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ORIGINAL ARTICLE

HCMV gB genotype and its association with cytokine levels in hematopoietic stem cell transplantation

JF Correia-Silva[1], RG Resende[1], TC Arão[1], MHNG Abreu[2], MM Teixeira[3], H Bittencourt[4], TA Silva[1], RS Gomez[1]

[1]*Department of Oral Surgery and Pathology, School of Dentistry, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais;* [2]*Department of Community and Preventive Dentistry, School of Dentistry, Universidade Federal de Minas Gerais, Belo Horizonte;* [3]*Department of Biochemistry and Immunology, Institute of Biological Sciences, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais;* [4]*Hematopoietic Stem Cell Transplantation Unit, Department of Hematology, Hospital das Clínicas, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil*

BACKGROUND: Glycoprotein B (gB) has been implicated in determining the pathogenicity and clinical outcomes of human cytomegalovirus (HCMV) disease.
OBJECTIVE: The purpose of this study was to assess the prevalence of gB genotypes in allogeneic hematopoietic stem cell transplantation (allo-HSCT) and the relationship between it and cytokine levels in saliva and blood samples. The impact of these parameters on patients' survival was also investigated.
METHODS: Samples were obtained from 63 patients receiving an allo-HSCT. HCMV gB genotyping was carried out by multiplex nested PCR. The cytokine levels were assessed using ELISA assay.
RESULTS: A single or mixed genotype infection was detected in the saliva and blood of 36/63 and 52/63 subjects, respectively. Patients with gB2 in their saliva showed lower IL-10 levels in comparison with patients without gB2. Reduced blood levels of IFN-γ and IL-1β were also found in recipients with the HCMV gB4 genotype compared with patients without it. Decreased IL-1β and increased IL-10 blood levels were associated with lower survival. However, HCMV gB genotypes have no impact on patient outcome.
CONCLUSION: Decreased IL-1β and increased IL-10 levels in the blood are associated with lower survival. HCMV genotypes are associated with different cytokine levels in saliva and blood.
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Keywords: human cytomegalovirus; glycoprotein B; cytokines; allo-HSCT

Correspondence: Prof. Ricardo Santiago Gomez, Departamento de Clínica Patologia e Cirurgia, Faculdade de Odontologia, Universidade Federal de Minas Gerais, Av. Antônio Carlos 6627, Pampulha, Belo Horizonte, Minas Gerais 31270-901, Brazil. Tel: +55 31 3409 2477, Fax: +55 31 34092430, E-mail: rsgomez@ufmg.br
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Introduction

Human cytomegalovirus (HCMV) disease remains an important cause of morbidity in immunocompromised patients, especially in allogeneic hematopoietic stem cell transplant (allo-HSCT) recipients (Allice *et al*, 2008). HCMV is a member of the betaherpesviridae subfamily; it has the ability to establish lifelong persistence, latent infection following primary exposure (Nichols and Boeckh, 2000) and alter cellular gene expression (Simmen *et al*, 2001). In addition, HCMV presents a characteristic salivary gland tropism of a prototype betaherpesvirus Mocarski (2002). The large number of immunomodulatory functions encoded by HCMV could contribute to its virulence in immunosuppressed individuals. HCMV infection has an impact on transplant outcome by its effects on the cellular and humoral immune responses, including lymphocyte subpopulations and cytokines (Nordøy *et al*, 2000). The pathogenesis of HCMV infection has been related to an interrelation of viral factors and host immune responses (Pang *et al*, 2008). The virulence of different HCMV strains may be an important factor in the occurrence of HCMV disease (Coaquette *et al*, 2004) because of genetic variation in genes that are involved in host cell penetration, tissue tropism, or replication (Halary *et al*, 2002), and polymorphism in the viral genome may play an important role (Yu *et al*, 2006).

