2008; **30**(6):475-479.*

Palavras-chave: *Citocina; polimorfismo; GVHD; transplante de células-tronco hematopoéticas.*

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