Human cytomegalovirus glycoprotein B (gB) is the major envelope glycoprotein of HCMV, and it is encoded by the *UL55* gene (Rasmussen *et al*, 2003). HCMV gB has been associated with host cell entry, cell-to-cell virus transmission, and fusion of infected cells (Navarro *et al*, 1993; Yu *et al*, 2006). HCMV gB is highly immunogenic in humans and is an important target of both humoral and cellular immune responses (Rasmussen *et al*, 2003).

Based on sequence variation in the UL55 gene, which encodes glycoprotein B (gB), HCMV can be classified

into five gB genotypes (gB1, gB2, gB3, gB4, and gB5) (Chou and Dennison, 1991; Shepp *et al*, 1998). The gB genotypes may be associated with different clinical manifestations or organ tropism (Tarragó *et al*, 2003). Patients with mixed-genotype infections present significantly higher HCMV viral loads than those with single-genotype infections (Pang *et al*, 2008). Co-infection of HCMV with multiple gB genotypes was associated with a higher virus load, a higher prevalence of HCMV disease, and a higher rate of graft rejection in immunocompromised patients, such as HSCT subjects (Coaquette *et al*, 2004). Glycoprotein B (gB) is very important in viral infectivity and in eliciting a protective immune response in patients with HCMV infection (Ludwig *et al*, 2006). The immunological components important for the control of HCMV are not completely understood.

During HCMV disease, several cytokines, chemokines, and adhesion molecules are expressed to recruit inflammatory cells for infection control (Nordøy *et al*, 2000). The deregulation of cytokine production seems to be involved in graft rejection and tissue damage in allo-HSCT (Ferrà *et al*, 1998). In addition, previous studies have indicated that interleukin concentrations may serve as a clinical parameter of the severity of HCMV infection (Zedtwitz-Liebenstein *et al*, 2009).

Studies have attempted to find a correlation between the gB genotype and the occurrence of HCMV-associated disease in immunocompromised patients. Pang *et al* (2008) showed that the viral load was significantly higher in patients with mixed-genotype infections *vs* those with a single-genotype infection in solid organ transplant recipients. However, it remains unclear whether certain gB genotypes or mixed gB genotypes are associated with distinct levels of cytokine production in HCMV infection. Salivary glands harbor latent HCMV infection and evidence suggests that immunosuppression is related to its reactivation. Therefore, a study of the presence and the frequency of gB HCMV genotypes in saliva and its relationship with saliva cytokines concentrations can clarify the HCMV-regulated immune responses and its impact on allo-HSCT patients' survival. The aims of this study were to assess the prevalence of the gB genotypes in saliva and blood of patients who underwent allo-HSCT and to investigate a possible relationship between these genotypes and IL-1β, IL-6, IL-10, IFN-γ, and TNF-α levels. In addition, we also evaluated the impact of gB genotype and cytokine production on patients' survival.

Methods

Patients and samples

The study was approved by the local Ethics Committee of the Universidade Federal de Minas Gerais. Sixty-three allo-HSCT recipients at Hospital das Clínicas of Universidade Federal de Minas Gerais (HC-UFMG) between October 2006 and October 2008 were eligible for and included in this study. Patients were conditioned for allo-HSCT according to specific protocols from the Stem Cell Transplant Unit at HC-UFMG, which varied according to the type and status of disease at the time of transplant. Cyclosporin (CSA) in combination with methotrexate (MTX) or mycophenolate mofetil (MMF) was used for graft *vs* host disease (GVHD) prophylaxis, and methylprednisolone (2 mg kg^{-1}) was used for GVHD treatment. Patient demographics as well as clinical and laboratory data were available through the database of HC-UFMG. Clinical information included underlying primary disease, source of stem cells, gender and age of the patient and donor, recipient and donor HCMV serostatus before transplant, conditioning regimen, HLA matching and HCMV reactivation (tested by pp65 antigenemia). HCMV disease was defined in according with Boeckh *et al* (2003).

Patients were followed until 1 year after allo-HSCT or until the death of the recipient. Saliva and blood samples were simultaneously obtained from each recipient once a week until day +100. Samples collections were previously submitted to HCMV analysis by real time PCR (Correia-Silva *et al*, 2010) and/or pp65 antigenemia assay for HCMV monitoring. Samples from the first positive assay for HCMV were used for gB HCMV genotypes identification and cytokine detection (median of 35 days; range 15–100 days). We attempted to obtain saliva and blood samples simultaneously from each allo-HSCT recipient with HCMV infection for use in multiplex nested PCR (M-nPCR) and enzyme-linked immunosorbent assay (ELISA). For the PCR test, the oral fluid was collected on swabs (Correia-Silva *et al*, 2007) from the normal oral mucosa of subjects using a sterile citobrush (Kolplast Ltda, São Paulo, Brazil), placed immediately in 500 μl of Krebs buffer, and then stored at −20°C until processing. For the ELISA assay, oral fluid was collected on Salivette neutral cotton swabs (Sarstedt, Nümbrecht, Germany). Salivette neutral cotton swabs were placed between the cheek and the gum or under the tongue until they were saturated with oral fluid. The cotton swab was sometimes chewed to stimulate oral fluid production. After collection, the swab was centrifuged to release the oral fluid. Subsequently, the oral fluid was transferred to tubes and diluted (1:1) in PBS (0.4 mM NaCl and 10 mM $NaPO_4$) containing protease inhibitors (0.1 mM PMSF, 0.1 mM benzethonium chloride, 10 mM EDTA and 0.01 mg ml^{-1} aprotinin A), and 0.05% Tween-20, and frozen at −20°C until analysis. Four milliliters of peripheral blood was collected in an EDTA tube for the PCR assay and stored at −20°C until processing. Four milliliters of peripheral blood was collected in anticoagulant-free tubes and centrifuged for 10 min at 800 *g* after being allowed to clot at room temperature for 30 min. The recovered serum was stored in aliquots at −20°C until cytokine quantification.

DNA extraction

Total genomic DNA was extracted from saliva and whole-blood samples using a QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA, USA) and was stored at −20°C until use. Two hundred nanograms of extracted DNA were used for HCMV gB multiplex nested PCR.

Multiplex nested PCR

gB genotypes of HCMV were determined by multiplex nested PCR, using primers published elsewhere (Tarragó *et al*, 2003). PCR was performed in an ABI Master Cycler (Applied Biosystems, Foster City, CA, USA). As a positive control for gB2, a DNA sample from the HCMV AD169 strain was used. Negative controls contained reagents only. The M-nPCR conditions were previously described (Tarragó *et al*, 2003). gB genotypes were determined by comparison of bands with size standards after electrophoresis in a 6.5% polyacrylamide gel and silver staining. The expected band sizes were 420, 613, 190, 465, and 139 bp for HCMV gB1, gB2, gB3, gB4, and gB5, respectively.

ELISA

The concentrations of IL-1β, IL-6, IL-10, IFN-γ, and TNF-α in saliva and blood were determined using commercially available quantitative sandwich enzyme linked immunosorbent assay (ELISA) kits (DuoSet, R&D Systems, Minneapolis, MN, USA), according to the manufacturer's instructions. The detection ranges were 3000–46 pg ml^{-1} for IL-1β, 600–9 pg ml^{-1} for IL-6, 4000–62 pg ml^{-1} for IL-10, 1000–15 pg ml^{-1} for IFN-γ, and 1000–15 pg ml^{-1} for TNF-α. Values below the detection limits were assumed to be zero. Concentrations were expressed as pg ml^{-1} for blood. Total protein in the saliva samples was measured using the Bradford method (Sigma, Saint Louis, MO, USA) compared with the BSA standard (Fermentas Life Sciences, Vilnius, Lithuania), and concentrations were expressed in mg ml^{-1}. Total protein concentration was used to correct saliva cytokine (IL-1β, IL-6, IL-10, IFN-γ, and TNF-α) values for each sample. Saliva sample values corrected for total proteins were expressed in pg mg^{-1} protein.

Statistical analysis

Univariate analyses were performed using the Mann–Whitney and Kruskal–Wallis tests. Time from transplant until death was displayed using the mean of the Kaplan–Meier method, and results were initially compared using the log-rank test. Variables showing $P < 0.25$ were included in a multivariate Cox proportional hazards model analysis. The records of each patient were reviewed 12 months after allo-HSCT to evaluate survival. Statistical analyses were performed using SPSS software (SPSS Inc., version 16.0, Chicago, IL, USA), and a P value ≤ 0.05 was considered statistically significant.

Results

The demographic characteristics of the 63 allo-HSCT patients are summarized in Table 1. A single or mixed genotype infection was detected in the saliva and blood of 36 and 52 subjects, respectively. The gB5 genotype was not detected in any of the samples. gB2 was the most common genotype in the saliva (19/36) and blood (33/52) samples. A single-genotype infection with gB1, gB2, gB3, or gB4 in the saliva was detected in 2/63 (3.0%), 11/63 (17.0%), 6/63 (9.5%), and 1/63 (1.5%) of the samples, respectively. A single-genotype infection with gB1, gB2, gB3, or gB4 in the blood was detected in 1/63 (1.5%), 14/63 (22.2%), 5/63 (7.9%), and 3/63 (4.5%) of the samples, respectively. A mixed-genotype infection was detected in 16/63 (25.4%) saliva samples and in 29/63 (46.0%) of blood specimens. Of these 16 mixed-genotype infections in saliva, 12 (19%) were dual gB genotype infections, three (4.5%) were three genotype infections and one (1.5%) showed four genotype infections. Of these 29 mixed-genotype infections in blood, 20 (31.7%) were dual gB genotype infections and nine (14%) presented three simultaneous genotype infections. Evaluating only samples with the presence of the gB genotype, infection with a mixed HCMV gB genotype was more often detected in blood (29/52–55.7%) than in saliva (16/36–44.4%) (Table 2).

Table 1 Demographic characteristics of the 63 allo-HSCT patients

Parameters	*Total (n = 63) (%)*
Median age in years (range)	30 (05–56)
Recipient/donor gender	
Male/male	16 (25.4)
Female/female	17 (27.0)
Male/female	11 (17.5)
Female/male	18 (28.6)
Recipient gender	
Male	35 (55.6)
Female	28 (44.4)
Donor gender	
Male	33 (52.4)
Female	29 (46.0)
Primary disease	
Malignant	42 (66.7)
Non-malignant	21 (33.3)
Stem cell source	
Blood stem cell	26 (41.3)
Bone marrow	37 (58.7)
HLA matching	
Matched related	53 (84.1)
Mismatched unrelated	5 (7.9)
Matched unrelated	5 (7.9)
HCMV serology status recipient/donor	
Positive/positive	50 (79.4)
Positive/negative	8 (12.7)
Negative/positive	2 (3.2)
Negative/negative	3 (4.8)
pp65 antigenemia	
Positive	40 (63.5)
Negative	19 (30.1)
HCMV disease	
Presence	3 (5)
Absence	60 (95)

HLA, human leucocyte antigen.

Table 3 shows cytokine levels in saliva and blood along with the presence or absence of HCMV gB genotypes. Patients with the HCMV gB2 genotype in the saliva ($n = 16$) showed lower IL-10 saliva levels compared with patients without this genotype ($n = 32$) ($P = 0.02$). IFN-γ and IL-1β levels in the blood of patients with the HCMV gB4 genotype ($n = 18$) were significantly lower than in individuals without this genotype ($n = 45/P = 0.04$ and $n = 43/P = 0.05$, respectively). There was no difference on IL-1β, IL-6,

Table 2 Comparison of HCMV gB genotypes prevalence in saliva and blood

Genotype classification	*Genotype(s) determined*	*No. patients genotyped*	
		Saliva	*Blood*
1	gB1	2	1
2	gB2	11	14
3	gB3	6	5
4	gB4	1	3
5	gB1 + gB2	1	6
6	gB1 + gB3	1	1
7	gB1 + gB4	5	8
8	gB2 + gB3	2	3
9	gB2 + gB4	2	2
10	gB3 + gB4	1	0
11	gB1 + gB2 + gB3	1	5
12	gB1 + gB2 + gB4	1	3
13	gB1 + gB3 + gB4	1	1
14	gB2 + gB3 + gB4	0	0
15	gB1 + gB2 + gB3 + gB4	1	0
–	Total	36	52

IFN-γ or TNF-α levels among patients who were gB negative, who had one gB genotype, or who had the simultaneous presence of two, three, or four gB genotypes (data not shown). However, patients with one or more than one gB genotype ($n = 26$) showed decreased levels of IL-10 in the saliva compared with recipients without the gB genotype ($n = 22$) ($P = 0.02$) (data not shown).

Thirty-eight of 63 patients (60%) were alive 1 year after allo-HSCT. The median survival of the 25 deceased patients after allo-HSCT was 68 days (range 17–240). Survival according to gB genotype and cytokine level is shown in Table 4. In univariate analysis, gB genotypes showed no effect on patients' survival. Only the IL-1β blood level was associated with survival rate 1 year after allo-HSCT in univariate analysis ($P = 0.009$). Multivariate analysis showed a reduced risk of death for patients with higher IL-1β blood levels (HR: 0.207; 95% CI 0.072–0.590; $P = 0.003$) and an increased risk of death for patients with higher IL-10 blood levels (HR: 2.813; 95% CI 1.117–7.086; $P = 0.03$) (Figure 1).

Discussion

The gene encoding gB has been detected as a highly polymorphic locus that can be clustered in different genotypes in clinical isolates (Chou and Dennison, 1991; Shepp *et al*, 1998; Landolfo *et al*, 2003). Previous reports suggested that HCMV gB genotypes are a determinant for viral virulence and pathogenesis (Pang *et al*, 2008; Manuel *et al*, 2009).

Virulence among HCMV strains could differ because of genetic variation in the gene UL55 that codifies glycoprotein B, which is implicated in host cell penetration, tissue tropism, and replication and is a target for both the humoral and the cellular immune response (Halary *et al*, 2002; Ludwig *et al*, 2006). In patients with HIV disease, several studies showed predominance of the gB2 genotype (Meyer-König *et al*, 1998). Studies with renal transplant patients and with other solid-organ transplant recipients showed that HCMV gB1 is the most frequent common genotype identified (Manuel *et al*, 2009; Nogueira *et al*, 2009). In HSCT recipients, gB3 was found less frequently than gB1 and gB2 (Vogelberg *et al*, 1996). However, another study found that gB type 2 virus was less frequent than gB type 3 (Fries *et al*, 1994). Differences in genotype frequencies may be attributed to variation in geographical distribution of HCMV genotypes in immunocompromised patients (Zipeto *et al*, 1998; Coaquette *et al*, 2004). Another possible explanation for gB genotype prevalence could be attributed to HCMV cell or tissue tropism given that studies had used different source of cells or tissues such as blood, bronchoalveolar lavage, biopsies, swabs, and urine (Vogelberg *et al*, 1996; Meyer-König *et al*, 1998; Nogueira *et al*, 2009). In this study, gB2 was the predominant genotype in the saliva and blood of allo-HSCT patients. The same prevalence of gB2 genotype could be attributed to a similar kinetics of HCMV infection observed in saliva and in blood reported previously by our research group (Correia-Silva *et al*, 2010). However, infection with a mixed HCMV gB genotype was detected more often in blood than in saliva. Therefore, in transplant recipients, HCMV infection may be caused by a variety of gB genotypes, including the patient's latent virus, as well as donor-acquired strains of HCMV (Pang *et al*, 2008). HCMV in saliva samples may represent the patient's own pretransplant latent virus, while the gB genotypes in blood samples may also be a latent virus reactivation experienced by the donor. However, further studies are necessary to confirm such assumptions. Despite a higher correlation between HCMV load in saliva and in blood samples of allo-HSCT recipients, the viral load in saliva tended to be lower than that in blood (Correia-Silva *et al*, 2010). A higher HCMV level and delayed viral clearance has been associated with mixed gB HCMV genotype infections (Pang *et al*, 2008; Manuel *et al*, 2009). In addition, it has been suggested that infection with mixed genotypes increases the risk of progression to HCMV disease (Tarragó *et al*, 2003; Coaquette *et al*, 2004). However, our data do not support the hypothesis that a specific genotype or mixed genotypes have an impact on allo-HSCT patient's survival. On the other hand, as gB genotypes influence cytokine levels, it may indirectly affect patient's survival.

Immune mechanisms involved in protection from HCMV reactivation or reinfection are complex and are far from being fully understood (Ludwig *et al*, 2006). HCMV might cause different immunological changes in immune function during and after recovery from viral infection. HCMV may modulate immunological status of host cells by altering expression of cellular genes coding for pro-inflammatory proteins and inducing local production of pro-inflammatory cytokines such as TNF-α, IL-1, IL-6, and IL-8 (Cinatl *et al*, 1999; Nickel *et al*, 2009). HCMV disease in solid organ transplant patients has been associated with increase of anti-inflamatory cytokine (IL-10) release (Jayaraman *et al*, 1993; Cervera *et al*, 2007; Sadeghi *et al*, 2007).

Table 3 Cytokine levels according to HCMV gB genotypes in saliva and blood samples of allo-HSCT patients

	gB1			*gB2*			*gB3*			*gB4*		
	Absence	*Presence*	P-*value*[a]	*Absence*	*Presence*	P-*value*[a]	*Absence*	*Presence*	P-*value*[a]	*Absence*	*Presence*	P-*value*[a]
Saliva (pg mg^{-1} protein)												
IFN-γ												
N	28	8	0.070	25	11	0.565	31	5	0.163	29	7	0.505
Median	5	0		2	0		0	0		0	0	
Range	0–717	0–8		0–609	0–717		0–717	0–11		0–717	0–164	
IL1-β												
N	33	10	0.107	31	12	0.947	37	6	0.262	35	8	0.592
Median	9	29		11	10		10	33		10	14	
Range	0–296	0–143		0–296	0–148		0–296	0–148		0–297	0–144	
IL-6												
N	35	11	0.722	33	13	0.278	39	7	0.590	37	9	0.207
Median	1	3		1	23		2	0		1	24	
Range	0–646	0–244		0–646	0–560		0–646	0–560		0–646	0–244	
IL-10												
N	37	11	0.235	32	16	**0.023***	41	7	0.422	39	9	0.603
Median	64	19		104	18		64	43		64	20	
Range	0–558	0–324		0–558	0–80		0–558	0–324		0–558	0–324	
TNF-α												
N	36	11	0.641	34	13	0.264	40	7	0.354	38	9	0.989
Median	0	0		0	0		0	0		0	0	
Range	0–111	0–20		0–111	0–44		0–111	0–2		0–111	0–21	
Blood (pg ml^{-1})												
IFN-γ												
N	38	25	0.227	30	33	0.479	48	15	0.340	45	18	**0.040***
Median	109	9		42	110		86	2		107	0	
Range	0–1507	0–2063		0–1507	0–2063		0–2063	0–1458		0–2063	0–937	
IL1-β												
N	36	25	0.547	29	32	1.000	46	15	0.712	43	18	**0.050***
Median	19	11		15	18		17	15		24	8	
Range	0–77	0–102		0–77	0–102		0–59	0–102		0–102	0–48	
IL-6												
N	37	24	0.266	30	31	0.223	46	15	0.699	43	18	0.436
Median	31	18		31	18		31	18		18	43	
Range	0–4193	0–2016		0–4193	0–1821		0–4193	0–856		0–4193	0–2016	
IL-10												
N	36	24	0.737	28	32	0.841	45	15	0.831	43	17	0.289
Median	0	0		0	0		0	0		0	0	
Range	0–5954	0–934		0–5954	0–1352		0–1352	0–5954		0–5954	0–362	
TNF-α												
N	37	25	0.238	30	32	0.904	47	15	0.993	44	18	0.167
Median	138	83		100	145		107	102		161	92	
Range	0–1364	0–862		0–1364	0–862		0–1364	0–1244		0–1364	337	

[a]Mann–Whitney test.
*Significance values.

Patients with the HCMV gB2 genotype in saliva samples showed lower IL-10 levels in saliva in comparison with patients without this genotype. In line with these findings, the reduced levels of the IL-10 may be associated with an effective cell-mediated immunity to HCMV in patients with the gB2 genotype (Essa *et al*, 2009). It is interesting to note that patients with the gB2 genotype died of infections associated with neutropenia less frequently when compared with subjects with the HCMV gB4 genotype (Torok-Storb *et al*, 1997). IL-10 is produced by multiple cell types, including T and B cells, monocytes, macrophages, and keratinocytes (Spits and de Waal Malefyt, 1992). IL-10 has been known for its potent anti-inflammatory effect and for its contribution to the immunosupressive properties of HCMV (Sadeghi *et al*, 2007). In this study, we also observed that IL-10 levels in saliva specimens were significantly higher in patients without the gB genotype than in recipients with one or more distinct genotypes. These results suggest that the presence of one or more different genotypes is associated with decreased anti-inflammatory response.

Patients positive for the HCMV gB4 genotype in the blood had reduced levels of circulating IFN-γ and IL1-β in comparison with patients without the gB4 genotype. A previous study indicated that HCMV genotypes such as types 3 and 4 are capable of escaping immune recognition in the marrow and therefore persist to cause more damage (Torok-Storb *et al*, 1997). In keeping with this, our result suggests that HCMV gB4 genotype elicits lower levels of protective immune responses mediated using IFN-γ and IL1-β. In addition, we observed that low levels of IL-1β in the blood are associated with increased risk of death after allo-HSCT. Finally, reduced levels of IFN-γ were reported to be involved

Table 4 Survival according to gB genotype and cytokine levels in saliva and blood as determined using uni- and multivariate analysis

Saliva			*Blood*		
Parameters	*Univariate* P-value	*Multivariate – HR (95% CI) and* P-*value*	*Parameters*	*Univariate* P-*value*	*Multivariate – HR (95% CI) and* P-*value*
gB genotypes			gB genotypes		
gB1 absence	0.37	–	gB1 absence	0.06	–
gB1 presence			gB1 presence		
gB2 absence	0.78	–	gB2 absence	0.92	–
gB2 presence			gB2 presence		
gB3 absence	0.44	–	gB3 absence	0.21	–
gB3 presence			gB3 presence		
gB4 absence	0.82	–	gB4 absence	0.06	–
gB4 presence			gB4 presence		
gB genotypes number			gB genotypes number		
Absence	0.78	–	Absence	0.55	–
One or more genotypes			One or more genotypes		
gB genotypes number			gB genotypes number		
Absence	0.73	–	Absence	0.25	–
One genotype			One genotype		
Multiple genotypes			Multiple genotypes		
IFN-γ (pg mg^{-1} protein)			IFN-γ (pg ml^{-1})		
0[a]	0.14	–	≤55*	0.26	–
>0[a]			>55*		
IL-1β (pg mg^{-1} protein)			IL-1β (pg ml^{-1})		
≤10[a]	0.75	–	≤16*	**0.009***	**0.207 (0.072–0.590)** $P = 0.003$*
>10[a]			>16*		
IL-6 (pg mg^{-1} protein)			IL-6 (pg ml^{-1})		
≤1[a]	0.41	–	≤28*	0.41	–
>1[a]			>28*		
IL-10 (pg mg^{-1} protein)			IL-10 (pg ml^{-1})		
≤58[a]	0.26	–	0*	**0.07***	**2.81 (1.12–7.09)** $P = 0.03$*
>58[a]			>0*		
TNF-α (pg mg^{-1} protein)			TNF-α (pg ml^{-1})		
0[a]	0.69	–	≤107*	0.81	–
>0[a]			>107*		

[a]Median cytokine levels.
*Significance values.

with reduced cell-mediated immunity to HCMV and development of viral infection (Essa *et al*, 2009).

In multivariate analysis, we observed that decreased IL-1β and increased IL-10 levels in the blood decrease survival of allo-HSCT patients. Recently, an association between decreased pro-inflammatory cytokines and increased anti-inflammatory cytokines with reduced cell-mediated immunity to HCMV has been demonstrated (Essa *et al*, 2009). Our study gives further strength to the theory that the imbalance of inflammatory response is not desirable for allo-HSCT patients.

Salivary glands may be important reservoir of HCMV (Correia-Silva *et al*, 2010). Further studies are necessary to delineate the contribution of HCMV gB genotyping in saliva for monitoring HCMV disease. Ours findings show that HCMV gB genotypes are associated with distinct cytokine levels and immune response

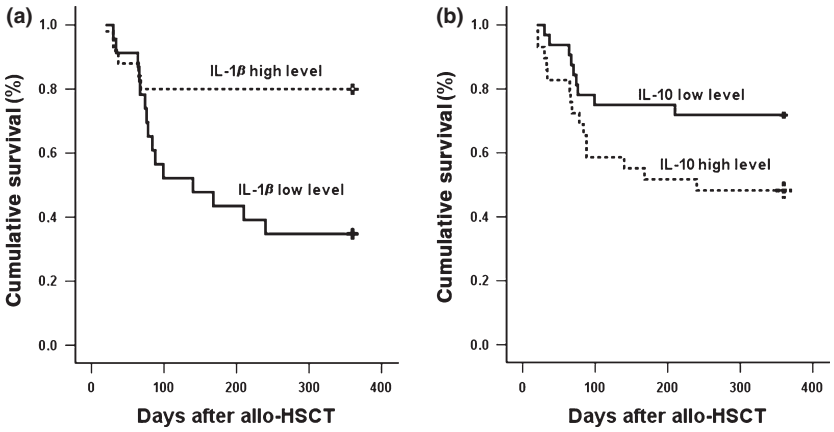

Figure 1 One-year overall survival according to IL-1β and IL-10 blood levels. Kaplan–Meier estimate of overall survival after allogeneic hematopoietic stem cell transplantation (allo-HSCT). (**a**) IL-1β levels in blood (High level, >16 mg ml^{-1}; Low level, ≤16 mg ml^{-1}). (**b**) IL-10 levels in blood (High level, >0 mg ml^{-1}; Low level, 0 mg ml^{-1})

Conclusion

In conclusion, the HCMV gB2 genotype is the most prevalent gB HCMV genotype in saliva and blood samples of patients who underwent allo-HSCT. We observed association between cytokine levels in saliva and blood of patients with different gB genotypes and overall survival.

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Author contributions

J.F. Correia-Silva, H. Bittencourt, T.A. Silva, R.S. Gomez designed the study concept. J.F. Correia-Silva, R.G. Resende, T.C. Arão responsible for data acquisition. J.F. Correia-Silva, M.H.N.G. Abreu, M.M. Teixeira, H. Bittencourt, T.A Silva, R.S Gomez analyzed the data. J.F. Correia-Silva, M.H.N.G. Abreu, H. Bittencourt analyzed the statistical data. J.F. Correia-Silva prepared the manuscript. J.F. Correia-Silva, M.H.N.G. Abreu, H. Bittencourt, T.A. Silva, R.S. Gomez reviewed the manuscript.

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